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An immunohistochemical study on the distribution of endocrine cells in the gastrointestinal tract of the musk shrew, *Suncus murinus*

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Summary. The endocrine cells in the gastrointestinal tract of the musk shrew were studied immunohistochemically. Eleven kinds of endocrine cells, immunoreactive for serotonin, somatostatin, gastrin, cholecistokinin, gastric inhibitory polypeptide, motilin, secretin, neurotensin, pancreatic glucagon, enteroglucagon and bovine pancreatic polypeptide, were revealed. In the stomach, serotonin-, somatostatin-, gastrin-, pancreatic glucagon- and enteroglucagon-immunoreactive cells were detected. The first three types of cells predominated and were more abundant in the pyloric glands than in the other stomach regions. In the small intestine, all types of endocrine cells were found, each having different distributions and relative frequencies. In the large intestine, 10 types of endocrine cells except cholecystokinin-immunoreactive cells were detected. Serotonin- and bovine pancreatic polypeptide-immunoreactive cells were more numerous in the large intestine than in the small intestine.

Key words: Immunohistochemistry, Endocrine cells, Gut, Shrew, Insectivore

Introduction

The musk shrew, *Suncus murinus*, is an insectivore which retains many primitive characteristics from the lowest order of eutherian. Therefore, it has been noted that the shrew is suitable as a new experimental animal as insectivore (Kondo, 1985). From the view point of these characteristics, some morphological studies have been done on the gastrointestinal tract of the shrew (Kurohmaru et al., 1980; Kurohmaru, 1985; Kiso et al., 1988). Its gastrointestinal tract is characterized by a short intestine where the large intestine, in particular, is extremely short and the cecum is absent. The presence of intestinal endocrine cells were reported briefly by conventional methods such as electron microscope and silver impregnation (Kurohmaru et al., 1980; Kiso et al., 1987). However, no immunohistochemical studies on the endocrine cells in the gastrointestinal tract of the shrew have been reported. The present study was designed to clarify the regional distribution and the relative frequency of occurrence of endocrine cells in the gastrointestinal tract of the musk shrew using specific immunohistochemical methods.

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Materials and methods

Fourteen adult musk shrew, *Suncus murinus* (5 males and 9 females) were killed by cervical dislocation. Tissue material from the gastrointestinal tract was sampled (Table 2). Tissues were fixed in Bouin's fluid, then processed routinely for embedding in paraffin and sectioned serially at 5 μm in thickness.

After deparaffinization and rehydration, the sections were stained immunohistochemically using the peroxidase-anti-peroxidase (PAP) method (Sternberger, 1979) for all antisera, except for gastrin and insulin which was done by the bridge method (Mason et al., 1969). Details of the specific antisera used are listed in Table 1. The specificity of each immunohistochemical reaction was determined as recommended by Sternberger (1979) including replacement of the specific antiserum with antiserum preincubated with an excess amount of corresponding antigen and related peptides. After immunohistochemical staining, the sections were counterstained with Mayer's hematoxylin.

The distribution and relative frequency of immunoreactive cells in the gut mucosa are summarized in Table 2. The immunoreactive cells were graded subjectively into five groups according to their relative frequency as confirmed using a light microscope. In this study, glucagon-immunoreactive cells were classified into two subtypes, according to their specific immunoreactivities to

the antiserum GL-5 or antiserum RPN 1602 (Table 1), as pancreatic glucagon- or enteroglucagon-immunoreactive cells, respectively.

Results

Eleven kinds of immunoreactive cells were revealed in the gastrointestinal mucosa of the musk shrew (Table 2).

In the cardiac glands, which consisted of a narrow circular zone around the cardia, only serotonin- and somatostatin-immunoreactive cells were detected in 2 of the 14 shrews (Fig. 1A). In the fundic glands, moderate numbers of serotonin-immunoreactive cells were found in all shrews examined, but only a few somatostatin-, pancreatic glucagon- and enteroglucagon-immunoreactive cells were recognized (Figs. 1B, C). In some of the shrews, they were rare or absent. In the fundic glands, endocrine cells had no luminal contact with their apical cytoplasmic processes but they had short or long basal processes running along the basement membrane (Fig. 1B). They appeared to be «closed-type» cells. The pyloric glands possessed numbers of serotonin- and somatostatin-immunoreactive cells as well as numerous gastrin-immunoreactive cells (Figs. 1D, E). These cells were generally distributed throughout the glands and were pyramidal, oval or barrel-like in shape. Their apical cytoplasmic process reached to the glandular lumen, and were classifed as «open-type» cells.

In the small intestine, moderate numbers of serotonin-immunoreactive cells and a small number of somatostatin-immunoreactive cells were usually found (Fig. 2A), while the other 9 types of immunoreactive cells had variable distributions. The immunoreactive cells were distributed throughout the intestinal villi and crypts. Only gastrin-immunoreactive cells were found to be distributed more frequently in the crypts than in the villi.

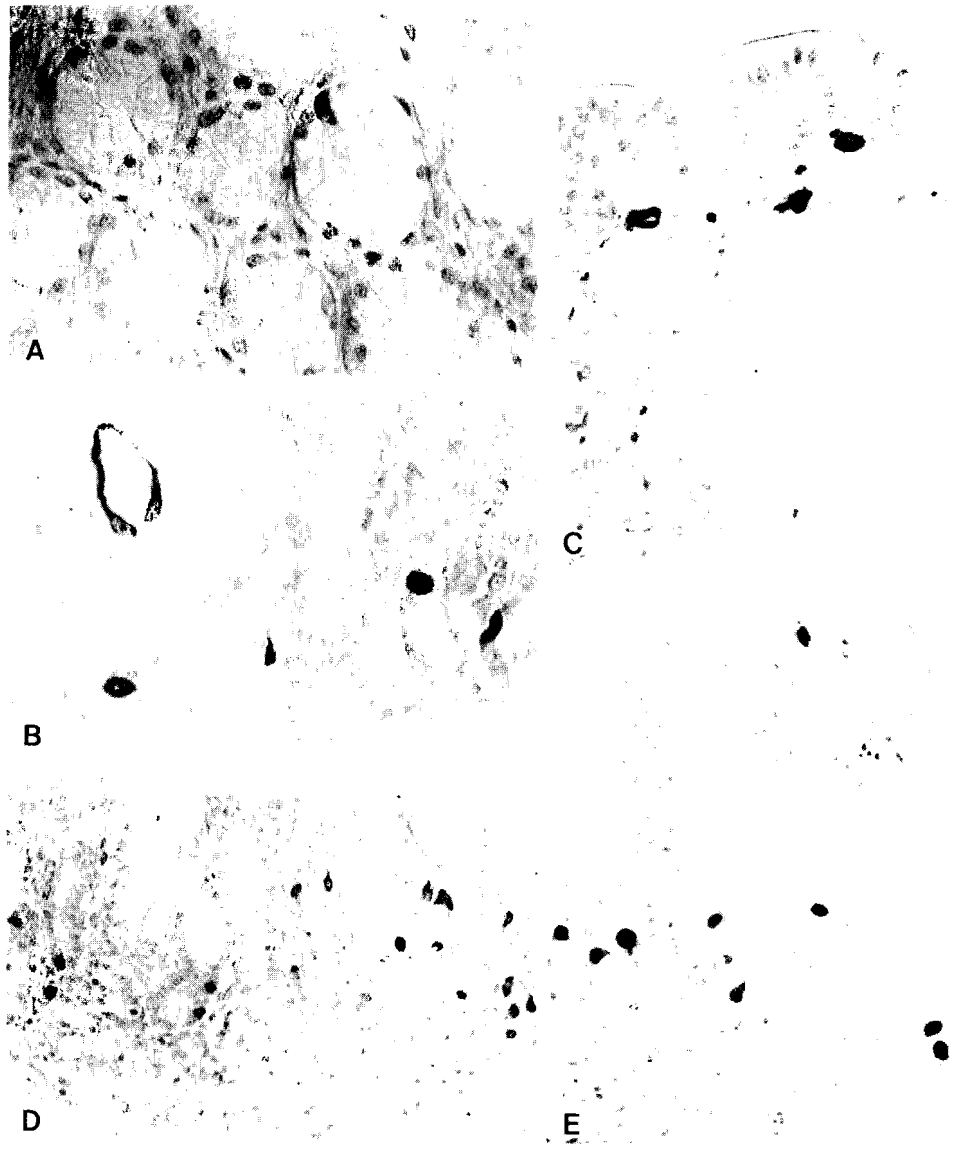

Fig. 1. Immunoreactive cells in the stomach. A. Somatostatin-immunoreactive cell in the cardiac gland. B. Serotonin-immunoreactive cells in the fundic glands. Note two cells bearing long cytoplasmic processes. C. Pancreatic glucagon-immunoreactive cells in the fundic glands. D. Somatostatin-immunoreactive cells in the pyloric glands. E. Gastrin-immunoreactive cells in the pyloric glands. A-C × 375, D, E × 188

Gastrin-immunoreactive cells were found more frequently in the initial portion of the small intestine (proximal duodenum) (Fig. 2B) and decreased in number distally along the intestine, and they were rarely found in the terminal portion of the small intestine (ileum). Although cholecystokinin (CCK)-, gastric inhibitory polypeptide (GIP)- and motilin-immunoreactive cells were located in every portion of the small intestine (Figs. 2D-F), they were more abundant in the proximal small intestine. Secretin-immunoreactive cells were also found consistently, but they were most frequent in the middle portion of the small intestine (Fig. 2G). Neurotensin- and enteroglucagon-immunoreactive cells which were distributed in every portion of the small intestine were most abundant in the distal small intestine (Figs. 2H, I). Pancreatic glucagon-immunoreactive cells were absent from the initial portion and rare in the remainder of the small intestine. A small

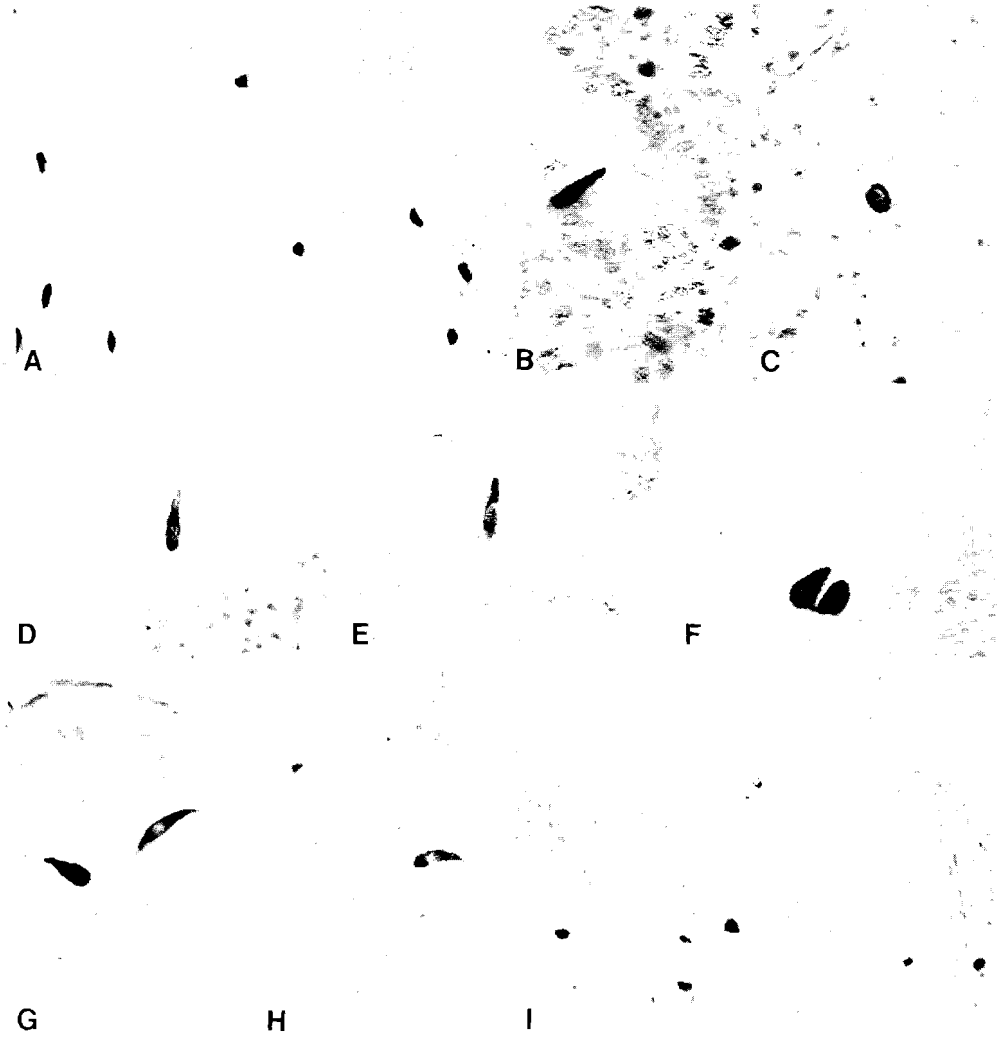

Fig. 2. Immunoreactive cells in the small intestine. A. Serotonin-immunoreactive cells in the middle region of the small intestine. B. Gastrin-immunoreactive cell in the initial part of the small intestine (proximal duodenum). C. Gastrin-immunoreactive cell in the duodenal gland. D. Cholecystokinin-immunoreactive cell in the middle region of the small intestine. E. Gastric inhibitory polypeptide-immunoreactive cell in the middle region of the proximal half of the small intestine. F. Motilin-immunoreactive cells in the initial part of the small intestine (proximal duodenum). G. Secretin-immunoreactive cells in the middle region of small intestine. H. Neurotensin-immunoreactive cell in the middle region of the proximal half of the small intestine. I. Enteroglucagon-immunoreactive cells in the middle region of the small intestine. A and I × 188, B-H × 375

number of bovine pancreatic polypeptide (BPP)-immunoreactive cells were only found in the distal small intestine.

In the duodenal glands, localized in a narrow circular zone just caudal to the pylorus, a few gastrin-immunoreactive cells were detected in 6 of the 14 animals (Fig. 2C).

In the large intestine, ten types of immunoreactive cells were found, but CCK-immunoreactive cells were absent. Serotonin- and BPP-immunoreactive cells were most numerous in this portion and were mainly located in the crypts (Figs. 3A, B). Small numbers of somatostatin-gastrin-, secretin-, and enteroglucagon-immunoreactive cells were detected in all animals, while GIP-, motilin-, neurotensin- and pancreatic glucagon-immunoreactive cells were very rare and not detected in every animal (Figs. 3C-I). In the restricted region just caudal to the anorectal junction, moderate numbers of «open» as well as «closed» types of serotonin-immunoreactive cells were recognized in the stratified squamous epithelium (Fig. 3J).

Discussion

In the present study, 11 kinds of endocrine cells, immunoreactive for serotinin, somatostatin, gastrin, CCK, GIP, motilin, secretin, neurotensin, pancreatic glucagon, enteroglucagon and BPP, were identified in the gastrointestinal mucosa of the shrew.

This study shows that the distribution and relative frequency of the gut endocrine cells of the musk shrew were somewhat different from those of other mammals. Somatostatin-immunoreactive cells were rare in the fundic glands. Yet there was a wide distribution of gastrin-, CCK-, GIP-, motilin-, secretin- and neurotensin-immunoreactive cells in the intestine. Both serotonin- and BPP-immunoreactive cells were numerous in the large intestine.

In the cardiac glands in the mammals, at least three types of immunoreactive cells were detected consistently (Kitamura et al., 1982, 1984, 1985; Kawano et al., 1983; Calingasan et al., 1984; Ohara et al., 1986; Ito et al., 1987). However, in the present study, a few serotonin- and somatostatin-immunoreactive cells were recognized but even then in only two of the 14 shrews investigated. In the duodenal glands, three to six types of immunoreactive cells, as small discrete populations, were identified and are found consistently in many mammalian species (Kitamura et al., 1982; 1984, 1985; Kawano et al., 1983; Calingasan et al., 1984; Ohara et al., 1986; Ito et al., 1987). In the present study, however, only a few gastrin-immunoreactive cells were found in 6 of the 14 shrews.

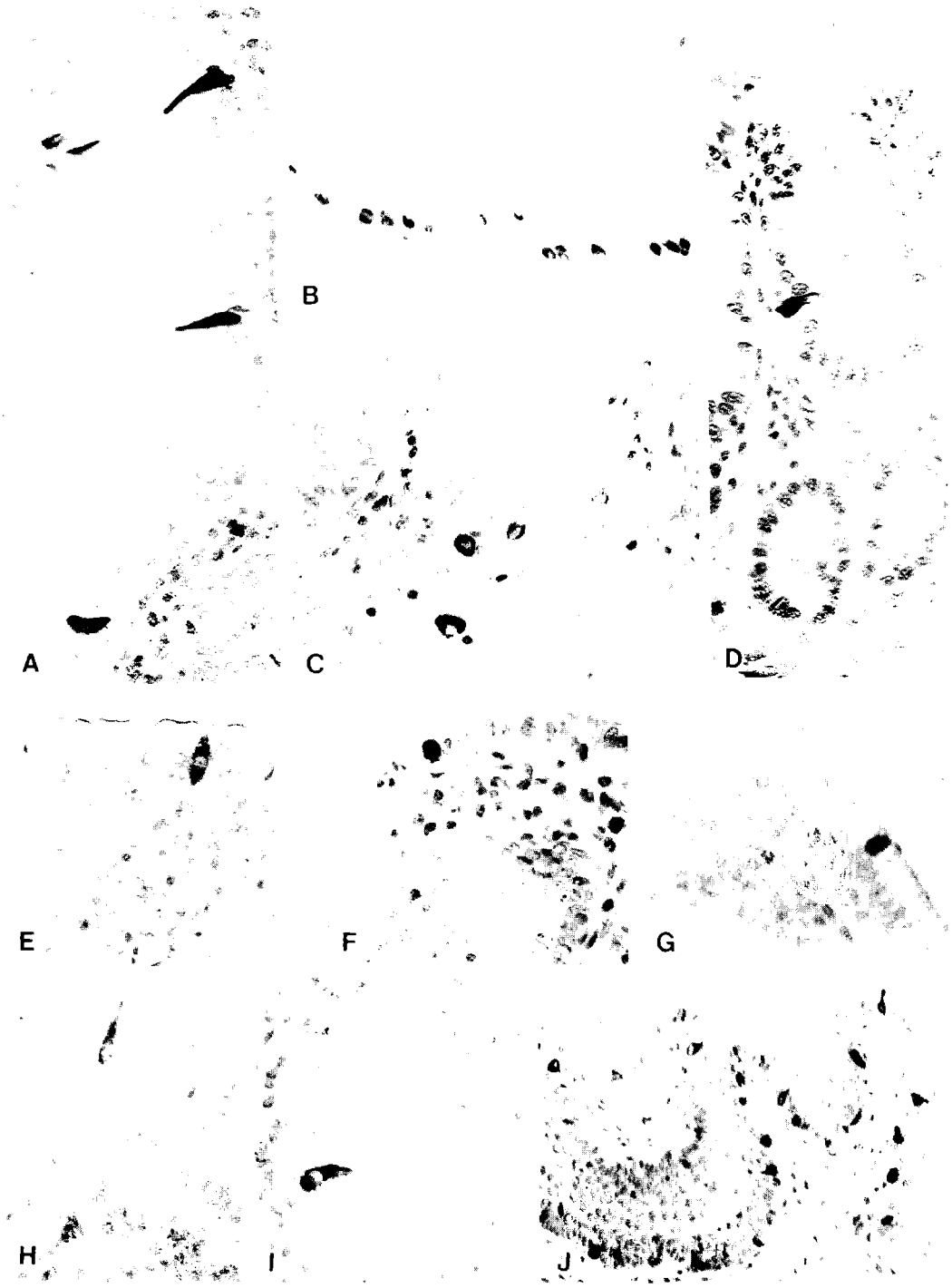

Fig. 3. Serotonin- (A), bovine pancreatic polypeptide- (B), somatostatin- (C), gastrin- (D), gastric inhibitory polypeptide- (E), motilin- (F), secretin- (G), neurotensin- (H) and pancreatic glucagon-immunoreactive cells (I) in the terminal part of the intestine (rectum). J. Serotonin-immunoreactive cells in the stratified squamous epithelium just caudal to the anorectal junction. A, C-I × 375, B × 188, J × 220

Somatostatin-immunoreactive cells of the gastric mucosa have been reported to be more numerous in the fundic glands than in the pyloric glands in the rat, dog, man, horse and Japanese field vole (Alumets et al., 1977; Kitamura et al., 1984; Ohara et al., 1986). In other mammals, they were detected in larger numbers or similar numbers in the duodenum (Kitamura et al., 1982, 1985; Kawano et al., 1983; Calingasan et al., 1984; Ito et al., 1987). Phillip et al. (1977) suggested that suppression of human gastric acid secretion largely resulted from a direct effect of somatostatin on the parietal cells and only to a minor extent from prevention of gastrin release. It could be presumed that in the shrew, which rarely has somatostatin-immunoreactive cells in fundic glands, direct inhibition of gastric secretion by somatostatin might share lesser extent than in other mammals.

As a rule, in most mammals studied to date, gastrin-, CCK-, GIP-, motilin- and secretin-immunoreactive cells

Table 1. Antisera used.

Antiserum raised to	Code	Specificity	Dilution	Source
Serotonin	Lot.16302	...	1:10000	Immuno Nuclear Corp., Stilwater
Synthetic human cyclic somatostatin	...	...	1:3000	S.Ito, Niigata
Synthetic porcine glucagon	GL-5	Reacts with pancreatic glucagon	1:2000	N.Yanaihara, Shizuoka
Porcine glucagon	RPN.1602	Completely cross-reacts with pancreatic and intestinal glucagon	1:1000	Amersham International pl, Amersham
Bovine pancreatic polypeptide	615-R-110-146-17	Cross-reacts with human pancreatic polypeptide	1:12000	R.E.Chance, Indianapolis
Synthetic porcine motilin	R-1104	Reacts against entire molecule	1:4000	N.Yanaihara
Synthetic human gastrin	GP-1304	No cross-reaction with CCK-8	1:5000	N.Yanaihara
Gastric inhibitory polypeptide	G/R/34-IIID	No cross-reaction with glucagon	1:10000	Guildhey antisera, Surrey
Natural porcine cholecystokinin	...	Reacts with CCK-11-20; No cross-reaction with gastrin	1:3000	D. Grube, Hannover
Synthetic porcine secretin	R-801	Reacts with the C- and N-terminals	1:1000	N.Yanaihara
Synthetic bovine neurotensin	R-3501	...	1:1000	N.Yanaihara

All antisera were raised in rabbits except that against insulin and gastrin which were raised in guinea pigs.

Table 2. Distribution and relative frequency of endocrine cells in the gastrointestinal tract of the musk shrew, *Suncus Murinus*.

Endocrine cells	Stomach			Intestine						
	Cardiac	Fundic	Pyloric	DG	1	2	3	4	5	6
Serotonin	±	++	++	−	++	++	++	++	++	+++
Somatostatin	±	±	++	−	+	+	+	+	+	+
Gastrin	−	−	+++	±	++	++	+	+	±	+
CCK	−	−	−	−	+	+	+	±	±	−
GIP	−	−	−	−	+	+	+	±	±	±
Motilin	−	−	−	−	++	+	±	±	±	±
Secretin	−	−	−	−	+	+	++	+	+	±
Neurotensin	−	−	−	−	±	±	+	+	+	±
PG	−	±	±	−	−	±	±	±	±	±
EG	−	±	−	−	±	+	++	++	++	+
BPP	−	−	−	−	−	−	−	±	+	+++

- not detected, ± rare and not detected in every animal, + few but detected in every animal, ++ moderate, +++ numerous, DG: Duodenal glands, CCK:Cholecystokinin, GIP:Gastric inhibitory polypeptide, PG:Pancreatic glucagon, EG:Enteroglucagon, BPP:Bovine pancreatic polypeptide, 1:initial part of the small intestine (proximal duodenum), 2:middle region of the proximal half of the small intestine, 3:middle region of the small intestine, 4:middle region of the distal half of the small intestine, 5:terminal region of the small intestine (ileum), 6:terminal part of the intestine (rectum)

are mainly localized in the proximal small intestine, while the neurotensin-immunoreactive cells are characteristically most abundant in the distal small intestine (Polak et al., 1975; Tobe et al., 1976; Helmstaedter et al., 1977; Larsson et al., 1977; Sundler et al., 1977; Kitamura et al., 1982, 1984, 1985; Kawano et al., 1983; Calingasan et al., 1984; Ohara et al., 1986; Ito et al., 1987). In the present study, however, these endocrine cells were distributed along the entire length of the intestine, except for the absence of CCK-immunoreactive cells in the large intestine. It is well known that gut endocrine cells are distributed more widely in fetal life than in adult life (Larsson, 1977). However, the present study was carried out on adults, so the extensive distribution of the gut endocrine cells does not appear to be due to ontogenic reasons. It is possible that there is a relationship between the extensive distribution of the gut endocrine cells and the short length of the intestine of the shrews. The large intestine of the shrew may have small intestinal nature to a certain extent at least in the aspect of endocrine regulation of gut function such as absorption. Morphological study of the mucosa suggested the possibility that the shrew large intestine has a high ability of absoprtion (Kurohmaru, 1985).

The total population of endocrine cells in the lower

gut have been reported to be more numerous distally (rectum) in other species as well, including man (Cristina et al., 1978; Kitamura et al., 1982, 1985; Calingasan et al., 1984). Kurohmaru et al. (1980) and Kiso et al. (1987) reported that in the shrew gut endocrine cells were more numerous in the crypts of the large intestine. These reports are consistent with the present immunohistochemical results. Why numerous gut endocrine cells are present in the terminal part of the intestine is not yet clear. However, it is possible that they may be involved in feed-back controls of secretory and motile functions as supposed by Kitamura et al. (1982). The importance of the colon and rectum as an endocrine organ has been considered (Lluis and Thompson, 1988). The lower gut as endocrine organ in the shrew may be also important in the aspect of the number and variety of endocrine cells.

It could be presumed that the distribution of the gut endocrine cells in the shrew, as a primitive insectivore, may represent the archetype of mammals, although further studies are needed to clarify this speculation.

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References

Alumets J., Sundler F. and Håkanson R. (1977). Distribution, ontogeny and ultrastructure of somatostatin immunoreactive cells in the pancreas and gut. Cell Tissue Res. 185, 465-479.

Calingasan N.Y., Kitamura N., Yamada J. and Yamashita T. (1984). Immunocytochemical study of the gastroenteropancreatic endocrine cells of the sheep. Acta Anat. 118, 171-180.

Cristina M.L., Lehy T., Zeitoun P. and Doufougeray F. (1978). Fine structural classification and comparative distribution of endocrine cells in normal human large intestine. Gastroenterology 75, 20-28.

Helmsteadter V., Taugner Ch., Ferule G.E. and Forssman W.G. (1977). Localization of nerutoensin-immunoreactive cells in the small intestine of man and various mammals. Histochemistry 53, 34-51.

Ito H., Yamada J., Yamashita T., Hashimoto Y. and Kudo N. (1987). An immunohistochemical study on the distribution of endocrine cells in the gastrointestinal tract of the pig. Jpn. J. Vet. Sci. 49, 105-114.

Kawano H., Yamashita T., Yamada J. and Kitamura N. (1983). A light microscopic study of the gastro-entero-pancreatic endocrine cells of the mink *(Mustera vision)*. Arch. Histol. Jpn. 46, 559-573.

Kiso Y., Oku K. and Yamauchi S. (1987). Four types of endocrine cells in the intestine of the suncus, *Suncus murinus*. Jpn. J. Zootech. Sci. 58, 714-717.

Kiso Y., Oku K. and Yamauchi S. (1988). Prenatal and postnatal development of the small intestine in the insectivore *Suncus Murinus*. Am. J. Anat. 183, 57-67.

Kitamura N., Yamada J., Yamashita T. and Yanaihara N. (1982). Endocrine cells in the gastrointestinal tract of the cat. Biomed. Res. 3, 612-622.

Kitamura N., Yamada J., Calingasan N.Y. and Yamashita T. (1984). Immunocytochemical distribution of endocrine cells in the gastrointestinal tract of the horse. Equine Vet. J. 16, 103-107.

Kitamura N., Yamada J., Calingasan N.Y. and Yamashita T. (1985). Histologic and immunocytochemical study of endocrine cells in the gastrointestinal tract of the cow and calf. Am. J. Vet. Res. 46, 1381-1386.

Kondo K. (1985). *Suncus murinus*. Biology of the Laboratory Shrew. Jpn. Sci. Soc. Press, Tokyo (In Japanease with English summary).

Kurohmaru M., Nishida T. and Mochizuki K. (1980). Morphological study on the intestine of the musk shrew, *Suncus murinus*. Jpn. J. Vet. Sci. 42, 61-71.

Kurohmaru M. (1985). Characteristics of the gastrointestinal tract of the house musk shrew, *Suncus murinus*. In: *Suncus murinus*, Biology of the laboratory shrew. Kondo K. (ed), Jpn. Sci. Soc. Press, Tokyo. pp 389-396 (In Japanese with English summary).

Larsson L.-I. (1977). Ontogeny of peptide-producing nerves and endocrine cells of the gastro-duodeno-pancreatic region. Histochemistry 54, 133-142.

Larsson L.-I., Sundler F., Alumets J., Håkanson R., Schaffalitzky de Muckadell O.B. and Fahrenkrug J. (1977). Distribution, ontogeny and ultrastructure of mammalian secretin cell. Cell Tissue Res. 181, 361-368.

Lluis F. and Thompson J.C. (1988). Neuroendocrine potential of the colon and rectum. Gastroenterology 94, 832-844.

Mason T.E., Phifer R.E., Spicer S.S., Swallow R.A. and Dreskin R.B. (1969). An immunoglobulin-enzyme bridge method for localizing tissue antigens. J. Histochem. Cytochem. 17, 563-569.

Ohara N., Kitamura N., Yamada J. and Yamashita T. (1986). Immunohistochemical study of gastroenteropancreatic endocrine cells of the herbivorous Japanese field vole, *Microtus montebelli*. Res. Vet. Sci. 41, 17-21.

Phillip J., Domschke S., Domschke W., Urbach H.-J., Reiss M. and Demling L. (1977). Inhibition by somatostatin of gastrin release and gastric acid responses to meals and to pentagastrin in man. Scand. J. Gastroenterol. 12, 261-265.

Polak J.M., Pearse A.G.E., Bloom S.R., Buchan A.M.J., Rayford P.L. and Thompson J.C. (1975). Identification of cholecystokinin-secreting cells. Lancet II, 1016-1018.

Sternberger L.A. (1979). Immunocytochemistry, 2nd ed. John Wiley & Sons. New York. pp 24-169.

Sundler F., Håkanson R., Hammer R.A., Alumets J., Carraway R., Leeman S.E. and Zimmerman E.A. (1977). Immunohistochemical localization of neurotensin in endocrine cells of the gut. Cell Tissue. Res. 179, 313-321.

Tobe T., Chen S.T., Henmi K. and Fukuchi K. (1976). Distribution of gastrin in canine, cat and human digestive organs. Am. J. Surg. 132, 581-582.

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