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Pancreatic Acinar Ultrastructure in Human Acute Pancreatitis

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Summary. Ultrastructural alterations in pancreatic acini from six patients operated for acute necrotizing pancreatitis are described. One of the patients suffered from biliary tract disease, the rest had excessive alcohol intake as the presumed aetiology. Areas of the pancreatic parenchyma showing oedematous inflammation in light microscopy were studied in the electron microscope. Findings in acinar cells included changes in zymogen granules and an increased autophagocytosis in addition to unspecific organelle alterations. Zymogen granules showed increase in size and number, loss or variation of electron-density and peripheral dissolution. Increased autophagic activity was indicated by several autophagic vacuoles and residual bodies. Acinar lumina were dilated showing effacement of microvilli and invaginations in the luminal plasma membrane of the acinar cells. In acinar lumina and in the interstitium fibrillar material was observed, with an increasing frequency in those areas showing severe cellular disintegration. These findings suggest: 1) an increased activity of zymogen granules, 2) an increased autophagocytosis, and 3) penetration of acinar luminal contents into the interstitium.

Key words: Pancreatitis – Human pancreatitis – Acute necrotizing pancreatitis – Acinar cells – Electron microscopy.

Introduction

Acute pancreatitis (AP) is one of the most common diseases of the pancreas and is thought to be an inflammatory response of the gland to a variety of injuries. The pathogenesis of AP is unknown (Kowlessar 1976). AP and chronic pancreatitis are considered to be different diseases (Carey 1975). In AP histopathological lesions of the gland vary from oedematous reaction to severe haemorrhagic-necrotizing alterations. Excessive use of alcohol, biliary tract disease and trauma, e.g., abdominal operations are regarded as the most usual

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predisposing aetiological factors (Glazer 1975; Kraft and Saletta 1976). It is assumed that these factors, through an unknown mechanism, initiate a premature intrapancreatic activation of the zymogens of the pancreatic juice which leads to inflammation and autodigestion of the gland, i.e., to AP (Myren 1977). Studies of the morphological basis of the pathogenetic process in AP, especially those involving ultrastructural examinations, have mostly been performed on experimental animals (Doerr et al. 1965; Schiller et al. 1974). We undertook an electron microscopical study of six cases of human acute pancreatitis with the aim of obtaining information that might help to clarify the obscure pathogenesis of human AP.

Patients and Methods

In our hospital the most severe cases of acute necrotizing pancreatitis not responding to conservative therapy are treated by surgical resection of the inflamed necrotic gland (Autio et al. 1979). The present study included six patients (Table 1) who were subjected to such an operation.

Tissue material for morphological evaluation was obtained at operation. Specimens were taken separately from areas showing haemorrhagic or only oedematous changes macroscopically. Parenchymal regions with frank necrosis were not included in the material processed for electron microscopy. For light microscopical examination the tissues were fixed in Bouin's solution or neutral formalin, embedded in paraffin and sectioned at 2–4 μm. The sections were stained with haematoxylin and eosin and with Weigert-van Gieson methods. For ultrastructural examination small blocks of pancreatic parenchymal tissue were fixed by immediate immersion in cold phosphate-buffered 3% glutaraldehyde, postfixed in phosphate-buffered 1% OsO_4, dehydrated in acetone series and embedded in Epon 812. Ultrathin sections were cut with a Reichert OM-U 3 ultramicrotome, stained with lead citrate and uranyl acetate and examined in a JEM 100C electron microscope. Before ultrathin sectioning, 1 μm sections were cut off the Epon blocks and stained for light microscopy with toluidine blue. For control tissue from the normal pancreas of a kidney donor suffering from subarachnoidal haemorrhage was processed as described above.

Table 1. Main clinical data on six patients operated for acute pancreatitis

Patient no.	Age/sex	Presumed etiology	Duration of symptoms h	Operation	Gross pathology	Outcome
1	64 M	biliary tract disease	50	resection 60% choledochotomy	ANP[a] with haemorrhage	died
2	49 F	alcohol	108	resection 80%	ANP with haemorrhage	recovered
3	33 M	alcohol	48	resection 95%	ANP with haemorrhage	recovered
4	37 M	alcohol	24	resection 95%	ANP with haemorrhage	recovered
5	47 M	alcohol	48	resection 65%	ANP with haemorrhage	recovered
6	54 M	alcohol	52	resection 80%	ANP	recovered

[a] ANP = acute necrotizing pancreatitis

Results

Gross Pathology

Induration with surrounding oedema and necrosis involving both the gland parenchyma and the extrapancreatic adipose tissue, were seen in each case. Macroscopical signs of haemorrhage appeared in five out of the six patients (s. Table 1).

Light Microscopy

The clinical and macroscopical diagnosis of acute necrotizing pancreatitis was confirmed in all six patients. Lesions varied from slight interlobular and interacinar oedema to structureless acidophilic necrosis. The degenerative and necrotic alterations were accompanied by a variable amount of haemorrhage or inflammatory cell infiltration, the latter consisting of neutrophils and mononuclear cells. Histological examination revealed haemorrhages in all six cases. Acinar changes of milder degree included dilatation of the lumina with occasional accumulation of cellular debris, pyknosis or necrosis of single cells.

Electron Microscopy

Sections for ultrastructural examination were prepared from areas showing mild to moderate inflammation in light microscopy of the semi-thin sections. Areas of advanced necrosis were not included. Thus electron microscopy at low magnification showed preservation of the basic acinar architecture which was accompanied by interacinar oedema and accumulation of inflammatory or red blood cells (Fig. 1). In the acini dilatation of the lumina was often seen (Fig. 2). Many of the dilated lumina contained amorphic material, presumably of cellular origin, residual bodies, polymorphonuclear leukocytes or fibrillar material. The latter consisted of irregular fibrils of medium electron density, arranged in parallel bundles or an interwoven network (Fig. 3A). Plump or completely effaced microvilli were observed in the luminal margin of the acinar cells accompanying the above luminal changes. In more severely altered cells invaginations of the luminal plasma membrane into the acinar cell cytoplasm were seen (Fig. 2B). In some cells this phenomenon gave an impression of newly formed acinar lumina.

Ultrastructural alterations seen in acinar cells varied in severity not only from one acinus to another but also within one acinus. Single necrotic cells or necrotic cells with nuclear pyknosis and marked cytoplasmic damage were detected in acini showing otherwise well-preserved ultrastructure. Acinar cell changes included cytoplasmic swelling, dilatation of the endoplasmic reticulum and mitochondrial alterations. The latter comprised swelling, myelin figures,

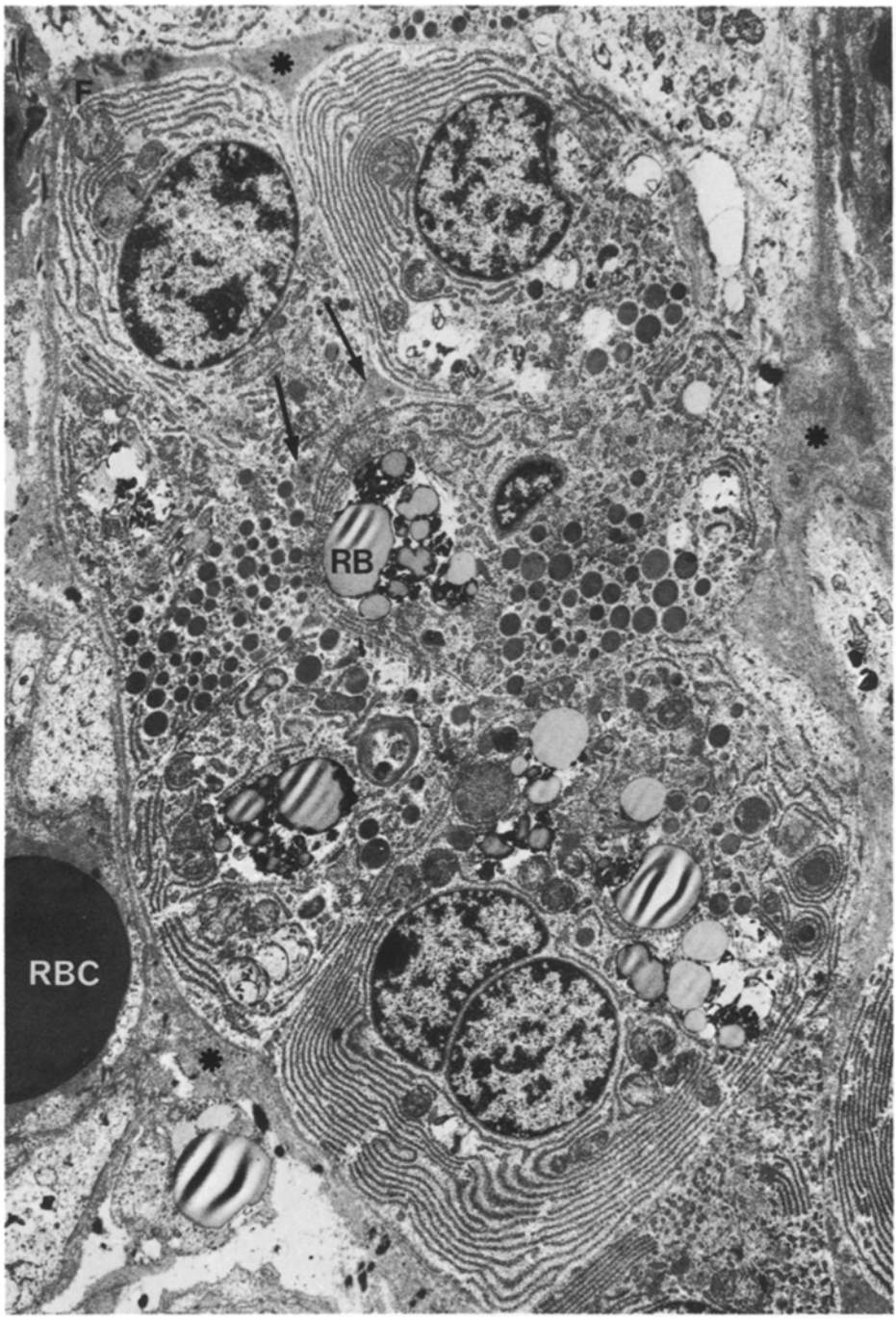

Fig. 1. Survey electron micrograph of a pancreatic acinus from an oedematous area of pancreas with acute necrotizing pancreatitis. Basic acinar architecture is preserved. Acinar cells contain large residual bodies (*RB*). Interstitial space shows accumulation of fibrillar material (*) which in some places is mixed with fibrin (*F*). The same material is seen between acinar cells (*arrows*). (*RBC*) red blood cell. × 5,360

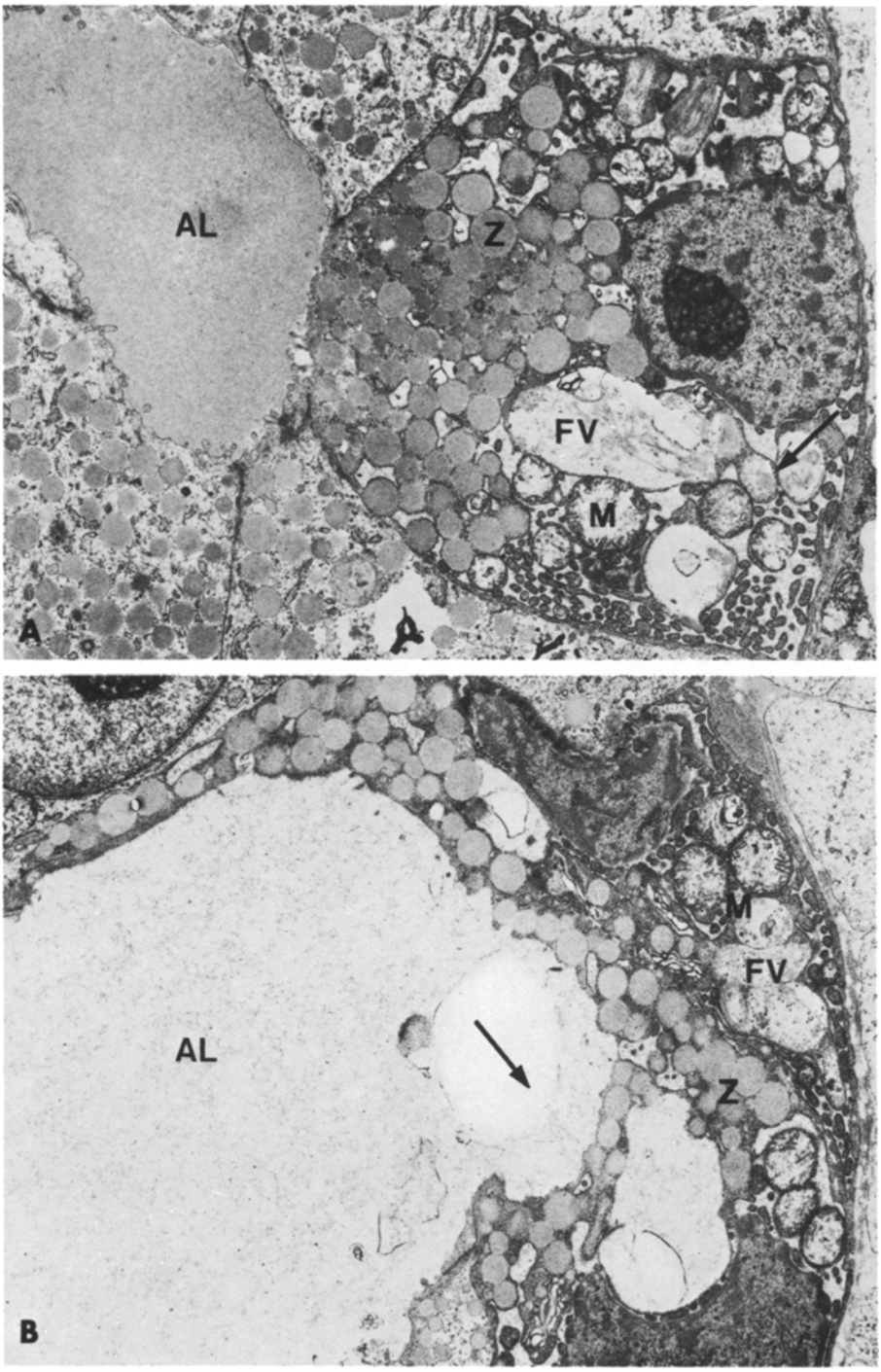

Fig. 2A and B. Sections from pancreatic acini showing marked dilatation of the acinar lumina (*AL*) and advanced acinar cell disintegration. In **B** protrusion of the acinar lumen (*arrow*) into the acinar cell. Note the swollen mitochondria (*M*) and the large fibril-containing vacuoles (*FV*). In **A** such a vacuole is merging with a cellular organelle (*arrow*). In (**B**) a few zymogen granules (*Z*) have fused. **A** ×6,200. **B** ×8,520

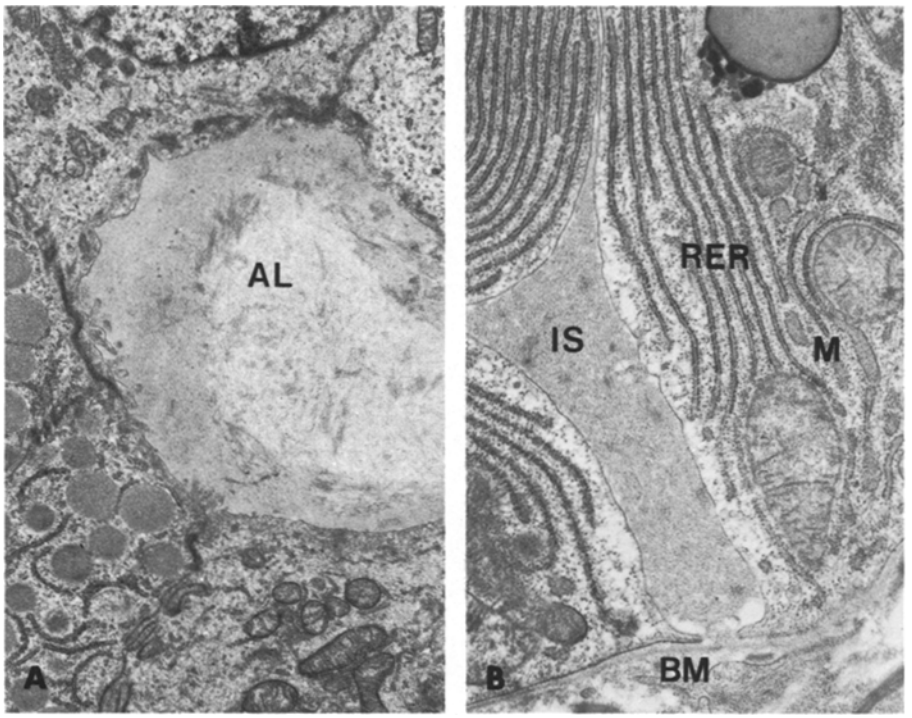

Fig. 3A and B. In **A** a dilated acinar lumen (*AL*) is filled with fibrillar material. Note the effacement of microvilli on the luminal margin of the acinar cells. In **B** the same type of material is seen in intercellular space (*IS*) between two acinar cells. Rough endoplasmic reticulum (*RER*) and mitochondria (*M*) show almost normal ultrastructure. (*BM*) basement membrane. **A** ×9,190. **B** ×13,570

flocculent densities or complete disruption (Fig. 4). Increased autophagic vacuoles and residual bodies were a fairly constant finding in acinar cells (Figs. 1 and 5). In addition, fibril-containing vacuoles, enveloped by a single-layered membrane, were observed in acinar cell cytoplasm (Fig. 2). These structures were often large and confluent and were more regularly encountered in cells with more severe damage. The fibril-containing vacuoles were detectable in both basal and apical portions of the acinar cells. In some cases they seemed to merge with remnants of cell organelles (Fig. 2A).

The zymogen granules were in general relatively well preserved. They were usually round in shape and showed a homogenous electron-dense content bordered by an intact membrane. In acinar cells with more advanced disintegration increase in size and number, peripheral electron-opacity and fragmentation of the bordering membrane were observed in the zymogen granules (Fig. 5A and B). Fusion or complete disruption of zymogen granules was not conspicuous even in most severely altered cells, the former alteration being very rarely seen and involving only a few granules (Fig. 2B). Zymogen granule and other organ-

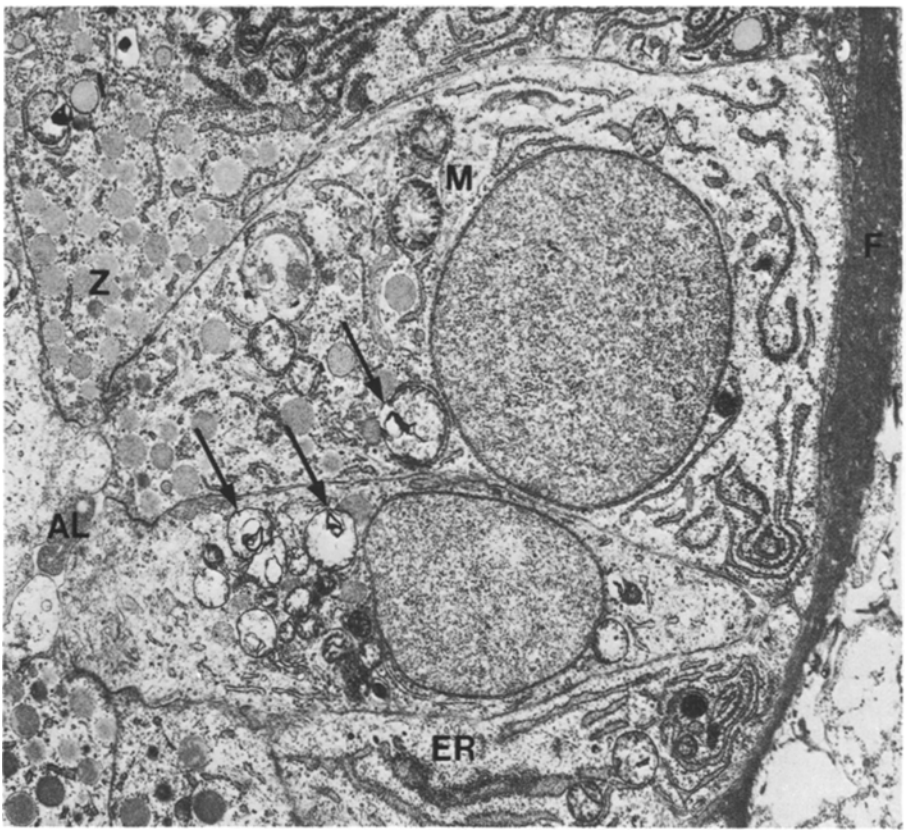

Fig. 4. Acinar cells showing altered cytoplasmic organelles. Mitochondria (*M*) are swollen and some of them contain myelin-like figures (*arrows*). Note also the dilated endoplasmic reticulum (*ER*). Zymogen granules (*Z*) appear electron-lucent but otherwise inconspicuous. (*AL*) acinar lumen. (*F*) fibrillar material. ×7,610

elle changes seemed to occur concomitantly, i.e., neither one appeared in the absence of the other.

Alterations in interacinar spaces were characterized by oedema and accumulation of inflammatory or red blood cells. In inter- and periacinar spaces fibrillar material, often intermingled with fibrin and morphologically identical with that found in dilated acinar lumina, was often seen and appeared to be continuous with the same kind of substance between adjacent acinar cells (Figs. 1 and 3 B).

No essential differences occurred in the pancreatic ultrastructural appearance between patients with different aetiologies or different duration of symptoms. It must, however, be pointed out that the areas of the inflamed gland selected for ultrastructural examination showed rather uniform alterations in light microscopy.

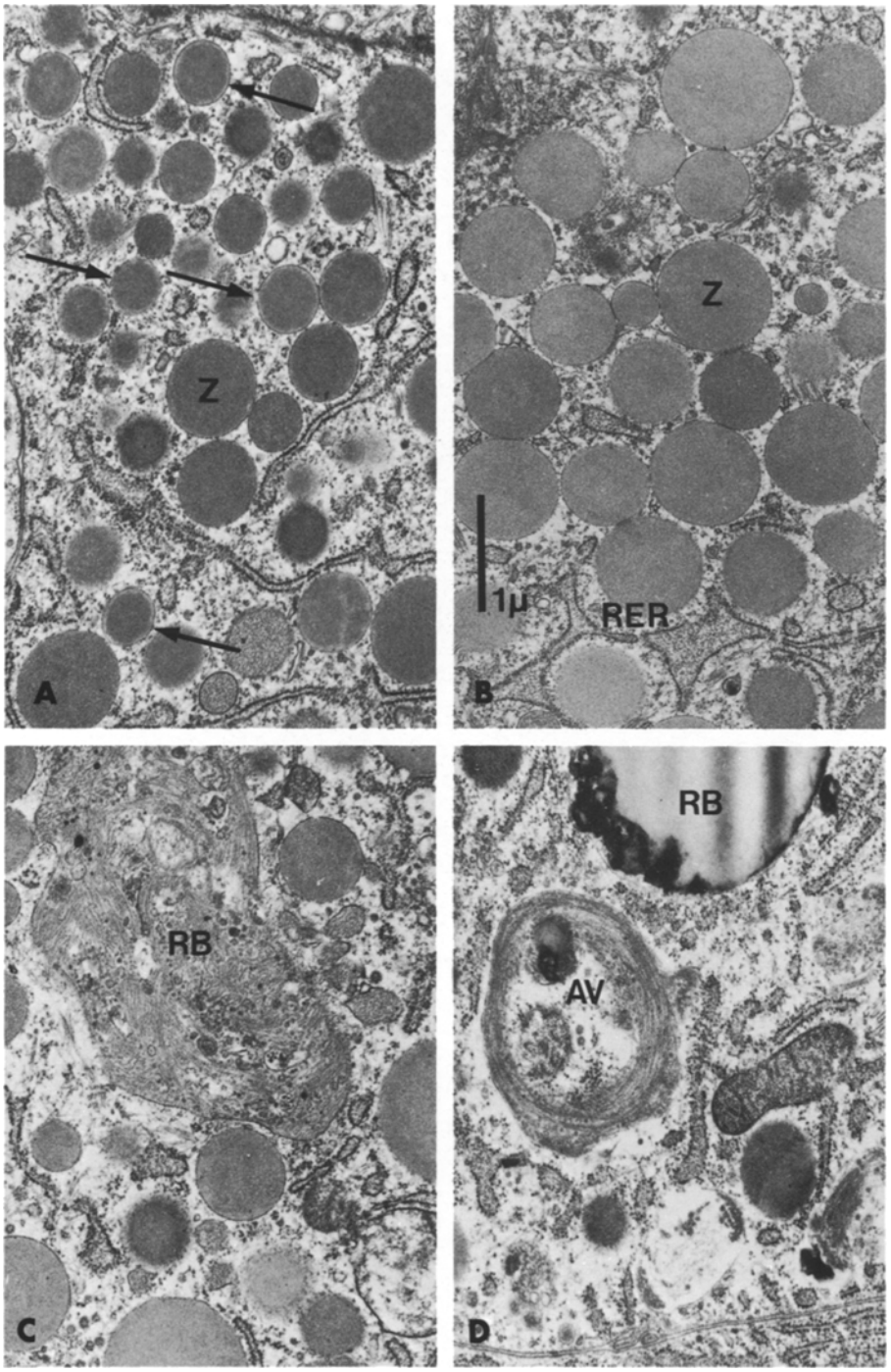

Fig. 5A–D. Zymogen granule (*Z*) alterations manifesting in **A** as peripheral loss of electron density and breaks in the enveloping membrane (*arrows*). In **B** marked increase in the size of the zymogen granules (compare with **5A**). Rough endoplasmic reticulum (*RER*) dilated. In **C** a residual body probably containing cellular debris. In **D** an autophagic vacuole (*AV*) with membranous residues and an osmiophilic residual body (*RB*). **A** and **B** ×15,400. **C** ×18,200. **D** ×17,500

Discussion

The purpose of this study was to evaluate the ultrastructural alterations found in human acute necrotizing pancreatitis. The limits of this kind of investigation are well recognized. It is not certain, for instance, whether areas of the acutely inflamed pancreatic tissue showing milder oedematous lesions and chosen for the object of this study might reflect early stages of acute necrotizing pancreatitis, although there are data to support the view that necrotizing pancreatitis is usually preceded by an oedematous phase (Doerr et al. 1965; Kowlessar 1976). Further, five of the six patients in our study had excessive alcohol intake as the presumed aetiology. Whether the lesions in pancreatic conditions initiated by other mechanisms of injury follow the same ultrastructural pattern is unclear. Nevertheless, no essential differences were noted in a comparison of the five cases with a history of alcohol ingestion with the sixth patient in the study who suffered from biliary tract disease.

Among the acinar luminal changes dilatation and accumulation of fibrillar material were the most prominent. In the luminal plasma membranes of acinar cells effacement of microvilli and invaginations were found, the latter with increasing severity of cellular disintegration. Disruption of the luminal plasma membranes did not occur except in cells with the most advanced damage. In this respect our results disagree with those presented in a previous case-report (Taura et al. 1975). In this paper penetration of acinar luminal contents into acinar cells by a breakthrough in the plasma membrane was postulated. Taura et al. (1975) found fibril-containing vacuoles in the cytoplasm of acinar cells and interpreted them as being protrusions of acinar lumina containing the fibrillar material. In the present study we did not observe direct connections between the cytoplasmic fibril-containing vacuoles and the acinar lumina. Rather, we regard the fibril-containing vacuoles as being derived from cytoplasmic constituents and share the opinion of Jones et al. (1975), who in their work describing alterations of the human pancreas in shock, interpreted similar-looking structures as residual bodies. A morphological feature favouring lysosomal origin of the fibril-containing vacuoles was the observation of engulfment of residues of cytoplasmic organelles by these vacuoles. We also looked for possible signs indicating an origin of the fibril-containing vacuoles from zymogen granules. However, no convincing structures interpretable as intermediate forms between zymogen granules and the vacuoles were seen. Along with the fibrillar vacuoles discussed above other types of lysosomal organelles occurred with high frequency in the injured cells. The lysosomal organelles included autophagic vacuoles, identified as such on the base of residues of membranous cytoplasmic organelles, and residual bodies containing electron-dense lipidic degradation product. Although it is known that a limited number of cytolysosomes may also be found in normal tissues, their increased number generally indicates a sublethal intracellular injury (Ghadially 1975). Increased autophagic activity has been encountered in various kinds of experimental pancreatic injury (Lombardi et al. 1975; Nevalainen 1975; Janigan et al. 1975), including that induced by long-term intake of ethanol in rats (Darle et al. 1970). In humans increased

autophagy has been reported in pancreatic acinar cells in shock (Jones et al. 1975).

A perhaps unexpected finding was the relatively good preservation of the basic structure of the zymogen granules. This is however well in keeping with observations made on human pancreatic tissue in shock (Jones et al. 1975). In pancreatic acinar cells of the rat morphologically intact zymogen granules have been observed after even 24 h autolysis (Nevalainen and Anttinen 1977). In our study we did not observe structures indicating engulfment of zymogen granules by autophagic vacuoles as did Longnecker et al. (1968) in puromycin-induced pancreatic necrosis. The main alterations in zymogen granules in the present study were an increase in size and number, occasional peripheral electron opacity and discontinuities of the enveloping membrane. The observation of a numerical increase and enlargement of the granules is based on comparison with the control tissue alone, since no attempt at exact quantitative analysis (e.g., morphometry) was made. Loss or variation in electron density of the granule contents was noted along with the changes described. Fusion or disruption was observed only in necrotic or severely disintegrated acinar cells and was by no means a frequent finding in the present study. The changes described might indicate an increased functional activity of zymogen granules. As regards the possible factors initiating the increase in pancreatic zymogen activity, alcohol in large doses has been shown to increase the enzyme and calcium concentration of the pancreatic juice in dogs (Hadorn 1974). We were not able to draw conclusions as to the primarity of the zymogen granule alterations in respect of other cytoplasmic organelle changes, since both of these seemed to appear concomitantly. Peripheral zymogen granule dissolution similar to that in our work has been described in experimental and in human pancreatic injuries (Janigan et al. 1975; Jones et al. 1975; Nevalainen 1977).

An interesting finding was the fibrillar material seen in acinar lumina and in peri- and interacinar spaces. Similar material was seen in both locations by Bockman et al. (1973) in pancreatic acini after ligation of the pancreatic duct with simultaneous intravenous secretin stimulation. It had previously been proposed that this material represents endogenous pancreatic enzymes penetrating from acinar lumina into periacinar spaces through minute disruptions in ductoacinar junctions (Bockman et al. 1971). In the present study we discovered no communications between acinar lumina and the periacinar space bridged by the fibrillar material. We did however find fibrillar material between acinar cells. This might suggest a route for acinar luminal contents from the acinar lumina between acinar cells into the interstitium. Such a route was shown to exist under apparently physiological conditions in the pressure experiments performed by Pirola and Davis (1970) on cats.

The conception that the acinar luminal contents in AP could contain prematurely activated enzymes is supported by both experimental observations and by clinical studies made on humans. Ohlsson and Eddeland (1975) found active trypsin, chymotrypsin and elastase in pancreatic exudate in dogs with bile-induced pancreatitis. Rao et al. (1976) were able to recover these activated enzymes within the pancreas during experimental murine pancreatitis. Pancreatic

juice collected from patients with AP through catethrization or external fistulae has been shown to contain active chymotrypsin (White and Allan 1974) or active trypsin, chymotrypsin and elastase with an apparent lack of trypsin inhibitor (Geokas and Rinderknecht 1974).

In conclusion, our results suggest the following. 1) An increased activity of zymogen granules, apparently reflecting hypersecretion of the exocrine pancreas. 2) An increased autophagic activity of pancreatic acinar cells. 3) Penetration of acinar luminal contents into the interstitium between the acinar cells. The usually early recognition of the fibrillar material and other acinar luminal alterations suggests a premature activation of pancreatic enzymes outside the acinar cells within the acinar lumina or the duct system.

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