

Reflections Syd Leach

Chemical Engineering to Protein Folding:

Syd Leach reflects on his early work in the UK then at CSIRO in Melbourne

Looking back on my scientific career from the vantage point of an Emeritus Professor in the Department of Biochemistry and Molecular Biology at the University of Melbourne, the journey that I have witnessed seems fantastic.

Little could I have envisaged at the start of my career the developments in all areas of science, let alone the explosion in our knowledge of the hitherto unknown area of Biochemistry. I certainly would never have imagined that as I approached eighty years of age that I would be using a Silicon Graphics workstation to perform molecular dynamics simulations.

It would not only be pretentious but also inaccurate to call my reminiscences "Wanderings of a Biochemist". Firstly that title was pre-empted by the eminent Fritz Lipmann in his 1971 memoirs. More important I had no intention nor possibility of playing a role in biochemistry when I started my scientific career.

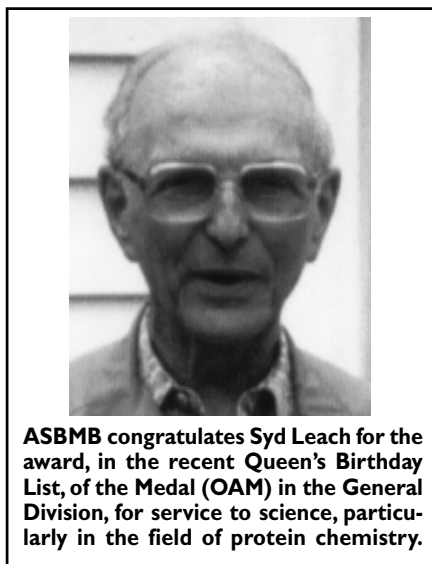
Biochemistry was virtually unknown as a discrete discipline when I graduated B.Sc.Tech at Manchester University in 1942. At that time, I believe that only at Cambridge could one take out a first degree (Tripos) which included a subject called Biochemistry – taught under Professor F.G. Hopkins.

With a degree in General Chemical Technology and having learned something about heat exchange, fluid dynamics, distillation and solvent extraction, it seemed logical to spend my war years in an oil refinery. I survived several oil fires as a sector captain during air raids on the industrial area of Trafford Park (where 50% of British manufacturing was located).

After a stint as a Chemical Engineer in a solvent extraction plant (using liquid sulphur dioxide under 15 atmospheres pressure), I moved into the more peaceful research atmosphere of oil refining. I had two projects. One objective was to test heavy (aromatic) petroleum oils as a substitute for imported plasticisers in the manufacture of the plastic insulation of electrical cables. This was at the height of the German bomb attacks on our trans-Atlantic shipping.

We needed the precious cargo space for foodstuffs from the USA. I and my research colleagues at other British refineries were able to achieve substantial replacement of the imported plasticisers by locally refined oils for cable manufacture.

My other project aimed to produce improved hydraulic oils for war planes flying at high altitudes. The hydraulic oils were used in certain WWII aircraft to activate brakes, flaps and undercarriages. Transmission of such hydraulic signals was inefficient at the low temperatures encountered at high altitudes because the



ASBMB congratulates Syd Leach for the award, in the recent Queen's Birthday List, of the Medal (OAM) in the General Division, for service to science, particularly in the field of protein chemistry.

oils in use increased in viscosity as the temperature decreased (i.e. they had a high "activation energy of flow").

Studying the E_{act} by plotting $\log(\text{viscosity})$ vs $1/T$, I noted that "light" oils suffered relatively smaller increases in viscosity at low temperatures (i.e. their E_{act} was lower.) More importantly, they retained these superior viscosity/temperature characteristics even when they were artificially "thickened" by dissolving suitably inert polymers in such oils.

I was able to produce suitably "thickened" hydraulic oils using polyoctyl methacrylate as solute. So much for my wartime research activities.

My induction into the world of Biochemistry came with my PhD project at Leeds University – surprisingly within a Department of "pristine" physical chem-

istry. This Department was a hotbed of reaction kinetics, electron-transfer reactions and redox potentials and provided me with my first insights into the mysteries and mechanisms of enzyme catalysis.

We were puzzled by the well-known fact that, in spite of the known low redox potential of NAD^+ (then usually called coenzyme I) the reduced coenzyme showed little tendency to autoxidise in the absence of intermediates. Lactate, malate, triosephosphate and numerous other substrates are oxidised by molecular oxygen in a cascade of reactions which are initiated by the reduction of NAD^+ .

Although the ΔG for the oxidation of NAD^+ was highly favourable, there appeared to be an equally important factor preventing the interaction of NAD^+ and oxygen. This factor was to be sought in the mechanism of oxidation of NAD^+ .

I therefore studied the redox properties of two "model" substances namely nicotinamide methochloride and N-methylacridan. Both were reduced electrolytically using a mercury cathode and the products shown to have properties similar to their flavin-type coenzyme analogs. Thus, they reacted only slowly with pure oxygen but reduced most other substances (dyes, iodine, ferric iron, ferricyanide) which had a higher E° . The E° of the nicotinamide methochloride was found, by potentiometric titration to be -0.36 V against the Normal hydrogen electrode at pH 9.1 and 32°C and this was very similar to that of the "parent" NAD^+ (-0.32 V).

The kinetics of oxidation of the reduced model compounds using 2:6-dichlorophenolindophenol proceeded by way of the conjugate acids $>N^+H-Me$. The first step in the oxidation of this acid was unlikely to involve the removal of an electron since the energy requirements to produce a double positive charge on the molecule would be prohibitive.

A hydrogen atom transfer step was therefore most probable and this was thought to account for the very slow reaction of both the nicotinamide, the reduced acridinium models and the parent dihydro NAD^+ with molecular oxygen. On this interpretation the function of

Reflections Syd Leach (contin.)

the flavin-type coenzymes would be to act as mediators, accepting the H atom from the dihydro-coenzymes and passing on an electron to cytochrome c in which the ferric/ferrous transformation may be effected by simple electron transfer. The components of the respiratory chain would thus be graded with respect to *chemical mechanism* as well as redox potential. The concept of the hydrogen atom transfer between NAD^+ and various substrates was supported by the data of others (Westheimer *et al.*) who showed conclusive evidence of a "direct" stereochemically specific transfer of deuterium atoms.

I enjoyed the stimulating academic atmosphere provided by Professor M.G. Evans and my fellow penurious research students. I even married in 1947, my wife (who was studying music and French) and I cheerfully sharing the simple life together. Our comfortable poverty was to be transformed by a fairy godfather called Sir Ian Clunes Ross. Sir Ian was visiting British scientific institutions and recruiting suitable young scientists for the CSIRO (or CSIR as it was then). He seemed to like what he saw and heard in my laboratory and in due course, I was offered an appointment at the CSIR Biochemistry Section – a unit of the Division of Industrial Chemistry located at Fishermen's Bend in Melbourne.

The prospect of a stint in Australia was something I had dreamed of. Thanks to a prolonged wharf strike at Tilbury Docks in London, the only transport available (air travel not being an option at that time) was a first class passage on the 75-passenger S.S. Gothic. We coped with six weeks of shipboard luxury and arrived in Melbourne via Sydney on the Spirit of Progress. The CSIR Biochemistry section was housed on the 4th floor of a grain warehouse also occupied by Parsons (John Bull) Oats located at the salubrious end of Flinders Lane. It didn't seem much like a setting for a scientific research department. How wrong I was.

The group was led by Dr F. Gordon Lennox. He was a most agreeable, kindly and shrewd research leader who had assembled a group of about a dozen young research scientists much like Noah had chosen candidates for his Ark – with a representative for each area of science. We covered organic, physical, biological, protein, enzymological and other sciences. Gordon brought out the best in each of us

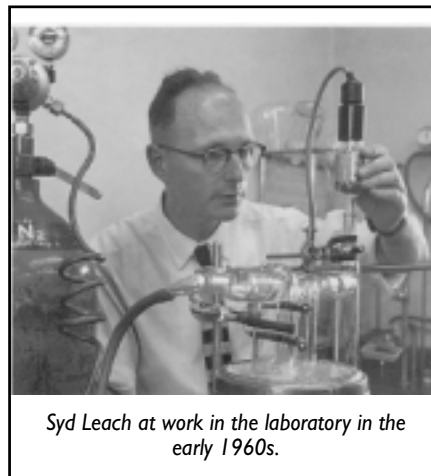
by giving us considerable latitude and individual responsibility and by leading us gently.

One of his preoccupations at that time was to replace the harsh chemical reagents (acids) then being used to dismantle keratins and other proteins for structure studies by the more gentle proteolytic enzymes to be isolated from, for example, cultures of *Aspergillus oryzae*. Indeed a crystalline protease was so isolated. Other preoccupations were with the chemical depilation of sheepskins - important in the Australian fellmongering industry. Without going into the politico-bureaucratic machinations which transformed CSIR to CSIRO in 1949 (the year that I arrived), the Biochemistry section became the Biochemistry Unit of the CSIRO Wool Textile Research Laboratories, with sister units of Wool Physics and Wool Processing in Sydney and Geelong respectively.

As a novice I was fortunate to be detailed to work with a Dr Hugh Lindley. Hugh had recently arrived from the Wool Industries Research Association in Leeds where he had worked alongside Nobel Laureates A.J.P. Martin and R.L.M. Syge using their famed chromatographic techniques. I learned, from Hugh, how to separate amino acid and peptide mixtures using chromatography on paper and on silica gel columns.

Our methods were unsophisticated but effective. For example, we did a primitive kind of amino acid analysis using 2-dimensional paper chromatography with solvent mixtures such as butanol-acetic acid-water and collidine. After drying we sprayed with ninhydrin and estimated the intensities of the coloured spot by comparison with serially diluted and separated standard amino acid mixtures run alongside the unknowns. Our silica gel columns were 10-15cm glass columns (1 cm diameter), packed by hand and "developed" with, for example, butanol-acetic acid mixtures using a hand-aspirated rubber bulb as pump! Using DNP-amino acid or DNP-peptide mixtures, we collected the bands by hand and eye as they emerged. A far cry from modern automated HPLC picomolar separative methods! I learned a great deal and we got results.

I worked on numerous projects during the keratinous or woolly phase of my career at CSIRO. The research personnel in Flinders Lane rapidly outgrew our cramped and dangerous quarters. (A wood fired pot-bellied stove which sometimes



Syd Leach at work in the laboratory in the early 1960s.

glowed red hot was hardly a suitable heating appliance where many litres of flammable solvents were in use).

W.G. Crewther, J.M. Gillespie and others concentrated on the extraction of soluble "kerateines" from wool, using thioglycolate to reduce the disulphide bonds which held the peptide chains together – the products then being S-carboxymethylated to prevent re-oxidation. Meanwhile J.M. Swan introduced the new methods of peptide synthesis learned from the laboratories of Vincent du Vigneaud in New York.

In 1950 we moved upmarket into newly acquired premises in Parkville. This 2-storey house in Royal Parade had been built in 1880 by Professor N.C. Kernot, the first Professor of Engineering at Melbourne University. It was rumoured that it had served as a brothel during the war. The premises were modified to accommodate our increasing numbers and wider interests (amino acid analysis by the new automated methods of Moore and Stein, electron microscopy, infra-red spectroscopy, x-ray diffraction and the increasingly sophisticated methods of peptide synthesis used to make insulin and other peptides).

In 1953, with W.G. Crewther as Assistant Chief to F.G. Lennox we hosted the first Wool Textile Research Conference which brought such luminaries as F. Sanger, A.J.P. Martin, R.L.M. Syge, J.C. Speakman, W.T. Astbury and lastly R.B. Corey who talked at length about his work with Linus Pauling on their model building studies on the newly described α -helix and the β -sheet.

(. . . to be continued in the next issue of the *Australian Biochemist*, December 2000)