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Ultrastructure of pancreatic exocrine cells of the rat during starvation

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Summary. Ultrastructural changes of the pancreatic exocrine cells after 3, 7, 14, 21, 28, 35 and 42 days of starvation were observed in male rats aged from 16 to 18 months weighing between 600 and 700 grams. The number of zymogen granules after starvation decreased to less than about 70 per cent of that of the control. Changes in the rough endoplasmic reticulum were hardly seen up to 14 days of starvation as compared with the control, but were observed in the apical and basal cytoplasm of the cell from 21 days after starvation. Particularly in 35- and 42-day starved rats, the rough endoplasmic reticulum was frequently shortened and dilated, and changed to disorganized membranous structures. The lysosomes in the apical cytoplasm of the cell gradually increased in number after starvation, and contact or fusion between the zymogen granules and lysosomes (viz. so-called crinophagy) was often seen at 35 and 42 days of starvation. Large autolysosomes especially those containing zymogen granules and rough endoplasmic reticulum were also marked in the basal cytoplasm of the cell after 35 and 42 days of starvation. Alterations in the basal cytoplasm of the cell appeared later than those in the apical cytoplasm. It was considered that, owing to its role in protein synthesis, the basal cytoplasm of the pancreatic exocrine cells in starved rats might be protected as far as possible during long-term starvation.

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Introduction

Since Jackson (1925) microscopically described in review the atrophy of pancreatic acini in human and animals during total inanition (or on water only), there have been many reports about the effects of starvation on mammalian pancreatic exocrine cells. At the light-microscopic level, diminution in the number and the change in the shape of mitochondria, loss of zymogen granules and vacuolar degeneration of rat pancreatic exocrine cells under short-term (150 hours) starvation have been observed (Paradisi and Cavazzuti, 1965). Also Bencosme and Lazarus (1956) have reported, that long-term (up to 48 days) starvation of rabbits resulted in vacuolation and loss of basophilic substance in pancreatic exocrine cells, but did not describe other changes in detail. At the electron-microscopic level, several papers have reported on the decreased numbers of zymogen granules, the depletion and irregular arrangement of rough endoplasmic reticulum (rER), the random detachment of ribosomes from ER, or the appearance of autophagic bodies in pancreatic exocrine cells of young adult rodents under starvation (Weiss, 1953; Kapeller et al., 1971; Nevalainen and Janigan, 1974; Watari, 1982). However, the lengths of starvation period in their experiments were short (up to 7 days) and there have been no detailed descriptions of the ultrastructure of the pancreatic exocrine cells in mammals under starvation of longer duration.

In the absorptive cells of the adult rat intestine during long-term (up to 21 days) starvation, short and sparse rER and many autolysosomes were found in the basal cytoplasm,

but the ultrastructure of the apical cytoplasm was unchanged compared with that of control rats (Sohma, 1983). In secretory cells such as the exocrine pancreas, the basal cytoplasm occupied by well-developed rER is known to play an important role in protein synthesis.

In view of the above, the present study was planned to observe the ultrastructure of the apical and basal cytoplasm in adult rat pancreatic exocrine cells following long-term (up to 42 days) starvation for various lengths of time. In addition, the number of zymogen granules was counted and acid phosphatase (AcPase) activity demonstrated at the electron-microscopic level in the pancreatic exocrine cells of control and starved rats.

Materials and methods

Thirty-two male rats of the Sprague-Dawley strain (aged from 16 to 18 months, weighing between 600 and 700 grams) were used in this study. Eight groups of four rats each were starved for 12 hours (control), 3, 7, 14, 21, 28, 35 and 42 days, respectively. They were placed singly, without food, in metal cages with wire-mesh floors (to prevent coprophagy) under normal laboratory conditions (room temperature: 20-25° C). During the periods of starvation, they were freely given tap water and Ringer solution. Prior to this, the same experiments had been carried out on 10-week-old rats (about 300 grams, Sprague-Dawley); however, as they died within 7 days, no data could be obtained.

All rats were anesthetized by an intraperitoneal injection of Nembutal at a dose of 3.0 mg per 100 g body weight between 9:00 and 11:00 a.m. After laparotomy and thoracotomy, the rats were perfused through the left ventricle of the heart with modified Karnovsky's fixative containing 4.0% paraformaldehyde and 1.25% glutaraldehyde in 0.1 mol/l cacodylate buffer (pH 7.4). The same fixative was also applied to the surface of the pancreas. The pancreatic tissue was removed, cut into blocks immediately and immersed in the same fixative for an additional 2 hours at 4° C. The blocks to be used for electron-microscopy were post-fixed in 1% osmium tetroxide in the same buffer solution for 1 hour, and then dehydrated with a graded series of ethanol solution and embedded in Epon 812. Ultrathin (0.05 μm) sections were cut with a Reichert OmU3 ultramicrotome, picked up on formvar-coated single-hole grids, stained with uranyl acetate and lead citrate, and examined with a JEM 100S type electron microscope. For light-microscopy, 1.0 μm-thin Epon sections were stained with 1% toluidine blue and photographed.

In addition, some samples for study of AcPase activity were fixed in the same fixative for 30 minutes. Blocks were washed overnight in the same buffer solution, and sectioned at 30-40 μm on a freezing microtome. The sections were incubated at pH 7.4 for 20 minutes at 37° C in Barka and Anderson's modification (1963) of Gomori's medium with 8.2 mmol/l β-glycerophosphate as substrate. In this case, the control sections were incubated with the substrate-free medium. The incubated sections were post-fixed in 1% osmium tetroxide in the same buffer solution for 1 hour, and then dehydrated and embedded in the same way. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined in an electron microcospe.

Some electron micrographs of the pancreatic exocrine cells were prepared at a magnification of ×4,000 in order to count the number of zymogen granules. Only cells which could be followed from the base to the acinar lumen and in which the nucleus could be seen, were used. About two hundred control cells and about two hundred cells from each starved group were selected; zymogen granules which had a round or oval shape and uniform electron density were counted regardless of size.

Results

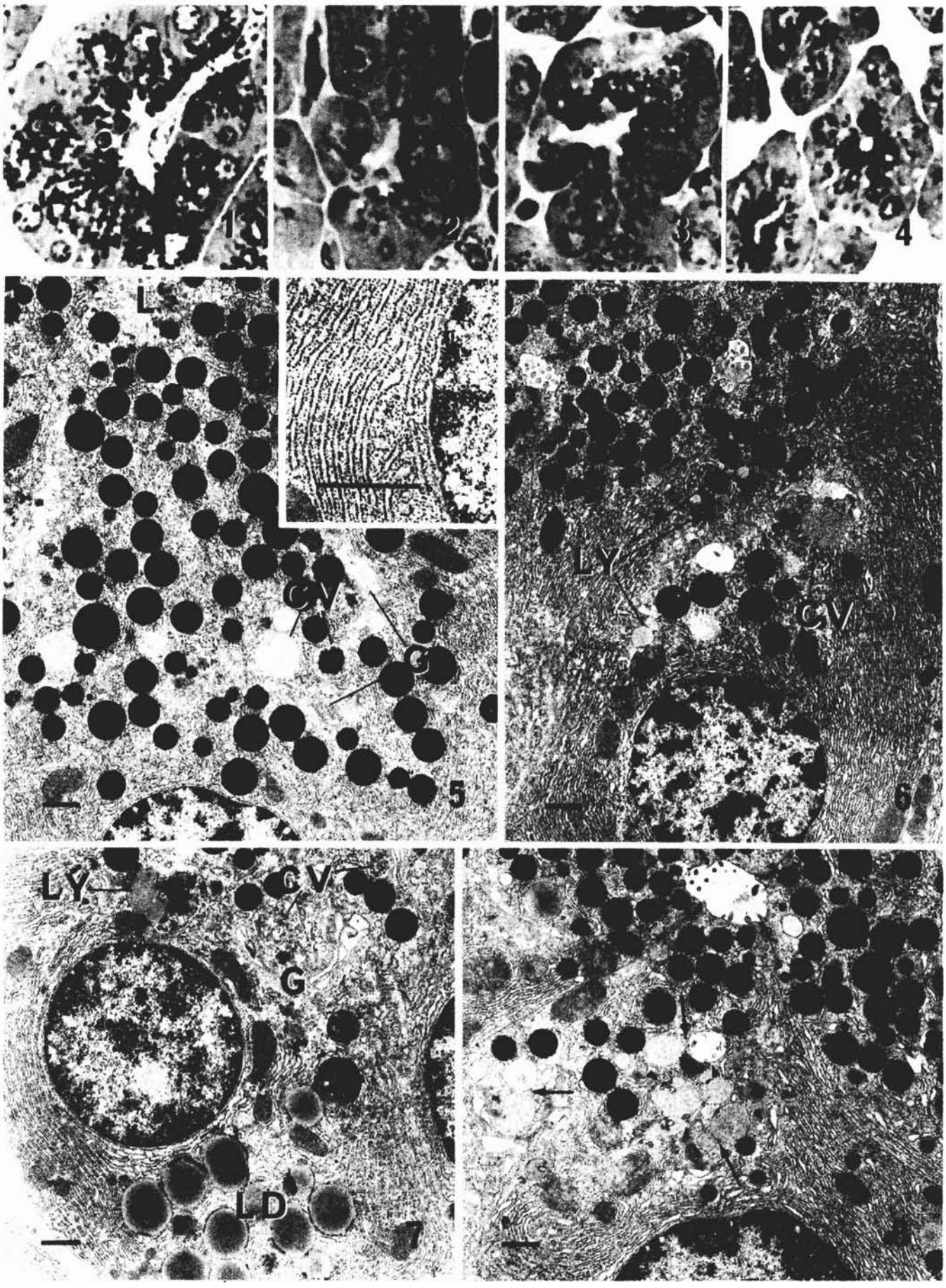
Light-rnicroscopic observations

The pancreatic exocrine cells of control rats aged from 16 to 18 months had numerous zymogen granules spread in the apical region of the cytoplasm (Fig. 1). The nucleus was usually situated in the basal half of the cell. In 3day starved rats, zymogen granules were markedly smaller in number than in control rats and were restricted to the apical portion of the cell (Fig. 2). The pancreatic acinus and its cells appeared to atrophy by degrees from 14 to 21 days after starvation (Fig. 3). Most of the acini and pancreatic exocrine cells were markedly reduced in size in the 35- and 42-day starved rats (Fig. 4), and some bulbous shaped exocrine cells isolated from acini were present. On the other hand, the acinar lumen appeared dominant due to the atrophy of the cells.

Electron-rnicroscopic observations

Control

As illustrated in Figure 5, pancreatic exocrine cells of control rats were characterized by numerous zymogen granules (smoothly rounded formations with a homogeneous content of high density) in the apical cytoplasm and by quantities of ribosome-attached rER, especially in the basal cytoplasm. The pancreatic exocrine cells contained an average of 47 zymogen granules (Fig. 16). Some condensing vacuoles containing secretory material of low density were seen in the vicinity of the Golgi apparatus, and a few lysosome-like bodies were detected in the apical cytoplasm of the cell. Oval and rod-like mitochondria were present in both the apical and basal parts of the cell.. The nucleus was smoothly rounded in general and tended to be slightly eccentric toward its basal portion. The acinar lumen, the surface of which formed varying numbers of microvilli, often contained secretory material.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3 days of starvation

The most prorninent ultrastructural change in the pancreatic exocrine cells of this group was a reduction in the number of zyrnogen granules (the average nurnber per cell: 17) (Figs. **6,** 16) and occasional absence of thern in the apical portion of the cell. Conversely, sorne lysosome-like bodies were observed scattered among the zymogen granules. In the basal cytoplasm of some cells, lipid droplets were detected, but they were srnall in size and nurnber. The rER and other cytoplasmic organelles were very similar to those of the control.

7 days of starvation

The average count of zymogen granules per cell was 23 (Fig. **16).** Lysosome-like bodies of almost oval shape were frequently observed in the apical portion of the cell, and occasionally contained srnall lipid inclusions. In rnost pancreatic exocrine cells, the lipid droplets lay in groups in the basal cytoplasm; moreover, the number in each group and their size had increased compared with those of 3-day starved rats. The rER, the Golgi apparatus and the rnitochondria remained apparently unchanged.

14 days of starvation

Some characteristic changes were recognized in this group. The acini began to atrophy and most of the exocrine cells were srnaller in size than those of the previous groups, although the number of exocrine cells per acinus was alrnost unchanged. The lysosorne-like bodies in the apical portion of the cell appeared to increase in number and to have various sizes, and frequently containedT large lipid inclusions. A cluster of lipid droplets which were elliptical or round, surrounded by a clear halo, were located basally in the cell (Fig. 7).

Fig. 1. Light micrograph of pancreatic tissue from control rat. Numerous zymogen granules spread widely in the apical cytoplasm of the ekocrine cells. x 1,000

Figs. 2-4. Light rnicrographs of pancreatic tissue from 3-day starved (Fig. 2), 21-day starved (Fig. 3) and 42-day starved (Fig. 4) rats. Pancreatic acinus is progressively smaller than that of control. Zymogen granules are also fewer and are located in the apical portions of the cells. x 1,000

Fig. 5. Pancreatic exocrine cells from control rat. Numerous zymogen granules containing homogeneous electron-dense material are scattered throughout the apical portion of the cell. A few condensing vacuoles (CV) are seen in the vicinity of the Golgi apparatus (G). In the apical and basal portions of the cell, a well-developed rER is dispersed. L: Acinar lumen. Stained with uranyl acetate and lead citrate. Bar: 1 µm. x 7,000

The inset shows the rER arranged roughly concentrically around the nucleus in the basal portion of the cell. Stained with uranyl acetate and lead citrate. Bar: 1 pm. x 20,000

Fig. 6. Pancreatic exocrine cells from rat starved for 3 days. A small number of zymogen granules can be seen in a localized area near the acinar lumen. There are a few lysosome-like bodies (LY) in the apical portion of the cell. CV: Condensing vacuole. Stained with uranyl acetate and lead citrate. Bar: 1 µm. x 7,000

Fig. 7. Pancreatic exocrine cells from rat starved for 14 days. A relatively large lysosome-like body (LY) with lipid inclusion lies superior to the nucleus, and a few condensing vacuoles (CV) are observed in the Golgi area (G). In the basal portion of the cell, there is a cluster of lipid droplets (LD). Stained with uranyl acetate and lead citrate. Bar: 1 µm. x 7,000

Fig. 8. Pancreatic exocrine cells from rat starved for 28 days. The condensing vacuoles vary in size and in the electron density of the secretory material, some of which are noted fusing with each other (arrows) and containing vesicles (arrowheads). Stained with uranyl acetate and lead citrate. Bar: 1 pm. x 7,000

Fig. 9. Pancreatic acinar cells from rat starved for 35 days. The cytoplasm of one exocrine cell(*) is coarse and low in density, and the rER is shortened and dilated in cisternae. The other pancreatic exocrine cells are relatively well maintained. CAC: Centroacinar cell. Stained with uranyl acetate and lead citrate. Bar: 1 µm. x 9,000

Fig. 10. A pancratic exocrine cell from rat starved for 35 days. A large autolysosome-like body containing a zymogen granule. and degenerated cytoplasm can be seen. Stained with uranyl acetate and lead citrate. Bar: 1 µm. x 12,000

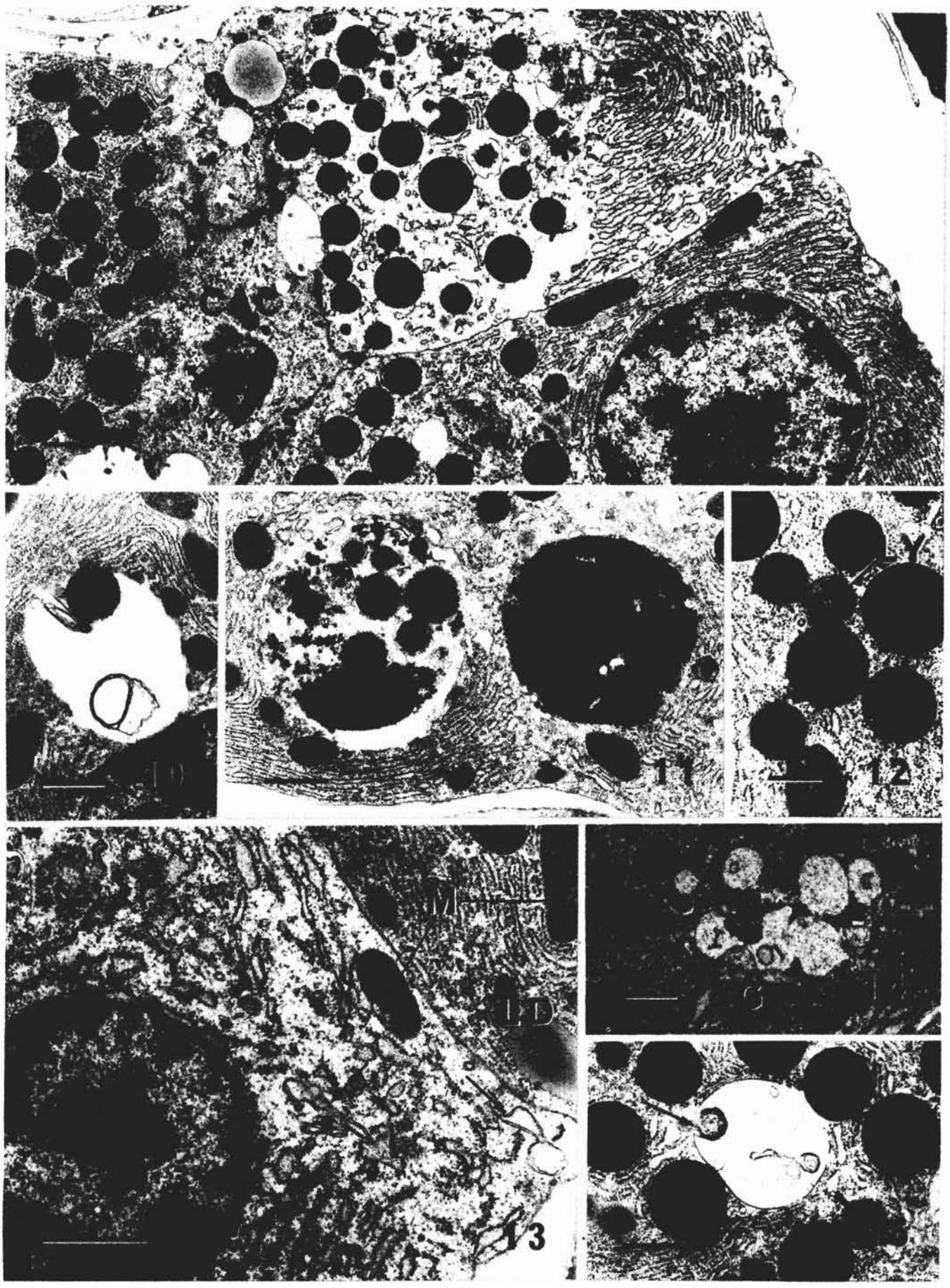
Fig. 11. The basal portion of a pancreatic exocrine cell frorn rat starved for 35 days. Two large autolysosome-like bodies containing some spherical structures of granules, vesicles and degenerated rER. Stained with uranyl acetate and lead citrate. Bar: 1 pm. x 12,000

Fig. 12. A pancreatic exocrine cell from rat starved for 42 days. AcPase activity. A lysosome (LY) having AcPase activity shows fusion with a zymogen granule. Stained with uranyl acetate and lead citrate. Bar: 0.5 pm. x 20,000

Fig. 13. Basal cytoplasm in pancreatic exocrine cells from rat starved for 42 days. The rER in the left cell is shortened and focally dilated; its arrangement is irregular. Disorganized membranous structures are noted (arrows). M: Mitochondria. LD: Lipid droplet. Stained with uranyl acetate and lead citrate. Bar: 1 µm. x 20,000

Fig. 14. The apical portion of a pancreatic exocrine cell from rat starved for 42 days. Some condensing vacuoles near the Golgi area (G) have fused with each other. Stained with uranyl acetate and lead citrate. Bar: 1 pm. x 10,000

Fig. 15. The apical portion of a pancreatic exocrine cell from rat starved for 42 days. A membrane-bounded vacuole, which fuses or contacts with zymogen granules and is invaginated into by degenerated cytoplasm (arrow), can be seen. Stained with uranyl acetate and lead citrate. Bar: 1 pm. x 16,000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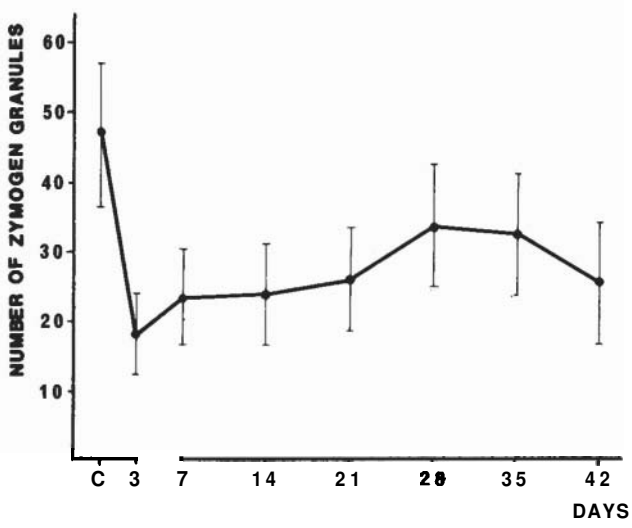


Fig. 16. Mean number of zymogen granules per cell of exocrine pancreas and standard deviation in control rats (C) and starved rats for 3, 7, 14, 21, 28, 35 and 42 days. The differences between the average counts in control and starved rats are significant at < 0.001 level *(t test)*.

They were most abundant at this stage of starvation. The average number of 24 zymogen granules per cell did not significantly differ from the 7-day starved group (Fig. 16). Condensing vacuoles were markedly present close to the Golgi apparatus. The Golgi region seemed to be more prominent but there was no consistent alteration in the rER, the mitochondria or the nucleus.

21 days of starvation

The gradual reduction in the size of pancreatic exocrine cells was clearly more evident and the acinar lumen appeared to be dominant. No obvious changes were seen in the shape and number of zyrnogen granules as compared with the 14-day starved group, the average number per cell in the 21-day starved group being 26 (Fig. 16). On the other hand, the condensing vacuoles had increased in number compared to the 14-day starved group. The vacuoles were sometimes elongated or oval and rarely appeared to be in contact with a lysosome-like body in the apical region of the cell. A further significant change was that in some cells the cisternae of the rER were shortened and arranged irregularly, not merely in the apical cytoplasm but also in the basal cytoplasm of the cell.

28 days of starvation

Under such long-term starvation most of the cellular organelles in at least half of the pancreatic exocrine cells underwent degenerative changes to some extent. However, the average count of zymogen granules was 34 per cell (Fig. 16). In addition, there was marked variability in the size of zymogen granules in one cell; smaller granules were rarely detected. Furthermore, as illustrated in Figure 8, the condensing vacuoles showed characteristic changes, increasing in number in the vicinity of the dilated Golgi apparatus and varying in size and in the electron densiiy of their secretory material. Vacuoles containing some vesicles, and which fused with each other, were often noted; the formation of large vacuolar structures was, however, seldom seen. Moreover, the rER gradually degenerated: the cisternae became focally dilated, appeared vesicular and saccular tangentially and the space between cisternae increased greatly in some sections.

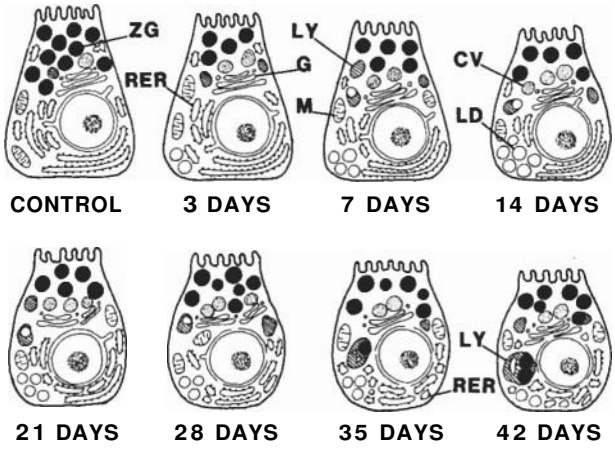
35 and 42 days of starvation

Figures 9-15 show the changes in pancreatic exocrine cells from 35- and 42-day starved rats. Between the two groups, there were some differences in the extent of degeneration of the cells; naturally, the damage to the cell in the 42-day starved group was slightly greater than that in the 35-day starved group. However, fundamentally the ultrastructural changes in the cell were extremely similar in both groups. As illustrated in Figure 9, in one and the same acinus there were cells maintaining their ultrastructure with relatively little alteration, and others severely damaged, the cytoplasm of which had become coarse, showed sparse electron density and had vacuolar changes.

The number of zymogen granules in the 35-day starved rats was very similar to that in the 28-day starved rats (the average number per cell: 33); however, that in the 42-day starved rats had decreased to 26 per cell (Fig. 16). The zymogen granules were of various sizes. Occasionally, zymogen granules in contact with each other were detected. The condensing vacuoles, which were immature granules with secretory material of low electron density, were observed near the Golgi area. Frequently, fusion of several vacuoles was observed, often producing a large and irregular vacuole (Fig. 14).

In the conspicuously degenerated cytoplasm, the rER was heavily damaged. The rER occasionally resembled a normal ultrastructure, but generally most of them underwent great changes which had not been found in previous Stages. The rER was reduced in amount and length, the cisternae were irregularly dilated, less compact and had changed to disorganized membranous structures and the elements of rER were widely separated (Figs. 9, 13). In addition, ribosomes detached from ER were observed.

Large lysosome-like bodies were found on the apical and lateral sides of the nucleus and in the basal part of the cell. As shown in Figure 15, a vacuole, which had fused or contacted with zymogen granules and was invaginated info by degenerated cytoplasm, was seen in the apical part of the cell. On the other hand, large lysosome-like bodies (about 4 μm in diameter) were found in the lateral and basal regions of the nucleus and they involved not only degenerated rER, vesicles and vacuoles but also many zymogen granules (Figs. 10, 11).



Lysosomes having the reaction products of AcPase activity which had fused with a zymogen granule were sometimes observed (Fig. 12). Moreover, they were often enclosed by an identical limiting membrane. Colgi apparatus, mitochondria and nucleus did not show significant changes in appearance.

Discussion

At the electron-microscopic level, some authors have reported, (without counting) a decrease in the number of zymogen granules in the pancreatic exocrine cells of young adult rodents under short-term (up to 7 days) starvation (Weiss, 1953; Nevalainen and Janigan, 1974; Watari, 1982). Nevalainen and Janigan (1974) have described a decrease in the number of zymogen granules under fasting for 3 days but this most probably reflected their decreased production rather than increased intracellular breakdown. In the present study, the average number of 47 zymogen granules per cell of exocrine pancreas in control rats is comparable to the findings of previous studies using young adult rats (Geuze et al., 1973, 1974; Ermak and Rothman, 1981). Moreover, in the starved rats, the number of zymogen granules decreased twice during the course of long periods (3 days: about 40 per cent; 28 days: about 70 per cent; 42 days: about 50 per cent of control). The present results are probably due to two factors. The early loss of zymogen granules was probably the result of release outside the cell, in spite of the presence of productive capacity. The later loss seems to be due to increased autophagic activities, as will be described below.

An increase in the number of condensing vacuoles (prozymogen granules, prozymogen vacuoles, irnmature zymogen granules) as compared with the control has previously been found in experiments with long periods of protein deprivation or deficiency in mammalian pancreatic exocrine cells (Weisblum et al., 1962, 28 days; Lazarus and Volk 1964, 1965, 21 weeks; Racela et al., 1966, 8 weeks; Svoboda et al., 1966, 85 days). On the contrary, condensing vacuoles have decreased, or not been observed, in pancreatic exocrine cells of children having Kwashiorkor, due to dietary deficiency, particularly of protein (Blackburn and Vinijchaikul, 1969). Also, fusion of condensing vacuoles (Racela et al., 1966) and discontinuity of the limiting membrane and fusion of condensing vacuoles (Blackburn and Vinijchaikul, 1969) have been reported. However, the condensing vacuoles have not been described in reports of starvation. In the present study, as the zymogen granules decreased in number, the condensing vacuoles were frequently seen in the Golgi region frorn 14 days after starvation. The condensing vacuoles then increased in number, varied in electron density, occasionally contained some vesicles, and often showed discontinuity of the limiting membrane. Furthermore, they contacted or fused with each other and became a large vacuole. It is known that the condensing vacuoles in the Colgi region can ultimately develop into zymogen granules (Caro and Palade, 1964). From the present results, it seems there rnay be little or no activity in the degenerated condensing vacuoles which would enable them to develop into mature granules in the pancreatic exocrine cells under long-term starvation.

Bencosme and Lazarus (1956) have described the loss of basophilic substance in the pancreatic exocrine cells of rabbits following long-term (up to 48 days) starvation under light-microscopic study. In electron-microscopic studies, it has been reported that short-term starvation led to alterations of rER in pancreatic exocrine cells, such as transformation into fingerprint-like structures, a reduction in quantity, irregular dilation of the cisternae and random detachment of ribosomes frorn the ER (Weiss, 1953; Nevalainen and Janigan, 1974; Watari, 1982). In this study, there is no doubt that the change in rER was the most significant influence of long-term starvation. Although degeneration of rER was hardly seen up to 14 days of starvation, in some cells the cisternae of the rER were shortened and irregularly arranged, mainly in the apical, though also in the basal cytoplasm, from 21 days of starvation. At 35 and 42 days of starvation, most rER had undergone severe degeneration. There were reductions in amount and length, and the cisternae were irregularly dilated, less cornpact and had changed into disorganized membranous structures. The rER elements were widely separated, the cells thus having a rarefaction of the basal cytoplasm. Furthermore, the number of attached ribosomes decreased. On the whole, the above results may be interpreted as indicating that long-term starvation of 35 or 42 days induced a hypoactivity of the pancreatic exocrine cells. However, in studies of protein deprivation or deficiency for long periods, some authors have reported that changes of rER were seen in the later part of the period (Weisblurn et al., 1962; Racela et al., 1966), while others have described no significant alteration in rER.

An increase of lysosomes or large autolysosomes (autophagic bodies, cytoplasmic bodies, cytoplasmic lesions) has been noted in the pancreatic exocrine cells of short-term starved rodents (Nevalainen and Janigan, 1974; Watari, 1982), in long-term artificially hibernated bats (Watari, 1968), in long-term protein deprivation or deficiency of mammals (Weisblum et al., 1962; Lazarus and Volk, 1964, 1965; Racela et al., 1966; Svoboda et al., 1966; Horie et al., 1971) and in children having Kwashiorkor (Blackburn and Vinijchaikul, 1969). Some authors have detected autolysosomes including, not only degraded cytoplasmic elements, but also structures resembling condensing vacuoles (prozymogen granules) (Lazarus and Volk, 1965; Racela et al., 1966; Blackburn and Vinijchaikul, 1969). Their descriptions, however, were not sufficiently detailed. Smith and Farquhar (1966) reported that the secretory granules in the cells of rat anterior pituitary gland were incorporated into the lysosomes by fusion. This is the process of so-called crinophagy as designated by De Duve (1969), the rnechanism being suggested as a means of degrading secretory material when exocytosis is inhibited. The existence of crinophagy has been previously described in endocrine gland cells and, more recently, reported in pancreatic exocrine cells of mouse administered with ethionine (Koike et al., 1982) and in exocrine pancreas culture cells of the hamster (Resau et al., 1983, 1984).

After starvation, some round or elliptical lysosome-like bodies were initially found scattered among the zymogen granules only in the apical cytoplasm of the pancreatic exocrine cells and to some extent having reaction products of AcPase activity. As the period of starvation increased, the lysosomes involving the structures resembling vacuoles increased in size and lay in the apical and basal parts of the cell. At 35 and 42 days of starvation, contact or fusion between the zymogen granules and the lysosomes was observed in the apical portion of the cell. On the lateral or basal sides of the nucleus, large autolysosomes containing vesicles, vacuoles, mitochondria, rER and secretory granules were noted. From the present study, it may be assumed that the lysosomes, the degenerated condensing vacuoles or the other cytoplasmic elements contacted and fused with each other in the supranuclear region, and then a large structure (autolysosome) began to form, in some cases containing some zymogen granules (able to be considered as so-called crinophagy). This structure was finally located in the basal region of the pancreatic exocrine cell.following long-term starvation.

In the present investigation, the state of the pancreatic exocrine cells of rat under long-term starvation was characterized by a decrease of the zymogen granule count, by the degeneration of rER and by autophagic activities including crinophagy. Furthermore, starvation in the early periods resulted in an increase of lysosomes in the apical cytoplasm of the cell. However, the basal cytoplasm, where well-developed rER is chiefly located and secretory proteins are synthesized, was relatively free from damage until later periods of starvation. This fact may reflect the principle of maintaining the most important elements of tissue under starvation in animals.

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