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IRON AND IRON DERIVED RADICALS

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It is well established that iron plays an important role in initiating and catalyzing a variety of free radical reactions that contribute to oxygen-dependent tissue injury. Mechanisms of iron involvement in the underlying processes are not fully elucidated, but there is a growing consensus that oxidative tissue damage is supported by submicromolar to micromolar levels of nonheme cellular iron, often mobilized from ferritin stores (1,2). Although an obligatory requirement for iron catalysis was disputed until recently (1,3), cyclical Fenton-like reactions with H_2O_2 to produce extremely reactive and potentially toxic hydroxyl radicals ($OH^{\bullet}$) are now recognized to be the driving force initiating cascades of cytotoxic free radical reactions.[2] We consider here three questions regarding iron's participation in oxygen radical reactions leading to tissue injury, two of them dealing with iron and lipid oxidation.

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1. Operated for the U.S. Department of Energy under Contract No. DE-AC-02-76CH00016.

2. The Fenton reaction is

   $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^{-}$.

   We use the term "Fenton-like" to refer to similar reactions of complexed or chelated iron. Fenton "cycling" occurs when the ferric product is reduced back to the ferrous form by superoxide radical or other one-electron reductants:

   $Fe^{3+} + (O_2^{\bullet}, AH^{\bullet}, DH_2) \rightarrow Fe^{2+} + {}^3O_2, A + H^{+}$, or $DH^{\bullet} + H^{+}$, respectively).

1. What is the role of valence in the initiation and/or catalysis of membrane lipid oxidation by iron?

While membrane lipid peroxidation is an important part of oxidative tissue injury, it can be an effect as well as a cause of reactions culminating in cytotoxicity (1,4,5). Initiation or amplification of the process by iron compounds has long been known, and the reactions responsible for modulation of lipid oxidation by iron are an active focus of current research. It is clear that OH• can initiate lipid peroxidation in homogeneous reaction systems (6) and that conditions consistent with Fenton-like generation of OH• often are associated with lipid peroxidation in vivo (5), but the precise nature of iron's role, if any, in initiating lipid oxidation remains elusive.

Lipid peroxidation is a well known free radical chain reaction which has no intrinsic metal requirement.[3] Nonetheless, iron compounds can increase the rate of lipid oxidation dramatically by converting lipid hydroperoxides (LOOH), the metastable products of the propagation cycle, to reactive alkoxyl or peroxyl radicals, and these can initiate new reaction chains, a process called chain branching (1,7).[4]

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3. Where L represents lipid, a representative initiating reaction is

   $LH + (OH^{\bullet} \text{ or } LO^{\bullet}) \rightarrow L^{\bullet} + (H_2O \text{ or } LOH)$,

   and the chain propagation cycle is

   $L^{\bullet} + O_2 \xrightarrow{\text{(very fast)}} LOO^{\bullet}$

   $LOO^{\bullet} + LH \xrightarrow{\text{(very slow)}} LOOH + L^{\bullet}$.

4. For example, where iron is assumed to be complexed but the ligands are not shown,

   $LOOH + Fe^{2+} \xrightarrow{\text{(fast)}} LO^{\bullet} + OH^{-} + Fe^{3+}$, and

In studying chain reactions in systems in which there are many potentially competing reactions for chain initiation, branching, and termination, one must take into account not only the relative but also the absolute concentrations of metals and other reactants because the balance between competing reactions depends strongly upon absolute concentrations. This is particularly critical when high concentrations of metals are used in experimental models of reaction systems which have access only to trace concentrations (i.e., fractional to a few micromolar) in their native states. Under such circumstances metals shift from being repeatedly cycled catalysts to serving as stoichiometric reactants, and they also may exhibit dominant

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$LOOH + Fe^{3+} \text{--(slow)--} > LOO^{\cdot} + H^{+} + Fe^{2+}$..

LO· and LOO· then can (re)initiate lipid peroxidation by reactions given previously.

chain termination behavior which is not seen at all at catalytic concentrations (8,9).

An important chain terminating reaction of ferrous complexes is

$Fe^{2+} + LOO^{\cdot} + H^{+} \text{ --(fast)--> } Fe^{3+} + LOOH,$

and other "antioxidant" actions of high levels of ferrous compounds may include

$Fe^{2+} + OH^{\cdot} + H^{+} \text{ --(very fast)--> } Fe^{3+} + H_2O,$

which reduces primary initiation reactions, and

$Fe^{2+} + LO^{\cdot} + H^{+} \text{ --(very fast)--> } Fe^{3+} + LOH,$

which inhibits chain branching. Increasing concentrations of H_2O_2 also can convert these same strong oxidants to less reactive species by way of

$H_2O_2 + OH^{\cdot} \text{ --(fast)--> } HO_2^{\cdot} + H_2O$ and

$H_2O_2 + LO^{\cdot} \text{ --> } HO_2^{\cdot} + LOH.$

When radicals, $L^{\cdot}$, can be oxidized by the ferric complexes present, high concentrations of the ferric species also can act as chain terminators because of

$Fe^{3+} + L^{\cdot} \text{ --> } Fe^{2+} + L^{+},$

with little competition from

$Fe^{3+} + H_2O_2 \text{ --(very slow)--> } HO_2^{\cdot} + Fe^{2+} + H^{+}.$

These considerations were largely overlooked in some recent investigations seeking to determine whether <u>initiation</u> of lipid peroxidation is caused by $OH^{\cdot}$ from Fenton reactions. Using brain synaptosomes in one case (10) and liposomes prepared from reconstituted microsomal lipids in another (11), these studies indicated that Fe^{3+}/Fe^{2+} ratios are critical determinants of iron pro-oxidant activity, and that this action appears to be unrelated to $OH^{\cdot}$ generation or decomposition of preformed lipid hydroperoxides. However,

the consequences of changing ferric:ferrous ratios were misattributed to effects on reaction initiation when, at concentrations of 200 micromolar (roughly 100 times physiological), chain branching and termination behavior dominates.

A second reaction characteristic that was insufficiently accounted for in these studies may have contributed further to underestimating the overwhelming dominance of chain propagating reactions relative to primary initiation reactions in determining the overall behavior of the lipid oxidation systems. Once lipid oxidation has commenced, the accumulating lipid hydroperoxides (LOOH) favorably compete with H_2O_2 for both ferrous and ferric iron because the reaction rates of iron in both valence states are much greater for LOOH (12) (see the chain branching reactions cited earlier). Only when iron interactions with the products of lipid oxidation are inhibited by separating the reactants (as by chelation with lipophobic EDTA) does the underlying primary initiation by OH$^{\bullet}$ become apparent (12).

Yet another factor must be taken into account when assessing the role of OH$^{\bullet}$ reactions with macromolecular or particulate targets: the extraordinarily high reactivity of OH$^{\bullet}$. Because of this, OH$^{\bullet}$ do not survive for more than a few collisions following their formation, and their reactions are intrinsically "site specific": i.e., characterized by the potential substrates for OH$^{\bullet}$ oxidation that are within but ten or a few tens of Angstroms of the loci of their production (1,13). This implies that Fenton-generated OH$^{\bullet}$ must react at the sites where iron is bound, and for attack on vesicles this means on the membrane surface or within the lipid environment. Fenton reactions forming OH$^{\bullet}$ from iron and H_2O_2 in the suspending medium are ineffective or may even be protective if the concentrations of H_2O_2 or of reactive reducing

equivalents are rate-limiting. This was clearly recognized in another study of metal effects on lipid oxidation wherein it was concluded that $O_2^{\cdot-}$ and H_2O_2 from xanthine oxidase plus iron bound to different complexing agents caused crosslinking of membrane proteins in erythrocyte ghosts through the intermediacy of site-specific OH$^{\cdot}$ production at the membrane surface (14).

Both sets of authors cited previously (10,11) concluded that OH$^{\cdot}$ initiation of lipid peroxidation had not occurred because interventions designed to alter Fenton-like generation of OH$^{\cdot}$ and actual measurements of OH$^{\cdot}$ fluxes in the suspending media correlated poorly or not at all with lipid oxidation. However, these conclusions cannot be accepted with confidence because there was insufficient appreciation that the characteristics of the primary initiation reactions were no longer reflected by the overall responses of their rapidly oxidizing, multibranched chain reacting systems to changing ratios of Fe^{3+} and Fe^{2+} or by the responses to OH$^{\cdot}$ scavengers or to amounts of OH$^{\cdot}$ formed in the fluid phase. All of these, to the extent they were effective at all, operated primarily by altering the reactions of Fe^{2+} and Fe^{3+} with chain-initiating LOOH or with chain-carrying LOO$^{\cdot}$.

Regardless of possible misinterpretations in terms of initiation reactions, the apparent requirements for Fe^{3+} are intriguing. At least to some extent they are illusory, because in both sets of experiments most protocols involved replacement of Fe^{2+} by Fe^{3+} with total concentrations of iron at an extremely unphysiological 200 micromolar. Hence the well known inhibition of iron-dependent oxidative chain reactions by excessive concentrations of either ferrous or ferric species (caused by their competition for chain-carrying peroxyl radicals or carbon-centered radicals, respectively (8,9), via the reactions given previously) could provide relief

from Fe^{2+} inhibition rather than actual stimulation by Fe^{3+} as the latter displaced the former from 200 micromolar levels.

To explain the apparent requirements for mixtures of valence states to optimize iron stimulation of lipid oxidation or to reconcile reaction kinetics apparently inconsistent with straightforward OH• chemistry, higher valence oxides of iron, such as ferryl (FeO^{2+} or $Fe(OH)_2^{2+}$) or ferrous peroxide ($Fe(H_2O_2)^{2+}$), have been invoked as the proximate reactants initiating chain reactions or causing lipid oxidation (8-11,15). Nevertheless, not until the past year (15) has spectroscopic identification been claimed for a putative nonheme ferryl reaction intermediate under physiological reaction conditions, and it was pointed out long ago that "kinetic analysis alone cannot produce direct proof for the existence and reactions of ferryl ion" (8). Even the chromophoric reaction intermediate recently detected by stopped flow spectrophotometry (15) in an alleged ferryl-producing reduction of H_2O_2 by iron(II) may be attributable to OH• instead. According to our analysis, a radical may have been formed on the organic ligand carrying the ferric product of the same Fenton reaction that gave rise to the OH•. This would represent site-specific back reaction of the OH• formed in the solvent cage of that reaction.

2. Can Fenton reactions occur within lipid phases to initiate lipid oxidation, or is attack of OH• or other radicals largely confined to the external membrane surface?

Up to this point we have commented, rather sceptically in some cases, on reports from other groups. Without undertaking to explain all the findings

reported,[5] we have expressed lack of conviction that a good case exists to reject Fenton-type OH• formation as the "primordial" initiator of lipid oxidation in the model systems studied, but we have not yet educed any positive experimental evidence in support of that notion. With that aim in mind, we reasoned that it was important to determine experimentally whether Fenton-like formation of OH• can occur in a lipid phase.

Although there are many reports of lipid oxidation initiated under conditions where Fenton-like reactions are thought to occur, it has been correctly noted that "the evidence that OH• are formed under conditions that exist biologically is indirect" (15). Furthermore, the shallow penetration of a lipid bilayer that would be expected before the highly electrophilic and indiscriminately reactive OH• extinguished themselves provides good reason to question whether OH• generated externally could penetrate to the hydrophobic membrane interior where the lipid chains are confined. There is evidence that iron on the membrane surface can lead to the production of <u>some</u> effective initiator of lipid peroxidation, however, because iron/ascorbate reactions that are prolific sources of OH• lead to liposome oxidation when the membrane's surface charge is neutral or negative and iron ions are not repelled from contact or binding. This does not occur when the charge is positive (16). It has also been concluded that most or all of the antioxidant ability of zinc ions to protect erythrocyte ghosts from undergoing lipid peroxidation in media containing soluble Fenton systems resides in the displacement of iron from membrane binding sites by zinc (14).

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5. Aspects of the measurement of OH• formation (Fig. 4) of the synaptosome study (10), however, strongly suggest unrecognized iron binding with site-specific OH• formation and autodestruction of salicylate, the substrate for the OH• assay that was used.

It was not feasible to confine strongly oxidizing Fenton systems within vesicles so that release to the medium secondary to lipid oxidation could be prevented. Although bulk lipids are weak surrogates for structurally organized membrane bilayers with hydrophobic centers of oriented lipids, exterior polar headgroups and, in cells, a further exterior thicket of glycoprotein filaments, they may provide experimental access to environments with hydrophobicity comparable to membrane interiors. Our earlier work (3), however, showed differences in the proticities of bulk lipids. In reacting with stilbene dibromide in aprotic media, superoxide acts as a strong nucleophile, causing dehydro-dehalogenation with the formation of a blue chromophore; but in protic environments superoxide is a weak electron transfer agent, and no color reaction occurs. By that criterion, bulk oleic acid is a weakly protic medium and its methyl ester and that of linoleic acid are aprotic (3). Although we assumed that the proticity of membrane interiors might resemble that of esters, we did not know, so we studied Fenton reactions in both bulk fatty acids and in lipid esters.

To have a Fenton reaction, hydrogen peroxide and ferrous iron, or ferric iron and a reducing agent, must be present. The questions to be answered, then, are 1) whether all the reactants can penetrate a membrane or a lipid phase, and 2) if so, whether the reaction "goes" in a lipid, hydrophobic environment.

We have shown that H_2O_2 diffuses through membranes (17), and our present research indicates that up to 10% of H_2O_2 in the aqueous phase partitions into bulk lipid. Iron salts and low molecular weight complexes partition from water into oleic acid at concentrations of about micromolar or less (Table 1). Although systematic studies of iron distribution as a function of iron

concentration have yet to be done, it is likely the data of Table 1 represent saturation values rather than partition coefficients.

When millimolar iron was present in the aqueous phase, the valence of the metal did not affect the solubility in lipid except for ADP chelates, where the ferric form was more lipophilic. As expected, EDTA and DTPA are only sparingly soluble in lipid. (In fact, EDTA washes can be used to demetal oils.) Optical absorption spectra indicate that iron ADP and DFO transfer as chelates to oleic acid. EDTA and DTPA concentrations were too low to measure.

To study Fenton reactions in lipid phases, we dissolved the spin traps DMPO or PBN[6] in neat fatty acids or esters, and separately partitioned H_2O_2, iron compounds, and hydroxylamine (as a lipid-soluble reducing agent when necessary) into the same lipids. All solutions and lipids were sparged and then maintained under argon. To start the reactions, aliquots from each of the lipid phases were mixed, and radical production was monitored by EPR. As we have reported (1,3), when Fenton reactions, either with Fe^{2+} or with Fe^{3+} and a reducing agent, were run in protic oleic acid, the well-known four-line 1:2:2:1 signal of the $OH^{\cdot}$ adduct was produced with DMPO. If oleic acid is incubated with iron before DMPO is added, the peroxyl radical is trapped (1,3), and if the Fenton reaction or iron incubation is allowed to proceed for some time, complex spectra are obtained with alkyl, alkoxyl, and peroxyl -- and in some cases also hydroxyl -- radical adducts. Thus the reaction system behaves as would be expected from classical chemistry of lipid oxidation.

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6. The abbreviations are DMPO, 5,5-dimethyl-1-pyrroline-N-oxide, and PBN, phenyl-t-butyl nitrone.

It is possible to follow the changes in OH• adduct concentrations with time by fixing the magnetic field on one of the center lines of the EPR signal, and we did this to follow Fenton-like reactions of ferric chloride: "free" and chelated by ADP, EDTA, and DTPA. Figure 1 shows that of the three chelates, ADP supported Fenton kinetics in oleic acid at rates approaching that of the ferric salt, while EDTA and DTPA chelates reacted much more slowly and provided lower total adduct concentrations. There are three possible explanations for this: 1) the absolute concentrations of chelates partitioned into the oleic acid are not the same (Table 1); 2) EDTA and DTPA are known to react rapidly with OH• and may have competed with DMPO for the hydroxyl radicals as they were formed close to the chelates; or 3) the redox characteristics of the three chelates may be different in oleic acid than in water. Probably all of these factors contribute to the observed behavior.

When the reactions were run in aprotic lauric, oleic, or linoleic methyl esters instead of the more protic acids, DMPO gave signals consisting almost entirely of lipid alkyl and sometimes peroxyl radical adducts, with weak, if any, contributions from hydroxyl radicals (Fig. 2). Especially with linoleate, the signals changed with time, indicating changes of radical species and varying proportions of different radicals (1). These experiments suggest either that the OH• adduct is not stable in aprotic media (and we are not aware of data which indicate this to be so) or that OH• reactions with aprotic lipids are much faster than those with protic lipids. This raises a more general question we are now investigating: what are the preferred targets for reaction with OH• in membranes, where most fatty acids are present as esters?

Have these results answered the question of whether Fenton reactions occur within lipid phases and initiate lipid oxidation? Clearly the chemistry occurs: all the "makings" for comparmentalized Fenton reactions are present in the model systems, and $OH^{\bullet}$ is produced. Oxidized species of lipid also can be spin trapped (Fig. 2), indicating that lipid oxidation occurs, and changing envelopes of spin-trapped lipid species over time (1) strongly suggest that the characteristic chain reactions of lipid peroxidation have been established with the traces of oxygen that remain. Quantification of this lipid oxidation currently is under way.

Hence intramembranous formation of $OH^{\bullet}$ also appears feasible *in vivo* provided that appropriately complexed nonheme iron is available and endogenous reducing sources can replace the hydroxylamine used in the models. However, whether chain propagation through lipid bilayers or free radical damage to embedded macromolecules will predominate in biological membranes depends upon the character of the nearby lipid and upon the microenvironment of the redox-active metal's binding site.

3. Can iron bound to desferrioxamine (DFO) support Fenton reactions?

Current dogma teaches that ferric iron bound to DFO to form ferrioxamine cannot participate in the reaction cycles that generate $OH^{\bullet}$ from H_2O_2. However, an apparently paradoxical enhancement of lung damage and mortality in an animal experiment designed to show DFO protection against oxidizing injury from the chemical toxin, paraquat (18), led us to re-evaluate DFO's redox chemistry in the presence and absence of traces of iron.

Although DFO itself showed no reduction wave by cyclic voltammetry on a glassy carbon electrode, its ferric chelate, ferrioxamine (FOA), was reduced

reversibly at $E^{o'}$ = -0.42 volts (vs. NHE, pH 7) (19). It is important to note that this is within the range of cellular reducing power, and, indeed, we found that under anoxic conditions FOA could be reduced stoichiometrically by paraquat radical and also by hypoxanthine/xanthine oxidase to a colorless product (19,20). Solutions of this reduced species reoxidized instantly upon exposure to air, regenerating orange-red FOA (absorption maximum at 430 nm) (19).

The low $E^{o'}$ suggested that the iron center in reduced FOA might directly reduce H_2O_2, affording a Fenton-like production of OH·. This was confirmed in two ways: 1) by monitoring disappearance of H_2O_2 with an oxidase-type electrode (see ref. 19) and 2) by observing the characteristic EPR spectrum of the OH· spin adduct of DMPO (19,20), as depicted by the uppermost tracing of Figure 3.

Other recordings in Figure 3 confirm the dynamic nature of reaction systems that present competitive pathways to highly reactive oxygen radical intermediates and how precariously their toxic potential may be balanced between excesses of either oxidizing or reducing capacity (21). The second spectrum shows an example of oxidizing capacity in excess: when an aliquot of anoxic, reduced FOA is added to an aerobic solution of H_2O_2 and DMPO, O_2 and H_2O_2 compete for its oxidation, and the production of OH· is markedly reduced. (With appropriate adjustments of reactant concentrations, one could arrange that OH· be altogether undetectable.) Insofar as OH· has a high cytotoxic potential when generated <u>in vivo</u>, this presents the apparent paradox of high oxygen tension serving as an <u>anti</u>oxidant in a reaction involving toxic oxygen radicals. The paradox is resolved upon recognizing that in this reaction system O_2 supports a detoxifying pathway.

The third tracing of Figure 3 was taken from a reaction wherein ethanol, as well as H_2O_2 and DMPO, was present in the solution added to reduced FOA, and it represents the superimposed spectra of two spin adducts of DMPO: that from OH• and that from the alpha-hydroxyethyl radical produced by reaction of OH• with ethanol. This helps confirm the presence of authentic OH• and also denotes the presence of a competitive reaction path for OH•. Even more instructive, however, is the absence of any signal when the reaction was run with a larger aliquot of reduced FOA. That is because excess reduced FOA scavenges oxidizing OH• and also reduces the spin adducts, which are nitroxide (aminoxyl) radicals, to hydroxylamines or even more reduced diamagnetic products. This illustrates reducing excess in the delicate balance cited in the previous paragraph (21).

Taking into account results like those discussed here plus some additional features of the experimental systems, we concluded that a pro-oxidant enhancement of oxidative cytotoxicity may occur when relatively high concentrations of DFO-chelated iron have access in vivo to mobile, strong reducing substances or to reducing enzymes under low oxygen tension (so that OH• production from H_2O_2 by reduced FOA can compete with its autoxidation) (20). Both hypoxia and elevated levels of the reducing enzyme xanthine oxidase exist following postischemic reperfusion, while localized hypoxia in the presence of very strongly reducing small molecules occurs during poisoning by paraquat and related compounds and in the presence of readily autoxidizable metabolites from various nitro compounds and other xenobiotics. Under these conditions, therefore, a biphasic antioxidant/pro-oxidant behavior of DFO as a function of dose is a real possibility (21), despite prevailing assumptions to the contrary. Such an outcome should be carefully considered when planning

treatment with DFO (19,20). Even in biochemical reactions, neither the failure of DFO to suppress an oxidative outcome nor the actual enhancement of such a response by DFO can be considered sufficient evidence to rule out an iron-dependent process without other supporting information (20).

CONCLUSIONS

We have discussed some reactions of iron and iron-derived oxygen radicals that may be important in the production or treatment of tissue injury. Our conclusions challenge, to some extent, the usual lines of thought in this field of research. Insofar as they are born out by subsequent developments, the lessons they teach are two: Think fast! Think small! In other words, think of the many fast reactions that can rapidly alter the production and fate of highly reactive intermediates, and when considering the impact of competitive reactions on such species, think how they affect the microenvironment (on the molecular scale) "seen" by each reactive molecule.

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Table 1

PARTITIONING OF IRON INTO OLEIC ACID AND METHYL LINOLEATE[a]

Concentrations (micromolar) and ferric/ferrous ratios:

	.....Oleic Acid.....			.....Methyl Linoleate....		
Iron complex	Ferric	Ferrous	Ratio	Ferric	Ferrous	Ratio
Ammonium sulfate	5.90	6.00	0.98	0.28	0.30	0.93
Chloride	3.50	3.50	1.00	-	-	-
Citrate	0.09	3.40	0.03	-	-	-
Sulfate	3.50	3.50	1.00	-	-	-
ADP[b]	>18	6.30	>~3	0.64	0.19	3.37
EDTA[c]	0.40	0.54	0.74	0.02	~0	-
DTPA[d]	0.11	~0	-	~0	~0	-
DFO[e]	>18	-	-	0.56	-	-

a. 1 mM solutions of iron with 10% excess of complexing agent were vortexed with an equal volume of lipid, the phases were separated by centrifugation, and the oil phase was analyzed by atomic absorption (using a furnace with deuterium arc background correction). Concentrations were calculated for the oil phase after corrections for the diluant used (tetrahydrofuran) and for solvent blanks (lipid vortexed with water).
b. Adenosine diphosphate (with ammonium sulfate)
c. Ethylenediaminetetraacetate
d. Diethylenetriaminepentaacetate
e. Desferrioxamine-B mesylate (deferoxamine)

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Fig. 1 EPR time curves for production of the OH• spin adduct of DMPO in oleic acid medium. 0.1 mM solutions of $FeCl_3$ or its chelates with ADP (1:17 Fe:ADP molar ratio), EDTA (1:1.1), and DTPA (1:1.1) were partitioned into oleic acid (see text). Final concentrations or reaction components: DMPO, 44 mM; H_2O_2 ~220 mM. EPR parameters: power, 5 mW; modulation amplitude 0.5 G (at 100 kHz).

Fig. 2 EPR spectra of DMPO spin adducts formed during Fenton reactions in neat methyl esters of lipids. Argon-purged $FeCl_3$ (1 mM), H_2O_2 (30%), and $NH_2OH \cdot HCl$ (10%) each were partitioned into equal volumes of lipid ester, the phases were separated by centrifugation, and aliquots of the lipid phases were mixed with DMPO (350 mM) dissolved in lipid ester.

Fig. 3 DMPO spin trapping of OH• produced in the reduction of H_2O_2 by ferrioxamine (FOA) previously reduced by paraquat radical cation ($PQ^{+\bullet}$). (0.1 mM FOA was reduced by >0.1 mM $PQ^{+\bullet}$, but no $PQ^{+\bullet}$ remained at the time of final mixing prior to the recording EPR spectra.) X-band EPR parameters: power, 5 mW; modulation amplitude, 1.6 G (at 100 kHz). Final concentrations of reaction components: FOA, 0.037 mM; H_2O_2, 80 mM; DMPO, 65 mM; and ethanol, when present, 3 mM.

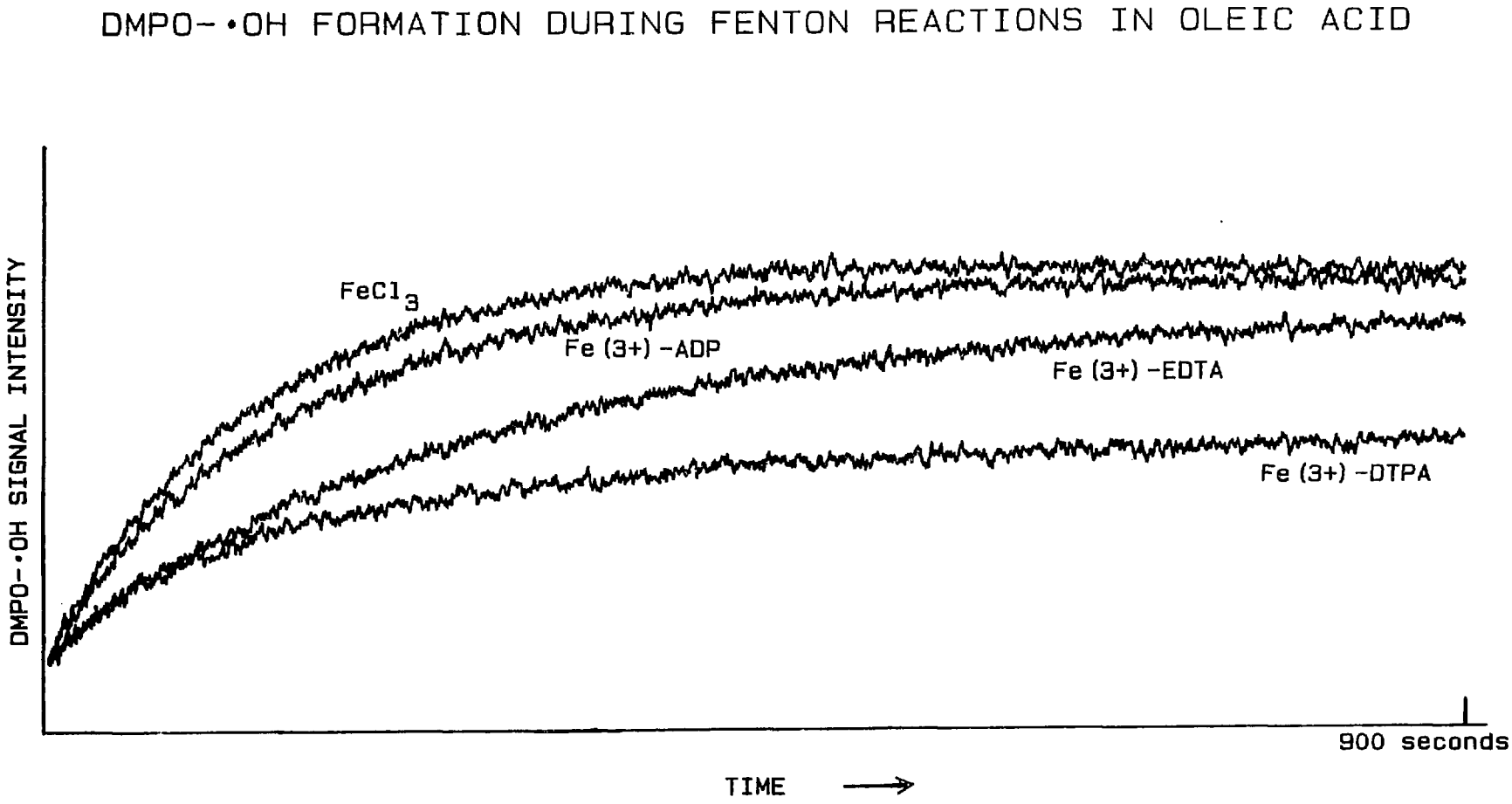
DMPO-•OH FORMATION DURING FENTON REACTIONS IN OLEIC ACID
DMPO-•OH SIGNAL INTENSITY
FeCl3
Fe (3+) -ADP
Fe (3+) -EDTA
Fe (3+) -DTPA
900 seconds
TIME

Fig. 1

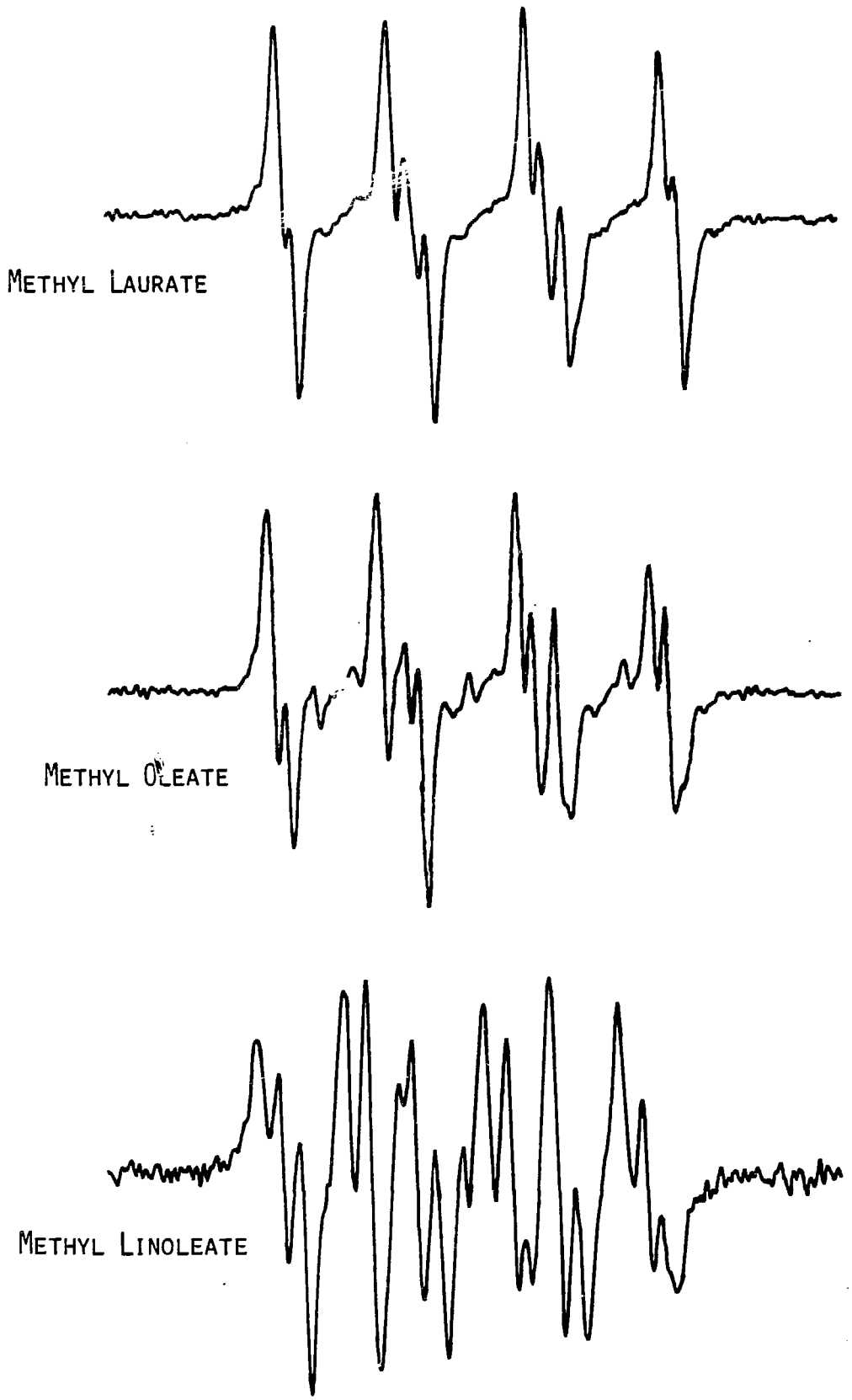
DMPO PLUS FENTON REACTIONS IN...
METHYL LAURATE
METHYL OLEATE
METHYL LINOLEATE

Fig. 2

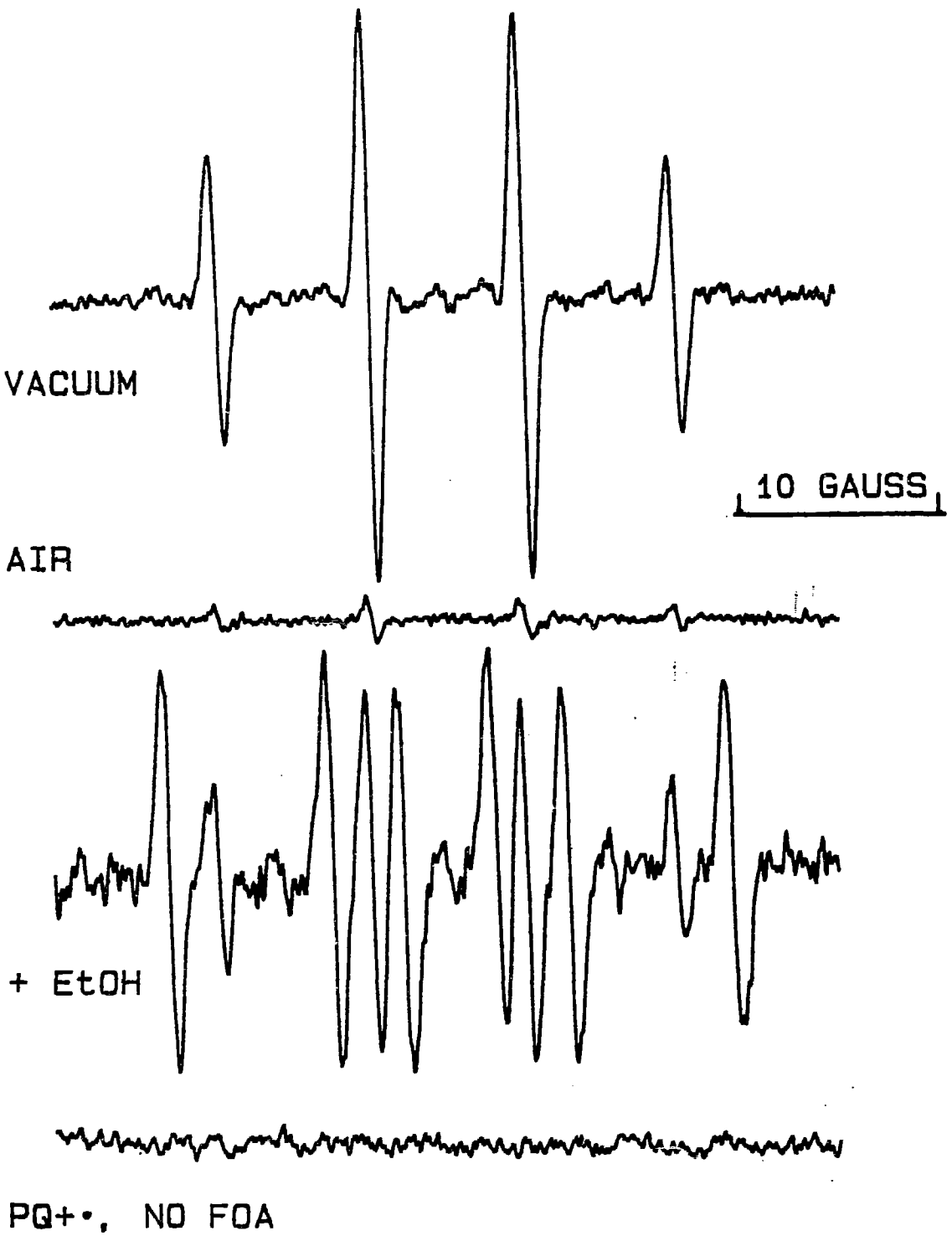

Fig. 3