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Ultrastructure of Rat Pancreatic Islets in Long Term Tissue Culture

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ABSTRACT

Islets of Langerhans in atrophic pancreas of duct-ligated rats have been explanted in organ culture and 47 explants have been studied by electron microscopy. The ultrastructure was essentially preserved for at least 4 weeks of cultivation leaving aside rapidly vanishing degenerative alterations during the first few days in vitro. Two different concentrations of glucose were used (0.1 and 0.4 g/100). At both levels, β cell granules showed increased variability in shape, size and electron density. Even at the higher glucose concentration the number of granules was about the same as at the lower one.

INTRODUCTION

We have previously studied long term insulin production in vitro by explants of pancreas from duct-ligated rats. In the light microscope surviving α and β cells showed an ordinary structure for at least one month. Production of insulin, which could even be modified by changing the extracellular concentration of glucose (1) occurred during the entire period of incubation. This extension of previous studies attempts to elucidate any ultrastructural modification which islet cells may undergo in long term organ culture where their function seems to be largely intact. Some of these findings have appeared in abstract form (2).

MATERIAL AND METHODS

Sprague-Dawley rats were "partially" duct ligated (3). After 4–6 weeks when atrophy of exocrine pancreas was well advanced, small explants embedded in a spongostan matrix (4) were placed in organ culture (1).

After various periods of time (15 min; 1, 3, 6, 24 and 48 hours; 1, 2, 3 and 4 weeks) the explants were fixed in 2.5% glutaraldehyde in a phosphate buffer (pH 7.4). After washing in 7.5% sucrose in phosphate buffer (pH 7.4), the tissue was postfixed in 2% osmiumtetroxide in the same phosphate buffer. After dehydration the specimens were embedded in Epon. One micron sections were scanned under phase optics. Selected areas were then cut out for electron microscopy (Zeiss EM 9, 60 kV) on an LKB ultrotome. Twenty and 27 explants grown in 0.1 and 0.4% glucose, respectively, were examined.

RESULTS

Explants incubated 15 min – 48 hours

Already at 15 min alterations were noted in the granulated endoplasmic reticulum (GER) of some β cells. The membranes were spread apart with finely granulated material between them. This often took the form of small vacuoles which were then limited by membranes the surface of which carried ribosomes, which sometimes seemed to peel off. The cores of the β cell granules were often rectangular or otherwise irregular in shape, sometimes resembling the analogous cores of human β cells. The space between core and limiting membrane could be dilated and even contain vacuoles. Occasional fusion of β granules was observed. The mitochondriae were commonly swollen with spots of diminished electron density and deformation of the cristae.

Later on autophagosomes containing secretion granules and a certain marginal clumping of nuclear chromatin could be noted.

The β cell changes were most pronounced at 6 hours when a few of them seemed completely necrotic. At 24 hours a certain recovery had taken place, which was obvious after 2 days. The granules were the last to revert to normal appearance.

The α cells reacted in a similar way as the insulin producing element, only that the degeneration started later. A certain relative decrease in the proportion of α to β cells seemed to be caused by selective α_1 cell damage since only very few cells of the latter kind remained viable.

Heterophagocytosis of β granules was seen in oval or spindleshaped fibroblastoid elements from 6 hours or possibly earlier. The remnants of the granules assembled in complexes with another unidentified material which often was very electron dense. This change reached its peak after 1–2 days. No auto- or heterophagocytosis of α granules could be definitely established.

Large quantities of lysosomes were noted in

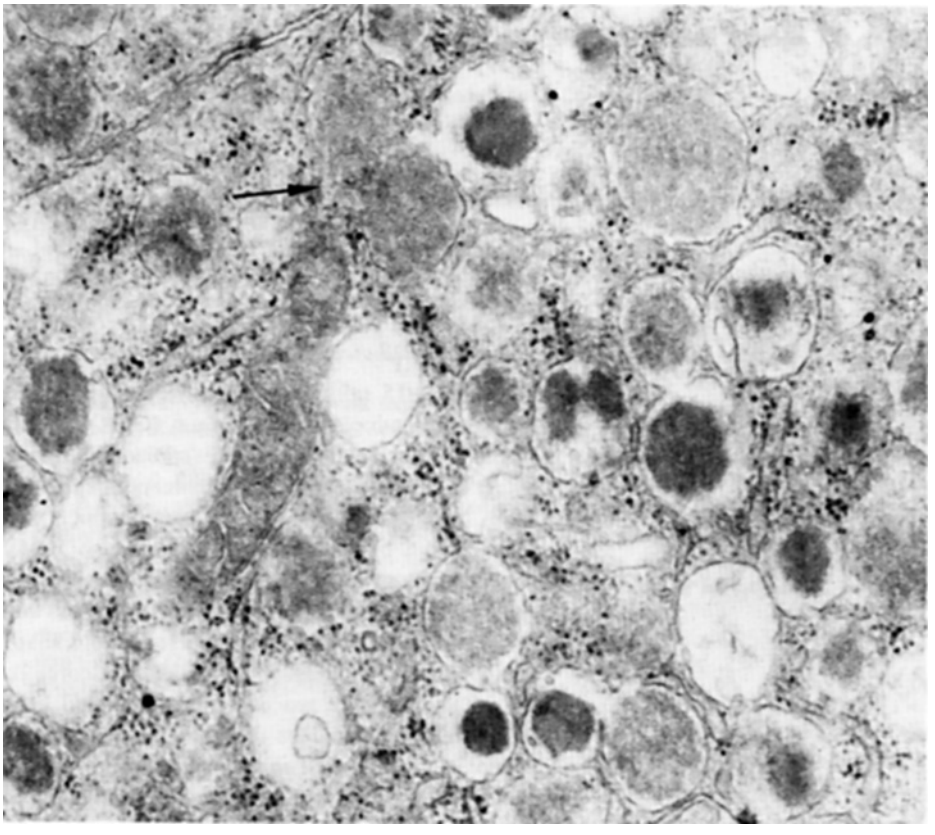

Fig. 1. Incubation time 2 weeks, glucose concentration 0.1%. β cell with an abundance of granules which vary considerably in size, shape and electron density. The limiting membrane of some granules is very indistinct (arrow) and there is also a fusion of granules. × 64 000.

islet cells, duct epithelium and macrophages already after a few hours incubation.

There was a suggestion of slightly more degeneration in the explants kept at the higher glucose concentration but this difference may not be significant.

Explants incubated 1–4 weeks

Individual islet cells. The degenerative alterations in GER and mitochondria seen shortly after explantation were only sporadic in the 1–4 weeks specimens. Generally the ultrastructure corresponded to that normally seen in vivo. Certain changes were, however, present, particularly in the β granules, which varied considerably in size, shape and electron density (Fig. 1).

One well represented type of granules had a low even electron density inside the limiting membrane. The very finely dispersed content filled the lumen more or less completely. Granules of low electron density sometimes showed indistinct limiting membranes which could even become undetectable.

Granules of high electron density in their cores were often characterized by a moderately large "empty" space between the core and the limiting membrane. This type of β granules could show considerable variations of the shape and density of the core.

The β cells could be divided into different types. One rather common variety showed no deviation from the picture in vivo with respect to the qualitative or quantitative appearance of GER, Golgi complex (GC) and granules.

Another type had well developed GER and GC with an abundance of granules showing all the individual variations referred to above (Fig. 2). Some of these β cells were darker than the rest.

A third type of β cells also contained well developed GER and GC, but here the concentration

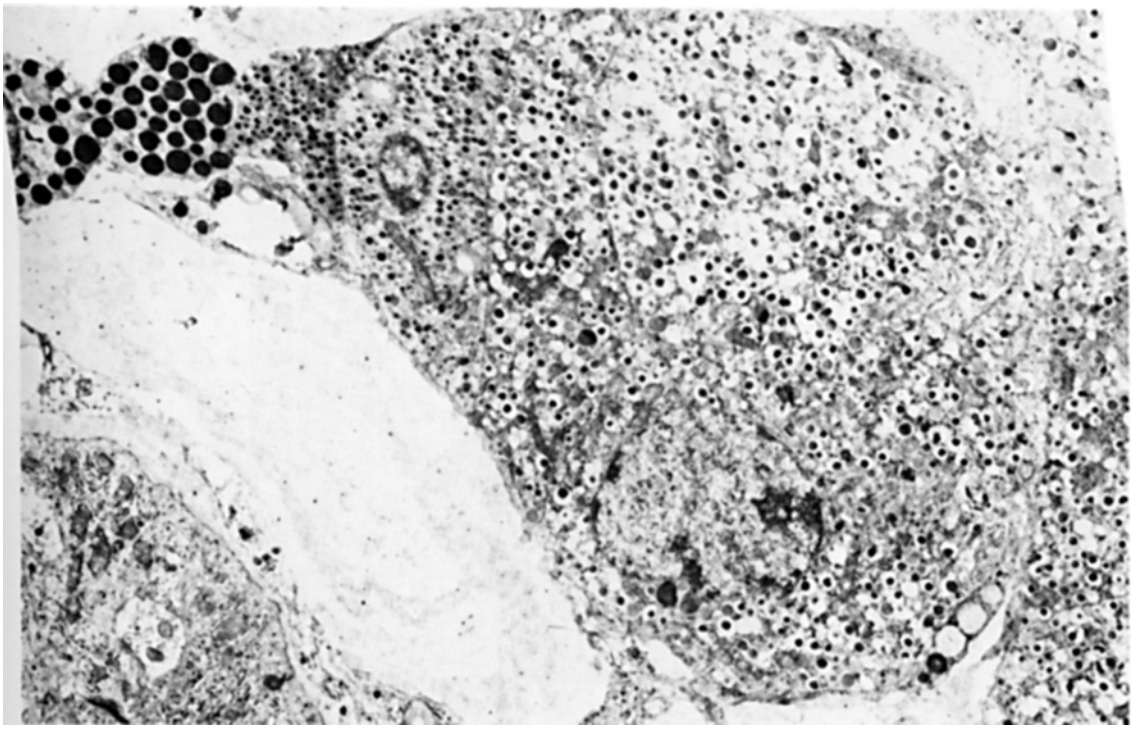

Fig. 2. Incubation time 3 weeks, glucose concentration 0.4%. Islet with an abundance of granules in the β cells. Two α_2 cells adjacent to mast cells (top left). Part of duct (bottom left) with parts of two clear cells. × 7 100.

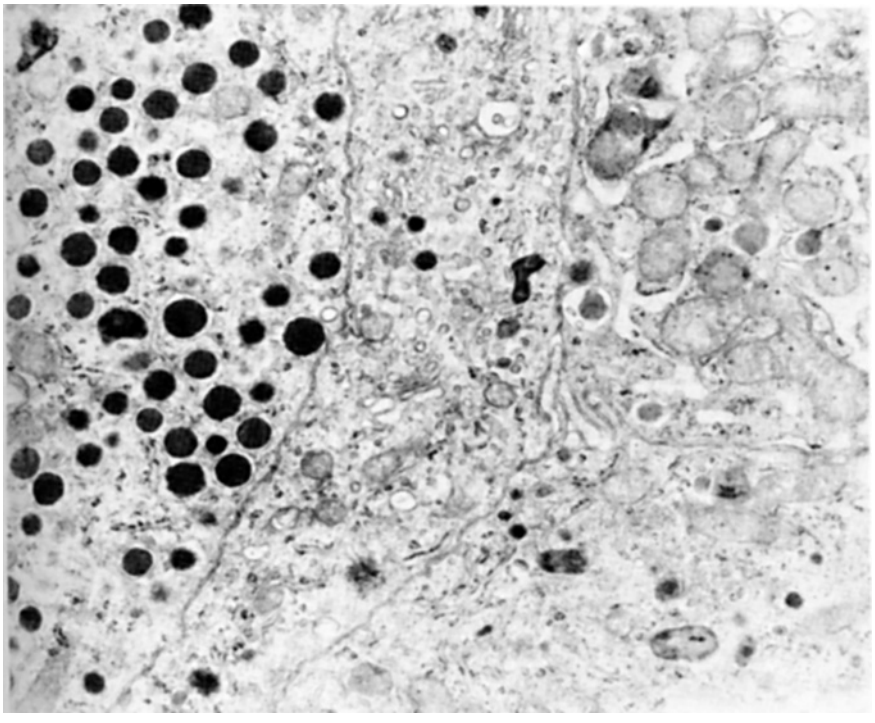

Fig. 3. Incubation time 2 weeks, glucose concentration 0.1%. α_2 cell (left) with ordinary structure on the whole. Part of B cell (top right) with sparse granules, GER-changes and numerous hypertrophic mitochondria. The other two cells (in the middle and bottom right) are probably β cells with sparse and fairly small granules. In the central cell numerous GCs with buds and coated vesicles. × 16 000.

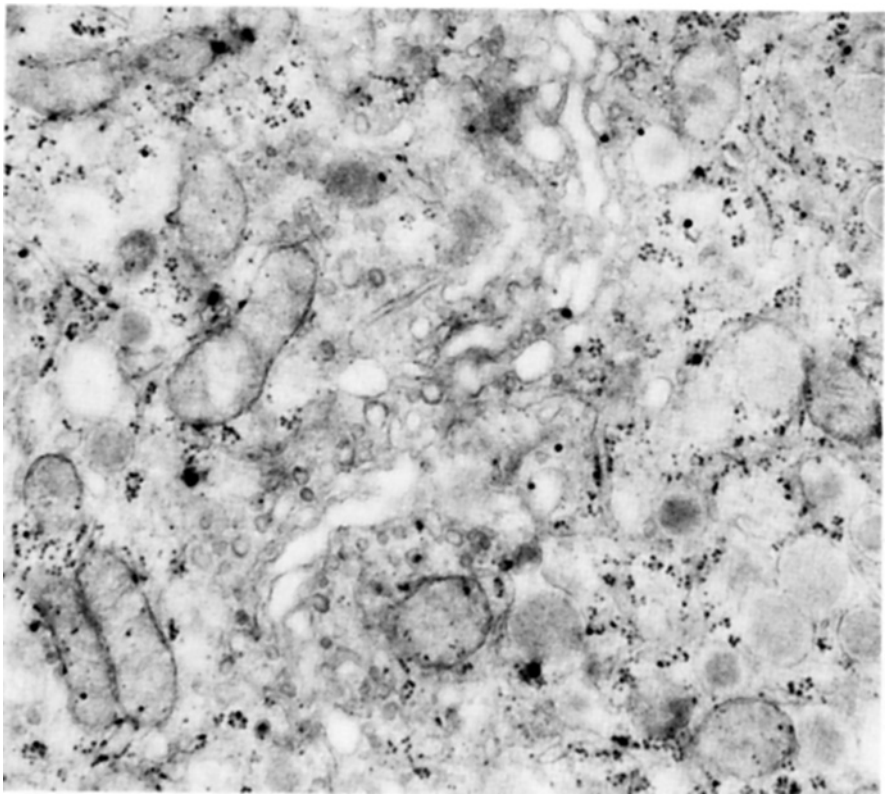

Fig. 4. Incubation time 1 week, glucose concentration 0.4%. Part of β cell with fairly abundant granules and well developed GCs with numerous coated vesicles and buds. × 26 800.

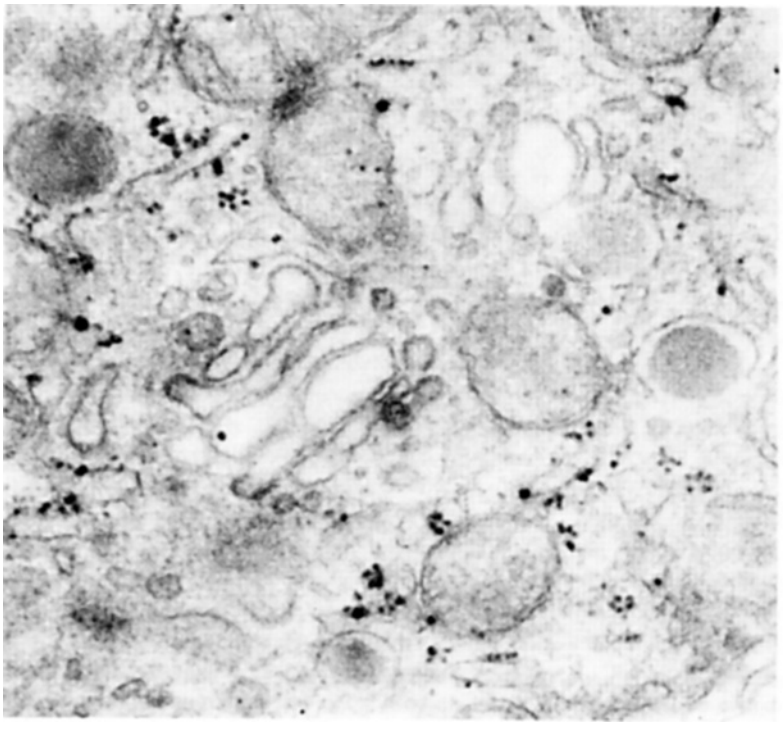

Fig. 5. Incubation time 1 week, glucose concentration 0.4%. Part of sparsely granulated β cell with buds and coated vesicle in GCs. × 67 000.

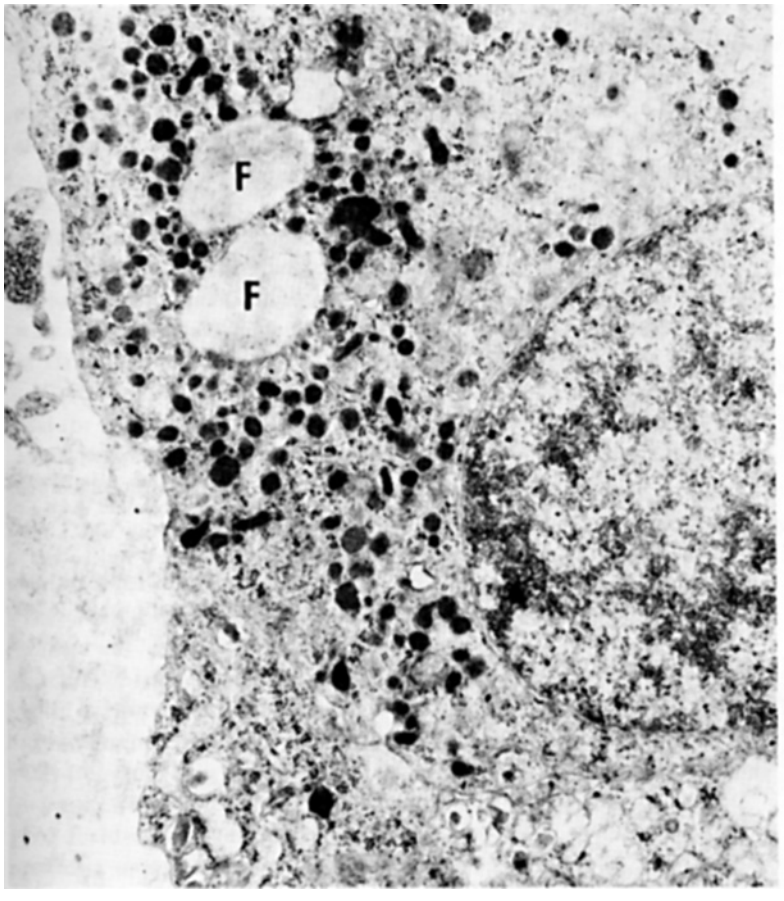

Fig. 6. Incubation time 1 week, glucose concentration 0.1%. Part of α_2 cell with pronounced variation in shape and size of the granules which also are smaller than normally (compare with Fig. 3). Two fat droplets (F). × 16 000.

of granules was less than in the other types (Fig. 3). The monomorphic electron dense cores were slightly smaller than normal.

Comparison of β cells at different concentrations of glucose disclosed that GER and particularly GC were often more abundant in cells bathed in medium with a high content of sugar. Numerous small buds from GC cisternae and coated vesicles were often noted (Fig. 4 and 5). A few hyperthrophic mitochondriae were found at both levels of glucose but these organelles seemed to be more numerous at the lower sugar concentration (Fig. 3). No clear-cut differences were established in the β granules and the third type of "degranulated" cells were thus found at 0.1 and 0.4% glucose.

Also the α granules were more variable than normally. In the oldest explants their classification became difficult and they could even be hard to distinguish from β granules. In an occasional α_2 cell with dense granulation the granules showed pronounced variation in shape and size and were sometimes considerably smaller than normally (Fig. 6). α_1 cells could not be identified later than 1 week after explantation.

The proportion of α cells relative to the β cells was less at high than low glucose concentration, otherwise no differences were noted.

Individual duct cells. These elements often had well developed and sometimes increased amount of GER and GC. Pronounced budding from the cisternae in GC and coated vesicles was common (Fig. 7). Cilia, bundles of fibrils and occasional centrioles were seen for instance after 4 weeks. A few duct cells, often with a partially "empty" cytoplasm (clear cells) were seen (Fig. 2). Some duct cells were rich in lysosomes (Fig. 8). In certain explants microtubuli were caught in the sections (Fig. 7) and mitoses were also discerned.

Fibroblastoid and other cells. Sometimes contacting peripheral islet cells numerous oval or spindle-shaped cells were seen. These elements had

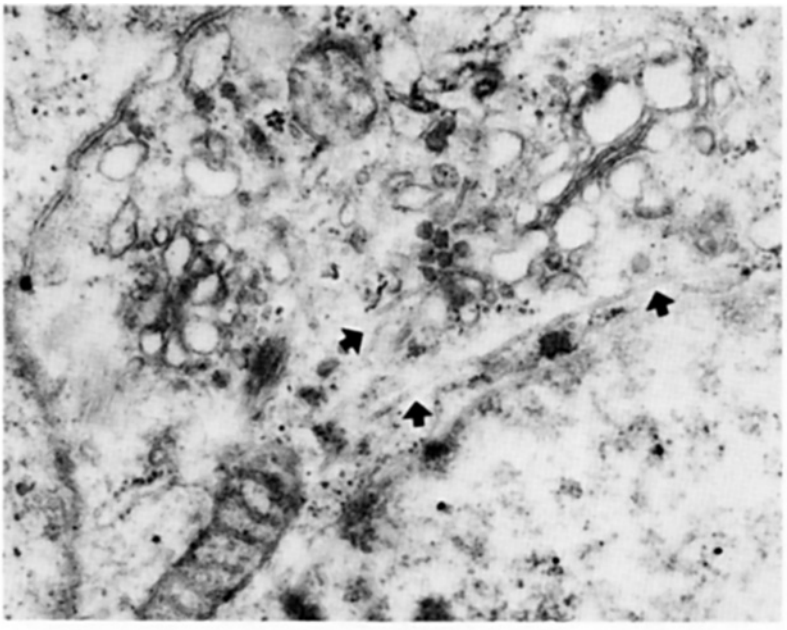

Fig. 7. Incubation time 3 weeks, glucose concentration 0.4%. Parts of duct cells with a few buds and coated vesicles and with bundles of fibrils and some microtubules (arrows). × 36 200.

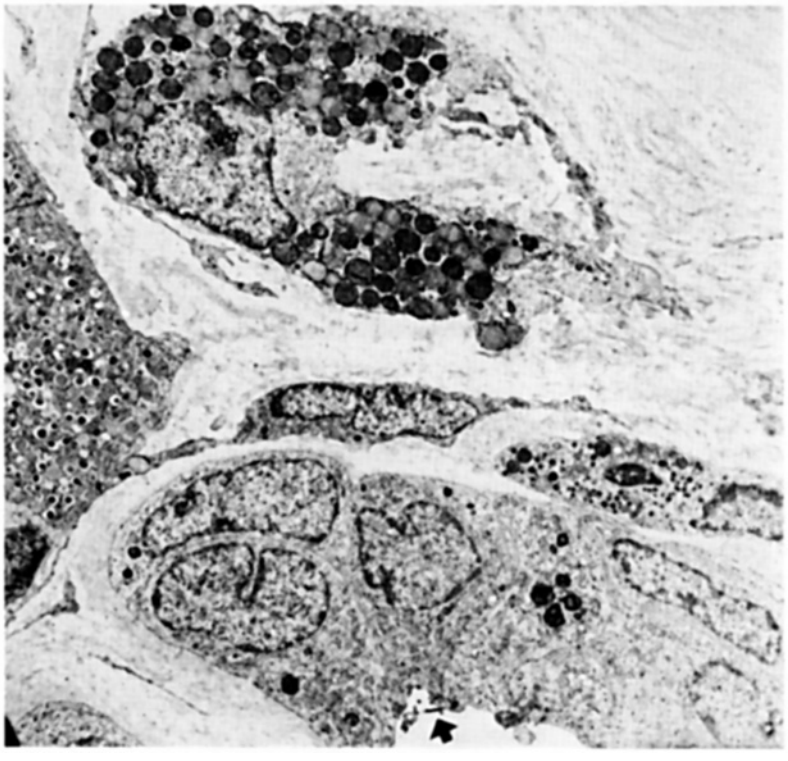

Fig. 8. Incubation time 2 weeks, glucose concentration 0.4%. Part of β cell (left) with an abundance of granules. Part of duct (bottom) with numerous lysosomes (residual bodies) and a few fragments of cilia (arrow). In the connective tissue three spindle-shaped cells with fat droplets and/or lysosomes. × 3 600.

often penetrated the spongostan meshwork surrounding the original explant. The surface of the fibroblastoid cells was often provided with microvilli and the cytoplasm contained GCs with abundant buds and coated vesicles.

Other cells had fat droplets and/or phagocytosed material in addition to a multitude of lysosomes (Fig. 8). In week-old explants heterophagocytosis of β cell granules was seen. Later on no indisputable remnants of granules were identified in the

numerous lysosomes and residual bodies of the phagocytes.

The presumably newly formed cells outside of the islet did not show any secretion granules at any point of time.

Islets. A certain decrease of identifiable islet cells—less pronounced in high glucose medium—occurred with time during the entire experimental period. The shape of the shrinking islets was essentially unaltered. Only during the first few days were certain islets fragmented into parts composed of viable and more or less necrotic cells.

The localization of the cells was often the normal for rats with β cells in the centre and α cells in the periphery (Fig. 2). Sometimes, however, this organization was reversed.

DISCUSSION

The labile morphology during the first few days, which was completely compatible with a non-specific (mainly hypoxic?) damage, precluded detailed early studies of the morphology of islet cells in equilibrium with their environment and reasonably free from progressive degeneration. After one week, however, the morphology stabilized and the EM features of such cells therefore presumably reflect a more physiologic in vitro condition. The discussion will therefore be restricted to this "post-degenerative" period.

The main result of the present report is a confirmation of our earlier light microscopic impression (1) that rat islet cells can be cultivated for long periods of time with retention of their differentiated structure and function.

The comparison between β cells grown at 0.1 or 0.4% glucose in the incubation medium failed to reveal any major differences. Sparsely granulated cells were thus few at both levels of sugar. This differs from the observations after free transplantation of similar explants to the anterior eye chamber, since in this case considerable degranulation was observed in hyperglycemic but not in normal animals (5). That β cell explants respond to increased insulin synthesis and/or release to an elevated concentration of glucose in the medium has been shown before (6). However, in that case, the increase was strongly influenced by the glucose concentration only up to 0.2%, above that level added sugar had only a small effect. It may be that β cells in vitro do not possess an entirely perfect mechanism for insulin release at superhigh sugar concentrations. The important question of β cell regeneration from duct cells has not been finally solved. On our explants a slow disappearance of β cells was evident. Concomitantly the duct cells displayed sign of active regeneration with mitotic figures etc. Although we could not observe any transitions from duct cells to β cells as has been claimed in vivo, the EM findings do not exclude such a process. The frequency of its occurrence may have been too low to be detectable. In this respect the results resemble those obtained with diabetic mutant mice (7) where regeneration of duct epithelium is present but no new β cells are produced in the advanced diabetic stage.

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