ULTRASTRUCTURAL STUDY OF GASTRIC ENDOCRINE CELLS OF HUNGRY AND FED IMMATURE RATS

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The connection between ultrastructure and functional activity of endocrine cells in the gastrointestinal tract is important for the understanding of the role of these cells in the regulation of physiological processes. The few data in the literature on functional morphology of gastric endocrine cells are mainly concerned with gastrin-producing cells, which have been most thoroughly studied, but the results and their interpretation given by different workers are contradictory [2, 3, 8, 11, 12].

EXPERIMENTAL METHOD

Immature albino rats weighing 100 g deprived of food for 48 h (but allowed free access to water) were used. Half of the animals were given a protein meal 30 min before sacrifice. After decapitation of the animals pieces of mucosa from the gastric fundus and pylorus were fixed in 3% glutaraldehyde and 1% OsO_4, and they were embedded in Epon-Araldite in the usual way. Sections were stained in uranyl acetate and lead citrate and examined in the JEM-100C electron microscope. The diameter of the granules was measured in sections through the cells on electron micrographs of strictly determined final magnification.

EXPERIMENTAL RESULTS

Six types of endocrine cells were distinguished in the gastric mucosa of the rats and, in accordance with the international classification, these were described as G, D_1, EC, D, ECL, and A-like. The G cells are known to produce gastrin and the ECL cells in rats contain histamine. Serotonin is found in granules of the EC cells but their protein product is not yet known. A-like cells have been shown to produce enteroglucagon, D cells somatostatin, and the D_1 cells produce vasoactive intestinal peptide (VIP) [9, 10].

Endocrine cells located in the chief glands of immature rats do not reach the lumen of the gland; cells of pyloric glands project into the lumen and their apical surface is covered with microvilli, and the polarity and distribution of the organelles are more clearly defined. A cell center, lamellar complex, and young and, less frequently, mature granules were found in the apical part of most cells. Microfilaments lay around the nucleus and most of the secretory granules were in the basal part. Mitochondria, vacuoles, and lysosomes with different types of structure, free ribosomes, and fragments of the endoplasmic reticulum were diffusely distributed.

The G cells were relatively large and the most numerous endocrine cells of the pyloric glands. The secretory granules of the G cells filled the greater part of the cytoplasm and their diameter varied from 170 to 370 nm (Fig. 1a). In the region of the Golgi complex and, less frequently, in the basal part of the cells young, immature granules with a moderately dense core and a distinct rim beneath the membrane could be seen. Granules with mature secretion, with a core of lower density and a close-lying membrane were classed as "dense." The process of discharge of the substance from the granules was accompanied by clearing of their matrix, with the consequent appearance of "empty" granules in the cytoplasm. All types of granules were found in each G cell, with predominance of certain types depending on the phase of the secretory cycle. The study of the composition of granules in G cells of the hungry animals (Fig. 2A) showed that about half of all cells contained predominantly mature granules

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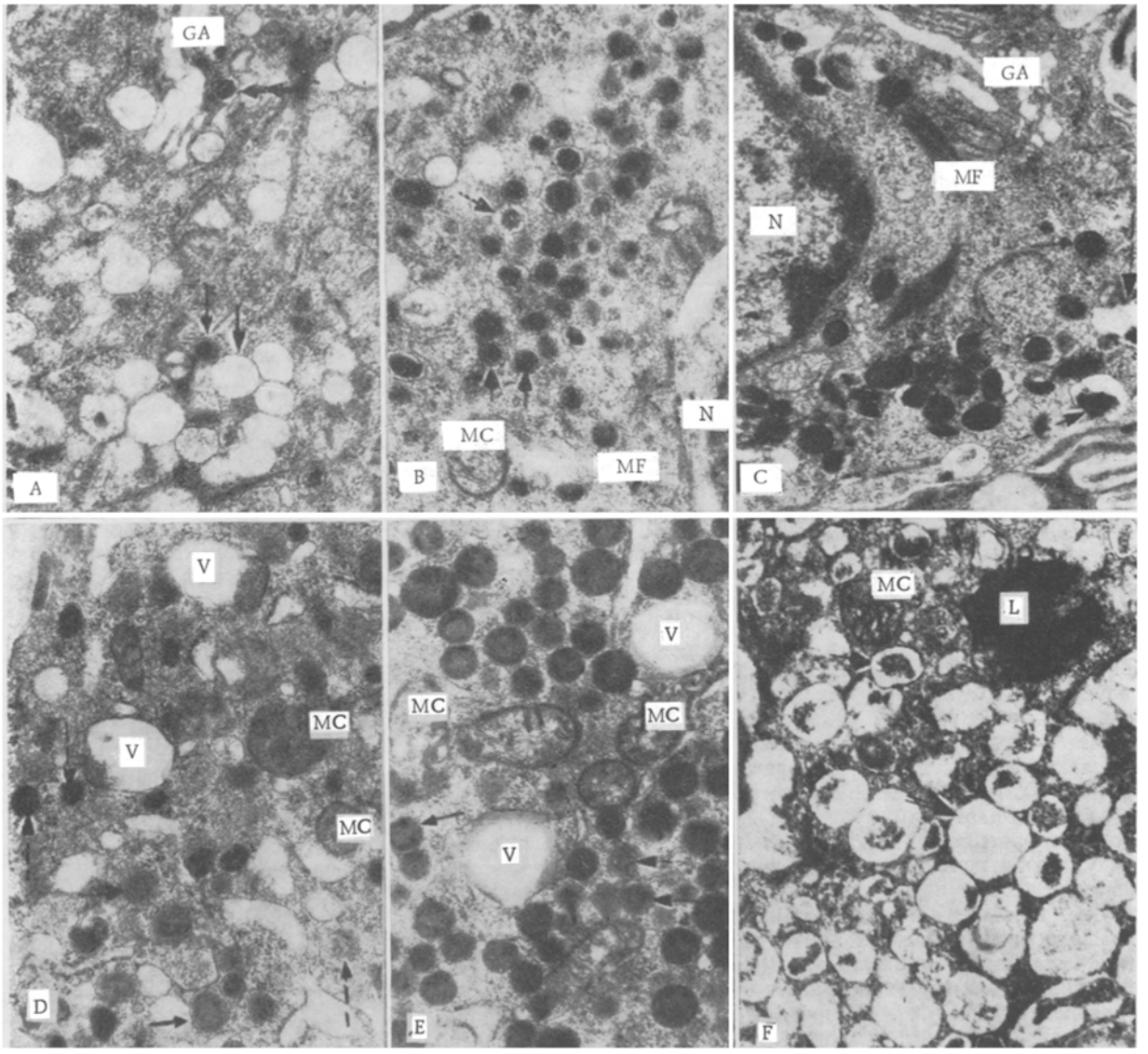

Fig. 1. Ultrastructural features of granules of gastric endocrine cells of immature rats (26,000 ×). A) G cell; B) D_1 cell; c) EC cell; D) D cell; E) A-like cell; F) ECL cell; N) nucleus; MC) mitochondria; MF) microfilaments; GA) Golgi apparatus; V) vacuoles; L) lysosomes; →) young granules; ⟶) mature granules; -⟶) lysed granules; ⇒) "empty" granules; - - -→) "filled" granules of ECL cells.

with secretion (so-called storage cells); some cells contained "dense" and "empty" granules in roughly equal proportions (actively functioning cells); other cells contained very large "empty" granules — cells without secretion. A protein diet is one of the most powerful stimulators of gastrin secretion [7], and for that reason cells with empty granules predominated in the fed rats (Fig. 2B), but their diameter was smaller than in hungry animals. As Fig. 2 shows, complete synchronization of the secretory activity of all G-cells did not take place either during prolonged starvation or in response to feeding. Changes in the other intracellular organelles of these cells could not be reliably established in response to feeding.

The D_1 cells were very numerous in the pyloric glands. They were characterized by thick bundles of microfilaments around the nucleus and by small (130-180 nm) granules with homogeneous, moderately dense core, pale rim, and distinct membrane (Fig. 1B). Changes in the granules began with clearing at the periphery and ended with complete lysis of the core.

The EC cells were distributed in the zone of transition between chief and pyloric glands and in the pyloric glands. They were distinguished by a well-developed rough reticulum and by the presence of vacuoles of different types of structure in the cytoplasm and bundles of microfilaments around the nucleus. In the lamellar complex and Golgi zone there were small round immature granules with low density. The mature granules were irregular in shape and

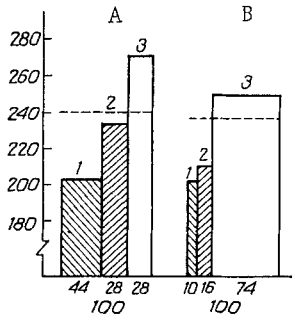

Fig. 2

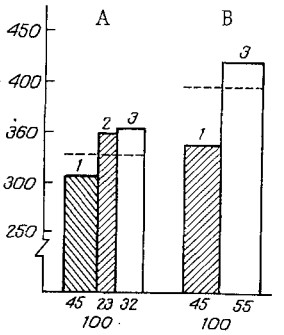

Fig. 3

Fig. 2. Characteristics of G cells according to composition of granules in hungry (A) and fed (B) immature rats. Here and in Fig. 3: abscissa, relative percentage of cells with a particular composition of granules; ordinate, diameter of granules (in nm); 1) cells with predominantly "dense" granules; 2) cells with mixed "dense" and "empty" granules; 3) cells with predominantly "empty" granules. Broken line indicates mean diameter (in nm) of granules of total cell population.

Fig. 3. Characteristics of ECL cells with respect to composition of granules in hungry (A) and fed (B) immature rats. 1) Cells with mainly "filled" granules; 2) cells with mixed ("filled" and "empty") granules; 3) cells with mainly "empty" granules. Remainder of legend as to Fig. 2.

the high-density core occupied the whole granule, leaving only a very narrow rim beneath the membrane (Fig. 1C). Gradually the granules swelled and the core was reduced through fragmentation or shrinkage.

D cells ere located mainly in the fundal glands. They contained round granules with a mean diameter of about 260 nm (Fig. 1D). Young granules only just detached from the cisterns of the Golgi apparatus had a core of average density and a thin rim beneath the fairly clear membrane. During maturation of the granules their membrane became wavy and interrupted, the density of the core decreased, and the granular structure became more clearly visible. This process ended with complete lysis of the granules in the cytoplasm.

No differences could be found in the structure, number, and size of the granules or other structures of the D_1, D, and EC cells in the hungry and fed animals. Because of the nature of their hormonal products they evidently function at later stages of digestion (when gastric secretion is inhibited).

The A-like cells were found only in chief glands and contained round granules whose membrane was close to the homogeneous high-density core (Fig. 1E). The diameter of the granules varied from 150 to 250 nm, with mean values of 209 nm in hungary animals and 185 nm in the fed rats. In both states studied, the formation of granules in the dilated cisterns of the Golgi apparatus could be observed in the A-like cells, and in the cytoplasm the mature granules were accompanied by young granules with an irregular, narrow rim beneath the membrane and by lysed granules. During digestion intensification of the secretory activity of the A-like cells evidently takes place, as shown by accumulation of small young granules. The functional role of these processes is not clear, for the physiological role of enteroglucagon had not been adequately studied.

The ECL cells also were found only in fundal glands. They contained large secretory granules, with a mean diameter varying from 230 to 630 nm depending on the functional state of the cell (Fig. 1E), and their density and size varied greatly even in the same cell. The densest granules, which also were the smallest, contained fine-grained material of average electron density, with indistinct borders and with a wide space between it and the limiting membrane. During maturation of the granules their diameter increased, but the size and density of the core decreased. Such granules were interpreted as "filled" to distinguish them from "empty" granules, which did not contain core material in the section or had only amorphous remains of it, distributed eccentrically in a large vacuole. During feeding activation of the lamellar complex took place in the ECL cells: Its volume increased, the lumen of the cisterns

was widened, and numerous small, smooth and coated vesicles and larger transparent vacuoles appeared. In the cytoplasm the network of microtubules and microfilaments was traced more clearly. The granules lost their contents and their mean diameter increased significantly from 327 to 395 nm (Fig. 3A, B). Consequently, during feeding the contents of the granules were secreted (with a change in their size and density) and, at the same time, new portions of the substance were synthesized. Many experiments have shown that gastrin is secreted from the pyloric portion during feeding [5, 7], and induces liberation of histamine from the depots, activates histidine carboxylase, and has a trophic influence [4, 5, 6]. Histamine, through its local action on the parietal cells, activates hydrochloric acid synthesis; through a feedback mechanism, hydrochloric acid inhibits activity of the gastrin cells [1]. Individual stages of the hypothesis of humoral regulation of gastric acidity, linking the taking of food, G (gastrin), ECL (histamine), and parietal cells (hydrochloric acid), have been elaborated by different workers by means of precise biochemical methods. The ultrastructural data now obtained are an illustration and also morphological confirmation of the initial stages of this chain.

Analysis of the results of these experiments with starvation followed by feeding revealed a different morphological and functional response of the various types of gastric endocrine cells, determined by the physiological role of their secretory products. This different response was most marked in the G and ECL cells, which produce stimulators of acid gastric secretion.

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