THE MAGNETIC PROPERTIES AND STRUCTURE OF THE HEMOCHROMOGENS AND RELATED SUBSTANCES

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In the course of a general study of the structure of hemoglobin and its derivatives we have investigated the magnetic properties of ferriheme, ferroheme, several hemochromogens and nickel protoporphyrin. The results of this investigation and their structural interpretation are discussed in the following paragraphs.

The prosthetic group of hemoglobin, ferrous protoporphyrin, we call ferroheme, ferriheme being ferric protoporphyrin combined with a negative ion (chloride in hemin, hydroxide in hematin). On denaturation hemoglobin forms a deep red substance called hemochromogen.[1] It was shown by Anson and Mirsky[2] in 1925 that hemochromogen is not ferroheme itself, as had been thought before, but a compound of ferroheme and denatured globin, and that hemochromogens in general are compounds of ferroheme and other substances, usually containing nitrogen. The characteristic property of the hemochromogens is their strong absorption spectrum of two sharp bands, α at about 5600 Å and β at about 5200 Å, with α stronger than β.

The magnetic measurements reported in the experimental part of this paper correspond to the following numbers of unpaired electrons per molecule of the substance studied: ferriheme, five; ferroheme, four; globin hemochromogen, none; pyridine hemochromogen, none; nicotine hemochromogen, none; dicyanide hemochromogen, none; nickel protoporphyrin, none.

The presence of five and four unpaired electrons in ferriheme and ferroheme, respectively, shows that in these substances the iron atom is attached to the four adjacent nitrogen atoms of the porphyrin not by covalent bonds but by ionic bonds;[3] that is, iron is present essentially as ferric ion in ferriheme and ferrous ion in ferroheme. The observed magnetic moments are incompatible with the assumption that four dsp^2 covalent bonds are formed, directed toward the corners of a square, whether or not additional bonds to the iron atom are present.

On the other hand, we have found that the four hemochromogens which we have investigated contain no unpaired electrons. This observation shows that two $3d$ orbitals of the ferrous iron atom are involved in the formation of covalent bonds, and that the iron atom is accordingly attached by essentially covalent bonds not only to the four porphyrin nitrogen atoms but also to two other atoms, giving an octahedral (d^2sp^3)

arrangement of six atoms about the iron atom.[4] In pyridine, nicotine and probably globin hemochromogen these six atoms are all nitrogen atoms; in dicyanide hemochromogen the cyanide radicals are probably attached by carbon rather than nitrogen.

From these results we conclude that the characteristic hemochromogen spectrum of a hemoglobin derivative is to be correlated with a structure in which the four porphyrin nitrogen atoms form covalent bonds with a central atom (iron). The same correlation of structure and spectrum can be made also for hemochromogen-like substances not containing iron. The absorption spectra of complexes of porphyrins and certain metals other than iron resemble those of the hemochromogens very closely, both as to positions and intensities of the two bands.[5] (Following Anson and Mirsky, we call these substances hemochromogen-like, restricting the word hemochromogen to substances containing iron.) Nickel protoporphyrin is a substance of this class. We have found it to contain no unpaired electrons, showing that the nickel atom is attached to the four surrounding nitrogen atoms by covalent dsp^2 bonds. We see that for the production of a hemochromogen-like spectrum it is not necessary that the central atom form two additional covalent bonds, as does iron in the hemochromogens themselves, but only that the bonds to the porphyrin nitrogen atoms be covalent.

(It is interesting to observe that the formation of dsp^2 bonds in nickel protoporphyrin shows that the four porphyrin nitrogen atoms are coplanar, or nearly coplanar, as is, indeed, certain for a conjugated system such as the porphyrin molecule.)

We have found that on heating palladous chloride and protoporphyrin in glacial acetic acid solution a substance (presumably palladous protoporphyrin) is obtained with an absorption spectrum similar to that of a hemochromogen, the bands being somewhat less sharp. (Observed bands: α, strong, 5700 Å; β, weak, 5250 Å.) In view of the fact that all palladous compounds which have been studied have been found to be diamagnetic[6] and to contain palladium forming four covalent bonds directed to the corners of a square,[7] it is highly probable that the bonds in palladous protoporphyrin are covalent. We have not yet carried out the magnetic study of this substance.

Cupric protoporphyrin also has a spectrum resembling that of a hemochromogen very closely.[5] Although the magnetic criterion for bond type is not applicable to cupric compounds, it is probable that in cupric protoporphyrin the copper atom forms four square dsp^2 covalent bonds with the porphyrin nitrogen atoms, its odd electron occupying the remaining $4p$ orbital, inasmuch as this structure has been found recently in several substances.[8]

We wish to thank Dr. A. E. Mirsky for advice during this investigation.

Experiments.—We have determined the magnetic susceptibilities of the substances studied by the Gouy method, which involves measurement of the change in weight of a vertical cylinder of substance when one end is placed in a field of strength H and the other in zero field. A large half-ring water-cooled electromagnet was used, the flat surfaces of the pole pieces being 38 mm. in diameter and 22.5 mm. apart. The field between the pole pieces, 7640 gauss at 10 amperes and 8830 gauss at 14 amperes current, could be kept constant by manual regulation to within 0.2%. In general weighings were made at both field strengths, the change in weight at 14 amperes being divided by the factor 1.337 and averaged with that at 10 amperes to give the reported value Δw (in milligrams).

Glass tubes selected for constancy of diameter were separated into two compartments by a glass septum, and provided with ground glass caps for the ends and suitable supports for suspension from the balance arm, the septum being between the pole pieces. The substances to be compared occupy the two compartments; our measurements of pure substances were usually made relative to air, and of solutions relative to the solvent. Solids were introduced in small portions and well tamped in the upper compartment; it was found by experiment that the apparent density was constant along the tube to within 2%. The tubes used varied in internal diameter from 6 to 18 mm., each compartment being about 15 cm. long.

In order to investigate solid substances formed by precipitation from aqueous solution, we found it convenient to determine the susceptibilities of suspensions in the liquid, settling being delayed by the use of 30% sucrose solution as the solvent. Gum arabic, suggested by Keilin,[9] was found to be less effective than sucrose.

The observed susceptibility values were corrected for the diamagnetic contribution with the use of susceptibility values for closely related diamagnetic substances. The magnetic moment (in Bohr magnetons) was then calculated from the molal paramagnetic susceptibility χ_{molal} by the use of the equation[10]

$$\mu = 2.84 \sqrt{\chi_{molal} T},$$

T being the absolute temperature, 293°K. unless otherwise noted. The specific susceptibility of water saturated with air was taken to be -0.719×10^{-6}.

Pyridine.—Water, $\Delta w = 15.75$; dried, redistilled pyridine, density 0.9830: $\Delta w = 13.18$, specific susceptibility $= -0.607 \times 10^{-6}$ (I. C. T. value, -0.623×10^{-6}).

Nicotine.—Water, $\Delta w = 15.75$; Eastman's nicotine: $\Delta w = 15.10$, volume susceptibility $= -0.689 \times 10^{-6}$.

Ferriheme.—(*a*) Water: $\Delta w = 11.45$; Eastman's crystallized hemin: apparent density 0.710 g./ml., $\Delta w = -223.2$, $\mu = 5.69$. (*b*) Water: $\Delta w = 10.52$; hemin prepared and recrystallized by the method of Willstätter and Asahina:[11] apparent density 0.821 g./ml., $\Delta w = -255.2$ at $T = 298°$K., $\mu = 5.93$. (*c*) Pyridine: $\Delta w = 13.18$; hemin dissolved in pyridine containing 0.01 mole/l. iodine: concentration of hemin = 105, 44.4 mg./ml.; $\Delta w = -26.2, -1.79$ within one hour after preparation; $\mu = 5.23, 4.98$. These are minimum values of μ for hemin, inasmuch as a slow decrease of paramagnetism of the solution with time was noted. (*d*) Water against air in differential tube: $\Delta w = -45.40$; hemin dissolved in sucrose-0.2 N NaOH solution (to form hematin), against solvent: concentration = 6.07, 7.94 mg. hemin/ml.; $\Delta w = 7.43, 9.69$; $\mu = 5.56, 5.56$.

Summary of results for ferriheme: crystalline hemin, $\mu = 5.69, 5.93$; hemin dissolved in pyridine, $\mu \geqslant 5.23, 4.98$; hematin in NaOH-sucrose solution, $\mu = 5.56, 5.56$.

Other work: Haurowitz and Kittel,[12] crystalline hemin and hematin, $\chi_{molal} = 16{,}000 - 17{,}000 \times 10^{-6}$, giving $\mu = 6.1 - 6.3$; Cambi and Szegö,[13] crystalline hemin at $T = 84°$ 192°, 294°K. (Curie's law satisfied), $\mu = 5.81$.

Spin moment for five unpaired electrons, 5.91; for three, 3.88; for one, 1.73. Conclusion: ferriheme contains five unpaired electrons.

Ferroheme.—Water against air in differential tube: $\Delta w = -45.40$; hemin dissolved in sucrose-0.2 *N* NaOH solution, reduced with solid $Na_2S_2O_4 \cdot 2H_2O$, against solvent: concentration = 6.07, 7.94 mg. hemin/ml.; $\Delta w = 6.00, 6.65$; $\mu = 5.02, 4.83$.

Spin moment for four unpaired electrons, 4.90; for two, 2.83; for none, 0.00. Conclusion: ferroheme contains four unpaired electrons.

Pyridine Hemochromogen.—(*a*) Pyridine: $\Delta w = -13.18$; solution of hemin in pyridine, reduced to pyridine hemochromogen by shaking for five minutes with mercury:[14] concentration = 105, 44.4 mg. hemin/ml.; $\Delta w = -12.75, -13.20$; calculated for two unpaired electrons, $\Delta w = -1.7, -8.4$; for none, $\Delta w = -13.18, -13.18$. (*b*) Water against air in differential tube: $\Delta w = -45.40$; hemin in sucrose-NaOH solution, concentration 5.11 mg. hemin/ml., plus 1 ml. pyridine and 2 g. $Na_2S_2O_4 \cdot 2H_2O$ per 60 ml., against sucrose-NaOH solution: $\Delta w = 0.88$; calculated for two unpaired electrons, with correction for diamagnetism of pyridine, $\Delta w = 2.2$; for none, $\Delta w = 0.3$. (*c*) Water against air in differential tube: $\Delta w = -45.40$; hemin in sucrose-NaOH solution, concentration 6.11 mg. hemin/ml., plus 2 ml. pyridine and 2.5 ml. 0.4 molal $Na_2S_2O_4$ per 60 ml., against sucrose-NaOH solution: $\Delta w = 1.03$; calculated for two unpaired electrons, $\Delta w = 3.3$; for none, $\Delta w = 0.8$. (A small amount of ferroheme may have precipitated simultaneously with the hemochromogen.)

Conclusion: Pyridine hemochromogen contains no unpaired electrons.

Dicyanide Hemochromogen.—Water against air: $\Delta w = -45.40$; hemin in sucrose-NaOH solution containing 30 mg./ml. KCN reduced with solid $Na_2S_2O_4 \cdot 2H_2O$ to form (soluble) dicyanide hemochromogen,[15] against solvent: concentration = 4.88 mg. hemin/ml.; $\Delta w = -0.28, -0.16$; calculated for two unpaired electrons, $\Delta w = 2.5$; for none, $\Delta w = 0.00$.

Conclusions: Dicyanide hemochromogen contains no unpaired electrons.

Globin Hemochromogen.—Defibrinated bovine blood, 100 ml. combining with 20.9 ml. O_2 S. T. P., was used in comparing globin hemochromogen with dicyanide hemochromogen. Water against air: $\Delta w = -45.40$; 32 ml. blood plus 1 g. $Na_2S_2O_4 \cdot 2H_2O$ and 10 ml. 6 *N* NaOH, against water: $\Delta w = -1.71, -1.53, -1.88$, average -1.71; 32 ml. blood plus 1 g. $Na_2S_2O_4 \cdot 2H_2O$, 1 g. KCN, and 10 ml. 6*N* NaOH (the 24-fold excess of cyanide ion being sufficient to replace denatured globin[15]), against water: $\Delta w = -1.68, -1.61, -1.30$, average -1.53. Globin hemochromogen relative to cyanide hemochromogen, $\Delta w = -0.18$; calculated for two unpaired electrons $\Delta w = 1.79$; for none, $\Delta w = 0.00$.

Conclusion: Globin hemochromogen contains no unpaired electrons.

Nicotine Hemochromogen.—Water: $\Delta w = -5.00$; sludge of 1.2 g. hemin and 10 ml. nicotine reduced with 2.4 ml. of 40% hydrazine; $\Delta w = -3.87$; calculated for two unpaired electrons, $\Delta w = -0.70$; for none, $\Delta w = -4.45$. (A small amount of unreduced hemin may have been present.)

Conclusion: Nicotine hemochromogen contains no unpaired electrons.

Nickel Protoporphyrin.—Water: $\Delta w = -4.62$; crystalline nickel protoporphyrin prepared from recrystallized protoporphyrin by the method of Fischer and Pützer:[5] apparent density = 0.77; $\Delta w = -2.69$; specific susceptibility = -0.54×10^{-6}. Calculated specific susceptibility for two unpaired electrons, $+5.6 \times 10^{-6}$; for none, about -0.5×10^{-6}.

Conclusion: Nickel protoporphyrin contains no unpaired electrons.

Other Work: in the course of independent magnetic studies of metal-porphyrin complexes, F. Haurowitz reported the nickel complex to be paramagnetic and L. Klemm found it to be diamagnetic. Haurowitz and W. Klemm have recently collaborated[16] in studying nickel dimethylmesoporphyrin, and have found it to be about as diamag-

netic as the porphyrin itself; they conclude, as do we, that the nickel porphyrins contain no unpaired electrons.

[1] For a summary of the history of the hemochromogen problem see M. L. Anson and A. E. Mirsky, *Physiol. Rev.*, **10**, 506 (1930).

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[3] L. Pauling, *Jour. Am. Chem. Soc.*, **53**, 1367 (1931); **54**, 988 (1932).

[4] This interpretation of the magnetic data is supported by chemical analyses, which correspond to two pyridine groups for each heme in pyridine hemochromogen (R. Hill, *Proc. Roy. Soc.*, **B100**, 419 (1925)) and to two cyanide ions for each heme in dicyanide hemochromogen (M. L. Anson and A. E. Mirsky, *Jour. Gen. Physiol.*, **14**, 43 (1930)).

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SEGREGATION OF COLOR AND GROWTH-REGULATING GENES IN SOMATIC TISSUE OF MAIZE

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A microscopical examination of mature seeds of *Zea mays* in a number of different lines has shown a surprisingly large number of color and growth mosaics in aleurone and endosperm tissue. Maize endosperm is unusually favorable material in which to study genetic changes in somatic tissue. It is a short-lived, food-storage structure. Changes can occur late in development with no serious injury to the young seedling. Consequently aberrations have not been selected against as severely as in other parts of the organism.

Mosaics do occur in plant tissues involving known genes but are rare. A sterile abnormal sector has been found in the pistillate inflorescence of maize that developed at a faster rate than the normal tissue on either side. This overgrowth has the appearance of a genetic mosaic. The normal