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MECHANISMS AND CONSEQUENCES OF LIPID PEROXIDATION IN BIOLOGICAL SYSTEMS

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INTRODUCTION

Nutritionists and food technologists have long been aware of the alterations that take place in foods during exposure to air. Although many food constituents may undergo oxidation, those containing fats are particularly susceptible to the changes in color, taste, and odor that accompany rancidity. The oxidation of lipids in biological systems also has profound consequences. For example, the peroxidative breakdown of membrane polyunsaturated fatty acids is involved in the pathogenesis of liver injury induced by several chemical agents. Such tissue damage is a consequence of the production and propagation of free radicals. Lipid peroxidation has also been implicated in hemolytic anemias and pulmonary damage subsequent to exposure to medicinal and environmental chemicals. It should be noted that radical-initiated lipid peroxidation may

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accompany physiological as well as pathological processes. The former events include prostaglandin synthesis as well as aging.

We attempt in this review to describe contemporary views of the chemical mechanisms involved in lipid peroxidation. We also describe the role of transition metals such as iron in initiating peroxidative events through the formation of oxygen radicals. It seems likely that iron plays a key role in such events in biological systems. In living systems, the breakdown products of peroxidized lipid may have unique functions and these products are described in some detail along with current concepts involved in their detection and quantitation. Finally, we discuss some of the biological consequences of lipid peroxidation.

We hope that it will be evident from even this circumscribed review of the literature that the significance of lipid peroxidation has moved far beyond early concerns with rancidity in foods. What has emerged in the past twenty years is the realization that the initiation and control of peroxidation reactions have far-reaching biological sequelae. We only begin to understand them.

CHEMISTRY

The peroxidation of lipids is commonly described as an oxidative, oxygen-dependent deterioration of fats, notably the unsaturated fatty acids. The remarkable susceptibility of unsaturated fatty acids is facilitated by the stabilization of a free radical adjacent to an olefinic group. Thus, an allylic hydrogen atom is relatively easily removed to produce a radical site subject to the addition of an oxygen molecule. The addition of oxygen yields a lipid peroxy radical, which is considered a hallmark of peroxidizing lipids.

The initiation, propagation, and termination processes of the autoxidation chain reaction for lipids are generally accepted as

initiation	$RH + \text{Initiator} \rightarrow R\cdot$	1.
propagation	$R\cdot + O_2 \rightarrow RO_2\cdot$	2.
	$RO_2\cdot + RH \rightarrow ROOH + R\cdot$	3.
termination	$RO_2\cdot + RO_2\cdot \rightarrow ROOR + O_2$	4.
	$RO_2\cdot + R\cdot \rightarrow ROOR$	5.
	$R\cdot + R\cdot \rightarrow RR$	6.

The influence of initiators on the rates and extent of peroxidation can be variably dependent on the initiation mechanism in question. Examples of such reactions are described below. Reaction 3 is known to be slower than Reaction 2; thus, over the range of oxygen partial pressures in air and tissues, the

concentration of oxygen is considered to be well above that required to support the propagation reactions (Reactions 2 and 3) and the concentrations of $RO_2\cdot$ will be greater than R·. With this qualification, the only significant termination reaction should be Reaction 4. If no other factors were operative (in fact there are a number), the rate of oxidation would be predicted by the equation

$$R = \frac{-d[O_2]}{dt} = \frac{d[ROOH]}{dt} \qquad 7.$$

The prevalence of ROOH species may be expected to diminish in certain tissues where oxygen tensions are low (<10 mm Hg) and under these circumstances the rate equation may not be predictive of the free radical process.

The improbability of spontaneous lipid radical formation from a thermodynamic point of view [rate of self-propagation reported to be 62 $mol^{-1}sec^{-1}$ (55)] presented researchers with the question of how the lipid peroxidation process is likely to be initiated in both biological and nonbiological systems. This query has been largely resolved, but by no means proven, through numerous experiments that elucidated the role of transition metals, or of transition metal–bearing compounds, as catalysts for free radical reactions involving oxygen. Additionally, cellular metabolism harbors a myriad of oxidation-reduction reactions from which it is possible to demonstrate the leakage of radical intermediates (2, 3) or metabolites (4, 5). The adventitious peroxidation of neighboring unsaturated lipids by these radicals is conceivable.

Transitions metals are thought to play a central role in the generation of free radicals that can initiate lipid peroxidation. Experimental systems used to produce lipid peroxidation often utilize iron salts, or with lesser frequency copper salts, as the transition metal catalysts (6–8). It is contended that the micromolar quantities of free iron used in the in vitro systems mirror similar reactions that are occurring in vivo. Although the existence of free or nonprotein-bound iron in biological fluids remains controversial (8, 9), Gutteridge et al (10) reported the ability of nonprotein-bound iron, prepared from various body fluids, to induce lipid peroxidation. It is unlikely that this iron exists in free form as there are a plethora of iron-binding protein and nonprotein compounds in tissue fluids. The sequestration of iron, however, need not suppress its ability to participate in redox or free radical–generating reactions. Accordingly, ferritin variably loaded with iron has been shown to stimulate lipid peroxidation in the presence of ascorbate (11). There are several instances where iron chelation imparts a more favorable redox potential to the metal complex producing an efficient coupling with oxygen reduction reactions. This was demonstrated for iron chelation by EDTA, which facilitates lipid peroxidation under certain conditions (12). The binding of iron by nucleotide phosphates such as ADP or ATP, also potentiates lipid peroxidation in several

systems (13–15). Of no lesser importance is the maintenance of iron in a stable soluble form by its chelation. The autoxidation of ferrous salts to their ferric forms in oxygenated media predisposes the latter to immediate precipitation as ferric hydroxides at neutral pH. Chelating agents such as EDTA or ADP overcome this potentially limiting process, and thus maintain an effective concentration of catalytic iron.

During the course of many redox reactions, oxygen can serve as the immediate electron acceptor, either forming radical species of oxygen or adding to the radical in question. If such reactions involve only lipid radicals and oxygen, the term "autoxidation" is applied. This description for autoxidation is necessarily limited to lipid peroxide (or lipid radical) induced lipid peroxidation since the involvement of another oxidizing species implies that the lipid is not experiencing self-oxidation. Such a situation on further consideration appears contrived because an initiating event must create the original lipid radical and thereby trigger the autoxidative process.

Although peroxidation of lipids is not kinetically favored, it is nevertheless a characteristic feature of oxidative tissue damage. This implies that reaction conditions must change in order to make the peroxidation process favorable and this is readily accomplished by radical chain reactions. In this regard, unsaturated lipids can be envisioned as secondary components in a cooxidative progression wherein lipid peroxidative chain reactions are kindled by the radicals formed through more readily oxidizable substances. A peroxidative scheme applicable to conditions in cells has been proposed by Borg et al (16). The sluggish reactivity of unsaturated lipids with oxygen can be viewed as being potentiated by radicals formed via the antioxidation of highly oxidizable compounds such as hydroquinones. Accordingly, 6-hydroxydopamine and dialuric acid rapidly autoxidize, catalyzing the oxidation of compounds such as ascorbate (16). In some cases the semiquinone radicals may be capable of initiating lipid peroxidation; it is equally feasible that radical species derived from semiquinone autoxidation, such as O_2^- and H_2O_2 (or more likely OH·), are the agents responsible for initiating lipid peroxidation.

The lipid peroxidation process is readily initiated by an abstraction of a hydrogen atom from the methylene between a *cis* double bond pair of an unsaturated fatty acid (18). Hydrogen abstraction from the *cis-cis* pentadiene center of an unsaturated fatty acid is purported to be the rate-limiting step in autoxidation (17). The predominant and thermodynamically most stable products are allylic radicals of the unsaturated fatty acids. In polyunsaturated fatty acids, there are multiples of these methylenic hydrogens available for abstraction. For example, arachidonic acid possesses three doubly allylic sites located at carbons 7, 10, and 13. By analogy the enzyme-catalyzed peroxidation of unsaturated fatty acids, for example lipoxygenase, selectively abstracts an activated hydrogen atom from a specific methylenic carbon to produce lipid

peroxy intermediates (19). Whether the process is enzymatic or not, the resulting lipid pentadienyl radical reacts with oxygen at carbons 5, 8, 9, 11, 12, or 15 as is the case for arachidonic acid. This is dictated in part by the carbon radical centers involved in the pentadienyl radical (see Figure 1). The ability of lipid dienyl radicals to react with molecular oxygen at nearly diffusion-controlled rates implicates lipid peroxyl radicals as the predominant radical species during microsomal lipid peroxidation (20). A similar mechanism applies to other unsaturated fatty acids.

There is at present considerable evidence that oxygen radicals, or species resembling them, are responsible in large measure for the induction of lipid peroxidation. Such evidence fostered a unifying concept for lipid peroxidation, one that incorporates reactions involving free or chelated transition metals, oxygen radicals, a variety of reducing species, and unsaturated fatty acids. Singlet oxygen and hydroxyl radical are considered as the two principal oxygen radicals capable of inducing lipid peroxidation (21–24), and it is widely held that hydroxyl radical can be generated by a Fenton-catalyzed Haber-Weiss reaction (25). In this instance, free or many chelated forms of iron serve as the catalytic mediator for the reaction between hydrogen peroxide and superoxide anion. The Haber-Weiss reaction is most effectively catalyzed by reduced iron

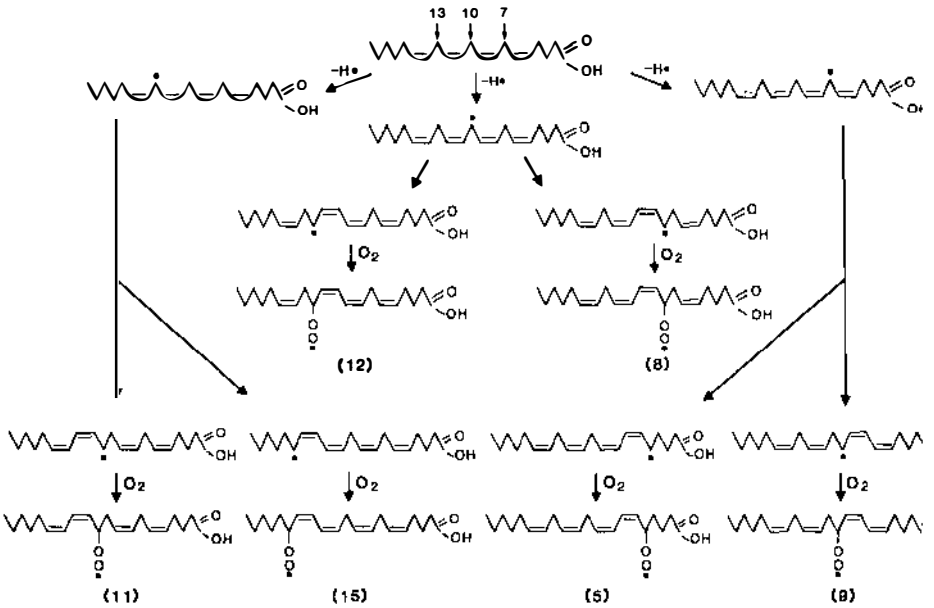

Figure 1 The formation of the six major peroxides derived by the autoxidation of arachidonic acid. The initi 10-, and 13-bisallylic radicals are depicted as rearranging to the 5-, 8-, 9-, 11-, 12-, and 15-pentadienyl radi which react with oxygen to form the isomeric peroxides.

complexes, which can be generated by the reaction of ferric complexes with superoxide radical.

In a manner similar to the controversy over the catalytic role of free iron in lipid autoxidation reactions, a dispute exists over the importance of superoxide for this reaction (26) in biological systems. There are several sources for hydrogen peroxide in biological systems, many of which involve a dismutation of superoxide. On the other hand, the reduction of iron need not require superoxide as many cellular reducing agents, including ascorbate and glutathione, are bountiful enough to carry out this function. Nevertheless, superoxide may acquire an important role as a biological reductant under circumstances such as oxygen toxicity induced by hyperoxia (27).

Although the major radical product of the Haber-Weiss reaction is hydroxyl radical, there is evidence for the formation of singlet oxygen (28). Both singlet oxygen and hydroxyl radical are capable of initiating lipid peroxidation, although the mechanisms of peroxidation differ. There are a large number of enzymatic reactions that generate oxygen radicals. Glucose oxidation by glucose oxidase produces hydrogen peroxide (29), while oxidation of xanthine by xanthine oxidase produces substantial amounts of superoxide, which subsequently dismutates to hydrogen peroxide (30). In the presence of reduced iron, both of these enzyme systems can generate hydroxyl radical via the Fenton reaction. A common in vitro method for producing lipid peroxidation takes advantage of the dual role played by enzymatically generated superoxide. In this reaction superoxide serves to maintain a pool of reduced iron as well as hydrogen peroxide, the essential components of the Fenton reaction.

Hydroxyl radical is of focal interest since its capacity to react at high rates ($\sim 10^9$ $mol^{-1}sec^{-1}$) with virtually any organic compound makes it among the most potent of oxidants. This suggests that the degree of lipid unsaturation provides no significant predisposition to peroxidation when initiated by hydroxyl radical. Empirically this is not the case, however, as the basis for the ready peroxidation of polyunsaturated fatty acids derives at least as much from the length of propagative lipid radical chain reactions that can be sustained following a given initiation reaction. The length of a lipid radical chain reaction, and therefore the propagative cycle of lipid peroxidation, is increased by the degree of lipid unsaturation and limited by the availability of these lipids. The observed rate constant per free radical initiation for a series of unsaturated fatty acids ranging from one to six double bonds increases by the order of 0.025:1:2:4:6:8 (31). Thus, the peroxide yield is expected to be large per initiation in a highly unsaturated system, with a significant contribution to overall peroxidation coming from free radical propagation or multiplication reactions; the latter is shown in the equation

$$2\ ROOH \rightarrow RO_2\cdot + RO\cdot + H_2O. \qquad 8.$$

Propagation reactions are a reflection of the likelihood for one-electron transactions to take place between lipid radicals (or peroxides) and olefins; however, the peroxidic chain reaction can be terminated by a number of radical annihilation or combination reactions considered as distinct from reactions involving antioxidants. Examples of radical combination reactions, all of which terminate the radical chain, are shown as Reactions 4–6 above. These reactions would be expected to become significant when lipid peroxide levels accumulate and/or when oxidizable substrates decrease in concentration.

Hydroxyl-radical-induced lipid peroxidation proceeds by hydrogen atom abstraction from the bis allylic carbon(s) of unsaturated fatty acids, but singlet-oxygen-induced peroxidations are thought to proceed by an "ene" (32) or peroxirane (32) mechanism. The singlet oxygen reaction involves either a "one-two" attack or direct addition of oxygen to the olefin with consequent migration of the double bond accompanied by a change from *cis* to *trans* configuration. Singlet oxygen reactions display from the onset of peroxidation time-dependent increases in peroxidation products, whereas an induction period is commonly seen for lipid peroxidation as initiated by other free radical mechanism. Differences between these two modes of peroxidation are evidenced by the nature of the fatty acid hydroperoxide products. Thus, hydroperoxides derived by an abstraction of a hydrogen from *oleic acid,* for example, occur as the 8 and 11 hydroperoxy isomers, and the 9 and 10 isomers. Singlet-oxygen-derived products, on the other hand, are almost exclusively the 9 and 10 hydroperoxides (33). Similarly, the peroxidation of *linoleic acid* produces the two predominant 9 and 13 hydroperoxides by abstraction from the bis allylic carbon 11 of the fatty acid molecule. These products possess the characteristic conjugated diene system. Hydrogen atoms can also be abstracted to a much lesser extent from the two monoallylic carbons 8 and 14, yielding the 8-, 10-, and 12-, 14-hydroperoxy isomer, respectively (34). Photo-oxidation of linoleic acid yields the 9- and 13-hydroperoxides as major products, but also significant amounts of the 10- and 12-hydroperoxides. The proportions of the hydroperoxide isomers derived from linoleic acid autoxidation thereby serve to reveal the preferred sites and mechanisms of oxidation.

Another common radical species capable of initiating lipid peroxidation is the ubiquitous air pollutant nitrogen dioxide. Lipid peroxidation as induced by low-level exposures to nitrogen dioxide appears to proceed either by hydrogen atom abstraction or by nitrogen dioxide addition to the olefin. The reaction course is largely influenced by the presence of radical trapping species, particularly oxygen (35). The favored process in air has been shown to be hydrogen abstraction, particularly from the doubly allylic hydrogens of polyunsaturated fatty acids (35, 36). Thus, a propagative chain reaction can be initiated for each nitrogen dioxide reacting with an unsaturated fatty acid with kinetic chain lengths varying between 3 and 200 molecules as determined by the population

of unsaturated lipids or the presence of antioxidants. The lipid peroxidation products would be characteristic of a free radical process.

Lipid peroxidation in cell membranes has been described as occurring by a process similar to those described above. The mechanism of NADPH-dependent peroxidation of microsomal lipids (13) was hypothesized to take place through the formation of oxygen radicals (24, 37) or alternatively as radical-bearing complexes (38). The key components are cytochrome P-450 and its associated flavoprotein reductase (39). One postulate ascribes the peroxidation to the dissociation of superoxide anion from the oxyferricytochrome complex, which serves as the oxidant in the hydrophobic milieu of the membrane or indirectly through conversion to hydrogen peroxide and in turn to hydroxyl radical (37). Alternately, the oxidizing species may be in the form of iron chelates, such as iron-nucleotide complexes (13), which are readily reduced by the P-450 reductase. The reduced iron-chelate is proposed to combine with oxygen to form a powerful oxidant via a ferryl system (40), with the possible oxidizing species being a ferryl radical [$Fe^{IV}O^{2+}$] (41). Here the iron is thought to exist at one oxidation state above the ferric form. Such oxidation states have been reported for iron-peroxide complexes [FeO_2H^{3+}] as in ferrimyoglobin (42).

The peroxidic chain reaction can also be sustained by a metal-catalyzed decomposition of hydroperoxides as shown in these examples:

$$ROOH + Fe^{+3} \rightarrow ROO\cdot + H^{+} + Fe^{+2} \quad 9.$$

$$ROOH + Fe^{+2} \rightarrow RO\cdot + OH^{-} + Fe^{+3}. \quad 10.$$

These reactions lead to further propagations facilitated by the peroxy or alkoxy radicals. Estimates for the rates of these reactions indicate that Reaction 10, i.e. ferrous-catalyzed decomposition to alkoxy radicals, is the more rapid process (43). This is demonstrated by the quantitatively greater yields of lipid peroxidation products measured when unsaturated fatty acids, or preformed hydroperoxides, are incubated in the presence of ferrous salts. It must be cautioned, however, that this effect of iron or other transition metals may be concentration dependent and that most metals within the milieu of the intact cell would be sequestered from reactions capable of facilitating lipid peroxidation. In this respect, several studies show that high concentrations of iron (21, 44) or the presence of cytosol (45, 46) provides an antioxidant effect. In one instance this effect was ascribed to a cytosolic protein (47), possibly related to the glutathione-dependent cytosolic factor described by Gibson et al (48), but distinct from glutathione peroxidase (49). We recently noted an inhibitory effect upon NADPH-dependent lipid peroxidation (see below), when samples are incubated in the presence of high concentrations of iron-ADP complex (50). This effect may have a bearing on the previously described role of iron in termina-

tion reactions (21) and under some circumstances may compete with the propagative generation of lipid peroxides. Alternately, the apparent inhibition of iron in some systems may be attributable to a suboptimal balance between free Fe^{+2} and Fe^{+3}. Accordingly, Ernster et al (51) suggest that a ratio of 1.0 for Fe^{+2}/Fe^{+3} supports maximal microsomal lipid peroxidation with any given concentration of iron.

A variety of iron-bearing compounds, e.g. hemoglobin (52), cysteine-$FeCl_3$ complex (53), and EDTA-Fe^{+3} (54), have been shown to facilitate lipid peroxidation largely by catalyzing the decomposition of hydroperoxides to alkoxy radicals, epoxides, and other fragmentation products. Many of these products may act as facile propagators of the free radical chain reaction. Heme compounds, as opposed to free or certain chelated forms of iron, appear capable of inducing lipid peroxidation in their oxidized state (55) by catalyzing the homolytic decomposition of preformed fatty acid hydroperoxides (55). This property may be due to the ability of iron within the heme to properly interact with organic peroxides or lipid peroxides residing in the hydrophobic environment of membranes. The degree of iron sequestration by heme compounds of increasing size appears to correlate inversely with the ability to catalyze lipid peroxidation (55), and may be reversed by damage to the heme protein causing a destabilization of the complex or incomplete coordination of the iron (55, 56).

Aust and colleagues (54) proposed that cytochrome P-450 participates in the *propagation* of lipid peroxidation and that this causes a destruction of the heme protein. The exact mechanism for this reaction remains unclear; however, the process resembles the organic hydroperoxide-dependent consumption of oxygen by cytochrome P-450, with hydroperoxide, cytochrome P-450, and oxygen as the only reactants (57). This reaction mechanism bears a resemblance to peroxidase(s) or pseudo-peroxidase that is a documented property of lipooxygenase (58, 59) and hemoglobin (60), and it is reported to cause the destruction of these catalysts. Insight with respect to these suicidal reactions may be gained by examining the products of the hydroperoxidase reaction. As in lipoxygenase reactions, the decomposition of fatty acid hydroperoxides by iron chelates, such as cysteine-$FeCl_3$ (61), is an oxygen-consuming process. A mechanism for this reaction is thought to involve a secondary peroxidation of the hydroperoxide to yield a mixture of keto acids, keto alcohols, and epoxy alcohols. This process is described in greater detail below. However, it should be noted that heme-protein-catalyzed decomposition of fatty acid hydroperoxides, with the associated consumption of oxygen and the propagation of lipid peroxidation by heme proteins, may have a mechanistic link that could be revealed by analysis of the lipid products.

Svingen and Aust (6, 54) suggested a mechanism for lipid peroxidation in microsomes that attempts to resolve some of the limitations of the oxygen radical hypothesis discussed above. NADPH-dependent microsomal lipid

peroxidation is considered to take place in two stages: initiation and propagation. Initiation reactions proceed by a NADPH-cytochrome-P-450-reductase-catalyzed reduction of ADP-Fe^{+3}, subsequently reacting with oxygen to form a ADP-perferyl radical (13). The perferyl radical is then responsible for initiating lipid peroxidation and forming lipid hydroperoxides. In this manner, the evanescent hydroxyl radical need not be invoked, nor is a significant hydrogen peroxide flux required. Propagation reactions proceed by the interaction of lipid hydroperoxides with cytochrome P-450, which catalyzes their decomposition to peroxy or alkoxy radicals. In this regard, EDTA or DTPA chelates of iron are also capable of catalyzing the propagation reaction. The cyclical reduction by P-450 reductase, and reoxidation of the iron chelates serves to maintain the propagation reaction.

The extent to which an initiation-propagation cycle such as this is operative in the cell is still open to serious question. Although this mechanism circumvents the need for superoxide and hydrogen peroxide in the lipid peroxidic chain reaction, and is thereby free of the anomalous action of catalase (62), it does not account for recent observations that NADPH at physiologic levels supports a peroxidase-like reduction of hydroperoxides (63). Thus, NADPH may just as readily act to eliminate lipid peroxides by serving as a cytochrome-P-450-dependent peroxidase cofactor and accordingly oppose its proposed activity in initiation and propagation reactions.

In addition to the greater disposition of polyunsaturated fatty acids toward peroxidation, a number of other features associated with peroxidation are influenced by the degree of lipid unsaturation. Chan et al (64) and others (65) described the reversibility of oxygen addition to a lipid radical as "oxygen scrambling," a process involving β-scission of the peroxide perpendicular to the diene system. The isomeric characteristics of hydroperoxides appear to be a consequence of the tendency for oxygen-scrambling reactions to compete with other possible reactions for lipid peroxides; including hydrogen abstraction, β-fragmentation, and cyclization. The determining factor for these events may be the total hydrogen-atom-donating capability of the system undergoing peroxidation (65). While in pure lipid preparations, this is a function of lipid unsaturation, in biological systems the hydrogen-donating capacity represents the composite of substrates including unsaturated fatty acids, phenolic antioxidants (66), and weak acids such as ascorbic and uric acids. Thus, the commonly encountered *cis-trans* fatty acid hydroperoxide products can give way to larger proportions of the more thermodynamically stable *trans-trans* isomers when hydrogen abstraction insufficiently competes with β-scission. The underlying feature accounting for this is proposed to be an inadequate supply of donatable hydrogens, which permits the β-scission of the peroxide instead of hydrogen abstracting. The resulting pentadienyl radicals are thus subject to isomerizations of sufficient lifetimes to allow the reforming of peroxides in either the *cis* or *trans* configurations. Porter et al (65) diagrammed this process.

Peroxides may continue along a number of reaction routes including cyclization reactions, or if donatable hydrogens are available, toward the formation of hydroperoxides. The demonstrated ability of fatty and peroxy radicals to undergo "self-attack" forming mono- and bicyclic peroxides (67) represents a potentially important reaction pathway, both mechanistically and as an influence on the characteristics of the measureable products. Figure 2 demonstrates a typical cyclization reaction for a polyunsaturated fatty acid. As mentioned above, reactions such as these may take precedence over hydrogen atom abstraction. The tendency to cyclize to compounds bearing five-membered rings also requires that a nonconjugated π system reside β-γ to the peroxy radical (67, 68). Hence, such reactions require that the fatty acid possess three or more unsaturated bonds.

The lower yields of internal hydroperoxide isomers of linoleic acid, for example, has been ascribed to the preferred cyclization of the 12- and 13-

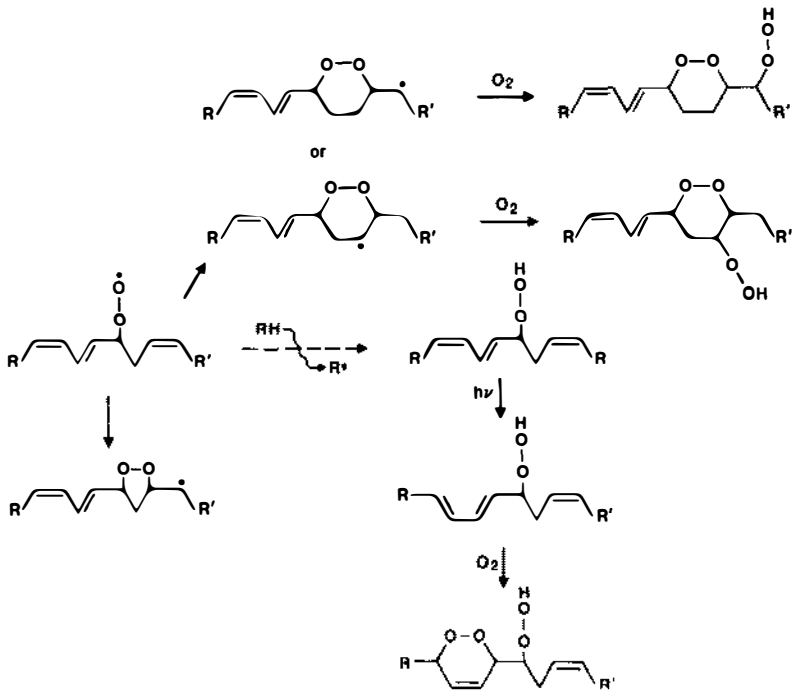

Figure 2 The formation of cyclic peroxides derived by the intramolecular attack of linoleic acid peroxy radical. Five- and six-membered cyclic peroxides may arise by 1,3- or 1,4-cycloaddition of the peroxide radical to a double bond. Radical migration and subsequent reaction with oxygen produces the isomeric hydroperoxy monocyclic peroxides and epidioxides. An isomerization of the *cis-trans* to a *trans-trans* hydroperoxide may also occur (a process apparently favored by photosensitization). This competes with or is followed by 1,4-cycloaddition of oxygen to form the six-membered hydroperoxy epidioxide.

hydroperoxides over that of the 9- and 16-hydroperoxides (69). This indicates that internal hydroperoxides experience cyclization reactions more readily, and such species are suggested to be the likely sources of malonaldehyde, believed to be a common decomposition product of lipid peroxidation. A number of cyclic peroxides (or endoperoxides) bear a remarkable resemblance to prostaglandins—notably PGG_2. The free radical cyclization reaction is, in fact, a chemical equivalent to the enzymatically controlled reactions catalyzed by cyclooxygenase. These enzymatic reactions represent an elaboration of the radical process wherein a concerted intramolecular radical attack produces a second peroxidation at a discrete carbon (specifically carbon 15 of arachidonate). It is significant to note that the decomposition of these cyclic peroxides can yield the three-carbon dialdehyde malonyldialdehyde (malonaldehyde).

DECOMPOSITION OF LIPID PEROXIDES

The thermolytic decomposition of hydroperoxides also produces numerous short-chain products including a number of oxygenated and nonoxygenated volatile hydrocarbons and aldehydes. The formation of pentane and ethane from ω-6 and ω-3 peroxides, respectively, is a well-known outcome of this decomposition process (70, 71). Another reaction course of potential significance involves the homolytic decomposition of hydroperoxides. For example, the iron-catalyzed reactions of hydroperoxides discussed above produce epoxides and epoxy alcohols (61). Gardner & Jursinic (61) proposed a mechanism whereby ferrous-catalyzed formation of the fatty acid alkoxy radical is followed by intramolecular attack to form an epoxy dienyl radical (see Figure 3). This radical can undergo a second attack by oxygen to yield isomeric epoxy alcohols as relatively stable products. Secondary peroxidation reactions are also known to give rise to 5- and 6-membered cyclic peroxides (67, 72). The formation of epoxides may occur through a *cis* 1,2-dioxolane radical intermediate that is then attacked by molecular oxygen at the peroxide bond (73). Alternately, hydroperoxides have been proposed to undergo a concerted intramolecular rearrangement to form epoxy alcohols (74). A reaction of this nature was proposed for the autoxidation of linoleic acid as a monolayer on silica gel; however, the reaction may be unique to this model system.

In recent years Mead and his associates demonstrated that epoxidation of unsaturated fatty acids can occur readily when these lipids exist in membrane-like arrangements (72, 74). In addition to displaying distinct kinetics of autoxidation, the mechanism of peroxidation also appears unique. It was suggested that unsaturated lipids that assume the ordered arrangements found in membranes undergo intermolecular addition reactions involving a peroxy radical and a neighboring unsaturated bond (75). This reaction appears to compete with hydrogen atom abstraction and produces significant quantities of

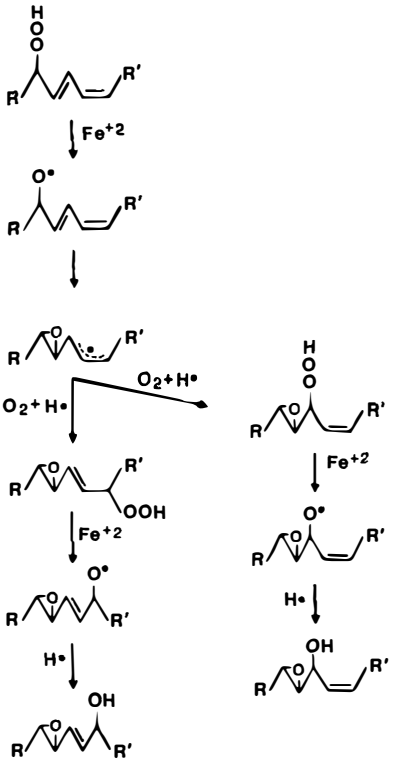

Figure 3 Decomposition of linoleic acid hydroperoxide to an epoxy alcohol is shown. A ferrous-catalyzed decomposition to the alkoxyl and epoxy allylic radicals is followed by a second reaction with oxygen and by hydrogen abstraction to yield the isomeric epoxy hydroperoxides. Formation of the epoxy alcohol is proposed to occur by an iron-catalyzed decomposition of the hydroperoxide to an epoxy alkoxyl radical intermediate.

isomeric epoxides and trihydroxy fatty acids notably lacking in diene conjugation (76). Variations in membrane fatty acid composition affect not only peroxidation rates but also the proportions of epoxides and other products. Thus, in highly saturated membranes, the formation of epoxides and hydroperoxides gives way to epoxy alcohols (76). These epoxy alcohols are believed to originate by an intramolecular attack of the hydroperoxide by one of the mechanisms noted above, a further indication of the influence of membrane structure on the course of lipid peroxidation.

By following the disappearance of unsaturated fatty acids, Wu et al (77) showed that lipid peroxidation in membranes proceeds via pseudo first-order kinetics, atypical of the autocatalytic reactions found in pure lipid systems or of lipids in organic solvents. Furthermore, peroxidation in membrane systems

appears to be distinguished by the absence of an induction period. This absence of an induction period again contrasts with lipid peroxidation in pure lipid systems and suggests there is an adequate original concentration of initiators so that an induction period is not needed and that unsaturated lipids in membrane arrangements support a facile propagation of the peroxidic chain reaction through the juxtaposed olefinic centers. In pure lipids it may be necessary to accumulate sufficient quantities of peroxides to induce peroxidation displaying typical first-order kinetics.

The kinetic characteristics of autoxidation for pure phospholipids in membranous states are in some cases notably different from the kinetics observed in natural membranes. Peroxide-induced or metal-catalyzed peroxidation of microsomal phospholipids usually follows a brief induction period. This induction period is likely afforded by membranous antioxidants, such as vitamin E, which prevents peroxidation of unsaturated lipids until the bulk of the vitamin has been expended. In fact, an induction period can be created in model membranes by the inclusion of vitamin E (76, 78) and lasts, with no significant loss of unsaturated lipid, until the vitamin is depleted.

MEASUREMENT

The selection of a suitable method for monitoring lipid peroxidation is ideally considered along with the state in which the system under study exists, components in the supporting medium, the duration of the reaction, and a reasonable idea of the nature of the peroxidized lipid. Moreover, in complex lipid systems, the products of peroxidation increase in variety, which makes the choice of methods for measurement especially critical. Taking a simple example of an unsaturated phosphatidylcholine, it is known that the kinetics and product profile following peroxidation differ considerably when this lipid exists in a membranous configuration as compared to a pure lipid. These differences were described in the preceding section but the question of selecting a measurement technique can be guided by the prevailing conditions during peroxidation. During the initial stages of lipid peroxidation, the major species to accumulate are lipid peroxides (79). This appears to be a feature common to both neat and aqueous preparations of lipids. The analytical implications of this are that the early phase of lipid peroxidation may be readily monitored by measuring peroxide levels (80–82). This measure is paralleled by the levels of diene conjugation (80, 83, 84) as the original pentadienyl radical rapidly rearranges to a *cis-trans* conjugated diene during the oxidation process (65). An effective method for measuring lipid peroxides and conjugated dienes is reviewed by Buege & Aust (85).

Lipid peroxides are, however, thermally unstable, decomposing via a number of pathways. Thus, numerous secondary products can form by several

mechanisms. These include (*a*) β-scission (thermal decomposition) of the carbon bonds adjacent to the peroxide; (*b*) β-scission of the carbon-oxygen bond of the peroxide, or of the weakened oxygen-oxygen bond making up the peroxide; and (*c*) cyclization of the peroxide. These are unimolecular events that are influenced by factors such as transition metals and the hydrogen-donating capacity of the system, e.g. peroxidizability. Included among the thermal decomposition products of peroxides are short-chain hydrocarbons and carbonyls usually of the length $n-1$ or $n+1$, where n is the number of carbons from the ω terminus to the first unsaturated bond of the fatty acid undergoing peroxidation. If the peroxide in question is derived from an ω-3 or ω-6 fatty acid, the decomposition products will include ethane or pentane, respectively (70). These volatile hydrocarbons have been widely used to monitor lipid peroxidation and, while their measurement does not lend itself well to quantitative determinations of lipid peroxidation, they do reflect the relative extent of the peroxidic process. Several investigators have demonstrated the utility of this measure of lipid peroxidation, particularly when the determination of lipid peroxidation in vivo is desired (71, 86, 87).

Ethane, pentane, and a number of other volatile hydrocarbons are trapped as components of exhaled air on molecular sieves, and are subsequently measured with gas chromatography by driving the gases off the sieves (usually by heating) (88). A similar analytical procedure, used to assay lipid peroxidation in vitro, requires the sampling of head-space gas in a sealed vessel using a gas-tight syringe (89). These methods for analyzing volatile hydrocarbons should be approached with care (90), and there is particular concern when in vivo measurements are attempted. The major limitations are the antioxidant status of the animal, or experimental preparation, and the requirement that oxygen tensions be low during peroxide decomposition. If the diet is deficient in vitamin E, the intestinal tract can be a major source of pentane, which is released either rectally or via the esophagus (90) (from dietary peroxidation products). Care must therefore be taken to avoid feeding diets containing lipid peroxides that can decompose in the digestive tract to yield pentane and other volatile gases (90).

It is widely accepted that lipid peroxides can readily decompose to alkoxy radicals, as in the ferrous-catalyzed process depicted in Figure 3. The alkoxy radicals are the immediate precursors to ethane or pentane via ethyl or pentyl radical intermediates. A scheme for this reaction is shown in Figure 4. Cohen (70) presented evidence to support the hypothesis that ethyl or pentyl radicals can either abstract hydrogen atoms from nearby unsaturated fatty acids or react with oxygen. Whereas, the former reaction produces ethane or pentane (Figure 4*a*), the latter yields oxidized ethyl or pentyl radicals (Figure 4*b*). These oxidized radicals do not produce ethane or pentane but rather yield ethylhydroperoxides, pentylhydroperoxides or related carbonyls. It is therefore critical

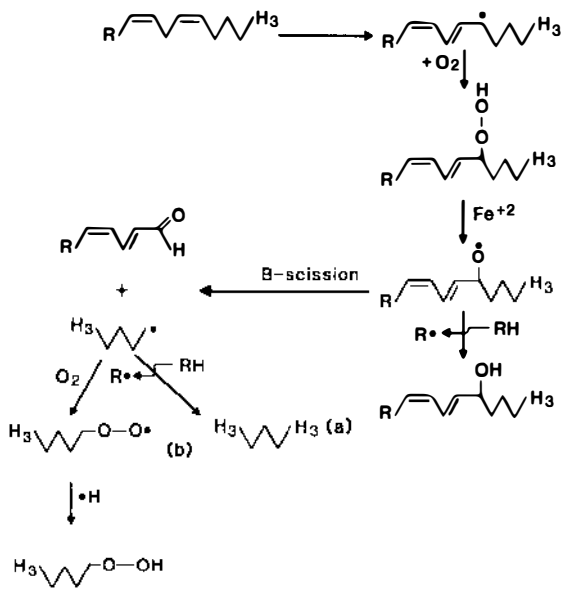

Figure 4 The origin of pentane from linoleic acid is described. The 14-hydroperoxide and alkoxyl radical intermediates are shown. Thermal decomposition of the alkoxyl radical yields the pentyl radical, which either abstracts a hydrogen or reacts with oxygen to form pentane or pentane hydroperoxide, respectively. A hypothetically alternate route for the alkoxyl radical is shown to yield a conjugated diene-bearing alcohol.

that oxygen tensions be minimal so as to avoid excessive formation of these short-chain volatile hydroperoxides.

Allowing for a momentary digression, it is an attractive theoretical notion that ethyl or pentyl radicals, and/or their oxidized counterparts, represent a class of mobile radical chain "carriers" that, by virtue of their volatility, migrate some distance from their site of origin to induce further lipid peroxidation (78). It is envisioned that an oxidized phospholipid undergoes thermolytic scission, yielding (under aerobic conditions) an oxidized pentyl radical. This volatile hydrocarbon then diffuses to a distal region of the membrane where it reacts with another unsaturated fatty acid to produce a lipid radical, thereby triggering further peroxidation. A process such as this may in part account for the dissemination of membrane lipid peroxidation, notably by carbonyl compounds, and resultant damage to other cell components (91, 92). It may also account for the ability of vitamin E to quench a peroxidic chain reaction occurring at remote sites. Since vitamin E is calculated to exist in a ratio of 1 to every 1000–2000 fatty acid molecules in membranes (93), its effectiveness as an antioxidant seems improbable on the proviso that a peroxidic radical chain reaction is quenched when the chain reaches a vitamin E molecule. The radical transfer-allylic hydrogen shift mechanism recently proposed by Ivanov (94) to

account for the membrane antioxidant action of vitamin E does not appear to explain satisfactorily the antioxidant efficiency of vitamin E noted at the remarkably low antioxidant-to-lipid ratios. As already discussed, the lipid radical chain reaction is probably short-lived, extending through no more than 4–10 successive hydrogen atom abstractions until a termination reaction takes place. Rather, the potency of this membrane antioxidant may be conceived by its ability to react rapidly with radical carriers at nearly diffusion-controlled rates. Its importance as an antioxidant is thus realized by its ability to interact with free-radical-evoked mobile chain carriers, such as volatile radicals, which could avidly destroy a membrane.

Malonaldehyde represents a common decomposition product of peroxides derived from polyunsaturated fatty acids. This three-carbon dialdehyde has been proposed to arise from fatty acid hydroperoxides when an unsaturated bond resides β-γ to the peroxide-bearing carbon. Under these circumstances, the peroxide can undergo cyclization to a five-membered endoperoxide. These cyclic peroxides have been proposed to be the precursors of malonaldehyde (95), and other volatile products (82).

The most common method for measuring malonaldehyde involves a reaction with thiobarbituric acid. The assay is intended to be specific for malonaldehyde, but in fact it is not. Products related to malonaldehyde and reactions with ribose sugars (96), bilirubin, or sialic acid (97) interfere in some cases in the standard assay. It is a misconception to assume that malonaldehyde is the only lipid peroxidation product capable of producing a positive thiobarbituric acid reaction. The presence of 2,4-alkdienals and 2-alkenals in oxidized lipids may produce a positive thiobarbituric reaction (68). Consequently, other pigments are known to be formed in the TBA reaction, absorbing maximally in the 450-nm range. Dienals and saturated aldehydes show absorption maxima in this region (98), as do alkanals and 2,4-alkdienals (99), but the latter also produce pigment that absorbs at 532 nm. Hence, it may be more appropriate to describe this assay as a measure for lipid peroxidation products reacting with thiobarbituric acid. Since these and perhaps other compounds can arise from the oxidative decomposition of nonpolyunsaturated fatty acids, they may account for the occasionally reported ability of unsaturated lipids (bearing fewer than three double bonds) to produce a positive TBA reaction (97). However, it is widely held that fatty acids such as linoleate, bearing fewer than three double bonds, produce no positive TBA color even at peroxide values exceeding 2000 (79).

The assay is most sensitive to detection of lipid peroxides when ferrous salts are present because reduced iron can accelerate decomposition of the hydroperoxides. One precautionary step in this assay involves the addition of an antioxidant to the thiobarbituric acid reaction. Thus, the addition of 0.5-mM BHT has been found to prevent further peroxidation of lipids carried over into

the assay preparation. This step serves to assure that only preformed peroxides are measured. As in the case of volatile hydrocarbon measurements, the analysis of TBA-reacting products is nonquantitative in that it represents only a few of the several possible decomposition products of polyunsaturated fatty acid hydroperoxides, which in turn represent a variable percentage of fatty acids undergoing peroxidation (see Table 1). In lipid systems containing low levels of polyunsaturated fatty acids, the lack of malonaldehyde detection may erroneously imply the absence of peroxidation.

Since lipid peroxidation involves the reaction of oxygen with a lipid, it should in principle be monitored by measuring oxygen consumption. Indeed, the peroxidation of microsomal lipids has been assayed by polarographic measurement of oxygen consumption (13). This measure is directly related to malonaldehyde formation with a molar ratio of 20 oxygen molecules consumed per malonaldehyde formed. It must be noted, however, that the ratio of oxygen consumed to lipid peroxidized can be greatly influenced by the presence of other autoxidizing species. Hence, the presence of ascorbate and/or other redox cycling compounds can lead to a significant level of oxygen consumption that need not be linked to lipid peroxidation. Nevertheless, a polarographic assay for lipid peroxidation has been used with artificial membrane systems with some facility (100).

In recent years a number of methodologic modifications have been developed that permit the measurement of lipid peroxides in vivo, i.e. in tissue and serum samples. Yagi (97) demonstrated that tissue and serum lipid peroxides can be measured without interferences from other TBA-reacting substances common in biological samples. The method he describes involves a reaction of sample peroxides (after precipitation away from other interfering substances) with an acetic acid/thiobarbituric acid reagent and subsequent measurement of the fluorescent product. This technique is reported to measure peroxides in 20-μl samples of serum.

The ability of malonaldehyde to form fluorescent products with a variety of

Table 1 Phospholipid fatty acyl content of liposomes[a]: effects of lipid peroxidation

	Expressed as μg fatty acid methyl esters									nmole[b]
	16:0	16:1	18:0	18:1	18:2	18:3	20:4	22:4	22:6	TBAR
Control	105.8	18.8	209.6	170.2	59.1	7.1	74.1	43.1	8.3	0.49
Fe^{2+} ascorbate (40 min)	102.4	15.9	204.3	165.1	45.7	4.6	59.3	32.5	6.8	3.75
Fe^{2+} ascorbate (90 min)	103.1	15.3	194.6	163.1	44.5	4.2	47.5	27.1	4.8	10.63

[a] Liposomes consisted of phosphatidylcholine and phosphatidylethanolamine in a molar ratio of 4:1. All values are based on a 1.0-mg original sample size.

[b] Thiobarbituric-acid-reacting (TBAR) substances are expressed in terms of nmole equivalents of malonaldehyde per mg of liposomal lipid.

organic and biological compounds represents a reaction purportedly occurring in several tissues that form a class of products referred to as "fluorescent chromolipids," "lipofuscin," and "aging pigments." These products are associated with tissue injury (101, 102) and aging (103, 104). The capacity of malonaldehyde and lipid peroxidation products to form a Schiff base with amino groups of phospholipids (103, 105) and proteins (106), yielding fluorescent chromolipids, serves as a rationale for the existence of similar reactions in vivo. Thus, Jain & Hochstein (107) used the characteristic fluorescence emission spectra exhibited by malonaldehyde reaction with erythrocyte membranes to demonstrate the role of these "polymerization" reactions in erythrocyte membrane peroxidation and aging. Reaction of linoleic acid hydroperoxide (a nonmalonaldehyde-producing peroxide) with specific amino acids has also been shown to produce fluorescent lipofuscin-like pigments (135).

Measurement of lipid peroxidation by the thiobarbituric acid test entails a common reaction of malonaldehyde (or related substance) with thiobarbituric acid to yield a chromophore or fluorescent product. In all these analytical methods, the procedure is in fact an indirect measure of lipid peroxidation since the substances detected are not lipid peroxides but rather decomposition products representing a small proportion of the peroxides originally present in the test sample. Cathcart et al (108) recently reported a fluorescence method for detecting hydroperoxides directly, a method that appears suitable for analyzing a variety of biological specimens. Several organic hydroperoxides and hydrogen peroxide are measured in an aqueous sample via hematin-catalyzed oxidation of dichlorofluorescein to the fluorescent dichlorofluorescein. As little as 25 pmol of peroxide is detected by this assay and stoichiometry is nearly identical (1 mole of dichlorofluorescein oxidized per mole of hydroperoxide) for all acylhydroperoxides tested.

Alkyl- and diacylhydroperoxides as well as cyclization products of hydroperoxides, e.g. PGH_2, are not readily detected by this procedure. Major limitations appear to be the suppression of the reaction by common biological antioxidants such as uric acid, ascorbic acid, and vitamin E. This suggests that biological samples should either be free of such compounds by a preliminary extraction of lipids, or that a molar excess of dichlorofluorescein be used in the assay.

It necessarily follows that the peroxidatic generation of thiobarbituric-acid-reacting compounds and the accumulation of conjugated dienes and other products described above must be accompanied by the loss of oxidizable fatty acids comprising the in vitro system subjected to lipid peroxidation. Careful measurements of phospholipid fatty acyl constituents during the course of lipid peroxidation reveal that polyunsaturated fatty acids diminish as the level of peroxidation increases. This is demonstrated in the Table 1, which shows the content of major fatty acids from liver phosphatidylcholine prepared as unilamellar liposomes. The quantities of these fatty acids are also shown follow-

ing 40 and 90 min of peroxidation, and are compared to the amounts of malonaldehyde equivalents formed. It can be seen that the polyunsaturated fatty acids, linolenic, arachidonic, and docosahexenoic acids are the major components lost during peroxidation. At 40 min there is also a significant loss of linoleic acid and a smaller decrease in oleic acid. It is further evident that the molar quantities of thiobarbituric-positive compounds (expressed as malonaldehyde) represent a small percentage of the fatty acids lost to oxidation. The rather subtle changes in fatty acid composition accompanied by relatively large increases in the red (ϵ_{max} 532 nm = 2.56×10^4 mole^{-1}) TBA-reaction pigment also demonstrate the insensitivity of this laborious analytical procedure. A number of other potentially useful measurements for a variety of lipid peroxidation products are reviewed by Gray (79).

The development of highly efficient high-performance liquid chromotography (HPLC) columns has enabled the characterization and isolation of many lipid peroxidation products. Notable among the isolated products are the hydroperoxides of unsaturated fatty acids, or their reduced alcohols (84). The eluted material is monitored for conjugated dienes (ϵ_{max} 234 nm); this imparts reasonably good sensitivity to the analysis. A chromatogram of the antoxidation products of arachidonic acid is shown in Figure 5. A gradient elution using acetonitrile:water:acetic acid, and an RP-18, 3-μm column allows resolution of several hydroperoxide isomers in less than 15 min. In this instance complete resolution was not accomplished for all isomers, particularly the *cis-trans* isomers of each positional hydroperoxide. Methods very similar to the above permit characterizations of epoxides (76) and cyclic peroxides (109).

BIOLOGICAL ROLES

The toxicity of oxygen, or of its radical derivatives, is often accompanied by the peroxidation of lipids. A vital question with respect to this rather consistent observation is whether lipid peroxidation represents simply a coincidental outcome of radical-mediated damage, being of consequence only when cumulative destruction of lipids produces deterioration of membranes and organelles, or if lipid peroxidation products are directly deleterious to the cell. One can draw upon a substantial body of evidence to support both a passive and an active role for lipid peroxidation products in free-radical-induced injury.

It was recently reported that the toxicity of oxygen radicals was unrelated to the content of peroxidizable unsaturated fatty acids in *E. coli* (110). This would suggest, with no surprise, that oxy-radical attack upon other cell components could lead to cell death. An interesting series of studies on reticulocyte lipoxygenase and the maturation of reticulocytes indicates that the seemingly

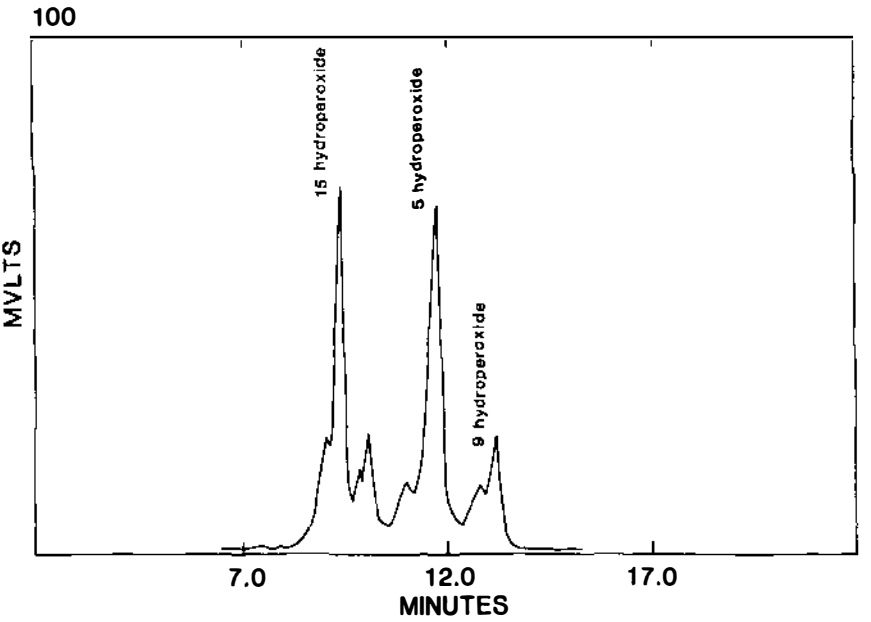

Figure 5 A high-pressure liquid chromatogram of the isomeric hydroperoxides formed by autoxidation of arachidonic acid. Pure arachidonic acid was exposed to a continuous stream of air for 40 hr at room temperature. The sample was dissolved in 10 ml of petroleum ether and extracted twice with 8-ml portions of methanol:water (3:1). The lower phases were pooled, flash evaporated under vacuum, and the dry residue dissolved in ethanol. The ethanolic solution was injected onto a 3-μm RP-18, 4.6 × 150 mm column (Chromanetics, Corp.), which was eluted with acetonitrile:water:acetic acid (65:34.9:0.1) at a flow rate of 1.0 ml/min. After 10 min a linear gradient was developed over the next 5 min to give a final acetonitrile concentration of 75% by volume. Elution with this solvent composition was continued for 15 min before cycling back to the original solvent composition. The eluent was monitored at 234 nm and only a portion of the chromatogram detailing the elution profile of the isomeric hydroperoxides of arachidonic acid is shown. Arachidonic acid elutes at 16.10 min under these conditions.

regulated peroxidation of membrane lipids results in an extensive decomposition of organelle membranes without evidence of cell death. Earlier studies demonstrated the existence of a potent respiration-inhibitory protein whose activity was maximal at the stage when the reticulocyte matures to an erythrocyte (111). This protein was later identified as a lipoxygenase capable of oxidizing the phospholipids of mitochondria (112). An extensive destruction of mitochondrial membranes appears to lead to the disappearance of mitochondria, and it is now more widely held that this enzyme plays a critical role in reticulocyte maturation. The enzyme's activity appears to be transient, reaching maximal activity just prior to mitochondrial destruction. Its activity

rapidly disappears, probably as a result of the suicidal nature of the reaction—the hydroperoxide products inactivate the enzyme after a single catalytic cycle (113). This process can be taken as evidence for a biochemical role for lipid peroxidation, essential for cell development and perhaps analogous to the function of lipoxygenase in plants, where it is a purported agent in the blanching of leaves (114). Events such as these can be viewed as biochemically intrinsic functions, analogous to the generation of prostaglandins, where the lipid peroxidation process normally plays an essential and noncytotoxic role.

The circumstances described above may not apply, however, when lipid peroxidation takes place via uncontrolled free radical chain reactions, particularly when the antioxidant capacity of the cell or organelle is exceeded. Under these conditions the products of lipid peroxidation may contribute to the cytotoxic cascade. These toxic reactions range from genotoxicity (115–117) to inflammatory events associated with cell damage and repair (see below).

Lipid peroxide formation appears to be associated, and may even be necessary, to the process of inflammation. Clearly cyclooxygenase- and lipoxygenase-derived products serve as potent vasoactive and chemoattractant factors (118, 119); however, peroxidation products of nonleukotriene and prostaglandin nature also display similar biological actions. Much of this activity may simply be the result of the "permissive" effect exerted by lipid peroxides upon prostanoid synthesis. Accordingly, peroxides are considered as activitors of prostanoid formation likely at the site of prostaglandin synthetase (120). Marshall & Lands (121) recently showed that lipid peroxide activators generated by neutophils stimulated with phorbol ester enhance the synthesis of specific prostaglandins. The accumulation of peroxides may thereby offset the homeostatic control of thromboxanes and leukotrienes, vs PGE_2 and prostacyclin, which supports synthesis of the former compounds while it suppresses formation of the latter (122, 123). For example, 12-hydroperoxy-eicosatetraenoic acid, a product of platelet lipoxygenase, inhibits prostaglandin and thromboxane production while stimulating lipoxygenase activity, and in turn its own biosynthesis (124). Similar, although less potent, properties are shared by 11-, 9-, and 8-hydroperoxy-eicosatetraenoic acids (124), which can be formed via arachidonic acid autoxidation. Furthermore, an assumed mixture of these peroxides has been shown to affect platelet aggregation (125), whereas arachidonic and other polyenoic fatty acid oxidation products appear to possess chemoattractant properties toward leukocytes (126).

The release of lipid peroxides from sites of injury is well documented under circumstances such as oxygen radical damage to the lung (127) and burn injury (97). McCord & Petrone (128) suggested that lipid peroxidation products formed via free radical reactions have the capacity to interact with serum components and form chemoattractant products. This may be a basis for the

inflammatory reaction evoked through injury of a nonimmunologic nature. Peroxidation of membrane lipids at the site of injury could lead to the release of lipid peroxide fragments from membrane phospholipids by mechanisms described in the preceding sections. Specific enzymatic reactions have been demonstrated to be capable of releasing peroxidized fatty acids from membranes. Some lipolytic reactions may represent a potentially important group of enzymes possessing the capacity to effect turnover of damaged lipids. In this respect, phospholipases were shown to hydrolyze oxidized lipids rapidly in variety of membrane systems (100, 129–131).

It is possible that this preferred activity against oxidized, or damaged membrane phospholipids is related to a general mechanism of membrane turnover and may therefore be an important component in maintaining a functionally intact membrane. Studies in our laboratory indicate that phospholipase A_2 activity may be linked to a hierarchy of self-defense and repair systems that either prevent or minimize peroxidative damage to cell membranes. For example, the ability of glutathione peroxidase to reduce hydroperoxides located in membranes is facilitated by their cleavage from phospholipids and release into the cytosolic fraction of the cell (100). In a membrane subjected to low levels of lipid peroxidation, this function of phospholipase could minimize the propagation of radical chain reactions by severing the propagating species from the membrane. On the other hand, membranes undergoing extensive peroxidation could be rapidly degraded by an active endogenous phospholipase whose hydrolytic action exceeds reacylation and other phospholipid synthetic systems. In this respect phospholipase may work in concert with the reticulocyte lipoxygenase to degrade mitochondrial membranes (112).

In some cases, degenerative diseases may become clinically manifested through peroxidative deterioration of tissues. One class of such diseases is associated with an accumulation of autofluorescent lipopigments, e.g. ceroid, which as described previously forms from lipid-peroxide-dependent polymerization reactions. Accumulation of these lipopigments is a hallmark of Batten's disease, otherwise known as neuronal ceroid-lipofuscinosis. Severe neuronal and visual impairment occurs progressively in individuals bearing the homozygous recessive trait. Although Batten's disease is classified as a lysosomal storage disease (132), there is recent evidence that a biochemical dysfunction associated with H_2O_2 and lipid peroxide metabolism may underly the disease process. In animal models the disease was found to be associated with a peroxidase deficiency (133), which suggests that lipid peroxides derived from the eicosanoid biosynthetic pathway accumulate and serve as the source of lipopigment (134). In this instance a specific disease process may be attributed to either an abnormal production of lipid peroxidation products or a deficiency in antioxidant protective systems. Given the range of products

formed during membrane lipid peroxidation, and the coordinated action of antioxidants and enzymes directed toward the metabolism or elimination of lipid peroxidation products, it is conceivable that a finely tuned system may operate in the cell whose functional roles have only recently been surmised.

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