

## Endocrine Cells in the Pyloric Region of the Black-tailed Gull (*Larus crassirostris*)

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ウミネコの幽門領域における内分泌細胞について

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### Introduction

Recently, LARSSON *et al.* (1974) revealed that in the chicken a narrow zone connecting the gizzard with the duodenum contained a large number of cells showing gastrin immunoreactivity and proposed the name antrum for this zone. In the chicken and quail, the gizzard-duodenal junction, which we call the pyloric region, has been reported to contain more endocrine cells than any other parts of the gastrointestinal tract (LARSSON *et al.*, 1974; SUNDLER *et al.*, 1977; ALUMETS *et al.*, 1977; ALUMETS *et al.*, 1977; YAMADA *et al.*, 1978, 1979). Most of the investigations on gut endocrine cells of birds have been restricted to the domestic birds of *Phasianidae*. Besides a few species of *Phasianidae*, GABE (1972) examined the pyloric region of the duck, pigeon, crow, siskin and starling, and OKAMOTO *et al.* (1976) the pyloric region of the duck, but their findings were rather insufficient.

This paper reports the occurrence and light and electron microscopic features of endocrine cells in the pyloric region of the black-tailed gull. Special reference is given to the occurrence of gastrin immunoreactive cells in this region. One of the reasons for selecting this bird of *Laridae* as the material of the present study is that it usually eats fishes and insects in contrast against the birds of *Phasianidae* which eat seeds and grains.

### Materials and Methods

Fifteen gulls of either sex weighing 500-670 g were used. For light microscopy, the

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pyloric region was fixed in BOUIN's fluid or neutral buffered formalin and embedded in paraffin. Sections, 3-5 $\mu$  thick, were treated with the following methods known to stain gut endocrine cells: (a) MASSON-HAMPERL's argentaffin reaction (SINGH, 1964), (b) GRIMELIUS' silver impregnation (GRIMELIUS, 1968), (c) HELLERSTRÖM-HELLMAN's silver impregnation (HELLERSTRÖM and HELLMAN, 1960), (d) SEVIER-MUNGER's silver impregnation (SEVIER and MUNGER, 1965), (e) lead-hematoxylin staining (SOLCIA *et al.*, 1969).

For electron microscopy, small tissue blocks were dissected from the pyloric region and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.3. Specimens were postfixed in 1% osmium tetroxide in the same buffer, dehydrated in graded ethanol solutions and embedded in Epon-Araldite mixture or Spurr resin. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined in a JEM-7 type electron microscope.

Some paraffin sections of the BOUIN-fixed tissue were processed for the immunohistochemical demonstration of gastrin. The gastrin-immunoreactive cells were stained with the peroxidase-labelled antibody method according to KAWARAI and NAKANE (1975). The gastrin antiserum (GP-1302) was donated by Dr. N. YANAIHARA, Shizuoka College of Pharmacy, Shizuoka, Japan, and used in dilution 1:40. Controls were run as recommended by STERNBERGER (1974).

### Results

The pyloric region of the gull is a very short canal (only 2 mm in length) between the gizzard and the duodenum. The pyloric region comprises concentration of tubular glands resembling those of the gizzard and is not easily distinguished from the gizzard. From the duodenum, on the other hand, it is clearly discerned by the absence of the intestinal villi.

A number of endocrine cells reactive to GRIMELIUS', HELLERSTRÖM-HELLMAN's, SEVIER-MUNGER's silver impregnation and lead-hematoxylin staining were found predominantly in the lower half of the glands (Figs. 1-4). The cells positive to HELLERSTRÖM-HELLMAN's method were half as many as the cells positive to other three staining methods. By comparing adjacent and alternately stained serial sections, it was shown that all the cells stained by GRIMELIUS' method were also reactive to SEVIER-MUNGER's method (Fig. 5). Endocrine cells were numerous in the part of the pyloric region close to the duodenum, but towards the gizzard the number of cells gradually decreased; the gizzard contained none of the endocrine elements. The cells positive to these staining methods occurred only in the epithelium, and could not be detected in the lamina propria, submucosa, Meissner's and Auerbach's plexuses. No cell reacting to MASSON-HAMPERL's argentaffin reaction was found in the pyloric region.

Numerous cells showing gastrin immunoreactivity were found in the epithelium of the pyloric region and predominated in the basal half of the glands (Fig. 6).

In the electron microscope, the mucosa of the pyloric region was found to contain a large number of endocrine cells, characterized by the presence of numerous basal granules of high electron density. On the basis of size and morphology of their secretory granules, the endocrine cells were classified into four types, namely, type I, II, III and IV. In every cell type, the cell base directly faced the basement membrane of the epithelium. The narrowed apical cytoplasm could be found to reach the glandular lumen (open type), except for type III cells which were few in number and difficult to be decided whether they were open to the lumen. The apical end of the endocrine cells of type I, II and IV was covered by microvilli. Sometimes, a cilium was found between them.

The nucleus of the endocrine cell was generally round or oval in shape and had a prominent nucleolus. Provided with only a small amount of chromatin granules, it showed a much clearer appearance than the nucleus of the adjacent exocrine cells. A bundle of filaments, intranuclear rodlet was frequently found in the nucleus (Fig. 12). A Golgi apparatus with well-developed lamellar and vesicular elements occurred in the supra- and paranuclear regions. Small, probably immature granules often were found in and around the Golgi apparatus.

The fine-structural characteristics of endocrine granules in the four different cell types will be described below.

Type I cell (Figs. 7, 8) contained numerous round granules, measuring 200–450 nm in diameter and displaying a varying, often low, electron density and a fine-granulated texture. The limiting membrane was tightly applied to the granule core. Type II cell (Figs. 9, 10) was characterized by round and electron-dense granules having an electron-lucent zone between the membrane and the core. Their granules measured 250–450 nm in diameter. Type III cell (Figs. 11, 12) contained small, membrane-bounded and electron-dense granules of 100–180 nm in diameter. They were mostly round in shape; occasionally pleomorphic granules were intermingled. Type IV cell (Figs. 13, 14) contained homogenous, uniformly electron-dense granules of 350–450 nm in diameter, which were round or slightly angular in shape.

In the above mentioned four types of endocrine cells, type I, II and IV cells were found numerous, whereas the occurrence of the type III cells was very low.

### Discussion

The black-tailed gull is a species of *Laridae*. The most frequent food is fishes and insects (LIVINENKO, 1975), while the chicken and quail usually eat grains and seeds. According to SIBLEY (1960), the gull is located lower than chicken or quail in the phylogenetic system of birds and rather near to ostrich and penguin.

The black-tailed gull has the pyloric region showing a high concentration of endocrine cells reactive to some staining methods as well as other birds reported.

GABE (1972) reported endocrine cells which were stained by lead-hematoxylin, HCl-

toluidine blue, GRIMELIUS' or HELLERSTRÖM-HELLMAN's silver impregnation in the pyloric glands of eight species of birds. In the duck pyloric region, OKAMOTO *et al.* (1976) described that cells stained with lead-hematoxylin or HCl-toluidine blue were more concentrated than in any other regions of the digestive tracts, although, there were no cell stained with MASSON-HAMPERL's and SEVIER-MUNGER's method. In addition, YAMADA *et al.* (1978) found in the quail a few cells reacting to SEVIER-MUNGER's method in the upper portion of the pyloric glands. The occurrence of numerous cells reactive to SEVIER-MUNGER's method seems to be characteristic of the gull pyloric region. As in other birds previously reported, enterochromaffin cells reactive to MASSON-HAMPERL's argentaffin reaction were not found in the pyloric region of the gull.

LARSSON *et al.* (1974) and YAMADA *et al.* (1979) demonstrated a number of gastrin cells in the pyloric region of the chicken and quail, respectively. Their findings suggest that the pyloric region of birds is analogous to the antrum of mammals, playing a leading role in the endocrine control of digestive functions. The gull also contained numerous cells showing gastrin immunoreactivity in this region. In addition to gastrin cells, somatostatin cells (ALUMETS *et al.*, 1977) and neurotensin cells (SUNDLER *et al.*, 1977) have been reported to occur abundantly in the chicken antrum.

By electron microscopy, LARSSON *et al.* (1974) distinguished three types of endocrine cells in the chicken antrum. The first type cell contained round, homogenous and uniformly electron-dense granules of 300–400 nm in diameter, with the membrane closely attached to the dense core. The second type contained somewhat smaller granules of varying electron density, measuring around 200 nm in diameter and having an electron-lucent zone between the membrane and the dense core. The third type cell contained small, membrane-bounded and electron-dense granules of about 100 nm in diameter. Endocrine cells resembling the three cell types in the chicken antrum were found in the pyloric region of the gull. We named them I, II and III type cells according to the classification in the chicken, though the difference in the granular size was recognized in some degree.

The I, II and III type cells of the gull correspond to I, II and III type cells in the pyloric region of the quail, respectively (YAMADA *et al.*, 1978). Type IV cell of the gull, however, seems to be a new cell type which has not been detected in the pyloric region of any birds. Its ultrastructural appearance is similar to that of L (EG) cell or X cell in the mammalian gut (SOLCIA *et al.*, 1975).

The first cell type in the chicken antrum was shown to secrete somatostatin (ALUMETS *et al.*, 1977). Ultrastructure of type I cell in the gull was identical with that of somatostatin cell in the chicken, and the pyloric region of the gull contained numerous endocrine cells stained by HELLERSTRÖM-HELLMAN's silver impregnation. For these reasons, it seems reasonable to suggest that the type I cell of the gull might produce somatostatin.

The second type cell in the chicken antrum was revealed by LARSSON *et al.* (1974) to be the source of gastrin. These authors demonstrated that gastrin granules of chicken

had homogenous, electron-dense core in contrast to the flocculent, less electron-dense core of the mammalian G-cell. This remarkable difference between mammal and bird in the ultrastructure of the gastrin granules was confirmed in several species of birds (YAMADA *et al.*, 1980).

The function of type III cells (the third cells in the chicken) which were rarely seen in every bird, is unknown.

The intranuclear rodlets observed in the present study apparently are identical in nature with those reported in B cell of the mouse pancreas (BOQUIST, 1969) and the enterochromaffin cells (EC cell) of the rabbit stomach (MÜLLER and RATZENHOFER, 1971). A detailed investigation of the rodlets is now in progress.

In this study, a few differences in the types and distribution of endocrine cells were found between the black-tailed gull and the chicken or quail. They are the presence of numerous endocrine cells reacting to SEVIER-MUNGER's silver impregnation and the occurrence of type IV cells. The interpretation of these differences, as to whether they may be due to the phylogenetically distant relation of the species or to different feeding habits of the birds, waits for further investigations.

### Summary

Endocrine cells in the pyloric region of the black-tailed gull were studied by light and electron microscopy. Gastrin cells were demonstrated immunohistochemically.

The pyloric region, measuring only 2 mm in length, between the gizzard and the duodenum contained numerous endocrine cells of open type, mostly in the lower portion of the tubular pyloric glands. The major part of the endocrine cells were demonstrated by either HELLERSTRÖM-HELLMAN's GRIMELIUS', SEVIER-MUNGER's or lead-hematoxylin staining. In addition, a number of cells showing gastrin immunoreactivity were found.

Ultrastructurally, four types of endocrine cells were identified on the basis of size and morphology of their endocrine granules.

The black-tailed gull differed from the chicken and some birds examined previously in that numerous endocrine cells reacted to SEVIER-MUNGER's silver impregnation, and that type IV cells, new type cells occurred in the pyloric region.

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### 摘 要

ウミネコの幽門領域を光学および電子顕微鏡で観察し、さらにガストリン免疫反応細胞について検索し、次の所見を得た。

- 1) 幽門領域は、幅約 2 mm の輪状帯として、筋胃と十二指腸の間に認められた。
- 2) 本領域には、ガストリン細胞を含む数種の内分泌細胞が高密度に集合して認められた。これらの細胞はすべて銀好性で、銀親和性細胞は認められなかった。SEVIER-MUNGER 法陽性細胞が多数存在していた点が他の鳥と比較して特徴的であった。
- 3) 本領域に認められた内分泌細胞は、果粒の形状から、4種に型別された。直径 350~450 nm で、高電子密度の均質な果粒を含む IV 型細胞は、鳥類ではウミネコで初めて観察された細胞である。
- 4) この領域のほとんどの内分泌細胞は、開放型であった。

## Explanation of Plates

## Plate I

- Micrographs showing the endocrine cells in the pyloric region of the black-tailed gull.
- Fig. 1. Numerous endocrine cells stain with GRIMELIUS' silver impregnation. BOUIN fixation. X 300
- Fig. 2. SEVIER-MUNGER's silver impregnation. The reactive cells show similar frequency to GRIMELIUS-reactive cells. BOUIN fixation. X 300
- Fig. 3. HELLERSTRÖM-HELLMAN's silver impregnation. The reactive cells occur half so many as GRIMELIUS-reactive cells. BOUIN fixation. X 300
- Fig. 4. Lead-hematoxylin method. Numerous cells stain also with this staining. Formalin fixation. X 300
- Fig. 5. Micrographs a and b are from adjacent sections. In a the section is stained with GRIMELIUS' silver impregnation, while b is with SEVIER-MUNGER's silver impregnation. All the GRIMELIUS-positive cells are reactive to SEVIER-MUNGER's method. BOUIN fixation. X 500
- Fig. 6. The gastrin-immunoreactive cells. Peroxidase-labelled antibody method. BOUIN fixation. X 600

## Plate II

- Fig. 7. Two type I cells found in the pyloric region of the gull. Many endocrine granules gather in the infra-nuclear portion of the cytoplasm. X 7,400
- Fig. 8. High magnification of Fig. 7. The granules of this type cell display a varying electron density and a finegranulated texture. X 24,000
- Fig. 9. Type II cell found in the pyloric region of the gull. X 6,600
- Fig. 10. High magnification of Fig. 9. This type cell contains the granules showing a clear space between the electron-dense core and limiting membrane. X 24,000

## Plate III

- Fig. 11. Type III cell in the pyloric region of the gull. X 9,600
- Fig. 12. High magnification of Fig. 11. This type cell contains small cored granules. A small bundle of the filaments, intranuclear rodlet is seen (arrow). X 24,000
- Fig. 13. A type I cell and a type IV cell in the pyloric region of the gull. Note the difference of the granule density. X 6,000
- Fig. 14. High magnification of the type IV cell in Fig. 13. This cell contains homogenous, electron-dense granules. 24,000

Plate I

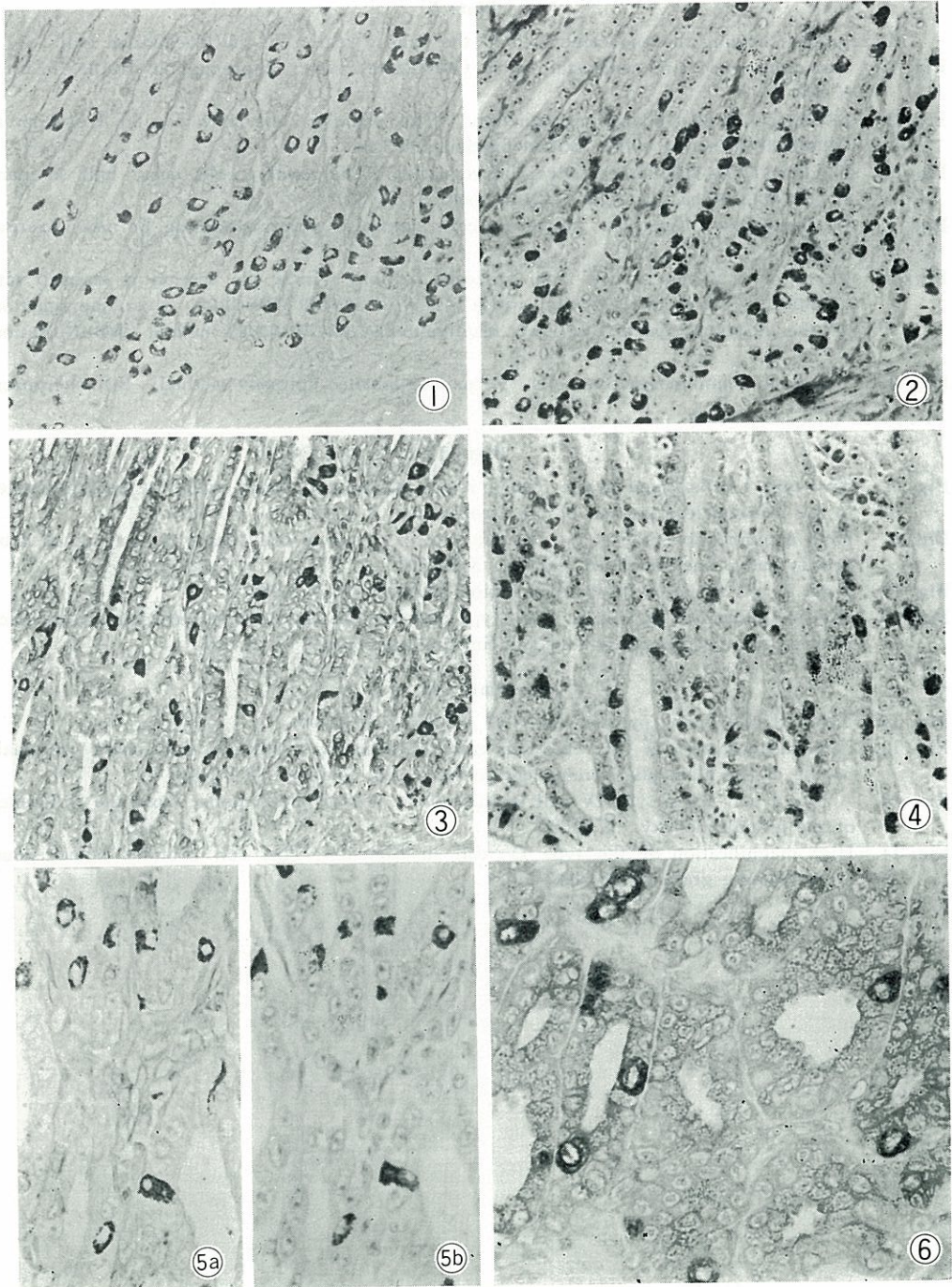




Plate II

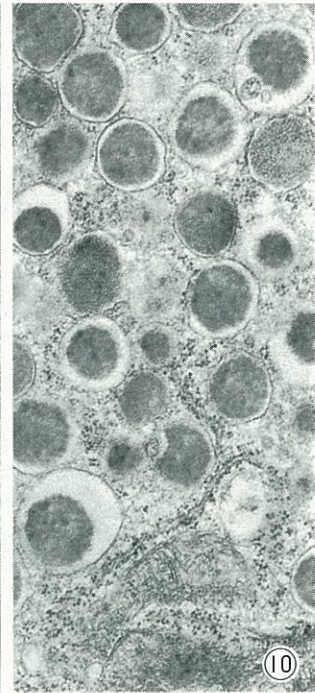
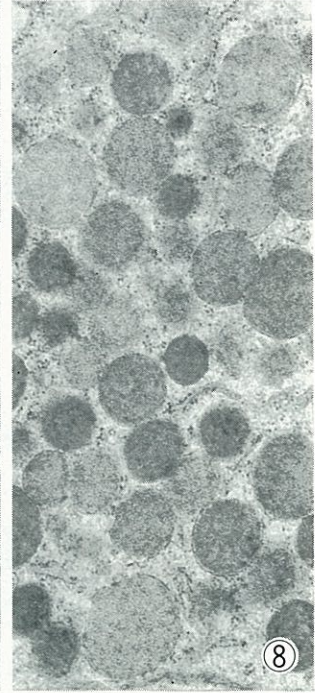
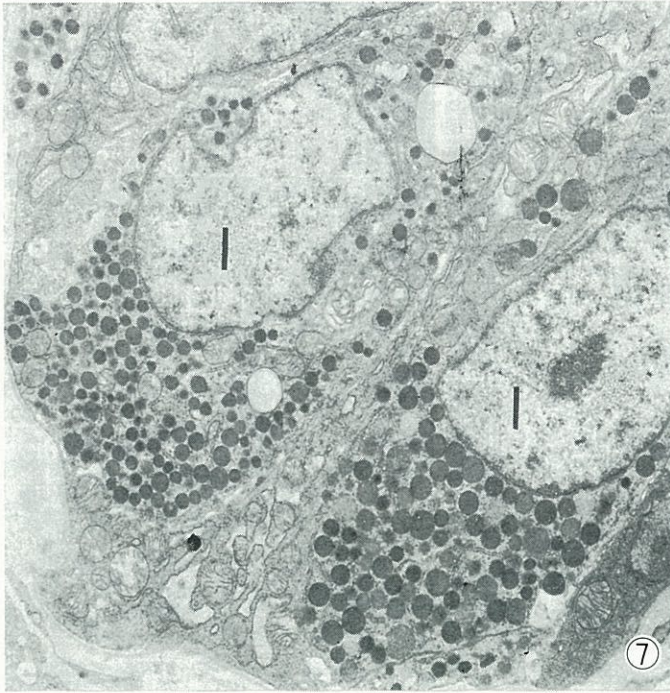


Plate III

