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The External Form of the Haemoglobin Molecule. II

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Considerations of packing of molecules in various forms assumed by mammalian haemoglobin agree with deductions as to the general overall dimensions of the hydrated molecules based on X-ray diffraction effects and described in a previous paper (Bragg & Perutz, 1952). The following figures for types of haemoglobin crystal listed at the beginning of this paper indicate the degree of consistency between various estimates:

Type 1.	Either $50\times50\times75$ Å or $55\times55\times65$ Å.
Type 12.	Same as above.
Type 4.	$56\times56\times72$ Å.
Type 10.	$54\times54\times69$ Å.

Though insufficient data are as yet available for deductions from the packing in the other types of molecule listed in this paper, the unit cells and symmetry are not inconsistent with these dimensions except in the case of foetal sheep haemoglobin, which would appear to have a different structure.

1

The haemoglobins crystallise in many different forms, and polymorphism is widespread. The unit cells and space groups of the forms shown in Table 1 have been determined.

The object of the present analysis is to define the outer form of the molecule by noting how the molecules pack into these different unit cells. The haemoglobins from different animal species resemble each other in their molecular weights and amino-acid composition; as will be shown in this paper they also appear to resemble each other as regards external form. The volume per molecule in the dry state is much the same for all crystals, varying between 97,000 Å^3 and 104,000 Å^3. (Type 9 appears to have a smaller volume of 84,500 Å^3, but the measurements were not reliable in this case as they were made on a very imperfect specimen dried from alcohol.) The volume in the case of the wet crystals varies over a much wider range, 138,000–189,000 Å^3. This is to be expected, since it has been shown (Boyes-Watson, Davidson & Perutz, 1947) that wet crystals of type 1 have a series of swelling and shrinkage stages with a corresponding range of unit cell volumes.

2

In order to test the packing of the molecules, it is necessary to know their positions and relative orientations in the unit cell. In type 1 the molecules are at lattice points; in the other cases their positions depend upon parameters which must be fixed by considering the X-ray diffraction and optical data. In some instances the crystal morphology provides a useful clue to the packing of the molecules. Types 1, 4, 10 and 12 are particularly favourable cases for examination and will alone be considered here. Even if the relative positions of neighbouring molecular centres are knwon, it is not possible to determine the form of the molecule uniquely by packing considerations alone. However, the analysis of low-order diffraction by crystals in water and in salt solution described in an accompanying paper (Bragg & Perutz, 1952) indicates that the hydrated molecule has equal overall dimensions of about 55 Å in two directions at right angles, and a longer overall dimension of 65 Å in the third direction. It will be shown here that a molecule of approximately these dimensions is in accord with the unit-cell dimensions and symmetry of the types of haemoglobin crystal considered, and is the simplest form which brings the molecules into contact along lines joining the centres of nearest neighbours. Most of the figures in the present paper are drawn for a molecule of overall dimensions $50\times50\times75$ Å, figures which seemed to be in the best accord with packing considerations, but a molecule $55\times55\times65$ Å fits almost equally well and we do not claim to distinguish between these alternatives. The molecules are represented as spheroids in the accompanying figures, but it is to be emphasized that this is done only in order to show

in a simple diagrammatic way how they pack together; no claim is made at this stage of the analysis to outline their precise form.

3

Met-, oxy- and carboxyhaemoglobin of horse. Type 1

$C2$. Wet $a=109$, $b=63{\cdot}2$, $c=54{\cdot}4$ Å, $\beta=111°$; dry $a=102$, $b=56$, $c=49$ Å, $\beta=134°$.

Perspective drawings of the packing for wet and dry crystals are shown in Fig. 1 (*a*) and (*b*). The analysis of diffraction referred to above leads to the conclusion that the short dimensions are in the *b* and *c* directions, and that the long axis lies somewhere in the arc between the *a* axis and the perpendicular to the *c* axis. Molecules of the dimensions $50\times50\times75$ Å can be packed into all the shrinkage stages of the wet crystals, as shown in Fig. 1 (*c*), but there is an overlap in the *c* direction in the final dry stage. Molecules of the dimensions $55\times55\times65$ Å pack well into the expanded and normal wet stages, but overlap slightly in the two shrunk stages (Fig. 1 (*c*) and (*f*); see also § 6 below). The packing in the dry crystal shown in Fig. 1 (*c*) is closest when the long axis makes an angle of about 20° with the *a* axis. The form shown in this figure is that for the crystal dried from 25% alcohol, which has cell dimensions $a = 102$, $b = 51{\cdot}4$, $c = 47$ Å, $\beta = 130°$, and a cell volume of 189,000 Å^3. The crystals show strong pleochroism of the negative uniaxial type (Perutz, 1939), the direction of weak absorption (α) making an angle of 16° with the *a* axis, in the obtuse angle between *a* and *c*. Thus α appears to be approximately parallel to the length of the molecule, a fact which is helpful in finding the molecular orientation in other unit cells. The only faces developed by the crystal are {001} and {110}, which are also the planes most densely packed with molecules.

4

Reduced haemoglobin of man. Type 12

$P2_1$. Wet $a=62{\cdot}5$, $b=83{\cdot}2$, $c=52{\cdot}8$ Å, $\beta=98°$; dry $a=59$, $b=70$, $c=47{\cdot}5$ Å, $\beta=97{\cdot}5°$.

This crystalline form is very interesting. As will be shown in a fortcoming paper by Liquori, Trotter & Perutz, the central portion of the Patterson projection on a plane perpendicular to the *a* axis is so similar to the corresponding projection for type 1 as to leave little doubt that the haemoglobin molecules of horse and man are closely similar in structure and are in identical orientations in these two projections. This conclusion is also in agreement with the pleochroism of the crystals which shows α, the direction of least absorption, to be parallel to *a*. If this interpretation is correct, other features of the Patterson define the vector relationship between two molecules related by the twofold screw axis shown in Fig. 2 (*b*). If a molecule A_1 is at the corner of the unit cell projected on the *b* face, the molecule *B* is arrived at by a translation of 26 Å parallel to *a*, 18 Å parallel to *c*, and of course $\frac{1}{2}b$ or 41·6 Å in a direction perpendicular to the plane of the diagram. Thus molecule *B* packs between $A_1\,A_2\,A_4$ above and three corresponding molecules below. The distances are A_1–B = 53 Å, A_2–B = 58 Å, A_4–B = 55 Å. The lattice is approximately body-centred so that faces of the type 011 and 110 are likely to be dominant, being the most densely packed.

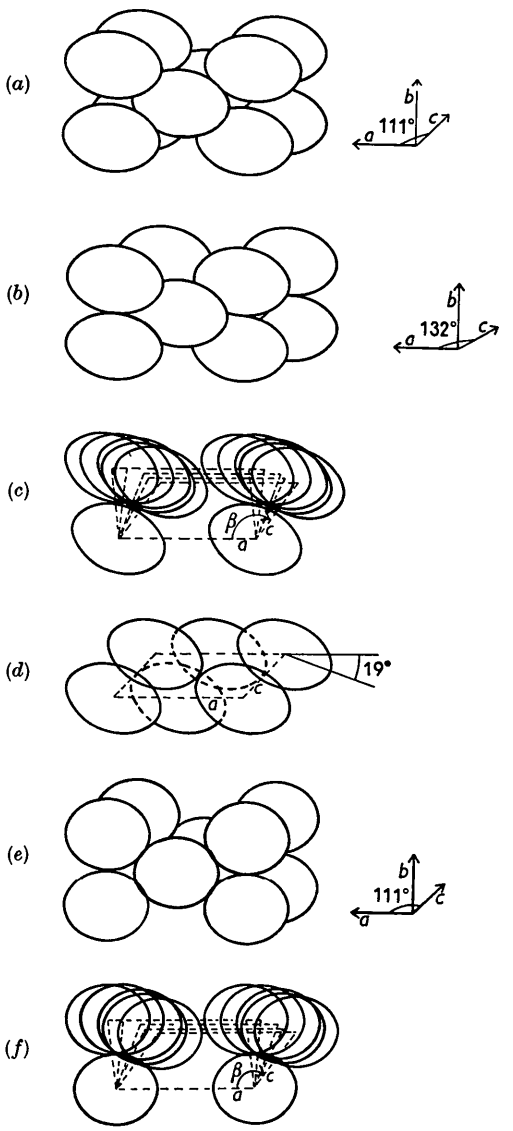

Fig. 1. Horse methaemoglobin. (*a*) Normal wet stage in perspective. In this and subsequent perspective drawings no attempt has been made to follow the rules of clinographic projection. (*b*) Dry stage in perspective. (*c*) Projection of successive shrinkage stages on *b* face. Molecules at the centre of the *c* face are not shown. (*d*) Projection of smallest dried cell (unit cell No. 1, Table 1, Boyes-Watson, Davidson & Perutz, 1947). (*e*) Normal wet stage in perspective, showing spheroids of $55\times55\times65$ Å, arranged with their long axes parallel to *a*. (*f*) Projection of successive shrinkage stages on *b* face showing spheroids of $55\times55\times65$ Å.

This is in accordance with observation. A perspective drawing is shown in Fig. 2(*a*).

In order to pack the longer type of molecules in the *b* plane, it is necessary to incline them at an angle of 30° to the *a* axis as compared with 20° in the crystals of type 1, although the Patterson diagrams indicate that the molecules have the same orientation relative to the *a* axis. This discrepancy can be avoided by choosing the shorter type of molecule and placing its

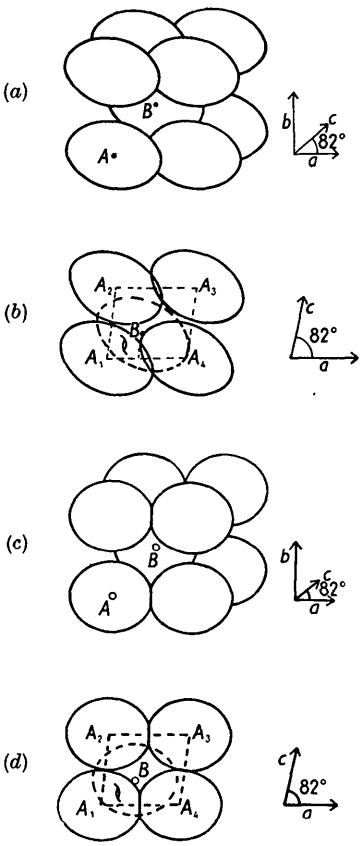

Fig. 2. Reduced haemoglobin of man. (*a*) Wet stage in perspective. (*b*) Projection of unit cell on *b* face. (*c*) Wet stage in perspective showing spheroids of 55×55×65 Å. (*d*) Projection of unit cell on *b* face showing shorter spheroids.

long axis parallel to *a* (Fig. 2(*c*) and (*d*)). Spheroids of 55×55×65 Å so placed pack well into the unit cell except for an overlap of about 3 Å where the molecules touch along the *a* axis. This implies a local sharing of water of hydration between neighbouring molecules which is not improbable in view of the exceptionally small unit-cell volume of the wet crystals (136,000 Å^3 per molecule as compared with 175,000 Å^3 in normal wet horse methaemoglobin). On the other hand, the arrangement of the molecules in Fig. 2(*d*) does not possess the attractive feature shown by the tilted long molecules in Fig. 2(*b*), where each molecule lies below the centre of a triangle formed by the three molecules immediately above.

Reduced haemoglobin of horse. Type 4

$C3_12$. Wet $a = 56{\cdot}1$, $c = 354$ Å;
dry $a = 47{\cdot}4$, $c = 308$ Å.

The *a* axes are so short that the molecules must be orientated with their long axes approximately in the *c* direction, and this is confirmed by the pleochroic properties of the crystals. Since they are in six layers (three pairs) perpendicular to the *c* axis of 354 Å,

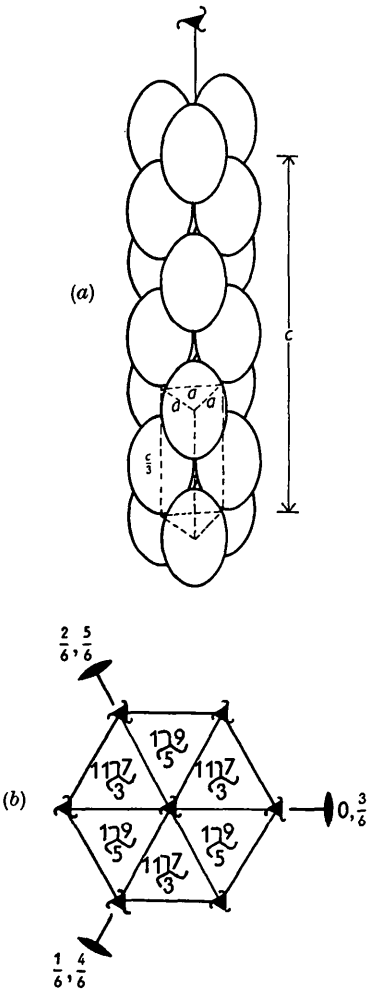

Fig. 3. Reduced haemoglobin of horse. (*a*) Wet stage, perspective drawing. (*b*) Symmetry elements of space group. Numbers represent heights of molecules in units of $\frac{1}{12}c$.

molecules of 65 Å length cannot be end-on in this direction. An approximation to hexagonal close packing elongated in the *c* direction such as that illustrated in Fig. 3(*a*) is indicated. This approximation is confirmed by the low-order spectra of the wet crystal, which are prominent because the crystals are salt-free so that there is a considerable contrast in scattering power per unit volume of the protein and of the water between the molecules. The spectra have the following characteristics:

(*a*) 0003 weak, 0006 very strong, 0,0,0,12 weak,

0,0,0,18 moderate, 0,0,0,9, 0,0,0,15, 0,0,0,21 absent. This indicates that the sheets of molecules are spaced rather exactly at intervals of $\frac{1}{6}c$.

(*b*) $10\bar{1}0$ and $11\bar{2}0$ very strong.

(*c*) $10\bar{1}3$ moderately strong.

The strong spectra are those of an elongated hexagonal close-packed structure with $a = 56$ Å, $c' = \frac{1}{3}c = 118$ Å, $c'/a = 2{\cdot}11$. For close-packed spheres $c'/a = 1{\cdot}63$. If it is assumed that the molecules are spheroids touching along a and that the ratio $2{\cdot}11/1{\cdot}63$ is a measure of their elongation, the dimensions deduced for the hydrated molecule are $56\times56\times72$ Å. The relation of the approximate molecular positions to the space-group $C3_12$ is shown in Fig. 3(*b*). The heights of the molecules are given in units of $c/12$. Each molecule at height $3c/12$ lies on three at height $c/12$, $5c/12$ on $3c/12$ and so on. The six molecules are in general positions and the co-ordinates of a typical centre and the orientation may have any values, but the spectra show that in actual fact they are in near accord with the simple structure shown in Fig. 3(*a*). The distance between molecular centres in the same sheet is, of course, $a = 56{\cdot}1$ Å, and that between nearest centres in successive sheets is 67 Å. The crystals grow in the form of large plates parallel to 0001, which is to be expected as this is the most densely packed plane.

If the conclusion that the molecules lie with their length roughly parallel to the c axis is accepted, an indication of the length of the molecule can be derived from the value of $F(0006)$. No reliable absolute values are as yet available but a preliminary estimate indicates that the value of $|F(\text{water})-F(\text{salt})|$ for this reflexion is not less, and probably more, than 2000 for $\varrho(w)-\varrho(s) = 0{\cdot}1$, which means that 0006 would have to lie within the central maximum. The position of 0006 in reciprocal space corresponds approximately to that of the point C in Fig. 4(*a*) of Bragg & Perutz (1952). This point lies on the first zero contour for the longer type of ellipsoid, but is well within the central maximum for the shorter type. In the air-dried crystals 0006 is absent. The reciprocal lattice point now comes to lie roughly on the points D in Fig. 4(*a*) (Bragg & Perutz, 1952) which is on the zero contour for the shorter type of molecule, but within the first minimum for the longer type. Thus the strength of $F(0006)$, in the wet state, and its absence in the dried, would furnish decisive evidence in favour of the shorter type of molecule.

5

Oxyhaemoglobin of man. Type 10.

$P4_12_1$. Wet $a = 53{\cdot}7$, $c = 193{\cdot}5$ Å;
dry $a = 47{\cdot}4$, $c = 174$ Å.

In this case the four molecules in the unit cell are in special positions on the twofold axes parallel to [110] shown in Fig. 4(*c*). They thus lie on four sheets equally spaced in the c direction. As in the previous case, the two short axes of the molecule must lie nearly in the equatorial plane and the long axis must be nearly parallel to c. This conclusion is in agreement with the pleochroic properties of the crystal. The low-

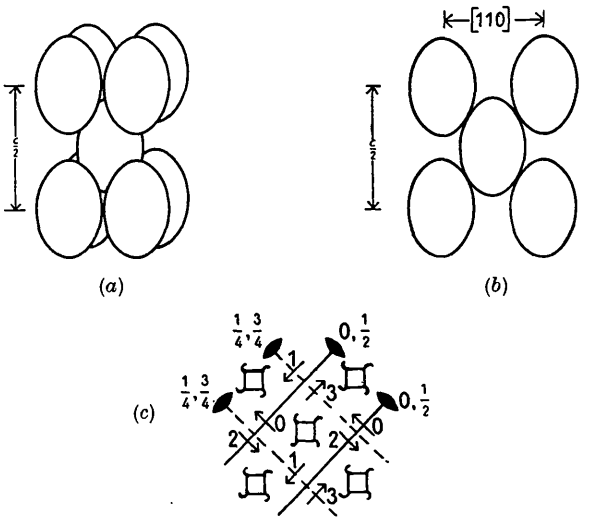

Fig. 4. Oxyhaemoglobin of man. (*a*) Simplified perspective drawing of one half the unit cell. (*b*) Section of unit cell along plane (110). (*c*) Symmetry elements of unit cell. The arrows denote the possible tilting of the molecules, at heights represented by the numbers in units of $\frac{1}{4}c$.

order spectra are of little assistance as a guide to the position of the molecules, because the crystals form only in a strong solution of potassium phosphate. The electron density (0·43 e.Å^{-3}) is little greater than that of the hydrated protein, and this lack of contrast makes the low-order spectra very weak.

The crystals form bipyramids of the form {102}, looking like regular octahedra somewhat drawn out along c. This suggests that the (102) face of the tetragonal unit cell corresponds to the (111) face of a larger, face-centred pseudo-cell, with $a' = [110]$ and $c' = \frac{1}{2}c$. This is equivalent to a simple body-centred tetragonal lattice with $a = 53{\cdot}7$, $c' = \frac{1}{2}c = 96{\cdot}7$ Å. Fig. 5 shows the relationship between this lattice and the octahedral crystals; Fig. 4 illustrates the packing of the molecules. Neighbouring centres in the equatorial plane are

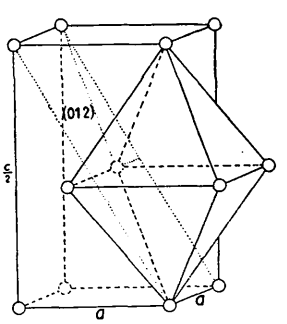

Fig. 5. Relationship between lattice and crystal habit in human oxyhaemoglobin (form 10).

Table 1. *Crystallographic data on haemoglobins*

Substance	Space group	State	Unit cell a (Å)	b (Å)	c (Å)	β (°)	Volume $\times 10^{-3}$ (Å^3)	Molecules	Molecular weight $\times 10^{-3}$	Magnitude of pleochroism	Direction of low absorption	Crystal habit (dominant habit bold)
1. Met-, oxy- and carboxyhaemoglobin of horse I[1]	$C2$	Wet	109	63·2	54·4	111	349	2	66·7	Strong	Near [100]	{001}, {110}
		Dry	102	56	49	134	206					
2. Met-, oxy- and carboxyhaemoglobin of horse II[1]	$P2_12_12_1$	Wet	122	82·4	63·7		640	4	66·7	Weak		**{110}**
		Dry	95	72	61		416					
3. Met-, oxy- and carboxyhaemoglobin of horse III[2]	$P2$	Wet	64·5	101	104	102	662	4	66·7			**{001}**, {010}, {110}
4. Reduced haemoglobin of horse[2]	$C3_12$	Wet	56·1		354		965	6	66·7	Medium	[0001]	**{0001}**, {10$\bar{1}$1}
		Dry	47·4		308		600	6				
5. Methaemoglobin of adult sheep[3]	$C2$	Wet	164	70	66	94·5	755	4	68	Strong	Near [100]	**{001}**
6. Methaemoglobin of foetal sheep I[3]	$B22_12$	Wet	112	108	56		677	4	68	Strong	[001]	**{100}**, {021}, {001}
		Dry	78	99	54		417					
7. Methaemoglobin of foetal sheep II[3]	$P2_1$	Wet	55	83	64	102	286	2	68	V. weak		
		Dry	48	71	58	101	194					
8. Met-, oxy- and carboxyhaemoglobin of man I[4]	$P2_12_12_1$	Wet	85·2	77·0	86·0		564	4	68	Isotropic		{110}, {111}
		Dry	70·4	73·3	77·0		393					
9. Met-, oxy- and carboxyhaemoglobin of man II[2]	$P2_12_12_1$?	Dry	50	55	123		338	4	68	Strong	[100]	**{001}**
10. Met-, oxy- and carboxyhaemoglobin of man III[6]	$P4_12_1$	Wet	53·7		193·5		55·2	4	68	Medium	[001]	**{102}**
		Dry	47·4		174		391					
11. Reduced haemoglobin of man I[2]	$P2_1$	Wet	66	98	110	98	700	4	68	Strong	[100]	**{001}**
12. Reduced haemoglobin of man II[5]	$P2_1$	Wet	62·5	83·2	52·8	98	272	2	68	Strong	[100]	**{011}**, {110}, {010}
		Dry	59	70	47·5	97·5	194					

1. Boyes-Watson, Davidson & Perutz, 1947.
2. Perutz (unpublished data).
3. Kendrew & Perutz, 1948.
4. Perutz & Weisz, 1947.
5. Perutz & Trotter (unpublished data).
6. Perutz, Liquori & Eirich, 1951.

at a distance $a = 53{\cdot}7$ Å apart, and those in successive planes at a distance 62 Å apart. A section of the simple tetragonal cell along the plane (110) is given in Fig. 4(*b*), and shows how the molecules pack. The space group shown in Fig. 4(*c*) allows them to be tilted around the twofold axes and displaced along them; the arrows represent the tilts of the molecules, the points of the arrows being directed in the positive *c* direction, and the numbers give their heights in units of $\frac{1}{4}c$. If the molecules are so displaced, a suitable tilt makes them pack almost as well as if they were in the ideal body-centred arrangement; for instance, the lower end of an arrow marked 1 in Fig. 4(*c*) lies at the centre of four upper ends of arrows at height 0. A Patterson projection of the crystal on (001) has two features of interest. In the first place the peaks immediately around the origin, corresponding to a 10 Å spacing, are very similar to those in a diagram obtained by compounding a Patterson of type 1 haemoglobin on a plane perpendicular to the *a* axis with the same Patterson turned through 90°. This suggests that the chains are parallel to the *c* axis in the tetragonal crystal. In the second place, it is clear from the Patterson that the centres of the molecules are not on the simple body-centred lattice; there must be some displacement as in Fig. 4(*c*). These results will be discussed in greater detail in a forthcoming paper by Liquori, Perutz & Trotter.

6. Comparison of wet and dry stages

There appear to be two types of shrinkage of the crystals:

(*a*) A series of stages for 'wet' crystals such as is found in type 1 is illustrated in Fig. 1(*c*) and (*f*). In these stages the *a* and *b* axes remain constant, but *c* varies slightly and the angle β varies greatly:

Stage	3	4	5
c (Å)	53·5	51·9	54·4
c sin β (Å)	42·3	46·1	50·7
β (°)	127·5	116	111
Cell volume (Å^3)	292,000	318,000	349,000

Stage	6	7
c (Å)	55·9	54·6
c sin β (Å)	54·9	54·4
β (°)	98	84·5
Cell volume (Å^3)	378,000	375,000

All these stages can accommodate molecules with dimensions $50\times50\times75$ Å, or $55\times55\times65$ Å, although the shorter molecules overlap slightly in the two shrunk stages 3 and 4. However, these stages were obtained in supersaturated ammonium sulphate solution where some reduction in protein hydration, with consequent overlapping of water layers or even direct contact between neighbouring molecules, could not be excluded, so that this overlapping would not be a reason for rejecting the shorter type of molecule.

(*b*) A passage from a 'wet' to a 'dry' state. Molecules with dimensions $50\times50\times75$ Å overlap when an attempt is made to pack them into the unit cell. For instance in type 1 the *c* axis shrinks to 49 Å in one dry state and 47 Å in another, in type 12 it shrinks to 47·5 Å. In the trigonal and tetragonal structures (4 and 10) the *a* axes shrink to 47·1 and 47·4 Å respectively. Seeing that the hydrated molecule has an envelope of bound water amounting to one-third of its volume, and so forming a coat of thickness about 3 Å around the protein, it seems very probable that the packing in the wet stages is one of hydrated molecules, whereas in the dry state the envelope of bound water is lost and a closer approach of the molecules is possible.

7. Other forms

As regards the other forms of haemoglobin listed in Table 1, it can only be stated that in no case are the cell dimensions and symmetry incompatible with a packing of molecules of the dimensions proposed in this paper. In order to determine the positions and orientations of the molecules in these crystalline forms, it would be necessary to make extensive experimental measurements of absolute intensities for low orders, and the figures are not yet available. It is hoped to undertake this investigation shortly.

In the two forms of foetal sheep haemoglobin (6 and 7) the arrangement of the molecules deduced from packing does not agree with that deduced from the optical pleochroism. Thus in form 6 the molecules can be packed with their long axes parallel to *a* and *b* but not *c*, while the pleochroism would indicate that their long axes are parallel to *c*. Form 7 has almost exactly the same unit-cell dimensions as form 12, both in the wet and the dried state, which would suggest that the two forms are similar in structure. Yet while form 12 is strongly pleochroic, form 7 is not, and a comparison of some of the X-ray intensities of the three principal zones showed no resemblance. This indicates considerable structural differences between foetal and adult sheep haemoglobin.

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