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Immunohistochemistry of Gastrointestinal Endocrine Cells in the Meckel's Diverticulum of the Bean Goose, *Anser fabalis* Latham

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The appearance of some gastrointestinal endocrine cells in the Meckel's diverticulum (MD) of the bean goose, *Anser fabalis* Latham was observed using specific antisera against serotonin, gastrin, cholecystokinin (CCK)-8, glucagon, secretin, somatostatin and human pancreatic polypeptide (HPP) with the peroxidase antiperoxidase (PAP) method. Among these specific antisera, serotonin-, gastrin-, CCK-8-, somatostatin- and HPP-immunoreactive cells were demonstrated in this study. Serotonin-, gastrin- and somatostatin-immunoreactive cells were detected at moderate frequency and CCK-8- and HPP-immunoreactive cells was rare and low frequencies, respectively. These immunoreactive cells were located in the superficial epithelium, intestinal crypt and intestinal glands with spherical or spindle shaped cells having long cytoplasmic processes (open typed-cell). Mucosal layer of MD was composed of simple columnar epithelium and numerous intestinal glands. In addition, numerous lymphatic tissues were also demonstrated. In conclusion, histological profiles of MD were similar to any parts of the large intestine, especially the cecum, but the appearance, distribution and relative frequency of gastrointestinal endocrine cells were similar to those of upper parts of the small intestine. Although the exact digestive functions were unknown, the finding that the appearance, distribution and relative frequency of gastrointestinal endocrine cells in MD is similar to small intestine may be considered as distinct evidence that this organ may have some digestive functions.

It is generally known that the Meckel's diverticulum (MD), unique organ of the avian species, is the remnant of the yalk sac and the yalk duct which are transferred into the body cavity before hatching. This rudimentary structure is found as a copolar-like appendage from 2 wk of age in chicken on the convexity of that part of the jejunum which represents the embryonic gut (Nickel et al., 1977; Olah et al., 1984; Olah and Glick, 1984). Gastrointestinal endocrine cells dispersed in the epithelia and gastric glands of the digestive tract synthesize various gastrointestinal hormones and play an important role in physiological functions of the alimentary tract (Bell, 1979). In addition, the regional distribution and relative frequencies of these endocrine cells varied with the animal species and feeding habits (Solcia et al., 1975) and they were divided into two types, open and closed type (Kobayashi et al., 1971; Solcia et al., 1975). Their regional distribution and relative frequency in the gastrointestinal tract of the bean goose were well defined (Park et al., 1999). Sugiyama et al. (1983) observed polycystic immature teratoma at the head of MD of a chick. MD resides consistently along the length of the chicken intestinal tract and this constancy in position supports the use of MD as a boundary point of the chicken intestine (Branton et al., 1988). Recently Olah et al. (1984) and Olah and Glick (1984) suggested that chicken MD was a novel lymphoepithelial organ. In addition, Jeurissen et al. (1999) have investigated cell types of intraepithelial leucocytes and M cells separately using double immunocytochemical staining in chicken MD and also reported that MD generally contained germinal centers from 12 wks of age (Jeurissen et al., 1989). However, no information seems to be available for digestive functions of MD with exception of few works. This possibility was indicated in mallard because their histological profiles resemble to large intestinal tract, especially cecum and appearance of gastro-intestinal endocrine cells, chromogranin-, serotonin- and

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Table 1. Antisera used in this study

Antisera*	Code	Source	Dilution
Serotonin	PUO680798	BioGenex, San Ramon	1 : 20
Gastrin	PUO190796	BioGenex, San Ramon	1 : 20
Cholecystokinin-8	75257	DiaSorin, Stillwater	1 : 500
Glucagon	PUO390699	BioGenex, San Ramon	1 : 20
Secretin	BO67122A	BioGenex, San Ramon	1 : 20
Somatostatin	PUO420198	BioGenex, San Ramon	1 : 20
Human pancreatic polypeptide	A619	DAKO, Santa Barbara	1 : 600

*All antisera were raised in rabbit.

somatostatin-immunoreactive cells generally detected in the gastrointestinal tract of various animal species (Ku et al., 1998).

In the present study, the possibility that MD serves some digestive functions in the bean goose, *Anser fabalis* Latham, was tested. Also, the appearance of gastrointestinal endocrine cells in this avian specific organ was demonstrated by peroxidase antiperoxidae (PAP) method using antisera against serotonin, gastrin, cholecystokinin (CCK)-8, glucagon, secretin, somatostatin and human pancreatic polypeptide (HPP). The results were compared to those of other parts of gastrointestinal tract in the same (Park et al., 1999) avian species.

Materials and Methods

Five adult bean geese, *Anser fabalis* Latham, without sexual distinction were used in this study. After anesthetizing with ketamine hydrochloride (Ketalar®, Yuhan Corp., Korea) and phlebotomy, samples from the MD 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 section was deparaffinized, rehydrated and immunostained by the PAP method (Sternberger, 1979). Background blocking was performed with normal goat serum prior to incubation with the specific antisera (Table 1). After rinsing in phosphate buffered saline (PBS, 0.01 M, pH 7.4), the sections were incubated in secondary antiserum. They were then washed in PBS buffer and finally PAP complex was prepared. Peroxidase reaction was carried out in 3,3'-diaminobenzidine tetrahydrochloride containing 0.01% H_2O_2 in Tris-HCl buffer (0.05 M, pH 7.6). After immunostaining, the sections were lightly counterstained with Mayer's hematoxylin and the immunoreactive cells were observed under a light microscope.

Table 2. Relative frequencies of some gastrointestinal endocrine cells in Meckel's diverticulum of the bean goose, *Anser fabalis* Latham

Antisera	Relative frequency
Serotonin	++
Gastrin	++
Cholecystokinin-8	+
Glucagon	-
Secretin	-
Somatostatin	++
Human pancreatic polypeptide	+

Relative frequencies: ++, moderate; +, low; ±, very low, -, not detected.

Results

MD of the bean goose were composed of three distinct layers, tunica mucosa, tunica muscularis and tunica serosa like other parts of the intestinal tract. Among these layers, tunica mucosa consisted of simple columnar epithelium and intestinal glands that were buried in numerous lymphocytes. In addition, invagination of epithelium, the intestinal crypt was also demonstrated (Fig. 1-4).

Among the seven antisera, serotonin-, gastrin-, CCK-8-, somatostatin- and HPP-immunoreactive cells were detected in varying frequencies. However, no glucagon- or secretin-immunoreactive cells were found (Table 2).

These immunoreactive cells were observed in the superficial epithelium, intestinal crypt and intestinal glands with spherical or spindle shaped having long

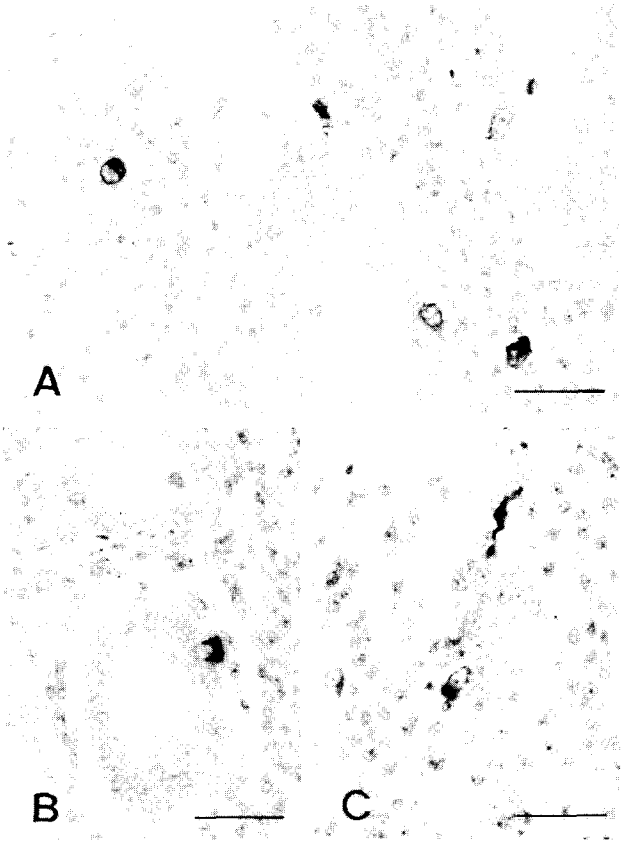

Fig. 1. Serotonin- and gastrin-immunoreactive cells in the Meckel's diverticulum of the bean goose. A, serotonin-immunoreactive cells located in intestinal glands and crypts. In addition, a few cells were also detected in interlymphocytic regions. B, gastrin-immunoreactive cells detected in intestinal glands. C, gastrin-immunoreactive cells demonstrated in intestinal crypts. PAP method. Scale bars = 20 μm.

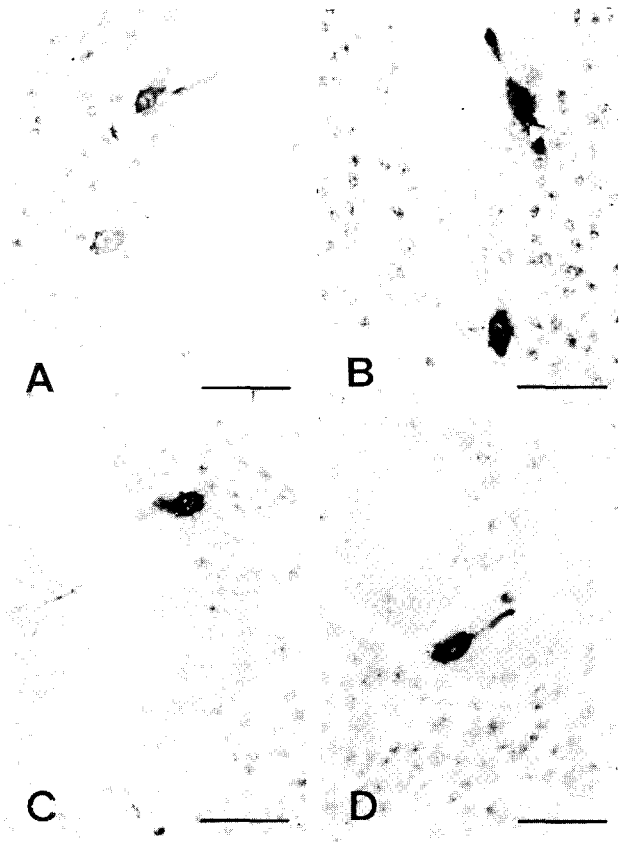

Fig. 2. Cholecystokinin (CCK-8)- and somatostatin-immunoreactive cells in the Meckel's diverticulum of the bean goose. A, CCK-8-immunoreactive cells having long cytoplasmic process located in intestinal crypts. B and C, somatostatin-immunoreactive cells having long cytoplasmic process detected in intestinal crypt. D, somatostatin-immunoreactive cells having long cytoplasmic process demonstrated in intestinal glands. PAP method. Scale bars = 20 μm.

cytoplasmic processes (open typed-cell).

Spherical to spindle shaped and open typed serotonin-immunoreactive cells were found in the basal parts of the intestinal crypts and glands at moderate frequency and a few cells were also detected in inter-lymphocytic regions with round to oval shaped closed types (Fig. 1A).

Gastrin-immunoreactive cells having spherical to spindle shaped (open type) were demonstrated in the basal parts of the intestinal crypts and glands at moderate frequency (Fig. 1B and C).

Spherical to spindle shaped, open typed CCK-8-immunoreactive cells having long cytoplasmic processes were detected in the basal parts of intestinal crypts at low frequency and their cytoplasmic processes were extended into intestinal lumen (Fig. 2A).

Spindle shaped and open typed somatostatin-immunoreactive cells having long cytoplasmic process were detected in the basal parts of intestinal crypts at moderate frequency and their cytoplasmic processes were extended into intestinal lumen (Fig. 2B and C). In addition, similar shaped immunoreactive cells were also detected in the basal parts of the intestinal glands (Fig. 2D).

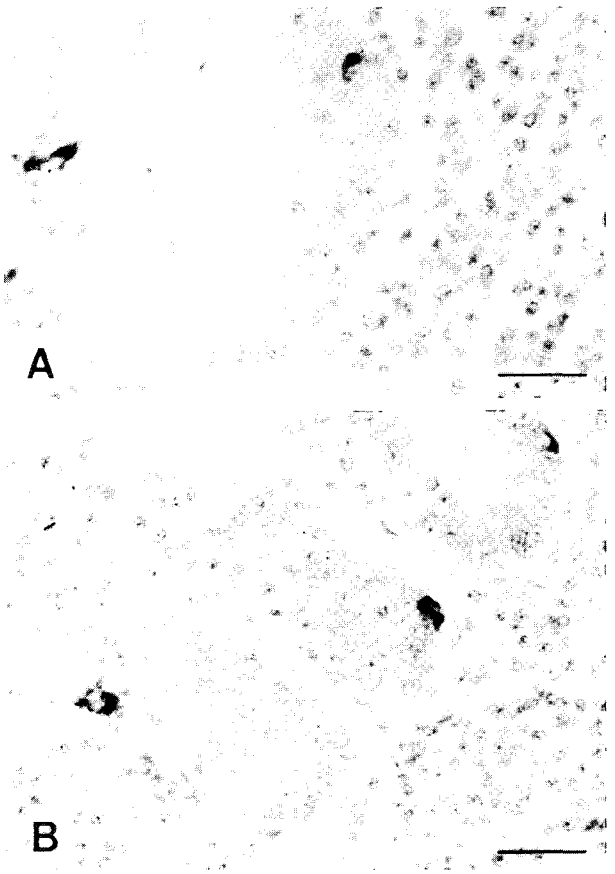

Fig. 3. Human pancreatic polypeptide (HPP)-immunoreactive cells in the Meckel's diverticulum of the bean goose. A, HPP-immunoreactive cells located in intestinal crypts. B, HPP-immunoreactive cells detected in intestinal glands. In addition, a few cells were also detected in interlymphocytic regions. PAP method. Scale bars = 20 μm.

Spherical to spindle shaped, open typed HPP-immunoreactive cells were demonstrated in the basal parts of intestinal crypts and glands at low frequency. In addition, a few cells were also detected in interlymphocytic regions. (Fig. 3A and B).

Discussion

Although histological profiles of MD were reported in some avian species, most of these reports observed lymphocytes located in this avian specific organs (Olah et al., 1984; Olah and Glick, 1984; Jeurissen et al., 1989; 1999). However, Ku et al. (1998) suggested that their histological profiles were similar to those of the large intestinal tract, especially the cecum in mallard. In the present study, corresponding to the previous report (Ku et al., 1998), MD of the bean goose was composed of three distinct layers, tunica mucosa, tunica muscularis and tunica serosa which were also observed in other parts of the avian gastrointestinal tract (Hodges, 1974; Banks, 1986). These three layers, especially tunica mucosa where numerous lymphocytes and germinal centers with goblet cells, contained simple columar epithelium, well-developed crypts and numerous intestinal

glands, which were demonstrated to be similar to those of the large intestine.

The regional distribution and relative frequency of the endocrine cells in the gastrointestinal tract showed remarkable differences depending on animal species but their location along the gastrointestinal tract showed some regularity (Gabe, 1972; Alumets et al., 1977). In addition, their shapes were subdivided into two types. One was round to spherical shaped close type cells which were located in the stomach regions, and the other the was spherical to spindle shaped open type cells which were situated in the intestinal regions (Kobayashi et al., 1971; Solcia et al., 1975). Although some exceptions existed, Park et al. (1999) reported that the open type cells were mainly located in the intestinal regions and the close type cells in the stomach regions of the bean goose. Similar to these previous studies (Kobayashi et al., 1971; Solcia et al., 1975; Park et al., 1999), open type cells were also found in MD of the bean goose.

Main functions of serotonin are inhibition of gastric acid secretion and contraction of smooth muscle in the gastrointestinal tract (Guyton, 1988). Generally, serotonin-immunoreactive cells are found throughout whole gastrointestinal tract in all animal species including avian species and are established in the gastrointestinal tract at early stages of vertebrate evolution (El-Salhy et al., 1985; Castaldo and Lucini, 1991; Lee et al., 1998). However, somewhat different from other avian species (Castaldo and Lucini, 1991; Lee et al., 1998), in the bean goose, serotonin-immunoreactive cells were detected in the intestinal tract but not in stomach regions (Park et al., 1999). Appearance of these immunoreactive cells at moderate to high frequencies in MD of mallard were reported (Ku et al., 1998). They were also found in MD of the bean goose at moderate frequency.

It is generally accepted that gastrin and CCK-8 are originated from the same ancestor. In human duodenum a large fraction of these cells, besides reacting with non-C terminal CCK antibodies and C-terminal gastrin/CCK antibodies, also show immunoreactivity with C-terminal gastrin-34 antibodies, colocalized with CCK in a variable portion of secretory granules. However, the CCK-producing cells have been characterised with non-C terminal reactive antibodies, lacking histochemical crossreactivity with gastrin (Solcia et al., 1989). Generally, some variations occurred, and gastrin-immunoreactive cells were found in the stomach and small intestine of avian species (Okamoto, 1980; Rawdon and Andrew, 1981). In the bean goose, gastrin-immunoreactive cells were restricted to the gizzard, pylorus and duodenum with low, high and very low frequencies, respectively. CCK-8-immunoreactive cells were found from the gizzard to ileum showing highest frequency in duodenum (Park et al., 1999). In the present study, gastrin- and CCK-8-immunoreactive cells were found in MD of the bean goose with moderate and very low frequencies, respectively. Thus, we demonstrated for the first time that gastrin- and CCK-8- immunoreactive cells are present in MD of avian species.

Glucagon is synthesized in the pancreatic and intestinal A cells. Glucagon regulates serum glucose levels and participates in contractation of stomach and/or inhibition of gastric acid secretion (Alumets et al., 1983). Although the distribution and relative frequency of those cells varied among the avian species, glucagon-immunoreactive cells were observed in the gizzard, pylorus, ileum, colon and rectum of the bean goose. However, they were not found in the present study.

In all vertebrates so far investigated secretin-immunoreactive cells proved to be exclusive to the small intestine, usually with preference for the duodenum and upper jejunum (Solcia et al., 1989; Baltzar et al., 1998; Ku et al., 2000). However, no data were available in the been goose. In the present study, no secretin-immunoreactive cells were demonstrated in MD of the bean goose.

Somatostatin inhibits the secretion of the other neuroendocrine hormones (Kitamura et al., 1984). It is known that somatostatin-immunoreactive cells show the widest distribution in the whole gastrointestinal tract except for large intestine of all vertebrate species investigated, including the primitive agnathans (Falkmer and Van Noorden, 1983). However, somewhat species-dependent variations on the distributional patterns were observed. They were found throughout whole gastrointestinal tract of avian species including bean goose except for large intestine where no cells were demonstrated (Rawdon and Andrew, 1981; Lee et al., 1998; Park et al., 1999). It is also reported that somatostatin-immunoreactive cells was detected in MD of the mallard at low frequency (Ku et al., 1998) and the bean goose at moderate frequency.

PP was isolated from insulin extraction of pancreas in 1961, and was quart to depress pancreatic and biliary secretion in laying hens with inhibition of gastric secretion and motility of avian species (Duke et al., 1988). Although the species-dependent variations on the distributional patterns of PP-immunoreactive cells were approved, they were generally detected in the pylorus and in lower part (jejunum and ileum) of the small intestine (Yamanaka et al., 1989). However, no data were available about the distribution of PP-immunoreactive cells in the gastrointestinal tract of been goose. In the present study these cells were demonstrated in MD of the bean goose with a few frequency. This is first time that HPP-immunoreactive cells were demonstrated in MD of avian species.

In conclusion, histological profiles of MD of the bean goose, *Anser fabalis* Latham, were observed to be similar to that of other parts of the large intestine, especially the cecum, but the appearance, distribution and relative frequency of gastrointestinal endocrine cells were similar to those of upper parts of the small intestine. Although its exact digestive functions were unknown, appearance, the present finding can be

considered as distinct evidence that this organ has some digestive functions.

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