tational devices to help plot this distribution were invented by Arnold Beevers and Henry Lipson in the form of their 'Beevers–Lipson strips' (Beevers & Lipson, 1934) and by J. Monteath Robertson with his 'Robertson sorting board' (Robertson, 1936). These devices were later supplemented by the XRAC electronic analogue machine of Ray Pepinsky (Pepinsky, 1947) and mechanical analogue machines (McLachlan & Champaygne, 1946; Lipson & Cochran, 1953) until electronic digital computers came into use during the mid-1950s.

A. Lindo Patterson, inspired by his visit to England in the 1930s where he met Lawrence Bragg, Kathleen Lonsdale and J. Monteath Robertson, showed how to use F^2 Fourier syntheses for structure determinations (Patterson, 1934, 1935). When the 'Patterson' synthesis was combined with the heavy-atom method, and (later) with electronic computers, it transformed analytical organic chemistry. No longer was it necessary for teams of chemists to labour for decades on the structure determination of natural products. Instead, a single crystallographer could solve such a structure in a period of months.

Improvements in data-collection devices have also had a major impact. Until the mid-1950s, the most common method of measuring intensities was by visual comparison of reflection 'spots' on films with a standard scale. However, the use of counters (used, for instance, by Bragg in 1912) was gradually automated and became the preferred technique in the 1960s. In addition, semi-automatic methods of measuring the optical densities along reciprocal lines on precession photographs were used extensively for early protein-structure determinations in the 1950s and 1960s.

1.2.3. The first investigations of biological macromolecules

Leeds, in the county of Yorkshire, was one of the centres of England's textile industry and home to a small research institute established to investigate the properties of natural fibres. W. T. Astbury became a member of this institute after learning about X-ray diffraction from single crystals in Bragg's laboratory. He investigated the diffraction of X-rays by wool, silk, keratin and other natural fibrous proteins. He showed that the resultant patterns could be roughly classified into two classes, α and β, and that on stretching some, for example, wool, the pattern is converted from α to β (Astbury, 1933).

Purification techniques for globular proteins were also being developed in the 1920s and 1930s, permitting J. B. Sumner at Cornell University to crystallize the first enzyme, namely urease, in 1926. Not much later, in Cambridge, J. D. Bernal and his student, Dorothy Crowfoot (Hodgkin), investigated crystals of pepsin. The resultant 1934 paper in *Nature* (Bernal & Crowfoot, 1934) is quite remarkable because of its speed of publication and because of the authors' extraordinary insight. The crystals of pepsin were found to deteriorate quickly in air when taken out of their crystallization solution and, therefore, had to be contained in a sealed capillary tube for all X-ray experiments. This form of protein-crystal mounting remained in vogue until the 1990s when crystal-freezing techniques were introduced. But, most importantly, it was recognized that the pepsin diffraction pattern implied that the protein molecules have a unique structure and that these crystals would be a vehicle for the determination of that structure to atomic resolution. This understanding of protein structure occurred at a time when proteins were widely thought to form heterogeneous micelles, a concept which persisted another 20 years until Sanger was able to determine the unique amino-acid sequences of the two chains in an insulin molecule (Sanger & Tuppy, 1951; Sanger & Thompson, 1953*a*,*b*).

Soon after Bernal and Hodgkin photographed an X-ray diffraction pattern of pepsin, Max Perutz started his historic investigation of haemoglobin.[1] Such investigations were, however, thought to be without hope of any success by most of the contemporary crystallographers, who avoided crystals that did not have a short (less than 4.5 Å) axis for projecting resolved atoms. Nevertheless, Perutz computed Patterson functions that suggested haemoglobin contained parallel α-keratin-like bundles of rods (Boyes-Watson *et al.*, 1947; Perutz, 1949). Perutz was correct about the α-keratin-like rods, but not about these being parallel.

In Pasadena, Pauling (Pauling & Corey, 1951; Pauling *et al.*, 1951) was building helical polypeptide models to explain Astbury's α patterns and perhaps to understand the helical structures in globular proteins, such as haemoglobin. Pauling, using his knowledge of the structure of amino acids and peptide bonds, was forced to the conclusion that there need not be an integral number of amino-acid residues per helical turn. He therefore suggested that the 'α-helix', with 3.6 residues per turn, would roughly explain Astbury's α pattern and that his proposed 'β-sheet' structure should be related to Astbury's β pattern. Perutz saw that an α-helical structure should give rise to a strong 1.5 Å-spacing reflection as a consequence of the rise per residue in an α-helix (Perutz, 1951*a*,*b*). Demonstration of this reflection in horse hair, then in fibres of polybenzyl-L-glutamate, in muscle (with Hugh Huxley) and finally in haemoglobin crystals showed that Pauling's proposed α-helix really existed in haemoglobin and presumably also in other globular proteins. Confirmation of helix-like structures came with the observation of cylindrical rods in the 6 Å-resolution structure of myoglobin in 1957 (Kendrew *et al.*, 1958) and eventually at atomic resolution with the 2 Å myoglobin structure in 1959 (Kendrew *et al.*, 1960). The first atomic resolution confirmation of Pauling's β structure did not come until 1966 with the structure determination of hen egg-white lysozyme (Blake, Mair *et al.*, 1967).

Although the stimulus for the Cochran *et al.* (1952) analysis of diffraction from helical structures came from Perutz's studies of helices in polybenzyl-L-glutamate and their presence in haemoglobin, the impact on the structure determination of nucleic acids was even more significant. The events leading to the discovery of the double-helical structure of DNA have been well chronicled (Watson, 1968; Olby, 1974; Judson, 1979). The resultant science, often known exclusively as molecular biology, has created a whole new industry. Furthermore, the molecular-modelling techniques used by Pauling in predicting the structure of α-helices and β-sheets and by Crick and Watson in determining the structure of DNA had a major effect on more traditional crystallography and the structure determinations of fibrous proteins, nucleic acids and polysaccharides.

Another major early result of profound biological significance was the demonstration by Bernal and Fankuchen in the 1930s (Bernal & Fankuchen, 1941) that tobacco mosaic virus (TMV) had a rod-like structure. This was the first occasion where it was possible to obtain a definite idea of the architecture of a virus.

[1] Perutz writes, 'I started X-ray work on haemoglobin in October 1937 and Bragg became Cavendish Professor in October 1938. Bernal was my PhD supervisor in 1937, but he had nothing to do with my choice of haemoglobin. I began this work at the suggestion of Haurowitz, the husband of my cousin Gina Perutz, who was then in Prague. The first paper on X-ray diffraction from haemoglobin (and chymotrypsin) was Bernal, Fankuchen & Perutz (Bernal *et al.*, 1938). I did the experimental work, (and) Bernal showed me how to interpret the X-ray pictures.'

Many of the biological properties of TMV had been explored by Wendell Stanley working at the Rockefeller Institute in New York. He had also been able to obtain a large amount of purified virus. Although it was not possible to crystallize this virus, it was possible to obtain a diffraction pattern of the virus in a viscous solution which had been agitated to cause alignment of the virus particles. This led Jim Watson (Watson, 1954) to a simple helical structure of protein subunits. Eventually, after continuing studies by Aaron Klug, Rosalind Franklin, Ken Holmes and others, the structure was determined at atomic resolution (Holmes *et al.*, 1975), in which the helical strand of RNA was protected by the helical array of protein subunits.

1.2.4. Globular proteins in the 1950s

In 1936, Max Perutz had joined Sir Lawrence Bragg in Cambridge. Inspired in part by Keilin (Perutz, 1997), Perutz started to study crystalline haemoglobin. This work was interrupted by World War II, but once the war was over Perutz tenaciously developed a series of highly ingenious techniques. All of these procedures have their counterparts in modern 'protein crystallography', although few today recognize their real origin.

The first of these methods was the use of 'shrinkage' stages (Perutz, 1946; Bragg & Perutz, 1952). It had been noted by Bernal and Crowfoot (Hodgkin) in their study of pepsin that crystals of proteins deteriorate on exposure to air. Perutz examined crystals of horse haemoglobin after they were air-dried for short periods of time and then sealed in capillaries. He found that there were at least seven consecutive discrete shrinkage stages of the unit cell. He realized that each shrinkage stage permitted the sampling of the molecular transform at successive positions, thus permitting him to map the variation of the continuous transform. As he examined only the centric ($h0l$) reflections of the monoclinic crystals, he could observe when the sign changed from 0 to π in the centric projection (Fig. 1.2.4.1). Thus, he was able to determine the phases (signs) of the central part of the ($h0l$) reciprocal lattice. This technique is essentially identical to the use of diffraction data from different unit cells for averaging electron density in the 'modern' molecular replacement method. In the haemoglobin case, Patterson projections had shown that the molecules maintained their orientation relative to the a axis as the crystals shrank, but in the more general molecular replacement case, it is necessary to determine the relative orientations of the molecules in each cell.

The second of Perutz's techniques depended on observing changes in the intensities of low-order reflections when the concentration of the dissolved salts (*e.g.* Cs_2SO_4) in the solution between the crystallized molecules was altered (Boyes-Watson *et al.*, 1947; Perutz, 1954). The differences in structure amplitude, taken together with the previously determined signs, could then map out the parts of the crystal unit cell occupied by the haemoglobin molecule. In many respects, this procedure has its equivalent in 'solvent flattening' used extensively in 'modern' protein crystallography.

The third of Perutz's innovations was the isomorphous replacement method (Green *et al.*, 1954). The origin of the isomorphous replacement method goes back to the beginnings of X-ray crystallography when Bragg compared the diffracted intensities from crystals of NaCl and KCl. J. Monteath Robertson explored the procedure a little further in his studies of phthalocyanines. Perutz used a well known fact that dyes could be diffused into protein crystals, and, hence, heavy-atom compounds might also diffuse into and bind to specific residues in the protein. Nevertheless, the sceptics questioned whether even the heaviest atoms could make a measurable difference to the X-ray diffraction pattern of a protein.[2] Perutz therefore developed an instrument which quantitatively recorded the blackening caused by the reflected X-ray beam on a film. He also showed that the effect of specifically bound atoms could be observed visually on a film record of a diffraction pattern. In 1953, this resulted in a complete sign determination of the ($h0l$) horse haemoglobin structure amplitudes (Green *et al.*, 1954). However, not surprisingly, the projection of the molecule was not very interesting, making it necessary to extend the procedure to noncentric, three-dimensional data. It took another five years to determine the first globular protein structure to near atomic resolution.

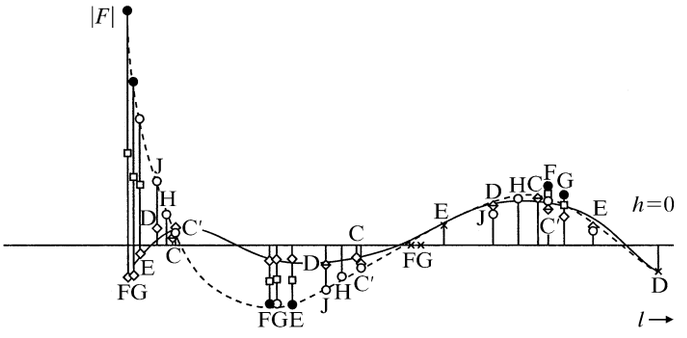

Figure 1.2.4.1
Change of structure amplitude for horse haemoglobin as a function of salt concentration in the suspension medium of the low-order $h0l$ reflections at various lattice shrinkage stages (C, C′, D, E, F, G, H, J). Reprinted with permission from Perutz (1954). Copyright (1954) Royal Society of London.

In 1950, David Harker was awarded one million US dollars to study the structure of proteins. He worked first at the Brooklyn Polytechnic Institute in New York and later at the Roswell Park Cancer Institute in Buffalo, New York. He proposed to solve the structure of proteins on the assumption that they consisted of 'globs' which he could treat as single atoms; therefore, he could solve the structure by using his inequalities (Harker & Kasper, 1947), *i.e.*, by direct methods. He was aware of the need to use three-dimensional data, which meant a full phase determination, rather than the sign determination of two-dimensional projection data on which Perutz had concentrated. Harker therefore decided to develop automatic diffractometers, as opposed to the film methods being used at Cambridge. In 1956, he published a procedure for plotting the isomorphous data of each reflection in a simple graphical manner that allowed an easy determination of its phase (Harker, 1956). Unfortunately, the error associated with the data tended to create a lot of uncertainty.

In the first systematic phase determination of a protein, namely that of myoglobin, phase estimates were made for about 400 reflections. In order to remove subjectivity, independent estimates were made by Kendrew and Bragg by visual inspection of the Harker diagram for each reflection. These were later compared before computing an electron-density map. This process was put onto a more objective basis by calculating phase probabilities, as described by Blow & Crick (1959) and Dickerson *et al.* (1961).

[2] Perutz writes, 'I measured the absolute intensity of reflexions from haemoglobin which turned out to be weaker than predicted by Wilson's statistics. This made me realise that about 99% of the scattering contributions of the light atoms are extinguished by interference and that, by contrast, the electrons of a heavy atom, being concentrated at a point, would scatter in phase and therefore make a measurable difference to the structure amplitudes.'