THE MAGNETIC PROPERTIES OF THE COMPOUNDS ETHYLISOCYANIDE-FERROHEMOGLOBIN AND IMIDAZOLE-FERRIHEMOGLOBIN

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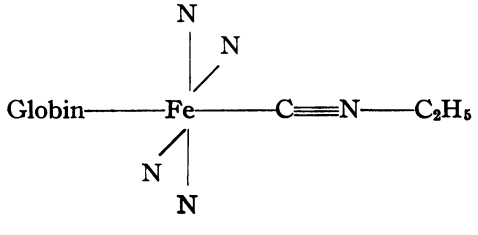
In the course of the series of magnetic investigations of hemoglobin being carried out in these Laboratories we have studied two new compounds, ethylisocyanide-ferrohemoglobin and imidazole-ferrihemoglobin, obtaining the results reported below.

Ethylisocyanide-ferrohemoglobin.—The compounds oxyhemoglobin, carbonmonoxyhemoglobin,[1] nitric oxide hemoglobin[2] and ferrohemoglobincyanide ion[3] have been shown to be diamagnetic, from which it is inferred that the iron atoms of the heme groups form octahedral covalent bonds in these molecules. Another compound of ferrohemoglobin, that with methyl isocyanide, has been reported by Warburg, Negelein and Christian,[4] and it would be of interest to determine from its magnetic properties whether or not the bonds formed by the iron atoms in this molecule also are of the octahedral covalent type. It is probable that similar compounds are formed with all of the lighter alkyl isocyanides, and we have found it convenient to study the reaction of ferrohemoglobin with ethyl isocyanide rather than that with the methyl derivative.

Ethyl isocyanide combines easily with ferrohemoglobin to give a compound with an absorption spectrum consisting of two narrow bands, at about 5540 and 5250 A.; this spectrum resembles closely those of the other compounds of ferrohemoglobin. Ethylisocyanide-ferrohemoglobin is completely diamagnetic, and accordingly contains no electrons with unpaired spins. The substance is shown by the magnetic titration described in the experimental part to have the ratio isocyanide to heme iron equal to unity. It is probable that the bond to the isocyanide molecule connects the iron and carbon atoms, corresponding to the structure



This structure is analogous to those proposed for the other ferrohemoglobin compounds.

Imidazole-ferrihemoglobin.—The interaction of imidazole and hemoglobin is of interest because of the likelihood that the bond between heme iron and globin involves the imidazole group of the side-chain of a histidine residue. We have found that imidazole combines with ferrihemoglobin to form a compound with spectrum differing little from that of ferrihemoglobin hydroxide (alkaline methemoglobin). The data from magnetic titrations at pH 6.86, 8.20, and 10.30 can be interpreted on the assumption of the simple equilibrium

$$HHbIm^+ \rightleftarrows HHb^+ + Im \qquad (1)$$

in which the symbol HHb^+ is used to represent the ferrihemoglobin cation (containing one heme); there is accordingly no evidence from our experiments for heme-heme interactions. The apparent equilibrium constants can be converted into equilibrium constants for Reaction 1 by use of data for the ferrihemoglobin : ferrihemoglobin-hydroxide equilibrium[6] and the imidazole:imidazilium-cation equilibrium (pK 6.95).[7]

The values found at 25°C. for the equilibrium constant $K = [HHb^+][Im]/[HHbIm^+]$ are the following:

pH 6.86	$K = 2.5 \times 10^{-3}$
8.2	2.0×10^{-3}
10.3	0.31×10^{-3}

The probable error in K is about 10% in each case.

The trend in the values of K with change of pH may be due to the presence in imidazole-ferrihemoglobin of an acid group with pK about 9.5, requiring consideration of the equilibrium

$$HHbIm^+ \rightleftarrows HbIm + H^+ \qquad (2)$$

Here we use the symbol HbIm to represent imidazole-ferrihemoglobin after loss of one hydrogen ion per heme. Assuming that this acid group is not effective for ferrihemoglobin itself (within the pH range 6.86 to 10.3), we calculate the ratios 2.3 : 3.2 : 0.31 for K at pH 6.86, 8.2 and 10.3, respectively, in satisfactory agreement with the values reported above.

The equilibrium between imidazole-ferrihemoglobin and its products of dissociation accordingly involves this heme-linked acid group with pK 9.5 as well as the acid group with pK 8.15 which corresponds to the addition of hydroxide ion to ferrihemoglobin ion to form ferrihemoglobin hydroxide. The latter acid group is absent in imidazole-ferrihemoglobin, since presumably imidazole competes with hydroxide ion for coördination with the iron atom.

The molal susceptibility of imidazole-ferrihemoglobin could be evaluated only roughly by extrapolation, the three values 2940, 2180 and 1290 $\times$ 10^{-6} c. g. s. u. being obtained. These correspond to the values 2.66, 2.29

and 1.76 Bohr magnetons for the magnetic moment of the heme group. Although the values are not very precise, there is little doubt that they show the presence of one unpaired electron per heme, with some orbital moment as well as spin moment. (It is possible that the trend of the values is the

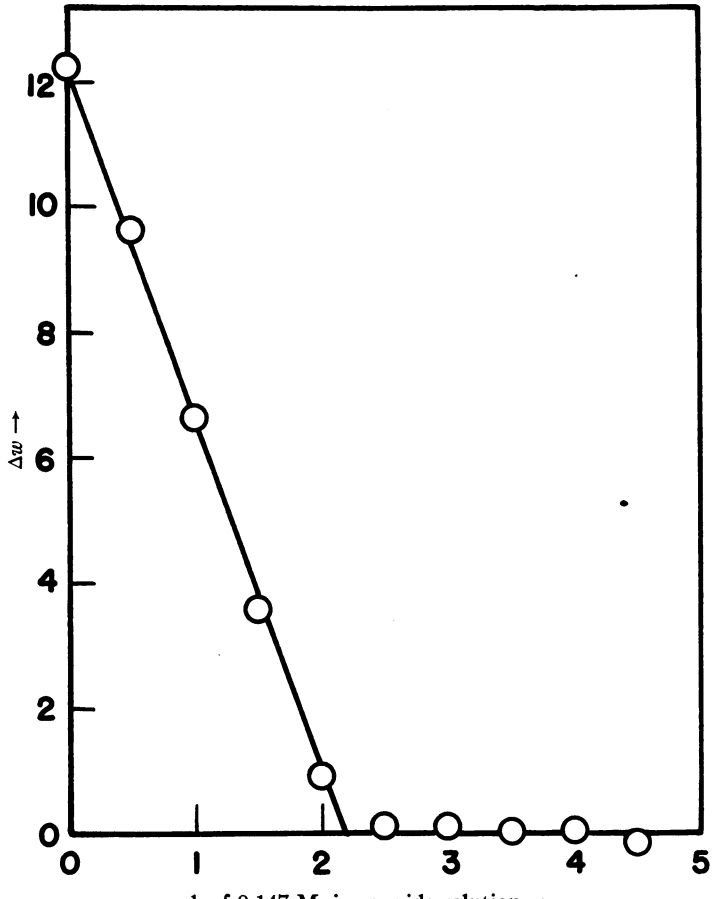


FIGURE 1

Magnetic titration curve for ferrohemoglobin and ethyl isocyanide, showing the formation of the compound ethylisocyanide-ferrohemoglobin.

result of a change in magnetic moment accompanying ionization of the heme-linked acid group.) The bonds to iron in this molecule are hence of the octahedral covalent type, as in ferrihemoglobin cyanide, hydrosulfide, and azide.

We plan to investigate the reactions of ferrihemoglobin with substituted

imidazoles, histidine, and other substances in order to obtain further information about the heme-linked acid group.

We are indebted to Dr. Harrison Davies and Dr. C. D. Coryell for advice and assistance during this work, and to Mr. Ray Clinton for the preparation of ethyl isocyanide.

EXPERIMENTAL PART.—The magnetic measurements were made in the way described in earlier papers.[1, 2, 3] Values of the force exerted by the magnetic field on the two-compartment tube were measured in each experiment for two standard field strengths; the values for the higher field strength were then multiplied by a suitable factor and averaged with those for the lower field strength to give the values of Δw (in mg.) reported in the tables. The values reported are corrected for diamagnetism by use of Δw values of the corresponding carbonmonoxyhemoglobin solutions obtained by reduction with sodium hydrosulfite ($Na_2S_2O_4$) and saturation with carbon monoxide, and are also corrected for dilution by added reagent solutions. The hemoglobin concentrations were calculated from the Δw values with use of data given by Taylor and Coryell[5] for ferrohemoglobin and Coryell, Stitt and Pauling[6] for ferrihemoglobin. Measurements were made at 20°C. for the ferrohemoglobin series and 25°C. for the ferrihemoglobin series.

Ethylisocyanide-ferrohemoglobin.—After a preliminary series of measurements had shown the compound ethylisocyanide-ferrohemoglobin to be largely undissociated under the conditions of the experiment, the following series was made: 20 ml. of a solution of bovine hemoglobin, concentration in heme iron 0.0160 mole/l., were placed in one compartment of a differential susceptibility tube and Δw determined. Successive additions of 0.50-ml. portions of an aqueous solution of ethyl isocyanide, concentration 0.147 mole/l., were then made from a 1-ml. glass syringe, Δw being determined after each addition. The corrected Δw values are given in table 1 and shown in figure 1.

TABLE 1

ADDITION OF ETHYL ISOCYANIDE TO FERROHEMOGLOBIN

TOTAL VOLUME OF ADDED ISOCYANIDE SOLUTION	Δw, CORRECTED
0.00 ml.	12.35 mg.
0.50	9.61
1.00	6.66
1.50	3.59
2.00	0.96
2.50	0.13
3.00	0.11
3.50	0.04
4.00	0.06
4.50	−0.14

Initial volume of ferrohemoglobin solution, 20 ml.
Initial concentration of ferrohemoglobin solution, 0.0160 mole of heme iron/l.
Concentration of ethyl isocyanide solution, 0.147 mole/l.
Temperature, 20°C.

It is seen that the decrease in Δw is linear in the volume of added isocyanide solution, showing the absence of an appreciable amount of uncombined isocyanide. The mole ratio isocyanide/heme iron at the break in the curve is 1.00. The average value relative

to carbonmonoxyhemoglobin of Δw for the last five points, 0.04 mg., differs from zero by less than the probable error of the measurements, showing ethylisocyanide-ferrohemoglobin to contain no unpaired electrons.

Imidazole-ferrihemoglobin.—After preliminary experiments had shown ferrihemoglobin to combine with imidazole, magnetic titrations were made with solutions buffered to

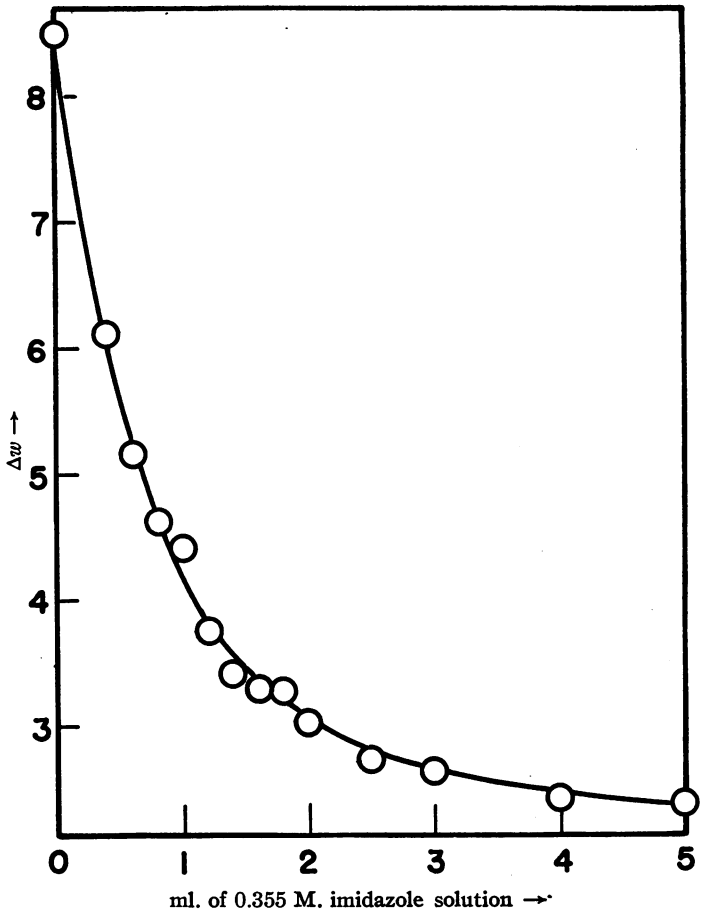


FIGURE 2

Magnetic titration curve for ferrihemoglobin and imidazole at pH 6.86. The full line is the theoretical curve for the value 2.5×10^{-8} for the dissociation constant of the compound.

pH 6.86, 8.20, and 10.30. In each experiment 20 ml. of bovine ferrihemoglobin solution containing phosphate or borate buffer were placed in a differential magnetic tube, Δw was measured, and then successive portions of imidazole solution were added, with measurement of Δw after each addition. In the experiment at pH 6.86 the imidazole solution was brought to this pH before the titration by addition of a small amount of hydrochloric acid. The data for this experiment are given in table 2 and represented in

figure 2. The second column of the table contains the values of Δw corrected for diamagnetism of all constituents and for dilution. In interpreting these values the asymptotic value 1.80 for Δw for the compound imidazole-ferrihemoglobin was selected as leading to no trend in the values of K. The third column contains the concentration of free un-ionized imidazole, calculated from the total added imidazole by correction for the amount combined with ferrihemoglobin and for ionization (44.8% un-ionized at pH 6.86, corresponding to $pK_A = 6.95$[7]). The fourth column contains values of the equilibrium constant K.

Similar experiments were carried out in duplicate at pH 8.2 and pH 10.3, with the following results:

pH 8.2: $\Delta w_{asymptote} = 1.62$ mg.; $K \times 10^3 = 1.7, 2.3$; average 2.0.
pH 10.3: $\Delta w_{asymptote} = 1.00$ mg.; $K \times 10^3 = 0.27, 0.34$; average 0.31.

TABLE 2

ADDITION OF IMIDAZOLE TO FERRIHEMOGLOBIN AT PH 6.86

TOTAL VOLUME OF ADDED IMIDAZOLE SOLUTION	Δw, CORRECTED	(IM)	$K \times 10^3$
0.00 ml.	8.50 mg.	0.00000	...
0.40	6.11	0.00159	2.7
0.60	5.16	0.00253	2.4
0.80	4.61	0.00369	2.5
1.00	4.41	0.00503	3.1
1.20	3.74	0.00611	2.4
1.40	3.42	0.00732	2.2
1.60	3.30	0.00866	2.4
1.80	3.29	0.0101	2.7
2.00	3.03	0.0112	2.4
2.50	2.74	0.0144	2.2
3.00	2.64	0.0175	2.4
4.00	2.42	0.0232	2.3
5.00	2.39	0.0286	2.7
		Average	2.5

Initial volume of ferrihemoglobin solution, 20 ml.
Initial concentration of ferrihemoglobin solution, 0.00974 mole of heme iron/l.
Concentration of imidazole solution, 0.355 mole/l.
Asymptotic value of Δw for imidazole-ferrihemoglobin, 1.80 mg.
χ_{molal} for imidazole-ferrihemoglobin, 2940×10^{-6} c. g. s. u.
Temperature, 25°C. Phosphate buffer.

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[3] F. Stitt and C. D. Coryell, *Jour. Am. Chem. Soc.*, **61**, 1263 (1939).
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