

## Immunohistochemical study on the gastro-entero-pancreatic(GEP) endocrine cells of the blue fox, *Alopex lagopus*

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### 북극여우의 위장체 내분비세포에 관한 면역조직화학적연구

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**초록** : 북극여우의 위장체 내분비세포의 부위별 분포, 출현빈도 및 세포의 종류를 밝히고자 면역조직화학적으로 관찰하였던 바 위장관에서는 6종, 췌장에서는 4종의 면역반응세포가 동정되었다.

5-HT 및 somatostatin 면역반응세포는 전 위장관에 다수 분포하였으나 이중 somatostatin 면역반응세포는 장관에서 소수로 관찰되었다. Gas/CCK 면역반응세포는 주로 유문부와 십이지장에 국한하여 다수 분포하였다. Glucagon 면역반응세포를 분문부와 위저부에서 다수 그리고 장관에서 소수 관찰되었다. BPP 면역반응세포는 분문부와 위저부에 중등도, 대장에 소수 분포하였다. 다수의 porcine CG 면역반응세포가 분문부와 위저부에서만 동정되었다.

한편 췌장에서는 somatostatin, glucagon, BPP 및 insulin 등 4종의 면역반응세포가 췌도 및 외분비부에서 관찰되었다.

이상의 결과는 위장체 내분비세포의 부위별 분포와 출현빈도가 동물종에 따라 매우 상이하다는 사실을 시사한다.

**Key words** : immunoreactive cell, GI tract, pancreas, endocrine cell, blue fox.

### Introduction

The blue fox, *Alopex lagopus*, belongs to Canidae in the order Carnivora. All of Canidae have anatomical features resembling the domestic dog.

The mucosa of the gastrointestinal (GI) tract contains many kinds of hormone-producing cells. Together with the cells of the endocrine pancreas, those cells are classi-

fied as the gastro-entero-pancreatic (GEP) endocrine paracrine cells.<sup>1</sup>

Although many reports on the GEP endocrine cells have been published by immunohistochemical method, the distribution and frequency of the GEP endocrine cells on the blue fox have not yet been investigated.

This study, therefore, reports the distribution and the relative frequency of occurrence of the several kinds of

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endocrine cells throughout the GI tract and pancreas of the blue fox investigated by specific immunohistochemical method.

### Materials and Methods

The five adult blue foxes, *Alopex lagopus*, of both sexes, sampled in winter were used in this study. Nine regions of the GI tract and one portion of the pancreas were dissected out and fixed with Bouin's fluid. The fixed specimens were dehydrated in graded ethanol solutions, cleared in xylene, embedded in paraffin and sectioned serially at 4~6  $\mu$ m. Representative deparaffinized and rehydrated sections were stained with hematoxylin-eosin or PAS for histological examination.

For immunostaining, the deparaffinized sections were immunostained with the peroxidase antiperoxidase (PAP) method.<sup>2</sup>

Background blocking was performed with normal goat serum prior to incubation with the specific antiserum (Table 1). After rinsing in PBS buffer, the specimens 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% hydrogen peroxide in Tris HCl buffer. After immunostaining, the sections were lightly counterstained with Mayer's hematoxylin.

The relative frequency of occurrence of each type of immunoreactive cells was allocated to one of five cate-

gories according to their frequency as seen by light microscopy.

### Results

**The GI tract :** The regional distribution and the relative frequency of occurrence of the immunoreactive endocrine cells in the GI tract are shown in Table 2. Six kinds of immunoreactive cells were identified with the antisera against 5-HT, somatostatin, Gas/CCK, glucagon, BPP and porcine CG in the GI tract of the blue fox. Generally, immunoreactive cells were flask-shaped or elongated spindle and their apical cytoplasmic processes were in contact with the lumen except for those in cardiac and fundic regions, which were usually oval or elliptical in shape. 5-HT- and somatostatin-immunoreactive cells were found throughout the GI tract. Cells with other kinds of immunoreactivities shows various distribution patterns.

Numerous 5-HT-immunoreactive cells were distributed in the whole GI tract. The number of these cells was observed to decrease distally from the stomach to the small and large intestine. In the GI tract, 5-HT-immunoreactive cells were distributed mainly in the basal portion, but were not detected in the duodenal glands (Fig 1a-f).

Somatostatin-immunoreactive cells were also scattered throughout the GI tract. Numerous of these cells were found in the stomach and duodenum but a few in the distal portion of the small and large intestine. In the stoma-

**Table 1.** Antisera used in this study

Antisera <sup>a)</sup>	Code	Source	Dilution
Somatostatin	CA325	Cambridge research Biochemical, Billerica	1 : 1,000
Gastrin/ Cholecystokinin (Gas/CCK)	i600/004	Union Chimique Belge (UCB)-bioproducts	1 : 100
Bovine pancreatic polypeptide(BPP)	i607	UCB-bioproducts	1 : 5,000
Glucagon	8635013	Immunonuclear Corp (INC), Stillwater	1 : 800
5-hydroxytryptamine (5-HT)	8535028	INC	1 : 10,000
Insulin	8622014	INC	1 : 2,000
Porcine chromogranin (Porcine CG)	8541012	INC	1 : 2,000

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

**Table 2.** Distribution and relative frequency of GEP endocrine cells of the blue fox, *Alopex lagopus*

	Ca	Fu	Py	Du	Je	Il	Ce	Co	Re	Pan
5-HT	++++	++++	+++	++++	+++	+++	+++	+++	+++	-
Somatostatin	+++	++++	++++	+++--++	+	+	+	+	+	+
Gas/CCK	-	-	++++	++++--+++	+	-	-	-	-	-
Glucagon	+++	++++	-	+	+	++	-	+	-	+
BPP	++	++	-	-	-	-	-	+	+	+
Insulin	-	-	-	-	-	-	-	-	-	+
Porcine CG	+++	+++	-	-	-	-	-	-	-	-

++++ : very numerous, +++ : numerous, ++ : moderate, + : a few, - : absent.

Ca : Cardiac, Fu : fundic, Py : pyloric, Du : duodenum, Je : jejunum, Il : ileum, Ce : cecum, Co : colon, Re : rectum, Pan : pancreas.

ch, they were found mainly in the basal portion of glands(Fig 2a-g). In the fundic region, typical paracrine-like cell could be found(Fig 2c).

Gas/CCK-immunoreactive cells were found in the pyloric region, duodenum and jejunum. These cells were distributed predominantly in the pyloric region. In the duodenum, they were more numerous in the proximal portion than in the distal portion. A few Gas/CCK-immunoreactive cells were found in the jejunum(Fig 3a-c).

In the stomach, a large number of glucagon-immunoreactive cells were detected, but a few were also found in the small intestine. They were scattered all over the epithelia predominating in the middle third of the glands. Most of the glucagon-immunoreactive cells were found apart from the gastric lumen(Fig 4a-d), but some reached the lumen with its apical process in the small intestine(Fig 4e).

Moderate number of BPP-immunoreactive cells were found in the cardiac and fundic regions(Fig 5a, b), but a few in the colon and rectum(Fig 5c). They were mainly located in the basal portions of the gastric and intestinal glands, but a few in the epithelia of the large intestinal glands. They could not be detected from the pyloric region to the cecum.

Numerous porcine CG-immunoreactive cells were demonstrated only in the cardiac and fundic regions. They were restricted to the basal portion of the gastric glands(Fig 6a, b).

**The pancreas :** The endocrine portion of the pancreas of the blue fox comprised typical islets of Langerhans as well as endocrine cells scattered singly or in groups in the exocrine tissue.

Endocrine cells immunoreactive for insulin, glucagon, somatostatin and BPP were detected in the blue fox pa-

necreas as shown in Table 2. Insulin- and glucagon-immunoreactive cells were the most prominent cell types. Somatostatin- and BPP-immunoreactive cells were fewer than insulin- and glucagon-immunoreactive cells.

Glucagon-immunoreactive cells were detected both in the islet and exocrine tissue, though they were more frequent in the islet. They were located mainly at the periphery of the islet, showing oval or spherical in shape. In the exocrine portion, these cells were scattered singly or in small groups inside and along the acini(Fig 7a).

Insulin-immunoreactive cells were distributed throughout the islet. These cells were distributed in the periphery or the capillary side of the islet seemed to be immunostained intensely(Fig 7b).

Somatostatin-immunoreactive cells were relatively fewer in number than glucagon- and insulin-immunoreactive cells. These cells were located at the periphery of the islet, and they could also be detected singly in the exocrine portion(Fig 7d).

BPP-immunoreactive cells were scattered singly or in small groups inside and along the acini of the exocrine portion(Fig 7d).

5-HT-, Gas/CCK- and porcine CG-immunoreactive cells were not detected in the pancreas of the blue fox.

## Discussion

The present data represented the regional distribution and the relative frequency of occurrence of the GEP endocrine cells throughout the GI tract of blue fox.

Generally, the distribution of immunoreactive cells obtained here corresponds to that of other reports in various mammals<sup>3-10</sup> including human. However, some differences in the regional distribution and relative frequencies of immunoreactive cells in the GI tract were

observed in this species.

Although 5-HT- and somatostatin-immunoreactive cells were distributed throughout the GI tract, the highest frequencies were in the stomach and duodenum, but somatostatin-immunoreactive cells were a few in the intestine. The basic pattern of distribution of these cells in the blue fox was similar to that in the other species.<sup>11-21</sup> However, present data are different from those in other species such as cow<sup>22</sup>, horse<sup>23</sup>, Korean native goat<sup>24</sup>, pig<sup>25</sup>, Rhesus monkey<sup>26</sup>, Korean hedgehog<sup>27</sup>, Korean squirrel<sup>28,29</sup>, toad<sup>30</sup> and turtle<sup>31</sup> reported numerous or absent in large intestine than in small intestine. The distribution pattern of the somatostatin-immunoreactive cells in the blue fox was most similar to that in the cat.<sup>11</sup>

Gas/CCK-immunoreactive cells were restricted to the pyloric gland region, duodenum and jejunum. Numerous Gas/CCK-immunoreactive cells were distributed in the pyloric region and duodenum, but a few in the jejunum. The blue fox was different from the Korean native goat<sup>24</sup> by a small numbers of Gas/CCK-immunoreactive cells in the fundic region and the large intestine, turtle<sup>31</sup> by the distribution of these cells from the pyloric region to the rectum. However, the basic pattern of distribution of these cells in the blue fox was similar to that in the Korean squirrel<sup>28</sup> and toad.<sup>30</sup>

Glucagon-immunoreactive cells were distributed in the GI tract except the pyloric region, cecum and rectum. Very numerous of these cells were observed in the fundic region, numerous in the cardiac, moderate numbers in the ileum and a few in the other regions. Larsson et al.<sup>6</sup> reported that glucagon-immunoreactive cells were rarely seen in the fundic mucosa of the pig, and somewhat more numerous in the cardiac region than in the oxyntic one. In the present study, they showed an inverse frequency of occurrence between the cardiac and fundic region. This distribution pattern of the blue fox was different from the other species.<sup>11, 13, 15, 18-20, 22, 24, 27-30, 32</sup>

BPP-immunoreactive cells were restricted to the stomach and the large intestine. Moderate numbers of these cells were distributed in the cardiac and fundic regions, but a few in the colon and rectum. This regional distribution of BPP-immunoreactive cells of the blue fox was different from the Korean hedgehog<sup>18</sup>, pig<sup>25</sup>, Korean squirrel<sup>30</sup>, mole<sup>32</sup> in the occurrence of these cells in the pyloric region, whereas honey possum<sup>19</sup>, musk shrew<sup>20</sup>,

cow<sup>22</sup>, Korean native goat<sup>24</sup>, in the absence of these cells in the cardiac and fundic regions. This distribution pattern of the blue fox was generally similar to that of hedgehog<sup>18</sup> except the presence of these cells in the pyloric region.

There were no insulin-immunoreactive cells in the GI tract of the blue fox.

Porcine CG-immunoreactive cells were restricted to the cardiac and fundic region. They were mainly found in the basal portion of the gastric glands. Recently, much attention has been given to the chromogranin<sup>33</sup> and chromogranin A is widely distributed in the endocrine cells of mammals.<sup>11,14,34</sup> This suggests a putative role for chromogranins as precursor of bioactive peptides. Ito et al.<sup>14</sup> reported that all of argyrophil cells by Grimelius method were chromogranin-immunoreactive cells in the overall GI tract and the pancreas. Furthermore, these cells were most numerous in the fundic and pyloric gland regions, and in the duodenum and primordial pancreas more numerous than glucagon-immunoreactive cells at the earliest fetal stage of porcine.<sup>14</sup> On the other hand, numerous chromogranin-immunoreactive cells were found throughout the GI tract in the Korean native goat<sup>24</sup> and Korean tree squirrel.<sup>28</sup> Comparing the present study with those reported for above mammals, quite differences in the regional distribution of porcine CG-immunoreactive cells in the GI tract were observed.

is region region

The discrepancies of the distribution and relative frequency were probably considered concerning with their environments, physiological characteristics and diet differences among the species, but the significance of these discrepancies are not yet understood.

The present study demonstrated four types of pancreatic endocrine cells-somatostatin-, glucagon-, BPP- and insulin-immunoreactive cells in the pancreas of the blue fox. Among these, glucagon- and somatostatin-immunoreactive cells were located at the periphery of the islet, and insulin-immunoreactive cells were distributed throughout the islet, and BPP-immunoreactive cells scattered singly or small clusters inside and along the acini in the exocrine tissue.

Many reports have done on concerning the distribution pattern and population of the cell types in the pancreatic islets of several species.<sup>14, 35-43</sup>

The distribution pattern and cell types in this species is quite similar to those of the African clawed toad<sup>30</sup>, Gray kangaroo<sup>42</sup>, carp, frog and ogolgae<sup>44</sup>. However, 5-HT-immunoreactive cells were found in the pancreatic islets of the other species.<sup>31, 38, 40, 41, 44</sup> Ding et al<sup>45</sup> reported that 5-HT-immunoreactive cells were identified in the pancreatic islets of the eels, toad, turtles, chicken, mice, guinea pig, cats, dogs and human. On the other hand, Grube and Yoshie<sup>34</sup> reported that chromogranin-immunoreactive cells were found in the canine pancreatic islet.

In conclusion, the distribution pattern, the relative frequencies and cell types of the GEP endocrine cells found in the blue fox may reflect, in part, its environments, physiological characteristics and diet. However, further comparative studies of this species should be undertaken to clarify the functional and phylogenetic significance of these findings in the GEP endocrine cells of the blue fox.

### Summary

The regional distribution and the relative frequencies of endocrine cells were studied in nine portions of the blue fox GI tract, and the distribution pattern and cell types of the pancreatic endocrine cells were also studied

in the pancreas by immunohistochemical method. Six kinds of immunoreactive cells were identified in the GI tract, and four kinds of immunoreactive cells were also identified in the pancreas.

Although numerous 5-HT- and somatostatin-immunoreactive cells were seen throughout the GI tract, somatostatin-immunoreactive cells were a few in the intestine. Very numerous Gas/CCK-immunoreactive cells were restricted generally in the pyloric region and duodenum. Numerous glucagon-immunoreactive cells were found in the stomach except the pyloric region, and generally a few in the intestine. Moderate number of BPP-immunoreactive cells were found in the stomach except the pyloric region, and a few in the large intestine. Numerous porcine CG-immunoreactive cells were restricted to the cardiac and fundic region.

In the pancreas, four types of pancreatic endocrine cells-somatostatin-, glucagon-, BPP- and insuline-immunoreactive-were identified in the pancreatic islet and exocrine portion.

These results suggest that the regional distribution, the relative frequencies and cell types of the GEP endocrine cells in the GI tract and pancreas varies considerably among the species.

### Legends for figures

**Fig 1.** 5-HT-immunoreactive cells in the GI tract.

a. Cardiac region, b. Fundic region, c. Pyloric region, d. Duodenum, e. Jejunum, f. Ileum, g. Cecum, h. Colon, i. Rectum, a, b :  $\times 120$ , c-i :  $\times 240$ .

**Fig 2.** Somatostatin-immunoreactive cells in the GI tract.

a. Cardiac region, b. Fundic region, c. This cell has typical cytoplasmic process in the fundic region., d. pyloric region, e. Duodenum, f. Colon(arrowheads), g. Rectum(arrowheads), a,b,d,e :  $\times 240$ , c,f,g :  $\times 480$ .

**Fig 3.** Gas/CCK-immunoreactive cells in the GI tract.

a. Pyloric region(arrowheads), b. Duodenum, c. Jejunum. a-c :  $\times 240$ .

**Fig 4.** Glucagon-immunoreactive cells in the GI tract.

a. Cardiac region, b. Fundic region, c. Jejunum(arrowheads), d. Colon(arrowheads), e. Ileum(arrowheads). a-e :  $\times 240$ .

**Fig 5.** BPP-immunoreactive cells in the GI tract.

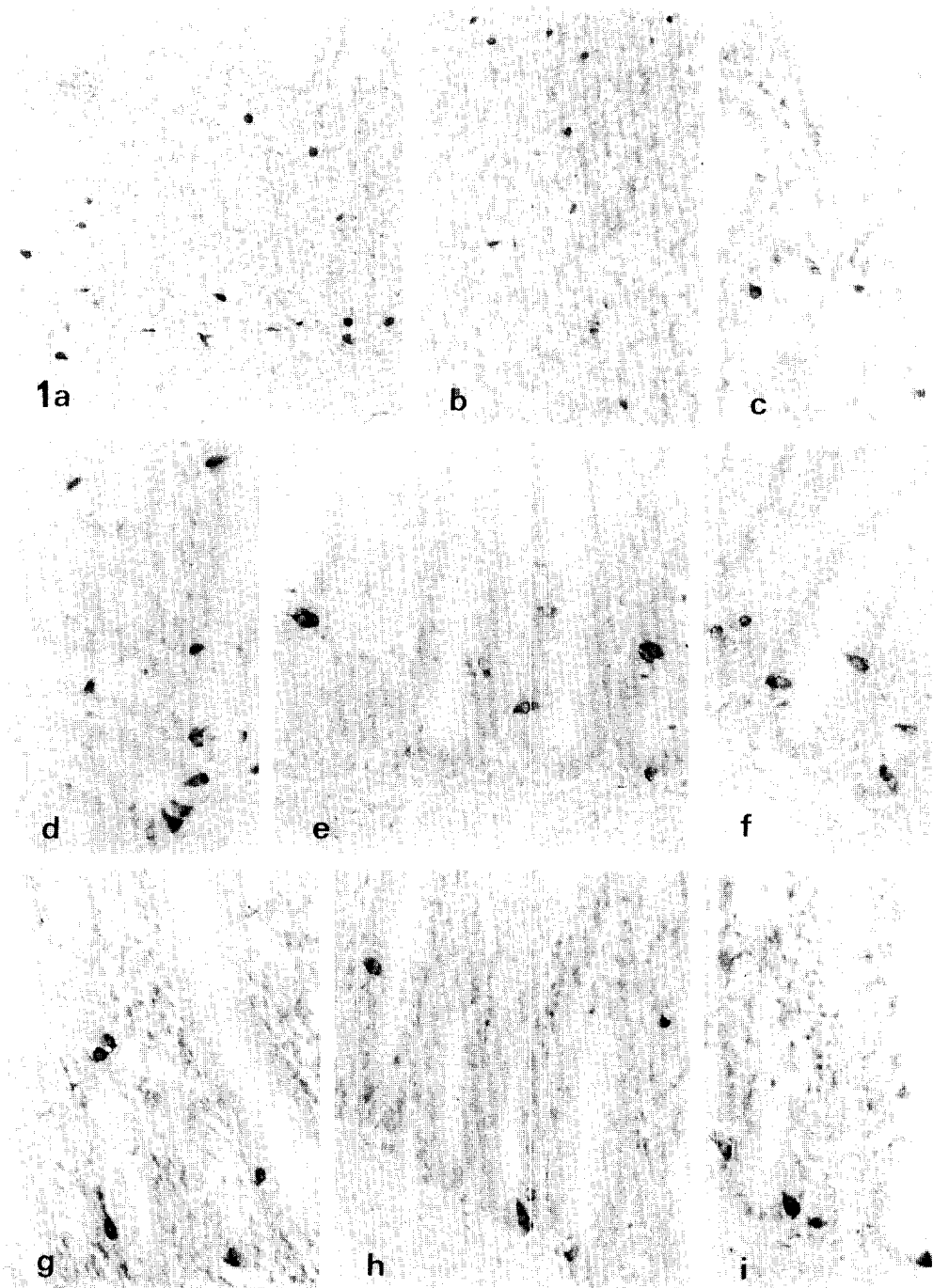
a. Cardiac region, b. Fundic region. c. Colon(arrowheads). a-c :  $\times 240$ .

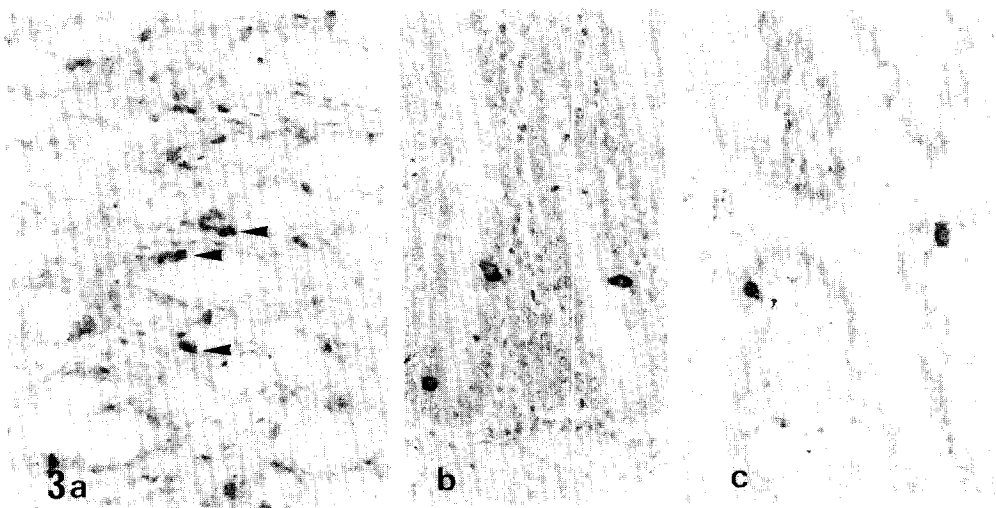
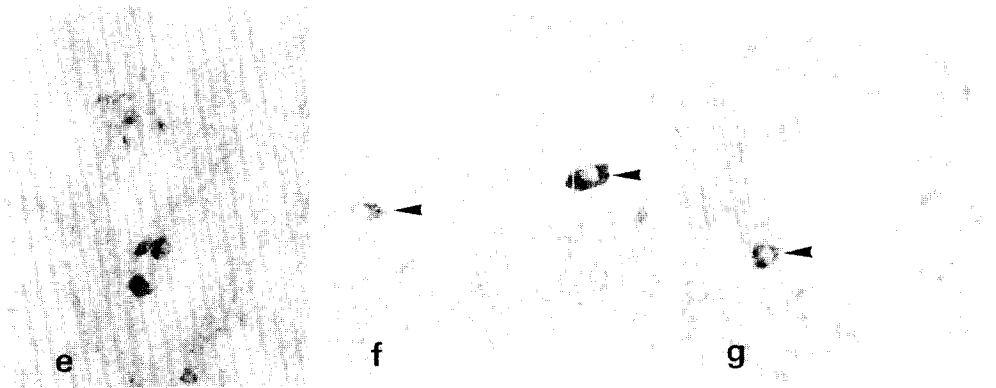
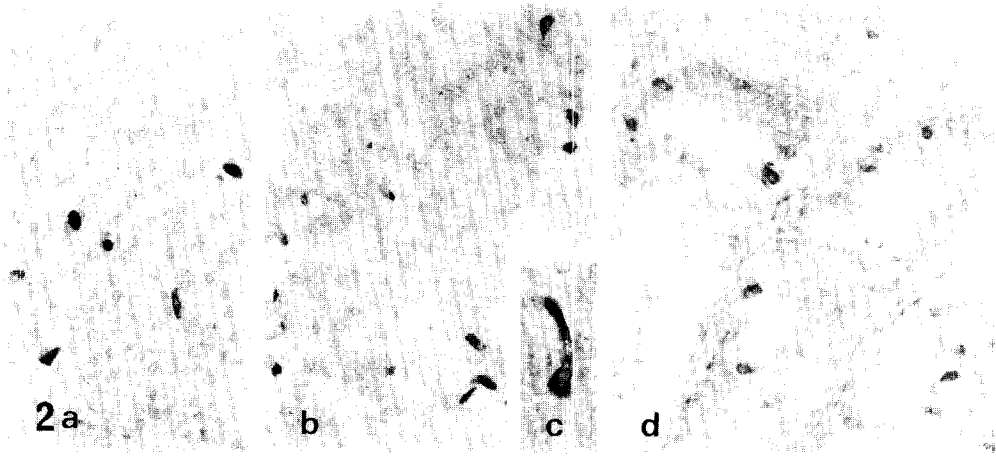
**Fig 6.** Porcine CG-immunoreactive cells(arrowheads) in the GI tract.

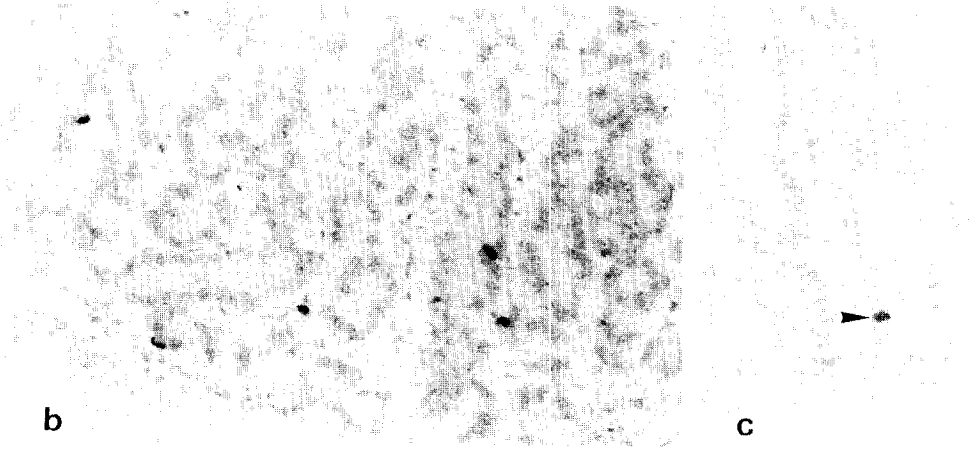
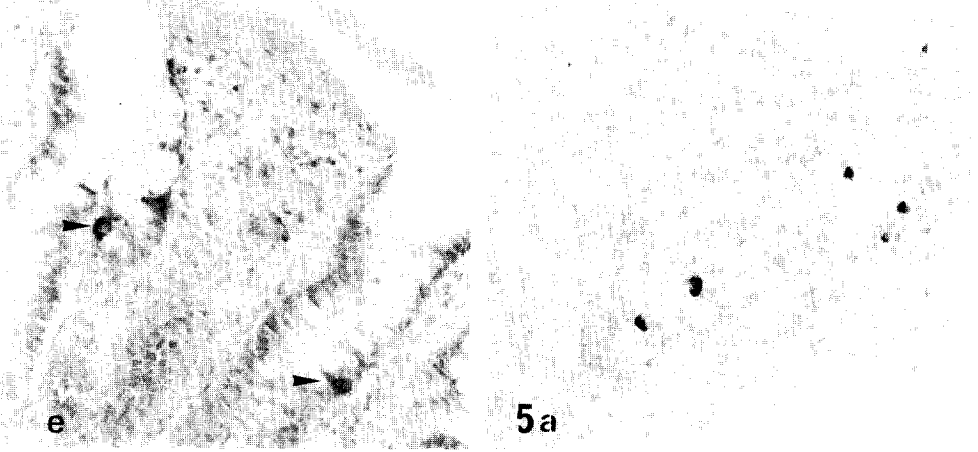
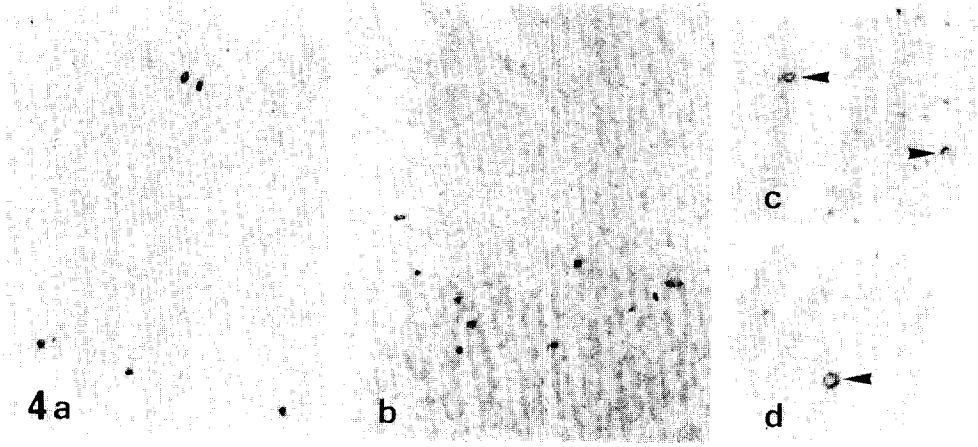
a. Cardiac region. b. Fundic region. a,b :  $\times 240$ .

**Fig 7.** Pancreatic endocrine cells.

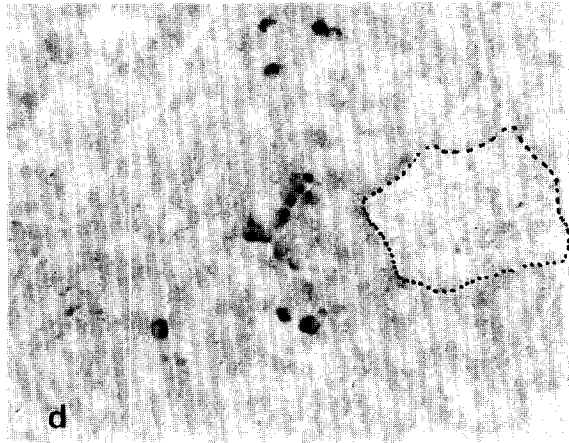
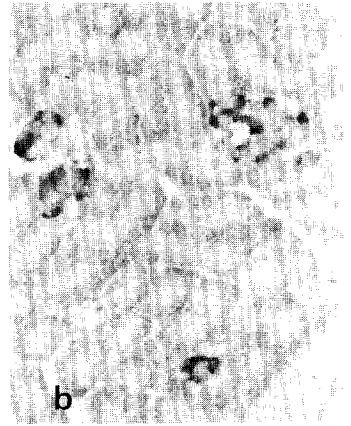
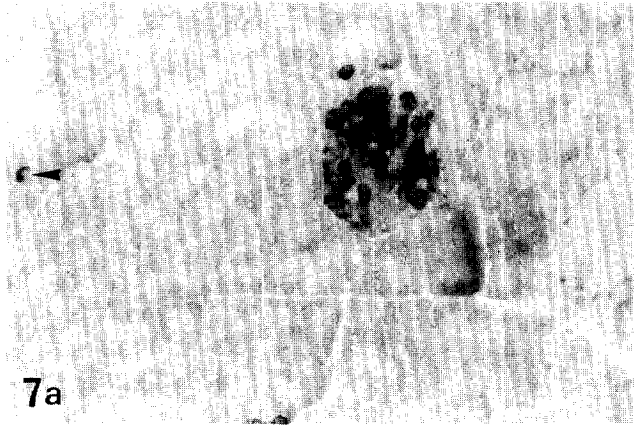
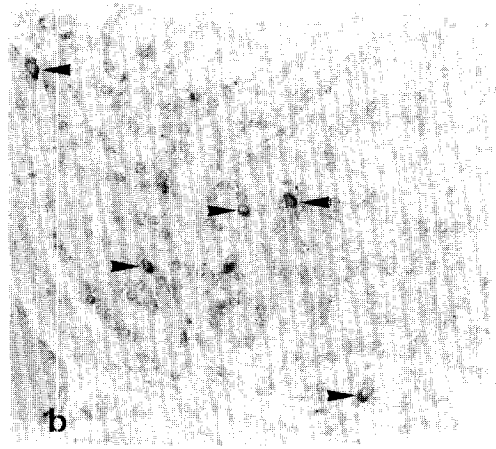
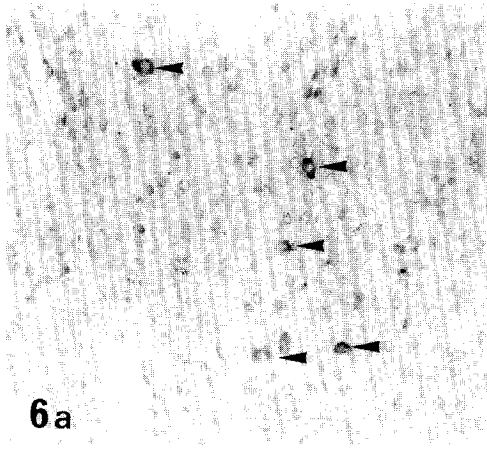
a. Glucagon-immunoreactive cells were detected in the islet and the exocrine portion(arrowheads), b. Insulin-immunoreactive cells, c. Somatostatin-immunoreactive cells were found in the islet and the exocrine portion(arrowheads), d. BPP-immunoreactive cell was not distributed in the islet(dots) except for the exocrine portion. a-d :  $\times 240$ .











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