Recalling His Contribution to the Crystallography of Vitamin B_{12}

I met the remarkable Dr Frank Moore in 1965 on commencing a research fellowship at UKAERE Harwell where I joined the Neutron Diffraction Group within the Ceramics Division under group leader Dr Terry Willis. I was fortunate to share an office with Frank and so began a life-long friendship. Frank was based at Harwell while doing a DPhil at Oxford University under the supervision of Professor Dorothy Hodgkin OM (Nobel Laureate, 1964). He was one of the many young international scientists who had travelled to Oxford to work with Dorothy. Frank is remembered especially for his doctoral work during the initial neutron diffraction investigation of a vitamin B12 (cyanocobalamin) derivative which at the time was the largest molecule imaged at near atomic resolution using neutrons. This important study was enabled by three considerations: first and foremost, Dorothy and team's pioneering work using X-ray diffraction to define the structures of insulin and vitamin B12; second, the development of an automated single crystal neutron diffractometer at Harwell by Uli Arndt and Terry; and then the collaboration between Dorothy and Terry on the use of neutron diffraction in biological structural crystallography. Critically important to the success of the neutron work was the prior X-ray diffraction structure determination which had been performed by other members of Dorothy's B12 team: Professsor Siv Ramaseshan (Bangalore), Dr Clive Nockolds (Oxford DPhil student from Perth), and Professors Joyce and Neil Waters (Auckland) [Refs 1-3].

Frank's B12 doctoral work was conducted almost exclusively at Harwell, with occasional trips to Oxford to consult Dorothy. My first recollection of him at Harwell was of a very happy person with a deep love of family and with a unique sense of wry humour which derived from his New Zealand roots. Frank loved the All Blacks and growing vegetables. He soon became very much a personal mentor and to some extent my 'older brother' in science. Frank had travelled to Harwell from New Zealand in 1959 to take up a research fellowship at Harwell, following which (1962-65) he worked under Dorothy's supervision for his DPhil at Oxford, plus a further year of post-doctoral research, on the neutron diffraction study of B12. He remembered with pride his Oxford DPhil examination viva with the father of neutron diffraction, Professor George Bacon of Sheffield, which was largely a conversation on the exciting prospects for neutron diffraction in biology.

The B12 neutron diffraction study was a landmark piece of research which pushed neutron diffraction to the very limits of its then capabilities for large molecule crystallographic research. The critical test for the neutron study was whether the B12 oxygen and nitrogen atoms in the amide/acid side chains could be differentiated in such a large molecule, as well as locating the hydrogen atoms. The definitive publication for the study was finally published in 1984 after two decades of patient and exhaustive data analysis [Refs 4-5]. The B12 study was conducted on the predominant propionic acid derivative produced by mild acid hydrolysis of vitamin B12, with the expectation from biochemical evidence, that the propionic acid group terminated the e-chain. Neutron diffraction was chosen to identify which of the three B12 propionamide groups had been converted to a carboxylic acid group by mild hydrolysis. This was very challenging for such a large molecule (formula $C_{63}H_{87}O_{15}N_{13}PCo.16H_2O$) comprising 228 atoms [Figure 1], with the aim being to define the entire molecule at atomic resolution. The study was conducted in two stages – the initial low resolution study (dmin = 1.3 Å) for which the data were measured and analysed by Frank, and subsequently a higher resolution study (dmin = 1.0 Å) for which the data were measured by Brian O'Connor and then analysed by Brian with Frank's support. The neutron data were acquired at the Pluto reactor using the 'automated' Ferranti Mk I instrument [Ref 6]. Data measurement was daunting by today's standards given the weak neutron flux and the high backgrounds from hydrogen incoherent scatter. Measurement of each reflection involved 30 minutes of scanning to define the Bragg peak and also the background either side of the peak. A staggering 2,500 hours of beam time were used for each data set. While the

instrument was automated, it was primitive by today's standards, especially the use of fragile paper tape to set the goniometer axes for each reflection.

Data analysis for the low resolution study was confined to using Fourier summations (neutron scattering density and difference maps) to define the atom positions, starting with the XRD structure. In retrospect, the task was daunting given the number of free parameters (*ca.* 900) versus the number of measureable reflections (*ca.* 1650), and this was made more challenging by series termination effects in the maps which were more severe than those normally experienced in X-ray diffraction due to the non-angular dependence of the neutron scattering factors. Figure 4 shows part of a Fourier summation for the low-angle data versus the superior definition subsequently observed with the high resolution data. Unfortunately, the series termination problem led to misidentification of the propionic acid group in the initial publication due to 'diffraction ripple' effects in the Fourier maps [Ref 7].

Atom definition in the Fourier maps constructed with Frank and Brian's merged data (*ca.* 3,000 observable reflections) clearly showed resolution of the atoms, with the surprising result that the propionic acid group terminated the b-chain rather than the e-chain which had been concluded from the low resolution work. Also, definitive interpretation was complicated by the e-chain termination being disordered. Then began some 15 years of work in proof-testing the revised model with Frank having moved to Sydney and Brian back to Perth. Importantly, Brian O'Connor ran extensive least squares calculations to optimise the positional and thermal parameters using a customised block-diagonal program written at the University of Western Australia. Optimisation of the thermal parameters was critically important in differentiating the oxygen and nitrogen sites for the amide/acid side chains. Subsequently, Frank spent many hours in examining the final difference maps to define the disorder in the propionamide e-chain termination. A relatively recent postscript to the study came in 2007 when Spingler *et al.* [Ref 8] published a synchrotron radiation study of B12 derivatives produced by mild acid hydrolysis. The work confirmed that, under mild acid hydrolysis, the acid group is most likely to form at the b-position.

Notwithstanding Frank's B12 work, an even greater contribution to neutron scattering science was his subsequent dedicated service over some 20 years as a senior professional officer with the Australian Institute of Nuclear Science and Engineering (AINSE) at Lucas Heights (1967-86). Frank worked tirelessly in developing and then delivering neutron diffraction capabilities for the Australian university research community, and his service was much valued by many university research staff and PhD students using the HIFAR reactor neutron scattering instruments. As a Queen Elizabeth II Research Fellow during 1968 - 70, I was fortunate to widen my collaboration with Frank in studies of electron density distributions in small molecules. As neutron diffraction using HIFAR became more challenging as the reactor neared the end of its competitive life, Frank was unswerving in his advocacy for a replacement reactor. His dream was eventually fulfilled in 2007 when the new OPAL research reactor was officially opened at Lucas Heights 20 years after Frank had departed AINSE. It was wonderful to see Frank at the official opening. Some of Frank's contributions at AINSE are recorded in the AINSE 50th anniversary history [Ref 9]

Brian O'Connor

Professor Emeritus, Curtin University

And

President Emeritus, Australian Institute of Nuclear Science and Engineering

References

[1] Nockolds, C K, Ramaseshan, S, Waters, T N M, Waters, J M Waters and Hodgkin, D C (1967). *Structure of a monocarboxylic acid derivative of Vitamin B12 – crystal and molecular structure from x-ray analysis*. Nature, 214, 129-130.

[2] Nockolds, C E and Ramseshan, S (1984). *X-ray and neutron diffraction studies of the crystal and molecular structure of the predominant monocarboxylic acid produced by the mild acid hydrolysis of cyanocobalamin. Part I. X-ray diffraction studies of air-dried crystals.* Journal of Chemical Sciences. 93, 195-196.

[3] Waters, J M and Waters T N M (1984). *X-ray and neutron diffraction studies of the crystal and molecular structure of the predominant monocarboxylic acid produced by the mild acid hydrolysis of cyanocobalamin. Part II. X-ray diffraction studies of wet crystals.* Journal of Chemical Sciences. 93, 195-196.

[4] Hodgkin, D C (1984). *X-ray and neutron diffraction studies of the crystal and molecular structure of the predominant monocarboxylic acid produced by the mild acid hydrolysis of cyanocobalamin. Outline.* Journal of Chemical Sciences. 93, 195-196.

[5] F.H. Moore, B.H. O'Connor, B.T.M. Willis and D.C. Hodgkin (1984). *X-ray and neutron diffraction studies of the crystal and molecular structure of the predominant monocarboxylic acid produced by the mild acid hydrolysis of cyanocobalamin. Part III. Neutron diffraction studies of wet crystals.* Journal of Chemical Sciences. 93, 235-260.

[6] Arndt, U.W. & Willis, B.T.M. (1963). *Automatic neutron diffractometer for three-dimensional structure-factor determination.* Review of Scientific Instruments, **34**, 224-230.

[7] Moore, F H, Willis, B T M and Hodgkin, D C (1967). *Structure of a monocarboxylic acid derivative of vitamin B12 – crystal and molecular structure from neutron diffraction analysis*. Nature, 214, 129-133.

[8] Spingler, B, Mundwiler, S, Ruiz-Sanchez, P, van Stavaren, D R and Alberto, R (2007). *Structures of the b-and d-acid derivatives of vitamin B12 and their complexes with [M(CO)3]+ (M = 99mTc, Re)*. European Journal of Inorganic Chemistry, 2007, 2641-2647.

[9] O'Connor, B H, Chivas, A R, Mather, D W, Studdert, J D C and Binnie, A. (2008). *AINSE – an institute for research and training excellence in nuclear science: The First 50 Years*. Pegasus

Figure Captions [reproduced from reference 5]

Figure 1

(Above). Structure found for B12 monoacid showing the amide/acid side chains.

(Below). Perspective view of the molecule highlighting the propionic acid group termination for the b-chain and the disordered propionamide group for the e-chain.

Figure 2. Projection views of the neutron scattering density for the final model. First contour at 2.5f/Å3; contour interval 2.5f/Å3.

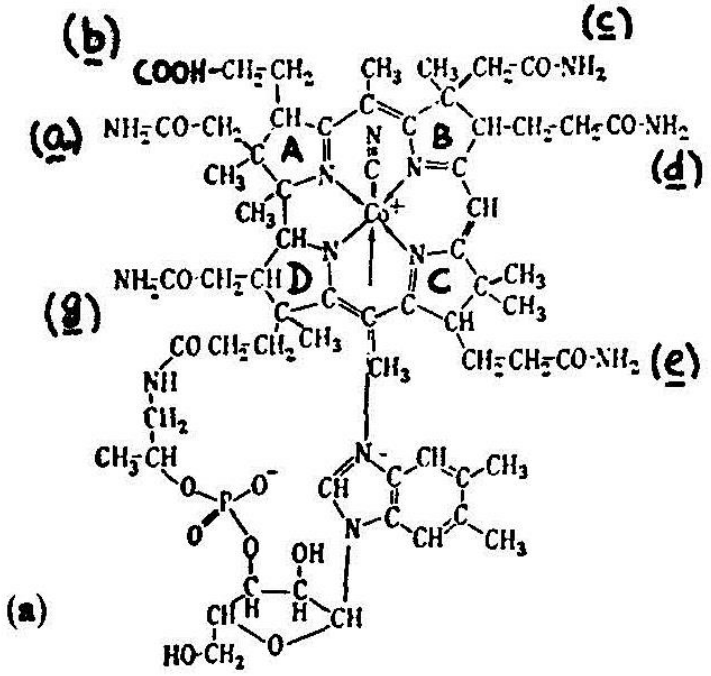
(Left) Corrin nucleus. (Right) Propanolamine, nucleotide and cyanide groups projected on the mean plane of the benzimidazole ring.

Figure 3. Neutron scattering density in the best plane for the a-acetamide side chain. First contour at 1.0f/Å3; contour interval 1.0f/Å3.

(Left) High resolution: d-spacing limit = 1.0 Å. (Right) Low-resolution: d-spacing limit = 1.3Å.

Figure 4. Fourier summation projected on the plane of the b-chain propionic acid group showing the hydrogen bonding links.

FIGURE 1


(b)
(c)
(a)
(d)
(g)
(e)
(a)
A
B
C
D
Co+

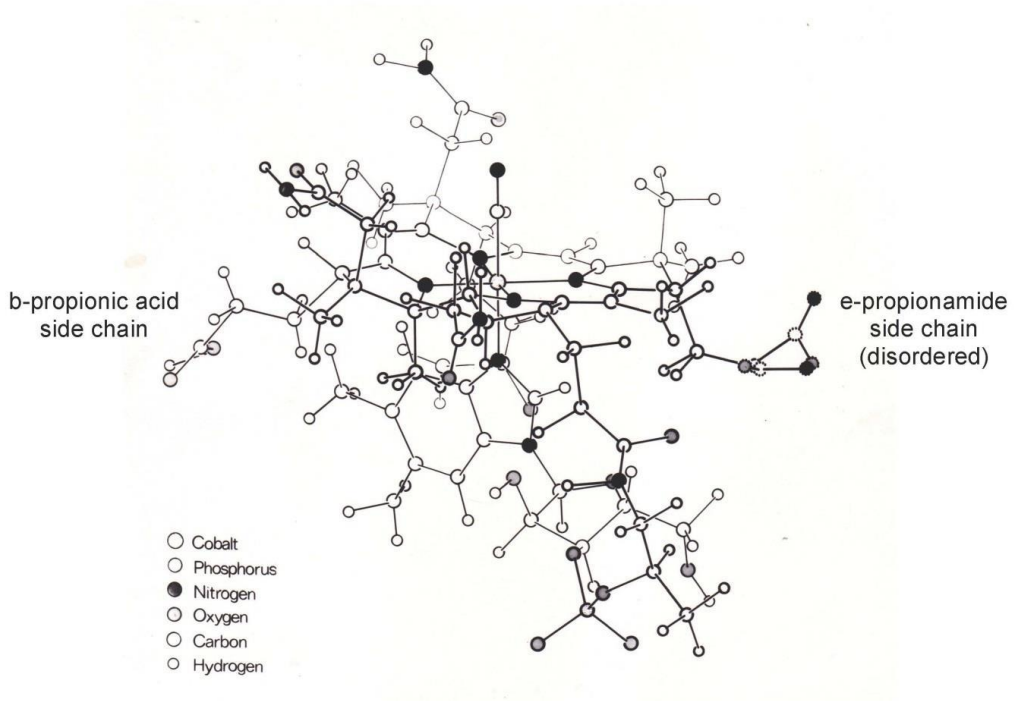

b-propionic acid
side chain
e-propionamide
side chain
(disordered)
Cobalt
Phosphorus
Nitrogen
Oxygen
Carbon
Hydrogen

FIGURE 2

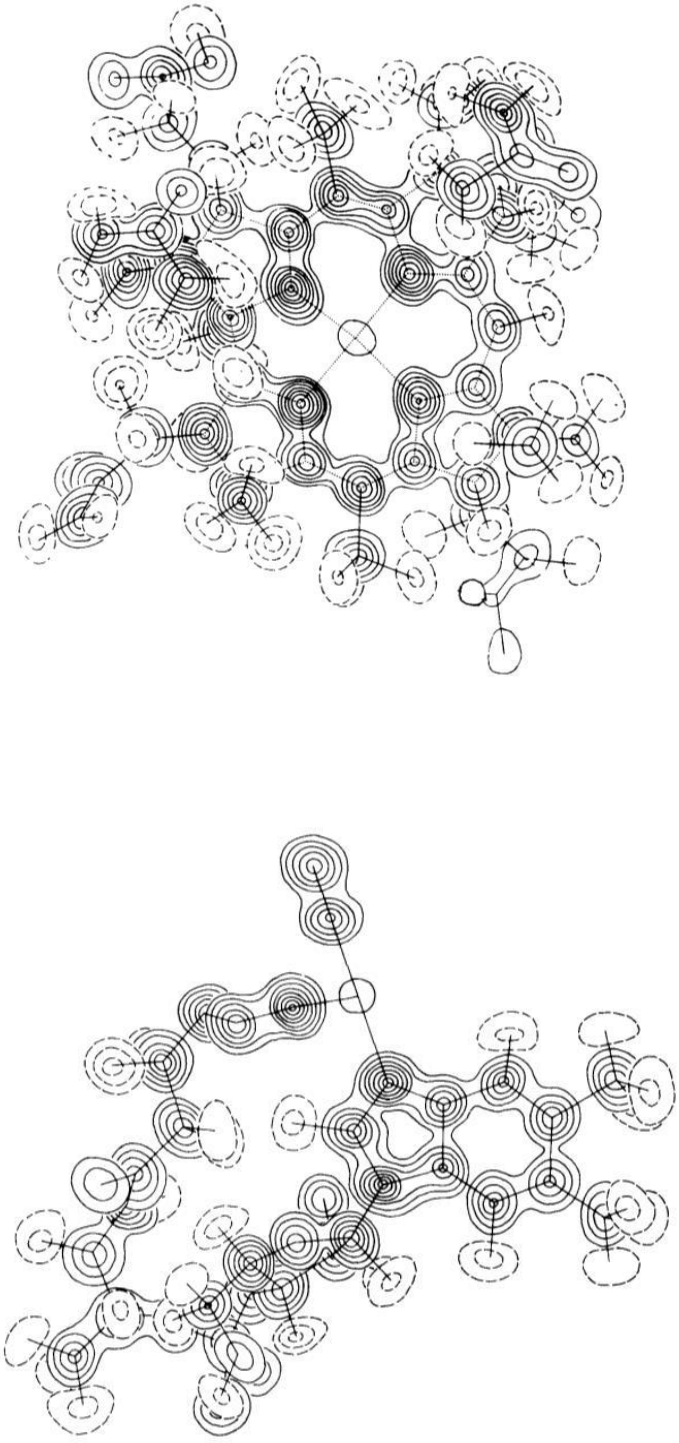


FIGURE 3

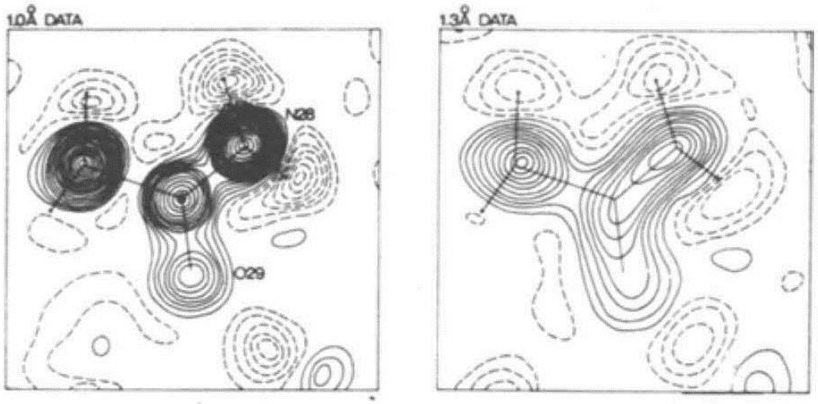


FIGURE 4