REVIEW

Lipid Oxidation: Biologic Effects and Antioxidants—A Review[1]

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ABSTRACT

The detection and measurement of lipid oxidation in biological systems and some biologic effects of this oxidation are reviewed. The role of lipid oxidation in the process of photocarcinogenesis and the protective effect of antioxidants against this process also are discussed. The mechanism of such protection is unknown and studies directed at elucidating the mechanism of antioxidant effect in photocarcinogenesis and in some other pathological conditions believed to involve lipid oxidation are needed. In addition to this, epoxidation of lipids observed in monolayer studies requires further investigation, particularly in the presence of some other unsaturated molecules. The possible significance of such a study—particularly in the presence of polycyclic aromatic hydrocarbon carcinogens, where formation of epoxides is generally accepted as active intermediates—is also discussed. In addition, present knowledge on the role of lipid peroxides in the destruction of proteins and biomembranes, in chemically induced toxicity and in generation of singlet oxygen is presented.

INTRODUCTION

It has long been known that fats go rancid by slow autoxidation during storage. In recent years, there has been a renewed interest in studying the mechanism of lipid oxidation and in the detection of oxidized products, particularly in biological systems. It is now recognized that lipid oxidation in biological membranes is a very destructive process. To date, lipid oxidation has been implicated in liver cell injury caused by chemicals (1-4) such as CCl_4, $BrCCl_3$, 1,1,2,2-tetrachloroethane, ethylene bromide and ethanol. Lipid peroxidation has been proposed as a possible mechanism in the clinically important phenomenon of ozone toxicity (5,6) in which lung damage induced by ozone and nitrogen dioxide results (7). In addition to these effects, reactions between peroxidized lipids and proteins have been shown to cause loss of enzyme activities (8,9), polymerization (10-14), polypeptide chain scission (15), accelerated formation of brown pigments (8,14,16) and the destruction of labile amino acid residues such as histidine, lysine, cysteine and methionine (12). Photosensitized oxidation of lipids has been invoked in the process of photocarcinogenesis (17-23). Black and Chan have reported that cholesterol-α-oxide is generated both in vitro and in vivo by irradiation of cholesterol in the presence of oxygen. Cholesterol-α-oxide is reported to show weak carcinogenic activity and was suggested as a proximate carcinogen in the process of photocarcinogenesis by these workers. In support of this postulate, Lo and Black (17,23) have reported that feeding a diet rich in antioxidants affords considerable protection against photocarcinogenesis and delays the growth of tumors in comparison to control animals fed unsupplemented diet.

In this article, lipid oxidation is reviewed in general with particular emphasis on some of its biologic effects. The role of antioxidants as protective agents against photocarcinogenesis and some other pathological conditions involving lipid oxidation also are discussed.

LIPID OXIDATION

Oxidation in Bulk Phase

"Dark" oxidation. Several studies on autoxidation of fatty acids have been reported previously (24-28). A mechanism which is now generally accepted is that autoxidation of lipids involves a free radical mechanism as shown in Figure 1. The oxidation is initiated by allylic $H^{\bullet}$ abstraction followed by oxygen attack on the carbon radical thus generated. In recent years, using gas chromatography (GC)-mass spectroscopy (MS), Frankel et al. (29-32) and others (33-35) have done a detailed

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$$RH + O_2 \longrightarrow R\cdot + {}^{\cdot}OH$$
$$R^{\cdot} + O_2 \longrightarrow ROO^{\cdot}$$
$$ROO\cdot + RH \longrightarrow ROOH + R\cdot$$
$$R\cdot + R\cdot \longrightarrow RR$$
$$R\cdot + ROO\cdot \longrightarrow ROOR$$
$$ROO^{\cdot} + ROO\cdot \longrightarrow ROOR + O_2$$

FIG. 1. Autoxidation of lipids: RH represents a fat molecule in which H is an allylic hydrogen.

study of the composition of autoxidation products of methyl oleate, methyl linoleate and methyl linolenate (Table I) and of their mixtures in different proportions. As shown in Table I, the major products obtained in autoxidation of methyl oleate include 8-, 11-, 9- and 10-hydroperoxides. It is interesting that concentrations of 8- and 11-hydroperoxides in these studies are higher than 9- and 10-hydroperoxides. Methyl linoleate, on the other hand, gives equal amounts of 9- and 13-hydroperoxides, indicating that initial $H^{\cdot}$ abstraction occurs at doubly allylic carbon 11. Bulk phase oxidation of methyl linolenate yields the expected products derived from abstraction of hydrogen radical from 9- and 11- carbons which are doubly allylic positions. However, the product distribution (9- and 16-OOH>>12- and 13-OOH) of hydroperoxides is not as expected by the general mechanism shown in Figure 1. On the basis of mechanistic studies reported up to this time, it appears that primary processes involved in autoxidation still conform to the general free radical mechanism shown in Figure 1; the final distribution of the products, however, would depend on secondary reactions such as rearrangement of the intermediate allylic radicals or of the final products, further oxidation and disproportionation reactions. In view of this, it has been suggested (32) that the reduced yield of 12- and 13-hydroperoxides (18-25%), in comparison to 9- and 16-hydroperoxides (75-81%) from methyl linolenate, may result from the unique 1,5-diene structure of the former group leading to the formation of the 6-membered cyclic peroxides or more likely by their tendency to cyclize into prostaglandin-like endoperoxides (Fig. 2). From the practical aspect, it implies that despite any secondary reactions involved, the bulk phase "dark" oxidation of lipids can be inhibited by free radical quenchers. The antioxidants commonly used in the food industry are 3(2)-*tert*-butyl-4-hydroxyanisole (BHA); 3,5-di-*tert*-butyl-4-hydroxytoluene (BHT); 4-hydroxy-methyl-2,6-di-*tert*-butylphenol (Ionox-100); mono-*tert*-butylhydroquinone (TBHQ), 3,3′-thiodipropionic acid (TDPA); 2,4,5-trihydroxybutyrophenone (THBP); dilauryl thiodipropionate (DLTDP); *n*-propyl gallate (PG); and nordihydroguaiaretic acid (NDGA).

Photosensitized Oxidation

Although most fats and lipids do not absorb visible or near untraviolet (UV) light, photosensitized oxidation caused by chromophore

TABLE I

Autoxidation of Fatty Acids

Fatty acid	Reaction conditions	Principal products	Ref. no.
1. Methyl oleate	bulk phase; "dark" 25-80 C	Hydroperoxides, 8,11 > 9,10	30
2. Methyl oleate	bulk phase; photosensitized (erythrosine); room temp.	9 and 10 OOH	39
3. Methyl oleate	bulk phase; photosensitized (riboflavine); room temp.	8,9,10 and 11 OOH	39
4. Methyl linoleate	bulk phase; "dark"; 40-50 C	9 and 13 OOH (1:1)	31
5. Methyl linolenate	bulk phase; "dark"; 25-50 C	9 and 16 OOH (75-81%) 12 and 13 OOH (13-25%)	32
6. Methyl linolenate	bulk phase; photosensitized (erythrosine), room temp.	9, 12, 13 and 16 conjugated diene OH	39
7. Methyl linolenate	bulk phase; photosensitized (riboflavine), room temp.	9, 12, 13 and 16 conjugated diene OOH	39
8. Linoleic acid	monolayer on silica gel, 60 C	*cis*-9,10- and *cis*-12,13-monoepoxy linoleic acids	51
9. Linoelaidic acid	monolayer on silica gel, 60 C	*trans*-9,10- and *trans*-12,13-monoepoxy linoelaidic acid	51

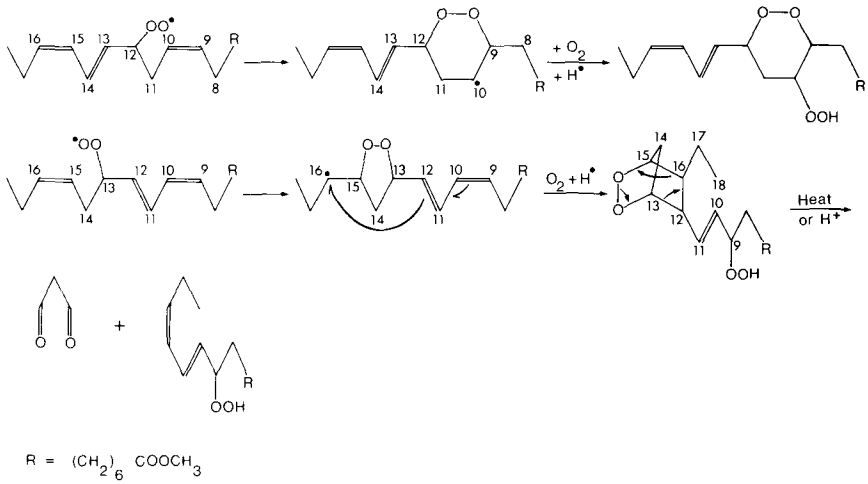


FIG. 2. Autoxidation of methyl linolenate: rearrangement of 12- and 13- hydroperoxides (32,69).

impurities present such as chlorophyll, porphyrins, myoglobins and pheophytins has long been known (36,37). In general, photosensitized oxidation of lipids is believed to involve singlet oxygen (37) produced by photosensitized excitation of triplet oxygen by chromophore impurities. Several mechanisms (38) such as "ene," radical-, ionic-, peroxirane- or dioxetane-intermediates have been proposed for addition of singlet oxygen to olefins. Of all the mechanisms proposed, the "ene" mechanism (Fig. 3) and peroxirane intermediates are most consistent with experimental facts (38). Chan (39) has reported, however, that some photosensitized oxidation of lipids may involve triplet state oxygen. Using 2 different sensitizers, erythrosine and riboflavine, for oxidation of methyl oleate and methyl linolenate, he showed that erythrosine sensitization involves singlet oxygen (Fig. 4, type II) whereas riboflavine-sensitized oxidations involve triplet oxygen (Fig. 4, type I). Since singlet oxygen reactions with nonconjugated olefins are known to involve 1,2-attack (Fig. 3) whereas oxidation by triplet oxygen involves free radicals, a distinction between 2 mechanisms was achieved by structural elucidation of the hydroperoxides formed, by the aid of GC-MS (39). Methyl oleate, upon photosensitized oxidation with erythrosine, afforded 9- and 10-hydroperoxides only whereas riboflavine-sensitized reactions gave a mixture of 8-, 9-, 10- and 11-hydroperoxides. Similarly, methyl linolenate generated a mixture of 9-, 12-, 13- and 16-conjugated diene hydroperoxides in both erythrosine- and riboflavine-sensitized reactions, except that erythrosine-sensitized reactions also afforded 10- and 15-nonconjugated diene hydroperoxides which were not observed at all in the riboflavine reaction. Since formation of nonconjugated diene hydroperoxides is possible only through singlet oxygen (attack at 10 or 15 position; Fig. 5), the erythrosine-sensitized reactions were considered to involve $^1O_2$. Riboflavine-sensitized oxidations primarily involve radical formation like "dark" oxidation but notable differences in the 2 processes were observed (39). Unlike "dark" oxidation, photooxidation involving triplet oxygen did not involve chain reactions. Besides, no induction period was observed in the photooxidations whereas the "dark" oxidations involve long induction period. This was supported by the relatively small inhibitory action of the antioxidant BHT in the riboflavine reactions. From the practical viewpoint, this implies that prevention of photosensitized oxidation involving type I or type II mechanisms should not be possible through the antioxidants commonly used to inhibit "dark" oxidation. Singlet oxygen quenchers such as carotene, triethylamine and nickel chelates are quite effective inhibitors of photooxidative deteri-

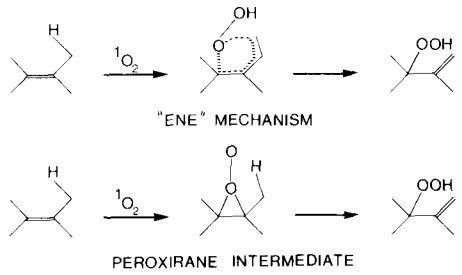


FIG. 3. Reaction of $^1O_2$ with olefins (38).

Sens. + A + hν → [intermediates I] Type I

[intermediates I] + O_2 → Products + Sens.

Sens. + O_2 + hν → [intermediates II] Type II

[intermediates II] + A → products + Sens.

Sens. + hν → ^{1}sens.

^{1}sens. → ^{3}sens.

^{3}sens. + $^3O_2$ → Sens. + $^1O_2$

FIG. 4. Proposed mechanism for photosensitized oxidation by triplet (Type I) and singlet (Type II) oxygen (39).

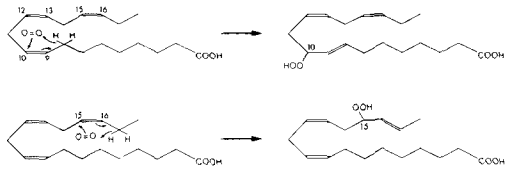


FIG. 5. Photosensitized (erythrosine) oxidation of linolenic acid. Formation of 10- and 15-nonconjugated diene hydroperoxides (39).

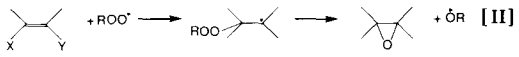
oration in lipids. Naturally occurring α-tocopherols quench singlet oxygen efficiently but are themselves oxidized in the process (40). Effective inhibitors for type I oxidation are not very well known.

Autoxidation in Monolayers

The use of adsorbed monomolecular films as models for the study of nonenzymatic membrane lipid autoxidation has been attempted by several investigators (41-51). Honn and coworkers (41) using silica gel as the support for soybean oil, were the first to correlate the effect of different ratios of substrate-to-solid support on autoxidation. Porter et al. (44,45) demonstrated that the maximal rate of autoxidation was exhibited by the linoleic acid-to-silica ratio close to that for a monolayer. Porter et al. (44,45) also studied the effects of prooxidants and antioxidants on the rate of lipid autoxidation.

Unlike oxidation in bulk phase, oxidation of linoleic and linoelaidic esters in monolayers form predominantly *cis* or *trans* epoxy compounds (51). Methyl oleate, however, does not give any detectable amount of the epoxy compound. Kinetically, the reaction is reported to be of first order in contrast to bulk phase oxidation where the reaction is kinetically more complex. Mechanistically, the reaction is rationalized as an addition of peroxy radical generated by initial abstraction of allylic hydrogen (Eq. I) on double bond. This is followed by loss of alkoxy radical from the intermediate, resulting in the formation of epoxides (Eq. II).

ROOH → ROO· + H· [I]



[II]

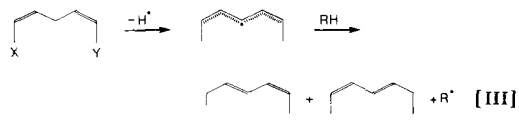
Since no detectable amount of the epoxide is observed in the monolayer oxidation of methyl oleate, it would be interesting to observe if methyl oleate epoxide is formed when a mixture of the esters of oleic and linoleic acids is oxidized. If interepoxidation reactions of this kind are observed in fatty acid esters, it will be of further interest to investigate their reactions with other molecules such as polycyclic aromatic hydrocarbons (52) and cholesterol (17-23), the epoxides of which have been implicated as "proximate" carcinogens in chemical and photocarcinogenesis, respectively. This kind of study may further shed light on the mechanism involved in enhancement of carcinogenic activity in the polycyclic aromatic hydrocarbons by unsaturated fatty acids (53).

DETECTION AND MEASUREMENT OF LIPID OXIDATION

Recently Gray (54) has reviewed the detection and measurement of lipid oxidation in vitro, specifically in food products. We shall therefore confine ourselves to biological systems only. Assays used in these systems can basically be divided into the following categories: (a) conjugated diene assay; (b) estimation of hydrocarbon gases; (c) detection of malonaldehyde and fluorescent products; and (d) loss of polyunsaturated fatty acids.

Conjugated Diene Assay

It has been observed by several workers that lipids containing dienes or polyenes on peroxidation show a shift in double bond position leading to conjugation (55-57). Mechanistically, it involves initial abstraction of H· from the doubly allylic position followed by double bond migration resulting in conjugated dienes which show an intense absorption at 233 nm (Eq. III). Similarly, conjugated trienes show an



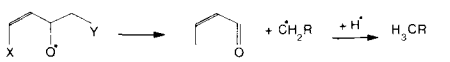
[III]

absorption at 288 nm. This has been widely used for detection and estimation of lipid peroxidation in liver cell injury by hepatotoxic agents. A limitation of the method is, however, that it is nonspecific and the extinction coefficients used for biological systems are only approximate.

Other methods based on estimation of conjugated dienes use classical Diels Alder reactions. Ellis and Jones (58) used this method for estimation of conjugated dienes in tung oil. Maleic anhydride was used as a dienophile and quantitation was done by estimating the unreacted anhydride. This method, however, requires high temperatures and long reaction times, and is thus not suitable for biological systems. Waller and Recknagel (59) have successfully extended the scope of this reaction to biological systems by using ^{14}C-labeled tetracyanoethylene (TCNE) as an extremely reactive dienophile. Quantitative determination of conjugated dienes is done by estimating the incorporation of radioactive labels in the adducts. Limitations of the method are that: (a) conjugated trienes and tetraenes also form adducts with the dienophile, whereas *cis-cis* dienes do not make an adduct because of their transoid configuration; (b) phosphate groups react with TCNE (this difficulty was overcome, however, by reducing phosphate groups with lithium aluminium hydride before analysis); and (c) preparation of labeled TCNE involves labeled KCN, which is a deadly poison and requires extremely careful handling.

Hydrocarbon Gases

Riley et al. (60) first reported in *Science* in 1974 that hydrocarbon gases of low molecular weight (MW) were released upon treatment of mice with CCl_4. Since then, several reports have appeared indicating measurement of these gases as an index of lipid peroxidation in biological systems (61-66). A general route for the formation of these gases as visualized by Evans et al. (67) is shown in Equation IV. Hydroperoxide decomposition to alkoxy radical is the key step in the proposed scheme which is followed by β-scission and hydrogen abstraction resulting in the formation of hydrocarbon gases. β-Scission of alkoxy radical is a well known process (68) and involves unpairing of electrons in the bond located *beta* to the free radical. This process generates hydrocarbon free radicals and stable carbonyl compounds. As a support for this mechanism, it has been observed that transition metals, particularly the iron and copper catalysts, help form these gases in relatively greater amounts. Since free radicals of hydrocarbons can follow other routes for their termination besides hydrogen abstraction, a variety of products are theoretically possible. Most important of these alternative routes include dimerization, unsaturation caused by loss of $H^{\bullet}$ radical and further β-scission followed by the processes just mentioned. In view of this, it would be difficult to develop a quantitative relationship between state of peroxidation and the amount of a particular gas generated. Besides, calculated molar ratios show that hydrocarbon gases are only minor lipid oxidation products (69). Since $^{\bullet}OH$ is more stable as ^{-}OH whereas $H^{\bullet}$ preferably stays as H^{+}, it is conceivable that transition metals in their lower oxidation states would aid the formation of alkoxy radicals (Eq. V) whereas their higher oxidation states would favor generation of peroxy radicals (Eq. VI). Since, in biological systems, transition metals are present mostly in higher oxidation states, routes leading to hydrocarbon gases are of only minor importance. Despite these limitations, Tappel et al. and other workers (64-67) have devised methods for quantitative measurement of hydrocarbon gases and have indicated their use in estimating the extent of oxidation of lipids.



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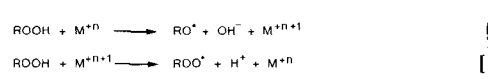


[V]

[VI]

Detection of Malonaldehyde and Fluorescent Products

Detection of malonaldehyde (Fig. 6), commonly known as the thiobarbituric acid (TBA) test, has been used widely both in vivo (68) and in vitro (54) for the detection of lipid peroxidation. Experimental procedure involves treatment of oxidized lipid with thiobarbituric acid which results in the formation of a highly colored complex that is measured by colorimetric method (λ max 532 nm). Limitations

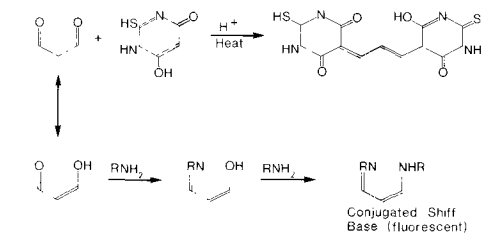


FIG. 6. Reaction of malonaldehyde with thiobarbituric acid and with compounds containing $-NH_2$ groups (69,74).

and pitfalls of this method in vitro are adequately covered by Gray (54) and may well hold for biological systems. Since malonaldehyde itself is a very reactive material and is known to make cross linkages with proteins in biological systems, it is questionable whether malonaldehyde is the ultimate product in lipid oxidation which reacts with TBA or if some other reactive material generates malonaldehyde under the conditions of TBA test. Pryor et al. (69) have suggested that, at least in part, the prostaglandin type of endoperoxides are a possible precursor of malonaldehyde under the test conditions. Since reaction of malonaldehyde with proteins and other cellular constituents results in fluorescent products, the detection of lipid oxidation in biological tissues by fluorescence has been found to be 10-to-100 times more sensitive than the TBA test (70-74). Although this method is very sensitive and is gaining wide acceptance, the chemistry of the formation of fluorescent products and their specific characterization needs further exploration.

Loss of Polyunsaturated Fatty Acids

This technique, developed by May and McCay (75), uses the loss of polyunsaturated fatty acids moieties as an index for the detection and measurement of lipid peroxidation. In this method, total fatty acid composition of the tissue lipid is determined by gas liquid chromatography (GLC) before and after lipid oxidation. The loss in amount of polyunsaturated fatty acids in oxidized lipid is then compared to that in the control. A major difference between this and other methods is that it involves a direct analysis of the tissue lipids themselves as opposed to the detection of products resulting from peroxidation. In this respect, this technique provides one of the most direct methods for detection and measuring the extent of lipid peroxidation in biological systems.

BIOLOGIC EFFECTS OF LIPID PEROXIDATION

Lipid Oxidation and Photocarcinogenesis

It is now widely accepted that UV light, specifically between 280-320 nm, can cause cancer (76). The mechanism of photocarcinogenesis is, however, not well understood. The basic mechanistic theories for photocarcinogenesis, DNA damage and repair, lysosomal destruction and photochemical mechanisms have been discussed in a recent review by Black and Chan (19). Although all these theories have their merits and demerits, we shall focus here on some aspects of photochemical theory which invokes sterol and lipid oxidation as the primary steps in the process of photocarcinogenesis. An intensive series of studies by Black and associates has centered around the observation that irradiation of skin, both in vitro and in vivo, leads to the photooxidation of sterols (77,78). One of the observed photoproducts, cholesterol-α-oxide, has been shown to be weakly carcinogenic (79), leading to the speculation that in vivo photooxidation might be the route to a "proximate carcinogen" of photocarcinogenesis. The mechanism for the formation of cholesterol-α-oxide is unknown and deserves further investigation as it may reveal the presence of some other active species involved in irradiation of skin. It has been suggested that the epoxide formation possibly involves free radicals because it was observed that cholesterol-5α,6α-epoxide levels in skin of animals fed an antioxidant- (free radical quencher) supplemented diet reached a peak 4 weeks after that of animals fed regular diet. Since formation of the epoxide is observed both in vitro (photolysis in water) and in vivo, its formation possibly involves an attack of peroxy radicals generated from cholesterol itself or from other lipids on the 5-6 double bond of cholesterol (Fig. 7). It may be recalled here that stereospecific formation of epoxy compounds is also observed in monolayer autoxidation of fatty acid methyl esters. Whatever the mechanism involved for the formation of the cholesterol epoxide, the subsequent studies of Black and Chan indicate there appears to be no direct relationship between light-induced formation of the epoxide and photocarcinogenesis. Moreover, the most effective wavelengths for the formation of the epoxide are reported to be 254 and 265 nm, which do not contribute to the solar spectrum reaching earth. Photosensitized reactions, however, involving triplet oxygen, as observed in riboflavine-sensitized oxidation of lipids (39), are possible in skin and deserve further exami-

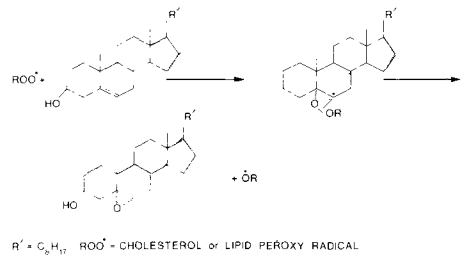


FIG. 7. Possible mechanism for the formation of cholesterol-5α,6α-epoxide.

nation. Without considering in detail the studies done by Black et al. and the associated interpretations, there are 3 specific observations in Black's work which invite further examination: (a) irradiation of normal skin with carcinogenic UV light led to oxidation of at least one normal constituent (78); (b) feeding a diet containing a mixture of added antioxidants delayed the appearance of photooxidation products (23); and (c) animals fed the antioxidant-containing diet were afforded considerable protection against photocarcinogenesis (17).

Whether or not a specific chemical mediator for carcinogenesis has been identified, the mechanism by which antioxidants could afford such protection is of interest. One possibility, certainly, is through direct or indirect screening of the skin. Since oral grooming is typical behavior in mice, and since the antioxidant mixture used made up 2% of the diet, detectable skin surface contamination is probable; alternatively, one or more of the components could have reached the skin indirectly through intestinal absorption and cutaneous excretion. Of the antioxidants used (BHT, ascorbate, reduced glutathione and α-tocopherol acetate), only the tocopherol (E max. 288 nm) is a likely candidate for chromophore status in normal light. DeRios et al. (80) have reported reduced erythema responsiveness in antioxidant-fed animals, but the light source in this case was a medium pressure mercury arc. Since shortwave (254 nm) UV light is a major contributor to the erythema effectiveness of this source, all components of the mixture could provide some protective screening.

That photochemical alteration of skin sterols has been observed in vitro (22) is evidence that such changes are not secondary effects of metabolic alterations. On the other hand, Black et al. (21) observed changes in metabolic activity of light-induced tumors compared to unirradiated skin, reflected in ^{14}C-acetate incorporation into various lipid classes. Whether the changes were tumor-specific or true of irradiated skin generally was not examined in this study. The possibility remains that any observed changes in cutaneous lipids of irradiated animals could involve direct or indirect photochemical effects or altered metabolic activity.

Most normal skin lipids are unlikely to function as chromophores for mid-UV light (> 290 nm); exceptions include carotenoids, some dehydrosterols and possibly other polyenes. Lipid peroxides were reported, however, by Dubouloz and Dumas (81) following irradiation of skin and others have reported enhanced free radical signals in irradiated skin (82). Thus, the likelihood of endogenously photosensitized reaction in skin does exist. Since oxidation of cholesterol and accumulation of its photoproducts reaches a maximum well before tumor appearance, it would be most reasonable to look for systematic cumulative lipid changes during early stages of irradiation.

Interaction of Lipid Peroxides with Proteins

The observation that malonaldehyde, an ultimate product in oxidation of polyunsaturated fatty acids, can make cross linkages with proteins (8,14,16,70) aroused a new interest in the study of lipid peroxides-protein interactions (8-16). Conversely, free radicals generated by peroxidation of lipids have been reported to initiate free radical formation in proteins which may, in turn, result in dimerization or polymerization (10-14). The polymerization process is considered to be more damaging to biomembranes. Most of the studies on lipid peroxide-protein interaction are done in vitro on sulfur amino acids because of the oxidative sensitivity of the sulfhydryl group. Roubal and Tappel (12) have reported that peroxidation can cause destruction, in varying degrees, of individual amino acids including arginine, serine, glutamic acid, methionine, tyrosine, phenylalanine and threonine. In studying the chemical nature of such interactions, Gardner et al. (83) has reported that N-acetyl cysteine, catalyzed by 10^{-5}M ionic iron in 80% ethanol, adds to linoleic hydroperoxide forming a thio bond. Reaction of a specific isomer of the hydroperoxide, 13-hydroperoxy-*trans*-11,*cis*-9-octadecadienoic acid, and N-acetylcysteine forms a number of products, 2 of which were identified as 9-S-(N-acetylcysteine)-13-hydroxy-10-ethoxy-*trans*-11-octadecenoic acid (I) and 9-S-(N-acetylcysteine)-10,13-dihydroxy-*trans*-11-octadecenoic acid (II) (Fig. 8). Yong and Karel (84) have reported that reaction of histidine with methyl linoleate

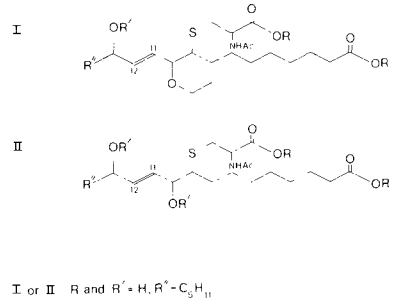


FIG. 8. Principal products of reaction between 13-hydroperoxy-*trans*-11, *cis*-9-octadecadienoic acid and N-acetylcysteine (81).

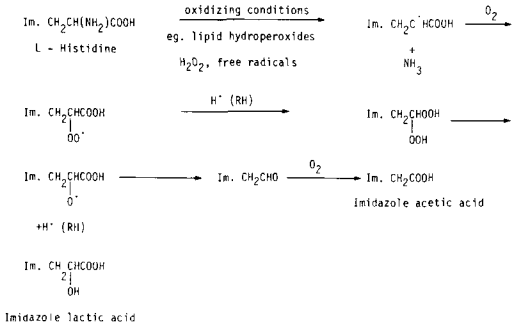


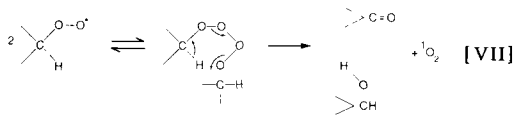
FIG. 9. Reaction of L-histidine and peroxidized lipids (82).

hydroperoxide (dispersed on a filter paper) affords imidazole lactic acid and imidazole acetic acid as major products. The reaction is considered to be taking place through free radical-mediated deamination and decarboxylation (Fig. 9). Nielson (85) has shown that interaction between peroxidized phospholipid (cardiolipin) and protein (albumin) results in covalent bonding.

The foregoing discussion clearly indicates that chemical interaction between peroxidized lipid and proteins is feasible but the nature of such interactions in vivo is not clear yet. More realistic model systems and further studies in vivo are required before any clear picture of these processes would emerge.

Generation of Singlet Oxygen

The finding by chemical means that singlet oxygen is generated in the self-reaction of *sec* butyl peroxy radicals (86) led to the speculation that peroxy radicals may be responsible for the generation of $^1O_2$ in the NADPH-dependent microsomal lipid peroxidation system (87). Nakano et al. have confirmed spectroscopically the generation of $^1O_2$ in such a system and have further demonstrated the generation of singlet oxygen from linoleic acid peroxy radicals. Peroxy radicals were produced from linoleic acid hydroperoxide by oxidation with ceric ion. The proposed mechanism (86,88) for the generation of singlet oxygen involves formation of tetroxide and seems to be in agreement with experimental observation (Eq. VII).



[VII]

Recently, it was shown that 2,5-diphenylfuran, when incubated with linolenic hydroperoxide in Tris buffer (pH 8.5) for 30 min, afforded *cis*-dibenzoylethylene (89), a product obtained by reaction of 2,5-diphenylfuran with singlet oxygen. It was inferred from these studies that a singlet oxygen-like factor was produced from linolenic acid hydroperoxide under the experimental conditions. Incubation of linolenic acid hydroperoxide, however, with diphenylanthracene (DPA) and 7,12-dimethylbenz[a]anthracene (DMBA) under the conditions described did not yield any detectable amount of endoperoxides (Logani, Austin, and Davies, unpublished results) or their rearranged products (90-92). Since it has been reported that formation of *cis*-dibenzoylethylene from 2,5-diphenylfuran may not necessarily involve singlet oxygen (93), the use of more specific traps is required to establish the generation of singlet oxygen from lipid hydroperoxides. Although at this time the hazards of singlet oxygen in biological systems are not very well defined (94), the presence of this active specie can be quite damaging to biological membranes and has been implicated in photocarcinogenesis (95) and photodynamic action (94,96).

Chemically Induced Toxicity

It has long been suspected that toxicity caused by several chemicals involve lipid peroxidation (1-4). The major limitation in these studies has been to detect and quantitate the amount of peroxidation directly in vivo. Because of the instability of organic peroxides, direct quantitation of lipid hydroperoxides cannot be relied upon in biological systems. Different analytical methods for quantitation and detection of lipid peroxides and their limitations have been discussed previously in this article and a combination of several techniques is therefore recommeded to obtain any reliable results. Despite these limitations, there is growing evidence that lipid peroxidation is induced by several chemicals, particularly by hepatotoxic agents. Based on conjugated diene assay, it has been shown that liver injury induced by CCl_4,$BrCCl_3$, 1,1,2,2-tetrachloroethane, ethylene dibromide and ethanol involves lipid peroxidation (50). The increase in expired ethane levels was further used by Riley et al. (60) to demonstrate the involvement of lipid peroxidation in CCl_4 toxicity. Participation of peroxides in liver injury caused by ethanol has been indicated by Di Luzio and Hartman (97) using increased formation of malonaldehyde in liver homogenates of rats as an assay for lipid peroxidation. More recently, Litov et al. (3) have supported these results using the increased levels of pentane as an index of lipid peroxidation. A similar increase in

levels of hydrocarbon gases (ethane and pentane) has been reported when rats were exposed to 1 ppm level of ozone for 1 hr (3).

The effect of antioxidants in modifying the influence of these chemicals appears to support the peroxidation (free radical) theory. Di Luzio (98) demonstrated that pretreatment with antioxidants inhibited ethanol-induced fatty liver. A similar effect of vitamin E on production of ethane and pentane has been observed in ozone and ethanol toxicity (3). In further support of the peroxidation theory, N,N′-diphenyl-*p*-phenylenediamine (DPPD), an efficient free radical quencher, was found to significantly delay the effect of vitamin E deficiency in male Sprague-Dawley rats (66). Despite all this evidence in support of peroxidation hypothesis, the mechanism and the significance of peroxidation induced by chemicals is unknown and deserves further investigation.

CONCLUSIONS

The implication that lipid peroxidation is involved in the process of photo carcinogenesis in the destruction of proteins in biological membranes and enzymes and in the chemically induced toxicity is supported by detection of free radical signals of inhibiting the effect of antioxidants and above all by detection of products considered to be originating specifically from lipid peroxides only. The use of modern sophisticated analytical techniques such as electron spin resonance (ESR), nuclear magnetic resonance (NMR), carbon magnetic resonance (CMR), GC-MS, isotopic labeling, high pressure liquid chromatography (HPLC), UV and fluorescent spectroscopy has greatly aided in detection and characterization of peroxidation products. Except for the characterization of lipid oxidation products, the conclusions based on detection of free radicals or on the effect of antioxidants are by inference only. Moreover, most of the studies are done in vitro where the effects of other components present in biological systems have not been taken into account. For instance, Wu et al. (99) have shown that the rate of disappearance of unsaturated fatty acids in the autoxidation as monolayers on silica gel per se changes considerably when palmitic acid, cholesterol or cholesteryl palmitate is incorporated in the system. Similarly, DNA has been reported to retard the rate of lipid oxidation in microsomal suspensions (100). Furthermore, at this stage, the significance of peroxidation in terms of different pathological conditions is not apparent. Although the significance of peroxidation in pathology is inferred from the inhibitory effect of antioxidants, the mechanism of such inhibition is unknown in most of the cases. For example, there is evidence from chemical carcinogenesis studies that antioxidants can inhibit aromatoc hydrocarbon carcinogenesis. The antioxidants, BHA and thio compounds (disulfuranne, dimethyldithiocarbonate, benzyl thiocyanate), have been reported to inhibit mammary tumorigenesis by benzpyrene (101). BHA reportedly inhibits binding of benzpyrene to DNA whereas the related compound BHT stimulates the activity of mixed function oxidases reputedly involved in carcinogenic activation (101). Studies directed at elucidating the mechanism of antioxidant effect are therefore needed to unravel the significance of lipid oxidation in the etiology of photocarcinogenesis and in other biological effects discussed here.

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