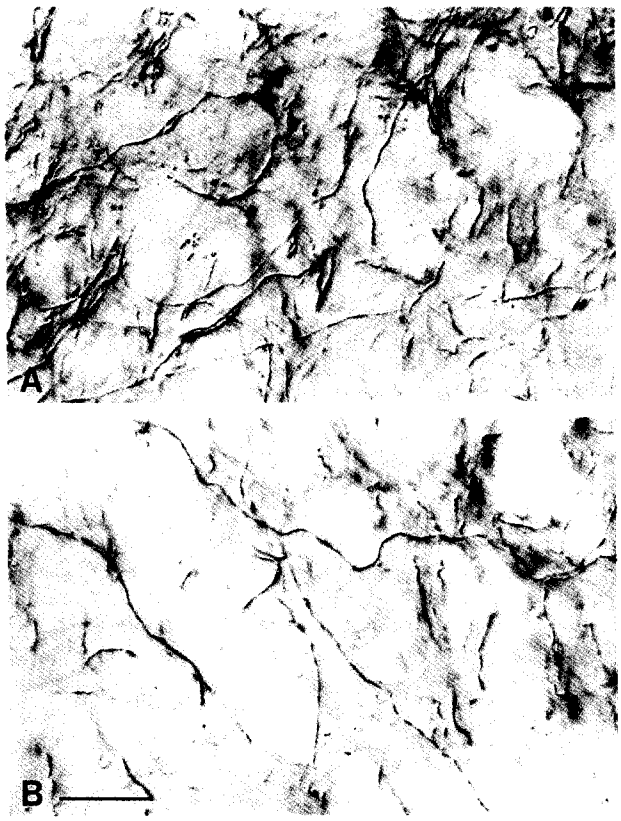

Fig. 2. Higher magnification of calretinin-immunoreactive fibers found within the superficial layers. Fiber density was more intense in the deep superficial gray layer (A) than the upper superficial gray and optic layers (B). Almost all these fibers were thin. DIC optics. Scale bar=20 μm.

noreactivity consisted of numerous, well-labeled fibers (Figs. 1, 2, 3A). This tier of labeled fibers was found throughout the rostral-caudal extent of the mouse superior colliculus. These fibers were found in the zonal, superficial gray and optic layers. In this plexus of anti-calretinin-labeled fibers, except for a few lightly labeled cells in the optic layer, no calretinin-immunoreactive cell bodies were localized.

The fibers in the superficial layers were not homogeneously distributed. Some areas contained more labeled fibers than other areas within the superficial layers. Fiber density was higher in the deep superficial gray layer (Fig. 2A) than in the upper superficial gray and the optic layers (Fig. 2B). Almost all calretinin-immunoreactive fibers in the superficial gray layer were small in diameter and had a few varicosities (Fig. 2). Some calretinin-immunoreactive fibers in the deep superficial gray layer were often loosely surrounded by small to medium-sized unlabeled neuronal somata (Fig. 2A).

Anti-calretinin immunoreactivity after enucleation

To determine whether the calretinin-labeled fibers in the superficial layers originate from the retina and

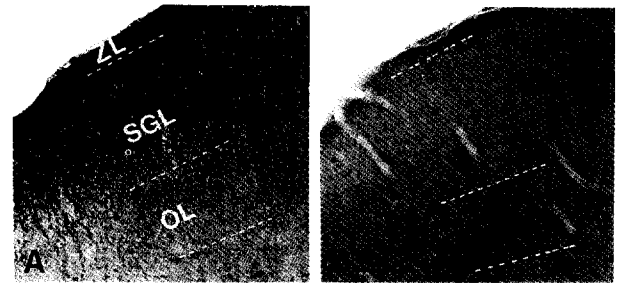

Fig. 3. Anti-calretinin immunoreactivity in the superior colliculus in normal (A) and enucleated (B) mouse. On the contralateral superficial layer to the enucleation (B), many calretinin-labeled cells were observed. The mouse had its right eye removed 10 days before sacrifice. ZL, zonal layer; SGL, superficial gray layer; OL, optic layer. Scale bar=100 μm.

whether the enucleation affects the distribution of calretinin immunoreactivity in the mouse superior colliculus, we performed enucleations in some animals. Figs 1A and 3A show calretinin immunoreactivity in the normal mouse superior colliculus while Figs 1B and 3B show calretinin immunoreactivity after monocular enucleation. A marked reduction of calretinin immunoreactivity was produced on the superficial layers of the superior colliculus contralateral to the enucleation. Calretininlabeled fibers were almost completely eliminated following enucleation. By contrast, in the superficial layers of the superior colliculus contralateral to the enucleation, many anti-calretinin-labeled cells were

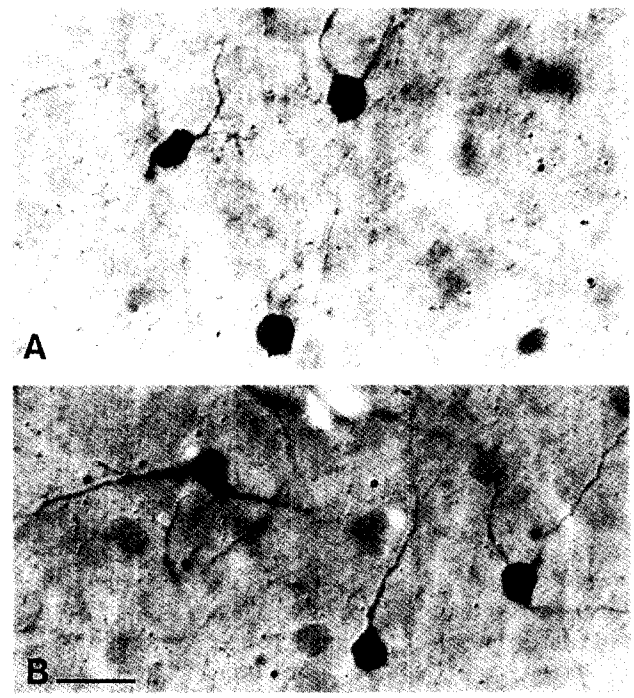

Fig. 4. Higher magnification of calretinin-labeled neurons on contralateral superficial gray layer to the enucleation. A, Most of these cells had small, round or oval or vertical pyriform cell bodies with main ascending dendrites toward the pial surface. B, A horizontal cell is also seen. Scale bar=20 μm.

observed (Fig. 3B). These cells were found in all superficial layers:zonal, superficial gray, and optic layers. The staining of calretinin was robust in certain small neurons in the superficial layers of the mouse superior colliculus. These anti-calretinin-labeled cells had small, round or oval cell bodies (Fig. 4). We identified at least two distinct morphologies based upon their dendrites. The first type in the superficial layer was composed of round or oval neurons with proximal dendrites projecting superficially toward the pial surface (Fig. 4). The majority of calretinin-immunoreactive neurons which appeared after eye enucleation, were this type of neuron. The second type also had round or oval cell bodies. However, these cells had horizontally oriented processes (Fig. 4B). Horizontal cells were very rarely found. We found that both 10 and 20 days of monocular enucleation had pronounced effects upon calretinin immunoreactivity. We also found no apparent differences in antibody labeling between 10 and 20 days of monocular enucleation.

Discussion

The patterned distribution of calretinin-immunoreactive fibers found in the mouse superior colliculus in the present study is similar to that observed in previous studies in the rat (Arai et al., 1993), cat (Jeon et al., 1996), mouse (Gobersztejn and Britto, 1996), and hamster (Jeon et al., 1997). In all these animals, the antibody against calretinin formed a dense plexus of labeled fibers in the superficial layers of the superior colliculus. The previous studies of the rat and mouse, however, did not describe the fiber morphology in detail. Our findings indicate that the type of calretinin fibers that exist in the superficial layers of the mouse superior colliculus are small diameter fibers with a few varicosities. The highest density of labeled fibers were located in the deep superficial gray layer. No large fibers were observed. Even though many retinal ganglion cells were labeled by anti-calretinin antibody (Pasteels et al., 1990), recently in our lab Jeon and Jeon (1998) found that large ganglion cells in the retina were not labeled by antibody against calretinin. These results combined with our present result suggest that subtypes of calretinin-containing retinal ganglion cells may project to the superior colliculus.

The pattern of calretinin-immunoreactive fibers found in the present and previous studies was different from that described in the rabbit. In recent studies from this laboratory (Jeon et al., 1998), we found that calretinin did not form a plexus of labeled fibers in the superficial layers of the rabbit superior colliculus. By contrast, many anti-calretinin-labeled neurons were localized in the superficial layers. This result indicates that there are some species with differences in calretinin immunoreactivity in the superior colliculus.

We have observed a new appearance in calretinin-immunoreactive cells in the superficial layers of the mouse superior colliculus after enucleation. Even though Goberstzjn and Britto (1996) also observed calretinin positive neurons on the experimental side of the mouse superior colliculus, they did not describe its cell morphology. Their study (see their Fig. 1) did not give any useful information regarding the detailed morphology of calretinin-labeled neurons. The present study is the first detailed description of the newly localized calretinin-immunoreactive cells found in the mouse superior colliculus after enucleation. We found that the newly appeared calretinin-containing cells were localized in all superficial layers. All of these cells were small rounded or oval-shaped cells. The large majority of these cells had dendrites projecting toward the pial surface. Horizontal cells with horizontally oriented dendrites were very rarely found. There are many morphologically different types of cells in the superior colliculus. It is important to know which types of neurons express calretinin for physiological roles in the future. No anti-calretinin-immunoreactive stellate-shaped or large neurons were observed in the superficial layers after enucleation. The present localization of calretinin-immunoreactive neurons after enucleation suggests that genes for calretinin in some neurons in the superficial layers may not work in a normal state. These cells may express calretinin genes after enucleation. Thus, the expression of calretinin genes may be activity-dependent in the superficial layers of the mouse superior colliculus. Localization of mRNA by in situ hybridization will be necessary to prove activity-dependent expression of calretinin gene in the mouse superior colliculus after enucleation. Activity-dependent changes of calretinin mRNA has been reported in the auditory system (Sans et al., 1995; Winsky and Jacobowitz, 1995).

The physiological role of the calcium-binding proteins is still unclear. It has been suggested that calretinin is involved in sharpening the timing of action potentials by having the capacity of calcium buffering and transport (Rogers, 1987). However, the function of calretinin is not clearly understood yet.

Acknowledgments

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Localization of Endocrine Cells in the Gastrointestinal Tract of the Manchurian Chipmunk, *Tamias sibiricus barberi*

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Immunohistochemistry
Manchurian chipmunk
Endocrine cells

The regional distribution and relative frequency of endocrine cells were studied immunohistochemically in the gastrointestinal tract (GIT) of the Manchurian chipmunk, *Tamias sibiricus asiaticus.* Six kinds of endocrine cells were identified in this study. 5-hydroxytryptamine (5-HT)-immunoreactive cells were detected throughout the GIT. These cells were observed in moderate numbers in the pylorus, duodenum, jejunum, ileum, fundus, colon, and rectum. Somatostatin- and bovine pancreatic polypeptide (BPP)-immunoreactive cells were also identified throughout the GIT. The former were abundant in the pylorus region while the latter were scattered in ileum and colon. Motilin-immunoreactive cells were rarely detected in the small intestine. A few neurotensin-immunoreactive cells were detected in jejunum, ileum and colon. Also, a few substance P-immunoreactive cells were observed to be restricted to duodenum and jejunum.

It is generally known that the Manchurian chipmunk, *Tamias sibiricus asiaticus,* belongs to the order Rodentia, and has been widely distributed in Korea. Hormone secreting cells of the gastrointestinal tract (GIT) are important in regulating digestive function (Bell, 1979). Although many studies have elucidated the distribution and relative frequency of different endocrine cell in the GIT of various vertebrates, a few works have been done on the endocrine cells of the Korean squirrel (Chung and Kwun, 1973; Chung, 1976; Lee and Lee, 1986; Lee et al., 1991; Lee et al., 1997a, b).

The purpose of the present work was to clarify the regional distribution and relative frequency of each endocrine cell type in the GIT of the Manchurian chipmunk, *Tamias sibiricus asiaticus*, by specific immunohistochemistry.

Materials and Methods

Both sexes of the five adult Manchurian chipmunks, *Tamias sibiricus asiaticus,* were captured in Chungsong, Kyungpook, Korea and used in this study. Samples from 7 portions of the GIT (fundus, pylorus, duodenum, jejunum, ileum, colon and rectum) were fixed in Bouin's solution. After paraffin embedding, 3-4 μm serial sections were prepared with routine methods. Each representative section was deparaffinized, rehydrated and immunostained with the peroxidase antiperoxidase (PAP) method (Sternberger, 1979). Background blocking was performed with normal goat serum prior to incubation with the specific antisera (Table 1). After rinsing in PBS buffer (0.01 M, pH 7.4), the sections were incubated in secondary antiserum. They were then washed in PBS buffer and the PAP complex was prepared. The peroxidase reaction was carried out in a solution of 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.

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Results

In this study, six kinds of the immunoreactive cells

Table 1. Antisera used

Antisera*	Code	Source	Dilution
5-hydroxytryptamine (5-HT)	8535028	Immunonuclear Corp., Stillwater	1:10,000
Somatostatin	CA325	Cambridge Research Biochemical Ltd.	1:1,000
Bovine pancreatic polypeptide (BPP)	i607	Union Chimique Belge Bioproducts	1:5,000
Motilin	R-017	Dr. Yanaihara, Shizoka	1:1,000
Neurotensin	R-3501	〃	1:1,000
Substance P	B9C 35	Sera-Lab, Sussex	1:1,000

*All antisera were raised in rabbits.

Table 2. The regional distributions and relative frequencies of the immunoreactive cells in the GIT of the Manchurian chipmunk

Immunoreactive cell	Fundus	Pylorus	Duodenum	Jejunum	Ileum	Colon	Rectum
5-HT	+	++	+++	++	++	++	++
Somatostatin	+	++	+	±	±	±	±
BPP	+	±	+	±	-	-	±
Motilin	-	-	±	±	±	-	-
Neurotensin	-	-	-	+	+	+	-
Substance P	-	-	+	+	-	-	-

+++; numerous, ++; moderate, +; a few, ±; rare, -; not detected.

were detected with the antisera against 5-hydroxytryptamine (5-HT), somatostatin, bovine pancreatic polypeptide (BPP), motilin, neurotensin and substance P. The regional distribution and relative frequency of these cells in the GIT of the manchurian chipmunk are showed in Table 2.

5-HT-immunoreactive cells, reaching a peak in the duodenum were demonstrated in the epithelia and mucosal glands of the entire GIT. A few number of cells were observed in the fundic gastric regions while moderate to numerous numbers of cells were found in the other regions. In addition, most of the 5-HT-immunoreactive cells were round or spindle-shaped (Fig. 1A-E).

Somatostatin-immunoreactive cells having spherical to spindle-shaped were observed in the epithelia and mucosal glands throughout the GIT. The frequency of the immunoreactive cells were moderate in the pyloric gland region, but gradually decreased distally along the large intestine (Fig. 2A-F).

A few number of BPP-immunoreactive cells were detected in the fundic gland region and duodenum, and they were rarely found in the pyloric gland region, jejunum and rectum. The immunoreactive cells, in moderate numbers were found in other regions. In addition, most of the 5-HT-immunoreactive cells were round or spindle-shaped (Fig. 1A-E).

Somatostatin-immunoreactive cells having spherical

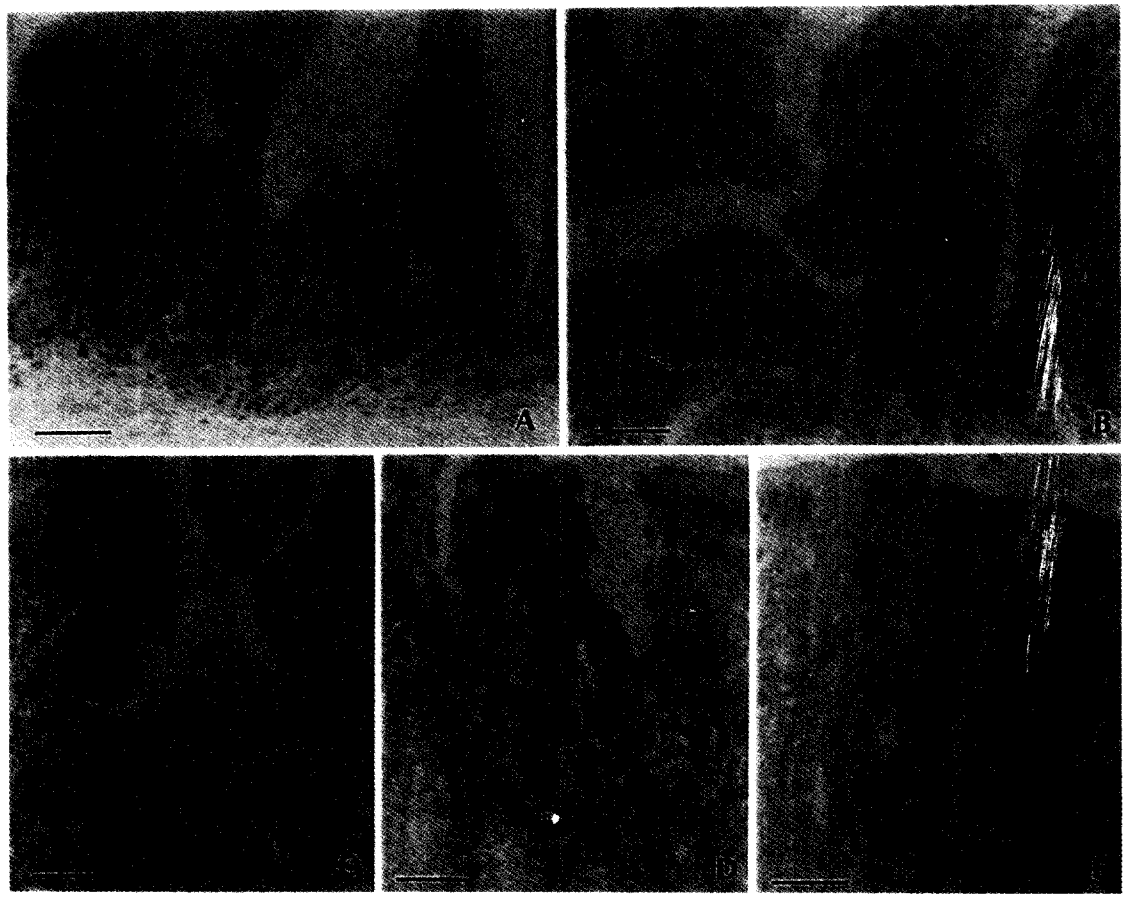

Fig. 1. 5-HT-immunoreactive cells in the gastrointestinal tract of the Manchurian chipmunk. These cells were observed in numerous or moderate numbers in the duodenum (A), jejunum (B), ileum (C), colon (D) and rectum (E). Scale bars=50 μm.

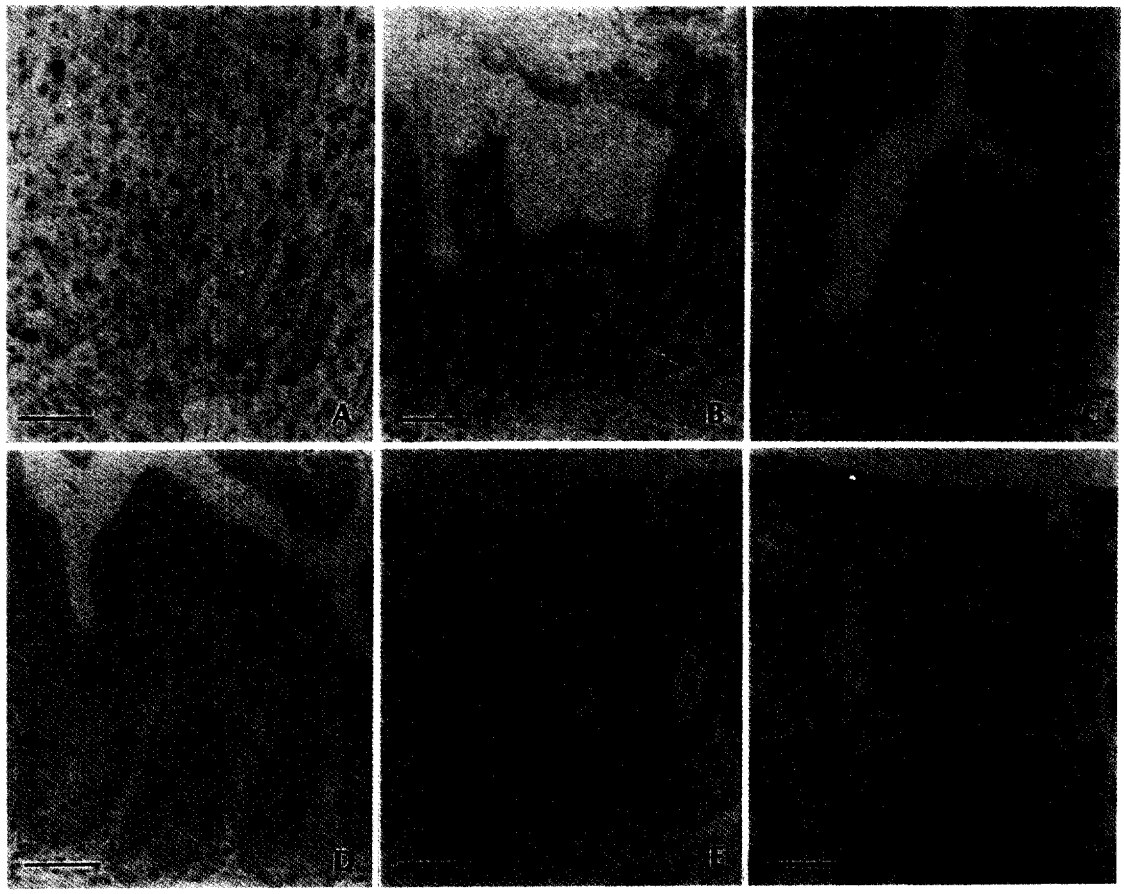

Fig. 2. Somatostatin-immunoreactive cells in the GIT of the Manchurian chipmunk. A few number of these cells were observed in the (A) fundus and (c) duodenum but rare in the (D) ileum (E), colon and (F) rectum. Moderate number of somatostatin-immunoreactive cells were detected in the (B) pylorus regions. Scale bars=50 μm.

to spindle-shaped were observed in the epithelia and mucosal glands throughout the GIT. The frequency of the immunoreactive cells were moderate in the pyloric gland region, but gradually decreased distally along the large intestine (Fig. 2A-F).

A few number of BPP-immunoreactive cells were detected in the fundic gland region and duodenum, and they were rarely found in the pyloric gland region, jejunum and rectum. While the immunoreactive cells were found in epithelia and mucosal glands of the stomach, they were observed in the submucosal glands of the duodenum and in the epithelia and mucosal glands of the other regions. These cells were spherical shaped (Fig. 3A-E).

Motilin-immunoreactive cells were spherical in shape and found only in the epithelia and intestinal crypts of the small intestine. Only a small number of these cells were found in the duodenum and jejunum, and rarely in the ileum (Fig. 4A-C).

Neurotensin-immunoreactive cells were distributed in the epithelia and mucosal glands of the jejunum, ileum and colon. These cells were spherical to spindle-shaped, and a few present in the jejunm and ileum, and rarely in the colon (Fig. 5A-C).

Also, a few number of substance P-immunoreactive cells were found to be restricted to the the duodenum and jejunum. These cells were present in the epithelia and mucosal glands (Fig. 6A and B).

Discussion

The regional distribution and relative frequency of the gastrointestinal endocrine cells in the manchurian chipmunk were essentially similar to those of other mammals (Bloom and Polak, 1978). However, some characteristic differences were observed in this species.

El-Salhy et al., (1985) reported that 5-HT-immunoreactive cells are found throughout the GITs of all species and established in the GIT at the early stage of vertebrate evolution. In the present study, the highest frequency of 5-HT-immunoreactive cells were found in the duodenum, however, these cells were also observed numerously in the other intestinal segments. This result shows that the regional distribution and relative frequency of 5-HT-immunoreactive cells are quite similar to those of previous works (Ito et al.,

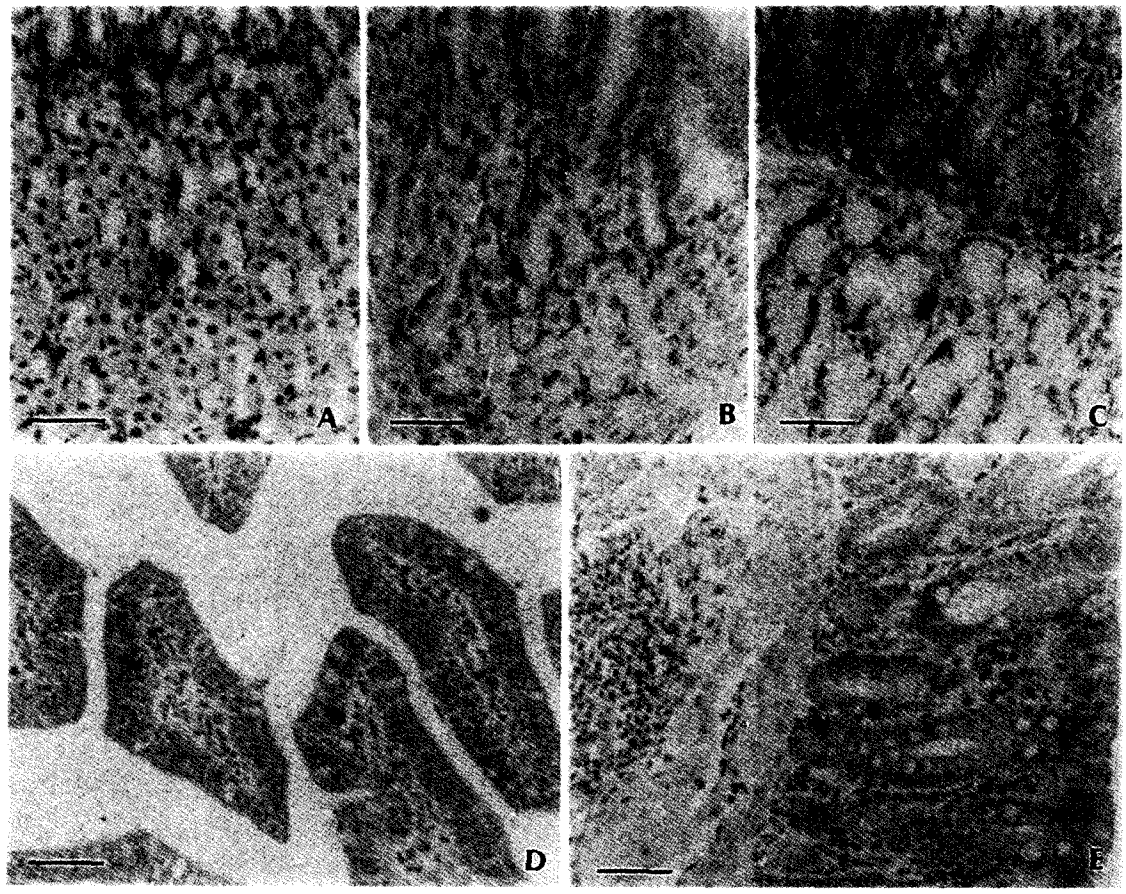

Fig. 3. BPP-immunoreactive cells in the GIT of the Manchurian chipmunk. A few number of these cells were observed in the fundus (A) and duodenum (C) but rare in the pylorus (B), jejunum (D) and rectum (E). Scale bars=50 μm.

1987; Cho and Kitamura, 1988; Lee and Lee, 1990; Lee et al., 1991). It is also known that these endocrine cells are predominant in the pyloric gland (Lee and Lee, 1996) or in the colon (Ohara et al., 1986; Lee, 1988).

It is known that somatostatin-immunoreactive cells show the widest distribution in the whole GIT of all vertebtrate species investigated, including the primitive agnathans (Falkmer and Van Noorden, 1983). On the

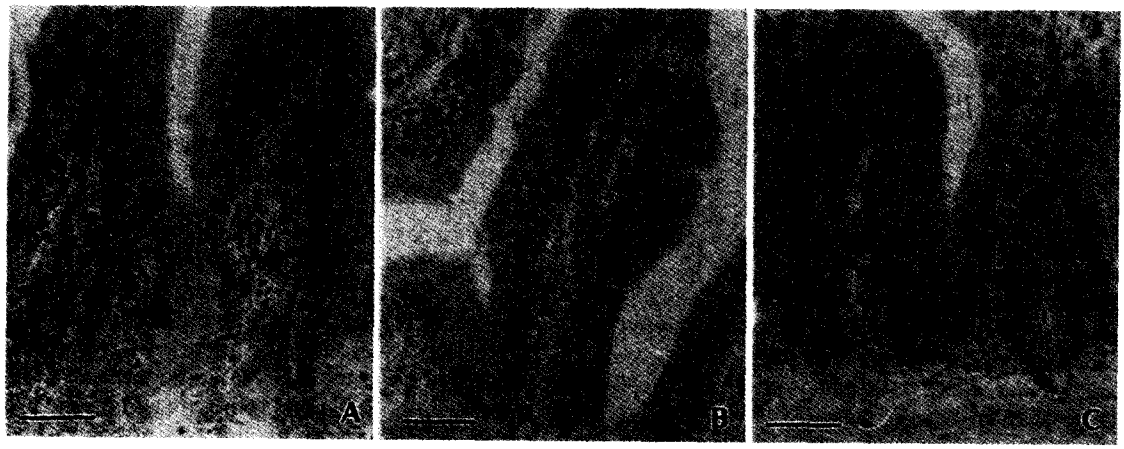

Fig. 4. Motilin-immunoreactive cells in the GIT of the Manchurian chipmunk. These cells were rarely observed in the duodenum (A), jejunum (B) and ileum (C). Scale bars=50 μm.

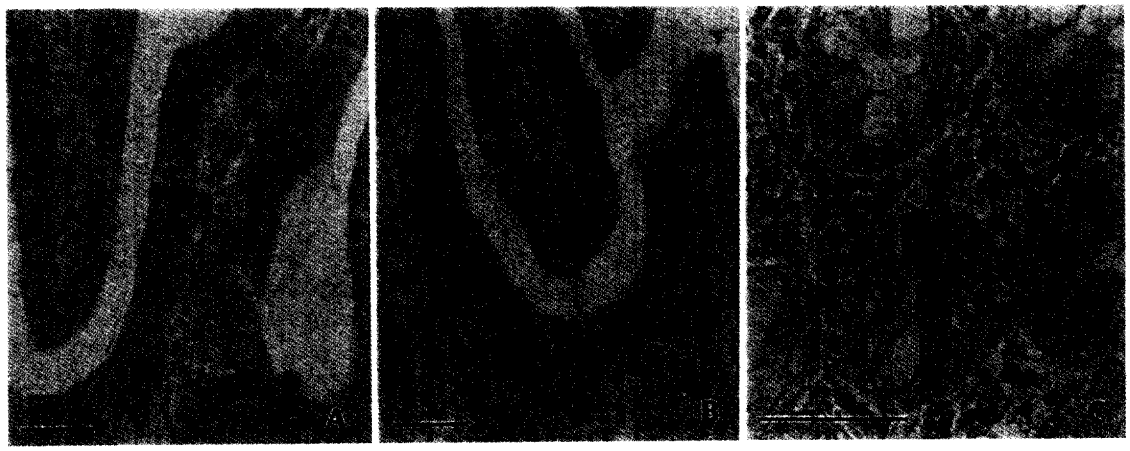

Fig. 5. Neurotensin-immunoreactive cells in the GIT of the Manchurian chipmunk. A few number of immunoreactive cells were observed in jejunum (A), ileum (B), and colon (C). Scale bars=50 μm.

other hand, Lee et al. (1991) suggested that the peak-pattern of the regional distribution of these cells could be divided into two groups: the fundic gland and the pyloric gland according to the species. The results for the somatostatin cells in the manchurian chipmunk support these findings.

Our results showed that BPP-immunoreactive cells were distributed throughout the GIT except for the ileum and colon. The distributions and relative frequencies of these cells were divided into three patterns, namely, the large intestinal pattern (Park and Chung, 1986; Cho and Kitamura, 1988; Lee and Lee, 1990), the stomach and large intestinal pattern (Lee and Lee, 1983), and the whole GIT pattern (Lee and Lee, 1996; Lee, 1988). In the Manchurian chipmunk, the pattern of distribution was correlated with the third pattern.

In the present study, a few motilin-immunoreactive cells were observed in upper parts of the small intestinal regions. These results corresponded with previous reports that these cells were detected in the small intestinal regions of the mink (Kawano et al., 1983), sheep (Calingasan et al., 1984), horse (Kitamura et al., 1984), opossum (Krause et al., 1985), musk shrew (Kanamori et al., 1989), lesser mouse deer (Agungpiryono et al., 1994) and rat (Sakai et al., 1994). In addition, these cells were observed throughout the GIT except for the rectum of the rabbit (Satoh et al., 1995), and no motilin-immunoreactive cells were detected in the vampire bat (Yamada et al., 1984) and the Japanese field vole (Ohara et al, 1986). These discrepancies might be due to differences in feeding behavior.

The relative frequency and distribution of neurotensin-immunoreactive cells were reported in the GIT of various animals (Kawano et al., 1983; Calingasan et al., 1984; Kitamura et al., 1984; Yamada et al., 1984; Krause et al., 1985; Ohara et al., 1986; Kanamori et al., 1989; Yamada et al., 1989a; Kitamura et al., 1990). According to these reports, these cells were

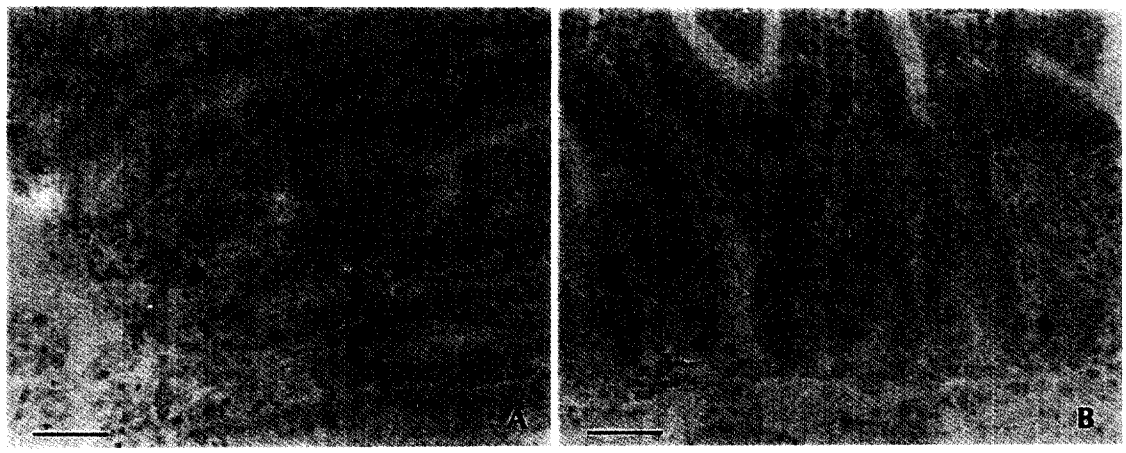

Fig. 6. Substance P-immunoreactive cells in the GIT of the Manchurian chipmunk. A few number of immunoreactive cells were observed in duodenum (A) and ileum (B). Scale bars=50 μm.

mainly distributed in the middle portion of the intestine, namely the ileum to colon regions except for the musk shrew (Kanamori et al., 1989). However, in spite of the same species, Kitamura et al. (1990) had failed to detect the immunohistological reactivity against neurotensin. It was also reported that the distribution and relative frequency of immunoreactive cells were somewhat different from the same species (Yamanaka et al., 1989). In the present study, neurotensin-immunoreactive cells were detected in the middle to distal portion of intestine. Except for in the musk shrew (Kitamura et al., 1990), these results corresponded well to previous studies.

The distributions and relative frequencies of substance P-immunoreactive cells in the GIT were reported in the cat (Feher and Wenger, 1981), human (Sjolund et al., 1983), sheep (Calingasan et al., 1984), Japanese field vole (Ohara et al., 1986), lesser mouse deer (Agungpiryono et al., 1994), and in other mammals (Pearse and Polak, 1975). In the present study, these cells were observed in the duodenum and jejunum. These results were similar to those of earlier works (Pearse and Polak, 1975; Feher and Wenger, 1981; Sjolund et al., 1983; Calingasan et al., 1984; Ohara et al., 1986; Castaldo and Lucini, 1991; Agungpiryono et al., 1994).

In conclusion, we have demonstrated the characteristic patterns of the distribution of six kinds of gastrointestinal endocrine cells and their relative frequencies in the Manchurian chipmunk. Some differences against other animals were also observed. According to previous reports (Solcia et al., 1975; Docray, 1977; El-Salhy and Grimelius, 1981; Ohara et al., 1986; Walsh, 1987; Yamada et al., 1989b), it can be concluded that these differences might be due to the differences of the antisera used or the method and/or feeding behaviors used in those studeis.

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