

쏘가리의 위장관 내분비세포에 관한 면역조직화학적 연구

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An Immunohistochemical Study on the Endocrine Cells in the Gastrointestinal Tract of the Mandarin Fish (*Siniperca scherzeri*)

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Abstracts : The regional distribution and relative frequency of neurohormonal peptides-producing cells were demonstrated in the gut of the stomach teleost, the Mandarin fish, *Siniperca scherzeri* Steindachner, using 7 types of specific antisera raised against mammalian regulatory peptides. The gastrointestinal tract of the Mandarin fish was divided into three portions from proximal to distal, stomach, small intestine and large intestine. Cells showing immunoreactivities against regulatory peptides were situated in the epithelial lining, between epithelial cells, and gastric or intestinal gland regions with various frequencies along with gastrointestinal tract. Most of immunoreactive cells in the epithelial lining portion were generally spherical or spindle shape having long cytoplasmic process that were reached to the lumen (open type cell) while cells showing round in shape (closed type cell) were found in the gastric gland of the stomach occasionally. Serotonin, somatostatin, gastrin, cholecystokinin (CCK)-8- and human pancreatic polypeptide (HPP)-immunoreactive cells were observed in this study. However, no insulin- and glucagon-immunoreactive cells were found. Serotonin- and somatostatin-immunoreactive cells were restricted to the stomach regions with moderate and numerous frequencies, respectively. Gastrin-immunoreactive cells were demonstrated in the stomach and small intestinal portions with a few and moderate frequencies, respectively and CCK-8-immunoreactive cells were restricted to the small intestinal portions with moderate frequency. In addition, HPP-immunoreactive cells were demonstrated in the stomach and small intestine with numerous frequencies, respectively. In conclusion, the distribution and relative frequency of these immunoreactive cells in the gastrointestinal tract of the Mandarin fish shows peculiar patterns compared to those of other stomach and/or stomachless teleost.

Key words : Immunohistochemistry, gastrointestinal tract, Mandarin fish, teleost, regulatory peptides, *Siniperca scherzeri*

초록 : 쏘가리 (*Siniperca scherzeri* Steindachner)의 위장관에 존재하는 내분비세포의 부위별 분포 및 출현빈도를 포유류의 peptide에 대한 7종류의 항혈청을 사용하여 면역조직화학적 방법으로 관찰하였다. 쏘가리의 위장관은 근위부에서부터 원위부까지 위, 소장 및 대장으로 3 등분 하였으며, 다양한 종류의

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항혈청에 면역반응성을 나타내는 면역반응세포들이 상피세포 사이와 위샘 또는 장샘에서 관찰되었다. 상피세포 사이에서는 대부분의 면역반응세포들은 장 내강까지 신장되어 있는 긴 세포질 돌기를 함유한 방추형의 개방형 세포 (open type cell)로 관찰되었으며, 세포질 돌기 없이 원형 또는 타원형의 형태를 나타내는 폐쇄형 세포 (close type cell)들이 위 부위에서 소수 관찰되었다. 본 실험에서는 serotonin, somatostatin, gastrin, cholecystokinin (CCK)-8 및 human pancreatic polypeptide (HPP) 면역반응 세포들이 관찰되었으나, insulin 및 glucagon 면역반응세포들은 관찰되지 않았다. Serotonin 및 somatostatin 면역반응세포들은 위 부위에 국한되어 각각 중등도 및 다수의 출현빈도로 관찰되었다. 또한 gastrin 면역반응세포들은 위와 소장에서 출현하였으며, 각각 소수 및 중등도의 출현빈도를 나타내었고, CCK-8 면역반응세포들은 소장에 국한되어 중등도의 출현빈도를 나타내었다. 한편 HPP 면역반응세포들은 위와 소장에서 다수 관찰되었다. 이상에서 쏘가리 위장관 내분비세포들의 부위별 분포 및 출현 빈도는 다른 경골어류에 비해 특이한 양상을 나타내는 것으로 관찰되었다.

Introduction

The stomach teleost, the Mandarin fish, *Siniperca scherzeri* Steindachner, belonging to the family Centropomidae in order Perciformes are stomach teleost fresh-water fish and their habitations are limited to Korea and a part of China. It is generally known that the Mandarin fish habited in Korea (50-60cm) was larger than that of China (30-40cm). Recently, the number of Mandarin fish habited in Korea was dramatically reduced because of over fish and destruction of ecosystems.

The gastrointestinal endocrine cells dispersed in the epithelia and gastric glands of the alimentary tract synthesized various kinds of gastrointestinal hormones and play an important role in the physiological functions of the alimentary tract¹. And they were divided into two types, open and closed type; open type cells were mainly located in the epithelial lining of the gut, showing spindle shape having long cytoplasmic process which were contacted with the lumen of the gastrointestinal tract, and closed type cells were mainly located in stomach and/or intestinal glands showing spherical to round in shape^{2,3}. Until now, the investigation of gastrointestinal endocrine cells is considered to be an important part of a phylogenic study⁴. In addition, the regional distributions and relative frequencies of these endocrine cells were varied with animal species and feeding habits³. Most recently intensive studies have been done on the Pisces because some endocrine cells were demonstrated in the skin, gills and airways⁵ and the alteration of regional distribution and relative frequency of these cells by heavy metal intoxication such as lead were also demonstrated⁶. In addition, the possibility of using the teleost fish endocrine

tissues for treatment hormonal disorder such as diabetes was suggested⁷.

Until now, the regional distribution and relative frequency of numerous types of regulatory peptides have been demonstrated in the gastrointestinal tract of the Pisces. Some endocrine cells in the gastrointestinal tract of the elasmobranch⁸, the *Barbus conchoniuis*⁹, the grass carp¹⁰, the Atlantic hagfish¹¹, the cod¹², the cartilaginous fish^{13, 14}, the leaping grey mullet¹⁵, the lamprey¹⁶; *Geotria australis*¹⁷; *Mordacia mordax*¹⁷, the rainbow trout¹⁸, the dogfish^{19, 20}, the tilapia²¹, the *Protopterus annectens*²², the turbot²³, the silver carp²⁴, the pumpkinseed sunfish²⁴, the gar²⁵, the Japanese flounder²⁶ and the Prussian carp²⁷ were studied by histochemical, electron microscopical and immunohistochemical methods. In addition, Al-Mahrouki and Youson²⁸ reported that immunohistochemical study of the endocrine cells within the gastro-entero-pancreatic system of Osteoglossomorpha, an ancient teleostean group and they detected some different distribution and frequency of endocrine cells between five species of the Osteoglossomorpha. However, there are no data or reports dealing the regional distribution and relative frequency of the endocrine cells in the stomach fresh-water teleost, the Mandarin fish, *Siniperca scherzeri*, except for pancreatic endocrine cells²⁹.

The present study aims to characterize the regional distribution and relative frequency of the endocrine cells in the gut of the stomach teleost, the Mandarin fish, *Siniperca scherzeri*, were investigated by immunohistochemical methods using 7 types specific antisera; serotonin, somatostatin, gastrin, CCK-8, human pancreatic polypeptide (HPP), insulin and glucagon raised against mammalian regulatory peptides.

Materials and Methods

Five adult stomach teleost, the Mandarin fish, *Siniperca scherzeri*, belong to the family Centropomidae in order Perciformes (about 50 ~ 60cm in length) were purchased from a merchant in Taegu, Korea and used in this study without sexual distinction. Because of their unique short alimentary tract compared to that of mammals, the gastrointestinal tract of Mandarin fish was divided into three portions proximal to distal, stomach, small intestine and large intestine. After anesthetizing with ethyl ether and phlebotomy from head, samples from gastrointestinal tract were fixed in Bouin's solution. After paraffin embedding, 3-4 μ m serial sections were prepared. Representative sections of each tissue were stained with hematoxylin and eosin for light microscopic examination of the normal gut architecture.

The each representative section was deparaffinized, rehydrated and immunostained with the peroxidase anti-peroxidase (PAP) method³⁰. Blocking nonspecific reaction was performed with normal goat serum prior to incubation with the specific antisera (Table 1). In case of control sections, they were reacted with 1.0%-non-immunized rabbit serum instead of primary antiserum. 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 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 Sternberger³⁰, including the replacement of specific antiserum by the same antiserum, which had been pre-incubated with its corresponding antigen. The relative frequency of occurrence of each type of IR cell was placed into one of five categories, not detected (), rare (; mean values were below 2/one filed), a few (+ ; mean values were below 5/one filed), moderate (++ ; mean values were below 10/one filed) and numerous (+++ ; mean values were up to 20/one filed), according to their observed mean numbers as seen under one filed of light microscope (\times 200).

Results

In the present study, five kinds of the immunoreactive cells were detected with the antisera against serotonin, somatostatin, gastrin, CCK-8 and HPP in the gastrointestinal tract. According to the location of the alimentary tract and situation in that regions, different regional distributions and relative frequencies of these immunoreactive cells were observed. These differences are shown in Table 2. However, no insulin- and glucagon-immunoreactive cells were demonstrated in this study.

Spindle shaped open typed serotonin-immunoreactive cells having cytoplasmic process were restricted to the inter-epithelial cell regions of the basal portion of the epithelial lining of the stomach portions with moderate frequency (fig. 1a, b). However, no serotonin-immunoreactive cells were demonstrated in the small and large intestine (Table 2).

Table 1. Antisera used in this study

Antisera raised*	Code	Source	Dilution
Serotonin	BO68082C	BioGenex Lab., San Ramon, USA	1 : 20
Somatostatin	PUO421295	BioGenex Lab., San Ramon, USA	1 : 20
Gastrin	PUO190796	BioGenex Lab., San Ramon, USA	1 : 20
CCK-8 ¹⁾	8643010	Immunonuclear Corp., Stillwater, USA	1 : 1,000
HPP ¹⁾	PUO660495	BioGenex Lab., San Ramon, USA	1 : 20
Insulin	PUO290395	BioGenex Lab., San Ramon, USA	1 : 24
Glucagon	PUO390598	BioGenex Lab., San Ramon, USA	1 : 20

*All antisera were raised in rabbits except for insulin which was raised in guinea pigs

¹⁾ CCK-8: cholecystokinin-8, HPP: human pancreatic polypeptide

Table 2. The regional distributions and relative frequencies of the endocrine cells in the gastrointestinal tract of the Mandarin fish, *Siniperca scherzeri*, belong to the family Centropomidae in order Perciformes

Antisera	Portion of gastrointestinal tract of the Mandarin fish		
	Stomach	Small intestine	Large intestine
Serotonin	++	-	-
Somatostatin	+++	-	-
Gastrin	+	++	-
CCK-8 ¹⁾	-	++	-
HPP ¹⁾	+++	+++	-
Insulin	-	-	-
Glucagon	-	-	-

¹⁾CCK-8: cholecystokinin-8, HPP: human pancreatic polypeptide; Relative frequencies; +++: numerous, ++: moderate, +: a few, ±: rare, -: not detected

Spindle shaped open typed somatostatin-immunoreactive cells having long cytoplasmic process were restricted to the inter-epithelial cell regions of the basal portion of the epithelial lining of the stomach portions, regions and occasionally round to spherical shaped close typed cells were also demonstrated in those regions (fig. 1c, d). Somatostatin-immunoreactive cells were restricted to the stomach portions with numerous frequency and no cells were demonstrated in the small and large intestinal portions

similar to that of serotonin-immunoreactive cells (Table 2).

Round to spherical shaped close typed gastrin-immunoreactive cells were mainly located in the inter-epithelial cell regions of the basal portion of the epithelial lining in the stomach portions with a few frequency (Fig. 2a) and in the small intestinal portions, spindle shaped open typed CCK-8-immunoreactive cells having long cytoplasmic process were situated in the inter-epithelial cells of the epithelial lining (Fig. 2b, c) and

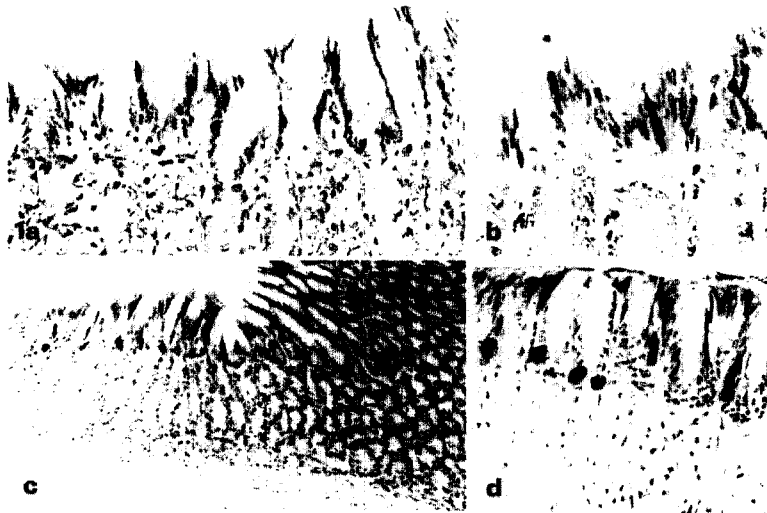


Fig. 1. Serotonin- (a, b) and somatostatin- (c, d) immunoreactive cells in the stomach portions of the Mandarin fish, *Siniperca scherzeri* (Centropomidae). Note that open type cells were observed in the inter-epithelial portion of the basal portion of the epithelial lining. a and d. $\times 300$, b. $\times 600$, c. $\times 150$, PAP methods.

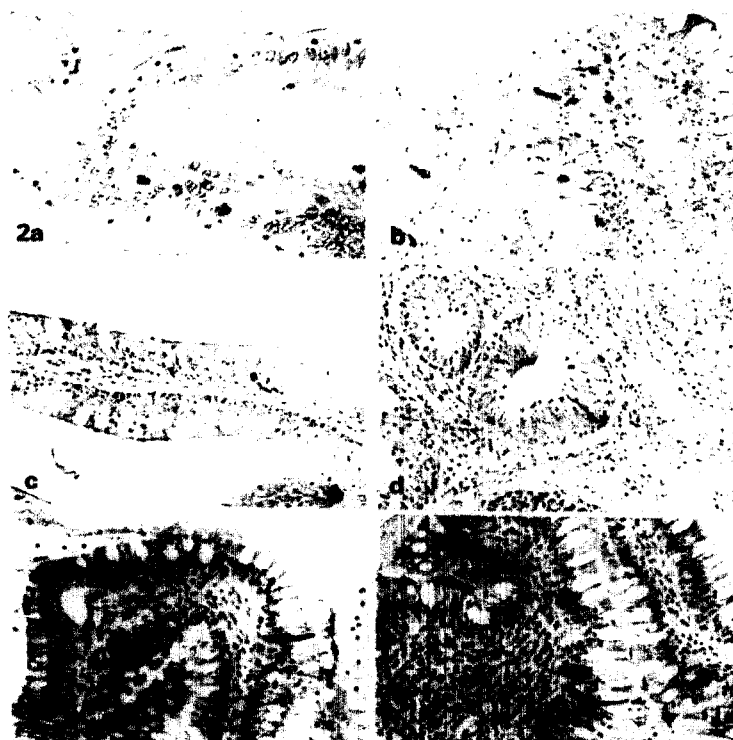


Fig. 2. Gastrin- (a ~ d) and CCK-8- (e, f) immunoreactive cells in the gastrointestinal tract of the Mandarin fish, *Siniperca scherzeri* (Centropomidae). Note that closed type gastrin- immunoreactive cells were restricted to the inter-epithelial portion of the basal portion of the epithelial lining in the stomach and open type gastrin- and CCK-8-immunoreactive cells were located in the inter-epithelial portion of the epithelial lining in the small intestinal portions. a. $\times 600$, b ~ f. $\times 300$, PAP methods.

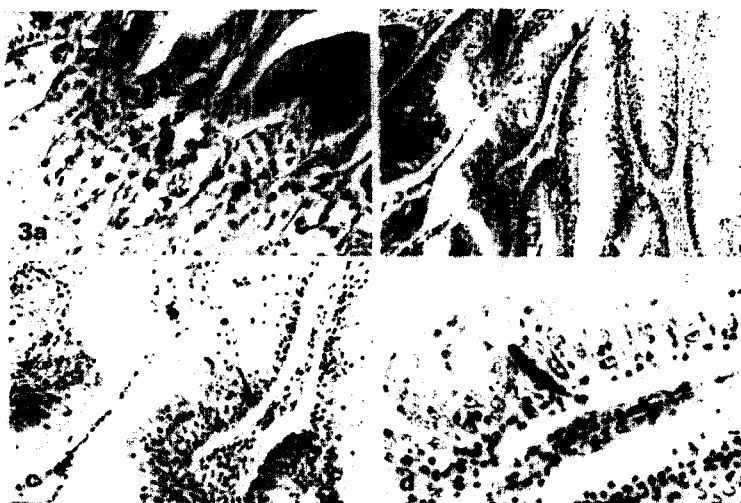


Fig. 3. HPP-immunoreactive cells in the gastrointestinal tract of the Mandarin fish, *Siniperca scherzeri* (Centropomidae). Note that close typed HPP-immunoreactive cells were located between parietal and chief cells in the stomach portions (a) and open type cells were demonstrated in the inter-epithelial portion of the epithelial lining in the stomach and small intestinal portions (a ~ d). a and c. $\times 300$, b. $\times 150$, d. $\times 600$, PAP methods.

inter-acinar cells of the intestinal glands (Fig. 2d) with moderate frequency but no cells were demonstrated in the large intestinal portions (Table 2).

Spindle shaped open type CCK-8-immunoreactive cells having long cytoplasmic process were mainly located in the inter-epithelial cell regions of the epithelial lining in the small intestinal portions (Fig. 2, f) with moderate frequency but round to spherical shaped close typed cells were not demonstrated in those portions. They were not observed in the stomach and large intestinal portions (Table 2).

Spindle shaped, having long cytoplasmic process, open typed HPP-immunoreactive cells were observed in the inter-epithelial cell regions of the basal portion of the epithelial lining of the stomach portions and occasionally round to spherical shaped closed type cells were also demonstrated between parietal and chief cells in those portions (Fig. 3a) with numerous frequency. In the small intestinal portions, spindle shaped open typed HPP-immunoreactive cells having long cytoplasmic process were detected in the inter-epithelial cell regions of the epithelial lining (Fig. b ~ d) with numerous frequency. However, no cells were found in the large intestinal portions (Table 2) and closed type cells were not detected in the small and large intestinal portions.

Discussion

The endocrine cells in the alimentary tracts appeared remarkably different in the regional distribution, relative frequency and cell types with animal species and each regional part of the alimentary tract^{31, 32} and among numerous types of regulatory peptides, serotonin consisted of monoamines and widely distributed in nervous system and GEP endocrine cells³³. El-Salhy *et al.*³³ reported that serotonin-immunoreactive cells were found throughout the gastrointestinal tract of all species and established in the alimentary tract at the early stage of vertebrate evolution. The appearance of serotonin-immunoreactive cells was well demonstrated throughout the whole gastrointestinal tract of the spiny dogfish⁸ and the rainbow trout¹⁸, and they were also detected in gill filaments of the goldfish and in there, they increased after one-week treatment of lead intoxication³⁴. In addition, ontogeny of serotonin-immunoreactive cells and their changes during development were also monitored in the gut epithelium of the cloudy dogfish³⁵ and the turbot²³. However, it is also reported that no serotonin-immunoreactive

endocrine cells were found in the gut of the goldfish⁶, the Prussian carp²⁷ and the arctic lamprey³⁶ but they were restricted in the nerve fibers in that species of fishes. According to these previous reports, it is considered that the appearances, distributions and frequencies of these serotonin-immunoreactive cells were somewhat varied along with species of Pisces. In the present study, the distributional patterns of serotonin-immunoreactive cells of the Mandarin fish showed unique patterns compared to other teleost^{6, 8, 18, 27, 23, 33-36}, they were restricted to the stomach portions.

Somatostatin, which consisted of 14 amino acids, was isolated from hypothalamus of sheep for the first and it could be subdivided into straight form and cyclic form³⁷. It is well known that somatostatin-immunoreactive cells show the widest distribution in the whole gastrointestinal tract of all vertebrate species investigated, including the primitive agnathans with serotonin-immunoreactive cells³⁸. The appearance of open and close typed somatostatin-immunoreactive cells were well demonstrated in the alimentary tract except for large intestinal tract of the *Anguilla anguilla*³⁹, the *Protopterus annectens*²², the southern-hemisphere lampreys¹⁷, the rainbow trout¹⁸, the lamprey¹⁶ and the elasmobranchs⁸. In addition, ontogeny during developmental stages of somatostatin-immunoreactive cells was also monitored in the digestive tract of the turbot²³ and the arctic lamprey³⁶. In the arctic lamprey, somatostatin-immunoreactive cells were not demonstrated in the gut of the adults but they were well documented in the gut of the larval lamprey³⁶. However, it is also demonstrated that no somatostatin-immunoreactive cells were found in the gut of the grass carp¹⁰, the leaping grey mullet¹⁵ and the *Barbus conchonus*⁹. In addition, they were restricted to the most proximal parts of the gut of the Prussian carp²⁷. According to these previous reports, it is considered that the appearances, distributions and frequencies of these somatostatin-immunoreactive cells were somewhat varied along with species of Pisces similarly to that of serotonin-immunoreactive cells. In the present study, somatostatin-immunoreactive cells were restricted to the stomach portions with numerous frequency. These results were quite differed from the results of the other species of the Pisces^{8-10, 15-18, 22, 23, 27, 36, 39}.

In the Pisces, it is well documented that the distribution and frequency of glucagon-immunoreactive cells were variable in the intestinal cells with the species²⁸. The appearance of these cells was detected in the proximal parts

of the gut of the *Protopterus annectens*²², the southern-hemisphere lampreys¹⁷, the grass carp¹⁰, the sea bream⁴⁰, the rainbow trout¹⁸, the Atlantic hagfish⁴¹, the salmonids⁴², the arctic lamprey³⁶, the cartilaginous fish¹³ and the *Barbus conchoni*⁹. In addition, the ontogeny during development of glucagon-immunoreactive cells was monitored in the digestive tract of the turbot²³ and the sea bream⁴⁰. However, it is also reported that no glucagon-immunoreactive cells were found in the gastrointestinal tract of the goldfish⁶ and the Prussian carp²⁷, and Tagliaferro *et al*²² reported that no open typed glucagon-immunoreactive cells were detected in the intestinal epithelium of the *Protopterus annectens*. In the present study, glucagon-immunoreactive cells were not demonstrated. These results are well corresponded to those of the goldfish⁶ and the Prussian carp²⁷. However, it was somewhat different from the results of other teleost^{9, 10, 13, 17, 18, 22, 23, 36, 40-42}.

It is generally accepted that gastrin and CCK-8 were originated from same ancestor and in the human duodenum of 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, and colocalised with CCK in a variable portion of secretory granules⁴³. In the Pisces, gastrin-, CCK-8- and/or gastrin/CCK-immunoreactive cells were restricted to the stomach and/or detected throughout the whole intestinal tract of the goldfish²¹, the rainbow trout¹⁸, the Atlantic hagfish⁴¹, the leaping grey mullet¹⁵, the arctic lamprey³⁶, the cod¹² and the elasmobranch⁸. In addition, ontogeny of CCK-8- and/or gastrin/CCK-immunoreactive cells was monitored in the gut of the Japanese flounder²⁶ and the turbot²³. However, no CCK-8-immunoreactive cells were also demonstrated in the gut of the grass carp¹⁰. These distributions of gastrin- and CCK-8-immunoreactive cells in present study are well corresponded to previous reports except for that of the grass carp¹⁰.

PP-immunoreactive cells were generally located in the proximal parts of the gut of the Osteoglossomorpha²⁸, the gar²⁵, the *Protopterus annectens*²², the goldfish⁶, the grass carp¹⁰, the rainbow trout¹⁸, the leaping grey mullet¹⁵, the Prussian carp²⁷, the arctic lamprey³⁶ and the *barbus conchoni*⁹, and similar to these previous reports, in the present study, they were found in the stomach and small intestinal portions. In addition, developmental changes of these PP-immunoreactive cells were well documented in the

gut of the Japanese flounder²⁶. However, it is also reported that no PP-immunoreactive cells were demonstrated in the intestinal tract of the turbot²³ and the lampreys¹⁶.

The appearance of insulin-immunoreactive cells in the gastrointestinal tract of the teleostean fish has been demonstrated⁴⁴ and especially Pan *et al*⁴⁵ reported that insulin-immunoreactive cells in the stomach and intestine of the 8 species of stomach-containing teleosts were different in distributions and types, but in the present study, quite different from this report, no insulin-immunoreactive cells were demonstrated in the gastrointestinal tract of the Mandarin fish.

In conclusion, the distribution and relative frequency of 7 types of immunoreactive cells observed in this study, were well corresponded to the previous reportes in the other teleost but somewhat peculiar patterns were also demonstrated.

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