THE PROPERTIES OF METALLOPORPHYRINS

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I. Introduction

The presence of a number of metal cations in biological systems is well authenticated (45, 95). In many of the enzymes and proteins in which they occur the immediate environment of the cation is known. For example, iron in hemoglobin, magnesium in chlorophyll, and cobalt in vitamin B_{12} are held in coördination with cyclic imines. These and other cations play an essential role in the biological activity of the enzymes containing them, for without the cations the enzymes become inactive. Despite the increasing study of the properties of the enzymes, however, there does not appear to have been much progress with the definition of the function of the cations. During the last fifteen years, on the other hand,

many new methods for the examination of metal complexes have become available and from the utilization of these methods, especially that devised by J. Bjerrum (7), a great increase in our understanding of the interaction between ligands and cations has developed (37, 53, 54, 96). It is the purpose of this review to compare this newly acquired information with the properties of a limited number of the metal compounds known to be present in biological systems. On grounds of length the review is restricted to a discussion of iron–porphyrin complexes, although an indication will be given of a possible extension of the discussion to other cation enzymes and proteins. Elsewhere a more general discussion of the specificity of cation action has been given (45, 95).

The iron–porphyrin complexes occur in peroxidases, catalases, hemoglobins, myoglobins, a range of cytochromes, and probably in certain flavoproteins (51). The method of this review will be to set down certain observations on carefully chosen model systems for these proteins and then to compare the properties of the models, which are now understood at least in part, with the properties of the iron-containing proteins. In particular, the complexes of cations with aromatic imines, such as phenanthroline, will be used as models. The discussion of even such a limited field of cation complexes will be shown to lead to conclusions which can be of considerable value in defining the functions of other cations —notably copper and zinc in certain enzymes. The latter topics will arise incidentally to the main theme.

A detailed understanding of the properties of all these complexes demands an acquaintance with current concepts used in the description of the bonds between cations and ligands. For a detailed account of this work the reader is referred to the literature (60, 61, 99).

II. Some Properties of Complex Ions

A. The Nature of the Bonds in Complexes between Cations and Different Ligands

Cations such as magnesium, calcium, strontium, and barium bind ligands largely through the electrostatic forces of attraction between opposite charges. Part of the binding, however, is undoubtedly of the donor–acceptor kind, in which the cation acts as acceptor. A proper description of this bonding requires a knowledge of the acceptor orbitals of the cation, but no thorough treatment of this problem has been offered as yet. In this article the acceptor orbitals of any cation will be assumed to be simple σ-orbitals developed from the hybridization of the atomic s-, p-, and d-orbitals of the cation. The description, σ-orbital, implies that the molecular orbital axis is coincident with a line joining the centers of the bonded atoms. Transition metal cations can develop bonding of this type, but there is also the further possibility of strong π-bonds (60, 64, 99).

Transition metals of the first transition series of the Periodic Table may have electrons in five $3d$-orbitals. The effect of a cubic force field, whether it be electrostatic or otherwise, is to split these five levels into two which point in the directions of maximum intensity of the field, d_γ-orbitals, and three which point

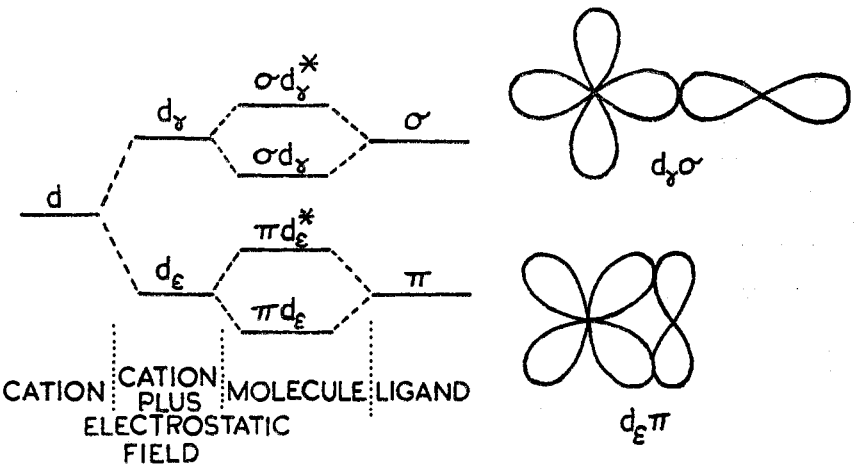


FIG. 1. The influence of the ligand field upon the d states of transition metal cations. The levels marked * are antibonding energy states and the other levels are bonding states (59, 60, 61, 62, 99). Illustrations to the right represent the overlapping molecular orbitals of the cation and the ligand.

along lines which bisect these directions, d_ϵ-orbitals. Now without detailed discussion it is evident that for a maximum stabilization of the electrons of the cation and of the ligands providing the cubic force field, it is advantageous if the d_γ-orbitals of the cation are empty or half-filled while the d_ϵ-orbitals are full. In this way the greater number of cation electrons can avoid the electrostatic field of the ligands which can simultaneously form σ bonding orbitals with the cation. If the ligand is an unsaturated compound, then the further possibility arises that the filled d_ϵ-orbitals of the cation can interact with empty π-orbitals of the ligand, which cannot be closely defined as yet, forming πd_ϵ bonding orbitals. The interaction of a transition metal cation with a ligand such as phenanthroline consists then of two parts: (*1*) the formation of σ molecular orbitals in which the cation acts as acceptor and (*2*) the formation of π molecular orbitals in which the cation acts as donor. These are shown in figure 1. Using this description the absorption spectra and stabilities of many cation complexes are open to interpretation (59, 60, 99).

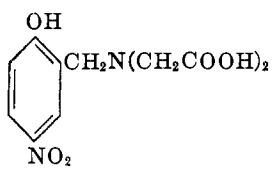
B. THE ABSORPTION SPECTRA OF SOME LIGANDS AND THE EFFECT OF CATIONS UPON THEM

The absorption bands of simple aromatic molecules are found in the region 250–400 mμ. They are due to transitions between different π states and perhaps also between different n and π states. The effect of substituents upon these bands is not easy to predict, for the substituent alters both the energy of the ground state and that of the excited state. Nor is it a simple matter to predict changes in intensity of the bands even along a graded series of simple substituents such as iodine, bromine, and chlorine. It might be thought then that the study of the spectra of metal complexes of these molecules would lead only to a

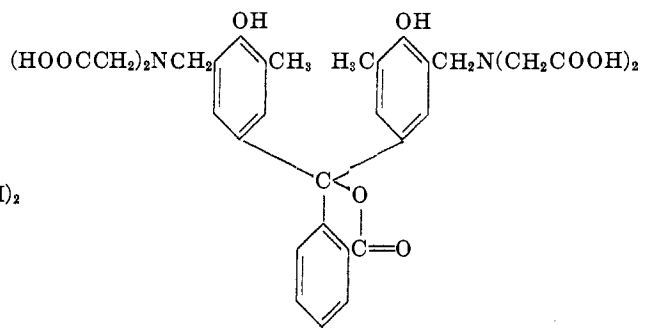
TABLE 1

The effect of cations on the absorption bands of ligands

Ligand	Absorption Maximum of Cation Complex						Reference
	Mn(II)	Co(II)	Ni(II)	Cu(II)	Zn(II)	Fe(II)	
Acetylacetone	273	282	294	296	292		(80)
8-Hydroxyquinoline	396	406	400	412	373		(80)
Phenanthroline	290	291	292	296	280		(79)
Dipyridyl	296	295	295	300	295	298	(79)
2-*p*-Dimethylaminophenylazopyridine	540	540	550	570	538		(43)
Riboflavin	447	445	450	455	450	455	(52)
	Zn(II)	Mg(II)	Ca(II)	Ba(II)			
Ligand I	380	385	396	403			(72)
Ligand II	560	565	565	570			(2)



Ligand I



Ligand II

collection of confusing data, for a cation can be considered to be a complicated substituent. Table 1 shows, however, that certain simple relationships exist between the nature of the cation and the position of the ligand absorption band of a complex. In several cases the wavelength maximum follows the stability of the complex. It must be noted, however, that table 1 contains two carefully chosen series of cations. In the first series the stability order, and the order of λ_{max}, is Zn(II) < Cu(II) > Ni(II) > Co(II) > Mn(II). Here the stability order reflects the electron affinity of the cation, but this is certainly not true of other stability orders (37, 96). The stability sequence Zn(II) > Mg(II) > Ca(II) > Ba(II) in the second group of data is also a series of diminishing electron affinity, and here the stability is controlled by this term rather than any other. If any other groups of cations had been chosen it would have been impossible to arrange them in an order independent of the ligand which followed the expected covalent character of their bonds, because of the mixture of covalent and ionic bonding in the complexes (96). It is also impossible to arrange the wavelengths of the absorption of complexes of other cations in any general

order independent of the ligand. It is concluded that the effective electron affinity of a cation is controlling the position of the absorption band of the ligand in the above limited series and may also do so quite generally.

In most of the complexes examined (table 1) the cation moves the absorption band of the ligand to longer wavelengths. It so happens that in all these cases the cation can act so as to extend the conjugation. All the ligands in this group are chelating compounds which can conjugate with the cation. In two groups, however, those of the complexes investigated by Schwarzenbach (2, 72), the wavelength of the absorption of the ligand falls the greater the electron-acceptor properties of the cation. These ligands do not form conjugating chelates. The direction of the shift of the absorption band may well depend upon the presence or absence of conjugation involving the cation, but no firm conclusion can be drawn without further experimental data. One point is worth recording here, however. The effect of the proton or a cation on the absorption of a phenoxide ion is to shift the ligand band to *shorter* wavelengths, while the effect of a cation upon a *chelating* and *conjugating* phenol is to shift the absorption to *longer* wavelengths.

It will be appreciated that if a cation undergoes a change of electron configuration on complex formation, making orbitals available for bonding which were not available in the normal state, then the ionization potential to the normal state for that cation does not reflect the electronegativity of the cation even qualitatively. The ferrous complex of dipyridyl is diamagnetic, and so is the nickel complex of bissalicylaldehyde ethylenediimine (55). In both cases the cation is not in its normal state, and the effect of the cation upon the absorption of the ligand is more marked (80) than would have been expected from its ionization potential to this normal state. The anomalous effect of ferrous cation upon the riboflavin absorption may be compared with the abnormal stability of ferrous riboflavin complexes (1, 52), and could have its origin in the mixed bonding state of ferrous cation in complexes with this type of ligand (98).

The changes of intensity of ligand bands on chelation with cations are less simple than the changes in wavelength, except in a few minor cases. The intensity falls regularly with increasing electron affinity in two series of Group II cation complexes: i.e., Ba(II) > Sr(II) > Ca(II) > Mg(II) > Zn(II) (2, 72). In the dipyridyl complexes, on the other hand, the change in intensity of the 290 mμ band follows no rational order of the cations. The intensity of the absorption of the ferrous complex is anomalously high (27). The ferrous dipyridyl complex differs from the other cation complexes of the Irving–Williams series in that it is formed with a change in the electron configuration of the cation. The ferrous complex is an inner orbital complex. A similar anomalously high absorption is found in the ferrous riboflavin complexes (51).

The above discussion will now be used in an examination of the absorption spectra of the complexes of metal porphyrins. The spectra of the porphyrins themselves are so complicated, however, that this examination would be confusing without a preliminary account of the absorption spectra of porphyrins (50, 68, 74).

III. Simple Porphyrin Complexes

A. The absorption spectra of porphyrins

The spectrum of a typical porphyrin (figure 2a) consists of an intense band at about 400 mμ, the Soret band, and four weaker bands at longer wavelengths. The assignment of these bands to definite electronic transitions is made the more difficult by the possible presence of tautomeric forms (74). It is generally considered that the Soret band is due to one $\pi \rightarrow \pi'$ transition, which is similar for both tautomers, and that the four bands in the visible (I, II, III, and IV in order of decreasing wavelength) arise in pairs, two from each tautomer, from a second $\pi \rightarrow \pi'$ transition which is twofold degenerate. All four long-wave bands move together to longer wavelengths on substitution at the peripheral positions of the pyrrole rings, confirming that they belong to the same electronic transition, but marked differences arise in the intensities of these bands. Whereas bands II and IV are little changed in intensity by substituents, bands I and III vary considerably from compound to compound. Their intensities are larger the larger the electron-acceptor properties of groups in diagonally opposed pyrrole rings: e.g., they are very large in oxorhodoporphyrin, a molecule with oppositely placed carbonyl groups in conjugation with the porphyrin nucleus (23, 46).

On the formation of the dihydrochloride of a porphyrin the four bands in the visible are replaced by two, the α- and β-bands, the former being at the longer wavelengths (figure 2b). It has been suggested that this change arises through the absence of tautomeric forms in the dihydrochloride. There is certainly a relationship between the intensity of either band II or IV and that of the β-band, for all three are hardly altered in intensity by change of substituent (5). The

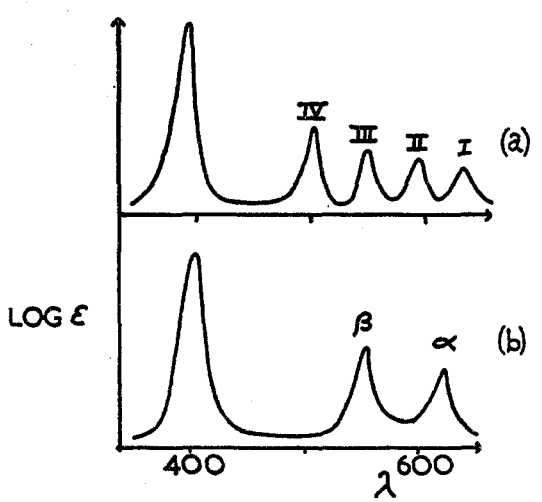


Fig. 2. The absorption spectrum (a) of a porphyrin and (b) of its dihydrochloride or one of its metal complexes. The figure is not intended to be the absorption spectrum of a particular porphyrin but rather is meant to show the method of labelling the individual absorption bands.

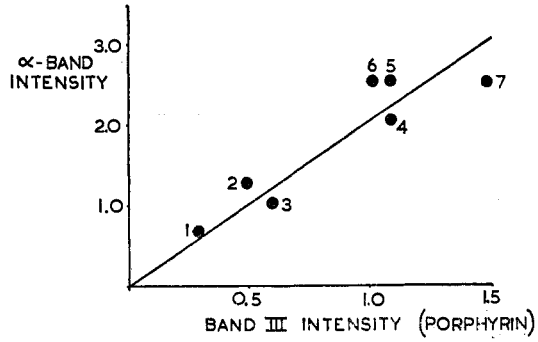


FIG. 3. Relationship between the maximum intensity of band III of the porphyrin and of the α-band of the cupric complex: (1) porphyrin; (2) porphyrin-1,4-dipropionic acid; (3) 1,4-diethyl-2,3-dimethylporphyrin (83); (4) mesoporphyrin dimethyl ester (81); (5) etioporphyrin (16); (6) coproporphyrin (83); (7) rhodoporphyrin (82).

α-band is similarly related to band III or I in its properties. Illustration of the second relationship is best given by reference to the spectra of metal porphyrin complexes which resemble the dihydrochloride in their symmetry and in having a two-band rather than a four-band spectrum in the visible region. Figure 3 is a plot of the intensity of band III of certain porphyrins against that of the α-band of the cupric complexes of these porphyrins, showing the close relationship. The β-band of the metal complexes does not change in intensity with change of substituent. Both the α- and the β-bands of dihydrochlorides and the metal porphyrins move to longer wavelengths with increasing electron-acceptor properties of the substituents in the porphyrin (5, 82), supporting the suggestion that they belong to the same electronic transition.

B. THE EFFECT OF DIFFERENT DIVALENT CATIONS UPON THE PORPHYRIN SPECTRA

The wavelengths of maximum absorption, shortest wavelengths first, of the α- and β-bands of the different metal complexes of several porphyrins and of the related phthalocyanines are in the order Pd(II) < Ni(II) < Co(II) < Cu(II) < Zn(II) < Fe(II) < Mn(II) < Mg(II) < Ba(II) (table 2). Below zinc the order is that to be expected from the already discussed effect of different cations upon the absorption spectra of other ligands: the polarizing power follows the ionization potential (table 1). The wavelength of the α- and β-bands falls with increasing electron-acceptor properties of the cation, a fact which suggests that the cation restricts the conjugation in the porphyrin (page 303).

The extension of the correlation of band characteristics with the ionization potential to the other cations, as in table 1, can only be expected provided the cations are in their normal states in the complexes, i.e., they are not in *promoted* rearranged electronic states. In the phthalocyanines it is known from measurements of paramagnetism that palladium, nickel, and cobaltous ions are not in their normal electronic configurations (42, 73). Furthermore, this is also true of

TABLE 2

Spectra of metalloporphyrins, λ_{max}

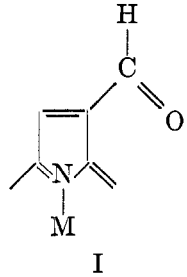
Porphyrin	Zn(II)	Cu(II)	Co(II)	Ni(II)	Reference
	mμ	mμ	mμ	mμ	
Mesoporphyrin IX	570	561	552	550	(81)
Protoprophyrin	586	578	573	567	(34)
Tetramethylhematoporphyrin		565	560	557	(32)
Tetraphenylporphyrin	550	540	530	530	(20)
Etioporphyrin II	572	560		550	(14)
Desoxyphylloerythrin	574	563	560	554	(88)
Phthalocyanine	649	648	642	641	(3)

The data for other metalloporphyrin derivatives are given in the individual papers.

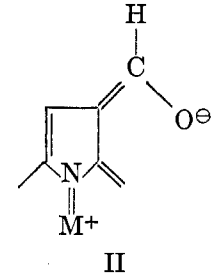
nickel protoporphyrin (67) and of a cobaltous mesoporphyrin (32). The cupric ion in a porphyrin complex may well have one of its atomic electrons elevated to a $4p$ state, for recent measurements (35) of paramagnetic resonance in crystals of copper phthalocyanine are strongly indicative of dsp^2 orbital binding. If this is so, then all the four ions which have the greatest effect upon the porphyrin spectrum have their electrons in promoted states and bind the porphyrin through empty σ-orbitals. The use of empty d-orbitals taken together with the possibility of strong double bonding through promoted d_ϵ electrons naturally enhances the electronegativity of the cations, and it is not surprising that these cations exert the greatest polarizing power upon the ligand, shifting the ligand absorption bands by the greatest amount (14).

The intensity of the α-band in the cation–porphyrin complexes, with the exception of tetraphenylporphyrins (20), follows the order Pd(II) > Ni(II) > Co(II) > Cu(II) > Zn(II) > Mg(II) > Hg(II).

Now, high intensity of the α-band in a porphyrin has been correlated with electron-acceptor properties of the peripheral substituents (page 304). How can cations introduce a similar change in polarity of the porphyrin to that due to an electrophilic substituent? The most reasonable explanation is that the cations become conjugated with the porphyrin to a greater degree in the excited state This can be written illustratively as follows:



I



II

Both ground and excited states are mixtures of I and II, but II is the more predominant in the excited state. Of course this description is equivalent to the statement that the d_ϵ electrons are more strongly donated in the excited

state. The order of α-band intensities of the different cation–porphyin complexes is not related to the electronegativity of the cations for, if it were, mercuric ion should be close to palladium. Instead, the intensity follows more closely the ability of the cation to back-coördinate, to act as an electron donor.

Two guiding principles to the interpretation of metal porphyrin spectra have now been stated: (*1*) the wavelengths of the absorption maxima are the shorter the more electronegative the cation; (*2*) the intensity of the α-band is greater the greater the electron-donor character of the cation. Other evidence supporting these generalizations will now be given.

C. THE SPECTRA OF TRIVALENT CATION–PORPHYRIN COMPLEXES

It is to be expected that a trivalent cation will act as a weaker donor of d_ϵ electrons than a divalent cation because of its greater positive charge. Taylor and Clark (85), who studied complexes of manganic and cobaltic mesoporphyrin IX, found that the α-band was weak in both complexes but much weaker in the former than in the latter. The β-band had its usual strength in both complexes. Now the manganic ion binds through square-coplanar dsp^2-orbitals without electron rearrangement, as the cation only has four $3d$ electrons. These electrons are not in a promoted state and cannot be expected to be donated strongly to the porphyrin. High intensity of the α-band is associated not with bonding through dsp^2-orbitals, σ-bonds, but rather with the arrangement of $3d$ atomic electrons in a promoted d_ϵ state. The electrons of the cobaltic ion, which is isoelectronic with the ferrous ion, are paired back in just such a promoted electronic state. It is not surprising then that the α-band in the porphyrin complex of this ion is much stronger than that in the manganic porphyrin. On addition of cyanide to the cobalt complex, however, the intensity of the α-band is strongly suppressed, although that of the β-band remains unaltered. This observation parallels the effects of cyanide upon the absorption spectra of ferric porphyrins.

In the ferric porphyrins (20, 81), which are "ionic" and have five unpaired electrons, the α- and β-bands are obscured for reasons to be discussed (page 314). Nevertheless it is clear that the α-band is of a very low intensity. The electrons in ionic complexes of ferric ion are not in a promoted state but occupy a state of maximum multiplicity. In the covalent hydroxy ferric porphyrin complexes the $3d$ electrons are in a promoted d_ϵ state, the cation has fewer unpaired electrons, and, as expected from the above discussion, the α-band is now of moderate intensity, i.e., comparable with that of the cobaltic porphyrins (85). The spectra of these covalent ferric complexes so resemble the spectra of certain ferrous porphyrin complexes that much confusion arose in the early literature. Although the α-band is strong in the covalent ferric porphyrin hydroxide and amine complexes, it is suppressed by the reaction of the ferric porphyrins with cyanide, although all the complexes have similar magnetic moments, indicating few unpaired electrons. An explanation which accounts for this observation is given in the further discussion of the ferric, cobaltic, and ferrous porphyrins (page 311).

IV. The Specific Properties of Some Ferrous Complexes

A. The anomalous absorption of some ferrous complexes

The absorption spectra of some cation phenanthroline complexes in the ultraviolet have been described (page 302). Apart from this absorption there is an additional band in the visible spectra of the complexes of ferrous, cuprous, and some other reducing cations (98). Most diamagnetic ferrous complexes show similar specific bands (table 3). These and other properties of the complexes, their stability for example, suggest that the ground state is stabilized by πd_ϵ double bonding (8, 60, 99), which implies a donation of d_ϵ electrons to the ligand. From a study of the effect of substituents upon the absorption in the visible of ferrous, cuprous, and cobaltous complexes, it is apparent that the degree of electron donation is further increased in the excited state (98). Thus a particular property of the metal porphyrins (page 306), the increased electron-acceptor properties of the ligand in the excited spectroscopic state, is common to certain other ferrous, cuprous, and cobaltous complexes. There is, however, an obvious difference between the porphyrin spectra and the spectra of these other complexes. The former are due to transitions of electrons of the ligand coupled with electron transitions of the cation, while the latter are due to transitions of cation electrons alone. That is to say, in the absence of the cation there are no absorption bands of phenanthroline corresponding to those in the ferrous complexes because the ground state π levels of the ligand which accept d_ϵ electrons are unoccupied in the free ligand. In the porphyrin, however, the free ligand has two electrons in addition to those needed to fill the lower orbitals, it has a negative charge of 2, and the extra electrons go into the energy states which can be further used in the binding of the d_ϵ electrons of the ferrous ion. Thus in the porphyrins there are already transitions of the free ligand corresponding to the transitions of the metal complexes, but in the phenanthrolines new transitions are made possible by the formation of complexes. Basically, however, the elec-

TABLE 3

The absorption spectra of some ferrous complexes

Ligand	λ_{max}	ϵ_{max}	Magnetic Moment	Reference
	$m\mu$		*B.M.*	
Dipyridyl 1:1 complex	435	3×10^2	5.2	
Dipyridyl 3:1 complex	523	10^4	0.0	
Phenanthroline 1:1 complex	425	3×10^2	5.2	
Phenanthroline 3:1 complex	510	10^4	0.0	
Cyclohexanedione dioxime	464	3×10^3		
Cyclohexanedione dioxime + ammonia	541	10^4		
Cyclohexanedione dioxime + pyridine	531	10^4		
Picolinic acid	440	3×10^2	5.2	
Picolinic acid + cyanide	440	10^4		
Quinaldinic acid	515	3×10^2	5.2	
Quinaldinic acid + cyanide	515	10^4		
Cysteine + carbon monoxide	438	7×10^2		(19)
Glutathione + carbon monoxide	490	14×10^2		(44)

Note the change of absorption on six coördination with strong donor ligands. A similar change is found in porphyrin complexes (page 309). References to this table are given in reference 98.

tron excitations are very similar. The phenanthroline complexes are excellent models for the study of the porphyrin complexes.

The model systems (table 3) serve to illustrate a second point. In many ferrous complexes absorption is observed which is apparently too weak to be assigned to a fully allowed transition. There is good reason for believing that this absorption is due to an allowed transition, however, and that the transition is very similar to that of the strong bands such as those of the ferrous trisphenanthrolines. In order to explain this contradiction it is suggested that in the latter all the ferrous ions are in a diamagnetic state but that only a small percentage of the ions are diamagnetic in those complexes which have weaker absorption bands. In other words, there is an equilibrium between two states of the ferrous ion. Such an equilibrium can lead to the observation of all intermediate properties of complexes between those of the extreme diamagnetic and extreme paramagnetic forms (table 3). Amongst the ferrous complexes worthy of particular note in the table are those of cysteine and glutathione (see page 323). With this information in mind the ferrous porphyrin complexes will be discussed.

B. THE FERROUS PORPHYRIN COMPLEXES

The simple paramagnetic ferrous porphyrins have absorption spectra very like those of the zinc complexes (47). In hem itself, for example, the α-band is so weak that it is obscured by the β-band. The position of the α-band is further to the red than 575 mμ (47). In zinc protoporphyrin the α-band is at 586 mμ and is of a low intensity compared with that in the nickel complex. When it accepts as additional ligands either pyridine or ammonia the paramagnetic ferrous protoporphyrin becomes diamagnetic (67), the α-band shifts to 558 mμ, and its intensity becomes twice that of the β-band. The absorption spectrum of the ferrous complex is now like that of a nickel porphyrin (table 2). From the change in band position it can be said that the ferrous ion behaves more like nickel ion than like zinc ion, although its natural position in the normal state makes its properties elsewhere more similar to the latter element. The position has been discussed (38).

C. THE EFFECT OF FURTHER COÖRDINATION UPON THE ABSORPTION SPECTRA OF FERROPORPHYRIN COMPLEXES

The intensity of the α-band in the ferroporphyrins is, of course, dependent upon the porphyrin substituents, just as in the case of the cupric complexes (figure 3). For example, in the spectrum of the paramagnetic ferrous chlorocruoro-hem the α-band can be easily resolved (57), but it is not so intense in the spectrum of hem. The former porphyrin has a rhodofying formaldehyde substituent (23). In the proteins which contain these ferrous porphyrins, chlorocruorin and hemoglobin, respectively, the ferrous ion is considered to be bound by one further group, probably an imidazole, and a water molecule. The intensity and position of the bands are but slightly affected by this additional coördination; these hemoproteins also are paramagnetic (67). On the addition of oxygen, carbon monoxide, cyanide, pyridine, nitric oxide, phosphorus trifluoride (94),

TABLE 4

The absorption spectra of some ferrous protoporphyrin complexes

Additional Ligand	Maximum Absorption λ_{max}		Extinction Coefficient ϵ_{mM}	
	α	β	α	β
	$m\mu$	$m\mu$		
Carbon monoxide	573	542	14.6	15.1
Cyanide	565–570	537–540	10.5–11.0	14.0–15.0
Pyridine	558	525	31.0–35.0	16.2
Ammonia	553	522	34.0	<20.0
None	585?	555	~5.0	15.0

The data are to be found in reference 47. Note the effect of cyanide on the intensity of the α-band (see text).

and very many organic bases there is a change in paramagnetic moment and a notable increase in the absorption of the α-band, which moves together with the β-band to shorter wavelengths. Now, although these changes in absorption upon additional coördination are similar in general aspect, there are certain marked differences (table 4). A base such as ammonia or pyridine moves the absorption bands to much shorter wavelengths, ammonia more so than pyridine (33), and increases the intensity of the α-band to a much larger value than that found in the complexes of the small unsaturated molecules, oxygen, carbon monoxide, nitric oxide, and cyanide. From the parallel discussion of the position of the bands and the relative intensity of the α-band in the different metal complexes of one porphyrin (page 306) it is suggested that the differing effects of the further coördination of the ferrous ion arise from the influence of the small coördinating groups upon the *effective electronegativity* of the ferrous ion in its bonding with the porphyrin. This electronegativity, it must be remembered, is made up of two parts: the acceptor properties of the ferrous ion, σ-bonds, and its donor, π bonding. The importance of the different parts of the overall interaction can be illustrated by reference to the models again.

D. METALLOPORPHYRIN COMPLEXES WITH UNSATURATED SMALL MOLECULES

Although complexes of the ferrous ion with aromatic bases, in which the cation is in the diamagnetic state, have intense absorption bands in the visible region (page 308), the ferrocyanide complex, which is also diamagnetic, does not show such a spectrum. Similar differences have been observed between the spectra of diamagnetic nickel, ruthenous, and osmous cyanides and their complexes with aromatic ligands. The bond distances in these transition metal cyanides are indicative of strong double bonding in which the cyanide accepts d_ϵ electrons from the cation in the ground state of the complexes (65). Cyanide acts then to stabilize the promoted d_ϵ electrons but does not provide a higher π acceptor state to which the electrons can be excited.

It is now possible to correlate the difference in spectra between the simple cyanide and the aromatic imine complexes with the differences between the spectra of metal porphyrin cyanides and, for example, metal porphyrin ammines. Cobaltic porphyrin provides the simplest example (85). In the cobaltic porphyrin

ammine complex the d_ϵ electrons are strongly donated only to the porphyrin. The intensity of the α absorption band is relatively high, and the α- and β-bands are at short wavelengths. In the cyanide the intensity is lower and the bands are at longer wavelengths. The cobaltic ion has an apparently lower effective electronegativity with respect to the porphyrin in the cyanide complex. From the consideration of the simple cyanide complexes one may conclude that the reduced electronegativity is due to the withdrawal of electrons, d_ϵ, from the porphyrin by the cyanide. A very similar shift in absorption spectrum is found on exchanging water with cyanide in the sixth coördination position of vitamin B_{12} (43a). In all these complexes the cobaltic ion is diamagnetic and has all its d electrons in a *promoted* d_ϵ state.

It has already been noted that the absorption spectra of the cyanide complexes of the ferric (page 307) and ferrous (page 309) porphyrins were different from the complexes with saturated amines. In the ferrous and the ferric porphyrins the situation is more complicated than in the cobaltic complex, however. The hydrated ferrous and ferric porphyrins have paramagnetic moments, indicating that the d electrons are in states of maximum multiplicity. Combination with cyanide produces a promoted arrangement of the d electrons in both ferrous and ferric porphyrins and, in accord with expectation, the intensity of the α-band is increased while both α- and β-bands move to shorter wavelengths. Neither of these changes is as great as that brought about by unsaturated amines, and it may be said that the iron cations have a greater effective electronegativity relative to the porphyrin ligand in the porphyrin amine complexes than in the corresponding cyanides. That part of the electronegativity of the cation due to the donation of d_ϵ electrons is reduced by the acceptance of d_ϵ electrons by cyanide.

Molecules which coördinate in a similar manner to cyanide are carbon monoxide, oxygen, nitric oxide (64), and phosphorus trifluoride (94). The intermediate intensity and position of the absorption bands in the diamagnetic ferroporphyrin complexes with these ligands is now seen as a consequence of the ability of all the small molecules to form double bonds, stabilizing the $3d_\epsilon$ atomic electrons of the ferrous ion as in the cyanide. By noting both the intensity of the α-band and the position of the α- and β-bands in ferrous porphyrin compounds of uncertain structure therefore, a number of interesting inferences may be drawn about the state of the iron cation.

V. Some Properties of Hemoproteins

A. The Oxygen-Carrying Proteins

Concentrating attention on the α-band, the spectra of myoglobin complexes, for example, can be considered relative to those of hemoglobin. The two proteins have the same hem unit and there are good grounds for thinking that the fifth and sixth coördination positions are occupied by imidazole and a water molecule (100). Their pyridine hemochromogens both have an α-band at 558 mμ of about the same intensity. The oxygen, carbon monoxide, and cyanide complexes of hemoglobin, however, have more intense bands at shorter wave-

lengths than the corresponding compounds of myoglobin (22, 28, 84). It is only possible to explain these observations in the same terms as used above by postulating that the imidazole groups can interact with the ferrous ion more effectively in a hemoglobin than in myoglobin. Such a postulate has been frequently offered to explain other differences between hemoglobin and myoglobin, such as the relative acid dissociation constants of the protein imidazole (101) groups, and would also give an explanation of the different affinities for oxygen.

As the temperature of a hemoglobin solution is raised, the affinity of the protein for oxygen falls and the absorption bands of the oxygen complex move out to the red (4). The movement of the bands to the red indicates that the electronegativity of the ferrous ion has fallen to a lower effective value. This change cannot be due to a change in the porphyrin but can readily arise from a change with temperature of the distance between the iron atom and the imidazole nitrogen. An increase in this distance would be expected to weaken the interaction of the ferrous atom with oxygen. There is much independent evidence which suggests that the effective electronegativity of the ferrous ion is extremely sensitive to the bond distance between it and the coördinating nitrogen atoms of simple model ligands. The effect, which will now be described, can hardly be termed surprising, since it is well known that the degree of double-bonding in, for example, aromatic molecules is very dependent upon interatomic distance.

A good example of the effect under discussion is provided by a comparison between the ferrous complexes of phenanthroline and 2-methylphenanthroline. The 2-methylphenanthroline complex, as judged by its absorption spectrum, is *paramagnetic* (38). The formation of the tris complex can be considered as taking place in three steps, and the equilibrium constants for the separate steps have been determined (36). The values are shown in table 5, together with the constants for the same step equilibria in the formation of the trisphenanthroline complex. When statistical differences are taken into account, the constants for the three steps in the formation of the tris(2-methylphenanthroline) complex are seen to be equal. This result implies that there is little steric hindrance to the formation of the *paramagnetic* complex. Now the three steps in the formation of the trisphenanthroline complex are very unequal, the third being much greater than the other two. This complex is known to be *diamagnetic*. It follows that the methyl groups of 2-methylphenanthroline effectively prevent the formation of a *diamagnetic* complex by steric hindrance. The Fe(II)–nitrogen bonds must be shorter in the diamagnetic than in the paramagnetic complex. It is interesting to speculate about the interaction between oxygen and hemoglobin in this respect.

TABLE 5

The stability of ferrous phenanthroline and 2-methylphenanthroline complexes

Ligand	log K_1	log K_2	log K_3	Reference
Phenanthroline	5.8	5.3	10.0	(38)
2-Methylphenanthroline	4.2	3.6	3.0	(36)

The constants are the stability constants for the successive steps (7).

Hemoglobin picks up four molecules of oxygen in spectroscopically indistinguishable steps (70), but the affinity for oxygen is much greater in the last step than in the other three (70). The last step cannot be different from the others as far as the interaction of iron with oxygen is concerned if the theory of the absorption spectra developed above is accepted. The difference must arise from the interaction of the protein with the iron. On uptake of the oxygen by the first iron atom it can be supposed that the interaction of the cation with the protein imidazole is changed by the formation of stronger double bonds and that therefore the imidazole moves closer to the ferrous atom. If each of the first three steps in the uptake of oxygen involves an energy of rearrangement of the protein, then this will be reflected by an apparently low interaction with oxygen. Providing the three rearrangements so adjust the stereochemistry of the protein that a much smaller rearrangement is required in the final step, the affinity for oxygen in this step will appear far greater than in the earlier steps. Differences between hemoglobins from different animal species can now be thought of in terms of the different stereochemistry of the imidazoles of the proteins relative to the positions of the iron atoms. The changes in stereochemistry of the different proteins will produce differences in the effective electronegativity of the iron in the same hem.

B. THE UPTAKE OF CARBON MONOXIDE

It has been observed that there is a relationship between the relative position of the α-band in the oxygen and carbon monoxide complexes, the span, and the relative affinity of a given hemoglobin for these two molecules (4). According to the above theory of the absorption spectra the span represents a measure of the relative electronegativity of the ferrous ion in the two complexes. A difference in electronegativity between the carbon monoxide and the oxygen complexes can only imply a similar difference in the binding of these two molecules, all other factors being constant. The relationship observed between these quantities is thus given a simple explanation.

The carbon monoxide complexes of hemoproteins are dissociated by the absorption of light (93). Now the absorption of light by such complexes has been correlated with a transfer of electrons from the ferrous ion to the porphyrin. This electron shift will induce a shift of electrons from the carbon monoxide to the ferrous ion, for it is easily seen that the d_ϵ electrons which are donated to the carbon monoxide are identical with those donated to the porphyrin in the excited state. Such a shift of bonding electrons must lead to the weakening of the bond to carbon monoxide. The relationship between the absorption of light and the dissociation of the complex is thus connected with the disposition of the $3d_\epsilon$ electrons in the ground and excited spectroscopic states of the complex.

The uptake of cyanide by peroxidase, myoglobin (41), and vitamin B_{12} is also photochemically reversible. Here again the argument that the absorption spectra and the binding of small unsaturated molecules by hem proteins depend in part upon the properties of the d_ϵ electrons of the ferrous (or cobaltic) cation provides a starting basis for a more detailed theory of these two properties. On

the other hand the uptake of oxygen by hemoglobin is not light-sensitive. There is an important difference between the uptake of oxygen and that of the other small molecules. On combination with hemoglobin there is a change of spin multiplicity of the electrons of the oxygen molecule. The dissociation of oxyhemoglobin may well be a very different process from the dissociation of, for example, carbon monoxide hemoglobin.

Several other iron complexes of carbon monoxide are also photosensitive. Notable amongst these are iron carbonyl, in which carbon monoxide is recognized to be bound by double bonds from the short iron–carbon bond distance (64), the carbon monoxide complex of cytochrome a_3 (39), and the carbon monoxide complexes of ferrous cysteine and glutathione (18, 44). All the ferrous complexes have absorption bands which can be interpreted in terms of an increased electron transfer (of d_ϵ electrons) to the ligand in the excited spectroscopic state (table 3). The same mechanism of photodissociation is therefore applicable to them all. It seems reasonable to extend the explanation to cover the light-sensitive uptake of carbon monoxide by cobaltous isocysteine (19). Finally it would not seem a far cry to the suggestion that the pick-up of oxygen by a variety of ferrous, cobaltous, and cuprous complexes has the same basis in the partial transfer of electrons from the cation (97). The very similar absorption spectra of the complexes of these three cations do provide firm ground for this argument (98).

VI. Further Comments on Ferric Complexes

A. The Absorption Spectra of Ferric Porphyrins

Many *ionic* ferric complexes have an absorption band at $\lambda_{max} = 550$–650 mμ. This band has been associated with the rearrangement of the $3d$ electrons of the cation and a concomitant increase in interaction with the bonds of the ligand (98). The band appears in the ferric phenanthroline complexes but not in the complex $Fe_2(phenanthroline)_4(OH)_2$, which is not strongly colored and is covalent (24). The band is so similar to that at about 600–650 mμ in the "ionic" ferric porphyrins, and yet it is absent in the covalent hydroxy complexes of the latter, as to indicate that the bands in both sets of ionic complexes have the same origin (95). No other cation–porphyrin complex has an absorption band in this region.[1]

The state of the ferric ion in phenanthroline complexes can be further discussed. The intensity of the absorption band is $\epsilon_{max} \simeq 10^3$ but the magnetic moment of the complex is only 2.43 B.M. (24). Now in ferric complexes a paramagnetism of 2.0 B.M. corresponds to a fully covalent complex, as in

[1] The excitation of ferric electrons in a $d - d$ transition involves a change of spin and is nominally strongly forbidden by selection rules. The excited state of spin $\frac{3}{2}$ has one empty d-orbital. Presuming that this orbital is of the correct symmetry to interact with the excited triplet state of the ligand the molecular orbital of the excited state has a nominal spin value of $\frac{5}{2}$, the same as that of the ground state of the ferric ion. The absorption spectra of the ferric porphyrins are very different from the spectra of other metalloporphyrins, for an optical transition to the triplet state of the porphyrin is also spin forbidden.

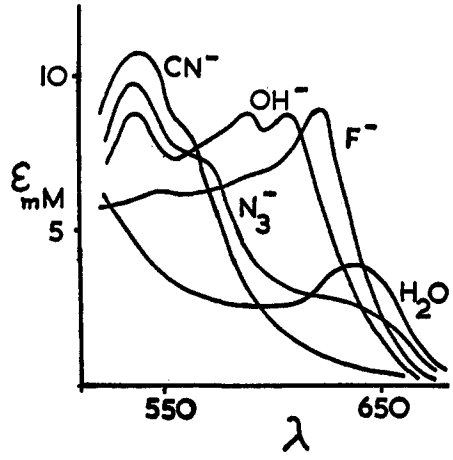


FIG. 4. The absorption spectra of some myoglobin complexes (27)

$Fe(CN)_6^{---}$, and such complexes have no strong absorption in the visible. On the other hand, the intensity of the ferric phenanthroline absorption is much lower than that expected of a six-coördinated ferric ion in a fully ionic complex (paramagnetic moment 5.9 B.M.) with such a strong donor ligand. In the ferric oxinate and acetyl acetonate for example, the intensity, ϵ_{max}, is about $10^{3.7}$, over five times as great. The acetyl acetonate is an "ionic" complex with a paramagnetic moment of 5.9 B.M. (13). It appears therefore that not all the ferric ion is in the "ionic" state in ferric phenanthroline, accounting for the low intensity of the absorption and the low magnetic moment. Turning to the porphyrin complexes these observations can be readily paralleled. In ferric hemoglobin or myoglobin there is a band of $\epsilon_{max} = 10^{3.3}$ at $\lambda_{max} = 640$ mμ (figure 4) and the complex has a paramagnetic moment of 5.80 B.M. This complex has been shown recently (28) to be other than simply "ionic." Now in the fluoride of this protein the paramagnetic moment is 5.94 B.M., the value for a fully ionic complex, and the intensity of the absorption band now at 625 mμ has increased to almost 10^4. The corresponding band in the hydroxides is less easily identified, but for reasons to be given below we suggest that it is at $\sim$595 mμ in ferric hemoglobin $\epsilon_{max} \simeq 10^3$, appearing only as an inflection on the band at $\sim$580 mμ, and at $\sim$605 mμ in myoglobin accounting for the *twin* peak where only a single peak appears in other myoglobin derivatives, e.g., the cyanide (table 5). In catalase, which has a paramagnetic moment of 5.98 B.M., the band at about 650 mμ is of the same intensity as that in the fluoride of methemoglobin. The fluoride of catalase has an absorption spectrum which differs but slightly from that of catalase itself and has the same paramagnetic moment. Catalase is a fully ionic ferric complex (table 6). Peroxidase and its fluoride are comparable with methemoglobin and its fluoride with regard to both paramagnetic moment and absorption spectra. The formation of azides and cyanides of all these proteins results in a fall of the absorption above 600 mμ or to its complete disappearance and a corresponding change in magnetic moment, proving that a more covalent ferric complex is

TABLE 6
The spectra of ferric porphyrin proteins

Protein	Ligand Other Than Porphyrin		λ_{max}	ϵ_{max}	Magnetic Moment
			mμ		*B.M.*
Hemoglobin	Imidazole	Fluoride	605	8.5	5.92
		Water	635	4.0	5.80
		Hydroxide	595	4.0	4.47
		Azide	625	1.0	2.84
		Cyanide	—	—	2.50
Peroxidase	Carboxylate(?)	Fluoride	615	7.0	5.90
		Water	645	3.0	5.48
		Hydroxide	595	?	2.66
		Azide	635	1.5	?
		Cyanide	—	—	2.67
Catalase	Carboxylate(?)	Fluoride	600	14.0	5.89
		Water	625	10.5	5.89
		Azide	620	12.0	5.86
		Cyanide	—	—	4.02
Myoglobin	Imidazole	Fluoride	615	7.0	5.90
		Water	640	4.0	5.85
		Hydroxide	605	?	5.11
		Azide	630	2.5	?
		Cyanide	—	—	2.50
Cytochrome *c*	Two nitrogen bases		No band		2.00

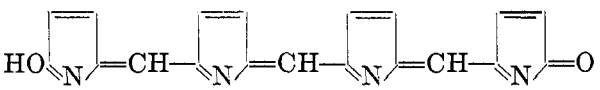
Spectrophotometric data from reference 40; magnetic data from reference 31.

formed (86). (The magnetic moment and absorption spectrum around 600 mμ of the azide of hemoglobin and peroxidase (table 6) are very similar to those of ferric phenanthroline.) It is seen that the fall in intensity of the 600–650 mμ bands together with the rise in intensity at 550–600 mμ in the spectra of the ferric complexes is to be associated with decreasing amounts of the ferric ion in the "ionic" state. The parallel between the phenanthroline and the hem complexes is extended to their oxidation–reduction potentials on page 320.

Another line of argument supports the idea that charge transfer to the ferric ion is responsible for the absorption band at 625 mμ. The position of this band has been studied in one ferric porphyrin protein bound by a series of additional ligands (table 6). No matter what the protein, the position of the band moves to shorter wavelengths along the series $H_2O > N_3^- > F^-$. This is the order in which these groups would be expected to oppose charge transfer to the ferric ion by electrostatic repulsion. Now in almost all respects the hydroxide ion is similar to the fluoride ion, although hydroxide ion is the better electron donor. The charge-transfer band in the hydroxide complexes should appear at a very similar wavelength to that observed in the fluoride. On examining the hydroxides of myoglobin and hemoglobin a weak band is found at about 595 mμ (28) (figure 4). It is suggested that this is the charge-transfer band obscured by the absorption due to a considerable percentage of the covalent form of the ferric ion in the hydroxide. The percentage of the ionic form in the hydroxide is about the same as that in the azide, as judged by the intensity of the charge-transfer band or by that of the β-band, $\lambda_{max} = 540$ mμ. On other grounds it is thought that the ferric ion in catalase is bound by a carboxylate anion, while the ion in hemoglobin is

bound by neutral molecules. It is satisfying that the long-wave band of hemiglobin (methemoglobin) is at longer wavelengths than that of catalase, for these enzymes contain the same porphyrin.

In passing, a brief comment on a reaction of hemiglobin is not out of place. The reaction has a close parallel in the reactions of ferric phenanthroline. Both complexes are attacked by molecular oxygen and in both cases the oxidation opens the rings of the ligands. *o*-Phenanthroline is oxidized to bipyridine-3,3′-dicarboxylic acid and hemiglobin is oxidized to a tetrapyrrole (48).

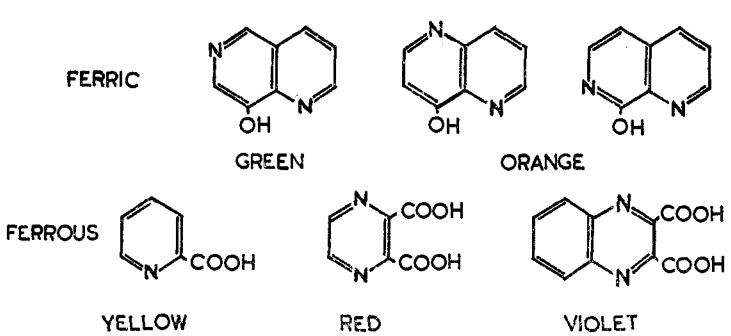


VII. Summary of Some Properties of Iron Complexes

It is useful at this stage of the review to stress two main conclusions which have been drawn from a study of properties of many models of the ferrous and ferric proteins and have been shown to be applicable to some iron porphyrin proteins themselves. The first proposes that certain properties of ferrous complexes are largely due to the donation of electrons to the ligand. The second proposes that some of the properties of ferric complexes are best described as arising from the acceptance of electrons from the ligand by the cation. A last example chosen from the models will be given to further illustrate these points, which the author considers to be of the utmost importance in the understanding of the function of iron in porphyrin–protein complexes.

Table 7 shows how the inclusion of electron-withdrawing nitrogen groups in aromatic chelating ligands alters the absorption spectra of the ferrous complexes of picolinic acid and of the ferric complexes of 8-hydroxyquinoline. The substitution shifts the ferrous bands to the blue, but the ferric bands to the red. Moreover, only when the substituent is ortho or para to the chelating nitrogen does it have this effect. It is concluded that the absorption spectra of the ferric complexes are at least in part charge-transfer bands in which the electron is donated to the cation and that the absorption spectra of the ferrous complexes are, at

TABLE 7



least in part, charge-transfer bands in which the electron is transferred to the ligand.

VIII. Properties of the Cytochromes as Compared with Other Hemoproteins

A. THE ABSORPTION SPECTRA

Table 8 includes data on the spectra and paramagnetism of many hemoproteins and of their complexes. There are two characteristic groups of these proteins. In the first are the oxygen-carrying proteins, hemoglobin, myoglobin, and chlorocruorin. The absorption spectra of these proteins differ very considerably from those of their pyridine complexes, hemochromogens. In this they are like the synthetic ferrous mesoporphyrin and coproporphyrin complexes of globin (21). On the other hand, there is hardly any change either in position or intensity of the absorption bands on going from the parent protein to the pyridine complex of cytochromes *b* and *c*. Cytochrome *b* and hemoglobin contain the same porphyrin, and the differences between the two proteins are known therefore to be due to the mode of further coördination of the ferrous ion. Iron in cytochrome *b*, and for that matter in cytochrome *c*, is most probably bound by two further nitrogen atoms (63) apart from the four nitrogens of the porphyrin, whereas in hemoglobin, and in all probability in all the other oxygen-carrying proteins, the iron is coördinated to only one further nitrogen. The two cytochromes have spectra and magnetic moments very similar to the pyridine hemochromogens of the oxygen-carrying proteins because the cytochromes are struc-

TABLE 8

The absorption spectra of ferrous porphyrin proteins

Protein	Further Ligand	α-Band		β-Band		Reference
		λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}	
		mμ		mμ		
Hemoglobin	Water	590	5	550	13	(84)
	Nitric oxide	575	13	545	12	
	Oxygen	577	15	541	14	
	Carbon monoxide	570	14	539	14	
	Isocyanides	570	11	542	13	
	Cyanide	562	10	533	15	
	Pyridine	558	30	525	16	
Cytochrome *b*		558	29	528	16	
Cytochrome *c*		550	29	520	15	(21)
Myoglobin	Water	?	?	555	15	(22)
	Oxygen	580	15	546	15	
	Carbon monoxide	580	13	548	15	
	Pyridine	558	30	525	15	
Chlorocruorin	Water	605?	12	565	12	(57)
	Oxygen	604	24	560	12	
	Carbon monoxide	600		557		(15)
	Pyridine	583?	25–30	533	12	
Cytochrome a_3	Water	605	High	560–570	Low	(15)
	Carbon monoxide	591	High		Low	(39)
	Pyridine	587	High		Low	
Cytochrome *a*	Water	605	High		Low	(23)
	Pyridine	587	High		Low	

turally very similar to the hemochromogens. In so far as the spectra and magnetism of the proteins reflect the electronegativity of the ferrous cation, it may be said that in the cytochromes iron is more electronegative than in the oxygen-carrying proteins.

The intensity of the α-band in the carbon monoxide, oxygen, nitric oxide, and cyanide complexes of the oxygen-carrying proteins is considerably greater than in the parent protein. The position of the band is intermediate between that of the protein and that of its pyridine hemochromogen, although nearer to that in the parent protein (table 8). On addition of such ligands to either of the two cytochromes the position of the α-band is but slightly affected, but the intensity of the band is considerably lowered. Whereas the two cytochromes have a very low affinity for carbon monoxide (or for oxygen), the affinity of hemoglobin for these molecules is large. These differences again reflect differences in the electronegativity of the ferrous ion in the original proteins. The ferric complexes of cytochromes *b* and *c* are covalent with no absorption band beyond 600 mμ, while the other groups of hemoproteins have ionic ferric complexes on the basis of either spectrophotometric or magnetic evidence (page 315). It is seen once more that there are two distinguishable groups of hemoproteins: the cytochromes *b* and *c* and the oxygen-carrying proteins.

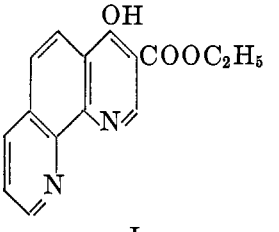
Turning to the cytochrome *a* group, in which the porphyrin has strong electron-attracting substituents (69), it is found that the shift of λ_{max} from 604 mμ in the proteins *a* and a_3 to 589 mμ in the pyridine hemochromogens is 15 mμ, a shift intermediate between that found in the oxygen-carrying proteins and that in the cytochromes *b* and *c* (15). From a comparison with chlorocruorin, another protein containing a porphyrin substituted with strong electron-attracting groups, a larger shift would have been expected. This difference would suggest that the ferrous ion in the cytochromes *a* and a_3 is further bound to strong basic groups, making it more electronegative. The position of the α-band in the carbon monoxide complex of cytochrome a_3 is 591 mμ, which is very close to that of its hemochromogen at 589 mμ, as in cytochrome *c*, although it is intermediate between this wavelength and that of the α-band in the parent cytochrome a_3, as in hemoglobin. The ferric cytochrome a_3 has a *weak* absorption band at 640 mμ which is clearly the consequence of ionic bonding, as is that in an oxygen-carrying protein, but this band is absent in ferric cytochrome *a* as in the other cytochromes. The combination of cytochrome *a* with cyanide results in the reduction of the intensity of the α-band (6), as in cytochrome *c*. The position of the band in the carbon monoxide, nitric oxide, and cyanide complexes of cytochrome *a* is at a slightly longer wavelength than in the protein itself (6). Cytochrome *a* is obviously more closely related to the other cytochromes than is cytochrome a_3. Cytochrome a_3 has an intermediate affinity for carbon monoxide. The properties of the group *a* cytochromes as a whole then suggest that the ferrous cation in these proteins is held by groups which impart an intermediate electronegativity upon the cation between that in the other cytochromes and that in the oxygen-carrying proteins. The oxidation–reduction potentials of the different proteins add further support to this argument.

B. THE OXIDATION–REDUCTION POTENTIALS OF HEMOPROTEINS

1. The potentials of iron–phenanthroline complexes

The problem of the reversible potentials of the ferrous–ferric couple in hemoproteins is best approached through a discussion of models. The first model systems to be examined are the phenanthroline complexes. Smith and his collaborators (10) have measured the potentials of very many substituted phenanthroline–iron complexes. The most electrophilic substituents, such as the nitro groups, give complexes with the lowest oxidation potential, e.g., −1.29 v. in the complex of 5-nitrophenanthroline. This is the weakest base in the series of these ligands (9). The ferrous complex is diamagnetic and the ferric complex only partly ionic, as judged by its absorption spectrum. The most basic of the phenanthrolines so far studied contains four methyl substituents and has a redox potential of −0.85 v. Thus the potentials rise toward more positive values as the basicity increases and as the stability of the ferrous and ferric complexes increases (9). Unfortunately there are no substituted phenanthrolines yet available of known lower basicity than that of the 5-nitro compound, but it must be expected that such compounds would yield iron complexes with a *lower* oxidation–reduction potential than the complexes of the 5-nitrophenanthroline. A plot of basicity against redox potential must finally go through the point for the ferrous–ferric hydrate couple, −0.77 v., so that a minimum in the plot must be observable. The factors which will be important in determining the potentials of ligands of lower basicity can be illustrated qualitatively despite the absence of quantitative information.

Mellon (30) synthesized the phenanthroline I and examined the reversible potential of the iron complex. He found a value of −0.71 v. The basicity of this ligand must be lower than that of phenanthroline on account of the electrophilic substituents. Now it was also observed that the ferrous complex was a pale pink in color and not the deep red of other ferrous phenanthrolines. This observation strongly suggests that the ferrous complex is paramagnetic, in which case the stabilization due to covalency (see table 3) is lost and it is not surprising that the ferrous complex is only of the same stability as the ferric complex. The redox potential is therefore low. A second example of a similar kind has already been mentioned (page 312) and will now be discussed in more detail.



I

2-Methylphenanthroline forms a 3:1 ferrous complex which is thought to be paramagnetic on account of its low stability and the absence of intense absorp-

tion in its spectrum (38). Its stability is less by about 10 kcal. than that of the corresponding trisphenanthroline complex. The greater part of this difference arises from the relative size of K_3, the formation constant of the last step, which is very large for the phenanthroline complex but not for that of 2-methylphenanthroline. On the addition of the third phenanthroline molecule the ferrous complex becomes diamagnetic. Recently Tomkinson[2] measured the oxidation–reduction potential of the 2-methylphenanthroline–iron complex and obtained a value close to that of the simple iron couple (hydrated ions), -0.7 v. The loss of the stability due to the formation of a paramagnetic complex has reduced the overall stability of the ferrous complex to that of the ferric, in agreement with the comments made above.

A slightly more complicated ligand, tetrapyridyl (or 6,6′-di-2-pyridyl-2,2′-bipyridine), also forms but a feebly colored ferrous complex (56), which is here assumed to be paramagnetic (table 3). As in the case of the complexes of I and of 2-methylphenanthroline the reversible potential of the tetrapyridyl complex is close to -0.7 v. (26). There are now three cases of ferrous imine complexes with low oxidation potentials which are weakly colored. They lend support to the suggested relationship between ligand basicity and redox potential.

2. The potentials of iron–porphyrin complexes

Martell and Calvin (54) have pointed out that as the substituents in the pyrrole rings of a porphyrin are made more electrophilic, the reversible potential of the iron complexes rises. These measurements were made on covalent ferrous (diamagnetic) and ferric complexes in pyridine. This change is strictly parallel to that observed by Smith in the phenanthroline complexes (page 320). If instead of changing the porphyrin, the ligands binding the fifth and sixth coördination positions of the iron in the porphyrin complex are altered in basicity, then it has been found that the potential of the complexes decreases to more negative values at first, e.g., with pyridine and α-picoline (16), but then rises again on further increase in ligand basicity, e.g., with pilocarpine and imidazoles (18, 54). The ferrous and ferric complexes of the porphyrins themselves are ionic, but both are covalent in the pilocarpine complexes (18). In the case of the ferric complexes covalency may also be presumed from the fact that the second ligand is added with a greater affinity than the first (18). The minimum in a plot of basicity of ligand against redox potential which can be observed in this series of complexes is parallel to that postulated in the phenanthroline complexes. The simple porphyrin complexes also serve to illustrate again the importance of steric hindrance in the formation of octahedral complexes (page 312). No complexes have been found between ferric porphyrins and 2-methylimidazoles (18).

3. The potentials of hemoproteins

In hem itself both ferrous and ferric ions are ionic and the redox potential is about $+0.2$ v. The addition of even a weak base such as the imidazole of hemo-

[2] To be submitted for publication.

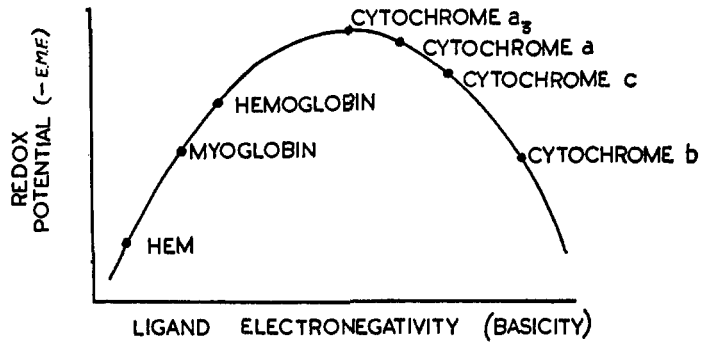


FIG. 5. The proposed relationship between the oxidation–reduction potential of iron porphyrin proteins and the electronegativity (basicity) of the ligand. The relationship is schematic, and the electronegativity of the ligand is dependent upon the properties of the porphyrin as well as those of the protein. Similar relationships are found in the phenanthroline and simple porphyrin iron complexes.

globin or myoglobin proteins shifts the potentials to more negative values without a change in the magnetic condition of the iron in either state. Addition of further neutral and *weakly basic* molecules such as carbon monoxide and oxygen to these proteins further stabilizes ferrous ion with respect to ferric and the redox potential reaches values around −0.25 v. The ferrous ion is now covalent, but the ferric ion remains "ionic." The binding of the iron by still stronger bases brings about the formation of covalent ferrous and ferric compounds, and in the cytochromes *c* and *b* the redox potential rises from −0.25 to +0.04 v. The redox potentials clearly go through a minimum as the donor character of the ligands is increased. Previously (page 319), on general grounds it has been found that cytochromes a_3 and *a* were intermediate between the oxygen-carrying and the electron-transporting enzymes. The oxidation potentials of the *a* cytochromes conform with this suggestion. Their values fall close to −0.3 v. Figure 5 illustrates the proposed relationship between oxidation–reduction potentials in the ferrous–ferric porphyrin protein and the average ligand basicity, where the latter takes into account the donor character both of the porphyrin and of the other coördinating bases. The proteins which fall before the minimum are characterized by (*1*) their ability to carry oxygen, (*2*) their ionic ferric complexes, and (*3*) the relatively long wavelength and small intensity of the absorption in the ferrous complex. The proteins which occur after the minimum have (*1*) covalent ferric complexes, as shown by magnetic moment and absorption spectrum, (*2*) covalent ferrous complexes, and (*3*) absorption maxima in the ferrous complex at a relatively short wavelength and of relatively great intensity.

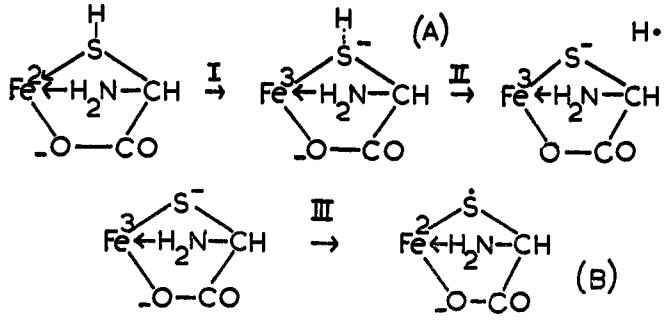
It is reasonable to ask why these differences cause such marked differences in the reactivity of the proteins.

C. POSSIBLE MECHANISM OF REACTIONS OF CYTOCHROMES

It is not entirely out of place in a discussion of the reactions of cytochromes to refer to the catalysis by iron and cysteine of certain reactions. Ferrous cysteine

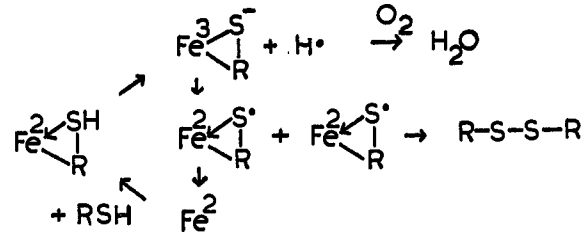
has an absorption band at around 500 mμ which can be attributed to partial charge transfer to the ligand from the cation, a band which can therefore be likened to the bands in porphyrin complexes (page 314). The excitation can be looked upon as the change, I, and the formation of a ferric complex as the further step, II.

The hydrogen atom on the sulfur is activated by the migration of charge to the sulfur. Now ferric cysteine also has a strong absorption around 550 mμ which is undoubtedly due to charge transfer in the opposite direction, III.

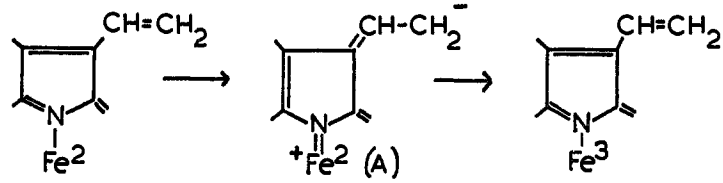


The sulfur is now in a position to receive a hydrogen radical from another group. The iron–cysteine system can clearly act as an oxidation–reduction catalyst by aiding in the transfer of hydrogen. The complexes A and B need only be visualized as activated states, of course.

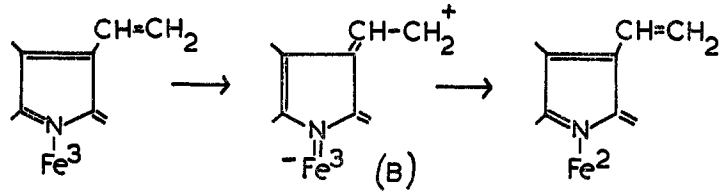
The further reactions in which iron cysteine plays a part become of considerable interest when it is remembered that transfer of hydrogen is equivalent to electron transfer, a property of the cytochromes. There is of course the simple reaction to cystine—an oxidation by molecular oxygen catalyzed by iron.



This reaction is further catalyzed by hemin, a catalysis which is reduced by carbon monoxide and increased by nicotine and pyridine. Now it has already been shown that in carbon monoxide complexes of hems the d_ϵ electrons are withdrawn from the iron, but in the nicotine and pyridine hemochromogens the d_ϵ electrons of ferrous ion are donated more strongly to the porphyrin. The catalysis is seen to depend upon the donation of electrons from ferrous ion to the porphyrin. It is possible to consider the reaction in the following way:



The $-CH_2^{\ominus}$ group acts as an electron donor to oxygen, giving O_2^-. Now the catalyst, ferric hem, reacts with ferrous cysteine, reverting to ferrous hem.



This type of reaction scheme is applicable to the reactions of cytochrome a_3, acting as the autoxidizable group in the electron transport to the other cytochromes, for it has a porphyrin which should be a particularly strong electron acceptor (stage A) from ferrous ion and its ferric form is at least partly ionic so that, through charge transfer, the central cation can act as an electron acceptor (stage B). Hemoglobin will not act as so good an electron-transporting catalyst, since the *paramagnetic* ferrous iron in hemoglobin is a weak electron donor (stage A), except when the hemochromogen is made by complex formation, with ammonia for example, which converts ferrous to the diamagnetic state. In the latter situation the state of the cation is no longer typical of hemoglobin but is now similar to that in cytochrome *b*, which does act as an electron-transporting protein (page 318).

It has already been seen that carbon monoxide reduces the catalytic effect of hemin in the reaction of cysteine. Now oxygen is a similar molecule to carbon monoxide, as far as coördination is concerned (page 310). Thus when oxygen adds to the iron of hemoglobin it converts the ferrous iron into a more electronegative diamagnetic cation but it does not permit strong donation of d_ϵ electrons from the cation to the porphyrin. The oxygen interacts with the d_ϵ electrons itself and thereby, like carbon monoxide, *prevents oxidation. The oxygen protects hemoglobin from oxidation* by taking the d_ϵ electrons to itself. If this view is accepted, then we might suspect that oxygen does not react directly with the iron of cytochrome a_3. The ferrous atom may be sufficiently electronegative in this complex to permit electron removal from the peripheral atoms of the porphyrin.

Some of the differences in reactivity between the oxygen-carrying and the electron-transporting hemoproteins are now beginning to correlate with physical properties of these molecules. There are of course many questions left unanswered by this analysis. To mention but one: What is the carrier which transports electrons from cytochrome to cytochrome? Is it the hydrogen radical?

Instead of attempting an answer to such a question attention will be drawn to some provoking parallels with other metalloenzymes.

IX. Some Other Enzymes Not Containing Iron

A. COPPER ENZYMES

It has already been indicated that iron–cysteine complexes can act as electron-transporting groups to oxygen. Substitution of cuprous ion for ferrous and cupric ion for ferric gives a similar parallel scheme for the copper-containing oxidases. If the cuprous complex picks up oxygen however, forming a complex with it, then the charge transfer of electrons from cuprous ion to the sulfur of the cysteine could be prevented. The oxygen acts, it is postulated, as the electron acceptor. It may well be that the relationship between the basicity of the sulfur and the redox potential of the copper complex has similar features to that drawn out in figure 5. Two types of cupric complex have been discussed in the literature, one covalent and the other ionic, which might well be a parallel for the two types of ferric complex. The oxygen-carrying proteins have the less basic sulfur and have "ionic" cupric complexes, while the oxidases have "covalent" cupric–sulfur links. Before accepting the idea, much experimental work remains to be done.

Other parallels are not hard to find. The cobalt–cysteine complexes are remarkably like those of iron. The cobaltous complex reacts with carbon monoxide and shows an absorption spectrum not unlike that of the ferrous cysteine complex with carbon monoxide (19). Although there are examples of oxygen-transporting cobalt complexes, the corresponding oxidase activity has not been discovered. All the parallels between cobaltous, ferrous, and cuprous complexes suggest that they arise largely from their readiness to donate d_ϵ electrons to the ligands. On the other hand, while charge-transfer absorption bands of cupric and ferric complexes in the visible have been observed, no corresponding bands of the cobaltic complexes are known. It is dangerous to conclude that the absence of such bands indicates that oxidase activity is impossible, for there are two "oxidase" systems in which the function of the cation is other than that described so far. The first is the hydrogenation catalysis by cuprous ion which has been studied by Calvin (11); the second is the recent discovery of the zinc enzymes.

B. SOME OTHER COPPER- AND ZINC-CONTAINING SYSTEMS

There has been an assumption in part of this discussion that the role of cation complexes in oxidation–reduction reactions is to go through oxidation–reduction cycles. Objections to this theory have become strengthened by the discovery of zinc in such enzymes as alcohol dehydrogenase and glutamic acid dehydrogenase (89, 90). These enzymes, plus the coenzyme diphosphopyridine nucleotide (DPN), oxidize their substrates, alcohol and glutamic acid. Zinc, which is essential for activity, plays a part in the reaction, but it clearly cannot act as a reversibly oxidizable or reducible cation. How then does it act? Undoubtedly it brings together the enzymes and the coenzymes and it probably activates the

coenzyme at the same time. A model can be built showing how a cation acting as a Lewis acid can activate an oxidation–reduction (91). The function of copper and iron in their reactions can be similar, and it is unwise to postulate valency changes unless there is some evidence to show that such changes are occurring. In the author's opinion there is but slight support for iron(IV) complexes in the reaction of hemoproteins with hydrogen peroxide (25). If a further case is to be quoted, then the study of the catalytic hydrogenation of quinone in the presence of cuprous quinoline acetate is one of the best. In this reaction there is no evidence at all for valency changes of the copper ion. It would seem more probable that this reaction is to be classed with the hydrogenations brought about by diphosphopyridine nucleotide, hydrogen being taken up by the aromatic heterocyclic bases (11).

The author wishes to point out that the basic ideas concerning charge transfer in complex ions were developed jointly with Dr. L. E. Orgel. For the particular expression and interpretation of these ideas, however, the author alone is responsible (98). The author wishes to thank Prof. R. Nyholm, Dr. J. E. Falk, and Dr. L. E. Orgel for reading the manuscript and for the many helpful suggestions which have found their way into the text.

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