

The regional distribution and relative frequency of gastrointestinal endocrine cells of the ICR mice : An immunohistochemical study

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Abstract : The regional distributions and relative frequencies of some gastrointestinal endocrine cells in the 8 portions (fundus, pylorus, duodenum, jejunum, ileum, cecum, colon and rectum) of the gastrointestinal tract of ICR mouse (ICR) with immunohistochemical method using 7 types of specific antisera against somatostatin, serotonin, glucagon, cholecystokinin (CCK)-8, secretin, pancreatic polypeptide (PP) and gastrin. In this study, somatostatin-, serotonin-, glucagon-, CCK-8-, secretin- and gastrin-immunoreactive (IR) cells were identified. Most of these IR cells in the intestinal portion were generally spherical or spindle in shape (open-typed cell) while cells showing round in shape (close-typed cell) were found in the stomach regions occasionally. Their relative frequencies were varied according to each portion of gastrointestinal tract. Somatostatin-IR cells were demonstrated throughout whole gastrointestinal tract except for large intestine. Serotonin-IR cells were detected throughout whole gastrointestinal tract and they were most predominant endocrine cell types in this species of mouse. Glucagon-IR cells were restricted to the fundus and rectum with moderate and a few frequencies, respectively. CCK-8-IR cells were observed in the pylorus, duodenum and ileum with numerous, moderate and rare frequencies, respectively. Secretin-IR cells were restricted to the duodenum and ileum with a few and rare frequencies, respectively. Gastrin-IR cells were restricted to the pylorus with numerous frequency. However, no PP-IR cells were found in this study. In conclusion, some peculiar distributional patterns of gastrointestinal endocrine cells were found in the ICR mouse compared to those of other mammals.

Key words : gastrointestinal endocrine cell, immunohistochemistry, ICR mouse

Introduction

The mouse, *Mus musculus*, is a rodent, order Rodentia, of the family Muridae¹. Commensal and wild mice have spread around the world, and the laboratory or house mouse is kept occasionally as a pet. Since mice are small and prolific breeders, maintained easily and economically in large populations, and possess great genetic diversity, characterized anatomically and physiologically, they are the most widely used vertebrates animal in biochemical research and testing. It is generally accepted that ICR, an abbreviated form of institute of cancer research, mouse was one of the most widely used strains and representative closed colony.

On the other hand, gastrointestinal endocrine cells dispersed in the epithelia and gastric glands of the digestive

tract synthesized various kinds of gastrointestinal hormones and played an important role in the physiological functions of the alimentary tract². And they were divided into two types, open and close type^{3,4}. Until now, the investigation of gastrointestinal endocrine cells is considered to be an important part of a phylogenetic study⁵. In addition, the regional distributions and relative frequencies of these endocrine cells were varied with animal species and feeding habits⁴. Many studies have elucidated the regional distribution and relative frequency of different endocrine cells in the gastrointestinal tract (GIT) of the various vertebrates including various species of rodents. Also the researches or data processing about gastrointestinal endocrine cells in the mice strains have been widely executed. Spangeus *et al*⁶ investigated the endocrine cells in the GIT of homozygous obese mouse, and Pinto *et al*⁷

showed that the gastrointestinal endocrine cells in genetically diabetic (db/db) mice had quite different distributional patterns compared to those of nondiabetic control (db/+) mice. In addition, the changes of regional distribution and relative frequency of some gastrointestinal endocrine cells in mice with ageing were reported^{8,9}. However, the regional distribution and relative frequency of the whole gastrointestinal endocrine cells of the ICR mouse, the most widely used strain, were uncommon except for some fragmented reports^{8,9}.

The objective of this study was to clarify the regional distribution and relative frequency of the endocrine cells in the GIT of the ICR mouse, one of most widely used strains, by specific immunohistochemistry using 7 types of antisera against somatostatin, serotonin, glucagon, cholecystokinin (CCK)-8, secretin, pancreatic polypeptide (PP) and gastrin.

Materials and Methods

Five adult ICR mouse (7 weeks old, 27-32 g body weight upon receipt) were acquired from the Charles River Laboratories (Yokohama, Japan) and were used in this study without sexual distinction after acclimatization for one week. Animals were allocated 5 per polycarbonate cage in a temperature (20-25°C) and humidity (30-35%) controlled room during acclimatization periods. Light : dark cycle was 12hr : 12hr and feed (Samyang, Korea) and water were supplied free to access.

The animals were anesthetized with ethyl ether. After food restriction about 24 hrs and phlebotomized, samples from the fundus, pylorus, duodenum, jejunum, ileum, colon, cecum and rectum were fixed in Bouin's solution. After paraffin embedding, 3-4 µm serial sections were prepared. Representative sections of each tissue were stained with hematoxylin and eosin for light microscopic

examination of the normal gastrointestinal architecture.

The each representative section was deparaffinized, rehydrated and immunostained with the peroxidase anti-peroxidase (PAP) method¹⁰. Blocking of nonspecific reaction was performed with normal goat serum prior to incubation with the specific antisera (Table 1). After rinsing in phosphate buffered saline (PBS; 0.01M, pH 7.4), the sections were incubated in secondary antiserum. They were then washed in PBS buffer and finally the PAP complex was prepared. The peroxidase reaction was carried out in a solution 3,3'-diaminobenzidine tetrahydrochloride containing 0.01% H₂O₂ in Tris-HCl buffer (0.05M, pH 7.6). After immunostaining, the sections were lightly counterstained with Mayer's hematoxylin and the immunoreactive (IR) cells were observed under light microscope.

The specificity of each immunohistochemical reaction was determined as recommended by Sternberger¹⁰, including the replacement of specific antiserum by the same antiserum, which had been preincubated with its corresponding antigen. The relative frequency of occurrence of each type of IR cells was placed into one of five categories according to their observed numbers as seen using light microscopy.

Results

In this study, six kinds of the IR endocrine cells were detected with the antisera against somatostatin, serotonin, glucagon, CCK-8, secretin and gastrin in the gastrointestinal tract of the ICR mouse. However, PP-IR endocrine cells were not demonstrated in this study. According to the location of the gastrointestinal tract, different regional distribution and relative frequencies of these IR cells were observed. These differences are shown in Table 2. The regional distribution and relative frequency of

Table 1. Antisera used in this study

Antisera raised*	Code	Source	Diluton
Somatostatin	PUO421295	BioGenex Lab., San Ramon.	1 : 20
Serotonin	BO68082C	BioGenex Lab., San Ramon	1 : 20
Glucagon	PUO390598	BioGenex Lab., San Ramon.	1 : 20
CCK-8 ¹⁾	8643010	Immunonuclear Corp., Stillwater.	1 : 1,000
Secretin	BO67122A	BioGenex Lab., San Ramon.	1 : 20
PP ¹⁾	PUO660495	BioGenex Lab., San Ramon.	1 : 20
Gastrin	PUO190796	BioGenex Lab., San Ramon.	1 : 20

* All antisera were raised in rabbits; ¹⁾CCK-8: cholecystokinin-8, PP: pancreatic polypeptide

Table 2. Regional distributions and relative frequencies of the gastrointestinal endocrine cells in the gastrointestinal tract of the ICR mouse

	Fundus	Pylorus	Duodenum	Jejunum	Ileum	Cecum	Colon	Rectum
Som ¹⁾	++	+	±	±	±	-	-	-
Serotonin	+	+++	+++	++	++	+++	+++	+++
Glucagon	++	-	-	-	-	-	-	+
CCK-8 ¹⁾	-	+++	++	-	±	-	-	-
Secretin	-	-	+	-	±	-	-	±
PP ¹⁾	-	-	-	-	-	-	-	-
Gastrin	-	+++	-	-	-	-	-	-

¹⁾ CCK-8: cholecystokinin-8; PP: pancreatic polypeptide; Som: somatostatin,

* Relative frequencies; +++: numerous, ++: moderate, +: a few, ±: rare, -: not detected

gastrointestinal endocrine cells were varied with GIT and some peculiar distributional patterns were found in the ICR mouse. Most of these IR cells in the intestinal portions were generally spherical or spindle in shape (open-typed cell), while occasionally round in shape (close-typed cell) cells were found in the stomach regions.

These IR cells were located between epithelial cells and/or in the intestinal glands of the intestine and located in the gastric glands of the stomach regions.

Somatostatin-IR cells (Fig 1, Table 2)

Somatostatin-IR cells were observed throughout whole

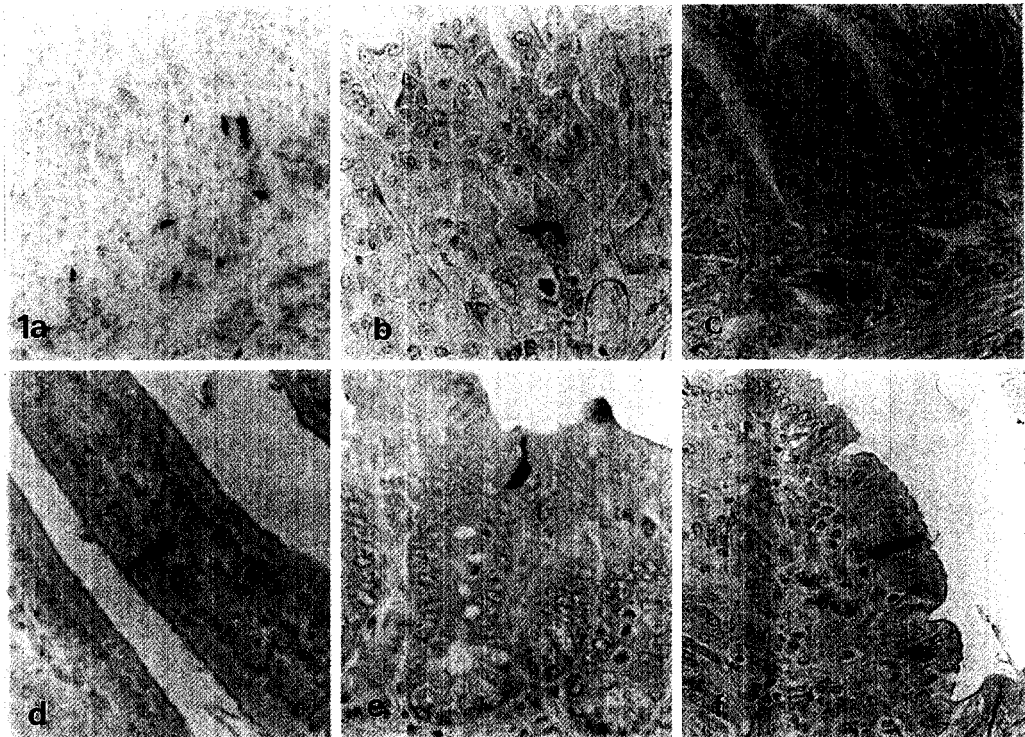


Fig 1. Somatostatin-immunoreactive cells in the gastrointestinal tract of ICR mouse. Note that various distributions and relative frequencies of these cells were observed throughout whole gastrointestinal tract except for large intestine. They were detected in the fundus (a, b), pylorus (c, arrow), duodenum (d), jejunum (e) and ileum (f). a: $\times 150$; b-f: $\times 300$.

GIT except for large intestine where no somatostatin-IR cells were demonstrated (Table 2). Close typed somatostatin-IR cells were found in the basal portion of gastric mucosa, between chief and parietal cells, of the fundus

with moderate frequency and also similar shaped IR cells were detected in the gastric gland mainly located in the basal portion of gastric mucosa of the pylorus with a few frequency (Fig 1a-c). In the duodenum, they

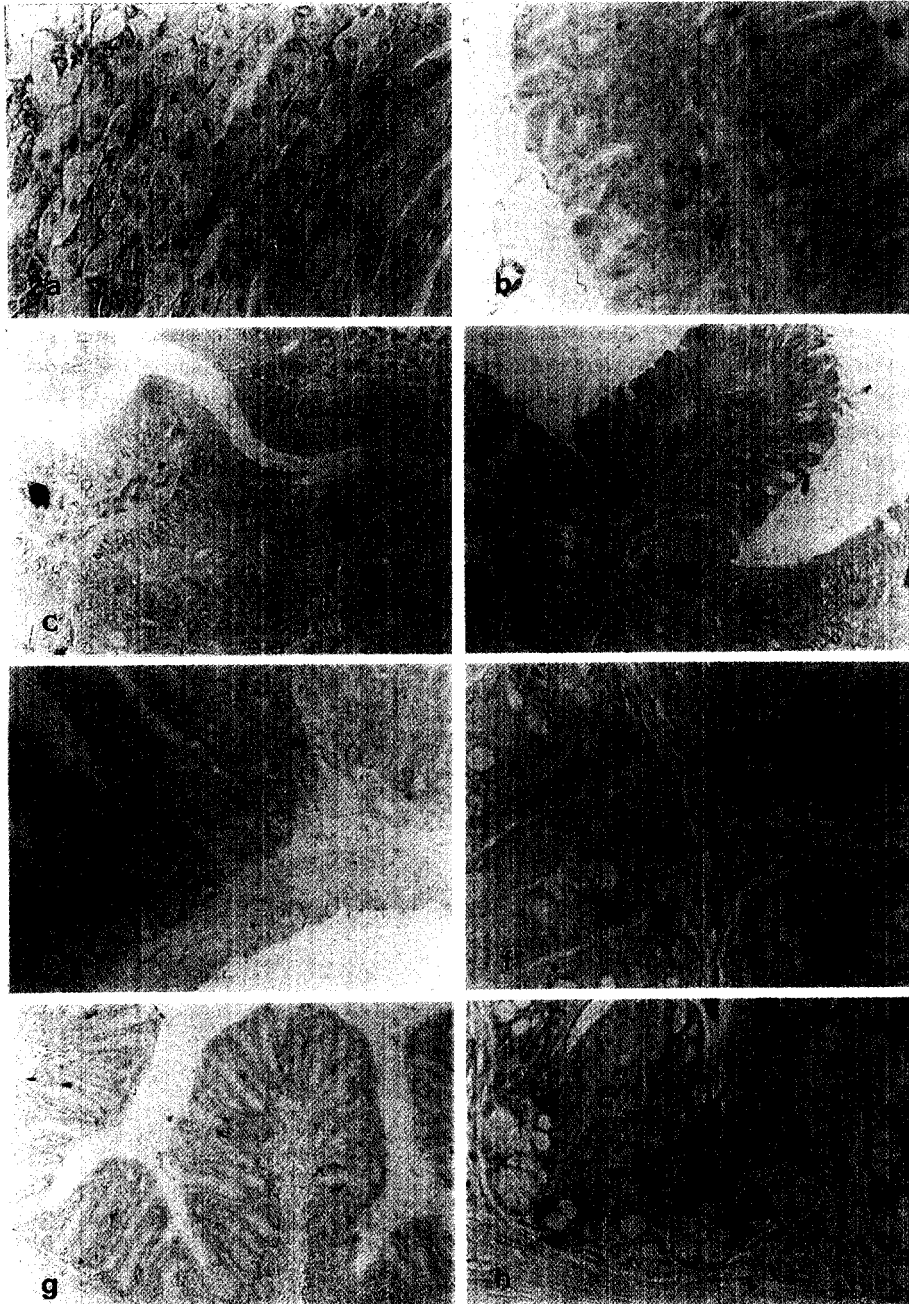


Fig 2. Serotonin-immunoreactive cells in the gastrointestinal tract of ICR mouse. Note that various distributions and relative frequencies of these cells were observed throughout whole gastrointestinal. They were demonstrated in the fundus (a), pylorus (b), duodenum (c), jejunum (d), ileum (e), cecum (f), colon (g) and rectum (h). a, c, d, f, & h: $\times 300$; b & e: $\times 150$; g: $\times 75$.

were located between epithelial cells or duodenal glands, which were located the basal portion of duodenal mucosa with rare frequency. Open typed cells were observed in the interepithelial cell regions (Fig 1d). In the jejunum and ileum (Fig 1e,f), open typed somatostatin-IR cells were observed in the interepithelial cell regions with rare frequencies.

Serotonin-IR cells (Fig 2, Table 2)

Serotonin-IR cells were observed throughout whole GIT and relative frequencies in each portion of GIT were shown in Table 2. Close typed serotonin-IR cells were dispersed in the gastric mucosa, between chief and parietal cells, of the fundus with a few frequency and some of these IR cells, which have long cytoplasmic process, are also observed (Fig 2a). Similar to those of the fundus, close typed serotonin-IR cells were detected in the gastric gland which were mainly located in the basal portion of gastric mucosa of the pylorus with numerous frequency, however, some open typed cells were also observed between epithelial cell regions (Fig 2b). In the duodenum, they were demonstrated between epithelial cells or in the intestinal glands, which were located in the basal portion of duodenal mucosa with numerous frequency. Open typed cells were restricted to the interepithelial cell regions while close typed cells were found in the intestinal gland regions (Fig 2c). In the jejunum and ileum (Fig 2d,e), the distributional patterns and appearance cell shapes were similar to those of the duodenum whereas the relative frequency was lower than those of the duodenum (Table 2). Similar to those of the small intestine, serotonin-IR cells were widely dispersed in the mucosa of the large intestine

but they were more numerously detected in these portions compared to those of the small intestine (Fig 2f-h, Table 2).

Glucagon-IR cells (Fig 3, Table 2)

Glucagon-IR cells were restricted to the fundus and rectum with moderate and rare frequency, respectively (Table 2). Close typed cells were found in the basal portion of the gastric mucosa of the fundus (Fig 3a) and open typed cells were located in the interepithelial cell regions of the rectum (Fig 3b). However, no glucagon-IR cells were observed in the remaining portions of GIT (Table 2).

CCK-8-IR cells (Fig 4, Table 2)

CCK-8-IR cells were detected in the pylorus, duodenum and ileum (Table 2). In the pylorus regions, these IR cells were located in the gastric gland, which were mainly located in the basal portion of gastric mucosa with numerous frequency and most of these cells were close typed cells but occasionally open typed cells were situated in that regions mixed with close type cells (Fig 4a). Open typed CCK-8-IR cells were demonstrated in the interepithelial cell regions of the duodenum with moderate frequency (Fig 4b). In addition, open typed IR cells were detected in the interepithelial cell regions of the ileum with rare frequency (Fig. 4c).

Secretin-IR cells (Fig 5, Table 2)

Secretin-IR cells were restricted to the duodenum, ileum and rectum (Table 2). A few frequency of open typed secretin-IR cells were located in the interepithelial cell regions of the duodenum (Fig 5a). In the jejunum,

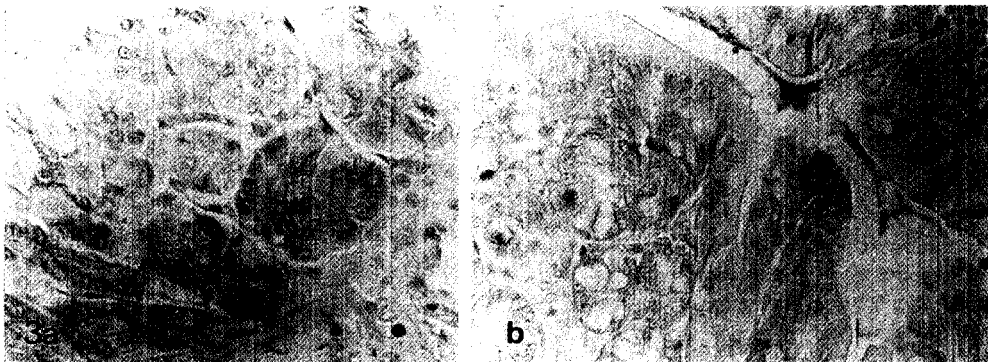


Fig 3. Glucagon-immunoreactive cells in the gastrointestinal tract of ICR mouse. Note that they were restricted to the fundus (a) and rectum. a & b: $\times 300$.

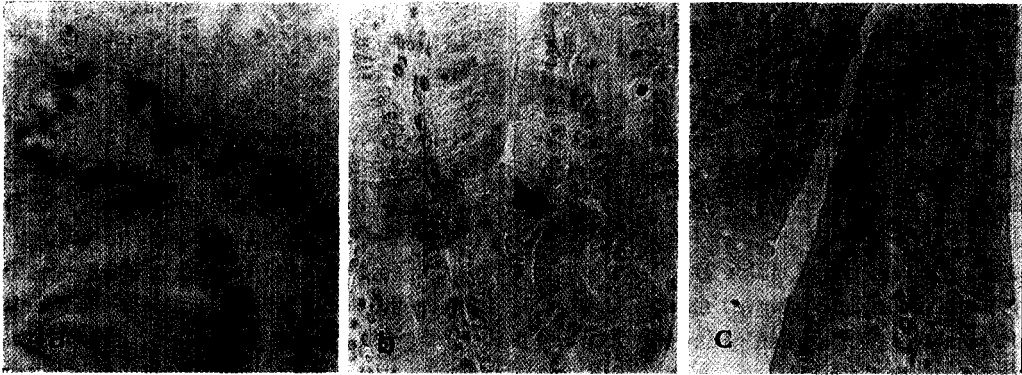


Fig 4. Cholecystinin-8-immunoreactive cells in the gastrointestinal tract of ICR mouse. Note that these immunoreactive cells were found in the pylorus (a), duodenum (b) and ileum (c). a: $\times 150$; b & c: $\times 300$.

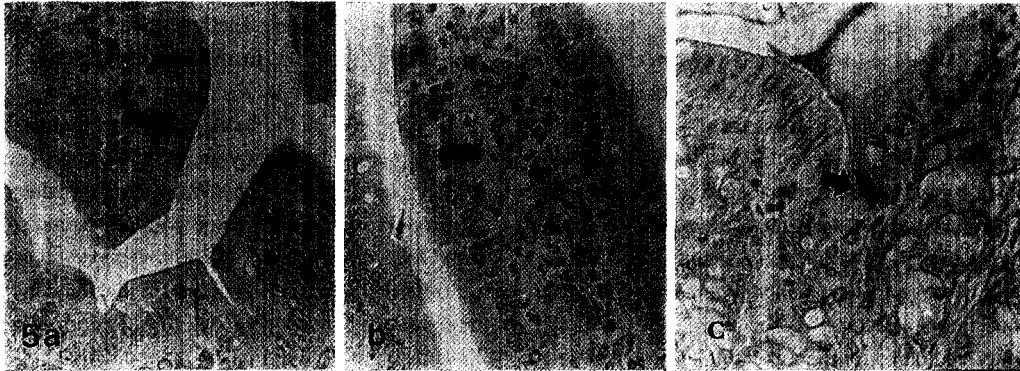


Fig 5. Secretin-immunoreactive cells in the gastrointestinal tract of ICR mouse. Note that these immunoreactive cells were restricted to the duodenum (a), ileum (b) and rectum (c, arrow). a-c: $\times 300$.

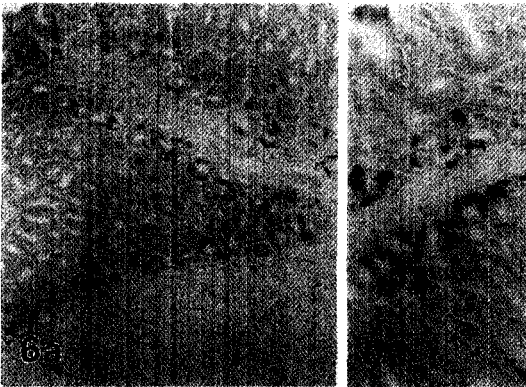


Fig 6. Gastrin-immunoreactive cells in the gastrointestinal tract of ICR mouse. Note that rare open type cells were located in the pylorus mixed with numerous close type cells (a, b). a: $\times 75$; b: $\times 150$.

detected in the ileum (Fig. 5b). In the large intestine, open typed secretin-IR cells were restricted to the rectum with rare frequency (Fig 5c, arrow).

PP-IR cells (Table 2)

No PP-IR cells were demonstrated in this study.

Gastrin-IR cells (Fig 6, Table 2)

Gastrin-IR cells were restricted to the pylorus with numerous frequency (Table 2). Close typed IR cells were exclusively located in the gastric gland, which were mainly located in the basal portion of gastric mucosa with numerous frequency cells but occasionally open typed cells were situated in that regions mixed with close type cells (Fig 6a,b).

Discussion

no secretin-IR cells were demonstrated in this portion of the GIT. However, open typed secretin-IR cells were

The endocrine cells in the alimentary tracts appeared

remarkably different depending on the regional distribution, relative frequency, cell types with animal species and each regional part of the GIT^{11,12}. In addition, many studies have elucidated the regional distribution and relative frequency of different endocrine cells in the GIT of the various vertebrates including various species of rodents. Generally, gastrointestinal endocrine cells were divided into two types, one was round to spherical shaped close type cells which were located in the stomach regions, and the other was spherical to spindle shaped open type cells which were situated in the intestinal regions^{3,4}. And also similar to those of previous study^{3,4}, open type cells were mainly located in stomach regions whereas most of close type cells were found in the intestinal tract in this study.

Somatostatin consisting of 14 amino acids was isolated from hypothalamus of sheep for the first time and it could be divided into straight form and cyclic form¹³. This substance inhibits the secretion of the other neuroendocrine hormones¹⁴. It is known that somatostatin-IR cells show the widest distribution in the whole GIT except for large intestine of all vertebrate species investigated, including the primitive agnathans with serotonin-IR cells¹⁵. However, somewhat species-dependent variations on the distributional pattern of these IR cells have been reported. It had been also reported that these IR-cells were restricted to the abomasums and small intestine of the *Philippine carabao*¹⁶ and restricted to the second stomach of the striped dolphin¹⁷. Well corresponded to those of previous report¹⁵, somatostatin-IR cells in the present study were detected from the fundus to the ileum with various frequencies.

Serotonin consisting of monoamines was widely distributed in nervous system and gastro-entero-pancreatic endocrine cells¹⁸. Main functions of serotonin were inhibition of gastric acid secretion and contraction of smooth muscle in the GIT¹⁹. El-Salhy *et al*¹⁸ reported that serotonin-IR cells were detected throughout the GIT of all species and established in the GIT at the early stage of vertebrate evolution. In addition, these IR cells were detected in the whole alimentary tract including esophagus of low vertebrates^{20,21}. Serotonin-IR cells were detected in the whole GIT of the common tree shrew²², *Philippine carabao*¹⁶, lesser mouse deer²³ and rat²⁴, but Domeneghini *et al*¹⁷ reported that these IR cells were not detected in the striped dolphin. In the present study, serotonin-IR cells were detected throughout whole GIT. These results considered as similar to most of other

mammals^{16,22,23,24}.

Glucagon is synthesized in the A cells of the pancreas and regulated serum glucose levels. These IR cells have been demonstrated in various mammals. They were demonstrated in the whole GIT of the common tree shrew²², lesser mouse deer²³ and musk shrew²⁵ but Baltazar *et al*¹⁶ persisted that these IR cells were only detected in the intestinal tract of the *Philippine carabao* and Lee *et al*²⁶ reported that they were restricted to the cardia and fundus of the Korean tree squirrel. Collectively it is considered that the distributional patterns of glucagon-IR cells in the GIT of the mammals showing species-dependent variation. In the present study, glucagon-IR cells were observed in the fundus and rectum with moderate and a few frequencies, respectively. These findings were quite different from those of previous studies^{22,23,25}, but the distributional patterns in SKH-1 were similar to those of Lee *et al*²⁶.

It is generally accepted that gastrin and CCK-8 originated from same ancestor and in the human duodenum a large fraction of these cells, besides reacting with non-C terminal CCK antibodies and C-terminal gastrin/CCK antibodies, also show immunoreactivity with C-terminal gastrin-34 antibodies, colocalised with CCK in a variable portion of secretory granules²⁷. Gastrin secreted by intestinal G cell, was promoted the gastric acid secretion and CCK secreted by intestinal I cell was stimulated the pancreatic enzyme secretion. In present study, gastrin-IR cells were restricted to the pylorus and CCK-8-IR cells were found in the pylorus, duodenum and ileum. Generally, it is well known that gastrin- and CCK-IR cells were located in the gastric mucoasa and whole small intestinal tract in mammals^{22,23,25}. However, Lee *et al*²⁶ reported that different from other mammalian species, gastrin/CCK-IR cells were abundant in the pyloric gland region but scarce in the duodenum and no cells were found in the other gastrointestinal regions of the Korean tree squirrel. Somewhat different from other mammalian species^{22, 23, 25}, these IR cells were showed more restricted distributional patterns in the present study, but these results were well corresponded to the reports in the Korean tree squirrel²⁶. These differences were considered that it might be due to the differences of the antisera tested or the methods and/or species differences used in the each study²⁸⁻³⁰.

In all mammalian animals so far investigated secretin-IR cells proved to be exclusive to the small intestine, usually with preference for the duodenum and upper

jejunum^{16,22,27} but Kitamura *et al.*²⁵ reported that these IR cells were detected in the small and large intestinal tract of the musk shrew. The distributional patterns in ICR were quite similar to those of mammalian species^{16,22,27}. Since PP was isolated from insulin extraction of pancreas at 1961, the regional distribution of PP-IR cells in the mammalian species was relatively well known but species-dependent differences were existed among the mammals^{16,22,23,26}. However, quite differing from those of previous reports^{16,22,23,26}, no PP-IR cells were demonstrated in this study.

In conclusion, some characteristic differences compared to those of previous reports due to the differences of the antisera tested or the methods and/or species differences used in the each study²⁸⁻³⁰, were observed in the present study.

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ICR 마우스 위장관 내분비 세포의 부위별 분포 및 출현 빈도 : 면역조직화학적 연구

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국문초록 : ICR 마우스 위장관 8개 부위(위저부, 유문부, 십이지장, 공장, 회장, 맹장, 결장 및 직장)에서 위장관내분비 세포의 부위별 분포 및 상대적 빈도를 somatostatin, serotonin, glucagon, cholecystokinin (CCK)-8, secretin, pancreatic polypeptide (PP) 및 gastrin 등 총 7종류의 항혈청을 이용한 면역조직화학적 방법으로 관찰하였던 결과 somatostatin, serotonin, glucagon, CCK-8, secretin 및 gastrin 면역반응세포의 7종류의 내분비세포가 관찰되었다. 본 실험의 결과 장관부위에서는 주로 타원형 또는 방추형의 개방형 세포(open-typed cell)들이 관찰된 반면 위저부와 유문부에서는 주로 원형의 폐쇄형 세포(close-typed cell)들이 관찰되었다. 이들 면역반응세포들의 부위별 분포는 위장관 각 부위에 따라 매우 다양하게 관찰되었다. Somatostatin 면역반응세포들은 대장을 제외한 위장관에서 전 부위에서 관찰되었고, serotonin 면역반응세포들은 전 위장관에 걸쳐 관찰되었으며, ICR 마우스에서 가장 높은 빈도를 나타내었다. Glucagon 면역반응세포들은 위저부와 직장에 국한되어 관찰되었으며, 각각 중등도 및 소수의 빈도를 나타내었다. CCK-8 면역반응세포들은 유문부, 십이지장 및 회장에서 각각 다수, 중등도 및 극소수의 빈도로 관찰되었다. 한편 secretin 면역반응세포들은 각각 소수 및 극소수의 빈도로 십이지장과 회장에 국한되어 출현하였고, gastrin 면역반응세포들은 유문부에 국한되어 다수 관찰되었다. 그러나 PP 면역반응세포들은 전 위장관에 걸쳐 관찰되지 않았다. 결론적으로 ICR 마우스의 위장관내분비세포의 부위별 분포 및 상대적 빈도는 다른 포유동물과 유사하게 관찰되었으나, 일부 특이한 양상을 나타내기도 하였다.

Key words : gastrointestinal endocrine cell, immunohistochemistry, ICR mouse