

THE MAGNETIC PROPERTIES AND STRUCTURE OF  
HEMOGLOBIN, OXYHEMOGLOBIN AND  
CARBONMONOXYHEMOGLOBIN

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Over ninety years ago, on November 8, 1845, Michael Faraday investigated the magnetic properties of dried blood and made a note "Must try recent fluid blood." If he had determined the magnetic susceptibilities of arterial and venous blood, he would have found them to differ by a large amount (as much as twenty per cent for completely oxygenated and completely deoxygenated blood); this discovery without doubt would have excited much interest and would have influenced appreciably the course of research on blood and hemoglobin.<sup>1</sup>

Continuing our investigations of the magnetic properties and structure of hemoglobin and related substances,<sup>2</sup> we have found oxyhemoglobin and carbonmonoxyhemoglobin to contain no unpaired electrons, and ferrohemoglobin (hemoglobin itself) to contain four unpaired electrons per heme. The description of our experiments and the interpretation and discussion of the results are given below.

*Note on Nomenclature.*—The current nomenclature of hemoglobin and related substances was formulated at a time when precise information about the chemical composition and structure of the substances was not available. Now that some progress has been made in gathering this information, especially in regard to chemical composition, it is possible to revise the nomenclature in such a way as to make the names of substances more descriptive than the older names, without introducing any radical changes. In formulating the following set of names we have profited by the continued advice of Dr. Alfred E. Mirsky.

The names whose use we advocate are given below, followed in some cases by acceptable synonyms. The expressions in parentheses are those whose use we consider to be undesirable.

Heme: an iron-porphyrin complex (generic term, used for either ferroheme or ferriheme).

Ferroheme (reduced heme): a complex of ferrous iron and a porphyrin.

Ferriheme (oxidized heme): a complex of ferric iron and a porphyrin.

Ferriheme chloride, hemin: a compound of ferriheme and chloride ion.

Ferriheme hydroxide, hematin: a compound of ferriheme and hydroxyl ion.

Ferrohemochromogen, hemochromogen: a complex of ferroheme and another substance, or two other substances, having the characteristic hemochromogen spectrum and involving covalent bonds from the iron atom to the porphyrin nitrogen atoms and the attached groups.<sup>2</sup> Individual hemochromogens may be designated by specifying the attached groups, as globin hemochromogen (ferroheme and denatured globin), dicyanide hemochromogen, dipyridine hemochromogen, carbonmonoxyhemochromogen, pyridine carbonmonoxyhemochromogen, etc.

Ferrihemochromogen (parahematin): a compound of ferriheme and another substance or two other substances, involving covalent bonds from the iron atom to the porphyrin nitrogen atoms and the attached groups.<sup>3</sup>

Hemoglobin: a conjugated protein containing heme and native globin (generic term, used for both ferrohemoglobin and ferrihemoglobin and also for closely related substances); specifically, ferrohemoglobin.

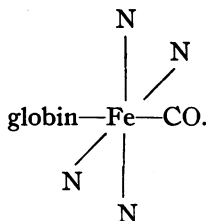
Ferrohemoglobin, hemoglobin (reduced hemoglobin): a conjugated protein formed by combination of ferroheme and native protein.

Oxyhemoglobin: a compound of ferrohemoglobin and oxygen.

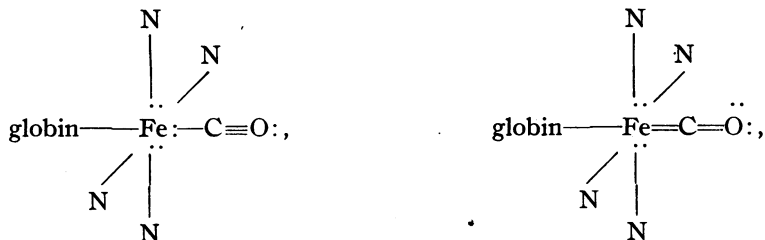
Carbonmonoxyhemoglobin, carbon monoxide hemoglobin (carboxyhemoglobin): a compound of ferrohemoglobin and carbon monoxide.

Ferrihemoglobin (methemoglobin): a conjugated protein formed by combination of ferriheme and native globin.<sup>3</sup>

*Carbonmonoxyhemoglobin.*—The magnetic measurements are described in the experimental part below. The carbonmonoxyhemoglobin molecule is found to have zero magnetic moment, and hence to contain no unpaired electrons. This is to be interpreted as showing that at least two  $3d$  orbitals of each ferrous iron atom are involved in covalent bond formation, the atom presumably forming six octahedral  $d^2sp^3$  bonds, four to the porphyrin nitrogen atoms, one to an atom (probably nitrogen) of the globin, and one to the carbon monoxide molecule:



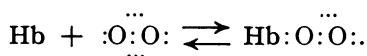
In view of the discovery of Brockway and Cross<sup>4</sup> that the nickel-carbon bond in nickel carbonyl has a large amount of double bond character, we may well expect this to be the case for the iron-carbon bond in carbonmonoxyhemoglobin also, the double bond being formed with the use of a pair of electrons conventionally assigned to the iron atom as  $3d$  electrons. To carbonmonoxyhemoglobin there would then be ascribed the resonating structure:



in which the dashes represent shared electron pairs and the dots unshared electrons.

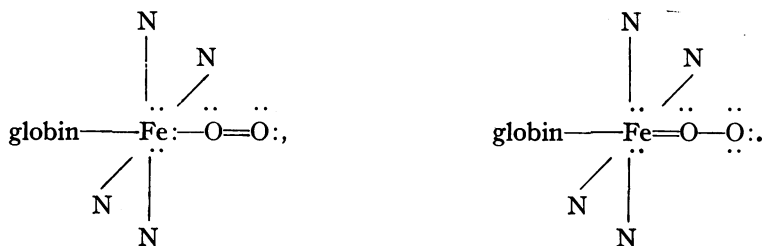
*Oxyhemoglobin.*—The molecule of oxyhemoglobin, like that of carbonmonoxyhemoglobin, is found to have zero magnetic moment and to contain no unpaired electrons. Each iron atom is accordingly attached to the four porphyrin nitrogen atoms, the globin molecule, and the oxygen molecule by covalent bonds.

The free oxygen molecule in its normal state ( $^3\Sigma$ ) contains two unpaired electrons. It might well have been expected, in view of the ease with which oxygen is attached to and detached from hemoglobin, that the oxygen molecule in oxyhemoglobin would retain these unpaired electrons, a pair of  $\sigma$  electrons of one oxygen atom, unshared in the free molecule, being used for the formation of the bond to hemoglobin:



However, this is shown not to be so by the magnetic data, there being no unpaired electrons in oxyhemoglobin. *The oxygen molecule undergoes a profound change in electronic structure on combination with hemoglobin.*

Of the structures of oxyhemoglobin compatible with the magnetic data, the most probable is the resonating structure analogous to that of carbonmonoxyhemoglobin:



The great similarity in properties of oxyhemoglobin and carbonmonoxyhemoglobin provides strong support for this structure. The structure in which each of the two oxygen atoms (connected with one another by a single bond) is attached to the iron atom by a single bond is rendered improbable by the strain involved in the three-membered ring.

*Ferrohemoglobin.*—In contrast to oxyhemoglobin and carbonmonoxyhemoglobin, hemoglobin itself contains unpaired electrons, its magnetic susceptibility showing the presence of a pronounced paramagnetic contribution. The interpretation of the magnetic data can be made only in conjunction with a discussion of the nature and magnitude of the mutual interactions of the four hemes in the molecule.<sup>5</sup> One possibility is that the heme-heme interaction is sufficiently strong to couple the moments of all electrons in the molecule into a resultant moment, with the same value for

all molecules. The magnetic data interpreted in this way lead to the value  $\mu = 10.92$  Bohr magnetons for the moment of the molecule. We reject this possibility on the following grounds. (1) The heme-heme interaction energy, as evaluated from the oxygen equilibrium data,<sup>5</sup> is hardly large enough to overcome the entropy advantage of independent heme moments. (2) The value 10.92 for the moment is not far from that (8.94) for eight unpaired electrons, two per heme, with parallel spins; however, it is about 22% larger, and this difference could be accounted for only as a surprisingly large contribution of orbital moment. (3) On this basis the magnetic susceptibility of partially oxygenated hemoglobin solutions would show large deviations from a linear dependence on the amount of uncombined heme; we have found large deviations not to occur. (These experiments will be described in a later paper.)

The other simple possibility, which we believe to be approximated in reality, is that the magnetic moments of the four hemes orient themselves in the applied magnetic field independently of one another. With the calculations made on this assumption, the experimental data lead to the value  $\mu = 5.46$  Bohr magnetons for the effective moment per heme. This shows that there are present in each heme four unpaired electrons, and that consequently the iron atom is not attached to the four porphyrin nitrogen atoms and the globin molecule by covalent bonds, but is present as a ferrous ion, the bonds to the neighboring atoms being essentially ionic bonds.

The resultant spin moment for four unpaired electrons is 4.90 magnetons. In compounds containing ferrous ion values of 4.9 to 5.4 are observed, the increase over the spin moment arising from a small orbital contribution. Complexes of ferrous iron with substances containing nitrogen (hydrazine, etc.) give values in the lower part of this range, the quenching of orbital moment being nearly complete.<sup>6</sup> It does not seem probable that the high value for ferrohemo-globin is to be accounted for as due to orbital moment, since the porphyrin nitrogen atoms should have a strong quenching effect on the orbital moment. We interpret this high value instead as due to a heme-heme interaction which tends to stabilize states with parallel heme moments relative to those with opposed heme moments, the oxygen-equilibrium value of the heme-heme interaction energy being of the order of magnitude required for this interpretation.

It is interesting and surprising that the hemoglobin molecule undergoes such an extreme structural change on the addition of oxygen or carbon monoxide; in the ferrohemo-globin molecule there are sixteen unpaired electrons and the bonds to iron are ionic, while in oxyhemo-globin and carbonmonoxyhemo-globin there are no unpaired electrons and the bonds are covalent. The change from ionic bonds to covalent bonds also occurs on formation of hemochromogen from ferroheme. Such a difference in bond

type in very closely related substances has been observed so far only in hemoglobin derivatives.

It is not yet possible to discuss the significance of these structural differences in detail, but they are without doubt closely related to and in a sense responsible for the characteristic properties of hemoglobin. For example, the change in multiplicity of the system oxygen molecule-heme in hemoglobin on formation of oxyhemoglobin need be only as great as two (from the triplet corresponding to the opposed oxygen molecule triplet and ferroheme quintet to the singlet of oxyheme), whereas the change in multiplicity on formation of carbonmonoxyhemoglobin is four; in view of the infrequency of transitions involving a change in multiplicity, we might accordingly anticipate that the reactions of hemoglobin with carbon monoxide would be slower than those with oxygen, in agreement with observation. The change in multiplicity may be related also to the photochemical reactivity of carbonmonoxyhemoglobin. The difference in bond type in hemoglobin and its compounds is probably connected with the preferential affinity of hemoglobin for oxygen and carbon monoxide in contrast to other substances. Further experimental information is needed before these questions can be discussed in detail.

*Experiments.—Solutions:* Defibrinated bovine blood (provided through the courteous cooperation of Cornelius Bros., Ltd.) was used as the source of material. Preparations *A* and *B* consisted of whole blood, collected and separately oxygenated by rotating 20 minutes in air in a large open vessel, and then packed in ice and used as soon as possible. For preparations *C* and *D* oxygenated blood was centrifuged, and the corpuscles washed three times with equal volumes of physiological sodium chloride. Ether was used to hemolyze the collected corpuscles, the stromata-emulsions were separated by centrifuging, and the dissolved ether removed from the oxyhemoglobin solutions by a current of air. The solutions were kept on ice until used.

Analyses were made for oxygen content in a Van Slyke-Neill constant-volume blood gas apparatus. The transfer pipet was calibrated for content and retention on the walls of whole blood or concentrated oxyhemoglobin solution corresponding to conditions of use; the gas pipet was also calibrated for volume. Correction was made for dissolved oxygen on the assumption that the quantity dissolved is proportional to the water present in the solution.

Corrected results of analyses: Blood *A*: 100 ml. combine with 20.20 ml. O<sub>2</sub> S.T.P.; formality of heme-iron, 0.00902. Blood *B*: 100 ml. combine with 20.59 ml. O<sub>2</sub> S.T.P.; formality of heme-iron, 0.00919. Solution *C*: 100 ml. combine with 37.15 ml. O<sub>2</sub> S.T.P.; formality of heme-iron, 0.01658. Solution *D*: 100 ml. combine with 41.26 ml. O<sub>2</sub> S.T.P.; formality of heme-iron, 0.01841.

*Apparatus:* The apparatus for magnetic susceptibility determinations has already been described.<sup>2</sup> All hemoglobin solutions were measured against water in a tube of about 18 mm. internal diameter. Fields of 7640 and 8830 gauss were used, the forces being reported as average  $\Delta w$  (in milligrams) for the former. A small correction to the observed  $\Delta w$  has been applied for blank on the tube, so that reported forces are for solution against pure water. Solutions were measured at approximately 20°C.

Calibration of field and tube with water against air:  $\Delta w = -49.59$ . (For hemochromogen and 6*N* NaOH the tube with  $\Delta w = -45.40$  for water against air was used.)

*Carbonmonoxyhemoglobin.*—Samples of blood *A* equilibrated with CO by rotation of 50 ml. in a liter tonometer filled with pure carbon monoxide:  $\Delta w = -0.56, -0.60, -0.76, -0.61$ , average  $-0.63$ . Samples of blood *B* equilibrated with CO:  $\Delta w = -0.84, -1.03, -0.80, -0.86$ , average  $-0.88$ . Samples of solution *C* equilibrated with CO:  $\Delta w = -0.28, -0.68$ , average  $-0.48$ . Samples of solution *D* equilibrated with CO:  $\Delta w = -0.36, -0.45$ , average,  $-0.41$ . (Completeness of saturation with carbon monoxide was generally tested by adding  $\text{Na}_2\text{S}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$  to the magnetic tube and measuring the increase in susceptibility due to formation of hemoglobin.)

We have established in the previous paper<sup>2</sup> the presence of no unpaired electrons in globin hemochromogen and dicyanide hemochromogen. For globin hemochromogen made by denaturing 32 ml. of whole blood with 10 ml. of 6*N* NaOH after reduction of the heme: average  $\Delta w = -1.71$ ; for dicyanide hemochromogen prepared in a similar manner: average  $\Delta w = -1.53$ ; average for the two,  $\Delta w = -1.62$ . Measurement of the 6*N* NaOH against water in the same tube gives  $\Delta w = -4.88, -4.95$ . Assuming the additivity of atomic diamagnetism (Wiedemann's rule), whole blood without paramagnetic constituent should give  $\Delta w = -0.58$  in the tube used for the hemoglobin series. This value is in satisfactory agreement with the  $\Delta w$  values given above. The calculated value for blood with two unpaired electrons per heme, and independent hemes, is  $\Delta w = +1.52$ , for four,  $\Delta w = +5.69$ ; the calculated values for the hemoglobin solutions are about twice as great.

Conclusion: carbonmonoxyhemoglobin contains no unpaired electrons.

*Oxyhemoglobin.*—Samples of blood *A*:  $\Delta w = -0.65, -0.40, -0.44$ , average,  $-0.50$ . Blood *B*:  $\Delta w = -0.58, -0.62, -0.62$ , average,  $-0.61$ . Solution *C*:  $\Delta w = -0.44, -0.55, -0.50, -0.50$ , average,  $-0.50$ . Solution *D*:  $\Delta w = -0.38, -0.36$ , average,  $-0.37$ . Oxyhemoglobin relative to carbonmonoxyhemoglobin: *A*,  $+0.13$ ; *B*,  $+0.27$ ; *C*,  $+0.02$ ; *D*,  $+0.04$ ; calculated for two unpaired electrons on oxyhemoglobin: *A*,  $+2.03$ ; *B*,  $+2.07$ ; *C*,  $+3.74$ ; *D*,  $+4.11$ .

Conclusion: oxyhemoglobin contains no unpaired electrons.

*Hemoglobin.*—35 ml. of blood *A* reduced in differential tube by addition of from 0.4 to 1.0 g.  $\text{Na}_2\text{S}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ :  $\Delta w = +7.32, 6.85, 6.98, 6.97$ , average,  $+7.03$ . Taking the mean of the oxy- and carbonmonoxyhemoglobin values ( $-0.57$ ) for  $\Delta w$  of diamagnetism of hemoglobin, the change on removing coordinating group ( $\text{O}_2, \text{CO}$ ) is  $+7.60$  gm., corresponding to paramagnetism and a magnetic moment of 5.48 Bohr magnetons per heme, assuming independent hemes. (The change in diamagnetism involved in loss of CO or  $\text{O}_2$  is negligible.)

Blood *B*, reduced:  $\Delta w = +7.21, 7.16, 7.51, 7.22, 7.50, 7.20, 7.51, 7.06$ , average,  $+7.30$ ; diamagnetic value,  $-0.74$ ; change,  $+8.04$ ;  $\mu = 5.58$ .

Solution *C*, reduced:  $\Delta w = +13.09, 12.83, 12.89, 13.28$ , average,  $13.02$ ; diamagnetic value,  $-0.49$ ; change,  $+13.52$ ;  $\mu = 5.38$ .

Solution *D*, reduced:  $\Delta w = +14.95, 14.33$ , average,  $+14.64$ ; diamagnetic value,  $-0.39$ ; change,  $+15.03$ ;  $\mu = 5.40$ .

Summary of results for hemoglobin: blood *A*,  $\mu = 5.48$ ; blood *B*,  $5.58$ ; solution *C*,  $5.38$ ; solution *D*,  $5.40$ ; average of the four,  $\mu = 5.46$ .

Spin moment for four unpaired electrons,  $4.9$ ; for two,  $2.83$ ; for none,  $0.00$ . Moment observed for ferrous ion in solution, about  $5.3$ ; moment observed for solid  $\text{Fe}(\text{N}_2\text{H}_4)_2\text{Cl}_2$ ,  $4.86$ . Conclusion: ferrohemoglobin has a susceptibility corresponding to four unpaired electrons per heme, with evidence for some magnetic interaction between the hemes.

*Summary.*—It is shown by magnetic measurements that oxyhemoglobin and carbonmonoxyhemoglobin contain no unpaired electrons; the oxygen molecule, with two unpaired electrons in the free state, accordingly under-

goes a profound change in electronic structure on attachment to hemoglobin. The magnetic susceptibility of hemoglobin itself (ferrohemoglobin) corresponds to an effective magnetic moment of 5.46 Bohr magnetons per heme, calculated for independent hemes. This shows the presence of four unpaired electrons per heme, and indicates that the heme-heme interaction tends to stabilize to some extent the parallel configuration of the moments of the four hemes in the molecule. The bonds from iron to surrounding atoms are ionic in hemoglobin, and covalent in oxyhemoglobin and carbonmonoxy-hemoglobin.

We have been helped a great deal by the advice and encouragement of Dr. Alfred E. Mirsky of the Hospital of the Rockefeller Institute. This investigation is part of a program of research on the structure of hemoglobin being carried on with the aid of a grant from the Rockefeller Foundation.

<sup>1</sup> A. Gamgee, *Proc. Roy. Soc. London*, **68**, 503-512 (1901), and one or two more recent investigators have reported blood to be about as diamagnetic as water, without discovering the difference between arterial and venous blood.

<sup>2</sup> L. Pauling and C. D. Coryell, *These PROCEEDINGS*, **22**, 159 (1936).

<sup>3</sup> We shall discuss the structure of ferrihemoglobin and the ferrihemochromogens in a later paper.

<sup>4</sup> L. O. Brockway and P. C. Cross, *Jour. Chem. Phys.*, **3**, 828 (1935).

<sup>5</sup> L. Pauling, *These PROCEEDINGS*, **21**, 186 (1935).

<sup>6</sup> L. Pauling, *Jour. Am. Chem. Soc.*, **53**, 1367 (1931).

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## STRUCTURE AND ARRANGEMENT OF SALIVARY GLAND CHROMOSOMES IN *DROSOPHILA SPECIES*

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The salivary gland nuclei in *Drosophila* contain two different chromosome derivatives, the long chromosome strands which correspond to the euchromatic parts of prophase chromosomes, and the chromocenter which arises from the heterochromatic regions (Heitz<sup>2</sup>). While most investigators now agree that the euchromatic strands are composed of a number of closely united chromonemata, the homologous chromomeres of which form discs (aggregate chromomeres), there is no uniformity of opinion regarding the chromocenter. Painter<sup>3</sup> considered it an aggregate of accessory material into which each chromosome sends a thin achromatic strand. Muller and Prokofjeva<sup>4</sup> believe that these regions show the same