Iron Dipyridyl Complexes as Models for Iron-Porphyrin Proteins

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The change of redox potential of the Fe(II)/Fe(III) couple with change of substituent in the ligand, dipyridyl, bound to the ions has been studied. It is shown first that the substituents affect the spin state of both the valence states. At high basicity the ferrous ion is in a low spin state, and at lower basicity it is in a high spin state. The *stable* forms of the ferric complexes are high spin, but there are also metastable, low spin forms. The redox potentials of low spin Fe(II)/low spin Fe(III), high spin Fe(II)/high spin Fe(III), and low spin Fe(II)/high spin Fe(III) couples are all specifically affected by the substituents. In the light of the results the properties of iron-porphyrin proteins are deduced to be grossly dependent upon steric hindrance in the coordination sphere in addition to the dependence on ligand basicity which is demonstrated in the model complexes.

The purpose of this paper is to show some specific ways in which the potentials of the ferrous/ferric couple depend upon the character of the ligands bound to the cations. In particular we wish to show how the spin state of the cations affects the redox potentials. A knowledge of the way in which spin states and thence redox potentials are controlled by ligand characteristics is an essential part of an understanding of many biologically important iron complexes, particularly those in which iron is bound in a porphyrin. The *o*-phenanthroline and dipyridyl complexes are extremely useful in this respect. Before we discuss these particular examples we wish to enlarge upon their relevance to biological systems.

Iron is known to occur naturally in at least four types of porphyrin complex: (1) in protoporphyrin compounds such as myoglobin, hemoglobin, and cytochrome *b;* (2) in meso- or hematoporphyrin compounds (cytochrome *c* has a porphyrin which is similar in kind to these); (3) in rhodoporphyrin compounds such as chlorochluorin and cytochromes *a* and a_3; (4) in dihydroporphyrin compounds such as cytochrome a_2. The four groups of porphyrins differ in the substituents in the pyrrole rings (Table I). These substituents can be expected to modify the properties of the porphyrin as a ligand for the iron atom in two ways. The basicity (σ-donor power) of the nitrogen atoms will fall in the order meso- or hemato- > proto- > rhodo- and dihydro-. At the same time the π-acceptor character of the porphyrin ring will change in the order rhodo- > proto- > meso- or hemato- $\geq$ dihydro-. Now modification of the porphyrin ligand in these ways will alter the relative stability of the two valence states of the iron atom, the redox potential, and the relative stability of the different magnetic spin states of each valence state. Unfortunately, it is very difficult to assess how these properties of the iron have been modified by the porphyrin substituents by a direct study of the biologically important compounds themselves, as the iron is further bound by the protein groups which will also differentially affect the iron atom. In order to resolve the separate effects of the substituents and the protein groups we have decided to study a series of model complexes in which the groups immediately coordinated to the iron atom are maintained constant while a change in substituents is made.

In an earlier paper (Tomkinson and Williams, 1958) we described the determination of the oxidation-reduction potentials of several iron (II)/iron (III) *o*-phenanthroline complexes, ML_3. The redox potentials were measured during the oxidative titration of the ferrous complexes by ceric salts in 1.0 M sulfuric acid. The resultant ferric complexes of formula ML_3 are blue and are not stable indefinitely. Blue ferric complexes cannot be prepared by the direct reaction of ferric ions and ligands. Under these circumstances brown-yellow complexes are obtained. The character of the brown-yellow complexes has been frequently discussed. Gaines *et al.* (1936) presented evidence showing that the compounds were to be formulated as $[Fe_2(phenan)_4(OH)_2]^{4+}$, while Harvey and Manning (1952) considered them to be $[Fe_2(phenan)_3]^{6+}$, with unknown groups completing the coordination sphere. Magnetic measurements on solids (Gaines *et al.*, 1936; Michaelis and Grannick, 1943) proved that the ferric iron in them is in a low spin state possibly with additional electron-electron coupling between the single odd electrons of the separate ferric ions. All these studies were made in the *p*H range 2–10. In nonaqueous media yellow 1:1 ferric *o*-phenanthroline complexes have been obtained. In the similar series of dipyridyl complexes both blue and yellow ferric complexes can also be obtained.

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TABLE I
PORPHYRIN SUBSTITUENTS

Porphyrin	Substituents
Proto-	Alkyl, vinyl, propionic acid
Meso-	Alkyl, propionic acid
Hemato-	Hydroxy-alkyl, propionic acid, alkyl
Cytochrome-*c*	Thio-ether, propionic acid, alkyl
Rhodo-	Formyl, hydroxy-alkyl, vinyl, propionic acid, alkyl
Dihydro-	Vinyl, hydroxy-alkyl, and one partly saturated pyrrole ring

The blue go over into the yellow forms much more rapidly than is the case for the phenanthroline complexes. The determination of interpretable redox potentials by the titration of the ferrous complexes with ceric salts is then considerably more difficult with dipyridyl rather than *o*-phenanthroline as a ligand. We decided therefore to reinvestigate the yellow ferric dipyridyl compounds before studying the redox potentials of the dipyridyl complexes.

The Yellow Complexes.—These complexes have been investigated by two procedures, spectrophotometric and magnetic. Both studies have been carried out over a wide range of *p*H. The ratio of iron to dipyridyl in the complexes is in principle determinable from Job's method of continuous variations. Table II contains the relevant information at two *p*H values. It would appear that at very low *p*H the ferric forms but a weak complex; at approximately *p*H 1.0 the only complex present is 1:1, but above this *p*H value the 2:1 form is present to increasing degree. These results are in agreement with earlier work. However, in the region of and above a *p*H of about 2.0 the absorption spectra of ferric/dipyridyl solutions change considerably, and clearly further absorbing species are involved. We have interpreted these changes in terms of two equilibria, an acid dissociation and a dimerization, present in the same solution.

$$[Fe(dipy)_2(H_2O)_2]^{3+} \underset{}{\overset{K_d}{\rightleftharpoons}} [(Fe(dipy)_2(OH)(H_2O)]^{2+} + H^+$$

$$2[Fe(dipy)_2(H_2O)(OH)]^{2+} \underset{K_m}{\rightleftharpoons} [Fe_2(dipy)_4(OH)_2]^{4+} + 2H_2O$$

The equilibrium constant for the dissociation, K_d, can be determined at low *p*H where the concentration of the iron complexes is low enough to prevent dimerization. The equation for K_d is shown in equation (1),

$$K_d = \frac{\left\{T_{Fe} - \frac{d}{\epsilon_2}\right\}}{[H] \cdot \frac{d}{\epsilon_2}} \qquad (1)$$

where T_{Fe} is the total iron in the system, d is the optical density, and ϵ_2 is the extinction coefficient of the hydroxide form at 450 mμ. The absorption of species other than the hydrolyzed one was negligible in the circumstances under consideration. A test of the equation up to *p*H 2.0 is shown in Figure 1, in which $d \cdot [H]$ is plotted against d. From the slope K_d is 210, a reasonable hydrolysis constant for a neutral ferric ion complex of this kind. At higher *p*H dimerization becomes predominant.

Magnetic measurements on ferric dipyridyl solutions of different *p*H were made covering the same *p*H range as for the spectrophotometric measurements. It was first noted that at high *p*H the susceptibility of the solution was very low. A dimerization equation was found to express the data. If the magnetic susceptibility of the dimer is very small compared with that of the monomer, χ_1, then the susceptibility of the solution, χ_c, in terms of the dimerization constant, is given by equation (2).

$$K_m = \frac{\frac{1}{2}\left\{T_{Fe} - \frac{\chi_c}{\chi_1}\right\} \cdot [H]}{\left\{\frac{\chi_c}{\chi_1}\right\}^2} \qquad (2)$$

$$\text{Plotting } \left[\frac{\chi_c}{\chi_1}\right]^2 \text{ against } [H] \cdot \left\{T_{Fe} - \frac{\chi_c}{\chi_1}\right\}$$

(Fig. 2), we obtain a straight line of slope 2 K_m, from which we can derive $K_m = 1.10 \times 10^{-2}$ (see the Experimental section). At a *p*H above 2.0, solutions of ferric dipyridyl complexes are increasingly hydrolyzed and polymerized, and it is hardly surprising that different authors have expressed different opinions about them. The solutions may contain still other species at different ferric/dipyridyl ratios than those discussed here. Our conclusion is that at higher acidity, greater than *p*H 1.0, iron-dipyridyl solutions con-

TABLE II
OPTICAL DENSITY AT 400 mμ, JOB'S METHOD

	Mole Ratio $\frac{\text{Fe (III)}}{\text{Dipyridyl}}$										
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
O.D. *p*H 0.8	0.0	0.025	0.043	0.065	0.071	0.070	0.065	0.061	0.050	0.03	0.00
O.D. *p*H 2.0	0.0	0.71	1.05	1.31	1.30	1.19	1.04	0.82	0.55	0.27	0.00

Total molarity Fe(III) + Dipyridyl = 0.01 M. The solutions were made up in 0.025 M potassium persulfate to prevent reduction of the ferric ions.

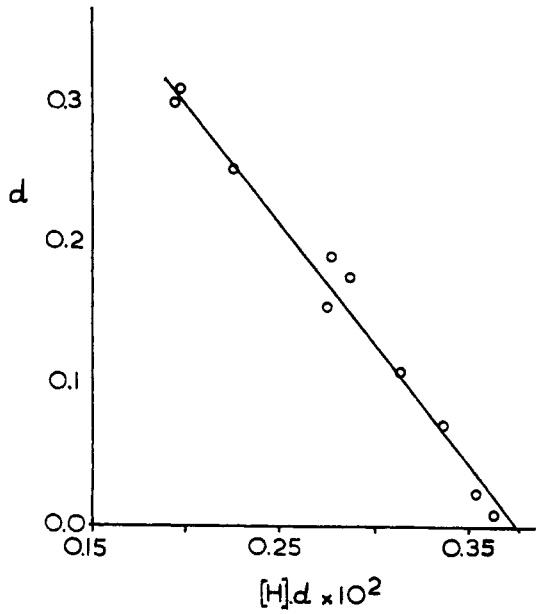

FIG. 1.—Plot showing dependence of optical density, d, on the product of d and the proton concentration [see equation (1)].

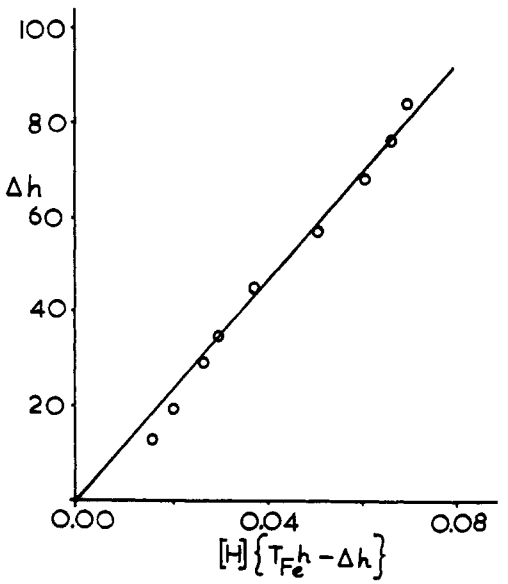

FIG. 2.—Plot of magnetic susceptibility in Δh units in accordance with equation (2).

tain some 1:1 and 2:1 dipyridyl-ferric complexes and some free ferric ions in *monomeric* forms only. The magnetic measurements show all these forms to be of high spin. The hydrolyzed monomeric species is also most probably of high spin, but the dimer has a moment between zero and one spin, in agreement with earlier work. We have repeated much of this spectrophotometric and magnetic work on the ferric complex with 5,5′-dicarbethoxy-2,2′-dipyridyl, the dipyridyl of lowest acid dissociation constant, pK_a, which was available to us, with very similar results.

The important observation from the point of view of this paper is that in the case of dipyridyl complexes there are simple complexes at low pH, and that at such pH values we can measure the redox potential of both the couple $Fe(II)X_3/Fe(III)X_3$ (blue, metastable, low spin ferric complexes) and the couple $Fe(II)X_3/Fe(III)X_{0-2}$ (yellow, stable, high spin ferric complexes). We can therefore examine the effect of spin pairing upon redox potentials. Moreover we can discover how the stability of two spin states changes as we change ligand along a series of substituted dipyridyls. Finally, because the complexes of the weakest base, the 5,5′-dicarbethoxy-2,2′-dipyridyl, with the ferrous ion are also of high spin (James *et al.*, 1961), and all the other ligands discussed here give ferrous complexes which are virtually 100% low spin, we can see the effect on the redox potential of a change to the third spin system, high spin/high spin.

Redox Potential Measurements.—The redox potentials were measured by a midpoint potential method in the cell described earlier (Tomkinson and Williams, 1958) with the same system of electrodes. In the case of dipyridyl complexes it is essential to measure the potential as soon as possible after the formation of the blue ferric complex, for this complex changes quite rapidly to a yellow form. Our procedure was to make up a 10^{-2} M solution of the ligand to which was added ferrous iron to give in all cases except that of the 5,5′-dicarbethoxy-2,2′-dipyridyl solutions a 10^{-3} M solution of the Tris ferrous complex. The solution was 0.1 M with respect to sulfuric acid. Just before commencement of potential measurements and while the cell solution was being stirred vigorously an amount of ceric solution was added from a microburet, the amount being exactly that required to oxidize half the ferrous complex to the ferric state. The change in redox potential with time was followed. A typical plot is shown in Figure 3. There is an initial period when readings fall but slowly and most of the ferric ion (> 75%) is present as the blue complex. Subsequent to this period the potentials fall very rapidly toward a steady value. When the steady potential is reached all the ferric complex is in the yellow form. The final potential is maintained indefinitely. The blue to yellow change is autocatalytic.

We shall only attempt to interpret two potentials here. First we consider the potential obtained by extrapolating the slow initial fall back to zero time. Very little error is involved in this extrapolation, as the initial rate of change of potential is relatively small. This potential we take to be that of the couple $Fe(II)L_3/Fe(III)L_3$. Values for different ligands, L, are given in Table III. Secondly we consider the steady potential read after an hour, when no further change in

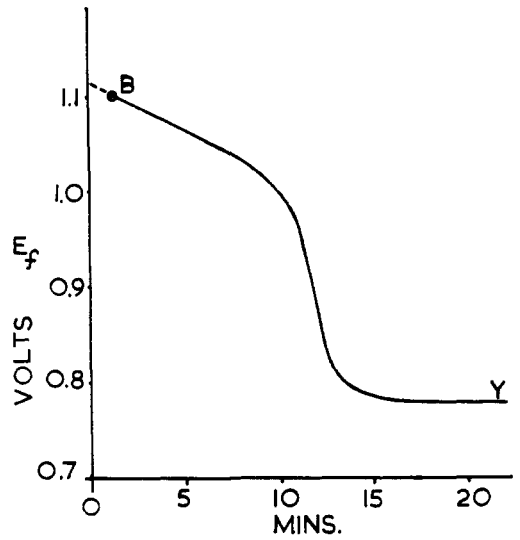

FIG. 3.—The variation of the redox potentials with time. The small circle is the first measured potential. *B* is the extrapolated potential of the Fe(II)/Fe(III) couple, blue ferric complex, and *Y* is the final potential of the Fe(II)/Fe(III) couple, yellow ferric complex.

potential is measurable. The interpretation of this potential is more difficult, for the composition of the ferric complexes is certainly not ML_3 but is a mixture of some free ferric ion and some 1:1 and possibly 2:1 ligand-to-metal complexes. We can proceed as follows:

If the concentration of free metal ions is $[M]$ in either valency, then [equation (3) of Tomkinson and Williams, 1958]

$$\frac{d\ ln\ [M]}{d\ ln\ [L]} = -n_M \qquad (3)$$

Now $[L]$ is given by equation (4)

$$[L] = \{T_L - \Sigma T_M \bar{n}_M\}/\{1 + [H]/K_a\} \qquad (4)$$

where T_L is the total ligand and T_M is the total metal. Ferric dimers and hydrolyzed species are assumed absent here and in the later discussion of the redox potentials, both on the basis of the evidence given above and from a study of the change of redox potential with solution composition. A plot of log [Fe(II)]/[Fe(III)] against E_f during an oxidative titration of the ferrous complex $Fe(II)L_3$, allowing time for formation of the yellow ferric form, has a slope of 0.058 ± 0.003 independent of ligand. There can be no dimerization. Now for all the ligands we are discussing and at the fixed acidity (pH ≃ 0.8) of the experiments, $[H]/K_a > 1$ and $T_L - T_m\bar{n}_M$ is very nearly constant and independent of ligand ($\bar{n}$[Fe(III)] ≃ 1.0 and $\bar{n}$[Fe(II)] = 3.0). Hence the concentration of free ligand is closely proportional to the acid dissociation constant of the ligand, *i.e.*, $[L] = Q \cdot K_a$, where Q is a constant independent of the ligand. Now the change in redox potential with change of ligand arises from two factors: (1) a term dependent upon the stability constants of the different complexes, and (2) a term arising from the different free ligand concentrations as we change ligand at fixed pH. The second term can be expressed for each ligand as follows. Starting with equation (5a)

$$\frac{dE_f}{d\ ln\ [M(II)]/[M(III)]} = RT/F \qquad (5a)$$

and substituting from equation (3) we have equation (5b).

$$dE_f = RT/F\ (\bar{n}_{III} - \bar{n}_{II})\ d\ ln\ [L] \qquad (5b)$$

The effect of this difference in the observed potential from one ligand to the next can be eliminated by correcting for this term as follows. In this series of experiments the change in $[L]$ from one ligand to the next is due to the change of ligand, all other conditions being kept constant. Therefore

$$dE_f = RT/F\ (\bar{n}_{III} - \bar{n}_{II})\ dln \cdot K_a + \text{constant} \qquad (6)$$

with [L] = QK_a. The potentials measured at constant pH do not reflect the stability of the complexes alone but include a term which is dependent upon the acid dissociation constant of the ligand. In other words, potentials measured at a fixed pH are not obtained for constant $[L]$ from one ligand to another, and are not therefore directly comparable. Direct comparison independent of pH is permissible only (1) when $\bar{n}_{II} = \bar{n}_{III}$, since then $dE_f/dln[L]$ is zero [equation (5), Table II], and (2) when $[L] = 1.0$. In order to obtain comparable formal potentials for the complexes of a series of ligands when the degree of complex formation is not the same for the two valence states, we must correct to *equal free ligand concentration*. This may be done with the integrated form of equation (6), adding the appropriate ΔE_f to each observed potential to give equation (7).

$$\Delta E_f = RT/F \cdot (\bar{n}_{III} - \bar{n}_{II}) \cdot ln\ K_a \qquad (7)$$

The integration is permissible, as $\bar{n}_{II}$ and n_{III} are virtually constant, and so the correction to be applied to each ligand system depends only upon the approximately fixed difference in degree of formation of the two species and the different $ln \cdot K_a$ values (Table II). From the Job method of continuous variations we know that $\bar{n}_{III}$ is not greater than 1.0 for dipyridyl itself and for the weakest base studied, 5,5′-dicarbethoxy-2,2′-dipyridyl. More precise information is difficult to obtain because the optical density changes on formation of the complexes are small and the complexes are formed in a region of fairly high acidity. In all the systems except that of the 5,5′-dicarbethoxy-2,2′-dipyridyl, $\bar{n}_{II}$ is 3.0 exactly. In the exceptional case $\bar{n}_{II}$ is known to be 2.4 under the conditions of the redox potential study. The difference $(\bar{n}_{III} - \bar{n}_{II})$ falls either within or close to the limits −2 to −3 in all cases. We have therefore corrected the observed E_f in the presence of the yel-

low ferric complexes in two ways; (*a*) by adding $\frac{2RT}{F} \ln K_a$ and (*b*) by adding $\frac{3RT}{F} \ln K_a$, both in accordance with equation (7). The values are given in Table II. The true E_f value at constant free ligand concentration for all the ligand systems certainly lies between these extremes. The effect of the correction term is to raise the potentials of the strongest bases.

Discussion

In Figure 4 we plot E_f (blue ferric complexes) against pK_a. We include some results of Smith and his co-workers and of our own work on the phenantholine complexes for purposes of comparison (see Smith and Bannick, 1959). The general trend is a fall in redox potential with increase in pK_a. The results for the dipyridyl complexes do not parallel the results for the *o*-phenathroline complexes. There is a noticeably greater difference between the two series at low pK_a. The general pattern of the new data is in keeping with earlier studies. In Figure 4 we also plot E_f (yellow ferric complexes) against pK_a. Three sets of values are given for each ligand. The values are taken from Table III. It must be remembered that the yellow complexes are the thermodynamically stable forms under the conditions of measurement. There is no simple relationship between pK_a and the redox potential. In fact, all the potentials increase toward a maximum value.

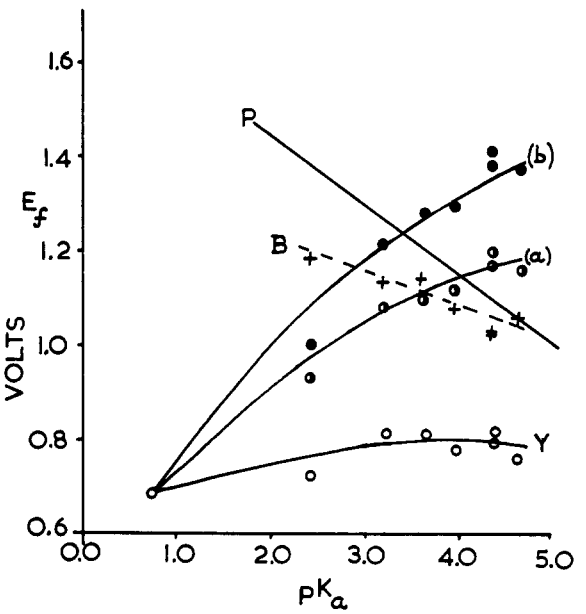

Fig. 4.—The change in redox potential with ligand substituent. *B* is the plot for the blue Fe(III)L_3 complexes, dotted line and individual points as crosses. *P* is the same plot for a series of phenanthroline complexes (aqueous solution). *Y* and open circles represent potentials for yellow ferric complexes and (*a*) and (*b*) are these potentials corrected as described in the text.

We interpret the two plots as follows. The blue complexes are *metastable* low spin ferric forms. On change of ligand to lower basicity the stability of these blue complexes falls more rapidly than the stability of the red low spin ferrous complexes. The redox potentials increase as pK_a falls. The yellow complexes are *stable* high spin ferric complexes. On change of ligand to lower basicity the stability of these complexes falls, though not as rapidly as with the blue complexes. The fall in stability is now less than the corresponding change in stability of the red low spin ML_3 ferrous complexes. In this pK_a region the potentials decrease as the pK_a falls. At the extreme end of this pK_a range, toward lower basicity, the ferrous complexes change from low to high spin states. Over the range of ligands where this change occurs their stability falls much more rapidly with decreasing basicity than that of the yellow ferric complexes. After the change, with both ions high spin, it would appear that the ferric stabilities are more sensitive to pK_a (Tomkinson *et al.*, 1958). The redox potential/pK_a plot is clearly dominated by the particular spin states. We note further that when both cations are in high spin states the redox potential falls below that of the hydrated ions so that the ligands stabilize the ferric form preferentially. No ligands are known to stabilize the ferrous state relative to the ferric state without a change of

Table III
Redox Potentials

Substituent in Dipyridyl	pK_a	E_f (blue)	E_f (yellow)	E_f (*b*)*	E_f (*a*)*
5,5′-Dicarboethoxy	0.85	(?)	0.67	0.67	0.67
4,4′-Dicarboethoxy	2.45	1.19	0.73	1.01	0.91
4,4′-Diphenyl	3.25	1.15	0.82	1.24	1.10
None (dipyridyl)	3.62	1.16	0.82	1.30	1.12
5,5′-Dimethyl	3.97	1.12	0.78	1.32	1.14
4,4′-Dimethyl	4.38	1.05	0.82	1.43	1.23
4,4′-Diethyl	4.40	1.05	0.80	1.41	1.21
4,4′-Diethyl-5,5′-dimethyl	4.59	1.08	0.77	1.41	1.20

* $E_f(a)$ and $E_f(b)$ are calculated potentials for yellow ferric complexes; see text. The formulae of the blue and yellow complexes are discussed in the text. All potentials were measured in 50% w/w dioxan–water solutions.

spin state of the ferrous ion. Finally there is a maximum in the plot of redox potential against the ligand basicity for a series of ligands which brings about changes in spin states of the iron valence states.

We shall now reconsider the biologically important molecules. We observe, first, that the order of their redox potentials is not related to the basicity of the porphyrins (Table IV). Thus the cytochrome with the highest redox potential, cytochrome *f*, has the same porphyrin as the cytochrome with the lowest potential, cytochrome c_3. Secondly we note that there is a correlation of redox potential with spin type of the same kind as that observed in the model dipyridyl complexes in that either completely high or completely low spin systems have lower potentials than systems in which the high and low spin states are in rough stability balance. The porphyrin substituents alone do not control the spin state equilibria. However we know from the models that the change in spin state, high to low spin, follows the ligand basicity for a series of similar ligands. We are forced to conclude that the spin state of a given porphyrin complex is largely dependent upon the protein groups bound to the iron and that their *effective* field strength increases from high to low spin porphyrin complexes. We imply not that there is no variation in field strength of the different porphyrins but that this particular parameter is not of decisive importance.

The spectra of all the iron porphyrin proteins under discussion suggest that the further groups binding the iron are either protein imidazoles or amines. If this is the case then the vast variety of cytochromes and oxygen-carrying pigments in Table IV can arise only if the effective ligand field due to these two groups is controlled by the relative geometry of the protein and the porphyrin. In other words the spin states and the redox potentials are largely controlled by steric restrictions and a change from imidazole to amine groups around the iron atom. The steric problems are of some complexity, for they involve not only the permitted iron to protein-nitrogen bond distances but also the angle the plane of the imidazole group makes with the plane of the porphyrin ring. It must also be remembered that the low spin states of both ferrous and ferric iron involve shorter bond distances than the high spin states. [It has been suggested (Williams, 1961) that the contraction in the iron-imidazole distance on change from high to low spin when hemoglobin absorbs oxygen causes the cooperative effects observed in this four heme protein]. Again change of environment may well alter this distance in a protein. In this fact alone could lie the explanation of the confusion about the cytochrome oxidase complex of proteins. Cytochrome a_3 may differ from cytochrome *a* only in the steric restrictions induced at the two iron atoms under the conditions existing in a living cell.

Another indication of the importance of steric restrictions is that many of the biologically important molecules of the cytochrome type have higher redox potentials than any of the model complexes of iron porphyrins. Now in ferrous complexes the equilibrium distance Fe-N is longer than in ferric complexes by 0.1 to 0.2 A. Steric restrictions will be more effective in reducing the stability of the ferric iron than in reducing the stability of the ferrous iron, which is the same thing as a raising of the redox potential. At the same time the effect will be further enhanced, as the ferric iron will be the more prevented from changing spin state. It does not appear that the ferric iron is more than about 90% low spin in other cytochromes than cytochromes *b* and perhaps c_3.

To summarize, the observed series of iron porphyrin proteins from myoglobin to cytochrome c_3 could well be produced by a gradual reduction in the steric restrictions around the iron atom, plus a change from imidazole to amine ligands. In this way a change of spin state of both valence states and a consequent variation of redox potential showing a maximum value could result. The function of the porphyrin substituents in effecting these properties of the iron directly may well be rather small but their general direction is known. Indirectly the substituents must be partly responsible for the steric restrictions themselves.

TABLE IV
THE IRON PORPHYRIN PROTEINS

Protein	Porphyrin	Spin State	Potential
Myoglobin	proto-	high	+0.02
Cytochromes *d*	hemato-type	high	±0.00
Hemoglobin	proto-	high	+0.15
Chlorocruorin	rhodo-	high	+0.20
Cytochrome a_3	rhodo-	high/low	+0.30 ?
Cytochrome a_2	di-hydro-	high/low	+0.30
Cytochrome *f*	hemato-type	high/low	+0.36
Cytochromes *a*	rhodo-	mostly low	+0.28
Cytochromes *c*	hemato-type	mostly low	+0.15–+0.25
Cytochromes *b*	proto-	low	±0.00–+0.08
Cytochrome c_3	hemato-type	mostly low	−0.20

The proteins are listed so as to bring out the relationship between spin type and redox potential which we discuss in the text.

TABLE V
THE MAGNETIC MEASUREMENTS

[H] × 10^2	h	Δh	$\{T_{Fe}h - \Delta h\}$
2.14	1644	89	5
1.55	1656	77	17
1.10	1667	66	28
0.81	1670	63	31
0.71	1681	52	42
0.65	1679	54	40
0.60	1688	45	49
0.50	1698	35	59
0.39	1694	39	55
0.33	1706	27	67
0.31	1711	22	72

$\Delta h = (1733 - h)$, where 1733 is the observed value of h for the 3.6×10^{-2} M solutions of dipyridyl used in the experiment. Ferric iron was 1.8×10^{-2} M, and in the absence of dipyridyl free high spin ferric ions gave a $T_{Fe}h$ against pure water of 94 units. h is measured in 10^{-4} cm.

EXPERIMENTAL

Materials.—Analar ferrous and ferric sulfates were used throughout. Ligands were prepared or obtained as described in a previous publication (James *et al.*, 1961). The dioxan used in making the solvent (50% w/w water–dioxan) for the redox potential work was purified by standard procedures.

Apparatus.—The measurements of *p*H, absorption spectra, and redox potentials were carried out as in our previous publications (James *et al.*, 1961). Magnetic measurements on solutions were made with a modified Quincke apparatus which will be described elsewhere. In the procedure the magnetic susceptibility of a solution is obtained as a difference in height, Δh, of a liquid in a narrow tube in the presence and absence of a magnetic field. Results pertinent to this study are given in Table V with the relevant experimental information.

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