Radiolysis of Carbohydrates and of Carbohydrate-Containing Foodstuffs

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Toxicological evaluation of irradiated foodstuffs requires knowledge of radiation-induced chemical changes. A review of the literature reveals much information on the radiation chemistry of pure substances, e.g., dilute solutions of individual carbohydrates. Much less is known about the interactions of food constituents during irradiation. In an effort to remedy this situation, radiation effects on various compounds have been studied in systems of increasing complexity. In one approach, gas chromatography was used to investigate the radiolysis of trehalose in pure solution and in the presence of amino acids or proteins. In another approach, radiation-induced aggregation of proteins and of [14C]tryptophan with proteins was studied in the absence and presence of (a) carbohydrates (trehalose, starch), (b) emulsified sunflower oil, and (c) a mixture of carbohydrates and emulsified sunflower oil. The extent of radiation-induced aggregation of proteins was reduced by (a) and remained unaffected by (b). Increasing amounts of lipid in mixture (c) increasingly counteracted the effect of the carbohydrates on aggregation of proteins.

This article will consist of two main parts. The first part will be a brief review of present knowledge concerning the radiation chemistry of pure carbohydrates. The second part will refer to some previously unpublished work on the radiation chemistry of mixtures of carbohydrates with proteins and lipids—model systems for carbohydrate-containing foodstuffs.

Our interest in this field is related to the use of radiation as a method of food preservation. Commercial uses of this promising process are still limited, mainly because existing legislation requires very extensive, very costly animal feeding studies to ascertain the wholesomeness of different irradiated food items. Even for those irradiated foodstuffs which have been submitted to animal tests of this kind, health agencies are still reluctant to grant clearances. A reason for this reluctance is, in our opinion, the feeling that not enough is known about what happens when foods are irradiated.

One approach to improving this knowledge is to analyze irradiated foods, to identify radiation-induced compounds, and to estimate their yields. This is being done in a number of laboratories, and the results of such studies have been documented in numerous publications. Another approach, one that has not yet received much attention, is to study the radiation chemistry of model systems of various degrees of complexity, ranging from pure carbohydrates, proteins, lipids etc. to multicomponent mixtures approaching the composition of meats, bread, fruits etc.

While an impressive amount of information on the radiation chemistry of pure substances is available, attempts to extrapolate this information, so as to permit prediction of radiation-induced changes in complex foodstuffs, are based on some uncertain assumptions (Diehl and Scherz, 1975). We still know too little about the mutual influences of the food constituents when they are irradiated simultaneously. The object of our studies described in the second part of this paper is to explore this avenue to a better understanding of radiation-induced changes in foods.

RADIATION CHEMISTRY OF PURE CARBOHYDRATES

A number of reviews on this topic are available (Phillips, 1963, 1972) and we can limit the discussion to some recent findings.

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Table I. G Values for Radiolysis of Glucose (10^{-2} M, N_2O Saturated) (Dizdaroglu et al., 1975)

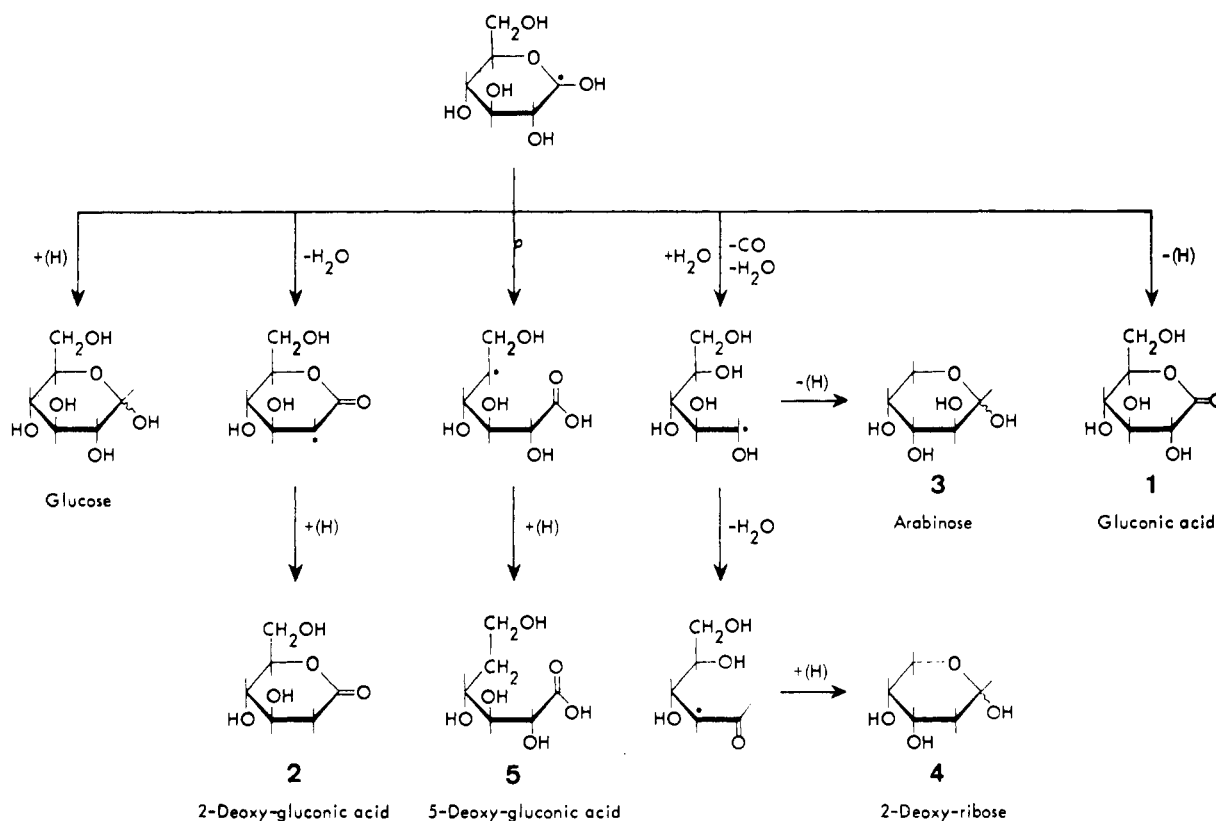| Product	G values
Glucose consumption	−5.6
2-Deoxygluconic acid	0.95
Glucohexodialdose	0.22
5-Ketoglucose	0.18
Gluconic acid	0.15
Glucosone	0.15
3-Ketoglucose	0.10
4-Ketoglucose	0.07
6-Deoxy-5-ketoglucose	0.05
2-Deoxyribose	0.04
Dihydroxyacetone	0.03
Butan-2-one-1,4-diol	0.02
Nine other products	0.35

Monosaccharides. Glucose is quantitatively the most important basic unit of food carbohydrates, such as starch and cellulose. As most foods contain a considerable percentage of water, a detailed study of the radiolysis of glucose in aqueous solution, as published by Dizdaroglu et al. (1975), is of particular interest.

It has been known for some time that OH· radicals (G = 2.7), hydrated electrons (G = 2.65), and H· atoms (G = 0.55) are formed when water is irradiated, and that of these the OH· radical is of primary importance in reactions with carbohydrates. It abstracts carbon bound hydrogen atoms, forming glucosyl radicals from glucose. The reaction is not very selective, and apparently all six possible glucosyl radicals are formed. According to Dizdaroglu et al. (1975) these primary glucosyl radicals give rise to a number of free radical reactions, such as illustrated in Scheme I for the C-1 glucosyl radical and in Scheme II for the C-2 glucosyl radical. Similar schemes for the C-3 to C-6 radicals were suggested. The more important ones of the reaction products which were identified by these authors are listed in Table I, together with G values (number of molecules produced/100 eV of energy absorbed).

The fact that the sum of the G values of product formation is less than the (negative) G value of glucose consumption indicates that not all reaction products have yet been identified. The unknown ones are presumably compounds with more than 6 (probably 12) carbon atoms, which are difficult to identify by gas chromatography.

In the aforementioned study, glucose solution was irradiated in the absence of oxygen (more exactly: in an atmosphere of N_2O which converts solvated electrons to

Scheme I. Product Formation after Radical Attack at C-1 or Glucose [(H) Denotes a Hydrogen Atom Transferred in a Disproportionation Reaction (Dizdaroglu et al. 1975)]

+(H)   -H$_2$O   +H$_2$O -CO -H$_2$O   -(H)

Glucose

3
Arabinose

1
Gluconic acid

+(H)   +(H)   -H$_2$O   +(H)

2
2-Deoxy-gluconic acid

5
5-Deoxy-gluconic acid

4
2-Deoxy-ribose

Scheme II. Product Formation after Radical Attack at C-2 of Glucose (Dizdaroglu et al., 1975)

+ (H)

Glucose

-H$_2$O   + (H)

7
3-Deoxy-glucoson

- (H)

6
Glucoson
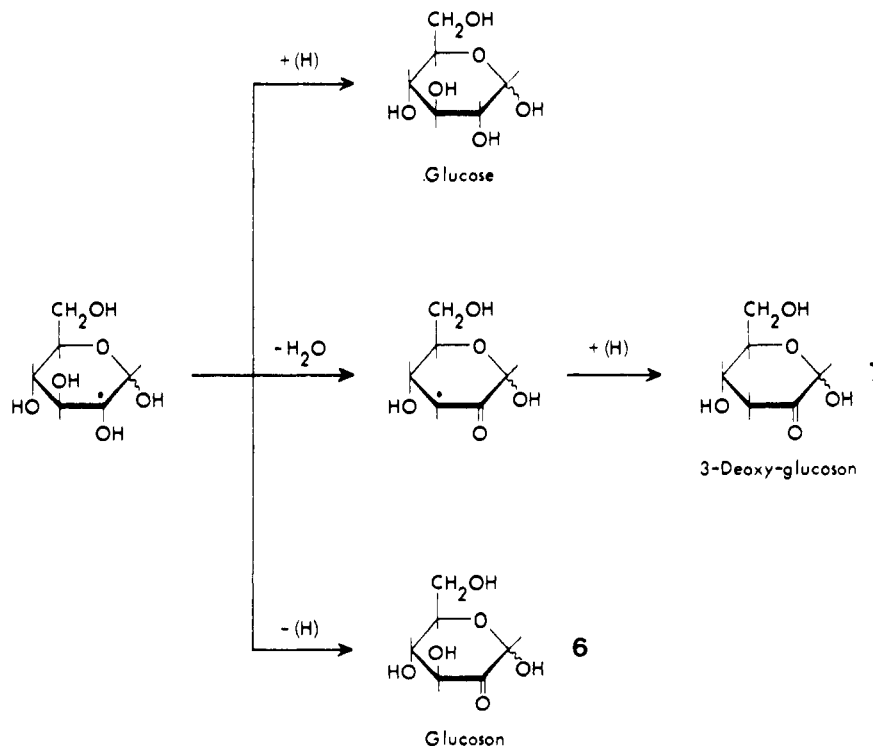
hydroxyl radicals). With respect to some applications of food irradiation, it is also of interest to know what happens in the presence of oxygen: water elimination and rearrangement processes are suppressed and no deoxy compounds are formed. Instead, the yields of sugar acids and of keto sugars are increased (Dizdaroglu et al., 1975). Another parameter which influences product formation is the pH of the sample. In glucose solutions, for instance, the G value for the formation of deoxy compounds is five times higher at pH 11 than at pH 2 (Scherz, 1970).

From the viewpoint of toxicological evaluation it is of interest to note that a certain amount of glucose destroyed does not mean an equivalent amount of one new product formed. Instead a very large number of compounds are

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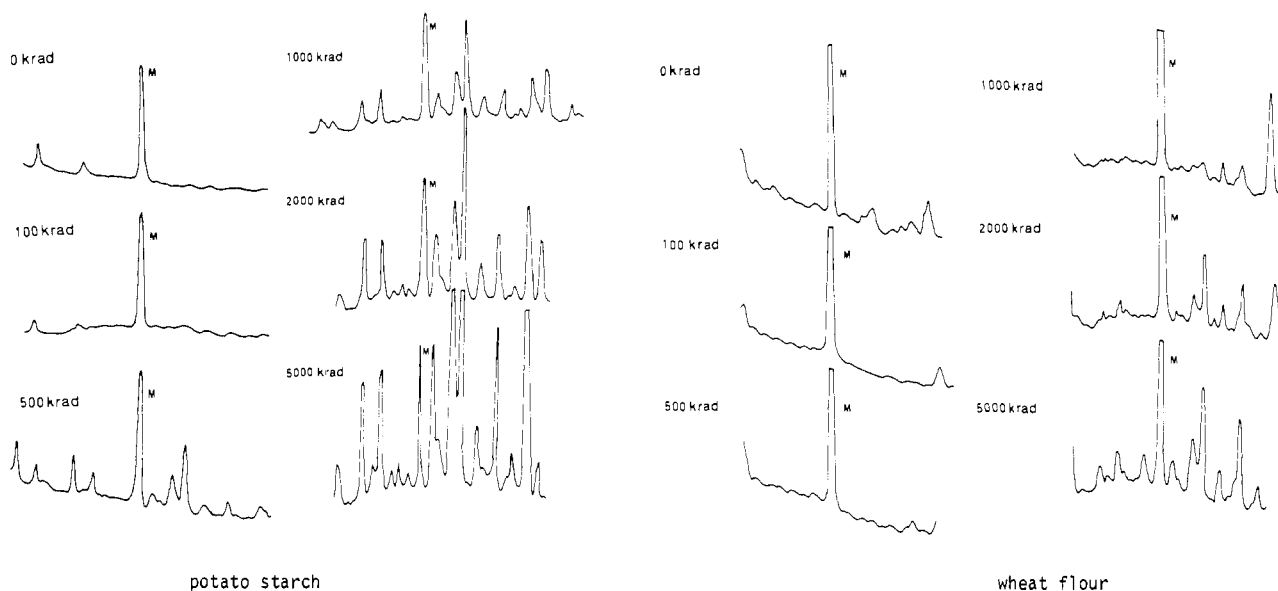
Figure 1. Gas chromatograms of extracts of unirradiated and irradiated potato starch and wheat flour (Scherz, 1975). Extracted with ethyl acetate–acetone–water 4:5:1; reduction with KBH_4; trimethylsilylation. M, mesoerythritol added as internal standard. For identification of other peaks, see Scherz (1971).

produced, all in minute quantity. This is not only true for glucose, but for the effect of ionizing radiation on food constituents in general, and this is what makes analytical studies on irradiated substances quite difficult (as indicated below, exceptions to this rule are observed with crystalline sugars).

A discussion of the various studies which have been carried out on monosaccharides other than glucose would go beyond the scope of this review.

Polysaccharides. Much work on irradiated starch has in recent years been reported by Saint-Lèbe's group in France. Lower molecular weight sugars such as glucose, maltose, erythrose, ribose, and mannose are formed in dose dependent yield, for instance 20 nmol of maltose/g of starch at 1 Mrad, 40 nmol at 2 Mrad (Berger et al., 1973). Further radiation-induced breakdown leads to the formation of formic acid (Dauphin et al., 1974), acetaldehyde, methanol, acetone, ethanol, and methyl formate (Berger et al., 1974). Measurement of the dose-dependent formation of colored products upon addition of 2-thiobarbituric acid, based on the radiation-induced formation of aldehydes and deoxysugars, has been suggested as a method for the identification of irradiated starch (Berger and Saint-Lèbe, 1969; Winchester, 1973). Studies carried out by Scherz (1975) at our institute have indicated that in the dose range practically used for insect control in cereals (less than 100 krad), the amount of deoxy sugars produced by radiolysis of starch is too small (less than 0.3 μg/g) to permit reliable identification of irradiated starch on that basis.

Gas chromatograms of the type shown in Figure 1 have also indicated that radiation doses of several hundred krad are required to cause the formation of products which can be measured in a reproducible way (Scherz, 1975). The presence of proteins in wheat flour suppresses product formation from carbohydrates, and much higher doses are required to produce measurable breakdown of starch in flour (Figure 1).

Less work has as yet been done on other irradiated polysaccharides, such as pectins or cellulose. Basic events must be the same as in the irradiation of starch and the spectrum of reaction products is probably not very different. With respect to food irradiation it is important to know how product formation is influenced by various

conditions such as temperature and gas atmosphere during irradiation and water content of the sample. Much of this information is already available (Berger et al., 1973).

Crystalline Sugars. While product formation in irradiated carbohydrate solutions and amorphous solid carbohydrates occurs with low G values, high yields have been reported for product formation from crystalline carbohydrates. For instance, when crystalline α-lactose monohydrate is irradiated, 5-deoxylactobionic acid is formed with $G = 40$. Dizdaroglu et al. (1973) have proposed a chain reaction mechanism for this reaction. There would be no practical reason or benefit in the irradiation of crystalline sugars, and foods which may benefit from radiation treatment usually do not contain crystalline sugars. However, in the radiation–sterilization of pharmaceutical preparations, where lactose is often used as a carrier material for the therapeutically active compound, the possibility of chain reactions of this type should be kept in mind.

RADIATION CHEMISTRY OF MIXTURES OF CARBOHYDRATES WITH PROTEINS AND LIPIDS

Starting with an investigation of the influences of proteins on radiation-induced product formation in carbohydrates, we have recently extended the scope of this work to include studies on the effects of carbohydrates and lipids on radiation-induced aggregation of proteins and radiation-induced binding of amino acids to proteins. The long-range goal of all of these investigations is a better understanding of radiation effects on all constituents of complex foodstuffs (Diehl, 1974).

Radiolysis of Trehalose. As a model carbohydrate for studying effects of proteins and amino acids on the radiolysis of sugars we have chosen trehalose. This non-reducing disaccharide is more stable to hydrolysis than saccharose. In the presence of amino compounds it therefore tends less to the formation of Maillard-type browning reactions which greatly complicate the analysis of the products of radiolysis.

Possible radiation-induced reactions of trehalose are shown in Scheme III. We have studied the destruction of trehalose and the formation of products by gas chromatography of silylated derivatives. As indicated in Figure 2, addition of amino acids protects trehalose against ra-

Scheme III. Radiolysis of Trehalose
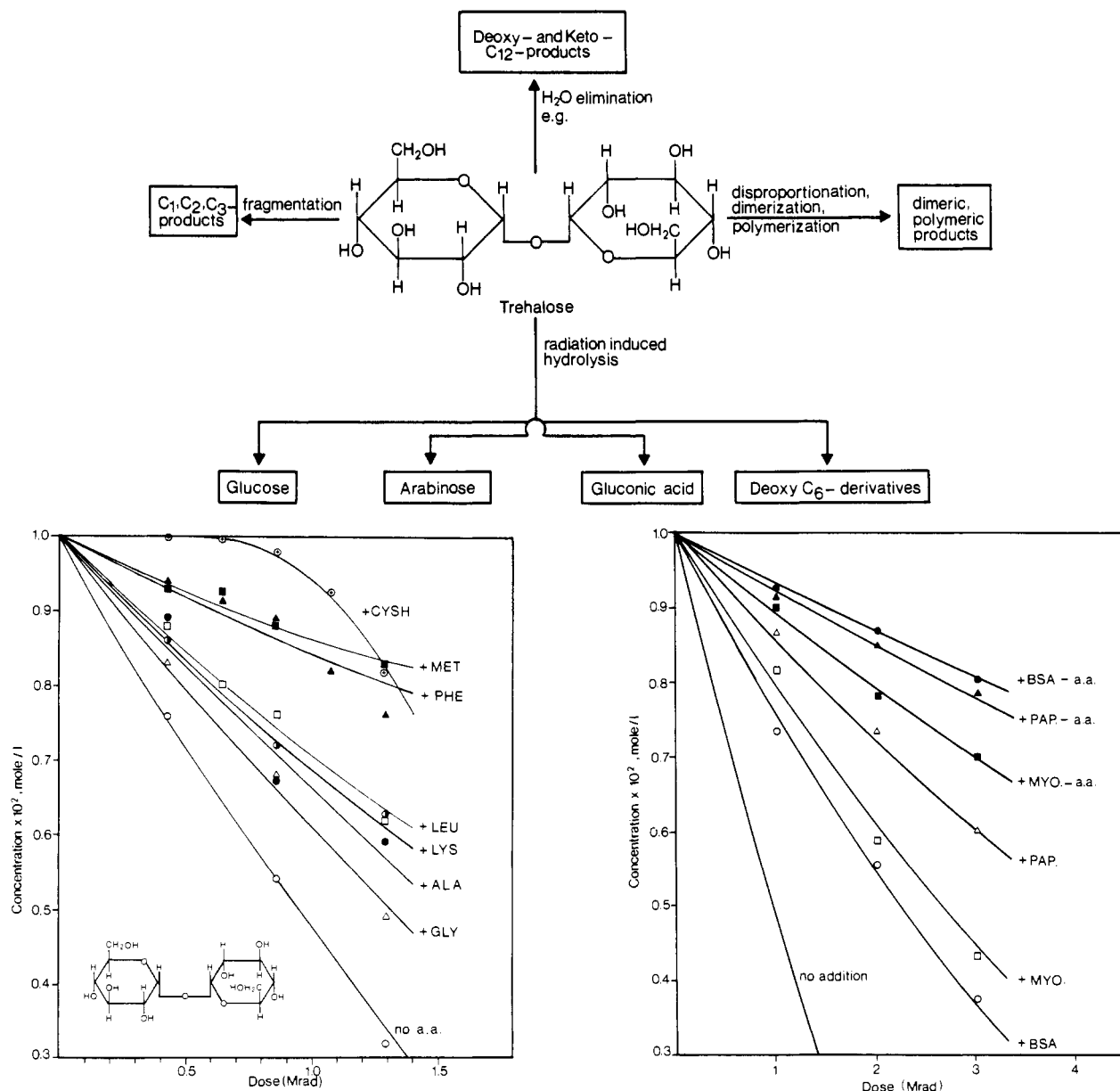
Figure 2. Dose dependence of the decomposition of trehalose upon γ-irradiation in air-saturated aqueous solution (10^{-2} M) with and without added amino acids (10^{-2} M).

Figure 3. Dose dependence of the decomposition of 0.36% aqueous trehalose solution (10^{-2} M) upon γ-irradiation with and without addition of 0.36% proteins or the mixture of constituent amino acids: BSA, bovine serum albumin; MYO, sperm whale myoglobin; PAP, papain; BSA-aa, amino acid mixture corresponding to the amino acid composition of BSA (PAP-aa and MYO-aa analogous).

diolytic destruction, with cysteine being most effective. This observation is not new. Tajima et al. (1969) have observed complete suppression of radiation-induced carbonyl formation from glucose by addition of cysteine at one-tenth the concentration of glucose. This may be explained by the higher reaction rate of cysteine (as compared to carbohydrates) with OH· radicals and by hydrogen donation from cysteine to glucosyl radicals.

In the experiments shown in Figure 3 we have gone a step further. Papain, myoglobin, and bovine serum albumin (BSA) were found to protect trehalose to an extent which cannot readily be related to the amino acid composition of these proteins. The protective power of BSA, which has the highest cysteine + cystine + methionine + phenylalanine content of the three proteins, should be expected to be the highest; it is actually the lowest. In contrast, if trehalose is irradiated in the presence of artificial mixtures of amino acids which correspond to the composition of these proteins, the "BSA" mixture has the highest protective effect, while the "myoglobin" mixture

(which contains no cystine/cysteine) has the lowest.

Obviously, the reaction of proteins with OH· radicals is determined not only by the amino acid composition but also the sequential and also the spatial arrangement in the protein molecule. The question of the relative importance of amino acid composition and protein conformation in determining the radiation sensitivity of proteins, particularly enzymes, has been much discussed (e.g., Burke and Augenstein, 1969). Studies with polyglutamic acid in random coil (less radiation sensitive) vs. helix conformation (more sensitive) have confirmed the great importance of the spatial arrangement (Ishikawa, 1970; Kunikane and Sugai, 1973).

It is also important to know whether or not the kind and amount of products formed upon radiation-induced carbohydrate destruction is influenced by the presence of

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Table II. Yield of Glucose upon γ-Irradiation of 0.36% Aqueous Trehalose Solution (10^{-2} M) with and without Addition of 0.36% Proteins or the Mixture of Constituent Amino Acids (Radiation Dose Adjusted to Cause 20% Trehalose Decomposition in Each Case)

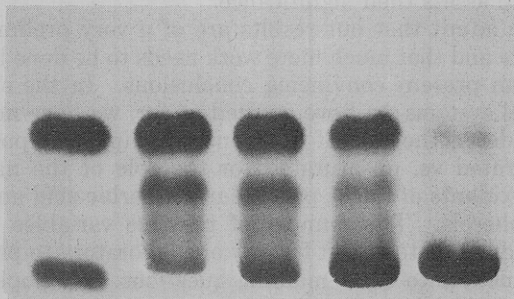| Irradiated sample	Yield of glucose, %
Trehalose without additive	19
Trehalose + BSA[a]	22
Trehalose + myoglobin	22
Trehalose + papain	22
Trehalose + BSA–amino acids	18
Trehalose + myoglobin–amino acids	20
Trehalose + papain–amino acids	22

[a] BSA = bovine serum albumin.

Figure 4. Radiation-induced aggregation of sperm whale myoglobin as a function of protein concentration. Thin-layer gel chromatography on Sephadex G-75 Superfine. Radiation dose 1 Mrad (mixture MYO + FER unirradiated). Protein staining with Amidoblack 10 B. MYO, myoglobin; FER, ferritin.

proteins or amino acids. Judging from the appearance of gas chromatograms we assume that this is not the case. Table II illustrates this for the particular case of glucose formation from trehalose. A radiation dose which causes 20% destruction of trehalose always yielded a reaction mixture which contained about 20% glucose, regardless of whether pure trehalose solution or mixtures with different proteins or amino acids were irradiated. The observation that the presence of proteins or amino acids affects the radiolysis of carbohydrates quantitatively but not qualitatively is in agreement with the notion that radiation-induced changes in aqueous carbohydrate systems are primarily caused by OH· radicals: proteins and amino acids appear to influence carbohydrate radiolysis only or primarily by influencing the availability of OH· radicals.

Radiation-Induced Aggregation of Proteins. It has been known for some time that irradiation of globular proteins causes the formation of protein aggregates. This can be demonstrated by thin-layer gel chromatography, which separates molecules according to size. In the chromatogram shown in Figure 4, an unirradiated solution of myoglobin and ferritin was applied on the left-hand side. Within a given time ferritin (mol wt about 500 000) migrates farther from the line of application than myoglobin (mol wt 17 800). Irradiation of a 1% myoglobin solution (right-hand side) causes almost complete disappearance of the monomeric myoglobin, which is converted to aggregates with higher molecular weights. At higher myoglobin concentrations a smaller percentage of the protein becomes aggregated by the same radiation dose. This is to be expected, as the amount of reactive species formed by radiolysis of water is constant for a given dose.

The results presented in Table III indicate that a comparable aggregate formation also occurs when solutions

Table III. Radiation-Induced Aggregation of Proteins (6% Aqueous Solution) in the Presence of Carbohydrates and Lipids (Dose: 2 Mrad)

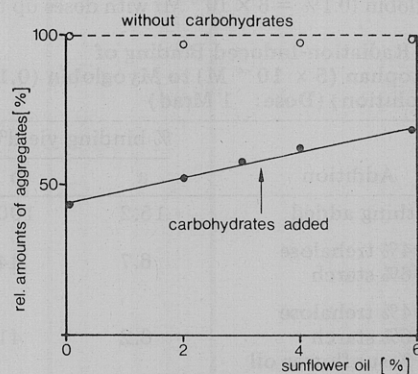|	% aggregation		
Addition	Sperm whale myoglobin mol wt 17 800	Ovalbumin mol wt 45 000	Bovine serum albumin mol wt 67 000
Nothing added	29.9 ± 0.7	51.1 ± 1.4	22.3 ± 0.7
2% trehalose 4% starch	12.9 ± 1.2	23.3 ± 1.3	6.4 ± 0.1
2% trehalose 4% starch 6% sunflower oil	20.5 ± 1.1	27.2 ± 0.4	10.8 ± 0.9
6% sunflower oil	29.4 ± 0.8	49.4 ± 0.8	21.1 ± 0.9

Figure 5. Radiation-induced aggregates in 6% myoglobin solution as a function of lipid concentration in the absence and presence of 2% trehalose and 4% starch. Radiation dose: 2 Mrad.

of ovalbumin or BSA are irradiated. The presence of an equal amount of carbohydrates causes a surprisingly large reduction in aggregate formation. Addition of an equal amount of sunflower oil (dispersed by homogenization with 1% Tween 80) to the protein solution has very little effect on aggregate formation. However, the decrease in aggregate formation caused by carbohydrate addition is counteracted by the lipid addition. The more sunflower oil is added, the smaller is the effect of carbohydrate addition (Figure 5). Addition of the detergent alone, without oil, had no influence on the effect exerted by addition of carbohydrates.

While in the absence of carbohydrates the radical sites in protein molecules can react with each other, leading to the formation of protein aggregates, the presence of carbohydrates interferes with the aggregation reaction. Whether this interference is due to competition between carbohydrate and protein molecules for OH· radicals or to reactions between protein radicals and carbohydrates or carbohydrate radicals is open to speculation. Also, we can only hypothesize on the reasons for the effect of addition of oil. It may be that in the presence of the detergent, protein "particles" are surrounded by an oil film, thus reducing protein–carbohydrate interaction and favoring inter-protein radical reactions. As we may expect similar influences of phase heterogeneity to play a role in foodstuffs of complex composition, it should be interesting to study these phenomena in more detail.

Radiation-Induced Binding of Amino Acids to Proteins. In another experimental approach we have investigated the radiation-induced binding of ^{14}C-labeled amino acids to myoglobin in the presence and absence of added carbohydrate and lipid. A number of amino acids were studied in the dose range up to 1 Mrad (Figure 6).

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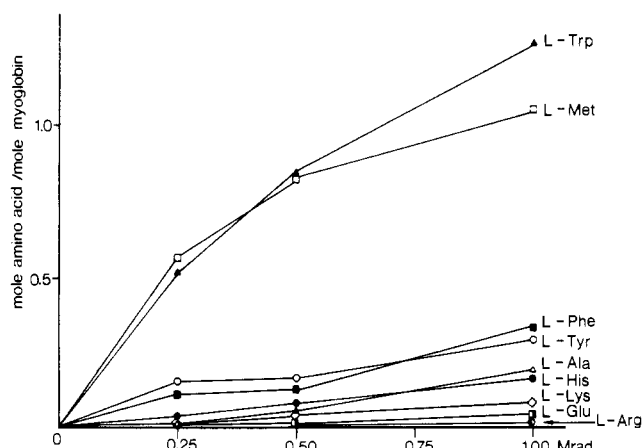
Figure 6. Radiation-induced binding of amino acids (5×10^{-4} M) to myoglobin ($0.1\% = 6 \times 10^{-5}$ M) with doses up to 1 Mrad.

Table IV. Radiation-Induced Binding of [^{14}C]Tryptophan (5×10^{-4} M) to Myoglobin (0.1% Aqueous Solution) (Dose: 1 Mrad)

	% binding yield[a]	
Addition	a	b
Nothing added	15.2	100
0.04% trehalose 0.06% starch	6.7	44
0.04% trehalose 0.06% starch 0.1% sunflower oil	6.2	41
0.1% sunflower oil	14.0	92

[a] a, expressed as percent of tryptophan radioactivity bound to myoglobin; b, related to "nothing added" = 100%.

With tryptophan, the most reactive of the amino acids here studied, about 15% of the added radioactive amino acid was bound to myoglobin after irradiation with a dose of 1 Mrad (higher doses did not increase this percentage). Yamamoto (1973) has studied the binding of some of these amino acids to serum albumin and has obtained similar curves relating radiation dose with the extent of binding.

As indicated in Table IV, addition of sunflower oil (homogenized with Tween 80) had little or no effect on radiation-induced binding of tryptophan to myoglobin. Also in agreement with the previous study on aggregation (Table III) is the observation that carbohydrate addition greatly reduced the binding of amino acid to protein. Not in agreement with the results on aggregation is the observation that addition of sunflower oil did not counteract the effect exerted by the added carbohydrate. This is perhaps related to the fact that more dilute solutions of protein and carbohydrate and correspondingly less oil were used in these experiments as compared to the aggregation studies. This was unavoidable because attempts to measure tryptophan binding to myoglobin in a 6% myoglobin solution had failed. The amount of tryptophan binding was too small at this protein concentration to measure it reliably. We are planning to carry out ag-

gregation studies on protein solutions of different concentrations in order to find out whether the lipid effect (or lack of it) depends on concentration.

OUTLOOK

With respect to possible conclusions concerning irradiated foodstuffs it should be pointed out that in all of these experiments radiation effects on one component of a mixture were quantitatively the same or, more often, less than the effects observed upon irradiation of the pure substance. This agrees with the general observation that radiation induced changes in complex foodstuffs are less extensive than might be expected from studies carried out on pure compounds. The literature contains numerous remarks about the role of protective substances in irradiated foods. Studies such as described here should help to gain a clearer understanding of these protective effects and to assess their significance.

We admit that our results are of a very preliminary nature and that much more work needs to be done before we can present convincing conclusions. In the simple model systems we have studied so far, we have not yet considered the role of differences in lipid composition (saturated vs. unsaturated) or the role of the natural antioxidants of foods, particularly ascorbic acid and the tocopherols. The number of possible variables to be considered is too great for any one laboratory to present the answers to the remaining questions. We hope that others will join us in this effort.

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