Biographical Review

Enzymes, Egg White and Eccentrics

Memories from 37 years of research in the brewing industry

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I was 10 years old and already I had a rather respectable chemistry lab in our home near Wigan, in Lancashire, England. It was shared with elder brother John who taught me the essentials of valence, balancing equations and much more at least two years before my first ever chemistry class at Up Holland Grammar School. From the age of 13 to 18, however, I was hardly a dedicated student of terribly much at school, being rather more focused on sport, especially playing rugby for the various school teams, and (in the latter part of the age span but still at a premature age in legal terms) the pub. However the very limited biochemistry that we studied in A Level Biology captured my imagination and accordingly I felt fortunate to be accepted by the University of Hull to study for my B.Sc in that subject.

Immediately after my arrival in Hull in 1970 I realized straightaway that I had chosen well. I loved the topic and worked exceedingly hard at it, with the theory coming to me much more comfortably than the more hands on aspects. The Biochemistry department at Hull was very much focused on matters microbial, and guided tours were offered past my bench as I did my sterile plating – to show how NOT to do it. I was never the most meticulous or tidy worker in the lab, no matter how much the science was intriguing me. I always seemed to be in a hurry – including on the day I dropped a titanium centrifuge rotor valued at £700 all those years ago. (They mounted a plaque on it and made it the prize for the dumbest act anyone did after that point.) The head of the department, Professor Eddie Dawes, was livid and never forgave me until I was awarded a D.Sc at Hull 20 years later. Dawes was a celebrated magician in his off duty moments – it disappointed me that he could not wave his wand and make the rotor whole again.
Someone who was quite the opposite of me when it came to laboratory dexterity was Chris Boulton, latterly of course of Bass, the University of Nottingham and even more recently SAB-Miller, who would become a close lab mate of mine at Hull and who has been a dear friend now for over 40 years.

Upon graduation I was much undecided on what to do next – there has never been a master plan at any stage of my life. I came from a long line of teachers and seriously thought of heading off to a college to study to become an instructor in Technical Colleges. Fortunately a junior faculty member at Hull, Melvin Midgley, convinced me that I could do research: “If you can cook and create in the kitchen you can do research.”

I was blessed with my choice of lab. Dr. Peter Large was exactly the right guy for me. Himself a somewhat chaotic presence when he ventured into the lab, he was however of a brilliant mind and by nature a lovable, placid Christian gentleman. The lab was tiny, in the attic of the oldest building on campus and, looking back, a veritable death trap. On a daily basis there were four of us in there: myself; Chris Boulton, who was Peter’s technical support; David Brook, a tremendously helpful post-doc whose lack of height caused Chris to call him “the Micro Biologist”; and an eccentric red-haired Scots lassie called Hazel McDougall who would drift away.

Methylotrophs
The focus of the lab was methylotrophs, bacteria that grow on single carbon compounds such as methanol and methylamine. At the time there was huge interest in exploiting these for their industrial value, in particular the production of what was then called Single Cell Protein: food as bacteria or yeasts grown on cheap and abundant feedstocks. It wouldn't pan out economically.

My PhD project was dedicated to studying the enzyme N-methylglutamate dehydrogenase, which plays a role in the oxidation of methylamine by the organism then known as *Pseudomonas aminovorans* (now re-named *Aminobacter aminovorans*). I couldn't have started my career as an enzymologist with a tougher challenge. The enzyme is membrane-bound and of low specific activity. I had to work up huge quantities of the organism in 5 gallon jars to render enough cells to give (after purification) sufficient enzyme for just a handful of experiments. The learning curve, however, was exponential and I was able to make a pretty good fist of the kinetic analysis of the enzyme, and for that I was grateful to another friend and fellow student who would go on to the brewing industry, Chris Dickenson. Chris was a couple of years ahead of me on the PhD path.

I was terribly nervous when I gave my very first presentation to a scientific meeting, to the Biochemical Society in Edinburgh in 1975. The audience comprised the people who had presented before me (too polite to leave), the people who were about to speak next (who were too busy thinking about their presentation to listen), Peter Large and a kindly American scientist by the name of Bill Shaw (head of the biochemistry department at the University of Leicester) who had once worked on a related problem. Nevertheless, it went well and the abstract became my first ever publication, surely the most exciting of one’s career (58). Two peer-reviewed papers emerged from the work (59, 60) and another abstract (61), the latter for my second presentation to the Society, this one in St Bartholomew’s Hospital in London.
remember to this day a member of the audience congratulating me on how I had described the complexities of 2-substrate kinetics. (Words of encouragement to young scientists can make a world of positive difference. The opposite is even truer.)

The UK system is different from that in the US in that a PhD comprises research alone – no classes – and takes 3 years, after which you write up your thesis, on which you are interrogated by an external and an internal examiner. I well recall Dr. Chris Anthony from the University of Southampton turning up and straightway putting me at my ease by saying "relax, you have passed", but he followed this with a warning that he had traveled a long way and was going to get his money's worth. He proceeded to interrogate me for over 3 hours, made me carve several pages out of the thesis because I had made a naive assumption at the start and he also caused me to give the most garbled description of oxidative phosphorylation that could ever have been delivered. (Years later I actually met Peter Mitchell, the genius behind the chemiosmotic theory, on a train down to my wife's folks in Cornwall. He had a lab in Glynn House in the beautiful valley of that name and he showed me the money that he minted himself in the basement. Scientists can be odd.)

By now I was married (to Diane, who I have now been with for well over 40 years) and for the only time in my career it was she who made the running on where we would live next. I did get offered a position with Burroughs Wellcome in Beckenham, Kent. I also was on the radar for a teaching job at Charterhouse School in Godalming, Surrey but I was turned off by only being asked three questions, one of which was specifically about my sexual predilections. (They were different times.) However Diane was in the midst of getting her nursing credentials in Sheffield and our first married home was going to be there. Very fortunately the godfather of methylotrophy had his lab at the University of Sheffield. Rod
Quayle was the most eminent scientist I ever worked with and the man with whom Peter Large had
pursued his PhD at Oxford University. Again I was blessed to step into a fabulous laboratory and a
department that included folks who had been part of Sir Hans Krebs’ team when he had been in
Sheffield. I was proud indeed to be the post-doc of such an eminence. I well recall the day that Rod
breezed into my lab beaming, showing me the letter that advised him that he would henceforth be a
Fellow of the Royal Society.

Immediately on arriving in Sheffield I set about characterizing several enzymes. Two of them were
alcohol dehydrogenases (ADH), however not like the ones from brewers yeast but rather those that
utilize cytochromes to receive the electrons and which are assayed using dyes such as phenazine
methosulfate and dichlorophenolindophenol (DCPIP, of course, has historic relevance in the brewing
industry to assess reducing power). The first enzyme was from a phototroph called *Rhodopseudomonas*
*acidophila* (71, 73). Alarming for me was the day I discovered a white scum growing in the vivid red
cultures in flat sided bottles that were positioned around the light bulb providing the light energy to the
organism. The scum represented an intruder, most likely arriving due to my shoddy technique. I was
distraught – perhaps the enzyme I had done extensive work on was not from my chosen organism at all.
Quayle told me to calm down, go off on vacation to my wife’s family in Truro while his excellent
technician Alan Hancock would get to the bottom of it. A couple of weeks later a letter arrived written in
Rod’s distinctive turquoise ink: "you can dispose of the red-and-white spotted handkerchief and the stack
of warm newspapers and come back. Your enzyme is indeed from *R. acidophila.*"

To be fair to myself, I was usually on the ball when it came to enzyme purification and characterization
per se. Rod used to say I had "enzymological green fingers" and would love to watch my isoelectric
focusing columns in which I separated the very yellow ADH from the very red cytochrome c, making for a splendid color show. Years later I worked with another Sheffield graduate, Michael Waites, to develop a bioelectrochemical cell that incorporated this very enzyme, a device that we proposed for the measurement of alcohol in beer (165).

The second ADH was from Paracoccus denitrificans (72), an intriguing bacterium which had been put forward as the likely forerunner of the mitochondrion, being engulfed by some protozoan or other to enter into a mutually beneficial symbiotic relationship. P. denitrificans was also the source of another enzyme I studied, hydroxypyruvate reductase (70).

Finally at Sheffield I did a joint piece of work with the molecular biologist Mary O’Connor (now Mary Lidstrom, Vice-Provost of Research at the University of Washington in Seattle). This was a throw back to my interest in P. aminovorans and involved the isolation of mutants thereof to explore which enzymes have an a priori role in the utilization of methylamine in that organism (67). Like I, Mary was a post-doc and a third was Hans van Dijken, who nowadays is Professor Emeritus in Delft University of Technology. Hans was always astonished by my predilection for secrecy and reluctance to be too forthcoming about my research findings until they were ready for presentation to a meeting or publication. That tendency remains with me.

Headed South
As a post-doc you at first beaver away doing as much high quality publishable research as you can, but later get distracted by scrutinizing the jobs pages of *Nature*, *Science*, *New Scientist* and the like. I was in the running for another post-doc at Oxford University but was more intrigued by an advertisement for an enzymologist at the Brewing Research Foundation (BRF) in Nutfield, Surrey. I was interviewed on the Thursday and the job offer came to my home on the Saturday. That impressed me. (Years later coming to the University of California the process took well over a year and had I not very much wanted the job I might easily have suggested where they might insert it. Universities can be SO slow and inefficient in this and so many other procedures. I love the university life but despair of the archaic way in which business is frequently conducted.)

I know that Diane was not desperately keen to move to the London area, but she supported me as she always has. And so I arrived in the Department of Biochemistry and Biophysics at BRF, a team headed by Trevor Wainwright, erstwhile of Guinness. His initial suggestion was that I should work on developing a high performance liquid chromatography procedure for separating dansylated amino acids. I made headway but the day I dissolved a sinter when trying to clean it in aqua regia was the day I told him that I was frustrated as heck and that I came to Lyttel Hall to do enzymology, not screw around with analytical methodology that held little interest for me. “So what do you want to do?” Trevor asked me and I said that there were two other projects in the lab that seemed to be screaming out for some enzymology and accordingly it was agreed that I would get involved in those. In each case we would encounter astonishing push back from others in the brewing industry, who simply did not believe what we were suggesting. Over the subsequent years we were proved to be right in both topic areas – you have to believe in yourself in the world of science and also must be less judgmental when others discover things that fall outside your belief set.
The first of these projects was on barley β-glucan (9, 18). Hilary Martin, a meticulous bench worker with copper plate writing and accomplished on the violin with the Croydon Symphony Orchestra, was struggling with an assay for measuring this polymer. The principle was to hydrolyze β-glucan to glucose using an enzyme complex from *Trichoderma reesei* and quantify the polymer on the basis of how much glucose was generated. Hilary had been trying unsuccessfully to remove the key contaminating enzyme amylglucosidase (AMG), whose presence would cause glucose to be generated from starch and which accordingly would greatly over-estimate the amount of β-glucan present. I suggested we look at the heat tolerance of the two enzymes and duly found that the β-glucanase in the *T. reesei* preparation was much more resistant to heating than was the AMG, which we could remove simply by warming the sample (139). In due course we would find that later batches of the commercial cellulase that we were using presented a little more difficulty in eliminating the AMG, so I turned to *Penicillium funiculosum* as a more reliable option (10).

Armed with this assay, I set about exploring (with Hilary’s technical assistance) a dogma that had existed since the pioneering work of Preece (149), Meredith (86) and, later, three others at BRF: Bob Scott (152), Ian Forrest (104) and Denise Baxter (87). The notion was that the breakdown of the starchy endosperm cell walls in barley during germination and in mashing was a two stage event: an initial solubilization of the glucan followed by the hydrolysis of the glucan by β-glucanases. I developed a simple assay to test for the existence of such an activity to which I gave the name β-glucan solubilase - and I have regretted
the name ever since. The legendary Professor Anna Macleod, an adviser to BRF, loved the work but hated the name I gave to it. The somewhat unapproachable Director of BRF, Charles Dalgliesh, who wasn't there terribly long in my first stint at Lyttel Hall, also was smitten with the work.

Sure enough, we confirmed what others had surmised and detected this enzyme in raw barley (indicating that it is not a β-glucanase, since these enzymes are produced during germination). Time to study in detail its properties. We showed that it is heat tolerant, meaning that it survives to a significant extent during kilning and was able to survive mashing at 65°C (82). Clearly it most definitely was not an endo-β-glucanase, one of the more thermo-intolerant enzymes. From the study of its migration on (my favorite tool of) isoelectric focusing, its rate of thermal destruction, inhibitor specificity and ability to hydrolyze certain substrates, we concluded that it was a carboxypeptidase, functioning by hydrolyzing ester linkages (82). Denise Baxter had already hinted that carboxypeptidase might work in this way (87).

In some quarters (for example the legendary German brewing professor, the true gentleman Ludwig Narziss) this discovery seemed to explain a great deal and the concept was embraced wholeheartedly. There were others, however, who saw no reason to invoke the existence of such an enzyme (169). Undaunted, we carried on with our investigations, investigating the switching on of additional quantities of the enzyme during germination (62), the relationship between levels of the enzyme and the quality of different barleys (138) and getting a feel for the true relationships between the so called gums (water-soluble β-glucans) and hemicelluloses (the water-insoluble ones) (8, 63). I was also struck by the imperative that Trichoderma must have a β1-3-glucanase if it was going to comprehensively convert the mixed linkage β-glucan all the way to glucose. Thus I made a study of an exo-β1, 3-glucanase from the fungus (7).
As part of the study of glucan degradation, we also made forays into endo-β-glucanase (64) and proposed an alternative assay for the enzyme (140). As a kid I had been struck by a tube of an odd substance in my Merit Chemistry set, namely Congo Red. Now, two decades later, I worked out what it was for: it specifically stains β-glucan! So we made agarose gels in Petri dishes containing β-glucan-Congo Red complexes and punched holes in them. Into the holes we put our enzyme solution. Overnight the enzyme would diffuse into the gel, break down the glucan and thus remove the red color. The diameter of the clear zones around the holes was logarithmically proportional to how much enzyme is present. I was rather proud of the method, but nobody has ever seemed to use it other than us. (You can't get too sensitive in this business.)

Yeast makes dimethyl sulfide

The second project that I muscled in on was being undertaken by a very slight red haired lad called Brian Anness, a Yorkshireman with an excellent talent as a cricketer. He had been trying to unravel the mystery of whether or not yeast makes dimethyl sulfide (DMS). Work from Allied Breweries' Kiwi Bob Anderson, working in conjunction with Roy Parsons and Harry White at Bass, had indicated that yeast does indeed make DMS, in addition to the amount that was released by thermal processes in the brewhouse, notably wort boiling (see 2 and 45 for reviews of the whole DMS story). White, another Yorkshireman and one who had arrived at Nutfield from a post-doc at UC Los Angeles, suggested that there were two precursors of DMS in malt: one called HADMS which released DMS upon strong heating in alkali (hence the HA) and one called 'active HADMS' which is converted by yeast into DMS. The latter was present in
significant amounts only after the curing stage of malting. He surmised that both were peptides containing S-methylmethionine. The aforementioned Chris Dickenson, by now working with Allied Breweries in Burton and still Morris Dancing, concluded that the former substance was simply SMM and intimated that the latter substance was actually non-existent. Brian had his own evidence, however, that yeast could make DMS and indeed Richard Wilson and Chris Booer working in Howieson Rennie’s team at BRF concurred. But nothing that Brian fed to yeast could be transformed into DMS, least of all SMM.

And so to the day that Brian and I were sitting chewing over the quandary. I can see us now. He was sitting at his chair gazing out to the lake and the cricket pitch beyond; I was sitting on the desk with my back to the window swinging my legs as we chatted. At that point another great batsman, Brian’s technical assistant Hamish Dunn, walked in holding a photocopy of a paper that he had been reading in the BRF library. It was by Zinder and Brock from the University of Wisconsin, Madison (172) and spoke of the reduction to DMS of dimethyl sulfoxide (DMSO). Nestling in the table of organisms (mostly bacteria) that the authors had listed was yeast, *Saccharomyces cerevisiae*, showing that it had a seemingly minor capability to get the job done. Brian and I looked at one another and wasted no time in looking into this.

Brian grew yeast on simple media that contained added DMSO and found that DMS was produced. Meanwhile he took suspensions of yeast and incubated them with DMSO and showed that they produced DMS. And I smashed open yeast, incubated the extracts with DMSO and NADPH and can see in my mind’s eye all these years later the huge peak of DMS appearing on the print out (loads of paper in those days) from the gas chromatograph. DMSO was indeed the mystery substance (3, 46), and Brian went on to measure it in malt and he unearthed the conditions under which it was produced – which was indeed in the curing stage of malting (see Harry White’s work mentioned earlier). I produced data
that suggested that the enzyme was actually methionine sulfoxide reductase working on DMSO instead of its primary substrate (6).

The redoubtable Acting Director, Dr. John Hudson, was at once excited (some extra puffs on the pipe) but also wary. "Good heavens, young man, DMSO is a bloody solvent in chemistry laboratories." However we were allowed to publish (3). In this first paper and the first publication on solubilase the head of the team insisted on having his name on the author list. When I became a head of section at BRF I refused to do this and at all subsequent stages in my career I have steadfastly avoided having my name on any publication to which I have not made a material contribution. I guess my publication list could have been many times longer but I would have gotten no satisfaction or pride from it. Certainly when I was Director of Research at BRF International years later I would have plenty to say about lots of projects leading to papers, but I don’t think that gives any head of department a right to claim the glory.

If people pooh-poohed the solubilase concept, then they were almost vitriolic about yeast making DMS. However Interbrew would report at the European Brewery Convention that 80% of the DMS in their beer came from labeled DMSO (136) and then Carlsberg produced mutants of yeast lacking in methionine sulfoxide reductase and which produced no DMS (113).

[This wasn’t to be the last time I took a look at the thioredoxin system. A formidable plant scientist, Bob Buchanan, at the University of California, Berkeley produced some evidence that the system may be involved in stimulating limit dextrinase, the enzyme that is barely functioning in conventional mashes because it is inhibited by attachment to a blocking agent (94). Try as we might, though, Craig Heisner and]
I could not demonstrate that the thioredoxin system could activate the enzyme, whereas the simple expediency of dropping the pH could (117). Throughout my career I have had a tendency to drift from topic to topic without necessarily dotting all the i’s and crossing all the t’s. This is one area that someone should go back to: take a proportion of a mash, drop its pH substantially to release limit dextrinase and then re-combine it with the main mash. A bit like decoction, but I digress. Apart from this study, my only contribution to the matter of starch degradation has been a review which threw up a number of areas that I would consider worthy of study had I not been heading in a twilight direction (30).]

Promotion

Around the time that Brian Anness and I were investigating DMSO, the nitrosamine problem raised its ugly head and Wainwright was moved over to take control of the studies on this headache. This freed up the position of Head of the Biochemistry and Biophysics team and I was invited to take that role. This meant that I was put in charge of a gifted group of scientists, including the excellent Denise Baxter who might fairly have expected to be given the role herself on the basis of years of service. Straightaway, though, I busied myself in looking at the team’s productivity, which was supposed to include work on foam, but in which area the output had been somewhat lacklustre. However my eagerness to get into the role coupled with their ability meant that for two of the team we were to embark on a period of some success.

Bubbles
Philip Slack is a loveable guy from Swindon, a chap who had the habit of wandering around in deep
thought before suddenly hitting upon a whiz of an idea. In the case of his new focus on foam, however, I
am not sure who came up with the idea of hydrophobic proteins, whether it was he or I, but it certainly
was a heck of a way to launch into a new research area. The concept for the new foam project was
simple: it might be anticipated that hydrophobic, water-hating polypeptides would prefer to head to and
linger in bubble walls than stay in the watery environment of beer. Phil fractionated the proteins from
barley and from beer by hydrophobic interaction chromatography and found that those with the most
amphipathic character were indeed the ones with the most foam-stabilizing capability (155).

As regards the idea for the other main foam project of the time, then I definitely know it was mine. It
was late one Friday and I was sitting in the library at Lyttel Hall doodling and contemplating how to
measure how much foam sticks to a beer glass. You do, don’t you? Within five minutes I had reasoned
that you could produce foam, have it stick to glass, then recover it from the glass as a solution and
measure how much protein is in the solution. The more protein in that liquid, the more had been
sticking to the glass. I collared Gordon Jackson before he left for the day, told him my idea and in the
course of a very few weeks his steadfastness in the lab and our verbal interchanges (I talk more than he
does!) led to the Lacing Index method (120), which we used to survey the impacts of various beer
components on cling (52). Hilary Martin wondered whether we should call the units of lacing “Klingons”.

In those days all papers went through the desk of John Hudson, who was a stickler for grammar. Many a
paper he ripped up (metaphorically), demanding a re-write. On this occasion, however, after 3 or 4
weeks he said to me “I have read it, re-read it and read it yet again. And I can’t find anything wrong with
t. Now that can’t be right, can it? So you’d best write it again.” It’s the only paper (120) that I ever
thanked him for input to in the acknowledgements at the end! In fact, I believe my most cited paper ever
was the review on foam that I wrote around that time (12). I also contributed another review later (20).

Radical stuff

By now we had a new Director-General at BRF, Professor Bernard Atkinson, who I immediately liked
immensely as a person, who was undoubtedly the brightest intellect I ever worked with, but who had a
very strange habit in the paper clip breaking department and somewhat of a brusque way of dealing
with staff. It was Bernard who insisted that I went on a course at his old establishment, the University of
Manchester Institute of Science and Technology, which was very much of a chemical engineering
persuasion. However one presentation captured my imagination and this was on oxygen. Not only did it
detail the impact of oxygen on rates of fermentation, but it also introduced me to the matter of oxygen
radicals. Was this, I wondered, the clue to achieving enhanced flavor stability in beer? Was it oxygen
radicals as opposed to ground state oxygen itself that was responsible for the deterioration reactions
that damage wort and beer?

That presentation had referred to the presence in aerobic organisms of enzymes that protect against
such reactive oxygen species, so I came back to Nutfield to work on one of these. (At that time, and
indeed for some while longer at my next employer, I still found time to work at the bench.) There
followed the first significant study of the enzyme superoxide dismutase in barley (11).
By now I had been at Nutfield for five years and was looking for the escape tunnel. I turned down the Research Manager’s job at a prominent malting company and I toyed with one of the new biotech companies, Biogen, in Geneva but that was not right for me. And then I interviewed successfully for a new lectureship position at Imperial College in London, but that felt even worse. However I reasoned that I would take it and went in to tell Atkinson that I was leaving. He confirmed that I was indeed on my way out – but it would be to Bass, because their Research Director Tony Portno wanted me. (I had been up to Burton just the once, given a talk about our DMS work, had parried and thrusted with Portno who did not believe the DMSO story, but seemingly I impressed him.) Thus it was to Bass in Burton in 1983. I was finally close enough to my beloved Wolverhampton Wanderers Football Club to get a season ticket!

In the ten months I was at Bass before being promoted to Research Manager I spent quite a bit of time myself at the bench, as well as being the line manager for a small team comprising Roy Parsons, Roy Cope and David Pantry. My focus continued to be foam and I had the crazy notion of bumping up the foam potential of beer by adding extra protein. Apart from the obvious and perhaps mundane possibility of getting more out of the spent grains, I also took a look at a range of well-known whipping proteins and hit upon egg white (48, 49, 163). We patented it (50) but soon came to realize that it was of limited value when added to beers produced from anything like a respectable amount of malt and which already contained ample foaming potential. It was only with our range of beverages made using a bland alcoholic base that it showed real potential and indeed we used it in a product called Flamingo that had a pink
hue, a strawberry flavor and a creamy head. However the product was short lived and we were concerned in any event about possible allergic reactions.

One approach that we did successfully employ to enhance the foam of many of our beers was to include a proportion of the bitterness as post-fermentation bittering (the higher levels of the more foam-stabilizing isomers is the key to this approach) but also to add zinc. Addition of this agent at an order of magnitude higher than might be added to a fermenter is quite effective for rendering a quasi-solid and cling-able foam. Doubts about whether we were strictly allowed to make such additions, even though zinc was added globally into fermenters, led us to abandon the practice, though not before we had seen whether the higher addition rates made pre-fermentation would carry through to the beer. They didn’t. Of course, the zinc saga was something we never published (although in one mega-analysis of beers by the Campaign For Real Ale the 2ppm stood out in our beers alone!). In light of the company changes induced by Thatcher’s Beer orders that saw Bass morph into InterContinental Hotels, I saw no restrictions on me re-exploring the zinc notion in one of our studies much later – nobody else had seemed to cotton onto it during the intervening years (150).

I did patent one other foaming invention: the concept of producing a foam stabilizing gas in situ (17). The demonstration experiment involved a beer containing the enzyme catalase and a glass rinse containing hydrogen peroxide. Pouring the beer into the freshly rinsed glass saw the extremely powerful enzyme convert the peroxide into oxygen and the foam not only grew and grew but it surged up above the rim of the glass and continued to grow whilst stabilizing into a solid mass that looked like a vast meringue atop the beer. We realized that this particular combination was not going to be the right one, but we thought the concept was pretty nifty!
Roy Cope worked with me on the egg white project. I am not sure he would claim it to be his favorite study in his long and prestigious career in the lab which started in the regime of the legendary Cyril Rainbow. But we did develop a technique based on albumin (48) that we still use here in the States to analyze foam problems (see later). Roy was one of the best brains I ever worked with – versatile, highly practical: he once was drummer in a band that warmed up for The Swinging Blue Jeans. With others, including Paul Hegarty, we worked on some fairly innovative approaches to foam, including expert systems incorporating scanning electron microscopy (115). Roy and I, together with a new product development guy, Kim Butcher, took a look at how individuals’ perception of beer flavor are heavily impacted by visual aspects of the product as well as by messages given to the consumer (77).

Roy Parsons, too, was capable of doing first class research, although it was difficult to get him to concentrate on much more than DMS, on which he had been devoting his attention for many years. (He also had another life as a marriage guidance counsellor specializing in sex therapy, but that is a story for another day.) However I was very keen to explore further the concept of oxygen radicals in the context of staling and Roy and I worked closely to do a study that firmly pointed the finger for the first time at these entities as being the underlying trigger of staling in beer. I presented the work at the very first Brewing Congress of the Americas, in St Louis in September 1984 (68). Since then there has been a plethora of work using highly sophisticated technology to confirm that our premise was correct and that such reactive species need to be prevented from arising by minimizing transition metal ions as well as oxygen. The technology we used was simple indeed, with test tubes, thiobarbituric acid and a simple spectrophotometer. I remain convinced that the secret to good science is the intellect more so than fancy flashing lights.
Meanwhile it was a delight to restore my collaboration with my old PhD supervisor Peter Large, through a series of studies funded through public agencies in the UK, but also through the co-authorship of my first book, one which took me back to my PhD and post-doc days (134). Our first PhD student was Richard Gibson (who would go on to a fine career with the confectionery company Rowntrees), who set to work to confirm my suggestion that the enzyme in yeast that reduces DMSO is a methionine sulfoxide reductase that uses the couple thioredoxin/thioredoxin reductase to feed it reducing power from NADPH (105, 106). It will be noted that I did not put my name on the papers as I really did not feel that my contribution was more than encouragement in that instance. However I did play a material role in three other elements of his work. We did labeling studies to show that even in beers that contained less DMS than was in the worts they were produced from, there was indeed ¹⁴C in the DMS measured, illustrating the fact that yeast is making DMS even when simple DMS analysis suggests otherwise (108). Secondly we demonstrated that there is an inhibitor of DMS production present in worts, especially those made from darker pale ale malts (107). We confirmed that this inhibitor was methionine sulfoxide, so strictly speaking we were dealing with a preferred substrate that was delaying the ability of yeast to make DMS. We also demonstrated that DMSO reduction by yeast was very much dependent upon the amount of assimilable nitrogen available to the organism, with the greater incidence of DMS production at lower FAN levels, presumably being a consequence of the greater availability of reducing power under conditions when yeast growth was impaired (109).

Much of what I was doing at Bass could, of course, not be published, including several internal advisory documents that were used by the company’s breweries to help control processes and troubleshoot problems. Perhaps the most significant of these was the document that Parsons and I wrote on...
strategy for controlling DMS levels in Carling Black Label, the flagship brand and biggest seller in our
portfolio. It has to be said that it downplayed the yeast side of things (Tony Portno still did not believe it!)
and that is quite reasonable. If you are controlling the SMM level in malt and are keeping all
fermentation conditions constant, including the shape and size of the fermenter, then the DMSO to DMS
route does indeed look after itself. It is the thermal degradation of SMM that generates the largest
quantities of DMS and which needs to be controlled by attention to SMM levels in malt and the length
vigor of the boil and the duration and temperature in the whirlpool.

One of our breweries, in Glasgow, was running well below target in the DMS level in their lager. Although
it was technically a different brand (Tennents) it was still expected to match the Carling from Burton, so I
was dispatched to the far North to sort them out. I came back with a flea in my ear, told by the Scots to
prove why a given level of perception of DMS in the Tennents occurred at the lower concentration that
they were running at. Roy Parsons hit upon phenylethanol and phenylethylacetate as agents that mask
the perception of DMS and these were at significantly lower levels in Tennents than in Carling (116). The
reason for this was that the Scots were fermenting at a temperature lower than the Group standard!

The real world – and back to BRF

Tony Portno was insistent that I needed to have the smooth edges of an ivory tower persona knocked off,
so off I went to be the QA Manager at the Preston Brook brewery in succession to Harry White. I
managed to keep my hand in on the research front by continuing my interactions with Peter Large. There
were two successive PhD students working on oxidation issues, notably furthering the studies on
superoxide dismutase, catalase and peroxidases. These were Simon Clarkson and, rather later, Chris Antrobus (4-5, 76, 78, 96-99). Simon went on to Whitbread and died tragically young.

Preston Brook was somewhat anarchic (42), so I was soon itching to get back to research as a fulltime activity. It was a relief, then, to accept the position of Director of Research back at BRF and to become part of the team (Atkinson, Derek Laws, Alastair Macleay and myself) that transformed the organization to BRF International. In this role I was obviously involved in all of the research output of BRFI, but I steadfastly refused to stamp my name on anything unless the project had more of my input than the customary oversight, nudging in the right direction, pressure on those who needed a bit more pushing and Hudson-like critiquing of papers. The majority of the scientists in a staff of around 100 of course needed very little pushing and we had outstanding folks in the various teams, including John Hammond pioneering the government acceptance of genetically modified yeast and Bill Simpson with his work on the impact of bitterness substances and later his development of flavor substances trapped in cyclodextrins. Bill and I had a blistering row when he announced his intention to take that invention private, but we got over it. There was Nigel Davies doing excellent work on malt and Robert Muller effectively pursuing various areas in the biochemical field. And many more.

Some projects, however, would not have happened without my ideas and those were the ones that resulted in papers featuring my name.

In foam we developed a method for quantifying the hydrophobic polypeptides, which involved measuring the protein content of beer before and after passage through tiny columns of phenyl
sepharose, the difference reflecting the amount of material that adsorbed to the column and which, accordingly, was hydrophobic (19). I have to say that I was never desperately happy with the procedure, as it involved subtracting two largish numbers to generate a smaller one, with those inherent inaccuracies. However others, including my friend Graham Stewart, did use the procedure to great effect and, indeed, BRF International commercialized the concept through a Dutch company.

I also felt it timely to re-visit the solubilase saga and set Joanne Moore (who was in Mike Proudlove’s Malt and Wort Production team) on to the task of firming up the identification of the enzyme. I will admit to being a much less tolerant person in those days (for sure, I should have been kinder to Mike). I recall well the unnecessarily negative way in which I received Joanne’s news that she had found not one solubilase peak on gel permeation chromatography but four. It was with skepticism that I suggested the necessary studies to take a further look at these supposed four fractions, but Joanne, with input from a temporarily-employed scientific whiz called Danny McKillop, came up with the scenario that there were indeed multiple enzymes that would solubilize β-glucan: a carboxypeptidase, a pentosanase and two separate esterases (83). In collaboration with Paul Kroon in Gary Williamson’s lab at the Institute of Food Research (IFR) in Norwich, we produced evidence that one of these enzymes is likely a ferulic acid esterase (146).

I love you, California – and back to bubbles

Once Bernard Atkinson retired from Lyttel Hall I quickly became disenchanted with what I saw happening around me in the new regime. It was therefore exciting to be confronted with the right academic
challenge at the right time, namely the new Anheuser-Busch Endowed Professorship in Malting and 
Brewing Sciences at UC Davis. My wife Diane and I debated long and hard the pro’s and con’s but the 
decision was finally made and early in 1999 we moved the family thousands of miles for my dream job. 

I inherited a dismal and grossly under equipped laboratory but, thanks to the generosity of Anheuser-
Busch, Sierra Nevada, Coors and others, we were able to progressively build up the facilities and the 
environment, culminating after a number of years in brand new buildings and state-of-the-art brewery 
and laboratories. 

In those initial days, though, in a few weeks of refurbishment when the old lab was shifted from being 
what I would categorize as “early fifties” to at least something that was late 1970’s in standard, the first 
piece of experimental work was centered on perceptions of foam. I was mindful of the supposition of 
many that people’s judgment of beer quality was strongly dictated by its head of foam but I was not 
aware of any published scientific study that actually gave concrete data in support of the premise. So, 
armed with a single beer, lots of glasses and a campus photographer called Sam Woo, I set about 
producing lots of images of beer with foams of diverse quality and analyzed people’s impressions of 
them (24). (I would go on to do rather more of this later, with a student called John Smythe, who liked to 
brake dance; 158, 160.) At this time Anheuser-Busch invited me to travel to St Louis as they intended 
loading a U-Haul with surplus laboratory equipment that I could drive the 1,992 miles back to California. 
(We would go from pycnometers to digital density meters almost overnight.) I was asked to present a 
talk whilst I was in Missouri and so I went through this study on foam that confirmed that even folks who 
confess to drinking beer straight out of the bottle do actually score higher those beers poured with a 
head as opposed to those that do not have a stable foam. When I finished, the top man, Doug
Muhleman, inquired why the beer I had used had come from their major competitor. I had not said what the brand was but he recognized the impacts of reduced iso-α-acids!

Once I had a half-decent lab in place – and supported by my steady and reliable part time right hand brewer and lab manager Candy Wallin - I recruited my first batch of students and embarked on some detailed studies on foam. One thing that happened was that suppliers of foam measuring instrumentation bombarded us with free equipment, in return for us using their instruments in our studies (119, 150, 166). However the Davis foam program started with a much simpler analytical tool.

Greg Kapp, a New York lad, was charged with the task of ascertaining the relative importance to foam of the water-soluble proteins from barley (the albumins) as compared to polypeptides solubilized from the water-insoluble fraction of barley protein (the hordeins) which of course comprise the greater quantity. I continued to ponder whether foaming was essentially determined by hydrophobic character in a polypeptide, this being more important than the absolute identity of a given polypeptide, which at the time was focusing people’s attention on Protein Z and, even more the case, Lipid Transfer Protein (LTP1).

The first thing Greg developed was a simple shaking test with which we could compare the foaming properties of the various polypeptide fractions, some of which were available in only small quantities. Straightway Greg found that there was indeed foaming power in both the albumin and hordein-derived fragments and that if you denatured the polypeptides using heat or acid you both increased their hydrophobicity and increased their foaming potential (132). This was entirely predictable and was in keeping with what Bech had found at Carlsberg in that LTP1 as isolated from grain really wasn’t a very good foaming protein but after boiling (a.k.a. denaturation) it became a strong foaming agent, on account of it being opened up to reveal the hydrophobic portion of the interior. It was also evident from
our work that the foam was superior from the albuminoidal polypeptides. (We did try to develop; a
flurochrome approach to assessing hydrophobic polypeptides but it wasn’t very successful, 81)

Next Greg simulated the protein degradation that occurs during barley modification by using a range of
defined proteolytic enzymes. Intriguingly he showed that limited proteolysis of hordein by several of
these enzymes caused an enhancement of foam performance, whereas some of the enzymes distinctly
damaged the foam from the albumin (132). Of particular relevance was the observation that Proteinase
A, the enzyme from yeast that Graham Stewart focused upon as being detrimental to the foam from
beers produced through high gravity brewing techniques, destroyed the ability of hordein-derived
polypeptides to stabilize foam and seriously depleted the foaming of the albumins.

Greg’s work was continued by a young Italian undergraduate, Cristian Milani. I reminded him that beer
is, of course, not a pure protein solution but rather a "soup" of many different molecules, including
diverse types of protein. What, then, were the foaming properties of mixtures of the isolated albumin
and hordein fractions? In a nutshell the conclusions were that the albumins were indeed superior
foaming polypeptides as compared to the hordeins but the presence of increasing quantities of the latter
decreased the ability of albumin to stabilize foam (65). We hypothesized that there is a competition for
places in the bubble wall and that if hordein-derived polypeptide squeeze out albumin then this is to the
detriment of the net foam performance. In other words, the observed foam stability is impacted by
competition between different polypeptides.

51
52
26
Soon after I arrived in Davis I agreed to accept into my laboratory a post-doc who came fully-funded from Japan. Early in 2000 there was a knock on the door to signal that Makoto Kanauchi had arrived. It was immediately obvious that he spoke not a word of English. Just a small problem – so I gave him a lab to share with French Cameroon native, Jean-Pierre Biawa, formerly of BRi and VLB Berlin, who was with me for a hugely enjoyable year (for me and I hope for him too) before he went on to do great things with the Miller Brewing Company. In that time he gave Makoto a smattering of the English language, but Makoto did learn to speak with a Cameroonian accent! Dr. Kanauchi and I retain our close collaboration all these years and still do much research together.

Makoto made further studies on the model foaming system, showing inter alia that ethanol enhanced the foaming potential of hydrolyzed hordeins but lessened that of albumins, that zinc had much more impact on albumin-based foams and that for both types of protein foam stability was higher as the pH was increased in the range 3.8-4.6 (55). Interestingly quite the opposite impact of pH on foam was seen in beers adjusted to different pH’s (80), highlighting that model systems may have their shortcomings. [pH, generally, is not the simplest of topics but its impacts are far ranging, as addressed in 27 and 74.]

Meanwhile another of the initial intake of masters students, the guitar-playing Dave Lynch, focused on the complexities of bubble nucleation. We devised a simple semi-quantitative technique for assessing the extent of bubble formation, featuring the definitive nucleation sintered glass bead which is in a case and guarded, a bit like the international prototype of the kilogram in the Bureau International des Poids et Mesures in Sèvres, France (137)! Mathematical models were developed to explain the factors that influenced the extent of foam formation, but it came as no surprise that the presence of a nucleation site per se is a necessary pre-requisite, nor that the amount of carbon dioxide in the beer had a very
substantial impact. I duly distilled my thoughts about the relative significance of physics and chemistry in determining the foaming properties of beer (31).

Time was when I was convinced that foam negative materials, notably lipids and detergents, were primarily introduced into the beer at the point of dispense. Work we had performed at BRF International on lipid binding proteins had led me to believe that any lipid endogenously emerging through the brewing process was adsorbed onto such proteins and rendered harmless (147). That this was not the case first arose from work we had performed at BRi (101), but I set Jonathan Goldberg, who came to us in Davis from a first degree at Heriot-Watt University, on investigating further the extent to which foam performance in commercial beers is dictated by the level of proteins as opposed to the presence of foam-negative species (110).

Jonathan approached the challenge in two ways. The first was based on the technique that we had used at BRi, assessing the extent to which the foam stability of a beer could be increased by passage of that beer through a Sepharose column to which a lipid binding protein (bovine serum albumin) had been attached. If the foam stability increased then this was indicative of the removal of foam-destabilizing lipid. Jonathan found that six out of ten beers examined displayed the presence of foam inhibitors.

The second approach was a development of the technique that Roy Cope and I had first proposed in 1987. The concept revolves around my old friend egg white. In the first instance albumin is added to the native beer. If this improves the foam stability it is indicative of an insufficiency of foaming polypeptide in the beer. Next, the egg white is added to a beer that has been passed through an ultrafiltration
membrane that removes everything with a molecular weight greater than 1,000 – which would mean removal of protein, for instance, but retention in the ultrafiltrate inter alia of the bitter substances and alcohol. The foam observed after different rates of addition of albumin to this ultrafiltrate is compared to the foam when the same amount of egg white is added to a simple solution containing the same amount of ethanol. Superior foam performance is expected in the former case, because of the presence of materials like the iso-α-acids. However if the foam is superior in the simple ethanolic solution this indicates that there is something foam negative in the ultrafiltrate. Again, Jonathan was able to demonstrate using this technique that many beers do indeed contain foam-inhibitory material.

Clues to the identity of these materials came later with the successive MS theses of Alex Combe and Justin Ang. In Alex’s case it was a classic example of how the vision that one has in response to a certain hypothesis can be turned completely upside down! I suggested to New Orleans-born Alex (someone capable of eating gigantic amounts of frighteningly spicy food) that he take a look at the long-standing hypothesis of the renowned Laurence Bishop that during kilning there is a melding of foaming polypeptides with polysaccharides, leading to complexes that are particularly adept at stabilizing bubble walls. I proposed that he make extracts of a range of pale and specialty malts and compare their foam performance, predicting that the more colored malts, as dogma dictated, would display superior foam performance that we could then dissect in terms of finding the Bishop complexes. It was a somewhat worried Alex who hesitantly showed me his initial data which revealed that, whilst the likes of roast barley and black malt did indeed have excellent foam performance, the various caramel and crystal malts for the most part displayed pretty paltry performance. In depth analysis using the Cope-Bamforth-Goldberg ultrafiltration approach and also dilution curve approaches indicated that indeed there is certainly foam-negative material in all the malts tested, but this is swamped by the foam positives in the
very dark malts. Furthermore the inhibitory material is much more exaggerated in the caramel malts as compared to pale malt (100).

Justin Ang, a Chicago native, built upon Alex’s work to use an old friend of a technique, thin layer chromatography, to reveal that there are various lipid species present in enhanced amounts in the particularly problematic malts, especially triglycerides and oxidized lipids (1). It seems that another dogma has been shot down – but too late for my book on how to solve foam problems (44)!

Unraveling cell walls

Makoto Kanauchi’s main pursuit at Davis was to try to unravel the solubilase saga. Initially we took two tacks. The first saw us turning to my old friend *Trichoderma viride*. (When I first started working on it I used to call it *T. reesei*, but the man it was named for, Elwyn T. Reese, wrote to me from the Army research place in Natick to tell me that that name was warranted for only a small range of *T. viride* species – and not mine.) Since first using cellulase isolates from this organism to measure β-glucan in the late seventies, I had realized that it was capable of totally hydrolyzing barley β-glucan, so the question was, would it grow on it as a carbon source? And, if it would, I hypothesized that it would release enzymes in the sequence that it needed them. So how would it be if we presented the organism with cell walls per se? What was the sequence of enzyme development? From that we should be able to get clues about the cell wall structure and the order in which enzymes were needed to digest them, all the time holding to my central dogma that it was at least a two stage process: solubilization followed by hydrolysis of glucans. Makoto duly did the experiment and found that, yes, *T. viride* grows on cell walls and that
Arabinoxylan (pentosan) was solubilized before β-glucan. Seemingly the first enzyme to be released was actually a xylanase, and it was demonstrated that an esterase, carboxypeptidase, and endo-β1-4-glucanase all preceded endo-β1-3, 1-4-glucanase (121).

The second approach was facilitated by the presence in Davis of a research group of the Novozyme company. Generously, their CEO, Glenn Nedwin, made available to us a range of highly purified enzymes that Makoto could use to dissect walls. Thus we showed that the most powerful effect was ascribable to a xylanase, further indicating that the glucan is somewhat trapped inside the pentosan in the cell wall (122, 123). There was some limited solubilase action due to esterases that remove ferulic acid and acetic acid from the arabinoxylan material – interestingly these enzymes seemed to smooth the fluffy surfaces of the cell walls as witnessed under an electron microscope. We duly came up with a model for the cell wall which featured a preponderance of pentosan on the outside of the wall, with the β-glucan concentrated inside (54). I was struck by the fact that Makoto had indeed confirmed the veracity of Joanne Moore’s observations from several years previously (146).

Two other Masters students delved further into these esterase enzymes. Bob Ward, who was my first ever MS student at UC Davis and who is now a Professor at Utah State (we recently edited a book together, 75), looked into acetylxyylanesterase (168), while our charming Brazilian colleague Alberto Sun focused on this and also on feruloyl esterase, a study in which I collaborated once more with the IFR, in the shape of Craig Faulds (164). However it was two other students who fitted two key pieces into the puzzle.
First was Anke Scheffler, one of three students who came to me to do their project within their studies at Weihenstephan. (Of the others, one found his reward in the shape of a bride, but the other we will come to later, in the form of Michaela Miedl.) Perhaps it was cynical of me to put a student from Germany to work on adjuncts and commercial enzymes, but Anke entered into the project with gusto. I proposed that she should use the BRF Mashing Bath to look at grists of 20% malt: 80% barley and with the addition of a range of enzymes. She found that, of course, β-glucanase additions had a major beneficial effect; however addition of a xylanase alone actually made matters worse in terms of an increase in viscosity and a decrease in filterability and extract yield. However the best performance of all came from mixtures of xylanase and glucanase (152). The most logical way to interpret this is in terms of xylanase releasing glucan trapped inside the wall (see above) which of course increases viscosity, but if glucanase was also present it could remove the glucan effectively, even more effectively than glucanase having to channel through the pentosan coat of the cell wall.

The second student was Robert Kuntz, who climbs mountains and is now with MillerCoors. I asked him to look at the sequence with which the enzymes are synthesized as barley germinates. Contrary to what others had claimed, namely that xylanase was relatively late in its synthesis, Robert found that the very first enzyme to switch on was indeed a xylanase – and there was a later appearance of still more xylanase (133). This early turning on of some of the xylanase was entirely in keeping with our model.

Notwithstanding this, it certainly does appear that there is significant retention of pentosan in malt and that this is not readily digested during mashing (unless an addition of heat tolerant enzymes is made). Substantial levels of pentosan were measured in beers by Kanauchi and I using a new procedure for pentosan that involved hydrolyzing the polymer using xylanase and measuring the xylose using xylose.
The most logical explanation of this survival of pentosan into beer is that there is an inhibitor of xylanase in grain (128, 129).

Young-Tack Lee, who came to my lab on sabbatical from Kyungwon University in Korea, showed that it truly is the malting process that most effectively deals with β-glucan – a low temperature mash does not fully hack it (135).

The other curiosity about cell wall degradation is that although there are enzymes present in malt that are in theory capable of completing the transformation of β-glucan into glucose, they do not seem to be primarily generating the ultimate end product and therefore oligosaccharides are present in sizeable amounts in beer (51). We proposed that the reason for this is that the enzyme likely to be most significantly employed to break down oligosaccharides actually has a very high \( K_m \) (low affinity) for the substrate (40, 125). The residual presence in beer of pentosans and β-glucan-derived oligosaccharides represent soluble fiber and putative prebiotics respectively (34, 51).

Getting fresh

A third major focus in our UC Davis program has been flavor instability and how to overcome it. The reality about studies on this topic is that they are extremely difficult to do and, for reasons eloquently aired by the late Morton Meilgaard (141), it is equally difficult to make a judgement about how reliable much of the work globally has been. An illustration of the problem comes from the work of my student
Sarah Bushnell (who became Mrs. Greg Kapp – there has been a history of match-making in the Bamforth Lab). The aim was to see whether we could demonstrate any significant difference in flavor stability in beer that had been treated with PVPP, which removes the polyphenols that some people stress are important for flavor life. We also threw in sulfur dioxide as a separate study. As Meilgaard explained, it is critical for trustworthy sensory reports to have properly conducted organoleptic trials. At Davis we are blessed to have three experts in this area, Jean-Xavier Guinard, Michael O’Mahony and Hildegarde Heymann. In this instance Sarah worked with J-X to develop a trained and trustworthy panel to evaluate the freshness of her various beers after storage. In the first instance we could find no significant difference between the beers treated or not treated with PVPP (92). However the “kicker” was in the work with SO$_2$. Although the average staling score was lower if SO$_2$ was added and higher if the air level in the bottle was increased, in this actual study these differences were not statistically significant. Now I am not at all saying that it is not important to keep O$_2$ levels as low as possible in the finished beer and I am not implying that SO$_2$ is without benefit. All I am stressing concerns the statistical significance of the reported data. It seems very easy for some people to report differences that suit their prejudice, without endeavoring to demonstrate that those differences are absolutely meaningful.

We also posed an even more fundamental question: just how important is it to keep beer fresh? In other words: does the customer care? And so Bill Stephenson (who came to me from Chicago with a first degree in Economic History but he crammed chemistry in Hollywood) took a famous North American lager and cooked it in an oven for a month. Then he took that beer (chilled) and the un-aged equivalent into Sacramento and sought the opinion of tasters (161). He presented pairs of beers, identifying them using different drip mats. When he put a fresh one and a stale one side by side on mats that showed the name of the brand, there was an equal split in declared preference. When he did the same thing using
glasses placed on plain white drip mats he got the same outcome. When he took the stale product in
both glasses and put one on a mat giving the brand name and the other on a plain mat, there was a two-
thirds preference for the one on the white mat. But when he put the fresh beer in both glasses, the one
placed on the mat giving the brand displayed a 66% preference over the one placed on an unlabeled
white mat. Our conclusion was that people make decisions primarily based on brand identity, but if a
product falls short in a characteristic (in this case freshness) then they move away from it. I recall the
phone call when we wrote up the paper from the head honcho of the company concerned, who didn’t
seem to be smiling when he said “why did you do that?”

Confident that we were indeed studying a worthwhile topic, we started to investigate the relative
importance of upstream oxidation (162), picking up from the work that I had been doing earlier with the
likes of Peter Large, Paul Hughes, Robert Muller, Martin Walker, Geoff Buckee and Mike Walters (79, 84,
167). It was all too apparent that oxygen could indeed be consumed in the brewhouse, notably in
mashing, but I remained unconvinced that lipoxygenase was quite the culprit that several people had
been insisting. From a theoretical perspective I had concluded that non-enzymic oxidation was at least as
damaging (21).

Indeed, Jean-Pierre Biawa did a fine piece of two substrate kinetic analysis on lipoxygenase in which we
showed that the $K_m$ for linoleic acid was really rather high, meaning that if this enzyme truly had
importance it was much more likely to be at the very onset of mash mixing, when the enzyme was still in
close proximity to the lipid-rich membranes of the embryo (88).
The reality is that there is plenty of competition for the oxygen in a mash: an abundance of chemical events that are seeking to remove the oxygen (22, 25, 26, 32, 41). These include reactions with thiol compounds and polyphenols, the latter probably by way of the hydrogen peroxide produced in this oxidation and the intermediacy of peroxidase enzymes. The oxidized polyphenols cause the insolubilization of proteins by cross-linking. Relatively recently my collaboration with Makoto Kanauchi has thrown up two “new” enzymes not reported previously in a malting and brewing context: ascorbic acid peroxidase (127) and ascorbic acid oxidase (131) which can use ascorbic acid to scavenge peroxide and oxygen respectively. The latter enzyme is particularly intriguing as it is phenomenally heat tolerant, probably because it is relatively tiny. The truly interesting outcome of this work is that we have shown that additions of ascorbic acid to mashes enable ascorbic acid oxidase to scavenge oxygen, thereby leading to less thiol oxidation (as measured by more – SH groups left behind in the wort) and less polyphenol oxidation (assessed by higher levels of polyphenol in the wort). Those folk who insist that lipoxygenase in the malt and mash is important might care to see if ascorbic acid will preferentially divert oxygen away from that enzyme, leading to enhanced flavor stability. But it could just as readily be argued that the decreased oxygen level means less base material for the production of reactive oxygen species.

Two other oxidases that we scrutinized were oxalate oxidase (130) and thiol oxidase (85). We were especially intrigued by the latter, notably the discovery that it is present in significant quantities in freshly kilned malt but that during 2-4 weeks of storage its levels decline to zero. We hypothesized that this is at least a part of the reason why malt needs to be stored prior to brewing. We speculated that if the enzyme is present it can catalyze the cross-liking of gel proteins leading to the sluggish separation of wort.
Discussion of these various enzymes amongst all the others addressed in my teams over the years brings home to me how I am fundamentally an enzymologist at heart, as evidenced by a range of contributions intended to clarify the significance of these fascinating entities to the production of great beer (13, 15, 26, 37, 39, 47, 114).

Looking at beer

Mention of the contribution of polyphenol oxidation to beer color and haze development leads to a discussion of the limited work that we have performed on color and haze at UC Davis. With John Smythe I took a look at the issue of how reliably color is really measured on the basis of simple absorption of light at 430 nm (156). He took four well known beers – an Irish stout, an English ale, a European lager and a North American lager – and diluted all of them so that their color as determined in the standard ASBC method matched that of the palest product, the North American lager. Then we presented them to observers in a series of pairs, asking people to pick out the ones that looked different. When it came to the two lagers the folks could not pick them apart. But they could certainly differentiate between pairs in all other instances. The two ales were distinctly different in perceived color, with pinkish hues. The study reinforced my belief that the standard color approach has its drawbacks when trying to make a full interpretation of the precise “shade” of coloring in darker beers.
As a teacher I must confess that color is one of the most difficult issues to get across – it is far more complex than you might imagine! To that end, Tom Shellhammer of Oregon State University and I made a fist at explaining it as simply as we could (154).

We were prompted to look at haze by a major brewing company who had concerns in a year in which the barleys were running high in protein. They wanted to know if their existing tolerance level on haze was at the appropriate level or whether it might be over-reaching. Dylan Clark tackled this one (before he went off to teach at college – getting rather more chili peppers for hotness on Rate My Professors than I have ever received). Our conclusion was that the turbidity specifications in the industry appear to err a tad on the cautious side of practicality and might possibly be adjusted upwards to a small degree (95). Indeed, for reasons that we didn’t explore further, it seemed that people actually prefer beer that had a level of turbidity just below the detection limit, as compared to rigorously bright product.

Actually my first brush with haze came in my first stint with BRF. A brewery within one of our member companies was having an invisible haze problem – in which a beer registered high on analytical haze as gauged with a Radiometer haze meter (90° scatter) whilst appearing bright to the naked eye. John Hudson really hated the term invisible haze and urged us to come up with an alternative, so we suggested ‘pseudo haze’ but that has never caught on because folks like to talk about having ‘invisible haze meters’ (i.e. those that detect scattered light at a forward angle), No matter what we tried to call it, the fact was that we needed a centrifuge running at 100,000 x g to collect the tiny particles, which upon enzymic digestion proved to be largely starch, protein and β-glucan, suggesting that the problem was due to inadequately degraded starchy endosperm (53). Not long after joining Bass, my immediate boss Stuart Molzahn asked me to write an advisory note on the angle at which the group should be measuring
haze. Twelve out of the thirteen breweries approved of my recommendation that it should be forward scatter. The exception was the Wellpark Brewery of Glasgow, who replied that they did not necessarily disagree with everything in my memorandum, for example I had spelled my name correctly.

In pursuit of achieving colloidal stability of beer (23), Michaela Miedl, an Austrian student in that cohort from Weihenstephan who would go on to do her PhD with Graham Stewart at Heriot-Watt, was charged with the task of looking at the relative importance of time and temperature in the cold stabilization of beer and our conclusions clearly demonstrated that it is the lowness of the temperature that is far more important than the duration of the storage (142, 143).

With Cale May and Christine Wright and my faculty colleague at Davis, Alyson Mitchell (who provided the polyphenol analysis), we compared "traditional" PVPP with a newer approach to removing the haze-forming tannic materials, showing the superiority of PVPP (145).

Although, as I have said, I primarily consider myself to be an enzymologist, I would like to think that we have made a worthwhile contribution generally to the study of some of the more challenging aspects of beer quality, which can be broadly grouped under the heading "non-biological stability" (43).
My friend Graham Stewart once joked that yeast was a bit of a mystery to me, yet over the years since our work on DMS we have dabbled with yeast in several ways, starting with a collaboration between Simon Clarkson and Peter Large in Hull and Chris Boulton and I at Bass. In this work we focused on the development of enzymes such as SOD and catalase in the protection of yeast against oxidative stress (99). I also critically discussed a frequently under-addressed issue, namely the extent to which variation in wort parameters can influence yeast performance (29).

Latterly in Davis there have been a series of studies focused on the impact of yeast on flavor. Thus came Evan Miracle’s investigation of sulfur volatile production on a commercial scale (144). The ongoing collaboration with Makoto Kanauchi fetched a short paper that presented evidence that the same enzyme (alcohol acetyl transferase) is responsible for producing the oxygen-containing esters but also the sulfur-containing thioesters (56). Furthermore the two of us scrutinized the enzymology of VDK removal by yeast and identified a selection of enzymes that serve the purpose of eliminating diacetyl, with differences between ale and lager strains (57). We also delved into the possibility that β-glucosidases from yeast might impact hop aroma (126).

Beer’s superiority over wine

The longer I worked in California the more irritated I became by people claiming a perceived and implied superiority of wine over beer. To be sure that this was not merely an irrational frustration born of my blunt-spoken and perhaps intolerant heritage, I set a charming MS student, Christine Wright, on the task of working with me and our resident consumer expert Christine Bruhn on comparing the public
perceptions of wine and beer (170, 171). It was clear that my feelings were justified and, allied to the
findings of John Smythe and myself concerning much ignorance of people when it comes to beer (159), it
was only too clear that there is a major education job to be done on the consumer in terms of
positioning beer fairly as a drink of respectability, interest and healthfulness. In a series of articles and
books I set about setting some facts straight (28, 33, 35, 36, 66), as well as delivering (with a succession
of co-workers) some explicit data on the composition of beer in relation to health. This included an
evaluation of whether beer really is a problem area when it comes to glycemic index (34).

Working with my charming Irish colleague from the Department of Nutrition, Andy Clifford, we produced
some realistic figures for the folic acid content of beers – lower than had been hitherto reported but still
significant (148). With Michael DiPietro I illustrated just how important the in vitro test is when
classifying beverages for their antioxidative capability, whilst at the same time showing that molecule for
molecule, the antioxidant potential of beer is superior to that of wine (102). And with Troy Casey, son of
Greg, we confirmed just how significant beer can be as a source of assimilable silicate (93).

For the longest time individuals with gluten intolerance (Celiac disease) have been warned off the
consumption of beer (38). However I was struck by the enormous losses of protein that generally occur
during malting and brewing, together with the fact that many a beer is brewed with significant levels of
grist materials that provide no problematic polypeptides. Surveying the literature for the state of the art
when it comes to measuring problematic peptides, Lindsay Guerdrum (who has an identical twin sister)
and I used the most reliable enzyme-linked immunosorbent assay that has so far become available to
quantify the levels of such materials in a range of commercial brews. We found that a substantial
proportion of beers – and not only the lighter ones – contained levels below the 20 ppm that represents
the guideline laid down in Codex Alimentarius (111). For anyone doubting the wisdom of claiming a
"normally" brewed beer to be low in gluten we were able to confirm the efficacy of the enzyme prolyl endoproteinase in removing any last trace of the undesirable sequences without jeopardizing foam (112).

Of course another strategy for ensuring that there is no gluten or gliadin in beer is to produce beer from materials that are devoid of it. This is widely adapted with the production of products from the likes of sorghum, buckwheat, quinoa and so on. However I took this opportunity to explore something that stemmed back to something Bernard Atkinson had once insisted would represent the future for brewing. As a chemical engineer he was frankly irritated by the malting and brewing processes, insisting that all that water in and water out and the use of huge amounts of energy in heating and cooling processes was illogical – I think his exact word was "stupid". He insisted that one day beer would be produced from a bland alcohol base with addition of color, foam and flavor from buckets. Sportingly, Jonathan Goldberg agreed to give it a shot in the lab and, armed with a bottle of Smirnoff vodka, he set about making an ersatz beer (118). Sensory guru Hildegarde Heymann agreed to run the taste tests and we presented the product twice to a panel of beer tasters, the first time without comment and the second time after fessing up about the approach taken. They didn’t like the product much either time, but it was accepted as a beer and as an initial attempt that could clearly have been hugely improved upon. Most importantly the tasters did not seem to be fazed by the approach taken which did not contradict an earlier study (157).

Another excellent student, Stephen Russell (who came to our attention from Doug Muhleman, whose son had been an on-line computer games pal of his) confirmed that the approach truly would be highly...
favorable from an environmental and all-manner-of-footprints basis (151). Let there be confusion, the
very notion of the approach horrifies me.

On a more traditional sensory platform, I also liaised with Dr. Heymann on the matter of hop aroma.
Again it stemmed back to my conviction that brewers were shooting themselves in the foot by not
articulating the diversity of aromas to be had from hops, unlike the wine folks who waxed lyrical about
the notes delivered from their diverse grape varietals. And so Bryan Donaldson, with his ever-present
bandana, worked with Hildegarde to develop panels able to describe in interesting terms the aromas to
be derived from various hops both as is and after dry hopping of a beer (103).

Wild stuff

And so I turn finally to Nick Bokulich and his astonishing productivity in the microbiological area of beer
and wine in his time as a MS student with me and later as a PhD student with David Mills. Nick came to
us from a liberal arts background, Hampshire College in Massachusetts, and proved himself to be a
totally exceptional student. He had gained good practical grounding as a summer intern with Anderson
Valley Brewing Company in Boonville CA and started their wild beer program (deliberately). His
fascination with the topic led to a detailed assessment of the successive emergence of microbes through
the life time of such products (89). I was also proud to co-author two reviews of the microbiology of beer
with him (90, 91). At the moment Nick is lost to the world of brewing as he pursues an academic career
in New York, but as I start to contemplate my life after brewing I fervently hope that somewhere out
there is another such individual with the passion, ability and determination to grab hold of the baton.
Living the dream

I started this reminiscence with my home chemistry laboratory of the early 1960's. It was fun – making hydrogen sulfide to stink out the place from lumps of ferrous sulfide and various acids and even worse generating ammonia and tricking my aunt into sticking her nose into the flask. It was small wonder that my widowed mother was generally happy when I wasn’t up to that sort of thing in doors, but rather was outside getting some exercise. And that for me was playing soccer with my brother and cousin, in which games I dreamed of being the Wolverhampton Wanderers goalkeeper. Although I had reasonable ability it remained that: a dream. Had I my time again I would have made it my business to put myself into a position where I might have made the vision a reality – even to the extent of knocking on the door of the club and asking for a trial. Years later, in 1985, when my playing career was in its final stages as keeper for a Bass Sunday League side was over, I did knock on the club’s door and offered my services as a writer for their match day program. Within a week I had seen my first article published – and for 30-plus years I have been writing for them, for other professional clubs in UK and US, for magazines, for a website (www.wolvesheroes.com) and also a book about Wolverhampton Wanderers goalkeepers (what else!). My published output on soccer far exceeds that on brewing and beer!

Thankfully, when it has come to my career in brewing, I have taken the opportunities as they have arisen and like to think that I have made the most of them, allowing me to rejoice in my current role which is the end of the journey. Sometimes it did not seem that the doors that I was invited to pass through were
necessarily the correct ones, but generally it has proved to be so. The QA Manager position at Preston Brook was one such, giving me invaluable “real world” experience.

Another valuable opportunity was afforded to me by John Hudson, who told me (I don’t think there was a choice in the matter) that I should become the recorder of the London Section of the (then) Institute of Brewing (IOB). The role entailed me journeying once a month into London and taking notes at the various meetings of the section, notably scribbling down the pearls of wisdom of guest speakers, at venues such as the famed Horseshoe Hotel pub on Tottenham Court Road and the London School of Economics. These notes were then published (anonymously) in the pages of the *Journal of the Institute of Brewing*. It was a marvelous introduction to this prestigious society, an organization that I have been proud to be a member of for over 35 years and to which I have contributed in diverse ways. This included being an examiner for the feared Paper II of the Diploma Exam, which was called the AME back in my time as the “interrogator” from 1988 to 1998. I chaired the Research Committee and the International Section, have been on the journal’s editorial board for many years and most recently have been truly proud to be the President of what is now the Institute of Brewing and Distilling. In 1984 I was the second recipient of the Cambridge Prize of the IOB (14). The first had been Chris Dickenson.

It is hugely important to give back and thus I have always felt the need to get involved in the various societies. I am honored, then, to have been Editor-in-Chief of the *Journal of the American Society of Brewing Chemists* since 2000. ASBC were kind enough to give me the Award of Distinction in 2011 (45). Equally I have been privileged to be Honorary Professor at Nottingham University since 2006 and to have previously been Visiting Professor at Heriot-Watt University, who recently bestowed an Honorary D.Sc on me, for which I am so grateful.
Which brings me to a final story. Years ago in England, with a degree of arrogance, my business card had a raft of post nominals after my name. I gave it to someone once, thinking they would be impressed. They returned their card to me, which simply gave their name after the prefix "Sir" and then underneath, "Ambassador to Norway". As my wife said: "I think simple cards are more effective, don't you, Charlie?"

But I can't resist pointing out that, with a PhD and two D.Sc post-nominals, I am strictly speaking "Doctor, Doctor, Doctor". To most people, including my students I remain simply: "Charlie".

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Footnotes

2. A D.Sc in the British University system is awarded for sustained and meaningful contribution to a field of study: one collates one's body of peer-reviewed work and the body is judged by a panel of D.Sc holders who determine whether that body of work represents a quality of delivery that warrants the degree.