the continuous transform of the protein at slightly different points, so that ΔI will be largest when the gradient of I in reciprocal space is greatest. Statistically this is likely to be correlated with the smaller values of I. In other words, on the average the extra atoms produce the bigger changes in the larger intensities, whereas shifts of the protein produce the bigger changes in the smaller intensities. We have not yet developed the exact theory for this approach.

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Ox Haemoglobin: Preliminary X-ray Studies

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Crystals of the carbon monoxide compound of ox haemoglobin, grown in ammonium sulphate, are orthorhombic; space group $P2_12_12_1$. The asymmetric unit consists of one haemoglobin molecule (molecular weight $\sim$ 68,000). A study of the very low order intensities shows the approximate positions of the molecules in the unit cell.

The Patterson projection along one axis resembles that of the a-axis projection of monoclinic horse haemoglobin, and suggests that the molecules of the two species may have certain broad features in common.

Introduction

This short paper presents preliminary X-ray work on ox haemoglobin. It forms part of a Ph.D. thesis accepted by the University of Cambridge (Crick, 1953). Further X-ray work on this protein is being undertaken by Dr D. W. Green, and will be reported elsewhere.

The crystals were very kindly supplied by Mr G. S. Adair. This appears to have been the first occasion on which crystals were grown successfully. The haemoglobin had been dissolved in half-saturated ammonium sulphate, and converted from oxyhaemoglobin to carbonmonoxyhaemoglobin by bubbling carbon monoxide through the solution. The saturated solution was then stored in a cold-room at 0° C. and as the solution became more concentrated, owing to slow evaporation, crystals were deposited.

1. Experimental results

In order to avoid taking X-ray pictures at 0° C. some mother liquor was first equilibriated at room temperature for a few days. As the protein is less soluble at higher temperatures, some of it precipitated and was filtered off. The crystals were then transferred to this new mother liquor. The density of the mother liquor was 1·159 g.cm.$^{-3}$, so the concentration of the ammonium sulphate was about $2\frac{1}{2}$ M.

More recently Dornberger-Schiff (1954) has obtained similar, but not identical, crystals by a method in which crystals were formed by allowing alcohol vapour to diffuse into a concentrated aqueous solution of the protein in the oxy form.

The crystals, some of which had dimensions of 1–2 mm., were tabular. Their shape and crystallographic properties are shown in Fig. 1. The most significant

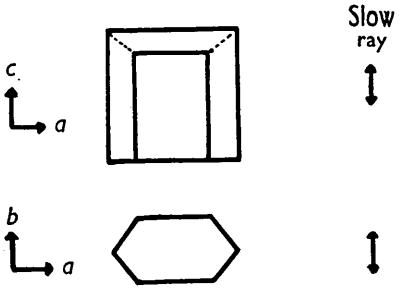

Fig. 1. Properties of crystals of ox haemoglobin.

feature of the crystals is their high pleochroism when viewed along the *b* axis. The high absorption occurred when the electric vector was parallel to *c*. Thus the haem groups must have their planes roughly perpendicular to *a*, and by analogy with horse haemoglobin one might expect the 'chain direction' to be parallel to *a* (Boyes-Watson, Davidson & Perutz, 1947; Perutz, 1949).

The crystals showed straight extinction in both views, and the X-ray photographs confirmed that the crystals were orthorhombic. The sloping faces developed proved to be (120) and (021).

Increasing the salt concentration to 4M altered the cell dimensions. The values for the two forms are given in Table 1, together with those for crystals which had been allowed to dry by exposing them to the atmosphere.

Table 1. *Unit-cell dimensions of ox haemoglobin*

	Form *A* ($2\frac{1}{2}$M Am_2SO_4)	Form *B* (4M Am_2SO_4)	Dry
a (Å)	64·7	64·4	60–61
b (Å)	160·0	156·3	136–137
c (Å)	55·2	55·2	49

The space group is probably $P2_12_12_1$.

By comparing the cell volume of the dry form (4×10^5 Å^3) with that of dry horse haemoglobin (2×10^5 Å^3), which has two molecules in the unit cell, it can be concluded that there must be four molecules in the unit cell, and therefore that the asymmetric unit is a single molecule. The intensities of the three principal zones of both forms were recorded using precession cameras, except for one case in which a Weissenberg camera was used. In most cases 8° precession pictures were taken, but in one or two cases 17° pictures were obtained. From these data Patterson projections were plotted. In all cases except one, a smoothing function was used to reduce diffraction effects. This was a slightly modified gaussian which fell to one-fifth at a spacing of 7·4 Å.

The intensities and the Patterson projections are available (Crick, 1953), but will not be reproduced here, with the exception of Fig. 2, which represents the Patterson projection along the *a* axis of lattice *B*. In all the Pattersons in this paper the 'zero' contour is dotted and the negative contours have been omitted.

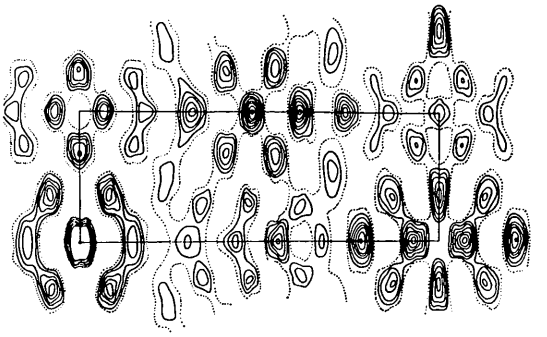

Fig. 2. The *a*-axis Patterson projection of lattice *B*. Origin bottom left. The rectangle is $\frac{1}{2}c$ high and $\frac{1}{2}b$ long.

In addition, three-dimensional low-order data were collected for Form *A* by taking a series of precession photographs, each of which contained the b^* axis. All spacings out to 20 Å were recorded, except for three reflexions with spacings close to this value. After applying a smoothing function, which reduced the intensity of the smallest spacing to about one-fifth, a three-dimensional Patterson was calculated, the sections of which are shown in Fig. 3. A schematic perspective drawing of this Patterson is shown in Fig. 4.

The pictures of the dry cell were rather poor, reflexions fading away around 15 Å, and intensities have therefore not been recorded. Qualitatively the *a*-axis projection is pseudo-double-faced centred, i.e. strong reflexions at $k/2+l$ = even. The *b* and *c* projections are rather featureless except that 200 is exceptionally strong.

2. Discussion

It was expected that the region near the origin of the 0*kl* Patterson projection would resemble the cor-

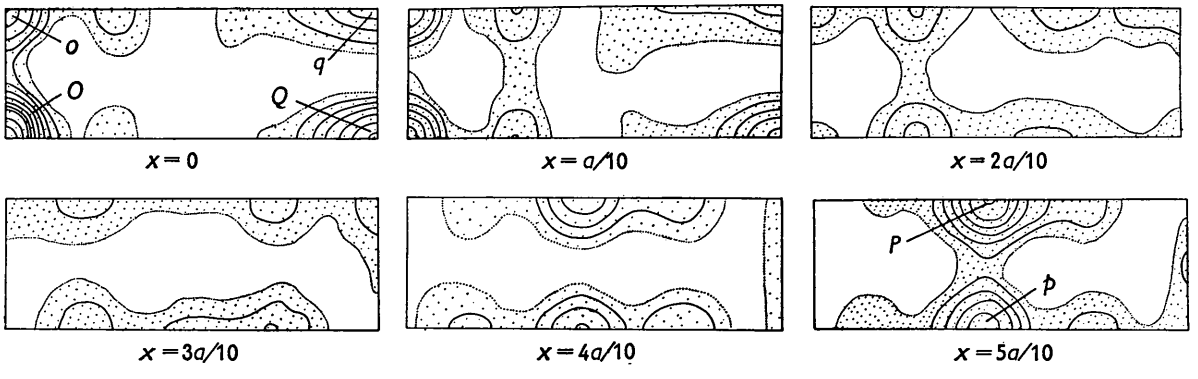

Fig. 3. Sections of a three-dimensional Patterson, using only the very low orders. Origin bottom left. The rectangles are $\frac{1}{2}c$ high and $\frac{1}{2}b$ long. The positive areas are speckled.

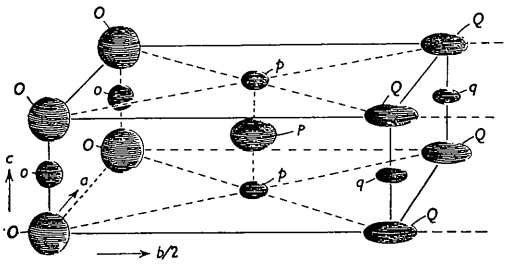

Fig. 4. Schematic perspective drawing of the Patterson of Fig. 3, to show the higher positive peaks.

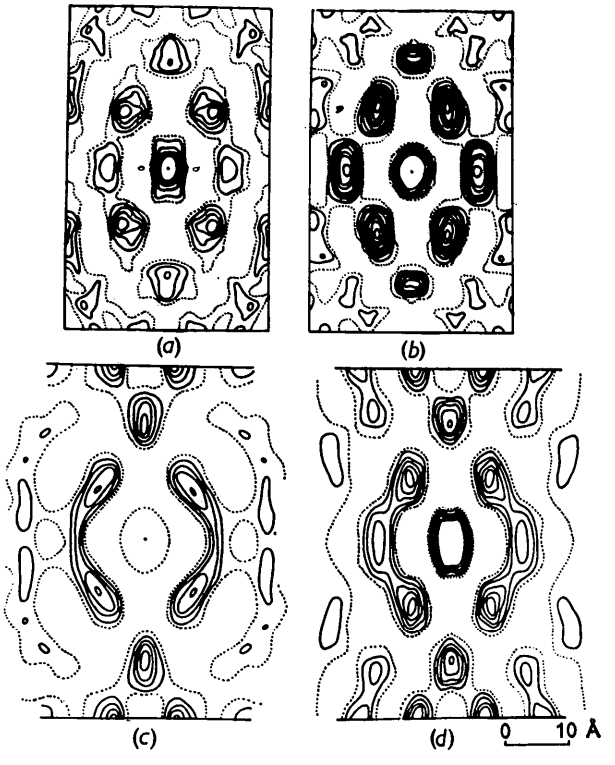

Fig. 5. Comparison of the origin regions of the a-axis Patterson projections of various haemoglobins. (*a*) Horse monoclinic; (*b*) human reduced; (*c*) ox, lattice A; (*d*) ox, lattice B.

responding region of monoclinic horse haemoglobin (Perutz, 1949). This comparison is made in Fig. 5, where part of the a projection of monoclinic human reduced haemoglobin (Perutz, Trotter, Howells & Green, 1955) is also shown. It can be seen that Form A might be considered a very disordered version of the horse haemoglobin pattern. Form B bears a rather closer resemblance to the horse pattern, and suggests that ox and horse haemoglobin resemble each other to some extent. This similarity is strengthened by the cell dimensions, which suggest (see Crick, 1953) that if ox haemoglobin is similar in shape to horse haemoglobin the packing will be pseudo-double-body-centered.

It can be seen from the three-dimensional Patterson (Figs. 3 and 4) that there are indeed large peaks at $(\frac{1}{2}a, \frac{1}{4}b, \frac{1}{2}c)$ and $(0, \frac{1}{2}b, 0)$, marked P and Q respectively in Fig. 4. Moreover, their peak height is in both cases just over half the origin peak height, and about three times larger than the largest negative value in the entire Patterson. The unexpected feature is the second set of peaks spaced a distance $\frac{1}{2}c$ above (and below) these major peaks, namely at $(0, 0, \frac{1}{2}c)$, marked o, and at $(\frac{1}{2}a, \frac{1}{4}b, 0)$ and $(0, \frac{1}{2}b, \frac{1}{2}c)$, marked p and q respectively. The peak at $(0, 0, \frac{1}{2}c)$ suggests that the molecule may be in two halves, one above the other. If this were true it would explain the other satellite peaks. Thus we may conclude that the cell is pseudo-double-body-centred. Other features of the Patterson projections (not reproduced here) support this. Thus the c-axis Patterson projections of both forms show a large peak near $x = \frac{1}{2}a$, $y = \frac{1}{4}b$ and a lesser peak near $x = 0$, $y = \frac{1}{2}b$. The b-axis Patterson projections have their highest peaks at $x = \frac{1}{2}a$, $z = \frac{1}{2}c$, and in each case the patterns round them are not dissimilar to those round the origin.

The change from lattice A to lattice B must involve a tilting of the molecules, since, although the a and c dimensions remain about the same, the intensities of *all* the projections change.

There is a further resemblance between ox and human reduced haemoglobin. The region of the ox a-axis projection Patterson (Fig. 2) around $y = \frac{1}{2}b$, $z = 0$, is similar to that near $y = \frac{1}{2}b$, $z = \frac{1}{2}c$ for the a-axis Patterson projection of human reduced haemoglobin (Fig. 5(*b*)), studied by Perutz *et al.* (1955). In particular, there is a 'hole' (a negative region) at the midpoint in both cases.

It seems unprofitable at the moment to attempt further interpretation. At a later stage in the investigation of haemoglobin structure the a projection of ox, and especially of Form B, should be very valuable, because the molecules are tolerably well resolved from one another and the projection has a centre of symmetry.

I should like to thank Mr G. S. Adair for providing me with the crystals, and Dr E. R. Howells for his assistance and for many helpful discussions.

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