somewhat more sensitive to the substituent on the diazonium ion, ranging from 244 Å^3 in the *p*-methoxy to 226 Å^3 in the *m*-chlorobenzenediazonium salt.

A complete structural analysis of the *p*-methoxybenzenediazonium fluoroborate and *p*-diazoniobenzenesulfonate is now in progress. No additional work is planned on the other salts.

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The relative orientation of molecules of crystallized human and horse oxyhaemoglobin.

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The horse oxyhaemoglobin molecule consists of four sub-units, namely two α and two β chains, packed in a tetrahedral array (Cullis, Muirhead, Perutz, Rossmann & North, 1962). Human reduced (deoxy) haemoglobin has a similar structure, except that the β chains are translated 7 Å apart (Muirhead & Perutz, 1963). This increased separation of the β chains is probably associated with deoxygenation, but the possibility exists that it merely reflects a difference between human and horse oxyhaemoglobin. The failure of human oxyhaemoglobin crystals to form isomorphous derivatives has hindered X-ray analysis by the isomorphous replacement method. However a method (based on the 'rotation function') of determining the relative orientation of two similar proteins in different crystal lattices was recently described (Rossmann & Blow, 1962). This note reports, as a preliminary step in the structural analysis of human oxyhaemoglobin, the application of the rotation function to a comparison of human and horse oxyhaemoglobin.

Horse oxyhaemoglobin crystals are monoclinic with space group $C2$ and cell dimensions $a=108{\cdot}9$, $b=63{\cdot}5$, $c=54{\cdot}9$ Å, $\beta=110{\cdot}9°$. The molecular twofold axis lies along the crystallographic twofold axis (*i.e.* parallel to *b*). In addition, the molecule possesses an approximate 222 point group symmetry with one of the pseudo twofold axes making an angle of about 5° with the *a* axis. On the other hand human oxyhaemoglobin crystals are tetragonal with space group $P4_12_12$ and with cell dimensions of $a=54{\cdot}3$, $c=196{\cdot}4$ Å. In this case the molecular diad must lie along the [110] and symmetry related directions (Perutz, 1953).

The rotation function program calculates the degree of concurrence arising when the Patterson vectors of one protein are superimposed, in a sphere around the origin, on those of another protein. In order to superimpose the self-Pattersons of human and horse oxyhaemoglobin correctly it is necessary to align the [010] direction of the horse oxyhaemoglobin molecule with the [110] direction of the human oxyhaemoglobin molecule. This result could be obtained by re-indexing the tetragonal unit cell. Maximum agreement between the Pattersons would then be obtained by rotating the tetragonal cell through an unknown angle (say θ) about the common axis. An alternative and more general procedure is to produce alignment of the [010] and [110] directions and rotation through an angle θ in one operation. That is, the tetragonal cell may be rotated through an angle $\varkappa$ about a rotation axis whose position in the monoclinic cell is defined in terms of the polar coordinates ψ and φ (Rossmann & Blow, 1962, Fig. 4).

Assume that initially the *b* and *c* axes of the first crystal are placed on top of the *b* and *c* axes of the second crystal, respectively. If the molecular twofold axes are coincident for a given rotation $\varkappa$, then it can be shown that the position of the rotation axis is given by:

$$y^2=\frac{1-\sqrt{2}\cos\varkappa}{\sqrt{2}(1-\cos\varkappa)}$$

where $\cos\psi=y$

and $$\cos\varphi=(\sqrt{2}-1)y/(1-y^2)^{\frac{1}{2}}$$

It is convenient to define the angle θ, which measures the amount of rotation of one crystal with respect to the other around the twofold axis, as the angle between the monoclinic *a* axis and the tetragonal *c* axis. Both these arbitrary directions are perpendicular to the molecular twofold axes (Fig. 1). The sign of θ is taken so that it is

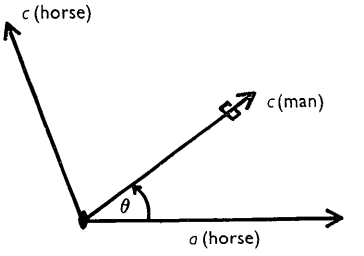

Fig. 1. Relationship between the horse and human oxyhaemoglobin groups viewed down the superimposed molecular twofold axes. θ defines the amount of rotation.

positive when the tetragonal *c* axis lies between the monoclinic *a* and *c* axes. The angle θ is related to $\varkappa$ by

$$\theta=110{\cdot}9°-\cos^{-1}\left[\frac{\sqrt{2}-1+2\sqrt{2}\cos\varkappa}{\sqrt{2}+1}\right].$$

Values of θ from 0° to 180° were explored using 6 Å intensity data and a radius of integration of 35 Å. The 'shaded *G*' function was used. The latter applies a weighting varying exponentially between 1 and 0·1 between the inside and outside of the sphere of integration. Smaller weights near the outside of the sphere emphasize that more cross-vectors between molecules might be found here. A simple sharpening was brought about by omitting

all terms whose spacing was greater than 10 Å. The results are shown in Fig. 2. The two curves correspond to unsharpened data (the dashed line) and sharpened data (the continuous line).

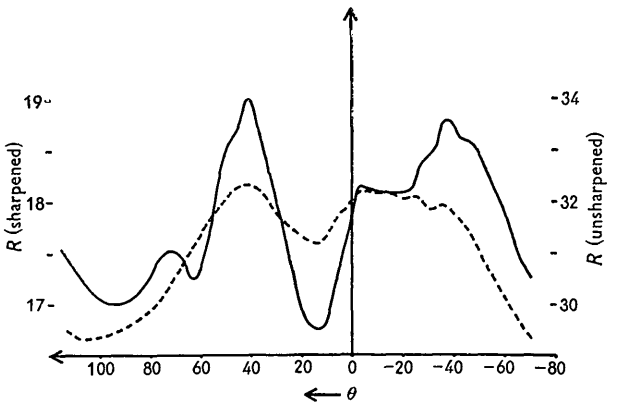

Fig. 2. Rotation function when the [010] direction of horse oxyhaemoglobin is rotated by the angle θ about the [110] human oxyhaemoglobin Patterson. The shaded line is the unsharpened 6 Å rotation function, while the full line represents the data sharpened by omitting all terms with spacing greater than 10 Å.

A check on the level of the background of the rotation function was made by calculating 28 points for values of $\varkappa$ between 0° and 360° in 10° to 15° increments, when $\psi = 35{\cdot}5°$, $\varphi = 54{\cdot}5°$. This section contains the peak at $\theta = 40°$. By averaging the rotation function values, omitting the points which lay within 7·5° of the large peak, the mean value (corresponding to the persistent origin overlap) of R was found to be 17·6 units, and the r.m.s. deviation from the mean was 0·4 units. Thus the peak at $\theta = 40°$, with $R = 18{\cdot}97$, is 3·6 standard deviations above the mean. The second largest peak was less than two standard deviations above background.

Fig. 2 shows two peaks, one of the peaks being rather broader and lower than the other peak. These two peaks arise as a consequence of the pseudo 222 symmetry of the haemoglobin molecule. That is, while one peak corresponds to the superposition of the self-Pattersons of an α onto an α chain and of a β onto a β chain, the other peak corresponds to the superposition of the self-Pattersons of the α onto β and β onto α chains. If the haemoglobin molecules were to contain exact 222 symmetry then the two peaks would be of the same height. Furthermore, in the Patterson the directions of the two different pseudo twofold axes of the molecule must make equal and opposite angles with the tetragonal fourfold axis. Thus if the monoclinic Patterson is positioned on top of the tetragonal Patterson, so as to superimpose the corresponding pseudo twofold directions, there will be good agreement, whereas at an equal and opposite angle with the tetragonal c axis the agreement will not be as good. As the arbitrary reference line from which θ is measured in the monoclinic system coincides to within 5° of the molecular pseudo twofold axis, the two peaks should occur at $\pm\theta$, approximately. Fig. 2 shows the two peaks to be at $\theta = 40°$ (sharp) and $-37°$ (broad).

The agreement of the position of the peak at θ with that at $-\theta$ to within 3°, and the agreement with the results of Perutz (1953) who showed from optical birefringence experiments that θ must be either 35° or —15° establishes the relative orientation of the two molecules as lying between $\theta = 35°$ and 40°. The large size of the peak at $\theta = -37°$ might suggest that the superposition of the 222 symmetrical parts of the molecular Pattersons agree better than the non-symmetrical parts.

We are grateful for discussions with Dr. M. F. Perutz, and to Dr. Hilary Muirhead for allowing us to use her human oxyhaemoglobin data. All calculations were made on the IBM 7090 computer, assisted by the IBM Endowed Time scheme.

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The Fourier transform of an assembly of helices.

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Many biological polymers have a helical conformation and it is possible to calculate the Fourier transform of a single molecule from the expressions of Cochran, Crick & Vand (1952) and Klug, Crick & Wyckoff (1958). It is evident however, from recent X-ray studies of the fibrous proteins α-keratin (Fraser, MacRae & Rogers, 1962) and paramyosin (Cohen & Holmes, 1963) that account must be taken of the interference which occurs as a result of the helices being assembled into bundles or fibrils. The present note describes a method of calculating the cylindrically averaged intensity from an assembly of N helices in which the helix axes are parallel to a unique direction Oz (the fibre axis). Following Klug *et al.*, we may express the transform of a right-handed helical molecule with helix axis coincident with Oz as

$$F_0(R, \psi, l/c) = \sum_n G_{n,l}(R) \exp\,[in(\psi + \tfrac{1}{2}\pi)] \qquad (1a)$$

where

$$G_{n,l}(R) = \sum_j f_j J_n(2\pi R r_j) \exp\,[i(-n\varphi_j + 2\pi l z_j/c)]. \qquad (1b)$$