either. He showed that changes in defocus during the exposure could cause certain periodicities in the image to be enhanced. Berry was inclined to favour an approach based on simple image transfer theory which he claimed means that the fringes in the image were merely random noise, spatially filtered by the objective aperture; provided a relatively small segment of the diffraction ring is used to form the image, groups of fringes would be observed, essentially independent of the detailed structure of the sample. Thus it is clear that the unravelling of the detailed structure of these quasi-amorphous films remains a difficult problem which is far from solved, though it is certain that electron microscopy has a part to play in its resolution.

Molecular solids

The application of electron microscopy and diffraction to the study of molecular solids and minerals was the subject of the last session at the meeting. C. A. English (University of Sussex) reviewed the work on solid rare gases, O_2 , N_2 and CO_2 , which has been carried out largely at the University of Sussex. An electron microscope fitted with a liquid helium stage is used and single crystals can be produced by annealing the thin films obtained by vapour deposition at a pressure of a few torr. Among the examples he described were the determination of the structure of α - N_2 , the measurement of the stacking fault energy of solid xenon, twinning in β - O_2 and radiation damage in solid O_2 , N_2 and CO_2 .

An elegant structure determination of a molecular solid, poly sulphur nitride, by electron diffraction, was described by M. Boudeulle and P. Michel (University of Lyon). Also, the complexities of graphite-halogen intercalation compounds, including structures with very long period order giving rise to the very beautiful diffraction patterns, shown by W. T. Eeles (UKAEA, Capenhurst), revealed that there is much to be learned about the bonding in all the molecular solids discussed. The advantages of combined use of electron microscopy and single crystal electron diffraction when the crystals are too small for single crystal X-ray work were well illustrated in all these contributions.

Minerals

This same theme was reinforced by P. E. Champness (University of Manchester) in her review of electron diffraction and microscopy as a means of characterising minerals. Analysis of the characteristic X rays by EMMA (combined electron microscopy and microprobe analysis) to obtain chemical information from regions as small as

1,000 Å in diameter is also invaluable. A particularly impressive example of the combined use of these techniques is the study of an igneous pyroxene containing lamellae around 0.1 µm thick spaced about 50 to 100 µm apart. In between the lamellae there are very fine precipitates with a precipitate free zone next to the lamellae. The orientation relationships of all the phases can be determined by electron diffraction. Analysis of the Ca/Si X-ray intensity ratio reveals that the lamellae are rich in calcium and the diffusion profile of the calcium content between adjacent lamellae can thus be determined. The fine precipitates can be imaged by lattice resolution of the 1.8 nm spacing and shown to be only one unit cell thick.

The conference clearly showed the range of opportunities offered by electron diffraction, particularly when it is used in combination with electron microscopy and other techniques such as X-ray analysis. The greater difficulty of interpretation *vis a vis* X-ray diffraction remains, but well-founded dynamical diffraction theory is being used with very considerable success for quantitative work on thin films with high energy electrons. At low energy, theoretical improvements are rapidly being made, but much remains to be done before the theory can be used with complete confidence. Direct structure determination by high resolution electron microscopy is now a fascinating possibility and will undoubtedly be explored further in the future.

If M haemoglobin, then R or T?

from a Correspondent

MUCH ingenuity has been shown in utilising the structural differences found in the genetically determined haemoglobin variants to assist the study of the relationships between structure and function of normal haemoglobin. A striking example of this approach is the work of Mayer, Ogawa, Shulman and Gersonde (*J. molec. Biol.*, **80**, 187; 1973) on high-resolution proton nuclear magnetic resonance (NMR) spectral studies of the quaternary state of haemoglobin M(Iwate).

Hb-M(Iwate) is an α -chain variant ($\alpha^{88}\text{His} \rightarrow \text{Tyr}$, β_2) and is identical with Hb-M(Oldenburg) and Hb-M(Kanakee). Apart from shortening the list of M-type haemoglobins, the locations in which it has been discovered provide an interesting example of the probable independent occurrence of the same mutation in three widely separated places. As with other M-type haemoglobins the chemical properties of the haems on the variant chains are abnormal. It has previously been shown that in the crystal the quaternary state

is deoxy (T) when the normal β -chain subunits are deoxygenated and also when they are oxidised to methaemoglobin and therefore ligated with H_2O . In view of the low oxygen affinity of the variant it seemed probable that after ligation it remains in the T quaternary structure in solution as well as in the crystal. Mayer and colleagues have therefore used Hb-M(Iwate) to test the extent to which three NMR spectral features are linked to the state of the quaternary structure.

These three features are (1) the paramagnetically-shifted resonances of the cyanide-ligated subunits in valency-hybrid haemoglobin, (2) the upfield resonance in oxy and carbonmonoxy-haemoglobin assigned to Val E11 near the haem group, and (3) the low-field resonance near -14 p.p.m. (referred to 2,2-dimethyl-2-silapentane-5-sulphonate) in deoxyhaemoglobin which comes from an exchangeable proton, and which disappears on ligation. Although these three features had been used to distinguish between oxy (R) and deoxy (T) quaternary structures, their validity had not been tested with a haemoglobin known to be in the T quaternary state in solution even when ligated.

A comparison of the 220 MHz NMR spectra of normal human adult haemoglobin valency hybrids and of Hb-M(Iwate) in the presence and absence of organic phosphates clearly shows that those NMR spectral features previously attributed to the deoxy (T) quaternary structure are present in Hb-M(Iwate) when the β subunits are ligated to O_2 , CO or CN^- as well as when they are unligated, with the quaternary structure remaining in the deoxy (T) state throughout. These results complement previous spectral studies on Hb-Kansas ($\alpha_2\beta_2$ 102 Asn $\rightarrow$ Thr) and on valency-hybrid haemoglobin.

In the authors' view, the increasing body of evidence that the quaternary structure is the most important factor in determining the functional properties of haemoglobin provides additional support for a two-state model, as proposed by Monod, Wyman and Changeux in 1965, as a suitable first-order description of haemoglobin function.

In another application of NMR spectroscopy to the study of haemoglobin, Manuck, Maloney and Sykes (*ibid.*, 199) have studied the binding of methyl isonitrile to the β chains by measuring the transverse relaxation times (T_2) of the isonitrile protons as a function of protein concentration, temperature and ^{14}N decoupling. Binding both at the haem iron and at non-specific site(s) affects the measured relaxation times. From the results a value of $57 \pm 12 \text{ s}^{-1}$ for the 'off' rate constant (k_{-1}) for specific binding, and an Arrhenius activation energy for k_{-1} of $14 \pm 3 \text{ kcal mol}^{-1}$, were obtained.