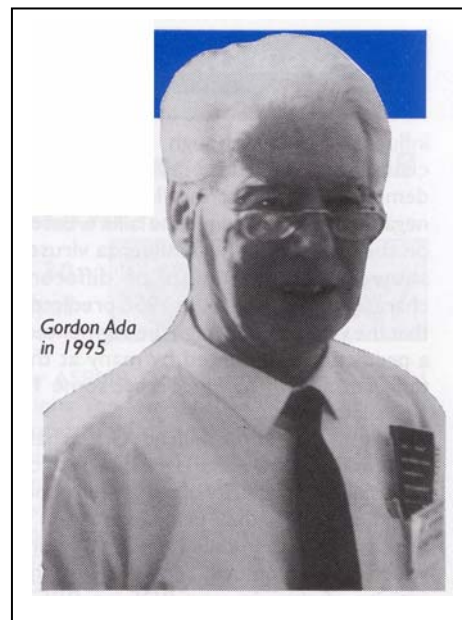


Reflections Gordon Ada

Life as a Biochemist Coming to Grips with Viruses

Foreword

It must be hard for recent graduates in many biological disciplines to appreciate what the frontiers of our knowledge were 50 years ago. The author majored in Biochemistry at the University of Sydney during the war and in 1948, joined the staff of the Walter and Eliza Hall Institute (WEHI) officially to help establish new biophysical techniques (moving boundary electrophoresis and ultracentrifugation), but spent most of the time doing research on virus-related topics. Macfarlane Burnet, a famous virologist, had become the Director of the Institute in 1942. This account describes some of the relevant biochemical findings made during the period 1948-60.

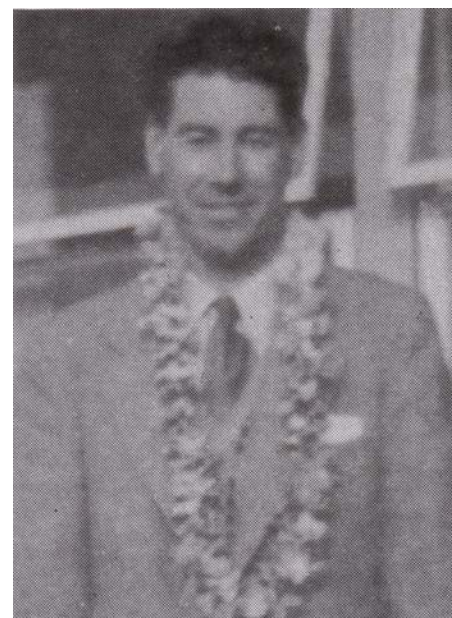


Discovering the Secrets of the Influenza Virus

The 1918-19 influenza pandemic killed at least 20 million people, more than the combined casualties of the two World Wars. Burnet, part way through his medical course at Melbourne University when it reached Australia, fortunately suffered only a mild infection, but the global and local effects remained a strong memory. On becoming Director of WEHI, and concerned that a similar pandemic might soon occur, he decided to make a determined effort to understand how the influenza virus infected and replicated inside cells and caused disease. Virtually all non-clinical scientists in the Institute were to become involved in this task. When I arrived in 1948, there were two other biochemists - Henry Holden, who earlier had achieved fame in the UK in elucidating the structure of haemoglobin, and Alfred Gottschalk (see **Box 1**), a carbohydrate specialist, who had escaped from Nazi Germany and joined the Institute in 1942. Holden, not far off retirement, was mainly responsible for establishing the biophysical techniques.

The influenza virus was first isolated and grown in ferrets in the early 1930s "the ferrets are sneezing" became a famous phrase. Burnet had since brought to a fine art the use of the embryonated egg for isolating and growing the virus. In 1942, George Hirst (New York) had reported that at 4°C, influenza viruses agglutinated red blood cells, but on incubation at 37°C, the virus subsequently eluted from the cells, but could still agglutinate fresh RBCs. This suggested that the virus had an intrinsic enzymic activity. Burnet found that different flu viruses could be placed in a 'gradient' according to their ability still to agglutinate RBCs from which another flu virus had previously eluted. In addition, he had shown that RBCs exposed to a *Vibrio cholera* culture could no longer be agglutinated by any flu virus. The culture contained a receptor-destroying enzyme (RDE). By using a simple piece of equipment put together by Henry Holden (who had learnt how to survive during the depression years), Joyce Stone and I showed that treatment of my RBCs with different flu viruses reduced their electrophoretic mobility by 30-50%, whereas RDE reduced it by about 90%. Clearly, this enzymic cleavage released an acidic component. I decided to try to purify RDE; this took some time but in partnership with Eric French, was finally achieved.

In the meantime, I realised that little was known about the biochemical properties of the virus. At that time, the role of nucleic acids in viruses was being debated. Two eminent British scientists especially, Norman Pirie and Fred Bawden, were reluctant to assign a central role to the nucleic acid. Based on colorimetric assays of the total viral sugar residues, C. A. Knight from Wendell Stanley's group (USA) had claimed that the flu virus contained mainly DNA and only traces of RNA. I thought it desirable first to extract the nucleic acid from the virus before attempting an analysis. A 10% NaCl solution was found to extract all the nucleic acid which, following acid hydrolysis and subsequent chromatography, showed only the four RNA nucleotides, and indicated that the virus contained about 1% RNA (molecular



Gordon Ada in 1956 at Honolulu Airport on his way back to Australia after the Ciba Foundation meeting in London

weight about 2 million per particle). I then showed that the ratios of different nucleotides differed between A and B strain viruses, and that viral preparations of low infectivity contained less RNA. The work was presented at the 1956 Ciba Foundation meeting in London on the Nature of Viruses, which was attended by many celebrities, including Francis Crick and James Watson. Many papers attested to the important role of the viral nucleic acids, but the reports at the meeting by A. Gierer and G. Schramm (Germany) and H. Fraenkel-Conrat (USA) that freshly isolated RNA from tobacco mosaic virus was itself infectious provided the decisive evidence.

On returning to Melbourne, I was able to obtain infectious RNA from Murray Valley Encephalitis Virus, which I had earlier purified, but not from influenza virus, although some others claimed to have success. The later demonstrations that the flu RNA was a negative strand explained the failure. Based on the ease with which influenza viruses showed recombination of different characteristics, Burnet in 1956 predicted that the viral genome might be segmented, a prediction discounted by many at the time but shown to be correct some 10 years later.

In the meantime, Alfred Gottschalk was making good progress towards analysing the specificity of the viral enzyme. We had found that urine contained a glycoprotein (then called a mucoprotein) which, like ovomucin, could block the activity of the virus or RDE on RBCs. Gottschalk suggested a configuration for the glycoprotein comprising a protein backbone with sugar side chains. He showed that the action of virus or purified RDE (see below) released a low molecular weight 'split product'. Others noted similarities between this split product and sialic (neuraminic) acid. In collaboration with an Australian, John Cornforth (see **Box 2**) working in London, the synthesis of neuraminic acid from N-acetyl-D-glucosamine and oxaloacetic acid was achieved. Using neuramin- (or sialyl -) lactose as substrate, the enzyme was shown to cleave an O-glycosidic-type linkage involving the keto group of neuraminic acid with another sugar molecule.

Eric French and I showed that N-acetyl neuraminic acid and sialyl lactose induced RDE production by *V. cholerae* and both were metabolised in the process. Of all the other compounds tested, only N-acetyl-mannosamine was active as inducer but, curiously, was not metabolised during culture.

Success in producing, purifying and crystallising RDE, called neuraminidase following Gottschalk's work, was only achieved when *V. cholerae* was grown in a synthetic medium

enriched with a dialysate of bovine colostrum, which we found contained sialyl lactose. Once produced, the enzyme was adsorbed onto RBCs, then eluted and passed through a chromatographic column. Obtaining the crystals slightly preceded the announcement that Burnet and Peter Medawar were to share the 1960 Nobel Prize in Medicine or Physiology, for their prediction and proof of the acquisition of immunological tolerance during foetal life. For a while, I supplied samples of the crystallised enzyme to investigators around the world until Sigma took over the production. Many years later, Graeme Laver, then at the John Curtin School, isolated the neuraminidase cleaved from the viral particle, and was successful in obtaining crystals suitable for X-ray analysis. This in turn has allowed the synthesis of a 'designer' drug, called Relenza, which inhibits the release of viral progeny from the surface of infected cells.

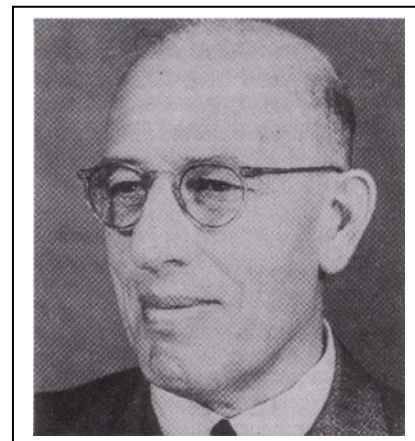
Gottschalk and I also devised a way to compare the carbohydrate components of the viral antigens with those of the cell substrate (cells in the allantoic membrane). Other than neuraminic acid, galactose, fucose, mannose and glucosamine were regularly found, and the relative amounts of each in the virus and from cells were similar, suggesting that cellular enzymes were responsible for the carbohydrate specificity of the viral glycoproteins. Ribose was the only pentose found.

Burnet and the Walter and Eliza Hall Institute

These biochemical achievements represent a significant contribution to the total research effort from the Institute. Over this period, the stimulus provided by Burnet and the superb collaboration offered by the other biologists was remarkable. Gottschalk later wrote "there must be few laboratories where 'the give and take' between biology and biochemistry was so closely wedded as at the Walter and Eliza Hall Institute".

Burnet, discouraged by his only partly successful studies to elucidate influenza virus genetics, turned to his interest in immunology. Stimulated by a new concept about antibody production published in 1955, he published his Theory of Clonal Selection of Antibodies in 1957, a concept which ten years later became regarded as the central paradigm in Immunology. Virology began to be phased out of the Institute from the late 1950s. In a recent article, Sir John Maddox, formerly the editor of *Nature*, nominated Burnet as tenth in a list of great scientists of this century. Those of us who got to know him well appreciate this recognition.

Box 1. Alfred Gottschalk (pictured) was born in Aachen in the Rheinland of Germany in 1894. During his work in Australia he became a member of Fellow of the Australian Academy of Science. Dr Gottschalk died in Tübingen, Federal Republic of Germany in 1973. The Gottschalk Medal commemorates his contributions to science and is awarded annually by the Academy. Its purpose is to recognise distinguished research in the medical sciences by young scientists (not over the age of 40 years) for research carried out mainly in Australia. *The Gottschalk Medal for 1999 was awarded to Dr Michael Parker (see page 37 of this issue)*



Box 2. John (later Sir John) Cornforth was born in Sydney in 1917. After graduating M.Sc. at the University of Sydney, he was awarded D.Phil. at the University of Oxford. He had a distinguished career in the United Kingdom and was awarded the Nobel Prize in Chemistry in 1975 for his work on the stereochemistry of enzyme-catalysed reactions. He was born deaf and attributed his success to teaching himself because he could not hear his lecturers. I once took him to a football match at Wembley Stadium. When the sole goal of the match was scored, the noise of the crowd was so great that he turned to me and said he could hear something for the first time!

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