

AN ABSTRACT OF THE THESIS OF

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Title RADIOLYSIS OF SUCCINIC ACID  
IN WATER

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The radiolysis of succinic acid in aqueous solutions induced by gamma irradiation from  $^{60}\text{Co}$  has been studied with respect to the identification and estimation of degradation products. Detailed information on the radiolysis of succinic acid is important in determining what effect two carboxyl groups in the same molecule would have on the reaction mechanisms and products relative to the degradation of the hydrocarbon chain resulting from gamma irradiation.  $^{14}\text{C}$ -labeled succinic acid was employed to facilitate product identification and estimation. In addition, specifically labeled succinic acid- $^{14}\text{C}$  and malic acid- $^{14}\text{C}$  were utilized for the elucidation of reaction mechanisms. The irradiation experiments were performed in both oxygenated and oxygen-free systems.

The principal means of identification of the radiolytic products were by paper chromatography and autoradiography, utilizing the technique of co-chromatography. The estimation of each radiolytic

product was determined by radiochemical assay using liquid scintillation counting techniques.

The oxidation reactions in both oxygenated and oxygen-free systems may possibly follow the same reaction mechanisms, since the degradation products resulting from these reactions are the same in the oxygenated and oxygen-free systems, although the yields are greater in the oxygenated system. The oxidation products identified from the radiolysis of succinic acid were malic, malonic, oxaloacetic, pyruvic, oxalic, mesoxalic, glyoxylic, propionic, acetic, and formic acids, glyoxal, and carbon dioxide. The products of molecular weight greater than succinic acid produced exclusively from radiolysis in the oxygen-free system were 1,2,3,4-butane-tetracarboxylic, 1,2,4-butane-tricarboxylic, tricarballic, glutaric, and adipic acids.

The radiochemical yields of the radiolytic oxidation products from specifically labeled succinic acid- $^{14}\text{C}$  were determined to be greater in the oxygenated system than in the oxygen-free system. This fact indicates that oxygen plays an important role in the radiolytic degradation of succinic acid. The graphical presentation of the degradation of succinic acid and the formation of malonic and malic acids with increasing cumulative dose follow the same course in both oxygenated and oxygen-free systems. However, the radiolytic

degradation of succinic acid and the formation of malonic and malic acids were greater in the oxygenated system.

The formation of malonic acid from radiolysis of succinic acid aroused considerable interest, since this acid was produced in unusually high yield. Therefore, studies were carried out to elucidate the mechanism for malonic acid formation. The results for radiolysis of  $^{14}\text{C}$  specifically labeled succinic acid indicated that malic acid probably was an intermediate in the formation of malonic acid. In order to verify this result, specifically labeled malic acid- $^{14}\text{C}$  was used in similar irradiation experiments. The results of this experiment indicated that malonic acid formation resulted from C-1 decarboxylation of malic acid followed by oxidation of the intermediate. Several reaction mechanisms were suggested as possible mechanisms for the formation of malonic acid. The most likely mechanism involved  $\cdot\text{OH}$  addition across the double bond of the enol form of oxaloacetic acid, in which decarboxylation of the adduct accompanied by oxidation resulted in the formation of malonic acid.

RADIOLYSIS OF SUCCINIC ACID  
IN WATER

by

FRED SHIGERU TANAKA

A THESIS

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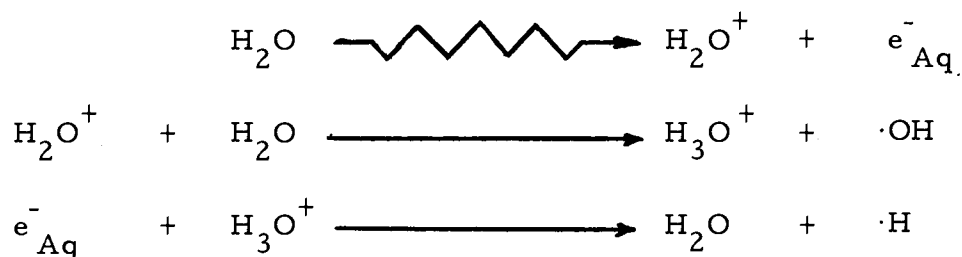
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# RADIOLYSIS OF SUCCINIC ACID IN WATER

## INTRODUCTION

The chemical action of ionizing radiation has recently aroused considerable interest. It has long been known that ionizing radiation affects the bonding in organic compounds, but not until recently has much work been done in this area. Findings of early investigations led one to believe that upon gamma irradiation of an organic compound, bond cleavage was random and non-specific. However recent studies have shown that there is some degree of specificity of action of radiation with respect to the types of bondings, especially in aqueous solutions (1, 47). In aqueous solutions most of the radiation effects inflicted on the solute molecules are due to secondary effects brought about by the action of ionizing radiation on the solvent molecules giving rise to ions, free radicals, and excited molecules.

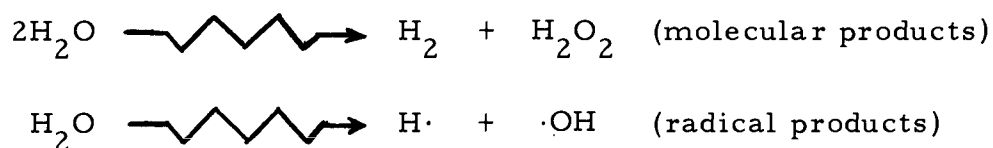
The exact chemistry of the radiolysis of water has not been completely ascertained, although considerable work has been done in this regard. In neutral or slightly acidic solutions ( $\text{pH} > 3$ ), the major reducing species has been recognized to be the solvated electron (47, p. 242-264).



The overall expression for these reactions may be written as:

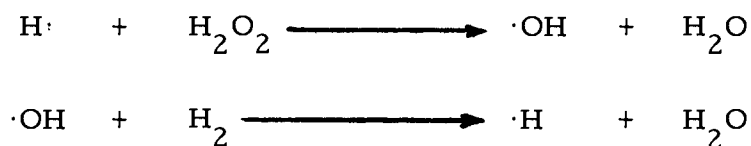


In most cases the solvated electrons and the hydrogen radicals eventually give the same products. But on the other hand, where both species react with the solute to give equivalent products, the rates of their reactions with the solute will in most cases be different. For the discussion of the present studies, reference will be made to the hydrogen radical as the primary reducing species, which will include the solvated electron. In general the most important species involved in the reactions of irradiated aqueous solutions are the radical products which are the hydrogen radicals and the hydroxyl radicals, and the molecular products which are molecular hydrogen and hydrogen peroxide.



The fate of the radical and molecular products in irradiated water depends on the substances present in solution with which they

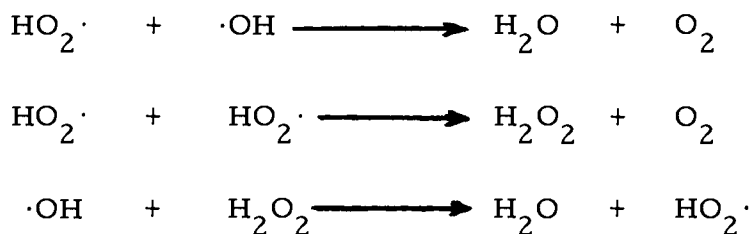
may react. In the absence of such substances, and in a closed system, the radicals will react with the molecular products of the radiolysis of water to reform water.



In an oxygenated system, molecular oxygen acts as a very efficient scavenger for hydrogen radicals, thus forming the perhydroxyl radical,  $\text{HO}_2\cdot$ .

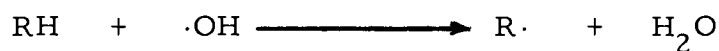


The  $\text{HO}_2\cdot$  is not a very reactive species, and rarely abstracts hydrogen from organic solutes. It is, however, a strong oxidizing agent and reacts with hydroxyl radicals and with other perhydroxyl radicals to produce oxygen.



When an organic solute is present in the aqueous solvent, the following types of reactions may occur.

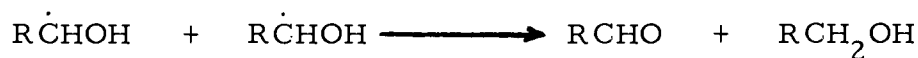
(Hydrogen abstraction)



(Dimerization)



(Disproportionation)



(Coupling)



Hence, one can visualize that the free radical reactions in aqueous solutions are initiated by the radicals formed by the primary interaction of radiation with water.

Numerous papers have appeared in recent years on the radiation chemistry of simple carboxylic acids, such as formic acid (16, 18, 24, 25) and acetic acid (14, 15, 17, 19) in aqueous solutions. Oxalic acid is the only dicarboxylic acid whose radiolysis has been studied very extensively (9, 10, 11). In 1938 Fricke et al. (13) studied the evolution of gaseous products from the radiolysis of some low molecular weight carboxylic acids in aqueous solutions. This group included malonic and succinic acids. Upon x-ray irradiation of 1.0 and 10 mM solutions of succinic and malonic acids, these

workers detected evolution of hydrogen from both acids and a trace of carbon dioxide from malonic acid.

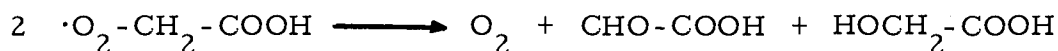
The electron spin resonance of an irradiated single crystal of succinic acid was studied by Hahn and Rexroad (23) and Heller and McConnell (28). Both groups of investigators found  $\text{HOOC-CH}_2-\dot{\text{C}}\text{H-COOH}$  to be the principal long-lived paramagnetic species produced in the irradiated single crystal of succinic acid. Cole, Heller, and Lambe (6) studied the electron nuclear double resonance (ENDOR) in an irradiated single crystal of succinic acid. Their data confirmed the presence of the  $\text{HOOC-CH}_2-\dot{\text{C}}\text{H-COOH}$  radical. Box, Freund, and Lilga (5) investigated the paramagnetic absorption of a single crystal of succinic acid irradiated at low temperature. These workers found that the principal paramagnetic species at 77°K had an unpaired electron in a 2p  $\pi$ -orbital of the carboxyl carbon atom, and they believed that this was due to an ionized succinic acid with the oxygen of the carboxyl group carrying a negative charge.

Barker et al. (4) found that upon irradiation of aqueous solutions of organic compounds in vacuo or under an atmosphere of nitrogen leads to radical dimerization, and under prolonged radiation to polymeric materials. Conversely, the presence of oxygen greatly reduces dimerization and produces no detectable polymers. In the presence of oxygen, oxidation products were formed with considerable

cleavage of carbon to carbon bonds and decarboxylation. Similar to the results obtained by Barker et al., Garrison et al. (14, 15) identified succinic, tricarballic, malonic, malic, and citric acids as some of the non-volatile products in the radiolysis of acetic acid. Succinic acid, which is the dimer of acetic acid, was the product of highest yield isolated from the radiolysis of aqueous acetic acid in vacuo. However, upon irradiation of oxygenated solutions of acetic acid, the products of oxidation were observed to be glyoxylic, glycolic, and oxalic acids, formaldehyde, and carbon dioxide (19). The primary radical intermediate for the oxidative degradation of acetic acid is believed to be  $\cdot\text{O}_2\text{-CH}_2\text{-COOH}$ . Consequently, this radical can be hydrolyzed to yield glycolic acid in accordance to the mechanism proposed by Sworski (48) and by Phung and Burton (43) for the formation of phenol in the radiolysis of aqueous benzene under oxygenated conditions.

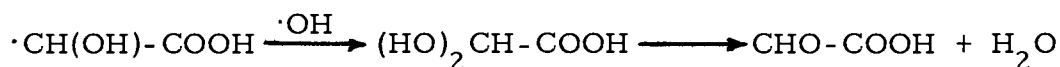


Another mode of removal of the  $\cdot\text{O}_2\text{-CH-COOH}$  radical would be by a disproportionation type reaction yielding glyoxylic and glycolic acids directly.

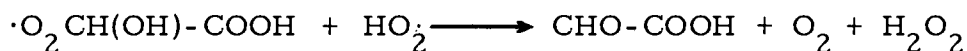


Hydroxylation is one of the first steps of oxidation in the radiolysis of aqueous solutions of organic compounds in the presence of oxygen.

Both qualitative and quantitative analyses were carried out by Grant and Ward (20, 21) to determine the effects of gamma radiation on aqueous solutions of glycolic acid under oxygenated conditions and in vacuo. The non-volatile products of glycolic acid irradiated in vacuo were isolated and identified as glyoxylic, oxalic, and tartaric acids. These authors postulated the following reaction as the mechanism for the oxidation of glycolic acid to glyoxylic acid under non-oxygenated conditions.



The gamma radiolysis of glycolic acid in the presence of oxygen gave higher yields of glyoxylic and oxalic acids, but the yield of tartaric acid was very much reduced. The oxidation of glycolic acid in the presence of oxygen can be explained by the following equation.

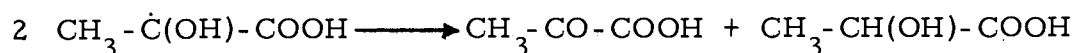


Four liters of a 0.1 percent solution of glycolic acid were irradiated with a total dose of  $5.5 \times 10^{19}$  ev per ml; 29.4 percent of the glycolic acid was destroyed in vacuo, and 23.5 percent was lost under oxygenated conditions.

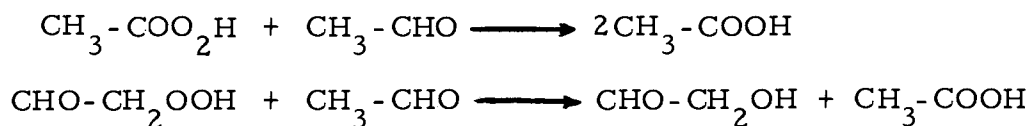
The action of free radicals, produced by x-rays, on lactic acid in aqueous solution with particular reference to the effect of molecular oxygen was reported by Johnson, Scholes, and Weiss (33). Lactic acid was found to be oxidized by the free radicals to produce pyruvic



acid and acetaldehyde. Pyruvic acid was produced in vacuo as well as in the presence of oxygen. However, the yield of pyruvic acid was approximately four times lower in vacuo. The formation of pyruvic acid in vacuo was explained as a disproportionation of two lactic acid radicals.

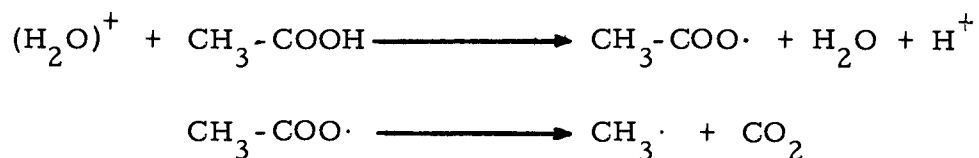


The photochemistry of pyruvic acid was studied by Leermakers and Vesley (34) in aqueous solutions with respect to the action of ultraviolet light. Considerable quantities of carbon dioxide was rapidly evolved, and the main product remaining in solution was acetoin. No acetic acid was detected and very small amounts of acetaldehyde were observed. Allen (2) investigated the action of gamma rays from  $^{60}\text{Co}$  and 2.5 Mev electrons from a Van de Graaff accelerator on oxygenated, aqueous solutions of acetaldehyde. Acetic acid and formaldehyde were the two organic products initially detected in this study. The oxidation of acetaldehyde was attributed to the action of organic peroxides.



The decomposition of ten aliphatic carboxylic acids by gamma radiation was reported by Johnsen (32) with emphasis on relative rates of decarboxylation. He found that iodine, which is a good

radical scavenger, had virtually no effect on the carbon dioxide output for acetic acid. However, the addition of iodine sharply decreased the output of methane. Similar results were observed by Garrison et al. (15) in their investigation of the radiolysis of aqueous acetic acid. These results indicated that approximately 80 percent of the methane production was due to radical reaction, and the rest resulted from molecular processes. The positive polaron  $(\text{H}_2\text{O})^+$  was suggested as a possible reacting species leading to the decarboxylation of irradiated aqueous solutions of acetic acid by Hayon and Weiss (27).



Therefore, this mode of decarboxylation could occur both in oxygenated systems and in vacuo, since the positive polaron is formed by the direct interaction of radiation with water.

The field of radiation chemistry is still in an early stage of development. For example, the radiolysis of water has been investigated for many years, and much is known about its behavior when it is subjected to the action of ionizing radiation, but even now many investigators are still probing many new problems dealing with the radiolysis of water. Similarly, as one can see from the papers previously cited, there is a great stress on the study of the effects of

of radiation on biologically important compounds. However, the effects of radiation on succinic acid, which is an important metabolic acid in the Krebs cycle, has not been systematically carried out. In the present work, the effects of gamma radiation on succinic acid in aqueous solutions has been thoroughly studied by means of radio-tracer methods aiming at both identification and estimation of the radiolytic products.

## EXPERIMENTAL METHODS

The present study made use extensively of radiotracer methods to facilitate detection and estimation of radiolytic products. In addition, specifically labeled carbon-14 compounds proved to be valuable in the elucidation of reaction mechanisms.

### Radiochemical Compounds

Succinic acid-1,4- $^{14}\text{C}$  and -2,3- $^{14}\text{C}$  and aspartic acid-4- $^{14}\text{C}$  were obtained from New England Nuclear Corporation. Malic acid-3- $^{14}\text{C}$  was purchased from Volk Radiochemical Company. Malic acid-4- $^{14}\text{C}$  was prepared in the laboratory from aspartic acid-4- $^{14}\text{C}$  by means of nitrous acid oxidation according to the procedure of Horberman and D'Adamo (29). All the radiochemical compounds were examined for purity by paper chromatography and autoradiography.

### Preparation of Samples for Irradiation

Distilled water which had been passed through a Barnstead deionizer was used in the preparation of the aqueous solutions of organic acids at the concentration of 0.005 M for the radiation experiments. The specific activity of the succinic acid- $^{14}\text{C}$  was 2  $\mu\text{c}$  per  $\mu\text{M}$  and that for malic acid- $^{14}\text{C}$  was 1  $\mu\text{c}$  per  $\mu\text{M}$ . The irradiation vessels were 5 ml Kontes drying ampoules (Kontes Glass Company,

Vineland, N.J.) with an attached side-arm as a means to introduce the desired sample atmosphere, and an attached break-seal to facilitate the analysis of the radiolytic gaseous product (Figure 1).

In a typical experiment, 1 ml of the organic acid solution was pipetted into an ampoule equipped with a side-arm, and then a break-seal was attached to the ampoule. The ampoule was placed in an ice bath, and was evacuated to a pressure of 0.25 mm of mercury. The vessel was then flushed with the desired gas which was either oxygen or nitrogen, and this procedure was repeated ten times to ensure that the desired atmosphere was fully established. Subsequently, the sample was frozen in liquid nitrogen to bring the entire system to a slightly reduced pressure. While maintaining the desired atmosphere with the ampoule in liquid nitrogen, the ampoule was sealed at the constriction in the side-arm. After the vessels were sealed, each sample was thawed and mixed vigorously on a Vortex Jr. mixer (Scientific Industries, Inc., Queens Villiage, N.Y.) to ensure an even distribution of the gas in the aqueous phase.

#### Radiation Source

The  $^{60}\text{Co}$  irradiator used in the present study was designed in this laboratory and was manufactured by the Budd Company (Figure 2). The irradiator contains approximately 3300 Curies of  $^{60}\text{Co}$  in the form of 12 pencil-size rods which are arranged in a circular

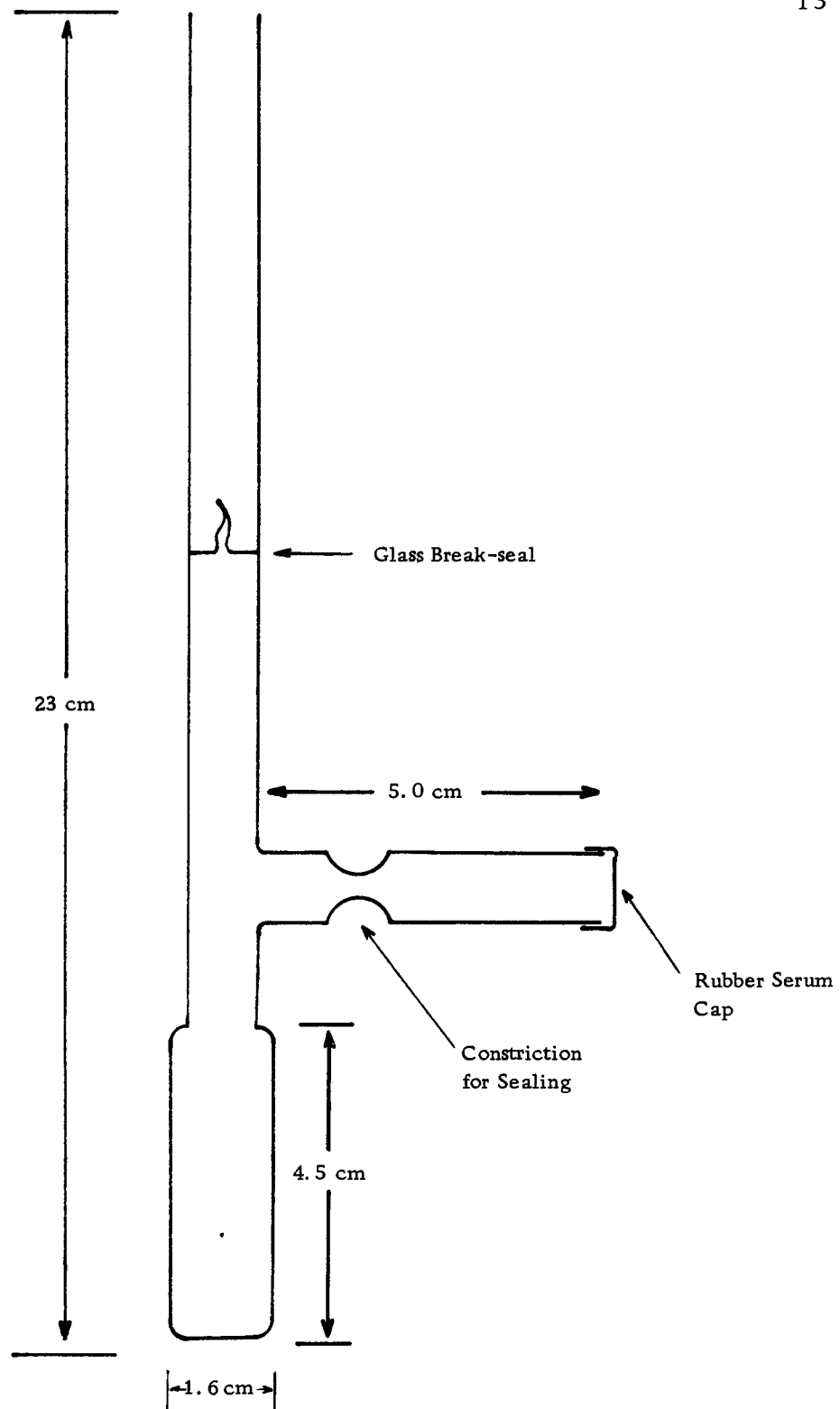


Figure 1. Irradiation vessel

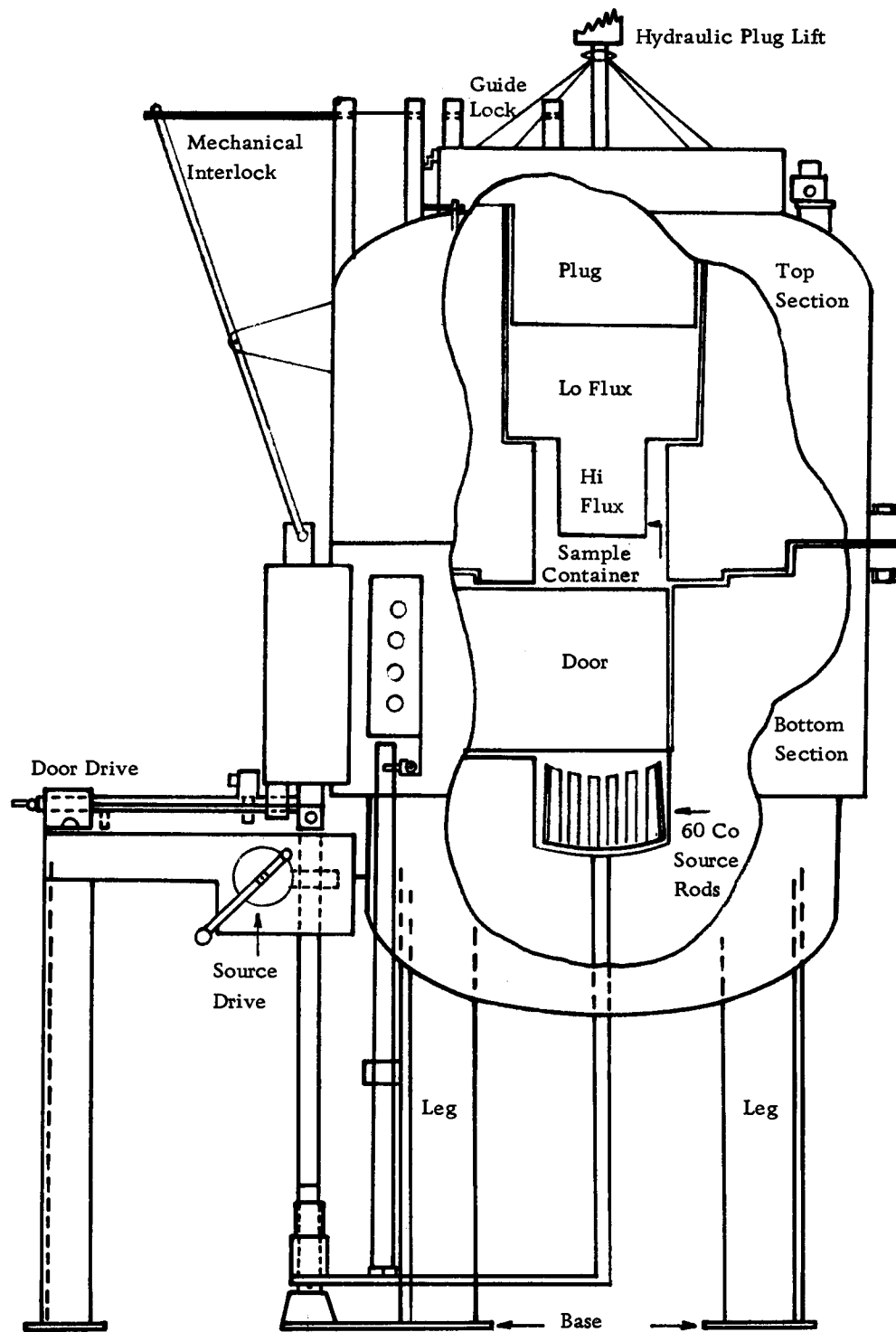


Figure 2.  $^{60}\text{Co}$  Irradiator at Oregon State University

configuration that surrounds the high flux chamber. The high flux irradiation chamber is cylindrical in shape and measures 5 inches in diameter and 5 inches in height. The total height of the sample chamber, which extends upward into the low flux chamber, is 11 inches.

The chemical dosimetry for gamma flux in the chamber was carried out by Edgerton, Germeshausen, and Grier, Inc. (EG&G). The radiation dosimeters consisted of stabilized tetrachloroethylene overlaid with an acidimetric dye-water solution. Each dosimeter was hermetically sealed to minimize the effects of environmental contamination and was appropriately lead shielded to provide relative energy independence. When the dosimeter was exposed to gamma radiation, tetrachloroethylene produced stable water soluble acids. These acids affect the acidimetric dye to produce a color change which was evaluated by spectrophotometric analysis techniques developed by EG&G. This type of dosimetry carries a precision of approximately  $\pm 10$  percent. The dose rate at the chamber wall was measured to be  $5.0 \times 10^{19} \text{ ev ml}^{-1} \text{ hr}^{-1}$ .

The homogeneity of the gamma flux in the irradiator was examined by the Budd Company at the time of installation, using the Fricke chemical dosimetry method. Samples were placed at 45 degree intervals around the irradiation chamber wall and were



irradiated for a period of 5 minutes. The results of this experiment showed a dose fluctuation of  $\pm 3$  percent.

### Procedure of Irradiation

For radiation exposure the samples were placed in the high flux chamber of the  $^{60}\text{Co}$  irradiator. Each ampoule was secured against the chamber wall. Sample irradiations were carried out at room temperature. The radiation exposure times ranged from 1.25 to 7.50 hours to yield total doses ranging from  $0.62 \times 10^{20}$  to  $3.75 \times 10^{20}$  ev per ml. After irradiation, the samples were immediately frozen to prevent further decomposition prior to analysis.

### Product Identification and Estimation

#### Possible Radiolytic Products

Sealed ampoules of succinic acid-1,4- $^{14}\text{C}$  and -2,3- $^{14}\text{C}$  solutions were irradiated with a dose of  $1.25 \times 10^{20}$  ev per ml in oxygenated and oxygen-free systems. After irradiation these samples were carefully freeze-dried to remove the steam distillable products and the volatile gaseous products. Each dry non-volatile residue was dissolved in 1 ml of distilled water. Then, 10  $\mu\text{l}$  of this aqueous sample were pipetted into a 20 ml glass counting vial containing 5 ml of 2-amino ethanol-absolute ethanol (1:2 v/v) and 10 ml of fluor solution. The 2-amino ethanol-absolute ethanol solution was used to

effect the solution of water in toluene. The fluor solution contained terphenyl (3 g/l) and 1,4-bis-2-(5-phenyl-oxazolyl)-benzene (30 mg/l) dissolved in toluene. These samples were counted in a Packard Tri-carb Scintillation spectrometer with the photomultiplier voltage set at 1000 volts and the pulse discriminator set at 100-1000 divisions. Counter efficiency was determined by the use of an aqueous solution of glucose-U-<sup>14</sup>C having a known radioactivity.

These analyses revealed that approximately 30 percent of the radiolytic products produced from aqueous succinic acid, both in oxygenated and oxygen-free systems were either steam distillable products or volatile gaseous products. Therefore, intact irradiated samples were analyzed in a different manner to identify these volatile products as well as the non-volatile products.

The analysis of the gaseous products was carried out by means of gas chromatography. The steam distillable acids were converted to the respective salts, separated and identified by means of paper chromatography. For the identification of the non-volatile radiolytic products remaining as residue in the distillation process, the technique of two-dimensional paper chromatography was utilized. The location of the products on the paper chromatograms was determined by means of autoradiography, and the estimation of each radiolytic product was determined by radiochemical assay using liquid scintillation counting techniques.

### Analysis of the Gaseous Radiolytic Product

To investigate the formation of the gaseous products sealed samples of unlabeled succinic acid were irradiated. After irradiation the volatile gases were transferred by means of vacuum transfer techniques (Figure 3). Any gaseous product present was transferred from the irradiation ampoule to a tube dipped in liquid nitrogen. The condensed gases were subsequently allowed to evaporate and pass through two specially designed silica gel traps connected in series. The first silica gel trap was immersed in an acetone- $\text{CO}_2$  bath ( $-77^\circ\text{C}$ ) which would remove  $\text{CO}_2$  and any anticipated volatile organic compounds other than methane. The second trap was immersed in a liquid nitrogen bath to trap any  $\text{CO}$  or  $\text{CH}_4$  that may be present. The volatile products in the respective silica gel traps were swept with helium into a Beckman GC-2 gas chromatograph for analysis. The analyzing column was made up of molecular sieve 4A and Beckman #70010 silica gel. No products were detected in the silica gel trap that was immersed in liquid nitrogen. Only one compound that had a retention time identical to the retention time for  $\text{CO}_2$  was detected in the silica gel trap immersed in the acetone- $\text{CO}_2$  bath.

Another test for  $\text{CO}$  was carried out in which aqueous succinic acid-1,4- $^{14}\text{C}$  was irradiated and analyzed. The gaseous sample was removed from the irradiation ampoule by vacuum transfer techniques.

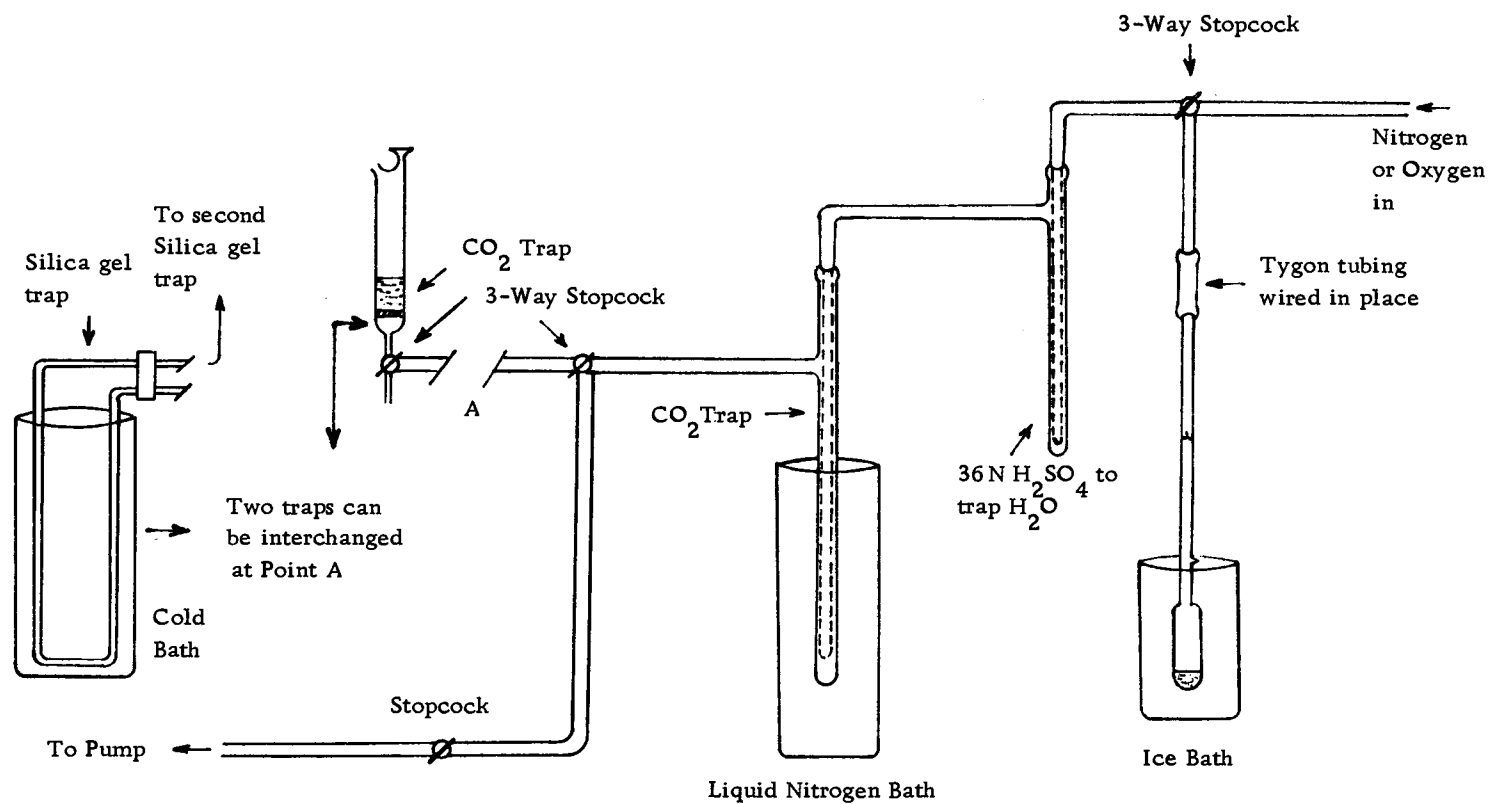


Figure 3. Apparatus for the trapping of gaseous products

This gaseous sample was passed through aqueous NaOH to remove  $^{14}\text{CO}_2$  and was subsequently passed through a tube containing CuO heated to  $520^\circ\text{C}$ . Copper oxide at this temperature will oxidize carbon monoxide to carbon dioxide. The effluent gas from the CuO tube was flushed through a  $\text{CO}_2$ -trap containing 2-amino ethanol-absolute ethanol. No radioactivity was detected in the  $\text{CO}_2$ -trap solution by means of liquid scintillation counting techniques. This observation confirms that CO is not a radiolytic product of succinic acid in dilute aqueous solution.

Irradiated aqueous solutions of succinic acid-1, 4- $^{14}\text{C}$  and -2, 3- $^{14}\text{C}$  were analyzed for the identification and estimation of carbon dioxide. The  $^{14}\text{CO}_2$  was transferred from the irradiation ampoule by vacuum transfer techniques to a carbon dioxide trap containing 10 ml of 2-amino ethanol-absolute ethanol solution. Then volumes of 0.1 or 1.0 ml, depending on the activity of the trap solution, were pipetted into a counting vial containing 5 ml of 2-amino ethanol-absolute ethanol and 10 ml of a solution of fluor in toluene. The counting of the radioactivity of these samples was carried out with a liquid scintillation spectrometer. For these analyses the photomultiplier voltage was set at 1000 volts, and the pulse discriminator was set at 100-1000 divisions. Toluene- $^{14}\text{C}$  purchased from the National Bureau of Standards was used to calibrate detection

efficiency of the counter for the analysis of the  $^{14}\text{CO}_2$  trapped in 2-amino ethanol-absolute ethanol.

#### Analysis of Steam Distillable Radiolytic Products

The identification of formic acid was achieved by utilizing the procedure of Osburn, Wood, and Werkman (40). This reaction is a specific oxidation reaction of formic acid to  $\text{CO}_2$  by the action of  $\text{HgO}$ . The volatile aqueous fractions that were trapped from freeze-dried, irradiated samples of succinic acid-1,4- $^{14}\text{C}$  and -2,3- $^{14}\text{C}$  were analyzed. The  $^{14}\text{CO}_2$  formed by the oxidation of formic acid was trapped and counted for the detection and estimation of formic acid.

For the identification of the steam distillable acids derived from irradiated succinic acid, several similarly irradiated samples were pooled together and distilled under reduced pressure. The distillate was made basic with ammonia, and this fraction was concentrated for paper chromatography. The three solvent systems reported by Osteux, Guillaume, and Laturaze (41), and the n-butanol: 1.5 N  $\text{NH}_4\text{OH}$  solvent system described by Reid and Lederer (3, p. 123) were used. All four solvent systems indicated the presence of propionic and acetic acids by means of one-dimensional co-chromatography.

The identity of glyoxal, as a radiolytic product of succinic acid,

was confirmed by isotopic dilution analysis. The presence of glyoxal was indicated by the chromatography of the 2,4-dinitrophenylhydrazone derivatives of the carbonyl containing radiolytic products of which the procedure will be described under the section on the analysis of the radiolytic keto acids. Non-radioactive 2,4-dinitrophenylhydrazone of glyoxal was added to the radioactive 2,4-dinitrophenylhydrazone of glyoxal which was isolated by paper chromatography from several irradiated solutions. This mixture was recrystallized seven times from boiling benzene until a constant specific activity of the crystalline compound was realized.

#### Analysis of Non-Volatile Radiolytic Products

The identification of the non-volatile acids was carried out by means of the technique of co-chromatography on paper. Several paper chromatographic solvent systems were used in order to identify the products resulting from the radiolysis experiments (12, 26, 30, 41, 42, 45, 46). Identical development patterns employing co-chromatography in three different two-dimensional solvent systems was considered as proof of the identity of the unknown acid.

The estimation of the non-volatile organic acids was carried out by means of liquid scintillation counting methods. One hundred  $\mu$ l of the irradiated sample were spotted on 17 x 17 inch sheets of Whatman 3MM filter paper. The samples were chromatographed in

two dimensions utilizing solvent systems 1 and 5 which were described by Hartley and Lawson (26). The autoradiograms of these paper chromatograms were obtained upon completion of a 5-day exposure to no-screen x-ray film.

In processing the radioactive acids on the paper chromatograms for liquid scintillation counting analysis, the acid spots on the paper were cut into small strips measuring 1.5 x 4 cm. As a guide, the autoradiogram was placed on the paper chromatogram in the exact orientation in which it was held during exposure in order to locate the radioactive acids on the paper chromatogram. Next a paper strip exactly the size desired for counting was placed over the spot, and holes were punched through the film and paper chromatogram at the corners of the paper strip with a dissecting needle. It was necessary to divide most of the acid spots into several strips in order to obtain a quantitative measure of the acid (Figures 4, 5, 6). The paper strips were cut from the paper chromatograms with small dissecting scissors. These chromatogram strips were analyzed in a liquid scintillation counter utilizing a counting vial equipped with a concentric well containing fluor-toluene solution, as described by Wang and Jones (49).

For the calibration of the detection efficiency of the counter for paper strip counting, a solution of glucose- $^{14}\text{C}$  of known radioactivity was spotted on several 1.5 x 4 cm strips of filter paper.



The counting of the radioactivity on the paper strips was carried out employing the method of Wang and Jones, with the photomultiplier voltage set at 975 volts and the pulse discriminator set at 100-1000 divisions.

#### Analysis of Radiolytically Formed Keto Acids

There was no evidence for the presence of keto acids in the initial analysis of the non-volatile products by paper chromatography. However, the possibility of keto acid formation was indicated with the identification of malic acid as a radiolytic product of succinic acid, since malic acid is known to yield oxaloacetic and pyruvic acids upon irradiation (44). Due to the instability of keto acids, it was necessary to convert these compounds into chemical derivatives prior to analysis. Therefore, 2.5 ml of 2,4-dinitrophenylhydrazine solution (0.1 g per 100 ml of 2 N HCl) were added to 0.5 ml of the irradiated succinic acid-1,4- $^{14}\text{C}$  and -2,3- $^{14}\text{C}$  solutions. The resultant 2,4-dinitrophenylhydrazones were extracted according to the procedure described by Rosan and Nieland (45). The hydrazones were spotted on Whatman 3MM filter paper, and were separated by means of co-chromatography in a solvent system of dioxane:0.5 N  $\text{NH}_4\text{OH}$ .

The remaining portion of the hydrazones were catalytically reduced with hydrogen gas to form the corresponding amino acids according to the procedure reported by Meister and Abendschein (36).

The reduced hydrazones were co-chromatographed with authentic samples of amino acids. Oxaloacetic, mesoxalic, glyoxylic, and pyruvic acids were identified by co-chromatography of their hydrazones and amino acids.

For the estimation of the keto acids, these compounds were converted to their 2,4-dinitrophenylhydrazones and were extracted from the irradiated solution. The extracted hydrazones were dissolved in 0.5 ml of ethyl acetate, and 200  $\mu$ l of the extract were spotted on Whatman 3MM filter paper. The solvent system used for the development of these paper chromatograms was dioxane:1.5 N  $\text{NH}_4\text{OH}$  (4:1). The radioactive hydrazone spots on the paper chromatograms were cut into 1.5 x 4 cm strips for liquid scintillation counting in the same manner as the non-volatile acid samples were prepared for counting. The counting of the radioactivity of the hydrazones on paper was carried out identical to the procedure used in the estimation of the non-volatile radiolytic products.

Insofar as calibration of counter efficiency for counting of paper strips containing the hydrazones was concerned, the procedures for calibration of counter efficiency for counting of the non-volatile acids on paper were carried out. Since the hydrazones impart a yellow color to the paper strip, experiments were carried out to determine if this color interfered with the counting of the radioactivity on the paper strip by means of liquid scintillation counting. The

findings of these experiments showed that color interference was negligible at the chemical levels that the hydrazones were counted. Also, leaching of the hydrazones from the paper strip by toluene in the fluor solution can affect the counting of the hydrazones. However, leaching was determined to be negligible for the period that the hydrazone was in contact with the toluene solvent during the counting of the radioactivity on the paper strips. Therefore, glucose- $U^{14}C$  of known radioactivity was used for the calibration of the counter, since the effect of color and leaching was determined to be negligible.

## RESULTS

The yield of the radiolysis products of succinic acid-1,4- $^{14}\text{C}$  and -2,3- $^{14}\text{C}$  in water irradiated with a cumulative dose of  $1.25 \times 10^{20}$  ev per ml of gamma radiation are given in Table I.

Data are given for experiments performed with oxygenated and oxygen-free systems. Table II contains the G-values for some of the non-volatile radiolytic products of higher yield. The G-value is defined as the number of molecules undergoing change per 100 ev of energy absorbed by the solution.

The paper chromatogram illustrating the separation of radiolysis products of succinic acid in the oxygen-free system is given in Figure 4. Figure 5 illustrates the paper chromatographic separation of the radiolysis products of succinic acid in the oxygenated system. The solvent systems used for the two-dimensional paper chromatography for Figures 4 and 5 were solvents systems 1 and 5 reported by Hartley and Lawson (26). The paper chromatographic separation of the extracted 2,4-dinitrophenylhydrazones of the radiolysis products of succinic acid in oxygenated and oxygen-free systems are given in Figure 6. The paper chromatogram of Figure 6 was developed in a solvent system of dioxane: 1.5 N  $\text{NH}_4\text{OH}$  (4:1). The radioactive spots that are not numbered represent contamination from other organic acids during the extraction process.

Table I. Radiochemical Yields of Radiolytic Products  
of Specifically Labeled Succinic Acid- $^{14}\text{C}$

Compound	Percent of Initial Radioactivity			
	Oxygenated System		Oxygen-Free System	
	Succinic Acid $^{14}\text{C}$ -1,4-	Succinic Acid $^{14}\text{C}$ -2,3-	Succinic Acid $^{14}\text{C}$ -1,4-	Succinic Acid $^{14}\text{C}$ -2,3-
Succinic Acid	58.0	55.5	74.0	72.0
1,2,3,4-Butane- tetracarboxylic Acid	--	--	4.3	4.4
Malonic Acid	5.6	11.8	2.4	4.9
Malic Acid	3.0	3.3	0.8	1.1
1,2,4-Butane- tricarboxylic Acid	--	--	0.7	1.1
Tricarballic Acid	--	--	0.5	0.7
Glutaric Acid	--	--	0.3	0.6
Adipic Acid	--	--	0.2	0.5
Glyoxylic Acid	0.2	0.7	0.03	0.3
Oxaloacetic Acid	0.6	1.0	0.1	0.2
Pyruvic Acid	0.4	1.0	0.1	0.1
Oxalic Acid	0.2	1.1	--	--
Mesoxalic Acid	0.0	0.02	--	--
Formic Acid	0.01	0.6	0.01	0.1
Carbon Dioxide	6.3	1.0	1.7	0.1

Products not estimated were propionic acid, acetic acid, glyoxal, and the unidentified compounds

Cumulative Dose-- $1.25 \times 10^{20}$  ev/ml

Concentration--0.005 M Succinic Acid

Table II. G-Values of Some Non-Volatile Compounds Associated with the Radiolysis of Succinic Acid in Oxygenated and Oxygen-Free Aqueous Solutions

Compound	G-Values	
	Oxygenated System	Oxygen-Free System
Succinic Acid	-1.1	-0.7
Malonic Acid	0.3	0.1
1,2,3,4-Butane-tetracarboxylic Acid	---	0.03

Cumulative Dose-- $1.25 \times 10^{20}$  ev/ml

Concentration--0.005 M Succinic Acid

G-value is defined as the number of molecules undergoing change per 100 electron volts of energy absorbed.

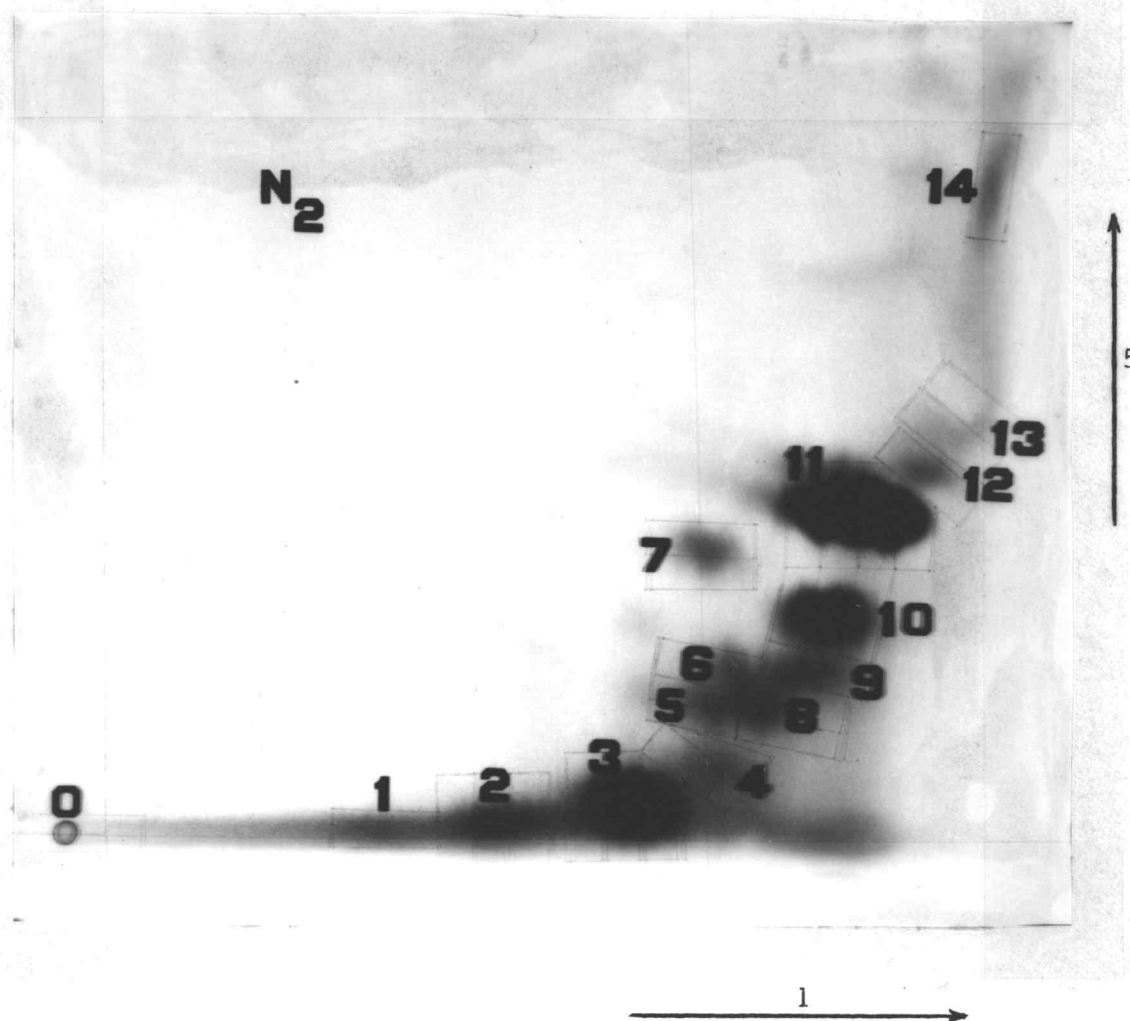


Figure 4. Separation of the radiolysis products of 0.005 M succinic acid in the oxygen-free system

Cumulative Dose- $1.87 \times 10^{20}$  ev/ml

1,2,3,4-Butane-tetracarboxylic Acid (3)

1,2,4-Butane-tricarboxylic Acid (8)

Tricarballic Acid (5)

Adipic Acid (13)

Glutaric Acid (12)

Propionic Acid (14)

Acetic Acid (14)

Malonic Acid (10)

Succinic Acid (11)

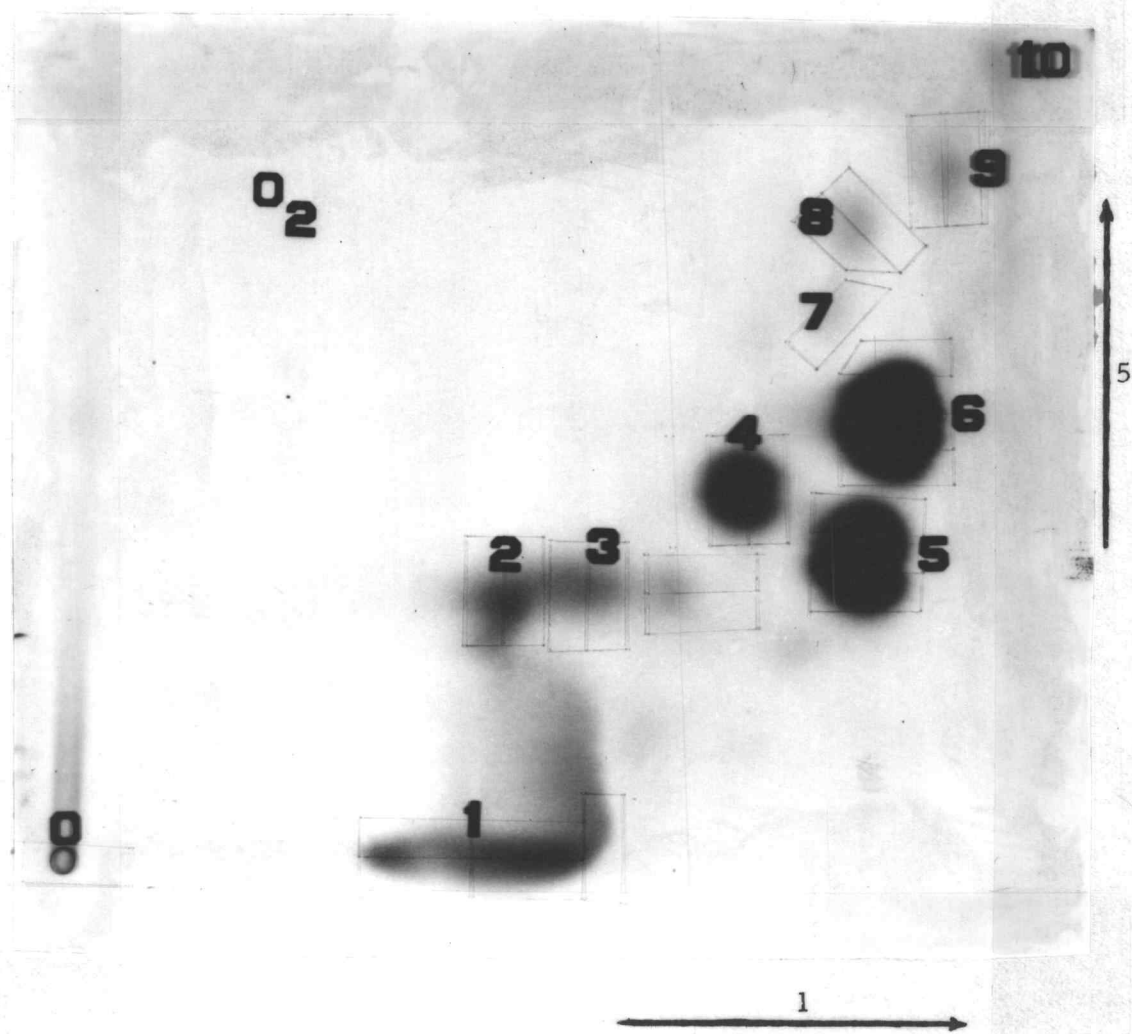


Figure 5. Separation of the radiolysis products of 0.005 M succinic acid in the oxygenated system

Cumulative Dose -  $1.87 \times 10^{20}$  ev/ml

- Succinic Acid (6)
- Oxalic Acid (1)
- Malic Acid (4)
- Malonic Acid (5)
- Propionic Acid (10)
- Acetic Acid (10)



Figure 6. Paper chromatographic separation of the extracted 2,4-dinitrophenylhydrazones of the radiolysis products of 0.005 M succinic acid in oxygenated and oxygen-free systems

Cumulative Dose -  $1.87 \times 10^{20}$  ev/ml

Solvent System - Dioxane:0.5 N  $\text{NH}_4\text{OH}$  (4:1)

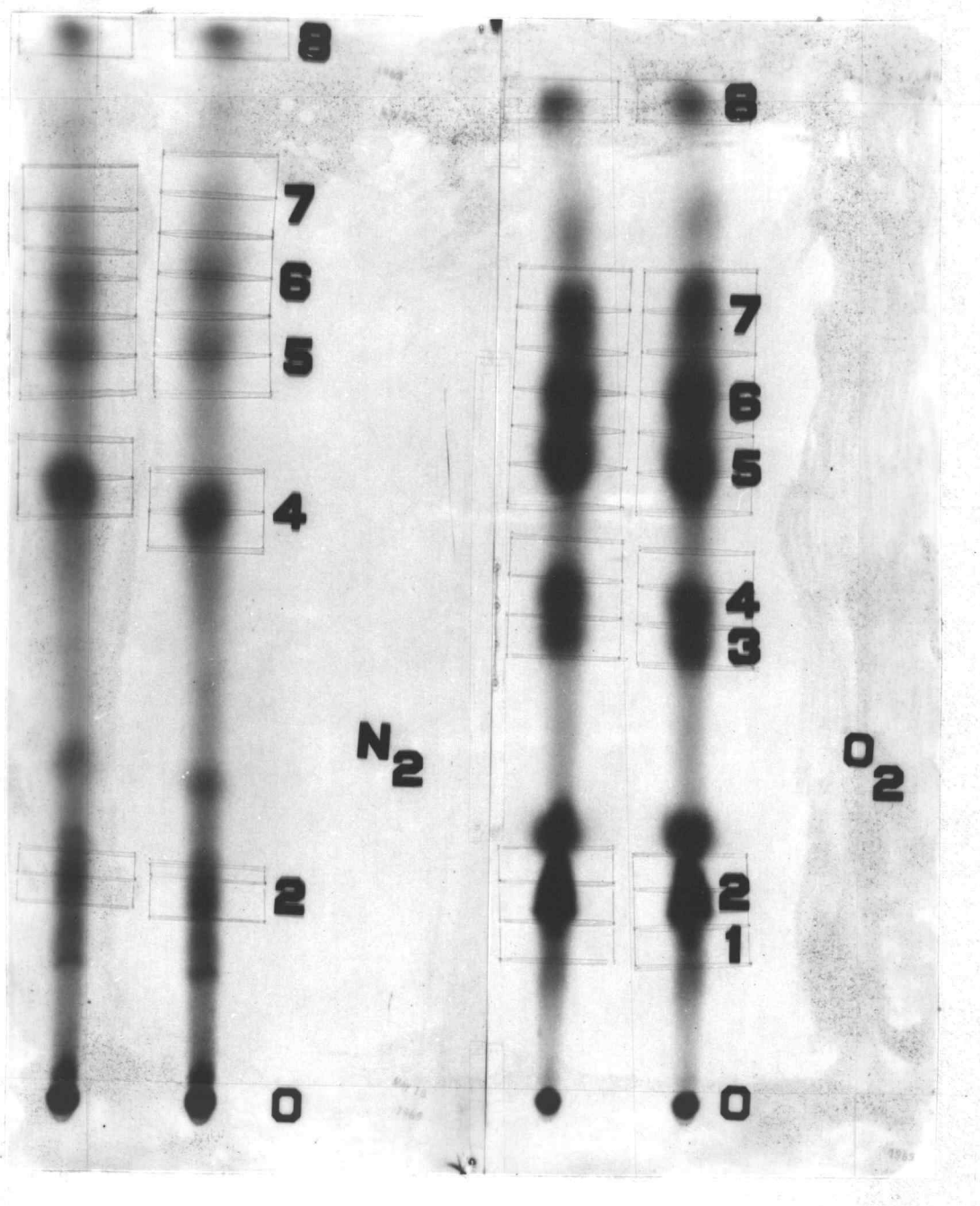
Mesoxalic Acid (1)

Oxaloacetic Acid (2)

Glyoxylic Acid (3 & 5)

Pyruvic Acid (4 & 6)

Glyoxal (8)



In Figure 7 and Table III are given the increase of yields of radiolytic  $^{14}\text{CO}_2$  from specifically labeled succinic acid- $^{14}\text{C}$  solutions with increasing cumulative dose. Figure 8 and Table IV show the degradation of succinic acid with increasing cumulative radiation dose for oxygenated and oxygen-free systems. The ordinates of the graph are expressed in both number of unreacted molecules of succinic acid per ml and fraction of unreacted succinic acid. The values in molecules per ml are used for the calculation of G-values. The yields of malic acid and 1, 2, 3, 4-butane-tetracarboxylic acid with increasing cumulative dose are given in Figure 9 and Table V.

The yields of malonic acid from radiolysis of succinic acid with increasing cumulative dose are shown in Figure 10 and Table IV. In Table VI the yields of  $^{14}\text{C}$ -labeled malonic acid from the radiolysis of  $^{14}\text{C}$  specifically labeled succinic acid are given. Since malic acid is probably the primary oxidation product formed by the radiolysis of succinic acid, malic acid may have been a key intermediate for malonic acid formation. To test this hypothesis, the radiolysis of aqueous malic acid-3- $^{14}\text{C}$  and -4- $^{14}\text{C}$  were studied and the yields of the radiolytic products under oxygenated and oxygen-free systems are given in Table VII.

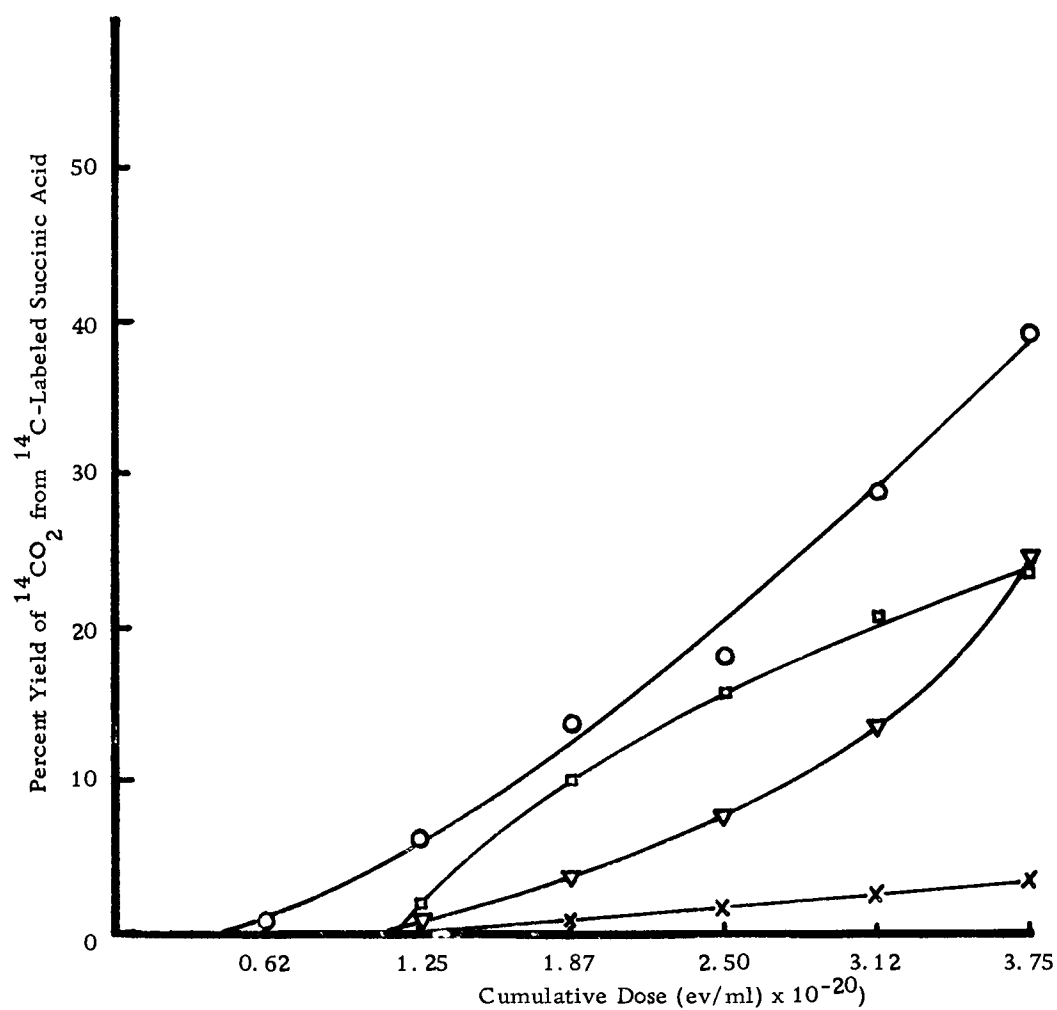


Figure 7. Yields of  $^{14}\text{CO}_2$  from specifically labeled succinic acid- $^{14}\text{C}$  irradiated in aqueous solution

- ▼ Succinic acid-2,3- $^{14}\text{C}$  in an oxygenated system
- Succinic acid-1,4- $^{14}\text{C}$  in an oxygenated system
- × Succinic acid-2,3- $^{14}\text{C}$  in an oxygen-free system
- Succinic acid-1,4- $^{14}\text{C}$  in an oxygen-free system

Table III. Yield of  $^{14}\text{CO}_2$  from Radiolysis of  $^{14}\text{C}$ -Labeled Succinic Acid

Cumulative Dose $\times 10^{-20}$ ev/ml	Oxygenated System		Oxygen-Free System	
	Succinic Acid -1,4- $^{14}\text{C}$	Succinic Acid -2,3- $^{14}\text{C}$	Succinic Acid -1,4- $^{14}\text{C}$	Succinic Acid -2,3- $^{14}\text{C}$
	Percent Yield of $^{14}\text{CO}_2$			
0.62	0.7	0.0	0.0	0.0
1.25	6.3	1.0	1.7	0.1
1.87	13.8	3.3	10.2	0.7
2.50	18.0	7.5	15.9	1.6
3.12	28.8	13.1	20.0	2.1
3.75	39.2	24.6	23.6	3.3

Concentration--0.005 M Succinic Acid

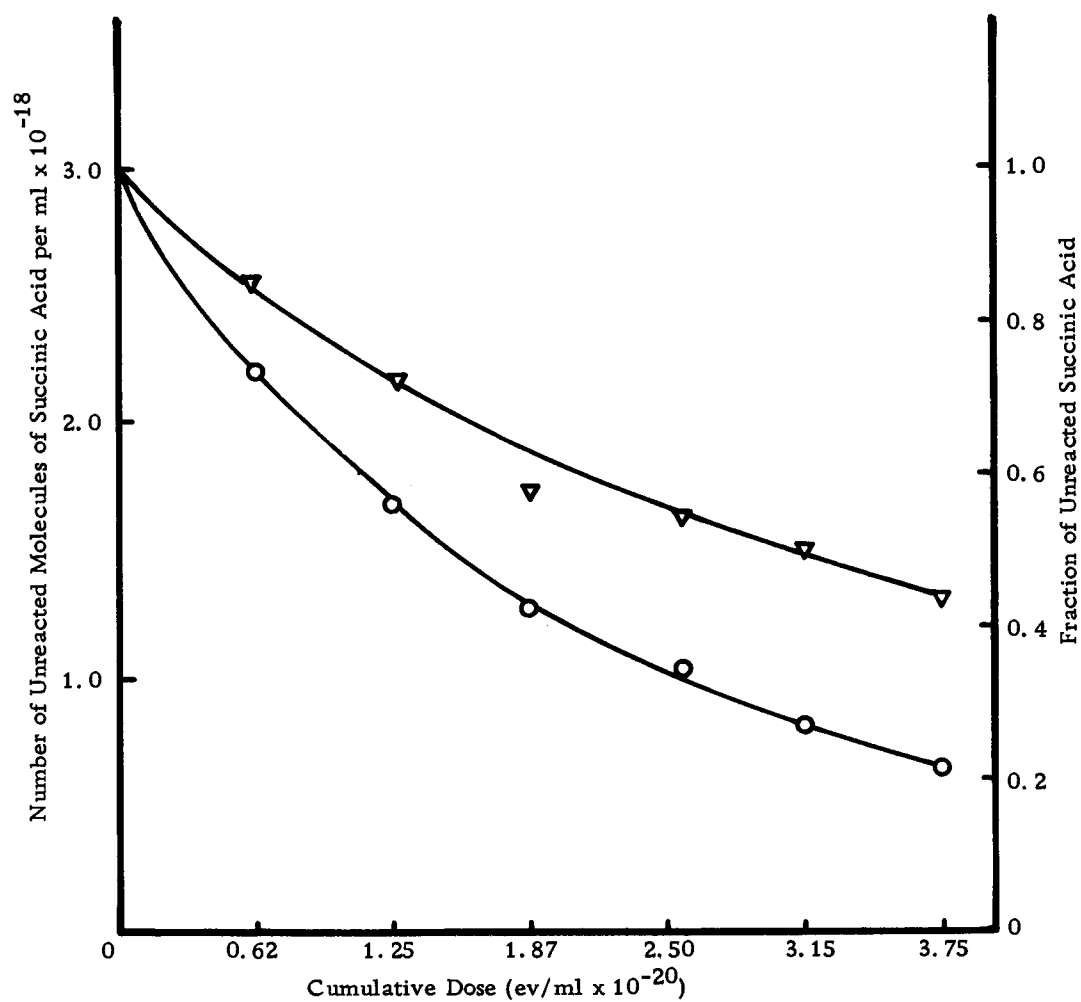


Figure 8. Degradation of succinic acid with increasing cumulative dose

- Succinic acid in an oxygenated system
- ▼ Succinic acid in an oxygen-free system

Table IV. Radiolytic Degradation of Succinic Acid and Formation of Malonic Acid in Oxygenated and Oxygen-Free Aqueous Systems

Cumulative Dose $\times 10^{-20}$ ev/ml	Succinic Acid		Malonic Acid	
	Number of Molecules/ml Unreacted $\times 10^{-18}$		Number of Molecules/ml Formed $\times 10^{-17}$	
	Oxygenated System	Oxygen-Free System	Oxygenated System	Oxygen-Free System
0.62	2.1	2.6	1.5	0.6
1.25	1.7	2.2	3.4	1.1
1.87	1.3	1.7	4.9	1.5
2.50	1.0	1.6	6.7	1.9
3.12	0.8	1.5	7.8	2.3
3.75	0.7	1.3	9.3	2.7

Concentration--0.005 M Succinic Acid

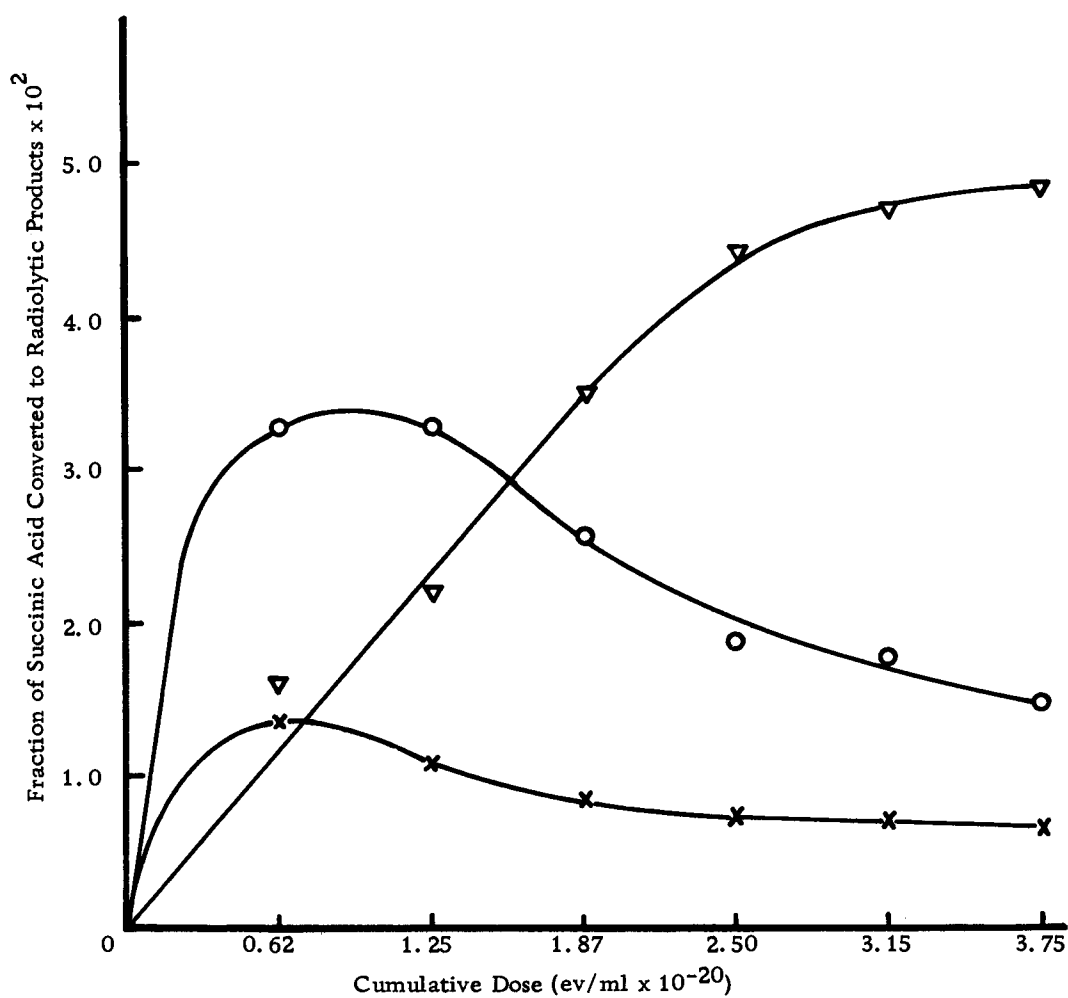


Figure 9. Product yields from the radiolysis of succinic acid with increasing cumulative dose

- ▽ 1,2,3,4-Butane-tetracarboxylic acid in an oxygen-free system
- Malic acid yield in an oxygenated system
- × Malic acid yields in an oxygen-free system



Table V. Radiolytic Formation of Malic Acid and 1, 2, 3, 4-Butane-tetracarboxylic Acid from Succinic Acid in Oxygenated and Oxygen-Free Aqueous Systems

Cumulative Dose  x 10 <sup>-20</sup> ev/ml	Malic Acid		1, 2, 3, 4-Butane-tetracarboxylic Acid	
	Number of Molecules/ml Formed x 10 <sup>-16</sup>			
	Oxygenated System	Oxygen-Free System	Oxygenated System	Oxygen-Free System
0.62	9.9	4.1	0.0	2.6
1.25	9.9	3.2	0.0	3.3
1.87	7.8	2.6	0.0	5.3
2.50	5.8	2.3	0.0	6.7
3.12	5.5	2.2	0.0	7.1
3.75	4.5	2.0	0.0	7.3

Concentration--0.005 M Succinic Acid

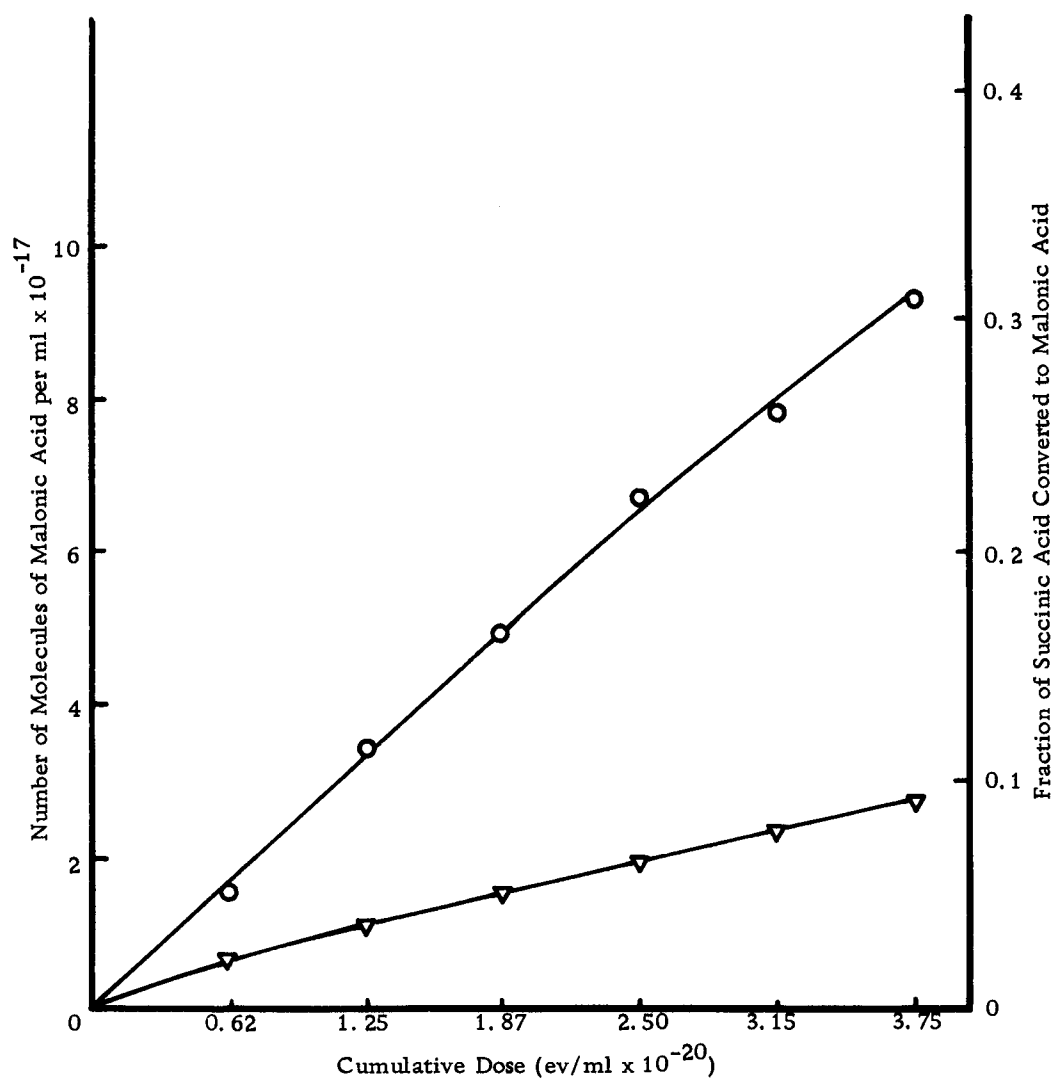


Figure 10. Yield of malonic acid with increasing cumulative dose

○ Malonic acid formation in an oxygenated system

▼ Malonic acid formation in an oxygen-free system

Table VI. Yield of Malonic Acid by the Radiolysis of Specifically Labeled Succinic Acid- $^{14}\text{C}$

Initial Compound	Percent Radiochemical Yield of Malonic Acid
	Oxygen-Free System
Succinic Acid-1,4- $^{14}\text{C}$	2.4
Succinic Acid-2,3- $^{14}\text{C}$	4.9
	Oxygenated System
Succinic Acid-1,4- $^{14}\text{C}$	5.6
Succinic Acid-2,3- $^{14}\text{C}$	11.8

Cumulative Dose -  $1.25 \times 10^{20}$  ev/ml

Table VII. Radiolytic Degradation Products of Malic Acid in Oxygenated and Oxygen-Free Aqueous Systems

Initial Compound	Percent Radiochemical Yield			
	Malonic Acid	Oxaloacetic Acid	Pyruvic Acid	Malic Acid
Oxygenated System				
Malic Acid-3- <sup>14</sup> C	30.1	1.4	1.2	23.8
Malic Acid-4- <sup>14</sup> C	26.1	1.2	0.0	24.8
Oxygen-Free System				
Malic Acid-3- <sup>14</sup> C	5.4	0.9	3.6	13.6
Malic Acid-4- <sup>14</sup> C	5.3	0.7	0.0	12.8

Cumulative Dose -  $1.25 \times 10^{20}$  ev/ml

Concentration - 0.005 M Malic Acid

Table VII. Radiolytic Degradation Products of Malic Acid in Oxygenated and Oxygen-Free Aqueous Systems

Initial Compound	Percent Radiochemical Yield			
	Malonic Acid	Oxaloacetic Acid	Pyruvic Acid	Malic Acid
Oxygenated System				
Malic Acid-3- <sup>14</sup> C	30.1	1.4	1.2	23.8
Malic Acid-4- <sup>14</sup> C	26.1	1.2	0.0	24.8
Oxygen-Free System				
Malic Acid-3- <sup>14</sup> C	5.4	0.9	3.6	13.6
Malic Acid-4- <sup>14</sup> C	5.3	0.7	0.0	12.8

Cumulative Dose -  $1.25 \times 10^{20}$  ev/ml

Concentration - 0.005 M Malic Acid

## DISCUSSION

Comparison of Radiolytic Products in Oxygenated  
and Oxygen-Free Systems

The results presented in Table I indicate that in radiolysis of aqueous succinic acid, products of greater molecular weight than succinic acid were observed exclusively in the oxygen-free system, while oxidation products were observed in both oxygenated and oxygen-free systems with greater quantity in the oxygenated system.

Since the mechanism for the formation of all the radiolytic products is unknown, the radiochemical yield could not be correlated to the molecular yield in all cases. Therefore the G-values were only calculated for some non-volatile radiolytic products of succinic acid in oxygenated and oxygen-free aqueous solutions (Table II), in which the molecular chain remained intact, or where just one carbon atom was removed from the four carbon atom chain of succinic acid. The calculations of G-values were made from the data of Tables IV and V at a cumulative dose of  $1.25 \times 10^{20}$  ev/ml. The G-value for degradation of succinic acid is higher for the oxygenated system than the oxygen-free system. This fact indicates that oxygen plays an important role in the radiolytic degradation of succinic acid. The G-values for malonic acid and 1,2,3,4-butane-tetracarboxylic acid are G-values of formation, since the early phase of the curves in

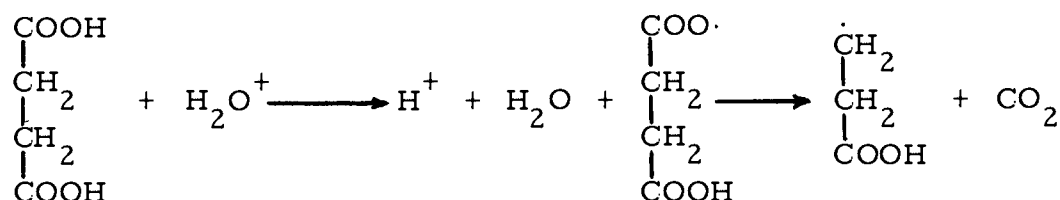
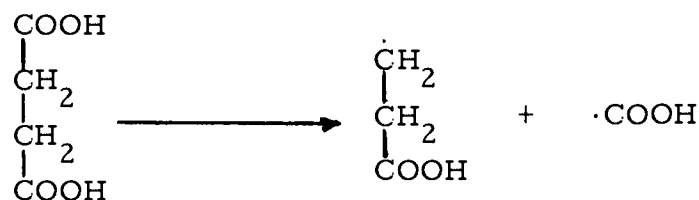
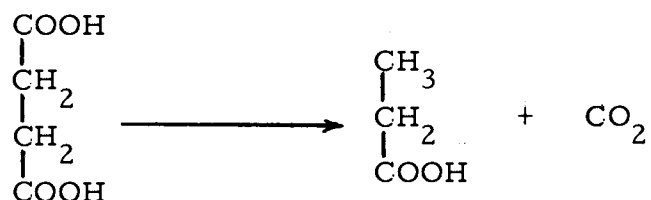
Figures 9 and 10 for these two compounds rise linearly with increasing cumulative dose.

Some of the products that were detected by paper chromatography and autoradiography were not identified (Figures 4, 5, 6). Although efforts were made in this regard, product identification was hindered because, in some cases, the product yields were very low (< 1 percent) or the products decomposed during the isolation steps. There was no indication of any dehydrogenation products of succinic acid such as fumaric and maleic acids. This finding may be accounted for by the results reported by Nofre, Le Roux, and Cier (39) on the radiolysis of aqueous solutions of fumaric acid. They found that  $\text{H}\cdot$  and  $\cdot\text{OH}$  derived from radiolysis of water, add readily across the double bond of fumaric acid, leading to the formation of succinic, malic, and tartaric acids.

The variation of keto compounds, as identified by chromatographic separation of their 2,4-dinitrophenylhydrazone derivatives, was the same for both the oxygenated and the oxygen-free systems except for the detection of mesoxalic acid in the oxygenated system (Figure 6). The multiple spots observed for the 2,4-dinitrophenylhydrazone derivatives were due to the cis and trans isomers of these compounds.

The decarboxylation of organic acids by the action of radiation has been demonstrated by other workers, and the mechanism involved could be either a radical process or a molecular process

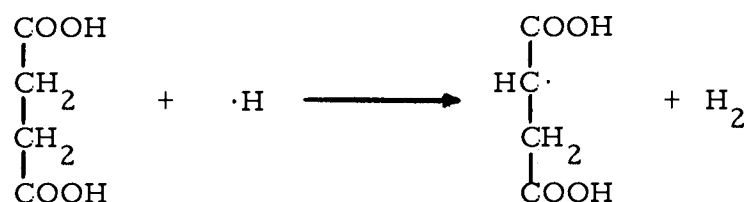
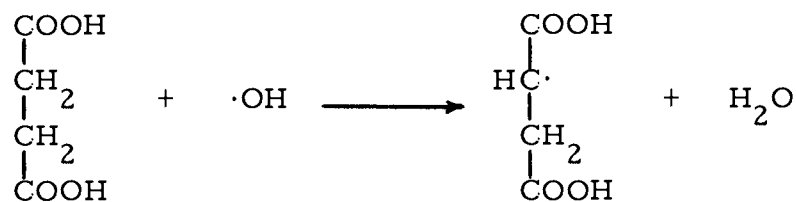
(15, 27, 32). Since propionic acid and carbon dioxide have been identified as radiolytic products, the decarboxylation of succinic acid may have taken place yielding the propionic acid free radical or propionic acid.



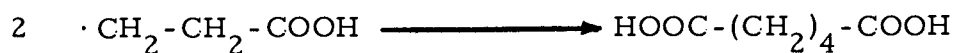
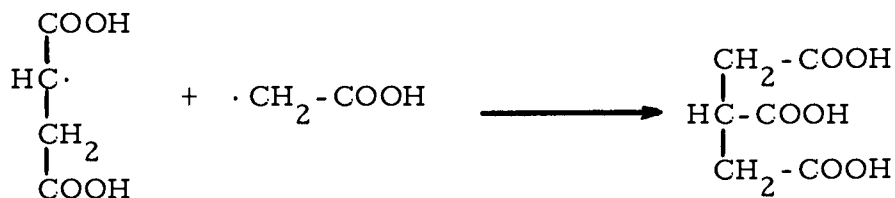
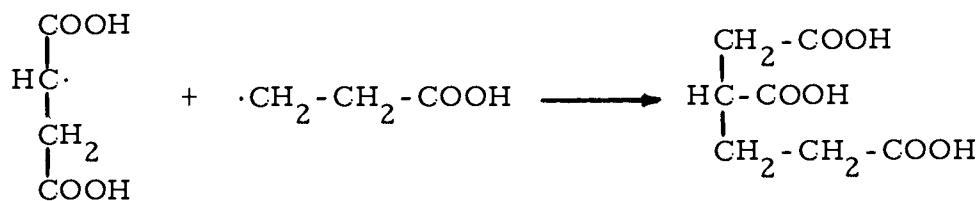
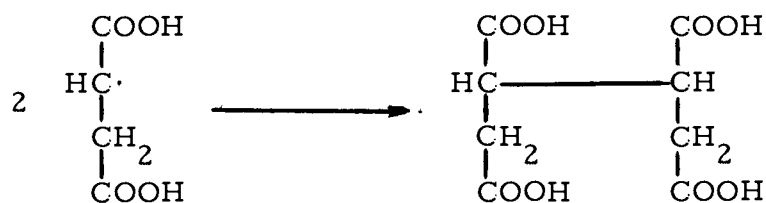
This decarboxylation of succinic acid cannot be an important process because the yields of  $^{14}\text{CO}_2$  in Figure 7 at low cumulative doses are very low, and yet the degradation of succinic acid at these doses yielding other radiolytic products is much higher (Figure 8). Consequently, the major mechanism of succinic acid radiolysis probably involves the formation of  $\text{HOOC}-\dot{\text{C}}\text{H}-\text{CH}_2\text{COOH}$ .

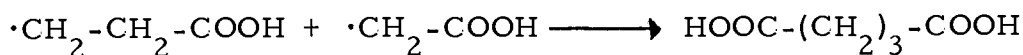
It appears that initial interaction of the free radicals derived from radiolysis of water with succinic acid gives rise to the formation of the succinic acid free radical.





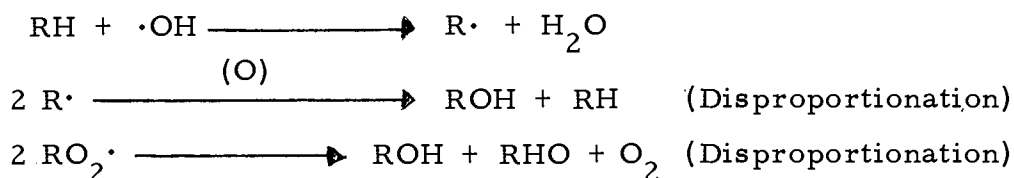
Once the organic free radicals are formed, radical coupling will occur in the absence of oxygen leading to the formation of compounds having molecular weights much greater than that of succinic acid, thus:





The rate of formation of the dimer of succinic acid, 1,2,3,4-butane-tetracarboxylic acid, is a linear function with increasing cumulative dose which subsequently levels off to a steady level as shown in Figure 9. The  $\cdot\text{CH}_2-\text{CH}_2-\text{COOH}$  can result from the decarboxylation of succinic acid, while the  $\cdot\text{CH}_2\text{COOH}$  may be formed from the decarboxylation of malonic acid and by oxidation of acetic acid resulting from pyruvic acid decarboxylation.

The disappearance of succinic acid with increasing cumulative dose qualitatively follows the same course in both oxygenated and oxygen-free systems (Figure 8). The results can be interpreted assuming that the initial steps of radiolysis of succinic acid were probably the same in either system. In the oxygen-free system radical coupling occurs leading to the formation of dimeric and polymeric molecules, in addition to the formation of oxidation products. In the presence of oxygen, reactions of oxygen with the initial radicals can lead to an approximate doubling of the rate of radiolysis of succinic acid, while still forming the same oxidation products.

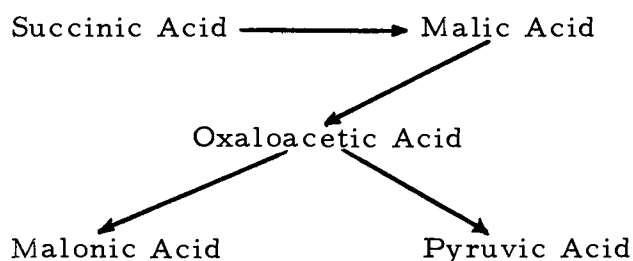


This change in yield must be attributed to the presence in solution of

molecular oxygen. The degradation products resulting from the oxidation reactions are the same in the oxygenated and oxygen-free systems, however the yields are greater in the oxygenated system. Therefore, the major sequence of reactions leading to the degradation products may be the same in both systems.

#### Mechanism for the Formation of Oxidation Products

The main oxidation products derived from the radiolysis of aqueous solutions of succinic acid are malic, malonic, oxaloacetic, and pyruvic acids. The general sequence of the oxidation reactions for both the oxygenated and oxygen-free systems appears to be the following.

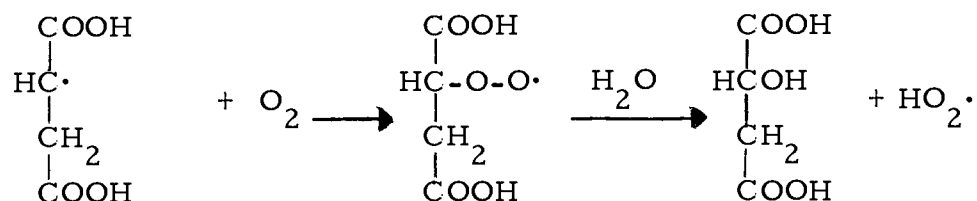


#### Mechanism for Malic Acid Formation

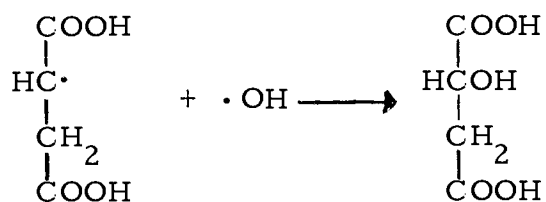
Malic acid appears to be the first four carbon acid resulting from the radiolysis of succinic acid, since hydroxylation is usually the first oxidation step in radiolysis of hydrocarbon chains. The results of Figure 9 show a very rapid formation of malic acid at low cumulative doses, but the concentration of malic acid drops very

rapidly due to the high sensitivity of this acid to radiolysis.

In the oxygenated system, the mechanism for malic acid formation involves an organic peroxide intermediate which can be hydrolyzed to yield malic acid (43, 48).



The formation of malic acid in the oxygen-free system cannot be explained by a coupling of a hydroxyl radical (21, 22) with the succinic acid free radical. The reaction probability of the  $\cdot\text{OH}$  is much less than that of molecular oxygen.



Therefore, the hydroxylation of succinic acid may result to the greatest extent from trace quantities of hydrogen peroxide that is formed during the radiolysis of oxygen-free water.

With the consumption of  $\text{H}_2\text{O}_2$ , the mechanism that takes place will be identical to the mechanism for malic acid formation in the oxygenated system.

#### Mechanism for Malic Acid Oxidation

In the presence of oxygen, malic acid is known to be oxidized to oxaloacetic acid by ionizing radiation according to the results of Putney and Pratt (44). This result may also be explained by utilizing mechanisms that Garrison et al. (19) used to explain the oxidation reactions that occurred when acetic acid was irradiated in the presence of oxygen.

In the absence of oxygen, the proposed mechanism for the oxidation of lactic acid to pyruvic acid was by disproportionation of two lactic acid free radicals (33). In turn malic acid could yield oxaloacetic acid by this mechanism. On the other hand, malic acid may be oxidized to oxaloacetic acid by the mechanism described by Jayson, Scholes, and Weiss (31) for the oxidation of ethanol to acetaldehyde. The mechanism for oxaloacetic acid formation may originate from the same radical in both oxygenated and oxygen-free systems.

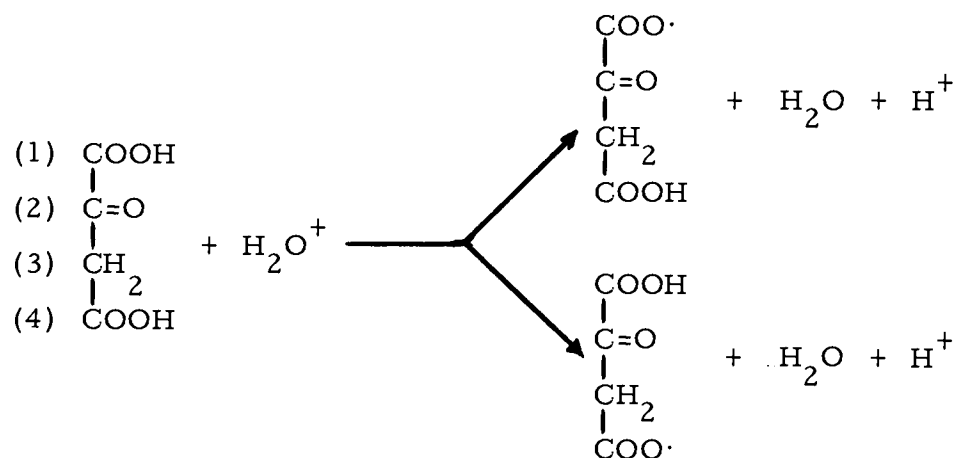
### Mechanism for Malonic Acid Formation

Malonic acid was found to be formed in substantial quantities under both oxygenated and oxygen-free conditions (Figure 10). Due to the stability of malonic acid toward radiolysis, the yield of this acid increased linearly with cumulative dose. From the results in Table VI, one notes that in both the oxygenated and oxygen-free systems the radiochemical yield of malonic acid was approximately one-half the value from succinic acid-1,4- $^{14}\text{C}$  as that from succinic acid-2,3- $^{14}\text{C}$ . This would be the expected result if the carboxyl carbon was lost as  $\text{CO}_2$  during this process. Hence, the formation of malonic acid appears to be due to the loss of one of the carboxyl groups as  $\text{CO}_2$  with a subsequent oxidation of the next carbon atom in the chain into a carboxyl group. The electron paramagnetic resonance studies of a single crystal of succinic acid showed that the main stable radical formed upon radiolysis of this crystal was  $\text{HOOC}-\dot{\text{C}}\text{H}-\text{CH}_2\text{COOH}$ , which supports the contention that malic acid was one of the first acids formed from succinic acid. As reported by Putney and Pratt (44) when malic acid was x-irradiated at low dosages in aqueous, oxygenated solutions, these workers found that approximately 65 percent of the malic acid was oxidized to oxaloacetic acid. The high yield of malonic acid from radiolysis of malic acid (Table VII) gives good evidence that malic acid probably was an intermediate

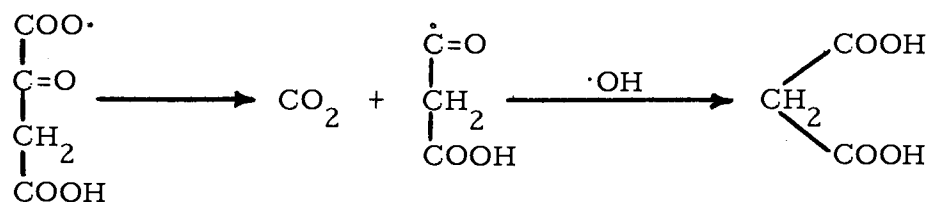
in the formation of malonic acid from radiolysis of succinic acid.

From the investigation of Putney and Pratt, malic acid is known to be oxidized to oxaloacetic acid in high yield; therefore, malonic acid appears to result from an oxidative decarboxylation of oxaloacetic acid.

A possible mechanism for the formation of malonic acid may be the mechanism proposed by Hayon and Weiss (27) in which the positive polaron attacks the carboxyl group of oxaloacetic acid.



The positive polaron may attack either the C-1 or C-4 carboxyl group yielding a carboxyl free radical. This carboxyl free radical intermediate is identical to the radical intermediate in the Kolbe electrolysis reaction. This intermediate will decompose to yield  $\text{CO}_2$  and an organic free radical. In the case of C-1 decarboxylation, this radical could couple with a  $\cdot\text{OH}$  to yield malonic acid.

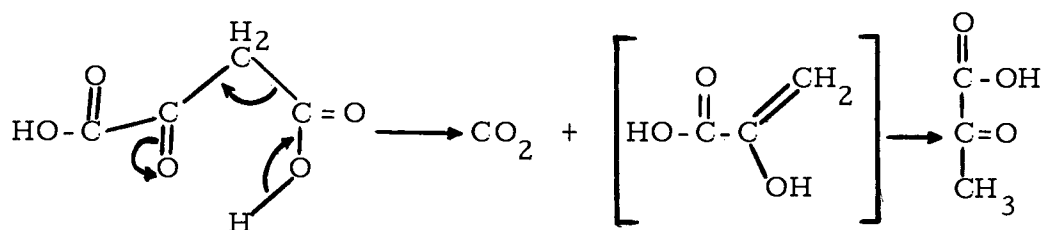


However, the C-1 carboxyl group of oxaloacetic acid would probably be found in the ionized state in aqueous solution. Therefore, the positive polaron would preferentially attack the hydrogen atom of the C-4 carboxyl group leading to C-4 decarboxylation. This mechanism for malonic acid formation would also be unfavorable, because the  $\cdot\text{OH}$  is not an efficient scavenger for organic free radicals.

Another mechanism in which malonic acid could be formed is by carboxylation of acetic acid in an oxygen-free system. Radiation induced carboxylation reactions of acetic acid and other monocarboxylic acids were reported by Liebster, Kopoldova and Babicky (35). These investigators irradiated 0.05 M acetic acid in the presence of 0.02 M  $\text{NaH}^{14}\text{CO}_3$ , however only 0.05 percent of the total radioactivity was recovered as radioactive malonic acid. Consequently, this mechanism cannot account for the high yield of malonic acid resulting from succinic acid in the irradiation experiments carried out in both oxygenated and oxygen-free systems.

In aqueous solution at room temperature, oxaloacetic acid decarboxylates at the C-4 position leading to the formation of pyruvic acid. This reaction takes place through a cyclic six membered ring.

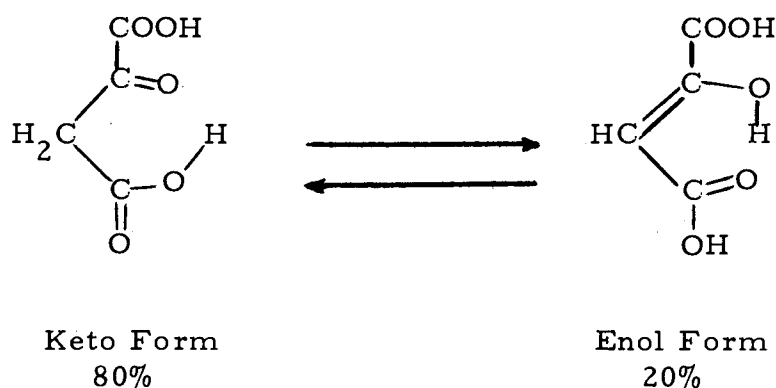




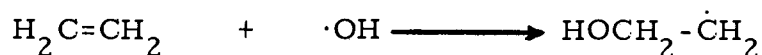
On the other hand, if oxaloacetic acid were to decarboxylate at the C-1 position accompanying an oxidation process, the resultant product would be malonic acid. In 1858 Dessaignes (8) oxidized malic acid to malonic acid by the action of aqueous potassium dichromate. Nofre et al. (39) oxidized succinic acid to malic acid and malonic acid by the action of Fenton's reagent. In view of these results, oxidative decarboxylation at the C-1 position of oxaloacetic acid can take place, since the oxidation of malic acid can be the source of oxaloacetic acid.

This mechanism involving C-1 decarboxylation is further supported by the data in Tables VI and VII. The recoveries of  $^{14}\text{C}$ -radioactivity in malonic acid were almost identical for malic acid-3- $^{14}\text{C}$  and -4- $^{14}\text{C}$ . This confirms that no C-4 decarboxylation occurred in the mechanism for malonic acid formation. Concurrently, the pyruvic acid derived from radiolysis of malic acid-4- $^{14}\text{C}$  was found not to be radioactive. This result illustrates that a portion of oxaloacetic acid could also undergo decarboxylation at the C-4 position to yield pyruvic acid.

As an alternative mechanism, the oxidative decarboxylation of oxaloacetic acid may involve the enol form of oxaloacetic acid. Meyer (37) determined by bromine water titration that 20 percent of oxaloacetic acid in aqueous solution exists in the enol form.

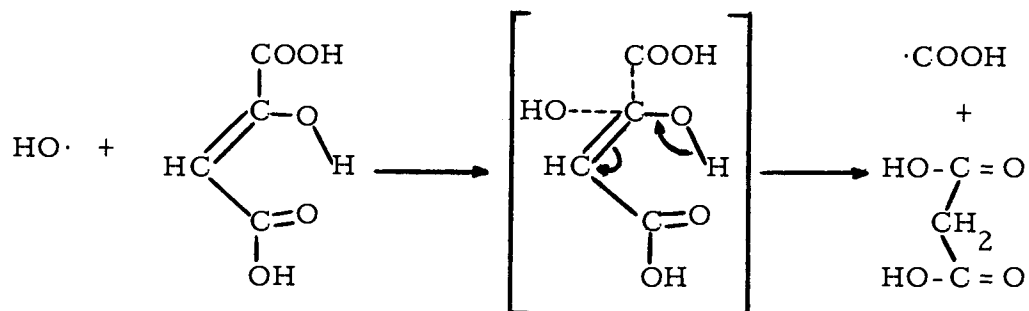


Nofre et al. (38) studied the effects of the  $\cdot\text{OH}$  from Fenton's reagent and from photolized, aqueous  $\text{H}_2\text{O}_2$  on the double bond of maleic and fumaric acids. The  $\cdot\text{OH}$  from these reagents was found to add very readily across the double bond of these acids. Cullis, Francis, and Swallow (7) reported a mechanism for the addition of the  $\cdot\text{OH}$  across a double bond in the radiolysis of aqueous solutions of ethylene yielding the  $\beta$ -hydroxyethyl radical.



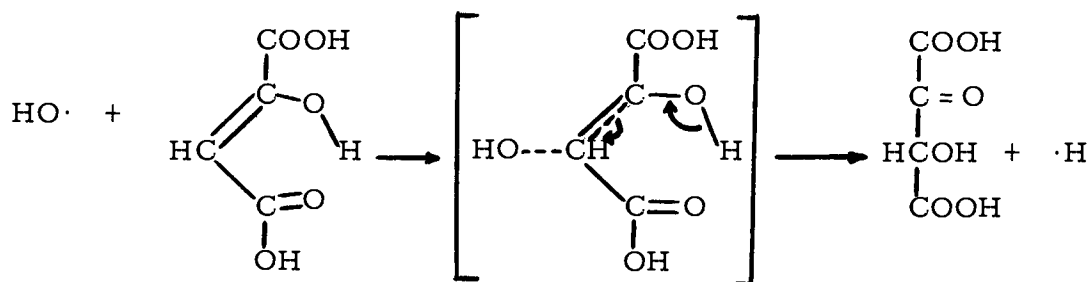
Therefore, one could postulate an initial attack of a  $\cdot\text{OH}$  on the double bond of the enol form of oxaloacetic acid leading to an addition reaction across the double bond at the C-2 position. But in this concerted mechanism, the adduct can be readily converted back to the

more stable keto form with the subsequent loss of the carboxyl radical to yield malonic acid.



This mechanism appears to be the most favorable of the proposed mechanisms for malonic acid formation resulting from the radiolysis of succinic acid.

Alternatively, the  $\cdot\text{OH}$  may add at the C-3 position rather than the C-2 position of the enol form of oxaloacetic acid.



This mechanism will lead to the formation of  $\alpha$ -hydroxy- $\beta$ -keto-succinic acid, which in turn could yield glyoxal by the mechanism proposed by Putney and Pratt (44). These workers suggested that glyoxal may be formed by a spontaneous decarboxylation during the handling and preparation of the 2,4-dinitrophenylhydrazone of  $\alpha$ -hydroxy- $\beta$ -keto-succinic acid. However, they did not identify this acid as one of the radiolytic products of malic acid.

Reaction mechanisms were given for the major compounds involved in the radiolytic degradation of succinic acid. Although other compounds of minor importance were identified, their mechanisms of formation were not given, since their formation can be explained by previously published mechanisms of other investigators.

## SUMMARY

The radiolysis of succinic acid in aqueous solutions induced by gamma irradiation from  $^{60}\text{Co}$  has been studied with respect to the identification and estimation of degradation products. The reaction mechanisms for the formation of the major radiolytic products, particularly malic and malonic acids, have been elucidated. The irradiation experiments were carried out in both oxygenated and oxygen-free systems.

The main oxidation products identified were malic, malonic, oxaloacetic, and pyruvic acids. The main products of greater molecular weight than succinic acid observed with oxygen-free irradiation experiments resulting from coupling reactions were 1,2,3,4-butane-tetracarboxylic, 1,2,4-butane-tricarboxylic, and tricarballylic acids. In addition to the identified compounds listed above, several compounds of minor importance were identified.

The radiochemical yields of radiolytic products of specifically labeled succinic acid- $^{14}\text{C}$  were measured for both oxygenated and oxygen-free systems. The degradation of succinic acid and the formation of malonic and malic acids with increasing cumulative dose were graphically presented for both oxygenated and oxygen-free systems. The graphical presentation for the formation of 1,2,3,4-butane-tetracarboxylic acid with increasing cumulative dose for the oxygen-free system was given.

The findings indicate that malonic acid is one of the major products resulting from the radiolysis of aqueous solutions of succinic acid. Several reaction mechanisms were suggested as possible mechanisms for the formation of malonic acid. The most favorable mechanism involved  $\cdot\text{OH}$  addition across the double bond of the enol form of oxaloacetic acid, in which decarboxylation of the adduct with oxidation resulted in the formation of malonic acid.

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