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Memories of a Senior Scientist

I was lucky, I was there at the right time

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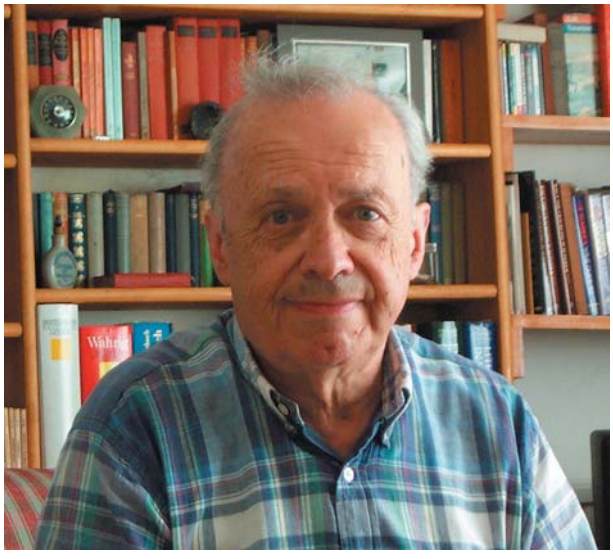

I was lucky, I was there at the right time

I was born in Vienna on 21 October 1921 and grew up in a professional middle-class environment until my teens. However, the above heading may appear inappropriate considering subsequent events. I emigrated as a penniless teenager in 1938 and made a living as a farm labourer in England for three years. I had not been conventionally successful at school but the economic and political upheaval of the 1930s that resulted in my emigration provided a good excuse for my unpromising early record. During my first few years in England I had a variety of unusual educational opportunities. Meeting the right people at the right time culminated in my getting to Cambridge in 1943 and in being awarded a PhD degree in 1947. From that time on my career progressed along more conventional lines with positions at Universities and research institutions.

In my childhood I was determined to become a physicist. I was much influenced by my mother's cousin Karl Weissenberg, a theoretical physicist who made considerable contributions to crystallography and rheology. He was also a very good teacher. Serendipity brought me later into a biochemical environment but at heart my approach is still that of a physicist. I feel more at home with an oscilloscope and a soldering iron than with a gel! When I had the pleasure of being elected to honorary memberships of both the ASBMB (American Society for Biochemistry and Molecular Biology) and the Biochemical Society I facetiously remarked that 'I can only compete with modern 'green fingers' biochemists because I can solve two simultaneous equations and know Ohm's law'. My move to Cambridge was due to my association with AC Chibnall who succeeded FG Hopkins as Sir William Dunn, Professor of Biochemistry. Apart from my PhD supervisor Gilbert Adair (Reader in Biophysics), my main contacts upon arrival in Cambridge were Fred Sanger, Peter Mitchell and Gregorio Weber – two future Nobel Laureates and the man who was principally responsible for the application of fluorescence to biochemistry. Other people in Cambridge at that time who are part of the History of Biochemistry were Malcolm Dixon, Marjorie Stephenson, Robin Hill and Dorothy Needham. A marvellous environment for a young man to grow up in.

Adair's influence left me with a life-long admiration for the work of J Willard Gibbs and an interest in the thermodynamic properties of protein solutions. Unfortunately Adair did not sympathise with my discoveries and continued interest in the reversible dissociation of oligomeric proteins. However, I managed to provide sufficient evidence for the importance of this phenomenon. In 1947, when the physiologist/biophysicist FJW Roughton became head of the Department of Colloid Science in Cambridge, he appointed me to an independent

research position. At that time scientists were returning to Cambridge from different wartime occupations eager to renew their academic/research careers. Biologists had gained experience with electronic instrumentation and many physicists decided to apply their talents to biological problems after having rubbed shoulders with biologists during joint projects. The resulting developments over the next twenty years or so made Cambridge a most exciting place to live. I must list some of the events that influenced me and provided me with standards to judge achievements – very humbling:

(1) Fred Sanger developed the method to obtain one-dimensional structures of proteins.
(2) Alan Hodgkin and Andrew Huxley established a model for nerve conduction.
(3) Francis Crick and Jim Watson solved the structure of DNA (not exclusively a Cambridge venture).
(4) Hugh Huxley and Jean Hanson established the sliding filament model for muscle contraction (not exclusively a Cambridge venture).
(5) John Kendrew and Max Perutz solved the first three-dimensional structures of proteins.
(6) The Laboratory of Molecular Biology (LMB) was founded and produced eight Nobel Laureates as well as other scientists of equivalent standing.

The scientific community at Cambridge was much smaller in the 1950s than it is now and I had continuous contacts in the laboratory and socially with many of the main players in the above developments. For instance, I daily had lunch at The Eagle and a post-prandial walk with Francis Crick and Jim Watson while possible structures for DNA were discussed. I also have vivid recollections of the arrival at the LMB of Pauling & Corey's 1951 paper on the α-helix. They postulated 3.5 amino-acid residues per turn, 1.5 Å per residue and consequently 5.41 Å per turn. This sparked off ideas in Max Perutz's mind. Max rushed to examine some x-ray photographs and to carry out some additional experiments. These endeavours convinced him that haemoglobin had a α-helical structure. As it turns out, haemoglobin and myoglobin have a larger proportion of α-helix along the peptide chains than most proteins. This was a fortunate coincidence that helped to encourage further work on the elucidation of this and other protein structures. My interest in solution thermodynamics led me to postulate among my Cambridge friends that a protein molecule would not have a completely rigid structure in an aqueous environment. Francis Crick called this 'Freddie's red herring'. However his optimism and confidence in me also made him write during 1952 'Freddie, when you come back (from the United States) next autumn we shall know the structure of proteins and then we can solve the problem of enzyme mechanisms' (I am known as 'Freddie' by all my friends).

From 1952 to 1955 I spent three periods totalling 18 months at Yale under the influence of Jack Kirkwood and Julian Sturtevant, while keeping my appointment in Cambridge. The Yale Chemistry Department was a marvellous place for improving one's education in solution physical chemistry through contacts with Lars Onsager and Herbert Harned, in addition to my sponsors. My later contributions benefited enormously through the experience gained from working with Julian Sturtevant, an outstanding experimentalist who was still running a laboratory in his late 80s. However, my interests shifted gradually from equilibrium thermodynamic to kinetic properties of protein molecules. Visits to centres of research in Enzymology in the USA taught me a lot about enzyme specificity and their ubiquitous role in intermediary metabolism. However, it also convinced me that steady state kinetics provides only limited information. For an insight into the molecular mechanism of enzyme catalysis the theory and practice of transient kinetics developed by Britton Chance in Philadelphia had to be expanded to a wider range of investigations into the kinetics of reactions of proteins. This subject remained my interest for the next 40 years and I documented its expanding applications in three books. The last of these 'Kinetics for the Life Sciences' [1] emphasises the application of transient kinetics to my later interests in the molecular physiology of muscle contraction and vision as well as to enzyme catalysis. In this essay I shall skip over a period of about five years when I worked on intra-cellular kinetics in a research institute run by the Agricultural Research Council.
From 1965, when David Phillips resolved the first structure of an enzyme (Lysozyme), elucidation of detailed reaction mechanisms became a healthy competition between kineticists and crystallographers. Until 1965 my work on enzyme mechanisms was carried out with pure crystalline proteins that could be bought in a bottle. From 1965 my studies were extended to pure proteins prepared in my laboratory. Early work on enzyme mechanisms was restricted, apart from Chance's work on haemproteins and his classical work on alcohol dehydrogenase, to the study of hydrolysis mechanisms. My own early development of methods for the elucidation and characterisation of reaction intermediates was carried out with chymotrypsin as a model. It was a very popular model at that time and much of my work was duplicated and later published again in the USA.
Two fortunate events made major contributions to my success in science. The first was that I lived in Cambridge during the 1950s and the second that outstanding students and research fellows joined me when I set up the Molecular Enzymology Laboratory in Bristol in 1965. All their names are in my list of publications but David Trentham, John Holbrook, Steve Halford and Mike Geeves deserve special mention because they have later set up similar

successful laboratories. I take credit for one major factor in the success of the Molecular Enzymology Laboratory. When I applied for funds to set it up I did not ask for expensive equipment (no six-figure items) but I set up a good workshop and got an electronic engineer and the services of a mechanical engineer. I took the example of my old friend Quentin Gibson, from whom I learned a lot, and I had a lathe next to my office. We could develop techniques that were not available to anybody else. We did not have to worry whether the same experiment was simultaneously performed in another laboratory. We developed novel stopped-flow, continuous flow, pressure jump and calorimetric equipment for designated kinetic experiments. Instrument development was directed towards investigations that provided important general conclusions. The systems studied in detail in our laboratory were glycolytic enzymes with special attention to NAD linked dehydrogenases, reactions of myosin-ATPase and interactions of other muscle proteins and reactions of rhodopsin. Between 1968 and 1972 studies on the subunit dissociation of haemoglobin and linkage with ligand binding were also in progress.

This is not the place to describe all the detail we obtained of reaction intermediates in enzyme turnover. However, I would like to answer the question: 'what novel principles have my colleagues and I added to what was known after the publication of JBS Haldane's book 'Enzymes' in 1930' [2]. We have characterised experimentally the intermediates postulated by the Haldane equation. 'On Enzyme' equilibria between enzyme-substrate and enzyme-product complexes were first identified and characterised in our laboratory with ATPases and NAD linked reactions. These results are linked to the fact that, unlike in classical models, in many enzyme reactions the chemistry is not rate limiting. Protein conformation changes controlling product release are often the slowest step and significant concentrations of prior intermediates accumulate during turnover and can be characterised. Conformation changes involved in product release are often the reversal of those introduced by substrate binding. The tight binding (slow release) of reactants, or ligands that modulate reactions, is now known to be significantly due to the conformational mobility of proteins. These are some of the lessons learned from the combined efforts of our kinetic studies and from the structural evidence, obtained in other laboratories. I was fortunate in that our achievements were recognised by my election to the Fellowship of the Royal Society in 1981.

David Trentham and I presented some of the above conclusions at a CIBA Symposium in honour of Fritz Lipmann at the occasion of his 75th birthday in 1974 [3]. During the discussion Saul Roseman expressed the views of several distinguished biochemists present when he asked the following question, combined with a comment: 'How does this elegant kinetic study relate to what we biochemists call the Michaelis constant of the enzyme reaction … in these reactions, the rate limiting step is the final dissociation, which is not the assumption a biochemist normally makes when talking in terms of Km'.

At my 80th birthday party Hugh Huxley asked me 'Freddie have you ever worked in only one place at a time?'. My answer was 'hardly ever during the last 50 years' and even before that I was connected with three departments in Cambridge. My collaborations with Julian Sturtevant at Yale, with Britton Chance in Philadelphia and with Sidney Bernhard in Eugene, Oregon gave me essential support before I moved to Bristol. During the 1960s I had very close connections with Manfred Eigen and Leo de Maeyer at the Max Planck Institute in Göttingen. This had a considerable intellectual influence on me and on the colleagues who helped to set up the Molecular Enzymology Laboratory. During the last 30 years the Max Planck Institute für Medizinische Forschung in Heidelberg was my second home and I still value the External Scientific Membership of the Max Planck Society. Ken Holmes and Roger Goody (now in Dortmund) have filled considerable gaps in my education.

For ten years after my official retirement from Bristol University in 1986 I maintained a base in the Biochemistry Department and spent some time as a Fogarty Scholar at NIH. During that time I wrote 'Kinetics for the Life Sciences' [1] and made some algebraic contributions to the simulation of postulated kinetic mechanisms. Having now finally retired to rural Oxfordshire I am continuing to help old colleagues and friends with advice on simulation projects. Like many retired people I find the ever-increasing power of desktop computers some recompense for the loss of secretarial and other assistance.

Many other friends and colleagues had considerable influence on my work and I hope they will accept this summary appreciation. However, it is a pleasure to record my admiration for John Edsall, who died recently in his 100th year. It was a great experience to write the monograph 'Biothermodynamics' [4] with him during the years 1980–1982. He was a true scholar and a wonderful human being.

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3 Roseman S. (1975) Energy Transformation in Biological Systems, CIBA Foundation Symposium 31 p. 82, Elsevier, Amsterdam
4 Edsall J. T. and Gutfreund H. (1980) Bio-thermodynamics. John Wiley & Sons, Chichester, UK