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Granule Release from Endocrine Cells in the Rat Stomach — An Electoron Microscopic Study —

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Introduction

Electron microscopic studies on the endocrine cells of the gastrointestinal tract under normal condition have been done by many investigators in humans and in several species of animals including rats [1)12)16)17)] They classified the endocrine cells into several types mainly according to the morphologic differences in their fine structures in an attempt to elucidate the relationships between cell types and hormones they produce.

It is also known that gastrointestinal hormones including a polypeptide (gastrin) and/or a monoamine (serotonin) are released from these endocrine cells by various stimuli, chemical[21)], nervous[23)], and mechanical[9)]. The mechanisms for release of these hormones, however, have not sufficiently been clarified.

In this study the fine structures of the endocrine cells in the rat stomach after refeeding and electric vagal stimulation were examined under the electron microscope and, as compared with those of non-stimulated condition which had been already reported[17)], the stimulation-induced morphologic changes in these cells, especially the secretory granules, were studied.

Materials and Methods

Male Wistar rats weighing between 200 to 300g were used as experimental animals after fasting for 24-48 hours.

(1) Ten rats were sacrificed from 10 minutes to 2 hours after refeeding with an ordinary rat diet. One rat was sacrificed 30 minutes after administration of 50% glucose solution into the stomach by the use of an oral tube. Each rat was laparotomized immediately after decapitation and the stomach removed.

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(2) Two rats were anesthetized with sodium pentobarbital (Nembutal, 30 mg/kg., i.p.). After laparotomy the bilateral vagus nerves were dissected free and cut at the diaphragmatic level. The peripheral ends of each nerve were placed on a bipolar platinum electrode connected to an electrical stimulator (Model ES 103, Sanei Sokki KK) delivering monophasic square wave stimuli of a duration of 5ms, an intensity of 4V and an impulse frequency of 5 Hz. The nerves were placed in paraffin oil to prevent drying during the experiment. Stimulation was continued for 30 minutes and then the stomach was removed.

Tissues taken from the antral and fundus mucosae of the stomach in two groups were fixed in 4% glutaraldehyde in 0.2M cacodylate buffer of pH7.3 at 4°C for 2 hours, transferred and washed in cold cacodylate buffer over 24 hours. Then, all samples were post fixed in a 1% solution of osmium tetraxoide in the same buffer for 1 hour at 4°C, dehydrated in graded alcohols and propylene oxide, and then embedded in Epon 812. Each sample was cut on a Portor Blum MT II with a glass knife. Thick sections were stained with toluidine blue and examined under the light microscope. Ultra-thin sections were stained with uranyl acetate and lead citrate and examined under a Hitachi HS7D electron microscope.

Results

The stomach removed after refeeding was dilated so that food was found in the lumen. Gastric juice also existed there for a brief period after refeeding.

Gastrointestinal peristalsis was observed during electric vagal stimulattion.

Light microscopic examination of the endocrine cells of the rat stomach under these conditions revealed no remarkable morphologic changes.

The findidgs obtained by electron microscopic examination of various types of the endocrine cells in the rat stomach after stimulation consisted of the following.

(1) *G Cells*

The plasma membrane partly looking like an omega or a dimple was observed in some of the G cells after refeeding (Fig. 1).

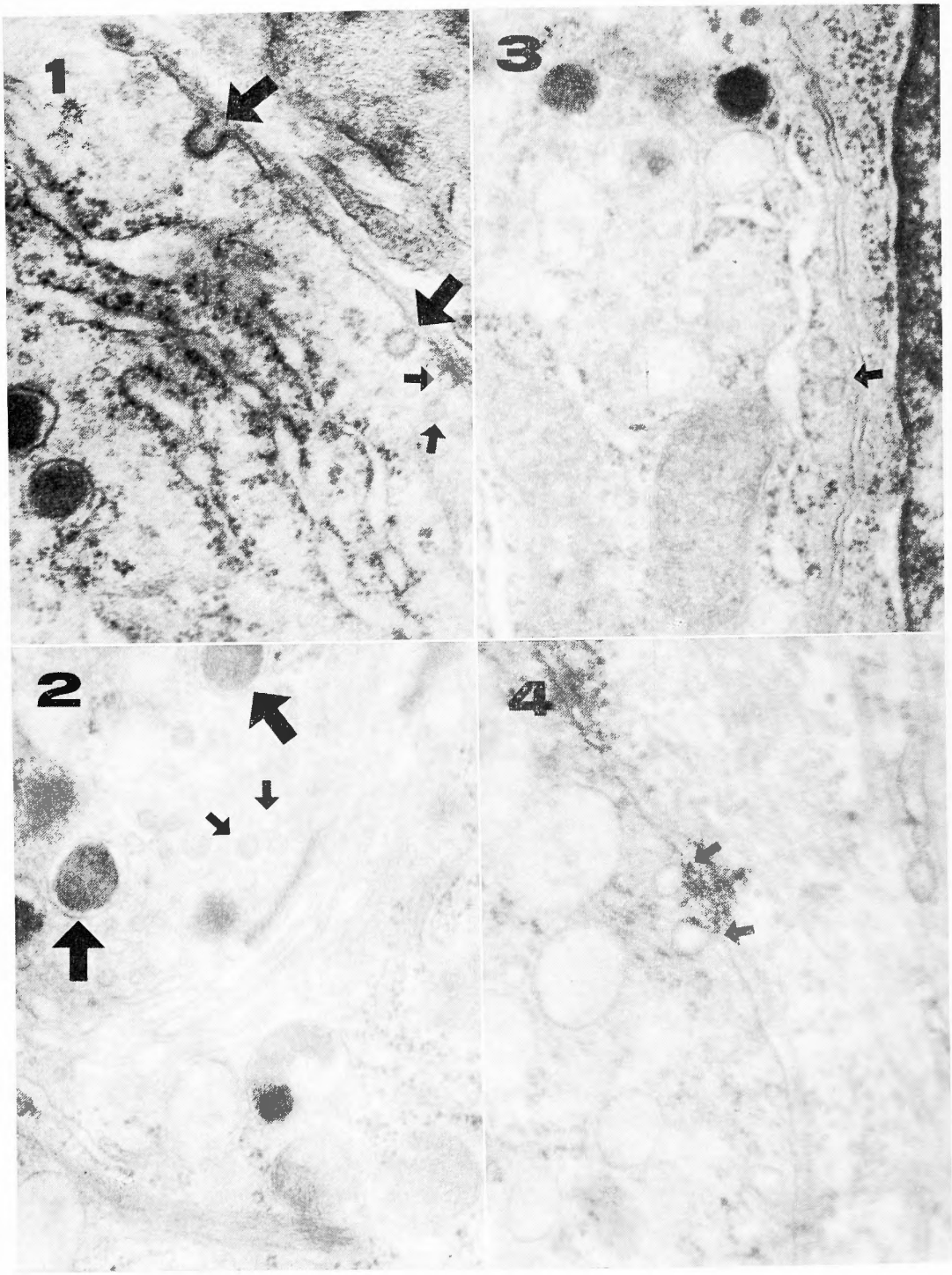
Enlargements of the rough endoplasmic reticulum in the cytoplasm, and the linear or round substances of high electron density in the Golgi complex, where a relatively large number of small corted vesicles existed, were also seen after refeeding (Fig. 2).

After electric vagal stimulation G cells were found to have small vesicles either appearing to be in contact with the cell membrane or to have omega-shaped dimplings of the cell membrane (Figs. 3 and 4).

The number and the electron density of the secretory granules in the cytoplasm of the G cell showed no remarkable changes after refeeding and electric vagal stimulation. Nor remarkable were the morphologic changes in the G cells after injection of a 50% glucose solution into the stomach.

(2) *EC Cells*

The EC cells showed finely granular contents within the membranous sacs and secretory granules with an eccentrically located small core in a relatively wide space within a



(Magnification approximately × 48000)

Fig. 1. Part of a G cell after refeeding showing omega-shaped dimplings(thick arrows) of the plasma membrane and microvesicles (thin arrows).

Fig. 2. Golgi complex of a G cell after refeeding showing a relatively large number of progranules (thick arrows) and corted microvesicles (thin arrows).

Fig. 3. and **4.** Parts of G cells after electric vaga[illegible] membranous vesicles which fuse with plasma membrane (arrows).

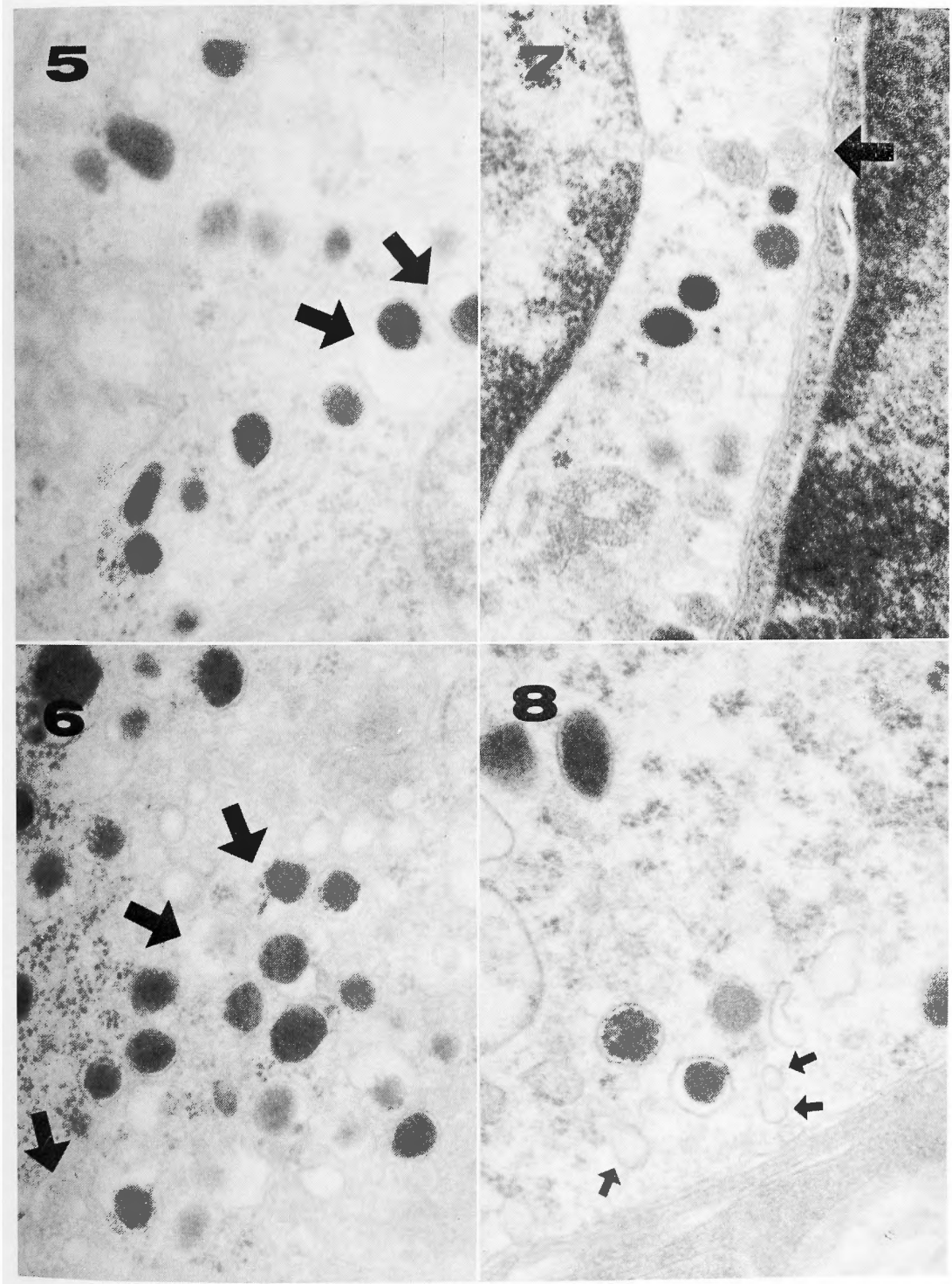


Fig. 5. and **6.** Parts of EC cells after refeeding containing some secretory granules showing a fine granular or cored-vesicular structure (arrows).

Fig. 7. An emiocytotic granule release (arrow) from the EC cells after electric vagal sti mulation.

Fig. 8. Part of an EC cell after administration of 50% glucose solution by an oral tube showing various shaped small vesicles (arrows) near the plasma membrane.

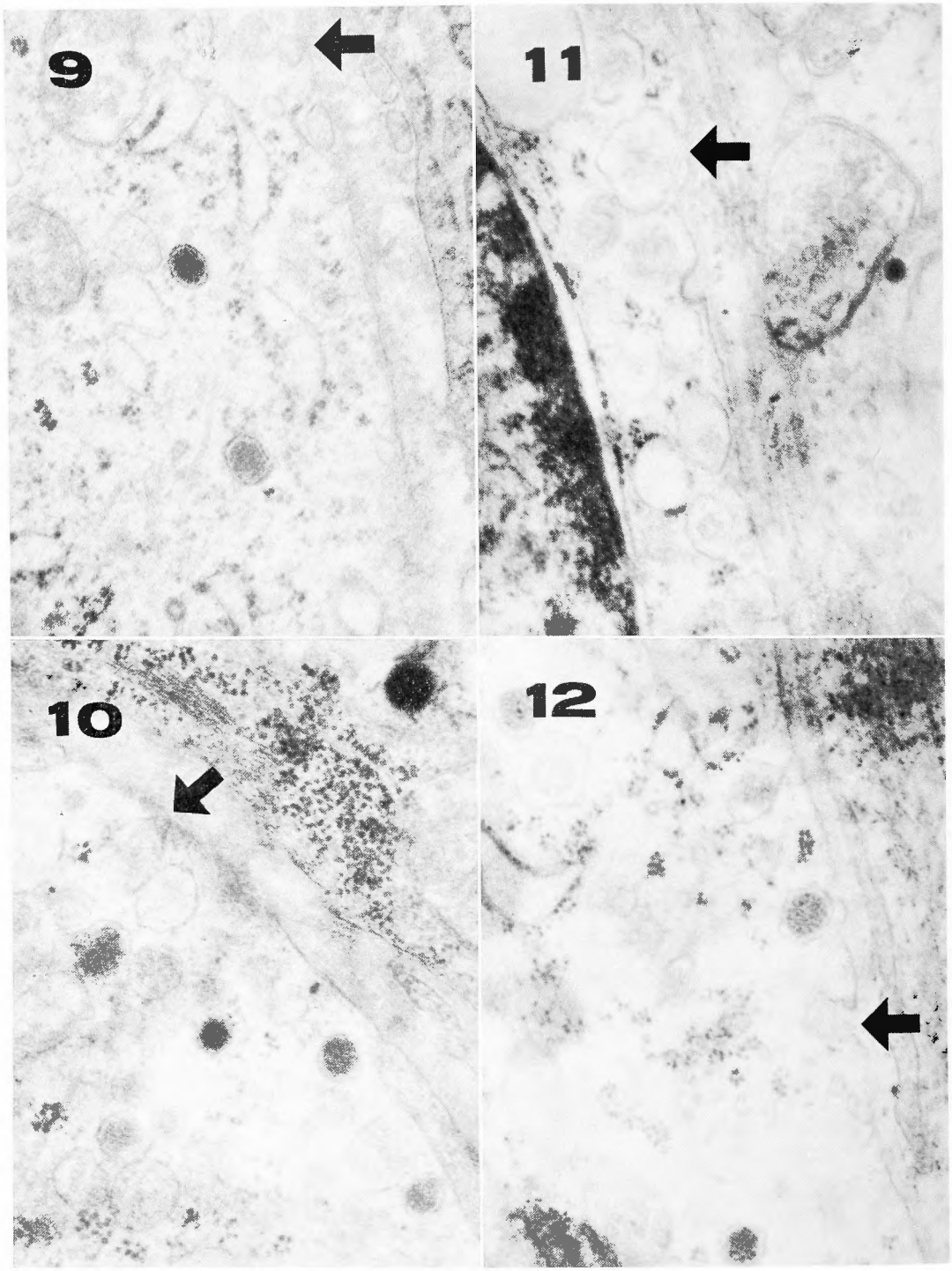


Fig. 9. and **10.** Parts of D_1 (Fig. 9) and D (Fig. 10) cells after electric vagal stimulation indicating an emiocytotic granule release (arrows).
Fig. 11. and **12.** Emiocytotic granule releases (arrows) from the ECL cells after refeeding.

membranous sac after refeeding (Figs. 5 and 6).

Morphologic changes indicating an emiocytotic granule release or a contact with the cell membrane of the secretory granules were observed after electric vagal stimulation (Fig. 7).

A relatively large number of microvesicles in the cytoplasm near the cell membrane were observed after injection of a 50% glucose solution into the stomach (Fig. 8.), although a definite figure showing an emiocytosis could not be found.

There were no remarkable changes in the number and the distribution of the secretory granules in the cytoplasm of the EC cell after refeeding. However, the secretory granules seemed to be marginated or translocated to the cell membrane after electric vagal stimulation and injection of a 50% glucose solution into the stomach (Figs. 7 and 8).

(3) *Other Types of Endocrine Cells*

Morphologic changes as in the above two types of endocrine cells were observed in some of these endocrine cells after stimulation.

Emiocytotic granule release from the D_1 cells (Fig. 9) and the D cells (Fig. 10) occured after electric vagal stimulation, although they could not be found after refeeding.

Some definite figures showing emiocytosis were frequently observed in the ECL cells after refeeding, especially after a brief period following refeeding (Figs. 11 and 12).

Discussion

The secretory mechanism of the endocrine cells in the gastrointestinal tract is not yet known in detail.

As to the other types of endocrine cells involving the islet β cell and the endocrine cell of the adrenal grand, the following model for hormone secretion was proposed[13)14)].

The hormone is synthesized in the endoplasmic reticulum, transferred to the Golgi complex where the hormone is encapsulated and stored as secretory granules in the cytoplasm. After specific stimulation, the secretory granules with their surrounding membranous sacs are translocated to the plasma membrane, where they are released by emiocytosis. After the release of the granules, the membranous sacs fuse with the plasma membrane and they are recaptured from the plasma membrane, and then they are returned as microvesicles either to the Golgi complex or lysosomes where they are degraded. The specific stimulus which results in the release of the hormone from the cells also initiates new formation of hormone in the endoplasmic reticulum.

Morphologic evidence for this mode of secretion as to the endocrine cells of the gastrointesinal tract was presented by FUJITA[6)] in the form of the D-like cells of the dog stomach observed after administration of 0.1N hydrochloric acid into the stomach by oral catheterization.

On the other hand, some other investigators suggested that intracytoplasmic dissolution or diacrine secretion of the granule contents with an intact membranous sac occurred in the G cells of the cat[5)] after refeeding or of the human[3)19)] with hypergastrinemia.

We could not obtain morphologic evidence of diacrine secretion in the G cells after stimulation, because the electron density of most of the secretory granules in the G cells of the rat stomach was usually low with our method for electron microscopy.

In the EC cells with usually high electron opaque secretory granules under normal condition, the secretory granules in the cytoplasm appearred to be finely granular or clear within the membranous sacs after stimulation, suggesting the decreased electron density of the granule contents.

It remained unknown, however, whether these findings might serve as morphologic evidence of intracytoplasmic dissolution indicating different functional stages of diacrine secretion of granule contents including 5-HT (Serotonin) which is a monoamine produced in the EC cell, or merely an artificial product resulting from the fixation procedure for electron microscopy, because these findings were also observed in some of the EC cells under normal condition.

On the other hand, the morphologic findings obtained time and again in several types of endocrine cells in the rat stomach after stimulation under various conditions, especially of the secretory granules, might be interpreted as morphologic evidence indicating various steps for emiocytotic granule release.

This indicates that the same secretory mechanism suggested by LACY[13)14)] in the other types of endocrine cells may be also present in the endocrine cells of the gastrointestinal tract as suggested by FUJITA[6)].

The degranulation indicating a definite dcfrease in the secretory granules in the cytoplasm and the morphologic evidence showing final steps of emiocytosis were not always observed in most of the endocrine cells under stimulated conditions, especially physiological condition. Some other investigators[10)18)] failed to observe any morphologic change in the endocrine cells of the gastrointestinal tract after refeeding. The endocrine cells seem to control the rate of release of the stored hormone after stimulation.

In our studies, emiocytotic granule release from the ECL cells and increased fromation of progranules in the G cells were often observed after refeeding. ECL cells in the rat stomach were indentified as the cells containing a definite gastric acid stimulating monoamine "histamine" and another unknown polypeptide which probably stimulates gastric secretion[2)17)]. G cell has been known as the cell containing a hormone "gastrin" which also stimulates gastric secretion[4)17)].

It may be reasonable to consider that refeeding, that is, a chemical, vagal or mechanical stimulus, is one of the specific physiologic stimuli which promote the release of the granules from these cells and, inevitably, the formation of the secetory granules in these cells. The cells seem to be responsible only for the specific stimuli.

The emiocytotic granule release from the ECL cells observed after refeeding also indicates that emiocytosis occurs in the endocrine cells of the gastrointestinal tract under not only the non-physiological but also the physiological condition.

In conclusion, the findings obtained in our mophologic studies seem to provide mor-

phologic evidence to indicate the secretion of the gastrointestinal hormones following refeeding[8)11)] and electric vagal stimulation[1)7)15)19)22)] demonstrated by biochemical studies. The results also indicate that emiocytosis is at least one of the physiological mechanisms for secretion in the gastrointestinal endocrine cells, although the possibility that secretion by the mode of diacrine, especially the release of other granule contents than polypeptide, may occur under a certain condition is not completely precluded.

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和文抄録

ラット胃内分泌細胞における顆粒放出について —電子顕微鏡的検索—

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給食刺激時，迷走神経電気刺激時におけるラット胃内分泌細胞の微細構造上の変化，ことに分泌顆粒の形態学的変化について電子顕微鏡的に検索した．

刺激後の各種の内分泌細胞においては，分泌顆粒の細胞膜周辺への局在傾向や，細胞膜との癒合，Ω型の開口分泌像など，開口分泌における顆粒放出形式の種々の段階を示すと思われる形態学的所見が観察された．以上の結果から，消化管内分泌細胞においても，非生理的あるいは生理的条件下をとわず，開口分泌による顆粒放出形式が存在することが判明した．