Lipid Oxidation: Mechanisms, Products and Biological Significance

E.N. FRANKEL, Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Peoria, IL 61604

ABSTRACT

This paper reviews our studies of fatty acid hydroperoxides, their secondary products and mechanisms for their formation in the context of some of their possible biological consequences. The uneven distribution of isomeric hydroperoxides in oxidized linolenate and photosensitized oxidized linoleate is related to the formation of hydroperoxy cyclic peroxides. Interest in the hydroperoxy mono- and bi-cycloendoperoxides from oxidized linolenate stems from their structural relationship to the prostaglandins. However, the biological activity of hydroperoxy cyclic peroxides formed by autoxidation has not yet been reported. Thermal decomposition studies of secondary lipid oxidation products show they are important precursors of volatile compounds. An acid-acetalation decomposition procedure establishes that 5-membered hydroperoxy cyclic peroxides and 1,3-dihydroperoxides are important precursors of malonaldehyde. This approach provides a more specific test than the thiobarbituric acid (TBA) color reaction to evaluate lipid oxidation products as sources of malonaldehyde and its biological effects due to crosslinking. A better understanding is needed of the biological effects of a multitude of lipid oxidation decomposition products other than malonaldehyde.

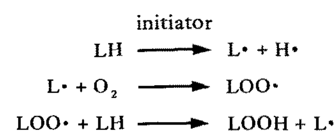
INTRODUCTION

The biological consequences of oxidized lipids that arise from in vivo reactions or from ingested foods long have attracted the attention of biochemists and food scientists. Many researchers are now working worldwide on a wide assortment of biological systems in which lipid peroxides and free radicals are implicated, including cancer, strokes, atheriosclerosis, inflammation and the aging process. Many lipid oxidation products are known to interact with biological materials to cause cellular damage. Bifunctional secondary products of lipid oxidation such as malonaldehyde are powerful crosslinking agents, and react with amino groups of enzymes, proteins and DNA. The resulting conjugated Schiff bases produced by crosslinking are fluorescent. The degree of fluorescence correlates with loss of template activity, which also is related to aging. The biological aspects of lipid oxidation have thus become the subject of a very active area of research, and many reviews have appeared (1-12).

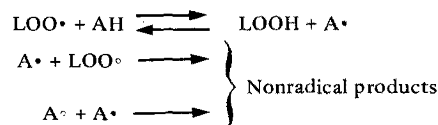
This paper reviews our structural studies of primary and secondary products of lipid oxidation, their volatile and nonvolatile decomposition products, mechanisms for their formation and some of their biological consequences. It must be emphasized at this point that much of the work on biological effects of lipid peroxidation is based on inferential evidence. Much more research is needed to establish a more direct causal relationship.

FREE RADICAL AUTOXIDATION

The reaction of oxygen with unsaturated lipids (LH) involves free radical initiation, propagation and termination processes (13). Initiation takes place by loss of a hydrogen radical in the presence of trace metals, light or heat. The resulting lipid free radicals (L•) react with oxygen to form peroxy radicals (LOO•). In this propagation process, LOO• react with more LH to form lipid hydroperoxides (LOOH), which are the fundamental primary products of autoxidation.

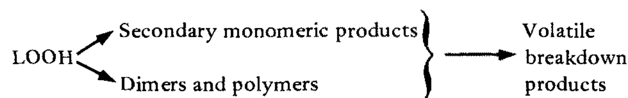


Antioxidants (AH) can break this chain reaction by reacting with LOO• to form stable radicals (A•) which are either too unreactive or form nonradical products.



Decomposition of lipid hydroperoxides constitutes a very complicated process and produces a multitude of materials that may have biological effects and cause flavor deterioration in fat-containing foods. This decomposition proceeds by homolytic cleavage of LO-OH to form alkoxy radicals LO•. These radicals undergo carbon-carbon cleavage to form breakdown products including aldehydes, ketones, alcohols, hydrocarbons, esters, furans and lactones (14,15).

Lipid hydroperoxides can react again with oxygen to form such secondary products as epoxyhydroperoxides, ketohydroperoxides, dihydroperoxides, cyclic peroxides and bicyclic endoperoxides. These secondary products can in turn decompose like monohydroperoxides to form volatile breakdown products. Lipid hydroperoxides also can condense into dimers and polymers that also can break down and produce volatile materials.

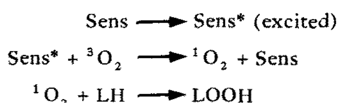


Finally, lipid hydroperoxides and some of their bifunctional breakdown products can interact with proteins, membranes and enzymes (16-21). These reactions with biological components are of most concern to biochemists because they can affect vital cell functions (1-12). Membrane deterioration caused by free radical mediated reactions contributes to the aging process (6). Age pigments known as lipofuscin are formed by this process and can be retarded by the administration of antioxidants such as vitamin E (10,22-28). The development of fat rancidity in complex food systems is also greatly affected by the interactions of proteins and amino acids with lipid oxidation products. Complex high-molecular-weight interaction products are formed during processing and cooking of foods, and their further degradation into volatile compounds is not well understood (29-32).

PHOTOSENSITIZED OXIDATION

Another important way that unsaturated lipids can be oxidized involves exposure to light and a sensitizer (sens) such as chlorophyll. By this non free radical process, oxygen becomes activated to the singlet state by transfer of

energy from the photosensitizer. The resulting singlet oxygen ($^1O_2$) produced by this process is extremely reactive. Linoleate is reported to react at least 1500 times faster with $^1O_2$ than with normal oxygen in the triplet ground state ($^3O_2$) (33).



This hydroperoxidation reaction is so rapid that it has been postulated as a process to initiate free radical autoxidation. Natural quenchers such as carotenoids protect lipids against photosensitized oxidation by interfering with this process (34). Further oxidation and decomposition of lipid hydroperoxides from photosensitized oxidation produce some of the same and some different breakdown products, which may have an impact on biological interactions similar to that of the corresponding hydroperoxides formed by free radical autoxidation.

In vivo LIPID OXIDATION

Much attention has been given to the problems of measuring lipid oxidation in biological systems (7,10,35,36). The formation of fluorescent conjugated Schiff bases by the interaction of amino acids, esters and amines with malonaldehyde has been suggested for a long time as a measure of in vivo lipid oxidation. The biological consequences of their reactions are the same as those of monohydroperoxides. Malonaldehyde has long been used as a model for secondary products of lipid peroxidation. However, as will be discussed later, a multitude of other bifunctional secondary oxidation products are known that can act as potential crosslinkers and may interact with proteins and DNA to cause biological damage.

The analyses of hydrocarbons in the breath of experimental animals has been used extensively as a sensitive index of in vivo lipid oxidation. This noninvasive method has received much attention lately (7,10). It is based on the observed increase in ethane and pentane in animals on a vitamin E-deficient diet, and by the effect of metal catalysts and oxidative agents such as ozone, carbon tetrachloride, ethanol and nitrogen dioxide. Antioxidants and selenium decrease the release of these respiratory hydrocarbons.

The most notable defense mechanisms that the body has against in vivo lipid oxidation include vitamin E and other natural antioxidants, and protective enzymes such as glutathione peroxides and superoxide dismutase. Vitamin E is the most effective in vivo inhibitor of lipid oxidation (23,28,37). In addition to its dual effects as a free radical and $^1O_2$ scavenger, vitamin E may have other cellular effects in protecting the integrity of membranes (25). Glutathione peroxidase catalyzes the reduction of hydroperoxides into innocuous alcohols, which are thus stabilized and no longer decompose into harmful aldehydes and other breakdown products (10). Superoxide dismutase removes superoxide (O_2^-), which is toxic to the cell, by converting it to hydrogen peroxide and normal $^3O_2$.

A metal-catalyzed interaction between O_2^- and H_2O_2 generates a potent oxidant postulated to be •OH, which can cause strand breaks in DNA; superoxide dismutase prevents this damage on DNA (38,39). During aging or under diseased conditions, the lowering of the concentration of protective enzymes may reduce these body defense mechanisms against the damage from free radicals and activated species of oxygen. Whether vitamin E would retard the aging process in higher animals is questionable (6).

HYDROPEROXIDATION OF UNSATURATED FATTY ACIDS

The mechanisms of hydroperoxide formation were reviewed previously for different unsaturated fatty acids (13,40). Further mechanistic studies have been published recently on the stereochemistry of linoleic and arachidonic acid oxidation (41,42). The formation of hydroperoxides by free radical autoxidation and photosensitized oxidation is summarized here to enable us to compare their isomeric distributions and understand the structures of the resulting secondary products.

According to the well-recognized mechanism of oleate autoxidation, hydrogen abstraction from the allylic methylenes on carbon-8 and carbon-11 produces 2 allylic radicals in which electrons are delocalized through 3-carbon systems (Fig. 1). These radicals react with O_2 at the end positions to produce a mixture of 8-, 9-, 10- and 11-hydroperoxide isomers. According to this mechanism, these 4 isomeric hydroperoxides would be formed in equal amounts. However, recent studies based on GC-MS (43,44) and HPLC (45) analyses show that the mechanism for oleate autoxidation is more complicated than that depicted in Figure 1, because the 8- and 11-hydroperoxide isomers are formed in small but consistently higher amounts (27%) than the 9- and 10-hydroperoxide isomers (23%). ^{13}C-NMR studies (44) also show that small amounts of *cis*-9 and *cis*-10-hydroperoxides and large amounts of *trans*-8 and *trans*-11-hydroperoxides are formed in autoxidized oleate. These results suggest a somewhat greater reactivity of carbon-8 and carbon-11 with O_2 and a change in the

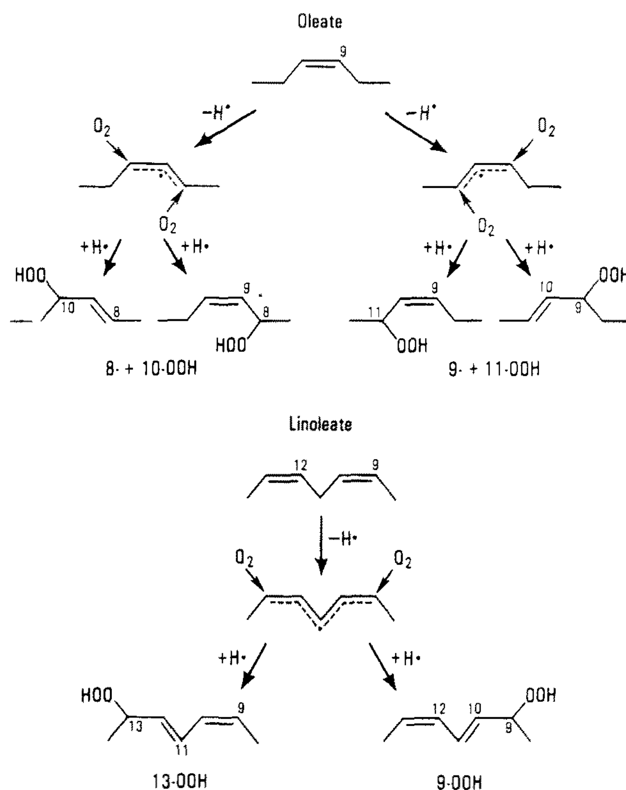


FIG. 1. Mechanism of oleate and linoleate autoxidation (40).

conformation of the allylic radical intermediates (40,46).

The classical mechanism of linoleate autoxidation proceeds by hydrogen abstraction from the doubly allylic methylene on carbon-11 to produce a delocalized pentadienyl radical. Oxygen attack at the end positions produces an equal mixture of conjugated 9- and 13-hydroperoxide isomers with the *trans,cis*-configuration (Fig. 1). Experimentally, a significant proportion of the conjugated hydroperoxides assume the *trans,trans* configuration, which increases with the level and temperature of autoxidation. The mechanisms for this change in hydroperoxide configuration have been discussed previously (40,42).

The mechanism of linolenate autoxidation is based on that of linoleate. Hydrogen abstraction on the doubly allylic methylenes on carbon-11 and carbon-14 produces 2 pentadienyl radicals. O_2 attack at the end positions of these radicals produces a mixture of 9-, 12-, 13- and 16-conjugated diene-triene hydroperoxide isomers (Fig. 2). Our early studies based on chemical cleavage analysis (47) were fully confirmed recently by GC-MS (48) and HPLC (49) studies in showing a significantly higher proportion of the outer 9- and 16-hydroperoxides than of the internal 12- and 13-hydroperoxides. This uneven distribution of isomeric hydroperoxides recently has been shown to be due to the 1,3-cyclization of the internal 12- and 13-hydroperoxide isomers into hydroperoxy cyclic peroxides (50). This cyclization of homoallylic hydroperoxides of linolenate also can be accompanied by a second cyclization to form bicycloendoperoxides structurally related to the prostaglandins (51).

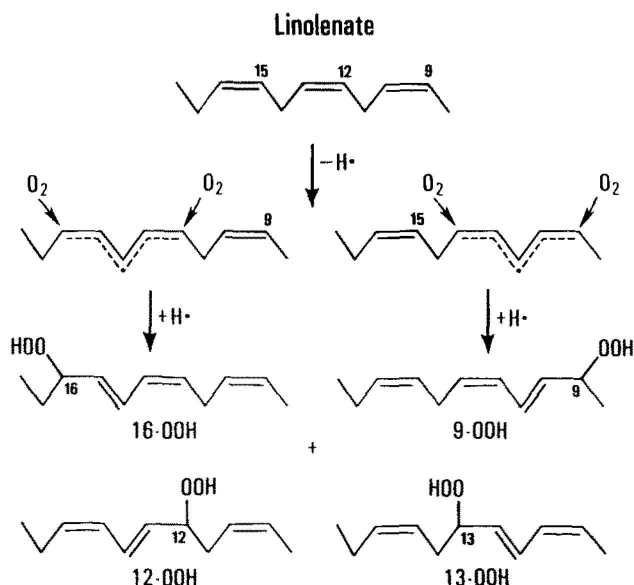


FIG. 2. Mechanism of linolenate autoxidation (47).

Autoxidation of arachidonate proceeds by the same mechanism as linoleate. Hydrogen abstraction at the three doubly allylic carbons −7, −10 and −13 produces 3 pentadienyl radicals. O_2 attack at the end positions of these radical intermediates produces 6 isomeric hydroperoxides with a conjugated diene system and 2 methylene-interrupted double bonds (Fig. 3). Like in linolenate, the external 5- and 15-hydroperoxide isomers are formed in relatively higher concentrations than the internal 8-, 9-, 11- and 12-hydroperoxide isomers (41), presumably because of their tendency to cyclize.

Photosensitized oxidation of unsaturated fatty acids proceeds by a different nonradical mechanism than autoxidation. There is a direct reaction of $^1O_2$ with the carbon-carbon double bond by a concerted "ene" addition, and hydroperoxides are formed at each unsaturated carbon. Thus, oleate produces 2 isomers: the 9- and 10-hydroperoxides with allylic *trans* double bond. Linoleate produces 4 isomers: 2 conjugated 9- and 13-diene hydroperoxides (as in autoxidation) and 2 unconjugated 10- and 12-diene hydroperoxides (different from autoxidation) (Fig. 4). Similarly, linolenate produces 6 isomers: the 9-, 12-, 13- and 16-isomers are the same as in autoxidation, and the 10- and 15- are different. According to the concerted ene addition mechanism for $^1O_2$, a statistical distribution of isomeric hydroperoxides would be expected in all unsaturated fatty acids. However, our results (52,53), which were confirmed by others (54), show an uneven distribution of hydroperoxide isomers in linoleate and linolenate.

The different distributions of hydroperoxide isomers produced by autoxidation and photosensitized oxidation are summarized in Table I. It is important to note again that the internal isomers of autoxidized linolenate (12- + 13-OOH) and of photosensitized oxidized linoleate (10- + 12-OOH) and linolenate (10- + 12- + 13- + 15-OOH) are

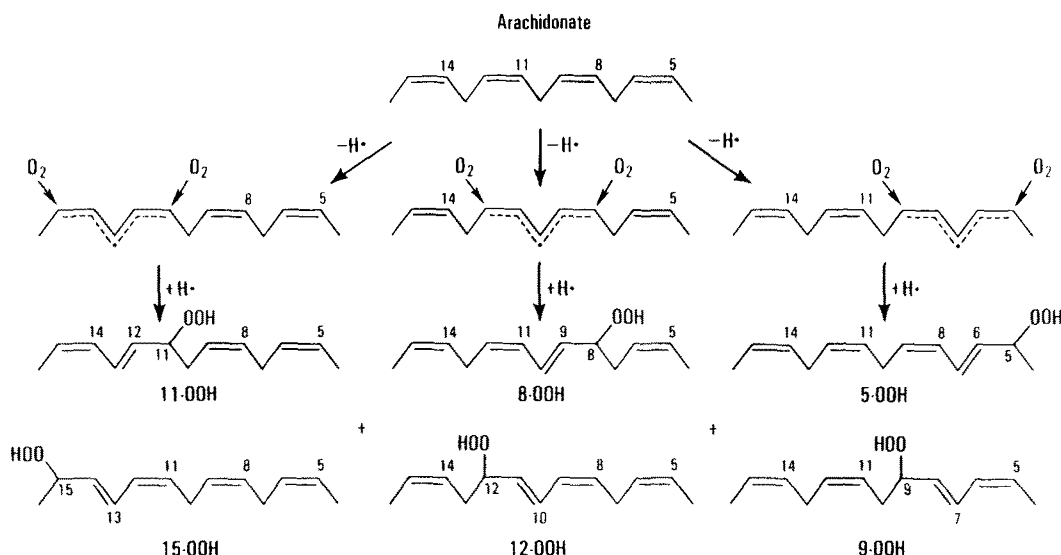


FIG. 3. Mechanism of arachidonate autoxidation.

formed in significantly lower concentrations than the external isomers. The reason for these uneven distributions of hydroperoxides is that the internal isomeric hydroperoxides have a homoallylic structure that permits 1,3-cyclization to form hydroperoxy cyclic peroxides.

SECONDARY OXIDATION PRODUCTS

Because of their structural relationship with the prostaglandins, much attention has been given recently to the cyclic peroxides formed from polyunsaturated fatty acids by autoxidation, enzyme oxidation and photosensitized oxidation (35,48,51,56-65). The precursors of materials that react with thiobarbituric acid (TBA) and prostaglandin E were shown to be mono- and bicycloendoperoxides formed during the autoxidation of linolenate and other fatty acids containing more than 2 double bonds (61). The mechanism first advanced for the formation of these cyclic peroxides involves 1,3-cyclization of the homoallylic hydroperoxide isomers (12- + 13-OOH) of linolenate (Fig. 5). Recent studies have confirmed the formation of monocyclic peroxides from autoxidized methyl linolenate (50,51,66) and from photosensitized oxidized methyl linoleate (65,67) and linolenate (68), and bicycloendoperoxides from autoxidized 13-linolenate hydroperoxides (51) as well as from photosensitized oxidized methyl linolenate (68). Bis-cyclic peroxides also were identified in photosensitized oxidized linolenate (68) and in a free radical-initiated autoxidation of the 15-hydroperoxide isomer of arachidonic acid (69). Six-membered hydroperoxy cyclic peroxides also have been prepared by the photosensitized oxidation of methyl linoleate hydroperoxides (70). Figure 6 summarizes the general structures of different hydroperoxy cyclic peroxides identified in autoxidized linolenate and photosensitized oxidized linoleate and linolenate.

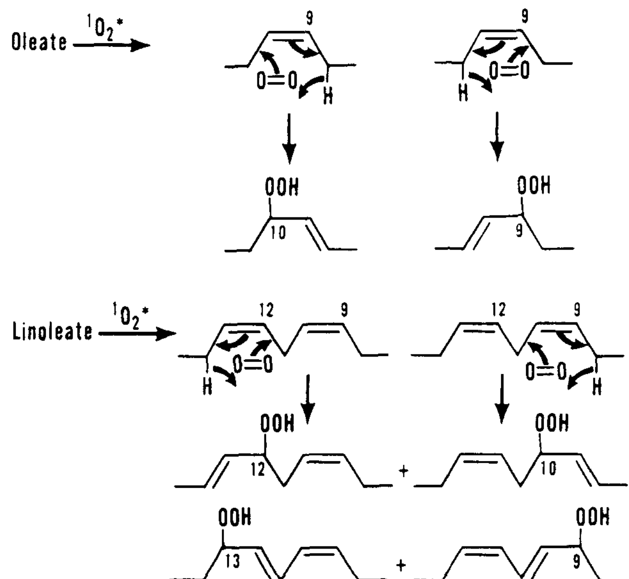


FIG. 4. Mechanism of photosensitized oxidation (13).

Much interest has been generated in the bicycloendoperoxides identified in oxidized linolenate because of their structural relationship to the prostaglandin endoperoxides formed biosynthetically from arachidonic acid (64) (Fig. 7). Prostaglandins have extremely potent physiological activities. PGH_2 and thromboxane aggregate platelets, whereas PGI_2 inhibit platelet aggregation. These materials thus have been implicated in the inflammatory process in heart attacks, strokes and smooth muscle contraction (64). The bicycloendoperoxides from linolenate were shown to have mainly *cis* substituents (51), in contrast to the *trans* stereochemistry of the prostaglandins derived enzymatically from arachidonic acid (64). The physiological importance of this difference in stereochemistry between the nonenzymatic and enzymatically produced bicyclic peroxides remains to be established.

TABLE 1

Isomeric Distributions of Fatty Acid Hydroperoxides

Fatty acids	Isomeric hydroperoxides,[a] %					
		Free radical autoxidation[b]				
Oleate		8-OOH	9-OOH	10-OOH	11-OOH	
		27	23	23	27	
Linoleate			9-OOH	13-OOH		
			50	50		
Linolenate		9-OOH	12-OOH	13-OOH	16-OOH	
		30	12	12	46	
		Photosensitized oxidation[c]				
Oleate			9-OOH	10-OOH		
			50	50		
Linoleate		9-OOH	10-OOH	12-OOH	13-OOH	
		31	18	18	33	
Linolenate	9-OOH	10-OOH	12-OOH	13-OOH	15-OOH	16-OOH
	21	13	13	14	13	25

[a]Mean values from GC-MS analyses of samples oxidized to different peroxide values and at different temperatures.
[b]References (43,48,55).
[c]Reference (52).

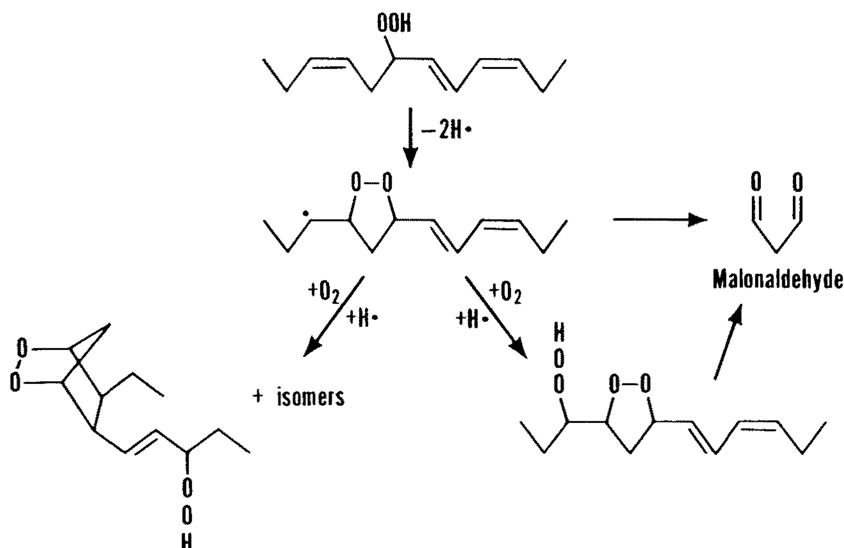


FIG. 5. Mechanism of 1,3-cyclization of 12- and 13-hydroperoxides of linolenate and formation of malonaldehyde (61).

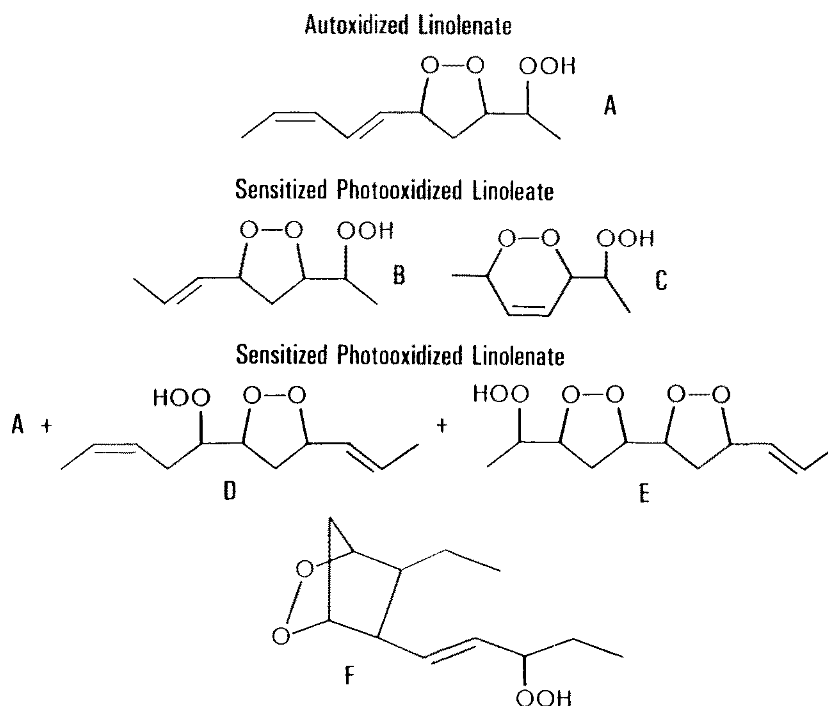


FIG. 6. Structures of hydroperoxy cyclic peroxides.

Other secondary products identified in highly oxidized oleate include saturated epoxy esters, allylic hydroxy- and ketoenes and saturated and monounsaturated dihydroxy esters. From highly oxidized linoleate, a multitude of di- and tri-oxygenated compounds also have been identified, including keto- or hydroxyepoxyene, epoxyenes, diketo- or dihydroxyenes as well as trioxygenated derivatives (71,72) (Fig. 8). The allylic ketoenes and ketodienes from oxidized oleate and linoleate respectively were shown to be particularly active in promoting the induction of nutritional encephalopathy in chicks (73).

In autoxidized methyl linolenate, hydroperoxy cyclic peroxides are formed in the same order of magnitude as the monohydroperoxides (Table II). These secondary products are formed so rapidly that, kinetically, they can be regarded as "primary" products in the sequence of events during autoxidation of linolenate. Dihydroperoxides are the next most important secondary products in autoxidized linolenate. In photosensitized oxidized linoleate and linolenate, the hydroperoxy cyclic peroxides are less important and constitute about 10% of the monohydroperoxides (Table III). Dihydroperoxides are also important secondary products of photosensitized oxidation (67,68). Bicycloendoperoxides and bis-cyclic peroxides are only minor products of linolenate.

Malonaldehyde is claimed to be an important biological

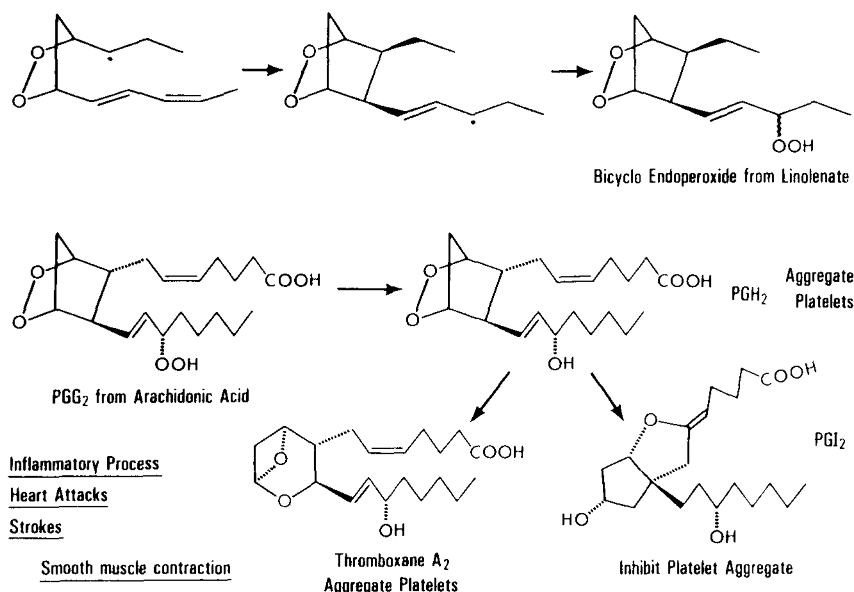


FIG. 7. Relationship between hydroperoxy bicycloendoperoxides and prostaglandins (51,64).

TABLE II

Autoxidation Products of Methyl Linolenate[a]

Compounds	Peroxide values (PV) 904	1286
Unoxidized ester	87.9%	74.8%
Epoxy esters	0.2	0.3
Monohydroperoxides	3.5	8.4
Hydroperoxy cyclic peroxides	3.8	7.7
Epoxyhydroxy dienes	0.1	0.1
Dihydroperoxides	0.9	2.9
Unidentified polar materials	3.7	5.9

[a]Based on weight-per cent composition of fractions isolated by silicic acid column chromatography (50).

TABLE III

Photosensitized Oxidation Products of Methyl Lineoleate and Methyl Linolenate[a]

Compounds	Linoleate[b] (PV 1947)	Linolenate[c] (PV 1956)
Unoxidized esters	70.2%	65.5%
Keto/epoxy esters	1.2	1.6
Monohydroperoxides	24.3	25.6
Hydroperoxy cyclic peroxides	2.8	2.2
Hydroperoxy bicycloendoperoxides	–	0.1
Dihydroperoxides	0.9	3.0
Hydroperoxy bis-cyclic peroxides	–	1.0
Unidentified polar materials	0.6	1.0

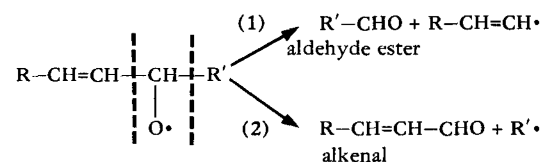
[a]Based on weight-per cent composition of fractions isolated by silicic acid column chromatography.
[b]Reference (67).
[c]Reference (68).

breakdown product expected from 5-membered cyclic peroxides of linoleate and linolenate (60,61) because of its crosslinking ability with amino groups of proteins, enzymes and DNA (10). Higher dialdehydes than malonaldehyde also may be derived from dihydroperoxides, 6-membered cyclic peroxides and other polyfunctional secondary products of linoleate and linolenate. The importance of these secondary oxidation compounds in crosslinking with amino groups and other functional groups of biological materials remains to be established.

VOLATILE DECOMPOSITION PRODUCTS

Hydroperoxide decomposition involves a very complicated set of reaction pathways. The volatile decomposition products have been studied extensively because of their impact on flavors and odors formed during deterioration of lipid-containing foods. More attention has been given to this problem recently by biochemists because the analysis of hydrocarbons in the breath of animals has proved to be a sensitive index of in vivo lipid oxidation. The mechanistic concepts for the formation of volatile lipid oxidation products were reviewed previously (15). Only a few of the fundamentals will be covered here and some of our more recent studies on the volatile decomposition compounds from secondary lipid oxidation products.

A generally accepted and authenticated scheme for the fragmentation of monohydroperoxides involves carbon-carbon cleavage on either side of the alkoxy radical to produce 2 types of aldehydes, an olefin radical and an alkoxy radical.



R' = ester end
R = hydrocarbon end

These radicals can, in turn, react with either •OH or H•. The vinyl alcohol derived from the reaction with •OH is unstable and tautomerizes to a saturated aldehyde.

$$R{-}CH{=}CH\bullet + \bullet OH \rightarrow R{-}CH{=}CH{-}OH \rightleftarrows R{-}CH_2{-}CHO$$

The product from the reaction with •H is either an α-

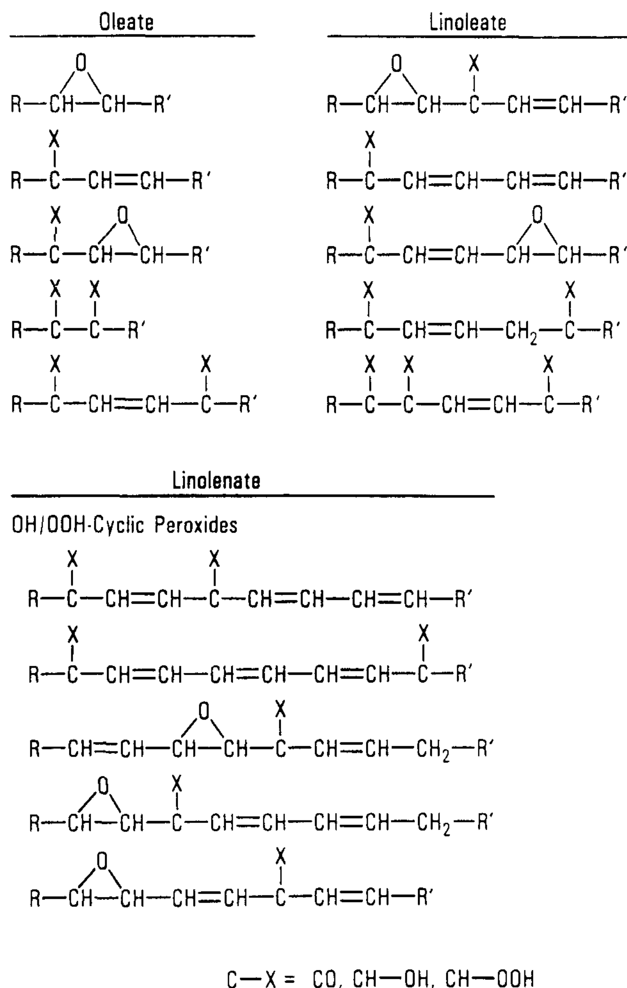


FIG. 8. Structures of secondary autoxidation products of oleate, linoleate and linolenate.

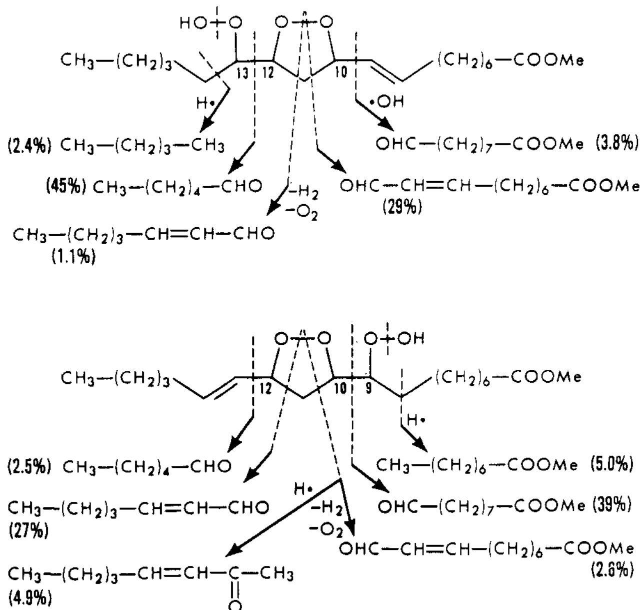


FIG. 9. Thermal decomposition of hydroperoxy cyclic peroxides from linoleate treated with $^1O_2$ (67).

olefin: $R-CH=CH\bullet + \bullet H \rightarrow R-CH=CH_2$, or a short chain ester: $R'\bullet + \bullet H \rightarrow R'H$. Cleavage reactions (1) and (2) explain most of the volatile products identified from the thermal decomposition of the hydroperoxides of oleate, linoleate and linolenate (15,74). The products include carbonyls, alcohols, esters and hydrocarbons. The formation of substituted furans, epoxy aldehydes, ketones, lactones, alkynes and aromatic compounds is difficult to explain, and the literature is full of speculative mechanisms (14,15).

More recently we have investigated the thermal decomposition of secondary oxidation products to determine their role as precursors of volatile oxidation products. The formation of bifunctional oxidation products of biological importance, such as malonaldehyde, also was studied because of their potential crosslinking reactions with amino acids, proteins and DNA (10). The thermal decomposition of cyclic peroxides from linoleate produced most of the same volatile cleavage products as the corresponding monohydroperoxides (Fig. 9). The most important cleavage between the hydroperoxide group and the cyclic peroxide produced aldehydes and aldehyde esters. Cleavage on the other side of the hydroperoxide group produced hydrocarbons and shorter-chain esters (67,75). Cleavage of the peroxide ring explains the formation of unsaturated aldehydes and aldehyde esters. Unsaturated methyl ketones are among some of the unique products of cyclic peroxides.

The thermal decomposition of hydroperoxy bis-cyclic peroxides from linolenate follows the same fragmentation pattern as the monocyclic peroxides (75) (Fig. 10). The most important cleavage A between the hydroperoxide

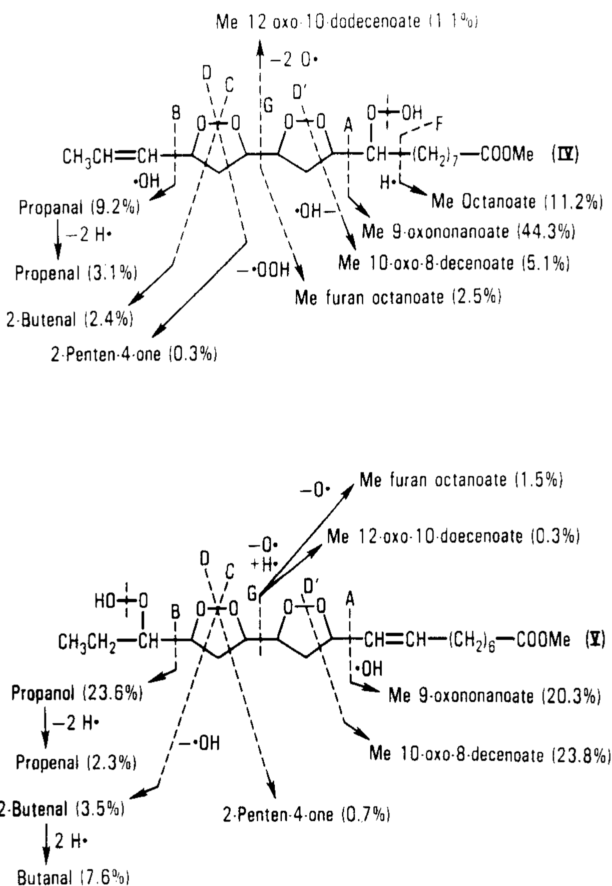


FIG. 10. Thermal decomposition of hydroperoxy bis-cyclic peroxides from linolenate treated with $^1O_2$ (75).

group and the first peroxide ring produces a C-9 aldehyde ester. Cleavage B on the other side of the second peroxide ring produces propanal. Other cleavages, C and D, produce volatile products similar to those from monohydroperoxides. Methyl furanoctanoate is a unique cleavage product that can be explained from cleavage E between the 2 peroxide rings. The bicycloendoperoxides isolated from photosensitized oxidized methyl linolenate also were thermally decomposed, and carbon-carbon cleavage around the hydroperoxide group was found to be the most important (76). We also have studied the thermal decomposition of dihydroperoxides from linolenate. The volatile products identified are those expected from cleavage on each side of the hydroperoxide group. Under our conditions used for thermal decomposition (gas chromatograph injector port at 200-210 C), in no case have we found evidence for the formation of either malonaldehyde or other dialdehydes expected from both cyclic peroxides and dihydroperoxides. Apparently, these difunctional products were too thermally unstable to be detected by gas chromatography.

Malonaldehyde has received much attention in the biochemical and food science literature, and it was reported to be mutagenic and carcinogenic (77-79). The TBA color reaction generally has been used to determine malonaldehyde in food and biological systems. Unfortunately, the TBA reaction is not specific for malonaldehyde, and many lipid oxidation products and their interaction products with other biological materials give positive reactions (7,10,31, 80-82). To determine malonaldehyde more specifically, we developed a milder procedure to decompose lipid oxidation products under acid conditions instead of using the elevated temperatures of a gas chromatograph. By using a dilute HCl solution in methanol, lipid oxidation products are readily cleaved and converted to stable acetals suitable for gas chromatography. Any malonaldehyde formed is converted to tetramethyl acetal derivatives, which can be determined quantitatively by gas chromatography (83). This acid decomposition-acetalation procedure was applied to different lipid oxidation products. As expected, 5-membered hydroperoxy cyclic peroxides and 1,3-dihydroperoxides were found to be the most important precursors of malonaldehyde (Fig. 11). 1,4-Dihydroperoxides were less important and monohydroperoxides were the least significant precursors of malonaldehyde. In contrast, the TBA test is known to give a positive test with all these lipid oxidation products (84). Therefore, our approach provides a more specific test than the TBA color reaction to evaluate the potential of lipid oxidation products to form malonaldehyde and its biological effects due to crosslinking.

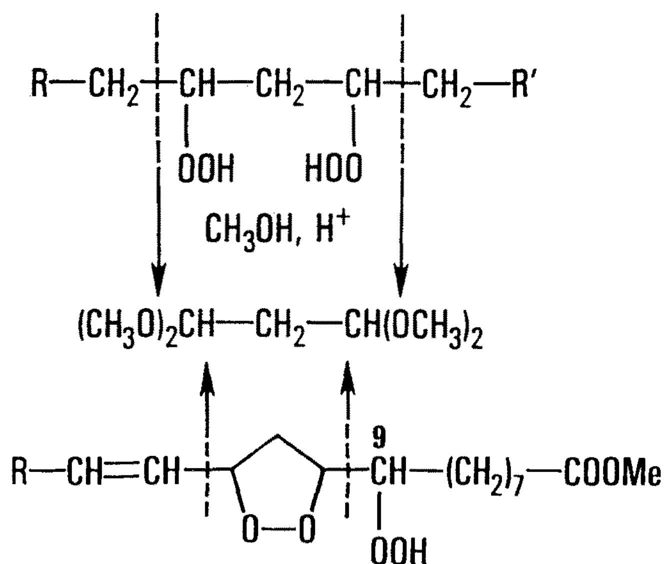


FIG. 11. Acetalation-acid decomposition of hydroperoxy cyclic peroxides and 1,3-dihydroperoxides (83).

BIOLOGICAL CONSEQUENCES

Many reviews have appeared on the biological effects of lipid oxidation products and their relevance to cancer (7,12,85-88). Lipid oxidation products are implicated in the disruption of biological membranes (1,6,7,9,10), the inactivation of enzymes and damage to proteins (10,16-21, 29), the formation of age pigments in damaged membranes (9,10,25,89), oxidative damage to lungs by atmospheric pollutants (4) and cancer. That free radicals are one of the important factors affecting cancer can be inferred by the beneficial effects of antioxidants such as vitamin E, BHA and BHT (12,86,90-93). These free radical scavengers apparently prevent the oxidation of chemical carcionogenic agents into more active forms. Chemical carcinogenesis may thus result from enzymatic or nonenzymatic oxidation of chemical agents into reactive intermediates formed either from stable free radicals or via singlet oxygen and •OH by metal complex catalysis (6,7,12,94).

Many examples are cited in the literature for the roles of free radicals in carcinogenesis. ESR evidence is reported for the formation of nitroxyl radicals as an intermediate from N-hydroxyacetyl-aminofluorene (N-OH-AAF) in its conversion to the more active carcinogenic species N-acetoxy-AAF and 2-nitrosofluorene (95-97). This conversion occurs also in the presence of 13-linoleate hydroperoxide and methemoglobin or hematin (98). A lipid hydroxy derivative was assumed to be important in this reaction. In view of the multitude of decomposition products expected to be formed by metal catalysis, it would be important to determine what particular functionality activates a carcinogen.

Benzo(a)pyrene [B(a)P] is another important chemical carcinogen that is converted to oxy radical either by photoirradiation or enzymatically by incubation with liver microsomes (87,99). The precursor of 6-oxy-B(a)P, 6-hydroxy-B(a)P, binds covalently with DNA in vitro, and the free radical of the bound complex is demonstrated directly by ESR studies. From these reports of free radical involvement in cancer, it can be readily deduced that any agent known to promote (e.g., metals and their active complexes) or inhibit (e.g., reducing agents and antioxidants) free radicals, would have a great impact on cancer formation. In view of the activity of polyaromatic hydrocarbons such as B(a)P as photosensitizers (34), the possible involvement of $^1O_2$ in activation of carcinogen also should be seriously considered (87).

Besides antixodants, what are some of the other biological defense mechanisms against lipid peroxidation? Enzymes such as peroxidase, catalase and superoxide dismutase remove different species of activated oxygen that promote lipid peroxidation (38). Any pathological or degenerative conditions such as aging may decrease the concentration of these protective enzymes, with consequent damage from the toxic effects of activated oxygen. Cell membrane integrity is another mechanism that the body has to separate biological catalysts and oxygen from lipid unsaturation and its resulting oxidation. Any factors that affect this structural separation will result in damaging lipid peroxidation. Finally, the relatively low intracellular concentration of oxygen is another defense mechanism against lipid oxidation (25,100).

REFERENCES

1. Mead, J.F., Free Radicals in Biology, edited by W.A. Pryor, Vol. I, Academic Press, New York, 1976, p. 51.
2. Kerr, J.A., J.G. Calvert and K.L. Demerjion, Free Radicals in Biology, edited by W.A. Pryor, Vol. II, Academic Press, New York, 1976, p. 159.
3. Mudd, J.B., Free Radicals in Biology, edited by W.A. Pryor, Vol. II, Academic Press, New York, 1976, p. 263.
4. Menzel, D.B., Free Radicals in Biology, edited by W.A. Pryor, Vol. II, Academic Press, New York, 1976, p. 181.
5. Wilson, R.B., CRC Crit. Rev. Food Sci. Nutr. 7:325 (1976).
6. Packer, L., and J. Walton, Chem. Tech. 276 (1977).
7. Pryor, W.A., Photochem. Photobiol. 28:787 (1978).
8. Korycka-Dahl, M.B., and T. Richardson, CRC Crit. Rev. Food Sci. Nutr. 11:209 (1978).
9. Simic, M.G., and M. Karel, editors, Autoxidation in Food and Biological Systems, Plenum Press, New York, 1980.
10. Tappel, A.L., Free Radicals in Biology, edited by W.A. Pryor, Vol. IV, Academic Press, New York, 1980, p. 1.
11. Rodgers, M.A.J., and E.L. Powers, editors, Oxygen and Oxy-Radicals in Chemistry and Biology, Academic Press, New York, 1981.
12. Floyd, R.A., editor, Free Radicals and Cancer, Marcel Dekker, New York, 1982.
13. Frankel, E.N., Prog. Lipid Res. 19:1 (1980).
14. Forss, D.A., Ibid. 13:177 (1972).
15. Frankel, E.N., Ibid. 22:1 (1983).
16. Chio, K.S., and A.L. Tappel, Biochemistry 8:2827 (1969).
17. Gamage, P.T., and S. Matsushita, Agric. Biol. Chem. 34:817 (1970).
18. Matsushita, S., J. Agric. Food Chem. 23:150 (1975).
19. Matsushita, S., and M. Kobayashi, Agric. Biol. Chem. 34:825 (1970).
20. Matsushita, S., M. Kobayashi and Y. Nitta, Agric. Biol. Chem. 34:817 (1970).
21. Mukai, F.H., and B.D. Goldstein, Science 191:868 (1976).
22. Bender, A.D., C.G. Kormendy and R. Powell, Exp. Gerontol. 5:97 (1970).
23. deDuve, C., and O. Hayaishi, editors, Tocopherol, Oxygen and Biomembranes, Elsevier, Amsterdam, 1978.
24. Desai, I.D., B.L. Fletcher and A.L. Tappel, Lipids 10:307 (1975).
25. Miquel, J.; J. Oro, K.G. Bensch and J.E. Johnson, Free Radicals in Biology, Vol. III, Academic Press, New York, 1977, p. 133.
26. Munkres, K.D., Age Pigments, edited by R.S. Sohal, Elsevier/North-Holland Biochemical Press, Amsterdam, 1981, p. 83.
27. Porta, E.A., and W.S. Hartroff, Pigments in Pathology, edited by M. Wolman, Academic Press, New York, 1969, p. 19.
28. Scott, M.L., The Fat Soluble Vitamins, edited by H.F. deLuca, Plenum Press, New York, 1978, p. 133.
29. Karel, M., K. Schaich and R.B. Roy, J. Agric. Food Chem. 23:159 (1975).
30. Matsushita, S., Ibid. 23:150 (1975).
31. Gardner, H.W., Ibid. 27:220 (1979).
32. Pokorny, J., Rev. Franc. Corps Gras 28:151 (1981).
33. Rawls, H.R., and P.J. van Santen, Ann. N.Y. Acad. Sci. 171:135 (1970); JAOCS 47:121 (1970).
34. Foote, C.S., Free Radicals in Biology, edited by W.A. Pryor, Vol. II, Academic Press, New York, 1976, p. 85.
35. Pryor, W.A., Molecular Basis of Environmental Toxicity, edited by R.S. Bhatnagar, Ann Arbor Science, Ann Arbor, 1980, p. 3.
36. Tappel, A.L., Fed. Proc. Fed. Am. Soc. Exp. Biol. 32:1870 (1973).
37. Witting, L.A., Free Radicals in Biology, edited by W.A. Pryor, Vol. IV, Academic Press, New York, 1980, p. 295.
38. Fridovitch, I., Free Radicals in Biology, edited by W.A. Pryor, Vol. I, Academic Press, New York, 1976, p. 239.
39. Brown, K., and I. Fridovitch, Autoxidation in Foods and Biological Systems, edited by M.G. Simic and M. Karel, Plenum Press, New York, 1980, p. 429.
40. Frankel, E.N., Fatty Acids, edited by E.H. Pryde, AOCS Monograph 7, American Oil Chemists' Society, Champaign, IL, 1979, p. 353.
41. Porter, N.A.; L.S. Lehman, B.A. Weber and K.J. Smith, J. Am. Chem. Soc. 103-6447 (1981).
42. Porter, N.A.; B.A. Weber; H. Weenen and J.A. Khan, Ibid. 102:5597 (1980).
43. Frankel, E.N.; W.E. Neff; W.K. Rohwedder; B.P.S. Khambay; R.F. Garwood and B.C.L. Weedon, Lipids 12:901 (1977).
44. Garwood, R.F.; B.P.S. Khambray; B.C.L. Weedon and E.N. Frankel, J. Chem. Soc. Chem. Commun. 364 (1977).
45. Chan, H.W.-S., and G. Levett, Chem. Ind. (London) 692 (1977).
46. Frankel, E.N.; R.F. Garwood; J.R. Vinson and B.C.L. Weedon, J. Chem. Soc. Perkin Trans. I, 2715 (1982).
47. Frankel, E.N.; C.D. Evans; D.G. McConnell; E. Selke and H.J. Dutton, J. Org. Chem. 26:4663 (1961).
48. Frankel, E.N.; W.E. Neff; W.K. Rohwedder; B.P.S. Khambay; R.F. Garwood and B.C.L. Weedon, Lipids 12:1055 (1977).
49. Chan, H.W.-S., and G. Levett, Ibid. 12:837 (1977).
50. Neff, W.E.; E.N. Frankel and D. Weisleder, Ibid. 16:439 (1981).
51. O'Connor, D.E.; E.D. Mihelich and M.C. Coleman, J. Am. Chem. Soc. 103:223 (1981).
52. Frankel, E.N.; W.E. Neff and T.R. Bessler, Lipids 14:961 (1979).
53. Neff, W.E., and E.N. Frankel, Ibid. 15:587 (1980).
54. Matsushita, S., and J. Terao, Autoxidation in Food and Biological Systems, edited by M.G. Simic and M. Karel, Plenum Press, New York, 1980, p. 27.
55. Frankel, E.N.; W.E. Neff; W.K. Rohwedder; B.P.S. Khambay; R.F. Garwood and B.C.L. Weedon, Lipids 12:908 (1977).
56. Nugteren, D.H.; R.K. Beerthuis and D.A. Van Dorp, Rec. Trav. Chim. 85:405 (1966).
57. Haverkamp-Begeman, P.; W.J. Woestenburg and S. Leer, J. Agric. Food Chem. 16:679 (1968).
58. Samuelson, B., Lipid Metabolism, edited by S. Wakil, Academic Press, New York, 1970, p. 107.
59. Porter, N.A., and M.O. Funk, J. Org. Chem. 40:3614 (1975).
60. Pryor, W.A., and J.P. Stanley, Ibid. 40:3015 (1975).
61. Pryor, W.A.; J.P. Stanley and E. Blair, Lipids 11:370 (1976).
62. Porter, N.A.; M.O. Funk; G. Gilmore; R. Isaac and J. Nixon, J. Am. Chem. Soc. 98:6000 (1976).
63. Roza, M., and A. Franck, Biochim. Biophys. Acta 528:119 (1978).
64. Porter, N.A., Free Radicals in Biology, edited by W.A. Pryor, Vol. IV, Academic Press, New York, 1980, p. 261.
65. Mihelich, E., J. Am. Chem. Soc. 102:7141 (1980).
66. Coxon, D.T.; K.R. Price and H.W.-S. Chan, Chem. Phys. Lipids 28:365 (1981).
67. Frankel, E.N.; W.E. Neff; E. Selke and D. Weisleder, Lipids 17:11 (1982).
68. Neff, W.E.; E.N. Frankel and D. Weisleder, Ibid. 17:780 (1982).
69. Khan, J.A., and N.A. Porter, Angew. Chem. Suppl. 513 (1982).
70. Neff, W.E.; E.N. Frankel, E. Selke and D. Weisleder, Lipids 18:868 (1983).
71. Neff, W.E.; E.N. Frankel; C.R. Scholfield and D. Weisleder, Lipids 13:415 (1978).
72. Terao, J., and S. Matusushita, Agric. Biol. Chem. 39:2027 (1975).
73. Budowski, P.; I. Bartor; Y. Dror and E.N. Frankel, Lipids 14:768 (1979).
74. Frankel, E.N.; W.E. Neff and E. Selke, Ibid. 16:279 (1981).
75. Frankel, E.N.; W.E. Neff and E. Selke, Ibid. 18:353 (1983).
76. Frankel, E.N.; W.E. Neff and E. Selke, JAOCS 60:686 (1983) (Abstract 33).
77. Shamberger, R.J.; T.L. Andreone and C.E. Willis, J. Natl. Cancer Inst. U.S. 53:1771 (1974).
78. Mukai, F.H., and B.D. Goldstein, Science 191:868 (1976).
79. Shamberger, R.J., Autoxidation in Food and Biological Systems, edited by M.G. Simic and M. Karel, Plenum Press, New York, 1980, p. 639.
80. Patton, S., and G.W. Kurtz, J. Dairy Sci. 34:669 (1951).
81. Shamberger, R.J.; B.A. Shamberger and C.E. Willis, J. Nutr. 107:1404 (1977).
82. Uchiyama, M., and M. Mihara, Anal. Biochem. 86:271 (1978).
83. Frankel, E.N., and W.E. Neff, Biochim. Biophys. Acta 754:264 (1983).
84. Porter, W.A.; J. Nixon and I. Ramdas, Biochim. Biophys. Acta 441:506 (1976).
85. Ts'o, O.P.; W.J. Caspary and R.J. Lorentzen, Free Radicals in Biology, edited by W.A. Pryor, Vol. III, Academic Press, New York, 1977, p. 251.
86. Floyd, R.A., Free Radicals in Biology, edited by W.A. Pryor, Vol. IV, Academic Press, New York, 1980, p. 187.
87. Nagata, C.; M. Kodama; Y. Ioki and T. Kimura, Free Radicals and Cancer, edited by R.A. Floyd, Marcel Dekker, New York, 1982, p. 1.
88. Hopkins, G.J., and C.E. West, Life Sci. 19:1103 (1976).
89. Koster, J.F., and R.G. Slee, Biochim. Biophys. Acta 620:489 (1980).
90. Shamberger, R., J. Natl. Cancer Inst. U.S. 44:931 (1970); 48:1497 (1972).
91. Wattenberg, L.W., Ibid. 48:1425 (1972); 50:1541 (1973).
92. Wattenberg, L.W.; W.D. Laub; L.K. Lam and J.L. Speier, Fed. Proc. 35:1327 (1976).
93. Sporn, M.B.; R.A. Squire; C.C. Brown; J.M. Smith; M.L.

Wenk and S. Springer, Science 195:487 (1977).
94. O'Brien, P.J., Autoxidation in Food and Biological Systems, edited by M.G. Simic and M. Karel, Plenum Press, New York, 1980, p. 563.
95. Floyd, R.A.; L.M. Soong; R.N. Walker and M. Stuart, Cancer Res. 36:2761 (1976).
96. Bartsch, H., and E. Hecker, Biochim. Biophys. Acta 237:567 (1971).
97. Bartsch, H.; M. Traut and E. Hecker, Ibid. 237:556 (1971).
98. Floyd, R.A., Free Radicals and Cancer, edited by R.A. Floyd, Marcel Dekker, New York, 1982, p. 361.
99. Lorentzen, R.J.; W.J. Caspary; S. Lesko and P.O.P. Ts'o, Biochemistry 14:3978 (1975).
100. Recknagel, R.O.; E.A. Glende, Jr. and A.M. Hruszkewycz, Free Radicals in Biology, edited by W.A. Pryor, Vol. III, Academic Press, New York, 1977, p. 97.

[Received February 13, 1984]

Sources and Consumption of Antioxidants in the Diet

JOHN G. BIERI*, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20205

ABSTRACT

Vitamin E is the most important tissue antioxidant in preventing or controlling non-specific reactions from various oxidizing species produced in normal metabolism. Through this action, the vitamin protects polyunsaturated fatty acid loss from phospholipids and consequent membrane damage. The U.S. diet has an abundance of vitamin E and normal individuals accumulate effective amounts in their tissues, which is consistent with the latest recommended dietary allowances for vitamin E as indicated by the National Research Council.

INTRODUCTION

One may reasonably ask why there should be an interest in antioxidants in our food supply. Are they beneficial or harmful? We know from biochemical studies that in the body's tissues there are chemical reactions which under some circumstances could lead to metabolic problems and tissue damage. These reactions produce free radicals or oxidizing species that may react readily with cell components. Such systems as the microsomal mixed function oxidases, the xanthine-xanthine oxidase system, cyclooxygenase and various other enzymes that produce hydrogen peroxide, superoxide or singlet oxygen, all may contribute to potentially damaging conditions in vivo. Fortunately, tissues also contain a varied system of defense against oxidant damage, and primary components of this system are antioxidants.

Foremost is vitamin E, since a deficiency of this vitamin leads to many cellular changes readily explained by its antioxidant action. Other nutrients which also can demonstrate an antioxidant effect, via metal scavenging and under limited conditions, are ascorbic acid, cystine, histidine, tryptophan and intact proteins. Numerous enzymes in tissues also will destroy oxidizing species: catalase, glutathione reductase, glutathione peroxidase (both selenium containing and non-selenium containing), and superoxide dismutase. It should be mentioned that the trace element, selenium, is not an antioxidant, but when incorporated into glutathione peroxidase it readily destroys peroxides.

Two types of antioxidants in the diet will be considered, the natural antioxidant vitamin E, and the synthetic antioxidants added in manufacturing of a multitude of food products. Of the many synthetic antioxidants available, only 2 were approved by the Food and Drug Administration for use in human food in the past. These are BHT (butylated hydroxytoluene) and BHA (butylated hydroxyanisole). These are permitted in fats and oils at a concentration of 0.02%, and also are added to packaging materials. However, BHT is no longer considered acceptable and its use has been stopped in most lipid-containing foods in the U.S. and other countries. In extensive animal testing 20 to 30 years ago, these compounds generally were found to be much less active than vitamin E in preventing the classical signs of vitamin E deficiency. The amounts required in the diet were 20-100 times that of vitamin E, and often bordered on a toxic level. Studies of the metabolism of BHT and BHA in man, using isotopic labeling, revealed that the compounds were rapidly excreted from the body, 80-90% in the urine within 7 days and the remainder in the feces. Furthermore, they are oxidized to 5 or more metabolites. In terms of the amounts that may be ingested daily by man from the U.S. food supply, probably only a few milligrams, it cannot be considered that these two antioxidants make a significant contribution to the body's overall antioxidant defense system. In preventing certain experimentally produced cancers in laboratory animals, these compounds must be in the diet at relatively high levels, 0.5% or more. This would be the equivalent of about 2.5-3 g per day for man.

Vitamin E is the most important dietary component contributing to antioxidant defenses in tissue. Vitamin E is a collective term comprising 8 compounds synthesized by plants. These fall into 2 classes, the tocols having a saturated side chain, and the tocotrienols having an unsaturated side chain. Within each class there are 4 "vitamers," designated alpha, beta, gamma and delta, which vary in the number of methyl groups on the chroman ring. Of these 8 compounds, only 4 have nutritional significance: alpha, beta, and gamma-tocopherols and alpha tocotrienol. When tested in animals for their vitamin E activity, the relative activities are: alpha tocopherol 100, beta tocopherol 30, gamma tocopherol 10, and delta tocopherol 1. In the tocotrienol series the activities are alpha 30, beta 5, and gamma and delta, < 1 (1).

According to their relative abundance in the diet and their relative biological activities, it can be estimated that of the total vitamin E activity in the U.S. food supply 75% comes from alpha tocopherol, 20% from gamma tocopherol, and 5% from beta tocopherol and alpha tocotrienol. Even though gamma tocopherol has only one-tenth the biological activity of alpha tocopherol, it is present in our diet at twice the amount of alpha tocopherol and thus makes a significant contribution. The U.S. diet may be unique in this regard compared with other western countries because of our relatively high consumption of soybean and corn oils, in which gamma tocopherol exceeds alpha tocopherol

*To whom correspondence should be addressed at Bldg. 6, Rm. B1-06, National Institutes of Health, Bethesda, MD 20205.