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.

LITERATURE CITED

Beke, H., Tobback, P. P., Maes, E., *Food Sci. Technol.* **7**, 291 (1974).
Dubravcic, M. F., Nawar, W. W., *J. Am. Oil Chem. Soc.* **45**, 656 (1968).
Faucitano, A., *J. Chem. Soc., Perkin Trans 2*, 1786 (1972b).
Faucitano, A., *J. Phys. Chem.* **76**, 801 (1972a).
Heller, C., McConnell, H. M., *J. Chem. Phys.* **32**, 1535 (1960).
Howton, D. R., *Radiat. Res.* **20**, 161 (1963).
Howton, D. R., Wu, G. S., *J. Am. Chem. Soc.* **89**, 3 (1967).
Jones, R., *Radiat. Res.* **47**, 35 (1971a).
Jones, R., *Radiat. Res.* **50**, 41 (1972).
Jones, R., *Radiat. Res.* **68**, 447 (1971b).
Leibler, K., Wozniak, J., Krauze, S., Checinski, K., *J. Chim. Phys.*, 743 (1970).
LeTellier, P. R., Nawar, W. W., *J. Agric Food Chem.* **20**, 129 (1972a).
LeTellier, P. R., Nawar, W. W., *J. Agric. Food Chem.* **22**, 693 (1974).
LeTellier, P. R., Nawar, W. W., *J. Am. Oil Chem. Soc.* **49**, 259 (1972b).
Meidani, J., Ph.D. Thesis, University of Massachusetts, Amherst, Mass., 1975.
Merritt, C., Angelini, P., Bazinet, M. L., McAdoo, D. J., *Adv. Chem. Ser.* **No. 56**, 225 (1966).
Tamura, N., Collins, M. A., Whiffen, D. H., *Trans. Faraday Soc.* **62**, 1037 (1966).
Vajdi, M., Ph.D. Thesis, University of Massachusetts, Amherst, Mass., 1976.
Williams, T. F., *Nature (London)* **194**, 348 (1962).
Wu, G., Howton, D. R., *Radiat. Res.* **57**, 390 (1974).
Wu, G., Howton, D. R., *Radiat. Res.* **61**, 374 (1975).

Received for review February 28, 1977. Accepted June 6, 1977. Presented at the Symposium on Current Studies on the Chemistry of Food Irradiation, First Chemical Congress of the North American Continent, Mexico City, Mexico, Dec 1975.

Chemical Consequences of Irradiating Nucleic Acids

John F. Ward

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

Laboratory of Nuclear Medicine and Radiation Biology, University of California, Los Angeles, California and the Department of Pathology, School of Medicine, University of California, Los Angeles, California 90024.

0021-8561/78/1426-0029$01.00/0 © 1978 American Chemical Society

by OH· radicals and 70% of the cell killing caused by radiation: the OH· radicals are produced following ionization of the intracellular water. It is suggested that OH· reactions with DNA are the significant events. (Of course, the significance of this damage in cell killing cannot be directly related to an association of OH· radical derived radiation products to products that may be significant as a food hazard.) Subsequent discussion is centered around products formed as a consequence of OH· radical reactions with nucleic acids and their constituent compounds.

OH· REACTIONS WITH DNA

The initial reactions of OH· radicals with DNA can be conveniently investigated by radiolysis of dilute aqueous solutions. The rate of reaction of OH· radicals with DNA has been shown by pulse radiolysis to be rapid (Scholes et al., 1965), occurring at a rate close to that calculated for the collision frequency (Michaels and Hunt, 1973). The DNA–OH· radical has a spectrum that is comparatively featureless and whose persistence appears to indicate that the radical is long lived (Scholes et al., 1969). However, the absorption spectrum does not decay even in the presence of oxygen, which is known to react with the transient. In the absence of oxygen, the DNA–OH· transient has been shown by a spin-trapping technique to have a long life time—10% remains even after ~10 min (Brustad et al., 1973). Van Hemmen and Meuling (1975) report that long-lived DNA radicals do not react with sensitizer molecules to cause lethality. Thus the life time of the DNA–OH· transient is still an open question.

In earlier work (Scholes et al., 1960), analysis of destruction of DNA bases indicated that the majority of radiation-induced DNA damage occurred on the base moieties; probably as a result of the high rate of OH· radical reaction with the bases compared with OH· + deoxyribose. More recent work (Ward and Kuo, 1973) using low-radiation doses causing less macromolecular breakdown showed that almost twice as much sugar damage occurs in double-stranded DNA, as in single stranded DNA, the latter is representative of a mixture of deoxynucleotides. Thus, base damage accounts for ~70% of DNA–OH· damage and 30% is by reaction with the deoxyribose moieties. In other words, even though the bases are protected inside the structure, they still represent the major type of damage to the DNA. But their occurrence within the double helix means that the subsequent reactions of radicals formed on them will be limited to those with molecules or radicals capable of penetrating to the radical site, i.e., small entities. Molecules bound in close proximity would, of course, be excellent candidates for reaction with the radical site.

In a consideration of possible food hazards from irradiated nucleic acids, breakdown of the macromolecular structure by the formation of single and double strand breaks is probably not important. Extrapolation of literature data (Corry and Cole, 1973) indicates that with a dose of 0.5 Mrad, a double-strand break is formed every 100 000 base pairs of cellular DNA. Also, assuming 30 eV per single-strand break (Painter, 1975) a single-strand break will be formed for every 20 000 nucleotides at 0.5 Mrad. In other words, nucleic acids will still exist as macromolecules after 0.5 Mrads. Monomeric products are formed, however (Ward and Kuo, 1976). We have shown that free bases and damaged bases are released from nucleic acids as a result of irradiation. Preliminary results (Ward and Kuo, 1973) indicate that a free base is released at every strand break.

It is difficult to postulate any potential food hazard from the production of strand breaks or the release of intact DNA bases caused by irradiation. What should be considered, however, are the radiation products of the nucleic acid bases, some of which are released upon irradiation of the polymer.

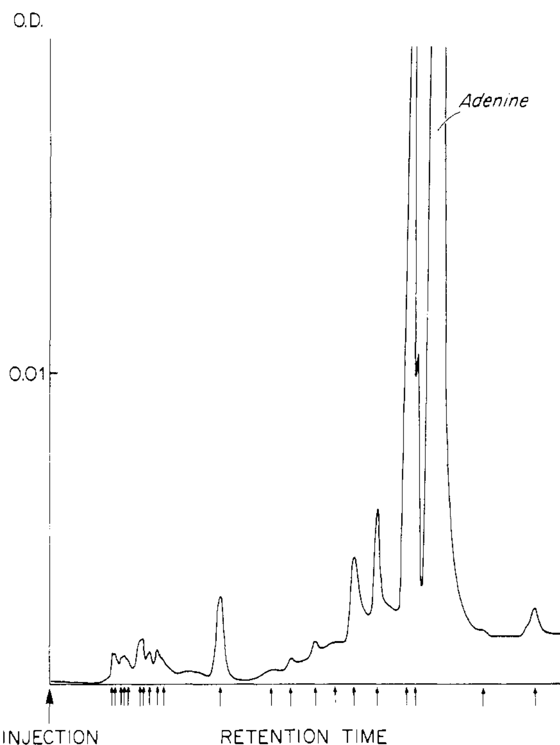


Figure 1. High-speed liquid chromatogram of irradiated adenine: instrument, Waters Model 660 fitted with 254 nm; column, μ Bondapak, 4 mm × 30 cm; eluent, linear gradient 0–50% methanol in water; sample, 2 μL of 10^{-3} M adenine irradiated to 5% destruction. The small arrows on the abscissa represent new UV-absorbing compounds formed by irradiation.

BASE PRODUCTS

The most widely studied nucleic acid base is thymine. The initial reaction with OH· radicals is fast (Scholes et al., 1965) as is the OH· reaction with all bases. Subsequent reactions of the OH· adduct lead to products. From thymine irradiated presence of oxygen, Teoule and Cadet (1973) have been able to isolate 21 radiation products in varied yields. The major products are the unstable hydroxy hydroperoxides (Schweibert and Daniels, 1971). The assignment of the peroxide structure by Teoule and Cadet (1973) was shown to be incorrect by Hahn and Wang (1973). Similar products are probably formed in thymidine, thymidylic acid, and in DNA. The relative proportions may be different in the macromolecule, however. Cerutti et al. (1974) have devised a technique that has enabled them to detect thymine products, formed by saturation of the 5–6 double bond, in irradiated cells. They have also been able to measure the abstraction of H atoms from the 5 methyl group, again in vivo.

A similar range of products is probably formed on the other base moieties. In our work we have been examining the radiation damage to adenine, again produced by OH· radical attack. We are specifically interested in radiation products that retain their UV absorption. Figure 1 shows a preliminary separation by liquid chromatography of UV-absorbing adenine radiation products. Approximately 20 unidentified products can be separated as indicated by the arrows in the figure.

Recently we have turned our interest to the detection and determination of radiation products of purines and pyrimidines that retain their ability to absorb ultraviolet

light. This property is an indication of the planar nature of the conjugated base. The rationale for searching for these products stems from an interest in damage that may not be repaired in vivo: Most types of damage in DNA shown to be repairable by enzymatic excision–repair processes are in the form of a gross distortion of the double helix, i.e., a recognition site for a repair endonuclease. Pyrimidine dimers, thymine base damage, alkylation damage, and strand breakage all cause physical distortion (Hanawalt and Setlow, 1975). The exception to this rule is an endonuclease activity isolated from *Micrococcus luteus* which specifically recognizes the RNA base uracil when it is present in DNA (Carrier and Setlow, 1974).

Radiation damage initiated by OH· addition to a purine or pyrimidine ring leading to saturation of the double bond will lead to buckling of the ring structure. Damage that does not cause ring buckling, such as damage to the exocyclic methyl group of thymine or addition of a new group such as formation of an N oxide, would leave the planarity of the ring intact. If these types of damage are not detected by repair enzymes, they may lead to lethality or mutagenesis, i.e., when replication or transcription of the altered base occurs. The significance of these considerations to food irradiation problems will be seen in the Discussion section.

Since UV-absorbing products are formed in low yield, and ideally should be measurable to biologically significant doses, we are developing immune assays for these products. Hydroxymethyl uracil (HMU), a radiation product of thymine (Teoule and Cadet, 1973; Myers et al., 1965), formed by damage to the methyl group, has been detected in γ-irradiated thymine by a serologic assay (Lewis et al., 1977). Developments of the techniques improving sensitivity and selectivity will permit detection of this product in vivo. Thus, it will be possible to determine whether cells can excise this damage by enzymatic methods.

A similar serologic assay has been developed for adenine N-1 oxide, a suggested radiation product of adenine (Lockmann, 1961). However, using this assay no adenine N-1 oxide has been detected in irradiated adenine solutions—the *G* value for formation must be less than 0.001, e.g., less than 0.03% of adenine destroyed is converted to this product.

A series of antibodies to other potential radiation products of the DNA bases is being developed (Lewis and Ward, in progress).

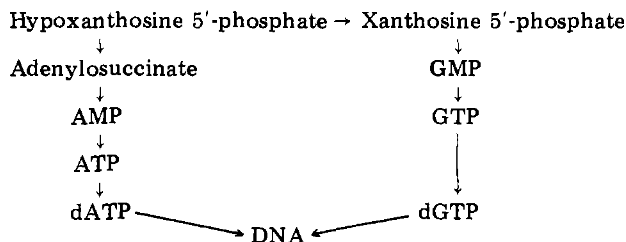
From the preceding discussion it can be seen that the potential for a variety of base products in DNA is high, but that few of those detected in the irradiated free base system have been pursued to the macromolecule or the in vivo situation.

SUGAR PRODUCTS

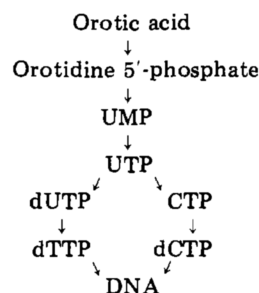
Most of the deoxyribose radiation product analyses have been carried out by von Sonntag et al. (1975). Radiolysis of deoxyribose in aqueous solution gives a variety of products stemming from the abstraction of a hydrogen atom from all possible positions in the sugar except the 2 position. Unimolecular and bimolecular decays lead to a variety of products. These data are not directly related to DNA since here the deoxyribose was not substituted and was present in a pyranose ring rather than a furanose (as in DNA). Most of the types of deoxyribose damage can lead to strand breakage either immediately or following β elimination of the phosphate. But damaged sugar moieties could remain in the DNA. Recently von Sonntag (1975) reported sugar products from irradiated DNA, showing that the three sugars 2,5-dideoxypentos-4-ulose ($CHOCH_2CHOHCOCH_3$), 2,3-dideoxypentos-4-ulose ($CHOCH_2CH_2COCH_2OH$), and 2-deoxypentos-4-ulose ($CHOCH_2CHOHCOCH_2OH$) were formed from the deoxyribose moieties of the DNA. Another sugar product formed from irradiated DNA, but only after heat treatment, is malonaldehyde (Ullrich and Hagen, 1971); this compound is also a product of lipid peroxidation (Goldstein and Balchum, 1967).

In vivo radicals formed on deoxyribose moieties of DNA, being on the outside of the macromolecule, may be expected to be more prone to form cross-links with neighboring molecules than base radicals.

Scheme I. Purine Metabolism (Sallach and McGilvery, 1967)



Scheme II. Pyrimidine Metabolism (Sallach and McGilvery, 1967)



DISCUSSION

Two potential hazards of irradiated nucleic acids can be envisaged: (1) acting as a poison or mutagen and (2) causing a mutagenic change by incorporation of a wrong or damaged base into host DNA.

Thymine hydroperoxide has been shown to cause mutagenesis of *Hemophilus influenzae* DNA (Thomas et al., 1976), keto sugars have been shown to have bactericidal activity (Schubert, 1974). However, as pointed out by Schubert, the reactivity of the keto sugars probably precludes their constituting a significant hazard. Similarly it could be argued that other reactive radiation products would be "mopped up" before reacting at a significant site. Some purine derivatives which may be radiation products have been shown to be oncogenic (Brown and Parkam, 1972). The mechanism of oncogenicity is not known.

As discussed above, a great variety of damaged DNA base products are formed upon irradiation. What are the possibilities of such a base being incorporated into host DNA? A consideration of the routes by which purines and pyrimidines are synthesized and subsequently incorporated make this a highly unlikely eventuality. Both the purines (Scheme I) and the pyrimidines (Scheme II) are incorporated by well-established routes. The parent purine hypoxanthine is not converted to the nucleic acid bases adenine and guanine until deoxyribose triphosphate has been added to it. Similarly, the parent pyrimidine orotic acid is not converted to thymine and cytosine until it too has been converted into uridine triphosphate. Thus, any radiation product would not compete directly with a pool of normal bases, but with precursors that are specifically acted upon by the converting enzymes.

It is possible to subvert this synthetic pathway: Cells can be labeled in their DNA by the addition of thymidine (labeled) or 5-bromodeoxyuridine or 5-bromodeoxycytidine to the growth medium (Szybalski, 1974). However, the levels necessary to accomplish this incorporation $\sim 10^{-5}$ M are very high when radiation yields are considered.

A further cellular protection against wrong base incorporation occurs at the level of the self-policing by the DNA polymerase (Brutlag and Kornberg, 1972). DNA polymerase I of *Escherichia coli* is almost error free in its replication of the DNA template because it has an exonuclease capability permitting back-tracking and excision of a mismatched base. Mammalian DNA polymerases (Chang, 1973; Springgate and Loeb, 1973) appear to be almost error free in their activity.

To conclude, it is evident that many radiation products of DNA constituents are formed and that mechanisms exist whereby these products can be hazardous. However, it is not possible to forecast the extent to which they present a hazard in food irradiation. More work is needed in the areas of product yield determination, metabolism of radiation products, and the extents to which the products can be incorporated into newly synthesized polymers. Testing of potential cell poisons or wrong base incorporation could best be carried out by methods developed by Ames (McCann et al., 1975) to screen for potentially mutagenic pollutants.

LITERATURE CITED

Brown, G. B., Parkam, J. C., *Jerusalem Symp. Quantum Chem. Biochem.* **4**, 550 (1972).

Brustad, T., Jones, W. B. G., Nakken, K. F., *Int. J. Radiat. Phys. Chem.* **3**, 55 (1971).

Brustad, T., Jones, W. B. G., Wold, E., *Int. J. Radiat. Biol.* **24**, 33 (1973).

Brutlag, D., Kornberg, A., *J. Biol. Chem.* **247**, 241 (1972).

Carrier, W. L., Setlow, R. B., *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **33**, 1599 (1974).

Cerutti, P. A., *Life Sci.* **15**, 1567 (1974).

Chang, L. M. S., *J. Biol. Chem.* **248**, 6983, (1973).

Corry, P. M., Cole, A., *Nature (London) New Biol.* **245**, 100 (1973).

Goldstein, B. D., Balchum, O. J., *Proc. Soc. Exp. Biol. Med.* **126**, 356 (1967).

Hahn, B. S., Wang, S. Y., *Biochem. Biophys. Res. Commun.* **54**, 1224 (1973).

Hanawalt, P. C., Setlow, R. B., Ed., *Basic Life Sci.* **5A,B** (1975).

Hartmann, V., von Sonntag, C., Schulte Frolhinde, D., *Z. Naturforsch., B* **25**, 1394 (1970).

Lewis, H. L., Muhlemann, D. R., Ward, J. F., in press (1977).

Lochman, E. R., Doctoral Dissertation, Technical University of Berlin, 1961, pp 24–31.

McCann, J., Choi, E., Yamasaki, E., Ames, B. N., *Proc. Natl. Acad. Sci. U.S.A.* **72**, 5135 (1975).

Michaels, H. B., Hunt, J. W., *Radiat. Res.* **56**, 57 (1973).

Myers, L. S., Jr., Holmes, D. E., Tsukamoto, W. T., Ward, J. F., *Nature (London)* **208**, 1086 (1965).

Neta, P., *Radiat. Res.* **49**, 1 (1972).

Painter, R. B., in Radiation Research, Biomedical, Chemical, Chemical and Physical Perspectives, Proceedings of the Fifth International Congress of Radiation Research, Seattle, Wash., Nygaard, O. F., Adler, H. I., Sinclair, W. K., Ed., Academic Press, New York, N.Y., 1975, p 735.

Sallach, H. J., McGilvery, R. W., Intermediary Metabolism, Sheet 2, Gibson Medical Electronics, 1967.

Scholes, G., Shaw, P., Willson, R. L., Ebert, M., in "Pulse Radiolysis", M. Ebert et al., Ed., Academic Press, New York, N.Y., 1965; p 151.

Scholes, G., Ward, J. F., Weiss, J. J., *J. Mol. Biol.* **2**, 279 (1960).

Scholes, G., Willson, R. L., Ebert, M., *Chem. Commun.* 17 (1969).

Schubert, J., Fifth International Congress of Radiation Research, Seattle, Wash., 1974; Abstract E-17-4.

Schweibert, M. C., Daniels, M., *Int. J. Radiat. Phys. Chem.* **3**, 353 (1971).

Smith, K. C., Ed., "Aging, Carcinogenesis and Radiation Biology = The Role of Nucleic Acid Addition Reactions", Plenum Press, New York, N.Y., 1976.

Springgate, C. F., Loeb, L. A., *Proc. Natl. Acad. Sci. U.S.A.* **70**, 245, (1973).

Szybalski, W., *Cancer Chemother. Rep.* **58**(4), 539 (1974).

Teoule, R., Cadet, J., *Chem. Commun.* **20**, 1269 (1973).

Thomas, H. F., Herriott, R. M., Hahn, B. S., Wang, S. Y., *Nature (London)* **259**, 341 (1976).

Ullrich, M., Hagen, U., *Int. J. Radiat. Biol.* **19**, 507 (1971).

Van Hemmen, J. J., Meuling, W. J. A., in "Fast Processes in Radiation Chemistry and Biology", Proceedings of the Fifth L. H. Gray Conference, Sussex, England, Adams, G. E., Fielden, E. M., Michael, B. D., Ed., The Institute of Physics, Wiley, New York, N.Y., 1975, p 264.

von Sonntag, C., Dizdaroglu, M., Schulte Frohlinde, D., Fifth International Biophysics Congress, Copenhagen, 1975; Abstract P222.

Ward, J. F., *Adv. Radiat. Biol.* **5**, 182 (1975).

Ward, J. F., Kuo, I., *Radiat. Res.* **66**, 485–98, May (1976).

Ward, J. F., Kuo, I., Radiation Research Society Meeting Abstract Ec4 (1973); paper in preparation.

Willson, R. L., *Int. J. Radiat. Biol.* **17**, 349 (1970).

Received for review February 28, 1977. Accepted July 5, 1977. Presented at the Symposium on Current Studies on the Chemistry of Food Irradiation, First Chemical Congress of the North American Continent, Mexico City, Mexico, Dec 1975. This work was supported by E(04-1) GEN-12 Contract, between the USERDA and the University of California, Los Angeles, and by a grant from the U.S. Public Health Service.