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Robert Donald Bruce Fraser 1924–2019

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Robert Donald Bruce (Bruce) Fraser was a biophysicist who gained world-wide distinction for his extensive structural studies of fibrous proteins. Bruce began a part-time BSc degree at Birkbeck College, London, while working as a laboratory assistant. In 1942, aged 18, he interrupted his studies and volunteered for training as a pilot in the Royal Air Force (RAF). He was sent to the Union of South Africa and was selected for instructor training, specialising in teaching pilot navigation. At the end of the war he completed his BSc at King's College, London, and followed this with a PhD. Bruce studied the structure of biological molecules, including DNA, using infra-red micro-spectroscopy in the Biophysics Unit at King's led by physicist J. T. Randall FRS. During that time Bruce built a structure for DNA that was close to the Watson-Crick structure that gained them and Maurice Wilkins at Kings College, the Nobel Prize in 1962. In 1952, he immigrated to Australia with his family to a position in the newly formed Wool Textile Research Laboratories at the Commonwealth Scientific and Industrial Research Organisation (CSIRO). Here, Bruce established a biophysics group for research on the structure of wool and other fibrous proteins that flourished until his retirement. Over that period he was internationally recognized as the pre-eminent fibrous protein structuralist world-wide. Having been acting chief, Bruce was subsequently appointed chief of the Division of Protein Chemistry and he remained in that role until he took retirement in 1987.

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Early days, family and education

Bruce Fraser (Fig. 1) was born on 14 August 1924 to parents Maggie née Wilson and Donald Fraser on a farm in Ickenham centred on an old village in Greater London. He was their only son but Bruce had two younger sisters—Margaret and Janet. They grew up in their family home in Harrow. Bruce's father worked for the Metropolitan Railways, and the Depression caused severe hardship for the young family until his father obtained a position with Eastman Kodak. This resulted in a significant improvement in their circumstances. Bruce's first school was considered to be very 'rough', because many of the pupils were from severely deprived families. He reflected on the fact that one of his friends had shoes riddled with holes, and that his family was so poor that they could not afford to have them repaired. Bruce left that school aged eleven but, crucially, he won a fees' scholarship to Harrow Weald County School, fees that his parents would have been unable to afford themselves. It was there that his passion for science was generated, especially in physics and mathematics, and this was largely a consequence of the excellent teachers available. After he had completed secondary school he needed funds to be able to continue his studies so he took a job as a laboratory assistant at Kodak, the same place as where his father worked. This was a significant step because Kodak had an enlightened scheme of providing support for technical employees to undertake further part-time studies. These were generally undertaken at Birkbeck College, University of London because this was the only college in London that offered part-time degrees. He was accepted into the BSc course and soon found that, because of the

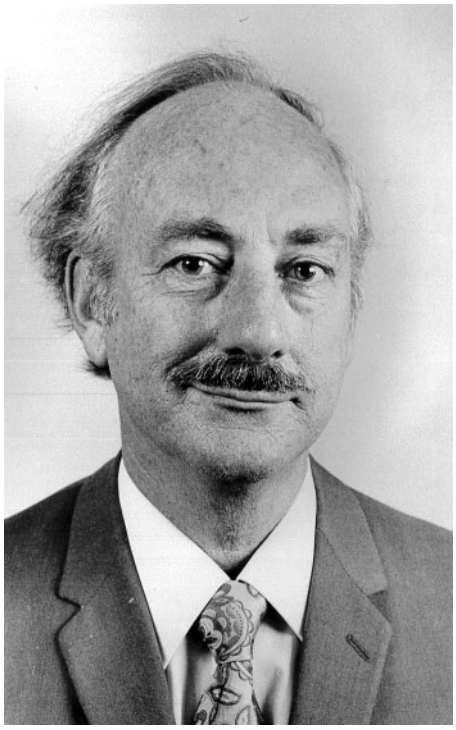

Fig. 1. Bruce Fraser, 1974 (Fraser family collection, reproduced with the permission of the family).

war, the teachers were older than normal. Nonetheless, they were experienced and excellent lecturers, one of whom was the distinguished Professor of Physics, J. D. Bernal.[1] Bruce recalled being inspired with his lectures and Bernal's description of the sine wave as the basis of much of physics. At the end of his first year Bruce sat and passed the Intermediate Examination at Birkbeck College in physics, chemistry, and pure and applied mathematics. At that stage (1942) Bruce was 18 years of age and able to enlist in the armed forces. Consequently, he decided to interrupt his studies and, as there was a shortage of pilots, he volunteered for flying duties with the Royal Air Force (RAF).

War service in the RAF

Bruce quickly found himself in uniform and after five months of pilot training he was sent to the Union of South Africa. His level of education in mathematics and physics was such that he was chosen to teach flying, navigation and meteorology to other recruits. He was appointed as a flight instructor, rising to Flight Lieutenant, and the pilots he trained in navigation later saw service in bomber command in the European sector. Like many others, Bruce suffered for the rest of his life from 'survivor guilt' since many of the friends and colleagues that he trained had not survived the conflict. After three years of service the war came to an end and Bruce returned to his studies supported by the British Government's post-war education scheme for ex-servicemen and women. It is of interest to note that Bruce retained his affinity for flying during and after his research years. He flew various small aircraft sometimes with two similarly minded ex-RAF CSIRO colleagues and that continued well into his retirement.

Kings College education and DNA research

Bruce continued his BSc at King's College on the Strand in London. The head of physics was Professor John Randall FRS FRSE (famed for the magnetron and radar) and he suggested to Bruce that he should take the special course in physics and mathematics that would give him the chance of making up for lost time. This meant taking the second and third years together. It was a very intense period but nevertheless, in 1948, he achieved first class honours. In the following year he took biology as a single subject, almost a prerequisite for his future in the Biophysics Unit at King's College. Professor Randall, who had established this unique research centre, diverted its research in 1949 to solving the structure of DNA. He firmly believed that DNA held the secret of the genetic code. Bruce's PhD research studies on biological molecules began at that time and his supervisor was the distinguished spectroscopist W. C. Price. Together they pursued the technique of infra-red micro-spectrometry, and this included an investigation of the spectra of DNA. The person in charge of the DNA team was Maurice H. F. Wilkins and included the PhD student Raymond Gosling. Together they used X-ray diffraction as the major tool in their studies. Their diffraction work was markedly advanced because Wilkins had obtained the highest quality DNA in 1950 from Rudolf Signer, a polymer chemist in Switzerland who had isolated it and provided minute samples to other scientists. Wilkins observed that the viscous DNA solution could be stretched, thereby allowing superb X-ray diffraction patterns to be obtained. These revealed a more crystalline form with helical symmetry. Unfortunately, Randall disrupted the DNA group when WiIkins was absent from the unit and transferred the research effort and the sample of Signer DNA to Rosalind Franklin. Raymond Gosling was also transferred to Franklin's supervision. Bruce found himself surrounded by this unfortunate conflict of scientific personalities. In 1951, he considered some ideas of DNA structure, being well aware of the publication of a DNA model by the chemist Linus Pauling. In that structure Pauling had proposed a triple helix but had mistakenly placed the negatively charged phosphate groups on the inside and the bases on the outside of the helix. Bruce temporally ceased his research to construct a new structure for DNA using Wilkins's X-ray results, his own spectroscopic data and the concept that the phosphate groups must lie on the outside of the molecule, where they could be hydrated. His model was a polynucleotide molecule with the pyrimidine and purine bases hydrogen-bonded and stacked in the centre and the phosphate groups on the outside, all of which were essential features of the subsequent Watson-Crick structure published in 1953 and for which the Nobel Prize was awarded in 1962 to James Watson, Francis Crick and Maurice Wilkins. Bruce's model was close but, unfortunately, he had adopted three chains for his model instead of two because both Franklin and Wilkins had insisted that the density measurements were consistent only with a three-chain structure. Bruce wrote up his model for publication in *Nature* with the idea that it would be published back to back with the Watson-Crick structure. Unfortunately, the Biophysics Unit was slow in supporting his structure and the paper was not submitted. However, in 2004, the year after the fiftieth anniversary of the Watson-Crick paper, Bruce's paper resurfaced from material discovered by Maurice Wilkins and it was then finally published in the *Journal of Structural Biology*.[2]

DNA, marriage and move to Australia

Since no more Signer DNA for on-going research was available for the King's team, a biochemist was appointed to prepare high quality DNA. Mary Jean Nicholls, a PhD graduate in biochemistry from Birmingham, was appointed to carry out the work using Signer's published protocol. Mary produced pure high molecular weight DNA but was disappointed because she was unable to achieve preparations that could be drawn out and stretched to produce the best diffraction patterns. It was suspected that there was a missing step in the published Signer protocol although a more likely reason could have been that the DNA had breaks in the chain caused by the lack of access to a cold-room during the isolation of the DNA. During this period Bruce and Mary formed a close friendship and they were subsequently married on 10 June 1950. They continued their research in the Biophysics Unit, supported by a Medical Research Council grant. Mary also collaborated with Bruce in the study of the DNA structure using infra-red dichroism techniques.

In 1952, Bruce and Mary had their first child. Salaries and research funding then prevailing in Britain for young scientists were very limited and they soon realized there were difficulties ahead in

[1] Hodgkin (1980).
[2] Fraser (2004).

supporting a family. This led Bruce to seriously consider moving elsewhere. He decided to immigrate to Australia and applied for a position with CSIRO's Biochemistry Unit of the Wool Textile Research Laboratories in Melbourne. His application was accepted and, together with Mary and their first-born child Susan, they travelled by ship to Melbourne arriving in late 1952. Thus began thirty-five years of highly productive research on wool and fibrous proteins in an Australian environment to which he readily adapted.

The Biochemistry Unit and the fibrous proteins structure group

Bruce started at the Biochemistry Unit in Parkville, Melbourne when the unit was in the process of developing a team with the necessary broad range of skills. Bruce's biophysical expertise provided an important balance to the unit, which was funded by the wool industry and federal government and headed by Dr F. Gordon Lennox.[3] In the first year or so, Bruce began studies of wool structure using his infra-red micro-spectroscopy techniques and he also initiated some collaborative light microscope investigations of the wool fibre structure using gold shadow-casting to improve the morphological detail. Another study that he undertook was on the bilateral organisation of the cortical cells in fine wool fibres less than 20 μm in diameter. Approximately half of the cortex is penetrated more rapidly by chemicals and dyestuffs and was called the orthocortex. The less penetrated region was termed the paracortex. The difference is related to the curliness of the fibre, and it was shown that its origin lay in the wool follicle.

During this period plans were established to understand the structural basis of wool properties at the molecular level. The main thrust was to use low-angle X-ray diffraction to obtain the dimensions of the component filamentous structures in the cortex of fine (20 μm) wool fibres. Bruce set about establishing such a facility with the required equipment and technical know-how that could also be applied to other keratins and fibrous protein structures. An important step was the appointment of Thomas (Tom) Perry MacRae in 1954. During the war, Tom also saw service in the RAF, as a navigator. After the war he graduated MSc in biophysics from the Bradford Technical College. The college did not have a PhD program. Tom's appointment was ideal because his MSc research training was in the operation and theory of X-ray diffraction equipment and he also had post-graduate experience with research on wool at Bradford. He became a vital colleague and friend with Bruce and their joint investigations flourished over thirty-two years.

X-ray crystallography and electron microscopy

The early work with low-angle X-ray diffraction involved improving the resolution of the X-ray patterns because the keratins are inherently weak diffractors. The technical task undertaken by Bruce and Tom was the successful but tedious redesigning of the diffraction-recording X-ray cameras. The other improvement of efficiency was in the digital interpretation of the reflections. An early result of the high-resolution data they obtained was that the axial repeat for α-keratin was indexed on a period of 47 nm instead of the 19.8 nm repeat originally described by researchers at Leeds University.[4] Another finding was on the fibrous protein, tendon collagen, in which Bruce described an assembly of collagen fibrils that would allow some elasticity in an otherwise inelastic structure.

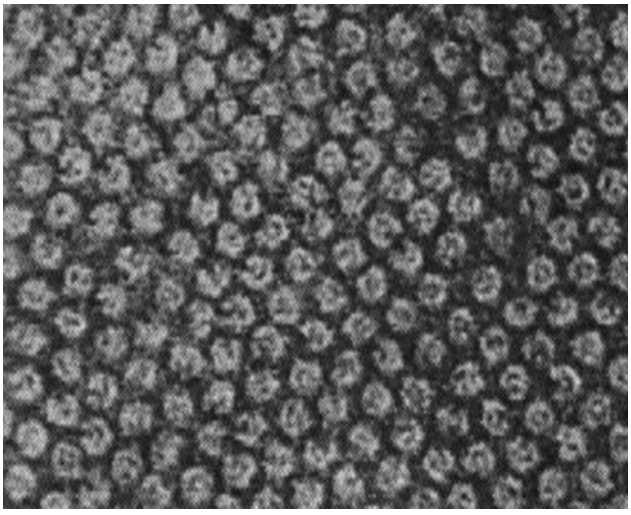

Fig. 2. Transmission electron microscope image of a cross-section of a wool fibre. The fine structure of approx 7 nm diameter filaments (intermediate filaments, IF) are in a quasi-hexagonal array and show some internal structure. The filaments are surrounded by matrix proteins with increased contrast produced by partial reduction of disulphide bonds and reacted with osmium tetroxide and lead hydroxide stains. Reprinted from Fraser and others (2003*a*), with permission from Elsevier.

As the studies of keratin progressed it was recognized that transmission electron microscopy (TEM) was needed, together with ancillary techniques for investigating biological material. The information from TEM would integrate with the X-ray results and with the increasing findings on the protein constituents of wool by protein chemists in the Biochemistry Unit. By 1957, the latest high-resolution Siemens TEM had been installed and thereafter new images from ultra-thin sections of wool began to provide new information on the organisation of filaments and matrix in the wool fibres. This advance was achieved by increasing the contrast in the TEM images by introducing heavy metals into the wool fibre to obtain adequate electron scattering. The heavy metal staining was enormously improved by reduction of disulphide bonds to sulphydryls. Dr Hugh Lindley, a protein chemist in the Wool Laboratories, had published chemical conditions for the partial reduction of the disulphide groups in the fibre cortex. It was thought that this fraction might be in the proteins of the fibre matrix and would bind preferentially more heavy metals. Using these conditions this indeed turned out to be the case as demonstrated by the production of the high-resolution images such as shown here.[5] This image was obtained in 1961 (Fig. 2).

[3] Rivett and others (1996) pp. 1–372.
[4] Fraser and MacRae (1971).
[5] Rogers (1959). Filshie and Rogers (1961).

Fig. 3. Reunion of the structural group in the X-ray lab in 1982. From left. Andrew Miller, David Parry, Barbara Brodsky—Rutgers University, Bruce, Tom MacRae and Eikichi Suzuki. Reprinted from Parry (2014), with permission from Elsevier.

The image revealed filaments about 7 nm in diameter (intermediate filaments, IF) surrounded by a denser matrix. The filaments were organized in a regular manner with quasi-hexagonal close-packing. Furthermore, the images revealed more information about the bilateral organisation of the cortex. The most regular packing of the filaments (later shown to belong to the large, intermediate filament family of cytoskeletal proteins) lay in the paracortex. The filaments of the orthocortex were differently organized and found to be twisted and packaged into larger clusters called macrofibrils. The amount of matrix between filaments was larger than in the paracortex. Potential mechanisms by which macrofibril assembly proceeded in vivo were subsequently modelled by Bruce and David Parry.[6]

Around this time several post-doctoral fellows arrived and these were to make significant contributions over their time with Bruce and Tom. The first in 1962, was Andrew Miller from the University of Edinburgh, who trained as a physical chemist but who had expertise in X-ray diffraction and the use of computers to analyse diffraction data. He became more of a biophysicist as a result of his work with Bruce. He was followed in 1966 by David Parry, from King's College, London who also had expertise in X-ray diffraction studies of fibrous proteins, including muscle. Both of these scientists later became distinguished researchers in their own rights and remained Bruce's life-long friends. A Japanese scientist Eikichi Suzuki also joined the group as a post-doctoral fellow and later as a permanent member of staff. In addition, a new physics graduate Barry Filshie was appointed to assist in the operation of the TEM facility.

The past years of the X-ray diffraction group were remembered in a special moment in 1982 when Andrew and David visited Melbourne and met up with Bruce and Tom in the diffraction laboratory (Fig. 3).

Bruce's contributions to knowledge of the structure of fibrous proteins

Our understanding of the complex structural hierarchy of wool and other keratins was markedly advanced by the work of the CSIRO Wool Textile Research Laboratories established in 1949. In turn, the biophysics structural group, formed by Bruce in 1952, was to prove a major contributor to the knowledge of the filamentous component of these important fibril-matrix composites.

When Andrew Miller arrived as a post-doctoral fellow in 1962, his first collaboration with Bruce involved the use of Tom MacRae's X-ray patterns from α-keratin of porcupine quill tip, to decide how many α-helical chains were coiled around each other in the intermediate filament molecules. Was it a two-chain coiled-coil or, as some of the chemists in the CSIRO lab thought, a three-chain coiled-coil? Bruce and Andrew published several papers on how to calculate the diffraction patterns from different molecular models.[7] After a couple of years of scanning and measuring the X-ray patterns they used the IBM computer at CSIRO Clayton, Melbourne, to calculate the patterns for different models. From that they hoped they would be able to deduce whether a two-chain model was feasible.

They took the approach used in X-ray diffraction studies of the 'catch' muscles in molluscs.[8] These authors had measured the axial

[6] Fraser and Parry (2003).
[7] Fraser and others (1964*a*). Fraser and others (1964*b*).
[8] Cohen and Holmes (1963).

distance between near-equatorial reflections to establish that α-helices formed a two-chain coiled-coil in the paramyosin. Bruce, Andrew and Tom worked on analogous reflections from α-keratin. This was difficult because the reflections were not so clearly resolved as those from molluscs because the muscle proteins exist in a low-density aqueous environment whereas in α-keratin, the α-helical molecules are embedded in a high-density sulphur-rich matrix. They worked out the effect of this on the diffraction pattern and made appropriate allowances for it. They then made comparisons of the equatorial regions in the X-ray diffraction patterns from several fibrous proteins—rabbit psoas muscle, oyster muscle, α-porcupine quill and tropomyosin. This strengthened the likelihood of two-chain coiled-coils in α-keratin. Today, cryo-electron microscopy could resolve this issue quite easily. The sulphur-rich matrix also made it tricky to calculate the effects of the fact that in keratin these two-chain α-helices were arranged in the filaments resembling the form of a 9+2 (or similar) structure. This arrangement was to prove very controversial and it took Bruce and David Parry more than fifty years to resolve it unequivocally. Bruce and Andrew worked out the effect of the filament arrangement on the X-ray patterns that Tom MacRae had obtained from porcupine quill. They were also able to explain a strong reflection on the equator at a spacing corresponding to 2.7 nm in terms of the filament dimensions. Many of Bruce's key structural conclusions were derived from fibre X-ray diffraction patterns recorded from highly oriented specimens of trichocyte keratin, mainly porcupine quill tip. These patterns show two characteristic meridional reflections corresponding to spacings of about 0.515 and 0.1485 nm, and an equatorial maximum corresponding to a spacing of about 1 nm. In addition, a strong near-equatorial layer line is observed that corresponds to an axial spacing of about 7 nm (Fig. 4).[9]

These features were characteristic of a coiled-coil rope structure with α-helical strands and a pitch length of about 14 nm. The observations were naturally associated with the ordered portion of the fibre structure, that is the filaments. The X-ray data, based on native specimens, also revealed both the diameters of the intermediate filaments (IF) and their separations. These were correlated with the electron microscope observations using sectioned and treated specimens (see Fig. 2).

Andrew's post-doc ended in 1965 but in 1974–5 he again joined up with Bruce for a further collaboration, initially in the Wool Laboratories. They worked on the arrangement of collagen molecules in fibrils of Type 1 collagen in rat tail tendons using focussing monochromatic X-ray cameras, the technique that Andrew had used in Oxford. He had followed up on a discovery that in the native condition, collagen fibrils yielded many sharp Bragg reflections indicating they were partially crystalline and hence might yield information on their 3-D arrangement.[10] Much clearer data sets had been obtained in Oxford and Melbourne and were subsequently used by Bruce and Andrew in further work. A key paper was published on the digital processing of the fibre diffraction patterns

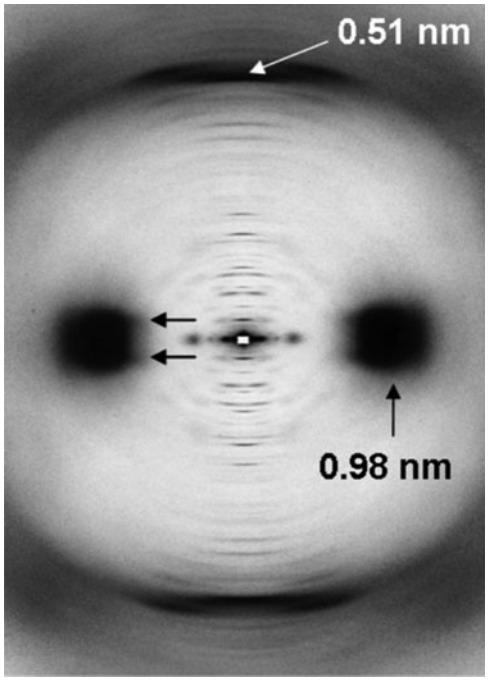

Fig. 4. X-ray diffraction pattern of porcupine quill, a well oriented example of a trichocyte keratin. This shows a meridional reflection that corresponds to a spacing of 0.51 nm and equatorial and near-equatorial maxima that correspond to a lateral spacing of about 0.98 nm. The axial spacing of the near equatorial layer line (indicated by arrows) corresponds to a value of about 7 nm and is a characteristic of a two-stranded coiled coil structure with a pitch length of about 14 nm. Reprinted from Parry and others (2008), with permission from Elsevier.

and the mathematics of extracting relevant data from the diffraction patterns obtained using X-ray cameras of different geometry, to test models of how collagen molecules were packed.[11] Funds were raised from the Royal Society, the European Molecular Biology Organisation (EMBO) and the European Molecular Biology Laboratory (EMBL) that enabled Bruce to spend more time with Andrew working on collagen, in Oxford, Grenoble, Edinburgh and Stirling. Since 1968, it had been widely accepted that collagen molecules in tendons packed to form microfibrils that might then be arranged in a tetragonal array.[12] Bruce, Andrew and David Parry suggested various arrangements. In 1979, David Hulmes and Andrew Miller proposed that collagen molecules were arranged in a quasi-hexagonal array,[13] because this gave a better fit with both the X-ray diffraction patterns and the density of collagen fibrils. Bruce accepted this solution in 1980 and he and Andrew, together with their colleagues, showed that the collagen molecules were kinked at the edge of the overlap and gap regions in the fibrils.[14] This collaboration continued until 1987.

[9] Fraser and others (1964*c*). Fraser and others (1965).
[10] North and others (1954).
[11] Fraser and others (1976).
[12] Smith (1968).
[13] Hulmes and Miller (1979).
[14] Fraser and others (1983). Miller (1984).

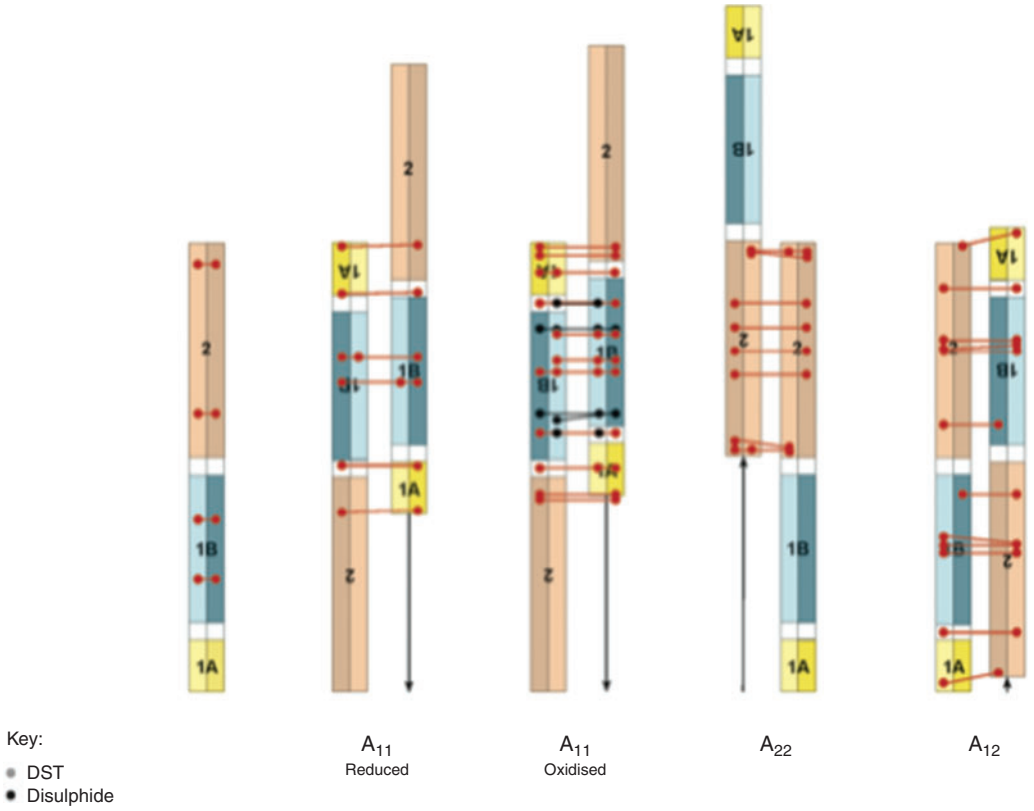

Fig. 5. Schematic representation of the induced crosslinks (red) characterized in (from left to right) a trichocyte keratin molecule, a pair of antiparallel molecules in the A_{11} mode (reduced) and then after molecular slippage has occurred (oxidized), a pair of antiparallel molecules in the A_{22} mode (reduced and oxidized), and a pair of antiparallel molecules in the A_{12} mode (reduced and oxidized). These data confirm that the molecule is a heterodimer and that the chains lie in axial register and are parallel (rather than antiparallel) to one another. Reprinted from Fraser and Parry (2018*b*), with permission from Springer Nature.

In the meantime, sequences of wool keratin proteins by Crewther and colleagues had revealed two homologous families of IF proteins. They were designated Type I and Type II chains and could be subdivided into an N-terminal (head) domain, an α-helix-rich, heptad repeat-containing central domain, and a C-terminal (tail) domain. The heptad regions encompassed about 284 residues (segments 1A, 1B and 2) and were joined by short linkers. Strong evidence was subsequently presented that the trichocyte keratin molecule was a Type I/Type II heterodimer about 46 nm long and that the chains lay parallel to one another (rather than antiparallel) and were in axial register. Three modes of molecular assembly were subsequently termed A_{11}, A_{22} and A_{12} by Steinert and Parry and were defined as (a) an antiparallel overlap of a pair of 1B segments, (b) an antiparallel overlap of a pair of 2 segments, and (c) an antiparallel overlap of a pair of molecules.[15]

Experimental proof came through the characterization of the chemical crosslinks induced between spatially adjacent lysine residues in intact IF. This approach was used successfully to mimic the initial formation of the IF in the hair follicle in a reducing environment, and in the fully developed (disulphide-bonded) oxidized form of the hair as it emerges from the follicle (Fig. 5).[16] In following up these results, Bruce developed a least-squares program that revealed that the A_{12} and A_{22} staggers were near identical for the reduced and oxidized structures (i.e. about $+2\ h_{cc}$ and $+187\ h_{cc}$ respectively, where h_{cc} is the mean axial rise per residue in a coiled coil conformation), but that the A_{11} stagger differed considerably from about $-112\ h_{cc}$ (reduced) to about $-131\ h_{cc}$ (oxidized).[17] The observation that hair keratin can adopt not one but two structurally distinct conformations, one formed in the living cells at the base of the hair follicle in a reducing environment and the second in the fully differentiated hair in dead cells in an oxidized state, was an eureka moment that had major implications for the mechanism of hair growth.

The molecular slippage of about 19 h_{cc} (about 2.82 nm) that occurs within the IF has several pronounced effects (Fig. 5). Firstly, it results in the positions of the cysteine residues in a pair of antiparallel 1B segments in the A_{11} mode that are not in axial alignment in the reduced form to become closely aligned axially in

[15] Steinert and others (1993).
[16] Wang and others (2000).
[17] Fraser and others (2003*b*).

the oxidized structure. The expectation was that these cysteine residues would then form intermolecular disulphide bonds that would stabilize the structure of the IF. Secondly, modelling of the molecular slippage in the A_{11} mode shows that this occurred along an hydropathic (apolar) stripe formed by a combination of both the Type I and Type II chains.[18] It was thus a simple axial translation that did not involve a relative rotation of either of the two contributing segments. The molecular packing was non-optimal in the reduced case with near node-to-node interactions of the helical molecules. In contrast, in the oxidized structure the molecules were packed closer to one another in near optimal node-antinode mode.

Regarding the stabilization of the IF that occurred as a result of disulphide bond formation it was possible to perform a least-squares analysis similar to that used for the crosslink data, but instead using the positions of the cysteine residues as the input data.[19] There were, of course, far fewer data available than in the crosslink analyses but nonetheless they led to more precise estimates of both A_{11} and A_{22}. The protofilament, defined as a pair of antiparallel molecular strands (four chains in section) with each molecular strand consisting of a linear array of similarly directed molecules with a small head-to-tail overlap, utilized the A_{11} and A_{22} modes of interaction. As the protofilaments thus contain all of the possible disulphide bonds between rod domain coiled-coil segments in the trichocyte keratin IF it followed that this sub-IF structure may indeed have a true physical existence.

The lateral dispositions of the constituent rod domains in the trichocyte keratin IF differ significantly between the oxidized and reduced structures. In the former case, Bruce and his colleagues used X-ray diffraction methods to show that the rod domains of the IF molecules lay on an annulus of radius 2.9 and 3.0 nm respectively for the dehydrated and hydrated forms.[20] This is markedly smaller than the value (3.5 nm) deduced from the maximum in the radial density function obtained from electron microscope observations of freeze-dried and vitrified specimens. It was clear that a radial compaction occurs between the reduced and oxidized states and that this happens at the same time as the axial rearrangement of molecules in the A_{11} mode.

From the positions of the meridional and near-meridional reflections in the X-ray diffraction patterns two possible dislocated surface lattices were derived by Bruce and Tom.[21] The first involved seven protofilaments and the second eight protofilaments. In both cases the protofilaments would assemble via the A_{12} mode. The former model consisted of seven protofilaments arranged on a constant radius—the (7+0) model—whereas the latter structure consisted of a ring of eight protofilaments arranged on a constant but slightly larger radius—the (8+0) model. A variant consisted of a central protofilament in a (7+1) structure. Although the nature of the dislocated surface lattice proposed by Bruce and Tom was somewhat controversial at the time no contrary evidence was ever proposed by others. Bruce and David, however, returned to this problem thirty years later and using a completely different technique (a Patterson function) were able to derive exactly the same dislocated structure.[22] Bruce was thrilled that his earlier conclusions could be confirmed independently and thereby dispel any lingering doubts as to the reality of the lattice structure.

Bruce and David Parry also ultimately unravelled the problem in deciding which of these models was correct for the ‘reduced’ and ‘oxidized’ states.[23] Suffice to say that Bruce’s own high-quality X-ray equatorial data allied to modelling were sufficient to show that the (8+0) structures were present in both states but that the protofilaments were closer packed in the oxidized form. A problem that had plagued the field for sixty years had finally been put to rest.

The structure of the ß-keratins—a major component of the epidermal appendages that include scale, claw and feather—was an on-going passion for Bruce. He would revel in the possibility of returning to them in the hope that he would discover something new. His interest started when he and Tom first studied the highly detailed X-ray diffraction patterns of feather keratin. A few years later Bruce and Suzuki studied the polarized infrared spectrum of feather rachis.[24] These studies established that small ß-sheets were present in the constituent chains and that they were in the antiparallel rather than the parallel configuration.

After the isolation of a purified feather keratin protein and the determination of its sequence by O’Donnell in 1973 Bruce and Tom analysed its ß- and coil-propensities using Fourier transform methods and identified a central domain where a high propensity for the ß-conformation alternated with a high propensity for coiling.[25] The Fourier transform of both propensities exhibited a peak in the vicinity of eight residues, thereby identifying the location of the small antiparallel ß-sheet in the sequence.

X-ray diffraction data collected from feather rachis were found to be inconsistent with a simple antiparallel ß-sheet but, instead, required pairs of such sheets, known as ‘sandwiches’, twisted together and arranged in a helical form with four-fold screw symmetry (pitch length 9.6 nm and unit height 2.4 nm) (Fig. 6).[26] The idea of a twisted sheet predated examples found in crystalline globular proteins. Given that a ß-sandwich has two sheets it seemed likely that the sheets would be related by a dyad, either parallel or perpendicular to the fibre axis. The latter was shown to be more likely on the basis of the fit of the model to the high-angle X-ray diffraction pattern and also to the lack of stereochemical interference between the sheets. From these considerations it proved possible to construct a model for the filamentous component of the filament-matrix texture observed in the electron microscope (Fig. 7). The matrix component corresponded to the N- and

[18] Fraser and Parry (2014*a*).
[19] Fraser and Parry (2012).
[20] Fraser and others (1965). Fraser and others (1968).
[21] Fraser and MacRae (1983). Fraser and MacRae (1985).
[22] Fraser and Parry (2018*a*).
[23] Fraser and Parry (2017).
[24] Fraser and Suzuki (1965).
[25] Fraser and MacRae (1976).
[26] Fraser and others (1971).

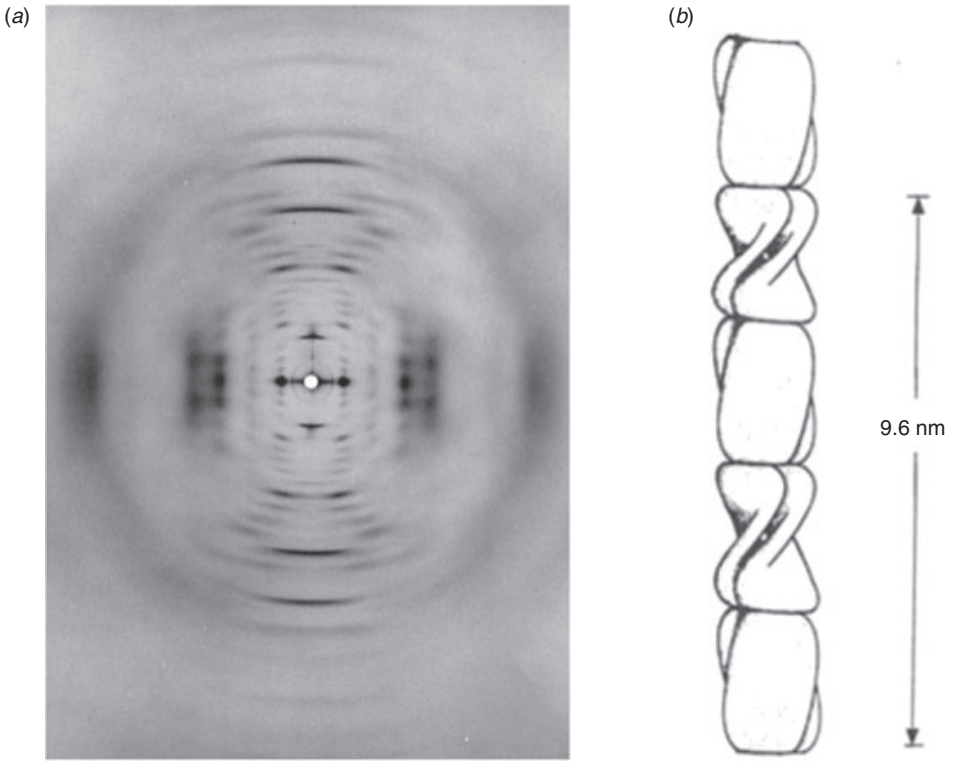

Fig. 6. (*a*) X-ray diffraction pattern of a feather rachis and (*b*) a schematic representation of a pair of twisted ß-sheets (a ß-sandwich) that are related to one another by a perpendicular dyad axis. In turn, the sandwiches are helically arranged with an axial rise of 2.4 nm and a (left-handed) pitch length of 9.6 nm This model fits the X-ray data quantitatively. (*a*) Reprinted from Parry (2014), with permission from Elsevier, and (*b*) reprinted from Fraser and Parry (1996), with permission from Elsevier.

C-terminal portions of the chains not involved in the central filament framework.

Interestingly, ß-keratin structures saw little progress for the next thirty years. However, at that time sequences began to appear in the literature and this provided Bruce and David with the opportunity to recommence structural studies. Since crystalline specimens of ß-keratin proteins were still not available it was necessary to resort once more to modelling. An important step in this process was to determine the phasing of the residues in the central domain with those in the ß-twisted sheets of the model. A preliminary assignment, based on a series of structural probes, identified a common 34-residue section of sequence[27] across the sauropsids in general (birds, crocodiles, turtles, snakes, lizards and tuatara). In each case the apolar residues lay primarily between the sheets in the sandwich and thus along the core of the filament, while the charged and cysteine residues were sited externally.

In addition, within any particular species the chains were shown to have a common overall sequence pattern in their N- and C-terminal domains. The former was described in terms of subdomain A in the archosaurs (birds and crocodiles) and the testudines (turtles), and subdomains A and B in the squamates (lizards and snakes) and the rhynchocephalia (tuatara). Subdomain A was about 25 residues in length and was cysteine- and proline-rich, whereas subdomain B was larger and in the range of 20–76 residues. It contained multiple sequence repeats based on glycine, serine, alanine, leucine and aromatic residues. Likewise, the C-terminal domain of all chains was conveniently subdivided into subdomains C and D in the archosaurs, testudines, squamates and rhynchocephalia. Subdomain C varied in length between about 20 and 128 residues, and was rich in glycine and tyrosine residues, often in sequence repeats. Subdomain D, that was 9–18 residues in length, was cysteine- and arginine/lysine-rich. Within that overall framework the individual chains displayed wide local variations in amino acid sequence. It was also possible to relate the sequences and compositions of these subdomains to the physical properties of these proteins in vivo.[28]

One particular chain, however, was shown to have a higher molecular weight (typically 33–41 kDa) than the majority of ß-keratin chains (typically 10–18 kDa), and this was found in vivo within all branches of the lepidosaurs.[29] It was argued that the four-repeat chain must have some special feature and, indeed, owe its existence to having a common and unique functional role

[27] Fraser and Parry (2011*a*).
[28] Fraser and Parry (2014*b*).
[29] Parry and others (2019).

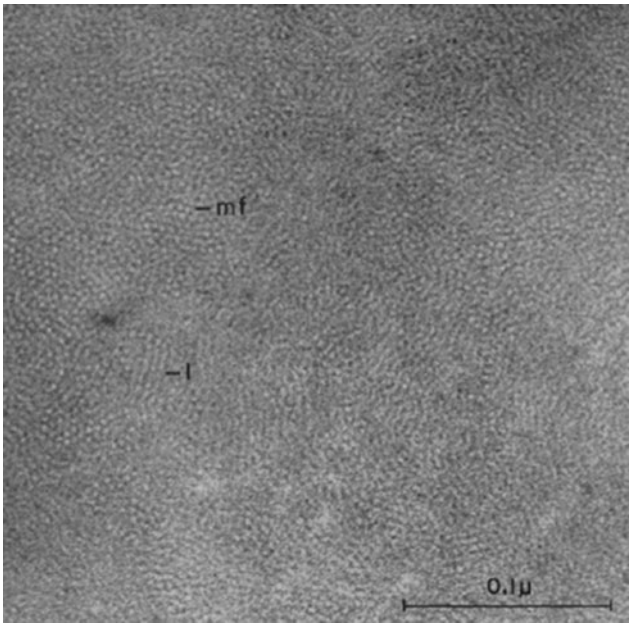

Fig. 7. Transmission electron micrograph of a cross-section of feather keratin rachis showing filaments about 3.4 nm in diameter. Reprinted from Fraser and Parry (2008), with permission from Elsevier.

that could not be achieved by assembly of chains with a single 34-residue repeat. Various possibilities were explored in Bruce's last paper in 2020 (Fig. 8).[30] These included those where the repeats lay in more than one filament.

The evidence suggested that a lateral role involving linking neighbouring filaments to one another was an attractive option, and one that would, if true, provide lateral integrity to the overall appendage. X-ray diffraction data had previously indicated that neighbouring filaments in feather keratin were not randomly organized but bore a specific orientation and axial alignment with respect to their neighbours,[31] though the details were unclear. Bruce and David returned to the problem some fifty years later and were able to show that one of the ß-sheets in one filament lined up with one of the sheets in the filament immediately adjacent to it.[32] The concept that a single four-repeat chain might have repeats in adjacent filaments and that a direct connectivity might therefore exist between oppositely directed β-sheets in these filaments was then seen as a potential explanation for the X-ray results. These four-repeat chains would not only provide short range order in line with electron microscope observations of small sheets in some of the sauropsids, but they would also act as a way of reinforcing the tissue through linking elements of structure together.

Bruce was the consummate scientist and an excellent leader of what was recognized internationally as the premier fibrous protein research group. A fundamental quality behind his success was his friendly attitude towards his colleagues in the structure group and interactions with all the staff in the wool laboratories. His central role in the teamwork of the Division of Protein Chemistry is apparent in the detailed history of the laboratories published in 1996.[33] He was an easy and stimulating colleague to work with. Never a cross word was spoken and he revelled in constructive discussion, preferably with a blackboard and chalk. Bruce had developed excellent skills as a communicator of scientific concepts and this likely originated from his time as a RAF instructor. It became very finely-honed over the course of time. He was also a very strong proponent of high quality and informative diagrams. Notably, he was responsible for the drawing of an exploded view of a wool fibre (Fig. 9) that has been widely used as a model in many wool-oriented publications.

By the end of his life Bruce had published 191 papers (some 100 were co-authored with his colleague Tom MacRae) and two books and numerous chapters for books on fibrous proteins and keratins. An outstanding work that was widely proclaimed was the book by Bruce Fraser and Tom MacRae that discussed the knowledge of fibrous proteins and polypeptides up to 1973 (see supplementary material).[34]

Family and retirement

When Bruce arrived in Melbourne, he first settled the family in a house in Flemington that was close enough to be within bicycle range of his job at the CSRIO laboratories in Parkville. Mary obtained a position as a chemistry tutor for University of Melbourne students. As the family increased to three children they moved to a house in Essendon, where they lived until his retirement. The three children—Susan, Andrew and Jane—were encouraged in their education, all achieved tertiary qualifications and all went on to professional careers. The four grandchildren in the Fraser family followed their parent's education in a similar fashion. Bruce and Mary couldn't avoid being helpful to their children's studies especially with mathematics, physics and chemistry.

Bruce's recreations included fly-fishing and acrobatic flying. When he and his ex-RAF colleague Tom MacRae needed a break from a frustrating experiment they would sometimes go to a local airfield and hire an aircraft. Through all his years flying Bruce never had an accident but there were close shaves in the Union of South Africa. Another nearly ended tragically when he and a wool laboratory member, also ex-RAF, were completing acrobatics and they experienced engine failure. Luckily, their joint skills enabled them to land safely. Andrew Miller recalls that on one of his visits to Australia, Bruce took him up in a two-seater plane and allowed him to fly it. When he took back the controls, he said 'You know I do aerobatics, don't you?'. He then looped the loop and did a vertical stall, restarting the engine on the descent, an experience enjoyed more in retrospect than at the time! Bruce's ninetieth birthday gift from his family enabled him to fly and successfully land a jet in a flight-simulator. A couple of years later he flew in a light-weight flying wing!

[30] Fraser and Parry (2020).
[31] Fraser and MacRae (1959). Fraser and MacRae (1963).
[32] Fraser and Parry (2011*b*).
[33] Rivett and others (1996).
[34] Fraser and MacRae (1973).

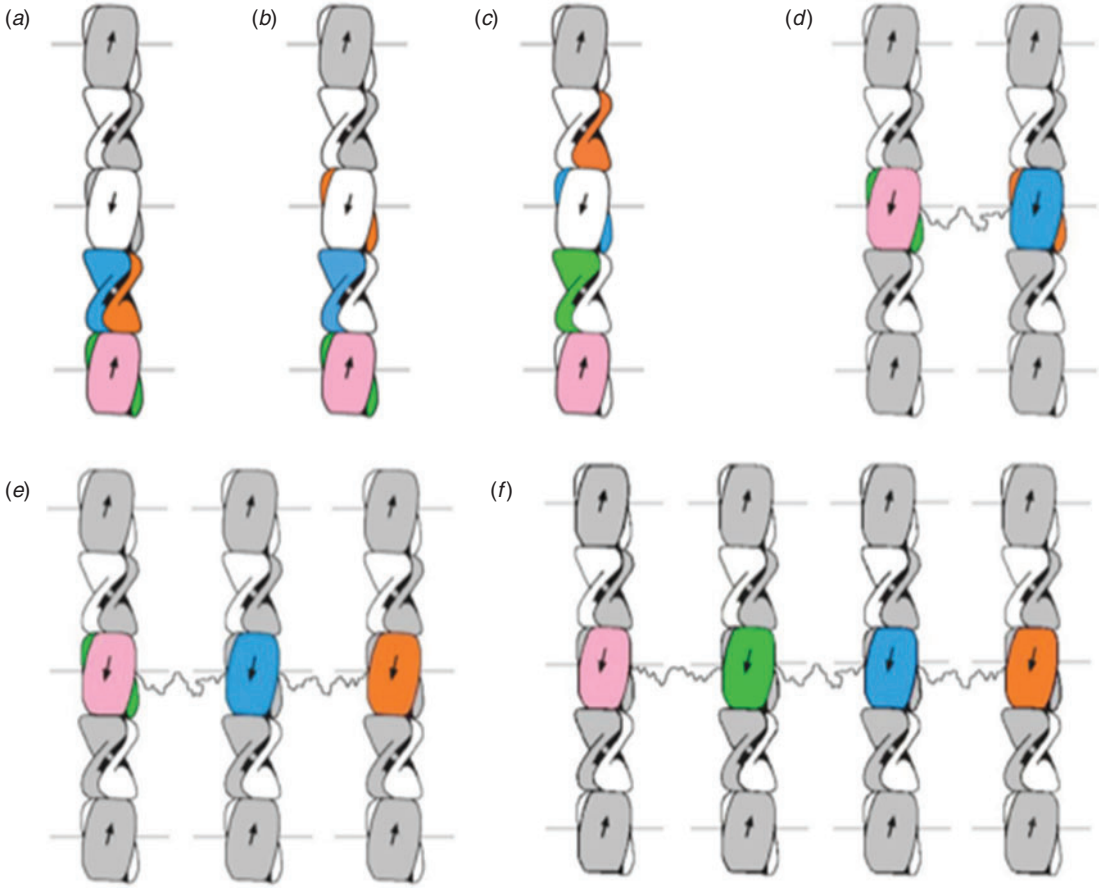

Fig. 8. Diagrammatic representation of the six classes of possible structures for the four 34-residue repeats in a lepidosaur ß-keratin chain. Reprinted from Fraser and Parry (2020), with permission from Elsevier.

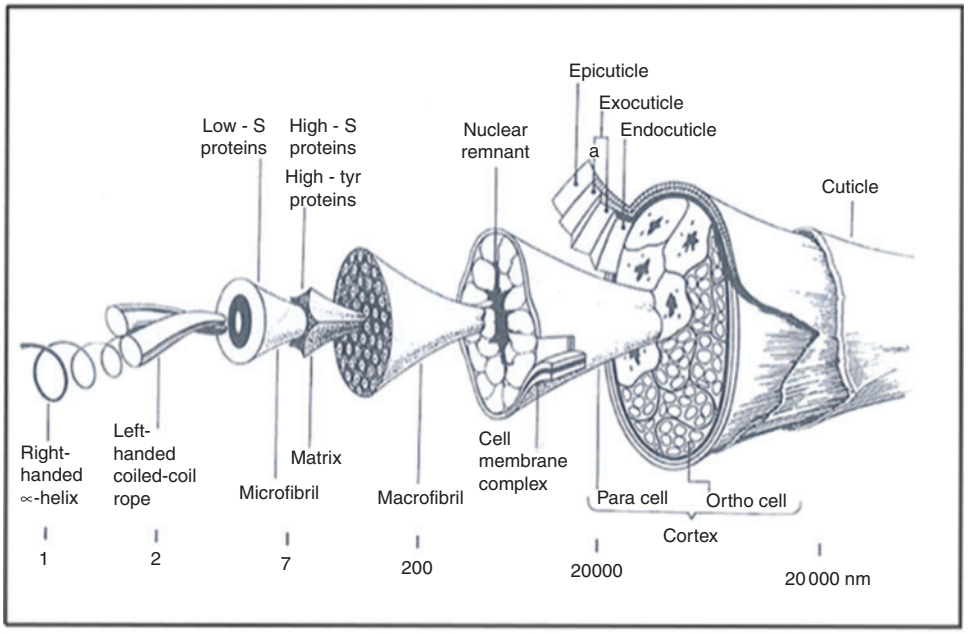

Fig. 9. An exploded view of a wool fibre from histological and macromolecular structure to molecular level. Reproduced from Rivett and others (1996) with permission from CSIRO Publishing.

When he retired in 1987 (and Tom MacRae retired at the same time) the work of the protein structure group ceased. Bruce and Mary's adult children had left home so he and Mary needed to decide where to spend their retirement years. They toured the east coast and chose Tewantin near Noosa in Queensland, and a house that had the advantage of abutting a national park.

Two of their children had also moved to Queensland. Bruce endured the loss of Mary in 2015, but he continued on with care from his family, and occupied his time with modelling molecular structures of hair and feather keratin with his New Zealand friend and colleague, David Parry. Amazingly, it was in these later years that Bruce and David were able to clear up a series of loose ends in keratin research. This partly arose from new data being available and partly from newer techniques that allowed a different approach to be made using existing data that had previously been uninterpretable. This was to give him great satisfaction. It also pleased him enormously that his on-going friendship with Andrew was maintained throughout his retirement years.

Bruce died suddenly, alone in his home in Tewantin on 16 June 2019 shortly after returning from a stay in Cairns with his elder daughter, Sue, and her husband, a grandchild and a great grandchild. The latter had recently been named 'Fraser', a tribute that gave Bruce enormous pleasure.

Selection of Honours and Awards*

1. The 1939–45 War Medal (1946)
2. The Defence Medal (1948–51)
3. Elected to Fellowship of the Australian Academy of Science (1978)
4. Royal Society of Victoria Science Medal for studies on Fibrous Proteins (1982)
5. S. G. Smith Memorial Medal of the Textile Institute, for studies of Fibrous Proteins (1984)
6. Fogarty Scholar, National Institutes of Health, Bethesda, USA (1988)

*Other awards are listed in the Supplementary Material

Conflict of interest

The authors declare no conflicts of interest.

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