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IMMUNOLOCALISATION OF SEROTONIN, GASTRIN, SOMATOSTATIN AND GLUCAGON IN ENTERO-ENDOCRINE CELLS OF THE GOOSE (*ANSER ANSER*)

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The processes of digestion in the avian gastrointestinal tract depend on sophisticated control systems that co-ordinate secretion of digestive juices and movement of the luminal contents. In the current study, the distribution of serotonin-, gastrin-, glucagon- and somatostatin-immunoreactive endocrine cells was investigated by immunocytochemical methods in the intestinal tract of the goose. The number of cells immunoreactive for each antiserum was evaluated in different regions of the intestinal tract. Serotonin-, glucagon- and somatostatin-immunoreactive endocrine cells were seen throughout the intestinal tract, but somatostatin-immunoreactive cells were not detected in the colon of the goose. Gastrin-immunoreactive cells were detected only in the duodenum, jejunum and colon mucosa. It is concluded that the distribution pattern of the entero-endocrine cells in the goose is similar to that of most of the mammalian and other poultry species.

Key words: Serotonin, gastrin, somatostatin, glucagon, intestine, goose

The endocrine cells of the digestive system and their chemical products constitute a very complicated system. The physiological function of this system is to regulate all the processes related to digestion and resorption of food (Grube, 1986). The recent developments of immunocytochemical procedures and the specific antibodies raised against peptides have led researchers to revealing the existence of different endocrine cells in the intestine, each of which synthesises and stores a distinct peptide hormone or biogenic amine.

The distribution of endocrine cells in the gastrointestinal system has been widely investigated in the gastrointestinal tract of many domestic (Capella and Solcia, 1972; Domeneghini and Castaldo, 1981; Peranzi and Lehy, 1984; Ceccarelli et al., 1985, 1987, 1990; Mimoda et al., 1998) and wild animals (Krause et al., 1985). The distribution of serotonin-immunoreactive cells has previously

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been studied in the proventriculus and intestinal tract of many avian species (Rawdon and Andrew, 1981; Usellini et al., 1983; Watanabe et al., 1987; Rawdon and Andrew, 1994). For instance, gastrin and somatostatin have been demonstrated in endocrine cells of the antrum mucosa (Wu et al., 1992). Also, serotonin (5HT) immunoreactivity has been detected in both adrenaline and noradrenaline cells of the chicken adrenal medulla (Ohmori et al., 1997).

Almost all gut endocrine cells are typically elongated with basal granules and termed open cells because of the presence of a tuft of microvilli on the apical surface, devoted to the recognition of chemical information from the gut content. Closed endocrine cells of round shape, lacking any luminal connection, are also present in the gastrointestinal tract (Fujita and Kobayashi, 1978). The present study was conducted by immunocytochemistry to determine the distribution and relative frequency of endocrine cells in the intestinal tract of the goose.

Materials and methods

In this study, ten 4-week-old geese (*Anser anser*) were used. Small pieces of tissues were dissected from the duodenum, jejunum, ileum, caecum and colon of these geese, immediately after they were sacrificed. Samples were fixed in Bouin's solution and then routinely processed for embedding in paraffin. Tissue blocks were cut into 6-micrometer-thick sections. The endogenous peroxidase and nonspecific binding sites for antibodies were suppressed by treating the sections with 0.5% hydrogen peroxide for 30 min and 10% normal rabbit serum for 10 min at room temperature, respectively. Furthermore, sections were processed for a standard immunocytochemical technique using peroxidase-antiperoxidase (PAP) method (Hsu et al., 1981). Antiserum specificity was determined in control experiments in which the primary antiserum was either omitted or preabsorbed with an excess of primary antiserum plus its own negative control. Tissue sections from the intestinal tract of cattle known to contain the hormones studied in the present work were used as positive control. The sections were incubated in primary antisera to serotonin, gastrin and glucagon (1:40 dilution, Signet, Dedham, MA) and somatostatin (1:100 dilution, Dako, Carpinteria, CA) for 1 h at room temperature. All the dilutions were made in PBS containing bovine serum albumin (2.5%) and Triton X-100 (0.2%). Subsequently, the binding of primary antiserum was detected using goat anti-rabbit antisera (1:100) and PAP complex (1:250, both from Zymed, San Francisco, CA). Finally, the chromogen protocol was used to reveal the distribution of bound peroxidase (Shu et al., 1988). The relative frequency was determined by the occurrence of the immunoreactive endocrine cells against that of the cells with the highest population in the sections. The number of immunoreactive cells in each antiserum used was evaluated and data were presented as 0.2 mm^2 of mucosa (Table 1).

Results

The presence of both closed and open entero-endocrine cells was determined throughout the intestinal tract of the goose and their number tended to decrease from duodenum to colon. All of them were immunoreactive for serotonin, somatostatin, gastrin and glucagon. The distribution and frequency of immunoreactive entero-endocrine cells varied among the four antisera examined. The immunoreactive endocrine cells were of either 'open' or 'closed' type. Open cells were triangular-, spindle- or flask-shaped, and they had luminal connection, whereas closed cells were round- or triangular-shaped and they lacked any luminal connection. Closed cells were distributed over the epithelium of both the crypts and the villi, mainly concentrated at the bases of the crypts. No immunoreactive endocrine cells were seen in the controls.

Although serotonin-positive immunoreactive cells were present throughout the intestinal mucosa, they were mainly localised in the basal third of the caecal mucosa. Serotonin-immunoreactive cells were of both open and closed types in the intestinal mucosa (Fig. 1), but they were only closed type in the caecal and jejunal mucosa.

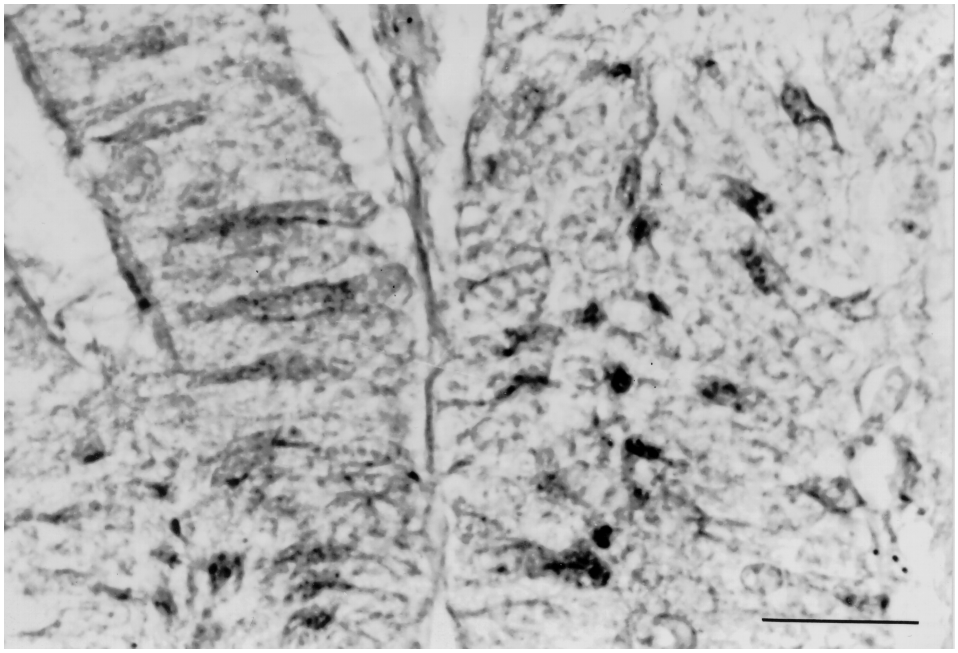

Fig. 1. High-magnification photomicrograph of duodenum immunostained using antibodies to serotonin. Serotonin-immunoreactive endocrine cells in the duodenum. Bar: 50 µm

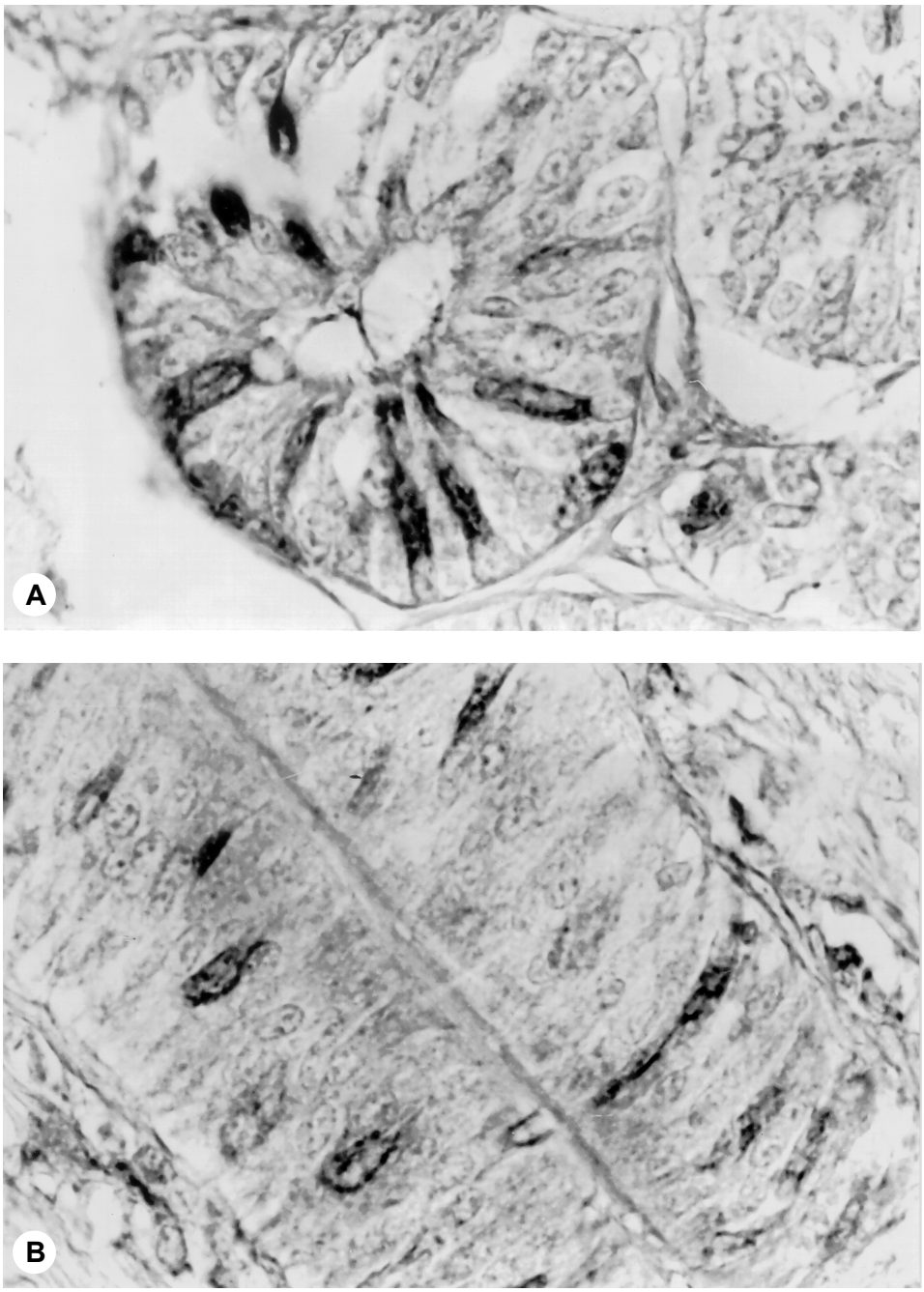

Fig. 2. High-magnification photomicrographs of a section of duodenum (A) and jejunum (B) to show somatostatin-immunoreactive endocrine cells in the intestinal tract. Bar: 50 μm

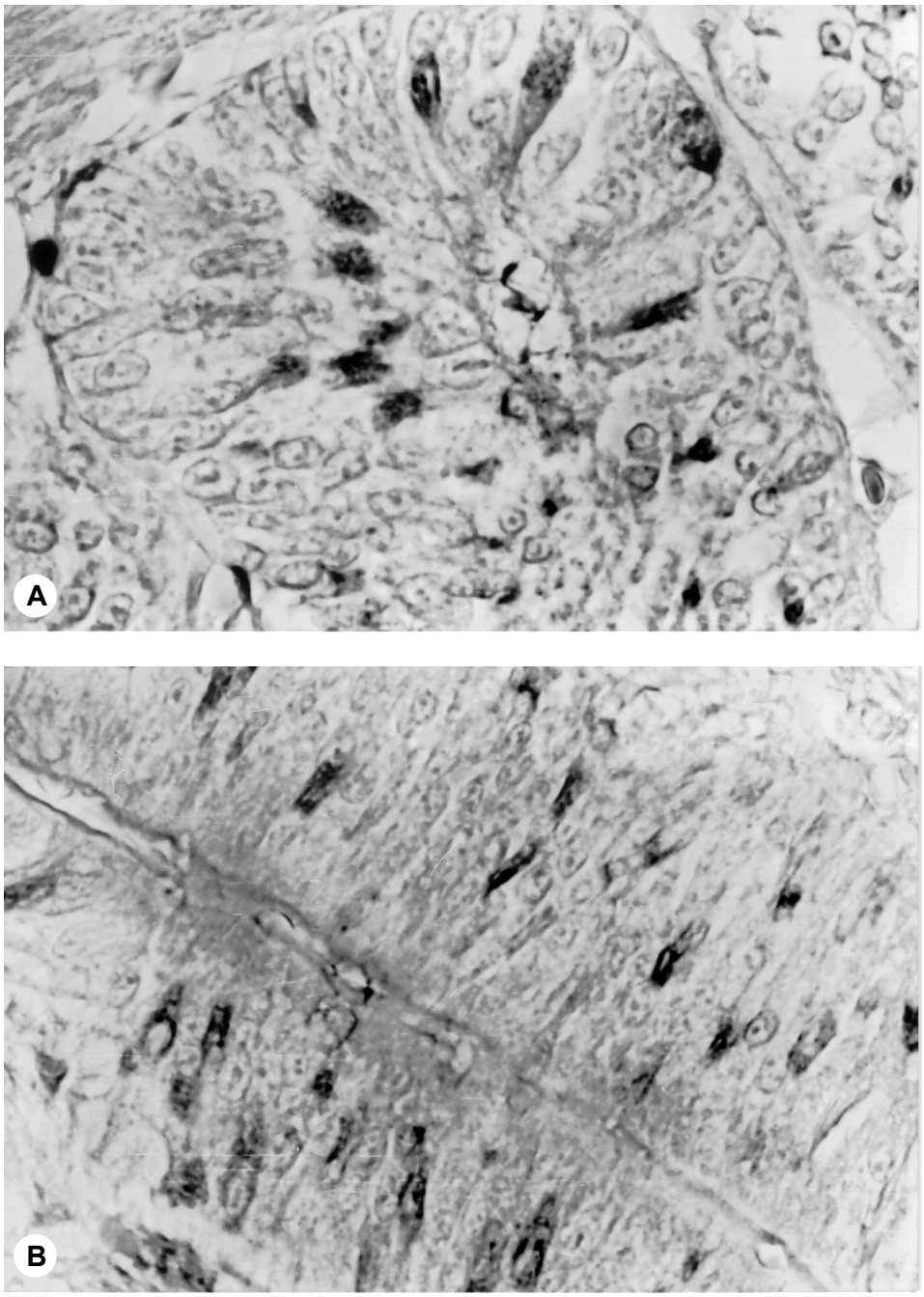

Fig. 3. High-magnification photomicrographs of gastrin-immunoreactive endocrine cells in the intestinal tract. (A) duodenum, (B) jejunum. Bar: 50 μm

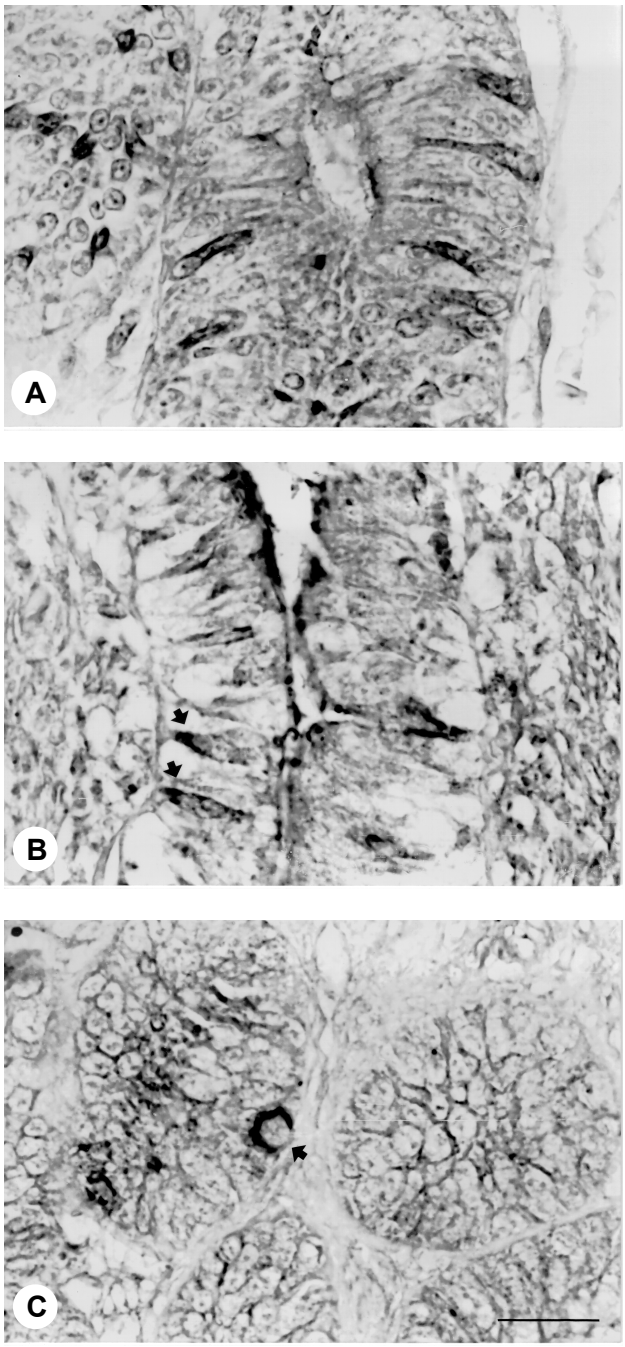

Fig. 4. High-magnification photomicrograph of duodenum (A), caecum (B) (arrows) and colon (C) (arrows) immunostained using antibodies to glucagon. Glucagon-immunoreactive endocrine cells in the intestinal tract. Bar: 50 µm

Somatostatin-immunoreactive cells were present throughout the intestinal mucosa except in the colon, while the small intestinal mucosa had both open and closed type cells (Figs 2A and 2B).

The caecum had closed type cells, particularly localised in the basal third of glands. Gastrin-immunoreactive cells were found in the mucosa of the duodenum, jejunum and colon. They were of both open and closed types (Figs 3A and 3B). Closed type cells were rarely found in the colon.

Glucagon-immunoreactive cells were seen throughout the intestinal mucosa. These cells were scattered in the jejunum and ileum, whereas they were mainly localised in the basal mucosa of the duodenum and large intestine. Glucagon-positive cells were of both open and closed types in the small intestinal mucosa (Figs 4A, 4B and 4C), while they were only of closed type in the large intestine (Figs 4B and 4C).

Table 1

Distribution and relative frequency of endocrine cells in the intestine tract of geese. The frequency of immunoreactive cells was classified subjectively into the following four grades: '–': not detectable; '+': 1–10 positive cells; '++': 10–25 positive cells; '+++': more than 25 positive cells

Cell type	Duodenum	Jejunum	Ileum	Caecum	Colon
Serotonin	+++	++	++	+	+
Somatostatin	+++	+++	++	+	–
Gastrin	+++	+++	–	–	+
Glucagon	+++	++	++	++	+

Discussion

In the present study, entero-endocrine cells immunoreactive for serotonin, somatostatin, gastrin and glucagon were identified in the intestinal tract of geese. Immunocytochemical studies on the intestinal tract of many avian species have revealed a range of endocrine cell types similar to that of mammals. Although different types of endocrine cells are detected in the small intestine, both variety and number are significantly reduced in the large intestine (Ceccarelli et al., 1995; Baltazar et al., 1998). In our study, we demonstrated that open type endocrine cells possessing luminal contact through their apical cytoplasmic process were observed throughout the intestinal tract of the goose while few closed type cells were noticed in the large intestine confirming those previous findings.

Serotonin-immunoreactive cells were detected throughout the intestine of the goose. A similar distribution has been described in mammals (Rawdon, 1984; Ceccarelli, 1995; Baltazar et al., 1998; Dall'Aglio et al., 1998). It has also been demonstrated that serotonin-immunoreactive cells are sparsely distributed in the proventriculus and the pyloric region of the stomach but are abundant in the in-

testine of newly hatched chicks (Polak et al., 1974; Rawdon and Andrew, 1994). Additionally, Pentilla (1968) found no enterochromaffin cells in the proventriculus and gizzard of the early chick embryo, but from 14 to 15 days of incubation such cells were seen in the small and large intestines. It has also been demonstrated that enterochromaffin cells are more common in the colon (rectum) than in the duodenum of the duck and three sparrows (Rutschke, 1976). In mammals, unlike birds, enterochromaffin cells are abundant in the pyloric region of the stomach and fairly common in the small and large intestine (Portela-Gomes et al., 1984). Interestingly, peptides in some of the endocrine cells are co-stored with serotonin (Usellini et al., 1983). The number of cells exhibiting co-storage is maximal at 17–18 days of incubation, but subsequently declines right before hatching (D'Este et al., 1986). It may be proposed that serotonin and neuropeptides are selectively secreted with adrenaline and/or noradrenaline from the adrenal medullary cells of the chicken (Ohmori et al., 1997).

In the present study, along with the findings of Ceccarelli et al. (1995) and Dall'Aglio et al. (1998), some entero-endocrine cells (primarily somatostatin immunoreactive cells) showed cytoplasmic processes that coursed along the basal membrane and made contact with neighbouring cells. These findings are also in agreement with the previous results described in the opossum (Krause et al., 1985) and other domestic mammals (Kitamura et al., 1984; Ceccarelli et al., 1995; Dall'Aglio et al., 1998). Somatostatin-immunoreactive cells were present throughout the intestinal mucosa of the goose. Their population decreased to a few in the duodenum and jejunum, while they were absent in the colon of the chicken, horse, carabao and wild boar (Rawdon, 1984; Ceccarelli et al., 1995; Baltazar et al., 1998; Dall'Aglio et al., 1998). Somatostatin-immunoreactive cells showed restricted distribution in the abomasum, duodenum and jejunum of the carabao (Baltazar et al., 1998). Immunocytochemical studies demonstrated that somatostatin-immunoreactive cells were present in the colon of the monkey and in the rectum of the cat and dog (Peranzi and Lehy, 1984).

Our findings are in general agreement with the data reported in most of the avian and mammalian species in terms of gastrin-secreting cell distribution. Cells reacting with antiserum to gastrin were present throughout the intestinal tract except in the caecum of quail and chicken (Polak et al., 1974). Gastrin-immunoreactive cells were detected in the mucosa of the small intestine and were absent from the large intestine of chicken, horse and carabao (Salvi and Renda, 1986; Ceccarelli et al., 1995; Baltazar et al., 1998). Additionally, the number of gastrin-positive cells tended to decrease from the small to the large intestine in wild boar (Dall'Aglio, 1998). However, we found a few gastrin-immunoreactive cells in the colon of the goose.

Glucagon-immunoreactive cells were predominantly localised in the duodenum, jejunum and ileum of the goose. They were not found in the colon or caecum of quail, chicken and wild boar (Polak et al., 1974; Rawdon, 1984; Dall'Aglio et

al., 1998). Immunocytochemical studies have shown that glucagon-immunoreactive cells are present in the stomach and in the intestine of chicken (Andrew, 1976; Usellini et al., 1983). Glucagon antiserum stained only a few cells in the mucosa of the colon (Peranzi and Lehy, 1984; Ceccarelli et al., 1995), rectum (Peranzi and Lehy, 1984) and caecum of horse, carabao, boar, cat, dog and monkey (Baltazar et al., 1998). Glucagon antiserum cross-reacted with both the pancreatic and intestinal glucagon-immunoreactive cells (Baltazar et al., 1998).

The intestine is reported to be one of the main targets of serotonin, which strongly stimulates the smooth musculature of the gut to release exocrine secretion (Furness and Costa, 1982; Fujita et al., 1988). It is possible that gastrin and somatostatin may co-operate or completely substitute serotonin in the control of the above-mentioned important functions. Throughout the intestine, somatostatin may intervene in regulatory phenomena connected with metabolism, permeability, vascular absorption and secretion (Keast et al., 1985). Somatostatin also suppresses the release of other hormones such as gastrin (Junqueira and Carneiro, 1992). Therefore, the distribution of somatostatin-immunoreactive cells in the intestinal tract of the goose might be due to these functions. The lack of enterochromaffin cells in the large intestine requires further explanation. It would also be necessary to know more about the types and distribution of nervous terminals in the intestinal tract. Our findings suggest that the expression and/or mechanisms for control of expression of regulatory peptides might differ not only between somatic and endocrine cells but also among animal species.

We are aware of the fact that it cannot be absolutely certain that mammalian-derived antibodies cross-react with goose counterparts without isolating antibodies from goose. However, based on the following well-established facts, we used peptide-specific antibodies isolated from mammalian species in the current study. First, there is very high amino acid sequence homology between mammalian-derived hormones used in our study and their avian counterparts (Rawdon, 1984; Bernard et al., 1997; Bossis and Porter, 2001). Secondly, previous studies demonstrated that mammalian-derived antibodies, such as rabbit anti-somatostatin and rabbit anti-gastrin as used in our investigation, cross-reacted with their avian counterparts (Polak et al., 1974; Salvi and Renda, 1989; Rawdon and Andrew, 1994). In conclusion, the pattern of distribution of entero-endocrine cells in the goose overlaps with that of most of the mammalian and other poultry species.

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