Early Experimental Techniques

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The Australian Biochemical Society was founded at the end of the post-World War II decade that saw the beginning of modern molecular biology. Underpinning the seminal discoveries of that decade were many techniques which, though fashionable then, are still with us today. X-ray crystallography gave us the double helix; the analytical ultracentrifuge and moving boundary electrophoresis told us that proteins were defined molecules and not vague colloidal entities. Sanger's sequencing of insulin at the end of this decade showed the way to the sequencing of much larger proteins. The electron microscope had already achieved a resolution of 6Å, enabling resolution of detail at the supramolecular level in viruses. In the background were the discoveries of electron paramagnetic resonance in 1944 in the Soviet Union and nuclear magnetic resonance in 1945 in the USA. Having no foreseeable impact then on biochemistry these still evolving techniques now occupy a central place in studies on molecular structure and dynamics and free radicals in biology.

My own undergraduate, postgraduate and postdoctoral years spanned that post-war decade. In 1949 I joined Gordon Ada's biophysics group at the Walter and Eliza Hall Institute (WEHI) as his first student. The group had been set up with a £20,000 major equipment grant from the NHMRC. The equipment was to consist of a Tiselius electrophoresis apparatus, an analytical ultracentrifuge and an electron microscope. While the latter was to be from Siemens in Berlin the centrifuge and Tiselius apparatus were to be built at the Hall Institute. The Institute was fortunate in having as its engineer and business manager a Melbourne mechanical engineering graduate, Arthur Hughes. Scientific expertise was provided by Henry Holden, a Cambridge Biochemistry graduate from the string and sealing wax era. Prior to joining the Institute, Arthur Hughes worked at the Maribyrnong Ordnance Factory and, using his contacts, turned the post-war 'swords into ploughshares' policy to good account in fabricating parts for the new instruments. Notably, the ultracentrifuge safety chamber was machined out of the breech end of an 8 inch naval gun blank. The simpler of the two, the Tiselius apparatus was finished quite quickly and turned to good account by John Pye who used it to monitor the purity of glycoprotein inhibitors of influenza virus haemagglutination and the reduction in their net negative charge following the action of neuraminidases from different virus strains.

The ultracentrifuge was not quite finished by the time I left the Hall Institute for my post-doctoral year in Sandy Ogston's laboratory at Oxford. The Hall Institute centrifuge was of the Beams and Pickels air turbine type whereas Oxford's was the original Svedberg oil turbine design built by LKB. An impressive machine, its quarters looked and smelt like a ship's engine room. As Sandy wrote years later in *TiBS*, "like a child among strangers," it was a great conversation starter. Both the Svedberg and Beams and Pickels designs were supplanted in time by the electric drive Spinco Model E. However, I acquired a Hungarian Metrimpex air turbine analytical ultracentrifuge at the Baker Institute in the mid-1960s. Elegantly packaged and smooth running on its air bearing, it was particularly suited to long sedimentation/diffusion runs on small molecules. Its drawback was a habit of regularly breaking its piano wire-thin drive shafts, which were imported and like all imports, were slow to arrive.

Unlike now, 50 years ago it was rarely possible to buy techniques off the shelf and in Australia, because of import restrictions brought about by economic mismanagement, acquisition of even the basics from overseas could be red tape-ridden and slow. A classic example was the travail of a colleague who needed highly pure sucrose, unavailable here. Unfortunately, importing even A.R. grade sugar was prohibited to protect local cane growers and it took her many months and an appeal to the Tariff Board to get what she needed.

The Tiselius moving boundary electrophoresis apparatus built at the Walter and Eliza Hall Institute, with Gordon Ada left and John Pye right. The schlieren optical system and cells came from Adam Hilger, London. The rest of the instrument was sourced locally. Image courtesy of WEHI Archives.

If you wanted to exploit a newly published technique you had to develop it yourself. There was rarely the critical mass of customers to support local kit/instrument manufacturers. One important exception was Geoff Frew's Techtron Appliances. Part of my PhD project was acid-base titration of glycoproteins and an

essential part of my rig was one of Techtron's decadic resistance boxes. Although I coveted a Cambridge Instruments box with its polished brass, wood and ebonite, I was quite happy to accept the readily available local product. Under its humble grey crackle finish it was just as precise as the import. Techron was involved in the development of Alan Walsh's CSIRO atomic absorption spectrophotometer, manufactured the early instruments and was absorbed by Varian in 1967 to become Australia's major instrument exporter.

The Walter and Eliza Hall Institute air turbine analytical ultracentrifuge, showing the massive safety chamber with the rotor suspended by its thin wire shaft. Image from author's collection.

Getting a new method started often meant going back to basics including creating one's own reagents. Immunofluorescence, which I got going at the Baker Institute in 1955, was a good example. The fluorochrome at the time was the highly unstable fluorescein isocyanate that had to be made as needed. It was synthesised by reacting fluorescamine with phosgene. The latter was prepared by dripping fuming sulphuric acid into boiling carbon tetrachloride. In the light of the inadequate fume cupboard designs of the day, the preparation fortunately went without a hitch and enough labelled antibody was prepared to last until a year or two later when the much more stable fluorescein isothiocyanate became available commercially.

Poor fume cupboard standards are only one reminder that OH&S regulations and committees were non-existent and we survived by the application of lots of commonsense and a little luck. Over the last 50 years, the biggest transition in biochemistry has been the move from bucket chemistry to microchemistry brought about a combination of the gene revolution and refinement of analytical techniques. Many of the classic isolations and characterisations involved huge amounts of starting material and near pilot plant scale operations using dangerous reagents, such as methanol, benzene and phenol. Mostly, things went well but sometimes unexpected equipment failures could create hazards. My closest call was caused by a centrifuge explosion. As part of my PhD project, I was using 80% phenol to extract a glycoprotein from meconium, centrifuging the mixture to remove the phenol insoluble residue. On one of these runs, the bottom blew out of a cup ejecting a fine spray of its unpleasant contents through the millimetre gap between the lid and the chamber. I was in the room at the time and had to cower behind a bench until the spray stopped and I could approach the madly vibrating machine to switch it off. Understandably, I have been paranoid about centrifuge safety ever since.

So, what has changed and what has remained the same? Although many of the basic techniques of separation and characterisation of 50 years ago are still in use, today they are a thousand to a million times more sensitive. This change was based partly on the gene revolution and partly on the much wider microelectronic revolution. The upside has been a much finer division of scientific labour which has led to much higher productivity; think sequencing, once a large team effort, now an Honours or PhD project. If there is a downside it comes from the 'black box' effect where the experimenter is simply not in command of all the variables. Fortunately the self-correcting nature of the scientific enterprise usually comes into play and somebody spots and explains the error or anomaly. Time wasting, sometimes ego-bruising, but a small price to pay for the immense power that we have gained from our present-day techniques.

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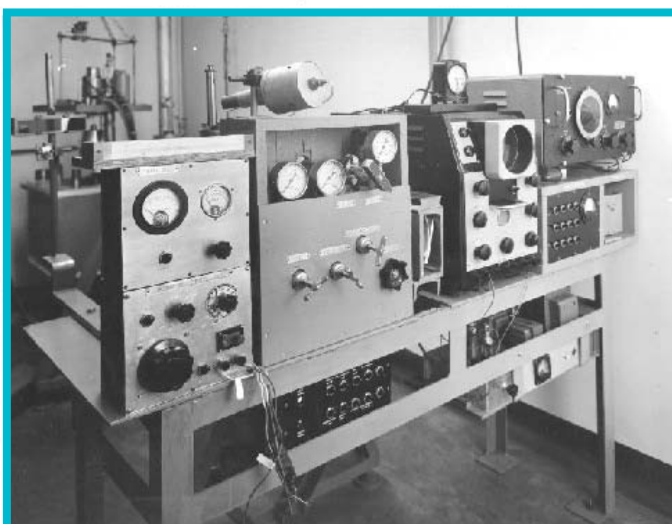

The control panel end of the ultracentrifuge. Before it was superseded by a Model E Spinco in the early 1960s the machine achieved some significant results, including the molecular weight of the neuraminidase of Vibrio cholerae *that had been crystallised by Gordon Ada. Image from author's collection.*