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# The Ultrastructure of the Gastro-endocrine Cells in the Gastric Mucosa of the Frog, *Rana rugosa*

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**The gastro-endocrine cells were examined in the fundic region of stomach of the frog, *Rana rugosa*, by transmission electron microscope. In the present paper, at least three kinds of cell type distributed in this region were identified according to their morphological characteristics based on the size, shape and electron density of the secretory granules.**

**Type I cells were characterized by the presence of round or oval granules (300-500nm in diameter) with high electron density. The granules showed a wide lucent between the contents and the limiting membrane. This cell was reminiscent of the ECL cell in the human alimentary mucosa.**

**Type II cells were characterized by the presence of spherical or oval granules (110-230nm in diameter) with low or high electron density. The granules showed a clear halo between the homogenous contents and the limiting membrane. This cell was reminiscent of the G cell in the human alimentary mucosa.**

**Type III cells were characterized by the presence of elongated oval or pheomorphic granules (50-200nm in diameter) with low or moderate electron density and abundant microfilament bundles in the cytoplasm. The granules contained a very thin halo.**

**KEY WORDS: Gastro-endocrine cell, *Rana rugosa*, ECL cell, G cell, Bombesin-producing cell**

It has been well known that the gastro-endocrine cells which are dispersed in the epithelia of the alimentary tract synthesize various kinds of gastrointestinal hormones and play an important role in the physiological functions of the gastrointestines.

In mammals including the human, the gastro-endocrine cells have been studied by using electron microscopic and immunohistochemical technics for their identifications (Solcia *et al.*, 1975; Grube and Forssman, 1979; Solcia *et al.*, 1980). In recent years, Solcia *et al.* (1981) demonstrated 18 different endocrine cells in the gastrointestinal tract by the Santa Monica 1980 classification.

According to the differences among species and each regional part of the gastrointestine, the gastro-endocrine cells appeared remarkably different in the distribution, relative frequences and cell types (Gabe, 1972; Chung, 1976; Alumets *et al.*, 1977; Grube and Forssman, 1979).

Although many reports have been made on the histochemical properties and structure of endocrine cells in the gastrointestinal mucosa of various vertebrates, among them only a few papers have been carried out on the amphibia including the urodele (Buchan *et al.*, 1980) and the anuran (Kim, 1967; Kim and Chung, 1973; Chung, 1976; Chung and Kwun, 1981, 1983; El-Salhy *et al.*, 1981). Lee and Lee (1989) reported on the characteristic morphological differences between the hibernating and the active phase of the gastro-en-

**Table 1.** Ultrastructural characteristics of various gastro-endocrine cell types in the stomach mucosa of the frog, *R. rugosa*

| Cell types | Granules | | |
|---|---|---|---|
| | Size (nm) | Shape | Contents |
| I | 300-500 | round or oval | high density, wide lucent |
| II | 110-230 | spherical or oval | low or high density, clear halo |
| III | 50-200 | elongated oval or pleomorphic | low or moderate density, clear halo |

docrine cells in *R. dybowskii* using light and transmission electron microscope (TEM).

In the present study, the gastro-endocrine cells of *R. rugosa* were examined by TEM and were described the fine structure of three kinds of cell type in the stomach mucosa.

## Materials and Methods

The frogs, *Rana rugosa* Schlegel collected in Hapcheon, Kyung Nam Province were used in this study. Samples were obtained from the fundic region of stomach. The tissue blocks were immediately fixed in 2% paraformaldehyde–2.5% glutaraldehyde mixture buffered with 0.1M cacodylate (pH 7.4) for 24 hrs, washed in the same buffer, postfixed with cacodylate buffered 2% $OsO_4$ for 2 hrs, dehydrated in graded ethanol solutions, and embedded in Epon 812 mixture. Ultrathin sections were stained with uranyl acetate and lead citrate, and observed under a TEM (Hitachi H-600 type).

## Results

The gastro-endocrine cells of *R. rugosa* were classified into three cell types by the size, shape, electron density of the secretory granules and fine structure of them (Table 1).

**Type I cell (Fig. 1):** This cell showed generally the light cytoplasm. The most common feature of the granules was round or oval shape and large-size (300-500nm in diameter) with high electron density. The granules showed a wide lucent between the contents and the limiting membrane. The development of the cell organelles was relatively poor, and a small amount of ovoid mitochondria and lipid droplets were found in the cytoplasm.

**Type II cell (Fig. 2):** This cell was characterized by appearing spherical or oval shaped granules of medium-size (110-230nm in diameter) with low or high electron density in the cytoplasm. The granules showed a clear halo between the homogenous contents and the limiting membrane. A few round or oval mitochondria, lipid droplets, vesicles and an infolded nucleus were also observed.

**Type III cell (Figs. 3, 4):** This cell was generally pyramidal shape extending toward the lumen, and the cytoplasm was relatively dark. The numerous secretory granules of small or medium-size (50-200nm in diameter) were mostly elongated oval or pleomorphic shape with low or moderate electron density and showed a very thin halo. In the cytoplasm a few of elongated mitochondria, especially abundant microfilaments and rER were found.

## Discussion

A few papers have been reported on the gastro-endocrine cells of amphibia detailing the distribution, relative frequences and fine structures of the gastrointestinal tract by light and transmission electron microscope (Kim, 1967; Geuze, 1971; Gabe, 1972; Kim and Chung, 1973; Kataoka, 1974; Chung, 1976; Buchan *et al.*, 1980; El-Salhy *et al.*, 1981; Chung and Kwun, 1981,

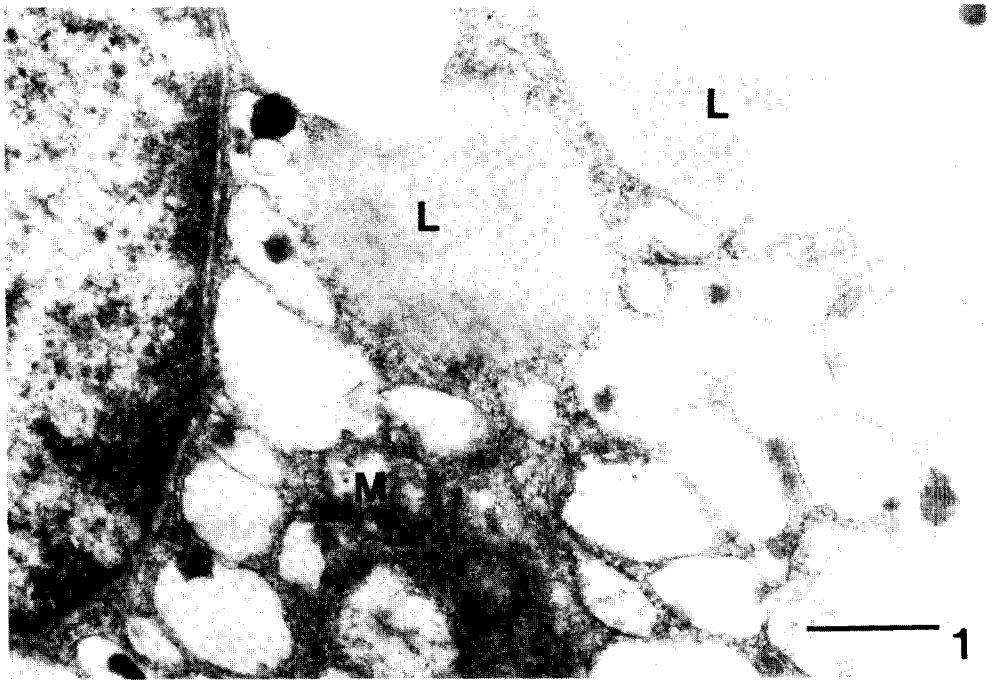



**Fig. 1.** A type I cell. Numerous round or oval shaped granules with high electron density and a wide lucent between the contents and the limiting membrane are found. Note a few of mitochondria (M) and lipid droplets (L). Bar = 0.5 $\mu$m

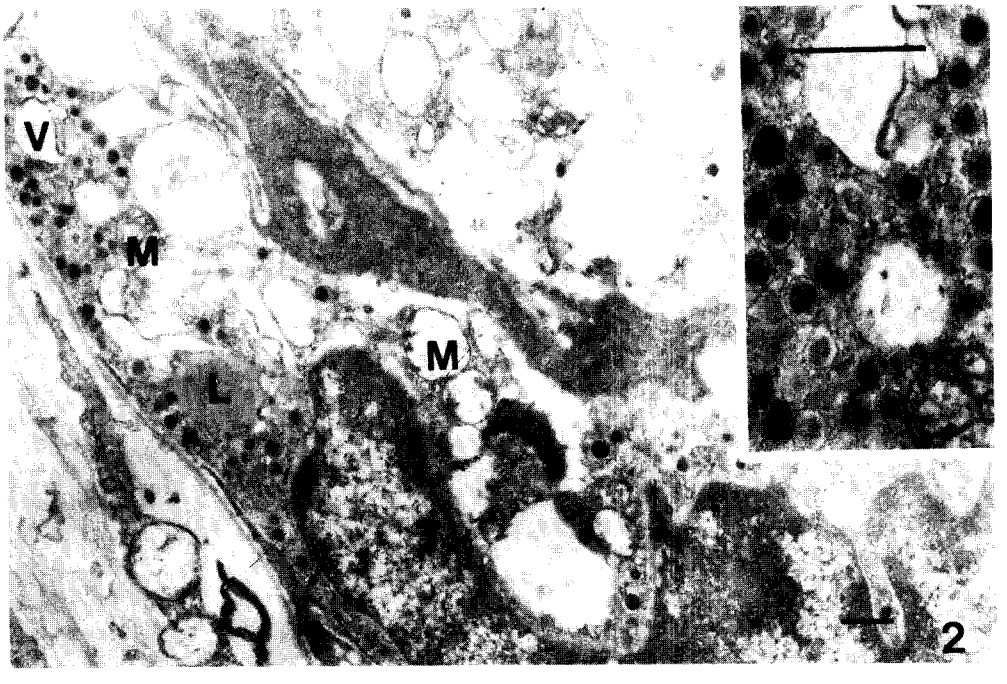
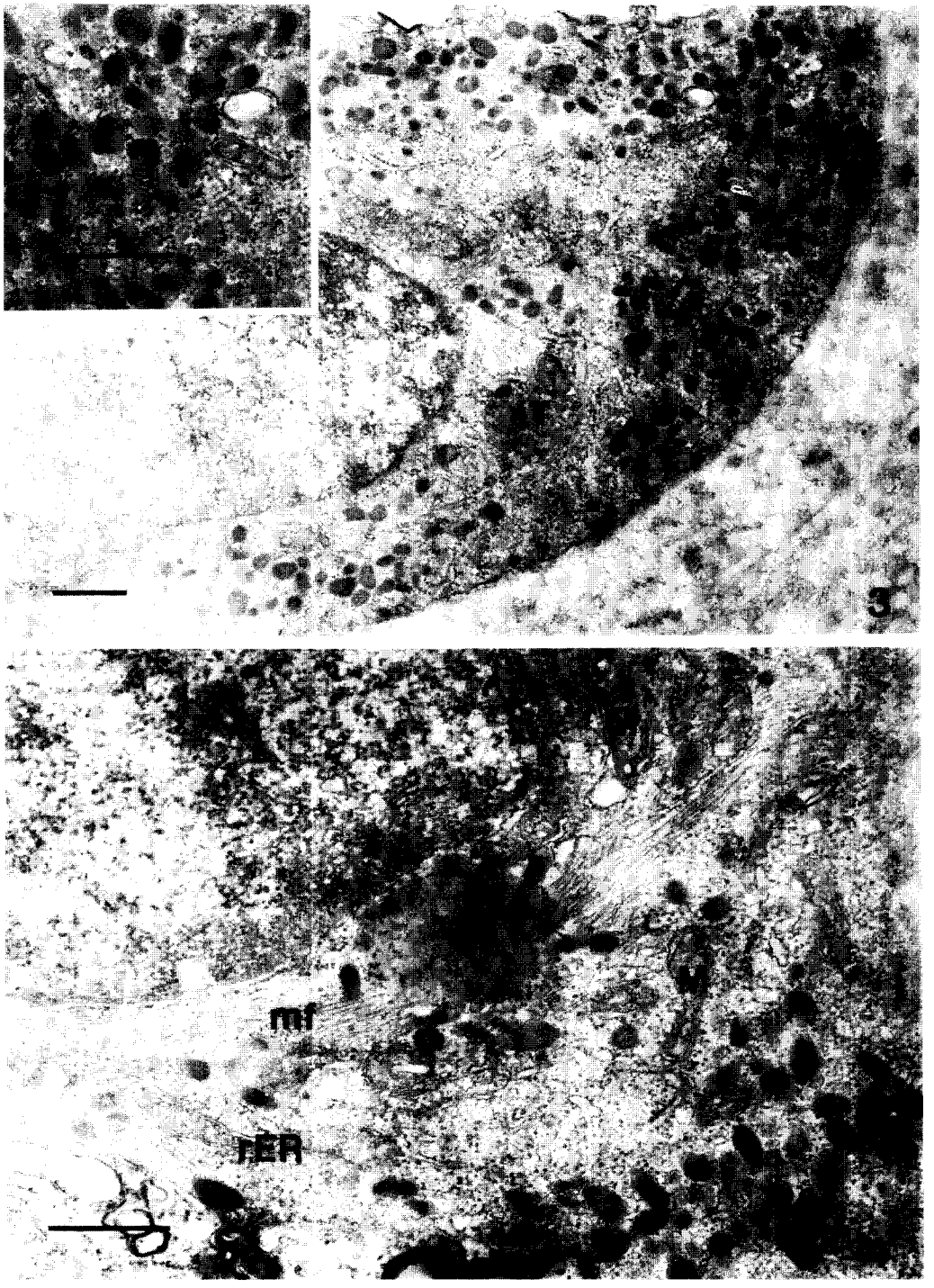



**Fig. 2.** A type II cell. A number of spherical or oval shaped granules with low or high electron density and a clear halo, a few mitochondria (M), vesicles (V) and few lipid droplets (L) are found. Insert: High magnification of a part of Fig. 2.




**Figs. 3, 4.** A type III cell. A number of elongated oval or pleomorphic granules with low or high electron density and a clear halo are shown. Note abundant microfilament bundles (Mf), the well-developed rER (rER), the elongated mitochondria (M) and somewhat dark of the cytoplasm. Insert: High magnification of a part of Fig. 3.

1983; Lee and Lee, 1989).

In amphibia, the gastro-endocrine cell which was quite similar to the EC cell of the mammalian alimentary tract was reported on the stomach of *R. esculenta* and the gastrointestinal tract of *R. nigromaculata* by Geuze (1971) and Kataoka (1974), respectively. In the urodele, *Salamandra salamandra,* Buchan *et al.* (1980) identified six endocrine cells which were somewhat different from those observed in the alimentary tract of mammal, and Chung and Kwun (1981, 1983) reported five types and four types of endocrine cells in the gastric fundus and the duodenum of *Bombina orientalis,* respectively.

In the present study, according to the granular size, shape, electron density and the fine structure of endocrine cell, three kinds of cell type could be identified in the fundic region of stomach of *R. rugosa.* Among them, type I cell was characterized by having round or oval granules (300-500nm in diameter) with high electron density and showed a wide lucent between the contents and the limiting membrane. These granules were quite similar in appearance to the type IV cell reported by Chung and Kwun (1983) and the type II cell of the hibernating phase in the gastric mucosa observed by Lee and Lee (1989). The type I cell, according to the classifications by Grube and Forssman (1979) and Solcia *et al.* (1974, 1981), was thought to be reminiscent of the ECL cell of the human gastrointestinal tract.

Type II cell was characterized by having spherical or oval granules (110-230nm in diameter) and showed a clear halo between the contents and the limiting membrane. These granules were quite similar in shape, size and electron density to the type III cell of the gastric mucosa in the hibernating phase (Lee and Lee, 1989) and also were considered the G cell of the human alimentary mucosa observed by Grube and Forssman (1979) and Solcia *et al.* (1974, 1981).

Type III cell was characterized by having elongated oval or pleomorphic granules (50-200nm in diameter), showing a very thin halo, and abundant microfilament bundles were scattered in the entire cytoplasm, especially. This cell was somewhat similar in appearance to the type IV cell of the gastric mucosa in the hibernating phase (Lee and Lee, 1989) and the type V cell (Chung and Kwun, 1983) in *Bombina orientalis.* And they regarded these cells as a bombesin (like)-producing cell observed by Lechago *et al.* (1978, 1979). The bombesin-producing cell reported by Grube and Forssman (1979) was characterized by having abundant microfilaments in the cytoplasm and mostly spherical shaped granules of small-size (120nm in diameter) with high electron density. In this study, type III cell was somewhat similar to the bombesin (like)-producing cell in amphibia, but the fine structures of this cell seem to be appeared the characteristic morphological differences in the granular shape and the dark cytoplasm. At present, because any attempt to identify the bombesin (like)-producing cell in amphibia would be difficult, phylogenetic and systematic studies in relation to lower vertebrates are required. Therefore, further investigations are necessary to reveal the differences between animal species and functional roles of the gastro-endocrine cells.

Previous immunohistochemical papers have identified the polypeptides hormones of some endocrine cell types in the gastrointestinal mucosa of various mammals; ECL cell producing histamin, G cell producing gastrin and bombesin (like)-producing cell producing bombesin, gatrin-releasing polypeptides in the frog, respectively (Lechago *et al.*, 1978; Grube and Forssman, 1979; Solcia *et al.*, 1980).

The present study demonstrated three types of endocrine cells in the gastric mucosa of the frog, *R. rugosa*, and it remained still to be elucidated as to what and how many endocrine cell types are distributed in the other intestinal tract in this species.

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## 옴개구리 ***Rana rugosa*** 위점막의 위장관 내분비세포에 관한 전자현미경적 연구

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본 연구는 옴개구리(*Rana rugosa*)의 위저부 점막을 전자현미경적으로 관찰한 결과, 내분비세포의 분비 과립의 크기, 형태, 전자밀도 및 세포의 형태에 따라서 다음과 같이 3종류의 세포를 분류하였다.

제 I 형 : 전자밀도가 높고 원형 또는 난원형의 과립을 가지며, 과립내용물과 한계막 사이의 편제하거나 공포상으로 출현하였다. 과립의 크기는 직경이 300-500nm였다.

제 II 형 : 구형 또는 난원형의 과립을 가지며, 전자밀도는 낮거나 높게 나타나며, 한계막과 과립내용물 사이에는 좁은 halo를 나타내었다. 과립의 크기는 직경이 110-230nm였다.

제 III 형 : 신장된 난원형 또는 다형태성의 과립을 가지며, 전자밀도는 낮거나 중등도이며 과립과 내용물 사이에는 명확한 halo를 형성하고 세포질내에는 미세섬유들이 풍부하게 출현하였다. 과립의 크기는 직경이 50-200nm였다.