A tribute to Professor Arnold L Demain – a lifetime in industrial microbiology

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Professor Arnold (Arny) Lester Demain is one of the few scientists who have witnessed the progress of biotechnology in a career that has spanned almost 60 years. He is one of the world's leading industrial microbiologists who has pioneered discovery in genetic and nutritional regulation of biosynthetic pathways leading to overproduction of a suite of primary and secondary metabolites, and their subsequent scale-up in manufacturing processes. These metabolites have huge economic value due to their application in the food, pharmaceutical and agricultural sectors. In this article, Arny's story is summarised and put in context of the changing face of biotechnology in the various 'golden ages' of biotechnology. A former Rubbo Orator in 1979, Arny will be visiting Australia again in 2010 to present the closing plenary address at the Genetics of Industrial Microorganisms Symposium (GIMS) in Melbourne, a role he played before at the first GIMS in 1970.

Pickles to PhD

Arny Demain's history was documented in a review article published in 2004 [1] and followed up in 2007 [2], the year of his 80th birthday: much of the following is sourced from these articles.

Born in Brooklyn, New York, in 1927, of parents from a Jewish Austro-Hungarian background, Arny finished high school – unlike his parents – at the age of 16, having attended five different elementary and three different high schools in Brooklyn and the Bronx. Being born into a world that was enduring a protracted depression then war, he admitted that times were tough, and that the support of his (divorced) parents and extended family in New York was important in his formative years. Spending much of his youth working afternoons and summer vacations in various jobs meant he had too little time to "feel sorry for myself".

Besides one uncle who was a pharmacist, he was the first of his extended family to consider a college education, although he saw himself as becoming a 'pickle man' like his father, who managed and commissioned pickles factories. However, his

father convinced him to become a food fermentation expert, and arranged for him to meet the 'pickle professor', Dr Frederick W Fabian, who ran an annual one-week summer course at Michigan State College (later University) entitled The Pickle and Kraut Packers School. After spending four quarters in his undergraduate training in microbiology, he decided to join the US Navy in 1945, eventually spending two years following medical training caring for amputees in Philadelphia, as war veterans returned from overseas duty. He resumed his studies at Michigan State in 1947, taking out his bachelor and master degrees by 1950, where the research topic for the latter was, not surprisingly, spoilage/softening of pickles during fermentation – which was most likely linked to pectic enzymes.

When Arny decided to return to study for his PhD, another 'pickle professor' advised him to go to the University of California's Department of Food Science in Berkeley, where his teachers and advisors were amongst the leading names in microbiology, biochemistry and chemistry of that time (Doudoroff, Adelberg, Barker, Pardee and Calvin). From his history of growing up in the aftermath of the Great Depression, Arny initially saw his studies as a means to eventually earn a reasonable and steady income, but admitted that with the great mentoring and tutelage at UC, he was "turned on to the magic of modern biology" – and stayed turned on for the rest of his life. When the department moved to UC Davis, he and his new wife (and lifelong companion, Jody) moved also, to commence his PhD on polygalacturonase of *Saccharomyces fragilis*. This work involved probably the first use of affinity chromatography, and one of the series of four resulting papers on pectic enzymes was published in *Nature* [3] – the current dream of every postgraduate student.

From Davis to Merck (1954–1969)

In early 1954, the Demain family moved to Pennsylvania to join Merck & Co., Inc, where Arny undertook research in the 'Cherokee' plant in Danville. Although the microbiology group at the penicillin factory focused on strain improvement and fermentation development in pilot-scale fermenters, Arny's role was basic studies on penicillin synthesis. He applied some of the approaches he had learned from Barker at Berkeley, which used starved and washed microbial suspensions to carry out enzymatic reactions in chemically defined fluids capable of supporting growth. This work was important in determining which amino acids formed the nucleus of penicillin and established some of the underpinning mechanisms of regulation that determined how primary metabolites (particularly amino acids) and carbon sources (through catabolite repression) could regulate secondary metabolite synthesis. He also showed that penicillin production was the net result of synthesis and inactivation during fermentation, a finding of significance in optimising final penicillin yields in industrial manufacture. His research on penicillin continued more than 10 years later at the Massachusetts Institute of Technology.

Moving to the Merck Sharp & Dohme Research Laboratories in Rahway, New Jersey, in 1956 saw Arny working on a diverse portfolio of projects that had in common fundamental studies on the regulation of synthesis of primary metabolites and growth factors, including amino acids (monosodium glutamate, MSG), nucleotides (including guanosine-5' – monophosphate, GMP, a potent flavour enhancer) and vitamin B_{12}, using auxotrophic mutants to determine nutritional regulation and precursors for synthesis of these commercially important products. He also studied the synthesis of cephalosporin C, a (then) newly discovered β-lactam antibiotic. In 1964, he founded the Fermentation Microbiology Department at Merck, which was involved in biosynthesis of Merck fermentation products and using mutagenesis to improve commercial strains. Work on MSG production by *Corynebacterium glutamicum* and vitamin B_{12} by *Pseudomonas denitrificans* continued, to achieve remarkable increases in productivity by characterising mutant strains plus manipulating the physical state of cells to enhance product formation, particularly by *C. glutamicum*. His department undertook research on optimising production of streptomycin by *Streptomyces griseus* (unravelling why the production organism is resistant to the antibiotic), riboflavin overproduction by *Ashbya gossypii* (later a commercial product of Merck), fosfomycin by a stereospecific bioconversion by *Penicillium spinulosum* and the development of a fermentation process for the interferon inducer, double-stranded RNA.

The level of innovation in this work (defining novel biochemical pathways, their regulation and determining the means to improve natural levels of production sometimes thousands of fold by mutation) is remarkable, both in its own right but also in the context of the times. The discovery of many of the glycolytic enzymes had occurred only during the 1950s; the first biochemistry pathways map was published by Boehringer Mannheim GmbH in 1965 [4]; the first edition of the biochemical engineering text by Aiba, Humphrey and Millis was published

in 1965 [5] (a hallmark text in this field); and the concepts of biochemical systems analysis and control of nutrient flux through metabolic networks in microbes was only later fully conceptualised [4]. His work built on the progress of traditional fermentations during the 20th century, which commenced with large-scale production of bulk chemicals and solvents (typified by commercial acetone/butanol fermentation before World War I) by natural isolates to the commercial manufacture of amino acids (from the late 1950s in Japan) [6] and antibiotics at large scale (penicillin and streptomycin), which heralded what Arny has described as the second golden age of industrial microbiology, predicated on biochemical engineering [7] and strain improvement through mutagenesis [8].

. . . to MIT (1969–2001)

In 1968, Arny was invited to interview for an academic appointment at MIT, in the Department of Nutrition and Food Science, a position he took up as Professor of Industrial Microbiology in 1969. The conditions of the appointment were that he would be paid 20% of his nine-month salary (0% for summer time), and he was expected to raise the rest of his salary from grants – which would have really focused the mind on achieving 'soft money' targets. He was obviously successful at this, as his laboratory was an exciting working environment for the next 32 years, with a constant flow of postgraduates, postdoctoral fellows (more than 100 during his time at MIT) and visitors from around the world.

In 1970, Arny was invited to present the closing Plenary Session address at the first Genetics of Industrial Microorganisms Symposium, held in Prague. This was an important meeting, as it was the first of 10 meetings held every four years, where the Symposium "is devoted to the future development of industrial microbiology in the light of recent progress in molecular biology and genetics" [9]. The main topics covered were:

- Transfer of genetic information and mechanisms of recombination.
- Mutagenesis and repair mechanisms.
- Genetic control of microbial metabolism.

The scope of papers from the international attendees (including Bruce Holloway from Monash University, with five other Australians) covered a spectrum of organisms and topics, from basic papers on microbial and fungal genetics, mechanisms of mutagenesis, regulation of enzyme and metabolite synthesis, strain improvement and identification of pathways for target compounds (emphasis on alkaloids), fermentation methods and barriers to high productivity, together with discussions on legal protection of strains, mutants and genetic procedures. What was striking about this meeting was the assumption that interdisciplinary skills were needed in industrial microbiology to be successful, and the willingness to learn and share across a diversity of microbes or systems – these were the days when specialist conferences on single microbes were yet to emerge.

Arny's paper was entitled *The marriage of genetics and industrial microbiology – after a long engagement, a bright future*. This title was somewhat prophetic. At that time, there had been considerable accumulation of knowledge of genetics in several microbes, as exemplified by the publication of the second edition of William Hayes's extensive text on *The Genetics of Bacteria and Their Viruses* [10], which mainly focused on *Escherichia coli* genetics (a text which was the bible of budding microbial geneticists at the University of Melbourne in the early 1970s). There were numerous genetic tools available, given that Hayes had first published on the mechanism of genetic recombination in *E. coli* in 1953 [11], and considerable gene mapping had occurred in *E. coli*, *Bacillus subtilis* and *Streptomyces*, amongst other species. DNA sequencing was in place, albeit manual. Gene mobilisation by plasmids was well-known, particularly by F-factors in *E. coli*, and the emergence of transferable drug resistance in the 1960s by the very mobile R factors was understood. However, it was still two to three years away until the key experiments associated with cutting and re-circularising plasmid DNA [12], and then cloning the first eukaryotic gene into a plasmid, occurred [13]. This was followed by the start of the ethical, environmental and legal debate surrounding the creation of recombinant organisms, commencing with the voluntary moratorium on recombinant DNA experimentation, the Asilomar gathering in 1975 led by Berg [14], which resulted in the foundations of the guidelines for experimentation involving recombinant DNA and the subsequent lifting of the moratorium soon thereafter [15]. This would usher in the next 'golden age' in industrial microbiology [7], reopening the quest for biodiscovery that had commenced following the discovery of penicillin.

Over his 32 years at MIT, Arny and his team, plus collaborators, pioneered research on the elucidation and regulation of the biosynthetic pathways leading to penicillins and cephalosporins. The list of projects is otherwise extensive, including research on: peptide antibiotics and sporulation in *B. subtilis*; mycotoxins; new antibiotic discovery through mutational biosynthesis (making idiotrophs); cellulases and ethanologenic clostridia; vitamin B_{12}; amino acids, organic acids and polymers; the immunosuppressant, rapamycin (sirolimus) and antitumour antibiotic, fumagillin; statins; pigments; vaccines (developing media with non-animal components for clostridial vaccines); and evaluating the impact of simulated microgravity on secondary metabolism [1]. He continues to add to his almost 500 published papers, 10 co-edited or co-authored books and 21 US patents.

He has received numerous honours, awards, and honorary degrees. His ability to 'hybridise' basic studies and industrial applications was recognised by his election to the presidency of the Society for Industrial Microbiology in 1990, membership in the National Academy of Sciences of the USA in 1994, the Mexican Academy of Sciences in 1997, and the Hungarian Academy of Science in 2002. Honorary doctorate degrees have been conferred by the University of Leon (Spain), Ghent University (Belgium), Technion (Israel), Michigan State University (USA),

and Muenster University (Germany). He is a member of the Board of Governors of the American Academy of Microbiology, has served as a member of the USA National Committee for the International Union of Microbiological Sciences (IUMS) and was a delegate to the 2002 General Assembly in Paris. He has been honorary consultant for the Fujian Institute of Microbiology and the Shanghai Institute of Pharmaceutical Industry in The People's Republic of China [16]. He was also a consultant to what was (arguably) the first biotechnology company, Cetus Corporation (later incorporated into Chiron Corporation, now Novartis), which was set up in Berkeley in 1971 [2].

After MIT (2001–) and final comments

After his retirement from MIT, Arny joined Drew University, New Jersey, as one of nine Research Fellows in the Charles A Dana Research Institute for Scientists Emeriti, or RISE. All of the Fellows have experience in industry and cover the chemical and biological sciences. The role of the scholars is to train undergraduate students to conduct research, mentoring the next generator of researchers, innovators and inventors. I hope they will, in the future, be as generous as Arny in recognising discovery by others and the impact that a mentor can have on a lifelong career, as Arny has done [1].

A feature of Arny's work has been an ability to undertake fundamental research on systems with clear industrial applications, recognising that biodiscovery is the start of the road that includes strain improvement to achieve levels of product synthesis that warrant further investment to take products into the marketplace. With the almost exponential growth in the number of microbial genomes sequenced, and their public release over time, the period between discovery and commercial uptake is shortening [4]. Metabolic engineering, the rational design of recombinant microbes based on modelling nutrient flux through known biochemical pathways to channel carbon into high yields of desired products, has already been used to develop new industrial processes. As predicted by Arny [17], this has occurred in the energy sector and is based on processes aiming to replace chemicals made currently via petrochemical conversions and lower the carbon footprint of manufacture – 'white biotechnology' [18]. The outstanding example of this is 1,3-propanediol production by *E. coli* and the commercialisation of this process for Bio-Based Materials (manufacture of polymers used for apparel, carpets, fibre – which are in the marketplace now), after a 10-year development investment by Du Pont, which leads the way to the future [4]. This future, however, will be realised only by annotation of the plethora of genes with unknown function and discovery of the biochemical pathways for yet undiscovered products – a blend of Arny's approach with the tools of 'industrial systems biology', biochemical engineering, process economics, and whole-of-lifecycle analysis to minimise environmental impact and ensure sustainability of manufacture. Arny's legacy will continue to be valued, as long as the history of this field is valued.

Arny's army and friends

The clan of former students, postdoctoral fellows, collaborators and friends of Arny have met six times since his retirement to honour their former mentor and colleague. The Seventh International Symposium on Industrial Microbiology & Biotechnology will be held in Melbourne, 1–3 July 2010, immediately after the 11th GIM Symposium. Information on this can be found on a link to the GIM Symposium, or by contacting the author of this paper. Australian friends would be very welcome to join the international visitors flying in for this event, present a paper, share the science and enjoy the social interchange.

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