The variety of structures that were being studied increased rapidly. The first tRNA structures were determined in the 1960s (Kim *et al.*, 1973; Robertus *et al.*, 1974), the first spherical virus structure was published in 1978 (Harrison *et al.*, 1978) and the photoreaction centre membrane protein structure appeared in 1985 (Deisenhofer *et al.*, 1985). The rate of new structure determinations has continued to increase exponentially. In 1996, about one new structure was published every day. Partly in anticipation and partly to assure the availability of results, the Brookhaven Protein Data Bank (PDB) was brought to life at the 1971 Cold Spring Harbor Meeting (H. Berman & J. L. Sussman, private communication). Initially, it was difficult to persuade authors to submit their coordinates, but gradually this situation changed to one where most journals require coordinate submission to the PDB, resulting in today's access to structural results *via* the World Wide Web.

The growth of structural information permitted generalizations, such as that β-sheets have a left-handed twist when going from one strand to the next (Chothia, 1973) and that 'cross-over' β-α-β turns were almost invariably right-handed (Richardson, 1977). These observations and the growth of the PDB have opened up a new field of science. Among the many important results that have emerged from this wealth of data is a careful measurement of the main-chain dihedral angles, confirming the predictions of Ramachandran (Ramachandran & Sasisekharan, 1968), and of side-chain rotamers (Ponder & Richards, 1987). Furthermore, it is now possible to determine whether the folds of domains in a new structure relate to any previous results quite conveniently (Murzin *et al.*, 1995; Holm & Sander, 1997).

1.2.6. Technological developments (1958 to the 1980s)

In the early 1960s, there were very few who had experience in solving a protein structure. Thus, almost a decade passed after the success with the globins before there was a noticeable surge of new structure reports. In the meantime, there were persistent attempts to find alternative methods to determine protein structure.

Blow & Rossmann (1961) demonstrated the power of the single isomorphous replacement method. While previously it had been thought that it was necessary to have at least two heavy-atom compounds, if not many more, they showed that a good representation of the structure of haemoglobin could have been made by using only one good derivative. There were also early attempts at exploiting anomalous dispersion for phase determination. Rossmann (1961) showed that anomalous differences could be used to calculate a 'Bijvoet Patterson' from which the sites of the anomalous scatterers (and, hence, heavy-atom sites) could be determined. Blow & Rossmann (1961), North (1965) and Matthews (1966) used anomalous-dispersion data to help in phase determination. Hendrickson stimulated further interest by using Cu $K\alpha$ radiation and employing the anomalous effect of sulfur atoms in cysteines to solve the entire structure of the crambin molecule (Hendrickson & Teeter, 1981). With today's availability of synchrotrons, and hence the ability to tune to absorption edges, these early attempts to utilize anomalous data have been vastly extended to the powerful multiple-wavelength anomalous dispersion (MAD) method (Hendrickson, 1991). More recently, the generality of the MAD technique has been greatly expanded by using proteins in which methionine residues have been replaced by selenomethionine, thus introducing selenium atoms as anomalous scatterers.

Another advance was the introduction of the 'molecular replacement' technique (Rossmann, 1972). The inspiration for this method arose out of the observation that many larger proteins (*e.g.* haemoglobin) are oligomers of identical subunits and that many proteins can crystallize in numerous different forms. Rossmann & Blow (1962) recognized that an obvious application of the technique would be to viruses with their icosahedral symmetry. They pointed out that the symmetry of the biological oligomer can often be, and sometimes must be, 'noncrystallographic' or 'local', as opposed to being true for the whole infinite crystal lattice. Although the conservation of folds had become apparent in the study of the globins and a little later in the study of dehydrogenases (Rossmann *et al.*, 1974), in the 1960s the early development of the molecular replacement technique was aimed primarily at *ab initio* phase determination (Rossmann & Blow, 1963; Main & Rossmann, 1966; Crowther, 1969). It was only in the 1970s, when more structures became available, that it was possible to use the technique to solve homologous structures with suitable search models. Initially, there was a good deal of resistance to the use of the molecular replacement technique. Results from the rotation function were often treated with scepticism, the translation problem was thought to have no definitive answer, and there were excellent reasons to consider that phasing was impossible except for centric reflections (Rossmann, 1972). It took 25 years before the full power of all aspects of the molecular replacement technique was fully utilized and accepted (Rossmann *et al.*, 1985).

The first real success of the rotation function was in finding the rotational relationship between the two independent insulin monomers in the *P*3 unit cell (Dodson *et al.*, 1966). Crowther produced the fast rotation function, which reduced the computational times to manageable proportions (Crowther, 1972). Crowther (1969) and Main & Rossmann (1966) were able to formulate the problem of phasing in the presence of noncrystallographic symmetry in terms of a simple set of simultaneous complex equations. However, real advances came with applying the conditions of noncrystallographic symmetry in real space, which was the key to the solution of glyceraldehyde-3-phosphate dehydrogenase (Buehner *et al.*, 1974), tobacco mosaic virus disk protein (Bloomer *et al.*, 1978) and other structures, aided by Gerard Bricogne's program for electron-density averaging (Bricogne, 1976), which became a standard of excellence.

No account of the early history of protein crystallography is complete without a mention of ways of representing electron density. The 2 Å map of myoglobin was interpreted by building a model (on a scale of 5 cm to 1 Å with parts designed by Corey and Pauling at the California Institute of Technology) into a forest of vertical rods decorated with coloured clips at each grid point,

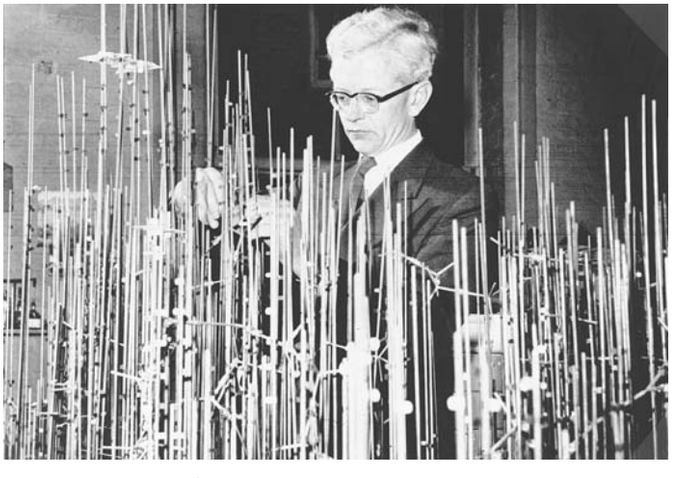

Fig. 1.2.6.1. The 2 Å-resolution map of sperm-whale myoglobin was represented by coloured Meccano-set clips on a forest of vertical rods. Each clip was at a grid point. The colour of the clip indicated the height of the electron density. The density was interpreted in terms of 'Corey–Pauling' models on a scale of 5 cm = 1 Å. Pictured is John Kendrew. (This figure was provided by M. F. Perutz.)

references