Three decades of gastroenterology in Soweto South Africa: from descriptive to scientific observations

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Chapter 15

Pancreatitis in Soweto, South Africa, Focus on Alcohol-related Disease

Segal I


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Abstract
Both acute pancreatitis and chronic pancreatitis now appear to be endemic at Soweto, South Africa, and they carry a substantial toll in terms of morbidity and mortality. Case-control studies identified the same three environmental factors in each disease, namely, heavy alcohol consumption, marked exposure to occupational chemicals and low intake of fruit (a major source of vitamin C). This congruity, and parallel trends on blood biochemical analysis indicating heightened free radical activity coupled with poor antioxidant status, suggest that the two diseases may be part of a pathobiological spectrum that is linked by pancreatic oxidant stress. Further, asymptomatic chronic alcoholics had plasma glutathione concentrations that were midway between the values in non-alcoholic controls and patients with chronic pancreatitis, being significantly different from each. And, finally, apparently healthy Sowetans were actually in a state of oxidant stress that was tied in with their very poor vitamin C status, and lower serum selenium concentrations than in the UK. These data, and evidence that both antioxidants mitigate against alcoholic toxicity in experimental studies, may offer scope for disease prophylaxis in this unprivileged community.

Background
Today acute pancreatitis and chronic pancreatitis are common diseases at Soweto, South Africa, and they cause considerable mortality and morbidity. Alcohol excess is the obvious aetiological factor but several observations question traditional interpretations on its modus operandi. Also in contrast to standard teaching, acute pancreatitis and chronic pancreatitis at Soweto appear to be part of a pathobiological spectrum, rather than being utterly different entities. This paper traces the emergence of pancreatitis in this area and briefly describes local peculiarities in presentation, before focussing upon theories for the development of alcoholic pancreatitis. Thereafter, collaborative studies in support of the new ‘oxidant stress’ concept are described in some detail.

Soweto, and the Emergence of Pancreatitis
The township of Soweto, on the outskirts of Johannesburg, is served by the Chris Hani Baragwanath Hospital and various polyclinics: the hospital with 3,200 beds is the largest in the world. The current population of Soweto is approximately 3.5 million, a complex polyglot of largely immigrant and mainly lower working class people, but with an emerging highly urbanised and better trained upper working class and middle class [1]. The population is
relatively balanced in terms of males and females and has a young age structure indicative of the current high population growth rate. Urbanisation and industrialisation is proceeding at an alarmingly high rate in South Africa. Soweto has a continuing influx of rural Black people who merge into and contribute towards an ever-growing permanently urbanised population. In a way it is analogous to that which occurred during the industrial revolution in England. Historically, African Blacks consumed traditional home-brewed beer of low alcohol content, approximately 3%, which was associated with tissue siderosis [2]. In 1950, Walker and Arvidsson [3] conclusively showed that the source of excess ingested iron was leaching of the element from utensils during the alcohol fermentation process. In 1962 the legislation was repealed which formerly forbade the sale of Western-type alcohol to Blacks [2,4], and African men markedly increased their consumption of spirits, notably brandy and fortified wine, with a corresponding decrease in traditional beverages. Now the pattern of liver disease changed. Whereas heavy deposits of haemosiderin were the hallmark of micronodular cirrhosis associated with iron overload, the new pattern conformed to alcoholic liver disease in Western societies, namely, alcoholic hepatitis or micronodular cirrhosis associated with steatonecrosis and alcoholic hyaline [2, 4, 5]. In keeping with these morphological differences, measurements of liver iron in autopsy studies showed that material obtained in 1976 had 40% less iron than that in material examined in 1959-1960 [5]. The emergence of pancreatitis at Soweto can be tracked from hospital admission reports over the past 70 years. Beyers [6], in a review of surgical diseases at Johannesburg during the 5-year period 1921-1926 stated that no cases of pancreatitis were observed in African Blacks. Thirty years later, Keeley [7] published a review of gastrointestinal diseases at Baragwanath Hospital and concluded that acute pancreatitis was frequently sought, but rarely encountered. A trickle of patients with calcific chronic pancreatitis began in the 1970s, such that 25 patients were identified by 1977 [8]. In the 3-year period 1981-1983 there were 55 new cases [9], the increased admission rate occurring some 20 years after Africans had access to Western-type alcohol [4]. Approximately two-thirds still drank mainly home-brews, in addition to Western-type spirits, as was evidenced by high serum ferritin levels in 65% and high liver iron in 64% of patients tested, in the other third, who mainly drank Western-type spirits, both these measurements were within normal limits [9]. A further survey in 1988 of admissions during the previous 4.5 years identified 90 new cases [4], with a male to female ratio of 6:1, a mean age of 40 years, and mean alcohol intake of 180 g/day for an average of 15 years. Nowadays, however, it is not uncommon to diagnose some 5 new chronic pancreatitis patients per month in the hospital which would amount to
>50 cases each year, leaving aside those who present with an attack of apparently acute pancreatitis. This later increase in disease frequency parallels urbanisation and industrialisation in Soweto.

**Clinical Idiosyncrasies**
The traditional classification of pancreatitis is into 'acute' and 'chronic' forms, wherein the descriptors are used in a pathological rather than temporal sense, although both diseases generally manifest with an episode of agonising abdominal pain with hyperamylaseaemia, a retrospective diagnosis of 'acute pancreatitis' is properly retained only when morphological and functional tests of the exocrine pancreas are within normal limits after full clinical recovery from the attack: if either modality is abnormal, the diagnosis is changed to 'chronic pancreatitis', implying a pathological picture of irregular inflammatory sclerosis with destruction and loss of exocrine parenchyma, whether focal, segmental or diffuse. Ductal distortion and intraductal calculi, although producing rather dramatic ERCP and computed tomography images, are not part of the diagnostic definition of chronic pancreatitis because the duct system is within normal limits in some 30% of patients [10, 11]. It can be extremely difficult to diagnose chronic pancreatitis pre-operatively in this subgroup, although suspicion may be raised by impaired exocrine secretory capacity in some of them [10]; random needle biopsies to look for the patchy lesions is like searching for a needle in a haystack [12]. Paradoxically, pancreatic pain is no less, and may be considerably greater among patients with 'small-duct disease' than in those with a hugely dilated duct system and while surgery remains the mainstay of treatment for intractable pain, total pancreatectomy may be the only option for these patients [13]. It has been considered axiomatic that acute pancreatitis does not lead to chronic pancreatitis except when the main pancreatic duct is disrupted during a vicious acute attack [14].

A 12-month audit of patients with a first attack of pancreatitis was undertaken at Baragwanath Hospital in 1994 [15]. The study group of 136 patients included 108 men and 28 women, of mean age 40 years. Alcohol was the predominant aetiological factor in 83% and biliary disease in 7%, while no cause could be identified in the others. Substantial morbidity was experienced by 32% of patients, from metabolic derangements, alcohol-related symptoms, respiratory impairment or, as in 10% cases, local complications of pseudocysts, pancreatic necrosis or abscess. The overall mortality rate was 8%. A follow-up after an average of 9.3 months revealed serious morbidity in two-thirds of patients: 52 suffered severe abdominal
pain, 36% had substantial weight loss, 18% had clear abnormalities on pancreatic ultrasound scans, and 31% had exocrine pancreatic impairment as gauged by faecal chymotrypsin levels. It could be estimated that 30-40% of these patients actually had underlying chronic pancreatitis, judging by the presence of at least two among the three criteria of weight loss, abdominal pain and low faecal chymotrypsin. It is very likely that the 100 or so patients with chronic pancreatitis, including those who present with an attack and others who arrive via the routine clinic, together with the further 100 cases of 'acute pancreatitis' each year represent the tip of an iceberg, in that Sowetans are stoical by nature and may neither seek medical therapy nor be admitted to hospital, and several still prefer traditional healers [16].

A clinical study in 1977 charted the presenting features of patients with chronic pancreatitis arriving via the clinic [8]. Pain was the dominant feature in 60% of patients, and obstructive jaundice 33% due to constriction of the intra-pancreatic portion of the common bile duct. Diabetes was identified in 23% of cases and was difficult to control because compliance with treatment was poor. Steatorrhoea was usually mild despite marked pancreatic insufficiency because traditional diets are high in carbohydrate but low in both fat and protein; gross fat malabsorption was only revealed when patients were deliberately tested with a high-fat load. Pulmonary tuberculosis was discovered in 25% of patients and was usually in an advanced stage [9].

Both internal and external pancreatic fistulas were not uncommon. The former presented with ascites, pleural effusions or pseudocysts, and sometimes with complex connections among these fluid-filled spaces. A significant correlation was noted between abnormalities on serum biochemistry at admission and the severity of ductal disease and rupture as gauged by ERCP. Whereas a tiny leak from the pancreatic duct and normal serum biochemistry, were usually accompanied by ready closure of the fistula in response to conventional medical treatment after a mean of about 30 days, sometimes assisted by octreotide, surgery was generally required when there were two or more sites of extravasation on the initial ERCP, and/or serum albumin was depressed and/or the ratio of fluid to total serum protein was high. In one study there were two deaths among 23 patients [17]. External pancreatic fistulas occurred after abdominal trauma, surgery to the pancreas, following catheter drainage of pseudocysts or as a complication of acute pancreatitis. Spontaneous closure was unusual, but octreotide therapy was highly efficacious, often achieving closure by 3 days; occasionally total parenteral nutrition was used as an adjuvant measure [18]. This experience concurs with that of others [19, 20].
This short description of some clinical features in patients with pancreatitis at Soweto serves to illustrate the ferocity of an attack, the lingering morbidity in a substantial number in whom underlying chronic pancreatitis was likely, and the high frequency of pulmonary tuberculosis and also diabetes diagnosed at initial presentation. A disquieting mortality rate of 15% over a 3-year period in one survey of patients with chronic pancreatitis [9] may still be an underestimate, because many patients are lost to follow-up. Patients with chronic pancreatitis are known to be at increased risk of pancreatic cancer [21], but the magnitude of this risk could not be assessed at Soweto because of the high early mortality rate and poor attendance at follow-up clinics.

**Alcohol and Pancreatic Injury**

Numerous experimental studies have been done and reviewed [22], as have clinical studies attempting to address key facets of the alcoholic pancreatitis problem in man [23, 24]. First, it is necessary to explain why only few people seem to be vulnerable among the thousands who consume excessive amounts of alcohol. Second, and in contradistinction to the first point, studies of patients with alcoholic chronic pancreatitis indicate that there is a threshold for alcoholic toxicity to the gland [14]. Third, there should be a plausible reason why alcoholism is a major aetiological factor for 'acute pancreatitis', and also for 'chronic pancreatitis' in the Western World, Japan, Brazil and South Africa [14]. Fourth, although the majority of patients with alcoholic chronic pancreatitis have the classical calcifying form of the disease, there are patients on record with the small-duct variant [10, 11, 13], or the newly characterised duct-destructive cancer-like variant which has autoimmune overtones [25]. Fifth, there is the enigma that the clinicopathological spectrum of chronic pancreatitis, including the composition of calculi [26], is identical in alcoholic disease and in non-alcoholic disease, irrespective of geography.

Any proposed mechanism must have the plasticity to accommodate all these findings, while also taking into account the absence of any convincing evidence to implicate a number of potentially predisposing factors. These include dietary macronutrients, drinking pattern and type of beverage, blood group antigens and HLA serotype, $\alpha_1$ antitrypsin level and phenotype, apolipoprotein E phenotypes [23, 24], distribution of lithostatin (pancreatic stone protein) [27], or cystic fibrosis genotype [23]. Any hypothesis should also accommodate the finding that 'trypsinogen activation' in the pancreas is a very early phenomenon in experimental acute pancreatitis [29], as can now be inferred from the release of the activation peptide. This finding has been taken as an endorsement for pancreatic autodigestion as the
all-important trigger in every attack of pancreatitis, whether clinical or experimental. The
discovery that a mutation in the cationic trypsinogen gene underlies hereditary pancreatitis,
and the further deduction that the abnormal form of the activated enzyme resists degradation
by trypsin-like proteases, has been seen as rubberstamping this orthodox philosophy [30].
This latest development has also been put forward in support of an emerging school of
thought that recurrent episodes of acute pancreatitis may, after all, give way to chronic
pancreatitis via a necrosis-to-fibrosis sequence [30-32].
Over the years, the same sets of mechanisms have been invoked in alcoholic acute pancreatitis
and chronic pancreatitis [22]. There are three main propositions: (1) the flow-reflow concept,
wherein sphincter of Oddi dysfunction allows reflux of enterokinase-rich bile or intestinal
juice into the pancreatic duct, with trypsinogen activation as a consequence; (2) the protein
plug philosophy, wherein precipitates of cell casts and protein are encouraged by the presence
of unduly low bicarbonate and citrate, trypsin inhibitor and lithostatin concentrations in
pancreatic juice and also by increased levels of lysosomal enzymes, lactoferrin and GP-2
(glycosyl phosphatidylinositol protein from zymogen granule membranes) [22, 33-37]; (3)
the toxic metabolite concept [38] in which alcohol is thought to be directly injurious to the
acinar cell, not least by altering lipid metabolism [22] so that small fat droplets appear in the
cytoplasm and also between membranes of the rough endoplasmic reticulum [33]. It is
probably fair to say that the first two sets of mechanisms are now out of vogue, turning the
focus on alcohol metabolism by the pancreas.
It is known that ethanol enters cells by diffusion, and that the rate of uptake in pancreatic
acinar cells is similar to that in hepatocytes [39]. However, in contrast, the pancreas has a very
low capacity for the oxidative metabolism of ethanol by the sequential action of alcohol
dehydrogenase and acetaldehyde dehydrogenase (fig. 1) [39, 40]. Oxidative metabolites that
are generated in the liver do appear in the bloodstream albeit at low concentrations: it is thus
of interest that acetaldehyde has been shown to damage the pancreas in an isolated perfused
pancreas model [41]. It is also possible that, as in injury to hepatocytes, pancreatic injury may
be initiated, or compounded, by antibody reactions against acetaldehyde adducts at the plasma
membranes [42]. Recently, there has been excitement over the possibility that the
non-oxidative metabolism of alcohol by esterification with fatty acids to yield fatty acid ethyl
esters (FAEE), may be responsible for the pancreatic toxicity of alcohol. In support of this
suggestion, the pancreas has been found to have the highest levels of FAEE and also of the
enzyme involved in FAEE production [43]. Further, the infusion into the carotid artery of
low-density lipoprotein particles reconstituted with FAEE were found to target the exocrine
pancreas selectively, producing the lesions that are associated with mild acute pancreatitis, including acinar cell vacuolation, 'trypsinogen activation' and oedema [44].

Another proposal [45-47] is that alcoholic damage to the pancreas results from its interaction with cytochrome P450 monooxygenases (CYP), specifically CYP2E1 which has been extensively examined in the context of alcoholic liver injury [48]. Whereas the metabolism of ethanol by CYP is a very minor pathway when doses of ethanol are small, it becomes increasingly important at large doses; further, even the smallest dose is a potent CYP2E1 inducer, thereby accelerating the processing of other xenobiotics by this CYP isoenzyme. Although CYP evolved to facilitate the passage of endogenous and exogenous (xenobiotic) lipophilic substrates out of cells, by increasing their hydrophilicity, it is increasingly realised that phase I metabolism via CYP may yield a toxic metabolite. Animal experiments show that in this event prior CYP2E1 induction by ethanol magnifies risk [49, 50], in clinical medicine, paracetamol poisoning is a good example. Unless phase II conjugating pathways are robust, cell viability is under threat, not only by reactive oxygen species that are linked to CYP function but, more importantly, by reactive xenobiotic metabolites that cause irreversible loss of glutathione (GSH). Studies of hepatocytes show that lipid membrane peroxidation, loss of calcium compartmentation, derangement of mitochondrial function and interference in vesicle movement interact to cause cell death [51, 52].

Fig. 1: Potential ways in which ethanol may injure the pancreas. Figure adapted by Braganza from Gut et al. [40]. ROS = Reactive oxygen species; FAEE = fatty acid ethyl esters; GSH = glutathione; iSO₄ = inorganic sulphate; CYP2E1 = ethanol-inducible form of cytochrome P450.
The Manchester Hypothesis ("Braganza")

When originally proposed 15 years ago [45], the hypothesis was that CYP induction in the liver and the entry of toxic xenobiotic intermediates into the pancreas, by way of refluxed bile or the bloodstream, initiated chronic pancreatitis, irrespective of putative aetiological factor, and also non-gallstone acute pancreatitis. In support of this concept, analysis of serum and duodenal aspirates, following full clinical recovery after a pancreatitis attack, showed high concentrations of lipid-based free radical oxidation products in both sets of patients [53]. When diversion of abnormal bile, laden with these products, failed to abort attacks in 3 young patients with Idiopathic chronic pancreatitis [54], the hypothesis was modified to include concurrent CYP induction and toxic metabolite stress in the pancreas itself [46, 47].

Over the years, a body of evidence has accumulated showing that the CYP machinery is present in the pancreas and that several isoenzymes are highly inducible [55]. This generalisation holds true for the human pancreas and immunocytochemical studies have confirmed that bioactivating CYP isoenzymes are indeed induced in pancreatic specimens from patients with chronic pancreatitis [56, 57]. Those isoforms include CYP1A which metabolises smoke constituents and also potential carcinogens such as benzo(a)pyrene, CYP3A which bioactivates aflatoxin, and CYP2E1 which processes numerous drugs and solvents. The last of these enzymes was clearly induced in a study in which all patients had alcoholic disease [57], but not in the earlier study in which several patients had idiopathic disease [56]. Animal experiments have now confirmed, pancreatic CYP2E1 induction by ethanol [58], and also show that ethanol administration causes pancreatic oxidant stress, with alteration in redox state and mitochondrial damage [59]. Finally, it has been shown that the toxicity of ethanol, especially when combined with high dietary unsaturated fat, is related to production of free radical intermediates [60] and that this combination now provides a reproducible animal model of chronic pancreatitis [61].

The Manchester hypothesis, as currently stated [62], envisages oxidant stress in pancreatic acinar cells as the initiator of pancreatic injury in non-gallstone acute pancreatitis and, almost without exception, in chronic pancreatitis. Three component causes of oxidant stress have been identified: (1) CYP induction, whether by ethanol, constituents of cigarette smoke, high dietary C18:2 fatty acids, or anticonvulsant drugs; (2) concurrent exposure to a volatile chemical, often in the occupational environment [63], which undergoes bioactivation via CYP; (3) above all, a shortfall in GSH because habitual diets fail to deliver sufficient amounts of methionine and vitamin C to meet the increased demand [64]. The first component is considered to be important in the development of large-duct calcifying disease, because
expansion of the endoplasmic reticulum accompanies induction of the broad CYP2 family, and results in a non-specific increase in the synthesis of a cell's normal secretory products, whether albumin and very low density lipoprotein by the hepatocyte, or digestive protein by the pancreatic acinar cell [46, 47]. The precipitation of protein in the duct system is known to be facilitated by the hypersecretion of (apo)lactoferrin and mucin; these excesses can be rationalised with the recognition of their antioxidant capability, but it has perhaps been insufficiently stressed that impaired bicarbonate secretion possibly as a result of oxidant stress in the centro-acinar space, could be a major contributor in the formation of intraductal protein plugs.

The oxidant stress concept envisages any attack of pancreatitis as representing a breakdown of the signal transduction pathway that normally results in exocytosis of enzymes from pancreatic acinar cells, such that the zymogens and also pro-inflammatory free radical oxidation products are re-routed into the interstitium [65]. Support for this philosophy has come with the recognition of the key role of pancreatic thiols in driving the regulated secretory pathway [66]. It has further been suggested that reactive xenobiotic metabolites predispose to chronic pancreatitis by causing the irreversible loss of cellular GSH, whereas GSH is quickly refurbished when it detoxifies biological oxygen metabolites that have been repeatedly implicated in experimental acute pancreatitis [55]. Within the oxidant stress template, increasing background pain is regarded as a consequence of persistently misdirected secretion of pro-inflammatory substances into the interstitial space, because of continued impedance to exocytosis, with the resultant increase in interstitial pressure, collagen synthesis and excitation of nociceptive nerve endings [62].

A range of pharmacokinetic, immunocytochemical, occupational, biochemical and secretory studies at Manchester helped to construct this disease model. The corollary that antioxidant supplementation should ameliorate symptoms has been realised in a placebo-controlled trial [67] and also by follow-up of some 100 patients for an average of 5 years [63].

Xenobiotic Stress and Pancreatitis:

Studies at Soweto

The rapid increase in alcoholic pancreatitis at Soweto in the past decade, in line with urbanisation and industrialisation, is philosophically in tune with the Manchester hypothesis. Further, the suggestion from hospital admission statistics that both acute pancreatitis and chronic pancreatitis may now be endemic at Soweto, and the aggressive nature of each disease could, in theory, be rationalised by inaffordability of antioxidant-rich foodstuffs. Accordingly,
collaborative pilot studies were undertaken in four groups: non-alcoholic controls, consecutive patients with newly diagnosed chronic pancreatitis, asymptomatic chronic alcoholics, and consecutive patients with a first attack of apparent acute pancreatitis.

The studies in non-alcoholic controls revealed two peculiarities: (1) subclinical oxidative stress, wherein heightened free radical activity was coupled with poor micronutrient antioxidant status [69, 70], (2) hypercoagulability, because of a low concentration of plasminogen activator inhibitor [Douglas, Segal, Braganza, unpubl. obs.]. In regard to the first outcome, plasma vitamin C levels were very poor, due to inaffordability of fresh fruit and vegetables, while serum selenium concentrations were also lower than in European centres. However, the concentrations of β-carotene, α-tocopherol and also linoleic acid in serum conformed to reference ranges from Manchester (table 1), indicating a good quality of dietary fat. Further, the concentration of urinary inorganic sulphate, which is a gauge of long-term sulphur amino acid intake conformed to levels in Manchester controls. Methionine is one source of cysteine, the rate-limiting component in the synthesis of GSH; thus, the plasma concentration of GSH, which is a rough-and-ready index of GSH homeostasis, was compatible with values in Manchester controls (table 1).

Ascorbate, the bioactive form of vitamin C, closely interacts with GSH in cells, quickly buttressing GSH concentration in times of need [71, 72]: the studies in Sowetan controls revealed that this back-up device would not be available should the demand for GSH increase, for example, as a result of CYP induction, in that a high percentage of the available ascorbate was already oxidised (table 1). Since ascorbate is a key protector against oxidation of plasma lipids [73], it was not surprising to find heightened lipid peroxidation in the control studies. It is now known that the pancreas has a very high rate of glutathione turnover and that it actively synthesises GSH; however, the concentration of GSH in the pancreas is only a quarter of that in the liver [74], such that the GSH requirements for normal functions of the acinar cell could be readily compromised if it took on the additional role of xenobiotic detoxification. In the group with alcoholic chronic pancreatitis, studied in the phase of background pain rather than during an acute exacerbation, there was evidence of further ascorbate oxidation and this was accompanied not only by a further increase in lipid peroxidation, but also by depletion of plasma GSH, suggesting that GSH in the acinar cell was being excessively utilised. This interpretation is supported by the reduction in urinary inorganic sulphates, and the mobilisation, probably compensatory, of the glucuronic acid phase II pathway of drug metabolism.
Table 1: Summary of pilot studies testing the oxidant stress concept at Soweto in 15 controls and 14 patients with chronic pancreatitis [40, 69, 70].

<table>
<thead>
<tr>
<th>Lipid isomerization (P)</th>
<th>Soweto controls</th>
<th>Soweto vs. Manchester controls</th>
<th>CP</th>
<th>Difference</th>
<th>CP</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linoleic acid, μmol/l</td>
<td>922</td>
<td>802</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td>&lt;0.05 (Mer↑)</td>
</tr>
<tr>
<td>9,11 isomer, μmol/l</td>
<td>17.0</td>
<td>22.1</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td>&lt;0.05 (Mer↑)</td>
</tr>
<tr>
<td>Molar ratio, %</td>
<td>1.80</td>
<td>2.89</td>
<td>&lt;0.002</td>
<td>NS</td>
<td></td>
<td>&lt;0.05 (Mer↑)</td>
</tr>
<tr>
<td>Lipid peroxidation (S)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipid peroxides, μmol/l</td>
<td>2.52</td>
<td>3.27</td>
<td>&lt;0.05</td>
<td>&lt;0.0001 (Swo↑)</td>
<td>&lt;0.001 (Mer↑)</td>
<td></td>
</tr>
<tr>
<td>Glutathione availability (P)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSH + GSSG, μmol/l</td>
<td>6.24</td>
<td>2.19</td>
<td>0.0001</td>
<td>NS</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Ascorbate consumption (P)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin C, μmol/l</td>
<td>17.6</td>
<td>10.5</td>
<td>&lt;0.02</td>
<td>&lt;0.0001 (Swo↓)</td>
<td>&lt;0.05 (Swo↓)</td>
<td></td>
</tr>
<tr>
<td>Ascorbate, μmol/l</td>
<td>13.6</td>
<td>4.54</td>
<td>&lt;0.001</td>
<td>&lt;0.0002 (Swo↓)</td>
<td>&lt;0.001 (Swo↓)</td>
<td></td>
</tr>
<tr>
<td>Molar ratio of inactive form, %</td>
<td>21.9</td>
<td>57.0</td>
<td>&lt;0.002</td>
<td>NS</td>
<td></td>
<td>&lt;0.0005 (Swo↑)</td>
</tr>
<tr>
<td>Selenium (S), nmol/l</td>
<td>1,329</td>
<td>848</td>
<td>&lt;0.001</td>
<td>&lt;0.01 (Swo↓)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>β-Carotene (S), nmol/l</td>
<td>130</td>
<td>45</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>α-Tocopherol (S), mmol/mol cholesterol</td>
<td>5.01</td>
<td>2.82</td>
<td>&lt;0.001</td>
<td>NS</td>
<td></td>
<td>&lt;0.05 (Swo↓)</td>
</tr>
<tr>
<td>Inorganic sulphate (U), mmol/l</td>
<td>1.40</td>
<td>0.80</td>
<td>&lt;0.05</td>
<td>NS</td>
<td></td>
<td>&lt;0.05 (Swo↓)</td>
</tr>
<tr>
<td>D-Glucaric acid (U), mmol/mol creatining</td>
<td>2.61</td>
<td>3.85</td>
<td>&lt;0.02</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Theophylline clearance (S), ml/kg/h</td>
<td>61</td>
<td>6.3</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td>&lt;0.001 (Mer↑)</td>
</tr>
</tbody>
</table>

Data as medians: comparisons against Manchester data from published reports [50, 67, 87, 88], using two-tailed non-parametric tests: P = plasma; S = serum; U = urine; Swo = Soweto; Mer = Manchester. The upward or downward arrow indicates the direction of the significant difference.

Studies of iron and iron-binding proteins confirmed that the heightened free radical activity was not due to iron overload [75], while pharmacokinetic studies of theophylline, a drug that is largely processed by CYP1A, suggested that environmental chemicals other than alcohol contributed to toxic metabolite stress in at least 2 among the group of 14 patients [40]. This deduction was reinforced when a structured questionnaire was administered to 35 consecutive patients, with 3 age- and gender-matched controls for each case. Univariate group comparison identified heavier alcohol intake (p = 0.0001), increased cigarette smoking (p < 0.017), marked exposure to occupational chemicals (p < 0.001) and lower intake of fruit (p = 0.01) in the patients. A logistic regression analysis underlined the high risk from combined exposure to alcohol and chemicals, higher still when cigarette smoking was considered in the equation, and extremely high when fruit intake was low [76].

In the group of asymptomatic chronic alcoholics, numbering 21 individuals who drank at least 150g ethanol daily, levels of vitamin C and selenium were as low as in the group with chronic pancreatitis, suggesting equally poor diets. However, the concentration of plasma GSH lay midway between the values in controls and patients with chronic pancreatitis, being significantly different from each (3.86 μmol/l, p < 0.01 vs. non-alcoholic controls, p < 0.05.
vs. chronic pancreatitis), while the concentrations of both inorganic sulphate and D-glucaric acid in urine remained within normal limits [77]. These data lend further support to the notion that the development of chronic pancreatitis in the heavily polluted city of Soweto may be linked with xenobiotics additional to alcohol [40, 76].

In groups with an attack of apparent acute pancreatitis several interesting findings emerged. (1) Studies of admission blood samples in 24 patients gave evidence of profound oxidant strain [78], with excessive activation of innate relative to immune inflammatory response [79]. (2) Heightened fibrinolysis indicating the presence of active plasmin, was a striking feature even in patients with mild pancreatitis, and seemed to be independent of both trypsinogen activation, as gauged by measurement of the activation peptide of carboxypeptidase B in urine, and thrombin activation as indicated by the concentration of soluble fibrin [80]. (3) Following recovery from the attack in a further group of 30 patients, the same questionnaire was administered as was used in the previous study of chronic pancreatitis, and the information was compared with that from 30 age- and gender-matched controls. The patients had markedly higher alcohol consumption, but also higher exposure to occupational chemicals ($p = 0.055$) and lower fruit intake ($p < 0.05$). Excessive alcohol consumption was for 15 years in 62% of the patients. Smoking, binge drinking, alcohol consumption for $>15$ years and vegetable intake did not discriminate between the two groups (Segal, Ally, Becker, unpub. Observations).

Collectively, these preliminary studies support the new oxidant stress concept for the evolution of pancreatitis, and they provide a rational explanation for the observation that when apparent acute pancreatitis develops in chronic alcoholics, the chance of underlying chronic pancreatitis is high, but the disease may only be revealed after further attacks over several years [32]. The poor antioxidant status and intrinsic hypercoagulability of outwardly healthy Sowetans helps to rationalise the aggressive nature of a pancreatitis attack [15], in that both oxidative stress and the deposition of fibrin degradation products contribute to multisystem organ failure, not least the adult respiratory distress syndrome [65]. The high frequency of pancreatic calculi at the time of diagnosis in patients with chronic pancreatitis is also potentially rationalised by heightened oxidation of ascorbate, leading to the compensatory mobilisation of lactoferrin and mucin. Prior deficiency of selenium in these circumstances may sow the seeds for painful disease, judging by observations in patients at Manchester [81], while multiple deficiencies may prejudice DNA repair mechanisms and thus sow the seeds for pancreatic cancer. The studies also confirm previous work from South
Africa showing low plasma vitamin E concentration that could not always be explained by exocrine pancreatic failure [82].

The results in the group of asymptomatic chronic alcoholics is particularly relevant to the development of chronic pancreatitis at Soweto in that the fall in plasma GSH could represent a 'half-way house' in the progression towards chronic pancreatitis of CYP-mediated oxidant strain (table 1). This interpretation rationalises the changes reported in pancreatic acinar cells and also in pancreatic juice composition in studies of asymptomatic chronic alcoholics in Europe and the USA. Those findings include small fat droplets in acinar cells [38], hypersecretion of lactoferrin [37] and an increase in concentration of lysosomal enzymes [361], which could reflect oxidant attack on lysosomal membranes. The reported increase in concentration and output of protein in chronic alcoholics [36, 37] is now readily understood on the basis of CYP induction, but it is not yet clear why trypsin inhibitor concentration is reduced in pancreatic juice, nor why the normal 2:1 ratio of cationic to anionic trypsinoen is reversed [36].

**Xenobiotic Stress and Tropical Chronic Pancreatitis**

As at Soweto, chronic pancreatitis is endemic in certain tropical zones of the Far East where alcohol is not implicated. In these areas, it is not uncommon for symptoms to begin in childhood and for many family members to be affected. Also as at Soweto, calcifying disease is predominant and chronic pancreatitis runs an aggressive course towards diabetes and premature death. The condition has been well described in studies from Kerala, South India, which were the first to suggest that hydrogen cyanide, derived from cyanogenic glycosides in the dietary staple, cassava, might be responsible for pancreatic damage [83]. More recent studies at Madras, in the neighbouring state of Andhra Pradesh, where dietary staples are not generally laden with cyanogenic glycosides, have produced results that are in keeping with the new oxidant stress philosophy.

As at Soweto, the community at large had very poor vitamin C bioavailability, but this could be traced to hostile culinary practices rather than to inaffordability of fruit and vegetables [84]. A predisposition to oxidant stress as suggested by this finding, explains the previous observation that the concentration and output of lactoferrin in duodenal aspirates from children at Kerala were as high as in patients with alcoholic chronic pancreatitis from Marseilles in France [85]. In the studies at Madras, serum selenium concentrations were far higher than noted in Sowetan controls, being as high as in controls at Manchester [86], but...
urinary inorganic sulphate levels were very similar in the three cohorts. The last finding undermines the proposed hydrogen cyanide connection, because sulphur amino acids hold the key to cyanide detoxification by producing thiocyanates. The paradox that experimental feeding of cassava leads to exocrine pancreatic injury [87], whereas hydrogen cyanide does not, is now rationalised by studies of another plant nitrile, which strongly suggest that the non-cyanide moiety is the true pancreatic toxin [88]. As with other xenobiotics, so too with plant nitriles, there is some evidence for bioactivation via CYP. In this context, drug metabolism studies showed significantly lower theophylline clearance levels in Madras controls [89], than in controls at Soweto [40] or Manchester [90], exemplifying the principle of polymorphism in CYP function.

In patients with chronic pancreatitis at Madras, theophylline clearance was significantly accelerated, and there was an increase in urinary concentration of D-glucaric acid, the combination suggesting toxic metabolite strain [89], the former finding was in line with studies at Manchester where the ratio of idiopathic to alcoholic disease is around 50:50 [90], but not at Soweto where alcoholic disease predominates [40]: the latter phenomenon was noted in all three centres [40, 89, 91] in line with the commonality of phase II pathways of drug metabolism. The implication from the Madras studies was that xenobiotic stress was both important and relevant to the aetiogenesis of chronic pancreatitis. Detailed social histories in 79 consecutive patients then identified regular close exposure to volatile petrochemical products, principally kerosene fumes in lamps and cookers, and also smoke constituents from burning firewood [92]. These exposures were accompanied by, and would account for, reduced concentrations of micronutrient antioxidants [93]. However, in contrast to data from Soweto [70] and Manchester [80], selenium levels were relatively preserved in the patients at Madras [93]. The observation that selenium concentration tended to be particularly low in patients with painful disease at Manchester [81], may help to explain why patients at Madras do not experience as much pain as they might be expected to have at a time when viable acinar tissue was still present [93].

**Concluding Comments**

The oxidant stress philosophy accommodates experimental and clinical observations on alcoholic injury to the exocrine pancreas. Some would say that the fatal flaw in the concept is that it does not attempt to accommodate pancreatic autodigestion, a concept that seems to have a wide appeal. However, as cogently argued by one prominent pancreatologist [94], 'trypsinogen activation' within crinophagic vacuoles in the acinar cell should not be equated
with 'autodigestion', because there is powerful evidence that this activation, en route to the complete degradation of trypsin by lysosomal enzymes, is a physiological phenomenon to rid the cell of excess material, as occurs when exocytosis is blocked at the inception of a pancreatitis attack [65]. The new observations linking hereditary chronic pancreatitis with a form of cationic trypsinogen that would resist complete degradation, should it become prematurely activated [30], is potentially rationalised in terms of free radical pathology, in that vulnerable members can seemingly be identified by their poor antioxidant profiles [95]. The similarity in free radical marker and antioxidant profiles in the pilot studies of acute pancreatitis and chronic pancreatitis at Soweto suggest that these two conditions may indeed be part of a pathobiological spectrum, linked by gradations in acinar cell GSH status, with a greater degree of GSH depletion in chronic pancreatitis as a result of conjugation reactions with xenobiotic metabolites [62]. The most exciting outcome of the studies at Soweto, and also at Madras, is that prophylaxis against chronic pancreatitis may be possible by the simple measure of a daily tablet of vitamin C, perhaps fortified with selenium at Soweto, and with β-carotene at Madras [70]. The protection conferred by these substances in experimental studies of alcoholic toxicity [55] provides scientific support for this proposal.

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