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Ultrastructure of Pancreatic Acinar and Islet Parenchyma in Rats at Various Intervals after Duct Ligation*

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Die Ultrastruktur des Acinus- und Inselzellparenchyms des Rattenpankreas zu verschiedenen Zeitpunkten nach Gangligatur

Zusammenfassung. Im Rattenpankreas kommt es nach einer Gangligatur zu einer Degeneration und Atrophie der Acinusepithelien. Das zugrunde gegangene Drüsenparenchym wird durch Fett- und Bindegewebe ersetzt. Zeichen einer Regeneration der Acinusepithelien lassen sich nicht beobachten.

Die B-Zellen des Inselsystems zeigen in den ersten postoperativen Tagen eine Degranulierung und eine Schwellung des endoplasmatischen Reticulum sowie des Golgikomplexes. Außerdem treten degenerative Veränderungen und auch Nekrosen einzelner B-Zellen auf. Die übrigen Zellen des Inselsystems zeigen keine Veränderungen. An den Ductuli lassen sich Zellproliferationen, Mitosen und Gruppen von agranulären oder granulierten Zellen beobachten, welche sich zu Inselkomplexen entwickeln. Die Ursache für die B-Zellveränderungen und die anschließende Neubildung von Inselzellen werden diskutiert.

Summary. Duct ligation of rat pancreas evoked degeneration and atrophy of the acinar cells. The atrophied parenchyma was replaced by adipose and fibrous tissue. No signs of regeneration of acinar cells were observed.

The islet β-cells showed degranulation and prominent endoplasmic reticulum and Golgi complex the first post-operative days. There were also degenerative changes and even necrosis of some β-cells. In the other islet parenchymal cells no obvious lesions were found. The ductules seemed to proliferate and showed mitoses and buds composed of agranular and granulated cells which appeared to develop into islets. The cause for the β-cell lesions and the subsequent neoformation of islet cells is discussed.

Introduction

It is well known that duct ligation of the pancreas gives rise to extensive atrophy of its exocrine portion. There are also alterations in the endocrine part of the organ (Mansfeld, 1924; Best, 1934; Larsson, 1956; Jönsson, 1965, 1968). The islets of duct-ligated animals have been stated to be of foetal nature and to be resistant to the diabetogenic effect of alloxan (Larsson, 1956). The newly formed islet parenchyma in duct-ligated pancreas may, however, have a considerable functional capacity (Zweens and Bouman, 1967, 1969).

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In preceding works the light microscopic and physiologic changes in duct-ligated rats were studied (Edström and Falkmer, 1967, 1968). Particular attention was paid to the pancreatic islets and the signs of islet neoformation. This neoformation seemed to originate from ductules and structures tentatively called ovoid or kidney-shaped bodies. Cells of agranular type appeared to be involved in the development of the granulated islet cells. The role of ductules and agranular cells for the neoformation and regeneration of the islet parenchymal cells has been emphasized in previous communications from our laboratory (*cf.* Boquist and Falkmer, 1969).

The present ultrastructural investigation was undertaken in order to obtain more information about the degenerative and regenerative changes in the exocrine and endocrine pancreas of duct ligated rats and also to compare these changes with those evoked in these two constituents of the pancreatic parenchyma after the administration of ethionine or excess methionine (Boquist, 1969b and c).

Material and Methods

The principles for ligation of the main pancreatic ducts and for the care of the animals were the same as those employed in preceding works (Edström and Falkmer, 1967, 1968). In all, 14 female Sprague-Dawley rats were used. At the beginning of the experiments they were about 2 months old and weighed about 200 g.

The animals were sacrificed at the following time intervals after duct ligation: 1, 3, 5 days, 1, 2, 3, 4, 6 weeks, and 6 months. One or two animals were investigated at every observation time. Specimens were taken from atrophic (distal to the ligature) and "intact" (proximal to the ligature) pancreas and were fixed by immersion in 1% osmium tetroxide in 0.34 M Veronal acetate buffer adjusted to pH 7.2—7.4. After fixation the specimens were rinsed, dehydrated with ethanol, and embedded in Epon 812. For trimming the blocks and for identification of appropriate areas for the thin sections, thick (1 μ) sections were stained with toluidine blue and studied under a light microscope. The sections were cut on an LKB Ultrotome III and were stained with uranyl acetate and lead citrate prior to examination in a Zeiss EM 9 and/or a Siemens Elmiskop I A.

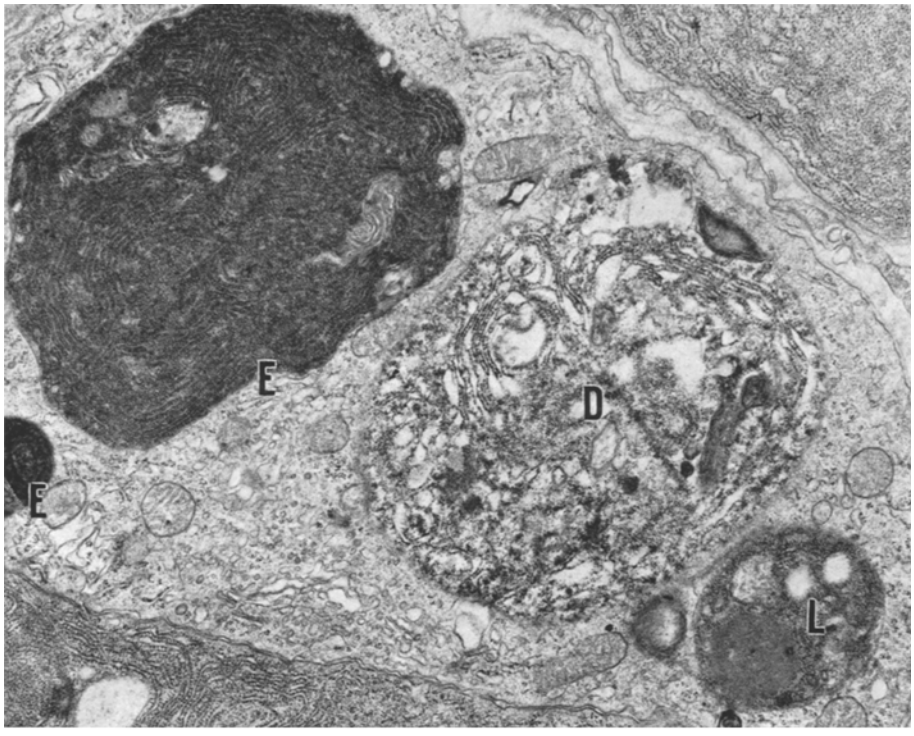
Results

No obvious changes were recorded in the acinar parenchyma, duct system, or islet parenchyma of the "intact" portion at any observation time.

Acinar parenchyma. During the first week there were progressive degenerative alterations in the acinar cells. The zymogen granules and ribosomes disappeared. The endoplasmic reticulum was widened and attained often a cystic appearance. The mitochondria exhibited loss of cristae, swelling, and disintegration. The Golgi complex was disorganized and the nuclei were pyknotic. Some acinar cells showed focal accumulations of altered cytoplasmic organelles and lysosome-like bodies containing structures interpreted as damaged mitochondria and membranes of endoplasmic reticulum (Fig. 1). The acinar lumina were dilated and subsequently there was atrophy of the acinar cells surrounding them.

Fig. 1. This and all subsequent electron micrographs are from the pancreas of duct ligated female rats. The time intervals elapsed after the operation are given within brackets. Exocrine cell with rather low electron density containing electron dense structures (*E*) mainly composed of condensed endoplasmic reticulum, a lysosome-like body (*L*), and a focal accumulation of degenerated cytoplasmic organelles (*D*). (3 days). × 17,000

Fig. 2. Exocrine pancreatic parenchyma exhibiting severe degenerative alterations. (1 week). ×6,000



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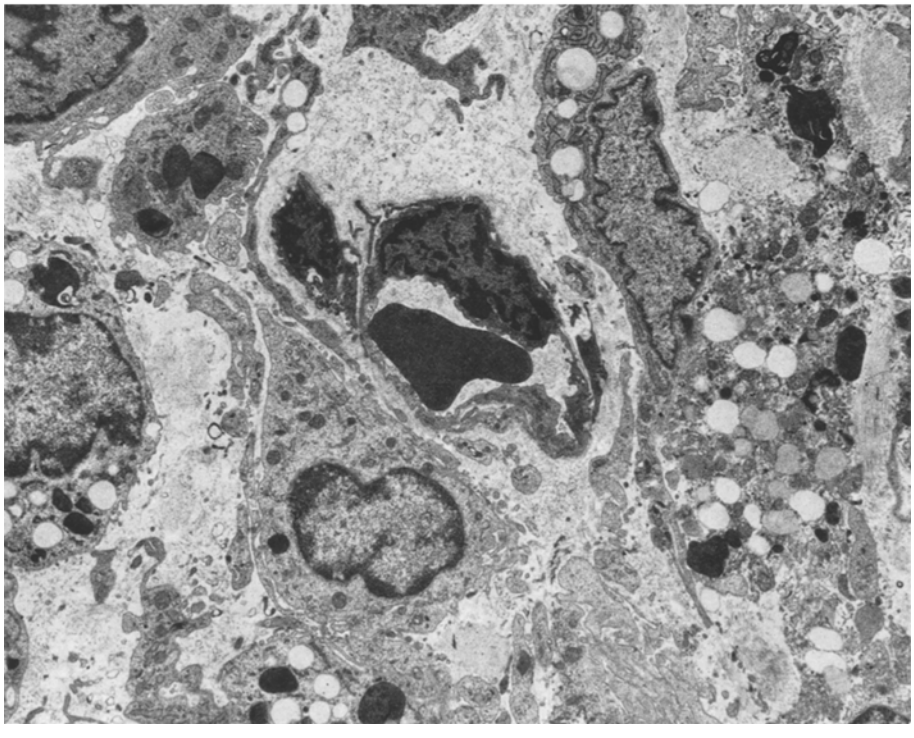

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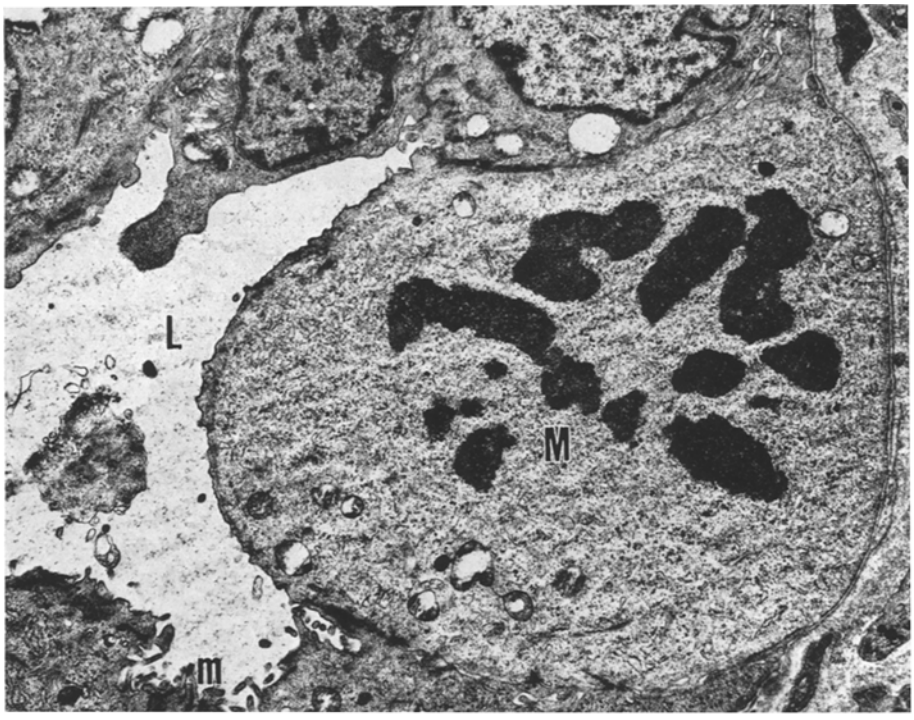
Figs. 1 and 2



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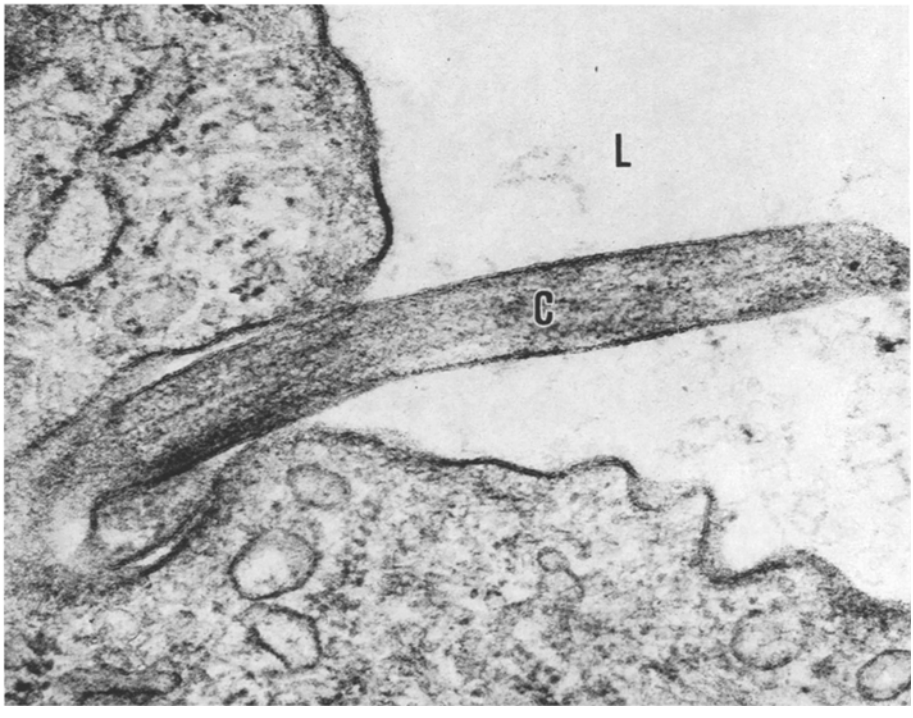

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Fig. 3. Pancreatic ductule demonstrating a mitotic figure (*M*) in one of the ductular cells. Microvilli (*m*) are seen in the lumen (*L*). (1 week). × 11,000

Fig. 4. Portion of ductule cell with a cilium (*C*) which protrudes into the lumen (*L*). At the base of the cilium there are invaginations of the cell membrane. The ciliary fibres are difficult to discern and possibly degenerated. (2 weeks). × 75,000

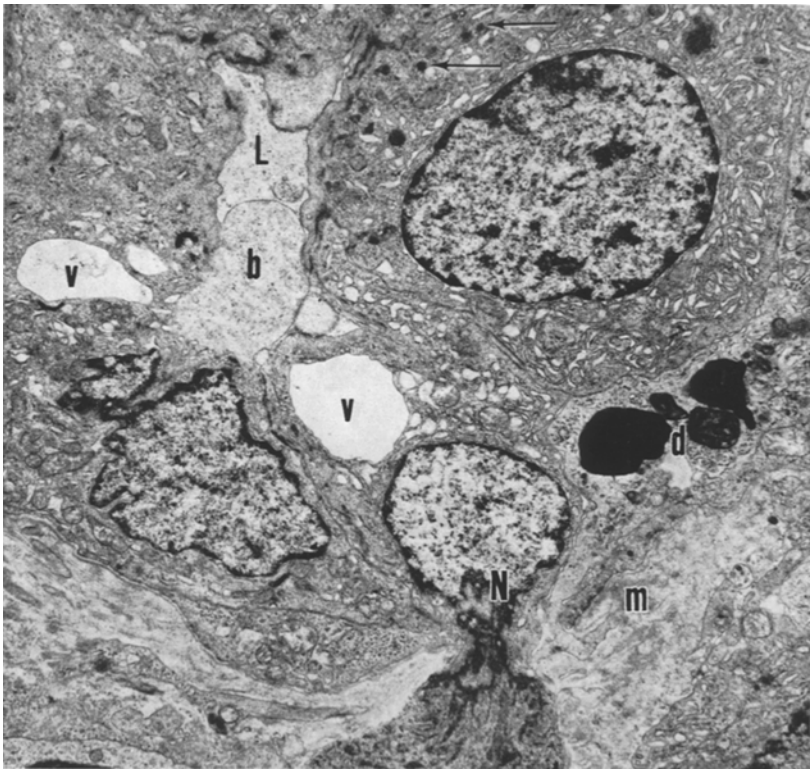


Fig. 5. Pancreatic ductule with amorphous rather electron translucent material in the lumen (*L*) and ductular cells showing large cytoplasmic vacuoles (*v*) and apical blebs (*b*). Two of the ductular cells have irregular nuclei, one of which is dumb-bell shaped (*N*), possibly denoting proliferation or division. In another ductular cell there are prominent endoplasmic reticulum and structures reminiscent of secretory granules (arrows). The basement membranes are partly fragmented and thick (*m*). Electron dense structures probably of degenerative nature are also seen (*d*). (2 weeks). × 9,000

By progression of these alterations the acinar parenchyma became almost completely atrophic and replaced by necrotic debris approximately one week after the operation (Fig. 2). After this time an increased amount of adipose and fibrous tissue appeared. At 6 months no acinar parenchyma was found.

Duct System. From the 5th day the ducts showed dilation and an increased number of tubular structures appeared during the following days. These structures that were interpreted as ductules were composed of cells with cytoplasm of low electron density devoid of zymogen granules. Mitoses were sometimes observed in the ductular epithelium (Fig. 3). Rather seldom the ductule cells possessed a cilium which was protruding into the lumen (Fig. 4). The Golgi complex and the endoplasmic reticulum of some ductule cells were inconspicuous whereas those of others were prominent. Vacuoles and structures resembling secretory granules were observed in the ductule cells (Fig. 5). The ductules showed buds of seemingly proliferating cells with cytoplasm of low or moderate electron density. Some of these cells were devoid of granules (agranular cells) (Fig. 6) whereas others were

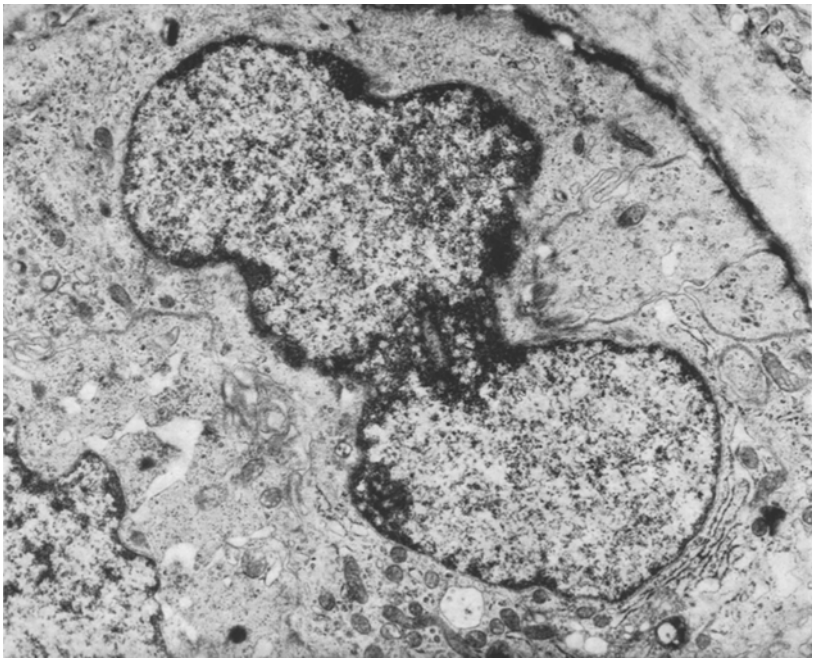


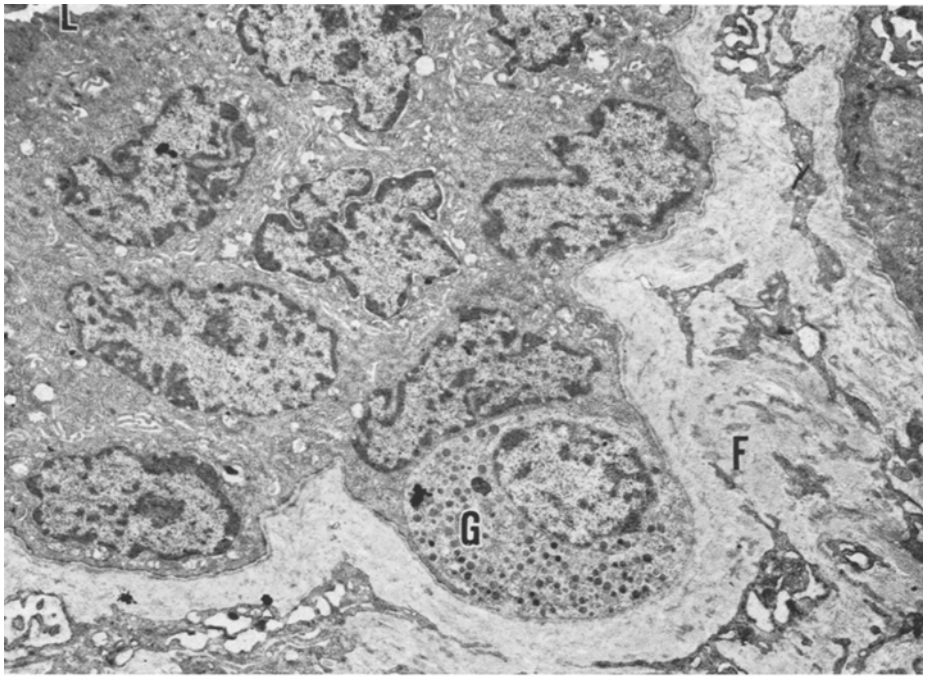
Fig. 6. Portion of bud of cells in a pancreatic ductule showing cells with cytoplasm that is moderately electron dense and devoid of obvious secretory granules. A nucleus with deep indentations is seen. (3 weeks) × 14,000

granulated (Fig. 7). Ductular buds of cells were found about 1—6 weeks after the duct ligation and were particularly numerous during the 2nd and 3rd week after the operation. At 4—6 weeks there were ductules close to or within small islets. Six months after duct ligation the number of ducts and ductules was decreased and there were no signs of cell proliferation from them.

Islet Parenchyma. During the first post-operative days there was moderate degranulation of the β-cells. The endoplasmic reticulum and Golgi complex of these cells were prominent. Mitochondrial swelling and disintegration and cytoplasmic vacuolization were sometimes recorded in the β-cells (Fig. 8). At one and two weeks there were extensive degenerative changes and necrosis in a few β-cells (Fig. 9). Fibrous tissue could be seen in some islets with damaged β-cells from the 2nd week after duct ligation. The cisternae of endoplasmic reticulum were sometimes dilated in the α_1-cells. Obvious degenerative changes were not observed in these cells at any observation time. Nor were such changes found in the α_2-cells (Fig. 10). The agranular cells were rather numerous in the ductules and islets,

Fig. 7. Pancreatic ductule in cells without granulation and a peripheral cell with cytoplasmic secretion granules (*G*). The ductular lumen (*L*) and fibrous tissue (*F*) are seen. (3 weeks). × 7,000

Fig. 8. Portion of islet β-cell showing typical secretory granules and slight vacuolization of the cytoplasm (*v*). The mitochondria (*m*) are swollen and their cristae are disrupted. (1 week). × 33,000



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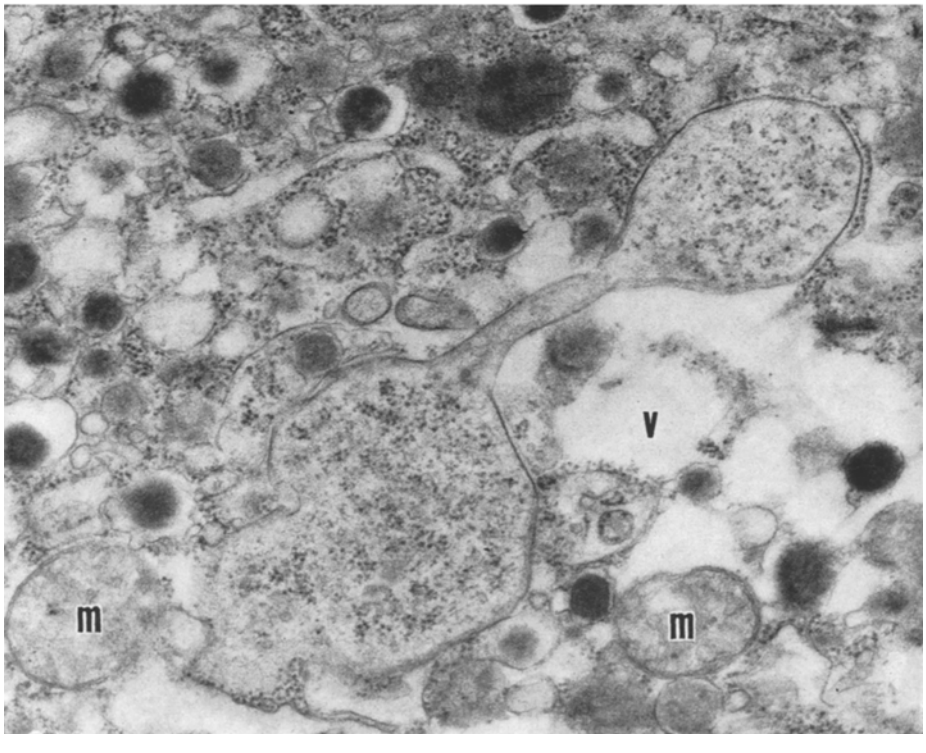

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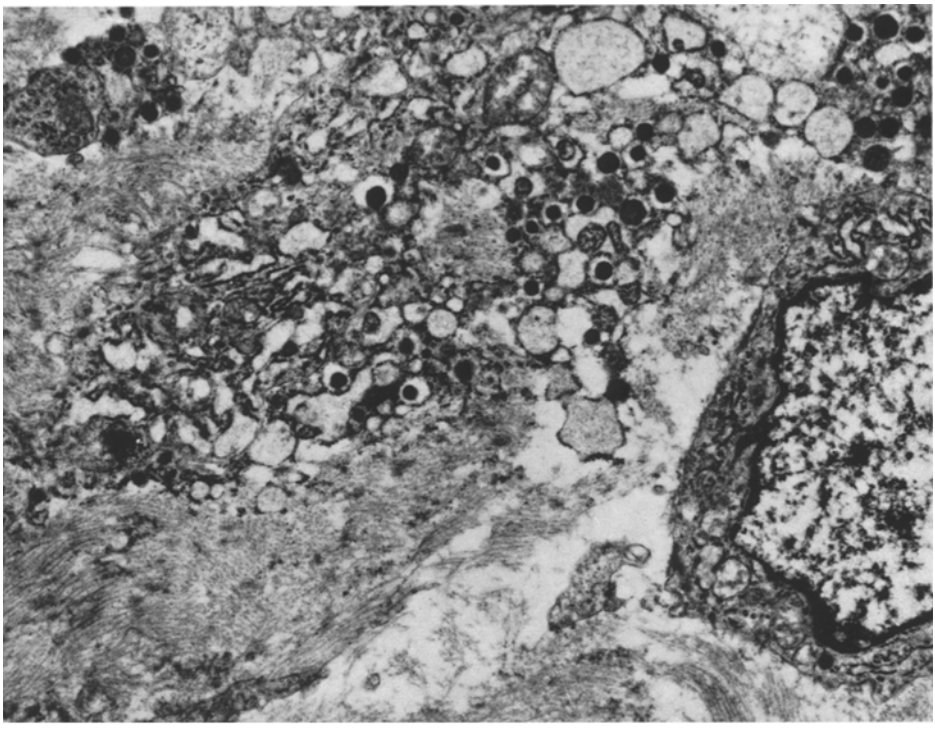
Figs. 7 and 8



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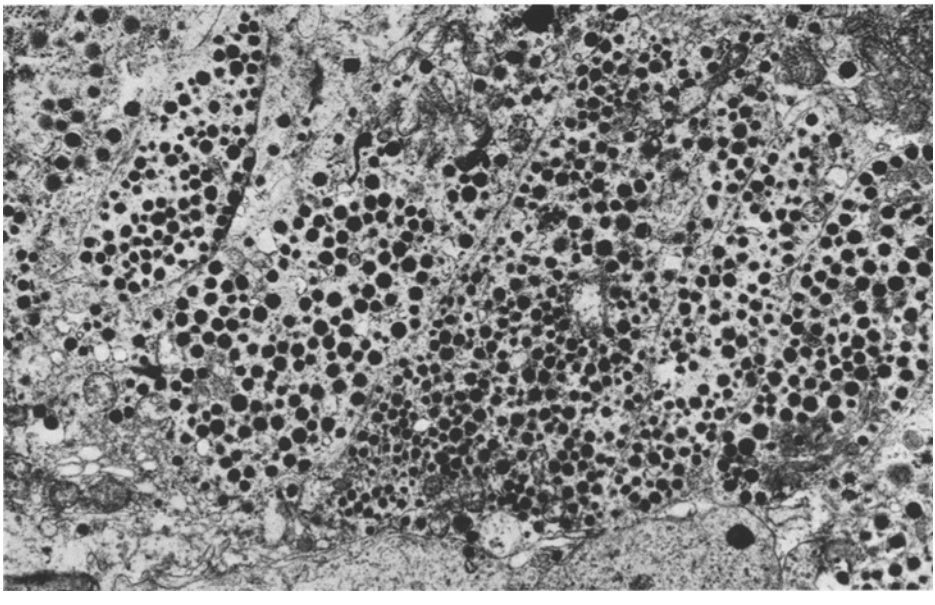

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Fig. 9. Islet β-cells showing severe degenerative alterations. There are some secretory granules which are structurally preserved. Fibrous tissue is seen among the damaged cellular components. (2 weeks). $\times$ 18,000

Fig. 10. Islet α_2-cells showing unaffected secretory granules and cytoplasm without vacuolization. Some of the mitochondria are swollen. (2 weeks). $\times$ 12,000

particularly at 1, 2, and 3 weeks. These cells showed no degenerative alterations. At 6 months the cellular composition of the islets was normal. Some of the β- and α_1-cells exhibited well developed endoplasmic reticulum and Golgi complex, but no degenerative changes were found in the islet cells.

Discussion

Atrophy of the exocrine pancreas with some similarity to that evoked by duct ligation can be elicited by the administration of ethionine (Boquist, 1969b) or excess methionine (Boquist, 1969c). The cause for this is not known but obstruction of the pancreatic duct system may at least play some role for the development of the lesions (Boquist, 1969c). In exocrine pancreas which has been damaged by the administration of ethionine there is sometimes regeneration of acinar parenchyma (Fitzgerald and Alvizouri, 1952; Kinney *et al.*, 1955; Fitzgerald *et al.*, 1968). Regeneration of damaged acinar cells has also been observed after the administration of excess methionine (Boquist, 1969d). In the present investigation there were signs of proliferation of ductule cells. Regeneration of acinar parenchyma was, however, not found in the present ultrastructural, or in the preceding light microscopic studies (Edström and Falkmer, 1967, 1968). This may be due to the fact that some acinar tissue was left between the ligature and the gut, giving possibilities for some pancreatic juice to reach the intestinal lumen, and consequently, there was no impetus for regeneration (Edström and Falkmer, 1968). If so, one would expect to find some signs of hyperactivity or hypersecretion in the small remaining "intact" portion. Though such signs were lacking it can not be excluded that the secretory activity in this portion was sufficient to supply the organism with adequate amount of pancreatic juice. The defective regenerative capacity of the duct ligated pancreas may also be due to the fact that the ligatures give a complete and permanent obstruction of the efferent passages from the acini. This might subsequently cause an "inactivity atrophy" of the acini. In animals treated with ethionine or excess methionine (Boquist, 1969b and c) there was no complete obstruction of the duct system which possibly can account for the occurrence of regeneration in their exocrine pancreas.

The endocrine pancreas of duct ligated rats showed β-cell degranulation the first days after the operation. There were also degenerative alterations and even necrosis in some β-cells during these days. The cause for these changes is not quite clear. However, they may be secondary to the extensive lesions in the acinar parenchyma. The degeneration can also be due to damage to the vessels of the ducts, since these vessels are of importance for the structural integrity of the ductal and periductal islets (Bunnag, 1966). The degranulation of the β-cells accords with the previous finding of hypoglycemia during the first post-operative week (Edström and Falkmer, 1968), as it can be anticipated that this signifies insulin release.

In the preceding light microscopic studies of duct ligated rats (Edström and Falkmer, 1967, 1968) an increased number of tubular structures were observed but it was not possible to decide with certainty whether these represented proliferating ductules and/or transformed acini. In the present study the tubular structures were found to be composed of ductular cells without zymogen granules. Intermediate stages between acinar cells and ductular cells were not recorded.

Thus no support for the transformation of acini into ductules was obtained. Consequently, there is reason to believe that the abundant occurrence of tubular structures indicates a real neoformation and proliferation of pancreatic ductules.

There is light microscopic evidence in duct ligated animals of neoformation of islets from ductules (Vranic, 1965; Zweens and Bouman, 1967, 1969; Edström and Falkmer, 1967, 1968). The presence of small islets close to duct epithelium was also observed by Jönsson (1968), although he did not state clearly that this could be interpreted as signs of islet regeneration. Ductules seem to play a role for the regeneration of pancreatic islet cells also in alloxan-treated animals (*cf.* Boquist, 1969a). In the present study there were some ductular cells showing structures resembling the secretory granules of β-cells. Granulated cells were also found in proliferating ductules and islets were observed in association with ductules. All these findings seem to support the hypothesis of development of islet cells from ductules. The cause for neoformation of islet cells in duct ligated rats may be that there is an initial damage to these cells with a subsequent need for formation of new cells.

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