The redox properties of metalloporphyrins have been studied extensively, and particular attention has been paid to the changes in oxidation states of the metal. In general, the most stable metalloporphyrins are those in which the metal is in the +2 oxidation state. However, most of the transition metalloporphyrins show a variety of oxidation states. Thus Co(II) porphyrins can, like the corresponding cobalt-containing vitamin B₁₂, be oxidized to the Co(III), or be reduced to the Co(I) complex; and like the Co(I) containing vitamin B₁₂ (B₁₂), the Co(I) porphyrin is nucleophilic and undergoes oxidative addition with alkyl halides to give the corresponding alkyl cobalt porphyrins. Mn porphyrins show +2, +3, and +4 oxidation states, and it has recently been shown that Pb(II) porphyrins can be oxidized to Pb(IV) while the more stable Sn(IV) can be reduced to Sn(II) systems. A particularly striking example of the stability of the divalent complexes is that of the Ag(II) porphyrins. Few examples of divalent silver complexes are known, but the planar tetradentate porphyrin ligand stabilized this unusual oxidation state which can, however, be oxidized to the trivalent state.

Although the redox properties of metalloporphyrins are fascinating and important in their own right, the initial focus and interest in the oxidation states of these systems stems from the redox properties of the cytochromes (which are enzymes containing iron porphyrins, and function catalytically via the Fe(II) ± Fe(III) couple) and the function of hemoglobin (an Fe(II) porphyrin which, unlike simple Fe(II) porphyrins, is not oxidized by oxygen to Fe(III) but reversibly binds oxygen at the Fe(II) oxidation level) as well as from the incompletely determined role of valence changes of iron in the peroxidase/catalase class of heme enzymes. The central role of the iron in these naturally occurring systems and the considerable efforts that have been expended on elucidating the roles of metals in these and other metalloporphyrins resulted in the widely held assumption that the macrocyclic porphyrin ligand serves merely to modify the redox potentials of the metals, and to act as a convenient bridge between the metal and the protein—an assumption which is far from true.
The chemistry of porphyrins and metalloporphyrins is, like that of most stable aromatic systems, somewhat limited. Electrophilic attack at both the β- and meso positions is facile, and it will take place with both metallo- and metal-free porphyrins provided that the metal-free porphyrin is not deactivated by N-protonation under the acidic conditions normally employed for electrophilic substitution. Nucleophilic attack at the neutral porphyrin nucleus is unknown, while a variety of oxidized porphyrins result from reactions with one-electron oxidants. Thus, the oxidation of metal-free porphyrins with lead tetraacetate gives meso-tetraoxoporphyrins (xanthoporphyrinogens), whereas oxidation with hydrogen peroxide in sulfuric acid gives an oxychlorin (FIGURE 1).

![FIGURE 1. Conversion of a porphyrin to an oxychlorin with hydrogen peroxide and sulfuric acid.](image)

Of biochemical importance is the oxidation of heme to the bile pigments that is thought to proceed by the intermediacy of a meso-hydroxyporphyrin. Although the biochemical mechanism is not yet known, it is of interest in that it has recently been shown that meso-hydroxylation can be achieved in high yield by the hydrogen peroxide oxidation of Fe(II), but not Fe(III), porphyrins, and that such meso-hydroxy iron porphyrins can be converted to bile pigments by oxygen.

In many of the above redox reactions, it has been generally assumed that the initial electron transfer occurs at the metal. We will show, however, that transfer of electrons to or from the π-system of the porphyrin ring is a facile process and may explain much of the redox chemistry and biochemistry of metalloporphyrins.

Polarographic studies led Stanienda to propose that metalloporphyrins and chlorophylls could be oxidized by two successive one-electron oxidations, and he suggested that the products were cations in which the positive charge was localized on nitrogen. Fuhrhop and Mauzerall oxidized MgOEP with iodine-methanol and showed this to be a reversible one-electron oxidation. The oxidation was only observed in methanol, ethanol and, to some extent, in n-propanol and these observations, coupled with the optical spectra, led to the suggestion that this stable, green oxidized product could be formulated as a phlorin derivative resulting from the attack of methanol or other nucleophilic solvents on the cation radical. The similarity between the optical spectrum of the oxidized MgOEP and those of chromophores such as the phlorins and bilatrienes supported this hypothesis. We had also expected interaction between solvent or ambient nucleophiles with highly oxidized metalloporphyrins, and in order to minimize such reactions we chose to bring about our oxidations by controlled potential bulk electrolyses, using dry methylene dichloride as solvent and tetraalkylammonium perchlorates as supporting electrolytes; tetraalkylammonium trifluoromethanesulfonates can also be used as supporting electrolytes. The trifluoromethanesulfonate ion is an even weaker nucleophile and poorer ligand than perchlorate and does not exhibit the oxidizing powers of perchlorate.
Electrochemical oxidation of magnesium octaethylporphyrin (Mg(II)OEP) proceeds to completion at 0.6 V vs. s.c.e., and simultaneous coulometry showed that 1.0 (±0.1) electrons/mole were removed. More than 99% of the initial metalloporphyrin was recovered by reduction at 0.0 volts (mild reducing agents such as KI also gave a quantitative reduction). The smooth course of the electrolysis was indicated by the well-defined isosbestic points in the absorption spectra that were measured at various stages of the oxidation (Figure 2). However, the optical absorption spectrum of the fully oxidized product resulting from the one-electron oxidation of Mg(II)OEP under these conditions was identical to the spectrum reported for what had been believed to be a phlorin derivative. Clearly, however, under the conditions of our electrolytic oxidations, quenching of an oxidized species by a nucleophile is unlikely. Moreover, metalloporphyrins are 18 π-electron aromatic systems, and the removal of an electron from the π-cloud, leaving an unpaired electron delocalized over the porphyrin macrocycle, should be a facile process, and a π-cation radical so generated could, as a result of the considerable delocalization, be stable.

![Figure 2. Oxidation of MgOEP in CH₂Cl₂. The solid line is the absorption spectrum of Mg(II)OEP, the broken line that of [Mg(II)OEP]⁺ · ClO₄⁻.](image)

The ESR spectrum (in CH₂Cl₂) of the oxidized Mg(II)OEP showed only a single line, at $g = 2.0025$, with a width of 2.5 G (peak to peak). As the solution cooled, the ESR signal diminished in intensity and, at the same time, the original blue-green solution turned red. In methanol, the optical spectrum remained unchanged even at $-50^\circ$, but the ESR spectrum was now partially resolved into 5 lines while, under the same conditions, the oxidized Mg(II)OEP-d₆ (deuterated at the meso positions) exhibited only a narrow singlet. Hence, the five-line spectrum can be assigned to a porphyrin π-cation radical where the remaining delocalized, unpaired electron couples strongly with four equivalent meso-protons. The fact that the four meso-protons in the oxidized species are equivalent excludes any possibility of meso-substitution by a nucleophile and characterizes this product as a simple π-cation.
radical. We pointed out earlier that upon cooling methylene dichloride solutions of [Mg(II)OEP]$^{+}$ ClO$_4^-$, the room temperature ESR signal disappeared and the color of the solution changed from blue-green to red (FIGURE 3). Experiments over a range of concentrations from 1-5 × 10$^{-4}$ M porphyrin gave an equilibrium constant of 4.7 (±0.3) × 10$^3$ liters/mole for the formation of a dimer at 0° in methylene dichloride.$^{22}$ No ESR signal at g = 2 or at half-field (as might be expected for a triplet-like, closely coupled diradical) was found for the red dimeric species, and Mauzerall$^{13}$ reported that the analogous dimeric zinc complex is diamagnetic and shows only one NMR signal for the meso-protons (suggesting that they are all equivalent). He suggested further that the equivalence of the meso-protons requires a face-to-face dimerization, so that electrons are paired in a molecular orbital extending over both rings. It should be noted, however, that the optical spectra of these dimers (FIGURES 3, 4) are somewhat similar to those of isoporphyrins (in which the porphyrin conjugation is broken at a meso-carbon atom), and this suggests a second formulation for these dimers in which a new bond is formed between two meso-carbon atoms. However, such a structure would have to be in rapid dynamic equilibrium with the monomer in order to account for the observed equivalence of the meso-protons.$^{13}$

This facile dimerization is not limited to magnesium porphyrins. Indeed, the published spectrum of [Zn(II)OEP]$^{+}$ ClO$_4^-$ prepared by the ferric-perchlorate oxidation$^{24}$ of Zn(II)OEP shows that even at room temperature this species is partially dimerized in methanol. On the other hand, none of the corresponding [Zn(OEP)]$^{+}$ ClO$_4^-$ exists as the dimer in methylene dichloride containing tetrapropylammonium perchlorate (FIGURE 4), and the extent to which dimerization occurs for a given species is a function of both solvent and counter ion.

The reversible one-electron oxidation of magnesium tetraphenylporphyrin, Mg(II)TPP, gives rise to an analogous π-cation radical but, in this case, the ESR spectrum shows$^{28}$ that the unpaired electron is coupled to four equivalent nitrogen atoms and eight equivalent hydrogen atoms (these eight hydrogen atoms are the eight ortho-protons of the four phenyl rings, rather than the porphyrin β-protons). Moreover, not only is the ESR spectrum of the TPP complex considerably different from that of the OEP complex, but the optical spectra for the π-cation radical of Mg(II)TPP (FIGURE 5) are quite unlike those exhibited by the [Mg(II)OEP]$^+$. 

![Figure 3. Optical absorption spectrum of the [Mg(II)OEP]$^+ \cdot$ dimer in CH$_2$Cl$_2$ at -60°.](image-url)
We have carried out self-consistent field molecular orbital calculations for porphyrin cation radicals$^{22}$ and find that such species may be described by either of two closely lying electronic ground states, a $^2A_{1u}$ or a $^2A_{2u}$ (calculations show that these ground states differ in energy by about 3,000 cm$^{-1}$). The $^2A_{2u}$ ground state is characterized by spin density on the meso-carbon atoms and at nitrogen, whereas the $^2A_{1u}$ state shows a small spin density at the meso positions and none at nitrogen. Hence, the $^2A_{2u}$ state will give rise to an ESR spectrum showing nitrogen hyperfine coupling, whereas the $^2A_{1u}$ will exhibit an ESR spectrum showing no nitrogen...
hyperfine coupling. This is exactly the situation we see with \([\text{Mg(II)TPP}]^+\), and \([\text{Mg(II)OEP}]^+\), respectively. Moreover, the optical spectrum we calculate for the former ground state corresponds closely to that we observe for \([\text{Mg(II)TPP}]^+\). Both the optical and the ESR spectra for \([\text{Mg(II)OEP}]^+\) are consistent with a \(2A_{1u}\) ground state, while those for \([\text{Mg(II)OEP}]^+\) suggest a \(2A_{2u}\) ground state. Our calculations show that the difference in energy between these two ground states is small, and this suggests that small changes in structure between one metalloporphyrin and another should be sufficient to determine which ground state will be occupied.

We have already seen that \([\text{Mg(II)OEP}]^+\) occupies a \(2A_{1u}\) state while \([\text{Mg(II)TPP}]^+\) occupies a \(1A_{2u}\) state. Not all oxidized metalloptetraphenylporphyrins exhibit a ground state formed by oxidation of a ring \(a_{2u}\) orbital, nor do all oxidized octaethylporphyrins exhibit a ground state formed by oxidation of an \(a_{1u}\) orbital (FIGURE 6). Nor does the change from TPP to OEP for a specific metal necessarily change the ground state as it did for magnesium, because both \(\text{Cu(II)OEP}\) and \(\text{Cu(II)TPP}\) exhibit, in their oxidized states, spectra characteristic of electron abstraction from an \(a_{3u}\) orbital (FIGURE 7). In this instance, exchange coupling between the hole on \(d^9\) \(\text{Cu(II)}\) and the hole in the porphyrin \(a_{3u}\) orbital results in a singlet ground state for \([\text{Cu(II)TPP}]^+\).

So far, the oxidation of a specific metalloporphyrin results in a \(\pi\)-cation radical exhibiting one of two ground states, and the question arises as to whether a cation

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**FIGURE 6.** The optical absorption spectra in \(\text{CH}_2\text{Cl}_2\) of \(\text{upper}\) \(\text{Ni(II)TPP}\) (solid line) and \([\text{Ni(II)TPP}]^+ \cdot \text{ClO}_4^-(\text{broken line}); and \(\text{lower}\) \(\text{Pd(II)OEP}\) (solid line) and \([\text{Pd(II)OEP}]^+ \cdot \text{ClO}_4^-\) (broken line).
radical in one ground state can be made to occupy the other closely lying ground state. The electrochemical oxidation\textsuperscript{32} of Co(II)OEP in methylene dichloride containing tetra-\textit{n}-propyl ammonium perchlorate as supporting electrolyte, at 0.77V vs. s.c.e., brings about a reversible one-electron oxidation to give [Co(III)OEP]\textsuperscript{+} ClO\textsubscript{4}\textsuperscript{-}, while continued electrolysis at 0.97V results in the removal of a second electron to give a green solution of the \pi-cation radical [Co(II)OEP]\textsuperscript{2+} 2ClO\textsubscript{4}\textsuperscript{-} (FIGURE 8). This new species requires two electrons/mole for a complete and quantitative reduction to the starting cobaltous octaethylporphyrin. These formulations are supported by the conversion of equimolar amounts of the cobaltic \pi-cation radical and cobaltous porphyrin to two moles of the diamagnetic cobaltic porphyrin.

\[
\text{Co(II)OEP} + \text{[Co(II)OEP]\textsuperscript{+}}} 
\rightarrow 2\text{[Co(II)OEP]\textsuperscript{+}}
\]

The formulation of the cobaltic porphyrin as a diamagnetic low-spin Co(III) is supported by the NMR spectrum which shows sharp lines with no paramagnetic broadening or contact shifts.

This same sequence of oxidations can be achieved chemically,\textsuperscript{26} rather than electrochemically, by means of molecular bromine. The first step, which requires 0.51 ± 0.02 moles of bromine, brings about the oxidation of the cobaltous porphyrin to [Co(III)OEP]\textsuperscript{+} Br\textsuperscript{-}. Further oxidation of this trivalent complex, using an additional 0.51 ± 0.02 moles of bromine gives the green cationic radical species [Co(III)
OEP$^{3+} \cdot 2\text{Br}^-$.

The optical changes observed during these chemical and electrochemical oxidations are shown in Figures 8 and 9. Of particular interest is the difference between the spectrum of [Co(III)OEP]$^{2+} \cdot 2\text{ClO}_4^-$ and that of [Co(III)OEP]$^{3+} \cdot 2\text{Br}^-$. It is clear that the former represents the $^2A_{1u}$ ground state, while the latter represents the $^2A_{2u}$ ground state. Mass spectrometric studies show that the two bromide ions are strongly coordinated to the cobalt in [Co(III)OEP]$^{3+} \cdot 2\text{Br}^-$, and the coordination of these two ligands is a sufficient perturbation to cause the $a_{1u}$ rather than the $a_{2u}$ orbital to be occupied. When, however, this dibromide, dissolved in CH$_2$Cl$_2$, is treated with silver perchlorate, the bromide ligands are removed and the resultant diperchlorate salt now shows the spectrum characteristic of the $^2A_{2u}$ state. We now have a species, [Co(III)OEP]$^{2+}$ which, as a function of its axial ligands, can be made to occupy either of the two ground states.

The chemistry of these $\pi$-cation radicals, although still somewhat limited, is undoubtedly important, especially in porphyrin-containing enzymatic systems. Their principal chemistry is that of electron transfer: either accepting electrons from a variety of reducing agents to regenerate the porphyrin from which the initial radical was derived by oxidation, or the loss of the second unpaired electron to generate porphyrin $n$-dications. The stability of these cation radicals (we routinely recrystallize samples of [Zn(II)TPP]$^{2+} \cdot \text{ClO}_4^-$, and such crystalline samples have remained unchanged over a period of more than two years) and their ease of preparation suggests that nature might make use of them for electron transport of biochemical systems. This is indeed the case, and we have shown$^{27}$ that the primary photochemical step in green plant photosynthesis is the loss of an electron from a photochemically excited chlorophyll-$a$ ($\Psi 700$) to give the $\pi$-cation radical of chlorophyll-$a$ (Katz's findings$^{28}$ suggest this may occur from a bridged chlorophyll dimer in vivo).

Similarly, the one-electron electrochemical oxidation of zinc meso-tetraphenyl-bacteriochlorin, Zn(II)TPBChl, generates a species whose ESR spectrum characterizes it as a $\pi$-cation radical. A comparison between the optical spectra of [Zn(II)TPBChl]$^{+}$ and the one-electron oxidation product of cell-free bacterio-
chlorophyll-a confirms the identity of the latter as a π-cation radical and, finally, comparison of the different spectra between cell-free bacteriochlorophyll-a and electrochemically generated bacteriochlorophyll-a with that of the in vivo difference spectrum between resting and photobeached (P 870, which is the bacterial analog of P 700), confirms that once again the first photochemical step in bacterial photosynthesis is the loss of an electron from the molecule of the bacteriochlorophyll-a (P 870) to generate the π-cation radical of bacteriochlorophyll-a.

The catalytic action of iron porphyrin-containing enzymes is usually associated with the redox properties of the iron. This is necessarily the case with the cytochromes, which function via the Fe(II) ⇌ Fe(III) couple and which must, at some stage in their catalytic cycle, transfer electrons to and from the metal. This role of the metal in the cytochromes suggests that the metal may play the same central role in the enzymes catalase and peroxidase. These two closely related systems contain trivalent iron in the resting enzymes, and both are oxidized by hydrogen peroxide through two reversible electron steps to give, at the highest oxidation level, enzymes containing iron in a formal pentavalent oxidation state.\(^{30,31}\) Initially these highest oxidation states of these enzymes, known as the primary complexes, were considered to be complexes between trivalent iron and hydroperoxide anion. Subsequently, however, these same primary complexes were obtained using nonperoxidatic oxidants, at which time it was widely assumed that the two oxidizing equivalents could be accounted for by the loss of two electrons from the metal to give a Fe(V) porphyrin.\(^{30,31}\)

The optical spectra of both the primary complexes of catalase (Cat I) and horseradish peroxidase (HRP I) (Figure 10) are unlike those of any other heme proteins. Note, however, the close similarity between the optical spectra of the catalase primary complex and that of \([\text{Co(III)OEP}]^{2+} \cdot 2\text{Br}^-\) and the spectrum of the horseradish primary complex with that of \([\text{Co(III)OEP}]^{2+} \cdot 2\text{ClO}_4^-\) (Figure 10). We contend that these optical spectra of the primary complexes characterize them as porphyrin π-cation radicals.\(^{32}\) Moreover, it is apparent that catalase I therefore

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**Figure 9.** Bromine oxidation of Co(II)OEP in CH\(_2\)Cl\(_2\). Solid line, Co(II)OEP; dashed line \([\text{Co(III)OEP}]^+ \cdot \text{Br}^-\); dotted line, \([\text{Co(III)OEP}]^{2+} \cdot 2\text{Br}^-\).
FIGURE 10. Upper spectra are those of \([\text{Co(III)OEP}]^{3+} \cdot 2\text{ClO}_4^−\) (solid line) and \([\text{Co(III)OEP}]^{2+} \cdot 2\text{Br}^−\) (dotted line). The lower spectra are those of the primary complexes of horseradish peroxidase (solid line) and catalase (dotted line).

exists in the \(^2\text{A}_{1u}\) ground state, whereas HRP I exists in the \(^2\text{A}_{2u}\) ground state, and it is interesting to speculate that differences in reactivity between these two enzymes (catalytic decomposition of \(\text{H}_2\text{O}_2\) by catalase and the peroxidatic action of horseradish peroxides) may be a function of the two different ground states. At the least we may conjecture that although catalase and peroxidase contain the same heme components, the apparently different ground states for Cat I and HRP I could reflect relatively minor changes in the axial ligands (from the two different protein components) analogous to the effects of the anions in the model \([\text{Co(III)OEP}]^{3+}\) compounds.

The identification of these primary complexes as \(\pi\)-cation radicals accounts for one of the two oxidizing equivalents. The other must be an oxidation of Fe(III) to Fe(IV), so that the overall electronic configuration for the primary complexes can be represented as \([\text{Fe(IV)HRP}]^{3+}\) and \([\text{Fe(IV)catalase}]^{3+}\). This formulation requires that a one-electron oxidation of an Fe(III) porphyrin, or an Fe(III) porphyrin \(\pi\)-cation radical, can give the corresponding Fe(IV) porphyrin. This is, in fact, what has been observed.\(^{33}\)

The continued oxidation of \(\pi\)-cation radicals, at potentials about 0.3V higher than those required for the first oxidation, brings about an additional reversible one-electron oxidation. As this oxidation proceeds, the ESR signal associated with the cation radical decreases in intensity and finally disappears. At the same time the
optical spectra change to give, at the end of the reaction, an almost featureless spectrum in the visible region and a single absorption in the ultraviolet region (Figure 11). Unlike the π-cation radicals from which they are derived, these π-dications are powerful electrophiles and they react with a variety of nucleophiles, such as water, methanol, and acetate. This reaction with nucleophiles is accompanied by a dramatic change in the absorption with an intense absorption occurring in the near infrared. (Figure 12). We propose that the nucleophilic attack on the π-dication occurs at one of the four equivalent meso-carbon atoms to give a cationic isoporphyrin. Isoporphyrins have the tetrapyrrole chromophore shown in Figure 13.

NMR observations support this formulation. The destruction of the aromatic system is apparent from the upfield position of the β-protons in the isoporphyrins compared to those in the parent Zn(II)TPP (9.06 ppm). Furthermore, the splitting
**Figure 12.** Optical absorption spectra, in CH$_2$Cl$_2$, of [Zn(II)TPP]$^{++}$ 2ClO$_4^-$, before (solid line), and after (dashed line) treatment with methanol.

**Figure 13.** Mechanism of formation of isoporphyrin from porphyrin π-dication.
of the β-protons into two AB quarters is consistent with an attack at the meso position to give the isoporphyran with $C_1$ symmetry. Attack at either the α- or β-carbon atoms of the pyrrole rings would result in the product having a much more complex spectrum than we observe (Figure 14). The characterization of isoporphyrans represents direct evidence for the nucleophilic attack upon the porphyrin nucleus and, at the present time, stands as the only such example.

Aside from their oxidation levels, the chlorophylls and bacteriochlorophylls differ from regular porphyrins as a result of the additional isocyclic ring in their chromophores. The biosynthetic origin of this ring has been the subject of much speculation, and both Kenner$^{15}$ and Woodward$^{19}$ have suggested that a nucleophilic attack of a β-keto ester-enol onto the meso position of a metal-free diprotonated porphyrin would result in the formation of this isocyclic ring (Figure 15), and Kenner strengthened this hypothesis by showing that dipyromethene salts were indeed attacked by nucleophiles at the meso position. It should be noted that these proposed cyclizations not only require nucleophilic attack at a meso position, but in order to regenerate a porphyrin an oxidative step is required (Figure 15). Moreover, despite the ease of nucleophilic attack on the dipyromethene salt, no such reactions have been observed with porphyrins.
Magnesium protoporphyrin is a biogenetic precursor of chlorophyll, and it seems likely that once magnesium has been inserted into this macrocycle it will be maintained through the subsequent biochemical transformation. Cyclization of a $\beta$-keto ester to give an isocyclic ring does take place, in vivo, on the magnesium complex. The initial suggestion that the one-electron oxidation product of Mg(II)OEP was a phlorin derivative prompted Kenner to react the magnesium complex of a $\alpha$-$\alpha$-keto ester containing porphyrin (derived from rhodoporphyrin) with iodine in 98% methanol containing $K_2CO_3$. After work-up and removal of magnesium, this gave a low but very significant yield of the porphyrin containing an isocyclic ring. It was proposed that this cyclization could be envisaged as taking place via the coupling of a radical derived from the one-electron oxidation of the magnesium porphyrin ring and the radical derived from the one-electron oxidation of the enolate ion (FIGURE 16).

This suggestion requires that the removal of two electrons from the $\pi$-system of the porphyrin should give rise to a diradical. We have found no examples (even in systems containing a $\beta$-keto ester side chain) in which the removal of a second electron from the $\pi$-system generates anything other than the dication. We propose that this cyclization observed with the iodine oxidation proceeds via the addition of an enol (or enolate) onto the magnesium porphyrin $\pi$-dication to give an isoporphyrin. This cationic isoporphyrin can then, by the loss of a proton from the tetrahedral meso-carbon atom, be converted into the porphyrin containing the isocyclic ring (FIGURE 17).

Experiments with Zn(II)OEP confirm that meso-substituted porphyrins can be prepared by nucleophilic attack on the dication. When [Zn(II)OEP]$^{2+} \ 2CF_3SO_3^-$ is treated with sodium acetate in acetic anhydride, followed by pyridine, monoacetoxy Zn(II)OEP is produced, and we envisage this process as an initial attack.
FIGURE 16. Kenner's proposed mechanism for the oxidative cyclization of a β-keto ester to the chlorophyll isocyclic ring.

FIGURE 17. Our mechanism for the oxidative cyclization.
of the nucleophile to give the isoporphyrin which, in the present case, loses a proton
to give the meso-substituted acetoxy zinc porphyrin (FIGURE 18).

Does this same mechanism apply to the cyclization of β-keto ester containing
porphyrins? We prepared a porphyrin carboxylic acid using the bromomethyl-
biladiene route, and converted it into both magnesium and zinc salts of a β-keto
ester containing porphyrin (FIGURE 19). At a potential corresponding to the first
oxidation wave, a clean reversible one-electron oxidation takes place with the zinc
complex to give a stable π-cation radical which, even over long periods of time,
shows no tendency to cyclize and can be quantitatively recovered by reduction.

![Figure 18](image18.png)

**FIGURE 18. Route to give meso-acetoxyoctaethylporphyrin.**

![Figure 19](image19.png)

**FIGURE 19. Conversion of a porphyrin carboxylic acid to the corresponding β-keto
ester. The reagents used for this transformation were: (1) oxaloyl chloride in benzene;
(2) t-BuO₂C-CH₂-CO₂Et, THF, NaH; (3) TFA; (4) ZnOAc, CH₃CO₂H, CHCl₃.**
Continued electrolysis at a higher potential gives an unstable π-dication and this, in the presence of pyridine, gives a complex mixture from which we have isolated (in approximately a 10% yield) the zinc complex of the isocyclic ring containing porphyrin. This porphyrin is characterized by its mass spectrum which shows inter alia peaks at 610, 566, and 525 m/e, which are consistent with the following cleavage pattern for the product (FIGURE 20).

![Diagram of mass spectral breakdown patterns of the isocyclic ring-containing zinc porphyrin.](image)

The same cyclization is observed when the β-keto ester containing zinc porphyrin is oxidized to its π-dication with an excess of [Zn(II)TPP]⁺⁺ 2ClO₄⁻. A similar oxidation and cyclization occurs with the magnesium complex, and it is interesting to note that the ease of oxidation of magnesium porphyrins is such that the potential required to oxidize a specific nonmagnesium metalloporphyrin to the π-cation radical state is usually sufficient to oxidize the corresponding magnesium complex to the π-dication stage. This ease of oxidation of magnesium porphyrins...
suggests that the biosynthesis of the isocyclic ring of chlorophyll may also proceed via the same mechanism.

A most unusual observation was made by Bonnett and Dimsdale\textsuperscript{1,40} who found that although ferrous OEP was oxidized (by hydrogen peroxide in pyridine) to give ferric meso-hydroxy OEP, the corresponding ferric OEP was unreactive. They proposed the following scheme, whereby the first step in the reaction is a one-electron oxidation of Fe(II)OEP by hydrogen peroxide to give $[\text{Fe(III)OEP}]^+ \cdot \text{OH}^-$ and a hydroxyl radical, which then attacks the porphyrin nucleus as follows (FIGURE 21).

Fenton-type reagents follow a similar initiation, $\text{Fe}^{2+}$ (or $\text{Ti}^{4+}$) + H$_2$O$_2$ $\rightarrow$ $\text{Fe}^{3+}$ (or $\text{Ti}^{4+}$) + OH$^-$ + OH$^\cdot$, and the hydroxyl radical so generated will oxidize benzene to phenol; thus, these observations support the above hypothesis. We should like to suggest an alternative mechanism for such meso-hydroxylations, but before doing so there is some further chemistry of porphyrin cation radicals which relates to this question.

Mg(II)OEP is oxidized by benzoyl peroxide in CH$_2$Cl$_2$ at room temperature to the $\pi$-cation radical $[\text{Mg(II)OEP}]^\cdot$ which in turn reacts under these conditions to give meso-benzoyloxy Mg(II)OEP. However, in the presence of an excess of benzoyl peroxide, the meso-benzoyloxy Mg(II)OEP reacts to give an isoporphyrin (FIGURE 22). In the case of Zn(II)TPP, oxidation with benzoyl peroxide follows the same initial steps as with the magnesium porphyrin, but once the isoporphyrin is formed, it is stable (unlike the corresponding isoporphyrin from Mg(II)OEP) and the reaction stops at this stage.

With hydrogen peroxide, however, a very different series of reactions is observed. Neither Mg(II)OEP nor Zn(II)TPP are oxidized by hydrogen peroxide at room
temperature. But when either of the corresponding \( \pi \)-cation radicals is treated with hydrogen peroxide, they are reduced to the neutral porphyrin and hydrogen peroxide is oxidized to oxygen: 

\[
2\text{[Mg(II)OEP]}^\cdot + \text{H}_2\text{O}_2 \rightarrow 2\text{Mg(II)OEP} + 2\text{H}^\cdot + \text{O}_2
\]

We believe that this reaction parallels that of catalase: the primary complex, which we have characterized as an Fe(IV) \( \pi \)-cation radical,\(^{32}\) accepts electrons from hydrogen peroxide to generate an Fe(III) porphyrin and oxygen.

In the case of peroxidatic oxidations the green primary compounds react with electron- or hydrogen-donor substrates, \( \text{AH}_2 \), to release free radicals, \( \text{AH}^\cdot \), and the red-brown secondary compound of the enzyme. This, in turn, can oxidize a second molecule of donor (since reactions with \( \text{AH}^\cdot \) are not favored) to regenerate the ferric form of the enzyme. We would represent this as follows:

\[
\begin{align*}
\text{[Fe(III)Per]} + 2\text{H}_2\text{O}_2 & \rightarrow [\text{Fe(IV)Per I}]^\cdot + 2\text{OH}^- \\
[\text{Fe(IV)Per I}]^\cdot + \text{AH}_2 & \rightarrow [\text{Fe(IV)Per II}] + \text{AH}^\cdot + \text{H}^\cdot \\
[\text{Fe(IV)Per II}] + \text{AH}_2 & \rightarrow [\text{Fe(III)Per}] + \text{AH}^\cdot + \text{H}^\cdot \\
2\text{AH}^\cdot & \rightarrow \text{A} + \text{AH}_2 
\end{align*}
\]

Catalatic action, however, has not been observed to release free radical products, and a reducing reaction of \( \text{H}_2\text{O}_2 \) on Cat I (as with \( \text{[Mg(II)OEP]}^\cdot \), above) provides an attractive explanation. Thus, it is interesting that detectable radicals are not generated with either the catalase enzymatic reaction with hydrogen peroxide or
our room temperature reactions, and it would appear that nature uses porphyrins, specifically catalase, to decompose hydrogen peroxide to oxygen and water via a nonradical pathway.

How then do we relate these oxidations to the observation that Fe(II)OEP but not Fe(III)OEP is oxidized by hydrogen peroxide to meso-hydroxy ferric OEP? It should be noted that if the analogy with Fenton's reagent is valid, then a trace amount of ferrous iron is needed to catalyze the radical reaction. In the ferrous porphyrin case, initiation of the radical chain can then proceed as follows:

\[
\text{Fe(II)OEP} + \text{H}_2\text{O}_2 \rightarrow [\text{Fe(III)OEP}]^+ \cdot \text{OH}^- + \text{OH}^-.\]

We suggest, however, that the next step will parallel that of Mg(II)OEP with benzoyloxy radicals, and that it may be the ferrous porphyrin (rather than the ferric porphyrin) which is attacked by the hydroxyl radical (Figure 23).

The principal difference between this mechanism and that suggested by Bonnett and Dimsdale\(^{40}\) is that the initial electrophilic attack takes place at the ferrous rather than the ferric porphyrin stage. This suggestion is supported by the observation that ferric porphyrins are not very susceptible to electrophilic attack at the meso position,\(^{11}\) that ferric OEP is not attacked by hydrogen peroxide (even in the presence of ferrous ion), nor by benzoyl peroxide at high temperatures, and that the oxidation of ferric porphyrins generates iron (IV) porphyrins,\(^{33,41}\) suggesting that the \(\pi\)-electrons in ferric porphyrins are not readily available for reaction. Moreover, it would appear that meso hydroxylation, as the first step in bile pigment formation, occurs with hemoglobin, where it is again unlikely that oxidation of the iron constitutes the initial step.

\[
\begin{align*}
\text{Fe(II)OEP} & \rightarrow [\text{Fe(II)OEP}]^+ \cdot \\
\text{Fe(II)OEP} & + \text{OH}^- \\
\text{Fe(II)OEP} & - \text{H}^+ \\
\text{H}_2\text{O}_2 & \rightarrow [\text{Fe(III)OEP}]^+ \cdot \text{OH}^- + \text{OH}^-.
\end{align*}
\]

**Figure 23.** Our proposed mechanism for the meso-hydroxylation of ferrous OEP.
This suggestion that oxidation of an Fe(II) porphyrin might take place to give an Fe(II) π-cation radical may also be extended to the function of the cytochromes. Dickerson and coworkers have shown that the iron in cytochrome c is effectively shielded from even the smallest electron carrier. Clearly then, the electron transfer which accounts for the overall Fe(II) ⇌ Fe(III) couple is unlikely to involve direct electron transfer at the iron, and we and others envisage that such electron transfer occurs initially at the porphyrin periphery followed by an internal electron transfer: i.e., Fe(II) + e− \[\text{Fe(II)heme}^+ \iff \text{Fe(III)heme}].

Acknowledgments

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Discussion

DR. SRIVASTAVA (Texas A & M University): You stated that two types of products are formed by oxidizing cobalt (III)OEP with bromine, one containing cation Co(III) and a single Br⁻ ligand and the other is a [Co(III)OEP]²⁺ 2Br⁻. Is there a possibility that bromide will coordinate with the cobalt, and you are getting two different types of effects?

DR. DOLPHIN: What I said was that if we take the dibromide and treat it with perchlorate it goes to the diperchlorate, whatever that is, and we have a change in the ground state. In the presence of the perchlorate I don't know what is coordinated. My guess is, probably nothing. But, in the case of the dibromide, we can isolate this material, crystallize it, run a mass spectrum; it shows quite clearly that the two bromide ligands are coordinated with the cobalt. So in one ground state, where bromide is present, these are coordinated. We then treat that with silver perchlorate; the bromides certainly come off. I don't know what goes on in their place.

DR. JOHN OLSON (Brookhaven): One of the secondary reactions of photosynthesis is, of course, the electron transfer from cytochrome to either the chlorophyll-a or bacteriochlorophyll. How would you visualize that in terms of what you presented?
Dr. Dolphin: In terms of our scheme, we assume that the oxidized chlorophyll loses an electron from the \( \pi \)-system to leave a \( \pi \)-cation radical. And so the transfer of that electron back from the cytochrome, I think, is simply into the empty orbital on the \( \pi \)-system.

Dr. Olson: Where in the cytochrome does it come from?

Dr. Dolphin: It might well come from the ring.

Dr. Bonnett (Queen Mary College, London): Perhaps I could make a comment on the reactions Dave referred to that we carried out some little while ago. Our reactions were with a different system with pyridine and hydrogen peroxide, originally used by Fischer and Lividisky many years ago. We felt that maybe valency change was occurring at an early stage simply because we could observe the formation of metal oxyfluorine derivatives with the ferrous, the cobaltous and the manganous octaethylporphyrins, but not with the ferric, the zinc, the nickel, and the copper octaethylporphyrins. In those last four cases, and in the case of octaethylporphyrin itself, we recovered the starting material in quite high yield; whereas with the three divalent transition metals—ferrous, cobaltous and manganous—we got a good yield of the meso-oxygenated porphyrin. We felt, and I still feel, that maybe this system is not quite the same as some of the systems you've been looking at, and that there is an action involving a change of valency and generating the hydroxyl radical very close to the site at which attack occurs. I think this is quite important. In making the analogy with the Fenton reagent, one has got the ferrous atom inside the aromatic system that's being attacked, if one looks at it in that way.

Dr. Dolphin: We have also found exactly what you found with hydrogen peroxide in pyridine, but I would like to suggest that the ease of oxidation of those systems you were discussing, particularly the ferrous, cobaltous and manganous, may still imply that the first oxidation is not an oxidation of the metal but rather an oxidation of the ring. Because in each of those cases in which you have the metal in the lower oxidation state, it is reasonable to assume that the ease of oxidation of the ring will be greater. In the other examples you gave, the ferric, the zinc, the nickel require a higher voltage to remove one electron. The zinc requires 0.9 volts to remove the electron which is somewhat higher than say magnesium, the example I gave, which is only about 0.5 or 0.6.

The reason that I don't like your mechanism and I do like mine is that we take a ferric OEP, treat it with hydrogen peroxide in pyridine and ferrous iron—the Fenton's analog—and get no reaction. Then we take hydrogen peroxide, pyridine and iron(III)porphyrin—no reaction. If we add ferrous iron, catalyze the decomposition of hydrogen peroxide, give hydroxyl radicals, we again see no reaction. Now, your explanation that you need to generate the radical near the site of attack may well be a valid one, except that Fenton's reagent will hydroxylate benzene and, clearly, in that case the radicals are not being generated near the benzene which they attack.

Dr. Bonnett: Yes, but in that case they're being generated in benzene as solvent.

Dr. Dolphin: The concentration is high, agreed.

Dr. Bonnett: A very important point, I think.

Dr. Dolphin: Yes. All I'm saying is that I think with these low oxidation states of the metal the ring will undergo ready oxidation. With the high oxidation states, is not so ready. So one possible way of explaining these results is that if you have a low oxidation state, you can oxidize the ring and hydroxylation will occur, if you have a high oxidation state, you can't. But I grant you that you can't initiate the reaction either in the way you suggest.

Dr. Jackson (Cardiff): I'd like to ask Dr. Dolphin, in view of his comments
about the relative ways in which these cyclizations might occur in nature and in
the laboratory, precisely under what conditions his zinc $\beta$-ketoester cyclized to give
the isocyclic ring?

DR. DOLPHIN: The conditions were: methylene dichloride as solvent with pyri-
dine as base, use of an electrochemical oxidation at a potential where we knew we
would take two electrons away, or use of an excess of an oxidant such as the zinc
tetraphenylporphyrin which had already had two electrons removed. An excess of
that, letting the solution sit for about half an hour, and then working it up.

DR. JACKSON: I see. In that case, I wonder why you showed the enol rather than
the enolate in your slides. I wonder whether that was significant or not?

DR. DOLPHIN: I don't know. Clearly, the enolate is going to be a more powerful
nucleophile. We know we have about 30\% enol, 70\% keto, and I don't know which
of those two react. I would assume it must be the enol, but it may be the enolate.

DR. JACKSON: I think it's quite clear from our experiments that it's the enolate
which reacts, because if we take magnesium ketoester and treat it with iodine, we
get the spectrum of the monocation radical very clearly present there. When we add
a spot of carbonate, the whole color of the solution changes immediately and
the reaction is over very quickly, and you get the keto ester. So we think it's essential
that the second electron is taken from the enolate anion system. This is very easily
oxidized and, therefore, one gets a diradical. Exactly how you write this diradical is
perhaps a philosophical question.

DR. DOLPHIN: No. There is a difference between a molecule which has lost two
electrons from two separate orbitals and a molecule which has lost two electrons
from the same orbital, but you're suggesting one and not the other.

DR. JACKSON: But they are not separate orbitals anymore, are they, since the
enolate anion is conjugated with the porphyrin.

DR. DOLPHIN: You're suggesting then that because it's conjugated, the two
electrons are taken to leave a diradical. I am saying we have not seen any examples
of this; even with the $\beta$-ketoesterporphyrin, where we can take two electrons away
to form the dication. There is a difference between the diradical and the dication.
In our case it would appear that it's the dication that is cyclizing. We have not seen
any evidence for a diradical.

DR. JACKSON: We haven't any evidence one way or the other. We know that the
thing cyclizes immediately.

DR. DOLPHIN: As soon as you add the base?

DR. JACKSON: Right. In other words, we don't think that the second electron
comes off the nucleus because, under the conditions which we used in our experi-
ments, we didn't take two electrons off a nucleus.

DR. DOLPHIN: If it requires the enolate to cyclize, and if you have only a small
concentration of dication which you wouldn't see, you might still not get a cycliza-
tion until you generate to the enolate, but it could still be the enolate cyclizing onto
the dication.

DR. JACKSON: I think we'll leave it at that.

DR. ADLER: I'd like to add one comment: one must always beware of the dif-
ference between kinetics and mechanisms and thermodynamics. When Mike talks
about some of the mass spectrometric work later, you'll see that in the excited states
we see in the mass spectrum of metalloporphyrins, it's very easy to relocalize
charge between the metal and the aromatic system. I think this is something that you
should bear in mind when talking of the differences between what you see and what
Ray sees. You may have the same overall reaction, but you may be looking at dif-
ferent pieces of the mechanism: in your case it may be kinetically possible to see one
intermediate, and in his case another one.