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THE CHEMISTRY OF BIOLOGICAL MANGANESE

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CONTENTS

ABBREVIATIONS

bipyr	bipyridine
cys	L-cysteinate ion
DMSO	dimethyl sulfoxide
DTC	N,N-diethyldithiocarbamate ion
EPR	electron paramagnetic resonance
Hb	hemoglobin
his	L-histidinate ion
Pc	phthalocyanine
phen	phenanthroline
PS II	photosystem II
8-Q	8-quinolinolate ion
salen	N,N'-bis(salicylaldehyde)ethylenediamine
SOD	superoxide dismutase
TPP	tetraphenylporphine

A. INTRODUCTION

"Mangania is the Greek word for magic or, in modern parlance, for voodooism. If this is the root of the metal's name, it reflects some reality in the biology of manganese, which is rich in phenomena and lacking in adequate guiding principles" [1].

Although made twenty years ago by Cotzias in a review of the clinical and nutritional aspects of manganese, this statement has not lost its veracity. Since then numerous enzymes have been found to contain manganese as a cofactor, but some are not specific for this metal and may substitute Zn^{2+} or Mg^{2+} for the Mn^{2+} ion. Several review articles have appeared in the past decade which include various biological systems that contain manganese, but we are not aware of any that focus on the chemistry of biological manganese.

This review attempts to bring together some of the biological systems that are specific in their requirement for manganese and to discuss the recent advances in the chemical understanding of manganese which may be pertinent to the biological function of this element. A full account of the biological aspects of manganese is far beyond the scope of this paper; therefore, we have restricted the discussion to those systems which have (or are believed to have) a unique requirement for manganese because of its chemical properties, especially oxidation—reduction reactions.

Although manganese is ubiquitous in nature, generally occurring at levels comparable to copper [2], only a few enzymes are known to contain tightly bound manganese. Pyruvate carboxylase was the first such enzyme discovered [3]. Later, Fridovich and coworkers [4] discovered a superoxide dismutase from *Escherichia coli* that contained manganese as the metal cofactor. Since then manganese containing superoxide dismutases have been isolated from a broad spectrum of organisms. A diamine oxidase enzyme that contains manganese has recently been discussed [5], but its role is not known.

The necessity of a manganese cofactor for photosynthetic oxygen evolution in green plants has been apparent for some time [6]; however, the chemistry and catalytic mechanism of this cofactor are not understood. Several reports on the role of manganese in various neurological and metabolic disorders are also summarized.

B. MANGANESE ENZYMES AND COFACTORS

(i) Pyruvate carboxylase

The first metalloenzyme shown to contain manganese was pyruvate carboxylase from chicken liver mitochondria [3]. The discovery of bound manganese in this enzyme was fortuitous because the experiments were designed to monitor the proton relaxation rate of water when manganese (and nucleotide triphosphate) was added to the enzyme as a paramagnetic probe. However, the enzyme had an inherent enhancement effect on the proton relaxation rate of solvent water even in the absence of added manganese [3].

Pyruvate carboxylase from chicken liver mitochondria has a molecular weight of 500 000—520 000, consists of four subunits and contains one biotinyl group and one Mn(II) ion per subunit [7]. The overall catalytic process is the sum of two reactions

$$E\text{—biotin} + MgATP^{2-} + HCO_3^- \overset{M^+,\ M^{2+}}{\rightleftharpoons} E\text{—biotin—}CO_2 + MgADP^- + P_i^{2-} \quad (1)$$

$$E\text{—biotin—}CO_2 + \text{pyruvate} \rightleftharpoons E\text{—biotin} + \text{oxaloacetate} \quad (2)$$

Proton relaxation rate studies with ^{13}C-enriched pyruvate have permitted estimates to be made of the interatomic distances between Mn(II) and the atoms of pyruvate. These indicate that the metal ion is in close proximity to the site of transcarboxylation (reaction 2), but the interatomic distances are too great to allow direct coordination of pyruvate to the metal ion [8]. A current view is that a molecule of water is tightly bound to the metal ion (which is buried in the protein) and that this coordinated water acts as an electrophile to activate pyruvate [8].

Although this enzyme does not show a strict specificity for manganese, the presence of bound manganese in pyruvate carboxylase from several sources has enabled some elucidation of the mechanism of action through proton relaxation studies. Such studies illustrate the utility of the Mn(II) ion as a probe for the study of enzymes that contain divalent ions.

(ii) The role of manganese in photosystem II

Several recent review articles [9—11] discuss the necessity of manganese in photosynthetic oxygen evolution. There is substantial convincing evidence that it is directly involved in the charge accumulation center of photosystem II (PS II) of green plants, although a manganese cofactor which exhibits Hill reaction activity (O_2 evolution) has not yet been isolated. Cheniae and Martin [6] found that there are two manganese pools in the chloroplasts of green plants and that the more loosely bound pool, which contains about two-thirds of the chloroplast manganese, is directly related to oxygen evolution. Treatment of chloroplasts with 0.8 M TRIS—HCl buffer at pH 8 depletes this two-thirds pool to give a complete loss of Hill reaction activity [12].

The scheme developed by Kok et al. [13] for charge accumulation in the oxygen evolving apparatus of PS II has gained wide acceptance since its inception several years ago (see Fig. 1). The current understanding of PS II is that manganese acts as a charge accumulator in the oxygen evolving apparatus and is an integral part of the enzyme catalyzed oxidation of H_2O to O_2. The oxidizing side of PS II has been characterized by means of magnetic resonance and chemical inhibitor studies. Oxygen evolving photosynthetic materials exhibit an EPR spectrum which is a composite of at least two components and probably more. The properties of two major signals, one associated with PS II and the other with PS I, have been reviewed [11]. More recently, Sauer and coworkers [14] have observed a transient radical species in oxygen-evolving

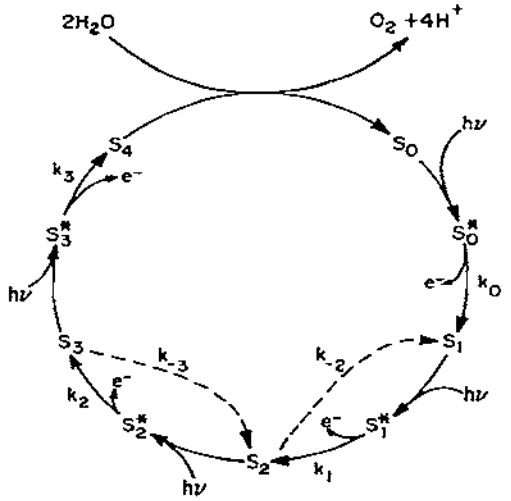


Fig. 1. Four-state, four-photon model of Kok[13] for photosystem II.

spinach chloroplasts which appears in less than 100 μs with flash illumination, and decays with a half-life of 400—900 μs. In contrast to the slow decaying signal, this transient radical does not readily saturate even at microwave power levels of 200 mW. Removal of manganese from these chloroplasts by TRIS treatment results in slower decay of the radical signal after a saturating flash, and the power saturation curve is greatly altered. Furthermore, addition of Ni(II) ion to the TRIS treated chloroplasts increases the power saturation curve to resemble that of untreated chloroplasts, but it does not restore oxygen evolving activity. The magnetic behavior and EPR lineshape of this transient radical indicate that it probably is a "quinoidal" species in close proximity to a paramagnetic ion, probably manganese [14].

Wydrzynski et al. [15,16] have recently monitored water-proton relaxation rates in spinach chloroplasts and have observed a correlation between the number of light flashes and the spin—spin relaxation rate. They conclude that the proton relaxation rates may be indicative of the manganese oxidation state in photosystem II. Their results are convincing, but must be interpreted with caution because the rates for proton relaxation of water are sensitive to many factors.

A wealth of data has been accumulated to discern whether water is indeed the source of the oxygen that is evolved in photosynthesis [17]. There is general agreement that the two oxygen atoms of the evolved molecular oxygen are ultimately derived from water, but scission of the O—H bond is not rate limiting [18].

Manganese is undoubtedly involved in the charge accumulation center of the water oxidizing system of PS II, and probably also acts as a template to bring two oxygen atoms into close enough proximity to form an O—O bond

[19]. Such a cofactor probably involves at least two, and perhaps as many as four, manganese ions per oxygen evolving unit.

Recent studies [20,21] of manganese–catechol complexes indicate that molecular oxygen is reversibly bound by the tris(3,5-di-*tert*-butylcatecholato)Mn(III) complex (vide infra). Such a process is equivalent to the terminal step of the PS II oxygen evolution reaction (Fig. 1). Quinoid species in the chloroplast membranes may bind to manganese ions in PS II in a similar manner to that for this catechol complex. The facile delocalization of unpaired charge by the Mn(III)–catechol–semiquinone–OH chelate system may be important for the water oxidizing process. Appropriate spacing of such membrane bound manganese ions would provide the template that is almost certainly required to promote formation of the O–O bond during the water oxidizing process.

A model for the PS II–water oxidation system is proposed on the basis of these considerations and is outlined in Fig. 2. The crucial step is the transition from the S_4 state to the S'_4 state to form the O–O bond. Both charge delocalization in the chelate rings and the atomic proximities imposed by the template

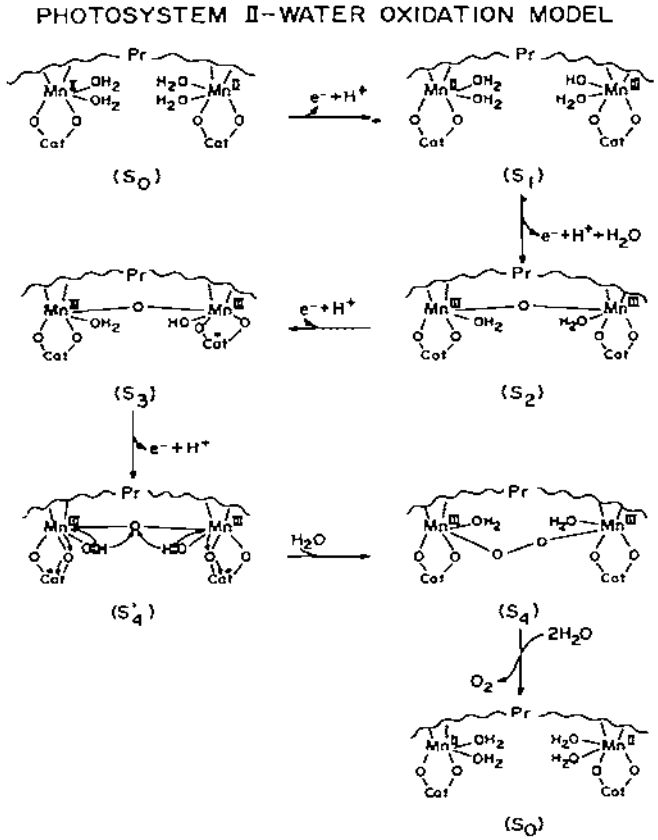


Fig. 2. Proposed model for the manganese cofactor of photosystem II.

favor formation of the peroxide bridge. In many respects the water oxidation scheme which is outlined in Fig. 2 is the reverse of the most recently proposed schemes for the four-electron reduction of molecular oxygen to water by cytochrome oxidase [22,23].

(iii) Superoxide dismutase

The role of superoxide anion radical (O_2^-) in metabolic processes has been realized only in the last decade. The development of a viable assay for superoxide dismutase (SOD) [24,25] has led to the isolation of this enzymatic activity from a broad spectrum of organisms. At least three forms of the enzyme have evolved, each with a different metal ion content. The properties of these three forms of SOD are discussed here only briefly to provide some points for comparison. The reader is referred to a recent monograph for a more comprehensive review of superoxide dismutases [26].

The first enzyme shown to exhibit superoxide dismutase activity was erythrocuprein, a copper—zinc metalloprotein isolated from mammalian erythrocytes [25]. This enzyme is composed of two identical subunits, each with one atom of copper and one atom of zinc; the molecular weight of the enzyme is 31 500 daltons. The activity is strongly inhibited by cyanide ion which forms a Cu(II)—CN complex [27]. It has been suggested that an open coordination site on the Cu ion is important for catalysis and complexing anions compete with O_2^- for this site [28].

An iron-containing SOD has been isolated from bacterial [29] and algal [30,31] sources. This enzyme resembles the manganese containing SOD from bacterial sources in subunit molecular weight, amino acid content and cyanide ion insensitivity. The Fe and Mn forms of the enzyme have distinct differences relative to the Cu—Zn SOD. These dismutases contain two apparently identical 20 000 dalton subunits per molecule and have one (or possibly two) metal atoms per molecule (dimer) [28]. The manganese enzymes are generally more stable to denaturation [30,31].

A manganese SOD has also been isolated from chicken liver mitochondria. This enzyme resembles the bacterial enzyme in subunit composition and molecular weight, but the native enzyme exists as a tetramer rather than a dimer [32]. Another manganese SOD which is also a tetramer [33] has been found in yeast mitochondria.

The manganese from bacterial SOD can be removed under strongly denaturing conditions [34,35]. When the *E. coli* apoenzyme is reconstituted with Co(II), Ni(II) and Zn(II) it remains catalytically inactive [35]. The SOD from *B. stearothermophilus* has been reconstituted with Co(II), Fe(II), Ni(II) and Cu(II), but each is catalytically inactive [36]. The *B. stearothermophilus* apoenzyme maintains the native dimeric structure, which indicates that the metal ion is not necessary for structural integrity [34]. Reconstitution of the apoenzyme with $MnCl_2$ restores full catalytic activity [36]. The fully active, manganese-reconstituted enzyme appears to contain a single manganese atom per

dimeric unit, which is a novel stoichiometry for an enzyme that contains two identical subunits. These results seem to establish unequivocally that manganese is essential for catalytic activity. That the iron-reconstituted enzyme did not exhibit catalytic activity is surprising because the iron SOD enzymes have similar subunit structures and amino acid content [37].

The mechanism of action of SOD has been studied by a number of groups [38—41]. The Cu—Zn enzyme [38] and the Fe enzyme [40] catalyze the disproportionation of O_2^- by a simple two-step mechanism that involves a one-electron reduction followed by oxidation of the metal ion by O_2^- to form O_2 and H_2O_2, respectively

$$E_A + O_2^- \xrightarrow{k_1} E_B + O_2 \tag{3}$$

$$E_B + O_2^- + 2\,H^+ \xrightarrow{k_2} E_A + H_2O_2 \tag{4}$$

The manganese SOD enzymes appear to operate by the same mechanism at low levels of O_2^-, but the kinetics become complicated at higher substrate levels [41] (levels which are probably not encountered in vivo).

Further consideration of the SOD catalyzed dismutation of superoxide ion has been the object of an ingenious stopped-flow investigation [42]. The results indicate that the initial complexation of the oxidized or reduced metal in SOD by O_2^- is followed by electron-transfer steps to give either O_2 or bound peroxide. The latter is displaced by proton abstraction from bound H_2O. The rate limiting process for the Mn—SOD enzymes appears to be diffusion of O_2^- to the active site.

To rationalize the energetics for the electron-transfer steps in the catalytic cycle for the dismutation of O_2^- by the SOD enzymes, an evaluation of their standard oxidation—reduction potentials, $E^{0'}$, has been achieved [43]. The measurements have been made by potentiometric titrations with coulometrically reduced methyl viologen. Table 1 summarizes the $E^{0'}$ values for several SOD enzymes and the effect of solution acidity. The sharp change in potential between pH 8 and 9 for the *E. coli* Mn—SOD enzyme is consistent with the greater than two-fold decrease in its catalytic rate constant when the medium is changed from pH 7 to 8.5 [44]. In the case of bovine erythrocyte SOD the greatest decrease occurs on decreasing the acidity from pH 6.0 to 7.8.

Because the potentials in Table 1 are more than adequate to oxidize O_2^- to molecular O_2 ($E^{0'} = -330$ mV) [45], the limiting process is probably the oxidation of the reduced SOD by O_2^-. In the absence of protons O_2^- is too weak an oxidizing agent to achieve the oxidation of reduced SOD by an outer sphere mechanism [46]. Hence, this part of the catalytic process must be accomplished by formation of an M_{ox}—peroxide complex via an inner sphere electron transfer. Formation of such complexes would enhance the oxidizing strength of O_2^- as well as the reducing strength of reduced SOD.

TABLE 1

Redox potentials for several SOD enzymes at 25°C in the presence of TRIS—phosphate buffers

Enzyme	$E^{0'}$ (mV vs. NHE)	pH
MnSOD (*B. stearothermophilus*)	+237	7.1
MnSOD (*E. coli*)	+311	7.0
	+320	8.0
	+285	8.5
	+180	9.0
Cu—Zn SOD (Bovine erythrocytes (BESOD))	+352	7.0
	+298	8.0
	+248	9.0

(iv) Diamine oxidase

Crabbe et al. [5] have recently discussed the purification and characterization of a diamine oxidase enzyme with unusual substrate specificity and metal ion content. The enzyme is isolated from human placenta and pregnancy plasma and is estimated to have a molecular weight of 70 000 daltons, in contrast to other amine oxidases which generally exist as dimers with subunit molecular weights of about 90 000 daltons.

The metal ion content of this enzyme, which has been studied by EPR and by atomic absorption spectroscopy, is apparently one Cu atom and one Mn atom per molecule of enzyme. Double integration of the EPR spectra yielded values of 0.7 atom of Cu and 0.3 atom of Mn per molecule of enzyme; the low values are attributed to spin—spin interactions. Addition of substrate to the enzyme does not apparently affect the spectral intensity or lineshape. Hence, there may not be any direct binding of substrate to the metal centers.

Clearly, more work needs to be done on this class of enzyme to determine the role of metal ions, as well as their oxidation states and the degree of specificity for manganese.

(v) Clinical aspects of manganese

Manganese toxicity and deficiency have been discussed extensively in two current monographs [2,47]. In recent years the role of manganese in neurological disorders has received increased attention. Hurley et al. [48] have shown that a relationship exists between the manganese levels in rats and their susceptibility to convulsions. More recently, Tanaka and Harpur [49] have discussed at least two cases in which children who had suffered numerous convulsions had blood manganese levels far below normal. Furthermore, the mothers of these children also had low blood manganese levels.

A correlation has been established between the levels of manganese and

catecholamines in the brain [50,51]. High levels of manganese in the diet of mice induce initial increases in levels of brain catecholamines.

Manganese also appears to play an important role in several metabolic processes such as bone growth, glucose tolerance, reproduction, and development of the inner ear [47].

C. COORDINATION CHEMISTRY OF MANGANESE

An adequate understanding of the chemistry of biological manganese must begin with the coordination chemistry of its various oxidation states. Until recently, studies of manganese coordination chemistry have been limited and directed primarily to the Mn(II) species. The high lability of ligand bonding of the latter has prompted most coordination chemists to look to more interesting systems.

The Mn(II) ion has a d^5 electronic configuration which, in the high spin state, corresponds to the spherically symmetric ^6S ground state for the free ion. This electronic configuration does not provide ligand field stabilization energy and thus, Mn(II) forms much weaker complexes than the succeeding divalent cations of the first-row transition series (Fe(II)—Cu(II)).

The Mn(III) ion is a strong oxidant and in the absence of complexing ligands disproportionates to Mn(II) and MnO_2. Even when coordinated, Mn(III) (and, likewise, Mn(IV)) remains a strong oxidant and most of its complexes decompose slowly due to oxidation of the ligand. As a result, the majority of the investigations of the higher oxidation states of manganese (>2+) has been with ligands that are resistant to oxidation. The visible and near IR spectral characteristics [52] of Mn(III) complexes and the properties of Mn(III) in aqueous solution [53] have been reviewed.

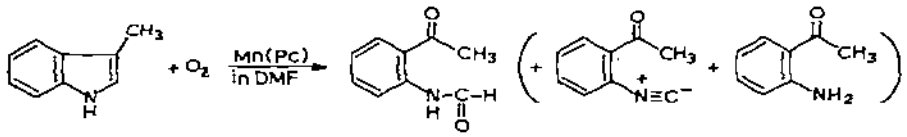
Little is known about the properties of the Mn(IV) ion because it is such a strong oxidant and complexes of this ion are usually unstable.

(i) Phthalocyanine and porphyrin complexes

The properties of transition metal complexes with this class of ligands have been of interest because of their utility as models for hemoproteins and their curious photochemical behavior. Almost 20 years ago the Mn(II)—phthalocyanine (Pc) complex was found to bind molecular oxygen reversibly in pyridine solution [54]. The final product of oxygenation in pyridine was shown by X-ray crystallography to be μ-oxo-bis(phthalocyanine manganese(III)) [55]. This binuclear species is formed from an intermediate which has been shown to be $Mn(Pc)O_2$ [56] and not a peroxo bridged species [54] or Mn(Pc)-(OH) [57], as previously suggested. Rigorous exclusion of water and protons in pyridine solutions of Mn(II)(Pc) inhibits formation of the oxygen adduct [56]. The absorption spectrum and the EPR characteristics of the Mn(Pc)—oxygen adduct suggest an $Mn(III)(Pc)(O_2^-)$ formalism [56].

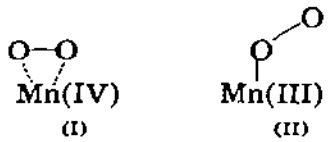
Recently, Uchida et al. [58a] have shown that Mn(II)(Pc) exhibits tryptophan-2,3-dioxygenase activity in DMF solution. The major product of the

catalyzed oxygenation of 3-methylindole is 2-formamidoacetophenone, with 2-aminoacetophenone and the 2-isonitrile derivative as minor products.



These results are significant because the Fe(III)heme—O_2^- adduct has been postulated to be the active component in the indoleamine 2,3-dioxygenase-catalyzed oxygenation of tryptophan and its analogs [58b].

Reversible binding of molecular oxygen also occurs with the Mn(II)—tetraphenylporphine (TPP) complex, although only at low temperatures (<—78°C) in toluene [59,60]. The oxygen adduct exhibits a spin state of 3/2 [60], which has generated much discussion concerning the electronic distribution formalism. The Basolo group [60,61] favors the Mn(IV)(TPP)(O_2^{2-}) formalism, which is consistent with the symmetrical, Griffith-type conformation(I) that has the Mn—O bond distances equal for both oxygen atoms. However,



the magnetic and spectroscopic properties are not inconsistent with an Mn(III)(TPP)(O_2^-) formalism(II); the analogous Mn(Pc)—oxygen adduct exhibits a similar magnetic moment and has been postulated as Mn(III)(Pc)(O_2^-) [56]. Recent molecular orbital calculations indicate that Mn(II)(TPP)(O_2) is a more reasonable formalism [62]. X-ray crystallographic evidence confirms that the Mn(II)(TPP) complex is five coordinate and that its out-of-plane geometry results in a high-spin electronic configuration (S=5/2) [59]. The observed physical characteristics are contraindicative of the Mn(II)(TPP)(O_2) formalism. The analogous Cr(II)(TPP)(py) complex in the crystalline state yields an oxygenated species upon exposure to oxygen, which, on the basis of spectroscopic evidence, is postulated to have the formula Cr(III)(TPP)(py)(O_2^-) [63].

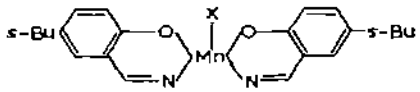
Substitution of Mn(II) for Fe(II) in hemoglobin (Hb) yields an Mn(Hb) species which does not reversibly bind oxygen, but is oxidized irreversibly to Mn(III)(Hb) [64]. These results indicate that the Fe(III)(O_2^-) formalism may be preferred for the oxyhemoglobin complex.

(ii) Schiff base and imine complexes

The Schiff's base, N,N'-ethylenebis(salicylaldimine) (salen), and its derivatives and analogs provide a relatively rigid, tetradentate coordination geometry with an N_2O_2 donor configuration. Mn(II)(salen) is readily oxidized by air

in organic solvents to an insoluble brown product which, on the basis of magnetic susceptibility measurements [65], appears to be an oxo-bridged dimer. Further studies [66], however, indicate that the oxidation yields several products with both Mn(III) and Mn(IV) centers.

The halogenated Mn(III)(salen) complexes exhibit high-spin magnetic moments with no apparent exchange interaction [67], and appear to involve five-coordinate Mn(III) with square pyramidal geometry.



The same coordination also is observed for the Mn(III)(Busalen)X complex (Busalen = 4-sec-butylsalicylaldehyde ethylenediimine and X = a variety of monovalent anions) [68]. Air oxidation of the Mn(III) Schiff base complexes in chloroform or methanol solutions yields a di-μ-oxo-bridged, mixed valence, Mn(III/IV) dimeric species [68].

The tris(bipyridyl)Mn(II) complex is high spin in contrast to the corresponding Cr(II) and Fe(II) complexes. Persulfate oxidation of the Mn(II)-(bipyr)$_3^{2+}$ complex yields a di-μ-oxo Mn(III/IV) complex which has been confirmed by X-ray crystallography [69] $[(bipyr)_2Mn^{III}(O)_2Mn^{IV}(bipyr)_2]^{4+}$.

The magnetic moment of this complex indicates a net spin of $\frac{1}{2}$, apparently as the result of strong antiferromagnetic coupling through the bridging oxygen atom [70].

The di-μ-oxo tetrakis(bipyridyl)Mn(III)Mn(IV) complex and the analogous 1,10-phenanthroline complex exhibit reversible, one-electron oxidation reactions with extremely positive redox potentials in acetonitrile for the (IV—IV)/(IV—III) couple, +1.61 V and +1.53 V vs. NHE, respectively [71,72]. The respective reduction potentials for the (IV—III)/(III—III) couple are +0.63 V and +0.72 V vs. NHE. Hence, the Mn(IV)—Mn(IV) di-μ-oxo bipyridyl and 1,10-phenanthroline systems represent prospective strong oxidizing agents for compounds in aprotic media.

The bis(phen)Mn(II) and bis(bipyr)Mn(II) complexes catalyze the decomposition of H_2O_2 [73]. Inhibition of the reaction by hydroquinone indicates that a radical chain mechanism is probably involved with $\cdot OH$ or $HO_2\cdot$ as intermediates [74].

The bis(8-quinolinato)manganese(II) complex (Mn(II)(8-Q)$_2 \cdot 2\,H_2O$) catalyzes the disproportionation of superoxide ion (O_2^-) in aprotic solvents [75]. The analogous Mg(II) and Zn(II) complexes do not exhibit any catalytic effect. Hence, the manganese of the Mn(II)(8-Q)$_2$ complex apparently acts as a redox catalyst for the disproportionation of $O_2^-\cdot$ to O_2 and H_2O_2, possibly by a mechanism that is similar to that for SOD enzymes. The Mn(II)(8-Q)$_2$ complex is also oxidized by air in the presence of excess ligand in 50% MeOH/H_2O solution to give a μ-oxo-bridged binuclear Mn(III) complex.

A proposed mechanism for the catalytic dismutation process is represented by eqns. (5)—(8) [75]. Reactions (7) and (8) occur in the presence of excess ligand

$$\underset{\text{(I)}}{Mn^{II}(8\text{-}Q)_2(H_2O)_2} + O_2^- \rightarrow \underset{\text{(II)}}{Mn^{III}(8\text{-}Q)_2(O_2H)(H_2O)} + OH^- \quad (5)$$

$$II + O_2^- \rightarrow I + O_2 + HO_2^- \quad (6)$$

$$Mn^{II}(8\text{-}Q)_3^- + O_2^- \xrightarrow{H_2O} Mn^{III}(8\text{-}Q)_3(O_2H)^- + OH^- \quad (7)$$

$$Mn^{III}(8\text{-}Q)_3(O_2H)^- + O_2^- \rightarrow Mn^{II}(8\text{-}Q)_3 + O_2 + HO_2^- \quad (8)$$

These results indicate that oxine-like ligands may indeed be a good model for the manganese environment in the MnSOD enzyme. One problem with the model system, however, is the proton requirement in the dismutation reaction, which limits the amount of O_2^- which can be destroyed in aprotic solvents. Nature obviously has provided a means to overcome this problem.

(iii) Amino acid complexes

Manganese(II) forms relatively weak complexes with most ligands, including the amino acids [76—79]. Studies of the latter are further complicated by the fact that most of the neutral amino acids form 2 : 1 (ligand : Mn) uncharged complexes which are insoluble in most common solvents.

The preparation of L-cysteine and dipeptide Mn(II) complexes and their subsequent oxidation has been discussed recently [80]. However, the oxidation products were not characterized with respect to manganese oxidation state and stoichiometry. The green Mn(II)(L-cysteine)$_2$ complex is oxidized to a red-violet complex (probably Mn(III)) on exposure to an oxygen atmosphere in methanol solution. The red-violet complex decomposes rapidly to form a white precipitate. The latter was concluded to be a binuclear complex with a disulfido bridge, (cys)Mn(II)(cys—S—S—cys)Mn(II)(cys) [80]. Spectrophotometric studies of other mercaptoamine and mercaptocarboxylate complexes indicate that Mn(III) probably coordinates with the sulfide group. The intense absorption bands ($\epsilon = 10^3$—10^4) in the visible region (600—700 nm) of the spectrum appear to be due to Mn(III)—S charge transfer bands [81].

The Mn(II)(his)$_2$ (his = histidine) complex also catalyzes the decomposition of H_2O_2 in a manner that resembles the catalase reaction [82]. This reaction, like the Mn(II)(phen)$_2$ catalyzed reaction, proceeds via a radical chain mechanism that probably involves HO· and HO_2· radicals. Although an Mn(I) intermediate was proposed [82], redox potential considerations for H_2O_2 and analogous Mn(II) complexes indicate that Mn(III)(his)$_2$ is a more likely intermediate that is consistent with the kinetic results.

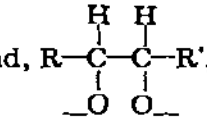
(iv) Oxygen donor ligands

Manganese complexes with oxygen donor ligands have been studied extensively. The Mn(III) complexes with several di- and polycarboxylic acids decompose readily via oxidation of the ligands; examples include the Mn(III) [83,84] and Mn(IV) [84] oxalate complexes. A mechanism that involves the CO_3^- radical has been proposed to account for the products and kinetics [83]. The decomposition of Mn(III) complexes with malonate [85] and tartrate [86] also have been investigated.

Asada and coworkers [87] have shown that the Mn(II) pyrophosphate ($H_2P_2O_7^{2-}$) complex is oxidized to the Mn(III) complex by O_2^-. The latter complex reacts with the H_2O_2 that is produced to yield O_2, but it apparently does not oxidize O_2^-. A recent report [88] indicates that Mn(II) forms a transient complex with O_2^- which is characterized by a red shift in the absorption maximum of O_2^- from 245 to 270 nm. However, the increase in molar absorptivity in the UV region of the spectrum and the presence of an absorption maximum around 420 nm indicate that this transient is rapidly converted to an Mn(III) species (cf. ref. 53).

That the Mn(II) ion inhibits lipid peroxidation [89,90] infers that O_2^- may be involved in the peroxidation mechanism. In the presence of Fe(III)—ADP, Mn(II) and superoxide dismutase accelerate lipid peroxide formation. This accelerative effect may be due to an initial reduction of Fe(III) to Fe(II) by O_2^-, followed by a "Fenton's reaction" whereby H_2O_2 (formed by Mn(II) or the SOD catalyzed dismutation of O_2^-) and Fe(II) produce ·OH. The latter would be the radical chain initiator for lipid peroxidation [90].

During the past four years electrochemical, spectrophotometric, and magnetic studies of the complexes that are formed by an extensive group of polyhydroxy ligands with manganese(IV), -(III), and -(II) ions have been made to obtain an understanding of their coordination chemistry [91—93]. A subsequent investigation [94] has made use of circular dichroic and spectrophotometric studies to characterize the equilibria and coordination chemistry of the Mn(III) and Mn(IV) complexes that are formed with D-glucarate ion, D-gluconate ion, L-tartrate ion, sorbitol, and *meso*-erythritol. These studies establish that polyhydroxy ligands form stable complexes with manganese ions in alkaline media and effectively solubilize the Mn(III) and Mn(IV) oxidation states. The complexes are the result of strong "hard acid—hard base" interactions between the strong Lewis acid metal ions and the oxo anions of the ligands. Formation of five-membered chelate rings by vicinal deprotonation of the



ligand, R—C(H)(O)—C(H)(O)—R', further stabilizes the complexes. The comparable stability of the Mn(III) and Mn(IV) complexes [93,94] that are formed by sorbitol, glucarate ion, and gluconate ion confirms that coordination is by the vicinal-secondary dioxo function of the ligand and does not involve the carboxylate

function. Such dioxo chelation competes effectively with hydroxide ion for the metal binding sites to give stable, soluble complexes. Hence, the dioxo anions represent the equivalent of "polyhydroxide ions" that yield soluble Mn(III) and Mn(IV) complexes because of their associated organic functionality.

Almost all the aliphatic polyhydroxy ligands that have been investigated [93] form two different Mn(III) complexes. Polarographic, circular dichroic, and spectrophotometric measurements [91,93,94] have established that the ligand-to-manganese stoichiometry is 2 : 1 for the $Mn(III)_A$ complex and 3 : 1 for the $Mn(III)_B$ complex. The magnetic moments for the $Mn(II)_B$ and Mn(IV) gluconate complexes [91] are close to their spin-only values, and, hence, are indicative of mononuclear complexes. In contrast, the magnetic moment for the $Mn(III)_A$ gluconate complex is significantly lower than the theoretical value, and indicates spin pairing within a binuclear complex.

On the basis of these observations as well as additional recent studies of Mn(III) and -(IV) complexes formed with sorbitol and *meso*-erythritol [94], the coordination chemistry of aliphatic polyhydroxy ligands (R—CH(OH)CH-(OH)—R') with manganese ions and the equilibria and redox reactions of the resulting complexes can be summarized by the expressions in Table 2A. In the case of the dimer—monomer equilibrium for Mn(III), the equilibrium constant when gluconate ion is the ligand is 3.13 M^{-1} at 25°C. The redox potentials which are included are specifically for the gluconate complexes, but all of the polyhydroxy complexes yield similar values in aqueous alkaline media.

The Mn(II) gluconate complex can be oxidized to the Mn(III) and Mn(IV) complexes in alkaline media with stoichiometric amounts of potassium ferricyanide [91]. In an oxygen atmosphere, the Mn(II) gluconate dimer complex is rapidly oxidized ($k = 10^4$ M^{-1} s^{-1}) to the Mn(III) dimer (see Table 2B), which reacts further with O_2, but more slowly ($k = 40$ M^{-1} s^{-1}), to form an Mn(IV) complex which probably incorporates a bridging peroxide ligand. This reaction is reversed under less alkaline conditions to yield an Mn(II) complex and molecular oxygen [92]. The Mn(III) polyhydroxy complexes are also oxidized by molecular oxygen, but the process is slower and can be reversed by lowering the pH [92]. Although the binuclear form of the Mn(III) gluconate appears to be the reactive species with O_2 under strongly alkaline conditions, detailed studies of the Mn(III)—sorbitol complexes indicate that the mononuclear tris(sorbitol dianion)Mn(III) complex may be the more reactive at pH 12 [94]. For this system the reaction with O_2 appears to be second-order in $[MnL_3^{3-}]$ and first order in P_{O_2}; at 25°C the approximate rate constant, k, is 50 M^{-1} atm^{-1} s^{-1}.

Hydrogen peroxide also oxidizes Mn(II) polyhydroxy complexes to the Mn(III) complexes by a process which is first order in H_2O_2 and Mn(II). The peroxide ion reacts with the Mn(II) complexes in a manner analogous to "Fenton's reagent" to yield the Mn(III) complex and $O^{\cdot-}$ [92]. The latter radical either reacts with the ligand or oxidizes a second Mn(II) (see Table 2B).

TABLE 2

Complexation reactions of manganese ions by polyhydroxy ligands (R—CH(OH)CH-(OH)—R'), the equilibria and redox reactions of the resulting complexes, and the interactions of these complexes with O_2 and H_2O_2 in alkaline media [a].

A. Complexation reactions, equilibria, and redox reactions [b,c]

$$Mn(II) + 2\ H_2L + 4\ OH^- \rightleftharpoons Mn^{II}L_2^{2-} + 4\ H_2O$$

$$2\ Mn^{II}L_2^{2-} + 2\ OH^- \rightleftharpoons [(Mn^{II}L_2)(OH)_2(Mn^{II}L_2)]^{6-}$$

$$[(Mn^{II}L_2)(OH)_2(Mn^{II}L_2)]^{6-} \rightleftharpoons [(Mn^{III}L_2)—O—(Mn^{III}L_2)]^{4-} + H_2O + 2e^-$$

$E^{0'} = -0.30$ V vs. NHE

$$[(Mn^{III}L_2)—O—(Mn^{III}L_2)]^{4-} + 2\ H_2L + 2\ OH^- \rightleftharpoons 2\ Mn^{III}L_3^{3-} + 3\ H_2O$$

$K = 3.13\ M^{-1}$ (L = gluconate)

$$Mn^{III}L_3^{3-} \rightleftharpoons Mn^{IV}L_3^{2-} + e^- \qquad E^{0'} = -0.04\ V$$

$$Mn^{III}L_3^{3-} + e^- + H_2O \rightarrow \tfrac{1}{2}\,[(Mn^{II}L_2)(OH)_2(Mn^{II}L_2)]^{6-} + HL^- \qquad E_{p,c} = -0.83\ V$$

$$[(Mn^{II}L_2)(OH)_2(Mn^{II}L_2)^{6-} + 4\ H_2O + 4\ e^- \rightarrow 2\ Mn + 4\ HL^- + 6\ OH^- \qquad E_{p,c} = -1.49\ V$$

B. Reactions and equilibria with O_2 and H_2O_2 [b,c]

$$[(Mn^{II}L_2)(OH)_2(Mn^{II}L_2)]^{6-} + O_2 \rightarrow [(Mn^{III}L_2)—O—(Mn^{III}L_2)]^{4-} + H_2O_2$$

$k = 2.8 \times 10^4\ M^{-1}\ s^{-1}$

$$[(Mn^{III}L_2)—O—(Mn^{III}L_2)]^{4-} + O_2 + H_2O \rightarrow [(Mn^{IV}L_2)—O—O—(Mn^{IV}L_2)]^{2-} + OH^-$$

$k = 4 \times 10^1\ M^{-1}s^{-1}$

$$(Mn^{III}L_3^{3-})_2 + O_2 + 2\ H_2O \rightleftharpoons [(Mn^{IV}L_2)—O—O—(Mn^{IV}L_2)_2]^{2-} + 2\ HL^- + 2\ OH^-$$

$k = 5 \times 10^1\ M^{-1}\ s^{-1}$; $K = 5.25\ atm^{-1}$

$$Mn^{II}L_2^{2-} + H_2O_2 \rightarrow Mn^{III}L_2(OH)^{2-} + \cdot OH$$

$$\cdot OH + H_2L \rightarrow H_2L\cdot + H_2O$$

$$2\ H_2L\cdot \rightarrow H_2L—LH_2$$

$$\cdot OH + Mn^{II}L_2^{2-} + HL^- \rightarrow Mn^{III}L_3^{3-} + H_2O$$

$$H_2L\cdot + Mn^{III}L_2(OH)^{2-} \rightarrow HLCHO + Mn^{II}L_2^{2-} + H_2O$$

[a] Conditions for the redox potentials are 1 mM manganese ion, 0.1 M gluconate ion, and 0.3 M NaOH. [b] L represents the dioxo dianion ligand (R—CH(—O—)—CH(—O—)—R') from vicinal deprotonated polyhydroxy molecules.

[c] In the case of Mn(II) the R of the ligand must be carboxylate ion to result in soluble complexes.

Aromatic polyhydroxy ligands such as the catechols form stable Mn(II) and -(III) complexes under alkaline conditions [93]. Because such ligands are more easily oxidized than gluconate, Mn(IV) complexes are not formed. Such ligand oxidation results in the formation of a tris(semiquinone)Mn(III) com-

plex which is more stable the tris(catecholato)Mn(III) complex. The semiquinone complex acts as a four-electron oxidizing agent.

Studies of the tris(3,5-di-tert-butylcatecholato)Mn(III) complex recently have been extended to aprotic media (dimethyl sulfoxide (DMSO), dimethyl formamide and acetonitrile) [20,21]. Oxidation of one of the ligands to a semiquinone (either by O_2 or $Fe(CN)_6^{3-}$) results in a species that reversibly binds molecular oxygen. In DMSO the $P_{1/2}$ value is approximately 0.2 atm. The system is especially intriguing because it involves a "quinoidal" radical and represents a model for the terminal step of the water oxidation process by the manganese cofactor of photosystem II (vide supra, see Fig. 2).

(v) Sulfur donor ligands

The chemistry of manganese complexes with sulfur donor ligands is much less understood than that for oxygen and nitrogen donor ligand complexes. This is due mainly to the facile oxidation of sulfur in such ligands. The X-ray crystal structure for the tris(N,N-diethyldithiocarbamato)Mn(III) complex indicates substantial distortion in the Mn coordination sphere as well as in the ligands [95]. An electrochemical investigation of a series of N,N-disubstituted dithiocarbamate (DTC) complexes in non-aqueous media indicates transient stability for the Mn(II), -(III), and -(IV) oxidation states [96]. Several Mn(IV) DTC complexes also have been isolated and characterized. There is not any evidence for manganese binding to sulfur donor groups in metalloproteins, but the chemistry of this class of complexes is interesting. The chemical similarity of Fe(III) and Mn(III) and the biological importance of iron—sulfur systems should prompt further investigation of manganese complexes with sulfur ligands.

D. REDOX CHEMISTRY OF MANGANESE COMPLEXES IN APROTIC MEDIA

Although Table 2A provides a reasonable indication of the oxidation—reduction potentials ($E^{0'}$) for the polyhydroxy complexes of manganese ions in aqueous solutions, it does not provide insight into the dramatic effect of aprotic media upon the redox chemistry for manganese coordination compounds. Whereas, an aqueous medium provides a leveling of electrostatic effects upon $E^{0'}$ values, aprotic media allow such effects to have a maximum influence. Because membrane bound manganese cofactors in biological systems probably experience an environment that is somewhat aprotic, the redox properties of manganese complexes in aprotic media should be more relevant to such biological manganese systems. Table 3 summarizes the oxidation—reduction potentials for a number of manganese coordination complexes in dimethyl sulfoxide and acetonitrile [20,71,75,97,98]. Although the tris(phen)Mn(III) and tris(bipyr)Mn(III) complexes are only transiently stable, their highly positive reduction potentials illustrate the effect of electrostatic

TABLE 3

Oxidation—reduction potentials for manganese coordination complexes in aprotic media (dimethyl sulfoxide (DMSO) or acetonitrile (AN) with 0.1 M tetraethylammonium perchlorate as supporting electrolyte)

Reaction [b]	$E^{0\prime}$(V vs. NHE) [a]
$Mn^{II}(8\text{-}Q)_2 \rightleftharpoons Mn^{III}(8\text{-}Q)_2^+ + e^-$	+0.38 (DMSO)
$Mn_2^{II}O(8\text{-}Q)_4(8\text{-}QH)_2 + 2\,e^- \rightleftharpoons 2\,Mn^{II}(8\text{-}Q)_3^- + H_2O$	+0.01 (DMSO)
$Mn_2^{II}O(8\text{-}Q)_4(8\text{-}QH)_2 \rightleftharpoons Mn_2^{IV}O(8\text{-}Q)_4(8\text{-}QH)_2^{2+} + 2\,e^-$	+0.97 (AN)
$Mn^{II}(phen)_3^{2+} \rightleftharpoons Mn^{III}(phen)_3^{3+} + e^-$	+1.52 (AN)
$Mn^{II}(bipy)_3^{2+} \rightleftharpoons Mn^{III}(bipy)_3^{3+} + e^-$	+1.60 (AN)
$Mn_2^{IV}O_2(phen)_4^{4+} + e^- \rightleftharpoons Mn_2^{III,IV}O_2(phen)_4^{3+}$	+1.58 (AN)
$Mn_2^{IV}O_2(bipy)_4^{4+} + e^- \rightleftharpoons Mn_2^{III,IV}O_2(bipy)_4^{3+}$	+1.57 (AN)
$Mn_2^{III,IV}O_2(phen)_4^{3+} + e^- \rightleftharpoons Mn_2^{III}O_2(phen)_4^{2+}$	+0.71 (AN)
$Mn_2^{III,IV}O_2(bipy)_4^{3+} + e^- \rightleftharpoons Mn_2^{III}O_2(bipy)_4^{2+}$	+0.62 (AN)
$Mn^{III}(bipyO_2)_3^{3+} \rightleftharpoons Mn^{IV}(bipyO_2)_3^{4+} + e^-$	+1.86 (AN)
$Mn^{III}(bipyO_2)_3^{3+} + e^- \rightleftharpoons Mn^{II}(bipyO_2)_3^{2+}$	+1.11 (AN)
$Mn^{III}(Cat)_3^{3-} \rightarrow Mn^{III}(Cat)_2(Cat\cdot)^{2-} + e^-$	+0.44 ($E_{p,a}$) (DMSO)
$Mn^{III}(DTC)_3 + e^- \rightleftharpoons Mn^{II}(DTC)_3^-$	+0.21 (AC) [c]
$Mn^{III}(DTC)_3 \rightleftharpoons Mn^{IV}(DTC)_3^+ + e^-$	+0.69 (AC) [c]

[a] $E^{0\prime}$ values represent the mean of the cathodic and anodic peak potentials ($E_{p,c}$ and $E_{p,a}$, respectively) that have been obtained at a platinum electrode at a scan rate of 0.1 V s^{-1}. [b] Ligands; 8-quinolinate anion (8-Q), 1,10-phenanthroline (phen), 2,2′-bipyridyl (bipy), 2,2′-bipyridyl 1,1′-dioxide (bipy O_2), 3,5-di-tert-butylcatecholate dianion (Cat), N,N-diethyldithiocarbamate (DTC). [c] A—C measurements in acetone with 0.1 M TEAP [97].

charge [97]. An even more dramatic example is provided by the (III)/(IV) and (III)/(II) couples for the TRIS 2,2′-bipyridyl-1,1′-dioxide complexes [99]. Clearly, charge and solvent medium have as much effect on manganese redox potentials as oxidation state or donor groups. Reference to Table 3 indicates that the redox potential for a given couple increases by +0.3—+0.7 V per increase in positive charge on the manganese complex. Similar effects should exist for membrane-bound manganese cofactors in biological systems.

E. DISCUSSION

The chemistry of biological manganese is only beginning to be understood. Further studies of the coordination chemistry of manganese should provide useful insight into the functional role of this transition metal in biological systems. Useful models for the superoxide dismutase reaction have evolved in

recent years as a result of the work done with manganese complexes [42,75]. However, the inability of the Mn(II)8-quinolinol model to provide adequate protons for formation of H_2O_2 from O_2^- in aprotic media is a serious deficiency. Because protons rapidly promote the disproportionation of O_2^- [45], this is a dichotomy.

The similarity of the coordination chemistry for iron and manganese ions indicates that the properties of manganese model complexes can be usefully extrapolated to iron-containing biological systems. Studies of Mn—porphyrins and Mn—hemoglobin have provided useful insight into the coordination chemistry of hemoglobin. That Mn(II)—phenanthroline and bipyridine complexes exhibit catalase activity may also provide insight into the structure—function relationships for this class of enzyme.

The environment and functional role of manganese in the oxygen evolving apparatus of green-plant photosynthesis remains elusive. Model systems which mimic the oxygen evolution reaction are being actively sought, but an effective model has not yet been developed. The experimental evidence strongly implicates water oxidation in photosystem II [18], although the immediate precursor may be other than two water molecules and could involve other inorganic or organic intermediates.

Although manganese appears to be a critical constituent in the brain and central nervous system [47—51], its function in those systems is not understood. Recent work with manganese catechol complexes in aprotic media indicates that they reversibly bind molecular oxygen [20], but the mechanism has yet to be elucidated. These studies have been extended to Mn—catecholamine complexes to ascertain whether these complexes also are capable of carrying oxygen. If so, there may be a direct interaction of manganese with the catecholamines in the brain, and the resultant complexes may play a role in oxygen transport or metabolism in the brain. That there is a secondary interaction between Mn and catecholamine levels in the brain has already been demonstrated [100].

The application of manganese model systems is far reaching. Because manganese has a unique redox function in several biological systems, it also may have useful applications in oxygen activation for oxidations, in solar energy conversion, or in treatment of various metabolic disorders. Hopefully, this review has summarized the coordination chemistry of manganese as it pertains to biological systems, what is known about the biological systems which require this metal, and what its function is in these systems.

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REFERENCES

1 G.C. Cotzias, Physiol. Rev., 38 (1958) 503.
2 E.J. Underwood, Trace Elements in Human and Animal Nutrition, 4th Edn., Academic Press, New York, 1977, p. 170.
3 M.C. Scrutton, M.F. Utter and A.S. Mildvan, J. Biol. Chem., 241 (1966) 3480.
4 B.B. Keele, J.M. McCord and I. Fridovich, J. Biol. Chem., 245 (1970) 6176.
5 M.J.C. Crabbe, R.D. Waight, W.G. Bardsley, R.W. Barker, I.O. Kelly and P.F. Knowles, Biochem. J., 155 (1976) 679.
6 G.M. Cheniae and I.F. Martin, Biochim. Biophys. Acta, 197 (1970) 219.
7 M.F. Utter, R.E. Barden and B.L. Taylor, Advan. Enzymol., 42 (1975) 1.
8 C.-H. Fung, A.S. Mildvan, A. Allerhand, R. Komoroski and M.C. Scrutton, Biochemistry, 12 (1973) 620.
9 R. Radmer and G.M. Cheniae, in Primary Processes, in press.
10 R.L. Heath, Int. Rev. Cytol., 34 (1973) 49.
11 A.J. Bearden and R. Malkin, Q. Rev. Biophys., 7 (1975) 131.
12 G.M. Cheniae and I.F. Martin, Plant Physiol., 44 (1969) 351.
13 B. Kok, B. Forbush and M. McGloin, Photochem. Photobiol., 11 (1970) 457.
14 J.T. Warden, R.E. Blankenship and K. Sauer, Biochim. Biophys. Acta, 423 (1976) 462.
15 T. Wydrzynski, N. Zumbulyadis, P.G. Schmidt and Govindjee, Biochem. Biophys. Acta, 408 (1975) 349.
16 T. Wydrzynski, Govindjee, N. Zumbulyadis, P.G. Schmidt and H.S. Gutowsky, Magnetic Resonance in Colloid and Interface Science, ACS Symposium Ser., 34 (1977) 471.
17 H. Metzner, J. Theor. Biol., 51 (1975) 201.
18 T. Arnason and J. Sinclair, Biochim. Biophys. Acta, 449 (1976) 581.
19 H. Metzner (Ed.), Photosynthetic Oxygen Evolution, Academic Press, New York, 1978.
20 K.D. Magers, C.G. Smith and D.T. Sawyer, J. Am. Chem. Soc., 100 (1978) 989.
21 K.D. Magers, C.G. Smith and D.T. Sawyer, Inorg. Chem., submitted for publication.
22 B.G. Malmstrom, Univ. of Göteborg, Sweden, private communication.
23 B. Chance, C. Saronio and J.S. Leigh, Jr., Proc. Nat. Acad. Sci. U.S.A., 72 (1975) 1635.
24 J.M. McCord and I. Fridovich, J. Biol. Chem., 243 (1968) 5753.
25 J.M. McCord and I. Fridovich, J. Biol. Chem., 244 (1969) 6049.
26 A.M. Michelson, J.M. McCord and I. Fridovich (Eds.), Superoxide and Superoxide Dismutases, Academic Press, New York, 1977.
27 G. Rotilio, L. Morpurgo, C. Giovagnoli, L. Calabrese and B. Mondovi, Biochemistry, 11 (1972) 2187.
28 J.A. Fee, A.M. Michelson, J.M. McCord and I. Fridovich (Eds.), Superoxide and Superoxide Dismutases, Academic Press, New York, 1977, p. 173.
29 F.J. Yost and I. Fridovich, J. Biol. Chem., 248 (1973) 4905.
30 K. Asada, K. Yoshikawa, M. Takahashi, Y. Maeda and K. Enmanji, J. Biol. Chem., 250 (1975) 2801.
31 J. Lumsden, R. Cammack and D.O. Hall, Biochim. Biophys. Acta, 438 (1976) 380.
32 R.A. Weisiger and I. Fridovich, J. Biol. Chem., 248 (1973) 3582.
33 S.D. Revindrath and I. Fridovich, J. Biol. Chem., 250 (1975) 6107.
34 C.J. Brock, J.I. Harris and S. Sato, J. Mol. Biol., 107 (1976) 175.
35 D.E. Ose and I. Fridovich, J. Biol. Chem., 251 (1976) 1217.
36 C.J. Brock and J.I. Harris, Biochem. Soc. Trans., 5 (1977) 1537.
37 J. Bridgen, J.I. Harris and F. Northrop, FEBS Lett., 49 (1975) 392.
38 K. Klug-Roth, I. Fridovich and J. Rabani, J. Am. Chem. Soc., 95 (1973) 2786.
39 E.M. Fielden, P.B. Roberts, R.C. Bray, D.J. Lowe, G.N. Mantner, G. Rotilio and L. Culabrese, Biochem. J., 139 (1974) 49.
40 F. Lavelle, M.E. McAdam, E.M. Fielden, P.B. Roberts, K. Puget and A.M. Michelson, Biochem. J., 161 (1977) 3.
41 M.E. McAdam, R.A. Fox, F. Lavelle and E.M. Fielden, Biochem. J., 165 (1977) 71.

42 J.A. Fee and G.J. McClune, in T.P. Singer and R.N. Ondarze (Eds.), Mechanisms of Oxidizing Enzymes, Elsevier, New York, in press.
43 G.D. Lawrence and D.T. Sawyer, Biochemistry, submitted for publication.
44 H.J. Forman and I. Fridovich, Arch. Biochem. Biophys., 158 (1973) 396.
45 D. Behar, G. Czapski, J. Rabini, L.M. Dorfman and H.A. Schwarz, J. Phys. Chem., 74 (1970) 3209.
46 D.T. Sawyer, M.J. Gibian, M.M. Morrison and E.T. Seo, J. Am. Chem. Soc., 100 (1978) 627.
47 Manganese — Medical and Biological Effects of Atmospheric Pollutants, Committee on Biological Effects of Atmospheric Pollutants, Nat. Acad. Sci., Washington, D.C., 1973.
48 L.L. Hurley, D.E. Wooley, F. Rosenthal and P.S. Timiras, Am. J. Physiol., 204 (1963) 493.
49 Y. Tanaka and E.R. Harpur, ACS Abstracts of Papers, 174th Meeting, Biol., (1977) 130.
50 G.C. Cotzias, S.T. Miller, P.S. Paperasiliou and L.C. Tang, Med. Clin. North Am., 60 (1976) 729.
51 G.C. Cotzias, P.S. Paparasiliou, I. Mena, L.C. Tang and S.T. Miller, Adv. Neurol., 5 (1974) 235.
52 R. Dingle, Acta Chem. Scand., 20 (1966) 33.
53 G. Davies, Coordin. Chem. Rev., 4 (1969) 199.
54 J.A. Elvidge and A.B.P. Lever, Proc. Chem. Soc., London, (1959) 195.
55 L.H. Vogt, A. Zalkin and D.H. Templeton, Inorg. Chem., 6 (1967) 1725.
56 A.B.P. Lever, J.P. Wilshire and E. Quan, J. Am. Chem. Soc., 1979, in press.
57 G. Engelsma, A. Yamamoto, E. Markham and M. Calvin, J. Phys. Chem., 66 (1962) 2517.
58 a) K. Uchida, M. Soma, S. Naito, T. Onishi and K. Tamara, Chem. Lett., (1978) 471; b) F. Hirata, T. Ohnishi and O. Hayaishi, in O. Hayaishi and K. Asada (Eds.), Biochemical and Medical Aspects of Active Oxygen, University Park Press, Tokyo, 1977, p. 101.
59 B. Gonzalez, J. Kouba, S. Yee, C.A. Reed, J. Kirner and W.R. Scheidt, J. Am. Chem. Soc., 97 (1975) 3247.
60 a) C.J. Weschler, B.M. Hoffman and F. Basolo, J. Am. Chem. Soc., 97 (1975) 5278; b) B.M. Hoffman, C.J. Weschler and F. Basolo, J. Am. Chem. Soc., 98 (1976) 5473.
61 R.D. Jones, D.A. Summerville and F. Basolo, J. Am. Chem. Soc., 100 (1978) 4416.
62 A. Dedien and M.M. Rohmer, J. Am. Chem. Soc., 99 (1977) 8050.
63 C. Bull, R.G. Fisher and B.M. Hoffman, Biochem. Biophys. Res. Commun., 59 (1974) 140.
64 S.K. Cheung, C.J. Grimes, J. Wong and C.A. Reed, J. Am. Chem. Soc., 98 (1976) 5030.
65 T. Yarino, T. Matsushita, I. Masuda and K. Shinra, Chem. Commun., (1970) 1317.
66 C.C. Patel and C.P. Prabhakoran, J. Inorg. Nucl. Chem., 31 (1969) 3316.
67 L.J. Boucher and M.O. Farrell, J. Inorg. Nucl. Chem., 35 (1973) 3731.
68 L.J. Boucher and C.G. Coe, Inorg. Chem., 15 (1976) 1334.
69 P.M. Plaksin, R.C. Stouter, M. Mathew and G.J. Palenik, J. Am. Chem. Soc., 94 (1972) 2121.
70 R.S. Nyholm and A. Turco, Chem. Ind. (London), (1960) 74.
71 M.M. Morrison and D.T. Sawyer, J. Am. Chem. Soc., 99 (1977) 257.
72 S.R. Cooper and M. Calvin, J. Am. Chem. Soc., 99 (1977) 6623.
73 Ya. D. Tiginyanu, A. Ya. Sychev and V.M. Berdnikov, Russ. J. Phys. Chem., 45 (1971) 1723.
74 Ya. D. Tiginyanu, A. Ya. Sychev and V.M. Berdnikov, Russ. J. Phys. Chem., 45 (1971) 1434.
75 J.K. Howie and D.T. Sawyer, J. Am. Chem. Soc., 98 (1976) 6698.
76 A. Albert, Biochem. J., 50 (1952) 690.
77 C.B. Monk, Trans. Faraday Soc., 47 (1951) 297.
78 H. Kroll, J. Am. Chem. Soc., 74 (1952) 2034.
79 C.B. Murphy and A.E. Martell, J. Biol. Chem., 226 (1957) 37.

80 H. Guntner and K.E. Schwarzhans, Z. Naturforsch. B, 31 (1976) 448.
81 G.D. Lawrence, unpublished results, 1977.
82 A.Ya. Sychev and V.G. Isak, Russ. J. Inorg. Chem., 47 (1973) 335.
83 H. Taube, J. Am. Chem. Soc., 70 (1948) 1216.
84 T. Uehiro, I. Taminaga and Y. Yoshino, Bull. Chem. Soc. Jpn., 48 (1975) 2809.
85 J.I. Bullock, M.M. Patel and J.E. Salmon, J. Inorg. Nucl. Chem., 31 (1969) 415.
86 T.N. Srivastava, Z. Phys. Chem. (Leipzig), 209 (1958) 22.
87 Y. Kono, M. Takahashi and K. Asada, Arch. Biochem. Biphys., 174 (1976) 454.
88 B.H.J. Bielski and F.C. Chan, J. Am. Chem. Soc., 100 (1978) 1920.
89 R.L. Heath and L. Packer, Arch. Biochem. Biophys., 125 (1969) 850.
90 K.L. Fong, P.B. McCoy, J.L. Power, B.B. Keele, J. Biol. Chem., 248 (1973) 7792.
91 M.E. Bodini, L.A. Willis, T.L. Riechel and D.T. Sawyer, Inorg. Chem., 15 (1976) 1538.
92 M.E. Bodini and D.T. Sawyer, J. Am. Chem. Soc., 98 (1976) 8366.
93 K.D. Magers, C.G. Smith and D.T. Sawyer, Inorg. Chem., 17 (1978) 515. Ligands that were studied included triethanolamine, diethanolamine, glycolate ion, L-tartrate ion, *meso*-tartrate ion, glycerate ion, mannitol, sorbitol, inositol (hexahydroxycyclohexane), *meso*-erythritol, tartronate ion, ethylene glycol, glycerol, 2,3-butenediol, D-gluconate ion, D-glucarate ion, catechol, 1,2-dihydroxybenzene-3,5-disulfonate ion, 3,5-di-tert-butylcatechol, 2,3-dihydroxybenzoate, tetrafluorocatechol, 4,5-dihydroxynaphthalene-2,7-disulfonate ion and salicylate ion.
94 D. Richens, C.G. Smith and D.T. Sawyer, Inorg. Chem., 1979, in press.
95 P.C. Healy and A.H. White, J. Chem. Soc. Dalton Trans., (1972) 1883.
96 A.R. Hendrickson, R.L. Martin and N.M. Rohde, Inorg. Chem., 13 (1974) 1933.
97 M.M. Morrison and D.T. Sawyer, Inorg. Chem., 17 (1978) 333.
98 M.M. Morrison and D.T. Sawyer, Inorg. Chem., 17 (1978) 338.
99 A.R. Hendrickson, R.L. Martin and N.M. Rohde, Inorg. Chem., 13 (1974) 1933.
100 E. Bonilla, M. Diez-Ewald and J. Medrano, J. Pharm. Pharmacol., 26 (1974) 261.