An X-ray study of horse methaemoglobin. I

By Joy Boyes-Watson, Edna Davidson and M. F. Perutz

Cavendish Laboratory and Molteno Institute, University of Cambridge

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[Plates 5 and 6]

The paper describes a detailed study of horse methaemoglobin by single crystal X-ray diffraction methods. The results give information on the arrangement of the molecules in the crystal, their shape and dimensions, and certain features of their internal structure.

Horse methaemoglobin crystallizes in the monoclinic space group $C2$ with two molecules of weight 66,700 per unit cell. In addition, the wet crystals contain liquid of crystallization which fills 52·4% of the unit cell volume. Deliberate variations in the amount and composition of the liquid of crystallization, and the study of the effects of such variations on the X-ray diffraction pattern, form the basis of the entire analysis.

The composition of the liquid of crystallization can be varied by allowing heavy ions to diffuse into the crystals. This increases the scattering contribution of the liquid relative to that of the protein molecules and renders it possible to distinguish the one from the other. The method is analogous to that of isomorphous replacement commonly used in X-ray analysis. It yielded valuable information on the shape and character of the haemoglobin molecules and also led to the determination of the phase angles of certain reflexions.

The amount of liquid of crystallization was varied by swelling and shrinkage of the crystals. This involves stepwise, reversible transitions between different well-defined lattices, each being stable in a particular environment of the crystal. The lattice changes were utilized in two different ways: the first involved comparison of Patterson projections at different stages of swelling and shrinkage, and the second an attempt to trace the molecular scattering curve as a function of the diffraction angle.

The results of the analysis can be summarized as follows. The methaemoglobin molecules resemble cylinders of an average height of 34 A and a diameter of 57A. In the crystal these cylinders form close-packed layers which alternate with layers of liquid of crystallization. The layers of haemoglobin molecules themselves do not swell or shrink, either in thickness or in area, except on complete drying, and lattice changes merely involve a shearing of the haemoglobin layers relative to each other, combined with changes in the thickness of the liquid layer. Thus the molecules do not seem to be penetrated by the liquid of crystallization, and their structure is unaffected by swelling and shrinkage of the crystal.

Space-group symmetry requires that each molecule consists of two chemically and structurally identical halves. Evidence concerning the internal structure of the molecules comes both from two-dimensional Patterson projections and one-dimensional Fourier projections. The former indicate that interatomic vectors of 9 to 11 A occur frequently in many directions, and the latter show four prominent concentrations of scattering matter just under 9 A apart along a line normal to the layers of haemoglobin molecules. No structural interpretation of these features is as yet attempted.

The liquid of crystallization consists of two distinct components: water 'bound' to the protein and not available as solvent to diffusing ions, and 'free' water in dynamic equilibrium with the suspension medium. An estimate of the 'frictional ratio' based on the molecular shape and hydration found in this analysis is in good agreement with the frictional ratio calculated from the sedimentation constant.

1. Introduction

(*a*) *Background and scope of research*

The molecular structure of the crystalline proteins is one of the major unsolved problems in biology to-day. During the last 25 years the recognition of their ubiquity and paramount importance in plant and animal metabolism has set in

4. Unit-cell dimensions and space group

(*a*) *Experimental technique*

For the purpose of taking X-ray pictures, wet haemoglobin crystals are mounted as follows. Capillaries of either borosilicate glass or quartz are prepared, with a wall thickness of 0·01 to 0·02 mm., a bore of about 1 mm. and a length of about 30 mm. A single protein crystal is isolated on a slide with the help of a dissecting needle and drawn into the capillary with a drop of mother liquor. A firm cotton thread, made by twisting cotton-wool between the fingers, is now introduced half-way into the capillary and the liquid drawn off very gently through the thread, leaving the crystal behind. The thread is now withdrawn and the liquid re-introduced into both

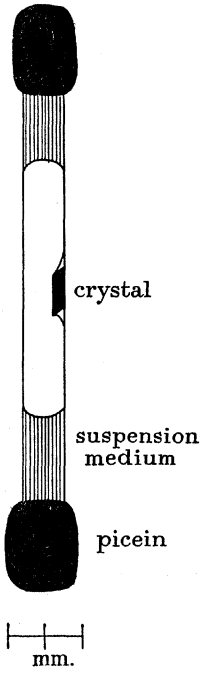


Figure 1. Wet protein crystal mounted for X-ray diffraction work.

ends of the capillary, if necessary with the help of a hypodermic syringe, but leaving the crystal itself clear. After this operation the ends of the capillary are sealed with picein (figure 1). This method of mounting has the advantage of keeping the crystal in equilibrium with its former suspension medium and yet avoids it actually being suspended in liquid; without this precaution it is difficult to keep crystals stationary during exposures.

The capillary is mounted on an X-ray goniometer in the usual way, and oscillation photographs are taken at specimen-to-film distances ranging from 5 to 10 cm. A typical series of oscillation photographs is shown in figures 14 to 19, plate 5. The

So long as complete drying of the crystals is avoided, swelling and shrinkage merely alter the thickness of the liquid layers without having any effect on the area or thickness of the layers of haemoglobin molecules. These molecules themselves do not seem to be penetrated by the liquid of crystallization, and their structure is unaffected by swelling and shrinkage of the crystal.

The unit-cell dimensions of dried crystals vary considerably according to the method of drying. In slowly dried crystals the individual layers of haemoglobin molecules only shrink in area by a small amount; not more, in fact, than would be

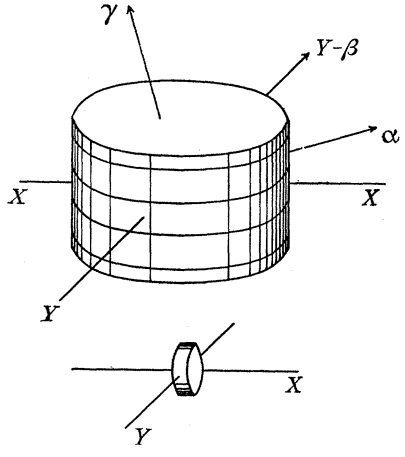


FIGURE 12. Diagrammatic model of haemoglobin molecule, showing its orientation with respect to the crystal axes. *Y* is the diad axis. The small disk underneath represents a haem group drawn on the same scale and in its correct orientation with respect to the crystal axes. The four lines on the cylinder surface indicate the positions of the concentrations of scattering matter deduced from the Fourier projections. The directions of the principal refractive indices are indicated by arrows.

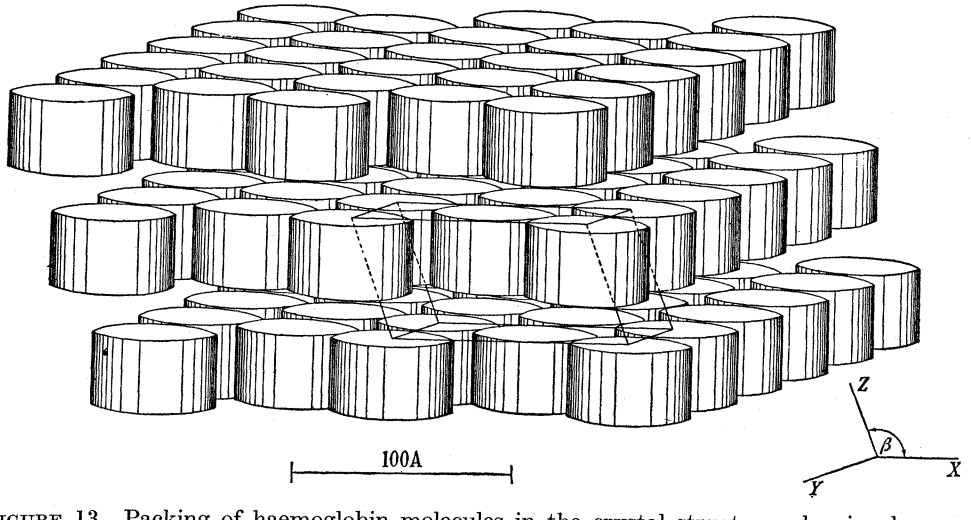
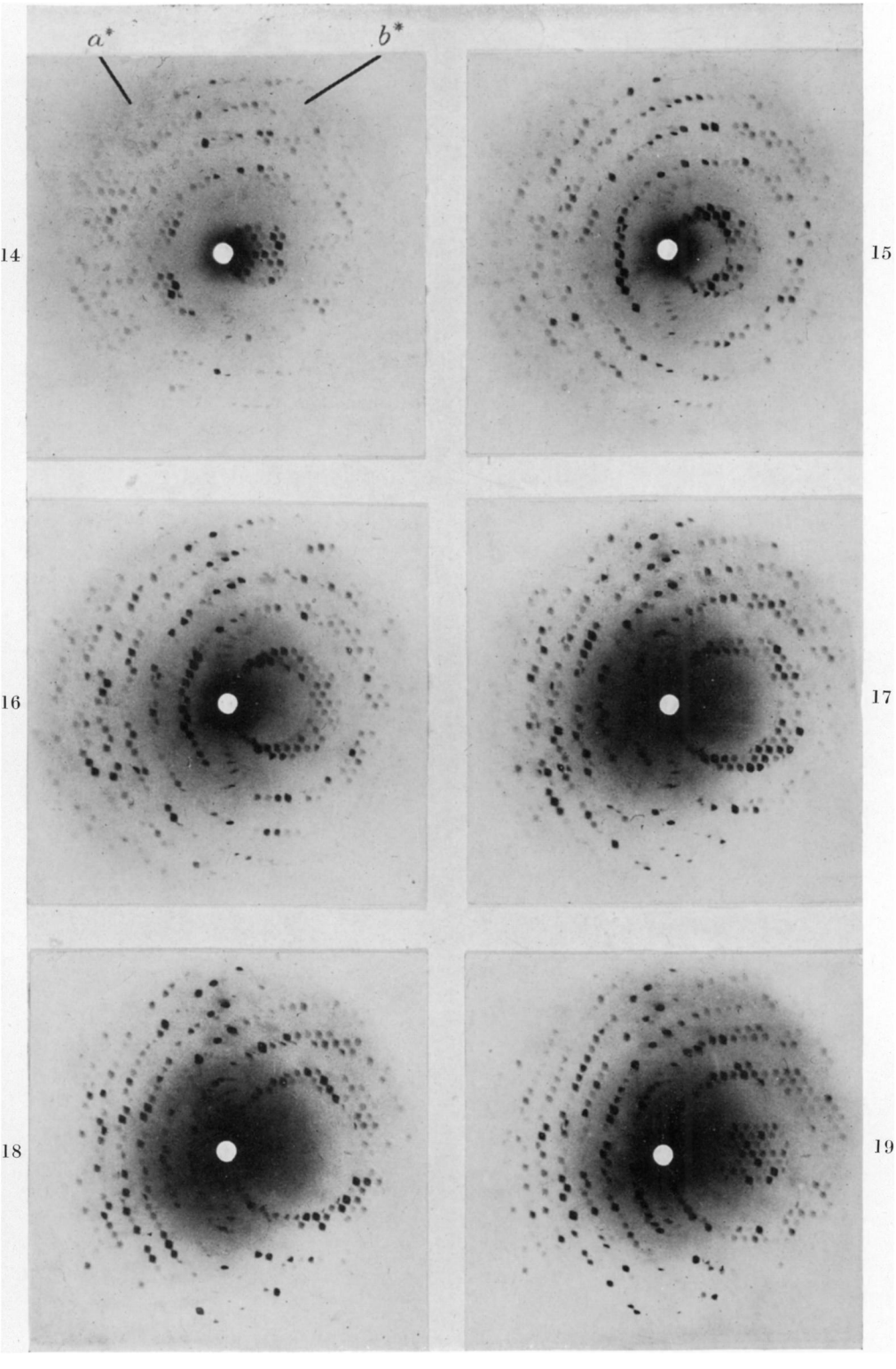


FIGURE 13. Packing of haemoglobin molecules in the crystal structure, showing layers of close-packed molecules separated by liquid. One unit cell is shown in the foreground on the right.



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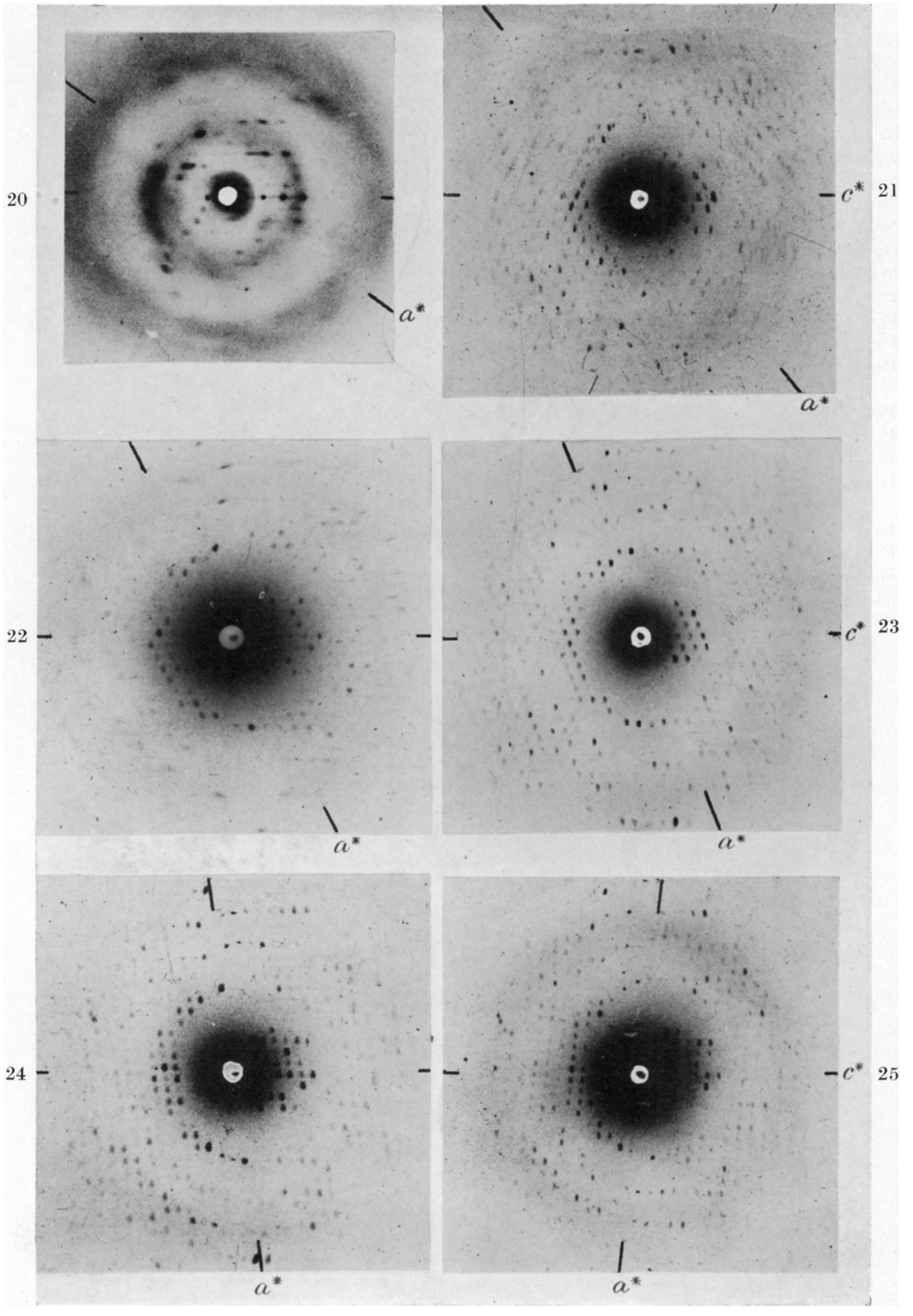
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