Reaction Mechanisms in the Radiolysis of Fats: A Review

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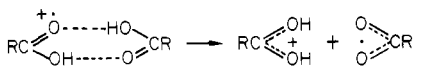
The general mechanism of the radiolysis of fats is thought to involve primary ionization, followed by migration of positive charges toward the carboxy groups and the double bonds, and cleavage at preferential positions near the carbonyl group. The resulting free radicals engage in various reactions leading to the formation of stable radiolytic products, which have been classified as primary, recombination, and secondary products according to the mode of their formation. In the case of unsaturated fatty acids, dimerization reactions appear to be of major importance. The course of radiolysis in fats is significantly influenced by the phase state and temperature.

The chemical changes induced in fats by high-energy radiation have been investigated under widely varied conditions. The sources of energy used in different studies included radiations from natural or artificial radioactive isotopes as well as machine-produced radiations and accelerated particles. Substrates varied from model systems of fatty acids, esters, and triglycerides, to natural fats or fat-containing biological systems. Some investigators employed relatively low radiation doses (e.g., 1 krad) while others used doses higher than 200 Mrad. Likewise, radiation temperature, atmosphere, medium, dose rate, postirradiation conditions, and methods of analysis were among the parameters which differed markedly among the various studies. In spite of such variations, considerable generalizations can be made regarding the radiolytic patterns. The purpose of this report is to present a general scheme for the radiolytic behavior of fats, based on the data available at present.

Irradiation-induced reactions are not the result of a statistical distribution of random cleavage of chemical bonds. The course of radiolysis follows preferred pathways which are largely influenced by molecular structure. The primary events involve the formation of excited molecules and molecule-ions. In the case of fats, as with all oxygen-containing compounds, the electron deficiency is highly localized on the oxygen atom and the ensuing reactions are strongly directed by the tendency of the oxygen atom to complete its valence shell of electrons (Williams, 1962). Restoration of the electron octet may occur via intra- or intermolecular hydrogen atom transfer to the electron-deficient atom as proposed by Howton and Wu (1967) for oleic acid (eq 1) or by a process in which a π bond is formed


(1)

between an oxygen atom and the adjacent carbon atom, a step which is accompanied by scission of C–H or C–C bonds so as to leave a free valence on the carbon to couple with the unpaired electron of the oxygen. The major radiolytic reactions in fats thus involve preferential cleavages in the vicinity of the carbonyl group, leading to the formation of specific, relatively abundant, free radicals. If a double bond is present in the fatty acid molecule, the electron deficiency is localized in both the carboxyl group and the center of unsaturation.

The various free radicals produced by irradiation may then engage in a number of reactions leading to the formation of stable radiolytic compounds.

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FREE RADICAL SPECIES

Based on qualitative and quantitative measurements of radiolytic products from a number of model systems, it can be concluded that the following free radical species are produced preferentially from triglycerides by irradiation. Obviously some of these radicals, those not involving the glyceryl moiety, are also formed from the radiolysis of free fatty acids and their esters. Evidence for the formation of some of the free radicals shown in Scheme I has also been obtained from electron spin resonance (ESR) studies of irradiated fatty acids.

Radicals I, II, and III result from scission at the acyl-oxy–methylene, or the acyloxy–methine bonds in triglycerides (LeTellier and Nawar, 1972a). Radical I can also be produced from a free carboxylic acid by hydrogen transfer to another molecule as shown above. ESR spectra attributed to carboxy radicals of the type $\sim CH_2C(=O)O\cdot$ were observed originally in γ-irradiated succinic and other dicarboxylic acids (Tamura et al., 1966), but more recently in γ-irradiated polycrystalline palmitic and oleic acids (Leibler et al., 1970). Decarboxylation of radical I yields radical VII.

Cleavage at the acyl–oxy bond of fatty acids gives rise to radicals IV, V, and VI. In their recent studies with palmitic and oleic acid Faucitano et al. (1972a,b) observed radicals of the type $\sim CH_2\dot{C}{=}O$ and suggested that these arise from the decay of the radical anion $\sim CH_2\dot{C}(O^-)OH$ which is also observed in the spectra of samples irradiated at low temperature and believed to arise from electron capture by carboxy groups. Radical IV may also lose CO to yield radical VII.

Scission between carbon 1 and carbon 2 produces radicals VII, VIII, and IX. It can be seen that the formation of radical VII (i.e., the alkyl radical with one carbon atom less than the parent fatty acid) is highly favored since it can arise from several sources. Faucitano and co-workers (1972a,b) presented experimental evidence to support the hypothesis that the participation of the reaction

$$\sim CH_2CH_2\dot{C}{=}O \rightarrow CO + \sim CH_2CH_2\cdot$$

and consequently contribution of the radical anion $\sim CH_2\dot{C}(O^-)OH$ to the mechanism of radiolytic decarboxylation is of minor importance. Loss of CO_2 from radicals VIII and IX leads to the formation of radicals II and III, respectively.

Cleavage between carbon 2 and carbon 3 of the fatty acid gives rise to radicals X, XI, and XII, while radicals XIII and XIV result from scission between the primary and secondary carbons of the glyceryl skeleton.

Formation of radicals of the type $\sim\dot{C}HCOOH$ corresponding to radical XV was concluded from ESR spectra

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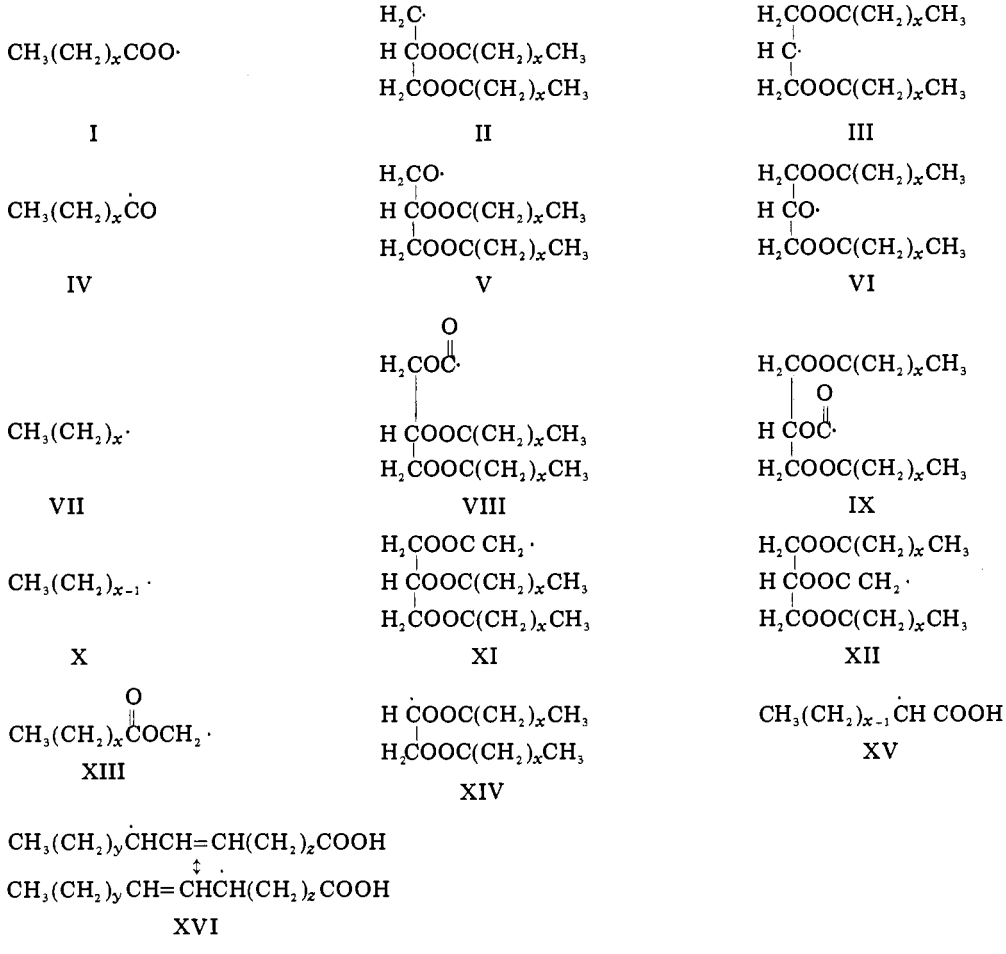
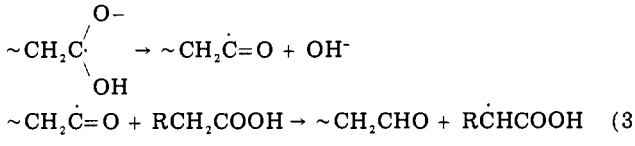
Scheme I



obtained from a single crystal of succinic acid (Heller and McConnell, 1960). The relative stability of such radicals, in the case of saturated fatty acids, was explained through resonance stabilization (eq 2). More recently ESR spectra

XV

corresponding to radical XV were observed in irradiated palmitic and oleic acids by Faucitano's group. These workers concluded that such radicals arise from the radical anion as shown in eq 3.



Radical XVI has been shown to arise from irradiation of monounsaturated fatty acids. Radicals of this type can be produced both by hydrogen atom abstraction and by C–H bond homolysis. In the case of oleic acid, ESR spectra obtained from irradiation at −196 °C appear to change at −78 °C to patterns corresponding to radical XVI, suggesting that hydrogen abstractions at methylenic groups α to the double bond are favored.

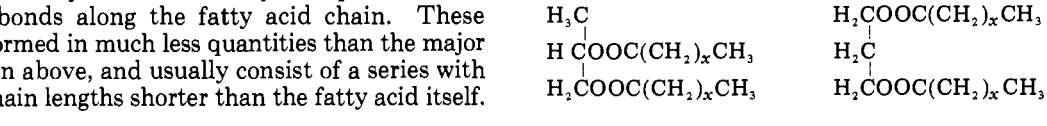
In addition to the free radicals listed here, alkyl radicals can be produced by random homolytic rupture of carbon–carbon bonds along the fatty acid chain. These radicals are formed in much less quantities than the major radicals shown above, and usually consist of a series with all possible chain lengths shorter than the fatty acid itself.

STABLE PRODUCTS OF RADIOLYSIS

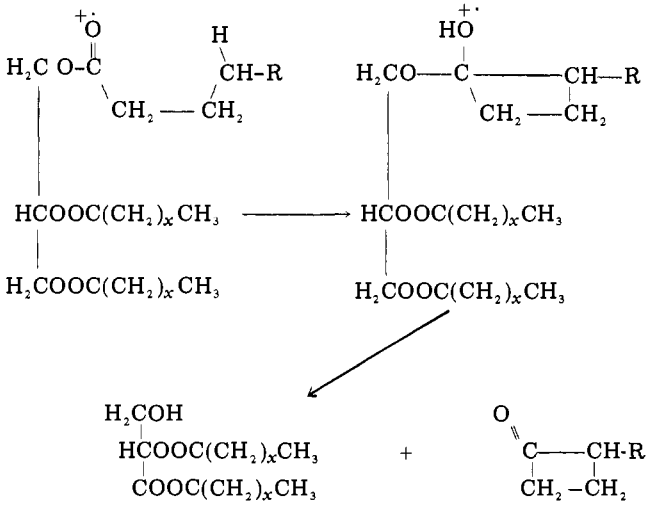
Termination of free radicals may take place via a number of pathways. These include abstraction, dissociation, radical–radical recombination, radical–radical disproportionation, and radical–molecule reactions. Since the free radicals shown above are produced preferentially in the radiolysis of fats, their reactions lead to the formation of products of relatively high abundance. On the other hand, much less quantities are formed of the compounds resulting from reactions of the radicals produced by random cleavage. We have classified the radiolytic products produced from free radicals upon irradiation of fats under three broad categories which we termed "primary products", "recombination products", and "secondary products" according to the mode of their formation.

Primary Radiolytic Products. These are the compounds formed by scission of only one bond in the parent molecule, followed by the abstraction or loss of a hydrogen atom.

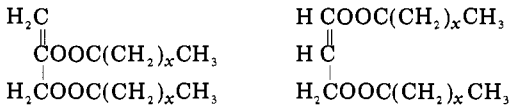
Quantitative data show that the free alkanoic acid, with a carbon number equal to that of the parent glyceride fatty acid, is the most abundant radiolytic product of triglycerides (G 1.9 for hexanoic acid from tricaproin. G value = the number of molecules (or ions) produced or destroyed per 100 eV of absorbed energy.) It arises from hydrogen abstraction by radical I. Abstraction of hydrogen by radicals II and III leads to the formation of propanediol diesters of the types:



Scheme II



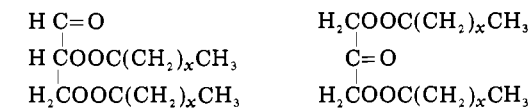
respectively, while loss of hydrogen produces the propenediol diesters of the type:



The saturated diol diesters are produced in larger amounts (G 0.35 for 1,3-propanediol dicaproate, and 0.07 for 1,3-propenediol dicaproate), suggesting that hydrogen abstraction is the preferential route for the termination of these free radicals. Among the radiolytic products involving the glyceryl skeleton, the propanediol diesters are the most abundant. Compounds of this type could only be isolated from the radiolysis of glycerides of low molecular weight (LeTellier and Nawar, 1972a; Meidani, 1975) but were recently observed in irradiated natural fats and triglycerides containing longer chains (Vajdi, 1976).

Radical IV is the precursor of the major aldehyde, produced from fats by irradiation. The latter, i.e., the aldehyde with the same carbon number as the parent fatty acid, was observed in irradiated triglycerides, G 0.28 for hexanal from tricaproin (LeTellier and Nawar, 1972a), and in irradiated free fatty acids, G 0.32 for octadecanal from tristearin (Wu and Howton, 1975).

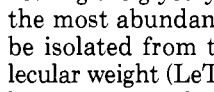
Abstraction of hydrogen by radicals V and VI produces 1,2- and 1,3-diglycerides, respectively. Termination of these free radicals by loss of hydrogen gives the isomeric oxopropanediol diesters.



As indicated above, radicals IV, V, and VI result from radiolytic cleavage of the acyl–oxygen bond in triglycerides. Cleavage at this location may also be responsible for the formation of 2-alkylcyclobutanones, containing the same number of carbon atoms as in the parent fatty acid, as observed in irradiated triglycerides. A pathway involving a six-membered ring intermediate was proposed for the production of these compounds (Scheme II).

Series of saturated and unsaturated hydrocarbons arise from termination of alkyl radicals (Merritt et al., 1966; Dubravcic and Nawar, 1968). Radical VII is responsible for the production of the major hydrocarbon in the radiolysis of fats, i.e., the decarboxylation product. It is formed in much greater quantities when the fatty acid is irradiated in the free form than when it is esterified on the triglyceride molecule. For example, the G value for pentadecane is 3.5 from the radiolysis of palmitic acid, but 0.14 from the radiolysis of tripalmitin. In addition, radiolytic decarboxylation is influenced by temperature, physical state, and chain length of the fatty acid. The amount of CO_2 as well as that of the major hydrocarbon produced is inversely proportional to the fatty acid carbon number (Beke et al., 1974; Jones, 1971a; Wu and Howton, 1974). In the case of oleic acid irradiated at −78 °C, the yield of the C_{17} hydrocarbons (G 0.56) compared well with that of CO_2 (G 0.61), while liquid state radiolysis at 35 °C resulted in a CO_2 yield (G 1.70) which exceeded that of the C_{17} hydrocarbons (G 0.65) by almost a factor of 3 (Faucitano, 1972b). This has been explained on the basis of the more restrictions on molecular motion in a crystalline lattice than in the liquid state. Thus in the liquid state radiolysis heptadecynyl radicals may recombine more easily with other radicals, but in the solid state they can only abstract hydrogen atoms from adjacent molecules to yield heptadecene. In a study with a homologous series of aliphatic carboxylic acids, Jones (1972) reported that in the solid state CO_2 yields are temperature dependent and that the solid acids above C_{10} show a striking alternation, those containing an odd number of carbon atoms being more extensively decarboxylated. On the other hand, the radiolytic yields of CO_2 were found to be independent of temperature in the 10–110 °C range (Jones, 1971b).

Termination of radical X produces the hydrocarbons with two carbons less than the fatty acid while radicals VIII, IX, XI, and XII yield triglycerides. The methyl ester of the parent fatty acid results from abstraction of hydrogen by radical XIII while radical XIV yields ethanediol diesters.



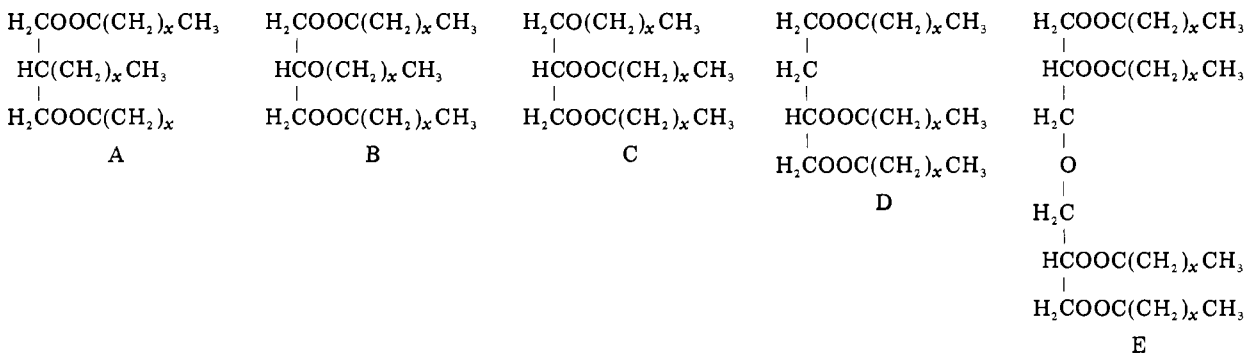
Recombination Products. These are the compounds believed to arise by combination of the primary free radicals discussed earlier. In general, those compounds resulting from recombination of radicals representing preferentially cleaved bonds were produced in greater amounts than those formed by reaction of radicals from random cleavage. In saturated systems the total concentration of the recombination products was much less than that of the primary radiolytic products. The recombination products which have been isolated from the radiolysis of fats can be summarized as follows:

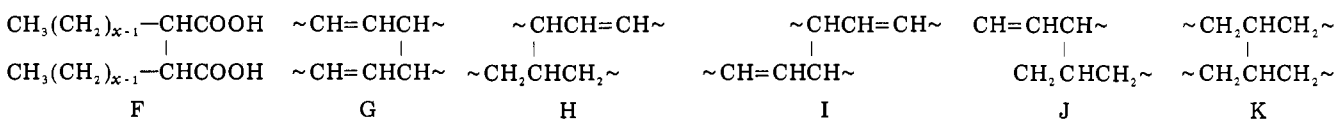
a. Dimeric or recombination hydrocarbons of the type $CH_3(CH_2)_x(CH_2)_xCH_3$ which result from dimerization of radical VII and the type $CH_3(CH_2)_x(CH_2)_yCH_3$ (where y is any number smaller than x) which result from recombination of radical VII with shorter chain alkyl radicals. Such compounds were observed in the radiolysis of low molecular weight triglycerides (LeTellier and Nawar, 1972b) and also from saturated and unsaturated free fatty acids (Howton and Wu, 1967; Wu and Howton, 1975). The G value for decane from tricaproin was found to be 0.1 and that for tetratriacontane from stearic acid was 0.06.

b. Ketones of the type $CH_3(CH_2)_xCO(CH_2)_xCH_3$ resulting from the recombination of radicals IV and VII. These were isolated from both irradiated triglycerides and irradiated fatty acids. In case of saturated fatty acids, the symmetric ketone is the recombination product produced in the largest quantity (G 0.47 for di-*n*-heptadecyl ketone from irradiated stearic acid).

c. Esters of the type $CH_3(CH_2)_xCOO(CH_2)_xCH_3$ which arise from recombination of radicals I and VII or radicals X and XIII.

Scheme III





d. Diketones of the type $CH_3(CH_2)_xCOCO(CH_2)_xCH_3$ produced from dimerization of radical IV.

e. Oxoalkyl esters of the type $CH_3(CH_2)_xCOOCH_2CO(CH_2)_xCH_3$ which result from recombination of radicals IV and XIII.

f. 2-Alkyl-1,3-propanediol diesters of type A (shown in Scheme III) from the recombination of radicals III and VII.

g. Alkanediol diesters which can result from recombination of radicals VII or X with radicals II or XIV.

h. Glyceryl ether diesters of types B or C. These arise from recombination of radicals V and VI with radical VII.

i. Erithritol tetraesters which can be produced by dimerization of radical XIV.

j. Butanetriol tetraesters, type D (Scheme III), which may form by recombination of radical II with radical XIII.

k. Glyceryl ethers of type E (Scheme III). These can result from recombination of radical II with radical V.

The radiolytic compounds listed under f, g, h, i, j, k above, have been isolated only from the radiolysis of glycerides containing short-chain fatty acids (LeTellier and Nawar, 1972b; Meidani, 1975). The corresponding products may be formed in the higher glycerides but their high molecular weights and extremely low concentrations make their analysis very difficult.

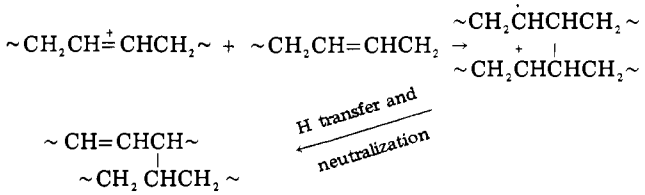
l. Dehydrodimers of type F (Scheme III) which result from the dimerization of radical XV. These were isolated from saturated fatty acids or esters. This α,α,-dehydrodimer (G 0.18) was recently isolated from stearic acid by Wu and Howton (1975). *n*-Docosane-11,12-dimethyl carboxylate (G 0.19) is similarly produced from methyl laurate.

m. Diunsaturated, monounsaturated and saturated dimers of types G–K (Scheme III). Such dimers have been shown to be major products in the radiolysis of oleic acid. When aqueous potassium oleate was irradiated at 1 Mrad, 16.5% of the original solute was converted to other products. Of the altered oleate 62% was converted to dimers (Howton, 1963). When crystalline oleic acid was irradiated at 56 Mrad, 6% of the initial product was transformed to various products among which 75% were oligomers (G 2.49).

Dimers may form by coupling of alkyl radicals of type XVI, by addition of the molecular ion $\sim CH_2CH{=}(+)CHCH_2\sim$ at the double bond of an intact olefinic molecule (Scheme IV) or by reaction of allylic and alkyl radicals.

The physical state of the fatty acid during irradiation has a definite influence on the radiolytic formation of dimers. In solid state radiolysis, the oligomers from oleic

Scheme IV



acid were reported to have a carboxy content appreciably lower than that of the pure acid and a different degree of unsaturation. Although liquid state dimerization is believed to proceed mainly via allylic radical coupling, this mechanism in the solid state appears to account for no more than 40% of the oligomers produced, indicating that other mechanisms are involved. Cage effects, lattice geometry, low temperature, and stabilization of the molecular ions by the crystalline matrix are among the factors which have been suggested to justify these differences.

To our knowledge no specific details are available on dimeric or polymeric products which are expected to be produced in the radiolysis of polyunsaturated fats.

Secondary Products. We have assigned this term to cover all radiolytic compounds which arise from more than one cleavage in the same triglyceride molecule or from the decomposition of "primary products". A number of compounds isolated from irradiated tricaproin were explained on the basis of double cleavage (LeTellier and Nawar, 1974). The formation of a 2-oxoethyl ester, for example, may arise from cleavages at a C–C bond in the glyceride moiety and at the acyl–oxygen bond of the fatty acid in the secondary position.

Quantitatively, the secondary products are produced in much less amounts than the primary or recombination products. It appears therefore that the probability of two or more radiolytic cleavages occurring in the same molecule is relatively low.

Radiolytic reactions other than those discussed above include hydrogenation and cis–trans isomerization. Thus, irradiation of oleic acid yields small amounts of stearic acid, elaidic acid, and heptadecane. In contrast to the dimerization or recombination reactions, radiolytic hydrogenation appears to be unaffected by the rigidity of the crystalline matrix, as indicated by the observation that the solid and liquid state yields of stearic acid and heptadecane, from oleic acid, are approximately equal. The presence of lactones in irradiated fats has also been reported but the mechanism of their formation remains unclear.

It must be evident that the radiolysis of natural fats is made significantly more complex than in the case of model systems, by the large number of fatty acids usually present and the wide variation in the distribution of these acids on the glyceride molecules. Furthermore, very little information is available regarding the radiolytic reactions of polyunsaturated fatty acids or those of triglycerides containing unsaturated fatty acids. Research in this area is indeed necessary for a more complete understanding of the radiolysis of natural lipid systems.

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Chemical Consequences of Irradiating Nucleic Acids

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The mechanisms by which DNA is destroyed by radiation are briefly surveyed and indicate that a large variety of products are possible. The products formed from DNA bases and from deoxyribose are discussed and mechanisms by which they could prove hazardous are summarized. It is concluded that more work is needed before any hazard can be identified.

The majority of the work on radiation damage to nucleic acids has been carried out as a background to determining the events in vivo that lead to cell death or mutation. In considerations of any hazards irradiated nucleic acids might cause in food irradiation, it is the chemical nature of the radiation products themselves that must be taken into account. Although the studies of nucleic acid damage have been comprehensive (Ward, 1975), it has not been possible to define precisely the lethal or mutagenic events in molecular terms. Thus, data are available which, though not obtained in food irradiation studies, will permit a discussion of the present topic.

Considerations of risks of radiation exposure have led to concepts that may prove useful in defining possible food irradiation hazard, e.g., addition to the genetic load, mechanisms of mutagenesis, repair deficient cells—all applicable to the food consumer.

In this paper, an attempt will be made to discuss DNA damage that would be produced in a cellular environment and to try to place this damage in perspective as a potential hazard in food irradiation.

RADIATION DAMAGE MECHANISMS

In an in vivo situation, damage produced in a molecule will be initiated by both direct and indirect effects: the former by deposition of the radiation energy directly in the molecule of interest either in the form of excitation or ionization, the latter by reaction of the molecule of interest with another molecule that has been ionized or excited by radiation energy. Hence, one of the problems of assigning mechanisms of nucleic acid alteration in vivo stems from the variety of molecules whose radicals, ion-radicals, or excited states are produced in close proximity and, hence, react with the target molecule.

Of course, once a radical is formed on a molecule, it must react further to produce a stable product. Several routes of radical decay are possible: (*1*) *Unimolecular.* Internal rearrangement or homolytic bond cleavage, both of which have been described for nucleic acid constituents (Hartmann et al., 1970; Neta, 1972). (*2*) *Biomolecular with a Molecule.* Many reactions of this type have been shown to occur (Smith, 1976). This mode of decay presents the possibility of multiple routes to a variety of radiation products. (*3*) *Bimolecular with Another Radical.* At normal temperatures and dose rates few bimolecular reactions with radiation-produced radicals are possible unless long-lived radicals are involved. Reactions with added free radicals such as oxygen (Willson, 1970) or nitroxyls (Brustad et al., 1971) have been widely characterized.

From these considerations, a myriad of possible reaction products can be forecast for any radical formed in a cellular environment. The variety of initial radical identities in a heterogeneous polymer such as a nucleic acid or a protein accentuates the complexity of any attempt to define the range of radiation products.

Faced with this complexity, what can be done to define a simple model system that can be validly used as a model for the in vivo situation. A correlation has been established (see Ward (1975) for summary) between damage produced

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