ULTRASTRUCTURE OF ENDOCRINE CELLS OF THE GASTRIC FUNDAL GLANDS IN DIFFERENT SEASONS OF THE YEAR

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An electron-microscopic study was made of the endocrine cells of the gastric fundal glands of the red-cheeked suslik in different seasons of the year. ECL, EC, D, and D_1 cells, differing in the structure of their granules, were found. Relatively unchanged cells, cells with weak function, and cells with various degrees of changes, some of them dying, were found in hibernating susliks. The presence of actively functioning EC cells in this period confirms the view that peripheral serotonin influences the mechanism of hibernation. On the 3rd or 4th day of awakening marked activation of rejuvenation of the endocrine cells was observed; the normal ultrastructure of the cells was restored by the second week of awakening from hibernation.

KEY WORDS: endocrine cells; granules; cisterns of the lamellar complex; tubules of the granular endoplasmic reticulum.

Several types of endocrine cells have been described in the fundal glands of the gastric mucosa in mammals. The EC cells contain serotonin which activates the secretion of mucus in the stomach and reduces the secretion of free hydrochloric acid [5, 6]. The ECL cells of most animals do not contain detectable biogenic amines, and only in rats and mice do they contain histamine [7, 8], a stimulator of gastric secretion and, in particular, the final mediator of the parietal cells [6, 8]. The D cells secrete the polypeptide hormone somatostatin, which inhibits gastric secretion, and the vasoactive peptide VIP [5, 12]. The D_1 cells produce GIP, a gastric polypeptide which inhibits the secretion and contractions of the stomach [11]. AL cells contain enteroglucagon, which evidently inhibits gastric secretion [3, 6].

The object of this investigation was the electron-microscopic identification of the endocrine cells of the fundal glands of the stomach in a hibernating rodent and to study their ultrastructure and changes in the course of the year.

EXPERIMENTAL METHOD

Areas of the greater curvature of the stomach in the region of the fundus (short glands) and body (long glands) were studied in the red-cheeked suslik *Citellus major* (Pallas), which is distinguished by a long period of hibernation (7-8 months) in the active summer period, during hibernation, and 1 and 3-4 days and 2 weeks after awakening. The mucous membrane was fixed in 3% glutaraldehyde in 0.1M phosphate buffer, pH 7.4, 1% OsO_4, and embedded in Epon-Araldite in the usual way. The sections were stained with uranyl acetate and lead citrate and studied in the IEM-100C electron microscope.

EXPERIMENTAL RESULTS

ECL, EC, D, and D_1 cells were discovered. No AL cells were present in the fundal glands of the suslik. The endocrine cells were located mainly in the terminal portions of the glands, on the basement membrane. ECL cells occasionally made contact with the lumen of the glands, but the EC, D, and D_1 cells never made contact in this way. The principal distinguishing feature was the presence of granules, characterized by a particular size, shape, and density (Figs. 1a, 2a, 3a, b). Differences also were found in the methods of formation of granules and liberation of secretion from the cell. The ultrastructure of the main organoids of the endocrine cells was similar.

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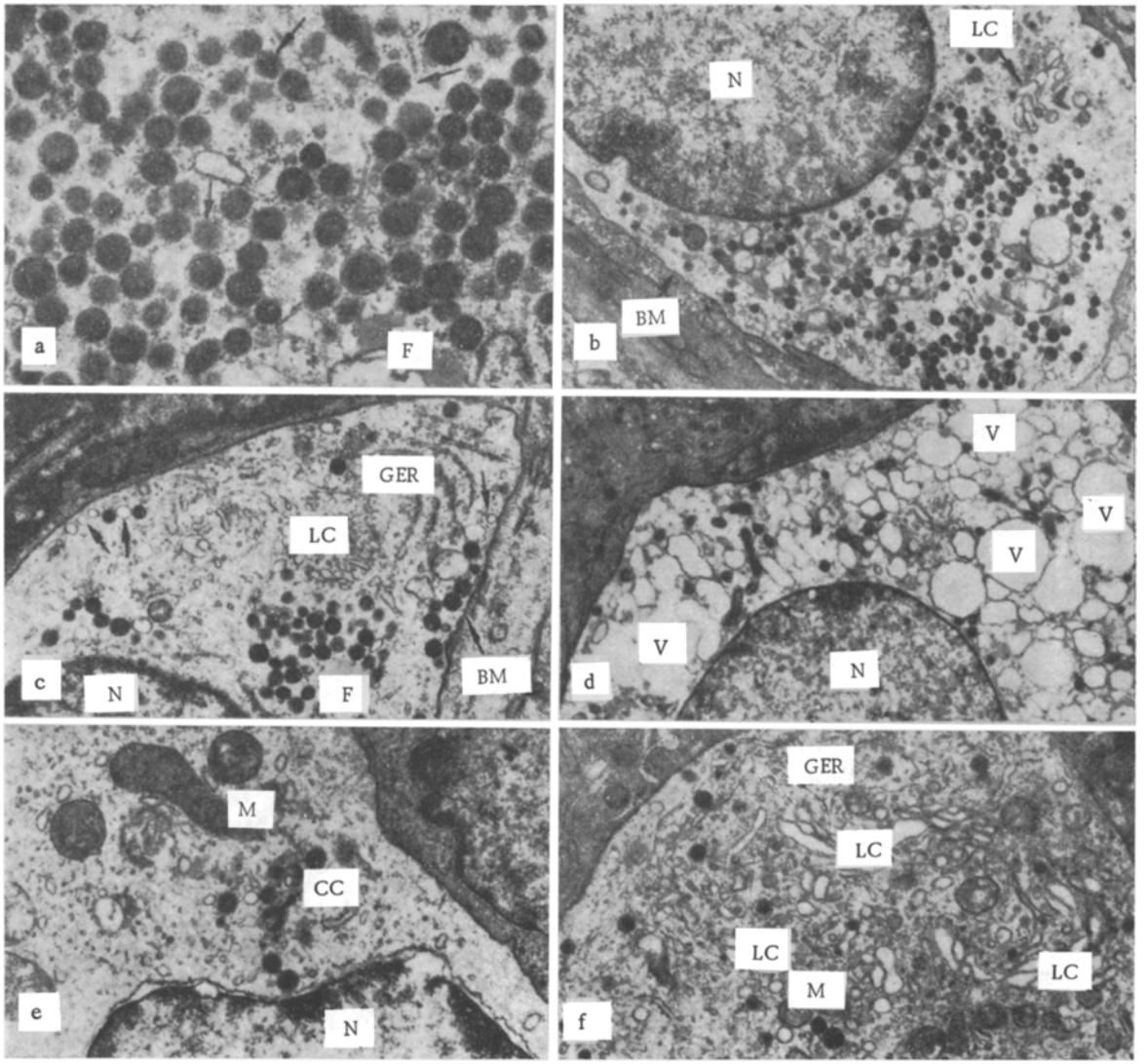


Fig. 1. ECL cells: a) granules (arrow indicates dissolving granules) (24,900 ×); b) cell in active summer period (7500 ×); c) weakly functioning cell during hibernation (arrow indicates progranules) (12,450 ×); d) vacuolated cell during hibernation (7500 ×); e) young cell on 4th day of awakening (12,450 ×); f) young active cell on 4th day of awakening (12,450 ×). Here and in Figs. 2 and 3: N) nucleus; LC) lamellar complex; GER) granular endoplasmic reticulum; M) mitochondria; BM) basement membrane; V) vacuole; CC) cell center; F) fat.

The contents of the mature granules of the ECL cells (Fig. 1a) of average electron density were surrounded by a membrane, which either was very closely apposed to them or separated from them by a narrow electron-translucent halo. The dimensions of the granules were 180-250 nm and they were round in shape. Liberation of secretion took place by emiocytosis and by lysis of the granules in the cytoplasm of the cell itself. The number of granules varied from 1 or 2 to large numbers, they were unevenly distributed (Fig. 1b), and formed clusters. The lamellar complex was located in the supranuclear part, and there were up to five complexes per section; each complex consisted of long parallel cisterns which could be either completely or partly dilated. They were surrounded by numerous tiny gray vesicles and by a few medium-sized electron-translucent vesicles which were evidently progranules. The granular endoplasmic reticulum (GER) consisted of long and short dilated tubules. Large vesicles containing single ribosomes were rare. The GER was located mainly in the apical part around the zone of the lamellar complex.

Many polysomes were present near the tubules of GER and the nuclear membrane. The mitochondria were narrow, long, and round, and medium-sized or large. Their matrix was electron-

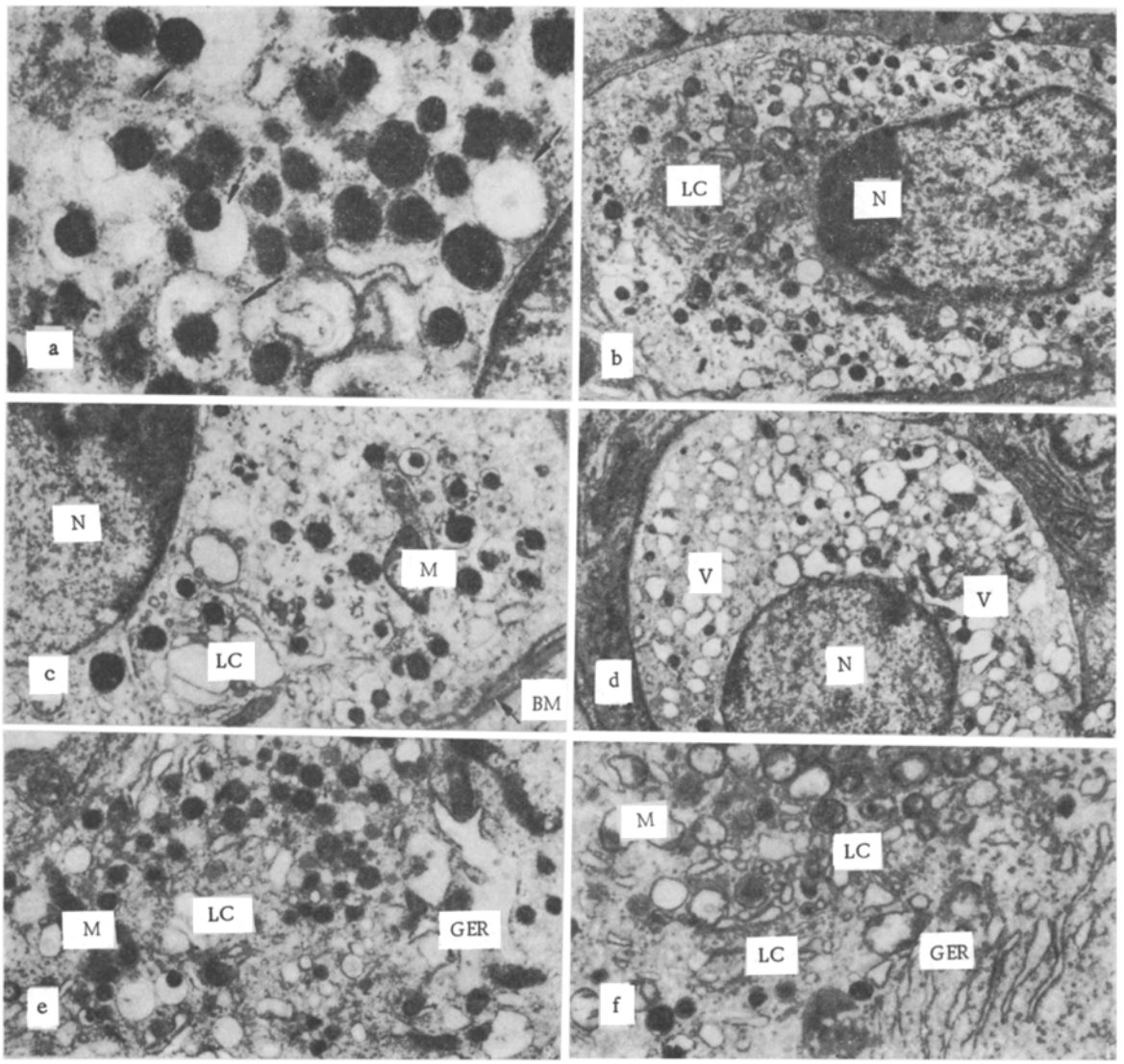


Fig. 2. EC Cells: a) granules (arrow indicates dissolving granules) (24,900 ×); b) cell in active summer period (7500 ×); c) weakly functioning cell during hibernation (12,450 ×); d) vacuolated cell in period of hibernation (7500 ×); e) actively functioning cell during hibernation (12,450 ×); f) young active cell on 4th day of awakening (12,450 ×).

pale and slightly granular, and the few cristae were arranged transversely, either singly or in large clusters. In some large mitochondria the matrix and cristae were completely destroyed and only double membranes were left (this was evidently the result of fixation). Lysosomes were observed in nearly all sections, a few loosely arranged myelin figures could be seen, and droplets of fat were present. A cell center and a few microtubules were present.

Mature granules of EC cells (Fig. 2a) contained material of high electron density; the granules were polymorphic but mainly round in shape and a halo was present. They measured 280-400 nm. Patterns of emiocytosis of mature granules were not observed. Several variants of lysis of the granules in the cytoplasm were noted. The cells contained different numbers of granules, most of which were either in a state of lysis or in a mature state, evidently depending on differences in the functional state of the cell. The EC cells (Fig. 2b) were characterized by a less clearly defined GER than the ECL cells and by the absence of fat.

A distinguishing feature of the D cells was the variation in size and density of the granules (Fig. 3a). They measured 300-400 nm. Compared with the EC and ECL cells, their electron-density was weaker, but within the group of D cells the granules varied considerably

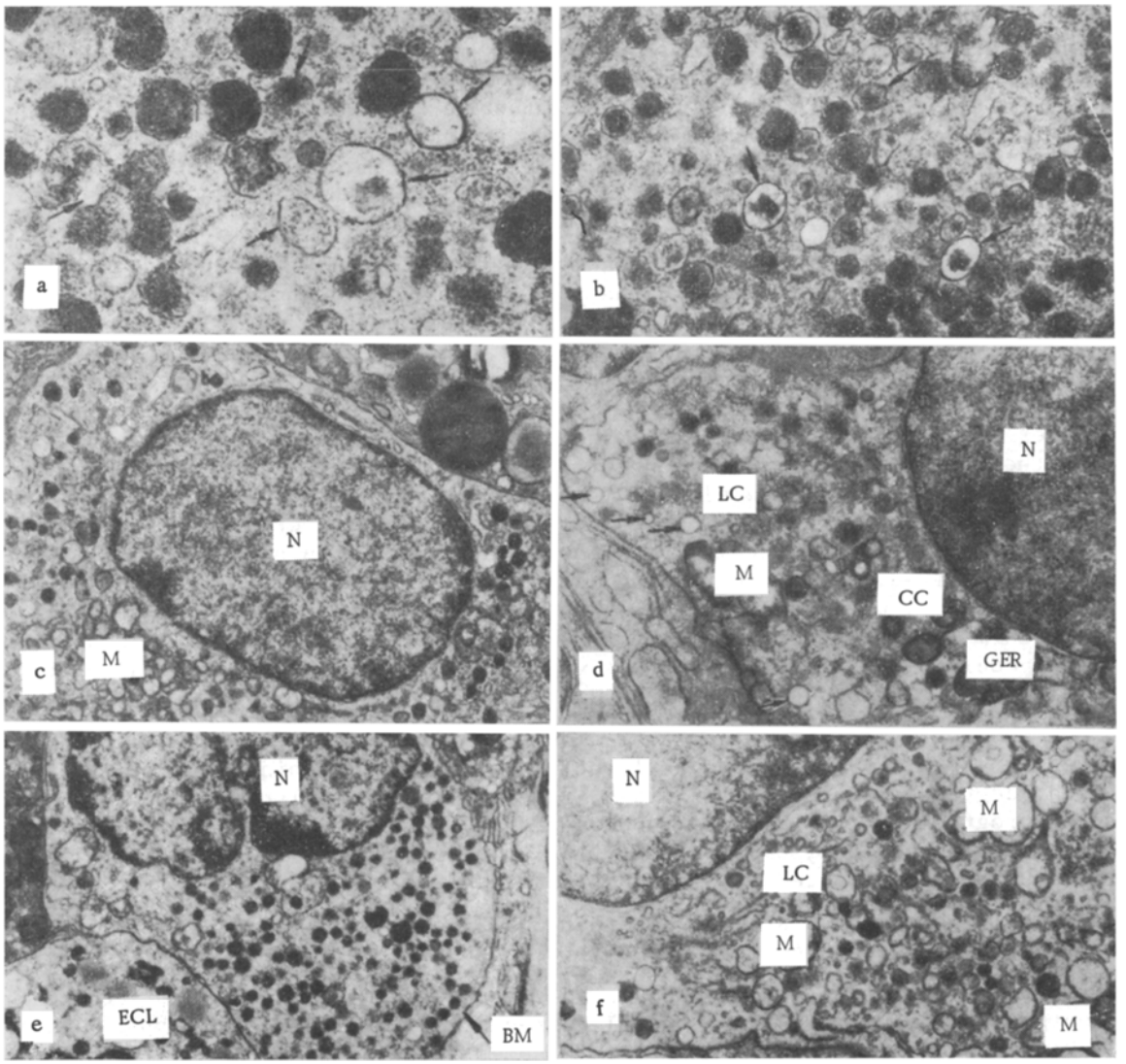


Fig. 3. D and D_1 cells: a) granules of D cells (24,900 ×); b) granules of D cells (arrow indicates dissolving granules) (24,900 ×); c) actively functioning D cell in summer period (7500 ×); d) weakly functioning D cell during hibernation (arrow indicates progranules) (12,450 ×); D storage cell in hibernation (7500 ×); f) active D cell on 4th day of awakening (12,450 ×).

in density. The D_1 cells contained granules like D cells, but they were smaller in size (190-280 nm; Fig. 3b). Since the D_1 cells were single and, morphologically, did not differ significantly from the D cells, they will be described together. Emiocytosis of the granules was observed in D and D_1 cells more frequently than in the others. Besides actively functioning cells (Fig. 3c), storage cells could also be distinguished in this group by the presence of many densely packed granules in the basal part, so that the granules themselves became polygonal in shape, the nucleus was displaced into the apical part, and the lamellar complex and GER were ill-defined.

The epithelium of the fundal glands of the stomach of the animals in a state of hibernation contained endocrine cells which ranged from those showing little change from cells in the active state to cells with well-marked, profound and, evidently, irreversible changes.

The relatively unchanged cells (and these were the majority) were evidently weakly functioning and contained a few granules at all stages of maturity and lysis (Fig. 1c, 2c, 3b). The cisterns of the lamellar complex were short, dilated, and few in number. The surrounding gray vesicles were smaller than in the active state and the number of progranules was increased, especially in the ECL and D cells; they became large and, together with

granules, they were aligned along the cell membrane (Fig. 1c). The GER consisted of a few flat tubules with occasional ribosomes, although some smooth branching membranes with solitary ribosomes also were seen. There were fewer free ribosomes, but among them there were some polysomes. The mitochondria were fewer in number, the large ones were vacuolated, and the matrix of the medium-sized and small mitochondria was flattened. The nucleus was pale on account of a reduction in the chromatin. The lysosomes were increased in number and size. The fat droplets were larger in the D cells and, in particular, in the ECL cells (in the EC cells, as before, they were absent). The cytoplasmic matrix was relatively translucent.

The more profound changes in the organelles (Fig. 1d, 2d) were expressed as follows. The number of granules was reduced and the young and mature granules disappeared. The cisterns of the lamellar complex were shortened and flattened and their number and the number of surrounding vesicles of all types were reduced. Vacuolation of the tubules of GER took place. The number of free ribosomes was reduced and the polysomes disappeared. The number of mitochondria was reduced, the matrix of those which were left was denser still, and the cristae were longitudinal in direction. The number of large vacuoles was increased: In most cells they were formed from mitochondria and the GER, only in EC cells through hyperplasia and hypertrophy of the lamellar complex, and in EC and D cells from membranes remaining after lysis of the granules. The nucleus was considerably enlarged, rounded, and pale. The perinuclear space was widened. Lysosomes were increased in number and size and dense myelin bodies appeared. The integrity of the cytoplasmic matrix was disturbed. As these processes progressed, ultimately the cells became greatly enlarged, their vacuoles joined together to form larger ones, and death of the cell took place. Profound changes and death of the cells could also take place without vacuolation, through clearing and disturbance of the integrity of the cytoplasmic matrix and organoids.

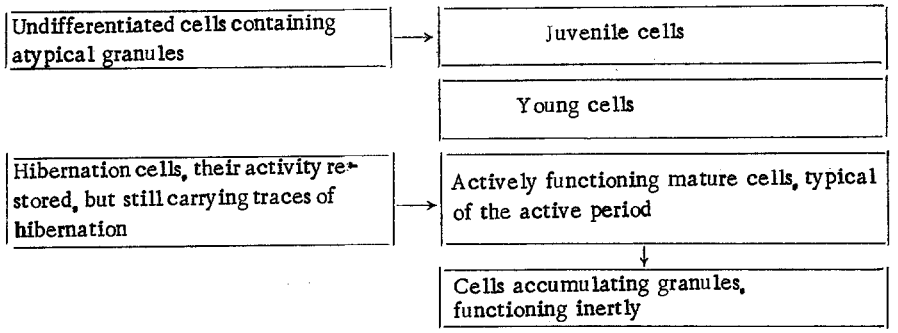
It must be specially emphasized that among the EC cells in the period of hibernation there were some (Fig. 2e) which were evidently actively functioning. They contained granules of different degrees of maturity and lysis, a well-marked lamellar complex with numerous surrounding progranules, and dilated tubules of the GER with many ribosomes. There were many free ribosomes and polysomes but only isolated lysosomes. The nucleus and cytoplasmic matrix were unchanged compared with the active state.

The D cells were most stable in hibernation. They showed the least marked changes and sometimes almost unchanged storage cells were found (Fig. 3e).

On the first day of awakening changes in the endocrine cells compared with the period of hibernation were minimal. Only a small degree of activation was observed: development of the GER and conversion of all the free ribosomes into polysomes. The number of residual bodies increased. By the 3rd or 4th day of awakening numerous undifferentiated cells and cells in various stages of differentiation appeared: juvenile cells (Fig. 1e) containing single typical granules, a developed lamellar complex, GER, numerous polysomes, large mitochondria only, a highly chromatized nucleus, cell center, microtubules; young cells (Figs. 1f, 2f, 3f) with hyperplasia and hypertrophy of the lamellar complex and GER and with young granules; actively functioning cells typical of the active period; mature endocrine cells with more or less well marked traces of hibernation.

In the group of D cells the degree of rejuvenation was not so marked as in the ECL and EC cells. Solitary juvenile D cells were observed and young cells were rare.

The formation and function of the endocrine cells during this period can be represented by the accompanying scheme:



After 2 weeks of awakening, compared with the active summer period, the number of young cells was a little greater. The remaining endocrine cells were typical of the active state and there were no cells with signs of hibernation.

Four types of endocrine cells — ECL, EC, D, and D_1, differing chiefly in the structure of their granules — were thus present in the gastric fundal glands of the red-cheeked suslik. During hibernation all underwent changes of different degrees of intensity. The presence of actively functioning EC cells in the period of hibernation is in agreement with earlier observation [1, 2] and with the observations of Popova and Naumenko [4], who found that peripheral serotonin affects the maintenance of hibernation. During awakening, on the first day, weak activation of the organelles was observed, and by the 3rd-4th day activity reached a peak and renewal of the endocrine cells took place from undifferentiated cells. After 2 weeks the endocrine cells were virtually restored to their state in the active period.

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