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The Ultrastructure of Pancreatic Tissue from Duct-ligated Rats Implanted into Anterior Chamber of Rat Eyes

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ABSTRACT

Pancreatic tissue from rats with ligated pancreatic ducts was implanted into the anterior chamber of the eyes of diabetic and non-diabetic rats, and the ultrastructure of the implants studied afterwards at inervals ranging from 15 min to 4 weeks. During the first, avascular, stage, lasting for 2 days at most, serious damage to a number of the islet cells was noted. During the next, revascularization stage, the ultrastructure, especially in the 2–4-week-old implants, pointed to recovery and/or regeneration of islet cells; on the whole, both the β and α cells in the implants from the non-diabetic hosts showed a normal ultrastructure at this time, whereas in the implants from the diabetic hosts the β cells showed signs of heightened activity. The cells in the excretory ducts showed largely the same changes with the passage of time as the islet cells, but to a less pronounced degree. The ultrastructure of the duct cells at the end of a few weeks exhibited greater signs of activity in the implants from the diabetic hosts than did the duct cells in the implants from the non-diabeic hosts.

INTRODUCTION

In our attempts to isolate pancreatic islet cells by implanting pancreatic tissue made atrophic by duct ligation into the anterior chamber of the rat eye, we observed that islet cells in the implants preserved a largely ordinary light-microscopic appearance for at least a year afterwards (1). The β-cells in implants in diabetic rats showed the same heightened activity, in the form of degranulation, as they do in the pancreatic islets of diabetic animals. The present study was made to determine what more we could learn with electron microscopy about the structure of the islet cells and on how they are able to function when isolated from their natural surroundings.

GC = Golgi complex
GER = Granulated endoplasmic reticulum

MATERIAL AND METHODS

Atrophic pancreatic tissue was removed from rats 4 to 6 weeks after "partial" duct ligation (2) and introduced into the anterior chamber of the eye (1). Homologous transplants were used for the litter-mates of the ligated animals. Both alloxan-diabetic and non-diabetic animals were used. Autologous transplants were also used for the ligated, non-diabetic animals. The transplantation was done 2 or 3 weeks after the alloxan injections when the diabetes had reached a steady state, the glucose in the blood and urine being checked continuously in the meantime.

Sixty-two transplants were examined, 20 from diabetic, 35 from non-diabetic, and 7 from alloxan-injected non-diabetic rats.

At various time points after transplantation—15 min; 1, 3, 6, 8 and 24 hours; 2, 3, 4 and 6 days; 2, 3 and 4 weeks—the transplant was removed and fixed in one of the following fluids:

2% osmium tetroxide in Veronal buffer at pH 7.2; 2.5% glutaraldehyde in phosphate buffer, pH 7.4, containing calcium chloride. After washing in 7.5% sucrose solution, post-fixation in 2% osmium tetroxide.

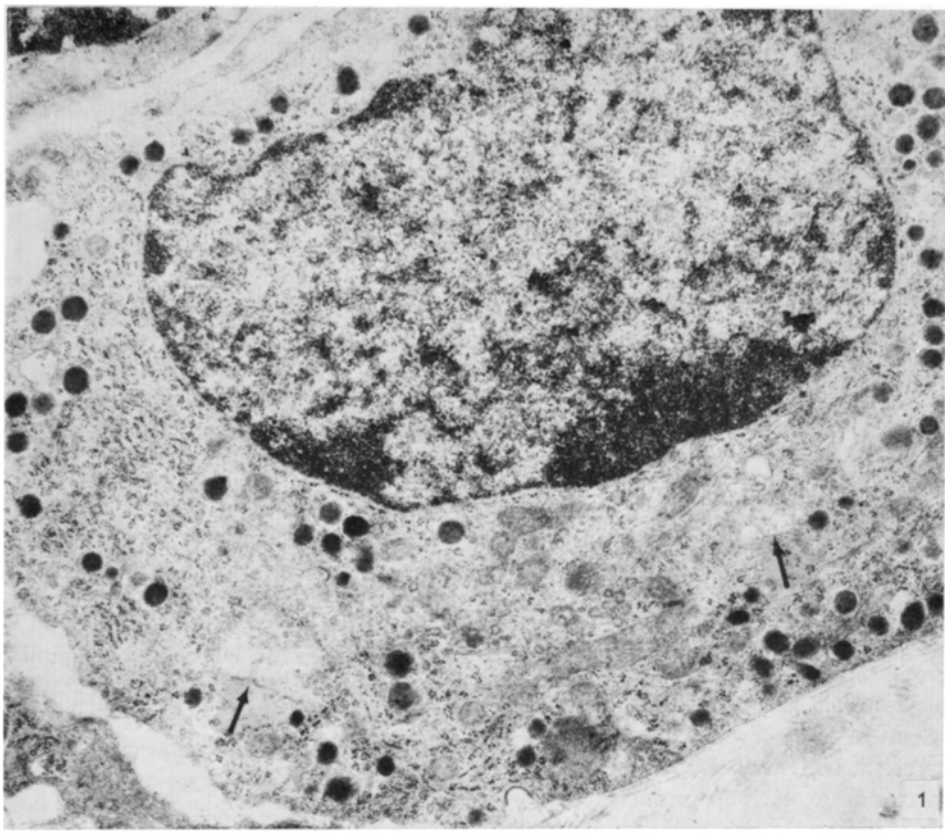
The tissue was embedded in Vestopal W or Epon. Suitable parts were selected in a phase contrast microscope from sections from the blocks, and ultrathin sections cut with an LKB Ultrotome. After contrast staining with uranyl acetate and lead citrate, the sections were examined in a Zeiss EM 9 electron microscope at 60 kV.

RESULTS

Pancreatic islet cells

Implant after 15 minutes to 24 hours

Non-diabetic rats. After only 15 min the β-cells had altered to varying degrees, containing vac-



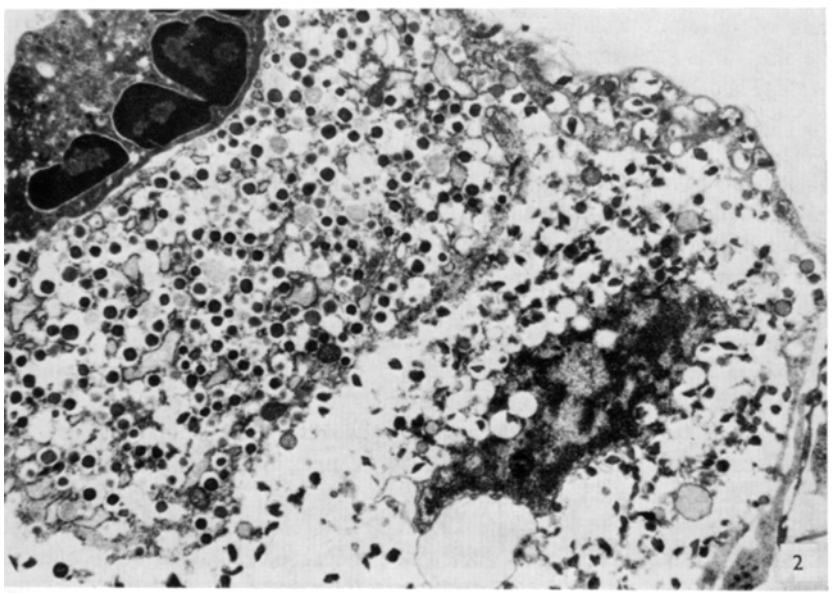


Fig. 1. Implant after 15 min, non-diabetic rat. Part of α_2-cell showing sparse granulation, fairly scant GER, occasional GCs, and numerous small vesicles and largely ordinary mitochondria. A few small vacuoles in the endoplasmic reticulum (*arrows*). Glutaraldehyde–O_sO_4, × 16 000.

Fig. 2. Implant after 3 hours, non-diabetic rat. Right: part of a β-cell showing intense changes in the granules and nuclear pyknosis. Left: part of a β-cell showing moderate changes, including vacuoles in the GER; beside it, part of a neutrophil leucocyte. Glutaraldehyde–O_sO_4, × 11 600.

uoles of various shapes and enclosed by a membrane coated on the outside with ribosome-like granules. At this stage the α-cells, too, began to show a suggestion of the same type of change (Fig. 1).

After an hour the changes in the β-cells had progressed.

After 3 to 8 hours the islet cells were still more altered, showing nuclear pyknosis (Fig. 2). The central core of the β-granules often had a rectangular or irregular profile instead of its normally spherical or oval outline. Their limiting membranes appeared to be greatly expanded and often fused. The α-cells showed less prominent alteration in their granules. No α_1-cells could be identified with certainty.

After 24 hours it was still possible to identify both the granulated endoplasmic reticulum (GER) and the mitochondria and granules in the β-cells, but more difficult to identify remains of the α-cells.

Besides the necrotic cells only a few leucocytes (Fig. 2) and phagocytes were seen, and sometimes single mast cells. Signs of more severe inflammation were rarely present, and whenever they appeared the transplants were excluded from the series.

Diabetic rats. The implants of different ages looked virtually the same as the same-aged implants from the non-diabetic rats (Figs. 3 and 4).

Implant after 2–4 weeks

Non-diabetic rats. The β-cells usually contained an ordinary or relatively scant GER and few Golgi complexes (GCs). Most of the β-granules, abundant in number, showed great variation in shape, structure, electron density and size (Fig. 5). Occasional β-cells with several GCs and few granules were seen (Fig. 6). The nuclei appeared undamaged.

The α-cells contained a large amount of GER and mitochondria. No changes could be seen in the granules.

Diabetic rats. The β-cells contained a profusion of granular and agranular reticulum and often prominent GCs (Fig. 7 *a*) and fairly numerous and large mitochondria (Fig. 7 *b*). The β-granules were often sparse in number (Fig. 7 *a, b, c*) and sometimes situated marginally. Occasional agranular cells were observed (Fig. 7 *c*).

The α-cells contained an essentially normal amount of GER and mitochondria. Several had largely normal granules but occasional irregularly shaped ones, sometimes split, and then generally undersized (Fig. 7 *a*).

Implants taken between the short-term and long-term implants, i.e., after 2–4 days

Only a few of several 4-day specimens showed distinct islet tissue. The cytoplasm of the β-cells was greatly changed, consisting of a coarse network made up of profuse, cord-like aggregates of ribosome-like granules interspersed by cytoplasm of very low electron density. Only occasional mitochondria were visible, and these were mildly changed. The β-granules were sparse and normal shaped.

The α-cells showed diffusely scattered areas of fine flocculation in the cytoplasm, which contained a very sparse but otherwise ordinary GER and ordinary mitochondria but patches of diminished electron density. In some places GCs with unusually small vesicles and cisternae were seen. The α-granules looked largely normal. Several lysosomes were seen in both types of cells.

Excretory Ducts

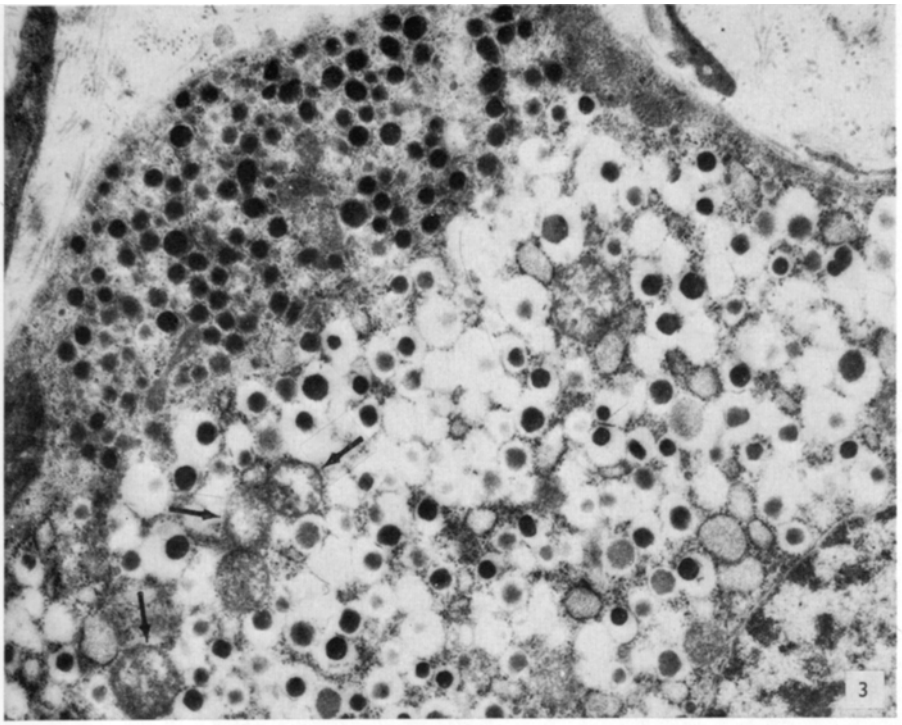
Implant after 15 minutes to 24 hours

Non-diabetic rats. The duct contained both dark and light cells, but the great majority were dark. The cells had a dense, finely flocculent cytoplasm and a fairly large amount of GER and GCs. Some contained vacuoles coated with granulated membranes of parallel membranes separated by a zone of finely granular substance. Here and there the mitochondria were swollen, partly devoid of cristae, and distorted, and contained patches of low electron opacity. Fibrils collected into bundles were seen, as well as occasional cilia.

Occasional cells showed nuclear alteration consisting mainly of marginal accumulation of the chromatin.

The alterations were most marked in the light cells, some of the dark cells looking quite normal.

Diabetic rats. The ducts of implants from the diabetic rats appeared essentially the same as those from the non-diabetic animals, though they differed distinctly in a number of details. Thus they contained more GER and GCs and a great number of fibrils in the dark cells. Granular



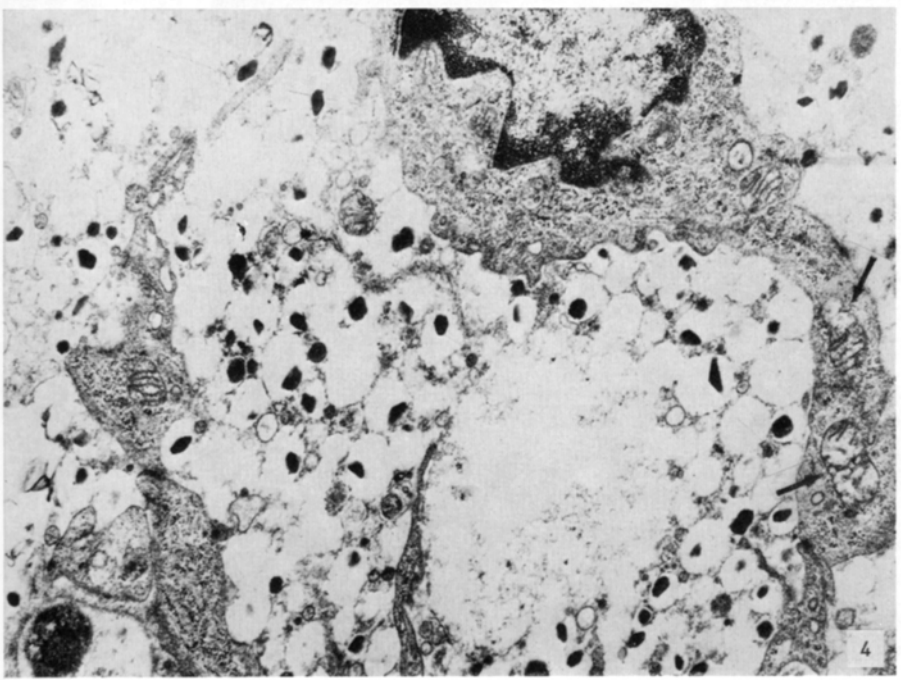
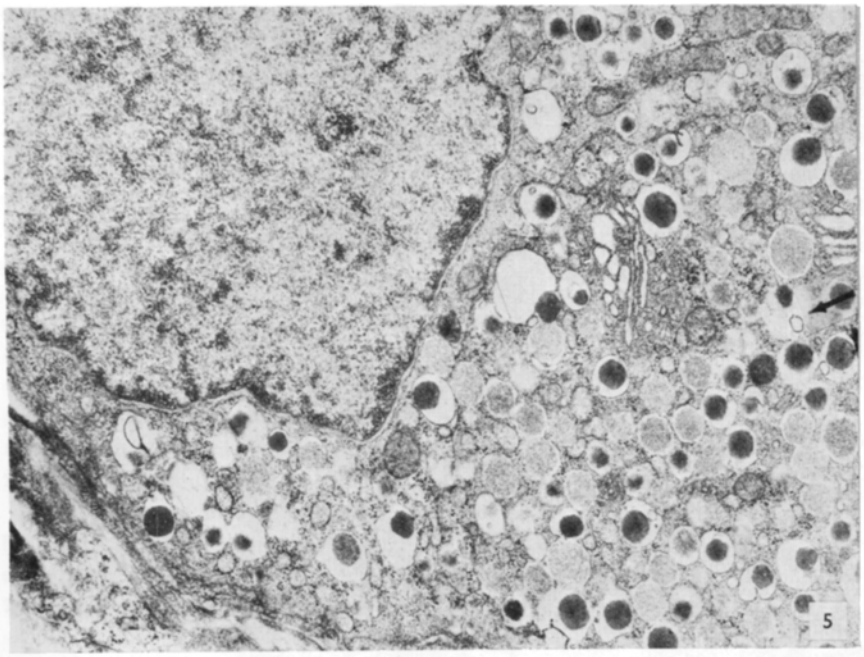


Fig. 3. Implant after 15 min, diabetic rat. Right: part of a β-cell showing an altered GER with ribosome-coated vacuoles containing a finely granulated substance; rounded mitochondria with patches of low electron density and altered cristae (*arrows*); fusion and expansion of the β-granules' limiting membranes; suggestion of clumping of nuclear chromatin. Upper left: part of an α-cell with a suggestion of vacuolation. Glutaraldehyde–O_sO_4, × 16 000.

Fig. 4. Implant after 24 hours, diabetic rat. A β-cell with a partly pyknotic nucleus and mitochondria showing patches of low density (*arrows*). Aggregates of greatly altered β-granules, apparently enclosed in limiting membranes, probably the initial stage of autophagosomes. Glutaraldehyde–O_sO_4, × 16 000.



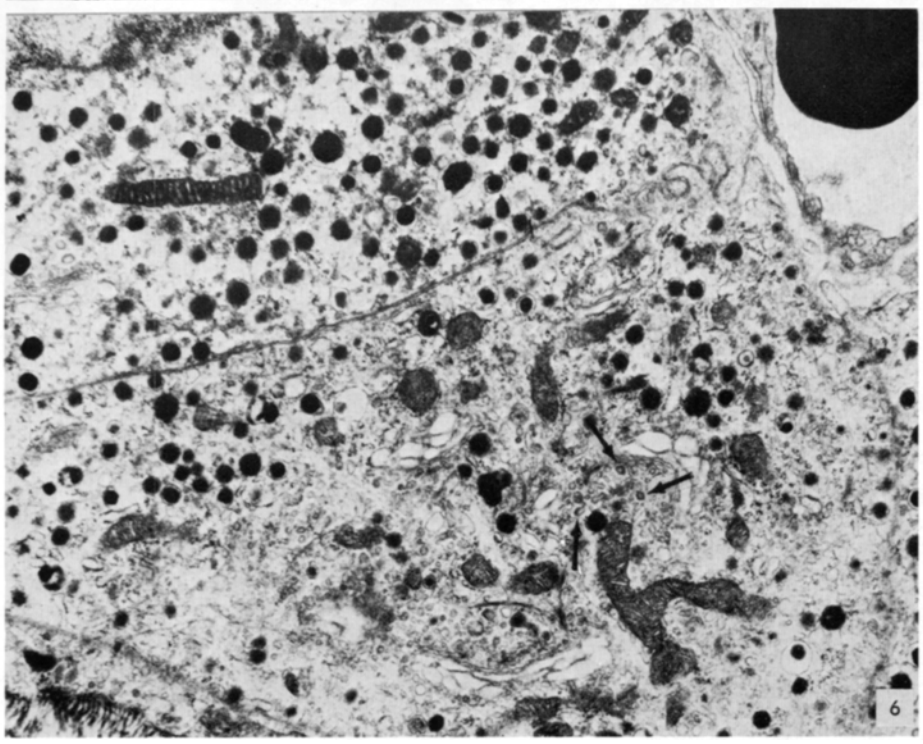


Fig. 5. Implant after 4 weeks, non-diabetic rat. Part of densely granulated β-cell showing great variation in the structure, electron density and size of the granules. A few of the granules contain small vacuoles between the core and limiting membrane (*arrow*). Glutaraldehyde–O_sO_4, × 16 000.

Fig. 6. Implant after 4 weeks, non-diabetic rat. Parts of two islet cells, probably β-cells. The upper cell is densely granulated, has a low density cytoplasm and contains only a few GCs and comparatively few mitochondria. The lower cell is more sparsely granulated and has several GCs, many agranular, partly coated vesicles (*arrows*), and a fair amount of mitochondria. In the upper right corner, part of a capillary with a red blood corpuscle. O_sO_4 fixation, × 16 000.

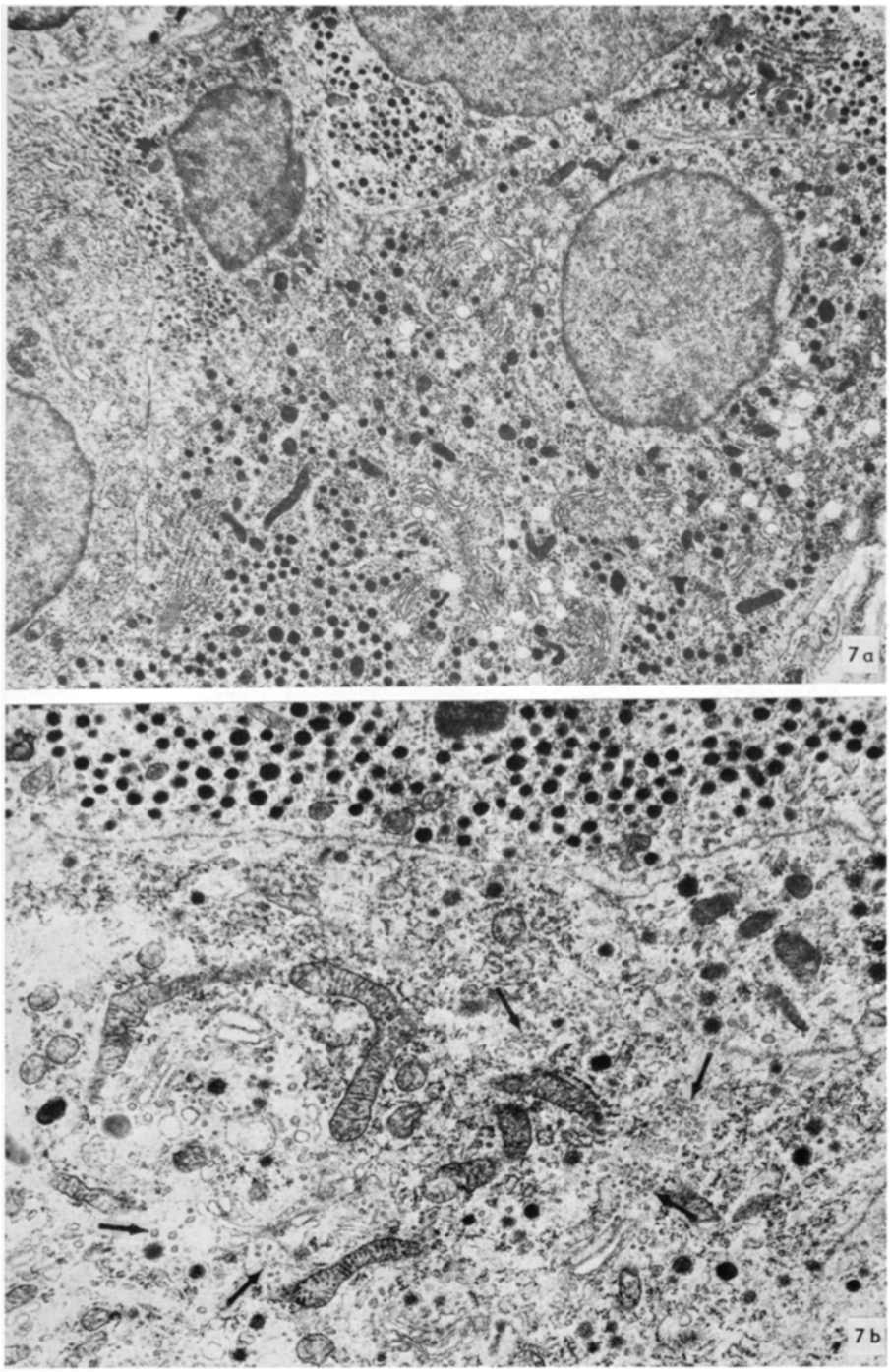
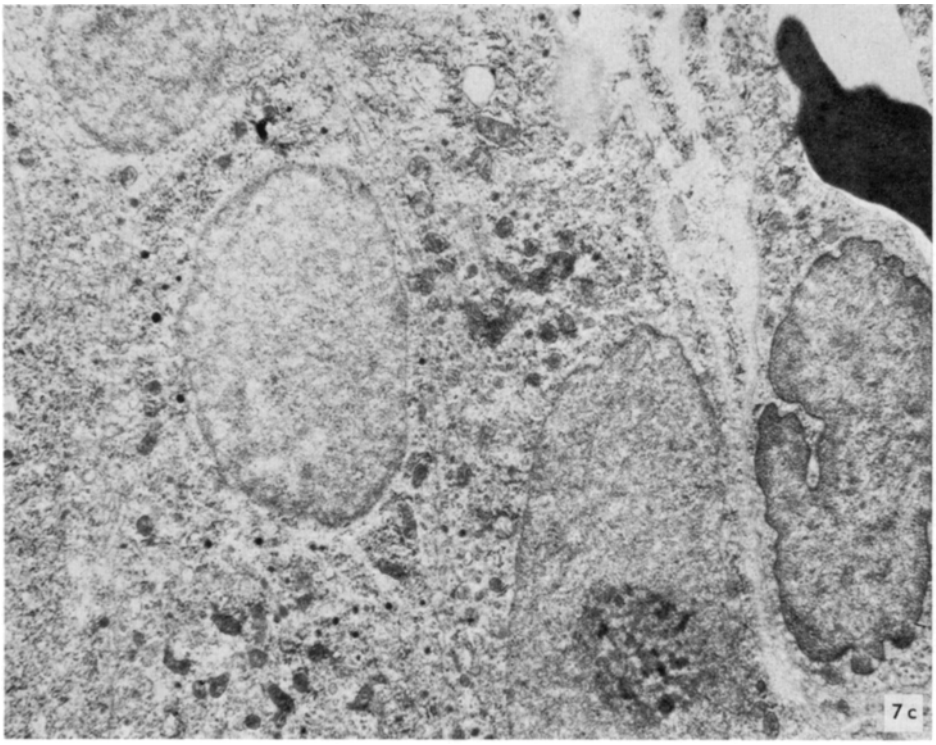


Fig. 7 a, b, c. Implant after 4 weeks, diabetic rat. O_sO_4 fixation. (*a*) Midway down the figure are parts of sparsely granulated β-cells containing numerous GCs, with part of a β-cell containing only occasional granules at the far left. Above are a few densely granulated α_2-cells, the left one containing remarkably small, irregular granules, a couple of dense bodies and a darker nucleus. × 13 600. (*b*) Above one sees a part of a densely granulated α_2-cell and below a sparsely granulated β-cell with GCs and many agranular, partly coated vesicles (*arrows*) and several elongated irregular mitochondria. × 16 000. (*c*) Part of pancreatic islet containing areas of extremely sparsely granulated β-cells. At the extreme left is what appears to be part of an agranular cell. At the extreme right a capillary with an endothelial cell and red blood corpuscle. × 16 000.



structures were also observed in occasional peripheral dark duct cells.

Implant after 2–4 weeks

Non-diabetic rats. The dark cells contained an abundance of GER and occasional cells showed the vacuolation formerly described. The GCs were abundantly developed, showing a large variety in vesicular structure (Fig. 8). The mitochondria appeared normal. The nuclei showed no changes. Fibrils were frequent and not infrequently accumulated in a zone near the surface of the cell facing the duct lumen. Fragments of cilia were present in the duct lumen (Fig. 9). Desmosomes and terminal bars occurred fairly often. Light cells occurred only occasionally and showed the same structure as in the dark cells with an altered GER, though of more marked degree. Only a few GCs were present and these showed little variation in vesicular structure. Mitochondria were less numerous than in the dark cells; they were often rounded and larger, and had spots of reduced electron density. Some nuclei showed a suggestion of marginal accumulation of chromatin. The light cells contained only a few fibrils.

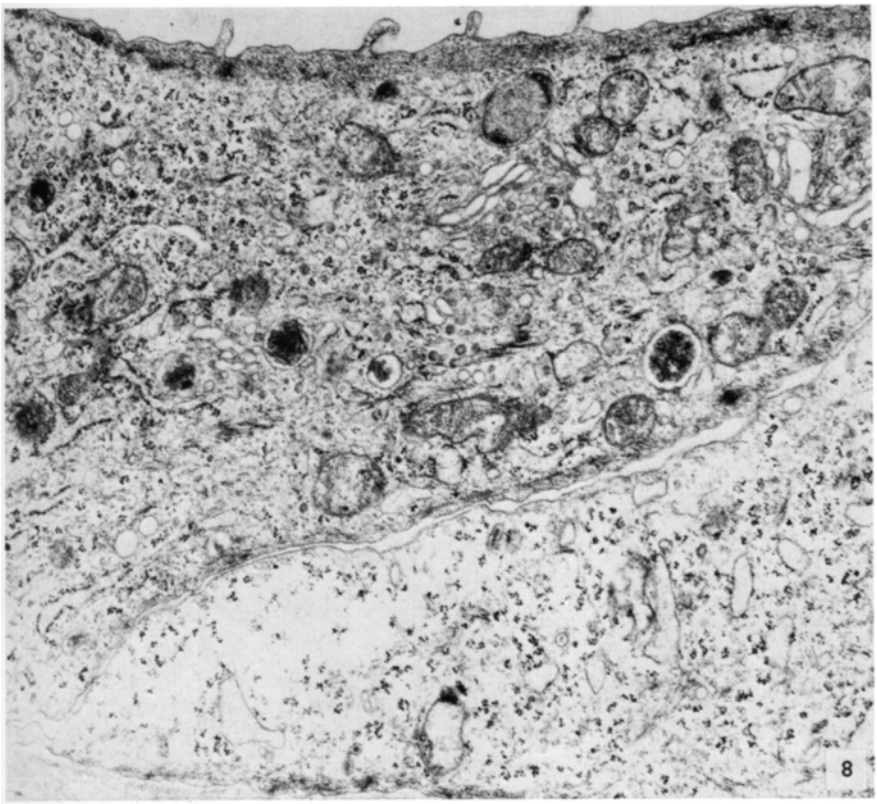
In a few implants mitoses were observed in the duct cells, as well as signs of the type of amitotic cell division described by Pehlemann (3).

Diabetic rats. The implants from the diabetic rats looked essentially the same as the implants from the non-diabetic animals. But there were a few differences. The light cells, for instance, showed less prominent signs of degeneration. The fibrils were more numerous and often more distinct, and the same was true of cilia. There were more distinct granule-like structures, and α- and β-cells were observed beside agranular cells in the duct epithelium without any basement membrane in between.

The implants from a rat with latent diabetes and an abnormal glucose tolerance test 2 weeks after the transplantation showed a remarkably large number of bundles of fibrils, several spindle fibrils (microtubules, Fig. 10), and some centrioles, and also fragments of cilia.

DISCUSSION

Directly after the transplantation the implant lay unconnected with the iris and had no vascular



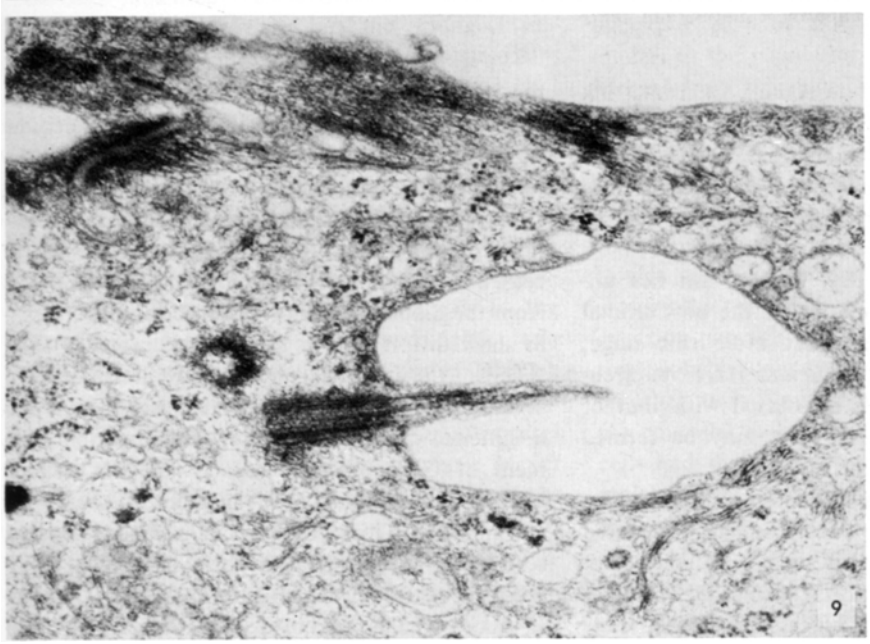


Fig. 8. Implant after 4 weeks, non-diabetic rat. Part of excretory duct with its lumen at the top of the photograph. Part of light cell with degenerative changes including vacuolation of the endoplasmic reticulum and patches of low electron density in the cytoplasm. The part of a dark cell adjacent to the lumen shows several GCs and vesicular structures with some coated vesicles. O_sO_4 fixation, × 20 500.

Fig. 9. Implant after 4 weeks, non-diabetic rat. Part of excretory duct with its lumen at the top of the photograph. Duct cell with a centriole in the centre of the picture and a cilium protruding into a large vacuole obviously an invagination from the cell surface. O_sO_4 fixation, × 38 000.

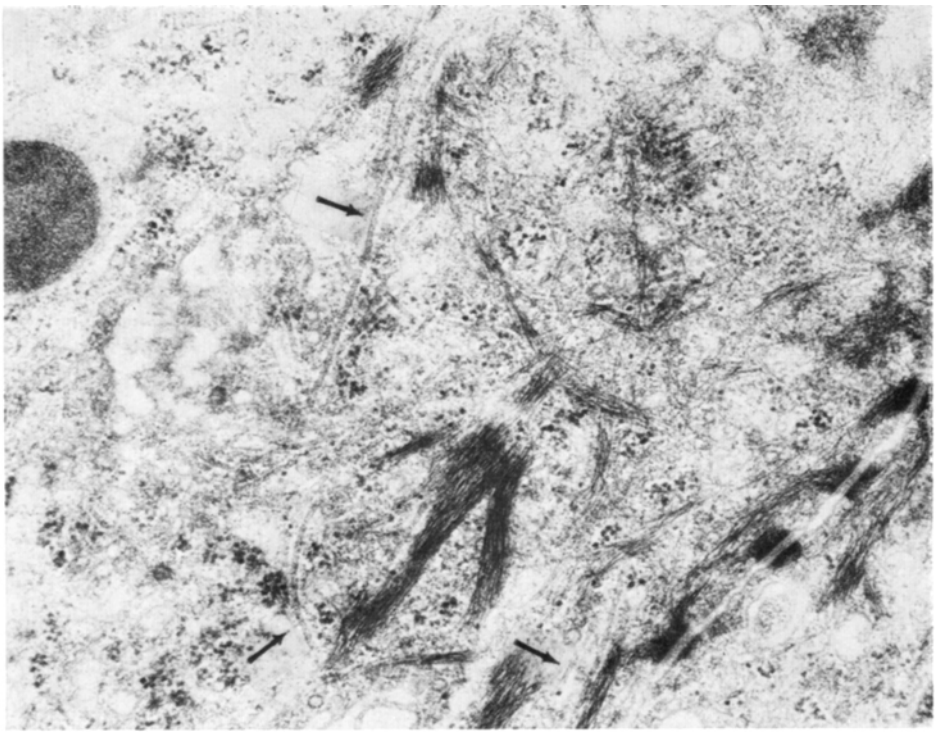


Fig. 10. Implant after 2 weeks, rat with latent diabetes (abnormal glucose tolerance test). Part of duct cell containing desmosomes, many bundles of fibrils, and fairly many spindle fibrils (*arrows*). Glutaraldehyde–O_sO_4, × 33 000.

continuity with it. But injections of India ink showed that 2 or 3 days afterwards it had acquired a certain amount of vascular continuity with the iris. The revascularization then progressed until 2 or 3 weeks later when the implants had an abundant vascular supply.

Thus it would seem that the implant lies unconnected with the circulation of the host animal for about 24 hours and that after this stage, which may be called the *avascular stage,* its circulation gradually becomes connected with that of the host animal, entering what may be termed the *revascularization stage.*

In the avascular stage, distinct degenerative changes were visualized. These changes progressed rapidly, leading to necrosis of several islet cells. Vacuoles seemed to develop from the GER.

One could follow this process up to the stage where the cells became necrotic, probably due to hypoxia. Naturally some details in the picture may have been due to another condition than hypoxia.

It is not possible to draw any conclusions on the reactive capacity of the islet cells during the avascular stage, during which one may assume that they are totally isolated, because of the picture being so distorted by the changes.

During the resvascularization stage, the degeneration predominant during the avascular stage ceased to dominate the picture. The implants from the diabetic and non-diabetic rats then began to show differences in the ultrastructure of their β-cells. In implants from the diabetic rats, several cells acquired the structure typical of heightened activity, including abundant development of GER and particularly of GCs as well as sparse granulation with the granules sometimes marginated. This picture corresponds to that seen after long-term adaptation of β-cells to increased functional demands in obese hyperglycemic mice (4), in steroid-treated diabetic or subdiabetic animals (5, 6, 7), and in mice injected with insulin antibodies (8). In the implants from the non-diabetic animals, the reticulation was usually more sparse and the granulation abundant. Occasional β-cells with sparse granules were seen.

Thus the ultrastructural picture verified the impressions earlier gained from light microscopy, that on the whole the β-cells retain their reactive capacity when isolated from their environment. It does not follow from this, however, that the β-cells isolated from their natural environment in free transplants are completely autonomic. There is good reason to assume that the circulation is restored during the stage of revascularization. In view of the re-innervation Malmfors et al. (9) observed in smooth muscle transplants into the anterior chamber of the eye, there are reasons to search for nerve elements in the implants.

Because it is hard to judge the functional state of the α-cells from the morphologic picture, it is virtually impossible to enter into a discussion on what happens to them in the transplants. Some of the α-cells in the implants from both the diabetic and non-diabetic animals showed a suggestion of changes in reticular development and granulation, but not enough to conclude that they functioned in another way.

The proportions of the different kinds of cells in the implants at the revascularization stage were not subjected to quantitative analysis. Subjective estimates indicated that, apart from the distinct absence of α-cells in all but the youngest implants, the α/β cell ratio remained essentially normal.

Alterations were also noted in the ducts of the implants. During the first days their cells showed changes characteristic of a regressive process, but on the whole they were not so severely or extensively changed as the islet cells.

Between the 2nd and 4th week after the transplantation, the most important changes in the ducts took place in the GER and particularly in the GCs, which showed considerable increase and differentiation, manifested in the development of several variants of vesicular structures ("coated vesicles") corresponding to those Meyer & Bencosme (10) classified and thought to be connected with the metabolism of the cells. These changes were accompanied by an increasing number of filaments. A suggested increase in the number of cilia and possibly granule-like structures was also observed. These changes seemed to reach their peak 2 or 3 weeks after the transplantation.

The implants from the diabetic animals nearly always tended to show better preserved β- and duct cells, or more progression in the cellular structure than did those from the non-diabetic animals.

The changes in the islet and duct cells seemed to progress in parallel. This raises the question whether there is any connection between the development of islet and duct cells.

The quantity of the islet cells fell directly after the transplantation and remained low until 3 or 4 weeks later when the revascularization had reached a steady state. During the first 48 hours the number of viable cells in the implants fell to a low level. After 1–2 weeks they seemed to multiply successively. This account is based on approximate estimation, but a sequence of events of this nature could be followed distinctly in a series of implants of different ages.

Thus, after experiencing a great reduction in number during the 24 to 48 hours after the transplantation, the islet cells seemed to recover and/or multiply. The increase in their number, or percentage of transplant surface occupied by islet tissue, pointed to regeneration. This regeneration may have originated either from the islet cells themselves or from the duct cells. It is hard to prove or to refute that the residual islet cells played a part in this regeneration. The same applies to the possibility of new cells having been formed from the duct cells. It is worthy of note, however, that the duct cells showed distinct ultrastructural signs of heightened activity for a few weeks after the transplantation. Heightened activity is not what one would expect of a tissue structure deprived of one of its main functions, in this case loss of ability to continue as an excretory duct because of being detached from the exocrine parenchyma (acini). On the other hand, this probably does not prevent it from exercising its other main function, that of acting as a matrix for new islet cells. The ultrastructure of the duct cells and the occurrence of mitoses and signs of amitotic cell division indicated that they could still produce new cells. This conforms with the observation of infiltration of islet cells outside the transplant in some cases (1).

A number of structures other than those just mentioned have been assumed to be correlated with the development of islet cells from the ducts. Thus an increased number of cilia has been taken as evidence of regeneration (11, 12). Fibrils have also been observed in connection with regenera-

tion and differentiation, e.g., adjacent to the GCs (11).

In the present investigation only a few cilia were observed in only a few duct cells.

The occurrence of fibrils was distinctly correlated with an increase in activity, as indicated by the GER and structure of the GCs. It is worthy of note that fibrils sometimes showed structural details much like those of spindle fibres, or microtubules, considered to be connected with mitosis (13, 14) and cellular division.

The granules or granule-like structures occasionally observed in the duct cells lend support to the assumption that granulated islet cells develop from agranular cells. Convincing proof of this has been furnished by studies of alloxan diabetes in Chinese hamsters (15).

The implants sometimes contained granule-like structures, mainly in peripherally situated duct cells. Distinct α- and β-granulated cells were also seen adjacent to duct cells without any basement membrane in between. The implants, however, came from pancreatic tissue made atrophic by ligating the ducts. It is possible that new islet cells can be produced from ducts in atrophic tissue of this kind (16). Thus the pictures observed in the implants may have already been present when they were transplanted.

When evaluating the present results, it must be remembered that the transplanted tissue differed in essential respects from normal pancreatic tissue, in that it was detached from the exocrine parenchyma, its vascular connections with the surrounding tissue were first severed and then re-established, and its neural connections were cut off.

ACKNOWLEDGEMENT

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