

## An immunohistochemical study of the endocrine cells in the stomach of the Korean hedgehog (*Erinaceus koreanus*)

Jae-hyun Lee, Hyeung-sik Lee\*, Nam-soo Lee, Jong-beom Kim  
College of Veterinary Medicine, Kyungpook National University,  
Dept of Biology, Daegu Oriental Medical College\*

(Received Aug 20, 1990)

### 고슴도치 위점막의 내분비세포에 관한 면역조직화학적 연구

이재현 · 이형식\* · 이남수 · 김종범

경북대학교 수의과대학, 대구한의과대학 생물학과\*

(1990. 8. 20 접수)

**초록** : 한국산 고슴도치의 위점막에 분포하는 내분비세포에 대해 면역조직화학적으로 관찰하였던 마, gastrin, somatostatin, 5-HT, glucagon, BPP, motilin, GIP 등 7종의 면역반응세포들이 동정되었으며, 이들 세포들의 부위별 분포와 출현빈도의 특징은 다음과 같다.

Gastrin 면역반응세포는 유문부에서만 아주 다수로 출현하였으며, somatostatin 면역반응세포는 유문부에서 다수, 분문부와 위저부에서는 중등도의 출현을 볼 수 있었다. 5-HT 면역반응세포는 분문부와 유문부에서 다수, 위저부에서는 중등도로 출현하였다. glucagon 면역반응세포는 위저부에서 소수로 그리고 유문부에서 극히 소수로 출현하였고, BPP 면역반응세포는 유문부에 다수로, 위저부에 중등도로 분문부에 소수로 분포하였다. motilin 면역반응세포는 유문부와 위저부에서 소수 또는 극소수로 출현하였으며, GIP 면역반응세포는 유문부에서만 소수의 분포를 볼 수 있었다.

**Key words**: immunoreactive cells, cardiac, fundic, pyloric, hedgehog.

### Introduction

Studies on the gastro-entero-pancreatic endocrine system have recently made a remarkable advance by the use of the immunohistochemical methods.<sup>1-3</sup> The Korean hedgehog, *Erinaceus koreanus*, is one of the order Insectivora that eats insects or invertebrates. Previously, we reported the regional distribution and relative frequency of twelve kinds of immunoreactive endocrine cells in the gut of Korean hedgehog.<sup>4</sup>

The purpose of the present study is to examine the distribution and relative frequency of endocrine cells in the cardiac, fundic and pyloric regions of the Korean hedgehog's stomach.

### Materials and methods

Five Korean hedgehogs (*Erinaceus koreanus*) of either sex were used in this study. Tissues were dissected out from the cardiac to pyloric regions along with great curvature. Tissues were fixed in Bouin's solution and embedded in paraffin. Sections were cut at 4~6 $\mu$ m thickness, deparaffinized and stained immunohistochemically using the peroxidase-antiperoxidase (PAP)<sup>5</sup> and the bridge technique.<sup>6</sup> Details of the antisera used are shown in Table 1. Controls were run as recommended by Sternberger.<sup>5</sup> After the immunohistochemical staining, the sections were counter-stained with Mayer's hematoxylin, dehydrated, cleared and mounted.

**Table 1.** Antisera used

Antisera* raised	Code	Dilution	Source
Synthetic human gastrin	GP-1304	1 : 5,000	N. Yanaihara, Shizuoka
Synthetic human cyclic somatostatin	—	1 : 3,000	S. Ito, Niigata
Serotonin(5-HT)	Lot 16302	1 : 10,000	Immuno Nuclear corp, Stillwater
Synthetic porcine glucagon	GL-5	1 : 2,000	N. Yanaihara, Shizuoka
Bovine pancreatic polypeptide	Lot-615- R110-146-17	1 : 9,000	R.E. Chance, Indianapolis
Synthetic porcine motilin	R-1104	1 : 1,000	N. Yanaihara, Shizuoka
GIP(Gastrin inhibitory polypeptide)	G/R/34-111D	1 : 10,000	Guildhey antisera, Surrey

\* All antisera were raised in rabbits except that against gastrin which are in guinea pigs.

The relative frequency of each immunoreactive cells in each region was divided by its degree of occurrence in the visual field ( $\times 300$ ) of light microscopy.

## Results

The endocrine cells reacted to gastrin, somatostatin, 5-HT, glucagon, BPP, motilin or GIP respectively were identified in the stomach of korean hedgehog.

The relative frequency and distribution of each immunoreactive endocrine cell in the cardiac, fundic and pyloric regions of the stomach are summarized in Table 2.

Gastrin-immunoreactive cells were numerously

**Table 2.** Distribution and relative frequency of stomach endocrine cells of hedgehog, *Erinaceus koreanus*

Cells	Region of stomach		
	Cardiac	Fundic	Pyloric
Gastrin	—	—	###
Somatostatin	+~#	+	##
5-HT	##	+	##~###
Glucagon	—	+	±~+
BPP	+	+	##
Motilin	—	±	+
GIP	—	—	+

—: absent, ±: rare, +: few, #: moderate, ##: numerous, ###: very numerous. BPP: Bovine pancreatic polypeptide, 5-HT: 5-hydroxytryptamine, GIP: Gastric inhibitory polypeptide.

detected only in the pyloric region(Fig 3d). No the cells were found in the cardiac and fundic regions. They were mainly located in the basal portion of glands.

Somatostatin-immunoreactive cells were diffusely found in the stomach. They were especially numerous in the pyloric region(Fig 3a) and moderate in the cardiac(Fig 1a) and fundic regions in degree(Fig 2a).

5-HT-immunoreactive cells were found in the entire stomach of the hedgehog to be examined. They were observed numerously in the cardiac(Figs 2b, c) and pyloric regions(Fig 3b) but moderately in the fundic ones(Fig 2b).

Somatostatin- and 5-HT-immunoreactive cells were mainly located in the basal portion of the pyloric glands.

Glucagon-immunoreactive cells were distributed few or rarely in the fundic and pyloric regions. No glucagon-immunoreactive cell was detected in the cardiac region.

BPP-immunoreactive cells were detected few in the cardiac region(Fig 1b), moderately in the fundic one(Fig 2c) and numerously in the pyloric one(Fig 3c). In the pyloric region, they were distributed only in the basal portion of the glands.

Motilin-immunoreactive cells were found rarely or few in the fundic and pyloric regions. These cells in the pyloric region had a luminal contact by mean of apical cytoplasmic process(Fig 3f).

GIP-immunoreactive cells were detected only in the pyloric region(Fig 3e). These cells with oval

or spherical in shape were found few.

## Discussion

In the present study, seven kinds of immunoreactive cells were identified as endocrine cells in the hedgehog stomach.

The distribution and relative frequency of the gastric endocrine cells in the hedgehog were basically similar to those of other animals.<sup>7-13</sup>

However, some characteristic findings were observed in this animal. Gastrin-immunoreactive cells were appeared very numerous in the pyloric glands, a finding similar to those for the other animals.<sup>7,9-23</sup>

In the study, somatostatin-immunoreactive cells were more numerous in the pyloric region than in the cardiac and fundic regions as reported in the other animals.<sup>9,10,12,16-18,23</sup> In the horse,<sup>11</sup> Rhesus monkey,<sup>21</sup> Japanese field vole<sup>23</sup> and rabbit,<sup>24</sup> however, there were reported that they were more numerous in the cardiac and fundic region than in the pyloric one. The reason for this discrepancy in the regional distribution of these cells is not yet clear.

5-HT-immunoreactive cells in this study were more numerous in the cardiac and pyloric regions than in the fundic one, a finding similar to those for the cow,<sup>10</sup> pig,<sup>12</sup> Japanese field vole,<sup>23</sup> whereas the reverse was true for the Korean native cattle<sup>18</sup> and honey possum.<sup>22</sup> Otherwise, these cells were numerous in the fundic region and rarely in the pyloric one of the Rhesus monkey.<sup>25</sup>

Larsson et al (1975) reported that glucagon-immunoreactive cells were rarely seen in the fundic mucosa of the pig<sup>12</sup> and somewhat more numerous in the cardiac region than in the oxyntic one. In this study, however, glucagon-immunoreactive cells could not detected in the cardiac region, but rarely or few in the pyloric and fundic regions. The basic pattern of distribution of the cells is similar to those of the sheep,<sup>9</sup> bat,<sup>17</sup> Rhesus monkey,<sup>21</sup> Japanese field vole.<sup>23</sup>

In the present study, BPP-immunoreactive cells were numerous in the pyloric region, moderate in the fundic one and few in the cardiac one. It was different that no BPP-immunoreactive cells were found in the stomach of the sheep,<sup>9</sup> cow,<sup>10</sup> Korean

native cattle,<sup>18</sup> Rhesus monkey,<sup>21</sup> honey possum.<sup>22</sup> However, in the bat<sup>17</sup> moderate numbers of these cells were found in the fundic region, and rarely of few in the cardiac and pyloric regions. In the pig<sup>12</sup> stomach BPP-immunoreactive cells were rarely found in the fundic and pyloric regions.

Motilin-immunoreactive cells were rarely found in the fundic region and few in the pyloric one. They could not found in the gastric mucosa of other animals<sup>9-12,17,22,23,26</sup> except Rhesus monkey<sup>25</sup> numerously appearing in the pyrolic region. The significance of motilin-immunoreactive cells in the gastric mucosa of the hedgehog is not yet understand.

Interestingly, GIP-immunoreactive cells were detected in the pyloric region of the hedgehog in this study because they were usually confined to the small intestine of man and the rat.<sup>27</sup> However, Ohara et al(1986) reported that the GIP-immunoreactive cells were moderately seen in the pyloric gland segment of the Japanese field vole's stomach. The significance of these cells in the pyloric region of the hedgehog is not yet explained.

The characteristic distribution patterns and frequencies of gastric endocrine cells in the hedgehog could be related to the species difference. The results of the study may contribute to further morphological and physiological investigation on the various animal's digestive system.

## Summary

The gastric endocrine cells of the Korean hedgehog, *Erinaceus koreanus* were studied immunohistochemically. Seven kinds of endocrine cells-, gastrin-, somatostatin-, 5-HT-, glucagon-, BPP-, motilin-and GIP-immunoreactive cells- were identified in this study. The characteristic findings of the regional distribution and relative frequency of them were examined.

Gastrin-immunoreactive cells were very numerously detected only in the pyloric region. Somatostatin-immunoreactive cells were more numerous in the pyloric region than in the cardiac and fundic regions. 5-HT-immunoreactive cells were more numerous in the cardiac and pyloric regions than

in the fundic one. Glucagon-immunoreactive cells were found few or rarely in the fundic and pyloric regions. BPP-immunoreactive cells were numerous distributed in the pyloric region, moderately in the

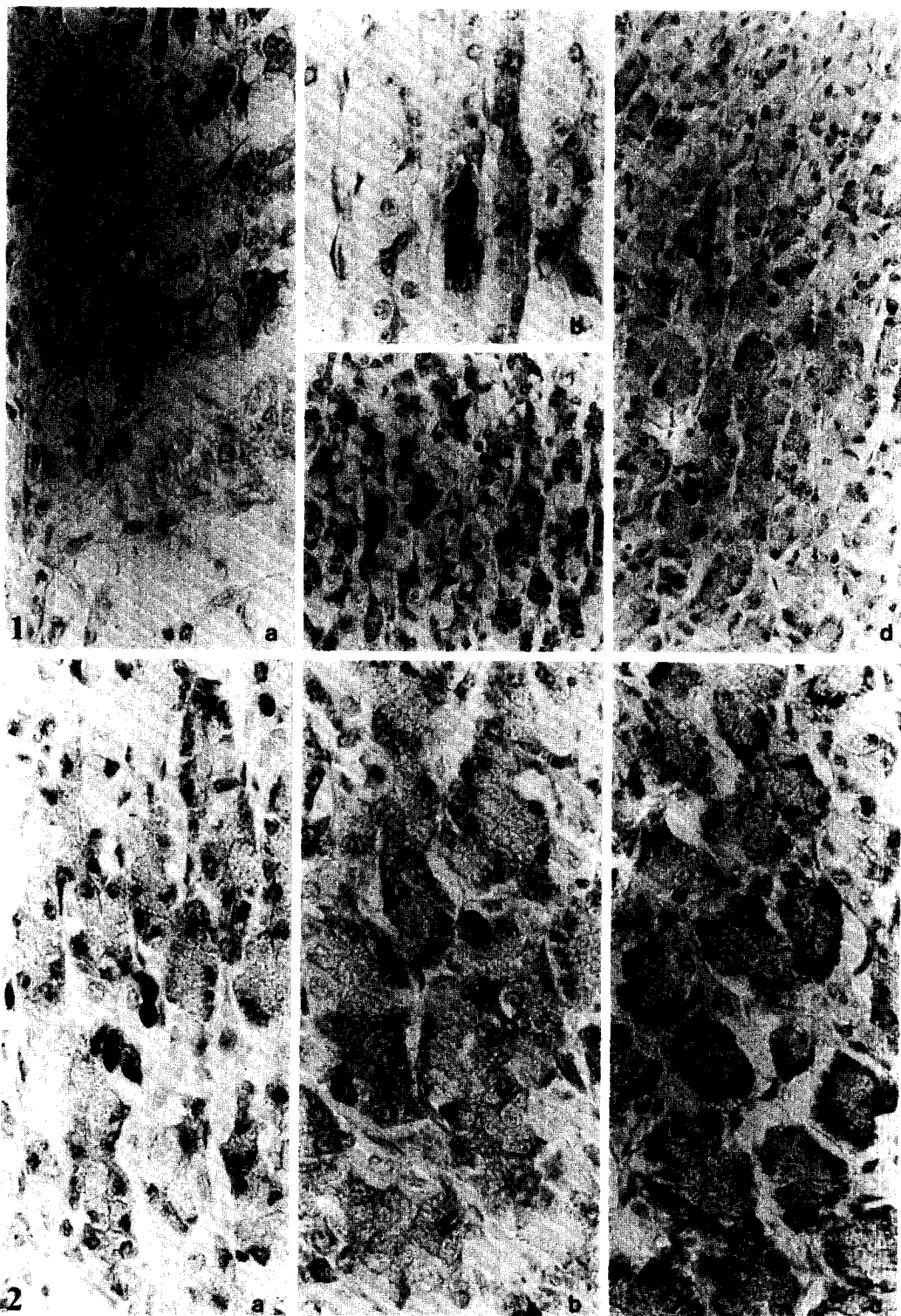
fundic region and few in the cardiac region. Motilin-immunoreactive cells were found rarely or few in the fundic and pyloric regions. GIP-immunoreactive cells were detected only in the pyloric region.

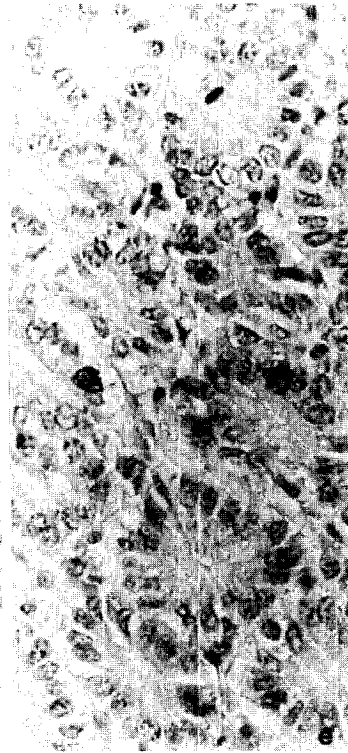
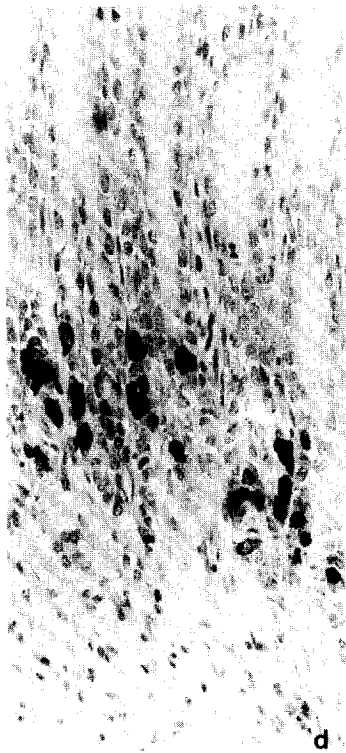
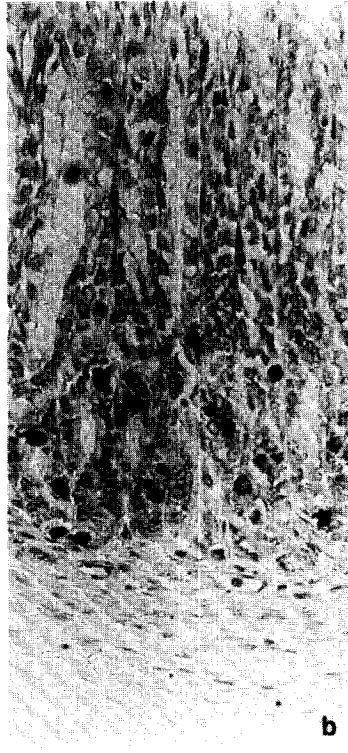
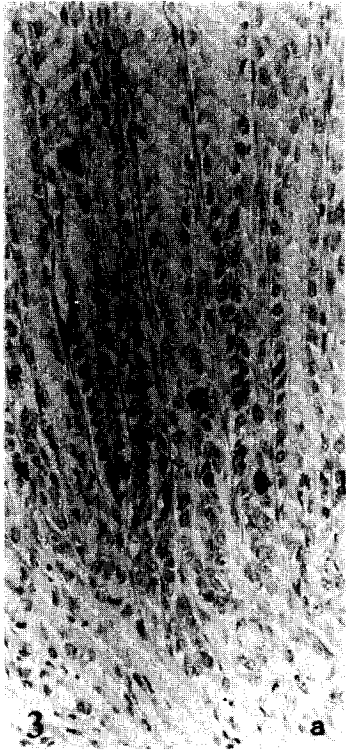
#### Legends for figures

**Figs 1a-d:** (a) Somatostatin-, (b,c) 5-HT- and (c) BPP-immunoreactive cells in the cardiac region.  
a, b:  $\times 600$ , c, d:  $\times 300$

**Figs 2a-c:** (a) Somatostatin-, (b) 5-HT- and (c) BPP-immunoreactive cells in the fundic region.  
a, b, c:  $\times 600$

**Figs 3a-f:** (a) Somatostatin-, (b) 5-HT-, (c) BPP-, (d) gastrin-, (e) GIP- and (f) motilin-immunoreactive cells in the pyloric region.  
a-d:  $\times 300$ , e, f:  $\times 600$





## References

1. Grube D, Forssmann WG. Morphology and function of the enteroendocrine cells. *Horm Metab Res* 1979;11:589-606.
2. Solcia E, Creutzfeldt W, Falkmer S, et al. Lausanne 1977 classification of gastroenteropancreatic endocrine cells. In: Bloom SR. *Gut hormones*. Churchill-Livingstone: Edinburgh, 1978;40-48.
3. Solcia E, Capella R, Buffa B, et al. Endocrine cells of the gut and related growths: Recent developments and classification. In: Bloom SR: *Gut hormones*. Churchill-Livingstone: Edinburgh, 1978;77-81.
4. 이재현. 한국산 고슴도치(*Erinaceus koreanus*)의 장관내분비세포에 관한 면역조직화학적 및 전자현미경적 연구. 한국전자현미경학회지 1988;18:59-76.
5. Sternberger LA. In: *Immunocytochemistry*. 2nd ed. New York: John Wiley & Sons, 1979.
6. Mason TE, Phifer RF, Spicer SS, et al. An immunoglobulin enzyme bridge method for localizing tissue antigens. *J Histochem Cytochem* 1969;17:553-569.
7. 최월봉, 원무호, 박형진 등. 고슴도치 위장관 gastrin(G)세포, glucagon(L)세포, somatostatin(D)세포 및 cholecystokinin(I)-8세포의 면역세포학적 연구. 동물학회지 1987;30:154-166.
8. Kobaru Y, Kitamura N, Yamada J, et al. Postnatal development of the stomach in the Japanese field vole, *Microtus montebelli*. *Anat Histol Embryol* 1988;17:138-148.
9. Calingasan NY, Kitamura N, Yamada J, et al. Immunocytochemical study of the gastroenteropancreatic endocrine cells of sheep. *Acta anat* 1984;118:171-180.
10. 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* 1985;46:1381-1386.
11. Kitamura N, Yamada J, Calingasan NY, et al. Immunocytochemical distribution of endocrine cells in the gastrointestinal tract of the horse. *Equine Vet J* 1984;16:103-107.
12. 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* 1987;49:105-114.
13. Kitamura N, Yamada J, Yamashita T, et al. Endocrine cells in the gastrointestinal tract of the cat. *Biomed Res* 1982;3:612-622.
14. Solcia E, Capella C, Vassallo G. Studies on the G cell of the pyloric mucosa, the probable site of gastrin secretion. *Gut* 1969;10:379-388.
15. Larsson L-I, Rehfeld JF. Distribution of gastrin and CCK cells in the rat gastrointestinal tract. *Histochemistry* 1978;58:23-31.
16. Tamate H, Yamada J. Histological and immunocytochemical observation of the hindstomach of the Collared Peccary, *Dicotyles tayacu* (Tayassuidae). *Jap J Vet Sci* 1983;45:547-559.
17. Yamada J, Li B, Deng Z, et al. An immunohistochemical study of gut endocrine cells in two species of insectivorous Vespertilinid bats (Chiroptera: *Pipistrellus abramus* and *Plecotus auritus sacrimontis*). *Gegenbaurs morphol Jahrb Leipzig* 1988;1:79-91.
18. Cho SW, Kitamura N. Immunocytochemical study of the endocrine cells in the gastrointestinal tract of the Korean native cattle. *Korean J Vet Res* 1988;28:251-259.
19. Lee J-H, Lee H-S. An immunohistochemical study of the endocrine cells in gastrointestinal tract of the Korean native goat. *Korean J Vet Res* 1990;30:261-270.
20. Kim J-B, Lee J-H, Lee H-S, et al. An immunohistochemical study on the gastro-entero-endocrine cells of the pond tortoise, *Amyda sinensis*. *Korean J Vet Res* 1990;30(4):383~394.
21. 최월봉, 원무호, 박형진 등. 붉은털원숭이 위장관 점막의 gastrin, glucagon, somatostatin 및 cholecystokinin-8 분비세포에 대한 면역세포화학적 연구, 대한해부학회지 1986;19:181-197.
22. Yamada J, Richardson KC, Wooller RD. An immunohistochemical study of gastrointestinal endocrine cells in a nectarivorous marsupial, the honey possum(*Tarsipes rostratus*). *J Anat* 1989; 162:157-168.

23. Ohara N, Kitamura N, Yamada J, et al. Immunohistochemical study of gastroenteropancreatic endocrine cells of the herbivorous Japanese field vole, *Microtus montebelli*. *Res Vet Sci* 1986;41:21-27.
24. Alumets J, Sundler F, Hakanson R. Distribution, ontogeny and ultrastructure of somatostatin immunoreactive cells in the pancreas and gut. *Cell Tiss Res* 1977;185:465-479.
25. 최월봉, 최창도, 원무호 등. 붉은털원숭이 위장관 접막의 motilin, pancreatic polypeptide 및 serotonin 분비세포에 대한 면역세포화학적 연구. 한림대학 논문집. 자연과학 의학편 1987;5:127-140.
26. Kobayashi S, Iwanaga T, Fujita T, et al. Do enterochromaffin (EC) cells contain motilin? *Arch histol jap* 1980;43:85-98.
27. Buchan AMJ, Ingman-Baker J, Levy J, et al. A comparison of the ability of serum and monoclonal antibodies to gastric inhibitory polypeptides to detect immunoreactive cells in the gastroenteropancreatic system of mammals and reptiles. *Histochemistry* 1982;76:341-349.