A PRELIMINARY X-RAY ANALYSIS OF HAEMOGLOBIN H

By Dr. M. F. PERUTZ, C.B.E., F.R.S., and L. MAZZARELLA

HÆMOGLOBIN H is an abnormal human hæmoglobin which consists of four β-chains[1,2]. Its oxygen equilibrium curve is similar in both shape and position to that of myoglobin, showing that the four hæm groups react with oxygen independently of each other and that their oxygen affinity is higher than normal. Like myoglobin, it exhibits only a negligibly small Bohr effect[3].

Crystals of oxyhæmoglobin H, suspended in 2·3 M phosphate buffer of *p*H 6·7, were given to us by Dr. Helen M. Ranney of the Albert Einstein College of Medicine in New York. They were strongly birefringent monoclinic prisms. Birefringence was negative, with the optic axial plane approximately normal to the prism axis. The crystals were only just large enough to be mounted in glass capillaries for X-ray analysis. Their unit cell dimensions and space group are shown in Table 1. The space group is the same, and the unit cell dimensions are closely similar to those of normal human reduced hæmoglobin, and quite different from those of any of the forms of human oxyhæmoglobin[4]. The habit of the hæmoglobin H crystals is different from those of normal human reduced hæmoglobin, the former being prisms elongated along [001], while the latter are elongated along [100].

Table 1. UNIT CELL DIMENSIONS OF NORMAL HUMAN REDUCED HÆMOGLOBIN AND OXYHÆMOGLOBIN H (β_4)
Space group $P2_1$; $n = 2$

	Reduced human hæmoglobin	Oxyhæmoglobin H
a	63·39	63·29
b	83·63	82·40
c	53·91	54·07
β	99·25°	91°

However, crystallization of oxyhæmoglobin H in a lattice similar to the normal reduced form suggests that the oxygenated and reduced forms of hæmoglobin H both have the same, or very similar, crystal structures, and that no major change in molecular structure accompanies its reaction with oxygen. We have not yet prepared crystals of reduced hæmoglobin H, but have tested the point by performing the following experiment.

Crystals of oxyhæmoglobin H were immersed in a solution containing 2·8 M phosphate buffer of *p*H 6·7 and 0·01 M ferrous citrate until their absorption spectrum had changed to that of reduced hæmoglobin. No other change was observed in the appearance of the crystals. They were then mounted in glass capillaries in an atmosphere of nitrogen, and X-ray diffraction pictures were taken. These showed sharp reflexions extending to a spacing of 3 Å. The unit cell dimensions were the same as those of oxyhæmoglobin H. The distribution of intensities in 9° precession photographs of the *h*0*l* and 0*kl* zones was very similar, though small alterations in the intensities of certain reflexions were noticeable.

If crystals of normal oxyhæmoglobin (human or horse) are reduced, or crystals of normal reduced hæmoglobin are oxygenated, the crystals develop numerous cracks and become opaque. Reflexions of more than 8 Å spacing are much weakened and those of less than 8 Å spacing fade out altogether. In these crystals the reaction with oxygen evidently introduces stresses which break up the regularity of the lattice. There is no sign of any such stresses in crystals of hæmoglobin H, where structural changes, if present, must be very slight.

The absence of structural changes accompanying the reaction with oxygen in hæmoglobin H was predicted from the absence of hæm–hæm interaction, and with the idea in mind that in normal hæmoglobin this interaction is linked to structural changes[3,5]. It is interesting to find this prediction confirmed by X-ray analysis.

However, there are two puzzling points arising from our observations. It is not clear why a hæmoglobin consisting of four β-chains should have the same structure as the normal reduced, since one might expect both pairs of β-chains to be equivalent and each pair to take up the relative position shown in Fig. 11 (left) of the preceding article, with a corresponding increase in width in the region of the molecule normally taken up by the α-chains. The second point concerns the kinetic properties. If the oxygen affinity of hæmoglobin H is as high as that of myoglobin, then its configuration should be that of normal oxyhæmoglobin, which is the fast-reacting, rather than that of reduced, which is the slowly reacting modification. These difficulties will need further study.

We thank Dr. Ranney for giving us the crystals and one of us (L. M.) thanks the Fondazione Alberto Beneduce of Naples for a fellowship.

[1] Rigas, D. A., Koler, R. D., and Osgood, E. E., *Science*, **121**, 372 (1955).
[2] Jones, R. T., Schroeder, W. A., Balog, J. E., and Vinograd, J. R., *J. Amer. Chem. Soc.*, **81**, 3161 (1959).
[3] Benesch, R. E., Ranney, H. M., Benesch, R., and Smith, M. G., *J. Biol. Chem.*, **236**, 2926 (1961).
[4] Perutz, M. F., Liquori, A. M., and Eirich, F., *Nature*, **167**, 929 (1951).
[5] Benesch, R., and Benesch, R. E., *J. Mol. Biol.*, **6**, 498 (1963).

THE NATIONAL PHYSICAL LABORATORY

MORE than 150 items from the research programme of the National Physical Laboratory were on display during Open Week, May 13–17, which was attended by several thousand visitors, including many from overseas. Lectures were given by Dr. W. P. Jones (superintendent of the Aerodynamics Division) on "The Work of Aerodynamics Division", and by Mr. A. Silverleaf (superintendent of Ship Division) on "Experiments with Ship Models".

In Standards Division, much interest was shown in an exhibit describing the method used to relate clocks over transatlantic distances by means of an active communication satellite. The inherent symmetry of the arrangement enables the classical method of comparing clocks to be applied, in which signals are transmitted from remotely situated clocks, at the speed of light, and the time differences between the received and local clock signals are observed. If these differences are equal then the clocks are regarded as synchronized; if not, the discrepancy indicates the magnitude and sense of the relative clock adjustment.

An experiment of this form was carried out in August 1962 by the National Physical Laboratory in co-operation with the U.S. Naval Observatory, making use of the *Telstar I* satellite to relate quartz clocks at the satellite ground station at Andover, Maine and Goonhilly Downs, Cornwall. It was determined that the clocks differed in their setting by 72·6 μsec. Due partly to the wide band-width of the radio circuit and its relatively high signal-to-noise ratio, the overall accuracy of the comparison was 1 μsec, an improvement of some three orders over existing methods of synchronization. The path via the satellite is continuously changing and means have to be provided for recording the reception time of the