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Serum-Induced Chronic Pancreatitis

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Summary. Clinical research into patients with idiopathic chronic pancreatitis points to a possible immunopathogenetic process in a number of cases.

In order to examine the behaviour between the exocrine pancreas under the influence of anti-rat-pancreas immune serum produced in rabbits, a 1.00 ml immune serum is administered once a week over a maximum 26 week period into Wistar-rats by intraperitoneal injection. By electrone-microscopy a much reduced production of enzymes apparently takes place, though to differing extent. There is also destruction of the basal membrane of acinocytes; the production of interstitial oedema, the new formation of collagen fibres and the proliferation of connective tissue cells. Under a conventional light microscope the first changes become noticeable after 8–12 weeks of study. These take the form of localised cell decay, deterioration and lysis of acinocytes; and an increasing non-specific inflammation. There is also the new formation of connective tissue. After 20–26 weeks the exocrine pancreas is characterised by reduction of parenchyma, acino-ductal metaplasia, chronic inflammatory infiltrates of differing density, fibrous and irregular calibres of the smaller and larger ducts.

The findings are almost identical to the structural changes of chronic idiopathic pancreatitis in human beings. The results support the view of an immuno-pathologic aetiology for human chronic idiopathic pancreatitis.

Introduction

The aetiology and pathogenesis of chronic inflammatory processes of the exocrine pancreas are unknown (Becker 1957; Doerr 1959, 1964; Becker 1973 and others). Various immunopathological phenomena have been invoked in causation.

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In the following experimental studies an antibody containing immune serum from the rabbit was injected into Wistar rats intraperitoneally and the immunologically-induced reactions of the exocrine pancreas were observed.

Material and Methods

The pancreas of healthy Wistar rats (Hand: Wist) was dissected by scissors into small pieces, washed several times in physiological saline solution to remove blood, fat tissue and other contamination as far as possible. The pieces were then homogenized with the Ultra-Turrax (Braun, Melsungen) and complete Freund's adjuvant was added in equal amounts. All steps were performed at a temperature of 4° Celsius.

The antigen was stored at a temperature of −18° C. This mixture of all pancreatic tissues had an average content of 6.7 g % proteins. The rabbits were actively immunized with 0.5–1.0 ml of the antigen once a fortnight. The protein content per injection was up to 34 mg %, at this dose enhancement of the immune response is not expected (Adler 1964).

After three injections the rabbits began to produce precipitating antibodies, demonstrable qualitatively and semiquantitatively with the immunodiffusion test of Ouchterlony (1965). In order to extract the anti-pancreatic antibodies from other contaminating antibodies – to fibrous, vascular and nervous tissue – the immune sera were adsorbed with tissues of the liver, red blood cells and with serum proteins of rats. The proteins were cross-linked with glutaraldehyde and used as an immunoadsorbent (Avrameas and Ternynck 1969). After this purification procedure no antibodies against tissues of the heart, liver or kidney were detected in control tests. Precipitation bands were seen with rat pancreatic tissue only.

1.0 ml of the adsorbed immune serum was injected intraperitoneally into rats once a week for 26 weeks. Applications of control sera were administered to Wistar rats in the same manner. A total of 120 Wistar rats were sacrificed, five per week, in ether anaesthesia. The following organs were investigated by light microscopy: pancreas, salivary glands, heart, lungs, liver, kidneys, gut and in some cases brain. The samples were fixed with 5% formaldehyde, embedded in paraplast, cut and stained with haematoxyline-eosine, elastic-van Gieson and PAS in the usual manner. For electron microscopy small 1 mm square tissue samples were removed immediately after death and fixed in glutaraldehyde 2.5% for 2.5 h. Further preparation was performed in usual manner, embedding in Epon 812, cutting slides with an Ultratom QmU3 (Reichert). Transmission electron microscopy was performed with a Philipps EM 101. For indirect immunfluorescence microscopy, carried out over a period of 30 min to 24 h after intraperitoneal injection of the sera, the pancreatic tissue was incubated with a FITC conjugated anti-rabbit IgG from sheep (Firma R. Paesel KG, Internationale Biochemica, Diagnostica, Pharmazeutica).

Certification of analysis:

Product:	FITC Conjugated Anti-Rabbit IgG
Prepared In:	Sheep
Code No.:	14-656-5204 Lot No.: S460
Preserved:	0.01% merthiolate
Analysis:	The ratio of O.D. at 280/495 = 1.2 Molar ratio F/P = 3.5 No free fluorescein present
Specificity by Immunoelectrophoresis:	The conjugate vs. normal rabbit serum – 1 precipitin line of rabbit IgG. Anti-sheep serum vs. the conjugate: 2 lines of sheep IgG.
Potency by Agar Block Procipit in Titration:	1/64 diluted conjugate vs. normal rabbit serum dilution of 1/640.
Working Diluation:	1/16 in direct staining on spleen cells. The conjugate is prepared from highly purified gamma globulin fraction (DEAE-cellulose chromatography) and high purity fluorescein isothiocyanate. The final product is solution in phosphate buffer saline at pH 7.4.

Results

Macroscopic Findings

Initial lesions were detected after a period of 8–12 weeks. The pancreas was swollen, reddish in color and of pasty consistency. A visible reduction in size of the peripheral areas of the organ appeared after a period of 16–18 weeks. At the end of the investigation, that is after 20–26 weeks, the pancreas was firm, uneven and plump. The tissue near the deformed ducts showed scars and a knotty surface. In several animals the pancreas appeared like a leafless tree. Some rats developed a variably intense alopecia.

In control animals treated with control serum no comparable lesions could be seen in the pancreas of the rats. There were some greyish-white spots, measuring up to 4 mm in diameter, on the peritoneum near the site of injection at the lower and ventral part of the abdominal cavity. The pancreas appeared to be of normal shape.

Histological Findings

Initial findings are focal necrosis of exocrine pancreas tissue, oedema, lymphocytic and granulocytic infiltration, marked acinar dilatation in a number of glandular lobules (Fig. 1), and intraductal fluid with high viscosity. These appear

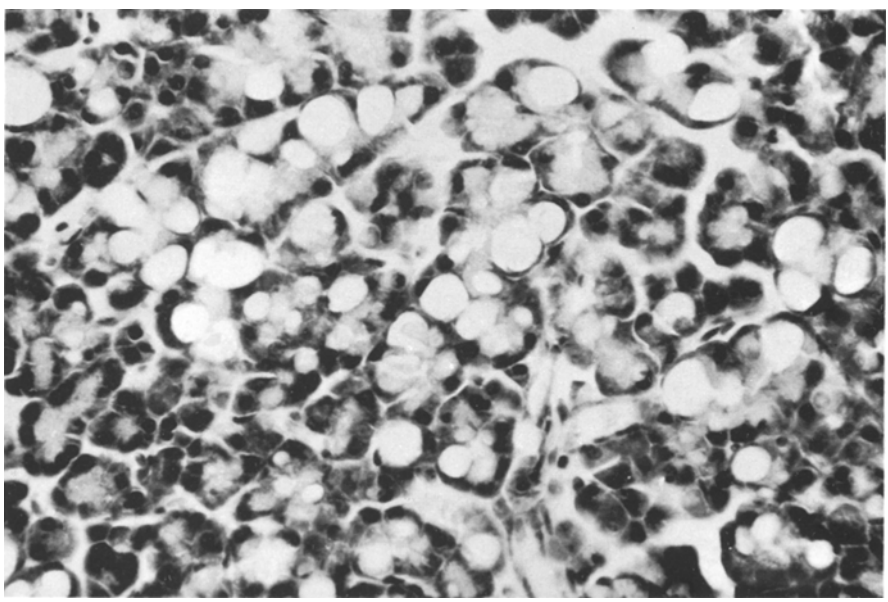

Fig. 1. Marked acinar dilatation. Viscous fluid within several wide acini. Mild interstitial oedema. Vacuolization of acinar cytoplasma. No inflammatory cells. 8 weeks of intraperitoneal injection of an anti-rat pancreas immune serum. H and E. ×310

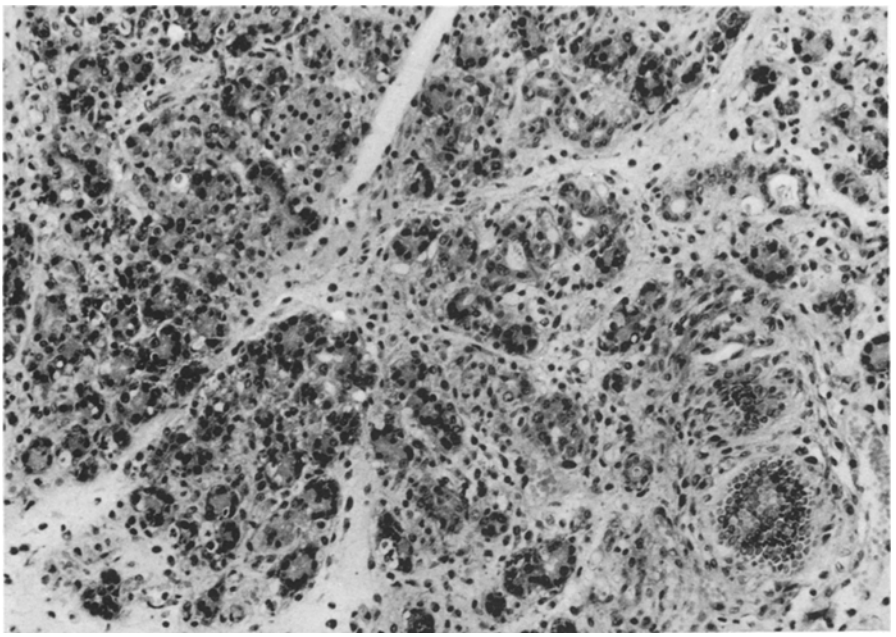

Fig. 2. Atrophy of specific exocrine pancreatic tissue. Dystrophic lesions of the secretory acinar cells. Acino-ductal metaplasia of former acini. Exudation of inflammatory cells and proliferation of fibrous tissue. 26 weeks of intraperitoneal injection of an anti-rat pancreas immune serum. H and E. ×130

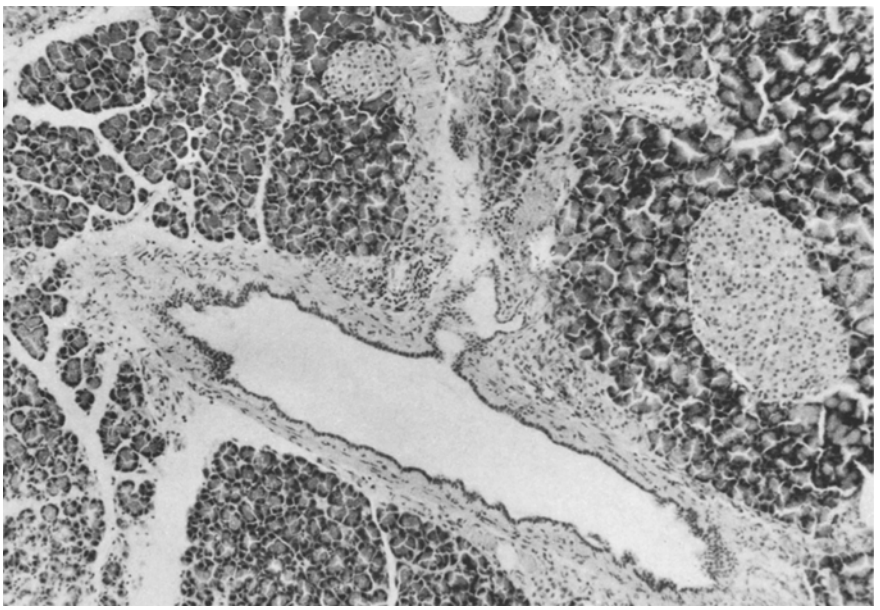

Fig. 3. Segmental ectasia and luminal irregularity of larger ducts and branches. Fibrous thickening of outer layers of ductal walls. No marked lesions of the periductal exocrine pancreatic tissue. Normal structure and shape of islands of Langerhans. Non-involvement of the peri-insular exocrine tissue (so-called Halo-phenomenon). 26 weeks of intraperitoneal injection of an anti-rat pancreas immune serum. H and E. ×40

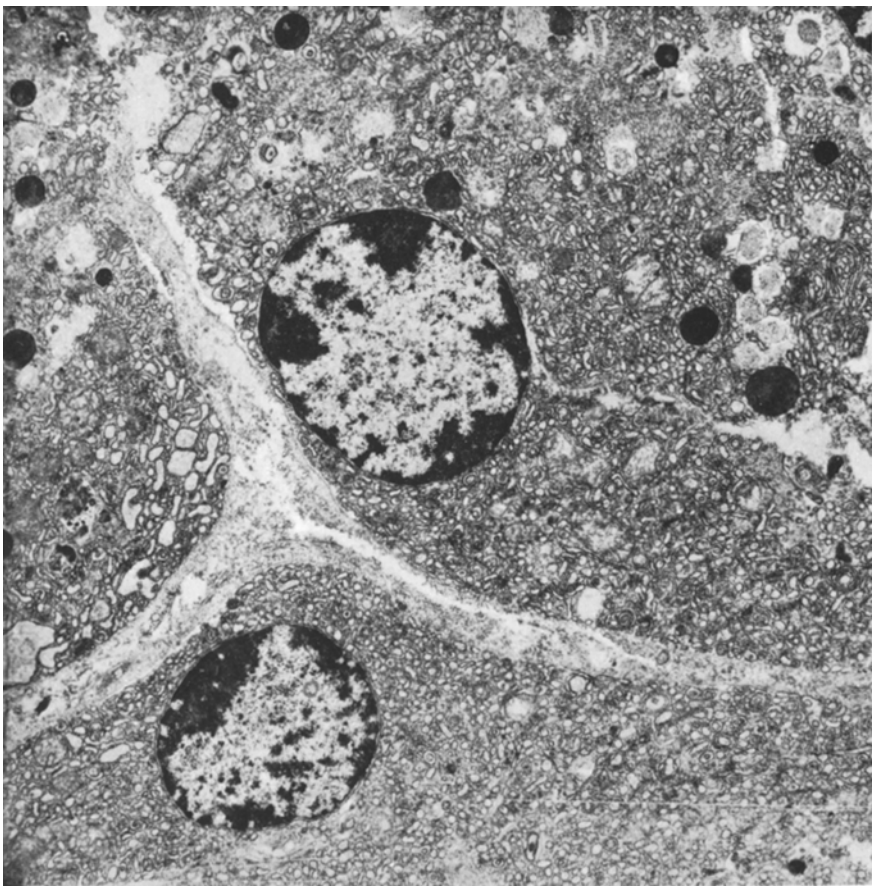

Fig. 4. Acinar epithelium of the rat pancreas. Marked decrease of enzyme granules. Focal lysis of the acinar cytoplasm and of cellular membranes. Granular protein substances within the interstitial spaces. 2 weeks of intraperitoneal injection of an anti-rat pancreas immune serum. Electron micrograph. ×4,500

after 8–12 weeks' application of serum. At the same time a marked proliferation of fibroblasts and an increase of collagen fibres are noticed. Almost every pancreas reveals dystrophic damage of the acinar cells. The exocrine epithelium is destroyed, often in the presence of lymphocytes. When the basal membrane is not damaged, the acinar cells are metaplastic. These metaplastic cells contain a large nucleus and a moderate amount of cytoplasm. Enzyme granules are not detected by light microscopy (Fig. 2).

As the experiment continues, it can be seen that more exocrine pancreatic tissue is replaced by inflammatory and fibrous tissue. The larger ducts are widened segmentally and reveal periductal fibrotic proliferation (Fig. 3). Sometimes there is a slight thickening of the epithelial layers of the ducts. All histological alterations are irregularly distributed and differ from lobule to lobule. There are, however, instances of severe inflammatory change adjacent to glands that

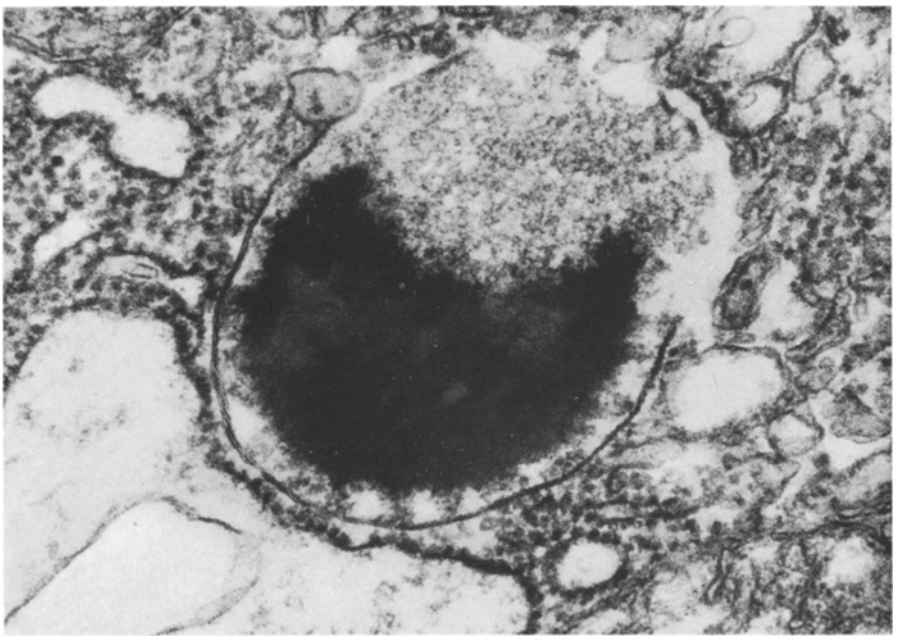

Fig. 5. Acinar cell of exocrine pancreas of the rat. Severe vesiculation and ectasia of the endoplasmatic reticulum. Focal lysis of cytoplasm. Large lysosomal structure with inhomogeneous osmiophilic and granular masses resembling enzyme proteins. No enzyme granules. 8 weeks of intraperitoneal injection of an anti-rat pancreas immune serum. Electron micrograph. ×45,000

seem to be normal. In contrast, no severe inflammatory or dystrophic lesions are observed in other organs. There is a moderate degree of fatty liver, irregular leukostasis in other organs, hyperplasia in lymph nodes and spleen and superficial dermal lymphohistiocystic infiltration in areas of alopecia only. There is no thickening of the basal membrane of the kidneys and no glomerulitis.

Immunofluorescence Microscopic Findings

Indirect immunofluorescence microscopy was performed on frozen sections by incubation with an FITC conjugated anti-rabbit immunglobulin G from sheep. A direct reaction between acinar cells and labeled antisera could be demonstrated after more than 10 h exposure of the animals with intact epithelium and more than 20 exposure hours in necrotic tissue of the exocrine pancreas. In organs other than the pancreas a mild spotty fluorescence was detected after a period of only 6 h exposure.

Electron Microscopic Findings

Following two to four intraperitoneal injections of immune serum one can observe a marked reduction of secretory granules within several acinar cells

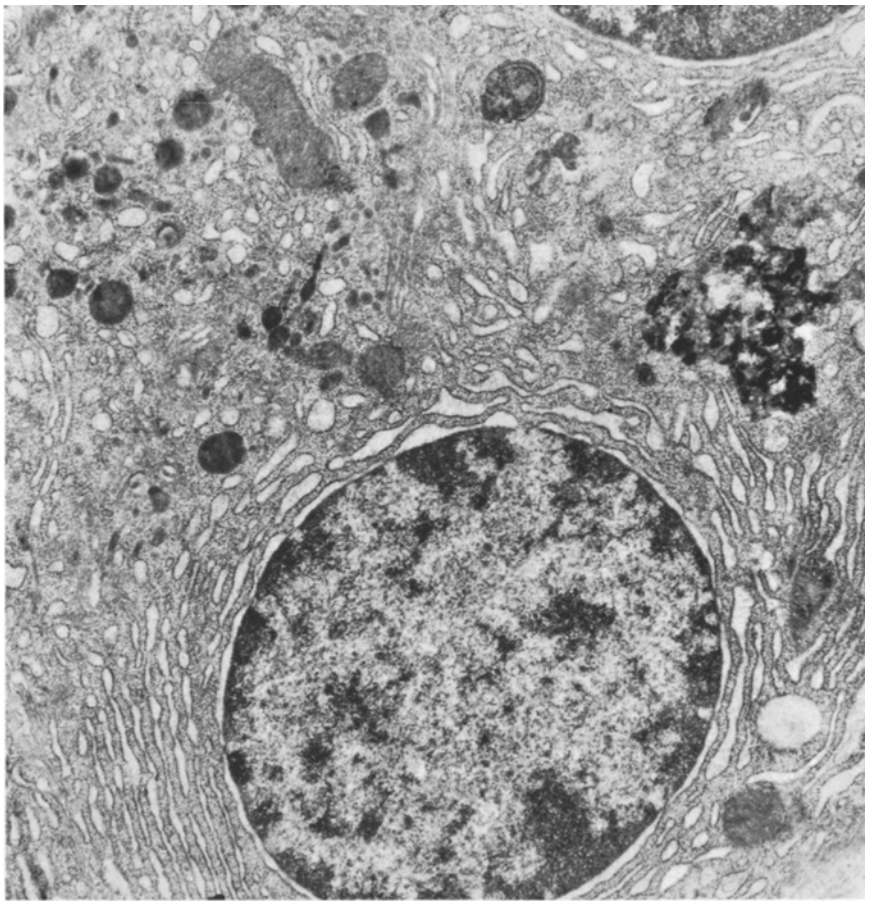

Fig. 6. Acinar cell of exocrine pancreas of the rat. Reduced production of enzyme granules. Variable size of the granules within the area of Golgi. Large residual body consisting of cloudy and dark coloured material. 26 weeks of intraperitoneal injection of an anti-rat pancreas immune serum. Electron micrograph. × 10,000

(Fig. 4). There is often a lack of membrane around the granules, so enzyme protein deposits are localized within the tubular and vesicular spaces of the endoplasmatic reticulum. At almost the same time a focal or total destruction of the basic cellular membranes begins. The longer the experiments are continued the more severe the cellular disarrangements and damage to acinar cells (Figs. 5 and 6). Cell death of the acinocytes results in the final stages. If the basal membrane remains intact, acinar epithelium may be replaced by a metaplastic epithelium (Fig. 7). In parallel with the epithelial alterations, degenerative and proliferative processes occur in the interstitial spaces. At an early stage neogenesis of collageneous fibres, slight oedema and proliferation of fibroblasts and fibrocytes are a common feature. An increase in newly formed connective tissue and scars can be registered till the final stages.

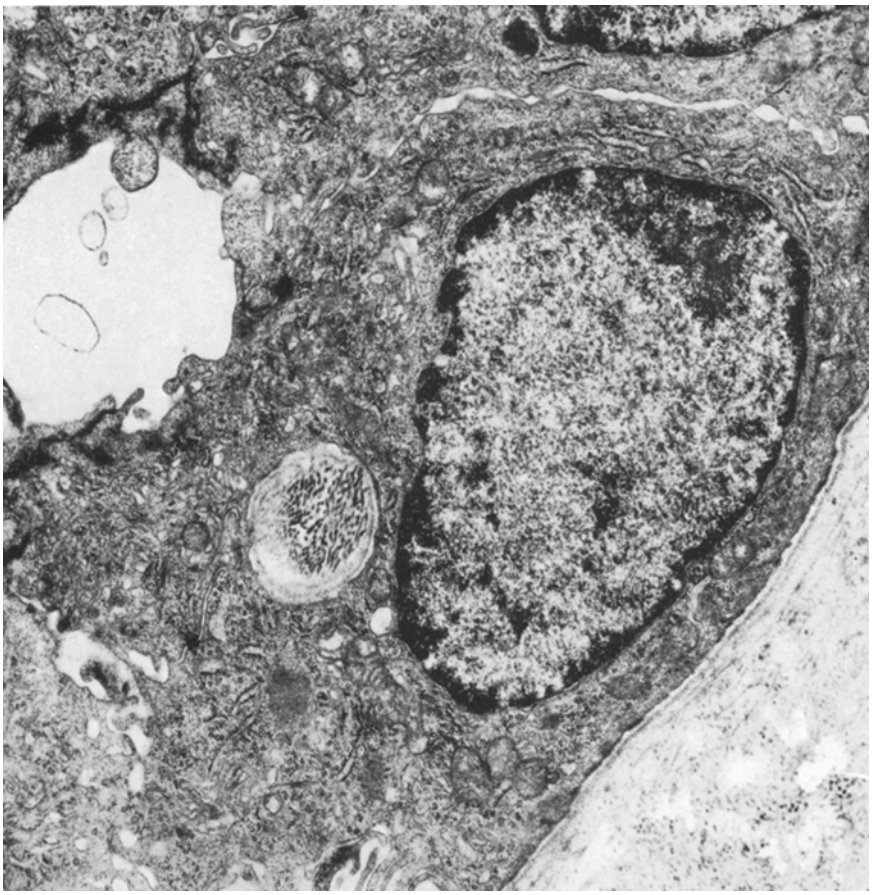

Fig. 7. Acino-ductal transformation of exocrine pancreas of the rat. Metaplastic acinar cells. Basal membrane in good condition and well preserved. Lots of collagenous fibres surrounding the ductal structure. 26 weeks of intraperitoneal injection of an anti-rat pancreas immune serum. Electron micrograph. × 10,000

Discussion

A great part of chronic pancreatitis in man is of unknown aetiology. The incidence depends on the environmental situation and medical factors (Ammann et al. 1973; Gastard et al. 1973; Marks et al. 1973; Sarles 1973).

In fully developed chronic pancreatitis or in the final stages of the inflammatory process, macroscopic and histological findings do not provide any typical or specific evidence for the nature of the primary lesion (Becker 1973). Primary chronic inflammatory lesions of any organ are indicative of intolerance of the immunological system which be of a local, generalized, primary or secondary nature. In the past 20 years antibodies in the blood and/or tissues have been demonstrated in several organs affected by inflammation. Several researchers have already focused their efforts upon immunological problems in chronic

inflammatory pancreatic lesions. Thal et al. (1957, 1959), Murray and Thal (1960), Schwarzmann and Julien (1960), Thal et al. (1960) and Rizetto et al. (1975) found several antibodies in the sera of patients suffering from acute or chronic pancreatitis. At the climax of an experimentally-induced pancreatitis in rabbits Alarcon-Segovia et al. (1964) and McGiven and Ireton (1970) demonstrated antibodies in the blood of rabbits. Often the binding capacity of these antibodies was multivalent, they reacted with pancreatic acinar cells and with smooth muscle antigens. Fonkalsrud and Longmire (1961) induced an inflammatory-process in the pancreas of animals experimentally by injections of human sera containing antibodies. In tissue from chronic pancreatitis Lendrum and Walker (1975) demonstrated antibodies with a granular distribution in acinar cytoplasma that correlated with antigens of the ABO-System of the blood. Walters (1966), Clemente et al. (1971), Band et al. (1973), Corrodi et al. (1975), Messer and Dean (1975), Sarda and Gupta (1975), Ludwig et al. (1977) and Takeuchi et al. (1977) all assume that the cause of the inflammatory pancreatic lesions seen is specific antibody pancreatic tissue reactions and further reaction from cross reactive antibodies. Greene et al. (1975) were able to isolate two "specific" antibodies against pancreatic tissue by means of absorption of sera from patients suffering from pancreatitis. Ballinger and Lacy (1972) observed an acute pancreatitis in the host following pancreaticoduodenal transplantation, without previous digestion of the exocrine tissue by collagenase. Dani et al. (1974) found increased levels of IgA and IgM in patients with chronic-calcifying pancreatitis. By the immunodiffusion test (Ouchterlony) these immunoglobulins had reactive determinants against smooth muscle, mitochondria, nuclear substances and parietal cells of the stomach. Richter (1974, 1978) observed, in Wistar rats, disturbances of the secretory kinetics and protein synthesis of pancreatic acinar cells, oedema in the interstitial tissue, slight inflammatory reaction and dystrophic acinar damage following multiple antibody containing sera injections intraperitoneally for a maximum period of three weeks. Janigan et al. (1975) and Nevalainen et al. (1977, 1978) achieved similar results after injecting intraperitoneally human sera. Seelig et al. (1975, 1978) and Seelig and Seelig (1975, 1975, 1976) stressed the importance of the complement system in initiating an inflammatory process in the pancreatic organ of mice.

These investigations and findings give reason for assuming the involvement of immunological phenomena in the pathogenesis of so-called idiopathic chronic pancreatitis. In order to investigate the acinar epithelium of the exocrine pancreatic tissue of rats after exposure to specific antibody immune serum was injected intraperitoneally into rats once a week for a period of 26 weeks maximum. Distribution of the immune serum over the whole pancreas was demonstrated by immunfluorescence microscopy. 30 min after injection the whole body contained the immune serum. Six hours later the immune serum was demonstrated in the pancreatic tissue only. Electron microscopy revealed an early spotty reduction of the production of enzyme proteins, destruction of the basic cellular membranes of the acinocytes, slight interstitial oedema, neogenesis of collageneous fibres and marked proliferation of cells of the connective tissue. The preservation of the basal membrane then determines the destiny of the acinus. Acinar epithelium may be replaced by metaplastic epithelium. By conventional light

microscopy the first lesions could be seen after 8–12 immune serum injections. There were focal necrobiosis, dystrophic damage, lysis of acinar cells, an increased inflammatory process and an early occurrence of fibrotic and fibrillar masses. Following 20–26 injections the organ was characterized by atrophy, wide areas of inflammatory tissue, scars and infiltrations of lymphocytic and histiocytic cells, some eosinophilic leukocytes, macrophages and other mononuclear inflammatory cells. The process was not distributed homogeneously over the pancreas, but differed greatly from lobule to lobule. Severely affected pancreatic areas were localized adjacent to lobules with very mild alterations. The larger ducts were segmentally widened and contained a fluid of apparently high viscosity. Acino-ductal metaplasia was a common feature in the final stage. Thus, we produced a chronic inflammatory process in the pancreatic tissue by the intraperitoneal injection of immune serum.

Inflammatory reactions were not observed in other organs. Acute pancreatitis was not induced by these experiments, only the chronic state was noted.

The morphological results in these animals, produced by the intraperitoneal injecting of anti-rat pancreas immune serum from the rabbit were nearly identical with the findings of chronic pancreatitis in man. We suggest that similar immunopathological phenomena occur in the chronic inflammatory process in the human pancreas.

References

Adler FL (1964) Competition of antigens. Progr Allergy 8:41–57

Alarcon-Segovia D, Herskovic T, Warkim KG, Bartholomew LG, Cain JC (1964) Immune mechanismus in chronic pancreatic disease. I. Occurence of precipitins to pancreatic homogenates following certain forms of pancreatic damage. An experimental study. Am J Dig Dis 9:845–855

Ammann RW, Hammer B, Fumagalli I (1973) Chronic pancreatitis in Zurich, 1963. Clinical findings and follow-up studies of 102 cases. Digestion 9:404–415

Avrameas S, Ternynck Th (1969) The cross-linking of proteins with glutaraldehyde and its use for the preparation of immunadsorbents. Immunochemistry 6:53–66

Ballinger WF, Lacy PE (1972) Transplantation of intact pancreatic islets in rats. Surgery 72:175–186

Bank S, Novis BH, Petersen E, Dowdle E, Marks IN (1973) Serum immunoglobulins in calcific pancreatitis. Gut 14:723–725

Becker V (1957) Sekretionsstudien am Pankreas. Georg Thieme, Stuttgart

Becker V (1973) Bauchspeicheldrüse. Inselapparat ausgenommen. Springer, Berlin, Heidelberg, New York

Clemente F, Ribeiro T, Colomb E, Figarella C, Sarles H (1971) Comparaison des proteins de sucs pancréatiques humains normaux et pathologiques. Dosage des protéins sériques et mise en évidence d' une protéine particulière dans la pancréatic chronique calcifiante. Biochem Biophys Acta (Amst) 251:456–466

Corrodi P, Knoblauch M, Binswanger U, Schölzel E, Largiadèr F (1975) Pancreatitis after renal transplantation. Gut 16:285–289

Dani R, Antunes LJ, Riboiro JEF, Nogueira CED, Ribeiro T (1974) Immunological participation in chronic calcifiying pancreatitis. Digestion 11:333–337

Doerr W (1959) Pankreatitis: Pathogenese, Formen, Häufigkeit. 76. Tagung Dtsch Ges Chir München

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

Fonkalsrud EW, Longemire jr WP (1961) The occurence of pancreatic antibodies and the experimental production of pancreatitis with pancreatic antiserum. Surgery 50:134–142

Gastard J, Joubaud F, Farbos T, Loussouarn J, Marion J, Pannier M, Renaudet F, Valdazo R, Gosselin M (1973) Etiology and course of primary chronic pancreatitis in Western France. Digestion 9:416–428

Greene EL, Halbert SP, Kiefer D (1975) Two pancreas "tissue-specific" antigens in normal human urine, one being an esterase. Int Arch Allergy APPI Immunol 48:764–775

Janigan DT, Nevalainen TJ, Macaulay MA, Vethamany VC (1975) Foreign serum-induced pancreatitis in mice. I. A new model of acute pancreatitis. Lab Invest 33:591–607

Lendrum R, Walker G (1975) Serum antibodies in human pancreatic disease. Gut 16:365–371

Ludwig H, Schernthaner G, Scherak O, Kolarz G (1977) Antibodies to pancreatic duct cells in Sjögren's syndrome and rheumatoid arthritis. Gut 18:311–315

Marks IN, Bank S, Louw JH (1973) Chronic pancreatitis in the western cape. Digestion 9:447–453

McGiven AR, Ireton HJC (1970) Natural rabbit antibody against rodent pancreas. Pathology 2:293–301

Messer M, Dean RT (1975) Immunochemical relationship between alpha-amylases of rat liver, serum, pancreas and parotid gland. Biochem. J 151:17–22

Murray MJ, Thal AP (1960) The clinical significance of circulating pancreatic antibodies. Ann Intern Med 53:548–555

Nevalainen TJ (1978) Rabbit serum-induced injury in rat pancreas in vitro. Exp. Pathol 15:67–73

Nevalainen TJ, Fowlie FE, Janigan DT (1977) Foreign serum-induced pancreatitis in mice. II. Secretory disturbances of acinar cells. Lab Invest 36:469–473

Ouchterlony O (1965) In: Immunchemie. 15. Coll Ges Physiol Chemie Mosbach 1964, Springer, Berlin-Heidelberg-New York

Richter K (1974) Experimentell immunpathologische Untersuchungen am exokrinen Pankreas der Ratte. Verh Dtsch Ges Pathol 58:518

Richter K (1978) Immunserum-induzierte Pankreatitis der Ratte. Verh Dtsch Ges Pathol 62:475

Rizzetto M, Bonino F, Verme G (1975) Antibodies against the human pancreas in acute and chronic liver disease. Ric Clin Lab 5:146–158

Sarda S, Gupta KG (1975) Antibody mediated injury to pancreas and susceptibility to infections. Indian J Exp Biol 13:527–531

Sarles H (1973) An international survey on nutrition and pancreatitis. Digestion 9:389–403

Schwarzmann V, Julien C (1960) Mise en évidence d'anticorps antipancréas, dans le sérum de malades atteints de pancréatite chronique et d'affections pancréatiques diverses. Acta Gastroenterol Belg 23:1002–1009

Seelig R, Ehemann V, Tschahargane C, Seelig HP: The serum complement system – a mediator of acute pancreatitis. Virchows Arch [Pathol Anat] 365:193–199

Seelig R, Lankisch PG, Koop H, Winckler K, Kaboth U, Seelig HP (1978) Complement system in sodium taurocholate pancreatitis in the rat. Res Exp Med (Berl) 174:57–65

Seelig R, Seelig HP (1975) The possible role of serum complement system in the formal pathogenesis of acute pancreatitis. I. Immunopathogenetic pancreatitis – local Schwartzman-Sanarelli phenomen – endotoxin induced pancreatitis. Acta Hepatogastroenterol (Stuttg) 22:263–268

Seelig R, Seelig HP: The possible role of serum complement system in the formal pathogenesis of acute pancreatitis. II. Cobra venom factor pancreatitis – sodium taurocholate and deoxycholate pancreatitis. Acta Hepatogastroenterol (Stuttg) 22:335–346

Seelig R, Seelig HP (1976) Complement-mediated acinar cell necrosis in pancreatitis induced by basement membrane antibodies. Virchows Arch [Pathol Anat] 371:69–77

Takeuchi T, Nakagawa Y, Ogawa M, Kawachi T, Sugimura T (1977) Immunoreactivities of alpha-amylase of humans and rats. Clin Chim Acta 77:203–206

Thal AP, Egner W, Murray MJ (1960) Circulating antibodies in chronic pancreatic disease. Surg Forum X –

Thal AP, Goott B, Margulis AR (1959) Sites of pancreatic duct obstruction in chronic pancreatitis. Ann Surg 150:49–56

Thal AP, Perry JF, jr, Egner W (1957) A clinical and morphologic study of forty-two cases of fatal acute pancreatitis. Surgery 105:191–202

Walters MN-K (1966) Pancreatitis induced by the graft-versus-host reaction. J Pathol Bacteriol 91:65–69

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