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On the meaning of the vector map of horse methemoglobin. By DOROTHY WRINCH, *Physics Department, Smith College, Northampton, Mass., U.S.A.*

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In a recent paper claiming to prove that a structure for horse hemoglobin composed of helical polypeptide chains is not incompatible with the particular features exhibited by its vector map in the regions approximately 5 and 10 Å from the origin, Howells (1954) criticizes my work in the following passage: "Wrinch (1953) describes the 5 Å vectors as an 'essentially three-dimensional distribution' and concludes that 'there is no confirmation of the hypothesis of rod-like polypeptide chains in the crystal'. The present investigation indicates that Wrinch's conclusion is unjustified."

I protest the misquotation and the misrepresentation of my work in the first sentence. I reject the claim in the second. In this note I explain the reasons for this rejection. I also offer a number of criticisms of Howells's approach to and interpretation of the vector map.

The question which Howells poses is precisely stated. Certainly the nature of Perutz's vector map (Perutz, 1949) at about 5 Å and at 10 Å and more from the origin is of great interest. Thus (a) the map shows a shell of high density, often referred to as the 5 Å shell, which extends from about 4–6 Å and completely circumscribes the origin. Further (b) beginning at about 10 Å and extending out to 13–15 Å according to the direction, there are various high-density regions. [We presume that (b) is the feature 'approximately 10 Å from the origin' referred to by Howells. But why does he talk of the 10 Å vector shell? There is no 10 Å shell.] These regions are arranged globally about the origin, but do not actually enclose it. However, the map has a number of other remarkable characteristics (see later). Have they no part to play in guiding interpretations of the map? Leaving this leading question aside for the time being, let us study the nature of the investigation reported in Howells's paper.

Though the question posed relates to polypeptide chains, the subject of this investigation is the helices of CF₂ groups found in polytetrafluoroethylene, a long-chain polymer with molecular weight of the order of a million (Bunn & Howells, 1954). As a result of the investigation, Howells makes various claims which, I judge, may be summed up as follows. It is possible to envisage a certain arrangement of CF₂ helices which will have a vector map containing shells of high density around the origin at about 3 and 5.5 Å. A similar arrangement of α-helices will give a vector map containing shells of high density around the origin at 5 and 10 Å.

Criticism 1.—Even if the situation with regard to the CF₂ helices is as stated, it does not follow that the situation regarding the α-helices is as stated. The transition from fluorocarbon to polypeptide raises a number of issues. The statement that the one situation follows from the other is asserted, not discussed. I have been unable to find any reason to believe it.

Criticism 2.—Were the statement regarding the α-helices correct, it would have no bearing on the structure of the horse hemoglobin entity.

This protein body is finite in mass and in volume. It has molecular weight *c.* 67,000 and is composed of

c. 580 amino acid residues plus 4 hemes. It has symmetry 2. It dissociates into halves on dilution. To understand its structure, we would seek for answers to the following questions among others: What is the vector relating one of the half-hemoglobins to the other and where is it in the unit cell of this particular crystal? What are the relative positions in space of the 290 residues and the 2 hemes in each half-entity or, as a start, into how many separate systems of covalent bonds are they subdivided, of what nature is the spatial pattern in each system? etc. etc.

A few of the characteristics of the extremely complex arrangement of helices envisaged by Howells suffice to show that his proposals fail to make contact with the requirements of the hemoglobin entity or the half-hemoglobins at any point. (1) Each helix is infinite. (2) The argument concerns the infinite array of helices parallel to an axis *c*, which lie at points of a hexagonal lattice. (3) This infinite array of infinite helices is pictured as being smoothed about the *c* axis with the result that infinite cylindrical shells replace the infinite chains. (4) 'Bundles of helices' are 'distributed uniformly in the surface of a cone of semi-vertical angle 10°'. (5) Two such 'bundles' are placed at right angles to one another . . .

Criticism 3.—The claims regarding vector maps obtained from CF₂ helices are, in any case, invalid.

(a) Let us for the moment adopt Howells's view of the meaning of his maps and study his assessment of the situation. Finally reaching the vector map of two bundles of CF₂ helices at right angles, he winds up his discussion with the remark: 'Summing up, the three-dimensional Patterson of the kind of structure we have envisaged is noticeably biaxial. However, the spread of vectors is larger than might be expected in view of the small departure from parallelism permitted in the chains of each half of the molecule.' In other words, it has not proved possible to get a structure composed of these helices which yields (apart from a change in scale) the non-uniaxial and non-biaxial 5 and 10 Å features of the hemoglobin vector map.

(b) But do the maps constructed by Howells actually have the meaning he imputes to them by the application of the MacGillavry–Bruins equations? So far as I can see, Fig. 2 is not the vector map of the CF₂ crystal smoothed about the *c* axis or indeed of any other smoothed atomic arrangement. It is the result of smoothing the vector map of the CF₂ crystal about the *c* axis. If this is so, the same point arises with the other maps.

§§ 1–3 of Howells's paper are concerned with what can and cannot be done with the CF₂ helices and their vector maps; as we pass on we find an abrupt change of subject. Criticism of § 4 arises on two counts.

Criticism 4.—There seems to be a lacuna between the last words of § 3, in which failure to attain the objective regarding the CF₂ groups is remarked, and the first lines of § 4, in which success is claimed with assertions regarding α-helices now included in the claim. (Incidentally, are not the *diameters* 3 and 5 Å in line 1 and the *diameters*

of 5 and 10 Å approximately in line 8 misprints for radii?) However let us, for the time being, proceed on the assumption that some particular set of α -helices with the required features in its vector map has been found. Even so, nothing of interest to the structure of horse hemoglobin follows. On the one hand, it is not claimed that, *mirabile dictu*, one of the particular sets happens to fit snugly with all the data we already have about horse hemoglobin and indeed it does not (see Criticism 2 above). This issue is not even raised. On the other hand, the fact that the one set of α -helices is not in conflict with the data implies nothing about any other set. Yet Howells talks freely about other sets of α -helices as if, in some undisclosed but well understood way, they, too, had been shown to be not in conflict with the data. It follows that, had it been proved that a structure for horse hemoglobin made up of some particular set of helical polypeptide chains was not in conflict with the data, nothing would have been added to our knowledge of the structure of this protein.

Criticism 5.—So far as I can see, it is no longer desirable, if indeed it ever was, to proceed to interpretations of the vector map via the polypeptide chain hypothesis. In one of my papers cited by Howells (see above) I showed that both the Perutz (1949) structure and the Bragg–Howells–Perutz (1952) structure for horse hemoglobin are disproved by the vector map itself. [These structures comprise bundles of rod-like polypeptide chains parallel to the a axis of the crystal, 7 to a bundle for the earlier structure, 11 to a bundle in the later structure.] We remark that, while Howells makes no mention of this fact, he talks about a uniaxial arrangement of helices only in passing, and quickly turns to a biaxial arrangement. But why is a biaxial arrangement worthy of discussion? In the other paper cited, I discussed structures of helical polypeptide chains in any number of directions, a matter also not mentioned by Howells. I showed that there is no way of getting the 5 Å shell from a set of helical polypeptide chains, no matter how varied the directions in which they run. I calculated the vector function $\varphi(x, y, z)$ for the $2_{14} \cdot \frac{1}{2}$ model (Bragg, Kendrew & Perutz, 1950) and for the α -helix. The function $\varphi(r)$ obtained by spherically smoothing the vector function about the origin was found, in both cases, to be monotonic decreasing up to and beyond 5 Å. This fact and the differentiation of $\varphi(r)$ from the radial distribution function $4\pi r^2 \varphi(r)$ are the kernel of the argument. So far as I can see, my claim that there is no support in the vector maps for a structure for horse hemoglobin made up of helical polypeptide chains, stands. So far as I can see, Howells's investigation of CF_2 helices and their vector maps has no bearing upon the claim.

These points and the closely related point that this investigation by its very nature contributes little or nothing to our knowledge of horse hemoglobin are made clear when we revert to the leading question posed earlier. It has long been my contention that a direct approach can be made to the interpretation of vector maps by studying the language of vector space (Wrinch, 1939) just as a direct approach can be made to interpreting intensity maps and is being made more and more frequently nowadays, e.g. Lipson (1954), by studying the language of structure factors (Wrinch, 1946). To illustrate

this contention in the case of protein vector maps and at the same time to counteract Howells's misrepresentation of my work, I devote a few lines to the results which emerge from a broader study of the vector map.

Such a study readily uncovers numerous clues to the nature of the horse hemoglobin entity in addition to the (a) and (b) features. We remark that (c) there is a large number of maxima spread over the cell as a whole, such as would result from interactions of numerous separate distributions also spread over the whole of the cell. [For diagrams illustrating this point see Wrinch (1939, Fig. 5(b and c)) or Bragg (1950, p. 44, Fig. 3).] This suggests that each half-hemoglobin is made up of a number of sub-units. To get information about these individuals and the manner in which they determine the orientation of small water clusters in this heavily hydrated crystal, we look at the vector map near the origin. We supplement the features (a) and (b) by remarking that (d) the origin peak is surrounded by a low-density region from, say, 3 to $4\frac{1}{2}$ Å, and that (e) there is, outside the 5 Å shell, another low-density region from, say, 6 to $9\frac{1}{2}$ Å, which almost circumscribes the origin. The nature of the vector maps of water clusters and of the vector maps and structure factors of spherical and cube shells make it possible to diagnose from the vector and intensity maps a type of sub-unit fitting the facts (b, d, e), and to infer from it a reasonable interpretation of the 5 Å shell (Wrinch, 1950, 1952a, b, 1953, 1954). On the one hand, the sub-units pictured are more or less equidimensional surface polycondensations of amino acids, one residue thick, with low-density interiors. On the other, the lengths of the oxygen-oxygen vectors in water clusters of tridymite type between second nearest and third and fourth nearest neighbors [say 4.51, 4.60, 5.28 Å if $OH \cdots O$ is of length 2.76 Å] readily account for high-density regions at about 5 Å from the origin. That these vectors are in a wide variety of directions, yielding a 5 Å shell, may be attributed to the globulite shapes of the individual molecules which make up each half-hemoglobin entity.

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