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An Immunohistochemical study of the gastro-entero-pancreatic endocrine cells of the insectivorous Korean mole, *Talpa micrura coreana*

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한국산 두더지의 위장췌내분비세포에 관한 면역조직학적 연구

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초 록 : 한국산 두더지의 위장관 점막과 췌장에 분포하는 내분비세포들의 분포와 출현빈도 및 종류를 알아보기 위하여 면역조직화학적으로 관찰하였던 바, 5-HT 면역반응세포는 극소수내지 중등도의 빈도로 전 장관에 고루 분포하였다. Glucagon 면역반응세포는 대장에 국한하여 출현하였다. 또 bovine CG, BPP 및 somatostatin 면역반응세포들은 각각 십이지장, 공장 그리고 위저부를 제외한 전 장관에 다양한 빈도로 고루 분포하였다. Gas/CCK 면역반응세포는 유문부와 소장에서만 다수 그리고 극소수로 관찰되었으나, insulin 면역반응세포는 전 장관에서 관찰할 수 없었다. 한편 췌도의 주변부에서는 glucagon, somatostatin과 BPP 면역반응세포들이, 중심부에서는 insulin 면역반응세포가 높은 빈도로 동정되었으며 또한 외분비에서는 이들 면역반응세포들이 단독 또는 소수의 집단으로 출현하였다. 이상의 결과로써 두더지의 소화관내분비세포의 분포패턴는 고슴도치의 그것과 유사하였으나 십이지장에서 세포의 종류와 출현빈도가 낮은 것을 알 수 있었다.

Key words : gastrointestinal tract(GIT), endocrine cell, Korean mole, insectivora, immunoreactive cell, pancreas.

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Introduction

The Korean mole, *Talpa micrura coreana*, is a kind of the order Insectivora, feeding almost on insects or invertebrates. Hormone secreting cell of the gastrointestinal tract(GIT) are important in regulating digestive function[1]. Although many studies have elucidated the distribution and frequency of different endocrine cell types in the GIT of various vertebrates, a few works have been done on the endocrine cells in the GIT of the Insectivora[2~5], including the mole using immunohistochemical method[6].

The purpose of the present study was to clarify the fundamental regional distribution and relative frequency of each endocrine cell type in the gastro-entero-pancreatic system of the Korean mole, *Talpa micrura coreana*, by specific immunohistochemistry.

Materials and Methods

Five adult specimens of both sexes of the Korean mole, *Talpa micrura coreana*, were used in this study. Samples from 8 portions of the GIT and pancreas were fixed in Bouin's fluid. After paraffin embedding, 4μm histological sections were prepared. The representative sections were then deparaffinized, rehydrated and immunostained with the peroxidase antiperoxidase(PAP) method[7]. Background blocking was performed with normal goat serum prior to incubation with the specific antiserum(Table 1). After rinsing in PBS buffer, the sections were incubated in secondary serum. They were then washed in PBS buffer and finally 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 buff-

Table 1. Antisera used in this study

Antisera*	Code	Source	Dilution
5-hydroxytryptamine(5-HT)	8535028	Immunonuclear Corp., Stillwater	1:10,000
Glucagon	8635013	Immunonuclear Corp., Stillwater	1:800
Insulin	8622014	Immunonuclear Corp., Stillwater	1:2,000
Bovine chromogranin(Bovine CG)	8541011	Immunonuclear Corp., Stillwater	1:500
Gastrin/cholecystokinin(Gas/CCK)	i600/004	Union Chimique Belge bioproducts	1:100
Bovine pancreatic polypeptide(BPP)	i607	Union Chimique Belge bioproducts	1:5,000
Somatostatin	CA325	Cambridge Research Biochemical Billerica	1:1,000

*All antisera were raised in rabbits except for insulin which was raised in a guinea pig.

Table 2. The regional distributions and relative frequencies of the gastro-entero-pancreatic endocrine cells of the Korean mole

	Fundus	Pylorus	Duodenum	Jejunum	Ileum	Colon	Rectum	Pancreas
5-HT	+	++	+	+	±	+	±	-
Glucagon	-	-	-	-	-	+	±	++
Insulin	-	-	-	-	-	-	-	+++
Bovine CG	++	++	-	±	±	±	+	-
Gas/CCK	-	+++	-	±	±	-	-	-
BPP	±	±	+	-	±	+	+	+
Somatostatin	-	+++	+	±	±	±	±	±

- : Not detected, ± : Rare, + : A few, ++ : Moderate, +++ : Numerous

er. After immunostaining, the sections were counterstained with Mayer's hematoxylin.

Results

In this study, seven kinds of immunoreactive cells were revealed with the antisera against 5-hydroxytryptamine, glucagon, insulin, bovine chromogranin, gastrin/cholecystokin, bovine pancreatic polypetide and somatostatin. However, no insulin-immunoreactive cells were found in the GIT, but reacted on the pancreas in the Korean mole. The regional distribution and relative frequency of the immunoreactive cells in the GIT and pancreas are shown in Table 2.

5-HT-immunoreactive cells were found in the epithelia from the fundic gland region to the rectum, reaching a peak in the pyloric gland region. In the fundic gland region(Fig 1a), the distribution of these cells were detected mainly in the basal portion, whereas in the pyloric gland region they were concentrated only in basal half(Fig 1b). In the intestine, these cells observed to decrease in number distally along the intestine, but seemed to increase in the colon(Fig 1e). They were located predominantly in the basal portion of the intestinal epithelia or glands(Figs 1c-e).

Glucagon-immunoreactive cells were found only a few number in the colon(Fig 2) and rarely in the rectum.

Bovine CG-immunoreactive cells were distributed in the whole GIT except for the duodenum. In the stomach, they were moderate in numbers in the fundic(Fig 3a) and pyloric gland regions(Fig 3b). In the intestine, these cells were rare in the jejunum, ileum(Fig 3c), colon and a few number in the rectum(Fig 3d). In addition, these cells were identified mostly in the basal portion of the stomach gland region, but sometimes scattered in the epithelia of the stomach and intestinal glands.

Gas/CCK-immunoreactive cells were found most numerously in the pyloric gland region(Fig 4a), and rarely in the jejunum(Fig 4b) and ileum. They were located mainly in the middle third of the pyloric gland region and in the intestinal glands.

BPP-immunoreactive cells were detected rarely in the stomach(Figs 5a,b), a few number in the duodenum(Fig 5c) and rarely in the ileum(Fig 5d). While the distribution of these cells were mainly limited in the basal portions of the stomach, they also observed in the intestinal epithelia and glands.

Somatostatin-immunoreactive cells were also detected throughout the GIT with the exception of the fundic gland region. They were numerous in the pyloric gland region(Fig 6a), gradually decreasing to the rectum. They were mainly detected in the basal half of the pyloric gland region(Fig 6a) and the intestinal epithelia(Figs 6b-d).

In the pancreas, glucagon-, insulin-, somatostatin- and BPP-immunoreactive cells were detected(Figs 7a-d). Glucagon-, somatostatin- and BPP-immunoreactive cells were usually confined to the periphery of the islets, in contrast to insulin-immunoreactive cells were demonstrated in the central portion of the islets in the endocrine portions. In exocrine portions, glucagon-, insulin-, somatostatin- and BPP-immunoreactive cells were detected singly or in small groups.

Discussion

In the present study, the regional distribution and relative frequency of the gastro-entero-pancreatic endocrine cells in the Korean mole were essentially similar to those of other mammals[8,9]. However, some characteristic differences were observed in this species.

Usually the predominant frequency of 5-HT-immunoreactive cells were found in the pyloric gland region[16], duodenum[10-14] and colon[3,4,15]. In the present study, these cells contain the highest number in the basal half of the pyloric gland, a finding similar to that for the African clawed toad[16]. Also this pattern of distribution would be agree with enterochromaffin cellls reported previous work[6].

In general glucagon-immunoreactive cells were found exclusively in the stomach[14,15,18,19] or throughout the GIT[3,4] of the mammals. Park and Chung[6] reported that these cells were distributed in the mucosa from the ileum to the rectum. However, in this study these cells were only detected in the large intestine. These discrepancies might be due to difference in the antiserum.

Our results showed that bovine chromogranin(BCG)-im-

munoreactive cells were distributed throughout the GIT except for the duodenum. On the other hand, chromogranin A(CGA) is widely distributed in endocrine cells of mammals[20,21]. This suggests a putative role for chromogranins(CGs) as precursors of bioactive peptides. Accordingly, CG-immunoreactive cells have been shown to be present in all identifiable endocrine cell types in the GIT[13,14,23-25]. This pattern of distribution is similar to those reported for the mammals[13,14,23-25] except for the duodenum which was devoid of these cells.

In mammalian species, Gas/CCK-immunoreactive cells were commonly restricted to the pyloric gland region and small intestine[26,27]. In the present study, these cells were demonstrated most numerously in the pyloric gland region, and rarely in the small intestine except for the duodenum. Similar finding has been reported for the Korean hedgehog[3,4], mole[6], Korean native cattle[10], Korean native goat[13], and the mouse, guinea pig and rabbit[28,29]. However, the absence of these cells in the duodenum was quite different from those of the previous reports.

We also observed that BPP-immunoreactive cells were distributed throughout the GIT except for the jejunum. It is known that the pattern of regional distribution of these cells are divided by three pattern, namely, mainly distributed to the large intestine pattern[6,10,12,13,30,31], distributed to the stomach and the large intestine pattern[32], and distributed to the whole GIT pattern[3,4,11,15]. This pattern of distribution was well correlated with the those reported for the whole GIT[3,4,11,15].

It is known that somatostatin-immunoreactive cells show the widest distribution in the whole GIT of all vertebrate species[33], and play important roles in gastric regulation, namely, the feedback control of gastric acid secretion[18]. Lee *et al*[14] suggested that the peaked pattern of regional distribution of these cells be divided of two groups, the fundic and pyloric glands according to species. The result for the somatostatin cells in the Korean mole support these findings, whereas the regional distribution of the rectum was quite different from the absence of the Korean hedgehog[3] and mole[6].

The discrepancies of distribution patterns and frequencies of the gastrointestinal endocrine cells could be related to the their enviroments, physiological characteristics and diet differences to the Korean mole.

In the pancreatic islets, the typical peripheral distribution of glucagon-, somatostatin- and BPP-immunoreactive cells and the central location of insulin-immunoreactive cells is well known in various species. In addition these cells were also scattered singly or small groups in the pancreatic exocrine portions. The results of the present study confirmed such findings but there were no 5-HT- and bovine CG-immunoreactive cells in the pancreatic islets, whereas these cells were described by Ding *et al*[34] and Grube and Yoshie[35].

In conclusion, we have demonstrated the characteristic patterns of distribution of seven kinds of the gastro-entero-pancreatic endocrine cells and their relative frequencies in the Korean mole.

Summary

The regional distribution and relative frequencies of the gastro-entero-pancreatic endocrine cells were studied immunohistochemically in the GIT and pancreas of the Korean mole. Seven kinds of endocrine cells were identified in this study. Although 5-HT- and somatostatin-immunoreactive cells were seen throughout the GIT, they were most predominant in the pyloric gland region. Glucagon-immunoreactive cells were restricted on the large intestine. Bovine CG-immunoreactive cells were more frequent in the stomach than in the intestines which were not detected in the duodenum. Numerous Gas/CCK-immunoreactive cells were found in the pyloric gland region, but rarely in the jejunum and ileum. BPP-immunoreactive cells were observed to be rare in the stomach and ileum but were a few in number in the intestines.

In the pancreas, four types, namely, glucagon-, somatostatin-, BPP- and insulin-immunoreactive cells were identified in the pancreatic islets and exocrine portion. These results suggest that although endocrine cells of the Korean mole is less abundant in the duodenum, the distribution pattern of its gastro-entero-pancreatic endocrine cells is similar to that reported for the Korean hedgehog.

Legends for figures

Fig 1. 5-HT-immunoreactive cells throughout the gastrointestinal tract.
a. fundus, b. pylorus, c. duodenum, d. jejunum, e. colon. a-e; × 240

Fig 2. Glucagon-immunoreactive cells in the colon. × 240

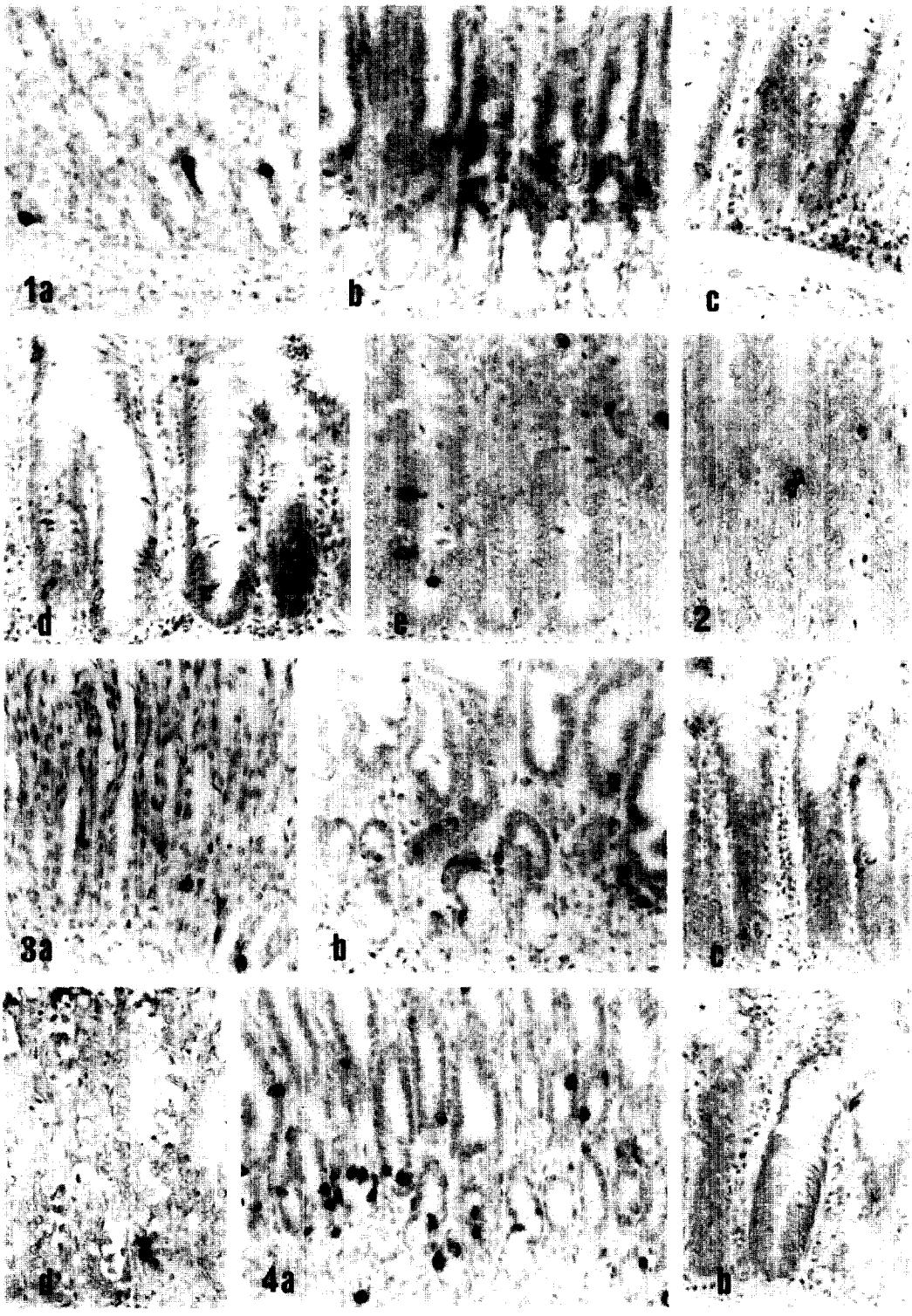
Fig 3. Bovine CG-immunoreactive cells throughout the gastrointestinal tract with the exception of the duodenum.
a. fundus, b. pylorus, c. ileum, d. rectum. × 240

Fig 4. Gas/CCK-immunoreactive cells in the pylorus(a) and jejunum(b).
a, b; × 240

Fig 5. BPP-immunoreactive cells throughout the gastrointestinal tract except for the jejunum.
a. fundus, b. pylorus, c. duodenum, d. colon. a-d; × 240

Fig 6. Somatostatin-immunoreactive cells the gastrointestinal tract except for the fundus.
a. pylorus, b. duodenum, c. ileum, d. colon, a-d; × 240

Fig 7. Immunoreactive cells in the pancreas.
a. glucagon, b. insulin, c. somatostatin, d. BPP. a-d; × 240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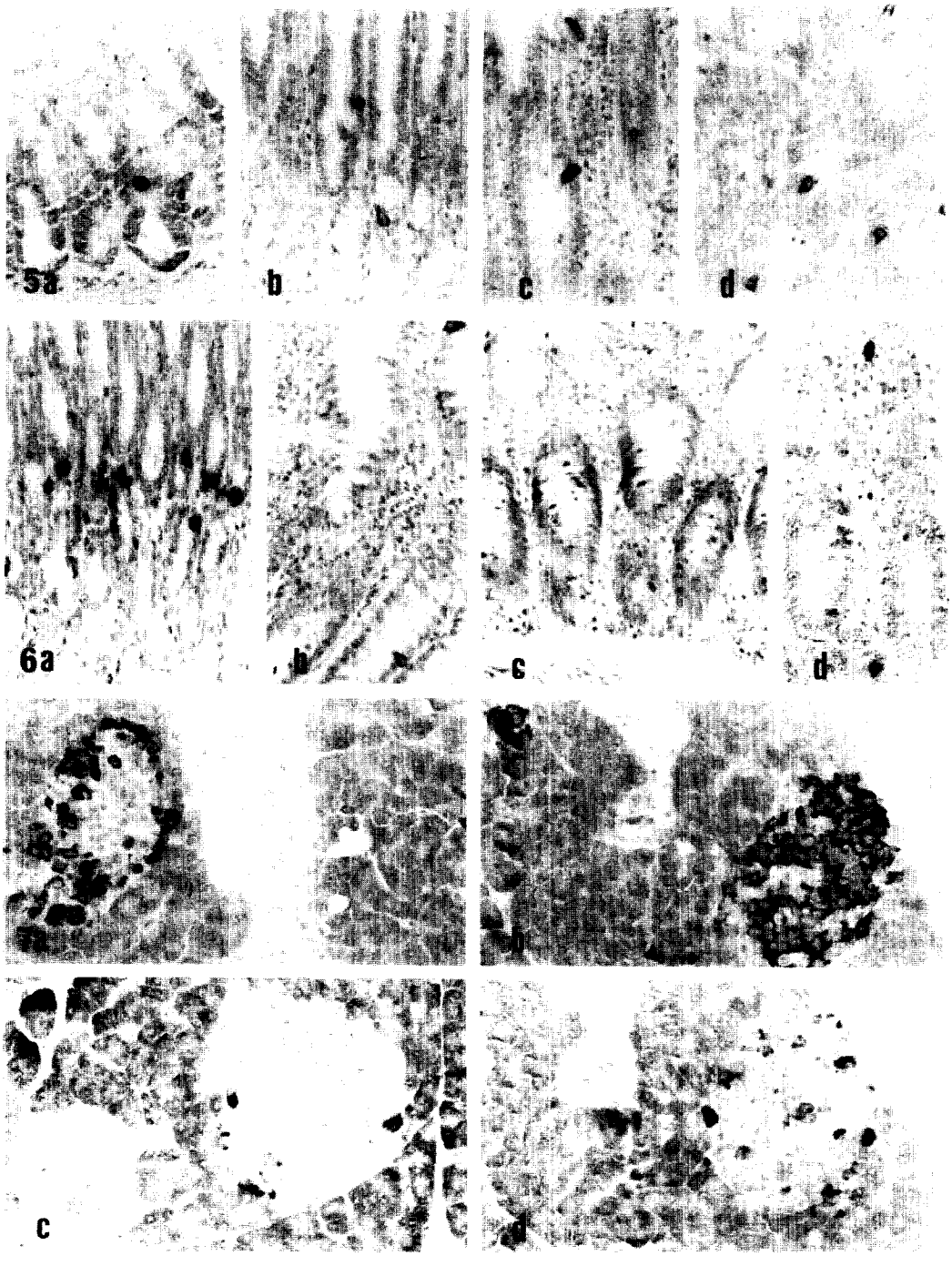
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References

1. Bell FR. The relevance of the new knowledge of gastrointestinal hormones to veterinary science. *Vet Sci Commun*, 2: 305-314, 1979.
2. Lee JH. An ultrastructural study on endocrine cells in the pyloric region of the Korean hedgehog(*Erinaceus koreanus*). *Korean J Electron Microscopy*, 17: 177-184, 1987.
3. Lee JH. An immunohistochemical and ultrastructural studies on the gut endocrine cells in the hedgehog, *Erinaceus koreanus. Ibid*, 18: 59-76, 1988.
4. Lee JH, Lee HS, Lee NS, *et al.* An immunohistochemical study of the endocrine cells in the stomach of the Korean hedgehog(*Erinaceus koreanus). Korean J Vet Res*, 31: 19-26, 1991.
5. Lee JH, Lee HS, Lee NS, *et al.* An electron microscopic study on endocrine cells in the cardiac and fundic regions of the Korean hedgehog *(Erinaceus koreanus). Ibid*, 30: 373-381, 1990.
6. Park IS, Chung JW. The topographic distribution of enterochromaffin, gastrin, somatostatin, secretin, glucagon and pancreatic polypeptide cells in the gastrointestinal tract of the mole, *Talpa micrura coreana. Korean J Anat*, 19: 91-103, 1986.
7. Sternberger LA. Immunohistochemistry. 2nd ed. New York: John Wiley & Sons, 104-169, 1979.
8. Bloom SG, Polak JM. Gut hormones. Edinburgh, Churchill Livingstone, 3-18, 1978.
9. Solcia E, Grossman MI, Grube D, *et al.* Cellular basis of chemical messengers in the digestive system. New York, Academic Press, 159-165, 1981.
10. Cho SW, Kitamura N. Immunocytochemical study of the endocrine cells in the gastrointestinal tract of the Korean native cattle. *Korean J Vet Res*, 28: 251-259, 1988.
11. Ito H, Yamada J, Yamashita T, *et al.* An immunohistochemical study on the distribution of endocrine cells in the gastrointestinal tract of the pig. *Jpn J Vet Sci*, 49: 105-114, 1987.
12. Kitamura N, Yamada J, Calingasan NY, *et al.* Histologic and immunocytochemical study of endocrine cells in the gastrointestinal tract of the cow and calf. *Am J Vet Res*, 46: 1381-1386, 1985.
13. Lee JH, Lee HS. An immunohistochemical study of the endocrine cells in gastrointestinal tract of the Korean native goat. *Korean J Vet Res*, 30: 261-270, 1990.
14. Lee HS, Hashimoto Y, Kon Y, *et al.* An immunohistochemical study of the gastro-entero-pancreatic endocrine cells in the alimentary tract of the Korean tree squirrel, *Sciurus vulgaris corea. Jpn J Vet Res*, 39: 117-131, 1991.
15. Ohara N, Kitamura N, Yamada J, *et al.* Immunohistochemical study of gastro-entero-pancreatic endocrine cells of the hervivorus Japanese field vole, *Microtus montebelli. Res Vet Sci*, 41: 21-27, 1986.
16. Lee HS, Lee JH. An immunohistochemical study of the endocrine cells on the gastro-entero-pancreatic system of the African clawed toad, *Xenopus laevie. Korean J Vet Res*, 32: 523-529, 1992.
17. Larsson L-I, Holst JJ, Hakanson R, *et al.* Distribution and properties of glucagon immunoreactivity in the digestive tract of various mammals: An immunohistochemical and immunochemical study. *Histochemistry*, 44: 281-290, 1975.
18. Kitamura N, Yamada J, Calingasan NY, *et al.* Immunocytochemical distribution of endocrine cells in the gastrointestinal tract of the horse. *Equine Vet J*, 16: 103-107, 1984.
19. Kitamura N, Yamada J, Yamashita T, *et al.* Endocrine cells in the gastrointestinal tract of the cat. *Biomed Res*, 3: 612-622, 1982.
20. Cetin Y, Müller-Köppel L, Aunis D, *et al.* Chromogranin A(Cg A) in the gastro-entero-pancreatic (GEP) endocrine system. II. Cg A in mammalian entero-endocrine cells. *Histochemistry*, 92: 265-275, 1989.
21. Rinidi G, Buffa R, Sessa F, *et al.* Chromogranin A, B and C immunoreactivities of mammalian endocrine cells. Distribution, distinction form costored hormones/prohormones and relationship with the argyrophil component of seretory granules. *Ibid*, 85: 19-28, 1986.

22. Facer P, Bishop AE, Lloyd RV, *et al.* Chromogranin: a newly recognized marker for endocrine cells of the human gastrointestinal tract. *Gastroenterology*, 89: 1366-1373, 1985.
23. Grube D, Aunis D, Bader F, *et al.* Chromogranin A (CGA) in the gastro-entero-pancreatic(GEP) endocrine system. I. CGA in the mammalian endocrine pancreas. *Histochemistry*, 85: 441-452, 1986.
24. Ito H, Hashimoto Y, Kitagawa H, *et al.* Distribution of chromogranin containing cells in the porcine gastroenteropancreatic endocrine cells. *Jpn J Vet Sci*, 50: 395-404, 1988.
25. Konecki DS, Benedum UM, Gerdes HH, *et al.* The primary structure of human chromogranin A and pancreastatin. *J Biol Chem*, 262: 17026-17030, 1987.
26. Dockray GJ. Gut hormones. Edinburgh: Churchill Livingstone, 129-139, 1978.
27. Solcia E, Grossman MI, Grube D, *et al.* Cellular basis chemical messengers in the digestive system. New York, Academic Press, 159-165, 1981.
28. Larsson L-I, Rehfeld J. Evolution of CCK-like hormones. In: Gut hormones. ed. Bloom SR, Edinburgh: Churchill Livingstone, 68-73, 1978.
29. Solcia E, Vassallo G, Capella C. Studies on the G cells of the pyloric mucosa, the probable site of gastrin secretion. *Gut*, 10: 379-388, 1969.
30. Sjölund K, Sanden C, Hakanson R, *et al.* Endocrine cells in the human intestine: an immunocytochemical study. *Gastroenterology*, 85: 1120-1130, 1983.
31. Calingasan NY, Kitamura N, Yamada J, *et al.* Immunocytochemical study of the gastroenteropancreatic endocrine cells of the sheep. *Acta Anat*, 118: 171-180, 1984.
32. Lee JH, Lee HS. Immunohistochemical study on the gastro-entero- pancreatic(GEP) endocrine cells of the blue fox, *Alopex lagopus. Korean J Vet Res*, 33: 369-379, 1983.
33. Falkmer S, Stefan Y. Pancreatic polypeptides(PP): phylogenetic aspects in gastrointestinal mucosa and endocrine pancreas. *Scan J Gastroenterol*, 13: (Suppl)49: 59, 1978.
34. Ding WG, Fujinura M, Tooyama I, *et al.* Phylogenetic study of serotonin-immunoreactive structures in the pancreas of various vertebrates. *Cell Tissue Res*, 263: 237-243, 1991.
35. Grube D, Yoshie S. Immunohistochemistry of chromogranin A and B, and secretogranin II in the canine endocrine pancreas. *Arch Histol Cytol*, 52: 287-298, 1989.