An Electron Microscopic Study on Endocrine Cells in the Pyloric Region of the Duck

Toshikazu OKAMOTO and Shunsaku FUJII

*Department of Animal Husbandry, Faculty of Applied Biological Science,
Hiroshima University, Fukuyama-shi, Hiroshima 720*

(Received for publication June 22, 1979)

Abstract. The endocrine cells of the pyloric region of the duck were observed by light and electron microscopy. This region was about 4 mm in width in its circular zone between the gizzard and duodenum. Many endocrine cells were present in it. They were represented by argyrophil cells. At least five types, I to V, of endocrine cells were identified by the electron microscopical morphology of granules in the cytoplasm. Type I cells were characterized by the presence of round and medium-sized granules (250–400 nm). Many of them, looked empty or contained a vague, cloud-like or spot-like core, and a few were dark and seemed to be solid. Type II cells were distinguished by the existence of small, round granules (100–200 nm) which possessed a dense core and a “halo”. Type III cells had small (100–300 nm in shorter diameter), characteristically polymorphous granules. Type IV cells contained rather large (200–400 nm in shorter diameter) polymorphous granules of moderate to high electron density. Type V cells were characterized by the presence of large round (200–500 nm) granules with no halo. The cells of all the five types of cells were identified as open-type cells. Type I cells were found at a frequency higher than 70% of 1,170 endocrine cells examined. Type IV cells were seen at a frequency of about 16%. Cells of any other type scarcely appeared. Endocrine cells in the pyloric region were compared between the duck and the chicken or quail.

The gastrointestinal endocrine cells of many mammals have been studied histochemically and electron microscopically in numerous papers. Those of birds, however, have been examined electron microscopically in only a few reports.

Recently, it has been clarified that the gizzard-duodenal junction or pyloric region of the domestic birds is a portion where endocrine cells are mostly condensed [9–11, 23, 24]. In this portion of the chicken and the quail, three and five types of endocrine cells, respectively, were identified by electron microscopic observation [10, 23]. Also in the duck, the frequency of appearance of endocrine cells was the highest in the pyloric region of all the regions of the digestive tract [11].

The present paper intends to describe some silver staining properties and ultrastructures of the endocrine cells in the pyloric region of the duck.

Materials and Methods

Six white Pekin ducks were used as materials. For light microscopy, the pyloric region was cut off widely from the gizzard and the duodenum. Samples were fixed in Bouin's fluid. Paraffin sections were subjected to the following methods known to stain endocrine cells: Masson's argentaffin reaction [16], Sevier-Munger's silver method [15], Grimelius' silver method [6], and Davenport's silver method [7].

For scanning electron microscopy, samples from the above-mentioned region were washed with buffered physiological saline, fixed in 2.5% glutaraldehyde and 1% OsO₄, dehydrated in ethanol series, and dried by a critical-point dryer. After drying, sputter-coating with gold was carried out. The superficial structure of the pyloric region was ob-

served under a JSM 35 and a Hitachi S-410 scanning electron microscope.

For transmission electron microscopy, small pieces of the pyloric region were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3), post fixed in 1% OsO₄ in the same buffer, and embedded in an Epon-Araldite mixture. Ultrathin sections were stained with uranyl acetate and lead citrate, and observed under a JEM 7 and an HU 12A electron microscope.

Results

Limitation of the pyloric region

The pyloric region of the duck is a narrow circular band (about 4 mm in width) between the central part of the gizzard and the duodenum (Fig. 1). This region is distinguished from the central part of the gizzard by the absence of the horny layer and thin musculature. It is also characterized by the absence of intestinal villi in contrast with the duodenum (Fig. 1).

Light microscopic observations of endocrine cells

Endocrine cells stained by Davenport's, Sevier-Munger's, or Grimelius' silver method were numerous in the epithelium of the pyloric region (Fig. 2). There were, however, no cells stained by Masson's argentaffin reaction.

Electron microscopic observation of endocrine cells

Electron microscopically, endocrine cells were numerous in the pyloric region, as shown in Fig. 5. Endocrine cells were mainly pyramidal or barrel-like in shape and connected with the neighboring exocrine cells of the pyloric glands by the terminal bar. The basal part of endocrine cells directly faced the basement membrane, while the narrowed apical cytoplasm reached the glandular lumen. The apical end of the endocrine cell was covered with more or less numerous microvilli.

The nucleus of the endocrine cell was generally round or oval in shape and had a prominent nucleolus. The rough-surfaced endoplasmic reticulum was relatively poor in development. Free ribosomes were scattered all over the cytoplasm. Mitochondria were more abundant in the supra-nuclear than in the infra-nuclear cytoplasm. Golgi complexes with well-developed lamellar and vesicular elements occurred mainly in the supra-nuclear portion. Their vesicular elements often enclosed small dense granules identical with immature specific granules.

The specific granules were generally gathered in the infra-nuclear portion of the cytoplasm. Some of them were often found in the supra- and para-nuclear cytoplasm. The endocrine granules were highly variable in size and in internal structure. In the present observation the figure of the releasing process of these granules was not found.

Five different types of endocrine cells were defined by the size and internal structure of endocrine granules contained. Characteristics of the fine structure of each cell type are described below.

Type I cells (Figs. 3, 5): These cells were characterized by the presence of many granules. The limiting membrane of these granules was a round sac which looked empty or contained a vague, cloud-like or spot-like core. These granules were 250–400 nm in diameter. A few of them were dark and solid-looking and had a limiting membrane which was separated from the core substance by a narrow and often hardly recognizable space. Sometimes, rather small solid-looking granules were found in the Golgi apparatus.

Type II cells (Fig. 4): These cells contained granules the smallest in size of all the five types of endocrine cells. The

granules were mostly round in shape and measured 100–250 nm in diameter. They showed high electron density with a small variation. They contained a round, dense core separated from the limiting membrane by a narrow space or a “halo”. Frequently, a large lipid droplet was visible in the cytoplasm.

Type III cells (Fig. 5): These cells were characterized by the presence of small, polymorphous granules. These granules were round, oval or disk-like in shape and measured 100–300 nm in shorter diameter. They showed moderate to rather high electron density.

Type IV cells (Figs. 5, 6): These cells were characterized by the presence of polymorphous granules which were larger than those of type III cells and which showed moderate to high electron density. The profiles of the endocrine granules were round, oval, disk-like or club-like in shape. These granules were 200 to 400 nm in shorter diameter, presenting remarkable polymorphism. They had a limiting membrane separated from the core substance by a narrow space (Fig. 6).

Type V cells (Fig. 7): These cells were characterized by the presence of rather large, round granules which were 200 to 500 nm in diameter. These granules showed moderate to high electron density, but rarely had a recognizable “halo” as compared with those of type IV cells. No typical “open type” cells could be found among type V cells at this time of writing. The terminal bar was observed in these cells.

Cells of all the types were found mainly in the lower half portion of the pyloric gland. A few cells of all the types except type III, were noticed in the apical portion of the pyloric region characterized by the presence of exocrine secretion. Cells and

Table 1. Observed number of endocrine cells of each type in the duck pyloric region

	Cell type					Total
	I	II	III	IV	V	
Number	835	77	39	192	27	1170
%	71.4	6.6	3.3	16.4	2.3	100

their mitochondria were larger in the upper half than in the lower half portion.

A total of 1,170 endocrine cells were examined and classified into the five types mentioned above. The cell population of each type is indicated in Table 1. Type I cells occupied more than 70% of the cells forming the duck pyloric region and type IV cells about 16%. Few cells of any other type were present.

Discussion

Okamoto et al. [11] studied the distribution of endocrine cells in the whole digestive tract of the duck and described that the frequency of appearance of endocrine cells was the highest in the pyloric region. They found no cells positive for Sevier-Munger's silver staining, although these cells were recognized in this study. This discrepancy may have been derived from the difference in fixation.

The number of types of gastrointestinal endocrine cells amounted to ten in man [4, 14], five in the cat [21], six in the rabbit [5], and eight in the pig [3] when determined by electron microscopy and some histological methods. According to the revised Wiesbaden classification [18], the gastrointestinal and pancreatic endocrine cells of mammals are classified into ten types: EC, ECL, A(A-like), D, D₁, G, EG(L), S, I and B. Later Solcia et al. [17] proposed that K and X cells should be added to this classification. Furthermore, Capella et al. [4] advocated that F and H cells should be listed in the classification

of cells of the human intestine.

As for the domestic fowls, Toner [20] identified only one type of endocrine cells in the proventriculus and the gizzard of the chicken, Kataoka [8] two types in the finch proventriculus, Larsson et al. [10] three types in the gizzard-duodenal junction of the chicken, Andrew [1, 2] seven types in the proventriculus, pyloric antrum, and intestine of the chick around the time of hatching, and Yamada et al. [23] five types in the pyloric region of the quail. The present study demonstrated five types of endocrine cells only in the pyloric region of the duck. It remains to be elucidated in future what and how many types other than the five of gastrointestinal endocrine cells are distributed in the other gut regions and intestine of the duck.

On the basis of the size and morphology of granules contained, the endocrine cells of the duck pyloric region were classified into five types in the present study. Types I, II, III, IV and V more or less closely resembled the human G, D₁, S, D and L cells, respectively [4, 14]. The type IV cell of the duck also resembled the mammalian EC cell, particularly because it contained polymorphous granules. Nevertheless, it was not identical with the mammalian EC cell, since argentaffin cells exist only in the intestine [11]. In this study, the type IV cell of the duck did not respond to Mason's reaction.

In the chicken, Polak et al. [12] demonstrated gastrin immunoreactive cells immunohistochemically in the gizzard and small intestine, while Larsson et al. [9, 10] showed them in the gizzard-duodenal junction and small intestine. Furthermore, Larsson et al. [10] presumed that the second type cell in the chicken gizzard-duodenal junction might be a gastrin-producing cell from an immunohistochemical point of

view. That cell contained rather small granules, about 200 nm in diameter, varying in electron density and having an electron-lucent zone between the membrane and the dense core. Yamada et al. [22, 23] elucidated by a consecutive semithin-ultrathin section technique that the type II cell of the quail pyloric region was a gastrin producing cell. That cell was characterized by the presence of round and medium-sized (250–350 nm) granules possessing a dense core and a clear halo.

The type I cell of the duck pyloric region analyzed in the present study closely resembled the mammalian G cell [5, 13, 19]. It did not so closely resembled the second type cell in the chicken gizzard-duodenal junction or the type II cell in the quail pyloric region. These chicken and quail cells were identified as gastrin-producing cells. If enzyme-labeled antibodies are used at an ultrastructural level in investigation, it will be more directly determined whether type I cells of the duck pyloric region are gastrin-producing cells or not.

References

- [1] Andrew, A. (1976). Intestinal endocrine cells of chicks around the time of hatching. *Cell Tissue Res.* **172**, 541–551.
- [2] Andrew, A. (1976). Endocrine cells of the stomach of chicks around the time of hatching. *Cell Tissue Res.* **172**, 553–561.
- [3] Capella, C., and Solcia, E. (1972). The endocrine cells of the pig gastrointestinal mucosa and pancreas. *Arch. Histol. Jpn.* **35**, 1–29.
- [4] Capella, C., Solcia, E., Frigerio, B., and Buffa, R. (1976). Endocrine cells of the human intestine. An ultrastructural study. In *Endocrine Gut and Pancreas*, Fujita, T., editor, Elsevier, Amsterdam, 43–59.
- [5] Capella, C., Solcia, E., and Vassallo, G. (1969). Identification of six types of endocrine cells in the gastrointestinal mucosa of the rabbit. *Arch. Histol. Jpn.* **30**, 479–395.
- [6] Grimelius, L. (1968). A silver nitrate stain for α_2 cell in human pancreatic islets. *Acta Soc. Med. Upsala* **73**, 243–270.

- [7] Hellerström, C., and Hellman, B. (1960). Some aspects of silver impregnation of the islets of Langerhans in the rat. *Acta Endocrinol.* **35**, 518-532.
- [8] Kataoka, K. (1974). An electron microscope study on a neuroendocrine complex in the pro-ventricular mucosa of the finch, *Uroloncha striata* var. *domestica*. *Ach. Histol. Jpn.* **36**, 391-400.
- [9] Larsson, L.-I., Sundler, F., Håkanson, R. (1976). Pancreatic hormones in the gut and gut hormones in the pancreas. In *Endocrine Gut and Pancreas*, Fujita, T., editor, Elsevier, Amsterdam, 133-143.
- [10] Larsson, L.-I., Sundler, F., Håkanson, R., Rehfeld, J. F., and Stadil, F. (1974). Distribution and properties of gastrin cells in the gastrointestinal tract of chicken. *Cell Tissue Res.* **154**, 409-421.
- [11] Okamoto, T., Sugimura, M., and Kudo, N. (1976). Distribution of endocrine cells in duck digestive tracts. *J. Fac. Fish. Anim. Husb., Hiroshima Univ.* **15**, 127-134.
- [12] Polak, J. M., Pearse, A. G. E., Adams, C., and Ceraud, J.-C. (1974). Immunohistochemical and ultrastructural studies on the endocrine polypeptide (APUD) cells of the avian gastrointestinal tract. *Experientia* **30**, 564-567.
- [13] Sasagawa, T., Kobayashi, S., and Fujita, T. (1970). The endocrine cells in the human pyloric antrum. An electron microscope study of biopsy materials. *Arch. Histol. Jpn.* **32**, 275-288.
- [14] Sasagawa, T., Kobayashi, S., and Fujita, T. (1973). Electron microscope studies on the endocrine cells of the human gut and pancreas. In *Gastro-entero-pancreatic Endocrine System. A Cell-biological Approach*, Fujita, T., editor, Igaku Shoin, Tokyo, 17-38.
- [15] Sevier, A. C., and Munger, B. L. (1965). A silver method for paraffin section of neural tissue. *J. Neuropathol. Exp. Neurol.* **24**, 130-135.
- [16] Singh, I. (1964). A modification of the Masson-Hamperl method for staining of argentaffin cells. *Anat. Anz.* **115**, 81-82.
- [17] Solcia, E., Capella, C., Vassallo, G., and Buffa, R. (1975). Endocrine cells of the gastric mucosa. *Int. Rev. Cytol.* **42**, 223-286.
- [18] Solcia, E., Pearse, A. G. E., Grube, D., Kobayashi, S., Bussolati, G., Creutzfeldt, W., and Gepts, W. (1973). Revised Wiesbaden classification of gut endocrine cells. *Rend. Gastroenterol.* **5**, 13-16.
- [19] Solcia, E., Vassallo, G., and Capella, C. (1969). Studies on the G cell of the pyloric mucosa, the probable site of gastrin secretion. *Gut* **10**, 379-388.
- [20] Toner, R. G. (1964). Fine structure of argyrophil and argentaffin cells in the gastrointestinal tract of the fowl. *Z. Zellforsch. Mikrosk. Anat.* **63**, 830-839.
- [21] Vassallo, G., Solcia, E., and Capella, C. (1969). Light and electron microscopic identification of several types of endocrine cells in the gastrointestinal mucosa of the cat. *Z. Zellforsch. Mikrosk. Anat.* **98**, 333-356.
- [22] Yamada, J., Iwanaga, T., Okamoto, T., Yamashita, T., Misu, M., and Yanaihara, N. (1980). Ultrastructure of avian gastrin cells. *Arch. Histol. Jpn.* **43** (in press).
- [23] Yamada, J., Kayamori, T., Okamoto, T., Yamashita, T., and Misu, M. (1978). Endocrine cells in the pyloric region of the Japanese quail (*Coturnix coturnix japonica*). *Arch. Histol. Jpn.* **41**, 41-52.
- [24] Yamada, J., Yoshino, M., Yamashita, T., Misu, M., and Yanaihara, N. (1979). Frequency and distribution of gastrin cells in digestive tract of the Japanese quail. *Arch. Histol. Jpn.* **42**, 33-39.

要 約

アヒル幽門部における内分泌細胞の電顕的研究：岡本敏一・藤井俊策（広島大学生物生産学部畜産科学講座）——アヒルの幽門部における内分泌細胞を光学および電子顕微鏡で観察し、次の所見を得た。

1) 幽門部は筋胃の十二指腸に連なる部位に幅約 4 mm の輪状帯として認められた。2) 本部位には多数の内分泌細胞が認められ、これらのすべては好銀性を示した。3) これらの内分泌細胞を電顕観察による顆粒の形状から、次の5種に型別した。I型：直径 250~400 nm の球型で、内容の抜けたような、あるいは雲状や点状の芯を持つ顆粒と、電子密度の高い内容の充実した顆粒を持つもの。II型：径 100~200 nm の小型球型で有芯の顆粒を持つもの。III型：短径 200~300 nm の小型多型性で有芯の顆粒を持つもの。IV型：短径 200~400 nm と比較的大型で多形性の有芯顆粒を持つもの。V型：径 200~500 nm の大型球型で、明調帯のほとんどみられない顆粒を持つもの。4) これら5型の細胞はすべて開放型であると判断した。5) 型別の判別できた細胞 1170 個における各型細胞の出現頻度は、I型が最も高くその 70% 以上を占め、次にIV型が約 16% と高く、他の型は低頻度であった。6) これらアヒル幽門部のそれら、および哺乳類消化管のそれらとの形態的異同について論じた。

Explanation of Figures

Fig. 1. Scanning electron micrograph showing the gizzard-duodenal junction. Pyloric region (P) is easily distinguished from the gizzard (G) and the duodenum (D) by its surface structures. $\times 18$.

Fig. 2, A-C. Photomicrographs of the mucosa of the pyloric region. Five or eight cells stained by Davenport's (A), Grimelius' (B), or Sevier-Munger's (C) silver method are observed. $\times 450$.

Fig. 3. Type I cell (I). The type I cell is open to the lumen. The apical cytoplasm of this cell is narrowed at the site of the terminal bar (arrow). The apical end is covered with numerous microvilli. $\times 6,000$. Insert: High power magnification of a part of another type I cell. Granules look empty or solid and are vague, cloud-like or spot-like with a core. $\times 20,000$.

Fig. 4. Type II cell (II). Type II cell is open to the lumen (arrow). $\times 6,000$. Insert: High power

magnification of a part of the type II cell. Small cored granules are visible. $\times 20,000$.

Fig. 5. Two type I cells (I), a type II cell (III) and two type IV cells (IV). Compare the granular size and shape among these cells. $\times 6,000$. Insert: High power magnification of a part of the type III cell. Small, rather polymorphous granules are visible. $\times 20,000$.

Fig. 6. Type IV cell (IV). Type IV cell is of open type. The apical end of this cell projects into the lumen. $\times 6,000$. Insert: High power magnification of a part of another type IV cell. Large polymorphous granules are visible. $\times 20,000$.

Fig. 7. Type V cell (V) in the upper part of the pyloric gland. $\times 6,000$. Insert: High power magnification of a part of another type V cell. Large round granules with a hardly recognizable "halo" are visible. $\times 20,000$.