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# The External Form of the Haemoglobin Molecule. I

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When a salt solution is substituted for water as the medium surrounding a haemoglobin crystal, a few spots of low order are reduced in intensity, while high orders are little affected. The salt enters the liquid surrounding the molecules in the crystal, reducing the contrast in density between protein and liquid. Absolute measurements of the changes in $|F|$ values are used to determine the approximate outer form of the hydrated haemoglobin molecule which is not penetrated by the salt. It is concluded that its width is about 55 Å in the $b$ and $c$ directions, and its length about 65 or 80 Å in the $a$ direction. Its volume is approximately 116,000 Å$^3$.

## 1. Introduction

This note describes an attempt to determine the approximate outer form of the haemoglobin molecule by comparing absolute values of $F$ in low orders for crystals bathed in salt-free and salt-containing liquid. It has been shown (Boyes-Watson, Davidson & Perutz, 1947) that the effect of substituting a solution of $(NH_4)_2SO_4$ for water is to reduce the strength of a few spots of low order in the rotation photograph, while higher orders are little affected. The salt enters into the crystal, as is shown by its increase in density, and seems to enter into the spaces between the haemoglobin molecules without, however, penetrating the molecules themselves. It thus reduces the difference in mean density between the protein molecules and the liquid between them, so that diffracted spots mainly due to this difference in mean density, and not to the intra-molecular structure, are weakened. It must be presumed that there is also adsorption of $NH_4^+$ and $SO_4^{--}$ ions on the protein surface, but they do not seem to have an appreciable effect on the diffraction pattern, and to a first approximation, at least, their effect can be neglected.

Suppose a region $V$ (Fig. 1) of constant shape to be occupied by the 'molecule', which for present purposes is defined as the region into which the salt does not penetrate when a salt solution is substituted for water. It is assumed that the density of the liquid outside $V$ is uniform. The effect on the value of $F$ of increasing the density everywhere outside $V$ is equivalent to that of decreasing the density by an equal amount everywhere inside $V$, since a uniform distribution of density throughout the unit cell has no effect on $F$. It follows that the values of $|F(\text{water})-F(\text{salt})|$ are the $F$ values for a region $V$ of uniform density equal to the difference between the electron concentration of salt solution and water.

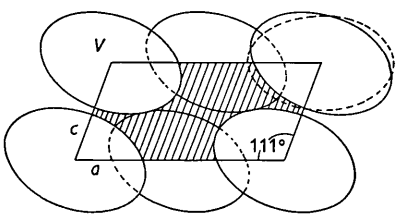


Fig. 1. Projection of the haemoglobin unit cell on the $b$ face. The molecules at 0, $\frac{1}{2}a$ are at the centre of the $c$ face. The shaded region is occupied by liquid. The broken molecule at the top right-hand corner is in an alternative orientation.

The space group $C2$ has twofold axes but no symmetry centres; hence in a strict interpretation of the above relation the differences are vectorial differences between $F$ values which may have any phase. The simplifying assumption will be made that the molecule can be regarded as centro-symmetric and the phase of $F$ as being 0 or $\pi$ in considering the reflexions of low order. It is significant that the initial effect of adding salt to the liquid is in each case to reduce the intensity, except for some higher orders where the effect is in any event quite small. Further, the reduction in the value of $|F|$ is proportional to the increase in electron density of the liquid. It follows that the phase of $F(P)$, the molecular scattering factor of the protein molecule with its complex internal structure is, at any rate as regards these low orders, the same as the phase of $F(V)$, the scattering factor of a region $V$ of uniform density, or that their signs are the same if centro-symmetry is assumed. Hence we may take $|F| = |F(\text{water})|-|F(\text{salt})|$ as the appropriate value for a uniform region $V$. The advantage of using these values for determining the shape of $V$ is that they are independent of the intra-molecular structure.

## 2. Data

The crystal is 'normal wet' horse methaemoglobin (Boyes-Watson, Davidson & Perutz, 1947).

Table 1. *$F^2$ values as function of salt concentration*

(Figures for intensities to be multiplied by $10^5$. Values of $\varrho$ in e.Å$^{-3}$.)

| Medium | Salt-free | | 2·2 M | 3·2 M | 4 M | 4 M + 10 % CsCl | |
|---|---|---|---|---|---|---|---|
| *h0l* | $\varrho = 0{\cdot}334$ | | $\varrho = 0{\cdot}379$ | $\varrho = 0{\cdot}395$ | $\varrho = 0{\cdot}408$ | $\varrho = 0{\cdot}422$ | |
| 200 | 3 | | 0 | 0·85 | 1·6 | 4 | *C* |
| 400 | 17 | | 8 | 5 | 2·6 | 5 | *C* |
| 600 | 3·5 | | 0·3 | 0 | 0 | 0 | *C* |
| 800 | 0 | | 0·3 | 0 | | 1·6 | |
| 001 | 70 | *G* | 8 | 2 | 0·2 | 2 | *C* |
| 201 | 0·3 | | 0 | 0·5 | 1·5 | 1·4 | |
| 401 | 5·5 | | 1·6 | 1 | 1 | 0·5 | *C* |
| 601 | 8·5 | | 7 | 6·5 | 5 | 7 | |
| 002 | 18 | *G* | 4 | 3·5 | 1·3 | 0·85 | *C* |
| 202 | 2 | | 1 | 1 | 0·4 | 1 | |
| 402 | 18·5 | | 10 | 9 | 7 | 8 | *C* |
| 602 | 0·5 | | 1 | 0·6 | 0·5 | 0·5 | |
| 003 | 3 | *G* | 2 | 2 | 2 | 2 | |
| 203 | 5·5 | | 5·5 | 6 | 4 | 4 | |
| 403 | 8 | | 7 | 7 | 5 | 7 | |
| 603 | 4 | | 4 | 4 | 4 | 4 | |
| 004 | 0 | | 1 | 1·7 | 1·6 | 2 | *C* |
| 204 | 4 | | 4 | 4 | 4 | 3 | |
| 404 | 3·5 | | 3 | 2 | | 1·7 | *C* |
| 20$\bar{1}$ | 0 | | 0 | 0 | 0 | 0 | |
| 40$\bar{1}$ | 0 | | 2 | 2 | | 3 | *C* |
| 60$\bar{1}$ | 0 | | 0 | 0 | | 1 | |
| 80$\bar{1}$ | 0 | | 0·3 | 1 | | 0 | |
| 20$\bar{2}$ | 18 | | 7 | 5 | 2·4 | 2 | *C* |
| 40$\bar{2}$ | 1 | | 0·4 | 0·25 | 0 | 0 | *C* |
| 60$\bar{2}$ | 37 | | 22 | 14 | | 14 | *C* |
| 80$\bar{2}$ | 4·5 | | 6 | 5 | | 5 | |
| 20$\bar{3}$ | 0 | | 1 | 1 | 1 | 1 | |
| 40$\bar{3}$ | 0·5 | | 0·3 | 0·4 | 0 | 0 | |
| 60$\bar{3}$ | 1·5 | | 3 | 3 | | 4 | *C* |
| 80$\bar{3}$ | 0 | | 0 | 0 | 0 | 0 | |
| 20$\bar{4}$ | 4 | | 3 | 2 | 1·3 | 0·5 | *C* |
| 40$\bar{4}$ | 2·5 | | 2 | 2 | 2 | 2 | |

Standard reflexions $F^2|006| = 16(G)$, $F^2(205) = 12{\cdot}5$, $F^2(10,0,0) = 16$.

| *0kl* | Salt-free | | 2·2 M $\varrho = 0{\cdot}379$ | | *hk*0 | Salt-free | 3 M $\varrho = 0{\cdot}39$ | |
|---|---|---|---|---|---|---|---|---|
| 020 | 7·0 | | 0 | *C* | 200 | 3·0 | 1·0 | *C* |
| 040 | 1·7 | | 5·3 | *C* | 400 | 17 | 6·0 | *C* |
| 060 | 0 | | 0 | | 600 | 3·5 | 0·3 | *C* |
| 080 | 19 | | 19 | | 800 | 0 | 0·3 | |
| 001 | 70 | *G* | 8 | *C* | 110 | 63·0 | 1·5 | *C* |
| 021 | 14 | | 2·7 | *C* | 310 | 4·3 | 2·5 | |
| 041 | 0·4 | | 0 | | 510 | 6·4 | 5·0 | |
| 061 | 10·5 | | 8·0 | | 710 | 1·4 | 1·5 | |
| 081 | 16 | | 18 | | 020 | 6·1 | 1 | *C* |
| 002 | 18 | *G* | 4 | *C* | 220 | 9·1 | 4·5 | *C* |
| 022 | 12 | | 3·5 | *C* | 420 | 4·9 | 4·5 | |
| 042 | 5·0 | | 7·1 | | 620 | 1·8 | 1 | |
| 062 | 45 | | 39 | | 130 | 6·4 | 1 | *C* |
| 003 | 3·0 | *G* | 2·2 | | 330 | 14·7 | 12 | |
| 023 | 15·0 | | 15·0 | | 530 | 3·0 | 1 | |
| 043 | 31 | *G* | 32 | | 730 | 2·0 | 1·5 | |
| 063 | 45 | *G* | 39 | | 040 | 2 | 5 | |
| 004 | 0·4 | | 1 | *C* | 240 | 2·0 | 3 | |
| 024 | 0 | | 0·9 | | 440 | 12·9 | 12 | |
| 005 | 7·7 | *G* | 9 | | 640 | 6·6 | 8·5 | |
| 025 | 0 | | 0·4 | | 150 | 2·2 | 1·5 | |
| 006 | 16 | *G* | 16 | | 350 | 2·0 | 1·5 | |
| 026 | 13 | | 13 | | 550 | 9·0 | 7·5 | |

Standard reflexion $F^2(006) = 16(G)$.

Values marked *G* have been determined by Geiger-counter measurements.

*C* denotes reflexions whose intensities undergo a change which is larger than the experimental error.

Space group $C2$.

$$a = 109,\ b = 63{\cdot}2,\ c = 54{\cdot}4\ \text{Å},\ \beta = 111°.$$

The cell dimensions are not altered by the substitution of neutral salt solution for water (Perutz, 1946), as long as the salt concentration does not exceed saturation point.

Absolute values of the intensities of $h0l$ reflexions are based on visual comparisons of intensities on precession photographs with those given on the same films by a standard anthracene crystal. These values were further checked by Geiger-counter measurements for certain reflexions from a salt-free crystal, using again comparison with a standard anthracene crystal. Intensities of $0kl$ and $hk0$ reflexions were estimated visually from precession photographs on a relative scale, and afterwards scaled to the standard values obtained from the absolute measurements. A list of but at this stage we are merely attempting to decide on the basis of X-ray diffraction observations between

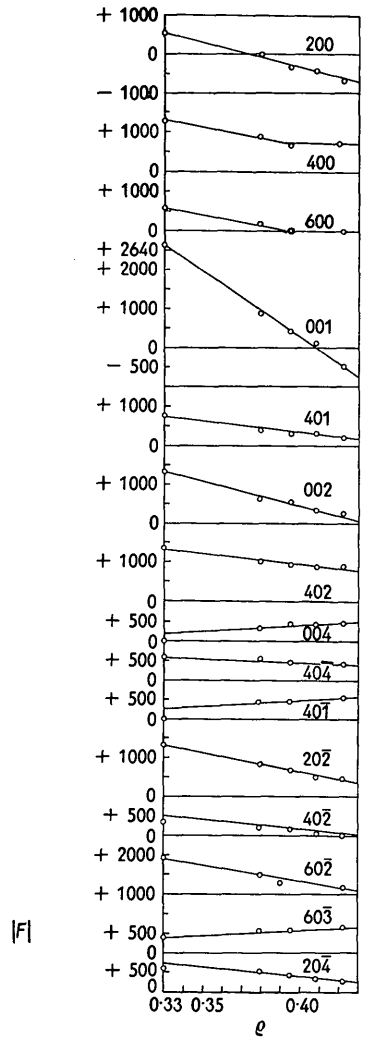


Fig. 2. Absolute values of $F(h0l)$ plotted against salt concentration.

$|F|^2$ values is given in Table 1. The values for the $h0l$ zone were carefully measured for five electron concentrations, and the consistency of the $|F(h0l)|$ values (Fig. 2) is evidence of their accuracy. The values for the $hk0$ and $0hl$ zones were measured only for water and for one salt concentration, and are less certain. The accuracy of a value of $|F|$ depends very sensitively on its magnitude and on the Lorentz factor. For instance, when a reflexion is not observable in the photograph we can in general only be sure that its $F$ value is less than 250, except for a case such as $F(20\bar{1})$ which is at so low an angle that its absence implies it must be less than 100. On the other hand, the difference between two large $F$ values can be measured with much greater accuracy.

### 3. The form of the region $V$

We wish to emphasize that in the present analysis we are only seeking to discover the general outer shape and volume of the molecule. It might, for example, be spheroidal, cylindrical or rectangular in outline, widely different alternatives, such as whether it is approximately equal in all three dimensions or much longer than it is wide. An estimate of the extent to which the precise form of the region $V$ occupied by the molecule may be expected to influence the values of $F(V)$ can be obtained from Fig. 3(*a*) which contrasts the scattering by circular and square apertures of equal area. The maximum at the centre is taken to be unity, and contours are plotted at intervals of 0·1, negative contours being shown as broken lines. The contours are practically identical for $F > 0{\cdot}5$, and not very different in the whole of the central maximum. The first negative region is of course continuous for the circular aperture, and broken up into four quadrants for the square aperture; on the whole, large negative values occur at the same distance from the origin. Beyond this point little correspondence can be traced.

Extending this comparison to three dimensions, it may be concluded that, unless the shape of the molecule is very irregular, a correspondence may be expected between the absolute $F(V)$ values and those of the nearest equivalent ellipsoid insofar as high values of $F$ in the central maximum and first negative area are concerned, but that departures from the ellipsoidal shape will make this correspondence progressively less as one passes to outer regions of the diffraction pattern. An ellipsoidal shape will therefore be assumed for the purpose of the present analysis, although it is clear from the measurements of $|F|$ that this is only a first approximation, and, indeed, it is hoped at a later stage to define the shape more closely when more observations are available.

The data for diffraction by a uniform sphere are given by James (1948) in connection with another problem. The amplitude passes thorugh a zero value when the maximum phase difference between centre

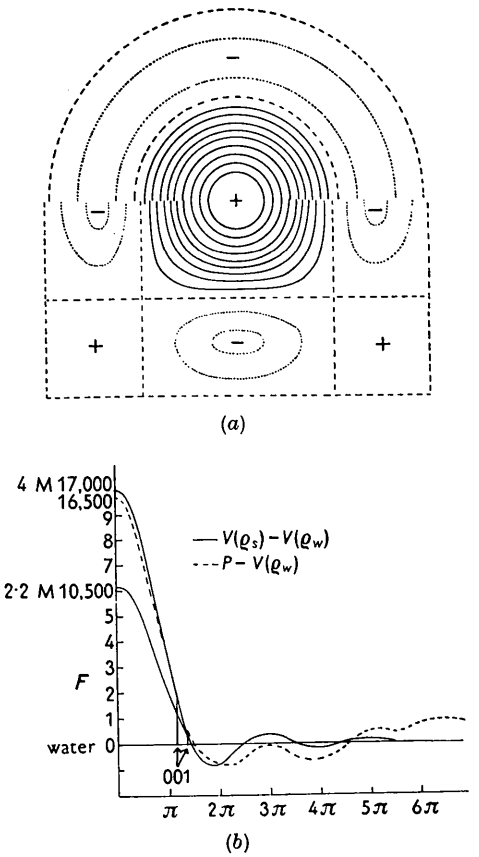


Fig. 3. (*a*) Contrast of Fraunhofer diffraction by circular and square apertures of equal area. Contours are drawn at intervals of 0·1. The broken lines mark the zero contours.
(*b*) Broken curve: Absolute values of $F$ for $\{P-V(\varrho_w)\}$. The curve is derived from the observed values of $F(00l)$ at different stages of swelling and shrinkage; the signs are chosen according to the molecular Fourier transform of haemoglobin derived by Bragg, Howells & Perutz (1952). Continuous curves: Absolute values of $F$ for $\{V(\varrho_s)-V(\varrho_w)\}$ for 2·2 M and 4 M $(NH_4)_2SO_4$. The curve corresponds to the Fourier transform of a spheroid of uniform density (James, 1948).

and surface has the values $1{\cdot}43\pi$, $2{\cdot}46\pi$, $3{\cdot}47\pi$, $4{\cdot}48\pi$, etc. (as compared with $1{\cdot}22\pi$, $2{\cdot}23\pi$, $3{\cdot}24\pi$ for a circular aperture, and $\pi$, $2\pi$, $3\pi$ for a slit). The maxima and minima are

| | | | | |
|---|---|---|---|---|
| Sphere | 1, | −0·085, | 0·036, | −0·020 |
| Circular aperture | 1, | −0·132, | 0·064 | |
| Slit | 1, | −0·224, | 0·13, | −0·09 |

Diffraction by an ellipsoid follows directly from that by a sphere, by scaling the axes appropriately, the fringes being closest in a direction parallel to a long axis, and vice versa.

The effect of altering the electron density of the liquid between the molecules is made clear by Fig. 3 (*b*). Values of $F$ for the crystal are due to a complex structure $P$ consisting of protein together with any water of hydration included in the region $V$, surrounded by liquid of constant density $\varrho$ occupying the rest of the unit cell. As has been seen, the effect of the latter is equivalent to that of a uniform density $-\varrho$ throughout $V$. Diffraction by a crystal in water of electron density $\varrho_w$ is due to $\{P-V(\varrho_w)\}$; a typical form is shown as a broken curve, the absolute values of $F$ chosen for the sake of comparison being actually those of the observed $F(00l)$ values for different stages of shrinking. If salt is substituted for water, the observed values of $F$ will be due to $\{P-V(\varrho_s)\}$, or

$$\{P-V(\varrho_w)\}-\{V(\varrho_s)-V(\varrho_w)\}\,.$$

Typical curves for scattering from $\{V(\varrho_s)-V(\varrho_w)\}$ are shown as full curves, assuming $V$ to be ellipsoidal. Any observed value of $F$ is the difference at the appropriate abscissa between ordinates of the broken curve and the full curve for that particular value of $\varrho_s$. At high angles $|F|$ for an ellipsoid is very small, while $|F|$ for the broken curve may be still quite large owing to the intramolecular structure; hence an alteration of salt concentration has little effect on the intensities at high angles. At low angles, on the other hand, the scattering is mainly due to the difference in average density between the molecule and the surrounding liquid; by increasing the density of the latter the difference in ordinates is reduced and at a sufficiently high value any given low-angle reflexion can be made to disappear. At still higher densities it appears again. The concentration at which this happens varies from one reflexion to another (see Fig. 2) because the $P$ and $V$ curves diverge in shape as the angle increases.

## 4. The volume of the hydrated molecule

In order to determine $F(000)$ it is necessary to know the volume of the region $V$ into which the salt does not penetrate, since $F(000)$ is equal to this volume multiplied by the difference in electron density of salt solution and water. The variation with salt concentration of the strong (001) reflection may be made the basis for a rather precise calculation of this volume.

$|F(001)|$ is outstandingly large in the normal salt-free crystal, being about 16% of $F(000)$ in the $\{P-V(\varrho_w)\}$ curve. In a shrunk form of the salt-free crystal, where $|F(001)|$ occurs at a larger angle, its value decreases. It can therefore be concluded that $F(001)$ is well within the central maximum, as in Fig. 3 (*b*) where the values for normal and shrunk crystal are shown. At this angle the forms of the $P$ and $V$ curves must be very similar. We may thus expect $F(001)$ to become zero when the density of the liquid in the crystal matches the average density of the molecule. Fig. 3 (*b*) shows why this comparison is justified in the case of $F(001)$ whereas it would lead to a false estimate if a higher order of reflection were used.

Now the total amount of salt which enters the unit cell for any given concentration of the surrounding liquid can be found by the increase in density of the crystal. We can deduce directly what volume in the

cell this salt must permeate in order that the density of the solution may match that of the molecule. For instance, the apparent density of anhydrous haemoglobin is 1·33 g.cm.$^{-3}$ (Adair & Adair, 1936), giving a volume of 83,000 Å$^3$ per molecule and electron density 0·43 e.Å$^{-3}$. On the other hand, there is evidence (Adair & Adair, 1936; Perutz, 1946) that the hydrated molecule has associated with it about 0·3 g. water per g. protein, forming an envelope about 3 Å thick which increases the volume to about 116,000 Å$^3$ and lowers the average electron density to 0·405 e.Å$^{-3}$. Perutz has further shown that if the known amount of salt permeates the water of hydration and is excluded only from the protein itself, its average concentration in the liquid of crystallization must be two-thirds that of the surrounding solution; on the other hand, if it is excluded from the hydrated molecule, the concentrations inside and outside the crystal are about the same. In the first case a density match with the protein would require a concentration of 6 M inside the crystal and 9 M outside, far beyond that of a saturated solution of $(NH_4)_2SO_4$. In the second case the density of the surrounding liquid which is the same as that inside the crystal should match the average density of the hydrated molecule, 0·405 e.Å$^{-3}$. Actually it can be seen from Fig. 2 that the match occurs at 0·407 e.Å$^{-3}$, showing definitely that the salt is excluded from the hydrated molecule of volume 116,000 Å$^3$.

The values of $F(000)$ are therefore $232{,}000(\varrho_s-\varrho_w)$ while $\varrho_s$ and $\varrho_w$ are electron densities of solution and water respectively.

## 5. Analysis of diffraction data

The absolute values of $|F(\text{water})|-|F(\text{salt})|$ may now be used to define the shape of the region $V$. They are plotted on reciprocal nets in Fig. 4, together with the values of $F(000)$ appropriate to the salt concentration in each case. When the reflections are very weak or absent, so that estimates of $F$ are uncertain, the spot is marked $w$. We are seeking to outline the first zero contour, because this reflects the general shape of the hydrated molecule. It is interesting to note that the $F$ values are quite large fractions of $F(000)$, an unusual feature in a problem concerned with diffraction by proteins and one which makes it easier to draw definite conclusions.

### (a) $F(h0l)$

The values of $|F|$ are deduced from the slopes of the curves in Fig. 2, and correspond to a change in electron density of 0·10 e.Å$^{-3}$ in the liquid inside the crystal, so that $F(000)$ is 23,200 e. The key reflexions are $F(001)$, $F(20\bar{1})$, $F(200)$. As shown above, $F(001)$ is definitely within the central maximum; using the full curve of Fig. 3(*b*) to extrapolate the position of the zero contour, the value of $F(001)$ of 15% of $F(000)$ defines the contour as passing through

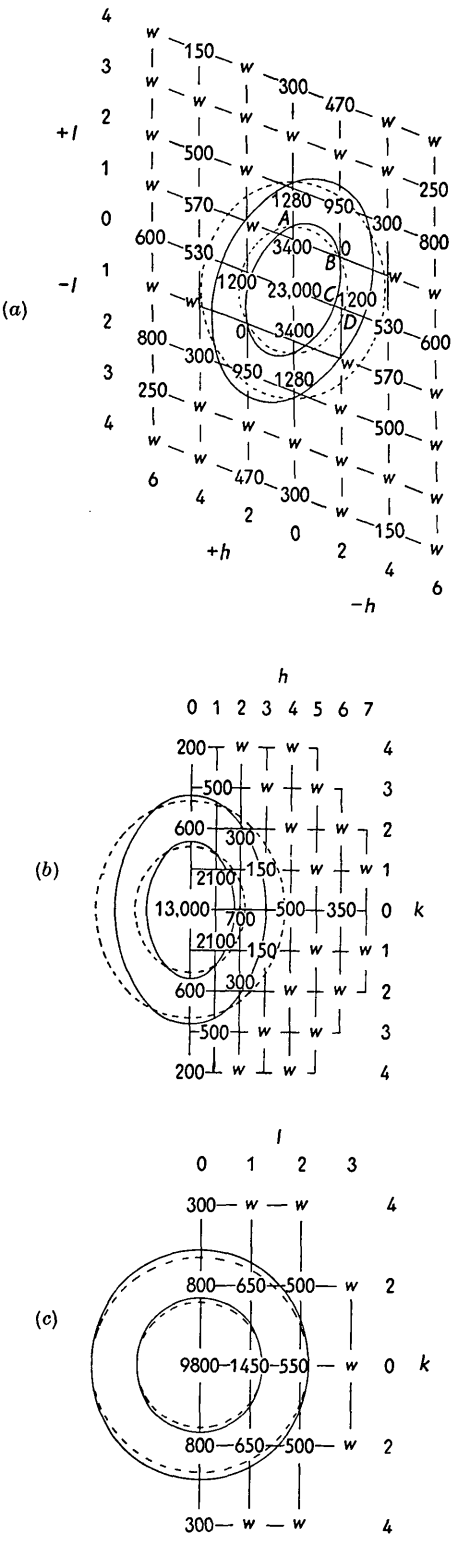


Fig. 4. Values for $(|F(\text{water})|-|F(\text{salt})|)$ for the (*a*) *h*0*l*, (*b*) *hk*0 and (*c*) 0*kl* reflexions. The full curves correspond to zero contours of first and second order of the Fourier transform of the longer type of ellipsoid, the broken curves to the corresponding contours for the shorter ellipsoid. Scale: $1/d = 1{\cdot}0 = 34$ cm.

the point marked $A$. $F(20\bar{1})$ is at all concentrations too weak to register, and as its Lorentz factor is large it must be extremely small; it is assumed to be zero, which means that the contour must pass through $B$. $F(200)$ is little more than one-third of $F(001)$. It must be near the zero contour, but it is not certain whether it is inside or outside this contour. Using, again, the full curve of Fig. 3(*b*) to find the position of the zero contour, the absolute value of $F(200)$ shows that the contour passes through either $C$ or $D$. Assuming the contours to be elliptical, the two alternative forms are shown in Fig. 4(*a*) and correspond to ellipsoidal molecules with axes

(I) ($F(200)$ positive) $a' = 33$, $c' = 29$ Å. $a'$ parallel to $a$;

(II) ($F(200)$ negative) $a' = 41$, $c' = 27$ Å. $a'$ perpendicular to $c$.

(*b*) $F(hk0)$

The figures are for salt-free and 3 M $(NH_4)_2SO_4$ concentrations. $F(110)$ is very strong and clearly inside the central maximum. There is the same uncertainty respecting the sign of $F(200)$. The two possible forms for the zero contour are shown in Fig. 4(*b*). Figs. 4(*a*) and (*b*) together define the alternative spheroids

($F(200)$ positive) $a' = 33$, $b' = c' = 29$ Å;
($F(200)$ negative) $a' = 41$, $b' = c' = 27$ Å.

(*c*) $F(0kl)$

The spheroid in this projection (Fig. 4(*c*)) is viewed along its long axis, and so appears nearly circular in shape. The contours are drawn for the two above alternatives. The low orders fit quite well into the nearly circular diffraction rings appropriate to this projection.

A molecule with $a' = 33$, $b' = c' = 29$ Å has a volume of 117,000 Å$^3$. One of $41 \times 27 \times 27$ Å has a volume of 125,000 Å$^3$. Though these measurements are very approximate, their rough agreement with the volume 116,000 Å$^3$ deduced for the hydrated molecule affords an independent check. On the other hand, the $c$ axis of the crystal is only 54 Å in length, so $c'$ cannot be greater than 27 Å. The difference between 29 Å and 27 Å is, however, well within the error of estimation.

To sum up, the diffraction measurements indicate a hydrated haemoglobin molecule of volume about 116,000 Å$^3$. The overall widths in the $b$ and $c$ directions are about 55 Å, and the overall length either about 65 Å in the direction of the $a$ axis or 80 Å perpendicular to the $c$ axis.

## 6. Alternative models of the molecule

As will be shown in a later paper, a spheroidal molecule of approximately the dimensions deduced above packs into the various shrinkage stages of this type of haemoglobin crystal, and also packs into some ten other types of haemoglobin crystal. Packing considerations alone, however, cannot determine the form uniquely. For instance, Dornberger-Schiff (1950) has proposed a very different type of molecule as regards its general outline, which packs equally well into the various shrinkage stages of horse methaemoglobin. An ellipsoidal shape corresponding to her model has overall dimensions of $101 \times 47{\cdot}2 \times 31{\cdot}5$ Å, the short axis being in the $b$ direction and the long axis making an angle of 49° with the $a$ axis. A comparison of the diffraction patterns for such a molecule with the observed $F$ values shows contradictions which are beyond any possible error of experimental measurement. For instance, in the $(0kl)$ projection $F(020)$ comes well inside the central maximum and should be very large, whereas $F(001)$ is only slightly outside the first zero contour and should be very small. It should be acknowledged that Dornberger-Schiff does not put forward her model as a correct or even a highly probable one, but merely as one which illustrates that there are possible alternative ways of explaining the packing in the unit cell. To sum up, if the reasoning in the present paper is sound, it definitely excludes all models except one for which nearest molecules are nearly in contact in both the $b$ and $c$ directions. In order to account both for the $|F|$ values and for the molecular volume it is necessary to assume a longer axis in the $a$ direction, and one is led directly to the general outline proposed here.

The model originally proposed (Boyes-Watson, Davidson & Perutz, 1947) for the anhydrous protein molecule was a circular cylinder of 55 Å diameter and 34 Å height with the base parallel to the $c$ plane. It has been apparent for some time that a modification of this model was necessary, especially as it does not pack into some of the unit cells which have since been discovered. The dimensions of the present model are greater, because it is hydrated, but in addition to this it differs in assigning to the molecule a longer axis in the $a$ direction and equal axes in the $b$ and $c$ directions.

## 7. Further refinements

It is tempting to continue the analysis of the observations in two directions. In the first place, one may seek to define the actual form of the hydrated molecule more closely by considering values of $|F(\text{water}) - F(\text{salt})|$ for higher orders, since it is clear from the observations that a spheroidal shape is only a first approximation. If such an analysis were successful it would determine the signs of several higher orders, and consequently the signs of $F$ for the protein molecule itself, so in the second place one could use these signs to determine large-scale departures from uniformity of the density of scattering matter within the molecule. It is proposed to continue along both these lines, but before embarking upon the

analysis it is desirable to improve the accuracy of the measurements, and to extend them to (*hkl*) values and to other shrinkage stages. These experimental measurements are in progress.

We wish to acknowledge the help of Mr K. K. Møller who determined the absolute intensities of certain reflexions, and of Mr F. H. C. Crick and Mr H. E. Huxley who took some of the required photographs.

*Note added in proof*, 4 *March* 1952. It has now been found that F(200) is positive, which excludes the longer of the two alternative models and indicates $55 \times 55 \times 65$ Å as the dimensiones of the hydrated molecule.

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## Short Communications

*Contributions intended for publication under this heading should be expressly so marked; they should not exceed about 500 words; they should be forwarded in the usual way to the appropriate Co-editor; they will be published as speedily as possible; and proofs will not generally be submitted to authors. Publication will be quicker if the contributions are without illustrations.*

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### A note on the calculation of the absorption factors for single crystals with high absorbing power.

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Recently Howells (1950) described a universal graphical method of estimating absorption factors for single crystals. This method is based on the application of the loci of points for which the optical path of X-rays is constant. The author (Grdenić, 1949) suggested a numerical method, based on the same principle, for calculating absorption factors for the zero-layer reflexions of a single crystal having the shape of a rectangular prism. This method, however, was intended for crystals of low or medium absorbing power. During the course of the structure analysis of mercury compounds (all having a high absorption coefficient) it was found that the absorption factors could be calculated more easily and rapidly by using simple formulae. It may be noted that the same is true of Howells's graphical method; it is very good when the absorption coefficient is not too high. For crystals with high absorbing power the use of formulae is much more practical. These formulae were obtained by a method similar to that of Hendershot (1937). Their application may best be explained using the same example as Howells (1950). All notations used here relate to Fig. 1 (p. 367) of his paper.

#### 1. Reflexion 'on the crystal face'

In this case area (1) makes the main contribution to the reflected intensity, areas (2) and (4) make small contributions, while area (3) makes practically no contribution. The integral

$$A_{hkl} = \int_S \frac{\exp(-\mu x) ds}{S}$$

for area (1) gives the formula

$$A(1) = \frac{1}{S} \frac{1}{\mu (\operatorname{cosec} \psi_2 + \operatorname{cosec} \varphi_2)} \left[ AB - \frac{\sin(\psi_2 + \varphi_2)}{\mu (\sin \psi_2 + \sin \varphi_2)} \right].$$

Substituting the values given by Howells for the reflexion 038 of mercury diphenyl we get $A(1) = 3{\cdot}45 \times 10^{-2}$. If we evaluate the integral for areas (2) and (4) the formulae

$$A(2) = \frac{1}{S} \frac{\sin \varphi_1 \sin \varphi_2}{\mu^2 \sin \delta_1} \frac{1}{1 + \sin \psi_2 / \sin \varphi_2}$$

and

$$A(4) = \frac{1}{S} \frac{\sin \psi_1 \sin \psi_2}{\mu^2 \sin \delta_2} \frac{1}{1 + \sin \psi_2 / \sin \varphi_2}$$

are obtained, where $\delta_1$ and $\delta_2$ are the interfacial angles, in this case 104° and 76° respectively. Substituting the appropriate values, we get $A(2) = 0{\cdot}11 \times 10^{-2}$ and $A(4) = 1{\cdot}18 \times 10^{-2}$. Thus the total absorption factor is

$$A_{038} = A(1) + A(2) + A(3) + A(4) = 4{\cdot}74 \times 10^{-2}.$$

The graphical method due to Howells gives $A_{038} = 4{\cdot}69 \times 10^{-2}$, which proves that the agreement is satisfactory.