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AND PREVENTION OF OFF-ODORS AND OFF-FLAVORS

Report No. 16, Sept. 14, 1962
34 pages
(Contract DA-19-129-QM-1172)
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CONTENTS

CONTRACT RESEARCH PROJECT REPORT

ARMED FORCES FOOD AND CONTAINER INSTITUTE, CHICAGO
U. S. Army Quartermaster Research and Engineering Center
Natick, Massachusetts

University of California
Davis, California

Project Nr.: 7X84-01-002
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Report No.: 16 (Final)
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Official Investigator: A. L. Tappel
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R. M. Cox

Title of Contract: Irradiation of Proteins to Study Cause and Prevention of Off Odor and Off-Flavor

SUMMARY

1. Radiation damage to ovalbumin, hemoglobin and cytochrome c has been studied under anaerobic condition and the degradation mechanisms has been discussed.

2. The radio-lability of free amino acids has been determined and evaluation methods for the radio-lability has been discussed. The radio-lability of amino acids as free amino acids or bound in peptide linkage in protein has been also discussed.

3. The radio-lability of methionine and its derivatives has been studied and the degradation mechanisms have been discussed.

4. Decrease of radiation damage to ovalbumin, hemoglobin and cytochrome c by cystine, AET and MEA has been studied and their protection mechanisms have been discussed.

5. Decrease of radiation damage to some of the typical amino acids by methionine has been studied and their mechanisms have been discussed.

INTRODUCTION

I. Radiation damage to proteins.

The use of ionizing radiation for food preservation and other biological applications will be dependent on a more thorough understanding of the basic biochemical effects of the radiation. One of the major problems in this field is the destruction of essential constituents of biological materials and the formation of undesirable compounds. Despite considerable work in the field of radiation chemistry of amino acids, peptides and proteins, the mechanism of radiation damage to proteins is not well understood and more knowledge of the generalities of radiation damage to protein is needed.

As solutions of proteins are exposed to different doses of radiation, both physical and chemical changes occur. Sowinski et al (1) demonstrated fragmentation and aggregation of dry fibrinogen after radiation. Aggregation and disorganization of secondary structure in bovine serum albumin has been reported by Alexander and co-workers (2). However, whether the aggregation of protein is due to cross-linking or to denaturation has not been ascertained. In a chemical study, Barron et al (3) pointed out to the oxidation of -SH groups as the principal effect of the aerobic radiation of protein. Sulfur groups and sulfur containing compounds have also been implicated as constituents of the radiation odors which present problems in the radiation preservation of meats.

In the research reported here aqueous solutions of hemoglobin, cytochrome c and ovalbumin are used as a model system to assess some of the over-all chemical changes in proteins exposed to ionizing radiation.

II. Comparative radio-lability of free amino acids to amino acids in proteins.

Our knowledge of radiation chemistry of free amino acids in aqueous solutions is limited to a few studies (4,5).

Very few experiments have been carried out to compare the radiation lability of various amino acids. Dale et al (6) compared the yield of ammonia from various radiated amino acids with glycine as the reference. However, the extent of deamination of amino acids by radiation did not reveal a definite pattern of lability of the amino acids. Barron et al (7) did not obtain ammonia from irradiated dilute solution of glycine. Similar discrepancies have been observed in the literature (8,9).

Critical appraisal of observations, by different workers, of the effects of radiation on amino acids is complicated because of several variables. Concentration, solubility and pH of amino acid solutions, presence or absence of buffers, oxygen tension of the medium and quantity and quality of radiation dose employed often modify the radiation effect. This report is concerned with the determination of radiation lability of free amino acids in aqueous solutions at pH 7, by measuring the amino acid retention, as a comparison to the radiation lability of amino acids of proteins.

III. Radiation damage to methionine and its derivatives.

Electron spin resonance studies indicate that the sulfur atom of the amino acids is the site of localization of radiation damage to proteins (10).

One of the major effects of ionizing radiations on cysteine (11,12,13) and methionine (14,15,16) is the scission of -SH and $-SCH_3$ groups. These reactions are of considerable importance in the study of radiation biochemistry of proteins and radiation preservation of foods (17). In our studies (18,19,20) with catalase, cytochrome c, hemoglobin and ovalbumin the sulfur amino acids were found to be among the most radiation labile amino acids. Markakis and Tappel (13) studied the radiation chemistry of aqueous solutions of

cystine and cysteine radiated over a wide range. As cystine and cysteine were degraded as a function of radiation dose, alanine and H_2S were formed in high yields. Grant et al (21) have identified cystine disulfoxide, cysteic acid and cysteine sulfinic acid as other radiation degradation products of cystine. Using methionine-S^{35} and methionine-$C^{14}H_3$, Kumta et al (15) determined the sequence of radiation degradation of methionine. In addition to the cleavage of $-SCH_3$ group to form CH_3SH, the $-CH_3$ group was also detached from radiated methionine forming a homocysteine-like compound. Also, ionizing radiations caused oxidation of methionine to form methionine-sulfoxide and methionine-sulfone and decarboxylation and deamination of methionine and its resulting products. Kopoldova et al (16) further identified methionine sulfoximine, α-amino-n-butyric acid, and homocysteic acid as some of the scission products. CH_3SH, H_2S, carbonyls and ammonia, the volatile products of radiated methionine, have been quantitatively determined (22).

Although the mechanism of radiation degradation of methionine is not completely understood, the mechanism by which methionine is degraded by various treatments to yield off-odor products has received considerable attention. In the processing of cheddar cheese (23), sunlight flavor of milk (24), radiated beef (25) the off-odor is attributed to the carbonyl compound - methional. This is formed by Strecker degradation of methionine (23,24,25). Ninhydrin reaction which is a special case of Strecker degradation resulted in the formation of CH_3SH, $(CH_3)_2S$, $CH_3-S-S-CH_3$, acrolein and isobutyraldehyde from methionine (26). Photolysis of methionine gives rise to methionine-sulfoxide, CH_3SH, α-amino-n-butyric acid, homocystine and homocysteic acid (27,28).

This report covers the radiolysis mechanism of methionine and its derivatives in aqueous solution.

IV. Decrease of radiation damage to proteins by known sulfhydryl protectors.

When aqueous solutions of proteins are radiated, several changes occur in the structure of proteins. Our studies with hemoglobin and cytochrome c showed complex types of interactions between the scission products and insoluble aggregates (18). Although the rank in terms of radiation lability of amino acids varied with the protein, in general, methionine, cystine, phenylalanine, histidine, threonine and serine seemed to be more degraded than other amino acids. The purpose of this study is to investigate the influence of known chemical protectors (29,30,31,32) in reducing the radiation damage to amino acids in hemoglobin, cytochrome c and ovalbumin.

V. Decrease of radiation damage to amino acids by methionine.

The -SH compounds, as a radio-protector, are known to function by different mechanisms, mainly (I) induced anaerobiosis, (II) free radical scavenger effect, (III) capacity to form mixed disulfides, and (IV) repair of chemical damage. Our study with the sulfhydryl protectors to the proteins suggests that the main protection is due to the free radical scavenger effect. Shields and Gordy (33) reported that radiation damage localized on the sulfur atom of methionine and that electron spin resonance of radiated methionine (solid, aerobic radiation) was quenched by oxygen or air. This evidence suggests the possibility of methionine functioning as a radio-protector.

Furthermore, our previous study on radiation damage to aqueous mixture of methionine and glycine or alanine indicates that glycine and alanine in the mixtures are greatly protected by methionine.

The purpose of this study is to measure the protection effect of methionine to other amino acids. As a model system, the aqueous mixtures of methionine and alanine, arginine, histidine, leucine, phenylalanine or serine are used.

MATERIALS AND METHODS

Protein solutions with and without sulfhydryl protectors.

In these experiments, cytochrome c (Sigma Chemical Company), hemoglobin (crystallized from fresh cattle blood) and ovalbumin (Nutritional Biochemical Corporation) were used. One tenth per cent solutions of these proteins were prepared in demineralized distilled water and pH was adjusted to 7 by addition of HCl or NaOH solution. Cysteine (Nutritional Biochemical Corporation), 2-aminoethylisothiuronium bromide HB (AET) and 2-mercaptoethylamine HCl (MEA) (both from K and K Laboratories, Inc.) were incorporated at a concentration of 1×10^{-3}M in the solutions. The solutions in polyethylene screw cap bottles were deoxygenated (except those under aerobic conditions) by repeated evacuation and gassing with N_2. Also, O_2 was used to create aerobic conditions. The bottles were then sealed in number 2 cans in N_2 (O_2 for aerobic). The canned bottles were kept frozen under dry ice during transit to and from the reactor.

Free amino acid and dipeptide solutions.

In these experiments, chromatographically pure amino acids and peptides (Nutritional Biochemical Corporation) were used. The concentration of aqueous solutions of amino acids and peptides was 0.01 M in all experiments unless otherwise specified. The solutions were prepared in demineralized distilled water and pH was adjusted by addition of HCl or NaOH solution. The solutions were anaerobically sealed in the polyethylene bottles and glass ampuls (20 ml) as previously described. During transit to and from the reactor, the bottles were kept frozen under dry ice and the ampuls were kept at ambient temperature.

Irradiation

The frozen samples were thawed by holding the cans in running water for 2 hours and then all samples were radiated, at ambient temperature, by exposure to 0.6 to 2 Mev γ-radiation from spent radioactive fuel rods (Material Testing Reactor, Idaho) and to Cobalt-60 γ-radiator (University of California, Davis, California) the dose range was up to 10^7 rads.

Separation of radiated proteins by solubility.

The solubility of proteins is decreased after radiation and in some instances proteins precipitate. The radiated proteins were separated into three fractions, insoluble protein aggregates, TCA precipitable protein and scission products, by filtration as described previously (18).

Elementary analysis.

Samples for elementary analysis, S, C, H, N and O, were thoroughly dried to constant weight by evaporating the solutions at low temperature and under reduced pressure. The results were expressed as percentage retention.

Qualitative and Quantitative analysis of amino acids.

All samples were analyzed for amino acids using paper chromatography as previously described (18,34,35,36). The results were expressed as percentage of amino acid concentrations of original samples.

The values for total ninhydrin negative non-volatile compounds were calculated from the differences in total solids and known amount of ninhydrin positive compounds.

Analysis of volatile compounds.

An estimate of total volatile compounds formed by radiation was made on the basis of differences in the dry weights of radiated and non-radiated samples.

Hydrogen sulfide and methylmercaptan were measured by the methods of Marback and Doty (37), and Slivinski and Doty (38) respectively. An absorption train was set up for trapping hydrogen sulfide and methylmercaptan using cadmium hydroxide and mercuric acetate solution in sequence. The ampul was broken in a closed vessel which was connected to the absorption train. The ebullition of nitrogen gas was used to transfer the volatiles from the vessel to the absorption train. After 4 hours of the ebullition at 65°C, hydrogen sulfide in cadmium hydroxide solution and methylmercaptan in both cadmium hydroxide and mercuric acetate solutions were measured calorimetrically.

Analysis of total nitrogen, amide nitrogen and ammonia.

Total nitrogen, amide nitrogen and ammonia were determined by the methods of Nesseler, Bailey and Kjehldahl respectively as described previously (39,40).

Analysis of TBA reactants, peroxides and carbonyl compounds.

Thiobarbituric acid reactants, such as malonaldehyde, and peroxides were measured by spectrophotometric method and iodometric method, respectively, and details were described in previous reports (41). The method of Lappin and Clark was used for determination of carbonyl compounds (42).

Functional properties of proteins.

The functional properties of hemoglobin, the formation of HbCO and HbO_2, and the chemical or enzymatic reduction of cytochrome c were determined by spectrophotometric method (18).

Analysis of sulfhydryl group.

Amperometric titrations were carried out as described by Benesch et al (43), molar urea being used to increase the -SH reactivity.

RESULTS AND DISCUSSIONS

I. Radiation damage to proteins.

(A). Ovalbumin.

Marked changes in the visual appearance and odor of the ovalbumin solution were associated with increases in radiation dose. The sequence of changes may be categorized as follows: 0.1 to 1 X 10^6 rad, increasing amounts of flocculent precipitate, increase in radiation odor; 2 to 10 x 10^6 rad, decrease in flocculent precipitate, marked increase in sulfide odor, and presence of a burnt odor somewhat similar to that detected in radiated raw beef; 20 to 80 x 10^6 rad, precipitate to a small amount of non-flocculent material, complete absence of any odor at 40 to 80 x 10^6 rad, and increasing yellow discoloration. These changes have been observed repeatedly in several experiments.

It is interesting to note that the main effects of low doses, i.e., flocculation of protein and odor formation, are completely transformed by continued radiation. The possible relation of these observations to measured chemical changes and the secondary effects of radiation is discussed below.

It has been shown that amperometric titration may be used to detect the loss of

-SH groups at the lower levels of radiation where the presence of odor is first detected (44). Results from the amperometric titrations of radiated ovalbumin are given in Table 1. Although ovalbumin contains five cysteine residue, one -SH group is normally not reactive. Thus, the value of 3.75 for the control indicates the absence of any oxidative effects. The loss of -SH groups may be due to the oxidation to S-S and also due to the cleavage of -SH groups from the proteins. On the other hand, the unexpected reappearance of titratable -SH groups at intermediate doses may be due to the availability of formerly masked -SH groups and reductive cleavage of S-S and $S-CH_3$ bonds.

The curves in Fig. 1 show remarkable decrease of sulfur and nitrogen in TCA precipitable protein fraction after radiation. The liberation of nitrogen and sulfur, especially nitrogen, suggests a rupture of larger fragments from the protein. Simple random deamination and loss of free -SH and $-SCH_3$ groups are not supported by our data, since it is unlikely that the nitrogen and sulfur groups would have the same reactivity necessary for the parallel rupture from the protein, and the free $-NH_2$ groups on the protein would account for only a part of the liberated nitrogen.

In contrast to localized radiation effects on the sulfur groups, the data above suggest a more general rupture of the protein molecule with predominant action at peptide bonds. Drake et al have demonstrated the destruction of amino acids on irradiation of insulin (45), and Jayko and Garrison have postulated a mechanism for the rupture of peptide bonds by aerobic radiation (46). To assess if such a mechanism were operative in anaerobic radiation of ovalbumin, measurements were made of the carbonyls, amide nitrogen and ammonia that are formed with increasing radiation dose. The results of these analysis are given in Table II. The formation of amide groups from ovalbumin is in agreement with the theory of Jayko and Garrison. However, if this were the only mechanism, a one-to-one ratio of carbonyl and amide formation would be expected. Such was not the case. It is impossible, with the present information, to predict whether secondary effects have obscured to the carbonyl-amide ratio, or whether other radiation scission mechanism are operative.

The overall radio-lability of amino acids, as constituents of ovalbumin, was measured chromatographically, and the results are shown in Table III. The results indicate that the radiation damage localizes mainly on sulfur amino acids and cyclic amino acids.

(B). Cytochrome c and hemoglobin.

Among the physical changes, decolorization of the radiated cytochrome c and hemoglobin solution was noted. Complete destruction of the color occurred at higher doses of radiation. Aggregation of the protein molecules was suggested by the formation of hairlike fibers. However, no off-odor was detected in the radiated samples.

The total nitrogen content of the radiated proteins was not decreased. However, as shown in Table IV, fragmentation of the protein molecule was evident by an increase in nitrogen content of the scission products. This increase was not accounted for by an increase in ammonia resulting from deamination. The scission products were analyzed for carbonyl compounds, but none were detected.

Damage to the functional properties of cytochrome c and hemoglobin is evident from the criteria employed in the present studies. As Table V indicates, samples exposed to 10^7 or 5×10^7 rad, there was complete destruction of the chromophore group and loss of functional properties. Although radiation may rupture the protoporphyrin moiety independent of its effect on polypeptide chains, the close relationship between the apoprotein and the prosthetic group makes this subject more interesting. Destruction of histidine and oxidation of cysteine by radiation would make the protoporphyrin moiety more labile.

The results presented in Table VI show the distribution of total amino acids in the three fractions of radiated proteins. The TCA-precipitable protein is ruptured as a

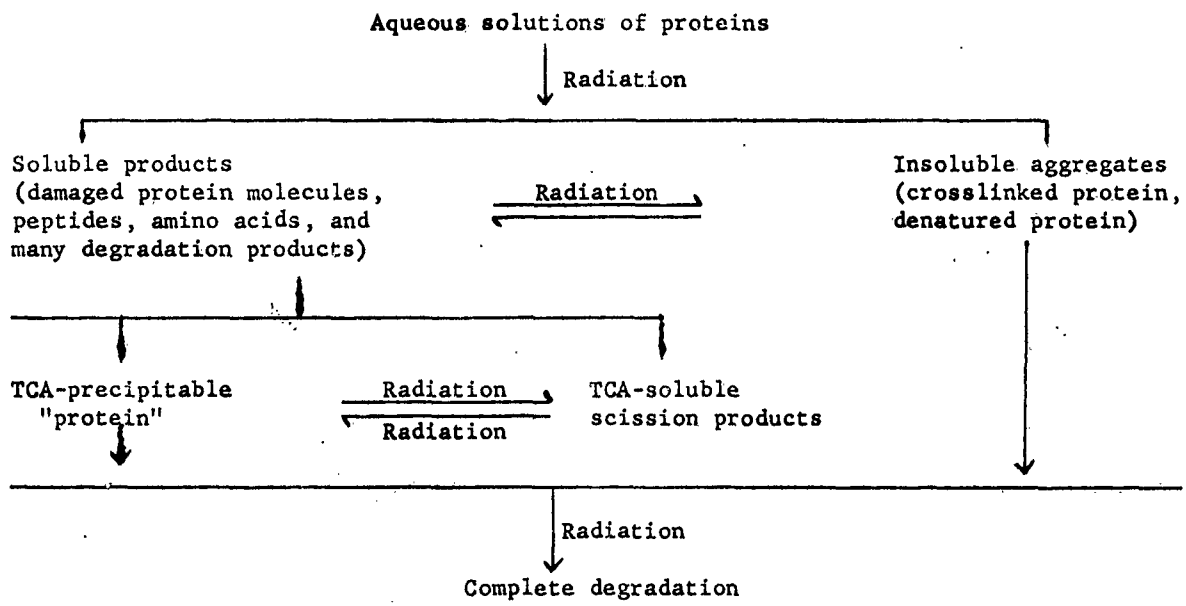
function of radiation dose, causing some amino acids to appear as "peptide" in the scission and in the insoluble fractions. Subsequently, the amino acids of those fractions also suffer radiolytic damage.

The amino acid analysis of the three fractions of the radiated proteins is given in Table VII and VIII. These results show that the sulfur amino acids are the most radio-labile ones, followed by cyclic and hydroxyl amino acids.

It is difficult to ascertain if the insoluble protein aggregates are derived from crosslinking of protein-protein chains, denaturation of the native proteins, or other mechanisms. However, it may be concluded from our data that protein aggregates are not formed by simple crosslinking or denaturation, but, rather, by more complex mechanisms.

On the other hand, there are many complex mechanisms for the production of scission products. Two tested in this research are radiation-induced hydrolysis of protein and random cleavage of protein into polypeptide chains. However, the results suggest that the mechanism is neither of these two simple cases. This is evident from the dissimilarity between the amino acid patterns of scission products and the original protein or TCA precipitable proteins for similar doses.

The general pattern of effects of radiation on these proteins may be summarized as shown.



II. Comparative radio-lability of free amino acids to amino acids in proteins.

The yield of a product as a measure for the comparison of radiation lability of amino acids has many disadvantages. The scission of C-S bond by radiation degradation of methionine, methionine-sulfoxide and -sulfone should give rise to the same product α-amino-n-butyric acid. However, in our studies (36) the yields of this compound did not serve as a good index of the stability of these sulfur amino acids. The linear relation between log of percent retention to radiation dose permitted calculation of the dose that would destroy 50% of the amino acid (half reduction dose, D 1/2). This formed a sound basis for the comparison of radiation lability of amino acids in relation to the known structural implications of the amino acids. Thus, the large differences in the D 1/2 for

these three amino acids showed that stability of methionine increased with higher oxidation states of the sulfur atom.

The values for D 1/2 are presented in Table IX. Some of the available values from the literature on the yields of ammonia (16) and peroxides (41) formed from amino acids have been included. The yields of ammonia do not reflect on the radiation lability of the amino acids as glycine, alanine and methionine have similar values (see Table IX). However, our studies (36) have shown that methionine is more radio-labile than glycine and alanine. The extent of deamination has no bearing on the number of amino groups or nitrogen atoms in the molecule. Similarly, the yields of peroxides formed do not reveal any definite pattern. The nature of peroxides formed is also unknown. This indicates that the radiation chemistry of amino acids is an important factor for defining the interaction between amino acids and free radicals (produced from radiolysis of water).

(A). Radiation lability of sulfur amino acids.

The D 1/2 values for methionine and its lower homologue S-methyl-cysteine are given in Table X. Since cystine and cysteine (0.01M) are insoluble in water at pH 7, the retention of these amino acids as a function of radiation dose was not determined in this study. However, the retentions of these amino acids determined under different conditions by Markakis and Tappel (13) were used for the calculations. The values are given in Table X. Both S-methyl-cysteine and methionine can be considered as radiation labile amino acids because of the low D 1/2 values. Degradation of methionine by the scission of -S CH_3 group, oxidation to form sulfoxide, sulfone, homocysteic acid and the decarboxylation of methionine and its resulting products have been documented in the literature (14,15,16). By similar mechanisms, S-methyl-cysteine would be degraded. Formation of CH_3SH and alanine from radiation of S-methyl-cysteine has been confirmed.

$$\underset{\text{S-methyl-cysteine}}{CH_3 \cdot S \cdot CH_2 \cdot \underset{\displaystyle NH_2}{\underset{|}{CH}} \cdot COOH} \xrightarrow{\text{free radicals}} \underset{\text{methyl mercaptan}}{CH_3SH} + \underset{\text{alanine}}{CH_3 \cdot \underset{\displaystyle NH_2}{\underset{|}{CH}} \cdot COOH}$$

Although cystine (and cysteine) were more degraded in hemoglobin, cytochrome c (18), and ovalbumin (see Table III), the D 1/2 values for such amino acids as free amino acids are very high. This poses an intriguing question on the influence of pH on the stability of amino acids towards radiation.

(B). Radiation lability of cyclic amino acids.

The low value of D 1/2 (1.1×10^6 rad) for phenylalanine shows that it is one of the most radiation labile amino acids. This is also substantiated from the study of proteins. Aqueous solutions of phenylalanine undergo radiation degradation forming phenylpyruvic acid (47) and hydroxylated products (48). In the present study tyrosine was not included because of the low solubility at pH 7.

It is interesting to note the high stability of tryptophan (D 1/2 = 14.5×10^6 rad). Tryptophan undergoes deamination, hydroxylation as well as opening of the ring to form formylkynurenine (44). Spectral studies (50) have shown complete disappearance of maxima and minima indicating complete damage of the indole ring. In our experiments, however, no appreciable change was detected in the spectral characteristics of radiated tryptophan solutions. This stability could be ascribed to formation of micelle and a effect of pH. The discrepancies observed between our results and those reported in the literature need further scrutiny.

Among other cyclic amino acids histidine was found to be radiation labile in the proteins we have studied. This amino acid is converted to imidazole acetaldehyde (51) and histamine-like compounds (52). The D 1/2 calculations for this amino acid shows

that compared to sulfur amino acid and phenylalanine, histidine is rather stable at this condition.

(C). Radiation lability of aliphatic amino acids.

Most of these amino acids form ammonia and carbonyl compounds on radiation. Among these amino acids, glycine and alanine have been well studied. Glycine is degradated into ammonia, methylamine, glyoxalic acid, acetic acid, formaldehyde, formic acid, carbon dioxide, hydrogen and hydrogen peroxide (4). Also irradiation of alanine yields ammonia, ethylamine, pyruvic acid, acetaldehyde, propionic acid, hydrogen and carbon dioxide (53). Leucine is converted to isoveraldehyde which contributesto the off-odor of radiated meat (4). The simplest amino acid, glycine, is the most stable one in the group of aliphatic amino acids (D 1/2 = 6.6×10^6 rad.). The D 1/2 values for the three structually similar amino acids, leucine, isoleucine and valine, are practically the same. Furthermore, our result shows a interesting trend that the radio-lability for simple aliphatic amino acids is inversely related to the aliphatic chain length. The same trend also observed in series of sulfur containing amino acids (S-methyl cysteine and methionine) and of dicarboxylic amino acids (aspartic acid and glutamic acid).

The radio-lability of dicarboxylic amino acids, aspartic acid and glutamic acid, is very similar to that of structually similar aliphatic amino acids. The D 1/2 value for aspartic acid (5.6×10^6 rad) is the same as that for alanine (5.4×10^6 rad). Also the D 1/2 for glutamic acid (3.4×10^6 rad) falls between that for alanine and valine (2.8×10^6 rad). The yield of carbonyl compounds from these dicarboxylic amino acids is not greatly different from the yield from alanine (22). This suggest the decarboxylation at β - and γ -carboxyl groups is not a predominant reaction. On the other hand, it is interesting to note the very low D 1/2 value for serine (2.0×10^6 rad). The reason could be due to the nature of the oxygen atom in the β -hydroxyl group.

Radiation lability of amino acids in proteins.

The trend of radio-labilities of aliphatic amino acids in proteins (see TableIII,VII, and VIII) is not exactly the same as that observed for free amino acids. At the highest radiation dose, 5×10^7 rad, all aliphatic amino acids are destroyed to some extent, however, at relatively low radiation doses, the amount of some aliphatic amino acids, especially alanine, is increased. This increase is considered to be due to the formation of this amino acid as a degradation product of radio-labile amino acids. This assumption is supported by the fact that alanine is formed from cysteine and cystine by radiation (13).

The rank in terms of radio-labilities of all individual amino acids varied with the proteins. On the other hand, our previous experiments indicated the influence of neighboring molecules on the radio-lability of amino acids in peptides (36). Therefore, the radio-lability of amino acids in proteins is not only dependent on the nature of amino acids, but also depends on the nature of neighboring amino acids.

III. Radiation damage to methionine and its derivatives.

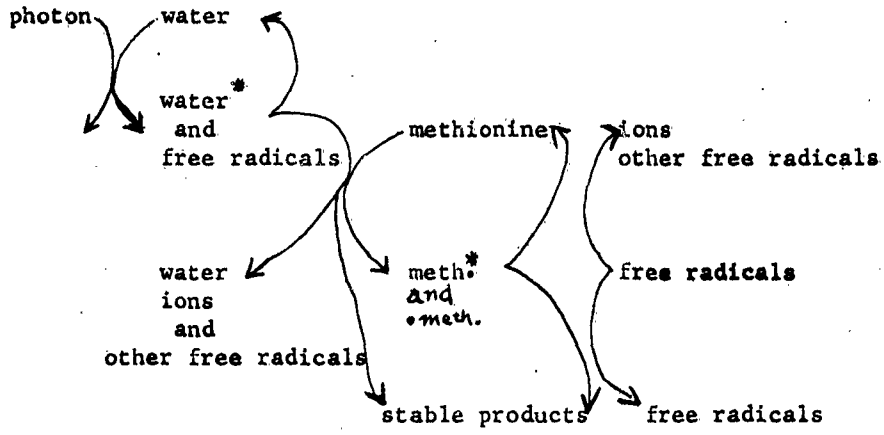
Typical patterns of radiation degradation of methionine and concurrent formation of products indicate that as methionine is degraded as a function of radiation dose, the resulting ninhydrin positive products are also degraded (36). A detailed consideration of some of these products was therefore undertaken in subsequent experiments. Radiation induced decarboxylation and deamination of the compounds will result in the loss of ninhydrin reactive groups. The marked increase in the volatile compounds is due to variety of products. Among the known compounds are CH_3SH, NH_3, CO_2, H_2S and carbonyl compounds (22). Dimethyl disulfide and CH_4 have been tentatively identified by gas chromatography[1]. The values for total ninhydrin negative non-volatile compounds would include several types of cleavage products including homocysteic acid, SO_4 (16), sulfonic

[1]Authors are grateful to Dr. R. A. Bernhard for his help in gas chromatography.

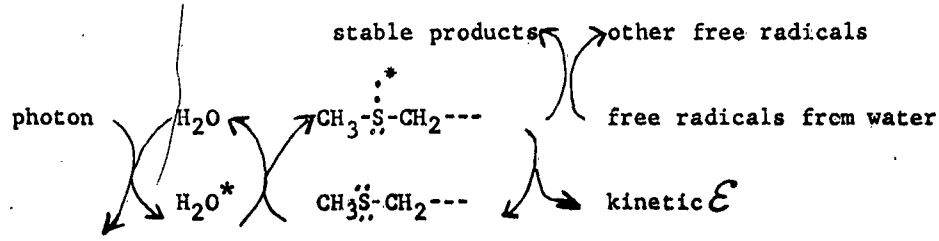
acids, sulfinic acids and short chain carbon residues. The percentage retention of sulfur, carbon, hydrogen, nitrogen and oxygen in radiated methionine (10^7 rad) was 56, 49, 60, 48, and 78 respectively. The retention for oxygen is higher than for other elements. Oxygen atoms of radiated methionine are lost as CO_2 and volatile carbonyl compounds. However, the free radicals produced from radiolysis of water will react with methionine to form highly oxidized products.

Although several ninhydrin positive products of radiated methionine solutions have been detected on paper chromatograms (14, 15, 16), only 2 products seem to be formed in measurable quantities. Table XI shows that as methionine is degraded, a.d methionine-sulfoxide and α-amino-n-butyric acid, as ninhydrin positive products, and methylmercaptan and hydrogen sulfide, as sulfur containing volatile products are formed. However, there is no simple stoichiometric relationship between the destroyed methionine and the formed products or between the formed products. In these experiments methionine-sulfone was not detected among the products formed. These results are in agreement with chemical degradation of methionine with H_2O_2 (54). Toennies and Callan (55) showed that H_2O_2 oxidizes methionine to methionine-sulfoxide but oxidation to methionine-sulfone requires presence of a catalyst (molybdate).

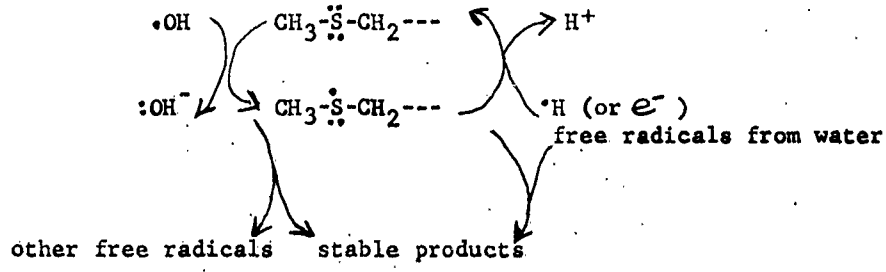
Schields and Gordy (33) reported that the sulfur atom is the site of localization of radiation damage and that electron spin resonance of radiated methionine (solid state, aerobic radiation) was quenched by the presence of oxygen or air. Therefore, it is interesting to compare the radiation stability of oxidized methionine derivatives to that of methionine. The results are shown in Table XII. Among these amino acids, methionine-sulfone is the most stable, methionines sulfoxide is intermediate while methionine is the least stable. The evidence that/the oxygen atom or atoms bound to the sulfur atom in methionine stabilize the whole methionine molecules against radiation damage is in agreement with the theoretical consideration described below. In the case of methionine, two pairs of unshared electrons/are located in the 3s and one of the 3p orbitals of the sulfur, where as methionine-sulfoxide has only one unshared electron pair in the 3s orbital and methionine-sulfone has no unshared electron pair (5 6). The double bonding character between the sulfur and oxygen as in methionine-sulfoxide arises from the $d\pi - p\pi$ bond (overlapping of a ultimate 3d orbital of the sulfur with a 2p orbital of the oxygen) as a result of the electronegativity of those atoms. Geometrically, the sulfur in methionine-sulfoxide bonds with three groups through a pyramidal p^3 bonding rather than a coplaner Sp^2 arrangement. Therefore, one would expect methionine-sulfoxide to be excited more readily than methionine-sulfone, because of the presence of/an unshared electron pair in 3s orbital. However, it would not be as easy as methionine, since/there is no unshared electron pair in the 3p orbital and the positive charge on the sulfur in methionine-sulfoxide would increase the energy required to excite an electron from the 3s orbital. The excitation energy (3p $\longrightarrow$ 3d for methionine and 3s $\longrightarrow$ 3d for methionine-sulfoxide, but the latter case is less possible) would come mainly from excited water molecules (and some of the free radicals) produced from radiolysis of water. Then some of the excited molecules would react with the free radicals from water and would consequently degrade into more stable molecules and/or other free radicals and the resting excited molecules would go back to the original molecules (see Example 1). On the other hand, some free radicals from water would subtract an electron directly from the unshared 3p orbital of methionine and also from the 3s orbital of methionine-sulfoxide (but the latter case is less possible), and the formed sulfur free radicals would stabilize by the presence of the vacant 3d orbitals. The sulfur free radicals would react with some free radicals from water and would form stable products and/or the initial molecules by abstraction of an electron (See Example 2 and 3). However, in the case of methionine-sulfone, the above reactions would not be the case, because there is no unshared electron pair on the sulfur. The generalized reaction sequences of methionine are summarized as shown.



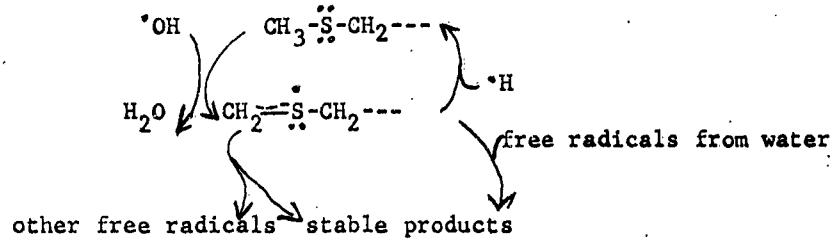
Example 1. Activated water scavenger mechanism.



Example 2. Free radical scavenger mechanism I.



Example 3. Free radical scavenger mechanism II.



In contrast to direct measurement of amino acid retained (Table XI and XII), the yield of ∝-amino-n-butyric acid by the scission of CH_3S-, CH_3SO- or CH_3SO_2- from these sulfur amino

acids is a poor criterion of the stability of these amino acids. With a radiation dose of 10^7 rad, methionine was cleaved to form 9 mole % of α -amino-n-butyric acid (maximum yield) but four times as much α-amino-n-butyric acid was formed from methionine-sulfoxide. However, no α-amino-n-butyric acid resulted from radiation of methionine-sulfone. The lower yields of α-amino-n-butyric acid from methionine can be ascribed to the several pathways through which methionine is degraded, whereas these pathways for methionine-sulfoxide are restricted by the electron configuration, electrical charge and/or steric hindrance.

The purpose of studying radiation damage to glycyl-methionine and alanyl-methionine was 3 fold: i) to determine if radiation causes hydrolysis of the peptide bond ii) to test if radiation chemistry of free methionine and methionine bound in the peptide linkage was similar and iii) to examine the competitive mechanism due to differences in the radiation lability of amino acids constituting the peptides. Samples of 0.01M glycyl-methionine and 0.01M alanyl-methionine radiated over a wide range were examined for products formed by paper chromatography. The formed products were as follows:

glycyl-methionine ⟶
- glycine
- methionine
- methionine-sulfoxide and/or -sulfone
- α-amino-n-butyric acid
- glycyl-methionine-sulfoxide and/or -sulfone
- glycyl- α -amino-n-butyric acid

alanyl-methionine ⟶
- alanine
- methionine
- methionine-sulfoxide and/or -sulfone
- α-amino-n-butyric acid
- alanyl-methionine-sulfoxide and/or -sulfone
- alanyl- α-amino-n-butyric acid

However, radiation induced hydrolysis of the peptides is a minor reaction as the products were formed only in trace.

The competitive reaction of amino acids with free-radicals produced from water was investigated using two criteria. As a function of radiation dose, the retention of individual amino acids constituting the peptide and the major products of the radiated peptides were determined. In another series of experiments, mixture of methionine and glycine or alanine were also radiated and the retention of individual amino acids and the major products were determined. The calculated half reduction dose for individual amino acids and the amount of the major products were summarized in Table XIII. The higher D 1/2 values for methionine in the peptides than that in the mixture indicate the influence of neighboring groups on the degradation of methionine. It is interesting to note the high yield of α-amino-n-butyric acid and the low yield of methylmercaptan from the peptides as compared to the mixtures or free methionine. These evidences suggest a difference or distriction in degradation pathways of methionine and, in case of the peptide, methione is mainly degraded to α -amino-n-butyric acid through the sulfoxide form rather than through the simple cleavage of CH_3S- group from methionine. The high D 1/2 values for glycine and alanine in the mixtures is due to the protection, effect by the free methionine, whereas methionine in the peptides has negative protection effect to the adjacent glycine and/or alanine residues. The probable reason is that the peptides would easily excite or radicalize by the presence of methionine, and glycine and alanine, as a part of excited or radicalized peptide molecule, would easily react with free radicals and would degrade into stable products whereas free glycine and alanine would not excite or radicalize so readily. Furthermore, the results suggest that radio-protectors should be free from the substances to be protected.

IV. Decrease of radiation damage to proteins by sulfhydryl protectors.

Amino acid analysis of the proteins radiated in presence of oxygen, nitrogen and/or chemical protectors are given in Tables XIV, XV, XVI. It is apparent that radiation in presence of O_2 increases damage and this is especially pronounced with methionine, cystine, histidine, phenylalanine, serine and threonine. This effect is less severe when the proteins are radiated anaerobically. Differences in the severity of radiation damage in these two cases can be attributed to the nature of free-radicals produced. Radiation of aqueous systems in the presence of oxygen produces more HO• and HOO• free-radicals than with anaerobic conditions. This would cause greater oxidative destruction of the amino acids of the proteins.

Cysteine, AET and MEA offer considerable protection to the radiation labile amino acids. Among the three chemical protectors tested, cysteine seems to be the most effective. Considering these data on amino acid destruction in three proteins, it is apparent that the protective effect is not specific for any particular amino acid. However, the most radiation sensitive amino acid in each protein received greatest protection. For example, in case of cytochrome c the addition of cysteine as a protector increased retention of methionine over the anaerobic conditions by 65%. However, the protection to other radiation labile amino acids, histidine, cystine and phenylalanine is less. With hemoglobin and ovalbumin, addition of cysteine protected methionine to the extent of 50% and 25%, respectively. The non-uniform response obtained with cysteine is also apparent with AET and MEA. These observations indicate that the -SH compounds may be functioning by more than one mechanism in counteracting the effects of ionizing radiation.

Although the amounts of protectors remaining after radiation were not measured, the results reported here indicate the extensive damage to -SH protectors by radiation. This is best illustrated for cysteine in Table XV. In the 0.1% hemoglobin, cystine (and cysteine) is equivalent to 6.2×10^{-5} M, while the concentration of cysteine added is 10^{-3} M (or 5×10^{-4} M cystine). This ten-fold excess of free cystine (as protector) makes it difficult to differentiate it from protein-bound cystine in these radiation experiments. Under anaerobic conditions, cystine of hemoglobin is not destroyed by a radiation dose of 10^6 rad. Therefore, the retention of cystine above 100% would reflect the amount of free-cystine remaining after radiation in samples containing cysteine as protector. However, the retention of cystine in control and radiated samples containing cysteine as protector is the same. This would indicate that free-cysteine reacted competitively with free radicals and thus protected the other radiation labile amino acids of the protein. The radiation chemistry of aqueous solutions of cysteine and cystine has been studied extensively (11,13, 57,58). Markakis and Tappel (18) found that 0.1M cysteine solution (pH 1.55) exposed to γ-radiation (2×10^7 rad) was 86% degraded, indicating the radiation lability of cysteine. Destruction will be greater with a lower concentration of free cystine (5×10^{-4} M) at pH 7. The disappearance of thiol groups and formation of H_2S has been shown to be dependent on pH and concentration of radiated cysteine solution (58).

Increased radiation damage under aerobic conditions has been observed by several workers (59,60,61). Higher yields of peroxides and carbonyls are found when amino acids were radiated aerobically as compared to anaerobic conditions (62). Alexander and Hamilton (63) attribute increased oxidative damage to proteins to HOO• radicals, peroxidation of organic radical and rupture of peptide bond. They reported increased destruction of amino acid residues especially cystine, tyrosine and side chain carboxyls in presence of O_2. Since in our experiments, aqueous solutions of proteins were radiated, the oxidative damage will be mostly due to the nature of free radicals produced (excess of HO• and HOO•).

The role of -SH compounds in decreasing radiation injury has been reviewed extensively (29,30,31,32). These compounds are known to function by different mechanisms. In considering the protection of radiated proteins the following functions seem to be most pertinent: i) induced anaerobiosis, ii) free radical scavenger effects, iii) capacity to form mixed disulfides, and iv) repair of chemical damage.

Induced anaerobiosis is a protective mechanism of great importance in radiated animals. This mechanism should not be operative here because these reaction systems were made anaerobic by repeated evacuation and gassing with N_2. Thus we are concerned mainly with the protection above that are due to anaerobic conditions.

The role of -SH compounds as free-radical scavengers has been supported from studies on the radiation chemistry of these compounds (64,65). In this aqueous system, the protein molecules (hemoglobin, 1.5×10^{-5} M; cytochrome c, 7.6×10^{-5} M; and ovalbumin, 2.3×10^{-5} M) would be encompassed by molecules of -SH protectors (10^{-3} M). The -SH compounds would be thus readily accessible to react with free-radicals produced from water. Also, the avidity with which these compounds combine with free-radicals, protects the most radiation labile amino acids of protein. Thus, there is a competitive mechanism between the chemical protector and the protein molecules to react with free-radicals. Fletcher and Okada (66) observed that addition of cystine, methionine, phenylalanine and histidine, which are the most radiation labile amino acids, competitively retarded the formation of dihydroxyphenylalanine from radiated tyrosine solution. In our experiments, if the free-radical scavenger mechanism was only operative, then, all the radiation labile amino acids would receive protection to the same extent. Results show that all sensitive amino acids are protected and that the increase in percentage of amino acid retained is inversely related to the sensitivity of that amino acid.

Experimental evidence of Pihl et al (67) and of Gordy and Miyagawa (6) favor the theory of mixed disulfide formation with protein molecules. According to this theory, one would expect cystine to receive greater protection than any other amino acid of similar radiation sensitivity. In case of ovalbumin, histidine and cystine have similar percentage retention under anaerobic conditions, yet the protection offered by -SH compounds, to cystine is of higher magnitude than that for histidine. In cytochrome c cystine is more protected than histidine and serine which have similar percentage retention as cystine under anaerobic conditions.

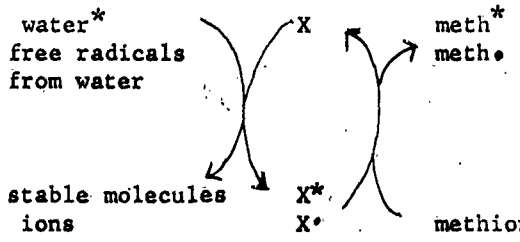
Another important mechanism is the ability of -SH compounds to repair the site of damage. Thus, if an amino acid becomes a free-radical by loss of a hydrogen, it could abstract hydrogen from the chemical protector. This mechanism of repair may apply to all amino acids but it is best known for cysteine which undergoes free radical oxidation-reduction readily.

From these considerations, the general protection of amino acids can be ascribed mainly to free-radical scavenger effects. The greatest protection to cystine is evidence for operation of disulfide formation and/or hydrogen abstraction repair mechanisms.

V. Decrease of radiation damage to amino acids by methionine.

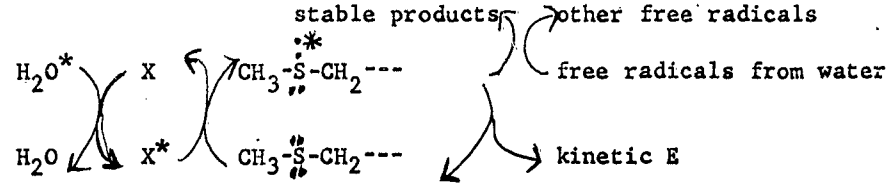
To test the protective effect of methionine to other amino acids, simple binary amino acid solutions (0.001M methionine + 0.01M other amino acid) were radiated over a wide range of radiation dose and the retention of individual amino acids were determined. The calculated half reduction doses are summarized in Table XVII. As the results indicate, methionine offer considerable protection to the secondary amino acids, except to histidine and phenylalanine.

The protection mechanisms by methionine can be ascribed mainly to the scavenger and repair mechanisms. The former mechanism is a competitive reaction between methionine and the secondary amino acids, such as alanine, leucine, arginine and serine, to react with free radicals and excited water molecules. The free radicals from water and excited water molecules would mainly react with methionine rather than with the secondary amino acids, because of the special reactivity of the sulfur atom (see Example 1, 2 and 3). Thus, the secondary amino acids have higher D 1/2 values. The latter mechanism is the ability of methionine to repair the damage site of the secondary amino acids and is summarized as below.

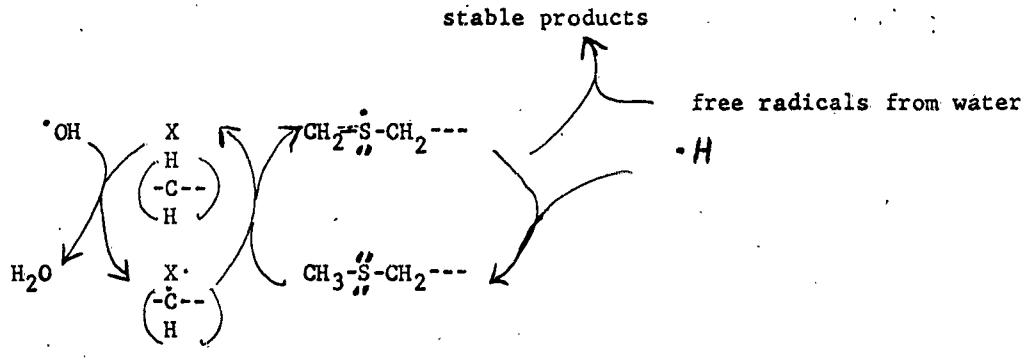


X = secondary amino acid

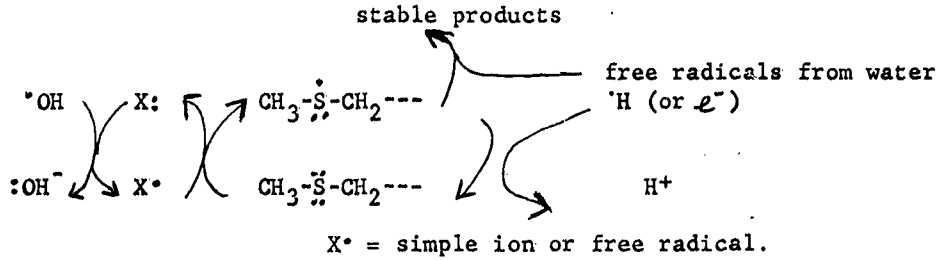
Example 4. Activated molecule repair mechanism



Example 5. Free radical repair mechanism I.



Example 6. Free radical repair mechanism II.



X• = simple ion or free radical.

Thus, when the secondary amino acid becomes a free radical and/or excited molecule, it would abstract a hydrogen atom or an electron form methionine molecule, and also it would transfer the excitation energy to methionine. This mechanism may apply to all secondary amino acids,but it is best known for serine in the mixture.

However, in the case of histidine and phenylalanine, practically no protection by methionine is observed. On the other hand, it is known that these amino acids are the most

radio-labile ones and their D 1/2 values are a little less than that of methionine. Therefore, it may be true that free radicals from water and excited water molecules would evenly react with methionine and histidine or phenylalanine, and intermediate histidine and phenylalanine free radicals would stabilize by their own cyclic structures.

Table 1

Amperometric Titration of Ovalbumin.

Dose in 10^6 rad	moles -SH per mole ovalbumin
0	3.75
0.1	3.2
0.2	2
0.5	0
1.0	0
5	1.6
10	1.4
20	.6
40	0
60	0

TABLE II

Formation of Ammonia, Amide N, and Carbonyls from Ovalbumin

Dose (x 10^6 rad)	Microequivalents per gram protein less control		
	Ammonia	Amide-N	Carbonyl
Control	(100)	(660)	(30)
0.4	50	70	
1	80	170	35
2	70	250	
5	310	850	45
10	600	900	110
25	1400	1100	200

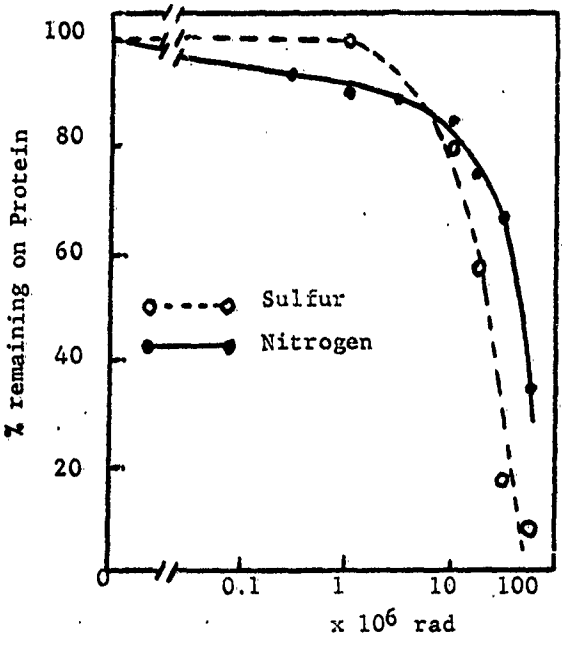


Fig. 1. The loss of sulfur and nitrogen from the TCA-precipitable ovalbumin after radiation.

Table III

Amino acids remaining after γ-radiation of ovalbumin

(as % of 0 rad)

Amino Acids	Radiation dose in rads				
	10^4	10^5	10^6	10^7	5×10^7
Histidine	90	0.0	0.0	0.0	0.0
Cystine	79	67	0.0	0.0	0.0
Methionine	94	73	56	0.0	0.0
Phenylalanine	93	73	58	0.0	0.0
Threonine	74	81	63	0.0	0.0
Leucine	98	95	69	35	0.0
Isoleucine	94	89	72	38	0.0
Tyrosine	114	97	78	0.0	0.0
Serine	97	94	79	65.5	0.0
Arginine	96	87	75	68	0.0
Lysine	95	90	85	71	7.4
Glutamic acid	100	105	97	97	34
Aspartic Acid	99	100	95	102	44
Glycine	95	81	73	87	46
Valine	114	95	84	76	16
Alanine	134	130	126	128	38

Table IV

Fractionation of Scission Products

Sample	Fractions	Radiation dose (rads): 0	10^5	10^6	10^7	3 x 10^7	6 x 10^7
Cytochrome c	Total nitrogen in scission (% of total nitrogen of whole protein)	2	5.5	6.6	7.6	7.8	8.8
	Ammonia (moles of N/mole of protein)[a]	0	2.0	3.5	7.7	10.0	11.5
	Amide (moles of N/mole of protein)	0	2.0	3.5	2.2	3.1	2.4
Hemoglobin	Total nitrogen in scission (% of total nitrogen of whole protein)	0	2.0	15	22	22	29
	Ammonia (moles of N/mole of protein)[b]	0	0	0	0	0	0
	Amide (moles of N/mole of	0	0	7.6	12.9	15.3	21.5

[a] Molecular weight of cytochrome c, 13,200.
[b] Molecular weight of hemoglobin, 67,000.

Table V.

PROPERTIES OF IRRADIATED SOLUTIONS OF CYTOCHROME c AND HEMOGLOBIN

Sample	Properties	Radiation dose (rads): 0	10^5	10^6	10^7	5 x 10^7
Cytochrome c	Chemical reduction (dithionite) (% of 0 rad)	100	100	84	0	0
	Mitochondrial reduction (% of 0 rad)	100	100	68.5	0	0
Hemoglobin	Total methemoglobin + HbO_2 (% of 0 rad)	100	80	68	0	0
	HbO_2 (% of 0 rad)	100	77	60	0	0
	Hb·CO (% of 0 rad)	100	64	55	0	0

Table VI

Distribution of Total Amino Acids in the Fractions of Irradiated Solutions of Cytochrome *c* and Hemoglobin

(Per cent of 0 rad)

Sample	Fraction	Radiation dose (rads): 0	10^5	10^6	10^7	5×10^7
Cytochrome *c*	TCA precipitate	100	63.1	47.0	4.5	7.3
	Scission	0	5.5	14.2	6.5	0.0
	Insoluble protein	0	0	0	41.2	33.7
	Total	100	68.7	61.2	52.2	41.0
Hemoglobin	TCA precipitate	100	88.5	22.7	23.4	15.7
	Scission	0	0	0	11.1	3.0
	Insoluble protein	0	0	60.5	34.8	2.7
	Total	100	88.5	83.2	69.3	21.4

Table VII.

Distribution of Individual Amino Acids in TCA-Precipitate, Scission and Insoluble Fractions of Irradiated Solutions of Cytochrome c (as % of 0 Rad)

Radiation Dose	10^5 rad			10^6 rad			10^7 rad				5×10^7 rad		
Amino acid	TCA ppt	Scission	Total	TCA ppt	Scission	Total	TCA ppt	Scission	Insol. protein	Total	TCA ppt	Insol. Protein	Total
Methionine	30	0.0	30	11	0.0	11	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Histidine	57	4.7	62	43	0.0	43	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cystine	62	5.6	68	53	0.0	53	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Phenylalanine	66	2.1	68	73	0.0	73	3.0	0.2	0.0	3.2	0.0	0.0	0.0
Serine	39	12	51	47	0.0	47	4.1	1.8	28	34	0.0	0.0	0.0
Threonine	38	2.4	40	7.0	0.0	7.0	0.0	0.0	33	33	2.0	26	28
Proline	55	7.8	63	7.0	27	34	2.3	27.5	48	78	6.0	15	21
Leucine	82	8.3	90	83	0.0	83	6.4	1.7	51	59	8.4	26	34
Isoleucine	76	9.6	86	69	0.0	69	4.8	1.8	57	64	5.0	24	29
Valine	91	5.7	97	68	32	100	5.4	2.6	52	60	13	35	48
Tyrosine	68	14	82	75	26	101	3.4	1.5	68	73	14	43	57
Alanine	86	12	98	56	25	81	7.1	3.2	100	110	22	99	121
Glycine	91	9	100	66	31	97	1.6	3.9	75	81	20	65	85
Glutamic acid	45	5	50	46	34	80	11	24	39	74	9	53	62
Aspartic acid	70	8.2	78	51	35	86	12	2.5	50	64	7.3	45	52
Lysine	84	10	94	49	27	76	9.5	1.0	45	56	13	41	54
Arginine	54	4.0	58	53	5	58	2.9	38	39	80	6.5	32	38

Table VIII

Distribution of Individual Amino Acids in TCA-Precipitate, Scission and Insoluble Fractions of Irradiated Solutions of Hemoglobin (as % of 0 Rad)

Radiation Dose	10^5 rad		10^6 rad			10^7 rad				5×10^7 rad			
Amino acid	TCA ppt	Total	TCA ppt	Insol. Protein	Total	TCA ppt	Scission	Insol. Protein	Total	TCA ppt	Scission	Insol. Protein	Total
Methionine	43	43	17	27	44	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Histidine	102	102	7.4	50	57	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Phenylalanine	69	69	11	62	73	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cystine	102	102	28	72	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Threonine	100	100	16	30	46	4.6	0.0	25	30	0.0	0.0	0.0	0.0
Serine	83	83	20	61	81	19	6.6	41	67	18	3.2	2.9	24
Proline	88	88	0.0	88	88	12	0.0	21	33	9.2	1.6	4.7	16
Leucine	62	62	31	73	104	14	0.0	33	47	9.9	0.0	0.0	9.9
Isoleucine	90	90	34	75	109	11	0.0	42	53	11	0.0	0.0	11
Valine	52	52	24	54	78	37	12	24	73	23	1.9	5.5	30
Tyrosine	74	74	0.0	44	44	21	16	25	62	0.0	0.0	0.0	0.0
Alanine	78	78	47	81	128	64	36	72	172	39	6.3	10	5.5
Glycine	106	106	27	64	91	53	51	59	163	52	18	4.3	74
Glutamic acid	53	53	23	47	70	31	25	37	103	24	4.6	4.8	33
Aspartic acid	93	93	34	76	110	65	43	82	190	46	14	12	72
Lysine	100	100	33	76	109	58	0.0	60	118	24	0.0	0.0	24
Arginine	83	83	37	56	93	16	0.0	20	36	0.0	0.0	0.0	0.0

Table IX. Comparison of radio-labilities of amino acids

Amino acid	D 1/2 (rad)	NH_3 formation* (Mg/ml)	G for peroxide** formation
methionine	1.4×10^6	6.6	0.03
phenylalanine	1.1×10^6	---	0.48
tryptophane	14.5×10^6	---	0.22
histidine	2.7×10^6	11.8	0.03
arginine	1.9×10^6	7.5	0.79
lysine	2.5×10^6	3.7	0.34
leucine	3.0×10^6	---	0.41
isoleucine	2.6×10^6	---	----
valine	2.8×10^6	---	0.55
serine	2.0×10^6	---	0.26
glutamic acid	3.4×10^6	---	0.53
aspartic acid	5.6×10^6	---	0.15
alanine	5.4×10^6	7.2	0.53
glycine	6.6×10^6	8.1	0.28

* ammonia yield from 0.13M amino acid solutions after 1.45×10^5 rep radiation (16).

**calculated G values fro peroxide formation from 0.1% amino acid solution (oxygenated pH 7) (41).

Table X. Radiation degradation of sulfur containing amino acids

	amino acid	initial concentration mM.	atmosphere	initial pH	D 1/2 (rad.)
A	methionine	10	N_2	7.0	1.4×10^6
	S-methyl-cysteine	9.5	N_2	7.0	1.5×10^6
B	cysteine	100.	N_2	1.55	6.6×10^6
	"	"	O_2	"	5.7×10^6
	cystine	10.	N_2	1.10	6.0×10^6
	"	"	O_2	"	6.0×10^6

Table XI. Analysis of radiated solutions of 0.01M methionine

dose (rad)	methionine ($M \times 10^4$)	methionine-sulfoxide ($M \times 10^4$)	α-amino-n-butyric acid ($M \times 10^4$)	methylmercaptan ($M \times 10^4$)	hydrogen sulfide ($M \times 10^4$)
0	100	0	0	0	0
1×10^6	55	4.8	4.1	6.8	0.42
3×10^6	23	5.3	8.2	10.3	0.58
10×10^6	0.7	0.6	9.0	12.5	0.81

Table XII. Radiation degradation of methionine-sulfoxide and -sulfone.

dose (rad)	methionine-sulfoxide ($M \times 10^4$)	methionine-sulfone ($M \times 10^4$)
0	100.	100.
1×10^5	98.	99.
1×10^6	83.	94.
1×10^7	13.	66.

Table XIII. Comparative radiation-lability of methionine, glycine and alanine.

amino acids and peptides	half reduction dose, $D_{1/2}$. methionine (rad x 10^{-6})	glycine (rad x 10^{-6})	alanine (rad x 10^{-6})	Products formed: α-amino-n-butyric acid (M x 10^4) 10^6 rad	α-amino-n-butyric acid (M x 10^4) 10^7 rad	methylmercaptan (M x 10^4) 10^6 rad	methylmercaptan (M x 10^4) 10^7 rad	hydrogen sulfide (M x 10^4) 10^6 rad	hydrogen sulfide (M x 10^4) 10^7 rad
glycyl-methionine 0.01M	3.1	5.3	------	5.0	31.	2.1	7.7	0.03	0.80
alanyl-methionine 0.01M	3.1	---	5.3	3.0	33.	1.5	5.7	0.09	0.55
glycine 0.01M + methionine 0.01M	4.4	19.2	----	---	7.0	6.0	12.5	0.20	0.77
alanine 0.01M + methionine 0.01M	4.4	---	19.2	---	7.0	4.8	10.3	0.23	0.89
methionine 0.01 M	1.4	---	---	4.1	9.0	6.8	12.5	0.42	0.81
glycine 0.01M	---	6.6	---	----	----	---	---	---	----
alanine 0.01M	---	---	5.4	---	---	---	----	---	----

Table XIV

Amino acids remaining after γ-irradiation of cytochrome c

Radiation Dose: 10^6 rad. (Calculated as % of 0 rad)

Amino acids	Aerobic	Anaerobic	Chemical protectors used		
			Cysteine	AET	MEA
Methionine	0	19.5	85	55	63
Histidine	25	48	64	50	44
Cystine	37	50	84	79	80
Phenylalanine	46	68	83	74	58
Serine	27	52	50	42	42
Threonine	62	74	86	69	67
Leucine	41	81	105	84	64
Isoleucine	56	87	105	67	63
Valine	95	95	106	83	94
Tyrosine	67	59	65	62	65
Alanine	76	82	88	77	80
Glutamic acid	89	89	93	98	85
Aspartic acid	89	89	100	87	89
Lysine	83	83	88	80	83
Arginine	64	64	109	98	74
Glycine	77	77	83	71	74

Table XV

Amino acids remaining after γ-irradiation of hemoglobin

Radiation Dose: 10^6 rad. (Calculated as % of 0 rad)

Amino acid	Aerobic	Anaerobic	Chemical protectors used		
			Cysteine	AET	MEA
Methionine	24	41	93	66	86
Histidine	35	53	71	71	83
Phenylalanine	49	73	87	76	81
Cystine	78	100	100	99	97
Threonine	39	50	78	89	64
Serine	60	80	85	92	90
Glycine	84	89	97	100	104
Leucine	84	107	100	112	100
Isoleucine	81	84	98	93	100
Valine	75	80	94	83	99
Tyrosine	49	80	85	85	90
Alanine	90	89	102	99	91
Glutamic acid	78	78	97	107	103
Aspartic acid	99	94	101	95	105
Lysine	83	100	107	98	98
Arginine	96	94	105	105	106

Table XVI

Amino acids remaining after γ-irradiation of ovalbumin

Radiation Dose: 10^6 rad. (Calculated as % of 0 rad)

Amino acid	Aerobic	Anaerobic	Chemical protectors used		
			Cysteine	AET	MEA
Histidine	0	7	56	46	52
Cystine	0	5	68	62	64
Methionine	20	54	79	70	73
Phenylalanine	48	60	85	82	81
Threonine	52	65	75	71	77
Leucine	68	71	84	82	80
Isoleucine	71	74	91	90	94
Tyrosine	60	73	89	85	88
Serine	55	77	91	85	94
Arginine	72	74	94	94	97
Lysine	69	81	97	96	97
Glutamic acid	91	92	100	100	100
Aspartic acid	94	91	99	100	100
Glycine	80	78	94	95	99
Valine	84	86	93	97	93
Alanine	114	120	126	120	123

Table XVII. Decrease of radiation damage to amino acids by methionine

System	half reduction dose, $D_{1/2}$ amino acid to be protected ($\times 10^6$ rad)	methionine as protector ($\times 10^6$ rad)
0.01M alanine + 0.01M methionine	44.5	2.3
0.01M leucine + "	22.0	2.6
0.01M arginine + "	21.0	2.0
0.01M histidine + "	3.7	3.0
0.01M serine + "	15.7	1.8
0.01M phenylalanine + "	3.0	3.0
0.02M alanine	8.0	----
0.02M leucine	7.6	----
0.02M arginine	3.6	----
0.02M histidine	3.5	----
0.02M serine	3.6	----
0.02M phenylalanine	3.3	----
0.02M methionine	-----	3.6

REFERENCES

1) R. Sowinski, L. Oharenko, and V. L. Koenig. Rad. Res. 9, 229 (1958).

2) P. Alexander, M.D.G. Hamilton, and K. A. Stacey. Rad. Res. 12, 510 (1960).

3) E.S.G. Barron, S. Dickman, J. A. Muntz, and T. P. Singer. J. Gen. Physiol. 32, 537 (1949).

4) C. R. Maxwell, D. C. Peterson and N. E. Sharpless. Radiation Res. 1, 530 (1954).

5) C. R. Maxwell, D. C. Peterson and W. C. White. Radiation Res. 2, 431 (1955).

6) W. M. Dale, J. V. Davies and C. W. Gilbert. Biochem. J. 45, 93 (1949).

7) E.S.G. Barron. Ann. N. Y. Acad. Sci. 59, 574 (1955).

8) G.R.A. Johnson, G. Scholes and J. Weiss. Science 114, 412 (1951).

9) G.R.A. Johnson, G. Scholes and J. Weiss. J. Chem. Soc. 3091 (1953).

10) W. Gordy. Symposium on Information Theory in Biology pp. 241-261 Pergamon Press. New York (1958).

11) W. M. Dale and J. V. Davies. Biochem. J. 48, 129 (1951).

12) S. L. Whitcher, M. Rotheram and N. Todd. Nucleonics 11, 30 (1953).

13) P. Markakhis and A. L. Tappel. J. Am. Chem. Soc. 82, 1613 (1960).

14) U. S. Kumta, S. U. Gurnani and M. B. Sahasrabudhe. J. Sci. Ind. Res. 15 c, 45 (1956) (India).

15) U. S. Kumta, S. U. Gurnani and M. B. Sahasrabudhe. ibid, 16 c, 25 (1957) (India).

16) J. Kopoldova, A. Kolousek, A. Babicky and J. Liebster. Nature 182, 1074 (1958).

17) M. P. Drake and J. W. Giffee. Radiation preservation of Food, pp. 133-158. U. S. Army Quartermaster Corps (1958).

18) K. S. Ambe, U. S. Kumta and A. L. Tappel. Radiation Res. 15, 709 (1961).

19) U. S. Kumta and A. L. Tappel. Nature 191, 1304 (1961).

20) U. S. Kumta, F. Shimazu and A. L. Tappel. Radiation Res. 16, 679 (1962).

21) D. W. Grant, S. N. Mason and M. A. Link. Nature 192, 352 (1961).

22) L. Duran and A. L. Tappel. Radiation Res. 9, 498 (1958).

23) E. A. Day, R. Bassette and M. Keeney. J. Dairy Sci. 43, 463 (1960).

24) S. Patton. ibid. 37, 446 (1954).

25) S. A. Goldblith and E. L. Wick. Report No. 4, May 1959-April 1960, Quartermaster Food and Container Institute.

26) P. E. Ballance. J. Sci. Food Agr. 12, 532 (1961).

27) G. Hirohashi. Nagasaki Igakkai Zassi 31, 706 (1956) (Japan). Chem. Abst., 51, 6358 i (1957).

28) G. Hirohashi. ibid, 12, 761 (1956).

29) Pihl, A., and Eldjarn, L. Pharmacol. Revs. 10, 437 (1958).

30) Patt, H. M. Fed. Proc. 19, 549 (1960).

31) Gordy, W., and I. Miyagawa. Radiation Res. 12, 211 (1960).

32) Maisin, J. R., and D. G. Doherty. Fed. Proc. 19, 564 (1960).

33) H. Shields and W. Gordy. J. Phys. Chem. 62, 789 (1958).

34) K. S. Ambe and A. L. Tappel. J. Chromatography 5, 546 (1961).

35) our report No. 10, Nov. 15, 1960 - February 14, 1961 Q.M.F.&C.I.

36) our report No. 13, Aug. 15, 1961 - Nov. 14, 1961 Q.M.F.&C.I.

37) E. P. Marback and D. M. Doty. Agr. and Food Chem. 4, 881 (1956).

38) R. A. Slivinski and D. M. Doty. Agr. and Food Chem. 6, 41 (1958).

39) our report No. 3, May 15, 1958 - May 14, 1959 Q.M.F.&C.I.

40) R. J. Romani and A. L. Tappel. Rad. Res. 12, 526 (1960).

41) K. S. Ambe and A. L. Tappel. J. Food Sci. 26, 448 (1961).

42) G. R. Lappin and L. C. Clark. Anal. Chem. 23, 541 (1951).

43) K. Bailey. Biochem. J. 31, 1406 (1939).

44) R. J. Romani and A. L. Tappel. Arch. Biochem. Biophys. 79, 323 (1959).

45) M. P. Drake, J. W. Griffee, D. A. Johnson, and V. L. Koenig. J. Am. Chem. Soc. 79, 1395 (1957).

46) M. E. Jayko and W. M. Garrison. Nature 181, 413 (1958).

47) J. Norworth and C. B. Allsopp. J. Colloid Sci. 11, 565 (1956).

48) C. Vermell and M. Lefort. C. R. Acad. Sci. Paris, 244, 889 (1957).

49) G. G. Jayson, G. Scholes and J. Weiss. Biochem. J. 57, 386 (1954).

50) B. E. Proctor and D. S. Bhatia. Biochem. J. 53, 1 (1953).

51) P. Szendrö. Arch. ges Physiol. (Pflügers) 228, 743 (1931). Chem. Abs. 26, 1862 (1932).

52) F. Ellinger. Strahlentherapie 38, 521 (1930).

53) N. E. Sharpless, A. E. Blair and C. R. Maxwell. Radiation Res. 2, 135 (1954).

54) G. Toennies and J. J. Kolb. J. Biol. Chem. 128, 399 (1939).

55) G. Toennies and T. P. Callan. J. Biol. Chem. 129, 481 (1939).

56) C. C. Price and S. Oae. Sulfur bonding, The Ronald Press Co., New York (1962).

57) M. Rotheram, N. Todd and S. L. Whitcher. Naturwissenschaften 39, 450 (1952).

58) S. L. Whitcher, M. Rotheram and N. Todd. Nucleonics 11, 30 (1953).

59) E. S. Guzman Barron, J. Ambrose and P. Johnson. Radiation Res. 2, 145 (1955).

60) E. S. Guzman Barron and P. Johnson. Radiation Res. 5, 290 (1956).

61) M. E. Jayko and W. M. Garrison. Nature 181, 413 (1958).

62) K. S. Ambe and A. L. Tappel. J. Food Sci. 26, 448 (1961).

63) P. Alexander and L.D.G. Hamilton. Radiation Res. 15, 193 (1961).

64) Z. M. Bacq and P. Alexander. Fundamentals of Radiobiology. Butterworths Scientific Pub. London (1955).

65) B. Shapiro and E. A. Dickins. Radiation Res. 13, 857 (1960).

66) G. L. Fletcher and S. Okada. Radiation Res. 15, 349 (1961).

67) A. Pihl, L. Eldjarn and T. Bremer. J. Biol. Chem. 227, 339 (1957).