

## 'Not Knowing Something Is Normally a Milestone on the Way to Knowledge'

An Interview with Joan M. Braganza, DSc, FRCP, FRCPath, Reader Emeritus, University of Manchester, UK

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### Abstract

Dr. Joan Braganza, a world expert in the field of chronic pancreatitis, proposed a new template for its pathogenesis based on the role of free radical pathology, in particular the heightened but unmitigated oxidative detoxification reactions via cytochromes P450. Dr. Braganza has gone on to show how pancreatic damage in cystic fibrosis, acute pancreatitis and pancreatic cancer fit into the scheme, paving the way for new treatment modalities. In this interview, Dr. Braganza shares her life experience as an investigator and provides a perspective for young researchers entering the field of pancreatology. Copyright © 2009 S. Karger AG, Basel and IAP

**M.E.F.-Z.:** What initiated you to work in pancreas research in the first place?

**J.M.B.:** Serendipity! I graduated in Bombay in 1966, having never seen a patient with chronic pancreatitis (CP), and in January 1968 found myself in a CP referral unit at the Manchester Royal Infirmary. Its chief was Henry Howat who had introduced pancreozymin – discovered by Alan Harper and colleagues at the University – as an adjunct in the classical secretin test. The finding of secretory impairment was the only way to diagnose CP pre-operatively, in the absence of calculi: simultane-



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ously, secretin's choleric effect and the cholecystokinetic property of pancreozymin gave information on hepatobiliary function. I did about two tests a week. They were cumbersome and made a heavy demand, too, on biochemists' time. However, they forced me to study pancre-

atic and bilirubin physiology, and to read exhaustively on CP and its relationship to acute pancreatitis, cystic fibrosis, gallstones and pancreatic cancer. It was clear that CP was equated with alcoholism, and that duct decompression or resective surgery was the mainstay of treatment for agonizing pain, in apparent support of the notion that calcifying protein plugs in the duct system were the seminal problem that led to strictures, compromising acinar function. I saw that resective surgery was no small feat, and for the patient no treat! I saw, too, the folly of submitting to resective surgery a patient already addicted to narcotic analgesics – all too often the result was an addict with brittle diabetes and malnutrition.

In May 1969, soon after I had obtained the MRCP, domestic tragedy forced me to resign. Howat had an ongoing research programme on gastric and pancreatic secretion in the anaesthetised cat in response to caerulein analogues. I was co-opted. My work involved cannulating the pancreatic duct, giving a priming infusion of secretin [Boots, Nottingham, UK, as used in the clinical test] and undertaking several assays. In November 1969, armed with the results, I made my first foray into a scientific arena – the European Pancreatic Club, that had been co-founded by Howat and Henri Sarles from Marseille.

In September 1970 I developed spinal tuberculosis and when, in December 1971, my husband had a stroke, I had to abandon research altogether and do a stint in his primary care practice. Later I resumed my clinical training but also synthesised the research data into an MSc thesis and wrote four papers for *The Journal of Physiology*. My new finding was that Boots secretin – but not the purer gastro-intestinal-hormone (GIH) product from Stockholm – had a potent pepsin-stimulating effect which was not due to a non-specific increase in blood flow, as shown by cannulating the hepatic artery. I went on to show that GIH secretin was four times as potent as the Boots product in stimulating pancreatic bicarbonate flow – thus enabling inter-centre comparison of clinical data [*Gut* 1975].

In May 1974, when my two children were very young, I was fortunate to secure the tenured post of University Lecturer, again in Howat's unit. These were heady times for a budding pancreatologist as isotope and ultrasound scans, endoscopic retrograde cholangiopancreatography and CT brought the pancreas into view as never imaginable. Realising that the labour-intensive SP test was no longer justified, I researched the simpler Lundh test – discovering that its high sensitivity was spurious, due to dilution of mean tryptic activity by gastric acid hypersecretion coupled with accelerated gastric emptying [*BMJ*

1978, *Digestion* 1986]. Thereafter, I introduced a range of tubeless tests and used bayesian statistics to document the efficiency of each diagnostic modality [*Lancet* 1981, *Medicine* 1982]. I also undertook the first comparison between pancreatogram and SP test data in the same group of CP patients and noted, with surprise, that excellent secretory capacity could co-exist with a grossly distorted duct system, and vice versa [*Gastroenterology* 1982].

Howat retired in 1976. Now, with responsibility for some 100 patients with CP, I switched focus to its aetiology. The threefold increase in annual admissions since 1955 was impressive, as was the younger age at presentation. Alcohol was not implicated in 50% of the cases. Instead, a threefold increase in the UK consumption of corn oil, essentially linoleic acid, had been documented. Could this be relevant?

Fortuitously, the opportunity arose to measure copper in SP test aspirates. In CP patients, there was a striking excess of copper and also bilirubin soon after secretin, and higher serum levels, too, of caeruloplasmin [*Clinical Science* 1981, *Clinica Chimica Acta* 1981]. The idea that these changes reflected a compensation for excessive copper absorption, in line with a failing pancreas, was supported by rat experiments. However, clinical studies using radioactive copper denied this explanation – despite the best efforts of an over-enthusiastic researcher to align the data! The flip side was that the copper disturbances in CP represented a primary aberration in hepatocyte function. The quest for an explanation led me to London's Thomas Dormandy – a pioneer in the field of free radical pathology. In patients with CP we found high concentrations in secretin-stimulated bile or duodenal aspirate of several lipid-based products of free radical oxidation [*Lancet* 1983] and went on to identify a particular isomer of linoleic acid (9,11,LA') as the predominant 'diene conjugate' in biological fluids [*FEBS* 1983]. Now, the copper aberrations could be interpreted as indicating the mobilisation of hepatic antioxidant defence. Moreover, secretin was known to increase the activity of microsomal cytochromes P450 (CYP) and bilirubin transferases in rat liver. Not only are CYP induced by alcohol and corn oil-rich diets, but they detoxify numerous xenobiotics, in the process generating reactive oxygen species and sometimes, as in paracetamol poisoning, also reactive xenobiotic species. Thus, I proposed that pancreatic disease – not only CP but also acute pancreatitis and cancer – may be a casualty of hepatic 'detoxification', when reactive material enters the gland in refluxed bile or duodenal juice [*Lancet* 1983].

David Dreiling was the first to see the potential merit of this hypothesis. He secured for me a visiting professorship enabling me to lecture at the Chicago AGA meeting in 1984 as well as at the Academy of Science and several hospitals in New York.

I felt like Alice in Wonderland! Fortunately, there were experts at hand to help test the hypothesis. Pharmacokinetic studies confirmed an induction of CYP – especially the CYP1 family – in the majority of patients, including those with idiopathic disease. This was rationalised by cigarette smoke constituents, but especially, by regular close exposure to occupational volatile hydrocarbons [*International Journal of Pancreatology* 1986, *Occupational and Environmental Medicine* 1994]. These would strike the pancreas directly, bypassing the protective liver sieve. Thus, CP – and also drug-related acute pancreatitis and pancreatic cancer – might actually reflect direct oxidant damage via reactivated pancreatic CYP [*Recent Advances in Gastroenterology* 1986]. That could be the reason why surgical diversion of toxic bile failed to abort attacks [*Gastroenterology* 1990]. The proof of pancreatic CYP induction in CP and also pancreatic cancer came from an immunolocalization study [*Journal of Pathology* 1993].

The corollary was that antioxidant intake fell short of need in the face of a xenobiotic assault in CP – and to a lesser degree in recurrent acute pancreatitis. Studies of habitual diets in patients with idiopathic CP, by reference to a CYP1-induced control group on anticonvulsants, underlined their lower intakes of selenium, vitamin C and methionine. These micronutrients interact in the methionine transsulphuration pathway that yields glutathione (GSH) and other detoxifiers. Several enzymes in this pathway are vulnerable to oxidative stress, as are the components of the signal transduction route towards exocytosis in the pancreatic acinar cell. Considering that a reversal in secretory polarity underlies the diagnostic rise in blood enzymes during an ‘attack’, it was logical to link disrupted GSH homeostasis with the pancreatic problem. These evolving concepts and their potential links to acute pancreatitis and to pancreatic damage in kwashiorkor and cystic fibrosis were discussed by luminaries at a symposium in 1990 which I organised when President of the British Pancreatic Society [*The Pathogenesis of Pancreatitis*. Manchester, Manchester University Press, 1991]. That year, too, saw the publication of our 20-week placebo-controlled switch-over trial of antioxidant therapy (AOT) in CP [*Alimentary Pharmacology & Therapeutics* 1990]. We found that pain reduction was accompanied by a fall in serum 9,11,LA<sup>c</sup> and correction of the

poor antioxidant status [*Alimentary Pharmacology & Therapeutics* 1992].

I am indebted to Rory McCloy, pancreato-biliary surgeon, for keeping an open mind while another bastion of gastrointestinal surgery was under threat. He oversaw a cross-sectional surgical audit in June 1992 of CP patients on AOT. In 94 consecutive patients followed up for an average of 30 months, AOT emerged as a safe and effective alternative to surgery for painful disease.

**M.E.F.-Z.:** You have pioneered pancreas research in so many directions. At the end of the day, what has given you most personal satisfaction?

**J.M.B.:** It has been marvellous to see CP patients become pain-free on AOT, as is increasingly recognised by other centres worldwide. The implication is that oxidative stress in the acinar cell, from reactive xenobiotic species in particular, underlies CP: the ductal changes are regarded as a disease-modifying factor, which is brought into play when the delivery of glutathione is compromised (see below) while the ancillary antioxidant defences of (apo)lactoferrin and mucin are mobilised. This sequence is the reverse of the orthodox ductal model [*The Quarterly Journal of Medicine* 1986]. It is also good to consider that a daily antioxidant tablet may offer community prophylaxis in areas where CP is endemic – as suggested by collaborative work with Isidor Segal on alcoholic disease in Soweto [*Clinica Chimica Acta* 1995], and with Mohan Vishwanathan’s diabetes centre in Madras where idiopathic painless calcifying disease predominates [*Scandinavian Journal of Gastroenterology* 1993]. These and other revolutionary concepts were reviewed at a symposium that I organized at the 1998 World Gastroenterology Congress in Vienna [*Digestion* 1998; 59(suppl 4)].

Another high point was the finding – in collaboration with Maurice Super and Martin Schwartz – of an increased frequency of mutations in the cystic fibrosis transmembrane regulator (CFTR) gene in patients with CP (and symptoms controlled by AOT) [*The New England Journal of Medicine* 1998]. Considering that cystic fibrosis is diagnosed in neonates by hypertrypsinogenemia and that pancreatic histology is akin to that of end-stage CP, our finding hints at the need for a full complement of CFTR protein in the acinar cell for exocytosis, and also that it may be a free radical target [*The Quarterly Journal of Medicine* 1986, *Digestion* 1988;59(suppl 4)].

It gives me immense pleasure to record the invaluable input during my 25-year ‘radical’ journey of numerous scientists – in physiology (Maynard Case, Sigrid Ru-

tishouser), transplant immunology (Ian Hutchinson), surgical science (Anders Borgstrom), bacteriology (Louis Quesnel), pharmacy (Brian Houston, Martin Jones, John Fell, Frank Leach), biochemistry (Frank Steven, Jop Ubink, Jessica Douglas, Lance Sandle, Iain Laing), pharmacogenetics (Jeffrey Idle), medical physics (Harbans Sharma), pathology (John Foster, Najeeb Haboubi, Iona Jeffrey), medical statistics (Linda Hunt, Roseanne McNamee, Chris Main), occupational health (Tim Lee, Ian Leck, Nicola Cherry), chemistry (Giocomo Sturniolo, George Smith, Philip Day), dietetics (Patricia Rose, Helen Worthington) and free radical pathology (Thomas Dormandy, John Gutteridge, John Butler).

Last but not least, it has been a privilege to work with young researchers – too many to name individually – and to rejoice in their attainment of senior posts as clinical academics, NHS consultants, clinical pathologists or within the pharmaceutical industry.

**M.E.F.-Z.:** Based on your experience as mentee and mentor, can you comment on the value of mentorship for the development of the new investigator?

**J.M.B.:** There is no substitute! Howat introduced me to animal experiments, found me bench space for biochemical assays, bought me my first calculator, underlined the need for research grant support and help from the pharmaceutical industry, showed how important it was to work closely with colleagues, demonstrated single-mindedness and involved me in writing book chapters. He was big enough, too, not to squash my emerging 'heretical' ideas on pancreatic disease.

In Thomas Dormandy I found a brilliant mind, the ability to think laterally, a wizard at biochemistry with a hypnotic turn of phrase in scientific writing and extraordinary generosity. His perspicacious essay on biological rancidification in the *Lancet* (1968) is a 'must-read'. His motto was that the original thinker should not be afraid to share research ideas as there would always be many more where those came from – as I sought to emulate when kick-starting the current interest in free radical pathology of liver disease in Manchester.

For me it was a high-wire act to balance family life on the one hand with study, securing grant support, supervising students and writing papers on the other. The path I had stumbled on was wholly untravelled and necessitated intensive study on copper metabolism, CYP, oxygen metabolism, antioxidant defence, inhalation toxicology and free radical pathology – in particular, how oxidative stress might compromise signal transduction and the solubility of secreted protein in pancreatic juice or cholesterol in bile.

**M.E.F.-Z.:** What is the best advice you have received during your career? What is your advice to the young investigators that are starting out in the field of pancreas research?

**J.M.B.:** I would say to young investigators, as and when appropriate: be true to yourself; you are responsible for your data; these must be transparent and indisputable but their interpretation is subjective; study hard; search out original papers instead of being seduced by the ease of internet access to abstracts; cultivate a curious mind; keep meticulous records of your experiments; focus, you can only answer one question at a time; if you find yourself in uncharted territory, seek help soon; your supervisor's door is (or should be!) open – knock and walk in; adversity makes you stronger; keep an open mind; don't be afraid to be wrong; you will enter many a cul-de-sac, just reverse out; frustration is par for the course; be dogged; think logically; do not arbitrarily eliminate data when they do not fit your supervisor's prediction; treat your patients with respect and remember that they do not have to cooperate in your research; time with your family is sacrosanct. If you should stumble upon a completely new concept, have the courage of your convictions and publish your hypothesis, but be prepared for brickbats, jealousy and vilification. Accept that it may be 10 years before your alien perception is even given a hearing. You owe it to the scientific community to publish your data even – perhaps especially – if negative. At the end of your research stint you are likely to know more about the subject than your supervisor, but that is the idea. Enjoy, have a drink in the pub after a scientific meeting for that is where ideas are generated and get cross-fertilised!

**M.E.F.-Z.:** What do you think are the big questions to be answered in pancreatology?

**J.M.B.:** Why is there no specific medical treatment for acute pancreatitis and, hence, little impact on early mortality? Could it be that the autodigestion theory is inherently flawed? And why aren't intravenous antioxidants useful when oxidative stress appears to be the seminal problem, as also an antagonist of platelet-activating factor when frustrated phagocytosis is an integral component. Is the mast cell the stealth bomber? [*Digestion* 2001, March, April].

Gallstones cause much morbidity and are aetiologically linked to acute pancreatitis, but is that link purely mechanical when a stone migrates, triggering a burst of pancreatic free radical activity, or might there also be a shared vulnerability through prior antioxidant deficiency [*Medical Hypotheses* 1995; *Clinica Chimica Acta* 2004]? If the latter, there may be scope for gallstone prevention.

There is no animal model of calcific CP – this should be rectified. Likewise, there is a dearth of information on inhalation toxicology of the pancreas, seemingly very important in the genesis not only of CP but also of pancreatic cancer. Of course, the quest must continue for screening tests to identify ‘early’ pancreatic cancer in at-risk groups.

**M.E.F.-Z.:** What do you think is the major need that a journal like *Pancreatology* should meet?

**J.M.B.:** Its frame of reference, as published on its website, is impressive. As with its predecessor, *The Inter-*

*national Journal of Pancreatology* launched by Parviz Pour, it provides a forum for new ideas e.g. Heinrich Rinderknecht’s brilliant proposal in 1988 that fatal pancreatitis may be a consequence of excessive leukocyte stimulation, my initial study hinting at a link between occupational volatile chemicals and CP, and my case report on the benefit from AOT in a lad with idiopathic disease. I note, too, the coverage of recent symposia deliberating on the similarity but also differences between recurrent acute pancreatitis and CP. The journal is clearly in tune with the needs of pancreatologists.