Virchows Arch [Pathol Anat] (1986) 409:791–803

Virchows Archiv A
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Human acute pancreatitis: its pathogenesis in the light of immunocytochemical and ultrastructural findings in acinar cells* **

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Summary. Human acute pancreatitis results from an autodigestive process frequently associated with alcohol abuse, gall stone disease and shock. Peripancreatic fat necrosis was identified as one of the earliest visible lesions, whereas acinar cell necrosis and haemorrhage were regarded as secondary changes. To examine the alterations in acinar cells in more detail, their enzyme content and fine structural features were studied immunocytochemically using antisera against α-amylase, lipase, trypsin, chymotrypsin and pancreatic stone protein, and electronmicroscopically in pancreatic tissues from patients with severe acute pancreatitis. Peripheral acinar cells in the immediate vicinity of fat necrosis were found to be heavily degranulated, while acinar cells at some distance of necrosis fully retained their enzyme content. Other frequent changes of the acinar cells included cuboidal transformation, loss of microvilli, increased occurrence of autophagosomes, and formation of enlarged acinar lumina. As there was no apparent cell membrane leakage or rupture of duct lumina, it is concluded that the acinar cells adjacent to fat necrosis release their granules by undirected basolateral extrusion. The findings thus suggest that one of the basic defects in acute pancreatitis is the uncontrolled release of enzymes from peripheral acinar cells into the interstitial space which, in turn, presumably by the action of lipase, leads to autodigestive fat necrosis.

Key words: Human acute pancreatitis – Enzyme immunocytochemistry – Ultrastructure – Acinar cell degranulation – Fat necrosis

* Dedicated to Prof. Dr. G. Seifert on the occasion of his 65th birthday

** Presented in part at the Second International Symposium on the Classification of Pancreatitis, Marseille, 1984, and at the Meeting of the European Pancreatic Club, Manchester, 1985

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Introduction

Autodigestion is the fundamental pathogenic principle underlying the sudden tissue destruction in most cases of human acute pancreatitis (Chiari 1906; Doerr 1964; Creutzfeldt and Schmidt 1970; Baggenstoss 1973; Becker 1973 and 1981; Wanke 1976; Dürr 1979; Gyr et al. 1984). Though all investigators in this field agree on the autodigestive hypothesis of acute pancreatitis there is much controversy about where and how the enzymes become activated. Moreover, it is a matter of debate which enzyme is most important for the production of tissue necrosis and whether the main aetiological factors, i.e. alcohol, biliary disease and shock, result in distinct forms of the disease.

From the "common channel theory" advanced by Opie (1901) at the beginning of the century and involving reflux of bile into the main pancreatic duct due to functional and mechanical obstruction, one would suggest that ductal and periductal necrosis is the initiating lesion in acute pancreatitis. This was demonstrated in a recent necropsy review of 37 cases of acute pancreatitis by Foulis (1980) who found periductal necrosis in patients with gall stone disease, ethanol ingestion and diabetes. In contrast, perilobular necrosis was observed particularly in patients dying of shock. In our series of 367 necropsy cases of acute pancreatitis of varying duration, severity and aetiological background, we were unable to distinguish between two distinct patterns of initial damage to the pancreas (Klöppel et al. 1984). We recognized only one pattern of injury which was dominated by peripancreatic fat necrosis. This lesion was encountered in all cases of pancreatitis, irrespective of their duration, severity and aetiology. In contrast, intraductal inflammation, ductal necrosis and subsequent periductal tissue damage were rather rare findings in our study and, when present, always occurred together with peripancreatic fat necrosis. Schmitz-Moormann (1981), in his study on the zonal distribution and characterization of lesions in acute pancreatitis, based on sequential mapping of the whole pancreas combined with ductograms, also emphasized the role of fat necrosis as the primary lesion in acute pancreatitis. He consequently suggested a key role for lipase in the production of pancreatic damage.

If autodigestive fat necrosis and not duct wall necrosis or acinar cell necrosis is the earliest visible lesion in acute pancreatitis, a number of questions arise: Firstly how do the pancreatic enzymes reach the fat cells, secondly do all compartments of the acinar cells loose their enzymes, and finally where does the enzyme activation occur?

The objective of the present study was to analyze the enzyme content of the acinar cells by immunocytochemical means and to examine the secretory apparatus of the acinar cells and the surrounding extracellular spaces by electronmicroscopy.

Material and methods

Surgically removed pancreatic specimens were obtained from four patients, two women and two men aged 30–71 years (mean: 44), operated upon for acute pancreatitis. Pancreatic tissue

from two post mortem examinations (two men aged 17 and 38 years) performed within 24 h after death and showing severe acute pancreatitis was also judged suitable for study. Alcoholism was the aetiological factor in four patients. One patient had an idiopathic aetiology and in another patient pancreatitis was presumably due to a treatment with valproic acid. Duration of symptoms varied between 3 and 7 days. Normal pancreatic tissue was obtained from patients operated upon for pancreatic tumours without concomitant obstructive chronic pancreatitis.

The tissue was fixed in 10% buffered formalin and processed to paraffin sections. Multiple blocks were cut and the first two sections were stained with haematoxylin and eosin, and periodic acid Schiff (PAS). Immunocytochemistry was carried out on subsequent deparaffinized sections using the avidin-biotin-peroxidase complex method. All sections were immunocytochemically stained for lipase, trypsin, chymotrypsin, alpha-amylase and pancreatic stone protein. The antisera against porcine alpha-amylase, porcine lipase, bovine trypsin and alpha-chymotrypsin (Sigma Chemicals, Munich, FRG) were raised in rabbits by injecting the animals with 300 μg enzyme preparation (highest commercially available purity), dissolved in 0.5 ml phosphate buffered saline and 0.5 ml Freund's complete adjuvant. After repeated injections at four week intervals the rabbits were bled one week after the last injection. The monoclonal antibody against pancreatic stone protein was purchased from Immunotech, Marseille, France. The histochemical reaction for peroxidase was performed using 3.3-diaminobenzidine tetrahydrochloride (0.05% w/v) and hydrogen peroxide (0.01% w/v) in Tris-buffer (0.05 M, pH 7.2). The sections were then postfixed in aequous osmium tetroxide (1%). The specificity of immunostaining of the four pancreatic enzymes was determined by preabsorption of the antisera with the appropriate antigen. Sections of normal pancreas were used as positive controls.

For electron microscopical examination small samples of pancreatic tissue were obtained at operative intervention in nine patients (six men and three women, aged 25–75 years (mean: 46) with acute pancreatitis of alcoholic (six patients) or biliary (three patients) aetiology. Duration of symptoms varied from 4 to 40 days. The samples were taken from different regions of the gland adjacent to necrotic areas. Normal pancreatic tissue was obtained from three patients operated upon endocrine tumours of the pancreas and from two cadaver kidney transplant donors. The tissues were fixed immediately after removal by immersion in a solution of 2.5% glutaraldehyde and 2.0% formaldehyde in 0.1 M cacodylate buffer at pH 7.5 for 2 h. After postfixation in 1% osmium tetroxide and dehydration in a graded series of alcohols, the tissue samples were embedded in Epon 812. From each pancreas multiple blocks were prepared and semithin sections from each block were analyzed at the light microscopical level to localize representative parenchymal regions outside necrotic areas. Ultrathin sections from these regions were stained with uranylacetate and examined using a Zeiss EM 109 electron microscope.

Results

Histology

The pancreatic specimens from the 15 patients included in this study showed severe acute pancreatitis. It was characterized by confluent fat necrosis on the surface of the organ (Fig. 1a) which also extended deeply along the interstitial septa into the gland. Extensive fat necrosis often involved directly adjacent acinar cells. The still intact acini of lobules in the vicinity of fat necrosis were composed of flattened cells lining enlarged lumina often filled with PAS positive material (Fig. 1b). Frequently the necrosis also affected the wall of venous and, more rarely, arterial vessels, which resulted in thrombosis and/or haemorrhage. The boundary between necrotic and viable pancreas was marked by a polymorphonuclear cell infiltrate. The still intact parenchyma showed marked interstitial oedema. Duct rupture was a rare finding, even in areas with advanced necrosis.

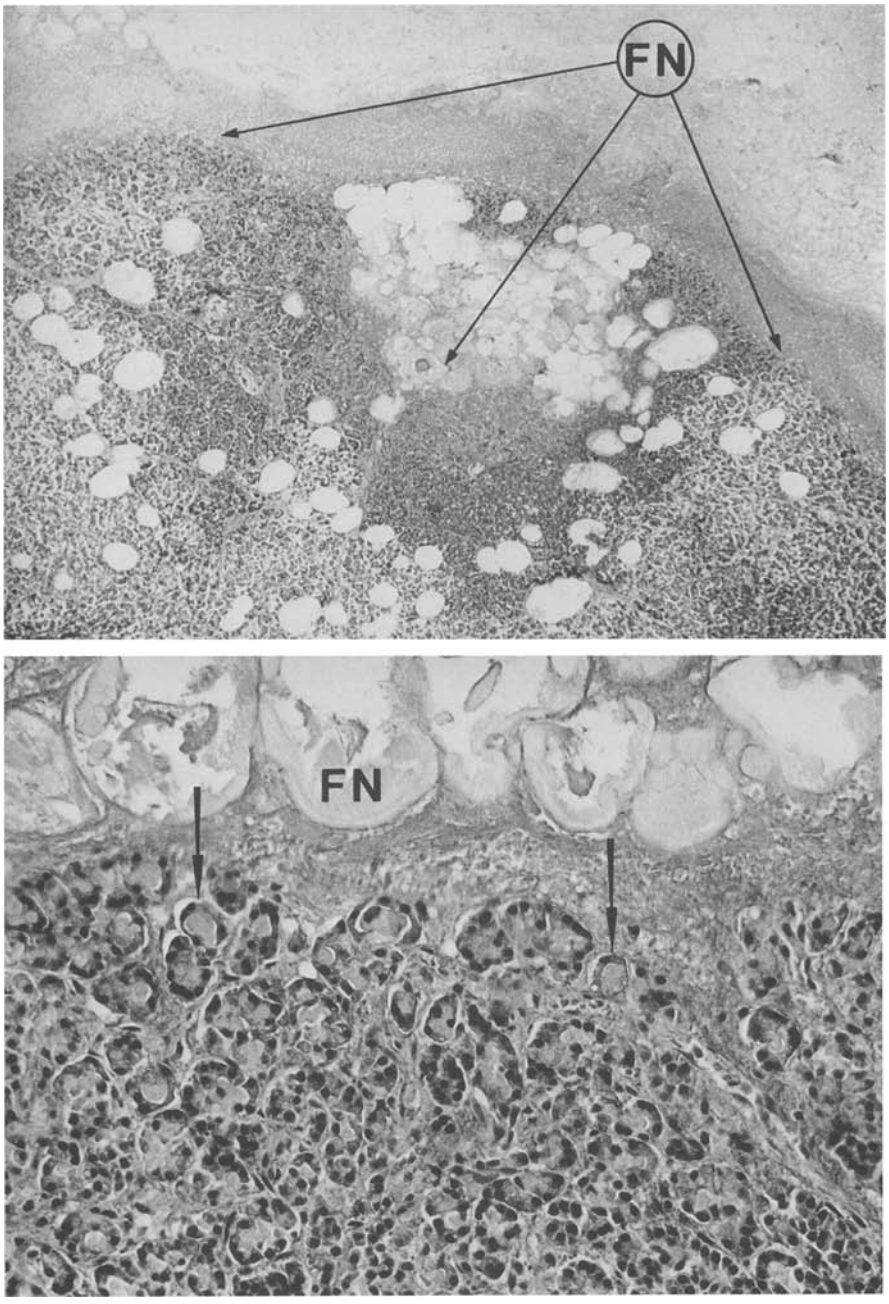

Fig. 1. Severe acute pancreatitis: (**a**) Confluent peripancreatic fat necrosis (*FN*) affecting the adjacent acinar cells at the margin of a lobule. H & E, ×40. (**b**) Intact acinar cells in the immediate vicinity of fat necrosis (*FN*) showing enlarged acinar lumina filled with secretion (*arrows*). H & E, ×250

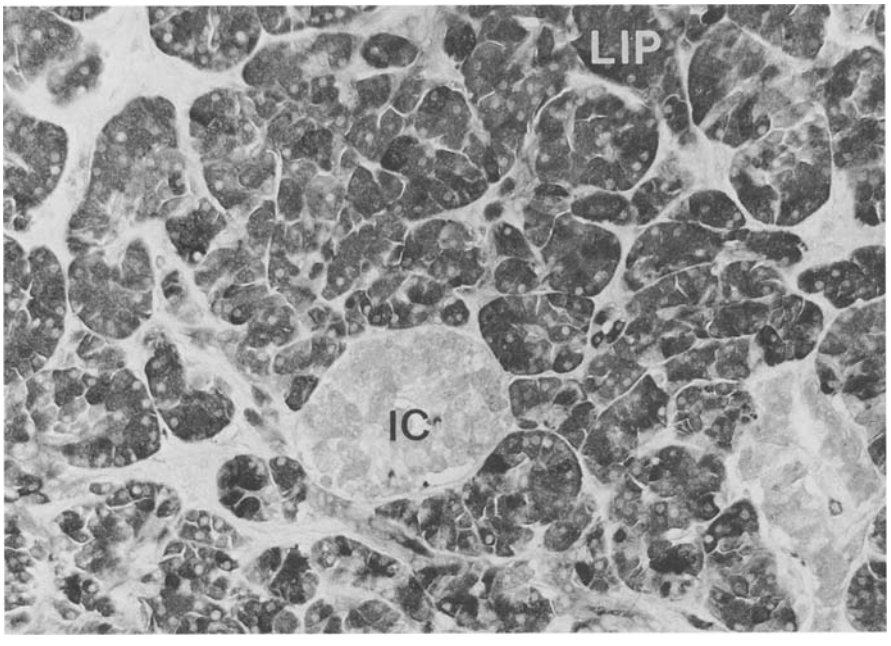

Fig. 2. Normal pancreatic tissue displaying intense immunostaining of acinar cells with antiserum against lipase (*LIP*). Islet cells (*IC*) remain unstained. × 250

Immunocytochemistry

Normal pancreatic tissue showed an intense immunoreactivity of acinar cells to the antisera against α-amylase, trypsin, chymotrypsin, lipase and pancreatic stone protein (Fig. 2). Islets, duct cells or connective tissue were not stained, apart from an occasional duct cell which was positive for lipase.

In acute pancreatitis the preserved acinar tissue showed an uneven immunoreactivity of the applied antisera. Peripheral acinar cells of lobules in the immediate vicinity of fat necrosis were almost completely depleted of amylase, trypsin, chymotrypsin, lipase and pancreatic stone protein, while many of the centrally localized acinar cells fully retained their enzyme content (Fig. 3a). In some of the enlarged acinar lumina immunoreactive material was found (Fig. 3b). The degranulated cells appeared to be preserved. No immunoreactive material was observed within ducts. Pancreatic lobules at some distance of fat necrosis displayed a largely intact enzyme positivity of the acinar cells. The margins of fat necrosis were often found to be stained. However, this positive reaction was regarded as an unspecific finding, since it was also seen to some extent in the slides checking the immunocytochemical specificity of the reactions.

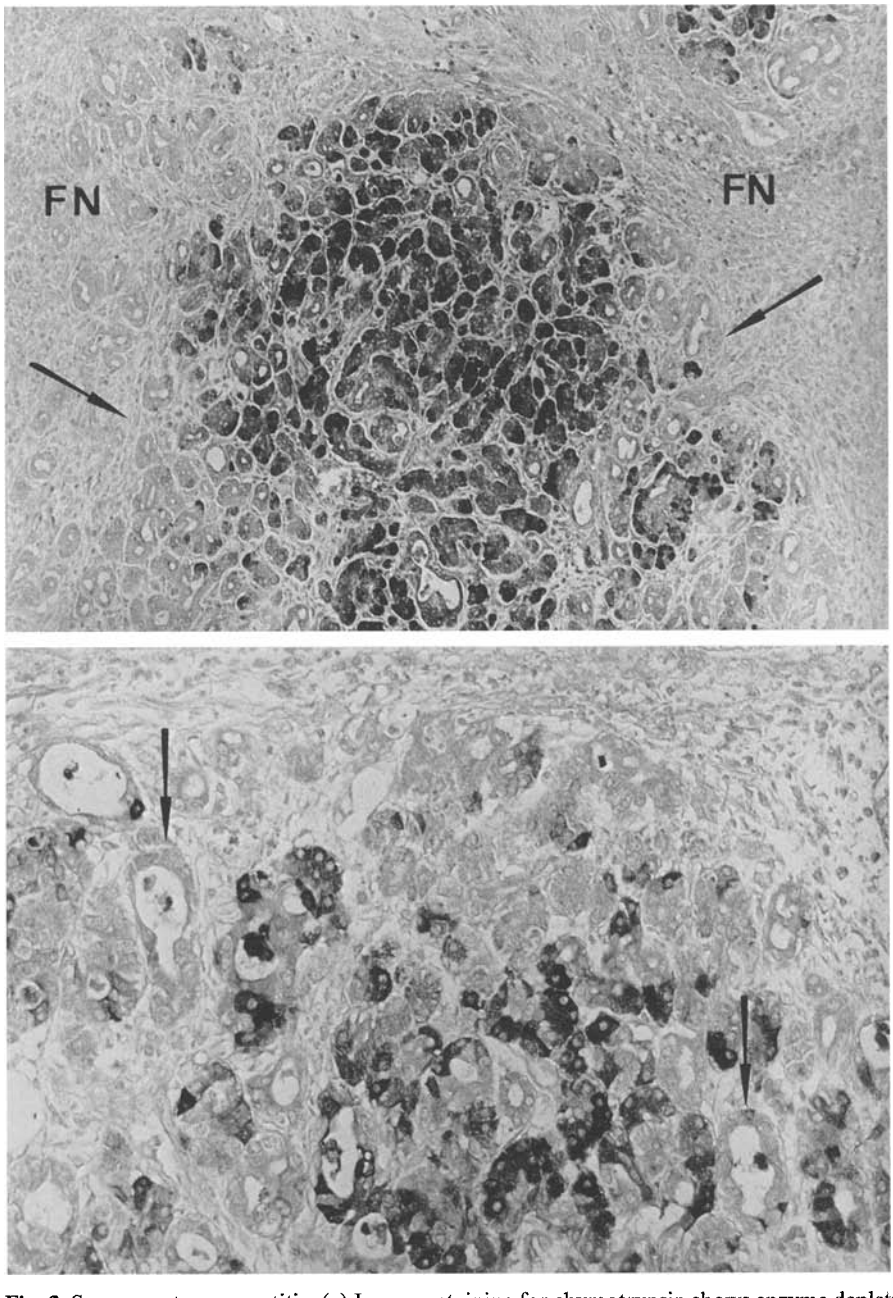

Fig. 3. Severe acute pancreatitis: **(a)** Immunostaining for chymotrypsin shows enzyme depletion of peripheral acinar cells (*arrows*) of a lobule surrounded by fat necrosis (*FN*). ×125. **(b)** Periphery of a lobule with degranulated but intact acinar cells occasionally forming tubular complexes (*arrows*). Immunostaining for trypsin. ×250

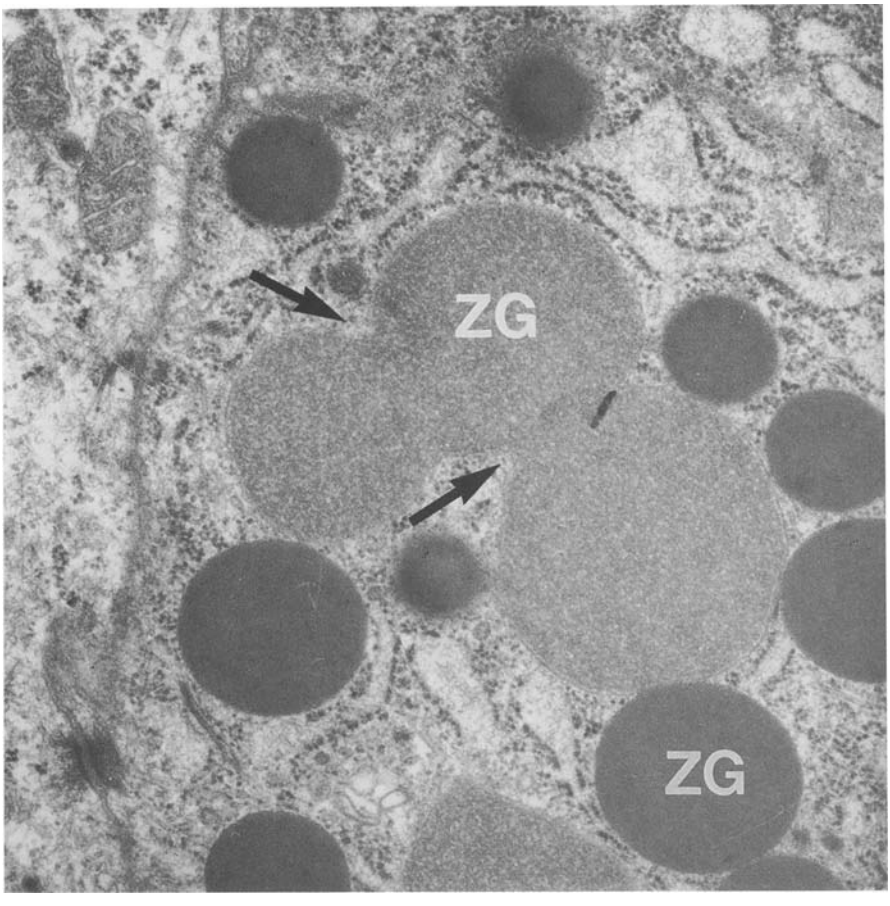

Fig. 4. Part of intact acinar cell in severe acute pancreatitis. Fusion of individual zymogen granules (*arrows*, ZG). × 32000

Electron microscopy

The general fine structural characteristics of the normal human pancreas have been reported previously (Kern and Ferner 1971; Kern 1986). Residual bodies containing lipid degranulation products increased with age.

The specimens from the patients with acute pancreatitis revealed normal acinar cells beside structurally altered acinar units and, of course, necrotic tissue. The changes in the abnormal but still intact acinar cells, on which we focused our analysis, included fusion, loss, irregular distribution and undirected discharge of zymogen granules. In addition, they showed occurrence of large autophagosomes and general dilatation of all cisternal compartments. Fusion between individual zymogen granules was frequently noted (Fig. 4). Loss of zymogen granules was observed predominantly in acinar cells lining enlarged lumina (Fig. 5). This enlargement of the lumina resulted

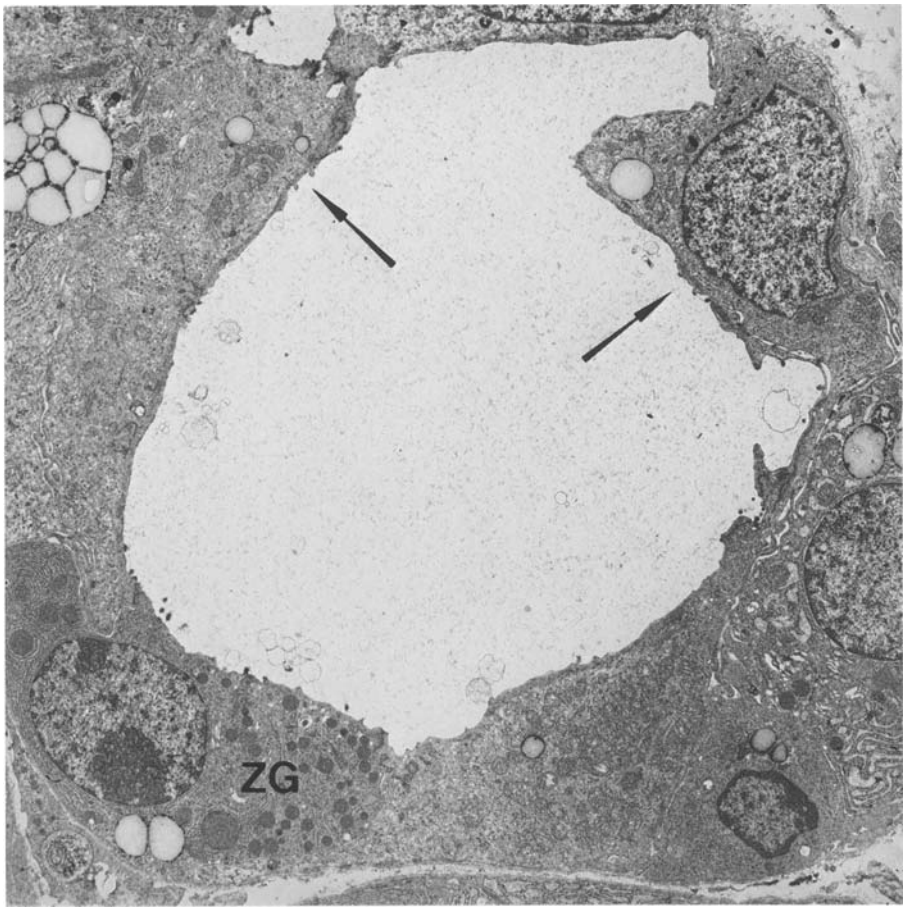

Fig. 5. Acinus with enlarged lumen from the periphery of a lobule adjacent to necrosis. The acinar cells show decreased cell height and loss of zymogen granules (*ZG*) and microvilli (*arrows*). ×4500

from a decrease in the acinar cell height and an irregular loss of microvilli. The disappearance of microvilli was occasionally combined with engulfment of the luminal plasma membrane. In the degranulated acinar cells the remaining granules were often found to be localized along the basolateral instead of the apical cell membrane (Fig. 6). Another frequent finding was the occurrence of large autophagic vacuoles, which contained floccular material, remnants of membranes and cell organelles including RER, mitochondria and zymogen granules (Fig. 7). The acinar cells with the largest autophagosomes also showed general dilatation of all cisternal compartments. Some of the acinar lumina were filled with fine fibrillar material. Occasionally they also contained lethally damaged acinar cells and cellular debris.

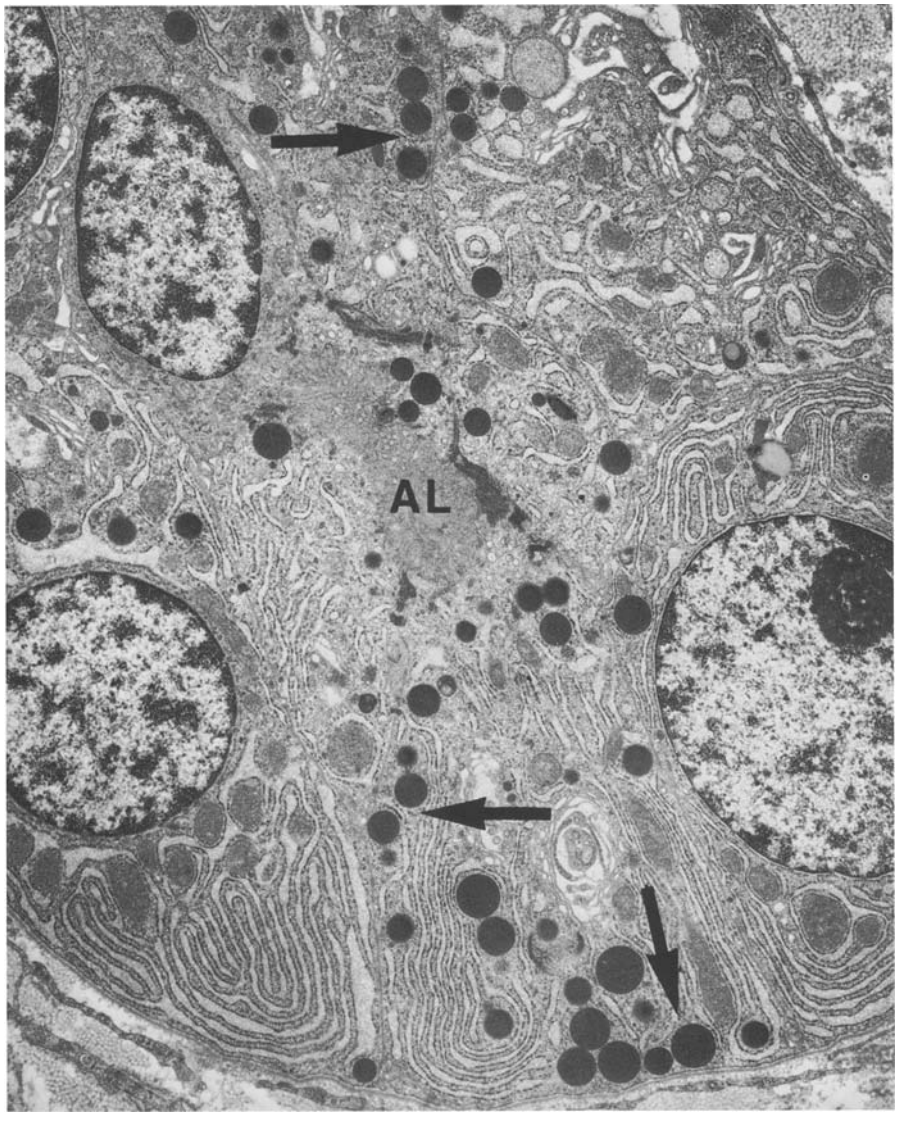

Fig. 6. Acinar cells with irregular distribution and reduced content of zymogen granules. Note the orientation of the granules along the basal cell membrane (*arrows*). Acinar lumen (*AL*). ×10100

The epithelial lining of the duct system appeared to be preserved even in those regions were most of the interstitial tissue and the acinar cells were damaged. Within the generally enlarged interstitial spaces there were inflammatory cells, erythrocytes, fibrin and small deposits of fibrillar material similar to that seen in the acinar lumina.

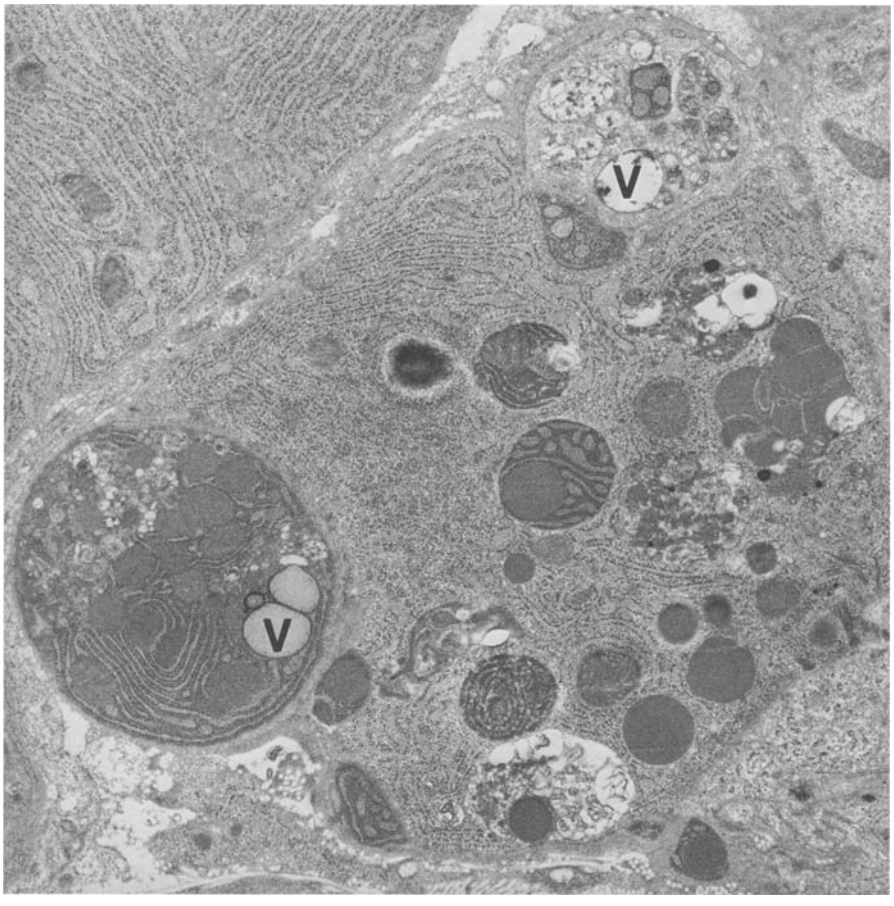

Fig. 7. Acinar cell filled with autophagic vacuoles (*V*) of varying size and content. ×12100

Discussion

Results of recent studies by our group (Klöppel et al. 1984) and by others (Schmitz-Moormann 1981) have suggested that fat necrosis is most likely the lesion which definitely marks the beginning of acute pancreatitis. This suggestion was based on the observation that fat necrosis was a constant feature of all cases with acute pancreatitis, irrespective of their duration, severity and aetiology. Since in most cases fat necrosis was present without evidence of a concomitant leakage of the duct system or a significant damage to the acinar cells, it was assumed that in acute pancreatitis a sudden release of acinar enzymes into the adjacent interstitial space initiates the mechanisms resulting in autodigestive necrosis.

Massive enzyme effusion from intact acinar cells into the interstitial space should result in degranulation of these cells. The enzymatic content of the acinar cells can be traced by immunocytochemistry using antisera

against pancreatic enzymes (Kraehenbühl et al. 1977; Bendayan et al. 1980). For the human pancreas this was recently shown by Nevalainen and his group, who demonstrated phospholipase A_2 and trypsin in acinar cells (Nevalainen et al. 1983; Aho et al. 1983). In acute pancreatitis they observed a patchy immunoreactivity at the border of necrotic and nonnecrotic exocrine parenchyma. Our study on the immunocytochemical distribution of α-amylase, lipase, trypsin and chymotrypsin confirms and extends these observations by the Finish authors. In particular, it was found that the peripheral acinar cells of a lobule in the immediate vicinity of fat necrosis were strongly depleted of enzymes while the acinar cells occupying the more central areas of that lobule remained granulated. Electron microscopy proved that the degranulated acinar cells had retained their fine structural integrity though they showed a reduced cell height and a loss of microvilli at their luminal surface. These findings strongly suggest that it is primarily the compartment of the peripheral acinar cells that delivers the enzymes for autodigestive necrosis of fat cells and that this cell compartment apparently represents the main target for those factors such as alcohol, biliary disease and shock, which are known to be causally related to acute pancreatitis.

As to the question how the enzymes reach the intercellular space it can be assumed that in the absence of any obvious cell membrane leakage or duct rupture a great deal of the zymogen granules leaves the cell through the basolateral cell membrane. This assumption is supported by the electronmicroscopical demonstration of an irregular intracellular distribution of zymogen granules which normally cluster in the apical cytoplasm, but in acute pancreatitis tend to assemble along the basolateral cell membrane. Whether the fibrillar material observed between acinar cells represents aggregates of secreted enzymes (Helin et al. 1980; Aho et al. 1982) remains to be established. With respect to the supposed basolateral granule release it is of interest that a similar process is observed in experimental pancreatitis induced by supramaximal stimulation with caerulein (Lampel and Kern 1977; Adler et al. 1982).

Although these findings seem to identify the undirected discharge of enzymes from peripheral acinar cells as the (or at least one of the) first visible basic defect(s) in acute pancreatitis, it remains poorly understood why such different factors as alcohol, gall stone disease or shock obviously cause the same functional disturbance. While in shock the preferential damage to the outer zones of the lobules may be explained by the fact that these regions belong to the microcirculatory periphery of the lobule and are thus most susceptible to ischaemia (Foulis 1980), it is so far unclear how alcohol and biliary disease interfere with the normal secretory process of the peripheral acinar cell.

So far the site and mechanisms of intrapancreatic enzyme activation are not known. Recently, lysosomes have been implicated with the activation of digestive enzymes (Scheele et al. 1984; Steer et al. 1984). Lysosomal hydrolases are known to be capable of activating trypsinogen (Greenbaum and Hirshkowitz 1961). Furthermore, an increased lysosomal activity was

found in acinar cells of animals with caerulein-induced pancreatitis (Adler et al. 1982; Watanabe et al. 1984). The acinar cells showed uncontrolled fusion of zymogen granules and large Golgi-derived autophagic vacuoles containing both lysosomal hydrolases and digestive enzymes. In human pancreatitis, strikingly similar changes seem to occur. Earlier studies by Finish authors (Helin et al. 1980; Aho et al. 1982) together with our own investigations have demonstrated accumulations of large autophagic vacuoles and fused granules in many acinar cells from non-necrotic areas. It is therefore conceivable that lysosomes play also a role in precocious activation of zymogens in human acute pancreatitis.

However, as autophagic degradation of cellular organelles may only reflect non-specific damage to the acinar cells and these cells, in addition, show no enzymatic destruction during enzyme effusion, extracellular enzyme activation must also be considered. The enzyme deserving greatest attention in this regard is lipase. Lipase is already secreted in active form and can cause massive necrosis of the abdominal adipose tissue experimentally when injected intraperitoneally (Schmitz-Moormann et al. 1978; Schmitz-Moorman 1981). We may thus speculate that from the uncontrolled release of enzymes from peripheral acinar cells, lipase reaches such a high concentration in the intercellular space that it is capable of damaging the fat cells. By this action lipase could then trigger all the other events which eventually lead to the full spectrum of acute pancreatitis.

Acknowledgements. We express our appreciation to Mrs. Ruth Malik, Susanne Peters, Lydia Perkowski, Roswitha Naumann and Dorothee Bonorden for skilfull technical assistance, and to Mrs. G. Lassen for typing the manuscript. We also thank Professor Dr. D. Maroske, Department of Surgery, University of Hamburg, and Professor Dr. H.W. Schreiber, Department of Surgery, University of Hamburg, for fruitful cooperation.

References

Adler G, Rohr G, Kern HF (1982) Alteration of membrane fusion as a cause of acute pancreatitis in the rat. Dig Dis Sci 27:993–1002

Aho HJ, Nevalainen TJ, Havia VT, Heinonen RJ, Aho AJ (1982) Human acute pancreatitis. A light and electron microscopic study. Acta path microbiol immunol scand Sect A 90:367–373

Aho HJ, Putzke H-P, Nevalainen TJ, Löbel D, Pelliniemi LJ, Dumller W, Suonpää AK, Tessenow W (1983) Immunohistochemical localization of trypsinogen and trypsin in acute and chronic pancreatitis. Digestion 27:21–28

Baggenstoss AH (1973) Pathology of pancreatitis. In: Gambill EE (ed) Pancreatitis. CV Mosby, St Louis, p 179–212

Becker V (1973) Bauchspeicheldrüse (Inselapparat ausgenommen). In: Doerr W, Seifert G, Uehlinger E (eds) Spezielle pathologische Anatomie, Bd 6. Springer, Berlin Heidelberg New York

Becker V (1981) Acute pancreatitis: Pathological anatomy and pathogenesis. World J Surg 5:303–313

Bendayan M, Roth J, Perrelet A, Orci L (1980) Quantitative immunocytochemical localization of pancreatic secretory proteins in subcellular compartments of the rat acinar cell. J Histochem Cytochem 28:149–160

Chiari H (1906) Über die Beziehungen zwischen dem Pankreas und der Fettgewebsnekrose. Zbl Pathol 17:798–799

Creutzfeldt W, Schmidt H (1970) Aetiology and pathogenesis of pancreatitis (current concepts). Scand J Gastroenterol 5 (Suppl 6):47–62

Doerr W (1964) Pathogenese der akuten und chronischen Pankreatitis. Verh Dtsch Ges Inn Med 70:718–758

Dürr GH-K (1979) Acute pancreatitis. In: Howat HT, Sarles H (eds) The exocrine pancreas. Saunders, London Philadelphia Toronto, p 352–401

Foulis AK (1980) Histological evidence of initiating factors in acute necrotising pancreatitis in man. J Clin Pathol 33:1125–1131

Greenbaum LM, Hirshkowitz A (1961) Endogenous cathepsin activation of trypsinogen in extracts of dog pancreas. Proc Soc Exp Biol Med 107:74–76

Gyr K, Heitz PU, Beglinger C (1984) Pancreatitis. In: Klöppel G, Heitz PU (eds) Pancreatic Pathology. Churchill Livingstone, Edinbourgh London Melbourne New York, pp 44–72

Helin H, Mero M, Markkula H, Helin M (1980) Pancreatic acinar ultrastructure in human acute pancreatitis. Virchows Arch [Path Anat] 387:259–270

Kern HF (1986) Fine structure of the human exocrine pancreas. In: Go VLW, DiMagno E, Lebenthal E, Brooks FP, Gardner J, Scheele G (eds) The exocrine pancreas: Biology, pathobiology and diseases. Raven Press, New York, pp 9–19

Kern HF, Ferner H (1971) Die Feinstruktur des exokrinen Pankreas beim Menschen. Z Zellforsch 113:322–343

Klöppel G, von Gerkan R, Dreyer T (1984) Pathomorphology of acute pancreatitis. Analysis of 367 autopsy cases and 3 surgical specimens. In: Gyr KE, Singer MV, Sarles H (eds) Pancreatitis – concepts and classification, pp 29–35
Excerpta Medica, Amsterdam New York Oxford

Kraehenbühl JP, Racine L, Jamieson JD (1977) Immunocytochemical localization of secretory proteins in bovine pancreatic exocrine cells. J Cell Biol 72:406–423

Lampel M, Kern HF (1977) Acute interstitial pancreatitis in the rat induced by excessive doses of pancreatic secretagogue. Virchows Arch [Pathol Anat] 373:97–117

Nevalainen TJ, Aho HJ, Eskola JU, Suonpää AK (1983) Immunohistochemical localization of phospholipase A_2 in human pancreas in acute and chronic pancreatitis. Acta Pathol Microbiol Immunol Scand [Sect A] 91:97–102

Opie EL (1901) The aetiology of acute haemorrhagic pancreatitis. Johns Hopkins Hospital Bull 12:182–188

Scheele GA, Adler G, Kern HF (1984) Role of lysosomes in the development of acute pancreatitis. In: Gyr KE, Singer MV, Sarles H (eds) Pancreatitis – Concepts and classification. Excerpta Medica, Amsterdam New York Oxford, pp 17–23

Schmitz-Moormann P (1981) Comparative radiological and morphological study of the human pancreas. IV. Acute necrotizing pancreatitis in man. Pathol Res Pract 171:325–335

Schmitz-Moormann P, Wedel R v, Agricola B, Himmelmann GW (1978) Studies of lipase induced fat necrosis in rats. Pathol Res Pract 163:93–108

Steer ML, Meldolesi J, Figarella C (1984) Pancreatitis. The role of lysosomes. Digest Dis Sci 29:934–938

Wanke M (1976) Pathogenese und morphologisches Bild akuter Pankreaserkrankungen. In: Forell MM (ed) Handbuch der Innerern Medizin, Band III, Teil 6: Pankreas, 5th edn. Springer, Berlin

Watanabe O, Baccino FM, Steer ML, Meldolesi J (1984) Supramaximal caerulein stimulation and ultrastructure of rat pancreatic acinar cell: early morphological changes during development of experimental pancreatitis. Am J Physiol 246:457–467

Accepted May 13, 1986