

## An immunohistochemical study of endocrine cells in the gut of the Prussian carp, *Carassius auratus*

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**Abstract :** The regional distribution and relative frequency of neurohormonal peptides-producing cells were demonstrated in the gut of the stomachless teleost, the Prussian carp, *Carassius auratus* Linnaeus, using 10 types of specific antisera raised against mammalian regulatory peptides. The gut of the Prussian carp was divided into five portions from proximal to distal (Segments I ~ V).

Most of immunoreactive cells in the epithelial lining portion, between epithelial cells, were generally spherical or spindle shape having long cytoplasmic process that reached to the lumen (open typed cell) while cells showing round in shape (close typed cell) were found in the basal portions of epithelial lining occasionally. Somatostatin-, cholecystokinin (CCK)-8- and pancreatic polypeptide (PP)- immunoreactive cells were observed in this study. However, no serotonin-, glucagon-, chromogranin A-, secretin-, vasoactive intestinal peptide (VIP)-, substance P- and bombesin-immunoreactive cells were found. Somatostatin-immunoreactive cells were restricted to most proximal segments of the gut (Segment I) with rare frequency and CCK-8-immunoreactive cells were demonstrated in the proximal segments of the gut (Segments I and II) with a few to rare frequencies. In addition, pancreatic polypeptide-immunoreactive cells were demonstrated in the proximal to middle segments (Segments I ~ III) with moderated to rare frequencies.

In conclusion, the distribution and relative frequency of these immunoreactive cells are well corresponded to the previous reports in stomachless teleost but somewhat peculiar patterns are also detected.

**Key words :** Immunoreactive cell, gut, Prussian carp, stomachless teleost, *Carassius auratus*, regulatory peptides

### Introduction

The stomachless teleost, *Carassius auratus* Linnaeus, belonging to the family Cyprinidae in order Cypriniformes is a fresh-water fish habited east Asia and Europe, and they are widely distributed and known in Korea. They were called as the goldfish in Europe.

The gastrointestinal endocrine cells that are dispersed in the epithelia and gastric glands of the alimentary tract synthesize various kinds of gastrointestinal hormones and play an important role in the physiological functions of the alimentary tract<sup>1</sup>. They were divided into two types, open and close type

- open typed cells were mainly located in the epithelial lining of the gut, showing spindle shape having long cytoplasmic process that contacted with the lumen of the gut and close typed cells were mainly located stomach and/or intestinal glands showing spherical to round in shape<sup>2,3</sup>. Until now, the investigation of gastrointestinal endocrine cells is considered to be an important part of a phylogenetic study<sup>4</sup>. In addition, the regional distributions and relative frequencies of these endocrine cells were varied with animal species and feeding habits<sup>3</sup>. Recently, intensive studies have been done on the Pisces because some endocrine cells were demonstrated in the skin, gills and airways<sup>3</sup> and the alteration of regional distribution

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and relative frequency of these cells by heavy metal intoxication such as lead were also demonstrated<sup>6</sup>. In addition, the possibility of using the teleost fish endocrine tissues for treatment hormonal disorder such as diabetes was suggested<sup>7</sup>.

Until now, the regional distribution and relative frequency of numerous types of regulatory peptides have been demonstrated in the gut of the Pisces. Some endocrine cells in the gut of the elasmobranch, *Squalus acanthias*<sup>8</sup>, *Barbus conchoniis*<sup>9</sup>, the grass carp, *Ctenopharyngodon idellus*<sup>10</sup>, the Atlantic hagfish, *Myxine glutinosa*<sup>11</sup>, the cod, *Gadus morhua*<sup>12</sup>, the cartilaginous fish, *Scyliorhinus stellaris*<sup>13,14</sup>, the leaping grey mullet, *Mugil saliens*<sup>15</sup>, the lamprey, *Petromyzon marinus*<sup>16</sup>; *Geotria australis*<sup>17</sup>; *Mordacia mordax*<sup>17</sup>, the rainbow trout, *Oncorhynchus mykiss*<sup>18</sup>, the dogfish, *Scyliorhinus torazame*<sup>19,20</sup>, the tilapia, *Oreochromis mossambicus*<sup>21</sup>, *Protopterus annectens*<sup>22</sup>, the turbot, *Scophthalmus maximus*<sup>23</sup>, the silver carp, *Hypophthalmichthys molitrix*<sup>24</sup>, the pumpkinseed sunfish, *Lepomis gibbosus*<sup>24</sup>, the gar, *Lepisosteus osseus*<sup>25</sup> and the Japanese flounder, *Paralichthys olivaceus*<sup>26</sup> were studied by histochemical, electron microscopical and immunohisto-chemical methods. In addition, Al-Mahrouki and Youson<sup>27</sup> reported immunohistochemical study on 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, except for vasoactive intestinal peptide (VIP)-, cholecystokinin (CCK)/gastrin-, neurotensin-, substance P- and bombesin-immunoreactive cells<sup>21,24,28</sup>, there were no data or reports dealing with the regional distribution and relative frequency of the endocrine cells except for mentioned five types of immunoreactive cells above in the gut of the stomachless fresh-water teleost, the Prussian carp, *Carassius auratus*.

The present study aims at characterizing the regional distribution and relative frequency of the endocrine cells in the gut of the stomachless teleost, the Prussian carp, *Carassius auratus*, which were investigated by immunohistochemical methods using 10 types specific antisera, serotonin, somatostatin, glucagon, CCK-8, pancreatic polypeptide (PP), chromogranin A, secretin, VIP, substance P and bombesin, raised against mammalian regulatory peptides.

## Materials and Methods

Five adult stomachless teleosts, the Prussian carp, *Carassius auratus*, belonging to the family Cyprinidae in order Cypriniformes (about 16 ~ 20cm in length) were captured around Kyungsan, Kyungpook, Korea and used in this study without sexual distinction. After anesthetized with ethyl ether, the intestinal tract of Pisces was divided into five portions from proximal to distal, Segments I, II, III, IV and V (Segments separated by equal lengths), because they don't have stomach and clear demarcation of intestinal tract like mammals. After phlebotomy from head, samples from the Segments I, II, III, IV and V were fixed in Bouin's solution. After paraffin embedding, 3-4 $\mu$ 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) method<sup>29</sup>. Blocking of nonspecific reaction was performed with normal goat serum prior to incubation with the specific antisera (Table 1). After rinsed in phosphate buffered saline (PBS; 0.01M, pH 7.4), the sections were incubated in secondary

Table 1. Antisera used in this study

Antisera raised*	Code	Source	Diluton
Serotonin	BO68082C	BioGenex Lab., San Ramon	1 : 20
Somatostatin	PUO421295	BioGenex Lab., San Ramon.	1 : 20
Glucagon	PUO390598	BioGenex Lab., San Ramon.	1 : 20
CCK-8 <sup>1)</sup>	8643010	Immunonuclear Corp., Stillwater.	1 : 1,000
PP <sup>1)</sup>	PUO660495	BioGenex Lab., San Ramon.	1 : 20
Chromogranin A	517210	Incstar Corp., Stillwater.	1 : 1,000
Secretin	BO67122A	BioGenex Lab., San Ramon.	1 : 20
VIP <sup>1)</sup>	B95 C	Sera Lab., Sussex.	1 : 1,000
Substance P	B9C 35	Sera Lab., Sussex.	1 : 1,000
Bombesin	8652015	Immunonuclear Corp., Stillwater	1 : 1,000

\*All antisera were raised in rabbits.

<sup>1)</sup> CCK-8: cholecystokinin-8, PP: pancreatic polypeptide, VIP: vasoactive intestinal polypeptide

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% H<sub>2</sub>O<sub>2</sub> in Tris-HCl buffer (0.05M, pH 7.6). After immunostained, the sections were lightly counterstained with Mayer's hematoxylin and the immunoreactive cells were observed under light microscope.

The specificity of each immunohistochemical reaction was determined as recommended by Sternberger<sup>29</sup>, including the replacement of specific antiserum by the same antiserum, which had been preincubated with its corresponding antigen and the relative frequency of occurrence of each type of immunoreactive cell was placed into one of five categories, not detected (-), rare ( $\pm$ ), a few (+), moderate (++) and numerous (+++), according to their observed numbers as seen using light microscope.

## Results

In the present study, three kinds of the immunoreactive cells were detected with the antisera against somatostatin, CCK-8 and PP in the intestinal tract. According to the location of the gut 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 serotonin- glucagon- chromogranin A-, secretin-, VIP-, substance P- and bombesin-immunoreactive cells were demonstrated in this study.

Spindle shaped open typed somatostatin-immunoreactive cells

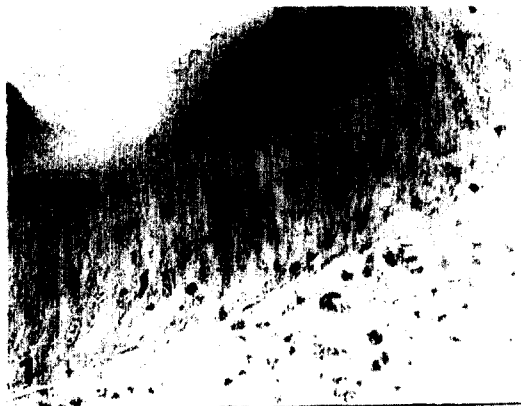


Fig 1. Somatostatin-immunoreactive cells in the Segment I of the Prussian carp, *Carassius auratus* (Cyprinidae). Note that open typed cells were observed in the inter-epithelial portion of the basal portion of epithelial lining.  $\times$  480, PAP methods.

having long cytoplasmic process were restricted to the inter-epithelial cell regions of the basal portion of the epithelial lining of the Segment I. However, no somatostatin-immunoreactive cells were demonstrated in the middle and distal portions of the gut, Segment II ~ V (Table 2). In the Segment I, only spindle shaped open typed somatostatin-immunoreactive cells were detected with rare frequency (Fig 1).

Table 2. The regional distributions and relative frequencies of the endocrine cells in the gut of the stomachless teleost, the Prussian carp, *Carassius auratus*, belong to the family Cyprinidae in order Cypriniformes

	Segments of the gut				
	I	II	III	IV	V
Serotonin	—	—	—	—	—
Somatostatin	$\pm$	—	—	—	—
Glucagon	—	—	—	—	—
CCK-8	+	$\pm$	—	—	—
PP	++	++	$\pm$	—	—
Chromogranin A	—	—	—	—	—
Secretin	—	—	—	—	—
VIP	—	—	—	—	—
Substance P	—	—	—	—	—
Bombesin	—	—	—	—	—

Relative frequencies; +++: numerous, ++: moderate, +: a few,  $\pm$ : rare, -: not detected

Spindle shaped open typed CCK-8-immunoreactive cells having long cytoplasmic process were mainly located in the inter-epithelial cell regions of the basal portion of the epithelial lining in the proximal parts of the intestinal tract, the Segments I and II (Table 2). In the Segment I, spindle shaped open typed CCK-8-immunoreactive cells were situated in the inter-epithelial cells of the basal portion of the epithelial lining with a few frequency but there was no cells showing round to spherical close type shaped (Fig 2a). Similar distribution and cell types were demonstrated in the Segment II compared to those of the Segment I (Fig 2b). However, the relative frequency of these cells in the Segment II was somewhat lower than in the Segment I.

Spindle shaped open typed PP-immunoreactive cells having long cytoplasmic process were observed in the inter-epithelial cell regions of the basal portion of the epithelial lining of the Segments I ~ III and occasionally round to spherical shaped close type cells were also demonstrated in proximal segments of the gut. However, no PP-immunoreactive cells were demonstrated in the distal portions of the gut, Segments IV

and V (Table 2). In the Segment I, spindle shaped open typed and round to spherical shaped close typed PP-immunoreactive cells having long cytoplasmic process or not were detected with moderated frequency (Fig 3a). Spindle shaped open typed somatostatin-immunoreactive cells having long cytoplasmic process, which were extended into the lumen and/or lamina propria, demonstrated in the inter-epithelial cell regions of the epithelia lining with moderated frequency in the Segment II (Fig 3b). Similar to that of the Segment II, spindle shaped open typed PP-immunoreactive cells having long cytoplasmic process were detected in the inter-epithelial cell regions of the epithelia lining with rare frequency in the Segment III but close typed cells were not demonstrated (Fig 3c).

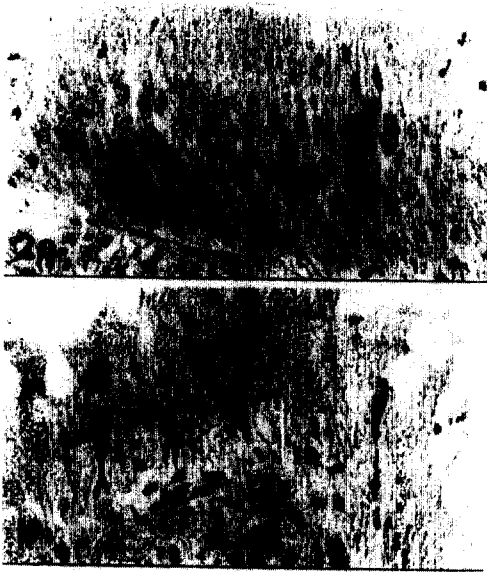


Fig 2. CCK-8-immunoreactive cells in the Segment I (a) and II (b) of the Prussian carp, *Carassius auratus* (Cyprinidae). Note that open typed cells were observed in the inter-epithelial portion of the basal portion of epithelial lining.  $\times 480$ , PAP methods.

## 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<sup>30,31</sup>.

Serotonin consist of monoamines and is widely distributed in nervous system and gastro-entero-pancreatic endocrine cells<sup>32</sup>. El-Salhy *et al*<sup>32</sup> reported that serotonin-immunoreactive cells

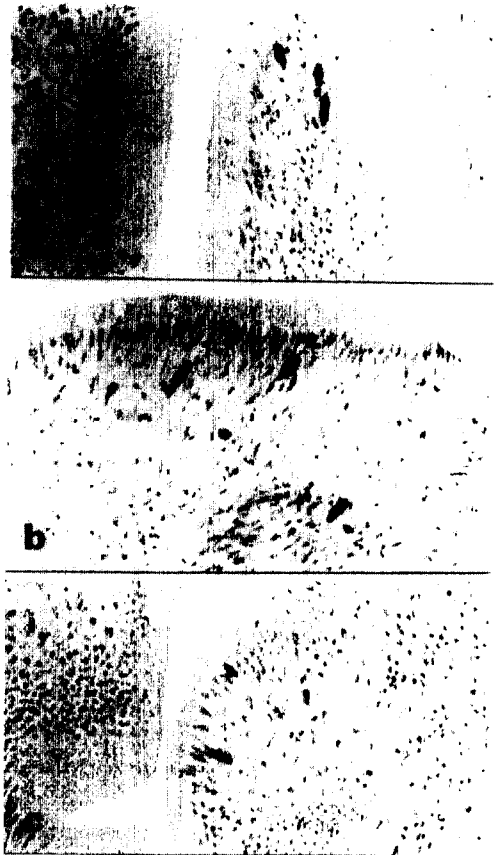


Fig 3. PP-immunoreactive cells in the Segments I (a), II (b) and III (c) of the Prussian carp, *Carassius auratus* (Cyprinidae). Note that open and close typed cells were observed in the inter-epithelial portion of the basal portion of epithelial lining.  $\times 240$ , PAP methods.

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 intestinal tract of the spiny dogfish<sup>8</sup> and the rainbow trout<sup>18</sup>, and they were also detected in gill filaments of the goldfish and in there, they increased after one-week treatment of lead intoxication<sup>33</sup>. In addition, ontogeny of serotonin-immunoreactive cells and their changes during development were also monitored in the gut epithelium of the cloudy dogfish<sup>34</sup> and the turbot<sup>23</sup>. However, it is also reported that no serotonin-immunoreactive endocrine cells were found in the gut of the goldfish<sup>6</sup> and the arctic lamprey<sup>35</sup> but they were restricted within 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, well corresponded to that of goldfish<sup>6</sup> and the arctic lamprey<sup>35</sup> but quite differed from that of the spiny dogfish<sup>8</sup> and the rainbow trout<sup>18</sup>, no serotonin-immunoreactive cells were demonstrated in this study.

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 form<sup>36</sup>. 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 cells<sup>37</sup>. The appearance of open and close typed somatostatin-immunoreactive cells were well demonstrated in the entire gut except for large intestinal tract of *Anguilla anguilla*<sup>38</sup>, *Protopterus annectens*<sup>22</sup>, the southern-hemisphere lampreys<sup>17</sup>, the rainbow trout<sup>18</sup>, the lamprey<sup>16</sup> and the elasmobranchs<sup>8</sup>. In addition, ontogeny during developmental stages of somatostatin-immunoreactive cells was also monitored in the digestive tract of the turbot<sup>23</sup> and the arctic lamprey<sup>35</sup>. 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 lamprey<sup>35</sup>. However, it is also demonstrated that no somatostatin-immunoreactive cells were found in the gut of the grass carp<sup>10</sup>, the leaping grey mullet<sup>15</sup> and *Barbus conchoni*<sup>9</sup>. 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, similar to those of serotonin-immunoreactive cells. In the present study, somatostatin-immunoreactive cells were restricted to the most proximal segments, Segment I with rare frequency. These results were quite different from the results of that of the other species of the Pisces<sup>8-10,15-18,22,23,35,38</sup>.

In the Pisces, it is well documented that the distribution and frequency of glucagon-immunoreactive cells were variable in the intestinal cells with the species<sup>27</sup>. The appearance of these cells was detected in the proximal parts of the gut of *Protopterus annectens*<sup>22</sup>, the southern-hemisphere lampreys<sup>17</sup>, the grass carp<sup>10</sup>, the sea bream<sup>39</sup>, the rainbow trout<sup>18</sup>, the Atlantic hagfish<sup>40</sup>, the salmonids<sup>41</sup>, the arctic lamprey<sup>35</sup>, the cartilaginous fish<sup>13</sup> and *Barbus conchoni*<sup>9</sup>. In addition, the ontogeny during development of glucagon-immunoreactive cells was monitored in the digestive tract of the turbot<sup>23</sup> and the sea

bream<sup>39</sup>. However, it is also reported that no glucagon-immunoreactive cells were found in the gut of the goldfish<sup>6</sup>, and Tagliaferro *et al*<sup>22</sup> reported that no open typed glucagon-immunoreactive cells were detected in the intestinal epithelium of *Protopterus annectens*. In the present study, glucagon-immunoreactive cells were not demonstrated. These results are well corresponded to that of the goldfish<sup>6</sup>. However, the results are somewhat different from those of other teleost<sup>9,10,13,17,18,22,23,35,39-41</sup>.

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 granules<sup>42</sup>. In the Pisces, CCK-8- or gastrin/CCK-immunoreactive cells were detected throughout the whole intestinal tract of the tilapia, the goldfish<sup>21</sup>, the rainbow trout<sup>18</sup>, the Atlantic hagfish<sup>40</sup>, the leaping grey mullet<sup>15</sup>, the arctic lamprey<sup>35</sup>, the cod<sup>12</sup> and the elasmobranch<sup>8</sup>. In addition, ontogeny of CCK-8- and/or gastrin/CCK-immunoreactive cells were monitored in the gut of the Japanese flounder<sup>26</sup> and the turbot<sup>23</sup>. However, no CCK-8-immunoreactive cells were also demonstrated in the gut of the grass carp<sup>10</sup>. These distributions of CCK-8-immunoreactive cells in present study are well corresponded to previous reports except for that of the grass carp<sup>10</sup>.

PP-immunoreactive cells were generally located in the proximal parts of the gut of the Osteoglossomorpha<sup>27</sup>, the gar<sup>25</sup>, *Protopterus annectens*<sup>22</sup>, the goldfish<sup>6</sup>, the grass carp<sup>10</sup>, the rainbow trout<sup>18</sup>, the leaping grey mullet<sup>15</sup>, the arctic lamprey<sup>35</sup> and *Barbus conchoni*<sup>9</sup>, and similar to these previous reports, in the present study, they were found in the proximal segments, Segments I ~III. In addition, developmental changes of these PP-immunoreactive cells were well documented in the gut of the Japanese flounder<sup>26</sup>. However, it is also reported that no PP-immunoreactive cells were demonstrated in the intestinal tract of the turbot<sup>23</sup> and the lampreys<sup>16</sup>.

The immunoreactivity of chromogranin was well documented in the gastric and duodenal mucosa of fish<sup>43</sup> and secretin-immunoreactive cells were detected in the gut of the goldfish<sup>6</sup>. Appearance of VIP-immunoreactive endocrine cells was generally accepted in the gut of the silver carp, the goldfish, pumpkinseed sunfish<sup>24</sup>, the turbot<sup>23</sup>, the cartilaginous fish<sup>14</sup> and *Barbus conchoni*<sup>9</sup>. In addition, substance P-immunoreactive cells were also demonstrated in the gut of the turbot<sup>23</sup>, the goldfish<sup>6</sup>, the grass carp<sup>10</sup>, the tilapia<sup>21</sup>, the rainbow trout<sup>18</sup>, the

Atlantic hagfish<sup>40</sup>, the leaping grey mullet<sup>15</sup>, the Atlantic cod<sup>44</sup> and *Barbus conchoni*<sup>9</sup>. Until now, localization of bombesin-immunoreactive cells was well documented in the gut of the goldfish<sup>6</sup>, the tilapia<sup>21</sup>, the rainbow trout<sup>18</sup>, the Atlantic cod<sup>45</sup> and the elasmobranch<sup>8</sup>. However, quite different from these previous reports, no chromogranin-, secretin-, VIP-, substance P- and bombesin-immunoreactive cells were detected in the gut of the stomachless teleost, the Prussian carp, *Carassius auratus*, belonging to the family Cyprinidae in order Cypriniformes in the present study.

In conclusion, the distribution and relative frequency of 10 types of immunoreactive cells observed in this study, are well corresponded to the previous report in stomachless teleost but somewhat peculiar patterns are also demonstrated.

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## 붕어(*Carassius auratus*) 장 내분비세포에 관한 면역조직화학적 연구

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**국문초록** : 붕어 (*Carassius auratus* Linnaeus)의 장에 존재하는 소화관 내분비세포의 부위별 분포 및 출현 빈도를 포유류의 peptide에 대한 10종류의 항혈청을 사용하여 면역조직화학적 방법으로 관찰하였다.

붕어의 장은 근위부에서부터 원위부까지 5 등분했으며 (Segments I ~ V), 대부분의 면역반응세포들은 상피부분의 상피세포들 사이 공간에서 관찰되었으며, 장 내강까지 신장되어 있는 긴 세포질돌기를 함유한 방추형의 개방형 세포 (open type cell)들이 주로 관찰되었으나, 세포질돌기 없이 원형 또는 타원형의 형태를 나타내는 폐쇄형 세포 (close type cell)들 역시 소수 관찰되었다. 본 실험에서는 somatostatin, cholecystokinin (CCK)-8 및 pancreatic polypeptide (PP) 면역반응세포들이 관찰되었으나, serotonin, glucagon, chromogranin A, secretin, vasoactive intestinal peptide (VIP), substance P 및 bombesin 면역반응세포들은 관찰되지 않았다. 한편 somatostatin 면역반응세포들은 장의 가장 근위부인 Segment I 에 국한되어 극소수 관찰되었으며, CCK-8 면역반응세포들은 장의 근위부인 Segment I 과 II 에서 소수 또는 극소수의 빈도로 관찰되었다. PP 면역반응세포들 역시 장의 근위부에서 중간 부위인 Segment I 에서부터 III 에 걸쳐 중등도 또는 극소수의 빈도로 출현하였다.

이상에서 붕어 위 내분비세포들의 부위별 분포 및 출현 빈도는 다른 위가 없는 경골어류 (stomachless teleost)에서의 보고들과 유사하였으나, 독특한 분포를 나타내는 면역반응세포들 역시 관찰되었다.