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.* 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. 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

* 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.

references