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Leading article

Pathogenesis of chronic pancreatitis

There have been few efforts to classify diseases of the pancreas by their specific pathological features and causes. In 1946 Comfort *et al*[1] observed that the changes of acute pancreatitis were often found in resected specimens of the pancreas from patients with chronic pancreatitis, suggesting that recurrent acute attacks were the basis for the development of chronic pancreatitis. It could be, however, that acute lesions complicated chronic lesions.

The hypothesis of Comfort *et al* was widely accepted and extended. Cattell and Warren[2] suggested that all possible transitions exist between the most acute haemorrhagic pancreatitis and the most chronic calcified pancreatitis, which represented two extremes of one disease: 'recurrent pancreatitis.'

Because pancreatic biopsy specimens are not readily available most clinicians attempted to classify their cases according to clinical or radiological criteria. The most recent attempt was the Cambridge classification[3] which defined changes in the pancreatic ducts as seen with endoscopic retrograde pancreatography. The Marseille classifications[4-6] represent an effort to isolate stable groups of pathological features and to study the statistical relationship between them and pathogenesis. The 1963 classification proposed that acute pancreatitis was not a common cause of chronic pancreatitis since the statistical studies showed that the average age of patients at first presentation with acute pancreatitis (the putative cause of recurrent pancreatitis) was 13 years more than the mean age at onset of chronic calcified pancreatitis. Further, the two diseases were statistically linked to different causes.[7 8] Recent experiments have shown that acute haemorrhagic pancreatitis after injection of trypsin[9-11] or after hyperstimulation[12 13] resolves after a phase of destruction and fibrosis of the exocrine parenchyma. These changes resemble the lesions of chronic pancreatitis but are reversible. Follow up of patients presenting with haemorrhagic necrosis by computed tomography has shown that even the most severe and extensive lesions will regress if the patient survives.[14]

The recent Marseille-Rome classification[15-16] defined acute pancreatitis as 'a spectrum of inflammatory lesions in the pancreas and also in peripancreatic tissues: oedema, necrosis, haemorrhagic necrosis, fat necrosis.' Chronic pancreatitis was defined as 'the presence of chronic inflammatory lesions characterised by the destruction of exocrine parenchyma and fibrosis and at least in the later stages, the destruction of endocrine parenchyma.' It is frequently complicated in the early stages of its evolution by attacks of acute pancreatitis which are responsible for recurrent pain and which may represent the only clinical symptom. After some years there is insufficiency, both exocrine (steatorrhoea) and endocrine (diabetes), and acute attacks decrease and disappear.

Two large computer based studies of chronic pancreatitis[17 18] distinguished two different types of chronic pancreatitis. A three dimensional reconstruction of the pancreatic ductal system using 7 μm sections drew the same conclusions.[19] The two populations defined by their pathological features were statistically linked to different and specific causes.[17]

In the first and smaller group chronic pancreatitis was due to obstruction of the pancreatic ducts predating the pancreatitis: tumours, papillary stenosis, cysts, or scars due to acute pancreatitis or trauma are the main causes. 'The lesions distal to the site of ductal obstuction are uniform, not patchy. The epithelium of the ducts in the obstructed part of the gland is usually preserved. Intraductal plugs may be observed but they are less frequent than in the following form and calcified plugs or pancreatic stones (calcifications) are not found.'

The usual form of chronic pancreatitis may be called chronic calcifying pancreatitis. Three studies have shown that after a variable time most chronic pancreatitis patients present with pancreatic calcifications visible on radiological films of the abdomen.[20-22] Calcification is intraductal pancreatic calculi.[23 24] Pancreatic calculi are composed of two materials: calcium carbonate, generally in the form of calcite which constitutes approximately 95% of stone weight[25] and a curious protein called PSP.[26] Radiolucent pancreatic stones composed of different degraded forms of PSP occur additionally.[27] We have described one example of a patient presenting with large pancreatic stones which she had had since childhood. These stones consisted of pure calcium carbonate without PSP.[28]

A morphometric ultrastructural study of exocrine pancreas of patients with chronic calcifying pancreatitis[29] was undertaken in those areas of the gland which were normal on light microscopy. The lobular distribution of the disease means that there are frequently such normal areas in a pathological pancreas. This study showed that in these areas acinar and ductal cells were intact but, compared to normal acinar cells, acinar cells from patients with chronic pancreatitis showed signs of increased protein secretion (larger diameter of the cell, nucleus, and nucleolus; increased length of endoplasmic reticulum; increased number of condensing vacuoles; decreased number of mature zymogen granules). Despite the normality of the cells, intra-acinar, or more frequently intraductal eosinophilic protein precipitates or plugs (which had been previously described at the optical microscope level) were found.[15 21] Therefore, even in its earliest stages, before there is any apparent damage to the cell, chronic calcifying pancreatitis seems to be a lithiasis.

These precipitates are mostly composed of protein fibrils and many transitions between plugs and calculi have been described.[30 31] Similar protein plugs are frequently observed in non-activated pure pancreatic juice collected from patients with chronic calcific pancreatitis. They comprise a degraded molecular form of PSP.[32] During the early stage of the disease pure pancreatic juice in addition to plugs contains crystals of calcium carbonate which are larger and more numerous than in normal subjects.[33]

Pancreatic lithogenesis seems to comprise precipitation of calcium salts and precipitation of different degraded forms of a special protein (PSP).

The original PSP molecules are found in pancreatic juice where they are termed PSP-S2-5. These molecules are glycosylated with a molecular mass of 16000 to 19000 daltons. They have the same 144 amino acid backbone, the different molecular masses being due to different glycosylation. The amino acid sequence of PSP and of its signal peptide and the nucleotide sequence of PSP mRNA are known.[34] This sequence does not resemble any other known

protein sequence and in particular it is completely different from trypsinogen and chymotrypsinogen.[35] PSP S2-5 is hydrolysed by trypsin but not by chymotrypsin to a long C-terminal 133 amino acid peptide which is not glycosylated, is insoluble, and polymerises to form fibrils. This has been called PSP-S1. A short N-terminal 11 amino acid peptide which is glycosylated and soluble is also formed.[36] PSP-S1 is easily formed in pure pancreatic juice when trypsin activation is not prevented by antiproteases. A similar fibrillar protein has been discovered in bovine[37] and human[38] pancreatic tissue by Gross *et al* with the name PTP. The partial amino acid sequence given by these authors strongly suggests that PTP is similar to PSP-S1, the degraded fragment of PSP-S2-5.

PSP-S is synthesised by the pancreatic acinar cell,[39] stored in the zymogen granules in the same way as enzymes,[40] and secreted in parallel with them.[41] PSP can prevent the nucleation and crystal growth of calcium carbonate in a saturated solution,[42 43] particularly PSP-S2-5 and the N-terminal glycosylated 11 amino acid peptide, but not in PSP-S1.

Pancreatic juice is normally saturated in calcium both in the dog[44] and in humans.[45] The observation that precipitation of calcium salts is one of the two phenomena observed in pancreatic lithogenesis and PSP prevents calcium precipitation in saturated solutions suggests that a deficiency of PSP in pancreatic secretion could play an important part in the disease. This is likely since the messenger RNA responsible for PSP biosynthesis is significantly decreased in the pancreas of patients with chronic calcifying pancreatitis (whether alcoholic or non-alcoholic),[39] PSP is severely decreased in the zymogen granules of acinar cells from these patients compared to normal subjects,[40] and PSP concentration related to total protein is significantly decreased in the pure pancreatic juice of these patients.[41 46] PSP estimation in pancreatic juice requires precise experimental conditions. When the juice is activated or when it is stored hydrolysis of the molecule produces the insoluble residue PSP S-1 which polymerises and precipitates. This probably explains why there is no clear distinction between patients and controls when juice is frozen and stored or when PSP is estimated with a monoclonal antibody prepared in Marseille and raised against PSP-S1.[46 47] However, with polyclonal antibodies, using immunodiffusion[41] or enzyme-linked immunosorbent assay,[46] the concentration of PSP related to secretory protein is decreased with almost no overlap between chronic calcifying pancreatitis patients and normal non-alcoholic controls.

In apparently normal alcoholic controls (mean daily consumption >100 g per day) PSP is decreased by half. This could be due either to an alcohol induced decrease of PSP biosynthesis or to increased denaturation in the acinar or duct lumen, or both.

Low PSP concentrations in pancreatic juice probably have at least two causes: one is independent of alcohol consumption since it is found in hereditary and idiopathic pancreatitis, the other is related to alcoholism.

The explanation for the precipitation of residues of PSP is more difficult to explain. The fibrillar protein present in pancreatic plugs is the degraded polymerised form PSP-S1. This suggests that a hydrolysis of PSP-S takes place in pancreatic juice in the earliest stages of chronic calcifying pancreatitis and as high as the acinar lumen (plugs are observed in the acinar lumen but more frequently in the ducts).[48] It is just possible, and has not been completely excluded, that an active protease which has the same specificity as trypsin, either activated trypsin itself or cathepsin B, is present in the lumen of the acinus. A careful morphometric study did not show any modification of lysosomes.[29] Spontaneous degradation of PSP-S2-5 is another possibility. Protein plugs are observed in 2% of normal pancreatic juices and are significantly more frequent in the samples collected from normal alcoholics and are found in more than 60% of patients with chronic calcifying pancreatitis, either alcoholic or abstinent[32]: plug formation is therefore increased by alcohol consumption, but this is not the only factor.

Plug formation by itself is not a sufficient condition for pancreatic stone formation. It is observed in normal and alcoholic controls but it seems to be responsible for some duct lesions: in contact with plugs the basal membrane of ducts vanishes,[49] which is responsible for the observed transudation of serum protein[50] and calcium[51 52] into pancreatic juice. This increased calcium concentration must facilitate calcium crystal formation and explains why calcifications visible on *x* ray films develop more rapidly after many years of clinical pancreatitis.[20-22] Spontaneous plug formation in normal juice could explain the frequent observation of tiny stones and duct lesions in localised areas of the pancreas at necropsy.[53 54] In chronic calcifying pancreatitis duct lesions are more severe. Atrophy of the epithelium followed by stenosis, cyst formation, and parenchymal atrophy distal to obstructed ducts are apparently the final consequences of plugs and stones.[17-19]

Chronic calcifying pancreatitis is significantly associated with alcohol consumption, fat, a high protein diet, tabacco smoking, hypercalcaemia, and life in some tropical countries where malnutrition is common. There are also inherited cases.[55 56]

Ethanol is metabolised by the pancreas[57] and it has been suggested that a direct toxic effect on the pancreas could explain alcoholic chronic calcifying pancreatitis.[58] But these lesions are an increased content of triglycerides and lesions of lysosomes in the ancinar cell. They are observed in all organs of alcoholics and are reversible. They do not explain the formation of plugs and stones.[59]

Two alcohol induced secretory modifications probably increase calcium crystal formation: a decreased secretion of citrate[60] and PSP.[46] This could be due to alcohol metabolism or to adaptation to alcohol.

The concentration and output of secretory protein is increased in the juice of alcoholic dogs and humans. This could play a part by increasing the viscosity of the juice. The same finding is observed in the juice of hypercalcaemic dogs, cats, and humans. As hypercalcaemia is also a cause of chronic calcifying pancreatitis, it could play a part, possibly by increasing the resistance to flow. The mechanism of this increased secretion is neural, cholinergic, and vagally mediated in alcoholics and neural and hormonal in hypercalcaemics (for review see Sarles[55] and Sarles *et al*[56]).

The cause of tropical pancreatitis is not known. It is seen in non-alcoholic children and young adults of both sexes living in some tropical areas such as South India, Indonesia, Zaire, and Nigeria but not in others (for review see Sarles *et al*[56] and Mohan *et al*[61]). Kwashiorkor[62] and cassava (manioc) consumption[63] have been implicated. Studies by Indian physicians[61] and ourselves,[64] conducted in different areas and comparing the type of diet and the incidence of tropical pancreatitis, have shown that neither factor plays a part. The nutritional disorders linked to the disease are a low protein diet (50 g per day or less) and a very low fat intake (less than 30 g per day).[56 64] It has previously been shown that a low as well as a high fat intake is a risk factor for chronic calcifying pancreatitis.[65] These disorders, if they have a role, could act directly on the pancreas of the child, but the disease begins early in life in Kerala, for instance, where restricted diets are common in adults as well as in children.[66] This is not the case in countries such as the Ivory Coast where malnutrition is mostly found during childhood.[64 67] Malnutrition of the mother may play a part in the disease of the child. We have shown an increased enzyme and particularly an increased protease content in the pancreas of young rats born of animals

submitted to a moderately restricted protein diet (9% of calories). This modification persists several months after weaning.[68] In any case the pathological features of tropical pancreatitis are identical to those of alcoholic and idiopathic pancreatitis in Western countries. The biochemical composition of stones is similar.[69]

Hereditary factors certainly play a part in the disease. There is a hereditary dominant form observed in children of both sexes.[70] The only known biochemical modification is a decrease of PSP in pancreatic juice.[41] However, there is an increased risk that in the family of a patient with chronic calcifying pancreatitis there will be another case of chronic calcifying pancreatitis when compared with normal control subjects,[71] which suggests that there is an underlying defect, probably different from the hereditary dominant pancreatitis, sometimes latent, sometimes shown by nutritional disorders, and sometimes sufficient to produce the disease by itself (idiopathic cases). The increased frequency of blood group O in chronic calcifying pancreatitis patients[72] is consistent with this hypothesis. The many reports dealing with HLA markers are contradictory and do not provide consistent results.[55]

Thus, although most cases of chronic calcifying pancreatitis in Western countries are related to consumption of alcohol, other rare causes exist (hypercalcaemia and heredity). In some tropical countries a form of chronic calcifying pancreatitis occurs which could be related to protein and fat malnutrition. All these forms are characterised by identical pathological changes in the gland and biochemical changes in the pancreatic secretion. When stones are present they have a similar composition independent of the cause of the chronic calcifying pancreatitis. We therefore have to conclude that chronic pancreatitis is due to stone formation consequent on changes in the composition of pancreatic juice.

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