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Boyd M. Weeks

Thesis

July 11, 1955
# INDIRECT AND DIRECT ACTION OF HEAVY-PARTICLE RADIATION ON GLYCINE IN AQUEOUS SOLUTION

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## Acknowledgments

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ABSTRACT

Glycine in aqueous solution, when irradiated with 30-Mev cyclotron-accelerated helium ions in the absence of dissolved oxygen, is shown to yield the following degradation products: hydrogen, carbon dioxide, carbon monoxide, ammonia, methylamine, glyoxylic acid, formaldehyde, acetic acid, and formic acid. About half a dozen compounds of greater complexity than the parent glycine are also formed, including: succinic acid and its amino-substituted derivatives, aspartic and diaminosuccinic acids. A method, based on ion-exchange chromatography, is described for the isolation, identification, and determination of the nitrogeneous products.

Product-yield information for various glycine concentrations and radiation doses is presented which, together with published data for the x-ray irradiation of glycine, forms the basis of a proposed mechanism of action.

Three initial net reactions for the direct radiolysis of glycine are proposed. In two of these, the radicals NH₂, CH₂COOH, NH₂CH₂, and COOH are formed. In the third, the stable compounds methylamine and carbon dioxide are formed. NH₂ and CH₂COOH, together with H and OH from the radiolysis of water, remove hydrogen atoms from glycine to form NH₂CHCOOH and CH₂COOH radicals and the stable products, hydrogen, ammonia, and acetic acid. NH₂CHCOOH, CH₂COOH, NH₂CH₂, and COOH combine or disproportionate to form a mixture of combination products plus acetic acid, carbon dioxide, and methylamine, as well as methyleneimine and iminoacetic acid. Hydrolysis of the imino compounds yields ammonia, glyoxylic acid, formaldehyde, and carbon dioxide. Hydrogen peroxide formed in the forward radiolysis of water is removed in a chain oxidation of NH₂CHCOOH, NH₂CH₂, and (or) COOH. An OH radical formed in each such oxidation reacts with glycine to yield another organic radical.

Observed radiation effects on glycine solutions saturated with oxygen are accounted for by this mechanism if it is assumed that initial H and CH₂COOH radicals are removed by oxygen. OH and NH₂ are assumed to oxidize glycine as in the absence of oxygen, but the organic radical formed (NH₂CHCOOH), instead of dimerizing or disproportionating, is oxidized to iminoacetic acid by oxygen with the formation of HO₂. Hydrolysis of the imino compounds yields the final products as in the absence of oxygen. Combination of HO₂ radicals yields hydrogen peroxide and oxygen. The chain oxidation of organic radicals by hydrogen peroxide is blocked by oxygen.
Factors influencing relative and absolute yields—such as glycine concentration, type of radiation, and dose rate—are discussed in detail in terms of several competing reactions. The possible role of excited glycine molecules is discussed.
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I. INTRODUCTION

When ionizing radiation is absorbed by living matter a remarkable series of chemical, physical, and physiological reactions is set in motion. The entire complex process of intermediary metabolism and the dynamic interrelationships of organ systems can become involved. The complexity of biological systems has so far made it impossible to unravel the many processes that lead to observed physiological effects, but a beginning has been made along several lines. The various approaches to the general problem range from physiological studies, involving intact cells or organisms, to purely chemical studies of synthetic systems. An intermediate approach of singular practical importance involves the concept of chemical protection against the injurious effects of ionizing radiation; greater injury usually is observed when oxygen is present. It can be argued that certain chemical agents providing protection against radiation injury do so by reducing the effectiveness of oxygen. It seems obvious that a search for chemical protective agents will be facilitated by improved knowledge of radiation-induced chemical reactions in the presence and absence of oxygen.

Largely on the basis of these considerations an extensive program was initiated at the Crocker Laboratory to study in detail the effects of cyclotron-produced radiations on aqueous solutions of organic acids. It was felt that a precise understanding of radiation-induced chemical reactions in aqueous solutions of biologically important compounds was basic to the entire field of radiation biology. Initial results in studies of acetic and formic acids led to the inauguration of this study of glycine (amino acetic acid) to test some of the proposed processes involving organic free-radical formation that were under investigation at that time.

Moreover, a radiation study of the simplest amino acid appeared to be a logical approach to the broader study of the radiation chemistry of protein, a study that is currently being expanded.
II. INITIAL CONSIDERATIONS

Primary Processes

Radiation energy is transferred to absorber molecules almost entirely by extranuclear interaction. The primary interaction of x- and \( \gamma \)-radiation results in the projection of fast recoil electrons. Each fast recoil electron gives up energy in successive interactions along its path to give individual ion pairs and the slow secondary electrons that are observed as "spurs" in cloud chamber photographs. Observed radiation effects result entirely from these recoil-electron interactions.

The primary interaction of fast charged particles results in individual ion pairs and recoil electrons of average energy comparable to the slow electrons that are responsible for the spur tracts of x- and \( \gamma \)-radiation.

Heavy charged particles lose energy rapidly in a series of frequent interactions in each of which only a small fraction of the total particle energy is given up. Fast electrons lose energy in a manner similar to that for heavy charged particles except that the successive interactions are not as close together.

Differences in the radiation effects of different types of radiation are due entirely to differences in frequency of the low-energy spurs and individual primary ion pairs for each type of radiation. For heavy charged particles spurs and individual ion pairs are formed so close together that the character of the entire primary track resembles that of the spur tracks. The ionization pattern important to radiation-chemical processes in the case of x- and \( \gamma \)-radiation is determined by the fast primary recoil electrons.

Initial Chemical Reactions

The ionization and excitation of absorber molecules along the track initiates the sequence of intermediate chemical processes that lead to observed radiation effects. In water the net initial chemical reactions are termed "radical" and "forward" reactions:

Radical Reaction: \( \text{H}_2\text{O} \rightarrow \text{H} + \text{OH} \)  
Forward Reaction: \( \text{H}_2\text{O} \rightarrow \frac{1}{2} \text{H}_2 + \frac{1}{2} \text{H}_2\text{O}_2 \)

It has been shown that the relative importance of the net radical and forward reactions depends only on the rate of energy loss of the radiation. If the rate of energy loss is high the forward reaction accounts for a larger fraction of the net water-decomposition yield (forward yield plus radical yield) than if the rate of energy loss is low.

In moderate concentration, organic and inorganic solutes capable of reacting with H or OH radicals do not appreciably influence the forward reaction. Regardless of the solute, the forward yield is essentially the same for a given type of radiation. It has therefore been concluded that formation of hydrogen and hydrogen peroxide in the forward reaction takes place by
combination of H and OH radicals respectively in regions of high radical concentration before other reactions can occur. The high momentary local concentrations of H and OH radicals in the tracks of heavy charged particles insure a relatively high proportion of radical combination reactions independent of the presence of a solute. Those H and OH radicals that diffuse out of the track become accessible for reaction with a solute. The radical reaction is thus defined in terms of the net decomposition of water to yield free H and OH radicals accessible for reaction with a solute. The forward reaction, then, is defined in terms of the net decomposition of water to yield H and OH radicals that, because of their high momentary local concentrations, combine respectively to form hydrogen and hydrogen peroxide regardless of the presence of a reactive solute. The importance of water-yielding back reactions under various irradiation conditions is discussed in a later section.

Indirect and Direct Effects

In studies of radiation effects on aqueous solutions terminology is based on the solute. The direct effect is the chemical change induced in the solute as the final result of the absorption of radiation energy directly in the solute molecules, whereas the indirect effect is the chemical change induced in the solute as the final result of the absorption of radiation energy in the solvent. In aqueous solutions the indirect effect results from the reactions of H and OH radicals with the solute.

When organic solute molecules absorb radiation energy directly, the resulting ionization or excitation leads to the formation of intermediates that may be different from those formed by indirect processes. Stable radiolysis products may also be formed directly by molecular rearrangement.

Both indirect and direct effects can involve a variety of interdependent processes in which short-lived intermediates are important.

Radiation Flux

The relative importance of the radical and forward reactions has been discussed in terms of the rates of energy loss for different types of radiation. Another variable that assumes importance in radiation studies involving organic solutes is dose rate or radiation flux. High radical concentrations brought about by high radiation flux permit a sequence of radical reactions different from the sequence that occurs with relatively low radical concentrations. Radicals capable of reacting with solute become available for reaction with one another.

The deliberate use of both high and low dose rates is fruitful in deducing mechanisms. Low dose rates simplify the reaction patterns and make quantitative evaluation of proposed mechanisms more precise. High dose rates involve a greater variety of chemical reactions and products and often provide qualitative evidence for reactions and intermediates that could not be detected otherwise. In practice fast electrons, whether originating in an electron accelerator, β emitter, or by interaction of x- or γ-radiation with the solution, usually combine the characteristically low rate of energy loss per unit distance along the electron track with a low rate of energy
absorption per unit volume in the solution. The radiation field can extend throughout the entire volume of the sample. Cyclotron radiations, on the other hand, usually involve intense, focused beams of energetic atomic nuclei which penetrate only a short distance into the sample. Extremely high dose rates per unit volume are attainable. High rates of energy loss per unit distance along the particle tracks are also characteristic of cyclotron radiations.

Background for this Study

When this study was initiated, the available information on glycine radiolysis was limited. Some quantitative information\(^5\),\(^6\) was available on the deamination of glycine in the presence and absence of oxygen and at various pH values. Hydrogen and aldehyde yields had also been reported.\(^6\) No previous attempt had been made to establish the complete pattern of reactions and resulting products.

Previous work in this laboratory with acetic acid\(^1\)\(^b\) had shown that the major product of indirect action (succinic acid) results from the dimerization of acetic acid radicals formed in the initial solute reactions with H and OH. We thought that reaction of glycine with H and OH radicals could similarly yield one or more analogous intermediate organic free radicals. Combination of these intermediates would lead to corresponding radical combination products. Work was therefore started on the isolation of possible higher-molecular-weight products. About half a dozen such products, most of which contained nitrogen, were isolated, but since the yields were low, attempts at identification were postponed until a preliminary investigation of the major products had been completed. A variety of methods eventually established hydrogen, ammonia, carbon dioxide, glyoxylic acid, formaldehyde, acetic acid, and methylamine as the major radiolysis products. Subsequent work then established that succinic acid and its amino-substituted derivatives, aspartic and diaminosuccinic acids, were among the higher-molecular-weight products. On the basis of these observations, a general mechanism of radiation action on aqueous glycine was formulated that involved several organic free-radical intermediates and both direct and indirect processes.

In the meantime, Maxwell, Peterson, and Sharpless\(^7\) published extensive data for the x-ray irradiation of aqueous, oxygen-free glycine. More recently, Maxwell, Peterson, and White\(^8\) published additional data on the x-ray and electron irradiation of glycine in the presence and absence of oxygen. Their reported spectrum of degradation products is qualitatively identical with that observed in this laboratory for 30 Mev helium ions although the relative and absolute yields are different. They made no attempt to investigate higher-molecular-weight product formation. Their data provided excellent independent support for the mechanism as a whole, and also permitted additional evaluation of the relative importance of separate radiolysis processes.

The mechanism is presented and discussed in the final section of this report. The effects of various radiation conditions—such as type of radiation, dose rate, and the presence and absence of dissolved oxygen—are dealt with in detail.
III. EXPERIMENTAL PROCEDURES

The immediate goal of the investigation reported here was to identify and determine products formed in a series of irradiations of aqueous glycine for which the radiation dose and the constitution of the solutions were accurately controlled.

The experimental procedures fell logically into several categories which are treated separately as follows:

A. Irradiation
   Equipment and procedures
   Dosimetry
B. Target solutions
C. Analytical techniques
   Gaseous products
   Carbonyl products
   Nitrogen-containing products
   Organic acids

Irradiation

Equipment and Procedures

The equipment and procedures employed in the irradiation of glycine solutions for the series reported here have been used in connection with other radiation chemical studies in this laboratory.

The radiation source for all but one of the glycine irradiations was the Crocker Laboratory 60-inch cyclotron. Helium ions were accelerated.

Target solutions were irradiated in all-glass cells external to the cyclotron vacuum. The cyclotron beam passed through an assembly attached to the cyclotron "snout" that provided: (1) a duralumin window through which the beam left the cyclotron vacuum, (2) a collimating aperture to reduce the diameter of the incident beam to about 1 cm, (3) suitable connections and geometry for monitoring the helium ion current absorbed by the solution, (4) a shutter for timing the exposure, (5) a support for the target cell, (6) an aluminum foil pack through which the beam passed to adjust the ion energy to ~30 Mev incident on the solution. The entire assembly has been described previously.\(^1\)

Two types of all-glass target cell\(^1 \text{d}\) were used for the cyclotron-irradiations. Cells of one type were used to irradiate 100-ml to 120-ml volumes of unlabeled glycine solution in vacuo. They were made from ordinary 200-ml Erlenmeyer flasks; the tops were sealed off, side arms for evacuation and gas sampling were attached, and thinned glass windows were drawn in from the sides. Before irradiation each cell was evacuated by means of a conventional mechanical vacuum pump through a dry ice trap,
and then sealed off. The evacuation process was continued, with agitation of the cell contents, until gas evolution ceased and the liquid made a sharp clicking sound against the inside of the cell when shaken. About 3 ml of water was distilled out of the solution in this operation. The solution was irradiated through the thinned glass window. A jig was constructed to shake the cell along the axis of the cyclotron beam at a rate of about 10 vibrations per second with an amplitude of 1.5 cm. After irradiation gaseous products that had accumulated in the evacuated space above the liquid were drawn off through a break seal in one of the side arms, measured by means of a gas burette, and sampled for subsequent analysis. Again approximately 3 ml of water was lost during the gas-sampling operation. Cells of the other type were used to irradiate 10-ml and 50-ml target volumes in which $^{14}$C tracers were used. They were tubular in shape, were open at the top, and were fitted with a glass frit at the bottom. The beam passed through a thinned glass window that was drawn in from the side. No attempt was made to collect product gases. The target solutions were agitated and kept free of oxygen by a stream of helium bubbled through the frit.

The one irradiation that did not involve cyclotron-accelerated helium ions was made with a $^{60}$Co $\gamma$-ray source. Samples of solution were sealed (in vacuo) into glass ampules. Three 5-ml ampules were irradiated in succession. They were then broken open, and the solutions were combined and analyzed.

**Dosimetry**

Ultimate yield information obtained depends equally on the accuracy of the radiation dose measurement and that of product determination. A dose-determination method was developed that is now used routinely for other radiation chemical studies, and which we believe is capable of accuracy to within $\pm 1\%$ of the absolute value.

Radiation dose was calculated from the integrated ion current absorbed by the target and from the average energy of the bombarding ions. The ion energy has been determined periodically by measuring total particle range in aluminum and then calculating the energy from theoretical range-energy relationships. **Correction was made for energy degradation caused by the several aluminum foils, air spaces, and the glass target-cell window. The cell-window thicknesses were individually measured with a calibrated microscope. Uncertainties in dose measurement arising from electrical pickup, secondary ionization currents, possible skewed distribution of ion energy about the median energy measured, possible day-to-day drift of average energy due to shifting cyclotron operating conditions, etc., were all painstakingly reduced to a minimum. Details of these practical considerations have been described previously.**

* The cobalt source was made available through the courtesy of the Bio-Organic Chemistry group of the University of California Radiation Laboratory.

** We are indebted to the Scatter Group of the University of California Physics Department for most of these range measurements and for use of range-measuring equipment.
To check the success of efforts to reduce error, a calorimeter was constructed to provide an absolute measurement of radiation dose. The calorimeter consisted of a solid copper sphere 2 in. in diameter in a highly evacuated glass vessel. Geometry electrically identical with that of the standard target assembly for the irradiation of solutions was maintained where appropriate. The beam passed through a 0.001-in. platinum window in the glass vessel and struck the copper sphere at the bottom of a milled slot. The walls of the slot trapped secondary electrons emitted at the site of beam incidence. Imbedded in dental amalgam* (for good thermal contact) in a hole drilled in the sphere, was a Western Electric Thermistor, type 14B, to monitor temperature changes. The Thermistor is a sensitive resistance thermometer with which, by means of a Wheatstone bridge, one can detect a 0.001°C temperature change. Also imbedded in dental amalgam in a hole drilled through the center of the sphere was a small electric heating element made from a 5-watt wire-wound resistor with all the outside insulation stripped off. A thin coat of Sauereisen (a porcelain-like insulating material) insulated the bare wires of the heater element from the copper sphere. Twenty watts of electrical power could be dissipated in this 5-watt resistor (mounted in the copper sphere) without causing a temperature-induced change in heater resistance greater than 0.2% of the "cold" resistance. This heating element was used to calibrate the thermal behavior of the intact calorimeter assembly. Comparison between measured beam and known electrical work necessary to produce a given change in temperature (Thermistor resistance) in the calorimeter could then be made.

The average discrepancy for several such comparisons was less than 1% on each of three different days, several months apart, on which these comparisons were made. The impracticability of making frequent range measurements somewhat impairs the realization of this precision, but a long-term study of the reproducibility of dose-yield measurements with the Crocker Laboratory cyclotron has shown that between range measurements average ion energy can be predicted with less than 3% error. The details of the calorimeter and its use have been described previously. 12

For the one experiment employing Co 60 γ-rays, dose was approximately determined from the known geometry of the source and its previous calibration against a ceric sulfate actinometer. Approximate product yields were then calculated on the assumption that ammonia yield for the conditions employed was 4 molecules/100 ev absorbed in the solution. The glycine concentration was 1 M.

Target Solutions

The purity of the target solutions was extremely important because radiation product was determined in concentration as low as 10⁻⁵ M. It was necessary to eliminate the possibility of confusing impurity or radiation product of impurity with radiation product of glycine.

Purification methods based on ion-exchange chromatography, crystallization from water and organic solvents, and sublimation in vacuo

* Assistance in the preparation of dental amalgams was given by Dr. Hollis Jean Hay, D. D. S.
were investigated. The method finally adopted and standardized for the preparation of relatively large amounts of unlabeled glycine was simply to crystallize commercially available glycine five or six times from doubly distilled water. All commercial glycine available to us showed a slight yellow tint in solution and contained one or more ninhydrin-positive contaminants. Nutritional Biochemicals Corporation glycine was used as the starting material in all but the $^{14}C$-labeled glycine preparations. After three or four crystallizations from water the glycine showed no suggestion of color in hot, saturated solutions or in the crystals. Single crystals, more than one-half inch long, commonly formed in the final crystallization steps. Approximately 75 g glycine were dissolved per 100 ml water (almost boiling); then, after cooling to room temperature, the solution was placed in the refrigerator overnight for each crystallization step. Final criteria of purity were established by subjecting unirradiated glycine to all the analytical procedures used for the actual targets. No impurities were detected in the final preparations.

For targets that incorporated a $^{14}C$ label, purification procedures were modified to improve recovery and to utilize glycine previously irradiated. The sample of labeled glycine was first chromatographed on a cation-exchange column by a method identical with that used in the analysis of irradiated targets (described later), and the glycine was recovered as the hydrochloride. The salt was then added to a short cation-exchange column (Dowex-50, hydrogen form, 0.8 by 20 cm) and eluted with 1 M ammonium hydroxide. The free glycine was obtained by drying the effluent in a warming oven at ~70°C. Carrier glycine, prepared as already described by crystallization from water, was then added, if necessary, to provide a little more than needed for irradiation. Because of the appreciable solubility of Dowex-50 resin in the eluant solutions, the glycine (containing both label and carrier) was then precipitated from a concentrated water solution by addition of methanol. The precipitate was washed with cold methanol and dried at ~70°C. Labeled glycine target preparations were irradiated within one week after purification in this manner.

The water used in all targets and preparative procedures was distilled once in a conventional Barnstead still and a second time in an all-Pyrex system from alkaline permanganate.

**Analytical Techniques**

**Gaseous Products**

After irradiation the gaseous products were measured and sampled as already described and then analyzed mass spectrographically.** Carbon dioxide, carbon monoxide, and hydrogen were the only gases found in measurable amounts.

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* $^{14}C$-labeled glycine was obtained through the courtesy of the Bio-Organic Chemistry Group of the University of California Radiation Laboratory.

** Mass spectrographic analysis of the gaseous products was made possible through the courtesy of Dr. Amos Newton.
Carbonyl Products

Carbonyl products, as 2, 4-dinitrophenylhydrazone derivatives, were separated chromatographically on paper by a method previously adapted from a method of Friedemann and Haugen. The hydrazones were formed by adding 2, 4-dinitrophenylhydrazine in acid solution to an acidified sample of the irradiated glycine solution. The mixture was warmed to about 50°C for 15 minutes and extracted with chloroform or carbon tetrachloride. Samples of the extract were dried in spots onto Whatman No. 1 filter paper along with similar samples from various glycine-carbonyl control mixtures. The chromatograms were developed with a 75% butanol--10% ethanol--15% water mixture. Color of the hydrazones was deepened for visual inspection by spraying the finished chromatograms with methanolic potassium hydroxide. The color and position of the hydrazone spots from irradiated glycine and simultaneously chromatographed controls indicated that formaldehyde and glyoxylic acid were the only carbonyl products formed.

Carbonyl products were determined colorimetrically by a method previously adapted from a method of Lappin and Clark. In a manner similar to that employed for the separation process, 2, 4-dinitrophenylhydrazone derivatives were formed in the glycine target solution. Then, without extraction of the hydrazones, methanolic potassium hydroxide was added to intensify the color in the solution. Total carbonyl yield was obtained by comparing the color photometrically with the color similarly formed in unirradiated controls containing glycine and known amounts of formaldehyde. Although the extinction coefficients for various carbonyl hydrazones differ, the differences are not great. Since formaldehyde was the major carbonyl product in most of the solutions tested, it was a suitable standard for the determinations made.

Glyoxylic acid product in irradiated glycine solutions was found to decompose completely in several days if no precautions for its preservation were taken. The total carbonyl determinations, just described, were therefore applied to glycine solutions at least one week after irradiation for formaldehyde determinations. The difference between a determination made immediately after irradiation and another made one week or more after irradiation was taken to be the glyoxylic acid yield. Present chromatographic methods were not available for independent glyoxylic acid and formaldehyde determinations at the time the glycine series were run.

Nitrogen-containing Products

The entire spectrum of nitrogen products was examined in a chromatographic procedure developed for the purpose from an early method of Stein and Moore. All of the nitrogen products plus the parent glycine were taken up on a column of Dowex-50 cation-exchange resin (sulfonic acid type) and individually eluted with controlled concentrations of hydrochloric acid. The column and associated equipment were conventional. Dowex-50 resin (hydrogen form, 200 to 400 mesh, 8% cross-linked) was used. After thorough washing in water by flotation, the resin was poured into the column in a water slurry such that after settling, it formed a column about 100 cm high by 0.8 cm in diameter. The column was washed with 1 to 2 liters of
4 N hydrochloric acid and then with water before the sample was added. The sample of irradiated glycine solution was poured gently onto the top of the column and allowed to drain slowly into the resin bed. The organic acid and neutral products were all washed out of the column with water and collected for future analysis before elution with hydrochloric acid was begun. The nitrogen products and parent glycine were retained at the top of the column. Hydrochloric acid in progressively increasing concentration was then used to elute the nitrogen products and glycine successively from the column.

Initially, stepwise increases in eluant concentration were employed, but it was found that improved resolution and increased elution rates could be obtained by continuously increasing the hydrochloric acid concentration as elution proceeded. Eluant was supplied to the column from a mixing reservoir, initially containing pure water but fed at a steady rate with hydrochloric acid from a second reservoir. A magnetic stirrer insured complete mixing. A flow rate of about 15 ml/hr for this column produced highest resolution. Faster flow rates were feasible for cruder separations. The desired flow rate was obtained by adjusting the air pressure in the hydrochloric acid reservoir or by adjusting its height relative to the delivery tip of the column.

Effluent was collected in small samples in beakers or counting dishes by means of an automatic fraction collector and dried in a gentle stream of warmed air. The hydrochloride salts of the isolated products were thus obtained. To locate the individual product peaks with respect to effluent volume, 2 ml of 0.001 N sodium hydroxide was added to each dried sample, and the pH was measured. Wherever product salt was present a lower pH was observed and the product location became immediately apparent. The samples of each peak were then combined. Nonvolatile product salts were titrated directly with sodium hydroxide. The volatile bases (ammonia and methylamine) were distilled from sodium hydroxide and titrated with acid. The organic acid fraction that was washed out of the column with water was titrated directly with sodium hydroxide without prior drying down. Complete titration curves were plotted for each product titrated separately. These curves aided in the identification procedures. Figure 1 shows a typical elution curve obtained when an irradiated target containing \( \text{NH}_2\text{C}^{14}\text{H}_2\text{COOH} \) is chromatographed in this way. Product activity is plotted against effluent volume. Overlapping of the diaminosuccinic acid and methylamine peaks is discussed later.

The principal nitrogen products identified were ammonia, methylamine, aspartic acid, and diaminosuccinic acid. Traces of ethylenediamine are also believed to have been formed. Ammonia was identified by its volatility, odor, lack of carbon, acid-base characteristics, chromatographic behavior compared with authentic ammonia, and by a positive Nessler reaction. Methylamine was identified by its volatility, acid-base characteristics, chromatographic behavior compared with authentic methylamine, mass-spectrographic spectrum, odor, and a positive color test based on the reaction of primary amines with lactose. Experiments using a \( ^{14}C \) label in both positions in the parent glycine showed that methylamine originates in the methyl end of the glycine molecule. Aspartic and diaminosuccinic
Fig. 1. Typical elution curve of nitrogen-containing products formed in the helium-ion irradiation of 0.25M glycine solution containing NH$_2$C$^{14}$H$_2$COOH. Standard Dowex-50 column procedure was used as described in text. Upper curve shows product activity plotted against effluent volume. Lower curve shows calculated HCl concentration in the eluant solution.

* Diaminosuccinic acid is eluted in two fractions, presumably the racemic and meso isomers; see text.
acids were identified by chromatographing C\textsuperscript{14}-labeled product simultaneously with authentic carrier so that product activity and carrier titre could be plotted individually on the same elution curve (Fig. 2). Exact correspondence of activity and titre were observed in both cases. Both these products also showed ninhydrin reactions identical in color with those of the authentic amino acids. Nutritional Biochemicals Corporation aspartic acid was used for the aspartic acid identification. Diaminosuccinic acid, synthesized for us by the Pilot Chemicals Corporation, Waltham, Mass., was used for the diaminosuccinic acid identification.

The diaminosuccinic acid product appears to be variably composed of two fractions. Properties of diaminosuccinic acid have been described\textsuperscript{19} and interconversion of the isomeric forms has been shown to take place readily. The meso and racemic forms appear to be separated completely by the chromatographic method employed here. Apparently the racemic forms are eluted with or just ahead of methylamine. The meso form is eluted after methylamine. The distribution of product between meso and racemic forms is variable and apparently depends, at least partially, upon postirradiation conditions and procedures. No thorough study of this problem has been attempted. When diaminosuccinic acid and methylamine product peaks overlap in the separation process, separate determination is possible because of the volatility of methylamine.

As can be seen in Fig. 1, several nitrogen products still await identification. The possible identity of Product I is discussed later.

Glycylglycine does not appear as a product under the irradiation conditions employed in the study reported here. It is separately eluted between aspartic acid and glycine, and corresponds to none of the product peaks so far observed when irradiated glycine solutions are chromatographed. Possible mechanisms for the radiation synthesis of the peptide bond are discussed briefly in a later section.

**Organic Acids**

When irradiated glycine solution was added to the top of the cation-exchange column, the organic acid products went through with little or no retention by the resin and were collected in a single fraction before elution with acid was begun. This fraction was titrated directly with sodium hydroxide to obtain the total non-nitrogen organic acid yield. The organic acids were separated chromatographically on silicic acid by a method adapted in this laboratory\textsuperscript{1b} from a method of Marvel and Rands.\textsuperscript{20} Formic, acetic, and succinic acids were identified. Cochromatograms of C\textsuperscript{14}-labeled product and added authentic carrier are shown in Fig. 3. Mass-spectrographic confirmation of acetic acid product has also been obtained. Appropriate volatility and acid-base characteristics of these products were observed. Glyoxylic acid product can be collected with the other organic acids in the water effluent fraction, but was not determined separately. Identification and determination of glyoxylic acid has already been discussed in connection with its properties as a carbonyl compound.
Fig. 2. Co-elution curves of authentic aspartic and diaminosuccinic acids with $^{14}C$-labeled product formed in the helium-ion irradiation of $^{14}C$-labeled glycine. Standard Dowex-50 column procedure was used as described in text.
Fig. 3. Co-elution curves of authentic acetic, formic, and succinic acids with C\textsuperscript{14}-labeled product formed in the helium-ion irradiation of C\textsuperscript{14}-labeled glycine. Chromatographic procedure involved the use of silicic acid columns as described in Ref. 1b, 20.
IV. RESULTS

In this investigation of the effects of helium-ion irradiation of glycine in aqueous solution, positive evidence was obtained for the following products: hydrogen, hydrogen peroxide, carbon dioxide, carbon monoxide, ammonia, methylamine, ethylenediamine, formaldehyde, and: formic, acetic, glyoxylic, succinic, aspartic, and diaminosuccinic acids. Several other nitrogen-containing compounds were isolated but have not yet been identified. Product yields obtained with helium ions under various irradiation conditions are given in Tables I and II. Some of the tabulated data are shown graphically in the next section in connection with a discussion of radiolysis processes.

Product yields obtained in the one γ-ray experiment are given in Table Ia.

Irradiation of glycine in which methyl and carboxy carbon atoms were successively replaced with C\textsuperscript{14} showed that methylamine, formic acid, and formic acid originate in the methyl position.

The carbon-hydrogen bond in glycine is a semi-labile bond; exchange of hydrogen at this bond with the hydrogen of water is slow.\textsuperscript{21} The other hydrogen atoms of glycine exchange freely with the hydrogen atoms of water. Glycine dissolved in D\textsubscript{2}O thus becomes ND\textsubscript{2}CH\textsubscript{2}COOD. Observation of product HD in concentration greater than that calculated on the basis of the equilibrium mixture of H and D in the water after the glycine is dissolved would be evidence for the abstraction of carbon-bonded hydrogen atoms of glycine by H radicals. (See discussion of Reactions 6a-b in the following section.) Relative yields of product D\textsubscript{2}, HD, and H\textsubscript{2} resulting from the helium-ion irradiation of 0.25 M, oxygen-free glycine dissolved in D\textsubscript{2}O were determined. The H-D ratio in the solvent D\textsubscript{2}O was also determined after the glycine was dissolved. The equilibrium distribution of D\textsubscript{2}, HD, and H\textsubscript{2} was calculated for this H-D ratio and compared with the observed D\textsubscript{2}, HD, and H\textsubscript{2} product yields. From this comparison it was evident that less than 5% of the product hydrogen contained hydrogen originally bonded to carbon in the glycine molecule.
### Table I

Product yields as a function of glycine concentration: Yield: number of product molecules formed per 100 ev absorbed in the solution. Radiation: 30 Mev helium ions. Nominal dose: $1 \times 10^{22}$ ev for 100 ml target volumes. Dose rate: $7 \times 10^{19}$ ev/sec. Instantaneous irradiated volume: $\sim 0.05$ cm$^3$. Data are for oxygen-free solutions.

<table>
<thead>
<tr>
<th>Product</th>
<th>Glycine Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0625 M</td>
</tr>
<tr>
<td>$\text{H}_2$</td>
<td>0.89, 0.90</td>
</tr>
<tr>
<td></td>
<td>0.98</td>
</tr>
<tr>
<td>Total volatile base</td>
<td>0.47</td>
</tr>
<tr>
<td>NH$_3$</td>
<td>0.66</td>
</tr>
<tr>
<td>NH$_2$CH$_3$</td>
<td>0.066</td>
</tr>
<tr>
<td>CO$_2$</td>
<td>0.19, 0.22</td>
</tr>
<tr>
<td></td>
<td>0.22</td>
</tr>
<tr>
<td>CO</td>
<td>0.007, 0.01</td>
</tr>
<tr>
<td></td>
<td>0.008</td>
</tr>
<tr>
<td>HCHO</td>
<td></td>
</tr>
<tr>
<td>HCOCOOH</td>
<td></td>
</tr>
<tr>
<td>Total organic acid*</td>
<td>0.23</td>
</tr>
<tr>
<td>CH$_3$COOH</td>
<td>0.06, 0.04</td>
</tr>
<tr>
<td>HCOOH</td>
<td>0.021, 0.020</td>
</tr>
<tr>
<td>(CH$_2$COOH)$_2$</td>
<td>0.03, 0.02</td>
</tr>
<tr>
<td>COOHCHNH$_2$-CH$_2$COOH</td>
<td>0.02, 0.06</td>
</tr>
<tr>
<td>(NH$_2$CHCOOH)$_2$</td>
<td>0.02, 0.02</td>
</tr>
<tr>
<td>(NH$_2$CH$_2$)$_2$</td>
<td>~0.01</td>
</tr>
<tr>
<td>Unidentified I</td>
<td>~0.02</td>
</tr>
<tr>
<td>Unidentified III</td>
<td>0.02, 0.04</td>
</tr>
<tr>
<td></td>
<td>0.03, 0.04</td>
</tr>
<tr>
<td>$\text{H}_2\text{O}_2$</td>
<td>~0.1</td>
</tr>
</tbody>
</table>

* Carboxyl groups/100 ev calculated from titre of water effluent fraction from ion exchange column. See Page 16.
Table Ia

Product yields for 1M glycine irradiated with Co\textsuperscript{60} γ-rays. Yield: number of product molecules formed per 100 ev absorbed in the solution. Yields are calculated on the assumption that ammonia yield was 4 molecules/100 ev. Dose: \(\sim 1.6 \times 10^{21}\) ev for 13.8 ml of target solution. Dose rate: \(\sim 6 \times 10^{15}\) ev/sec. Instantaneous irradiated volume: \(\sim 3\) ml. Data are for oxygen-solutions.

<table>
<thead>
<tr>
<th>Product</th>
<th>Yield (molecules/100 ev)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH\textsubscript{3}</td>
<td>4 (assumed)</td>
</tr>
<tr>
<td>NH\textsubscript{2}CH\textsubscript{3}</td>
<td>0.36</td>
</tr>
<tr>
<td>Total organic acids*</td>
<td>2.7</td>
</tr>
<tr>
<td>Unidentified I**</td>
<td>0.31-0.62</td>
</tr>
<tr>
<td>Unidentified III</td>
<td>0</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>0.23</td>
</tr>
<tr>
<td>Diaminosuccinic acid</td>
<td>0.07</td>
</tr>
</tbody>
</table>

* Carboxyl groups/100 ev, calculated from titre of water effluent fraction from ion-exchange column. See Page 16. (This yield value is undoubtedly low owing to the decomposition of glyoxylic acid during the 3 days that elapsed between the beginning of irradiation and analysis on the cation-exchange column.)

** Yield is calculated from titre of hydrochloride salt. Lower figure is based on the assumption that the product salt is in the form of the dihydrochloride. Higher figure assumes the monohydrochloride form.
Table II

Product yields as a function of radiation dose: Yield: number of product molecules formed per 100 ev absorbed in the solution. Radiation: 30 Mev helium ions. Dose is for 100 ml target volumes. Dose rate: $2 \times 10^{19}$ ev/sec. Instantaneous irradiated volume: $\sim 0.05 \text{ cm}^3$. Glycine concentration: 0.25 M. Data are for oxygen-free solutions.

<table>
<thead>
<tr>
<th>Product</th>
<th>Nominal dose (ev/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$0.33 \times 10^{22}$</td>
</tr>
<tr>
<td></td>
<td>H$_2$</td>
</tr>
<tr>
<td></td>
<td>0.99, 0.74</td>
</tr>
<tr>
<td></td>
<td>0.97</td>
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<tr>
<td></td>
<td>Total volatile base</td>
</tr>
<tr>
<td></td>
<td>NH$_2$CH$_3$</td>
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<tr>
<td></td>
<td>CO$_2$</td>
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<tr>
<td></td>
<td>CO</td>
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<td></td>
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<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HCHO</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total organic acid*</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CH$_3$COOH</td>
</tr>
<tr>
<td></td>
<td>HCOOH</td>
</tr>
<tr>
<td></td>
<td>(CH$_2$COOH)$_2$</td>
</tr>
<tr>
<td></td>
<td>COOHCHNH$_2$-CH$_2$COOH</td>
</tr>
<tr>
<td></td>
<td>H$_2$O$_2$</td>
</tr>
</tbody>
</table>

* Carboxyl groups/100 ev calculated from titre of water effluent fraction from ion exchange column. See Page 16.
V. DISCUSSION

It was indicated in the early pages of this report that certain types of free-radical processes were anticipated when the glycine study was initiated. The experimental results just presented provide evidence for several organic radical intermediates and permit a more-or-less complete mechanism of radiation action on aqueous glycine to be formulated. Such a mechanism is proposed in the following discussion. First, the complete sequence of proposed reactions is briefly presented. Then the separate processes, together with substantiating evidence, are discussed in detail.

The mechanism as given is built on the reactions of five intermediate organic radicals. The observation of succinic, aspartic, and diaminosuccinic acids is taken as evidence for the organic radicals \( \text{NH}_2\text{CHOOH} \) and \( \text{CH}_2\text{COOH} \). The radicals \( \text{NH}_2, \text{NH}_2\text{CH}_2, \) and \( \text{COOH} \) are postulated on the basis of observed product yields obtained from this study as well as from the published work of others.\(^5-8\) Observed effects of dissolved oxygen on net product yields are fully accounted for by the proposed reactions.

Reactions in the Absence of Oxygen

The primary interaction of radiation with water molecules has already been discussed in terms of the radical and forward reactions:

Radical Reaction  \( \text{H}_2\text{O} \rightarrow \text{H} + \text{OH} \)  \( (1) \)

Forward Reaction  \( \rightarrow \frac{1}{2}\text{H}_2 + \frac{1}{2}\text{H}_2\text{O}_2 \)  \( (2) \)

We propose that the initial direct reactions resulting from the primary interaction of radiation with glycine molecules in aqueous solution involves the rupture of the \( \text{N}-\text{C} \) and \( \text{C}-\text{C} \) bonds as follows:

Initial Direct Reactions: \( \text{NH}_2\text{CH}_2\text{COOH} \rightarrow \text{NH}_2 + \text{CH}_2\text{COOH} \)  \( (3) \)

\( \rightarrow \text{NH}_2\text{CH}_2 + \text{COOH} \)  \( (4) \)

\( \rightarrow \text{NH}_2\text{CH}_3 + \text{CO}_2 \)  \( (5) \)

Of the products formed in Reactions (1) to (5) above, only the radicals \( \text{H}, \text{OH}, \text{NH}_2, \) and \( \text{CH}_2\text{COOH} \) are assumed to react with glycine:

\( \text{H} + \text{NH}_2\text{CH}_2\text{COOH} \rightarrow \text{H}_2 + \text{NH}_2\text{CHCOOH} \) (or \( \text{NHCH}_2\text{COOH} \))  \( (6a) \)

\( \rightarrow \text{NH}_3 + \text{CH}_2\text{COOH} \)  \( (6b) \)

\( \text{OH} + \text{NH}_2\text{CH}_2\text{COOH} \rightarrow \text{H}_2\text{O} + \text{NH}_2\text{CHCOOH} \)  \( (7) \)

\( \text{NH}_2 + \text{NH}_2\text{CH}_2\text{COOH} \rightarrow \text{NH}_3 + \text{NH}_2\text{CHCOOH} \)  \( (8) \)

\( \text{CH}_2\text{COOH} + \text{NH}_2\text{CH}_2\text{COOH} \rightarrow \text{CH}_3\text{COOH} + \text{NH}_2\text{CHCOOH} \)  \( (9) \)
NH$_2$CHCOOH can be oxidized by hydrogen peroxide as can also the radicals NH$_2$CH$_2$ and COOH formed in Reaction (4):

\[
\begin{align*}
H_2O_2 + NH_2CHCOOH &\rightarrow NH=CHCOOH + H_2O + OH \quad (10) \\
H_2O_2 + NH_2CH_2 &\rightarrow NH=CH_2 + H_2O + OH \quad (11) \\
H_2O_2 + COOH &\rightarrow CO_2 + H_2O + OH \quad (12)
\end{align*}
\]

The OH radical formed for each hydrogen peroxide molecule consumed reacts with glycine as in Eq. (7) yielding another MH$_2$CHCOOH radical. Thus, no net decrease in organic radicals results from this catalytic removal of hydrogen peroxide.

In the absence of other oxidizing species, the remaining radicals (including some CH$_2$COOH) can enter into a variety of combination and disproportionation reactions as follows:

\[
\begin{align*}
2NH_2CHCOOH &\rightarrow NH=CHCOOH + NH_2CH_2COOH \quad (13a) \\
\text{combination product(s)} &\quad (diaminosuccinic acid) \quad (13b) \\
NH_2CHCOOH + CH_2COOH &\rightarrow NH=CHCOOH + CH_3COOH \quad (14a) \\
\text{combination product(s)} &\quad (aspartic acid) \quad (14b) \\
NH_2CHCOOH + NH_2CH_2 &\rightarrow NH=CHCOOH + NH_2CH_3 \quad (15a) \\
\rightarrow NH=CH_2 + NH_2CH_2COOH \quad (15b) \\
\rightarrow \text{combination product(s)} \quad (15c) \\
NH_2CHCOOH + COOH &\rightarrow CO_2 + NH_2CH_2COOH \quad (16a) \\
\rightarrow \text{combination product(s)} \quad (16b) \\
2CH_2COOH &\rightarrow (CH_2COOH)_2 \quad (17) \\
CH_2COOH + NH_2CH_2 &\rightarrow NH=CH_2 + CH_3COOH \quad (18a) \\
\rightarrow \text{combination product(s)} \quad (18b) \\
CH_2COOH + COOH &\rightarrow CO_2 + CH_3COOH \quad (19a) \\
\rightarrow COOHCH_2COOH \quad (19b) \\
2NH_2CH_2 &\rightarrow NH=CH_2 + NH_2CH_3 \quad (20a) \\
\rightarrow \text{combination product(s)} \quad (ethylenediamine) \quad (20b)
\end{align*}
\]
The relative importance of the individual combination and disproportionation reactions listed above depends on many factors and is discussed later.

The two reactive imino compounds, NH=CHCOOH and NH=CH₂, formed by radical disproportionation and by reaction of hydrogen peroxide with organic radicals, hydrolize to form ammonia, aldehyde, and carbon dioxide:

\[
\begin{align*}
\text{NH}=\text{CHCOOH} + \text{H}_2\text{O} & \rightarrow \text{NH}_3 + \text{HCOOCH}_2 \quad (23a) \\
& \rightarrow \text{NH}_3 + \text{HCHO} + \text{CO}_2 \quad (23b) \\
\text{NH}=\text{CH}_2 + \text{H}_2\text{O} & \rightarrow \text{NH}_3 + \text{HCHO} \quad (24)
\end{align*}
\]

The above series of reactions describing the proposed mechanism for the radiolysis of glycine in oxygen-free aqueous solution can be thought of as proceeding in several stages as follows:

**Initial Direct Reactions**

The direct absorption of radiation energy by the glycine molecules results in the rupture of the N-C and C-C bonds according to proposed Reactions (3) through (5). Rupture of the N-C bond--Eq. (3)--yields NH₂ and CH₂COOH radicals. Rupture of the C-C bond yields NH₂CH₂ and COOH radicals--Eq. (4)--or the molecular rearrangement products, methylamine and carbon dioxide--Eq. (5)--.

**Radical-solute Reactions**

The proposed reactions of solute glycine with H, OH, NH₂, and CH₂COOH radicals--Eqs. (6) through (9)--yield the radical NH₂CHCOOH (or NHCH₂COOH which is discussed later) and the stable products hydrogen, ammonia, and acetic acid.

**Radical-radical Reactions**

In the absence of oxygen it is proposed that NH₂CH₂, CH₂COOH, NH₂CHCOOH, and COOH radicals are long-lived and eventually react with one another (CH₂COOH reacts with glycine also). They can either combine or disproportionate--Eqs. (13) through (22)--. Combination results in a variety of compounds some of which have been isolated and identified as products of irradiation (succinic, aspartic, diaminosuccinic acids, and ethylenediamine). Disproportionation yields acetic acid, methylamine, carbon dioxide, and the reactive imino compounds, iminoacetic acid and methyleneimine.

* Formic acid, although a potential product of radical disproportionation reactions, is apparently formed in other ways; its formation is discussed later.
Radical-hydrogen peroxide Reactions

Hydrogen peroxide is observed as a product in only trace amounts from oxygen-free glycine solutions. Removal of hydrogen peroxide formed in the forward reaction—Eq. (2)—can proceed as a chain oxidation of organic radicals—Eqs. (10) through (12)—. NH₂CHCOOH, NH₂CH₂, and COOH are each potentially able to react with hydrogen peroxide to yield iminoacetic acid, methyleneimine, and carbon dioxide. An OH radical is also formed in each case which, upon reaction with solute glycine—Eq. (7)—, provides a replacement (NH₂CHCOOH) for the organic radical consumed.

Hydrolysis of Imino Products

Ammonia, glyoxylic acid, formaldehyde, and carbon dioxide result from the spontaneous hydrolysis of iminoacetic acid and methyleneimine.

Reactions in the Presence of Oxygen

If, during irradiation, the glycine solution is saturated with oxygen, all organic and inorganic radicals capable of reacting with oxygen are removed from the reaction sequence just described. H and CH₂COOH have been shown to react with oxygen as follows:

\[ \text{H} + \text{O}_2 \rightarrow \text{HO}_2 \]  \hspace{1cm} (25)

\[ \text{CH}_2\text{COOH} + \text{O}_2 \rightarrow \text{O}_2\text{CH}_2\text{COOH} \rightarrow \text{products} \]  \hspace{1cm} (including HCOCOOH)

\[ \text{See Ref. 1d-e.} \]  \hspace{1cm} (26)

NH₂CHCOOH, NH₂CH₂, and COOH are oxidized by oxygen as follows:

\[ \text{NH}_2\text{CHCOOH} + \text{O}_2 \rightarrow \text{NH=CHCOOH} + \text{HO}_2 \]  \hspace{1cm} (27)

\[ \text{NH}_2\text{CH}_2 + \text{O}_2 \rightarrow \text{NH=CH}_2 + \text{HO}_2 \]  \hspace{1cm} (28)

\[ \text{COOH} + \text{O}_2 \rightarrow \text{CO}_2 + \text{HO}_2. \]  \hspace{1cm} (29)

As in the absence of oxygen, the imino products are hydrolyzed to ammonia, aldehyde, and carbon dioxide. Reaction of NH₂ with glycine—Eq. (8)—is assumed to be unaffected by oxygen. An alternative to this assumption is discussed later. HO₂ radicals react to form hydrogen peroxide:

\[ 2\text{HO}_2 \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \]  \hspace{1cm} (30)

Product Evidence

The most extensive qualitative evidence in support of the mechanism just presented is found in the helium-ion data given in the preceding section of this report. Degradation products indicated by the mechanism (ammonia, glyoxylic acid, formaldehyde, acetic acid, carbon dioxide, methylamine, and hydrogen) were observed. In addition, the observation of the combination products succinic, aspartic, diaminosuccinic acids, and ethylenediamine provides evidence for the proposed intermediate organic radicals NH₂CHCOOH, CH₂COOH, and NH₂CH₂. Reactions (3) and (4) are postulated to account for
their initial formation. Moreover, specific product evidence for Reaction (3) (NH₂CH₂COOH −−→ NH₂ + CH₂COOH) is found in the observation that ammonia⁵,²² and acetic acid²² are formed in the radiolysis of dry glycine crystals.

Although methylamine may be formed in part from NH₂CH₂ radicals, a consideration of product stoichiometry indicates that at least a portion must be formed in a direct reaction independent of the reactions of intermediates or of the presence or absence of oxygen. Reaction (5) (NH₂CH₃COOH −−→ NH₂CH₃ + CO₂) is postulated on this basis. Product stoichiometry is discussed in detail later.

Formic acid is indicated as a possible product in the disproportionation of COOH radicals—Eq. (23)—but arises principally from the methyl carbon of glycine. It has been shown²³,¹⁰ that formic acid can be at least partially accounted for by reaction of glyoxylic acid product with hydrogen peroxide:

\[
\text{HCOCOOH + H₂O₂} \rightarrow \text{HCOOH + CO₂ + H₂O.} \tag{31}
\]

Mass spectrographic²⁴ and radiation chemical¹⁶ data for various carboxyl compounds indicate that carbon monoxide can arise directly from processes involving high-energy rupture of two bonds simultaneously.

Product evidence for the proposed sequence of reactions initiated by OH oxidation of glycine is also found in experiments employing Fenton's Reagent²⁵ in which the major products from glycine (ammonia, glyoxylic acid, formic acid, and formaldehyde) are formed in sufficiently high yield to indicate a chain oxidation of organic radicals by hydrogen peroxide.

The product spectrum reported by Maxwell, Peterson, and Sharpless⁷ in the x-ray irradiation of aqueous oxygen-free glycine is in complete agreement with that reported here.

**Product Stoichiometry**

It can be shown that the product yields obtained in this study with cyclotron-accelerated helium ions conform to the requirements of the proposed mechanism if account is taken of various competing reactions (discussed later) that assume importance under the irradiation conditions employed. Independent quantitative support for the mechanism is found, however, in the data reported by Maxwell, Peterson, and Sharpless⁷ and Maxwell, Peterson, and White.⁸ Their data, obtained with 50-kv x-rays for both oxygen-free and oxygen-saturated solutions, is considered in the following discussion of product stoichiometry. It can be assumed that under the irradiation conditions they employed, the separate steps given in the mechanism are quantitative, and that the influence of additional competing reactions is negligible.

The relative importance of the various reactions in the mechanism just presented is indicated in Tables III and IV for oxygen-free and oxygen-saturated solutions respectively. Table V gives the individual product yields derived from the reaction yields in Tables III and IV. R and F denote the
yields of the radical and forward reactions respectively and express for each reaction the number of water molecules decomposed per 100 ev radiation energy absorbed in water. Similarly, \( d_1, d_2, \) and \( d_3 \) express for the initial direct reactions -- Eqs. (3) through (5), respectively -- the number of glycine molecules decomposed per 100 ev radiation energy absorbed in glycine. Of the total number of H radicals reacting with glycine, \( f_1 \) is the fraction yielding hydrogen -- Eq. (6a) -- and \((1-f_1)\) is the fraction yielding ammonia -- Eq. (6b). Of the total number of organic radicals entering combination or disproportionation reactions, \( f_2 \) is the fraction disproportionating and \((1-f_2)\) is the fraction combining.

The initial yield of formic acid in both oxygen-free and oxygen-saturated systems is small and can be ignored in this treatment of product stoichiometry. Although accounted for earlier by reaction of glyoxylic acid and hydrogen peroxide -- Eq. (31) --, formic acid derived from the methyl position can also be formed in other ways, for example, by radical oxidation of glyoxylic acid. Its exact role in product stoichiometry is not yet fully understood.

Calculated and Observed Product Yields for 50-kv x-Rays

To make actual comparison of calculated and experimentally determined product yields, numerical values must be assigned to the various "constants" in Tables III through V. \( R \) and \( F \) have been measured for several different kinds of radiation under various conditions. Hart\(^6\) gives 3.0 and 0.88 as the most likely values for \( R \) and \( F \) respectively for Co\(^{60} \) \( \gamma \)-rays. He gives 2.35 and 1.02 for \( R \) and \( F \) respectively for tritium \( \beta \) particles. Maxwell, Peterson, and White\(^8\) have shown that initial product yields from glycine solutions irradiated with 50-kv x-rays are not appreciably different from initial yields similarly obtained with 160-kev electrons. It is thus impossible to assign precise independently determined values to \( R \) and \( F \) in order to predict product yields for irradiations with 50-kv x-rays. It can be argued, however, that the forward and radical yields for 50-kv x-rays would be expected to lie between the corresponding values reported for high-energy \( \gamma \)-radiation and tritium \( \beta \) particles.

Numerical values for \( R, d_1, f_1, \) and \( f_2 \) can be calculated by equating observed ammonia, hydrogen, and acetic acid-plus-combined-CH\(_2\)COOH yields in Table VI to the yield expressions for the same products in Table V. The linear increase of ammonia yield with respect to glycine concentration (Fig. 4) is assumed to be due entirely to Reaction (3) (\( \text{NH}_2\text{CH}_2\text{COOH} \rightarrow \text{NH}_2 + \text{CH}_2\text{COOH} \)). \( F \) is taken equal to 0.9 molecules of water decomposed via the forward reaction per 100 ev absorbed in the water. Solution of the resulting simultaneous equations gives:

\[
R = 2.5 \text{ water molecules decomposed via the radical reaction per 100 ev absorbed in water (H}_2\text{O} \rightarrow \text{H} + \text{OH}).
\]

\[
d_1 = 9.3 \text{ glycine molecules decomposed via Reaction (3) per 100 ev absorbed in glycine (NH}_2\text{CH}_2\text{COOH} \rightarrow \text{NH}_2 + \text{CH}_2\text{COOH}).
\]

\[
f_1 = 0.69 = \frac{\text{number of H radicals reacting with glycine to form hydrogen}}{\text{total number of H radicals reacting with glycine}} \quad \text{-- Eq. (6a-b)}
\]

\[
f_2 = 0.72 = \frac{\text{number of organic radicals disproportionating}}{\text{total number of organic radicals interacting}} \quad \text{-- Eqs. (13-22)}
\]
Table III

Reaction yields in the radiolysis of oxygen-free glycine solutions

<table>
<thead>
<tr>
<th>Reaction number</th>
<th>Reaction</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>6a</td>
<td>H+NH₂CH₂COOH → H₂+NH₂CHCOOH</td>
<td>f₁R</td>
</tr>
<tr>
<td>6b</td>
<td></td>
<td>(1-f₁)R</td>
</tr>
<tr>
<td>7</td>
<td>OH+NH₂CH₂COOH → H₂O+NH₂CHCOOH</td>
<td>R+(1/2)F</td>
</tr>
<tr>
<td>8</td>
<td>NH₂+NH₂CH₂COOH → NH₃+NH₂CHCOOH</td>
<td>d₁</td>
</tr>
<tr>
<td>9</td>
<td>CH₂COOH+NH₂CH₂COOH → CH₃COOH+NH₂CHCOOH</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>H₂O₂+NH₂CHCOOH → NH=CHCOOH+H₂O+OH⁺</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>H₂O₂+NH₂CH₂ → NH=CH₂+H₂O+OH</td>
<td>(1/2)F</td>
</tr>
<tr>
<td>12</td>
<td>H₂O₂+COOH → CO₂+H₂O+OH</td>
<td></td>
</tr>
<tr>
<td>13-22</td>
<td>Radical disproportionation</td>
<td>f₂(R+d₁+d₂)</td>
</tr>
<tr>
<td></td>
<td>Radical combination</td>
<td>(1-f₂)(R+d₁+d₂)</td>
</tr>
<tr>
<td>23a</td>
<td>NH=CHCOOH + H₂O → NH₃+HCOOCH</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>f₂(R+d₁)+(1/2)F</td>
</tr>
<tr>
<td>24</td>
<td>NH=CH₂+H₂O → NH₃+HCHO</td>
<td></td>
</tr>
</tbody>
</table>

R = number of water molecules decomposed via Reaction (1) per 100 ev absorbed in water (H₂O→H + OH)  
F = number of water molecules decomposed via Reaction (2) per 100 ev absorbed in water (H₂O→(1/2)H₂ + (1/2)H₂O₂)  
d₁ = number of glycine molecules decomposed via Reaction (3) per 100 ev absorbed in glycine (NH₂CH₂COOH→NH₂ + CH₂COOH)  
d₂ = number of glycine molecules decomposed via Reaction (4) per 100 ev absorbed in glycine (NH₂CH₂COOH→NH₂CH₂ + COOH)  
f₁ = number of H radicals reacting with glycine to form hydrogen  
f₂ = total number of H radicals reacting with glycine  
f₂ = number of organic radicals disproportionating  
        total number of organic radicals interacting
Table IV

Reaction yields in the radiolysis of oxygen-saturated glycine solutions

<table>
<thead>
<tr>
<th>Reaction number</th>
<th>Reaction</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>$H + O_2 \rightarrow HO_2$</td>
<td>R</td>
</tr>
<tr>
<td>7</td>
<td>$OH + NH_2CH_2COOH \rightarrow H_2O + NH_2CHCOOH$</td>
<td>R</td>
</tr>
<tr>
<td>8</td>
<td>$NH_2 + NH_2CH_2COOH \rightarrow NH_3 + NH_2CHCOOH$</td>
<td>$d_1$</td>
</tr>
<tr>
<td>26</td>
<td>$CH_2COOH + O_2 \rightarrow O_2CH_2COOH \rightarrow$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>\hspace*{1cm} products including $NH_3 + HCHO + CO_2$</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>$NH_2CHCOOH + O_2 \rightarrow NH=CHCOOH + HO_2$</td>
<td>$R + d_1$</td>
</tr>
<tr>
<td>28</td>
<td>$NH_2CH_2 + O_2 \rightarrow NH=CH_2 + HO_2$</td>
<td>$d_2$</td>
</tr>
<tr>
<td>29</td>
<td>$COOH + O_2 \rightarrow CO_2 + HO_2$</td>
<td>$d_2$</td>
</tr>
<tr>
<td>23a</td>
<td>$NH=CHCOOH + H_2O \rightarrow NH_3 + CHOCOOH$</td>
<td>$R + d_1$</td>
</tr>
<tr>
<td>23b</td>
<td>\hspace*{1cm} $\rightarrow NH_3 + HCHO + CO_2$</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>$NH=CH_2 + H_2O \rightarrow NH_3 + HCHO$</td>
<td>$d_2$</td>
</tr>
<tr>
<td>30</td>
<td>$2H_2O \rightarrow H_2O_2 + O_2$</td>
<td>$R + (1/2)d_1 + d_2$</td>
</tr>
</tbody>
</table>

$R, F, d_1, d_2, f_1,$ and $f_2$ as defined in Table III
Table V
Product yields in the radiolysis of aqueous glycine

<table>
<thead>
<tr>
<th>Product</th>
<th>Oxygen-free</th>
<th>Oxygen-saturated</th>
</tr>
</thead>
<tbody>
<tr>
<td>$H_2$</td>
<td>$f_1 R + (1/2)F$</td>
<td>$(1/2)F$</td>
</tr>
<tr>
<td>$NH_3$</td>
<td>$(1-f_1 + f_2)R + (1/2)F + (1+f_2)d_1$</td>
<td>$R + 2d_1 + d_2$</td>
</tr>
<tr>
<td>Combination products</td>
<td>$(1-f_2)(R+d_1)$</td>
<td>0</td>
</tr>
<tr>
<td>$CH_3COOH +$ combined $CH_2COOH$</td>
<td>$(1-f_1)R + d_1$</td>
<td>0</td>
</tr>
<tr>
<td>HCOCOOH + HCHO</td>
<td>$f_2 R + (1/2)F + f_2 d_1$</td>
<td>$R + 2d_1 + d_2$</td>
</tr>
<tr>
<td>$H_2O_2$</td>
<td>0</td>
<td>$R + (1/2)F + (1/2)d_1 + d_2$</td>
</tr>
</tbody>
</table>

$R$, $F$, $d_1$, $d_2$, $f_1$, and $f_2$ as defined in Table III
### Table VI

<table>
<thead>
<tr>
<th>Product</th>
<th>Yield (molecules/100 ev absorbed in the solution)</th>
<th>Oxygen-free</th>
<th>Oxygen-saturated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calculated</td>
<td>Observed</td>
<td>Calculated</td>
</tr>
<tr>
<td>H₂</td>
<td>2.02</td>
<td>2.02</td>
<td>0.45</td>
</tr>
<tr>
<td>NH₃</td>
<td>3.97</td>
<td>3.97</td>
<td>4.01</td>
</tr>
<tr>
<td>CH₃COOH + combined CH₂COOH</td>
<td>1.4</td>
<td>1.4 **</td>
<td>0</td>
</tr>
<tr>
<td>HCHO + HCOOCH</td>
<td>2.59</td>
<td>2.63</td>
<td>3.4 - 4.0 †</td>
</tr>
<tr>
<td>Combination products</td>
<td>0.8</td>
<td>0.6 - 1.2</td>
<td>0</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>0</td>
<td>0</td>
<td>3.4</td>
</tr>
</tbody>
</table>

R = 2.5 water molecules decomposed via Reaction (1) per 100 ev absorbed in water (H₂O $\rightarrow$ H+OH).

F = 0.9 water molecules decomposed via Reaction (2) per 100 ev absorbed in water (H₂O $\rightarrow$ (1/2)H₂+(1/2)H₂O₂).

d₁ = 9.3 glycine molecules decomposed via Reaction (3) per 100 ev absorbed in glycine (NH₂CH₂COOH $\rightarrow$ NH₂+CH₂COOH).

\[ d₂ = \sim 4 \text{ glycine molecules decomposed via Reaction (4) per 100 ev absorbed in glycine (NH₂CH₂COOH $\rightarrow$ NH₂CH₂ +COOH).} \]

\[ f₁ = \frac{0.69}{\text{total number of } H \text{ radicals reacting with glycine}} \]

\[ f₂ = \frac{0.72}{\text{total number of organic radicals interacting}} \]

* Yields in parentheses are the observed yields and were used in the calculation of R, d₁, f₁, and f₂ as described in text.

** Includes the yield of aspartic acid (no. of molecules/100 ev) as determined in this laboratory from one experiment in which Co⁶⁰ gamma rays were used.

† Value depends on yield of glyoxylic acid from O₂CH₂COOH that is formed because of Reaction(4). Glyoxylic acid is probably the principal product, and the higher value indicated is favored.
Fig. 4. Ammonia yield from oxygen-free solution as a function of glycine concentration. $G = \text{molecules ammonia formed per } 100 \text{ ev absorbed in the solution}$. Upper curve is for 50-kv x-rays as reported by Maxwell, Peterson, and Sharpless. Lower curve is for 30-Mev helium ions and is obtained from data in Table I.
Formaldehyde yields, obtained with cyclotron helium ions (see Tables I and II and Fig. 5), are taken as a measure of $d_2$. For reasons that are discussed later, formaldehyde formation via indirect processes is small compared with formation via direct processes at the higher glycine concentrations for the irradiation conditions employed. Methylamine and possible combination products involving the NH$_2$CH$_2$ radical are ignored because their yields resulting from Reaction (4) (NH$_2$CH$_2$COOH $\rightarrow$ NH$_2$CH$_2$ + COOH) are small as well as uncertain. Accordingly, $d_2 = \sim 4$ glycine molecules decomposed via Reaction (4) per 100 ev absorbed in glycine.

Table VI compares observed product yields from 1M glycine solutions (irradiated with 50-kv x-rays in the presence and absence of oxygen) with yields calculated from the expressions in Table V by means of the above values for $R$, $F$, $d_1$, $d_2$, $f_1$, and $f_2$. It is seen that there is virtually complete internal agreement between the calculated and observed yield patterns. If the same values for the initial reaction yields and the fractions $f_1$ and $f_2$ are used for other glycine concentrations, a linear relationship with respect to glycine concentration is obtained for calculated ammonia yield which coincides with the experimental curve above 0.5M (Fig. 6). Below this concentration the observed yield falls below the calculated yield because of competing reactions.

A substantial portion of the available data for oxygen-free systems was used for the estimation of the initial reaction yields and the fractions $f_1$ and $f_2$. Independent substantiation of the mechanism is found, however, in the agreement between calculated and observed yields for total carbonyl products and total radical-combination products from oxygen-free solutions, and for all observed product yields from oxygen-saturated solutions. The calculated value of $R$ compares appropriately with values determined independently for other types of radiation.

The Oxygen Effect

The presence of dissolved oxygen in the glycine solutions during irradiation has a profound effect on the radiolysis processes. A comparison of reactions and product yields in the presence and absence of oxygen has been made already (Tables III through VI). The initial consequences of adding oxygen to glycine solutions during irradiation were described as the blocking of three groups of reactions: H and CH$_2$COOH reaction with glycine, radical disproportionation and combination reactions, and the chain oxidation of organic radicals by hydrogen peroxide. Accordingly: formation of acetic acid and combination products is prevented; hydrogen peroxide becomes a major product; carbonyl product formation is increased; and hydrogen formation is reduced to that resulting from the forward reaction. Ammonia yield is singularly unaffected for 1M glycine solutions. This absence of an observed oxygen effect on ammonia yield is, however, the result of several pronounced effects that tend to cancel one another. From a consideration of the reaction yields given in Tables III and IV, the influence of oxygen on the different ammonia-yielding processes can be treated separately.
Fig. 5. Formaldehyde yield from oxygen-free solution as a function of glycine concentration. $G$ = molecules formaldehyde formed per 100 ev absorbed in the solution. Curve is obtained from data in Table I for 30-Mev helium ions.
Fig. 6. Predicted ammonia yields as a function of glycine concentration. 
G = molecules ammonia formed per 100 ev absorbed in the solution. 
Calculations are based on irradiation conditions as given in Table VI. 
(50-keV x-rays, R = 2.5, F = 0.9, d_1 = 9.3, d_2 = 4, f_1 = 0.69, and 
f_2 = 0.72.)
In the absence of oxygen, ammonia yield from Reaction (3) is \((1+f_2)cd_1\) ammonia molecules per 100 ev absorbed in the solution. Of the total amount of radiation energy dissipated in the solution, \(c\) is the fraction absorbed by glycine and \((1-c)\) is the fraction absorbed by water. In practice \(c\) can be assumed equal to the glycine fraction by weight in the solution because the mass stopping powers of water and glycine are very nearly equal. For every molecule of glycine initially decomposed in Reaction (3) one molecule of ammonia results immediately from the reaction of \(\text{NH}_2\) with glycine. One more ammonia molecule (or combination product) ultimately results from reactions of \(\text{NH}_2\text{CHCOOH}\) (formed by reaction of \(\text{NH}_2\) with glycine) and initial \(\text{CH}_2\text{COOH}\).

In the presence of oxygen the ammonia yield from Reaction (3) is \(2cd_1\) ammonia molecules per 100 ev absorbed in the solution. Again, one molecule of ammonia and one \(\text{NH}_2\text{CHCOOH}\) radical result from the reaction of \(\text{NH}_2\) with glycine. The \(\text{NH}_2\text{CHCOOH}\) radical ultimately yields another molecule of ammonia upon oxidation to the imino acid by oxygen. \(\text{CH}_2\text{COOH}\) is removed by reaction with oxygen without the formation of ammonia.

Initial Reaction (3): \(\text{NH}_2\text{CH}_2\text{COOH} \rightarrow \text{NH}_2 + \text{CH}_2\text{COOH}\)

In the absence of oxygen, reactions of \(\text{NH}_2\text{CH}_2\) and \(\text{COOH}\) radicals from Reaction (4) have virtually no net effect on total ammonia yield. Ammonia formation via hydrolysis of methyleneimine is largely compensated by the addition of hydrogen to organic radicals by both \(\text{NH}_2\text{CH}_2\) and \(\text{COOH}\). In the presence of oxygen, however, no such compensation occurs. All \(\text{NH}_2\text{CH}_2\) radicals are oxidized to the imine by oxygen. The ammonia yield from Reaction (4) is, then, \(cd_2\) ammonia molecules per 100 ev absorbed in the solution.

Initial Reaction (4): \(\text{NH}_2\text{CH}_2\text{COOH} \rightarrow \text{NH}_2\text{CH}_2 + \text{COOH}\)

In the absence of oxygen every \(\text{H}\) and \(\text{OH}\) radical reacts with glycine to yield \(\text{NH}_2\text{CHCOOH}\) or \(\text{CH}_2\text{COOH}\). Subsequent reactions of these organic radicals ultimately lead to ammonia via disproportionation, or to combination products. Disproportionation yields one molecule of ammonia for every two organic radicals disproportionating. Of the organic radicals reacting with one another \(f_2\) is the fraction disproportionating. Another molecule of ammonia results from \(\text{H}\)-radical deamination of glycine. Of the \(\text{H}\) radicals reacting with glycine, \((1-f_1)\) is the fraction yielding ammonia and \(\text{CH}_2\text{COOH}\). Therefore the yield of ammonia from the radical reaction is \((1-f_1+f_2)(1-c)R\) ammonia molecules/100 ev absorbed in the solution. As it turns out, \(f_1\) and \(f_2\) are approximately equal for the x-ray experiments discussed. Thus, in the absence of oxygen, for every pair of \(\text{H}\) and \(\text{OH}\) radicals formed in the radical reaction, approximately one molecule of ammonia is formed.

In the presence of oxygen little difference in net yield is observed, although the formation process is quite different. All \(\text{H}\) radicals are removed as \(\text{HO}_2\). \(\text{OH}\) reacts with glycine to form \(\text{NH}_2\text{CHCOOH}\), which quantitatively
yields ammonia via oxidation to the imino acid by oxygen. The net ammonia yield, then, from the radical reaction in oxygen-saturated solutions is \((1-c)R\) ammonia molecules/100 ev absorbed in the solution. The yield is exactly one molecule of ammonia per radical pair produced in the radical reaction.

\[
\text{Forward Reaction: } \text{H}_2\text{O} \rightarrow (1/2)\text{H}_2 + (1/2)\text{H}_2\text{O}_2
\]

In the absence of oxygen every molecule of hydrogen peroxide oxidizes an organic radical (predominantly \(\text{NH}_2\text{CHCOOH}\)). Both an imino compound and an OH radical are formed. The OH radical, by reaction with glycine, replaces the organic radical removed. The imino compound yields ammonia. Ammonia yield, then, from the forward reaction is \(1/2(1-c)F\) ammonia molecules per 100 ev absorbed in the solution (the forward yield of hydrogen peroxide). In the presence of sufficient oxygen the chain oxidation of organic radicals by hydrogen peroxide is blocked and no ammonia results from the forward reaction.

**Net Direct and Indirect Effects**

Yields for the separate ammonia-forming processes can be combined to obtain net ammonia yields for the direct and indirect effects. The yields for indirect formation of ammonia in the absence and presence of oxygen are \((1-f_1+f_2)(1-c)R+1/2(1-c)F\) and \((1-c)R\) respectively. The direct ammonia yields, in the absence and presence of oxygen, are \((1+f_2)d_1\) and \(2d_1+cd_2\), respectively. Adding oxygen to the solution decreases the indirect formation of ammonia by an amount approximately equal to the forward yield of hydrogen peroxide. Simultaneously the direct formation of ammonia is increased by an amount equal to the yield of Reaction (4) plus \((1-f_2)\) times the yield of Reaction (3). The total net oxygen effect on ammonia formation thus depends on glycine concentration. At low glycine concentration ammonia yield is less for oxygen-saturated solutions than for oxygen-free solutions. For high glycine concentrations, ammonia yield is higher for oxygen-saturated solutions. At some intermediate concentration, ammonia yield must be the same for oxygen-free and oxygen-saturated solutions. In any case the net oxygen effect is not large. Figure 6 shows predicted ammonia yields plotted against glycine concentration for oxygen-free and oxygen-saturated solutions. Calculations are based on irradiation conditions as given in Table VI (50-kv x-rays\(^7\),\(^8\)). The same values of \(R\), \(F\), \(d_1\), \(d_2\), \(f_1\), and \(f_2\) are used. The calculated yield curves correspond to observed yields only between approximately 0.5 and 3.0 M glycine concentration. Below 0.5 M, competing reactions can cause actual ammonia yield to fall below the calculated value. If the curves are extrapolated to zero glycine concentration the absolute indirect ammonia yields are obtained as molecules ammonia formed via indirect action per 100 ev absorbed in water. Observed ammonia yields\(^7\) for glycine concentrations between 0.5 and 2 M fall on the calculated curve for the oxygen-free case. No study of ammonia yield with respect to glycine concentration has yet been reported for oxygen-saturated solutions.

**Effect of Oxygen in Small Amount**

Maxwell, Peterson, and White\(^8\) have observed a 30% increase in volatile base yield over the oxygen-free value for 1 M glycine solutions in
which oxygen partial pressure is 5 mm Hg. For oxygen-saturated solutions the ammonia yield is about equal to the value for oxygen-free solutions. At low oxygen pressure, radical combination and disproportionation is apparently blocked before H-radical reaction with glycine or organic-radical reaction with hydrogen peroxide is blocked. The addition of small amounts of oxygen thus increases the efficiency of ammonia production from organic radicals before it decreases the total number of organic radicals formed.

Other Interpretations of the Oxygen Effect

A mechanism involving a group of compensating reactions has just been described that accounts for the observed fact that oxygen has little effect on ammonia yield. At the same time this mechanism accounts for the pronounced effects of oxygen that are observed for other products.

It is known that oxygen successfully competes with most solutes for reaction with H radicals. Therefore, some reaction has to take the place of H-radical oxidation of glycine in the presence of oxygen if ammonia formation is not decreased.

The suggestion has been made that HO₂ radicals might be able to react with glycine and thus provide for equal ammonia yields in the presence and absence of oxygen. This reaction would satisfactorily compensate the removal of H radicals by oxygen. The proposal that oxygen reacts with organic radicals, however, would then have to be abandoned. Otherwise, calculated ammonia and hydrogen peroxide yields would differ markedly from observed yields for oxygen-saturated solutions.

An alternative suggestion was made that glycine may react with OH rapidly enough to "reach into the track" and suppress the forward formation of hydrogen peroxide. It has been shown that certain inorganic ions possess this ability. As was discussed already, the radical pair, NH₂CH₂ and COOH contributes to the net ammonia yield only if oxygen is present. Likewise, reaction of glycine with OH in the track would contribute to the net ammonia yield only in the presence of oxygen by decreasing the importance of the blocked chain oxidation of organic radicals by hydrogen peroxide. Reaction (4), which yields the radical pair NH₂CH₂ and COOH, and reaction of glycine with OH in the track are equally successful in accounting for observed ammonia and hydrogen peroxide yields. Both reactions increase in importance as glycine concentration is increased. Both reactions may occur to some extent. Data obtained with cyclotron-accelerated helium ions, however, indicate a primarily direct process in the formation of formaldehyde (Fig. 5). Moreover, Barron, Ambrose, and Johnson have observed that in very dilute oxygen-saturated glycine solutions (10⁻³ M), ammonia yield is virtually zero. It is evident, then, that glycine cannot be so reactive with OH that it competes successfully for removal of OH in the track at this concentration.

Explanation of the observed effects of dissolved oxygen in terms of the proposed free-radical intermediates requires two assumptions that cannot at present be proved: (1.) that NH₂ radicals react with glycine in the presence of oxygen, and (2.) that NH₂CH₂ radicals react preferentially
with \( \text{NH}_2\text{CHCOOH} \) in the presence of large amounts of glycine. Evidence for the reaction of \( \text{NH}_2 \) radicals with oxygen has been obtained \(^{30}\) in studies of the photolysis of liquid ammonia, but there are no available data on the relative rates of reaction of \( \text{NH}_2 \) with oxygen and glycine. In the photolysis of ammonia, the only fate for \( \text{NH}_2 \) radicals—other than reaction with oxygen—is dimerization to hydrazine which was also observed.

A modification of the proposed mechanism has been suggested \(^{19}\) that avoids the necessity of making these two assumptions. Reactions of excited glycine molecules are involved. In the presence of oxygen, all the stoichiometric requirements of the proposed mechanism are met if it is assumed that the common precursor to the radicals \( \text{NH}_2, \text{NH}_2\text{CH}_2, \text{CH}_2\text{COOH}, \) and \( \text{COOH} \) formed in the initial direct Reactions (3) and (4) is an excited glycine molecule capable of reacting with hydrogen peroxide:

\[
\text{NH}_2\text{CH}_2\text{COOH}^* + \text{H}_2\text{O}_2 \rightarrow \text{NH}_2\text{CHCOOH} + \text{H}_2\text{O} + \text{OH}
\]

Ultimately two ammonia molecules and two aldehyde molecules result from reactions of the radicals formed in Reaction (32). Some carbon dioxide is also formed. The hydrogen peroxide yield is not affected; \( \text{HO}_2 \) radicals formed by reaction of \( \text{NH}_2\text{CHCOOH} \) with oxygen combine to form hydrogen peroxide—Eq. (30)—. In the absence of oxygen, hydrogen peroxide is catalytically removed by \( \text{NH}_2\text{CHCOOH} \) and it can be postulated that the excited glycine molecules predissociate into \( \text{NH}_2, \text{NH}_2\text{CH}_2, \text{CH}_2\text{COOH}, \) and \( \text{COOH} \) radicals as already described by Reactions (3) and (4). \(^{\ast}\)

In the absence of oxygen, yields for Reactions (3) and (4) show an apparent dependence on radiation type and dose rate. This dependence can be accounted for by a competition between excited glycine molecules and organic radicals (predominantly \( \text{NH}_2\text{CHCOOH} \)) for reaction with hydrogen peroxide under conditions which favor high local concentrations of hydrogen peroxide and excited glycine molecules.

Although it has been shown \(^{29}\) that glycine in low concentration reacts too slowly with \( \text{OH} \) to suppress the forward formation of hydrogen peroxide, the reaction of excited glycine molecules with \( \text{OH} \) and \( \text{H}_2\text{O}_2 \) in the track may be fast and is stoichiometrically equivalent to the suggested suppression of forward hydrogen peroxide formation.

It is beyond the scope of this paper to discuss in detail all the possible reactions of excited species. An investigation of excited-molecule processes is now in progress in which irradiations of glycine solutions containing known amounts of added hydrogen peroxide are being made.

\(^{\ast}\) Predissociation is here used to denote a delayed bond rupture that occurs more than one molecular vibration period after the absorption of energy, and that may occur at a site different from that involved in the absorption act. (See G. K. Rollefson and M. Burton, "Photochemistry and the Mechanism of Chemical Reactions," Prentice-Hall Inc., New York, 1939, p 70.)
Influence of Competing Reactions

Interaction of Intermediate Products

The mechanism, as it has been described so far, assumes the quantitative reaction of the initial radicals H, OH, NH₂, and CH₂COOH with glycine in oxygen-free systems. These radicals can become involved in other reactions, however, before they have a chance to encounter or react with glycine molecules. Low glycine concentration and (or) high radiation flux permit products of water radiolysis to be removed in the following reactions:

\[
H + H₂O₂ \rightarrow H₂O + OH \quad (33)
\]
\[
OH + H₂ \rightarrow H₂O + H \quad (34)
\]
\[
H + OH \rightarrow H₂O \quad (35)
\]

Organic radicals can capture H radicals under the same conditions:

\[
NH₂ + H \rightarrow NH₃ \quad (36)
\]
\[
CH₂COOH + H \rightarrow CH₃COOH \quad (37)
\]
\[
NH₂CHCOOH + H \rightarrow NH₂CH₂COOH \quad (38)
\]
\[
NH₂CH₂ + H \rightarrow NH₂CH₃ \quad (39)
\]

Increased glycine concentration tends to decrease the importance of the above "back" reactions--Eqs. (33) through (39)--, but if the radiation flux is high enough, at least some of them will occur at all concentrations. Radiation composed of a focused beam of cyclotron-accelerated helium ions completely absorbed in ~0.05 cm³ of solution at the very high dose rate of 2 x 10¹⁶ ev/sec has been observed to result in extensive reaction of this type in acetic acid solutions. Under the same conditions, but in glycine solutions, hydrogen yield was observed to be independent of glycine concentration over the range 0.0625 to 3.0 M. The average yield (0.95 molecules hydrogen per 100 ev absorbed in the solution) is about equal to 0.5 F as indicated by independent experiments in this laboratory under similar radiation conditions. Analysis of HD and D₂ yields from D₂O solutions of glycine shows that little, if any, H-radical dehydrogenation of glycine occurs at the C-H bond under the irradiation conditions employed. Virtually complete removal of H in one or more reactions of the type shown in Eqs. (33), (35) through (39) is indicated.

For every H radical removed via Reactions (33), (35) through (39), an additional radical or molecule capable of completing an oxidation step toward ultimate radiolysis products is also removed. The stoichiometric consequence of this, as regards net radiolysis yield, can be described as an equivalent decrease in the value of R. Actually, R is determined by the radiation type but can, in effect, be partially or completely counteracted by reactions in which H radicals are removed by other oxidizing species. Thus, for the very high radiation flux employed in this study, forward hydrogen peroxide or its
OH equivalent (depending on which of the Reactions (33), (35) through (39) are involved in the removal of H radicals) accounts for practically the entire indirect effect. Only a small amount of initial-radical reaction with glycine is necessary for complete catalytic removal of hydrogen peroxide.

Dose-yield Considerations

Another consideration important for conditions of high radiation flux as well as for prolonged exposure is the competition of accumulating products for reaction with intermediates. Of the major products, glyoxylic acid is probably the most sensitive to attack by oxidizing radicals and undoubtedly "protects" glycine to some extent. It has been shown 7,8 that product glyoxylic acid reaches a limiting concentration for x-ray doses comparable to the doses used in the helium-ion irradiations reported here. Thus, radiolysis of glycine is decreased, and some product yields are correspondingly decreased. Glyoxylic acid yield is decreased because of reduced formation resulting from reduced glycine radiolysis and also because of radical attack on glyoxylic acid itself. Carbon dioxide and formic acid yields increase. Oxalic acid may appear as a secondary product.

A detailed analysis of calculated and observed product yields from the helium-ion study reported here, similar to that presented for x-ray studies, can be made by making corrections in calculated product yields appropriate to the described competing processes. Product yields are comparatively low, and high precision in calculations is impossible because of the magnitude of the necessary corrections, but no significant conflict exists between observed and calculated yield values.

Initial Reaction (3): $\text{NH}_2\text{CH}_2\text{COOH} \rightarrow \text{NH}_2 + \text{CH}_2\text{COOH}$

The value of $d_1$, calculated from the helium-ion data of this study in the same manner as from the x-ray data, is less than the value for x-rays. This can be explained in terms of processes occurring in the individual tracks. A "composite forward reaction" involving initial radicals (or excited molecules) from both water and glycine could reasonably account for smaller numbers of NH$_2$ and CH$_2$COOH radicals available for reaction with solute. Reaction of NH$_2$ and CH$_2$COOH with H in the tracks would lead to product stoichiometry indistinguishable from that resulting from H-capture reactions already presented as important in the beam-absorption zone—Eqs. (33), (35) through (39)—. Reaction of NH$_2$ and CH$_2$COOH with OH in the tracks would be recognized as an apparently decreased value for $d_1$. Similarly, reaction of excited glycine molecules with OH or H$_2$O$_2$ in the track would result in a lowered value of $d_1$.

Hydroxylamine was not observed in this study, but its decomposition products (nitrogen and nitrous oxide) cannot be excluded as possible products at the higher glycine concentrations studied. Hydroxylamine itself has been reported 32 as a product from presumably acid solutions of alanine irradiated with x-rays.
Initial Reaction (4): \[ \text{NH}_2\text{CH}_2\text{COOH} \rightarrow \text{NH}_2\text{CH}_2 + \text{COOH} \]

The relative ineffectiveness of the radical reaction in causing observable radiolysis under conditions of high radical concentration facilitates the separate consideration of direct and indirect processes. There are two sources of product formaldehyde:

\[
\begin{align*}
\text{NH}=\text{CHCOOH} + \text{H}_2\text{O} & \rightarrow \text{NH}_3 + \text{HCHO} + \text{CO}_2 & (23b) \\
\text{NH}=\text{CH}_2 + \text{H}_2\text{O} & \rightarrow \text{NH}_3 + \text{HCHO} & (24)
\end{align*}
\]

Iminoacetic acid is formed as a consequence of both direct and indirect processes, whereas methyleneimine is formed solely as a consequence of Reaction (4). Since only a small fraction of the iminoacetic acid yields formaldehyde, and the principal product is glyoxylic acid, a negligible amount of product formaldehyde is formed from iminoacetic acid under conditions that minimize net indirect effects. Methyleneimine is the principal product of the \( \text{NH}_2\text{CH}_2 \) radical. Only small amounts of \( \text{NH}_2\text{CH}_2 \) react to yield methylamine or combination products. Conditions favoring high radical concentrations would therefore be expected to result in a net formaldehyde yield that was roughly proportional to glycine concentration. Figure 5 shows that formaldehyde yield is indeed proportional to glycine concentration under the conditions employed in this study. Maxwell, Peterson, and Sharpless\(^7\) report a declining rate of increase of formaldehyde yield as glycine concentration is increased. This is to be expected under the irradiation conditions they employed, where contribution to the total formaldehyde yield by indirect processes was of greater relative importance.

**Future Experiments**

The foregoing discussion of mechanisms involved in the radiolysis of aqueous glycine points to a program of future experiment. Verification or clarification of a number of the separate processes is possible.

The evidence for \( \text{CH}_3\text{COOH} \) and \( \text{NH}_2\text{CHCOOH} \) intermediates is good. It is not certain, however, just what their exact place is in a possible series of intermediates leading to combination products or to iminoacetic acid. The possible role of excited glycine molecules has already been discussed. There are several radical combination products not yet identified, and the imino radical, \( \text{NHCH}_2\text{COOH} \), may be important in their formation. With high-energy helium ions, diaminosuccinic acid is formed in excess of unidentified Product I (see Fig. 1). With \( \gamma \)-rays, however, unidentified Product I was the principal radical combination product. It is reasonable to wonder, then, if low radical concentrations permit the following reaction before combination or disproportionation can occur:

\[
\text{NH}_2\text{CHCOOH} + \text{NH}_2\text{CH}_2\text{COOH} \rightarrow \text{NH}_2\text{CH}_2\text{COOH} + \text{NHCH}_2\text{COOH} \quad (40)
\]

The dimerization of \( \text{NHCH}_2\text{COOH} \) radicals would then yield hydrazoacetic acid (\( \text{COOHCH}_2\text{NNHCH}_2\text{COOH} \)) and perhaps account for unidentified Product I. The disproportionation reaction would be the same as for \( \text{NH}_2\text{CHCOOH} \).
The COCOOH radical possibly formed in secondary reactions of glyoxylic acid would lead to a peptide upon combination with NHCH₂COOH. Combination of COCOOH with NH₂CHCOOH would yield α-keto, β-amino-succinic acid which is also a possible candidate for unidentified Product I. The radiation-induced synthesis of a peptide bond in an aldehyde-glycine mixture would be strong evidence for the formation of NHCH₂COOH from glycine in aqueous solution.

Possible formation of organic radicals by initial radical attack at the carboxy carbon cannot be entirely dismissed. It is beyond the scope of this paper to discuss in detail the various factors that influence the relative probabilities of radical attack at different loci or to discuss the precise mechanism of such attack. If organic radicals are formed as a result of initial radical attack at the carboxy end, they may react rapidly with glycine, yielding one or both of the nitrogen-containing radicals already discussed. There is a larger number of opportunities for radical reaction at the methyl end than at the carboxy end. Furthermore, both the N-H and C-H bonds are weaker than the RO-H bond. Possible radical attack at the carboxy carbon of acetic acid was dealt with in some detail in a previous study in this laboratory.³

The introduction of additional solutes to the glycine solutions irradiated will be helpful. The detailed fates of both CH₂COOH and NH₂CHCOOH are subject to clarification by controlling their concentrations relative to each other, and the concentrations of both relative to glycine in studies involving mixtures of acetic acid and glycine. For example, the reaction between CH₂COOH and glycine can be effectively studied by irradiating solutions of acetic acid containing C¹⁴-labeled glycine. Radiation studies of glycine-formic acid mixtures in the absence of oxygen will help determine the specific fate of COOH.

Clarification of the relative importance of the initial Reactions (3) through (5) is needed. Irradiation of solid glycine, and of solutions in which the practical glycine concentration range is extended by reduced pH or elevated temperature, will be undertaken. The use of additional solutes, (e.g., I or I⁻) that are known to "quench" certain radical reactions will help to establish the validity of the proposed initial reactions.

Irradiation of glycine dissolved in D₂O has already shown that very little, if any, H-radical dehydrogenation of glycine occurs under the irradiation conditions employed in the study reported here. Similar experiments with other types of radiation would clearly show the fraction of total hydrogen arising from H-radical oxidation of glycine and that arising from the forward yield only. The pattern of deuterated products from D₂O solutions of glycine would also help clarify the details of H-radical-capture reactions.

The chain oxidation of organic radicals by hydrogen peroxide has been proposed to account both for observed product yields and for the absence of forward hydrogen peroxide in irradiated glycine solutions. The obvious way to test the validity of this proposal is to irradiate oxygen-free glycine
solutions containing added hydrogen peroxide. Increased yields of ammonia, glyoxylic acid, formaldehyde, and carbon dioxide would be expected. The companion experiment (oxygen-free), in which Fenton's Reagent is used, would also be helpful. The addition of hydrogen peroxide to oxygen-saturated solutions at high glycine concentration has already been mentioned in connection with reactions of excited glycine molecules.

Perhaps the most important approach to take is a continued investigation of processes important in the radiolysis of aqueous glycine is a thorough study, over the entire practical glycine concentration range, of oxygen and pH effects. The effects of oxygen have been discussed in detail, but actual investigation of product yields for various conditions involving the presence of oxygen in the solution is very limited. Maxima in ammonia yield at pH 3 and 9, and a minimum at pH 6, have been reported for glycine and alanine. Although there are several possible explanations, including varying reactivity of glycine in its different ionic configurations and altered conditions for H-radical-capture reactions, there is not enough experimental evidence to convincingly suggest any one process. Knowledge of the effect of pH on various product yields may well be crucial to a detailed understanding of the complete radiolysis mechanism.
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