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FESTA, John Lucian, 1940-
A STUDY OF THE RADIATION CHEMISTRY OF
AQUEOUS CYSTEINE SOLUTIONS.

University of New Hampshire, Ph.D., 1967
Chemistry, physical

University Microfilms, Inc., Ann Arbor, Michigan

A STUDY OF THE RADIATION CHEMISTRY
OF
AQUEOUS CYSTEINE SOLUTIONS

BY

JOHN LUCIAN FESTA
B. A., University of Connecticut, 1962

A THESIS

Submitted to the University of New Hampshire
In Partial Fulfillment of
The Requirements for the Degree of
Doctor of Philosophy

Graduate School
Department of Chemistry
August, 1966

This thesis has been examined and approved.

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August 18, 1966
Date

ACKNOWLEDGEMENT

The author is indebted to Dr. Alexander R. Amell for his guidance and encouragement, which helped to make this thesis possible.

The author would also like to thank the National Institutes of Health and the Atomic Energy Commission for financial assistance.

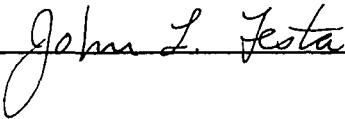


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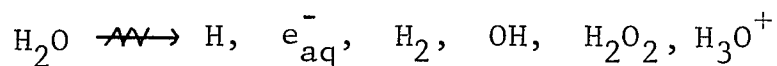
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INTRODUCTION

A. Radiation Chemistry of Dilute Aqueous Solutions

The radiation chemistry of pure water has been studied extensively, and it is now generally agreed that the net radiation-induced decomposition may be expressed by^(1,2)



where e_{aq}^- represents the hydrated electron. The radiation chemistry of dilute aqueous solutions is concerned with the nature of the reactions between these species and solute, and with the nature of subsequent reactions between products and solute.

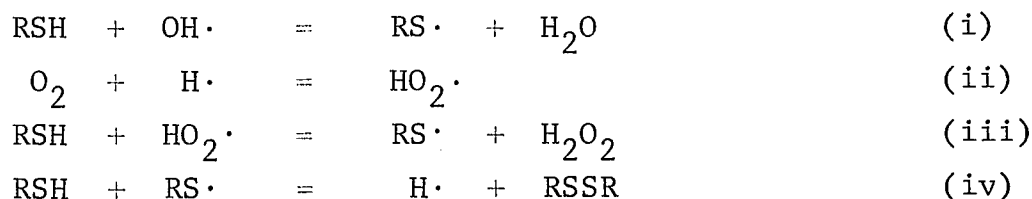
This thesis is a detailed study of the effect of cobalt-60 gamma radiation on dilute aqueous solutions of cysteine.

B. Previous Irradiation Studies

Cysteine has been the object of radiochemical investigation mainly because information from such studies may help to explain its effect in protecting living systems against lethal radiation.⁽³⁾ The radiation chemistry of cysteine in aqueous solution shows how it may react with the products of water radiolysis, a possible means of protection.⁽⁴⁾ In addition, the effectiveness of cysteine as a free radical scavenger in aqueous solution makes it an excellent tool for studying the means by which ionizing radiation produces its effect.

Products resulting from the radiolysis of cysteine in aqueous solution have been previously determined.^(5,6,7,8) The major decomposition product is cystine, but smaller quantities of alanine, hydrogen, hydrogen sulfide, and hydrogen peroxide^{*} are also formed. In addition trace amounts of ammonia, carbon dioxide, alaninesulfinic acid, cysteic acid, sulfate and sulfur are detected at high radiation doses, and are probably formed in secondary reactions. (Ammonia, and cysteic acid are major degradation products of the radiolysis of cystine in aqueous solution,^(8,9) while carbon dioxide and ammonia are formed in the radiolysis of alanine.⁽¹⁰⁾) It is clear from the results of the product analyses that the primary reaction site on cysteine is at the thiol group. This is in marked contrast to the radiolysis of other amino acids where the amino group is usually the reaction site.⁽¹¹⁾

Swallow⁽¹²⁾ found that oxygen greatly enhanced the yield of thiol loss in the irradiated solution. The exceptionally high value obtained for the yield could not be explained without postulating a chain reaction. The author suggested the following partial mechanism for such a chain reaction.

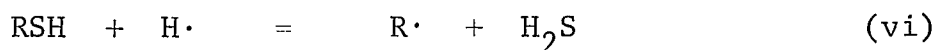
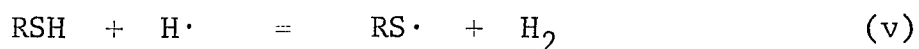


(RSH = cysteine)

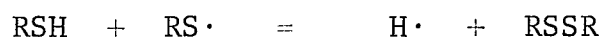
* not formed directly from cysteine

Reactions (ii), (iii), and (iv) are the chain propagating steps. The chain may be terminated by dismutation of $\text{HO}_2\cdot$ radicals and by combination of $\text{RS}\cdot$ radicals.

Littman, Carr, and Brady⁽¹³⁾ investigated the reaction between hydrogen atoms from an electrical discharge and cysteine in solution. The main products in acid solution were cystine and hydrogen, but smaller quantities of hydrogen sulfide were also formed. The following reactions were suggested to explain product formation:



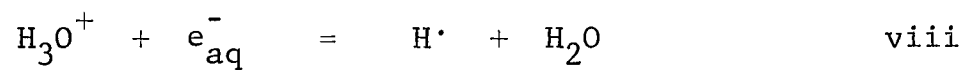
Packer⁽¹⁴⁾ has indicated that since reaction (v) occurs readily, then in Swallow's mechanism a chain reaction should proceed in the oxygen-free system viz.



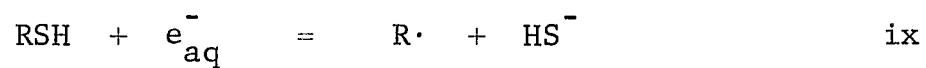
There is sufficient evidence, however, that a chain reaction does not occur in the absence of oxygen. Such evidence and the fact that reaction (iv) is approximately 17 kcal per mole endothermic make Swallow's mechanism seem unlikely.

Armstrong and Wilkening⁽¹⁵⁾ irradiated aqueous solutions of cysteine in the pH range 0.72 to 8.0 and quantitatively measured the formation of hydrogen and hydrogen sulfide. It was found that increasing the pH lowered the yield of hydrogen but increased the yield of hydrogen sulfide.

These results provide strong support for a competition between reaction (viii)



and (ix)



in the irradiated solution.

EXPERIMENTAL

A. Materials

L-cysteine ($\text{HSCH}_2\text{CH}(\text{NH}_2)\text{CO}_2\text{H}$) was purchased from Nutritional Biochemicals Corporation and used without further purification.

Pure water used in the preparation of solutions to be irradiated was obtained by first passing distilled water through a Barnstead Demineralizer, and finally through an organic removal cartridge (Barnstead No. 8904). Water purified in this manner is comparable to triply distilled water.

Nitrogen (Airco-prepurified) and oxygen (Linde, U.S.P.) were used as supplied.

All other chemicals were analytical reagent grade, and were used without further purification.

B. Irradiations

Samples were irradiated with gamma-rays from a 62-Curie cobalt-60 source obtained under a grant from the Atomic Energy Commission. A description of the source, except for a slight modification described in this section, has been given elsewhere.⁽¹⁶⁾

Two types of radiation vessels were used. One of these is a round bottom double-walled beaker having a capacity of 100 ml. Its cylindrical shape ensures uniform absorption of radiation by the liquid sample (Fig. 1-a). Solutions

were also irradiated in test tube shaped vessels of 7ml. capacity with a break seal fashioned at one end to facilitate removal of gaseous products. (Fig. 1-b). Several of these ampoules could be mounted around the inside of a metal can, and exposed to the radiation in the usual manner. However, despite symmetric disposal of the ampoules about the cylindrical source, the amount of radiation absorbed by each sample was not uniform. Therefore, it was necessary to have the ampoules rotate about the source. This was accomplished by placing the can containing the ampoules on a small turntable, which could be driven at the rate of four r.p.m.'s by a motor mounted on the rear of the sample carrying cart. The assembly is shown in Fig. II.

All radiation vessels were thoroughly cleansed prior to use by rinsing them successively with nitric acid, distilled water, and finally several times with pure water.

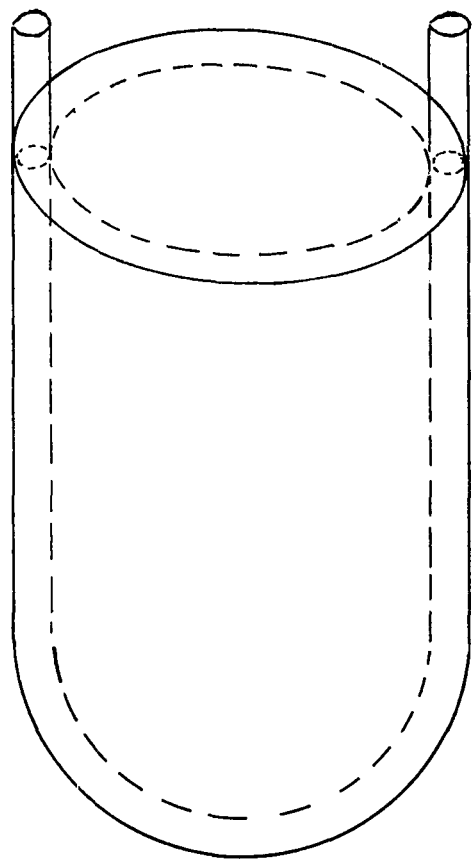
C. Dosimetry

The absorbed dose rate was determined by the Fricke dosimeter ($G(\text{Fe}^{3+}) = 15.5$; $\epsilon_{305} = 2201$ at $25^\circ\text{C}.$). Over the period of this study the dose rate was ca. 6×10^{16} e.v. $\text{g.}^{-1} \text{ min.}^{-1}$ using the large radiation vessels, and ca. 7×10^{16} e.v. $\text{g.}^{-1} \text{ min.}^{-1}$ using the ampoules.

D. Preparation of Solutions

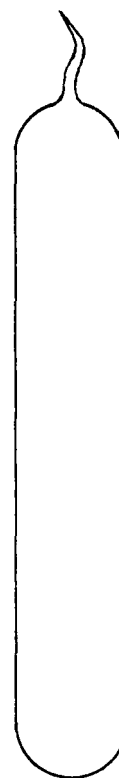
In neutral or basic solutions, cysteine is air oxidized quite readily to the disulfide. Acid solutions were used for the most part, and were prepared fresh immediately prior to each run.

FIGURE I-a



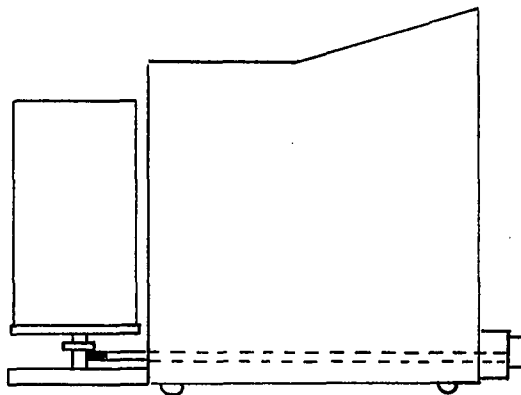
Rabbit-ear Irradiation Vessel

FIGURE I-b



Irradiation Ampoule

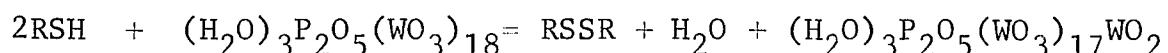
FIGURE II
Cart and Turntable Assembly



Solutions of cysteine were irradiated in both the presence and absence of dissolved air (oxygen). Deaerated solutions were prepared by bubbling nitrogen gas through the solution in a flask for one hour. At the end of the bubbling period, the flask and its contents were removed to a glove bag where the radiation vessels were filled under a nitrogen atmosphere. Oxygen-saturated solutions were prepared by bubbling pure oxygen gas through the solution for 30 minutes. Solutions saturated with air were prepared by simply shaking the liquid in atmosphere.

E. Methods of Analysis

Cysteine was determined by the method of Shinohara⁽¹⁷⁾ adapted for use with a Beckman model Du spectrophotometer. The deep blue color of the complex formed when a solution of cysteine is treated with phospho-18-tungstic acid reagent is the basis of a colorimetric method of analysis. The color production is caused by thiol reduction of phospho-18-tungstic acid according to the following stoichiometric equation:

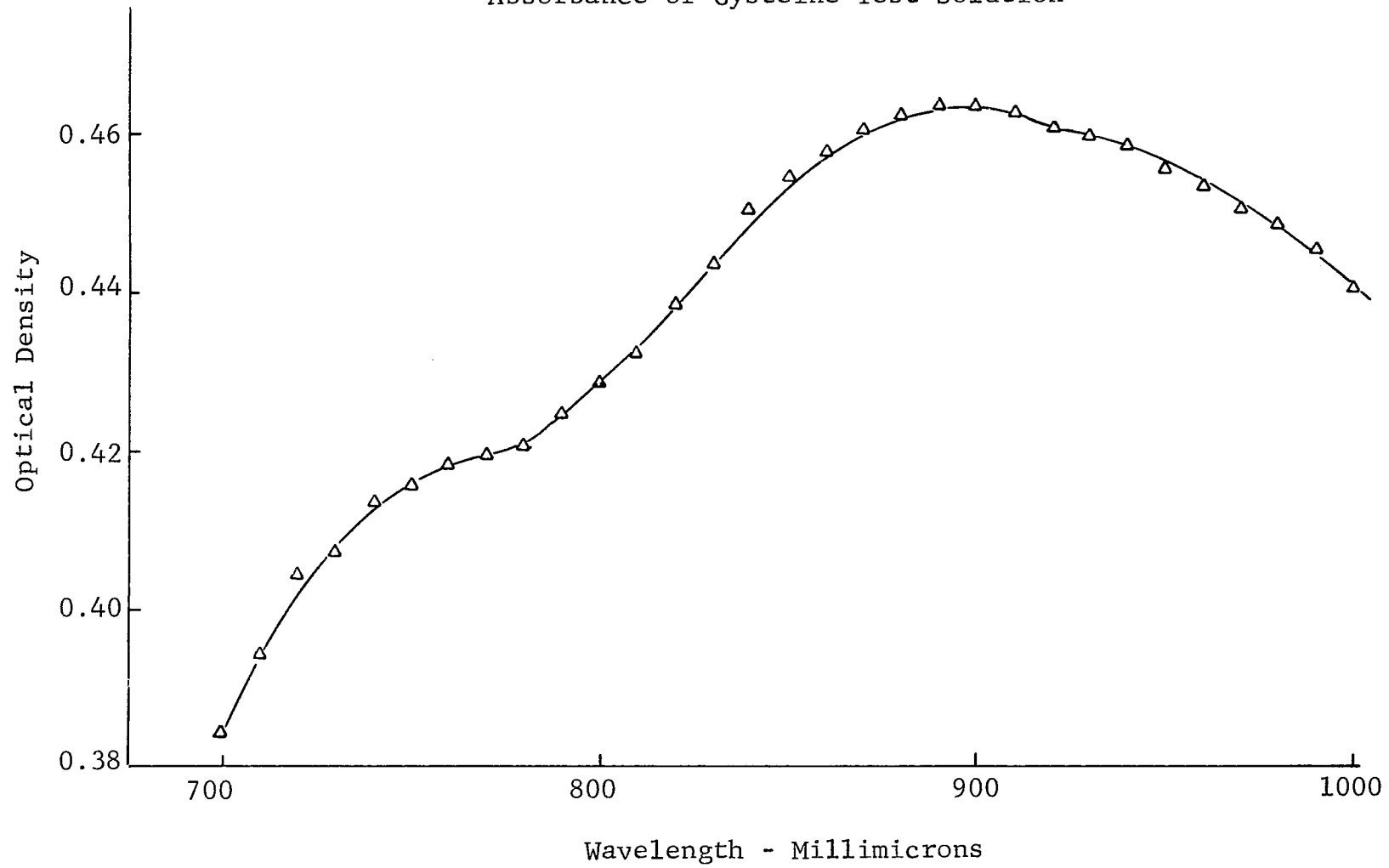


The coloring reagent was prepared according to the directions given by Folin and Marenzi⁽¹⁸⁾ except that the step involving the removal of interfering molybdates was omitted, being entirely unnecessary with the grade of reagent used. (Sodium tungstate (Folin) molybdenum low Fisher Certified).

The absorption spectrum for a solution of the blue colored complex compared to a blank solution is shown in Fig. III. The wavelength of maximum absorption was taken at

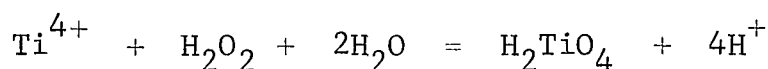
FIGURE III

Absorbance of Cysteine Test Solution



900 mu. Several standards were made up in the described manner, and a calibration curve was prepared by measuring the optical densities of the standards, and plotting the values obtained against the corresponding cysteine concentrations. The resulting curve, shown in Fig. IV, is linear over the specified concentration range.

Hydrogen peroxide was determined by the titanium sulfate method⁽¹⁹⁾ employing a Beckman Du spectrophotometer. The method is based on photoelectric measurement of color intensities of hydrogen peroxide solutions treated with titanium sulfate reagent. The reaction may be expressed by the following equation:



The wavelength of maximum absorption of a developed solution was obtained by measuring its optical density at various wavelengths. The results are shown graphically in Fig. V with λ_{max} appearing at 405 mu. A calibration curve that conformed to Beer's law was obtained, and this is shown in Fig. VI.

It was found that cysteine could interfere with the determination when present in the developed solution at a concentration of 10^{-2}M or more. At these concentrations cysteine caused the color of the solution to fade rather quickly once it had developed. Fortunately, the color develops immediately upon adding the reagent to the solution, and the optical densities may be measured at once.

FIGURE IV

Cysteine Calibration Curve

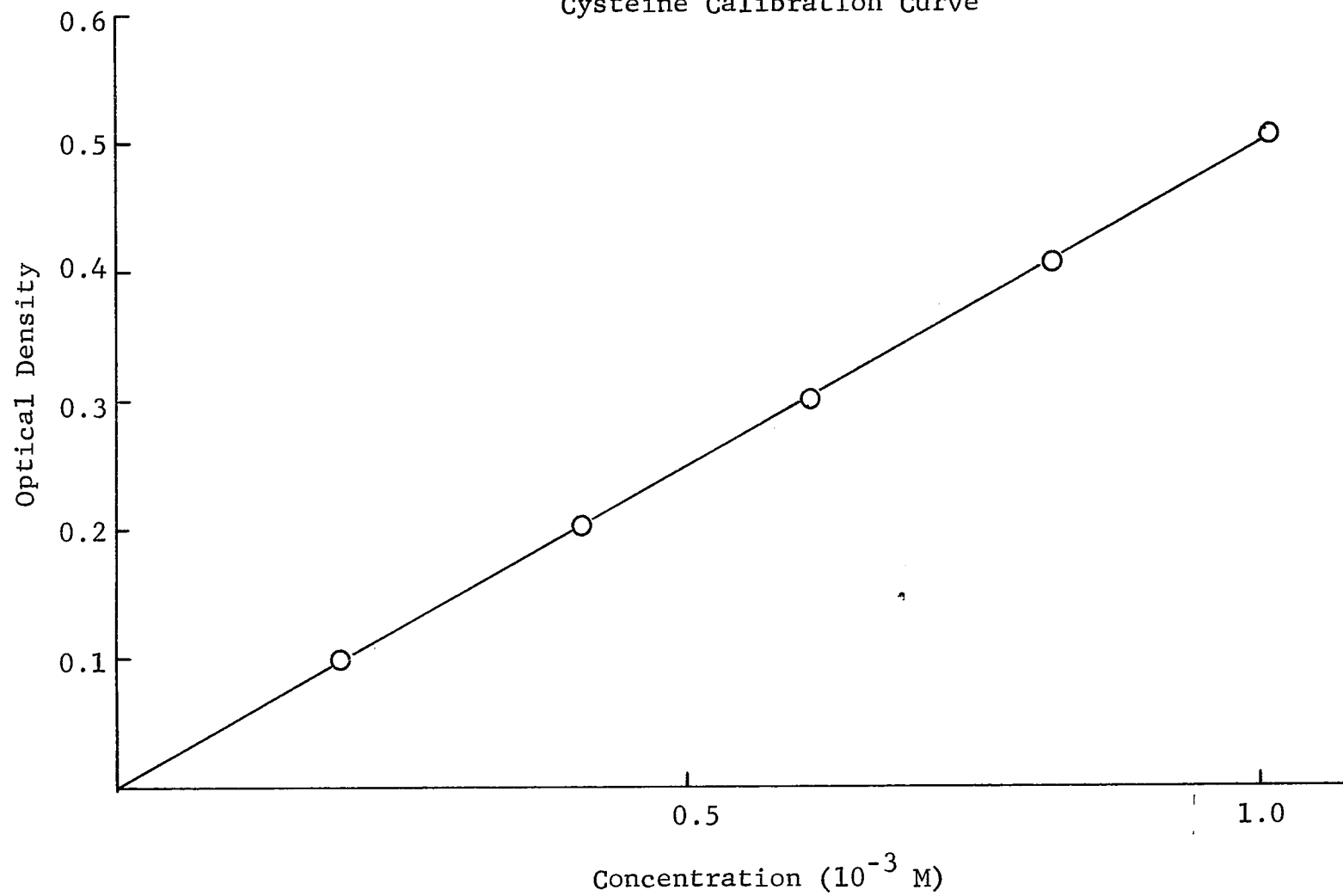


FIGURE V

Absorbance of H_2O_2 Test Solution

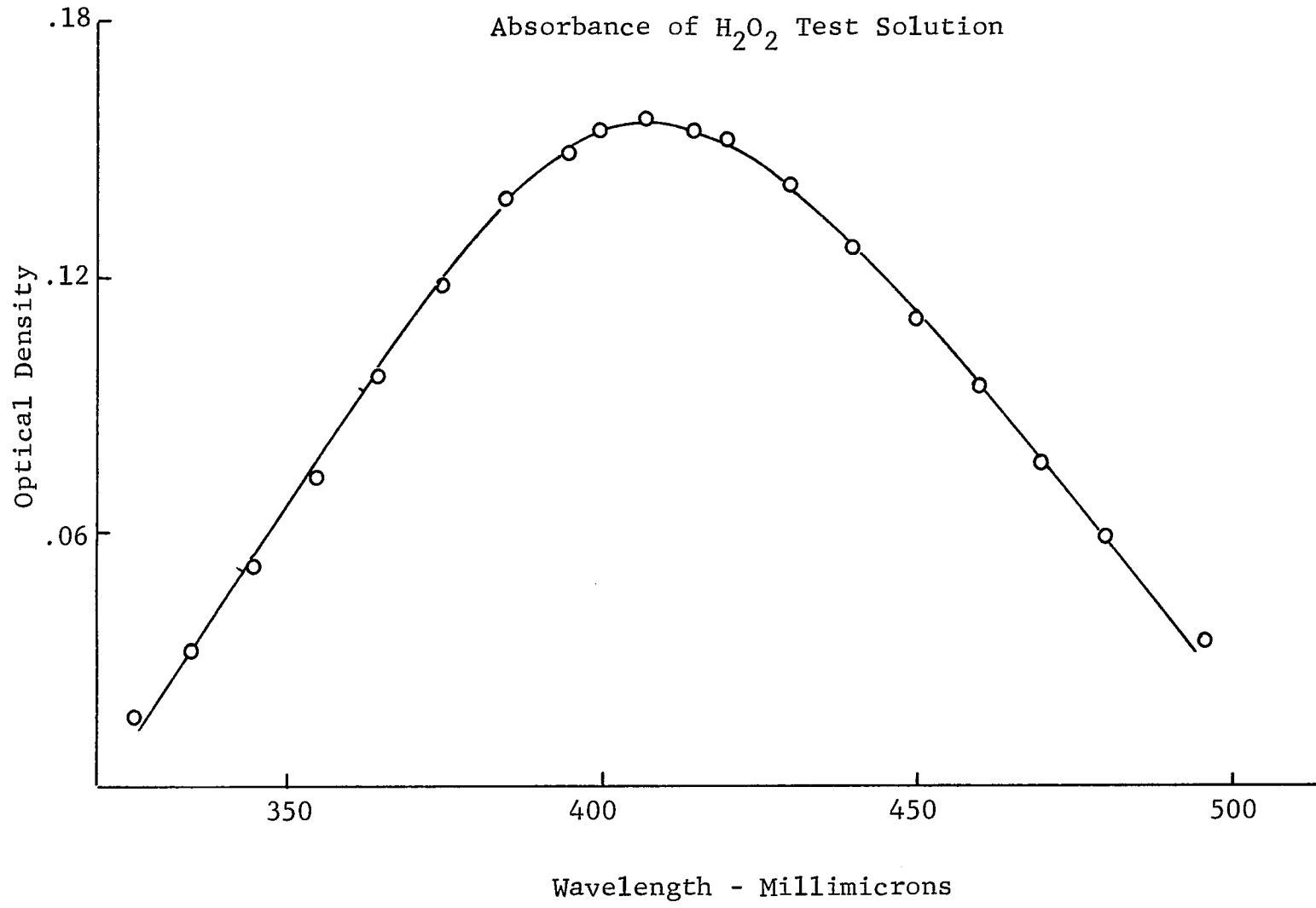
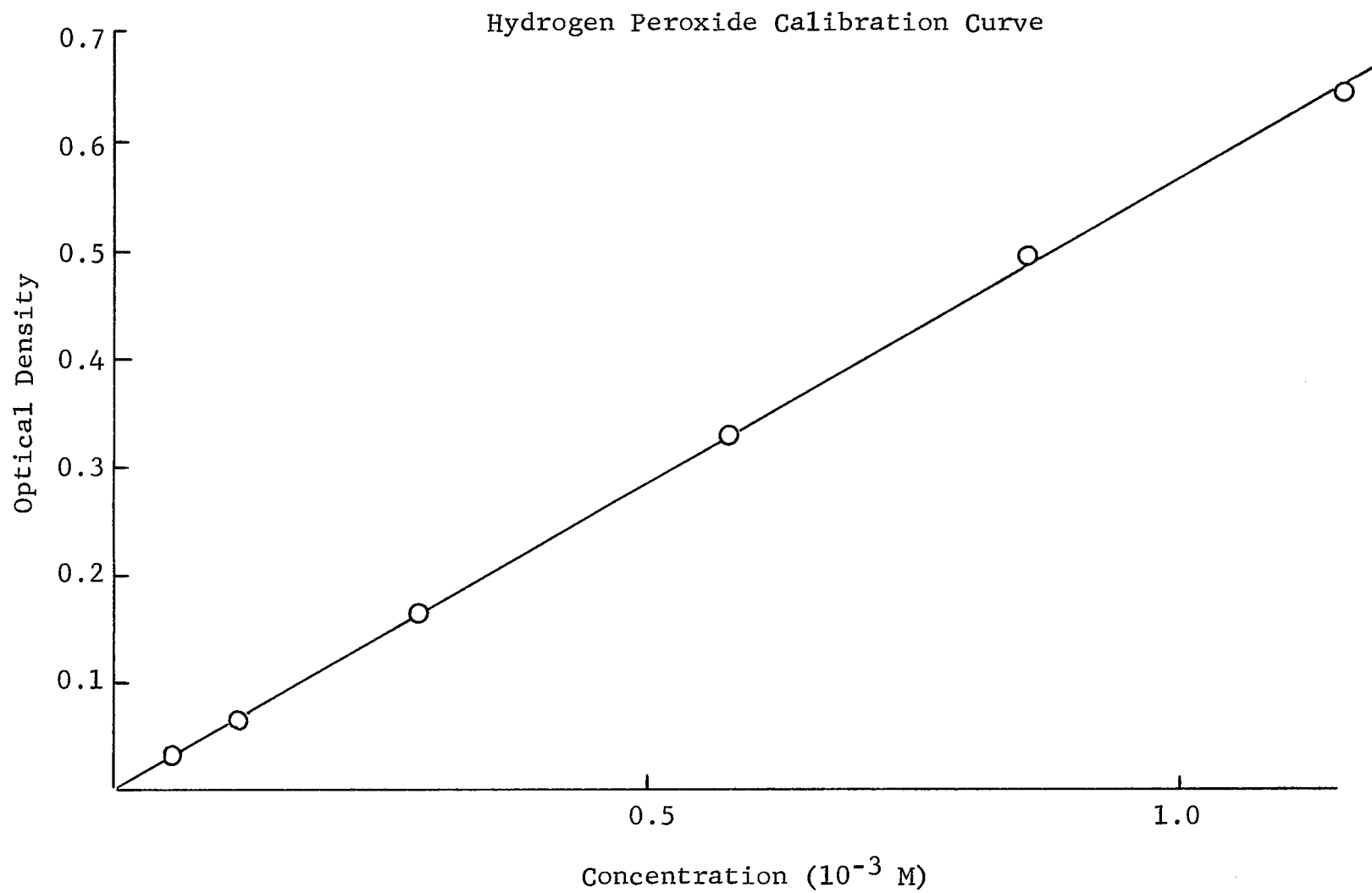


FIGURE VI



Hydrogen was determined by gas chromatography. The instrument used for this purpose was a Perkin-Elmer model 154B Vapor Fractometer, which employs a dual type thermistor thermal conductivity cell as the sensing device. Two vertical U-shaped glass columns packed with molecular sieve (5A) were used in series. Nitrogen was used as the carrier gas, and the column pressure was maintained at 10 psig. The samples of hydrogen gas were injected into the carrier gas stream at room temperature. A Photovolt Microcord Model 44 millivolt recorder was used to record the signal voltage.

A calibration curve was prepared for hydrogen by injecting known amounts of the gas into the instrument, measuring the peak heights (m.v. deflection), and plotting the values obtained against the corresponding quantities of hydrogen. Peak heights were reproducible to within 1%. A linear calibration curve was obtained, and this is shown in Fig. VII.

Figure VIII-a shows the Toepler pump arrangement used to transfer hydrogen gas from the irradiated solutions to a gas sample holder. The transfer was accomplished by the usual procedure of repeated cycles of freezing, pumping and thawing. The design of the gas sample holder (Fig. VIII-b) was such that it could be easily attached to the gas sampling valve of the vapor fractometer.

Hydrogen sulfide was determined by the molybdate method.⁽²⁰⁾ This method consists essentially of the removal of H_2S from solution by a stream of inert gas, the H_2S being absorbed in zinc acetate solution and determined colorimetrically as molybdenum blue. The experimental arrangement for stripping the gas from the irradiated liquid sample and trapping it is shown in Fig. IX. The trapping chamber (A), containing 17 ml. of the absorbing solution, was kept in an ice bath to increase

FIGURE VII

Calibration Curve for Hydrogen

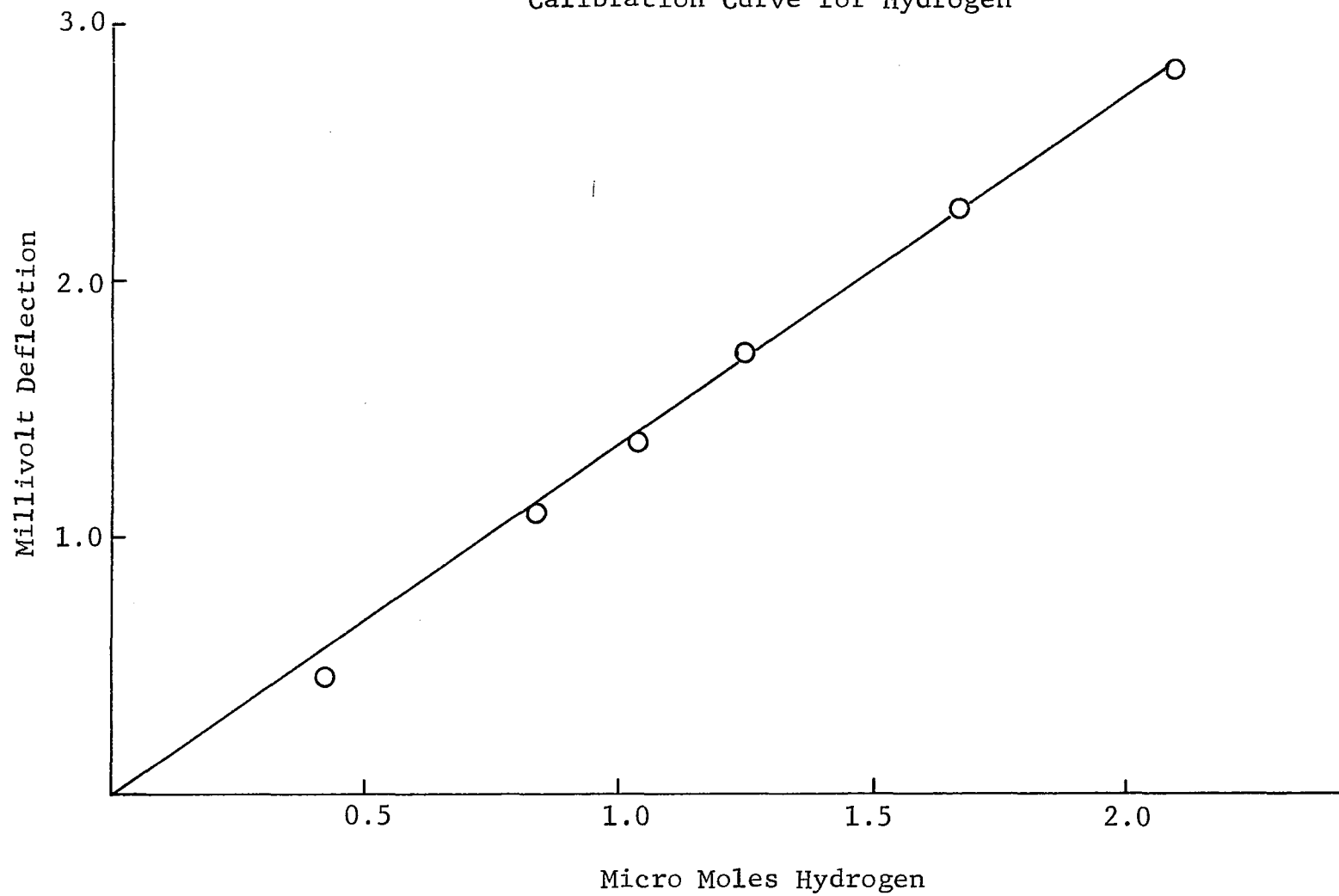


FIGURE VIII-a

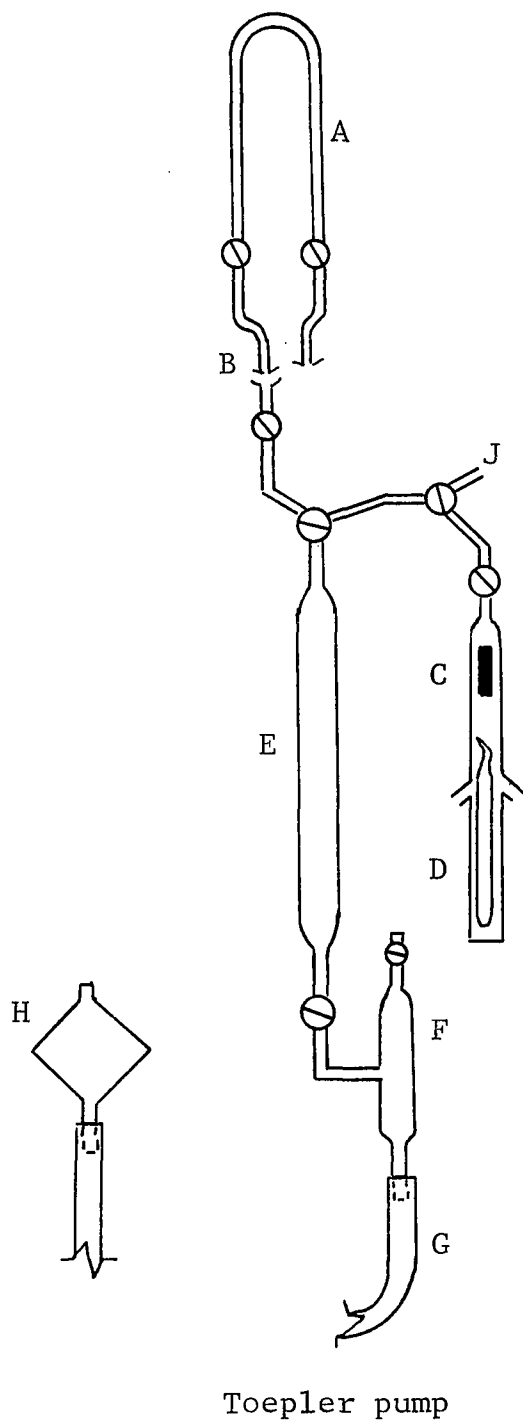
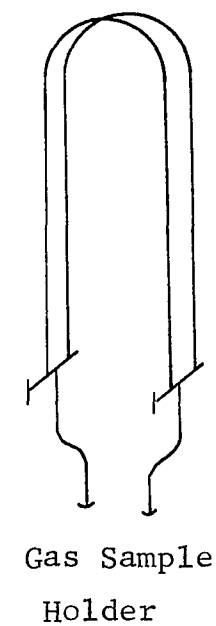


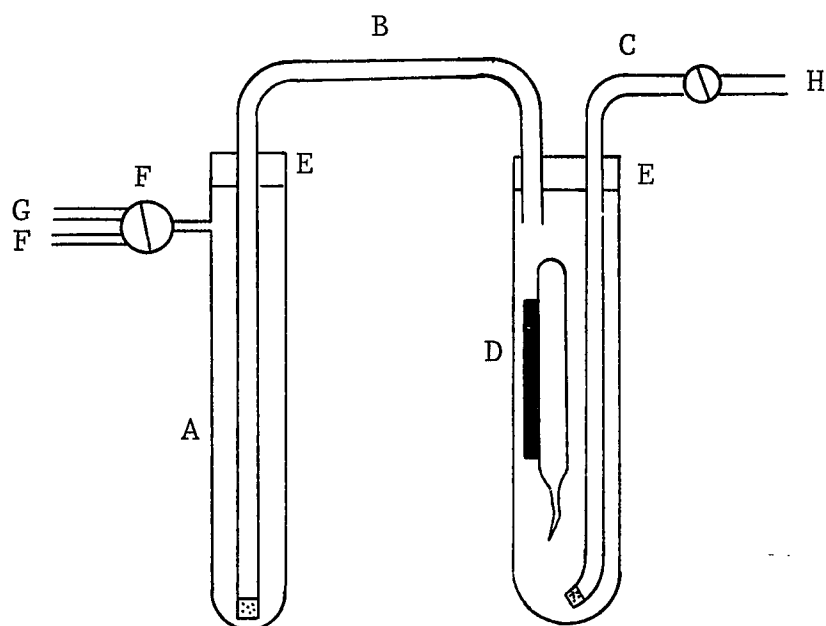
FIGURE VIII-b



- ⊖ - stopcock
- A - gas sample holder
- B - 12/5 ball joint
- C - nail
- D - irradiation ampoule
- E - mercury piston
- F - air trap
- G - tygon tubing
- H - leveling bulb
- J - to vacuum line

FIGURE IX

Assembly for Trapping Hydrogen Sulfide



- A - trapping tube
- B,C - delivery tubes
- D - ampoule with affixed iron nail
- E - rubber stopper
- F - to aspirator
- G - to atmosphere
- H - to nitrogen cylinder
- ▣ - sintered glass
- ⊙ - stopcock

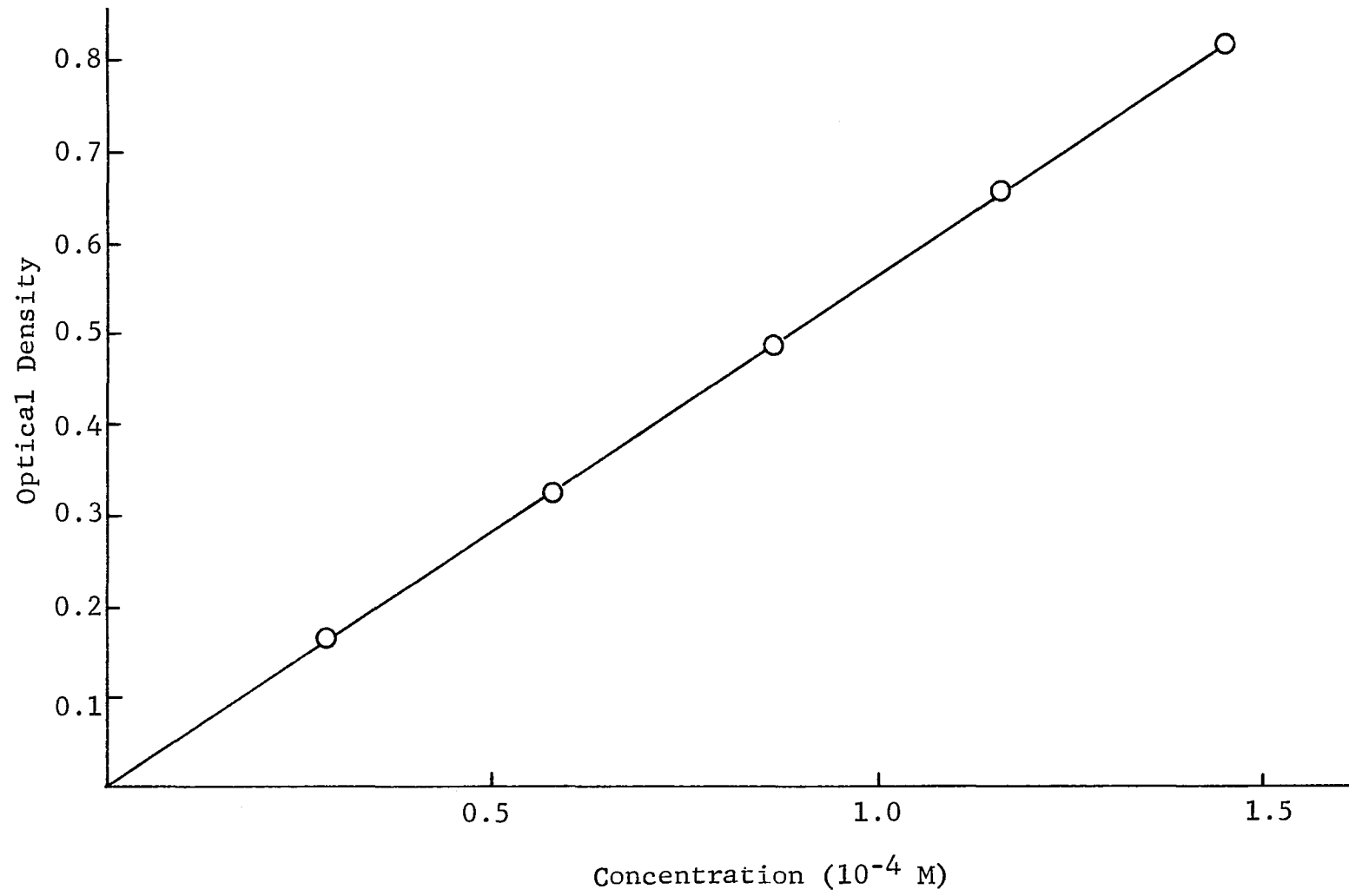
the solubility of hydrogen sulfide. The ampoule was suspended in the sample chamber by means of an affixed iron nail and an external magnet. After the system had been stoppered tightly, the ampoule was broken by dropping it. The small bore opening made in the ampoule prevented the liquid from draining freely. Therefore, it was necessary to apply a slight vacuum by means of an aspirator connected to the system through the two-way stopcock. After the liquid had completely drained into the sample chamber, it was stripped of H_2S by bubbling dry nitrogen gas through for two hours. The gas delivery tubes, (B) and (C), were fitted with porous sintered glass discs to disperse the gas through the solutions. At the conclusion of the bubbling period, the test reagents were added directly to the trapping tube, and the color was allowed to develop for the prescribed time. The volume of the developed solution was then made up to 50 ml., and the optical density measured at 670 μ .

Several standards were made up by adding the color reagents to carefully measured quantities of sodium sulfide solution, and a calibration curve was prepared by plotting the optical densities of these standards against the corresponding sulfide concentration. A straight reference line, shown in Fig. X, was obtained.

The amount of H_2S liberated from Na_2S and measured by this technique was found to be on the average only 65% of that corresponding to an equivalent amount of Na_2S added directly to the color reagent. Thus, there appeared to be considerable loss of H_2S in the process of transferring the gas from the sample chamber to the absorbing solution. The amount of gas transferred was not always reproducible.

FIGURE X

Calibration Curve for Hydrogen Sulfide



Consequently, a corrected value of the H₂S yield, reliable to within \pm 20%, was obtained by dividing the measured value by 0.65. Part of this large experimental error resulted from having to measure extremely small quantities of H₂S. Although the colorimetric method was sensitive to as little as 1.7 micrograms of the gas, the maximum amount determined was only slightly larger than this. Consequently, the precision was rather poor.

F. G-value Measurements.

Reaction yields are expressed in terms of the G-value, which is defined as the number of molecules formed or destroyed per 100 e.v. of energy absorbed by the system. Each G-value measured, except that for H₂S, is the result of four or five irradiations carried out at different times to give linear yield-dose curves. In the case of H₂S, samples had to be exposed to radiation for the longest possible time in order to generate enough gaseous product for analysis. Consequently, the G-value of H₂S had to be taken as the average result of a number of single point determinations.

RESULTS

A. Preliminary - Yield-Dose Curve

Figure XI shows the loss of thiol as a function of radiation dose for air-free 1.0×10^{-3} M cysteine. The curve is seen to be linear up to about 16% destruction of the thiol and then begins to slope off gradually, typical for a yield-dose plot. Initially, the reactive intermediates produced by the radiolysis of water are scavenged only by the primary solute. However, as the product concentration builds up, secondary reactions become important, resulting in partial protection of the primary solute. Radiolytic yields (G-values) refer to the initial yields, and are usually determined from the initial slope data.

B. Scavenger Studies - Results and Discussion

The effects of various added agents on the radiolytic destruction of cysteine in aqueous solutions were studied. Additives were chosen on the basis of their reactivity with the intermediates produced by the radiolysis of water. The agents employed for this purpose are listed below together with the rate constants for the scavenging reactions.*

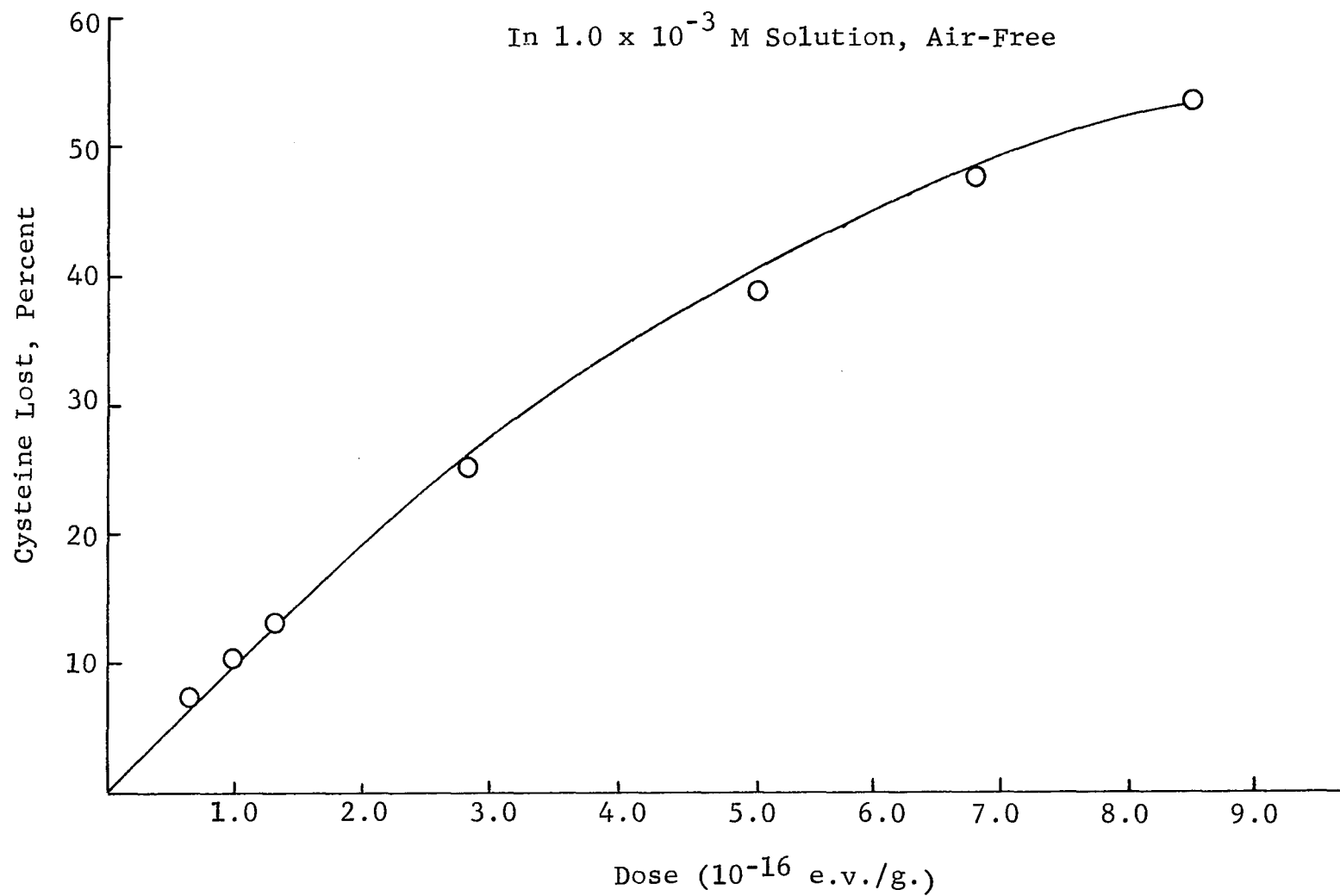
Br^- (KBr)	$k(\text{OH}+\text{Br})$	=	3.6×10^{10}	$\text{M}^{-1} \text{sec}^{-1}$
Cl^- (HCl)	$k(\text{OH}+\text{Cl})$	=	3×10^8	$\text{M}^{-1} \text{sec}^{-1}$
HOAc	$k(\text{H}+\text{HOAc})$	=	1×10^5	$\text{M}^{-1} \text{sec}^{-1}$
NO_3^- (HNO_3)	$k(\text{H}+\text{NO}_3^-)$	=	1.8×10^8	$\text{M}^{-1} \text{sec}^{-1}$

* See ref. (27)

FIGURE XI

Yield Dose Curve for Loss of Cysteine

In 1.0×10^{-3} M Solution, Air-Free



Protection of the thiol by any one of these added agents should be indicated by a decrease in the rate of thiol destruction. The results are shown in Table I. Radiolytic yields for the disappearance of cysteine in solutions of radiation stable sulfuric acid and phosphoric acid are included for comparison. Contrary to what was expected, neither chloride, acetic acid nor bromide were found to have any effect on the rate of thiol destruction, and surprisingly enough, nitrate actually enhanced the yield in the deaerated solution.

It is possible to assume that chloride and bromide ions are converted by $\text{OH}\cdot$ radicals to chlorine and bromine atoms, ⁽²¹⁾ which like $\text{OH}\cdot$ may abstract hydrogen atoms from the thiol group to produce the same over-all effect. The ineffectiveness of added acetic acid in reducing the yield of thiol loss may be explained by the fact that cysteine itself is an extremely efficient H-atom scavenger. The value of the rate constant for the reaction between cysteine and $\text{H}\cdot$ is of the order $10^{10} \text{ M}^{-1} \text{ sec.}^{-1}$, indicating a nearly diffusion-controlled rate. (See Discussion) Since the rate constant for the reaction between acetic acid and $\text{H}\cdot$ is considerably smaller, it should not be surprising that cysteine competes favorably for the radical in these solutions.

Mahlman and Sworski ⁽²²⁾ studied the effect of Co-60 γ -rays on aqueous solutions (0.4 M H_2SO_4) of sodium nitrate. The main products determined were nitrite (NO_2^-) and oxygen. These results are noteworthy since experiments in this laboratory showed that nitrite reacts quite readily with cysteine in 0.1 M sulfuric acid. Thus, the increase in the yield of thiol destruction in solutions with added nitrate may at least

TABLE I

Effect of Various Additives on the Yield of Thiol Loss

$$[\text{RSH}]_0 = 1.0 \times 10^{-3} \text{ M}$$

Concentration of Added Reagents	pH	$G(-\text{RSH})_{\text{air}}$	$G(-\text{RSH})_{\text{N}_2}$
1. 0.1 M H_2SO_4	0.95	11.5	6.5
2. 0.2 M HCl	0.70	11.5	6.7
3. 0.1 M H_2SO_4 + .010 M KBr	0.95	---	6.7
4. 0.2 M HNO_3	0.70	11.8	11.8
5. H_2SO_4 - solution	3	---	5.9
6. 0.0011 M H_3PO_4	3.0	---	6.0
7. 0.057 M $\text{HC}_2\text{H}_3\text{O}_2$	3.0	---	6.0

in part be due to this reaction. Of course, enhancement of the yield may also result from the presence of oxygen (formed from NO_3^-) in the solution.

C. Reaction Yields

Initial yields were determined for the disappearance of cysteine, and for the formation of hydrogen, hydrogen peroxide, and hydrogen sulfide in the radiolysis of oxygen-saturated and oxygen-free acidic (0.1 M H_2SO_4) solutions of cysteine.

Figure XII

Shows the loss of cysteine as a function of dose for 4.0×10^{-3} M cysteine.

Figure XIII

Shows the formation of hydrogen as a function of dose for 4.0×10^{-3} M cysteine.

Figure XIX

Shows the formation of hydrogen peroxide as a function of dose for 1.0×10^{-2} M cysteine. (In this case the more concentrated solution of cysteine allowed a longer sample exposure time, which was necessary to insure formation of a sufficient amount of peroxide for analysis).

Yield dose curves for hydrogen sulfide were not obtained for the reasons indicated in the experimental section.

The measured G-values are given in Table II. The quantity of hydrogen sulfide formed in the oxygen-saturated solutions was too small to be measured accurately, and was simply estimated as being less than the yield in deaerated solutions.

FIGURE XII

Yield-Dose Plot for Thiol Loss

In 4.0×10^{-3} M-Cysteine

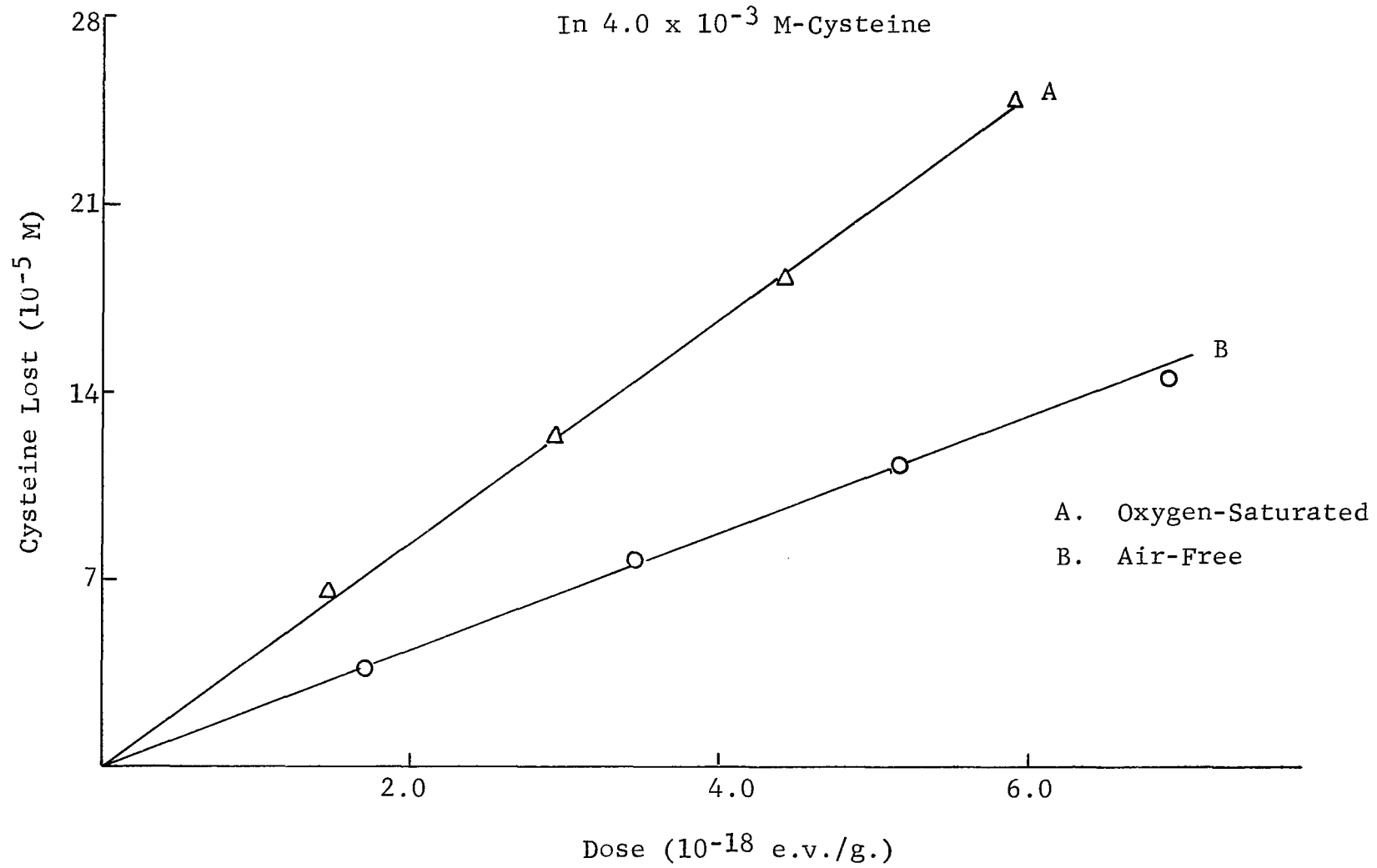


FIGURE XIII

Yield-Dose Plot for H₂ Formation

In 4.0×10^{-3} M-Cysteine

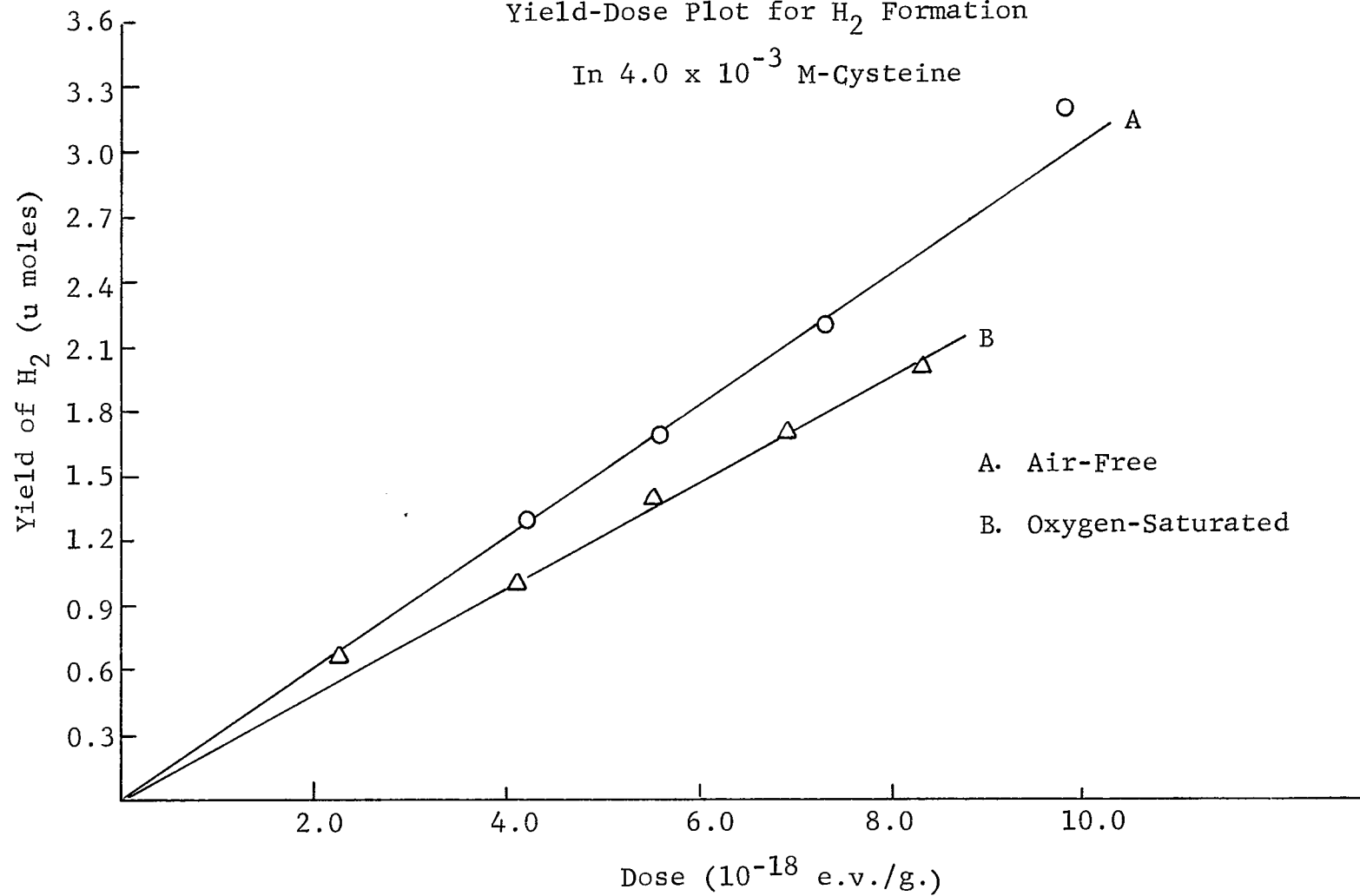


FIGURE XIV

Yield-Dose Plot for H_2O_2 Formation
In 1.0×10^{-2} M-Cysteine

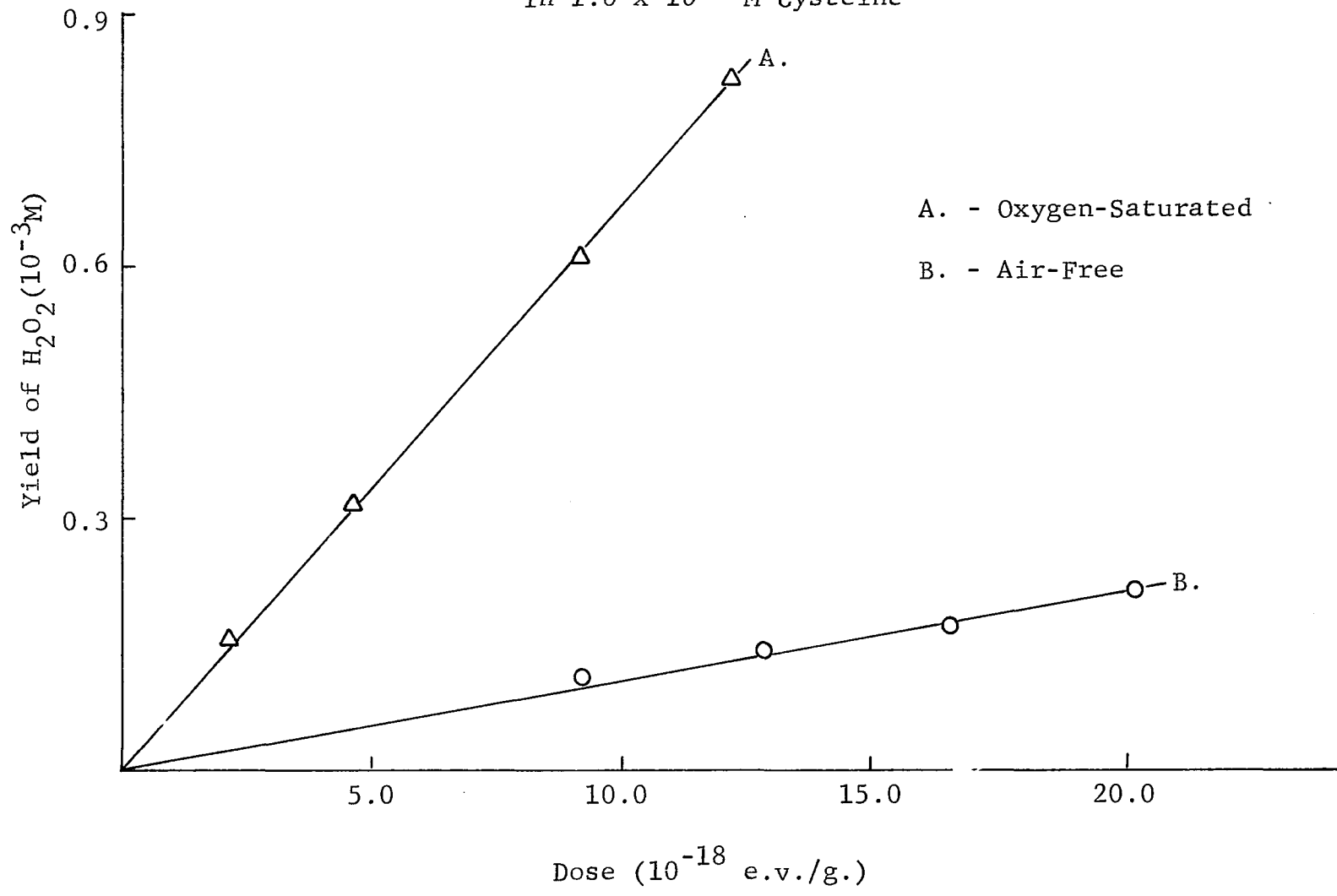


TABLE II
 Measured Yields in the Radiolysis
 of Aqueous Cysteine Solutions
 pH 0.95

[RSH] ₀ , M			Deaerated Solutions	Oxygen-Saturated Solutions
4 x 10 ⁻³	G(H ₂)	=	2.8	2.1
4 x 10 ⁻³	G(H ₂ S)	=	0.6	<0.6
1 x 10 ⁻²	G(H ₂ O ₂)*	=	0.6	4.0
4 x 10 ⁻³	G(-RSH)	=	6.6	12.3

* G_{H₂O₂} = 0.7 at pH ~ 0.95⁽²⁷⁾ —See page 36

D. Dependence of G(-RSH) on pH, and on Initial Cysteine Concentration.

The yields for the disappearance of cysteine in both deaerated and oxygen-saturated solutions were found to increase with thiol concentration. The effect was greater in the oxygenated system, wherein a ten-fold increase in thiol concentration (from 10^{-3} M to 10^{-2} M) almost doubled the value of G(-RSH). (Table III).

The yields were also found to be quite sensitive to pH. Figure XV shows the pH dependence of G(-RSH) in deaerated solutions. Measurements were made only below pH 6 because of the inherent instability of cysteine in neutral or basic solutions. Dilute sulfuric acid was used to adjust the acid concentration of the solutions. No attempt was made to use buffers, since these could conceivably alter the course of the reactions. At any rate, buffering was unnecessary since irradiation caused no change in pH.

E. The Yield of Thiol Loss In Air-Saturated and Oxygen-Saturated Solutions.

Figure XVI shows the disappearance of cysteine as a function of dose for 1.0×10^{-3} M cysteine solutions saturated with air (curve A) and with pure oxygen (curve B). From the slope of the plots the values of $G(-RSH)^{\text{air}}$ and $G(-RSH)^{\text{O}_2}$ are 11.5 and 8.4 respectively.

TABLE III - a,b
Effect of Initial Cysteine Concentration
On the Yield of Thiol Destruction

<u>a- Deaerated Solution</u>		<u>b- Oxygen Saturated Solution</u>	
[RSH] ₀ , M	G(-RSH)	[RSH] ₀ , M	G(-RSH)
1×10^{-3}	6.5	1×10^{-3}	8.4
4×10^{-3}	6.6	5×10^{-3}	12.3
7×10^{-3}	7.1	1×10^{-2}	16.3
1×10^{-2}	7.4		

FIGURE XV

Effect of pH On The Yield of Thiol Destruction
in Deaerated Solutions

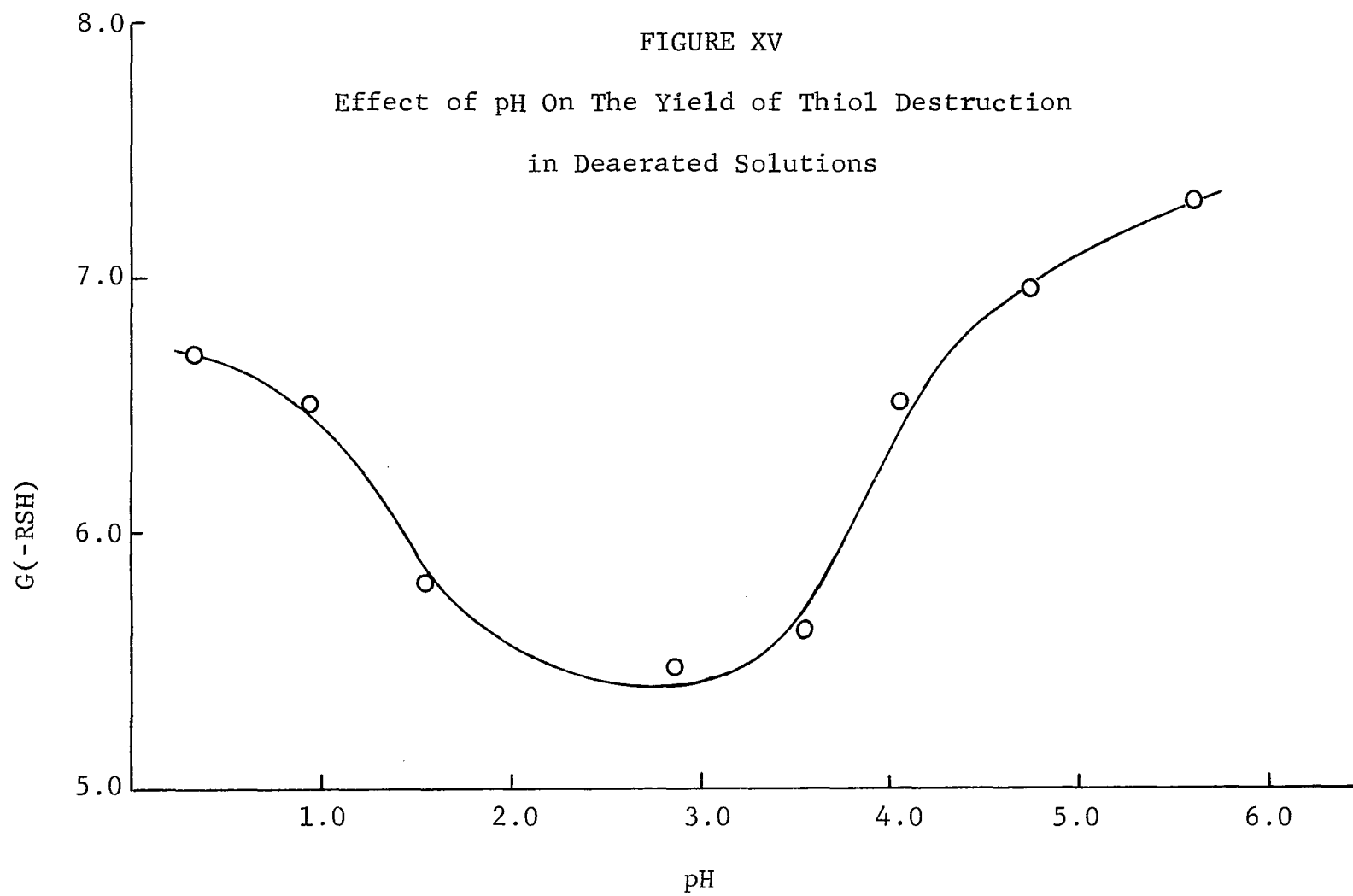
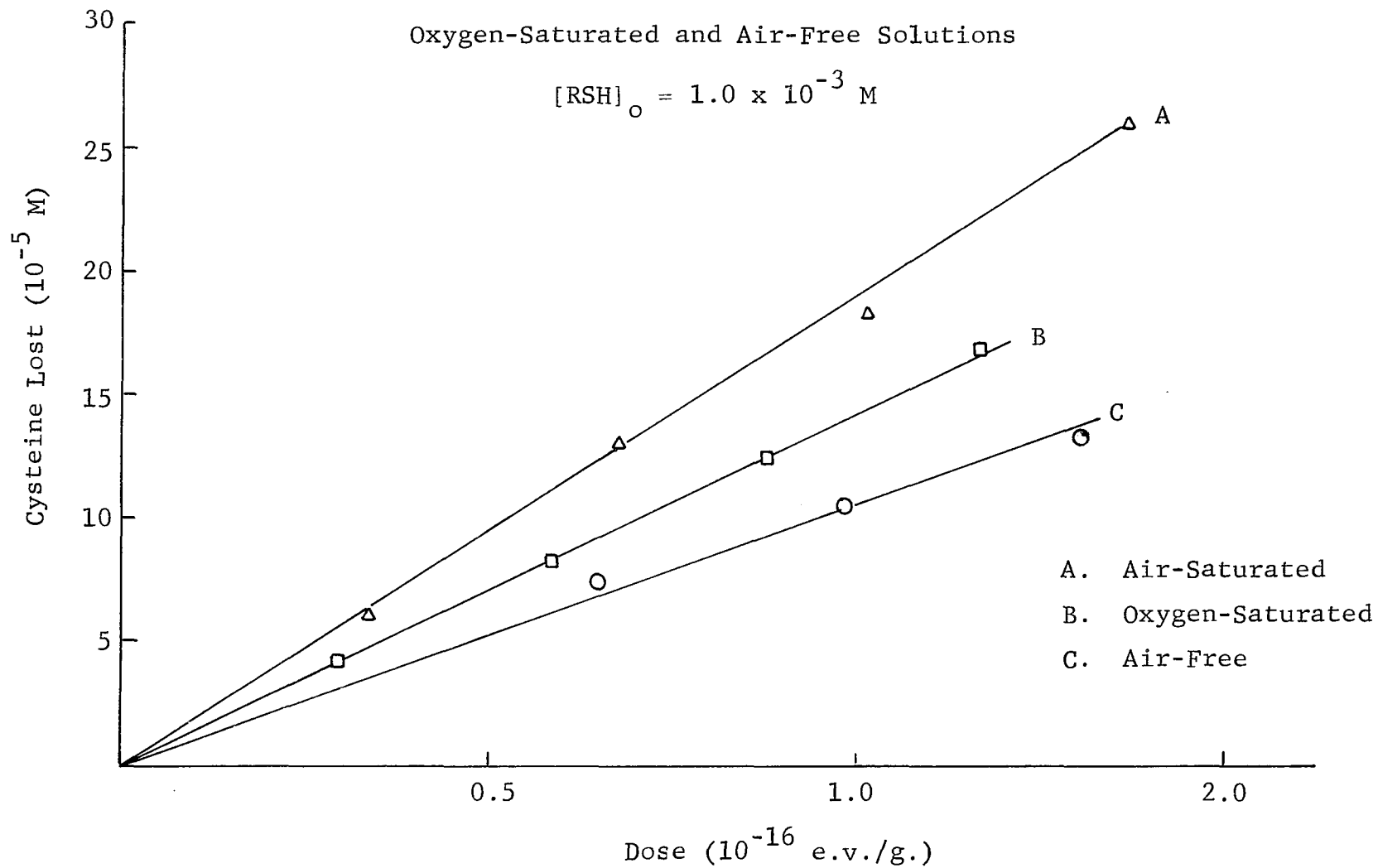


FIGURE XVI

Yield-Dose Curves for Thiol Loss in Air-Saturated,
Oxygen-Saturated and Air-Free Solutions

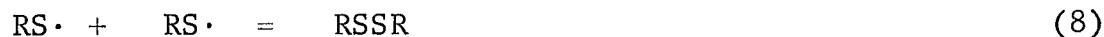
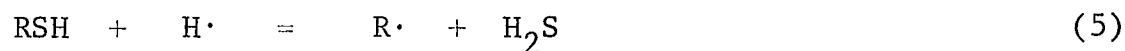
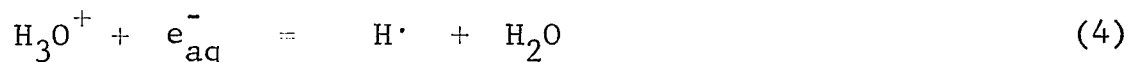
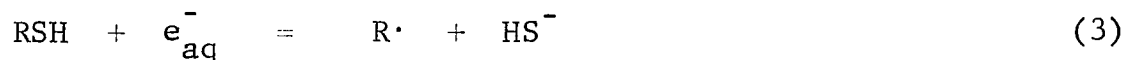
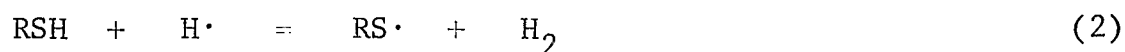
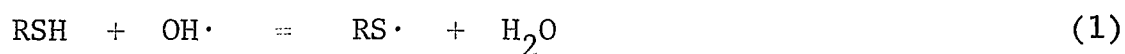
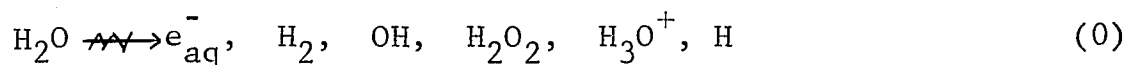
$$[\text{RSH}]_0 = 1.0 \times 10^{-3} \text{ M}$$



DISCUSSION

In the following discussion the observed radiolytic yield of a substance is denoted by G with the formula of the substance in parentheses; e.g. G(H₂S). The yields of substances formed initially from water (primary yields) are denoted by G with the formula of the substance as a subscript; e.g. G_{H₂O₂}.

The results obtained in oxygen-free solutions can be best explained on the basis of the following reaction sequence:*



(RSH = cysteine)

* For 10^{-3} M RSH at pH ~ 7 , the thiol group is less than 5% ionized. (23)

It is assumed that formation of peroxide in irradiated acidified solutions (pH 0.95) does not affect the decomposition of cysteine. This assumption is valid since hydrogen peroxide does not react with cysteine in acid solutions.⁽²⁴⁾ Furthermore, it has been shown in this study, and in other work as well,⁽²⁵⁾ that the concentration of peroxide in irradiated oxygen-free acid solutions of cysteine is consistent with the primary yield, $G_{H_2O_2}$. (See Table II).

Since the rate constant for the conversion of e_{aq}^- to $H\cdot$ by hydronium ion is extremely large ($k = 2 \times 10^{10} \text{ M}^{-1} \text{ sec}^{-1}$), equation (4) has been included in the reaction scheme.

In deriving the expression for the reaction kinetics from such a scheme, the assumption is made that each free radical formed must disappear. Thus the sum of the number of radicals of any given kind formed per 100 e.v. of radiation dose by reactions in the above scheme may be equated to the sum of the number consumed in other reactions. It is customary to denote the number of times a reaction occurs per 100 e.v. by the number of the equations enclosed in parentheses. Then,

$$\text{for } OH\cdot, \quad G_{OH} = (1) \quad (a)$$

$$\text{for Red}^*, \quad G_{RED} = (2) + (3) + (5) + (7) \quad (b)$$

$$\text{for } R\cdot, \quad (3) + (5) = (6) + (7) \quad (c)$$

$$\text{The observed yield of hydrogen } G(H_2) = G_{H_2} + (2) \quad (d)$$

$$\text{of thiol disappearance } G(-RSH) = (1) + (2) + (3) + (5) + (6) \quad (e)$$

$$* \quad G_{RED} = G_H + G_{e_{aq}^-}$$

and of hydrogen sulfide formation

$$G(\text{H}_2\text{S}) = (5) + (3) \quad (\text{f})$$

Substituting (f) into (b) and (e), and (a) and (f) into (e), we obtain the following equations:

$$(2) + (7) = G_{\text{RED}} - G(\text{H}_2\text{S}) \quad (\text{b}^1)$$

$$(6) + (7) = G(\text{H}_2\text{S}) \quad (\text{c}^1)$$

$$(2) + (6) = G(-\text{RSH}) - G_{\text{OH}} - G(\text{H}_2\text{S}) \quad (\text{e}^1)$$

also

$$(2) = G(\text{H}_2) - G_{\text{H}_2} \quad (\text{d})$$

subtracting (c¹) from the sum of (e¹) + (b¹), and substituting (d) into the resulting equation, we have finally

$$3 G(\text{H}_2\text{S}) + 2G(\text{H}_2) - G(-\text{RSH}) = 2G_{\text{H}_2} + G_{\text{RED}} - G_{\text{OH}} \quad (\text{g})$$

Since the values of $G(\text{H}_2\text{S})$, $G(\text{H}_2)$ and $G(-\text{RSH})$ have been measured (Table II), it is possible to obtain a "calculated" value for each of the three observed yields. For example, from the measured values of $G(\text{H}_2\text{S})$, $G(-\text{RSH})$ and the known values of the primary yields on the right hand side of equation (g), $G(\text{H}_2)$ is calculable. A test of the validity of the proposed mechanism can then be made by comparing the calculated value of the yield with the measured value. Actually, in view of the large uncertainty associated with the measured value of the hydrogen sulfide yield,* the only meaningful

* See experimental section.

comparison which can be made is that between $G(\text{H}_2\text{S})$ -calculated and $G(\text{H}_2\text{S})$ -measured. -- Equation (g) may be rearranged to give

$$G(\text{H}_2\text{S}) = \frac{2G_{\text{H}_2} + G_{\text{RED}} - G_{\text{OH}} + 2G(\text{H}_2) - G(-\text{RSH})}{3}$$

The values of the primary yields in the above equation are to some extent pH dependent (Fig. XVII). In 0.1 M sulfuric acid (pH 0.95) the values of G_{H_2} , G_{RED} and G_{OH} are 0.40, 3.6 and 2.9 respectively. ⁽²⁶⁾ From Table II, $G(\text{H}_2) = 2.8$, and $G(-\text{RSH}) = 6.6$. Substituting these values into the above equation, we obtain $G(\text{H}_2\text{S})$ -calculated = 0.8, in reasonable agreement with the measured value of 0.6 (Table II).

If equations (b) and (e) are added, and (c) subtracted from the resulting equation, the expression for $G(-\text{RSH})$ becomes

$$G(-\text{RSH}) = 2(2) + 3(3) + 3(5) + G_{\text{OH}} - G_{\text{RED}} \quad (\text{h})$$

From the reaction kinetics,

$$(2) = \frac{G_{\text{H}} + (4)}{1 + \frac{k_5}{k_2} + \frac{k_7 [\text{R}]}{k_2 [\text{RSH}]}}$$

$$(3) = \frac{G_{\text{e-}}^{\text{aq}}}{1 + \frac{k_4 [\text{H}_3\text{O}^+]}{k_3 [\text{RSH}]}}$$

$$(4) = \frac{G_{\text{e-}}^{\text{aq}}}{1 + \frac{k_3 [\text{RSH}]}{k_4 [\text{H}_3\text{O}^+]}}$$

$$(5) = \frac{G_{\text{H}} + (4)}{1 + \frac{k_2}{k_5} + \frac{k_7 [\text{R}]}{k_5 [\text{RSH}]}}$$

Then, by substitution into (h)

$$\begin{aligned}
 G(-RSH) = & \frac{2 \left(G_H + \frac{G_{e_{aq}^-}}{k_3 [RSH]} \right)}{1 + \frac{k_5}{k_2} + \frac{k_7 [R\cdot]}{k_2 [RSH]}} + \frac{3 G_{e_{aq}^-}}{1 + \frac{k_4 [H_3O^+]}{k_3 [RSH]}} \\
 & + 3 \left(\frac{G_H + \frac{G_{e_{aq}^-}}{k_3 [RSH]}}{1 + \frac{k_4 [H_3O^+]}{k_3 [RSH]}} \right) + G_{OH} - G_{RED} \quad (i) \\
 & \frac{1 + \frac{k_2}{k_5} + \frac{k_7 [R\cdot]}{k_5 [RSH]}}
 \end{aligned}$$

Now, according to the reaction scheme, R· radicals formed in the irradiated solution will disappear via reactions (6) and (7). Radical combination reactions such as (7) are extremely rapid, having rate constants that approach $10^{10} \text{ M}^{-1} \text{ sec.}^{-1}$. With this rate constant it can easily be shown* that the concentration of R· radicals would not be likely to exceed 10^{-7} M . Therefore, for 10^{-3} M RSH , the value of $[R\cdot]/[RSH]$ in (i) should not be larger than 10^{-4} . Also, the values⁺ of the rate constants, k_2 and k_5 , are of the order $10^{10} \text{ M}^{-1} \text{ sec.}^{-1}$; consequently, the ratios $\frac{k_7}{k_2}$ and $\frac{k_7}{k_5}$ are approximately equal

* See Appendix

+ See later discussion, p. 44

to unity. Armstrong and Wilkening⁽¹⁵⁾ have estimated the values of $\frac{k_2}{k_5}$ and $\frac{k_4}{k_3}$ to be about 1.5 and 2 respectively.

In view of these results it may be concluded that the ratios $\frac{k_7 [R\cdot]}{k_2 [RSH]}$ and $\frac{k_7 [R\cdot]}{k_5 [RSH]}$ are small in comparison with

$(1 + \frac{k_5}{k_2})$ and $(1 + \frac{k_7}{k_5})$ respectively, and may be ignored.

Substituting the above values of the rate constant ratios into (i) and rearranging, we obtain the following equation:

$$G(-RSH) = \frac{7}{5} G_H + G_{OH} + \left(\frac{2[RSH] + \frac{14}{5} [H_3O^+]}{[RSH] + 2[H_3O^+]} \right) G_{e\bar{a}q} \quad (i)$$

This expression derived on the basis of the proposed mechanism predicts a complex dependence of the reaction yield on the initial acid and thiol concentrations. To determine just how the quantity, $G(-RSH)$, would be expected to vary with changes in either $[H_3O^+]$ or $[RSH]_0$ it is necessary to examine the components of the above equation analytically. The matter is complicated to some extent by the fact that hydrogen peroxide is quite reactive with cysteine above pH 4⁽²⁴⁾, and consequently, its effect on the reaction yield must also be considered. The parenthetic term in equation (j) was evaluated at several pH values with $[RSH]$ equal to 1.0×10^{-3} M. The results are given below.

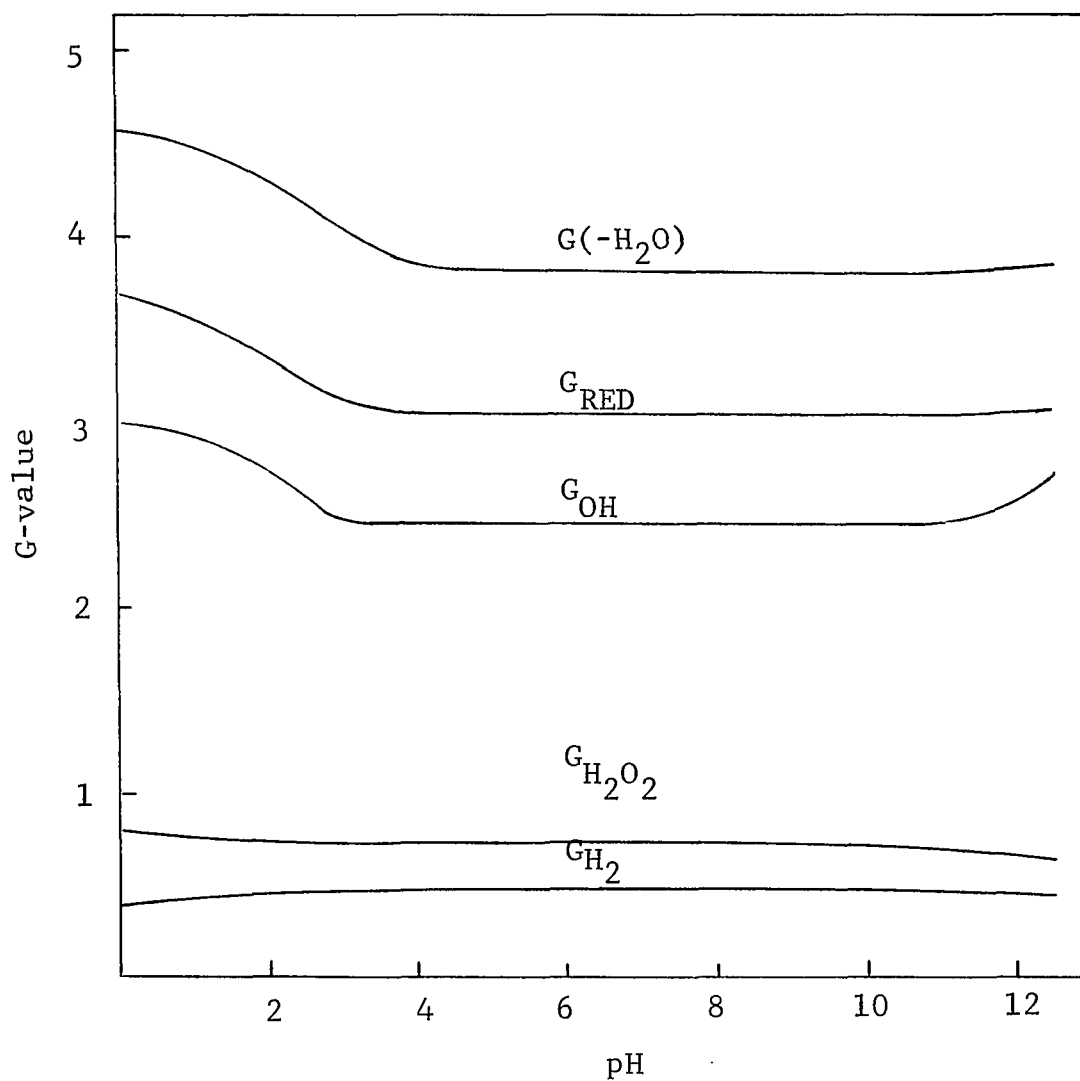
<u>pH</u>	<u>f(H⁺, RSH)[*]</u>
0.00	1.4
0.30	1.4
1.0	1.4
2.0	1.4
2.1	1.5
2.3	1.5
3.0	1.6
3.3	1.7
3.5	1.8
4.0	1.9
4.3	1.9
4.7	2.0
5.0	2.0
6.0	2.0

It may be seen that $f(\text{H}^+, \text{RSH})$ attains a lower limiting value below pH 2.0, and an upper limiting value above pH 4.7. Hence as the value of the pH is increased from 2.0 to 4.7, $f(\text{H}^+, \text{RSH})$ will tend to increase the value of $G(-\text{RSH})$ given by (j). Fig. XVII shows that the primary yields, G_{RED} and G_{OH} , decrease with pH in the range 0 to 4, then remain nearly constant up to about pH 11.3. Accordingly, the quantity $G(-\text{RSH})$ might be expected to vary with pH as indicated below

$$^* f(\text{H}^+, \text{RSH}) = \left(\frac{2[\text{RSH}] + 2.8 [\text{H}_3\text{O}^+]}{[\text{RSH}] + 2[\text{H}_3\text{O}^+]} \right)$$

FIGURE XVII

Dependence of Yields of $G(-H_2O)$, G_{RED} , G_{OH} ,
 $G_{H_2O_2}$ and G_{H_2} as a Function of pH. (26)

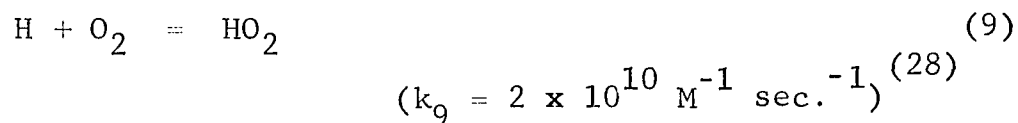


- a.) decrease with pH in the range from 0 to 2.
- b.) may increase or decrease with pH in the range from 2 to 4
- c.) above pH 4 reaction between cysteine and hydrogen peroxide would lead to an increase in G(-RSH).

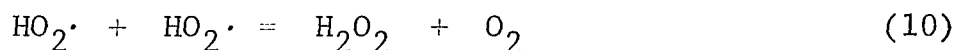
Experimentally (Fig. XV), it was found that as the pH was increased starting from 0.32, G(-RSH) decreased from a value of 6.7 to a minimum value around pH 2.8, then rose to a value of 7.3 at pH 5.6. This behavior of the reaction yield is consistent with the expected behavior over the pH range investigated.

With $[H_3O^+] = 0.1$ M and $.0010 \leq [RSH] \leq .010$ M, the quantity $f(H^+, RSH)$ in (j) is a constant. Hence, in this same concentration region the value of G(-RSH) given by (j) also remains constant. From Table III-a it may be seen that the measured yields of thiol loss vary from 6.5 to 7.4 in the concentration range 1.0×10^{-3} to 1.0×10^{-2} M [RSH]. This dependence of the decomposition yield on $[RSH]_0$ is not entirely unexpected, since the thiol may react with free radicals in the spur; and the reaction probability increases with thiol concentration.

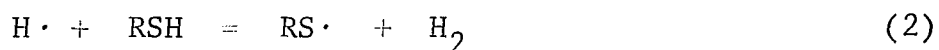
When oxygen is present in the irradiated solution, the following reaction is expected to occur readily:



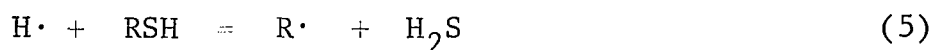
The $\text{HO}_2\cdot$ radicals are not as reactive as the hydrogen atoms which they replace, and unlike the latter, rarely abstract hydrogen from organic solutes.⁽²⁷⁾ The usual fate of the $\text{HO}_2\cdot$ radical is disproportionation



Since reaction (9) is competitive with reactions (2)



and (5)

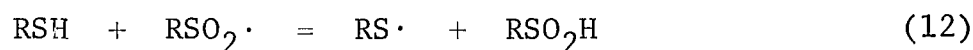


it is clear that oxygen, by removing hydrogen atoms from the solution, can confer a measure of protection on the thiol group. This is indicated by the lower yields of hydrogen and hydrogen sulfide in the oxygen-saturated solutions (Table II). (Since the thiol is able to compete with oxygen at a comparable concentration for hydrogen atoms, the rate constants for reactions (2) and (5) must be about the same order of magnitude as k_9 . This fact appears noteworthy since few other solutes are known to be as reactive as oxygen toward hydrogen atoms.⁽²⁷⁾)

Oxygen, being a diradical, adds readily to free radicals in solution. Hence, in the oxygenated system, the following reaction is likely to occur:

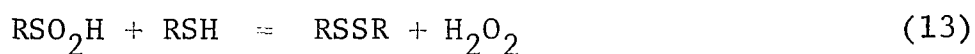


The peroxy radical so formed may easily abstract a hydrogen atom from the thiol group.⁽¹⁴⁾ Thus,

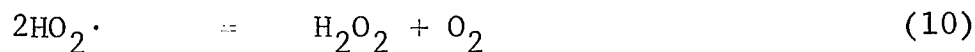
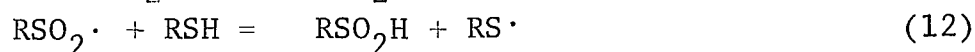
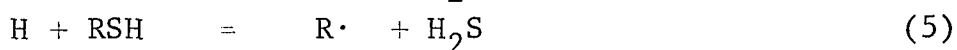


Equations (11) and (12) constitute a chain reaction. Such a chain is probably responsible for the high yields of thiol destruction in the presence of oxygen (Table II).

If hydrogen peroxide in excess of the primary yield were formed only via reaction (10), its maximum yield in acid solution (assuming that all $H\cdot$ radicals are converted by oxygen to $HO_2\cdot$) would be equal to $G_H/2 + G_{H_2O_2} = 2.6$. Since the value of $G(H_2O_2)^{O_2}$ is equal to 4.0 (Table II), hydrogen peroxide must be formed in some other reaction as well. The sulfinic acid intermediate (RSO_2H) possibly reacts with cysteine to give hydrogen peroxide and the disulfide:



Equations (1) through (13) constitute the proposed reaction scheme for the oxygenated system. Some of these equations are rewritten below:



From equations (9), (2) and (5) it is clear that an increase in thiol concentration, at constant oxygen concentration, is expected to increase the rate of thiol destruction. This is shown in Table III-b for the oxygen-saturated system. It may also be seen that the extent of the increase is too large to be attributed to just a spur effect. (Compare data in Tables III-a and III-b).

The dependence of the reaction yield on oxygen concentration is somewhat more complex. For 1.0×10^{-3} M cysteine the values of $G(-RSH)^{O_2\text{-free}}$, $G(-RSH)^{\text{air}}$, and $G(-RSH)^{O_2}$ are 6.5, 11.5 and 8.4 respectively. Evidently, at sufficiently low concentrations, oxygen acts primarily in propagating the chain via reactions (11) and (12). However, at high concentration, oxygen can effectively remove hydrogen atoms from the solution, thereby impeding the chain.

It is well known that anoxia decreases the radio-sensitivity of biological systems.⁽²⁹⁾ Thus chemical compounds which are capable of reducing intracellular oxygen tension are generally found to be excellent protective agents. It is suggested that the apparent ability of cysteine to use up oxygen (i.e. $RS\cdot + O_2 = RSO_2\cdot$) may at least partly explain its effect in protecting living systems against lethal radiation.

SUMMARY

Dilute aqueous solutions of cysteine were irradiated with gamma rays from a cobalt-60 source. Radiolytic yields (G values) were determined for the disappearance of cysteine and for the formation of hydrogen, hydrogen sulfide, and hydrogen peroxide in both deaerated and oxygenated solutions. In the absence of air the yield of thiol loss was found to be dependent on pH and on the initial thiol concentration, while in the presence of oxygen the yield was sensitive to both the initial thiol and oxygen concentrations. Oxygen reduced the yields of both hydrogen and hydrogen sulfide, but enhanced the yield of hydrogen peroxide.

The effects of various solutes on the rate of thiol destruction in the irradiated solutions were investigated. The results obtained indicate that cysteine can react quite readily with the intermediates produced by the radiolysis of water.

Complete mechanisms have been postulated for the radiation induced decomposition of cysteine in the deaerated and oxygenated solutions. In addition, a possible explanation has been given for the mechanism of radiation protection of biological systems by cysteine.

BIBLIOGRAPHY

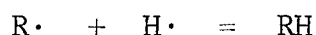
1. Hart, E. J., Science, 146, 19 (1964).
2. Allen, A. O., Radiation Res. Suppl., 4, 54 (1964).
3. Patt, H.M. et al, Science, 110, 213 (1949).
4. Thomson, J. F., "Radiation Protection in Mammals", Reinhold Publishing Corp., New York, 1962.
5. Dale, W. M. and Davies, J. V., Biochem. J., 48, 129 (1951).
6. Whitcher, S. L., Rotheram, M. and Todd, N., Nucleonics, 11, 30 (1953).
7. Markakis, P. and Tappel, A. L., J. Am. Chem. Soc., 82, 613 (1960).
8. Forbes, W. F., Rivett, D. E. and Savige, W. E., Photochem. Photobiol., 1, 97 (1962).
9. Grant, D. W. and Armstrong, W. A., Can. J. Chem., 41, 1882 (1963).
10. Maxwell, C. R., Blair, A. E. and Sharpless, N. E., Radiation Res. 2, 135 (1955).
11. Swallow, A. J., "Radiation Chemistry of Organic Compounds", Pergamon Press Inc., New York, 1960.
12. Swallow, A. J., J. Chem. Soc., 1334 (1962).
13. Littman, F. E., Carr, E. M. and Brady, A. P., Radiation Res., 7, 107 (1957).
14. Packer, J. E., Nature, 194, 81 (1962).
15. Armstrong, D. A. and Wilkening, J. G., Can. J. Chem., 42, 2631 (1964).

16. Lamprey, P. S., Ph.D. Thesis, University of New Hampshire, August, 1960.
17. Shinohara, K., J. Biol. Chem., 110, 263 (1935).
18. Folin, O. and Marenzi, A. D., J. Biol. Chem., 83, 109 (1929).
19. Eisenberg, G. M., Ind. Eng. Chem., Analyt., 15, 327 (1943).
20. Snell, F. D. and Snell, C. T., "Colorimetric Methods of Analysis", Vol. II, 3rd. ed., D. Van Nostrand Co., Princeton, New Jersey, 1959, p. 663.
21. Allen, A. O., "The Radiation Chemistry of Water and Aqueous Solutions", D. Van Nostrand Co., Princeton, New Jersey, 1961, p. 63.
22. Mahlman, H. A. and Sworski, T. J., Presented at 150th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept. 1965. Abstract of papers p. 21V.
23. Benesch, R. E., J. Am. Chem. Soc., 77, 5877 (1955).
24. Pirie, N. W., Biochem. J., 25, 1565 (1931).
25. Packer, J. E., J. Chem. Soc., 2320 (1963).
26. Hayon, E., Trans. Faraday Soc., 61, 734 (1965).
27. Baxendale, J. H., Radiation Res. Suppl., 4, 119 (1964).
28. Anbar, N. and Neta, P., "Tables of Bimolecular Rate Constants of Hydrated Electrons, Hydrogen Atoms and Hydroxyl Radicals with Inorganic and Organic Compounds", Israel Atomic Energy Commission, Soreq Research Establishment, IA-992, 1964.
29. Dowdy, A. H., Bennett, L. R. and Chastain, S. M., Radiology, 55, 879 (1950). See also ref. (4) p. 161.
30. Spinks, J. W. T. and Woods, R. J. "An Introduction to Radiation Chemistry", John Wiley and Sons, Inc., New York, 1964, p. 248.

APPENDIX

Estimation of the Equilibrium Concentration of R·
Radicals in the Irradiated Solution of RSH.

The rate constant for the radical combination reaction



is assumed to be of the order $10^{10} \text{ M}^{-1} \text{ sec}^{-1}$. The rate of removal of R· radicals is then given by,

$$[R\cdot] [H\cdot] \times 10^{10} \text{ M}^{-1} \text{ sec}^{-1}$$

The maximum concentration of hydrogen radicals at the dose rate used* is 10^{-8} M . (30) Thus,

$$\text{Rate of removal of } R\cdot = [R\cdot] \times 10^2 \text{ M}^{-1} \text{ sec}^{-1}$$

For a dose rate of $10^{17} \frac{\text{ev}}{\text{g sec}}$ and a value of $G(R\cdot)$ as high as 10, the rate of radical production is given by,

$$\frac{10 \text{ (radicals)}}{100 \text{ ev}} \times \frac{1}{6.03 \times 10^{23}} \frac{\text{(mole)}}{\text{(radical)}} \times 10^3 \frac{\text{(g)}}{\text{(liter)}} \times 10^{17} \frac{\text{(ev)}}{\text{(g sec)}}$$

Applying the steady state approximation, we find

$$[R\cdot] = 10^{-7}$$

* See page 6

BIOGRAPHICAL DATA

Name: John Lucian Festa

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Place of Birth: Waterbury, Connecticut

Secondary Education: Crosby High School, Waterbury, Connecticut

Collegiate Institutions Attended:	Dates	Degree
University of Connecticut	1958-1962	B.A.
University of New Hampshire	1962-1966	Ph.D.