Micronutrient ('Antioxidant') Therapy for Chronic Pancreatitis: Basis and Clinical Experience

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1. Introduction

Chronic pancreatitis is an on-going fibro-inflammatory process that causes patchy loss of acinar cells while at the same time favouring the formation of intraductal calcium carbonate stones. It often presents as an attack that is indistinguishable from acute pancreatitis, representing paralysis of apical exocytosis in acinar cells, 'pancreastasis' (17, 19, 34), which in animal experiments is tied in with a burst of electron transfer reactions (loosely called free radical activity, FRA) (19, 129). Until all secretory parenchyma is obliterated, agonizing pain is usually the predominant symptom, whether accompanying recurrent attacks, or constant and disabling. Its treatment is largely empirical, such that addiction to narcotic analgesics is a compounding menace, because there is no consensus on disease pathogenesis (34). The concept that electrophilic stress is the detonator and inflammatory motor (23, 24) offers the opportunity for corrective micronutrient therapy (11, 20, 30, 66, 140, 154) and, thereby, pain control (28). This usage of micronutrients exploits more than 'antioxidant' properties.

2. Stresses and Stressors

Electrophilic stress
The phrase indicates the threat when electrophilic compounds (ie with a relative electron deficit) steal electrons from nucleophiles, as are most biological macromolecules. Xenobiotics (ie exogenous lipophilic substrates) are the major pathological source of electrophiles, by way of reactive xenobiotic species (RXS) that are inadvertently generated upon processing by cytochrome P450 monooxygenases (CYP). Highly reactive carbonyl products derived from oxidation of polyunsaturated fatty acids in cell membranes are the most relevant endogenous source (21).

Oxidative stress
This descriptor points to the threat from an unusually high concentration of reactive oxygen species (ROS), of which many are free radicals (ie. with an unpaired electron) (111). The best known are superoxide ($O_2^{-\bullet}$) which is quenched by superoxide dismutase, hydrogen peroxide (H_2O_2) which is removed by catalase and glutathione (GSH) peroxidase, and the highly reactive hydroxyl radical ($OH^\bullet$) - leaving aside products of interaction with nitric oxide (46, 87). About 10% of molecular oxygen undergoes ROS-yielding stepwise reduction during such physiological processes as mitochondrial respiration, CYP-mediated processing of endogenous lipophilic compounds, phagocytosis, and synthesis of disulphide (S-S) bonds from cysteine that are needed for proper protein folding in the endoplasmic reticulum (ER) (143). Evidently cells can tolerate the burden, deliberately allowing low-grade oxidative stress for the cited

and many other vital roles including signal transduction, calcium homeostasis, membrane turnover, redox control and genomic stability. A pathological excess of ROS - as is associated with CYP induction (65), ultraviolet irradiation, xanthine oxidase activity under conditions of ischaemia-reperfusion, and so on - threatens cell viability by jettisoning just those homeostatic mechanisms that physiological oxidative stress secures (21). Transition metals, iron in particular, promote electron transfer reactions (67). Insofar that ROS are integral to CYP function, the degree of electrophilic stress might be thought to mirror oxidative stress, but studies in the context of ageing show that the level of electrophilic stress can be disproportionately greater than that of its oxidative drive (179).

Reductive stress

This idiom describes abnormally high electron (reducing) pressure behind a blockade of an enzymic step in the ATP energy production staircase. The blockade may be due to absence of an enzyme, or to malfunction. When electron pressure is sufficiently high some of the electrons may react with O_2 directly and generate ROS. Swings in electron pressure (redox potential) mimic and are reciprocally linked to swings in pH (proton pressure) (30, 58, 59). In fact, just as alkalosis is rarely if ever a problem unless deliberately induced because all metabolic processes tend to be acid-generating, so too reducing pressure / reducing stress seems to be the main route to oxidative stress, at least in the long term. The problem is epitomised by alcoholism, hypoxia, redox cycling compounds such as doxorubicin that cause electron dislocation, and uncouplers of electron flow, such as NSAIDs, cyclosporine, and cytokines.

Endoplasmic reticulum stress

If not quickly rectified, any of the above stresses activates the ER stress- unfolded protein response (UPR) which, in turn, exacerbates oxidative stress and elicits inflammation by activating stress response genes such as NF-kB

(9, 122). The exocrine pancreas with its huge rate of protein synthesis is particularly vulnerable when subjected to congestion in the busy protein-trafficking lanes - an inevitable consequence of pancreastasis episodes, despite the acinar cell's best efforts to compensate by endocrine re-routing of newly synthesised enzymes; removal of zymogen granules via the three-pronged strategy of centripetal dissolution, crinophagy and basolateral redirection; and down-regulation of enzyme synthesis (19, 30, 34, 52). The close integration between oxidative, electrophilic, ER, and inflammation stress is now regarded as the basis for many chronic diseases (177) and, increasingly, for chronic pancreatitis (97, 112, 123).

3. Electrophilic Stress Template

Component clauses

Since it was first mooted in 1983 (15), this disease model has evolved in line with new observations (16, 23). The 1998 version (24) views the acinar cell as the site of mounting electrophilic stress that steadily erodes methyl (CH_3) and thiol (SH) - principally glutathione (GSH) - moieties, as a result of CYP induction, concurrent exposure to a toxicant that yields RXS, and insufficiency of refurbishing micronutrients **(Figure 1)**. A fourth factor must now be built into the equation, namely, gene mutations that might favour the cytoplasmic presence of trypsin (97): the enzyme, as also chymotrysin, is readily inhibited by GSH via SH-SS exchange (24) should it break loose of constraint by SPINK1 (serine protease inhibitor Kazal type 1) (118), but less GSH is then available for control of electrophilic / oxidative stress and other vital roles (24, 30, 162). The qualifying clauses help to explain why patients on CYP-inducing anticonvulsant drugs rarely develop chronic pancreatitis, or why profound electrophilic / oxidative stress but with low CYP activity in children with kwashiorkor results in painless loss of acini, not chronic pancreatitis (64).
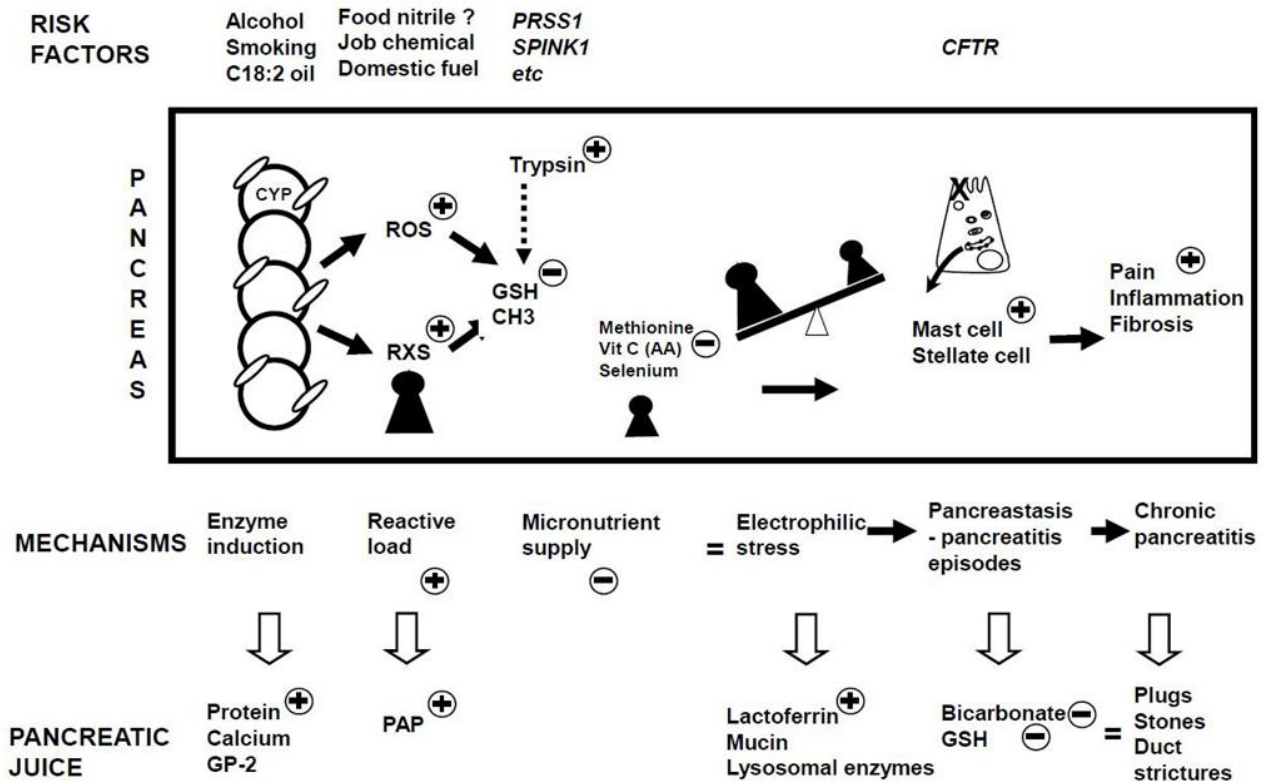
Figure 1. A framework for the pathogenesis of chronic pancreatitis, showing how risk factors interact to generate electrophilic / oxidative stress while also promoting lithiasis. Note that the supply of critical micronutrients might be subnormal in absolute terms - due to inaffordability of source foodstuffs (eg in Soweto), problems associated with senility (22), or hostile culinary practices (eg as in India, resulting in destruction of ascorbic acid) - or relative to increased oxidant load. Abbreviations: C18:2, oils rich in bi-unsaturated fatty acids; CYP, cytochrome P450 mono-oxygenases;   *PRSS1*, *SPINK1, CFTR,* mutation(s) in genes for cationic trypsinogen, the serine protease inhibitor Kazal type 1, and the cystic fibrosis transmembrane conductance regulator, respectively;   ROS reactive oxygen species; RXS reactive xenobiotic species; GSH, glutathione in bioactive form; CH3 activated methyl groups; Vit C (AA) the bioactive ascorbic acid form of vitamin C; GP-2, secreted component of zymogen granule membranes analogous to the renal cast protein (34); PAP, pancreatitis associated protein activated by electrophilic stress (34). Encircled plus or minus symbols represent increases or decreases, respectively.

The concept does allow for a steady build-up of ROS alone, as in elderly people (22), and patients with hereditary pancreatitis (57, 98, 162).

Within this framework, each burst of electron transfer reactions hinders apical exocytosis to trigger an attack of pancreatitis by interfering with the methionine-to -GSH metabolic pathway, which interacts closely with ascorbate and selenium. The diversion of free radical oxidation products (FROP) into the interstitium causes mast cells to degranulate **(Figure 1)** (25, 34), thereby provoking inflammation, the activation of

nociceptive mechanisms that promote a chronic pain syndrome (28), and pro-fibrotic interactions: here it is worth noting that RXS (including from opiates), bile salts and radiocontrast media evoke a non-IgE anaphylactoid response (25). Meanwhile, the acinar cell generates its own pro-inflammatory mediators under the influence of redox-sensitive signalling cascades (87), but pancreatitis is said not to ensue when basolateral exocytosis is prevented (52).

Cystic fibrosis, usually due to severe mutation in both alleles of the *cystic fibrosis transmembrane*

conductance regulator (*CFTR*) gene, causes an accelerated non-calcific form of chronic pancreatitis that begins *in utero*: oxidative stress and inflammation are now regarded as integral features of the disease, driven by unfolded CFTR via the ER stress-UPR system (121, 177). This is not the position depicted in **Figure 1**, which instead seeks to understand the increased frequency of *CFTR* mutation(s), with or without mutation in *SPINK1*, among patients with idiopathic chronic pancreatitis (97), especially the tropical variant (102).

Thus, neonatal hypertrypsinogenaemia in *CFTR* carriers, and the enhanced susceptibility to experimental pancreatitis so conveyed, suggests hindrance to CFTR-facilitated apical exocytosis in the acinar cell under conditions of excessive FRA (30). Moreover, as predicted (24), CFTR is easily inactivated by oxidants (30), which would have the same impact as pancreas-selective mutations in *CFTR* (85), compromising the delivery via ductal cells of bicarbonate into pancreatic juice and, thereby, contributing to lithogenicity (34, 81). The ability of the antioxidant curcumin to rescue DF508-CFTR localization in cell lines (88) suggests that oxidants might be responsible for the cytoplasmic mislocalization of CFTR observed in alcoholic, idiopathic and autoimmune pancreatitis (81). Of interest, the CFTR channel also transports the antioxidants GSH (30, 121) and thiocyanate (174).

The template **(Figure 1)** envisages permutations and combinations among the aforesaid factors plus oxidant attack on CFTR in ductular epithelium as determining outcome - whether recurrent acute pancreatitis, small-duct chronic pancreatitis, or large-duct disease with or without calculi; age at onset; and rate of progression. The worst combination appears to be in patients with tropical pancreatitis (30, 102, 103). The populist notion of pancreatic autodigestion by prematurely activated trypsin in acinar cells has no part in the philosophy (17, 25, 26, 118), and is increasingly challenged by its former proponents (124). Although not in the schema, it is conceivable that RXS via CYP might be involved in the genesis of autoimmune pancreatitis, as in autoimmune hepatitis (60): this becomes plausible with the finding from studies in hepatocytes that newly synthesised CYP enters the secretory pathway to arrive at the outer surface of the plasma membrane (119). As to a connection with ER stress, many xenobiotics have been shown to influence the UPR signalling route, with either pro-survival or pro-death features - unsurprising given that resident CYP straddle ER membranes (84).

CYP induction / Concurrent toxicants

The liver is the main site of xenobiotic processing by CYP although since around 1986 it has become increasingly evident that many organs including the pancreas have the archaic, dormant but inducible machinery (16, 21). The detoxification of xenobiotics and excretion of hydrophilic metabolites is brought about by an initial oxidative step that utilises ROS, followed by conjugation of the intermediate metabolite with glucuronic acid, inorganic sulphur, acetyl groups or GSH via GSH transferases (GST) (70, 154). The phenomenon of 'enzyme induction' ensures increased availability of the particular CYP isoform that is appropriate for the substrate in question. This is accomplished by increased synthesis of haem for incorporation into CYP and haem oxygenase (90). The latter degrades excess toxic haem with release of ferritin, bilirubin (via biliverdin) and carbon monoxide. Haem oxygenase is up-regulated by numerous other stressors that share a capacity to decrease tissue GSH. The enzyme is carried in blood and is a potent antioxidant, as also are the catalytic end-products: moreover, it strongly inhibits mast cells (27). Membrane lipids are integral to proper CYP function (21). So too is the trace element selenium, a deficit of which causes hepatic haem to be wasted down the bilirubin route (47).

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Table 1.

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	Hepatocyte	Acinar cell
Compatible with CYP induction	↑ Cell size Ground glass hepatocytes ↑ SER mass ↑ Phospholipids in bile	↑ Cell size ↑ RER mass ↑ Protein in pancreatic juice ↑ Calcium in pancreatic juice

	↑ Drug metabolism
	  BSP-κ_1 [= ↑ligandin]
	  Antipyrine clearance [= ↑ CYP overall]
	  Theophylline clearance [=↑ CYP1A]
	  Urinary D-glucaric acid [=↑ phase-2]
	↑ CYP by immunocytochemistry
		
		? Pancreatic contribution
		
		↑CYP by immunochemistry

Compatible with electrophilic / oxidative stress	Microvesicular steatosis Dilated SER ↑ Lipofuscin in tissue [= ↑lipid peroxidation] ↑ FROP in bile ↑ Bilirubin in bile [= ↑ haem oxygenase in liver] ↑ Copper in bile ↑ serum caerulopalmin [= ↑ ferroxidase 1] ↑ serum ferritin Sclerosing cholangitis-like lesions	Microvesiculation Pancreastasis episodes Tubular complexes Dilated RER ↑ Lipofuscin in tissue ↑ FROP in pancreatic juice ↑ Lysosomal enzymes in pancreatic juice ↑ Mucin and PAP/*regIII* in pancreatic juice ↑ Lactoferrin in pancreatic juice ↑ Albumin in pancreatic juice ↑ serum PAP Sclerosing ductal lesions

Abbreviations: SER = smooth endoplasmic reticulum; RER = rough endoplasmic reticulum; BSP-$_{\kappa 1}$ = first -phase corrected disappearance curve after injection of sulphobromophthalein; CYP =cytochrome P450 ; CYP1A= the isoform inducible by polycyclic and other hydrocarbons; FROP = free radical oxidation products; PAP/*reg111* = secretory stress, pancreatitis- associated protein subtype. Upward arrows indicate increases.

The information in **Table 1** is a distillate of many studies in patients with chronic pancreatitis, itemised in earlier reviews (16, 18, 23, 34), now incorporating more recent observations in patients with mainly alcoholic disease (138, 5). The findings are readily rationalized by the electrophilic stress concept **(Figure 1)**, but not by any other theory on pathogenesis **(34)**. The information reveals that induction of the xenobiotic-processing machinery in liver and pancreas is not innocuous, despite mobilization of several natural antioxants (67, 111). Studies on surgically resected specimens afford direct evidence of CYP induction (55, 147, 168), and also on-going oxidative stress : structural aberrations by microscopy **(Table 1)** (24), FRA signals (155), increased FROP with decreased GSH (5, 133), increased concentrations of pro-oxidant metals (copper, iron) but decreased levels of antioxidant metals (zinc, selenium) (5); and markers of the ER stress-UPR (123). The last finding might be expected to involve disrupted calcium homeostasis (177), but studies in isolated rat acini indicate that this is not a factor in toxicity from induced CYP (37). As to whether oxidative stress contributes to the sclerosing ductal lesions,

its involvement in primary sclerosing cholangitis is worth registering (43), in that similar lesions are not infrequently present in patients with ordinary chronic pancreatitis (18). Moreover, pancreatic juice (130), bile (86), and duodenal aspirates (56, 68) from patients with chronic pancreatitis have high concentrations of irritant lipid oxidation products.

The key point is that the pancreas falls clinical victim while liver injury is generally silent   - but why? The best explanation is that xenobiotics hit the gland directly via the arterial route, whereas ingested toxicants first encounter the liver which is best equipped to deal with RXS, via its huge complement of GSH and GST (21, 170). This is illustrated by experimental studies in the 1950s using a subcutaneous dose of carbon tetrachloride, which is processed by CYP to yield RXS : damage in advance of liver injury; lesions that could be "produced at will" by varying the dose, ranging from patchy lesions of early chronic pancreatitis with or without concretions, through to 'pancreatic cirrhosis' or a cystic fibrosis- like picture (165). The theme is reinforced by more recent studies with nitriles akin to those in dietary

cassava (manioc, tapioca), and the occupational chemical dibutyltin (170), showing that prior induction of CYP2E1 by a small dose of ethanol augments dibutyltin injury (101) -   as is also true for hepatotoxicity from volatile hydrocarbons (131, 149). As to chronic exposure to ethanol, laboratory studies show that increased FRA precedes pancreatic injury (71). In the drug metabolism studies from Manchester, UK **(Table 1)**, heightened theophylline clearance was the predominant finding in patients with idiopathic or alcoholic disease (18), indicating induction of CYP1A (70) -   as by C18:2 fatty acids (eg in corn, peanut or linseed oil), polycyclic aromatic hydrocarbons and halogenated hydrocarbons (94).

Since a proportion of arterial blood first enters islets cells, it is likely that RXS generated in CYP-induced islets are delivered to some acini by the insulo-portal conduit, so adding to their RXS burden, and potentially explaining the patchy distribution of lesions (24, 147, 165). The abundance of GST in islet cells affords insurance against injury in the short term, whereas a dearth of GST in acinar cells -   whether absolute (161), or relative to increased need from CYP induction (55, 147, 168) -   renders them vulnerable (30). Moreover, relatively long-lived FROP and RXS generated in the CYP-induced liver **(Table 1)** could aggravate pancreatic injury if they find their way there via refluxed bile (128), or the bloodstream (18).

Of all the findings in **Table 1**, the increase in bilirubin is most revealing because it indicates induction of haem oxygenase to combat severe oxidative stress. A further surge accompanies a pancreatitis relapse (18), mimicking the abrupt enzyme rise when phenobarbitone-treated rats are exposed to RXS from halothane gas (152). The combination of induced CYP1A, increased copper, and induced haem oxygenase is a unique exposé of environmental toxicology in humans (83, 110). The three findings cannot be dismissed

as a consequence of impaired pancreatic function because there was no correlation with its degree as gauged by secretin-pancreozymin tests (18). However, the normalization of copper and bilirubin data by long-term treatment with pancreatic extracts (18) is of the utmost interest, now that a paper documenting the antioxidant potential of such extracts has been unearthed (see below). Both bile and pancreatic juice inhibit copper absorption in experimental work, but studies using radioisotopes did not show any difference in copper absorption by healthy volunteers, patients with untreated chronic pancreatitis or those on pancreatic extracts (H Sharma, P Tasker, JM Braganza, unpublished).

Reports from the UK (Manchester) (33, 100), south India (Madras) (32) and south Africa (Soweto) (73, 138) have revealed regular close exposure to volatile hydrocarbons in patients with chronic pancreatitis, whether in the occupational environment, domestic setting (from kerosene or paraffin lamps and cookers in confined spaces), or both. In the first UK study, patients noted freedom from attacks when away from the workplace (33). The six-fold decline between 1962 and 1987 in annual hospital admissions with the disease in Kerala province, south India, coincides with the introduction of electricity, which removed the dependence on traditional lighting (34). An investigation at Soweto concluded that exposure to occupational chemicals distinguishes patients labelled as 'alcoholic chronic pancreatitis' from alcoholic controls with similar cigarette usage and equally poor diet (138). These observations add weight to case reports cited in previous reviews (21, 79, 91). Although the direct pancreatic toxicity of petrochemicals is documented in lower species (21), and hepatotoxicicity from kerosene was recorded some time ago (148), the field of inhalation toxicology to the pancreas has been a vacuum until recent evidence of injury from cigarette smoke in rodents (34).
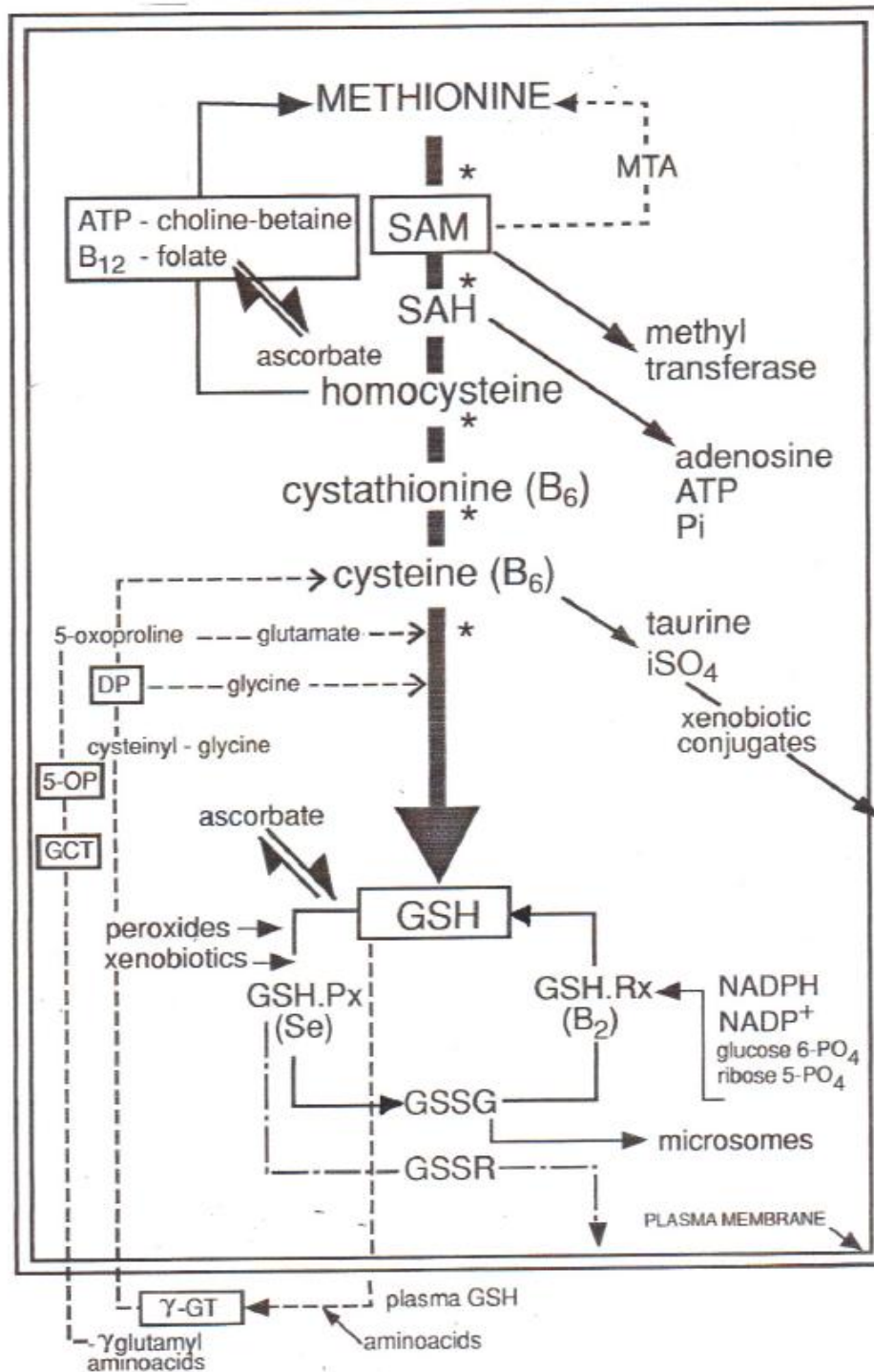
Figure 2. Reproduced from reference (25) with kind permission from the publisher (S. Karger AG, Basel, Germany). The pathway of methionine metabolism wherein key metabolites are SAM, sulphadeosnsyl methionine; SAH, sulph adenosyl homocysteine; and GSH, glutathionine. Other abbreviations: MTA methylthioadenosine; ATP adenosyl triosephosphate; Pi, activated phosphate groups; iSO$_4$, inorganic sulphate; B$_2$, B$_6$, B$_{12}$, riboflavin, pyridoxine and cobalamin, respectively; GSH.Px, glutathione peroxidase; GSH.Rx, glutathione reductase; Se, selenium; GSSG, oxidised glutathione on engaging with peroxides; GSSR, conjugates of glutathione with electrophiles from xenobiotics; NADPH and NADP, reduced and oxidized forms of nictotinamide adenosine phosphatase, respectively; DP, 5-OP and GCT, enzymes involved in the synthesis of glutathione (170). Asterisks indicate enzymes that are known to be vulnerable to electrophilic / oxidative stress.

This should soon be rectified, scepticism notwithstanding (144), because the health risk from volatile petrochemicals is currently under intense scrutiny (109, 117, 156).

Methyl / Thiol insufficiency

In theory, there are many ways in which a burst of electron transfer reactions can impede apical exocytosis in acinar cells (21), but the evidence in patients with chronic pancreatitis points to a breakdown in the delivery of CH_3 and SH moieties **(Figure 2)** (21, 23-25). The concept of methionine-dependent exocytosis was enunciated in the 1950s (77, 165, 166) and is now pinpointed to methylation of membrane components, probably of a prenylated cysteine residue (40).

The supply of CH_3 moieties depends on *de novo* synthesis from S-adenosylmethionine (SAM) via a folate-dependent enzyme which catalyses the production of S-adenosyl homocysteine (SAH), or by re-methylation of the next metabolite, homocysteine, via choline-betaine and vitamin B_{12}-folate cycles that need ATP and are facilitated by ascorbic acid , the bioactive from of vitamin C (30). Hence folic acid lack inhibits secretion too (6). GSH derived from homocysteine by the transsulphuration pathway is another absolute requirement for apical exocytosis, not least by protecting participating enzymes in the methionine metabolic route (24) while also sparing critical protein thiols (93). Furthermore, *in vitro* studies identify the need of CFTR for exocytosis (115), while *in vivo* experiments show that a surfeit of magnesium stabilizes the exocytosis machinery by antagonizing calcium (132).

Both steps in the onward route from homocysteine to cysteine are powered by vitamin B_6 (pyridoxine)-dependant enzymes. Pyridoxal-5'-phosphate is also a co-factor for two other enzymes involved in the synthesis of the gaseous mediator hydrogen sulphide (H_2S) from homocysteine and cysteine (172), seemingly provoked when the progression to GSH is impeded. Cysteine is pluripotent : rate-limiting component in GSH synthesis, source of taurine and inorganic sulphate that facilitate the removal of RXS **(Figure 2)**, key to proper protein folding in the ER (143), and seemingly even more important than GSH for redox control (105). The same is true for GSH: besides facilitating exocytosis and inhibiting proteases, it helps in redox control, serves as a reservoir for cysteine, mops up hydrogen and lipid peroxides, detoxifies RXS and contributes to the extracellular antioxidant shield. Whereas its utilisation in peroxide control is soon made good via interlocking GSH peroxidase-GSH reductase, NADPH-NADP, and glucose 6 phosphate-ribose 5 phosphate shuttles, it is permanently excreted from cells in conjugates with RXS **(Figure 2)** (21, 170). In these circumstances, the ability of ascorbic acid to substitute for GSH by redox and non-redox pathways is invaluable (30), as is heightened activity of gamma glutamyl transpeptidase (γGT) in the plasma membrane, which enables the uptake of reconstituting amino acids from the plasma GSH pool (138, 170) - but these resources are finite.

The pathway of methionine metabolism also impacts on the correction of reductive stress by biomolecules with electrophilic methyl groups. These include SAM, phosphatidylcholine, betaine and carnitine (58).They appear to act by binding to positively charged nitrogen or sulphur moieties, a poising mechanism that is demonstrable *in vitro* when the reaction mix includes catalytically active iron, H_2O_2 and ascorbic acid: carbon dioxide and carbon monoxide are formed from the ascorbate molecule in parallel with generation of methane gas. It is now recognized that albumin acts as a sacrificial antireductive protein which when modified by OH• radicals emits a signal to proteolytic degradation and elimination (89).

In patients with chronic pancreatitis there is clear evidence of oxidant-associated breakdown in methionine metabolism. Thus, during a relapse, neutrophils show low GSH but an increase in the

oxidized form (96), while urine and / or blood analysis point to a metabolic block in the transsulphuration pathway distal to cysteine - leading to surges in cysteine and more proximate metabolites (96, 160), but a fall in inorganic sulphur. By the third day subnormal methionine and a further decline in sulphur levels hint at poor pre-morbid intakes of sulphur amino acids (30). These twin problems of hindrance to methionine metabolism within an oxidative environment and methionine insufficiency are shown in studies of patients with quiescent disease, whether alcoholic or idiopathic. (a) Peripheral blood displays a strong tendency to produce ROS (51, 151, 167). (b) Plasma / serum contains excessive amounts of protein carbonyls (167), and also lipid-based FROP as reported in papers that are too many to cite individually. (c) Erythrocytes have subnormal levels of certain antioxidant enzymes, and GSH (10, 62, 82). (d) Transmethylation and transsulphuration pathways remain fractured (63). (e) ^{11}C methionine scanning demonstrates good pancreatic uptake of the amino acid but then its regurgitation coupled with impaired enzyme secretion into the duodenum (150, 153). (f) Subnormal plasma concentrations are reported of sulphur amino acids (61) and thiols derived via the transsulphuration route (125, 167), including GSH (138, 167). Plasma homocysteine level may remain normal (167), or increase in conjunction with subnormal folic acid (63), vitamin B_6 (35) or vitamin B_{12} (138). (g) H_2S appears in exhaled air (107). It is not known whether any of these aberrations has a bearing on displacement of Munc18c into the cytosol of intact acinar cells noted in the resected specimen of a patient with stable disease: this 'SM protein' is involved in pathological basolateral exocytosis (52).

4. Towards Treatment

Clues for a prescription

Non-enzymic endogenous defences to electrophilic/oxidative stress are already up-regulated in patients with chronic pancreattis **(Table 1)**, and it is known that the acinar cell has

liitle copper-superoxide dismutase (30). The inference is that micronutrient antioxidants fall short in the face of the persisting assault from RXS / ROS (31, 47). Many trace elements, the sulphur amino acids, and several - perhaps all - vitamins contribute in one or more ways to the antioxidant repertoire of tissues (64). Analysis of habitual diets is the only way to glean which items might be crucially lacking in the face of an increased oxidant load, not merely less than in healthy controls. The axiom - which extends to blood levels - cannot be overstated, while appreciating the difficulty in estimating that load (21, 111). Low blood levels reflect the net result of intake, absorption, tissue sequestration and excretion.

Studies at Manchester, UK, identified lower habitual intakes of selenium, vitamin C, riboflavin and vitamin E in patients with idiopathic chronic pancreatitis than in age and gender-matched controls, Selenium was the best discriminator on step-wise analysis, and when examined against theophylline clearance (as marker of CYP1A-related oxidant load) (70), afforded good separation between patients and controls (120). When the studies were extended to a control group with similarly high theophylline clearance - patients with epilepsy on anticonvulsant CYP inducers - a second discriminant function emerged that was equally weighted on lower methionine and vitamin C by the chronic pancreatitis set (158). This finding accords with *in vitro* studies showing heterosynergism between ascorbic acid and sulphur antioxidants (135). Moreover, whereas the epilepsy group was in a care centre cocooned from environmental toxicants, regular exposure to volatile hydrocarbons was noted in four others who were in employment and developed chronic pancreatitis (158). There are no comparable studies of habitual diets in patients with chronic pancreatitis.

The antioxidant role of selenium is generally linked to its presence at the active centre of enzymes that are redox catalysts, of which the

best known are GSH peroxidase which removes H_2O_2 and lipid peroxides, and thioredoxin reductase which is homologous to GSH reductase and critical for redox regulation of protein function and signalling (4). However, there is evidence that the element serves other important roles in the detoxification of xenobiotics (31). Ascorbic acid is pluripotent in combating electrophilic / oxidative stress. It can substitute for GSH; facilitates the homocysteine remethylation cycle **(Figure 2)**; scavenges electrophiles; acts as 'Michael donor' in reactions with acrolein and genotoxic FROP (30); protects against $OH^{\bullet}$ in plasma (67); and quenches mast cell histamine (76) which generates H_2O_2 (108). Not only does the last factor rationalize the virtual absence of ascorbic acid in plasma samples from patients admitted with a pancreatitis attack (14, 136), but it also underlines the involvement of mast cells (25-27).

Lower concentrations of selenium have been noted in serum / plasma of groups with chronic pancreatitis compared to control groups in diverse geographic areas (5, 31, 106, 116, 138, 163, 164, 175), with subnormal GSH peroxidase activity when selenium level is very low (163). At Manchester the lowest selenium values accompanied painful disease, and levels fell progressively over four days upon repeated exposure to CYP substrates used for drug kinetic studies (31). There is debate as to whether (116), or not (173, 175), malabsorption contributes to the decrease. However, treatment with pancreatic extracts is expected to augment the intake of selenium, zinc, magnesium and methionine, because the gland is a repository of these: the metals should survive the purification procedure and sulphur amino acids might, whereas the vitamins could be lost. This deduction is supported by a hitherto buried study in patients with cystic fibrosis, in whom increases in plasma selenium and erythrocyte GSH peroxidase activity could be attributed to substantial amounts of selenium in commercial preparations (173). The finding has obvious repercussions on usage of pancreatic extracts to ease pancreatic pain in patients with small-duct chronic pancreatitis, indicating micronutrient antioxidant therapy by proxy (29).

Vitamin C assays by spectrophotometric methods do not indicate the percentage in the bioactive ascorbic acid form, as is enabled when an HPLC assay is run in parallel. Thus, the respectable control levels of plasma vitamin C reported in India - at Madras (36), Delhi (10), and Cochin (62) - are misleading if the Madras results can be generalized. Here the samples contained very little ascorbic acid, the discrepancy likely due to hostile culinary practices that might also destroy β-carotene. By contrast, in controls at Soweto the low level of ascorbic acid in plasma was proportionate to that of vitamin C, reflecting inaffordability of fresh fruit and vegetables, and fell further in the oxidizing milieu of chronic pancreatitis (138). At Manchester, ascorbic acid values were negligible in patients with calcific disease or cysts/pseudocysts (20). Against this background, the good value for ascorbic acid reported in French patients seems anomalous (116), and begs the question as to what fraction this represented of total vitamin C.

The triple whammy in the genesis of pancreatic electrophilic stress - CYP induction, concurrent exposure to volatile toxicants, and insufficiency of particular micronutrients - was highlighted by a report on patients with idiopathic disease at Manchester (128). Low protein intake, as in Madras and Soweto, impairs CYP induction: thus, the rate of theophylline clearance in Madras controls was lower than in Manchester controls, but significantly increased in local non-alcoholic patients (44), alongside regular close exposure to kerosene fumes and little ascorbic acid (36, 103). At the time of the studies at Soweto, it was not known that chlorzoxazone is a probe of CYP2E1 induction, as by ethanol (92): in these patients, with predominantly alcoholic disease, theophylline clearance was similar to that in healthy controls, but the impact of RXS evident from a fall in

plasma GSH, increased oxidation of plasma ascorbic acid, decreased urinary inorganic sulphate and increased D-glucaric acid (138). A subnormal concentration of zinc in serum (5, 138) and erythrocytes (62, 116) correlates with reduced exocrine secretory capacity (62), rather than poor intake, and has no bearing on CYP function.

Potential benefit

Treatment with a combination of methionine, vitamin C and selenium should, in theory, help patients with painful chronic pancreatitis by several means - protecting the acinar exocytosis machinery; controlling rogue trypsin; removing RXS from halogenated hydrocarbons (166) and petrochemicals (157) ; shielding CFTR (30); curbing NF-kB activation and cytokine production (13); rectifying reductive stress, as by cyclosporine (137); inhibiting ER stress and activation of the UPR (74, 75, 95); reducing the oxidative drive to stellate cells (49); and stabilizing mast cells (26, 28, 29), mediators from which are not only pro-fibrotic, but also are implicated in converting peripheral pain sensitization to the unrelenting pain from central sensitization (28).

Insofar that recurrent acute pancreatitis (ie with histological restitution six or more weeks after the last episode, as gauged by normal secretory and imaging studies (16) and chronic pancreatitis are now regarded as a disease continuum (34), the following areas of overlap as well as subtle differences are interesting. Genetic studies show an increased frequency of *CFTR* with or without *SPINK1* mutation among patients with pancreas divisum, type-1 hyperlipidaemia or hyperparathyropidism (30). A pilot study disclosed regular exposure to volatile hydrocarbons in patients without gallstones (33). Drug disposal studies indicated induction of CYP and ancillary systems in several of them (18). Analysis of secretin-stimulated duodenal aspirates identified Increased lipid peroxidation, albeit less than in chronic pancreatitis (68). Dietary inventories showed that datapoints lay on or close

to the aforementioned discrimination line based on selenium intake versus theophylline clearance (54). Oxidative stress is recognized in the pathogenesis of recurrent pancreatitis due to deficiency of lipoprotein lipase (69). Plasma / serum profiles of micronutrients were within normal limits, in contrast to the deficiency profiles in chronic pancreatitis (106). In other words, there seems to be a better match between the availability of micronutrient antioxidants and the degree of electrophilic / oxidative stress in patients with recurrent acute pancreatitis, despite the need to protect a larger mass of functional parenchyma. However, this might not be the only explanation for why recurrent acute pancreatitis does not always progress to chronic pancreatitis (30, 69, 106, 123, 176).

Pilot studies

In the 1980s there was no commercial preparation that would deliver methionine, vitamin C and selenium simultaneously. Methionine tablets were available from the Evans Medical Ltd, Horsham, UK (to treat paracetamol poisoning, which is caused by CYP-derived RXS), as was a nutraceutical from Wassen International, Leatherhead, UK that would provide the other two micronutrients, but along with β-carotene and α-tocopherol (ie vitamin E). By trial and error in 23 patients (20) - including five described in case reports of small-duct chronic pancreatitis or large-duct disease without or with huge calculi (128, 158) - total daily doses that most often reduced attack frequency and / or background pain were identified. These were 2 gm methionine (although patients exposed to occupational chemicals tended to need twice as much), 600 μg organic selenium, 0.54 gm vitamin C and, inevitably, also 9000 IU of β-carotene and 270 IU of vitamin E. Side effects were usually minimal (eg. nausea, skin discolouration from β-carotene). Schizophrenia has been reported when methionine dose exceeds 10 gm/day: a patient with a strong family history developed symptoms on 4 gm/day.

A number of exclusion criteria for future placebo-controlled clinical trials could be delineated: suspected pancreatic cancer; over-the counter vitamins / antioxidants; children; pregnant women; pain that could be explained by concurrent illness such as gallstones, peptic ulcer or somatic causes which are neither expected to nor do respond to micronutrient therapy (JM Braganza unpublished); large pseudocysts, or bile duct stones that need invasive intervention; such advanced disease that oxidants have no target (51), as evident from steatorrhoea, secretin tests and nowadays by assay of faecal elastase (29, 34,62); family history of schizophrenia; chronic renal failure or liver failure; addiction to narcotic analgesics in that the associated pain mimics that of chronic pancreatitis and because addicts might have ulterior motives for sickness behaviour (28). Clearly, the earlier that micronutrient therapy starts in patients with (non-gall stone) relapses, the greater should be the chance of success. These exclusion criteria can be relaxed outside trials when regular clinical and biochemical monitoring is possible (99).

Assessing outcome

The goal of treatment is to correct electrophilic / oxidative stress and thereby to control pancreatic pain (28). Hence it stands to reason that there must be evidence of such stress pre-recruitment, or its confident expectation on the basis of numerous published reports - provided that ineligible patients are excluded and that patients do not change their lifestyles for the duration of a trial. Furthermore, any reduction in pain on active treatment should ideally be shown to occur *pari passu* with correction of such stress, so as to distinguish true improvement from the 20% rate of amelioration by placebo (41). Low plasma/serum level of one or more micronutrients in isolation merely indicates a propensity to oxidative stress. Likewise, increases in plasma levels upon supplementation thereof without reference to oxidant load are not only meaningless, but in healthy controls is without benefit (3), and could

be harmful by abolishing low-grade oxidative stress that is so essential physiologically.

In order to assess oxidant load, clinical investigators seek biochemical 'fingerprints' of persisting stress. The choice, from the immense library (111), must be guided by the perceived primary target of attack, and practicality. If lipids are the all-important target, the best current marker is F_2 isoprostanes (86), and 'thiobarbituric acid reacting substances (TBARS)' the least specific albeit most popular. These are products of the classical lipid peroxidation pathway, but there is another route that accounts for the bulk of so-called 'diene conjugates' in biological fluids. This is the isomerisation pathway, and is easily mimicked *in vitro* by irradiating linoleic acid (9, 12 LA) in the presence of albumin. Moreover, the route seems to be under the control of a selenium-dependent enzyme, in that only one isomer (9 *cis*,11 *trans*) is present in bile, duodenal juice and serum when four are possible (42, 146), and also that consideration of serum selenium alongside the percent molar ratio of the isomer (9,11 LA') to the parent fatty acid (% MRLA) enabled good discrimination between data from patients with cystic fibrosis and controls (126). The isomer's stability under ordinary freezing conditions and ease of batch analysis by automated HPLC makes it an attractive marker, but potential invalidation by food sources and bacterial contamination must be remembered.

As argued recently (28), the triggering attack in pancreatitis is on enzymes and receptors that are protected by ascorbic acid interacting with GSH. Hence, useful measures in plasma/serum might include the percent oxidized ascorbic acid relative to total vitamin C (138); GSH coupled with y-GT activity (138); protein carbonyls (167); and allantoin, signifying oxidation of uric acid which, along with albumin, glucose and bilirubin constitute the bulk antioxidants. Convenient tests that measure 'total plasma antioxidant activity' by a commercial kit, or so-called 'TRAP' and 'FRAP' assays that have been used in clinical trials (10,

80), are misleading in monitoring micronutrient therapy because they are largely influenced by those compounds : ascorbic acid contributes 20% to the FRAP reading, vitamin E 5%, β-carotene very little and thiols none (30). If a nomogram is available to monitor treatment (126, 160), so much the better. Since dysregulated methionine metabolism due to RXS seems to underlie most cases of chronic pancreatitis, an index of its repair by treatment would be very helpful - as by analysing a metabolite(s) (63, 96, 160) , and / or ^{11}C methionine scanning (150,153). These resources are scarce, but a sustained increase in erythrocyte GSH upon micronutrient therapy appears to be an indirect pointer (10). It is generally accepted that the identification of oxidative stress and its correction should involve more than a single index of attack on a single target (21).

As to clinical recording, exclusion criteria should be specified, as also criteria for diagnosing chronic pancreatitis, recognizing that minimal changes on endoscopic pancreatography or endoscopic ultrasonography are insufficient without a test of secretory capacity (34). The frequency of attacks, pain scores using visual analogue scales for the most common local descriptors of pain, the best available quality-of-life measure that befits pancreatic pain (139), and analgesic usage are indices of therapeutic efficacy, or otherwise. Questionnaires should be kept to a minimum and applied by the same clinician, given that these patients co-operate voluntarily although they may be in much pain. Monitoring of compliance is best achieved by an objective measure: since the detection of ascorbic acid by urinary dipstick is not quantitative, compliance might only be determined retrospectively when the results of blood analysis in the post-active treatment phase are known. The wide inter-individual variability in disease pattern

favours a switch-over trial design which, however, is foiled by the carry-over effect of today's high potency material eg. 'Antox' (Pharmanord, Morpeth, UK), although the average daily dose of selenium is half that used initially (134).

5. Clinical Trials

Meta-analyses
The first independent appraisal of antioxidant therapy for pain control in patients with
chronic pancreatitis covered reports on randomized controlled trials (RCT) up to 2009, and concluded that the identified micronutrient combination (Section 3) improved outcome in each of three placebo-controlled studies (159, 80, 10), but that meta-analysis was impossible because different instruments were used to measure pain (104). In the past 12 months, three meta-analyses have appeared, covering studies until October '12 (1), December '12   (39), and February '14 (178). Despite the indiscriminate inclusion of RCTs judged satisfactory on mechanistic grounds, without considering the basis for treatment or legitimacy, each report concluded that active treatment reduced pain, especially using the micronutrient combination (39), and that although side-effects in up to 19% were usually mild (eg headache, nausea, allergy, constipation, diarrhoea), they did cause some patients to withdraw from the trial. The first trial, published in 1990 (159), was the only one to tick all the boxes in the report under the Cochrane banner (1) : an attempt to gauge quality-of-life used questionnaires based on patients with chronic backache, inappropriate in retrospect but the best available at the time. Interestingly, reports that were deemed fit for inclusion were not the same in these meta-analyses. All authors called for further large-scale studies.

Table 2.

Author	Clinical	Trial type/Active treatment	Outcome	Biochemistry
Uden [159, 160] Manchester 1990 / 1992	n=20 ACP 7, ICP 8 RAP 5 Opiates none Steatorrhoea none	20 week switchover double-dummy double-blind Rx: 6/day Se-βcar-C-E (Wassen,UK) 8/day methionine (Evans,UK) Dose/day : Se 600µg, βcar 9000 IU (= 5.4mg), C 540mg, E 270 IU (=270mg), methionine 2g	Attacks ↓ 11 pain words VAS ↓ Pain diaries 2nd 5-wk ↓ Pain psychology ↔ Placebo effect	Baseline Se, βcar < controls; E, A ↔; post-Rx all ↑↑ Baseline SAM < controls; downward drift post-Rx Baseline MRLA > controls; post-Rx ↓ Baseline pGSH:Px, uSO4 = controls; post-Rx ↔ pSAM iin attack↑; Se vs MRLA nomogram useful No carry-over at 10 week
De las Heras Castano [48] Santander 2000	n=19 ACP 11, ICP 5, RAP3 Opiates? Steatorrhoea ?	Open trial for 12 months Rx: 4/day (hospital preparation) Dose/day : Se 300µg, βcar 12mg, C 600mg, E 188mg, methionine 1.6g	Admissions vs previous year ↓ Pain word VAS vs previous year ↓ Pancreatic function vs previous year ↔	Not done
Uomo [162] Naples 2001	n=3 children with HP	2 year open in 4 blocks of 6 months, Rx 2nd & 4th Rx: 2/day SAM, 3/day multivitamin (sources ?) Dose/day :Se 75µg, A 2.4g, C 180mg, Mg 300mg, E 30mg, SAM 800mg	Painful days ↓ Analgesics ↓	Not done
Kirk [80] Belfast 2006	n=19 all ACP ? Opiates ? Steatorrhoea ?	Trial type: as Manchester 1990 Rx: 4/day Antox (Pharmanord, UK) Dose/day : Se 300µg, βcar 12mg, C 600mg, * E 152mg, methionine 1.6g	SF-36 Quality of life score ↑ Pain↓ Physical/social well-being ↑ (Nausea)	No control data; post-Rx Se,βcar,C & E > placebo Post-Rx A,αcar & TAC ↔ Post-Rx Fox1,MDA, & pGSH ↔ Carry-over at 10 week
Bhardwaj [10] Delhi 2009	n=127 study power 80% ACP 35, ICP 92 Calculi ? Opiates ? Steatorrhoea 20%	6 month parallel placebo or active Rx: ?/day Belamore G (Osper, India) Dose/day : as Manchester 1990 Plus all on pancreatic enzyme supplements	Admissions↓ Pain free: active > placebo Pain days/month ↓ Analgesics ↓, man-days lost ↓ Improvement by 3 months (Headache, constipation)	Baseline A,E < controls, C = controls Baseline FRAP, E-TGSH & E-SOD < controls, Post-Rx all these ↑, vitamins by 1 month Baseline S-SOD & TBARS > controls, post-Rx ↓ 3-month data not given
Shah [139] Manchester 2010	n=137 ACP 84 Antox 68, non 69 28 disease-duration matched pairs	Prospective cross-sectional study of concecutive patients already on Antox (Pharmanord,UK) versus standard Rx. Antox dose not stated, likely variable	VAS: Antox vs Non (p=0.01) EORTC QLC C-30 &QLQ PAN-28 Quality of life. Antox group better on all counts (p<0.001-<0.0001) Opiates Antox vs Non (p<0.01)	Not done
Siriwardena [145] Manchester 2012	n=70 study power 80% ACP 51, ICP 19 CT calculi 66 Opiates? mean 88mg/d Marked EPI in many Prior intervention 54%	6 month parallel placebo or active Intended 4-block design ie +/- intervention Rx: 6/day Antox v1.2 (Pharmanord, UK) Dose/day: Se 300 µg, C 720 mg *E 228 mg, methionine 2.88g *βcar from coating 2.5mg	Clinic & Diary numeric pain score↔ Brief pain inventory ↔ 4 Quality of life scores↔ In-patient days↔Out-patient visits↔ 4-block data not given (Bad taste, heartburn, diarrhea, nausea)	No baseline data; no raw data post-active or placebo All data as change from baseline No evidence of micronutrient lack at baseline No evidence of oxidative stress by MRLA at baseline GSH and MRLA change post-active =post-placebo Post-active Se, βcar, E, C >> post-placebo Increments post-active suggest major reporting errors
Dhingra [50] Delhi 2013	n=61 ACP 21, ICP 40 strict exclusion criteria Steatorrhoea 15	3 month parallel placebo or active Rx 8/day Belamore G (Osper, India) Dose/day : as Manchester 1990, Delhi 2000	Pain days/month Pain diary, Analgesic use Pain less on active, data sparse	Baseline TBARS > controls, FRAP < controls Baseline TGF-β1 & PDGF-AA > controls (p 0.07) Post-active ↓drift in TBARS & ↑FRAP Post-active PDGF-AA ↓, TGF-β1↔

Abbreviations alphabetically. Clinical ACP: alcoholic; HP: hereditary; ICP: idiopathic; RAP: recurrent acute; EPI: exocrine pancreatic insufficiency by faecal elastase assay. Trial type βcar: β carotene; C, E: vitamins C & E; Mg: magnesium; Rx: active treatment; SAM: sulphadenosylmethionine; Se: selenium. Outcome EORTC QLC-30: European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core questions 30, and Pancreatic Modification questions 28; VAS: visual analogue score; vs: versus. Biochemistry A: retinol; AA: ascorbic acid; αcar: α carotene; E-TGSH, E-SOD: erythrocyte total glutathione and superoxide dismutase respectively; FRAP :free radical trapping ability of plasma; Fox1: ferrous oxidation; MDA: malondialdehyde; MRLA: molar ratio of linoleic acid isomer to parent acid; pGSH: plasma glutathione; ROS: reactive oxygen species; S-SOD: serum superoxide dismutase; TAC total antioxidant capacity, TBARS thiobarbituric acid reacting products of lipid peroxidation; TGF-β1: transforming growth factor-beta1; PDGF-AA: platelet-derived growth factor-AA. Arrows signify direction & degree of change. * doses from senior technical officer at Pharmanord, Denmark
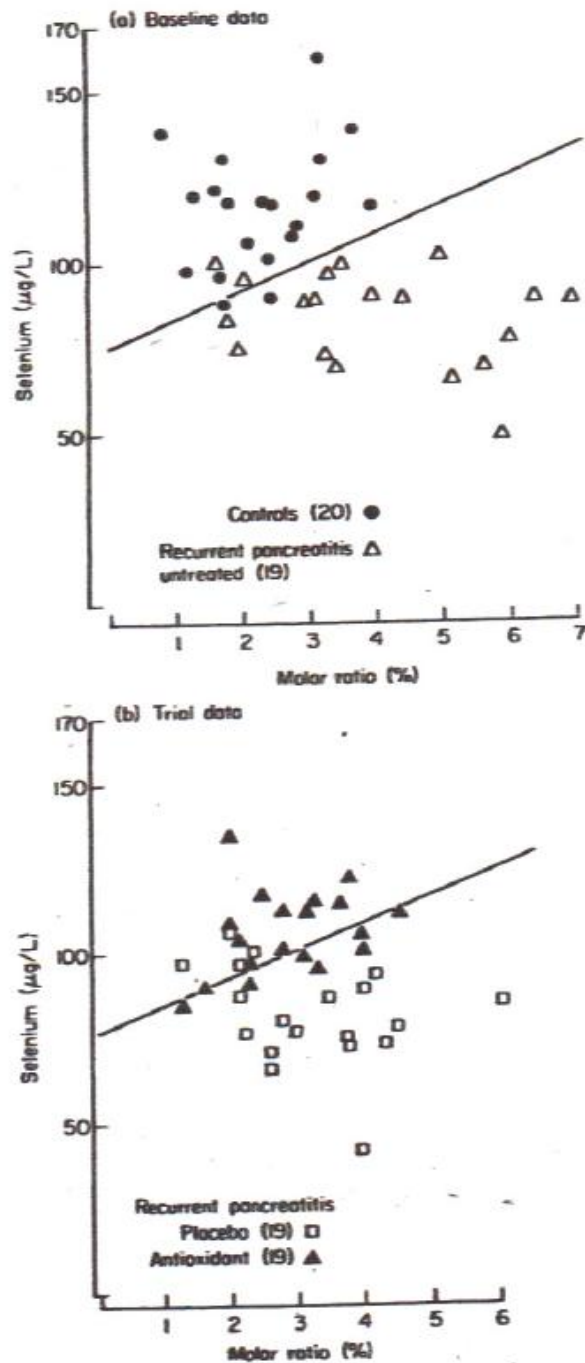
Figure 3. Reproduced from reference (160) with kind permission from the publisher (Wiley, West Sussex). Discriminant analysis of serum selenium and the % molar ratio of the 9 *cis* 11 *trans* isomer of linoleic acid to the parent compound in controls and patients with recurrent pancreatitis. Discriminant function: selenium= 8.76 x % MR + 75.74. Note that this function also applies to data from placebo-treated patients but fails to distinguish between controls and antioxidant-treated patients. Moreover, the discriminant function is not dissimilar to that recorded in patients with cystic fibrosis (126).

Since the *raison d'etre* for a micronutrient prescription is to boost the supply of CH_3 and SH moieties (Section 3), the inefficacy of the micronutrient curcumin (despite curbing lipid

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peroxidaton and being a potent inhibitor of mast cells) and allopurinol (inhibitor of xanthine oxidase) in clinical trials indicates that these substances do not achieve the goal: of note, curcumin did not increase GSH in erythrocytes. Yet, the curcumin trial (53) was included in each meta-analysis, and the allopurinol trial (8) in two (1, 39). The Cochrane report (1) correctly separated a study showing the benefit of allopurinol or another antioxidant, dimethyl sulfoxide, in patients with a pancreatitis relapse (127), wherein many other factors such as pancreatic ischaemia come into play (23, 25), but was included in the other two appraisals (39, 178). However, that analysis included two studies published in abstract only (1), and a Polish language report of vitamin C / E treatment versus no treatment (ie. not the full micronutrient package) (72), which was also included in another meta-analysis (178).

Subjective assessments during this period concluded that micronutrient antioxidant therapy was convincing (11, 154); had potential (66, 140); could be useful as adjuvant therapy (45); was poor, based on very limited experience (38); or useless (113).

Micronutrient combination

Table 2 summarises information on studies of the micronutrient combination, whether (10, 50, 80, 145, 159, 160) or not (48, 162) placebo-controlled, excluding studies reported only in abstract. In the original cross-over trial clinical improvement was accompanied by migration of datapoints towards the control zone in the nomogram relating serum levels of a lipid oxidation marker and selenium **(Figure 3)** (160). Amelioration of oxidative stress concurrently with clinical improvement was shown in two other trials (10, 50), of which one also noted reduction in markers of fibrosis (50). Clear benefit from active treatment accrued in all but one trial (145), although its authors argued strongly in favour just two years earlier **(Table 2)**, in a cross-sectional study of patients already on micronutrient

supplements versus no supplements under a later policy (see below) (139). Unfortunately, serious flaws render the second report invalid (28), while diluting the value of micronutrient therapy in each meta-analysis.

Of special note in **Table 2** is the observation that the combination of SAM (instead of methionine) **(Figure 2)**, plus vitamins A, C, E, and magnesium was beneficial in three children with hereditary pancreatitis (162). Combination therapy was also highly successful in abolishing attacks in patients with lipoprotein lipase deficiency (69), interesting because in this instance xanthine oxidase rather than induced CYP is the likely source of increased FRA (23). Intravenous treatment with N-acetylcysteine - in lieu of methionine - as a more immediate precursor of GSH **(Figure 2)**, plus the other micronutrients by appropriate routes, resulted in rapid pain relief and contraction of the inflammatory calcific head mass in an emaciated patient with impedence to gastric outflow and silent haemochromatosis (142).

Two RCTs are in progress : NCT01528540 is testing the micronutrient cocktail plus pregabalin (a presynaptic voltage-gated blocker of the calcium channel) or placebo in all-comers; EUROPAC-2 is a three-armed trial involving a potent commercial preparation of the antioxidant combination ('Antox version 1.2', Pharmanord, UK), versus magnesium , versus placebo in patients with hereditary or idiopathic disease.

Other observations

(a) SAM alone, or with selenium and vitamin E, was ineffective in double-blind placebo-controlled RCTs from the UK (12). (b) This was also true for selenium and vitamin C in a single-blind RCT from India, the report of which gave no diagnostic criteria, stated gall stones / common bile duct stones as the commonest aetiological factor, did not specify if these problems were on-going, and administered proton pump inhibitors plus pancreatic extracts - both with antioxidant potential - to treatment and no-treatment

groups (78). (c) By contrast, in an open observational study from the Czech Republic, vitamin C (0.5 gm/day) plus vitamin E (100 mg/day) for 12 months resulted in substantial pain reduction, to the point of abolition in 44% of 70 patients with mainly alcoholic disease. This was largely attributable to vitamin C, in that vitamin E levels were inexplicably unchanged by treatment, and with the greatest decrements in ROS generation and lipid peroxides among patients with the most functional parenchyma, judging by grade of pancreatogram abnormality (51). (d) A study reported in Polish (72) is cited in two meta-analyses (1, 178). The prescription (C 0.4 gm/day, E 300mg/day) or no treatment was administered in an open RCT for six months in 91 patients with alcoholic disease, while blood antioxidant levels were monitored. The English abstract reports that 68% of the group of 46 on active treatment became pain-free versus 31% of 45 untreated (p=0.002). The treated group also had fewer pancreatitis relapses (p=0.03) and their weight improved (p= 0.001), as did pancreatic exocrine and endocrine function (p=0.001, p=0.015 respectively). (e) An anecdotal account from the USA showed impressive improvement in three patients treated with a grape-seed extract (7).

Clinical improvement in the latter three studies suggests that methionine intake in those patients was adequate, such that ascorbic acid or potent antioxidants in the grape seed extract protected enzymes in the methionine metabolic route **(Figure 2)**. Moreover, the Czech study shows that it is illogical to expect relief from oxidative stress-induced inflammatory pain in patients whose exocrine pancreatic function is severely compromised.

Long-term treatment
The long-term value of combination micronutrient therapy has been documented in reports from Delhi (102) and Manchester (99, 141). However, the importance of correcting reductive stress is illustrated by the finding that in three Manchester patients with recurrent acute pancreatitis, who

were referred after the trials, standard treatment failed: the addition of folate to provide more methyl groups did not help, but a choline supplement did (JM Braganza unpublished). This is in tune with the experimental observation that polyenylphosphatidyl choline protects against pancreatic oxidative stress in alcohol-treated rats (2). When choline intake falls short, this phospholipid can be synthesised from phosphatidyl ethanolamine, but incurs severe pressure on 'the universal methyl donor', SAM **(Figure 2)** (169). There is now a convergence of thought on mechanisms of liver and pancreatic damage from a protracted excess of alcohol. The combination of three methyl donors -   SAM, folate, betaine **(Figure 2)** -   alleviated alcoholic liver injury, while at the same time lowering the elevated SAM / SAH ratio and homocysteine level (114), but the aforesaid evidence also points to the critical importance of ascorbic acid in protecting the exocrine pancreas, as shown by its ameliorating effect in animal models of mild and severe pancreatitis (30). The inescapable conclusion is that the choline-deficient ethionine-supplemented dietary (CDE) model of acute pancreatitis, which is easily modified to cause inflammatory fibrosis, is highly relevant to clinical pancreatitis.

A surgical audit in 1992 involved 94 patients attending the Manchester Pancreato-Biliary Unit, with a mean follow-up period of 30 months on micronutrient therapy, and more than five years in 22% of cases (99). Imaging studies revealed that 85 % had 'large-duct disease' (moderate or advanced change pancreatitis by endoscopic pancreatography), with or without calculi , and 15 % had 'small-duct disease', usually identified by secretin-pancreozymin tests. No patient needed duct decompression or resective pancreatic surgery during 248 patient-years of follow up; the total number of days spent in hospital while on treatment was significantly lower than in the preceding year; 78% of patients became pain-free and a further 7 % had a substantial reduction in pain (although several continued to take simple

analgesics as fearful of an attack); just two patients had continuous pain compared to 29 before micronutrient therapy; and of the 76 patients previously in employment, 88% were back at work and 80 % of these were doing the same job. Of the 42 patients who drank alcohol excessively, a third continued to drink as previously, half had abstained altogether, and the others had reduced their intake to 'safe' limits (99).

This excellent result, which was continued through to 1998, such that surgery to treat the pain of chronic pancreatitis was virtually obsolete, accrued through strict guidelines: patient-controlled devices to deliver morphine forbidden; routine endoscopic sphincterotomy, pancreatic duct stents or attempts at clearance of pancreatic calculi firmly rejected; psychiatric help sought early when dependence on narcotic analgesics or despair at social upheaval loomed; input of primary care practitioners solicited; nutritionist, social worker and pharmacist engaged from the outset. The prescription of opiates in patients who were already dependent at referral was devolved to the pain team. A weekly medical-surgical clinic was preceded by an interdisciplinary discussion on patients to be seen that day, co-ordinated by a medical registrar, nurse specialist, and biochemist. Each doctor had a printout of previous antioxidant and % MRLA data, so that selenium dosage could be adjusted with reference to the nomogram **(Figure 3)**, while whole blood GSH helped to assess methionine adequacy, and that of vitamin C by reference to the % oxidation of contained ascorbic acid (JM Braganza, unpublished).

The full prescription was usually needed for six months while dietary advice was given on antioxidant -rich foodstuffs (99). Negotiation with executives from Pharmanord, UK, resulted in a combination tablet, 'Antox', that reduced the number to an average of four per day compared to 14 per day in the original trial. Further

improvements were made by the company in order to increase the daily dose of methionine, while limiting β-carotene to the shell, because of cosmetic distress from a yellow hue, resulting in 'Antox version 1.2'. Treatment failure in around 10% of about 300 patients was associated with large cysts / pseudocysts, non-compliance in unreformed alcoholics, undiagnosed neoplasia in two (adenocarcinoma, papillary mucinous) and in the described patients with recurrent acute pancreatitis (JM Braganza, unpublished).

After 1998, new Consultants brought to bear their previous experience, such that patient-controlled pumps to deliver morphine were introduced, morphine prescriptions soared, invasive intervention increased, and the micronutrient prescription ceased. This change in practice is witnessed by the high daily dose of morphine and 54% prior intervention rate in the recent negative trial of combination micronutrient therapy **(Table 2)** (145), compared to none on either count in the 1990 report (159).

6. Conclusion

Chronic pancreatitis seems to represent 'hepatization' of the gland - a reversion to its ancestral roots, as a result of chronic exposure to xenobiotics that strike parenterally (16, 165). Choline intake and status in patients on their habitual diet were unfortunately not assessed in the Manchester studies, but are needed urgently. In the meantime, the addition of a choline supplement to the successful micronutrient cocktail is judicious, and probably should take precedence over calls to prescribe zinc, folate or magnesium. The perception of electrophilic / oxidative stress in acinar cells as the 'obligate intermediate phenotype' in the pathogenesis of chronic pancreatitis **(Figure 1)** fulfils a set of postulates derived from Koch's classical work on tuberculosis, as modified for a polygenic disease (23,24). Hence, it is difficult to see the need for 'a new framework for 21st century medicine' (171).

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