THE RADIOLYSIS OF CYTOSINE AND OTHER BIOLOGICAL SOLUTES

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (Continued)

CHAPTER I

INTRODUCTION

The general aim of this thesis is to contribute some new experimental results, interpretations, and theoretical concepts to existing knowledge concerning the effect of ionizing radiation on living organisms. More specifically, the thesis deals with a phenomenological formulation of the radiolysis of organic compounds in aqueous solutions and with the radiolysis of cytosine.

Chapter II presents a literature survey of the radiolysis of water and dilute aqueous solution of the importance of DNA and DNA bases in radiation biology, and of the radiolysis of cytosine. Chapter III concerns the radiolysis of organic compounds in aqueous solution and presents a phenomenological formulation as well as mechanistic models. Chapter IV examines the radiolysis of cytosine in both oxygen-saturated and helium-saturated systems. A spectrophotometric method for the determination of the $\underline{G}$ value for decomposition of cytosine is described. Some of the phenomenological formulations and mechanistic interpretations developed generally in Chapter III are here applied to a specific case. Chapters III and IV have been written in a form suitable for publication.

Finally, additional experimental results, which could not be included in the preceding chapters, are given in Chapter VI.

CHAPTER II

LITERATURE SURVEY

Introduction

The harmful effects of ionizing radiation on living cells are ultimately due, in large part if not wholly, to the alteration of DNA. Consequently, this substance and its related compounds are of special significance and interest from the radiobiological point of view. Many investigators, have discussed the close relationship that exists between radiobiological injuries in living organisms and the radiolysis of their constituents. This aspect of the subject may be called molecular radiation biology. In this chapter, first there is presented some general background to the subject, and then a detailed literature review of work done previously in the specific topics investigated in this thesis.

Some General References

The chronological development of the radiation chemistry of aqueous solutions has been described by Haissinsky (1). The book by Spinks and Woods (2) gives a good general introduction to the subject. The book by Allen (3) and the chapter by Schwarz (4) deal specifically with the radiolysis of water and aqueous solutions; more recent are the books by Draganic and Draganic (5) and by O'Dannell and Sangster (6) and the chapters by Anbar (7), by Thomas (8), and by Hart (9). Some of the books

concerning more specialized topics are The Hydrated Electron by Hart and Anbar (10), Pulse Radiolysis by Matheson and Dorfman (11), and Pulse Radiolysis, edited by Ekert and others (12). The book by Pikaev (13) contains a list of the rate constants for more than 600 pulse radiolysis reactions.

Good general references on radiobiology are the books by Bacq and Alexander (18), Casarett (19), and Okada (20), as well as the serial edited by Ebert and Howard (21). The chapter by Alexander and Lett (22) is concerned with the effect of radiation on biological macromolecules. The importance of DNA in radiobiology has been discussed extensively in the literature (19,20,24,25,59). Kanazir (23) has reviewed specifically the role of DNA in radiation damage to living systems. The chapters by Weiss (26), by Scholes (27), and by Swingle and Cole (28) deal with the chemical effects of radiation on nucleic acids and related compounds.

Radiolysis of Water and Dilute Aqueous Solutions

Water is the predominant component of most living organisms. In the radiolysis of water, chemically reactive species are formed, which then react rapidly with most biological compounds. The radiolysis of water and dilute aqueous solutions, consequently, has been intensively studied. To some extent, the process depends on the kind of radiation used; unless stated otherwise, the discussion below refers to cobalt^{60} γ-rays.

There are three major stages in radiolysis. In the first stage, within 10^{-15} - 10^{-13} second, the events that occur are conventionally described as "physical". They are ionization, excitation, and super-excitation of water molecules. Energy deposition has been described as

involving the formations of spurs, blobs, and short tracks (2,3,5,6,8, 14). Co^{60} γ-rays have a low linear energy transfer (L.E.T.) and the energy deposition is mainly in the form of spurs which are widely separated.

In the second stage, physico-chemical reactions take place. The important process is the formation of primary radicals in the spurs, within 10^{-11} second. They are hydroxy radicals (OH.), hydrated electrons $(H_2O.)^-$, and hydrogen atoms (H.). The rate at which they are produced is usually expressed as number of radicals per 100 eV of energy absorbed by the system and called the G value. In pure water, the G values for (OH.), $(H_2O\cdot)^-$, and (H.) are about 2.6, 2.6, and 0.5, respectively (10). The mechanisms of the radiolysis can be represented as follows:

$$3\ H_2O \xrightarrow{\sim\sim} (OH.) + (H_2O.)^- + H_3O^+ \qquad (I\text{-}1)$$

$$4\ H_2O \xrightarrow{\sim\sim} (H.) + 3/2\ H_2 + 2\ H_2O_2 \qquad (I\text{-}2)$$

$$(H_2O.)^- + H_3O^+ \rightarrow (H.) + H_2O \qquad (I\text{-}3)$$

$$(H.) + (OH.) \rightarrow H_2O \qquad (I\text{-}4)$$

$$(OH.) + (OH.) \rightarrow H_2O_2 \qquad (I\text{-}5)$$

$$(H.) + (H.) \rightarrow H_2 \qquad (I\text{-}6)$$

$$H_2 + (OH.) \rightarrow (H.) + H_2O \qquad (I\text{-}7)$$

$$H_2O_2 + (H.) \rightarrow (OH.) + H_2O \qquad (I\text{-}8)$$

Reactions (1) and (2) occur independently. The rate constants are in the order of 10^{10} M^{-1} sec^{-1} for reactions (3) - (6), and of 10^7 M^{-1}

sec^{-1} for reactions (7) and (8). According to the scheme, the concentrations of H_2 and H_2O_2 attain a steady state when H_2 is kept within the system. There is extensive recombination of the radicals, as shown in reaction (4), to regenerate water molecules. Because hydrogen, H_2, and hydrogen peroxide, H_2O_2, are converted back to water by reactions (7) and (8), in the radiolysis of pure water little net decomposition occurs.

The radiolysis may be enhanced by the presence of solutes; the G values for $(H_2O\cdot)^-$ and (OH.) may increase to 3 or more (15,16,17). A third stage, then, takes place, in which the chemically reactive primary radicals react with the solute in various ways. With organic solutes, some important processes are addition to carbon-carbon double bonds, abstraction of hydrogen from C - H or C - OH groups, and transfer of electrons onto solute molecules.

By the technique of pulse radiolysis, the spectrum of the resulting organic radicals often can be observed directly. The role of these radicals can then be more easily determined. Finally, these organic radicals combine together to form products. The destruction of solute and the formation of products are generally studied in steady state radiolysis. If oxygen is present in a system, it also can react with the hydrated electrons, hydrogen atoms, and organic radicals.

The Importance of DNA and of DNA Bases in Radiation Biology

The interaction of ionizing radiation with living organisms can lead to various types of damage, such as loss of the selective permeability of cell membranes, gross structural chromosome change, and subtle chemical changes in the structure of DNA. Death of the organ-

isms can result from this damage.

Evidence that DNA is the most radiosensitive material in cells has been extensively discussed in the literature (19,20,22-24). Some of the evidence is as follows:

(1) The DNA bases are among the most radiosensitive biological compounds.

(2) The radiosensitivity of DNA can be varied by chemical changes in the molecule. For example: 5-bromouracil can be incorporated into bacterial DNA in the place of thymine and the bacterial cells are more radiosensitive than those with non-substituted DNA.

(3) Precisely focused microbeams of ionizing radiation can be used to irradiate specific areas of individual cells. Much smaller doses are required to produce cell death when the beam is focused on the nucleus then on the cytoplasm.

These findings, along with many others, make DNA of particular significance and interest from a radiobiological point of view.

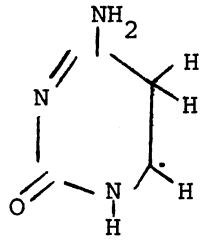
The effects of radiation on DNA have been studied for decades both in solution (3,32-35) and in the solid state (37,38). The effects in solution comprise: deamination, hydroxylation, ring opening of the DNA bases, the formation of urea, and the liberation of bases and inorganic phosphate. These chemical changes are "indirect", i.e., caused by reaction with radicals produced in water by the radiation. Since the molecular weight of DNA is at least several millions, it contains a large number of sites which can undergo reaction with the radicals, giving in turn a great variety of products. The decomposition process must accordingly be very complex.

Although the polymer, DNA, may behave differently from the sum of

its parts, it is not unreasonable to expect that study of the four DNA bases--cytosine, thymine, adenine, and guanine--could serve as a model for the radiation damage in DNA. Recently, much attention has been paid to how radiation interacts indirectly with each one of the four bases. Cytosine was chosen for this study because relatively little was known about its radiolysis. In addition, some of the products, uracil and 5,6-dihydrocytosine derivatives, may be involved in mutagenesis (29-31).

Literature Review on the Radiolysis of Cytosine

The early work on the radiolysis of cytosine was primarily concerned with the qualitative identification of the products formed. More recently, quantitative measurements of the destruction of cytosine have also been reported. Experiments have been done both in the solid state and in solution. The latter are more numerous, and also more interesting from the radiobiological point of view, because water is the predominant constituent of cells. It is, however, pertinent to note one result obtained in the solid state. Powdered cytosine was exposed to a stream of hydrogen atoms, produced in a pyrex tube by an electric discharge, and this gave a radical, that has been identified by ESR measurements; it has the structure (39,61):



This breaking of the C5 - C6 double bond agrees well with the nature of the major products obtained in solution, dihydrocytosine derivatives and 5-hydroxycytosine, which also indicate primary radical attack at the C5 - C6 positions.

The radiolysis of cytosine in solution has been described in many papers (40-57) and reviews (26,27,34,60). Reported values for G(-cytosine) and G(product), as determined by ultraviolet and chromatographic methods, are listed chronologically in Tables I and II; the former lists data obtained in presence of oxygen, the latter data in absence of oxygen. A variety of conditions and methods were used in the quantitative determinations, which makes it difficult or impossible to make comparisons. In the following discussion, first we consider the facts, then attempt to organize them as well as possible.

System Containing Oxygen

(A) Value of G(-cytosine). Cytosine has an intense absorption maximum at 266 nm at pH 6 - 11, which decreases on exposure to radiation. The first determination of the initial G value, G_o, made by Scholes, et al., in a 2×10^{-4} M cytosine solution, and the result was reported to be about 2.2. The determination was done both by chromatographic and ultraviolet methods. Later, much higher values of G, 3.0 - 3.8, were reported by other groups (42-45). This indicates that the products must have substantial absorbance at the peak and that Scholes' value is too low.

The value of G(-cytosine) was also reported to be about 3 by Holian and Garrison (46). In this investigation, Cu^{+2} was used in the place of O_2; the metal ion is a good $(H_2O.)^-$ scavenger, so that only oxidized cytosine radicals would be produced. On the other hand, Kamal and Garrison (47) produced only reduced cytosine radicals by using either sodium formate or ethanol in a de-aerated system. These compounds are good (OH.) scavengers while they are relatively inert toward $(H_2O.)^-$.

TABLE I

VALUES OF G(-CYTOSINE) AND G(PRODUCT) IN OXYGENATED SOLUTIONS

O_2 or air content	Initial cytosine concn., M	pH	G(-Cytosine)	G(Product)	Comments, Reference
O_2,1 atm	2×10^{-4}	5.2	G_o = 2.05 - 2.28 G_o = 2.16		UV Spectrophotometry chromatography Scholes et al., 1960
O_2,1 atm or air equil.	10^{-3}			Uracil glycol 0.2; 5-hydroxy-cytosine 0.34	150 krad dose Ekert and Monier, 1960
O_2-Saturated before irradiation	10^{-3}	7.4	3.54 (80 krad) 3.26 (300 krad) 3.84 (80 krad) 3.06 (300 krad)	Cytosine Hydroperoxide 0.05 0.29 0.06 0.07	0.01 M phosphate 0.01 M phosphate unbuffered unbuffered Barszcz and Shugar, 1961
Continuous stream O_2	3×10^{-2}		3.6	H_2O_2 1.3	Krushinskaya, 1965

TABLE I (Continued)

O_2 or Air content	Initial cytosine concn., M	pH	G(-Cytosine)	G(Product)		Comments, Reference
				Cytosine hydroperoxide	H_2O_2	
Air equil.	3×10^{-3}	2		0.10	1.28	
O_2,1 atm	3×10^{-3}	2	3.3	0.52	2.58	
Air equil.	3×10^{-3}	2		0.11	1.22	Daniels and Schweibert, 1967
O_2,1 atm	3×10^{-3}	2	3.2	0.58	2.88	
	2×10^{-4}	6.5	3			$+10^{-3}$ M, 10^{-2} M NaCl
	2×10^{-4}	2	6.5			$+10^{-1}$ M, NaCl
	2×10^{-4}	1	8.8			$+10^{-2}$ M, NaCl
						Ward and Kuo, 1969
				1-Carbamylimidazolidone		
	1.9×10^{-2}	6		0.075		Hahn, et al., 1973

TABLE II

VALUES OF G(-CYTOSINE) AND G(PRODUCT) IN DE-OXYGENATED SOLUTIONS

Initial cytosine concn., M	pH	G(-Cytosine)	G(Product)		Comments, Reference
10^{-3}			Cytosine glycol	Uracil	
			low	0.1	Latarjet, et al., 1961
0.1 (%)		Decrease of cytosine	Uracil		
		9.8 %	2.7 %		10^3 krad dose
		10.2 %	3.7 %		2×10^3 krad dose
		39.0 %	0.2 %		5×10^3 krad dose
		53.5 %	0.05 %		10×10^3 krad dose
					Ponnamperuma, et al., 1962
			Dihydrocytosine derivatives		
5×10^{-2}	7 ± 0.3		2.4		+Sodium formate, 0.5M
					Kamal and Garrison, 1965

TABLE II (Continued)

Initial cytosine concn., M	pH	G(-Cytosine)	G(Product)		Comments, Reference
			Cytosine glycol	5-hydroxy-cytosine	
1.0×10^{-3}	3.7		2.28	0.42	$+2 \times 10^{-3}$ M Cu^{+2}
2.0×10^{-3}	3.1		2.25	0.45	$+1 \times 10^{-3}$ M Cu^{+2}
					Holian and Garrison, 1966
			Cytosine glycol	5-hydroxy-cytosine	
10^{-2}		$G_o = 1.8$	$G_{-o} = 0.1$	$G_{-o} = 0.46$	
			6-hydroxy-cytosine trace	Uracil $G_o = 0.3$	Khattack, and Green, 1966
			Dihydrocytosine derivatives		
5.0×10^{-2}	7		0.9		No other solute
5.0×10^{-2}	7		2.8		$+2.5 \times 10^{-3}$ M cysteine
5.0×10^{-2}	7		2.9		$+1.5 \times 10^{-3}$ M ascorbic acid
					Holian, and Garrison, 1969

In this case, G(-cytosine) was also reported to be about 3.

(B) The Products. Ekert and Monier (48) identified uracil glycol, 5-hydroxycytosine, and cytosine glycol from an irradiated 1.0 x 10^{-3} M unbuffered solution of cytosine. Chromatographic and ultraviolet methods were used for the analysis. The G values of the first two products at 150 krads were found to be 0.2 and 0.34, respectively. The yield of cytosine glycol was not given. The authors speculated that the three products were derived from cytosine hydroperoxide; however, they could not obtain direct evidence for the formation of that intermediate.

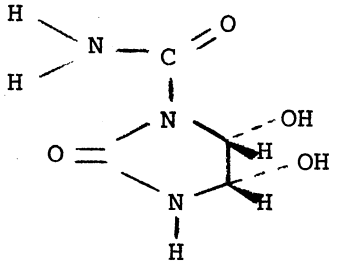
Later, Barszcz and Shugar (42) did find cytosine hydroperoxide when 1.0 x 10^{-3} M cytosine was irradiated in 0.01 M phosphate buffer at pH 7.4. G(peroxide) was reported to be dose dependent; 0.05 at 80 krads and 0.29 at 300 krads. The product was not detected when the same concentration of cytosine was irradiated in the absence of the buffer. It is not clear why the G value increased at the higher dose and how 0.01 M phosphate buffer is involved in the reaction. Daniels and Schweibert (44) observed the formation of cytosine hydroperoxide at pH 2. The amount of the product was dependent on the concentration of oxygen during irradiation. The G value was 0.52 under O_2 at 1 atm, and 0.11 in air. The rate constant for the hydrolysis of cytosine hydroperoxide was 0.73 x 10^{-4} sec^{-1} in acidic medium and 7 x 10^{-4} sec^{-1} in neutral medium. The authors suggested that the earlier results by other groups as well as their own could be explained by the rapid hydrolysis of cytosine hydroperoxide in neutral solution; alternatively, the reaction mechanism might be different in acidic and in neutral medium.

Kamal and Garrison (47) reported nearly quantitative yield of dihydrocytosine derivatives when 5.0×1.0^{-2} M cytosine was irradiated in the presence of either sodium formate or ethanol in de-aerated solution. Holian and Garrison (46) also obtained a similar result in de-aerated solution of pH 3.5, containing Cu^{+2}.

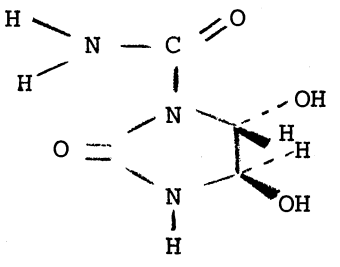
The sum of the products is nearly equivalent to the (OH.):

$$\underline{G}(\text{-cytosine}) = \underline{G}(\text{cytosine glycol}) + \underline{G}(\text{5-hydroxycytosine})$$
$$= 2.3 + 0.5 = \underline{G}(\text{OH.})$$

Most recently, Hahn et al. (49) have identified cis and trans 1-carbamylimidazolidone-4,5-diols as stable products. The G(cis + trans) value was only 0.075. These products were determined both by spectral and x-ray diffraction methods. The structures are:



Cis



Trans

De-aerated Systems

Kattach and Green (50) calculated the initial destruction of cytosine, $\underline{G}_o$(-cytosine), to be 1.8 in an unbuffered system. The G value was found to decrease rapidly with dose. The yield is only about 60% of that in an aerated system. It has been proposed that the low decomposition yield is due to the reconstitution of cytosine by recombination reactions of the cytosine radicals (26,27).

The major products were uracil, G_{-o} = 0.3, and 5-hydroxycytosine, G_{-o} = 0.46 (50). The formation of uracil had been reported previously by other groups (51,52). Plots of the yields vs. dose shows that G(-cytosine) and G(uracil) decrease with dose while G(5-hydroxycytosine) increases with dose. It is reasonable to expect uracil and 5-hydroxycytosine would be as radiosensitive as cytosine, if so the G values should decrease with dose because of the competition between cytosine and the products for the primary radicals. But it is difficult to understand why G(5-hydroxycytosine) increased with dose. Kattach and Green also reported the formation of dihydrocytosine derivatives, G_{-o} = 0.25, and of a trace amount of cytosine glycol at pH 6. Latarjet et al. (52) also found a low yield of cytosine glycol (the G value is not given). However, Holian and Garrison (53) obtained a much higher yield of dihydrocytosine derivatives; the G value is reported to be around 0.9 at pH 7. This discrepancy concerning the yield of dihydrocytosine derivatives must mainly come from the instability of the products and variation in the irradiation conditions.

Factors Affecting the Radiolysis in Solution

As discussed above, there is not good agreement among the reported values for G(-cytosine) and G(product). At least in part, the discrepancies may be due to variations in the concentration of oxygen, the pH of the system, and the dose; effects of these factors are considered below.

(A) Concentration of Oxygen. Cytosine reacts rapidly with the primary radicals as follows:

$$\text{cytosine} + (H_2O\cdot)^- \rightarrow (\text{cytosine}\cdot)^- + H_2O \qquad \text{(II-1)}$$

$$\text{cytosine} + (H\cdot) \rightarrow (\text{cytosine}H\cdot) \qquad \text{(II-2)}$$

$$\text{cytosine} + (OH\cdot) \rightarrow (\text{cytosine}OH\cdot) \qquad \text{(II-3)}$$

The rate constants are 8×10^9, 2×10^9, and 4×10^9, in units of M^{-1} sec^{-1}, respectively (27). In the presence of oxygen, there is rapid reaction with $(H_2O\cdot)^-$ and (H.) as follows:

$$O_2 + (H_2O\cdot)^- \rightarrow (O_2\cdot)^- + H_2O \qquad \text{(II-4)}$$

$$O_2 + (H\cdot) \rightarrow (HO_2\cdot) \qquad \text{(II-5)}$$

The rate constants are 2×10^{10} and 10^{10} M^{-1} sec^{-1}, respectively (13). Oxygen also attacks the oxidized cytosine radicals (26,27,54), produced by reaction (II-3), and cytosine hydroperoxide is formed.

Clearly oxygen can play a major role in the determination of reaction mechanism, and the presence or absence of oxygen in the system should be carefully controlled. However, in the literature the oxygen content is not always clearly stated.

Oxygen concentration is approximately 2×10^{-4} M in air-equilibrated solutions at $20^{\circ}C$ (58). In a 1.0×10^{-3} M cytosine solution that has been equilibrated with air, oxygen will be exhausted when the cytosine is about 20% decomposed, if diffusion of oxygen into the sample is slow. In a 1.0×10^{-3} M cytosine solution, adequate oxygenation might be maintained by bubbling oxygen through the solution during radiolysis.

(B) pH. The total yields of the reducing primary radicals, $(H_2O\cdot)^-$ and (H.), are relatively constant over the range of pH 4 - 9

(9). However, the rate constant of the reaction between cytosine and $(H_2O.)^-$ in neutral solution (Equation II-1) is more than ten times faster than that with (H.) (Equation II-2). The pK values for the ionization of cytosine are 4.5 and 12.2; therefore, cytosine exists in different forms in acidic, neutral, and alkaline solutions. Cytosine hydroperoxide, one of the major products in oxygenated solution, is relatively stable in acidic medium but quite unstable in neutral medium. For these reasons, one must expect the mechanisms of radiolysis of cytosine to vary with changing pH.

(C) Dose. The values of G(-cytosine) and G(product) are dose-dependent. This dependence is especially marked in the absence of oxygen. It is important to keep in mind that the reported G values are in some cases extrapolated to zero dose, G_o; in others they are the integral values at certain doses. It is also pertinent to know the dose rate and the method of product analysis--since the major products, dihydrocytosine derivatives, are chemically unstable, a higher dose rate and quick measurement of the products will give a higher G value.

CHAPTER III

RADIOLYSIS OF ORGANIC COMPOUNDS IN AQUEOUS SOLUTION: GENERAL CONSIDERATIONS

(To be Submitted for Publication By: George Gorin, Nobuko Ohno and L. M. Raff)

(Abstract)

The radiolysis of compounds in solution is usually a very complex process, that gives rise to many products and is affected by multifarious factors. A general approach is proposed, which facilitates the study and interpretation of the phenomenon. (1) A power-series expression is formulated to describe the rate of solute decomposition observed empirically. (2) General mechanisms are considered for the formation of products and a limit expression is derived for the total mass of products, regardless of their number and the specific mechanism by which they are formed. (3) A formulation is given for the decomposition yield $\underline{G}(-S)$ in a medium containing any number of constituents and, in particular, dissolved oxygen. (4) Curves are derived for the decomposition of S given a simple mechanism in which product U competes with S for the radicals derived from the solvent. (5) A comparison is made with $\underline{G}(-S)$ values reported in the literature.

Introduction

When the solution of an organic compound in water is exposed to ionizing radiations, decomposition of the solute always takes place. This phenomenon is of great practical and theoretical importance, and it has been extensively investigated (62-65). Unfortunately, however, the problem has proven to be quite complicated. Even with relatively simple compounds, many products may be formed, in amounts that are not directly proportional to the energy absorbed. Moreover, the amounts, and even the nature, of the products may be affected by diverse factors, such as the presence of other solutes, the dose-rate, and the absolute concentration. Although many empirical results have been obtained, so far little has been developed in the way of theory to systematize, correlate and rationalize the results.

Especially challenging is the task of discovering the relation that must exist between the chemical changes that take place in the constituents of irradiated living organisms and the biological effects which eventually ensue: an increased frequency of mutation, injuries and, possibly, death (19,20). But most biological substances have complex chemical structures; indeed many of them are polymers, containing several hundred to millions of atoms per molecule. In such cases, a myriad of products may be produced, and to study them individually becomes impossibly difficult.

One aim of the present paper is to present and develop an approach which focuses attention on the decomposition yield of some solute S, $G(-S)$, and on the extrinsic factors that modify it. Of course, the decomposition of S and the formation of products are inextricably related aspects of the same process. But it will be demonstrated that, for many

purposes, the products can be treated as a unit, without specifying their individual identities.

This approach is especially applicable to biologically "active" substances, because such activity is, in general, critically dependent on chemical structure; in many instances, any and all chemical changes destroy or radically alter that activity, and then, of course, it makes little difference whether S is converted into some product P_1, or some other product or mixture of products, $P_2 \ldots P_i$.

Conventionally, radiolysis reactions have been studied in dilute solutions, in which the mass fraction of water is 0.99 or greater. It is reasonable to expect that, in such systems, the absorption of energy and the chemical processes which follow within a short time, say < 1 ns, will be quite similar. However, dilute solutions containing one, or at most a few, solutes differ very markedly from the conditions found *in vivo*. It is therefore an open question to what extent the mechanisms worked out for dilute solutions may be applied to radiobiological problems.

For this reason, the approach developed in this paper emphasizes phenomenological interpretations, that do not depend on knowing the identity of the species, $Z_1 \ldots Z_i$, formed by the radiolytic decomposition of water. Admittedly, the interpretation which can be developed on such a basis is limited, because in most respects the nature of Z *does* have a determining influence on the chemical and biological consequences.

Pertinent Aspects of Water Radiolysis

Since water is the predominant constituent of all the systems to be considered in this paper, it is appropriate to discuss at the outset

the interaction of ionizing radiations with water itself. This is a huge subject, on which many hundreds of papers and entire books have been written (3,5,7); in this section, therefore, only a few salient points are summarized briefly.

As has been intimated, there is still considerable uncertainty concerning the nature of the processes that occur in water within <1 ns from the time energy is "deposited" (5,7). But there is, on the other hand, fairly general agreement that the main products found in water after that time are (OH·), hydrated electrons $(H_2O\cdot)^-$, and (H·). The overall process can be represented approximately by the equations[1]:

$$3H_2O \rightsquigarrow (OH\cdot) + (H_2O\cdot)^- + H_3O^+ \qquad (1)$$

$$(H_2O\cdot)^- + H_3O^+ \rightarrow (H\cdot) + 2H_2O \quad k_2 = 2 \times 10^{10} \text{ m}^3 \text{ mol}^{-1}\text{s}^{-1} \qquad (2)$$

About three (OH·) radicals and three $[(H_2O\cdot)^- + (H\cdot)]$ are produced per 100 eV of energy absorbed. It is useful to convert this quantity into SI units and, for solutions of density 1 kg dm^{-3}, into mol rad^{-1} dm^{-3} (50):

$$(6 \text{ radicals/100 eV}) \times (1 \text{ mol}/6.02 \times 10^{23} \text{ radicals}) \times ([100 \text{ eV}/1.602 \times 10^{-17} \text{ J}]) = 6.23 \times 10^{-7} \text{ mol J}^{-1} \qquad (3)$$

$$(6.23 \times 10^{-7} \text{ mol/J}) \times (1 \text{ J}/100 \text{ rad-kg}) \times (1 \text{ kg}/1 \text{ dm}^3) = 6.23 \times 10^{-9} \text{ mol rad}^{-1} \text{ dm}^{-3} \qquad (4)$$

The above mentioned species are very reactive, and they undergo the following reactions spontaneously, with the spectific-rate constants

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[1] This value, and other data constants, are taken from Ref. (10).

indicated[1]:

$$2(OH\cdot) \rightarrow H_2O_2 \qquad \underline{k}_5 = 6 \times 10^9 \text{ dm}^3 \text{ mol}^{-1}\text{s}^{-1} \tag{5}$$

$$2(H_2O\cdot)^- \rightarrow H_2 + 2OH^- \qquad \underline{k}_6 = 6 \times 10^5 \tag{6}$$

$$2(H\cdot) \rightarrow H_2 \qquad \underline{k}_7 = 1 \times 10^{10} \tag{7}$$

$$(OH\cdot) + (H_2O\cdot)^- \rightarrow OH^- + H_2O \qquad \underline{k}_8 = 3 \times 10^{10} \tag{8}$$

$$(OH\cdot) + (H\cdot) \rightarrow H_2O \qquad \underline{k}_9 = 3 \times 10^{10} \tag{9}$$

Some other reactions may be taking place, in addition (5,7). But those listed suffice, by themselves, to limit the accumulation of the aforementioned radicals to very low concentrations.

Exact calculations are complicated, but an order of magnitude result can be obtained fairly simply from the following scheme:

$$H_2O \rightsquigarrow 2(Z\cdot) \qquad G(Z\cdot) = 6/100 \text{ eV} = 6.2 \times 10^{-9} \text{ mol rad}^{-1} \text{ dm}^{-3} \tag{10}$$

$$2(Z\cdot) \rightarrow H_2O \qquad \underline{k}_{11} = 10^{10} \text{ dm}^3 \text{ mol}^{-1}\text{s}^{-1} \tag{11}$$

If $\dot{\underline{E}}$ is the rate of energy absorption, we may write for the steady-state:

$$6 \times 10^{-9} \dot{\underline{E}} = 2\underline{k}_{11} [(Z\cdot)]^2_{ss} \tag{12}$$

$$[(Z\cdot)]_{ss} = (3 \times 10^{-19} \dot{\underline{E}})^{\frac{1}{2}} \tag{13}$$

i.e., at a dose rate of, say, 100 rad s^{-1}, the steady-state concentration of $(Z\cdot)$ would be 5.5×10^{-9} mol dm^{-3}.

The radiolysis products H_2O_2 and H_2 formed in reactions (5) and (6,7), are qualitatively different from the radical products, $(Z_{\underline{i}}\cdot)$, in

that they do not react spontaneously. They would, therefore, accumulate as the radiolysis proceeds, except for the fact that they are themselves susceptible to attack by ($Z_i \cdot$) radicals (5):

$$H_2O_2 + (H_2O\cdot)^- \rightarrow (OH\cdot) + OH^- + H_2O \qquad k_{14} \sim 1 \times 10^{10} \qquad (14)$$

$$H_2O_2 + (H\cdot) \rightarrow (OH\cdot) + H_2O \qquad k_{15} \sim 10^8 \qquad (15)$$

$$H_2 + (OH\cdot) \rightarrow (H\cdot) + H_2O \qquad k_{16} = 5 \times 10^7 \qquad (16)$$

These reactions, in essence, reverse the radiolysis and consequently the interaction of radiation with pure water produces very little net decomposition, as is well known.

Since in the presence of a solute S the regeneration of water is usually prevented, in whole or in part (see below), it is pertinent to estimate the amount of water which would be decomposed if no regeneration took place. Expressed in atomic mass units, or daltons (1 dalton = $1/L = 1.66 \times 10^{-24}$ g) that amount is, approximately, 54 dalton per 100 eV or 56 ng rad^{-1} dm^{-3}.

Phenomenological Description of the Radiolysis of S

When a dose D is administered at a constant rate, $\dot{D}$, to a solution containing solute S in water, we may write:

$$E_V = E/V = Dm/V = D\rho = \dot{D}\rho t = (E_S + E_w)/V = (D/V)(m_S + m_w); \qquad (17)$$

where m is the total mass of the solution, ρ its density, and V its volume, t the time of exposure, and E_V the absorbed energy per unit

volume, while the subscripts $\underline{w}$ and S refer to water and solute, respectively. In dilute solution $\underline{m}_w >> \underline{m}_S$ and we shall assume in most of what follows that $\underline{E}_S$ is negligible in comparison to $\underline{E}_w$.

If irradiation converts S into products, the phenomenon can be described quantitatively by the expression:

$$[S] = [S]_o - \underline{a}E_V + \underline{b}E_V^2 + \underline{c}E_V^3 + \ldots \qquad (18)$$

where $[S]_o$ is the molar concentration at $\underline{E}_V = 0$, $[S]$ the concentration remaining after $\underline{E}_V$ has been absorbed, and $\underline{a}$, $\underline{b}$, $\underline{c}$, etc., are phenomenological constants.

Irradiation may cause diverse changes in the composition of the system. If these do not affect the mechanism, whatever it may be, by which $\underline{E}_V$ causes the chemical alteration of S, we may expect that the phenomenological coefficients, $\underline{b}$, $\underline{c}$, etc., will be zero and that $[S]$ will accordingly decrease in direct proportion to $\underline{E}_V$. The decomposition yield $\underline{G}(-S)$ is defined[2] as the amount of substance $\underline{n}_S$ decomposed per unit energy, and will be given by:

$$\underline{G}(-S) = (\underline{n}_S^o - \underline{n}_S)/\underline{E} = ([S]_o - [S])/\underline{E}_V = \underline{a} \qquad (19)$$

If, on the other hand, $\underline{b}$, $\underline{c}$, etc., are finite, the plot of $[S]$ vs. $\underline{E}_V$ will exhibit curvature. The value of the derivative $d[S]/d\underline{E}_V$ at $\underline{E}_V = 0$ is of special significance, in that it represents the relation between $[S]$ and $\underline{E}_V$ when the composition of the medium is only infinite-

---

[2] Often $\underline{G}$ has been defined in terms of specific units, such as molecules per 100 eV, but a general definition is preferable. Factors can be obtained from Equation (3) and (4) to convert the aforementioned units into SI units, or mol rad^{-1} dm^{-3} if ρ = 1 kg dm^{-3}: 1 molecule/100 eV = 103.6 nmol J^{-1} = 1.036 nmol rad^{-1} dm^{-3}.

simally different from that taken initially:

$$\left|d[S]/d\underline{E}_V\right|_{E=0} = \underline{a} \equiv -\underline{G}_o(-S) \qquad (20)$$

If a set of experimental results is sufficiently well represented with two coefficients, i.e., if $\underline{c}$ etc. is zero, rearrangement of (18) gives:

$$\underline{G}(-S) = ([S]_o - [S])/\underline{E}_V = \underline{G}_o(-S) - b\underline{E}_V \qquad (21)$$

i.e., a plot of $\underline{G}(-S)$ vs. $\underline{E}_V$ will be a straight line of slope $-\underline{b}$ and extrapolation of the line to the abscissa will give $\underline{G}_o$. Further analysis of the radiolysis process requires the formulation of a detailed mechanism for it. A simple example will be presented below, and a more complex one in the succeeding paper (67).

It is generally agreed that the decomposition of S in dilute solution is almost completely due to an indirect mechanism, i.e., S reacts chemically with one or more of the reactive radicals ($Z_{\underline{i}}\cdot$) mentioned in the preceding section.

An important generalization is that the reaction of a radical with an even-electron molecule necessarily produces another radical. The radicals obtainable from organic compounds are usually quite reactive in their turn, and to produce an even-electron product (which may be stable, but need not be so necessarily) two radicals must react together.

The following equations are examples of mechanisms commonly encountered (68,69) (for the present purpose, the solute will be symbolized by RH_2):

$$(RH_2) + (OH\cdot) \rightarrow (RH\cdot) + H_2O \quad (22)$$

$$(RH_2) + (H\cdot) \rightarrow (RH\cdot) + H_2 \quad (23)$$

$$2(RH\cdot) \rightarrow RH\text{-}RH \quad (24)$$

If unsaturation is present in the solute, normally addition will occur, instead of abstraction; e.g.:

$$RCH{=}CHR' + (OH\cdot) \rightarrow (RCHOH\dot{C}HR') \quad (25)$$

The scheme may be generalized to any and all reactive radicals from water, and also for the possibility that a given radical may attack S at different sites, giving diverse radicals $(T_1\cdot)\ldots(T_j\cdot)$:

$$S + (\underline{Z}_i\cdot) \rightarrow (\underline{T}_j\cdot) + \ldots \quad (26)$$

From them, in turn, there can arise a much greater number of products:

$$(\underline{T}_i\cdot) + (\underline{T}_j\cdot) \rightarrow \underline{U}_k + \ldots \quad (27)$$

If $(\underline{k}_{26}[S])$ is much larger than $[\underline{G}(\underline{Z}_i\cdot)\rho\underline{\dot{D}}]$, essentially all $(\underline{Z}_i\cdot)$ will be converted to $(\underline{T}_j\cdot)$, and if $\underline{k}_{27}$ is, in turn, as large as $\underline{k}_{11}$, or even two or three orders of magnitude smaller, $[(\underline{T}_j\cdot)]_{ss}$ will be comparable to the result obtained in (13):

$$[(\underline{T}_j\cdot)]_{ss} = (3 \times 10^{-9}\ \underline{\dot{E}}_V/\underline{k}_{27})^{\frac{1}{2}} \quad (28)$$

i.e., for moderate values of $\underline{E}_V$, $[\underline{T}_j\cdot]_{ss}$ will only attain a low value, $10^{-8} - 10^{-10}$ mol dm^{-3}, and quickly decay to zero if irradiation is stopped.

In such circumstances, from processes of the type exemplified by

(22-27), in which one molecule of S is consumed per radical, the yield $\underline{G}(-S)$ will equal the sum of the radical yields, $\Sigma\underline{G}(Z_{\underline{i}}\cdot)$. It is generally agreed that the latter is 6-8 per 100 eV.

That maximum yield will only be realized if no reactions occur that can regenerate the original solute. But diverse such reactions may in fact take place. For example, in addition to reaction (24) considered above, one may expect the reaction:

$$2(RH\cdot) \rightarrow R + RH_2 \tag{29}$$

or

$$RH_2 + (H_2O\cdot)^- \rightarrow (RH_2\cdot)^- + H_2O \tag{30}$$

$$(RH_2\cdot)^- + (RH\cdot) + H_2O \rightarrow 2RH_2 + OH^- \tag{31}$$

To the extent that such regenerative reactions occur, $\underline{G}(-S)$ will be lowered. From reactions like (29), $\underline{G}(-S)$ can, at most, be halved; but from reactions like (30) and (31) the yield can be reduced, in principle, to any value between 6-8 and 0.

On the other hand, the yield will be raised by reactions in which a radical derived from $(Z_{\underline{i}}\cdot)$ reacts with a second molecule of S:

$$S + (T_{\underline{j}}\cdot) \rightarrow (T_\alpha\cdot) + \ldots \tag{32}$$

In a subsequent Section, Comparison with Experiment, a number of empirical $\underline{G}(-S)$ values will be considered, in the light of the above mechanisms.

Because the radiolysis product may be formed by diverse concurrent and consecutive reactions, no simple relation may be expected to exist between the stoichiometric coefficients ν of S and of the products:

$$\nu_s S + \nu_{H_2O} H_2O \rightarrow \nu_1 U_1 + \ldots\ldots \nu_{\underline{k}} U_{\underline{k}} \tag{33}$$

However, summing reactions of all the types considered above consumes <u>at most</u> one molecule of H_2O per molecule of S. The ratio is lowered by reactions such as (22) and (30), which regenerate water, and by reactions such as (26 - 32), in which one radical ($Z_{\underline{i}}\cdot$) consumes more than one molecule of S. Therefore, one may write the inequality:

$$0 \leq \nu_{H_2O}/\nu_S \leq 1 \tag{34}$$

If we let $m_{\underline{k}}$ be the mass of products $U_{\underline{k}}$, we have by mass conservation

$$\sum_{\underline{k}} m_{\underline{k}} = (m^{o}_{S} - m_S) + (m^{o}_{H_2O} - m_{H_2O}) = \Delta m_S + \Delta m_{H_2O} \tag{35}$$

where the superscript zero represents the mass present initially. We may also write

$$\Delta m_{H_2O} = \Delta m_S \ (\nu_{H_2O} M_{H_2O}/\nu_S M_S) \tag{36}$$

where $M_{\underline{k}}$ represents the molar mass of substance $U_{\underline{k}}$. Combination of Eqs. (35) and (36) yields

$$\sum_{\underline{k}} m_k = \Delta m_S \ (1 + \nu_{H_2O} M_{H_2O}/\nu_S M_S) \ . \tag{37}$$

The summation in Eq. (37) runs over all stable products, $U_1 \ldots U_{\underline{k}}$, it being implied that all reactive intermediates $T_{\underline{j}}$ have disappeared when the determination is made.

At any point in the radiolysis in which no appreciable amounts of secondary products derived from $U_{\underline{k}}$ have yet been formed, we may easily obtain upper and lower bounds on $G(-S)$. From Eqs. (19) and (37), we

have

$$\Delta m_{-S} = \underline{G}(-S)\ \underline{E}_{V}\underline{M}_{S} = \sum_{\underline{k}} m_{-k}/(1 + \nu_{H_2O}M_{H_2O}/\nu_{S}M_{S}) \tag{38}$$

Since $(1 + \nu_{H_2O}M_{H_2O}/\nu_{S}M_{S}) \geq 1$, we can write

$$\underline{G}(-S) \leq \sum_{\underline{k}} m_{-k}/E_{V}M_{S}\ , \tag{39}$$

which is the upper limit to $\underline{G}(-S)$. Furthermore, from Eqs. (34) and (38), we also have

$$\underline{G}(-S) = (1/E_{V}M_{S}) \sum_{\underline{k}} m_{k}/(1 + \nu_{H_2O}M_{H_2O}/\nu_{S}M_{S})$$

$$\geq (1/E_{V}M_{S}) \sum_{\underline{k}} m_{k}/(1 + M_{H_2O}/M_{S}) \tag{40}$$

which therefore gives a lower limit for $\underline{G}(-S)$. These limiting values may be useful in obtaining an estimate of $\underline{G}(-S)$ without having to formulate a mechanism for the conversion of S into the products.

Radiolysis of S in the Presence of Oxygen and/or Other Solutes

The treatment presented in preceding sections may be generalized to solutions containing solutes $S_1 \ldots S_i$. The yield $\underline{G}(-S_1)$ then applies to the condition that $S_2 \ldots S_i$ have certain initial values:

$$\underline{G}_{o}(-S_1) = (\partial[S_1]/\partial E_{V})_{S_2 \ldots S_i} \tag{41}$$

The experimental determination of this quality poses no particular problem if $[S_2] \ldots [S_i]$ can in fact be kept approximately constant while $[S_1]$ decreases. If this is not possible, $\partial[S_1]/\partial[S_i]$ may be determined by making measurements of both S_1 and S_i, or of $\underline{G}_{o}(-S_1)$ at various

values of $[S_i]_o$.

One may anticipate a priori that radiolysis of such solutions might produce all the products that would be formed if each solute were radiolyzed separately. In addition, one may expect "cross-reactions", i.e., the T_j-, and possibly the U_k-, products from S_1 might react with S_i, and also the derived products from each S_i might react with each other.

In other words, the radiolysis of a mixture of solutes may be much more complicated than that of a single solute. However, this is not necessarily the case. If some particular type of cross-reaction predominates over others, the number of products and the course of radiolysis may be as simple as, or even simpler than, those observed with a single solute. But, of course, the products will then be different.

One special case is quite often encountered in practice, that of solutions which have been equilibrated with air. Such solutions contain, in addition to S, a substantial concentration of dissolved O_2 and N_2. The latter can, in most cases, be neglected. However, the former reacts very rapidly with $(H_2O\cdot)^-$ and $(H\cdot)$ (63)

$$O_2 + (H_2O\cdot)^- \rightarrow (HO_2\cdot) + OH^- \qquad k_{-42} = 2 \times 10^{10} \qquad (42)$$

$$O_2 + (H\cdot) \rightarrow (HO_2\cdot) \qquad k_{-43} = 1.5 \times 10^{10} \qquad (43)$$

and may therefore be expected to exert a profound influence on the radiolysis. If the oxygen present initially should become depleted during the radiolysis, a gradual change may then also occur in the product being formed. In that case, a considerable simplification may be realized by bubbling oxygen continuously through the solution during the irradiation; this will maintain $[O_2]$ constant and near the saturation value (1.3 mM for pure water at 25° and 1 atmosphere) (70).

The presence of O_2 will not affect the reactions of S with (OH·). But very commonly one may get reaction between O_2 and (R·):

$$(R\cdot) + O_2 \rightarrow (RO_2\cdot) \qquad (44)$$

Such reaction would not, *per se*, increase the value of $G(-S)$, but the product(s) obtained will be more highly oxygenated.

On the other hand, the oxygen will in many cases completely consume $(H_2O\cdot)^-$ and (H·), and thus eliminate the contribution that those radicals may make to the total $G(-S)$. Regeneration reactions like (30-31) would also be prevented, however. Because of these potentially countervailing tendencies, no generalization concerning the effect of oxygen can be made.

As we have seen, the interaction of S with reactive products from the solvent, $(Z_i\cdot)$, produces products $(T_j\cdot)$ which, in turn, react spontaneously to give products U_k. U_k may also be susceptible to reaction with (Z·), giving products $(V_i\cdot)$ and W_m; and so on. The overall process may be represented as follows:

$$S + H_2O \qquad [T_1 \ldots T_j] \rightarrow U_k \qquad [V_1 \ldots V_1] \rightarrow W_m \qquad \ldots \qquad (45)$$

Let us now consider a simple, hypothetical mechanism and the results which it will give. We postulate that, after reaction (10), we have:

$$S + (Z\cdot) \xrightarrow{k_S} (T\cdot) \qquad (46)$$

$$2(T\cdot) \xrightarrow{k_T} 2U \qquad (47)$$

$$U + (Z\cdot) \xrightarrow{k_U} (V\cdot) \qquad (48)$$

$$2(V\cdot) \xrightarrow{\underline{k}_V} 2W \qquad (49)$$

Furthermore we postulate that the pertinent concentrations and the values of $\underline{k}$ are such that steady-state conditions can be assumed for the species $(Z\cdot)$, $(T\cdot)$ and $(V\cdot)$. As shown in the Appendix, these premises lead finally to the following expressions for [S] and [U], in terms of $\underline{k}_U/\underline{k}_S = \sigma$, provided $\sigma \neq 1$:

$$\underline{G}(Z\cdot)\underline{D}\rho = 2([S]_o - [S]) + \{[S]-[S]_o([S]/[S]_o)^{\sigma}\}/(1-\sigma) \qquad (50)$$

$$[U] = \{[S]-[S]_o([S]/[S]_o)^{\sigma}\}/(\sigma-1) \qquad (51)$$

Figure 1 represents the limiting case, in which $\underline{k}_U = 0$, i.e., U does not compete with S for the radicals $(Z\cdot)$. In that case, as has already been pointed out, [S] decreases linearly with $\underline{E}_V$. To avoid the complications that would ensue as [S] is reduced to very low values, the calculations have been truncated at a point short of complete decomposition.

As $\sigma = \underline{k}_U/\underline{k}_S$ is made larger than 0, i.e., if we postulate that U has some tendency to react with $(Z\cdot)$, the calculated plots of [S] become curved, and the curvature depends on the value of σ. Let us first assume, for example, that $\underline{k}_U$ be relatively small relative to $\underline{k}_S$, e.g., $\sigma = \underline{k}_U/\underline{k}_S = 0.25$. In this case, the competition by U only becomes marked in the later stages of the reaction, around and after the point where [U] reaches its maximum, and the first part of [S] *vs.* $\underline{E}_V$ is nearly linear. The results are depicted in Figure 2.

The curvature in the plot of [S] does not increase monotonically with σ. On the contrary, as σ is increased above 1, the plots of [S]

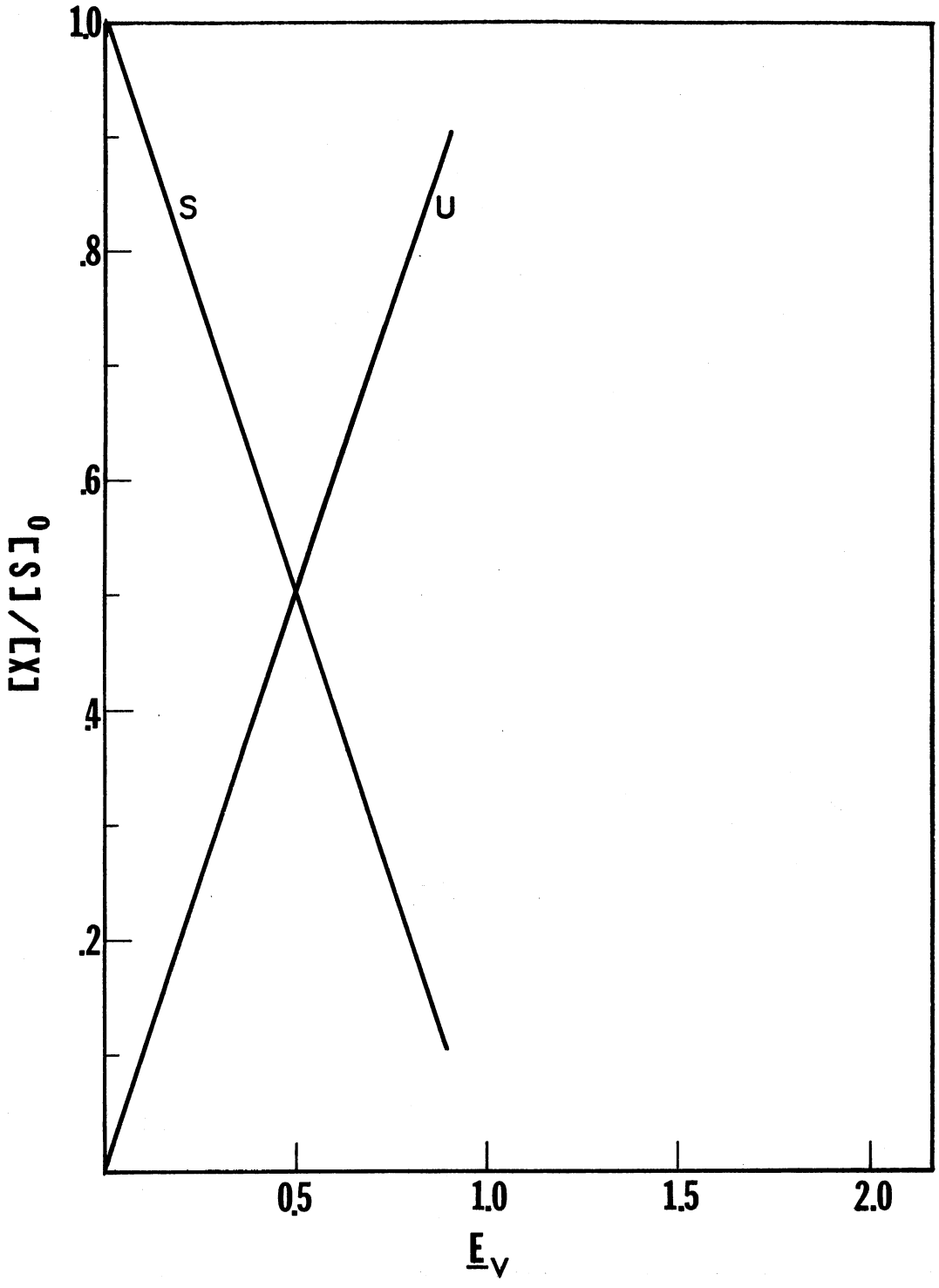

Figure 1. Decomposition of S and Formation of U; Curves Calculated for Mechanism (46-49), assuming $\underline{k}_U = 0$. Ordinate represents fraction radiolysed; X = S or U. Abscissa is $\underline{E}_v$, in units of $([S]_o/\underline{G}(Z)\rho)$

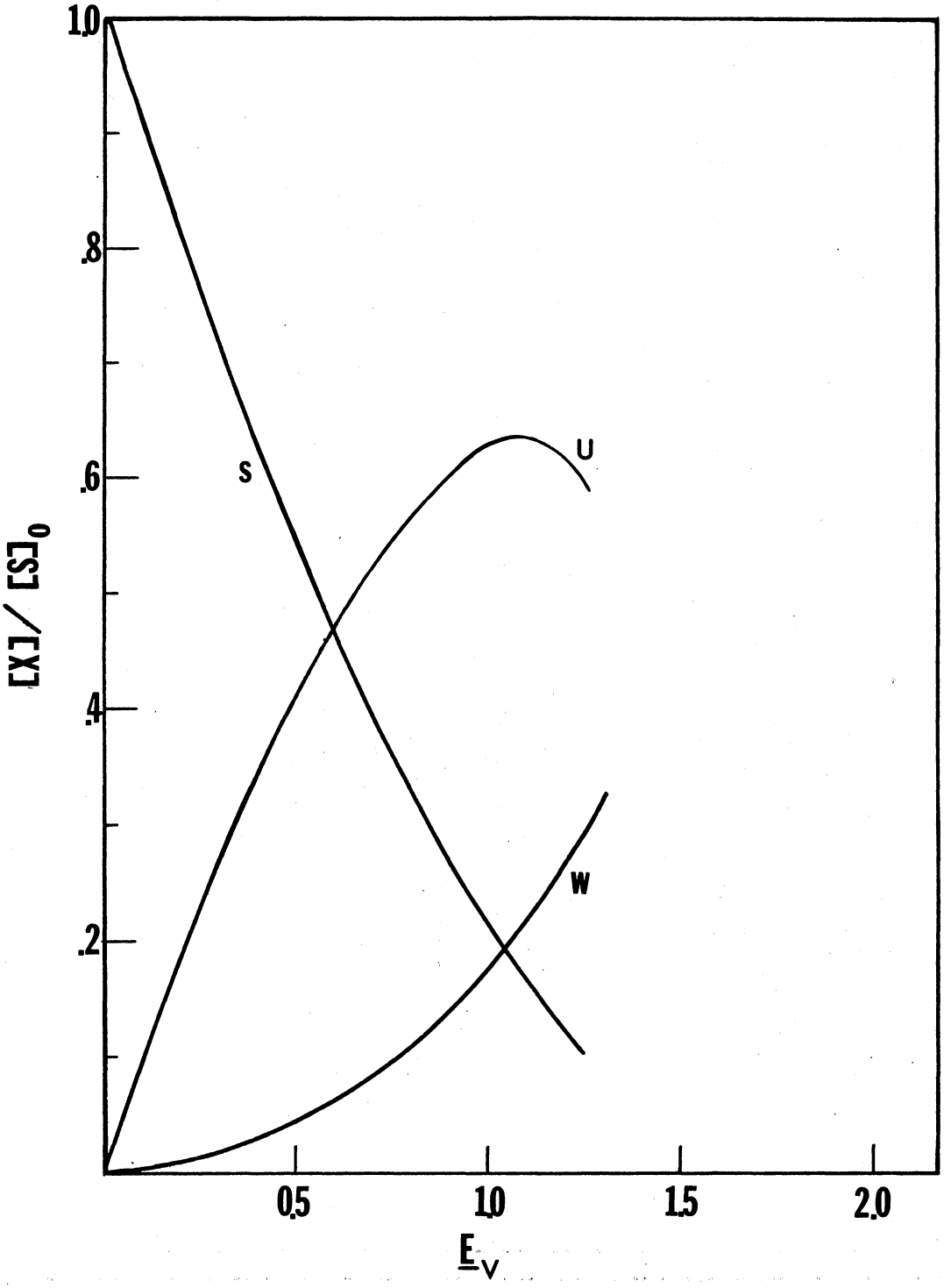

Figure 2. Decomposition of S and Formation of U and W; Curves Calculated for Mechanism (46-49), Assuming $\sigma = 0.25$. Ordinate represents fraction radiolysed; X = S, U or W. Abscissa as in Figure 1

again approach a straight line, but with a smaller slope. This is exemplified by Figure 3, which represents the results for $\sigma = 4$. In this case, $[U]_{max}$ is shifted to a lower value of $\underline{E}_V$ and, of course, only attains a lower magnitude than in the previous case.

For large values of σ, say > 100, U becomes itself an "unstable" intermediate; $[U]_{max} < 0.01[S]_o$, and one observes the "direct" conversion of S into W. The yield $\underline{G}(-S)$ is in this case one half of that represented in Figure 1, since two $(Z\cdot)$ radicals are used up in the conversion.

For the special case of $\underline{k}_S = \underline{k}_U$, equations (50-51) become indeterminate, and the solution must then be expressed in logarithmic terms (see Appendix):

$$\underline{G}(Z)\underline{D}\rho = 2([S]_o - [S]) - [S] \ln ([S]_o/[S]) \tag{52}$$

$$[U] = [S] \ln ([S]_o/[S]) \tag{53}$$

Figure 4 shows the results obtained in this case. The maximum in [U] now occurs when its plot intersects that of S; both its magnitude and its position are, naturally, intermediate between those in Figure 2 and Figure 3.

It is interesting to note that the first part of the curve in Figure 4 approximates fairly closely an exponential. This is most easily demonstrated by plotting values of (ln S) against $\underline{E}_V$; the line is sensibly straight to (ln S) = -0.45, i.e., 37% decomposition (Figure 5).

The foregoing treatment can be generalized to the formation of several products, $U_1 \ldots U_{\underline{k}}$, provided all have the same reactivity. Then

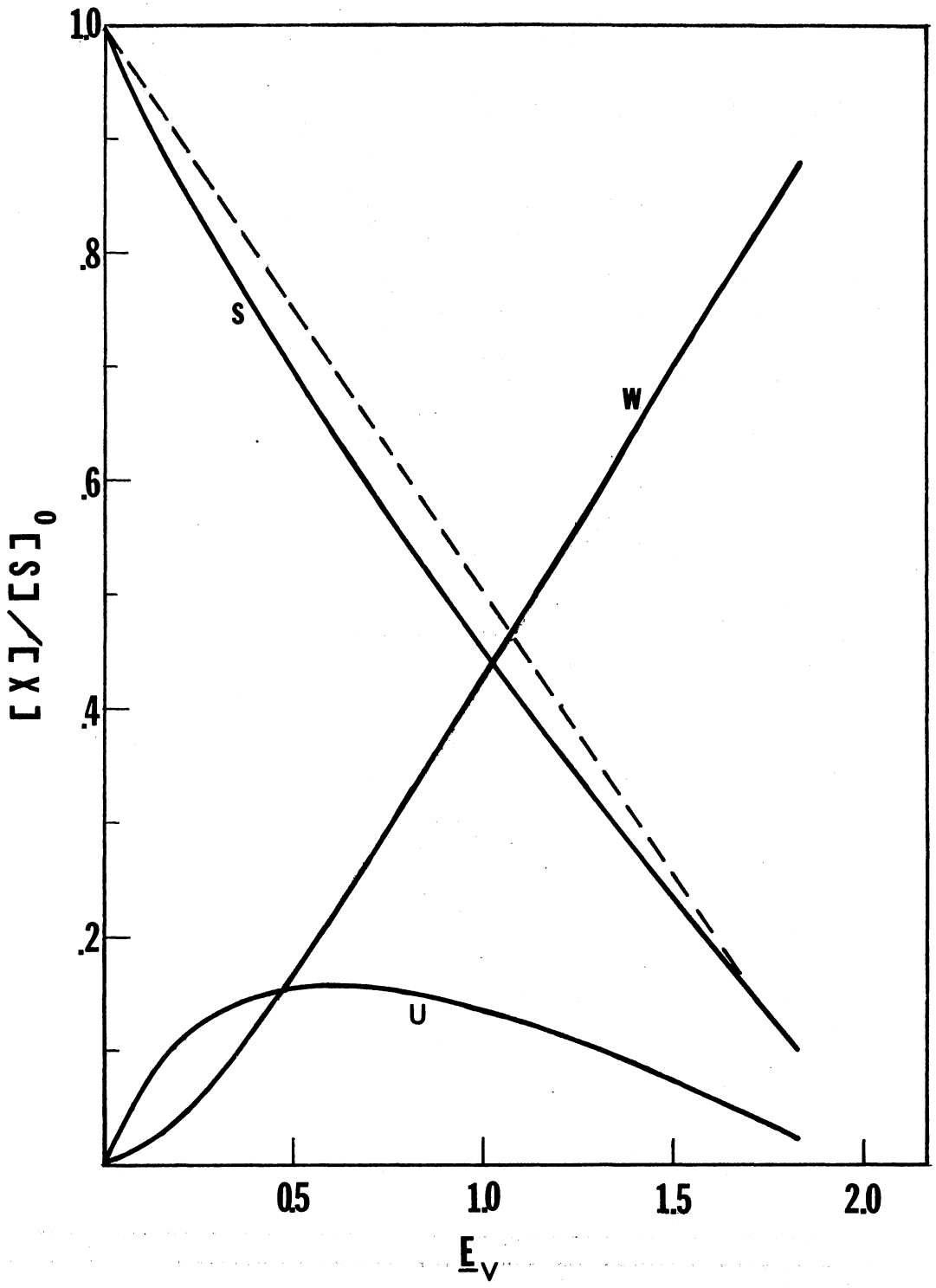

Figure 3. Decomposition of S and Formation of U and W; Curves Calculated for Mechanism (46-49). Solid lines represent values for $\sigma = 4$. Stippled line represents $[S]/[S]_o$ for the limiting case of $\sigma > 100$. Axes are as in Figure 2

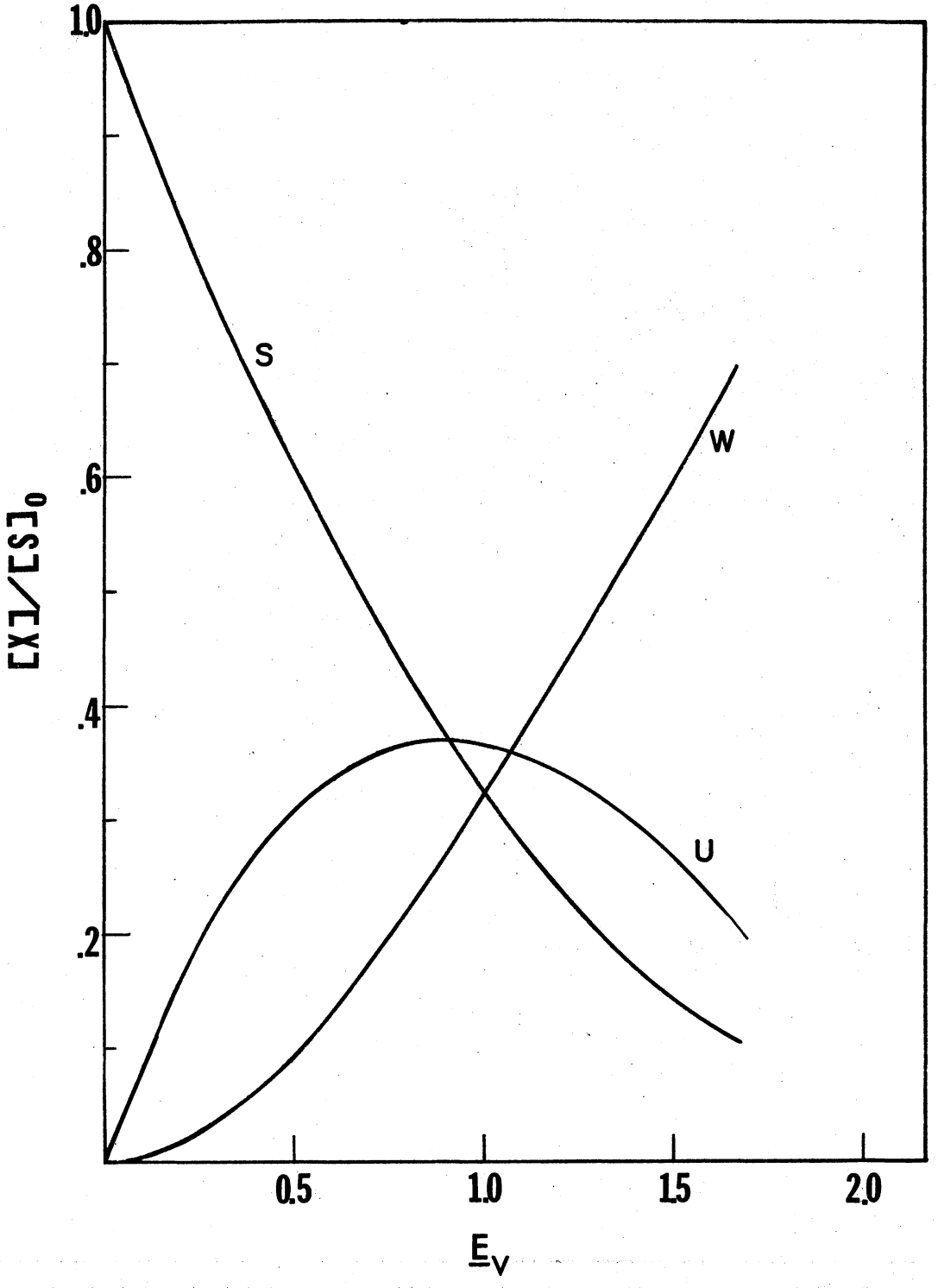

Figure 4. Decomposition of S and Formation of U and W; Curves Calculated for Mechanism (46-49), Assuming $\sigma = 1$. Axes are as in Figure 2

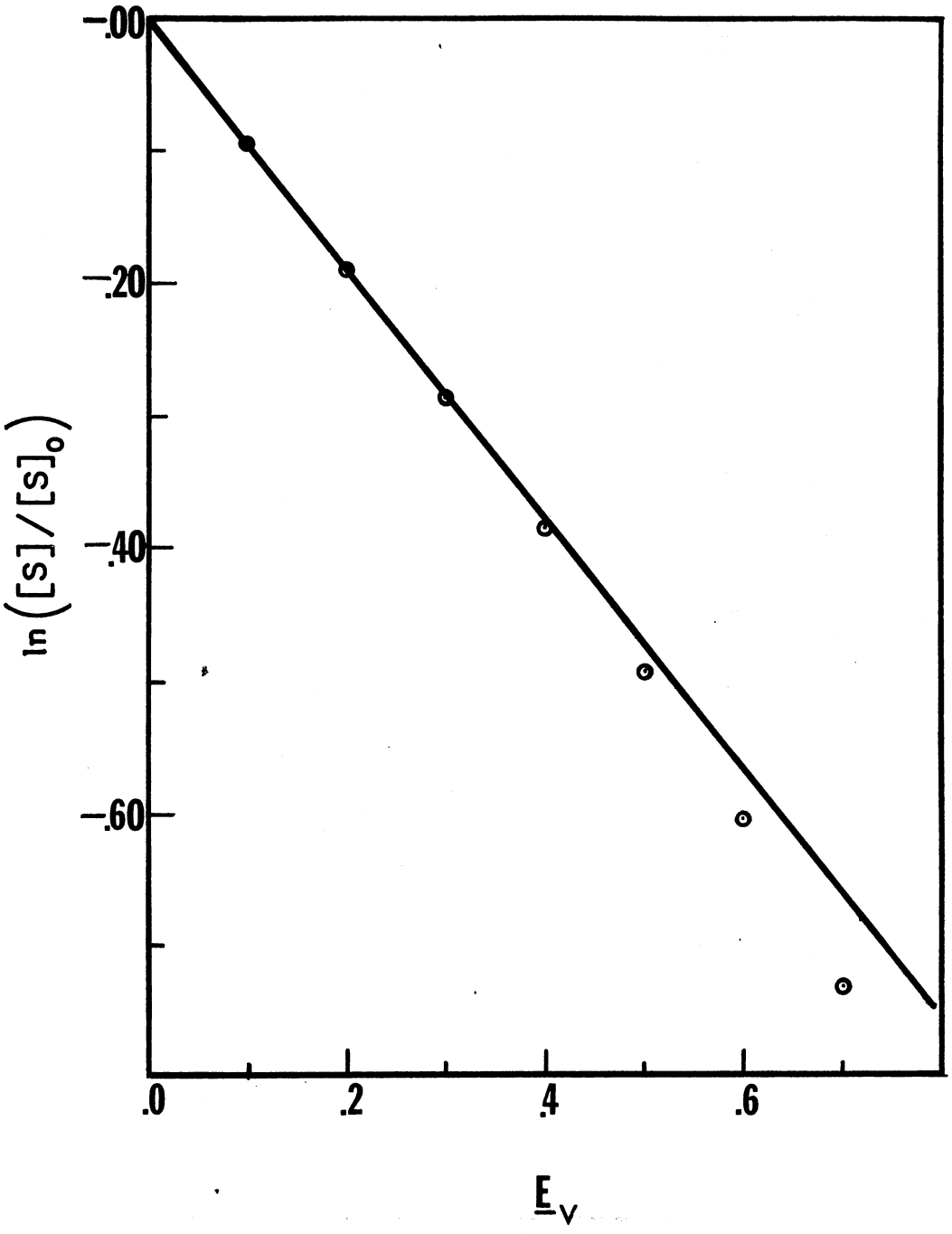

Figure 5. Dependence of [S] on E_v for Mechanism (46-49), Assuming $\sigma = 1$. Values of $\ln([S]/[S]_o)$ Plotted Against E_v, units of latter as in Figure 1

they would accumulate in some constant proportion to one another, the limiting slopes, $\underline{G}_o(U_{\underline{k}})$, being proportional to the magnitude of the respective $\underline{k}$ values for their formation. Such a mixture of products would behave as a single entity, the effective properties of which would be the weighted sum of those of its constituents.

Such a postulate is, however, quite unrealistic--it must be anticipated that, in general, products $U_1 \ldots U_{\underline{k}}$ will have different reactivities toward ($Z\cdot$). If so, then they would accumulate in continually changing proportions, the less reactive products increasing in relation to the more reactive ones. If all the products were less reactive than S, this would tend to make the plot of [S] more nearly linear than for a single U-product which had the same reactivity throughout.

If, on the other hand, some products were less reactive than S and some were more reactive, the decreasing proportion of the latter would tend to keep the plot of [S] more nearly exponential than the curve represented in Figure 4.

The mechanisms assumed above are simpler than those likely to take place in actual radiolyses. But the calculated results resemble the empirical results that have been obtained in many cases. The comparison is then helpful in suggesting possible interpretations. More realistic but complex models will be considered in subsequent papers.

Comparison With Experiment

Hundreds of compounds have been investigated, but $\underline{G}(-S)$ values have been determined only in relatively few cases. This situation is reflected in the extensive compilation assembled in 1961 by Haïssinsky and Magat (71)--some two hundred compounds are listed, but only about

twenty $\underline{G}(-S)$ are quoted. Table III presents a selection of data from the cited compilation, supplemented by some more recent values taken from two other reviews (72,73). No attempt has been made to review all the data available, which is not necessary for the present purpose. The compounds have been arranged in order of increasing molecular complexity.

It can be seen from the Table that $\underline{G}(-S)$ in the absence of oxygen is, in most cases, 6 or less, in accordance with the general mechanistic considerations discussed in a previous section.

In the presence of oxygen, $\underline{G}(-S)$ values are, in general, not larger than in the absence of oxygen, and they may be smaller. This also is in accordance with the preceding discussion. The DNA bases are exceptions, but even in this case the effect of oxygen on the magnitude of $\underline{G}(-S)$ is not large.

Appendix

The mathematical derivation of equations (50-53) is given below. In this section, to simplify the symbolism, S, T, and U represent the concentrations of those substances, with square brackets omitted, Z and T are shown without the dot designating the unpaired electron and, finally the symbol G represents the yield of Z formed by radiolysis of H_2O.

From equations (46-49) we can write, for the rate of change in S and U:

$$(dS/dt) = \underline{k}_S SZ \tag{55}$$

$$(dU/dt) = 2\underline{k}_T T^2 - \underline{k}_U UZ \tag{56}$$

TABLE III

SELECTED RADIOLYTIC DECOMPOSITION YIELDS[a]

Empirical Formula and Name of Compound	In Deoxygenated Solns.		In O_2 -contg. Solns.		Ref.
	pH	G(-S)	pH	G(-S)	
$C_2H_4O_3$, glycolic acid	?	6.1	?	4.5	(71)
$C_2H_2O_4$, oxalic acid	-	-	2	2.9	(71)
C_6H_6, benzene	5-7	2-2.8	-	-	(71)
$C_6H_4O_2$, benzoquinone	5.5	5-9.5	5.5	2.8-8	(71)
$C_4H_4N_2O_2$, uracil	7	2.7	5.2-7	1.9-2.5	(72)
$C_4H_3N_2O_2Br$, 5-bromouracil	7	4.6	6.5	3-6	(72)
$C_4H_5N_3O$, cytosine	7	1.8	5.2-7	2.2-3.3	(72)
$C_5H_6N_2O_2$, thymine	7	1.0-2.5	6.2-7	1.7-3.1	(72)
$C_4H_6O_5$, malic acid	7.3	2.2	7.3	2.6	(71)
$C_7H_6O_2$, benzoic acid	-	-	4-9	2.1-2.6	(71)
$C_5H_{11}NO_2$, valine	?	5.5	?	7.2	(73)
$C_4H_9NO_2$, α-amino butyric acid	?	5.7	?	7.8	(73)
$C_9H_{11}NO_2$, phenylalanine	-	-	2.3-2.8	3.9-4.3	(73)
$C_6H_{12}O_6$, glucose	-	-	?	3.5	(71)
$C_{10}H_{12}N_4O_5$, inosine		1.5		2.5	(71)

[a] The values of G(-S) are expressed in molecules/100 eV.

The rates of change in the concentrations of Z and T are given by:

$$(dZ/dt) = G\rho(dD/dt) - Z(\underset{-}{k}_S S + \underset{-}{k}_U U) \tag{57}$$

$$(dT/dt) = \underset{-}{k}_S SZ - 2\underset{-}{k}_T T^2 \tag{58}$$

By setting equations (57) and (58) equal to zero, we can obtain the following expressions for the concentration of Z and T in the steady state:

$$Z_{ss} = G\rho(dD/dt)/(\underset{-}{k}_S S + \underset{-}{k}_U U) \tag{59}$$

$$\underset{-}{k}_S SZ_{ss} = 2\underset{-}{k}_T T^2_{ss} \tag{60}$$

By substituting equation (60) into equation (56), we obtain:

$$(dU/dt) = Z_{ss}(\underset{-}{k}_S S - \underset{-}{k}_U U) \tag{61}$$

Dividing equation (55) by equation (61) gives:

$$(dS/dU) = -\underset{-}{k}_S S/(\underset{-}{k}_S S - \underset{-}{k}_U U) \tag{62}$$

By making the substitution $\sigma = \underset{-}{k}_U/\underset{-}{k}_S$ and multiplying equation (62) by $S^{-\sigma}$ we obtain:

$$(1/S^{\sigma})dU - \sigma U dS/S^{(\sigma+1)} = -(1/S^{\sigma})dS \tag{63}$$

<u>σ Not Equal to One</u>

Equation (63) is integrated from S_o to S and U is not present initially, from zero to U. The result is :

$$U = [1/(\sigma - 1)][S - S_o(S/S_o)^{\sigma}] \tag{64}$$

Substitution of equation (59) into equation (55) gives:

$$dS = -G\rho dD/[1 + \sigma(U/S)] \tag{65}$$

and by substitution of equation (64) into (65) we obtain:

$$-G\rho dD = [1 + \sigma/(\sigma - 1)]dS - [\sigma/(\sigma - 1)]S_o^{(1-\sigma)}S^{(\sigma-1)}dS \tag{66}$$

Integration of equation (66) between the limits S_o to S and O to D gives

$$G\rho D = [1 + \sigma/(\sigma - 1)] (S_o - S) - (\sigma - 1)^{-1} S_o^{1-\sigma} (S_o^{\sigma} - S^{\sigma}) \tag{67}$$

Equation (67) can be rearranged into:

$$G\rho D = 2(S_o - S) - (\sigma - 1)^{-1} S - S_o(S/S_o)^{\sigma}) \tag{68}$$

<u>σ Equal to One</u>

In this case, equation (63) can be written as

$$(1/S)dU - (U/S^2)dS = -(1/S)dS \tag{69}$$

Integrate equation (69) over S_o to S and zero to U. We obtain:

$$U = S \ln (S_o/S) \tag{70}$$

Substitution of equation (65) into equation (70) gives:

$$dS = -G\rho dD/(1 + \ln (S_o/S) \tag{71}$$

Equation (71) can be rearranged into:

$$1 + \ln (S_o/S)\ dS = -G\rho dD \tag{72}$$

By integration of equation (72) between the limits S_o to S and zero to D we obtain:

$$G\rho D = 2(S_o - S) - S \ln (S_o/S) \tag{73}$$

CHAPTER IV

RADIOLYSIS OF CYTOSINE IN DILUTE NEUTRAL AQUEOUS SOLUTIONS

(To be Submitted for Publication By: G. Gorin, N. Ohno and L. M. Raff)

(Abstract)

Cytosine, 0.1 to 10 mM, has been radiolyzed in 0.02 M phosphate buffer, pH 7. If the pH is then adjusted to 3, the absorption of the radiolysis products at 275 nm becomes negligible, and the radiochemical decomposition yield $\underline{G}(-\text{cyt})$[3] can be calculated from the decrease in absorbance. In oxygen-saturated solutions it is described by the expression ($\underline{D}^*$ = dose in krad): $\underline{G}(-\text{cyt}) = 3.3 - 0.024\ \underline{D}^*$ (to at least 40% decomposition). The radiolysis products react with the radicals derived from the solvent about five times slower than cytosine. The radiolysis products have appreciable absorbance at <260 and pH 3, and at 266 nm and pH 7 or 11. The predominant products are dihydrocytosine derivatives ($\underline{G} \sim 2$). In solutions containing >1 mM cytosine and initially saturated with air, the oxygen is soon depleted; in absence of oxygen, different radiolysis products are formed, that have a higher absorbance.

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[1] All $\underline{G}$ values are in the units molecules $(100\ \text{eV})^{-1}$.

Introduction

This paper describes an investigation of the radiolysis of cytosine in dilute, neutral aqueous solution. For the most part, the systems studied were saturated with oxygen, but some experiments also were done in deoxygenated solutions. As we shall see, oxygen has a critical effect on the course of the radiolysis.

Cytosine is one of the four bases found in DNA, which spell out the so-called genetic code. There is considerable evidence to indicate that the alteration of DNA by radiation plays an important part in the development of radiation injuries (23) and, for this reason, it is pertinent to investigate the effects of radiation on the constituent parts of DNA. In general, radiation is exceedingly harmful to living organisms and, consequently, stringent precautions must be taken to minimize exposure (19). But, on the other hand, controlled exposure to radiation has one important medical use; it is, after surgical resection, the next most widely used means of treating cancer (74). The mechanisms that underlie radiation injury are, consequently, the subject of widespread and pressing interest.

A substantial amount of work has already been done on the radiolysis of cytosine, which has recently been reviewed by Infante *et al.* (60). Their paper should be consulted for details that will not be repeated here. Unfortunately, the problem is very complicated and many questions have not yet been satisfactorily resolved. The reported values of the radiochemical yield for decomposition, $G(-cyt)$, range from 0.9, found in a degassed unbuffered solution (51,60) to 8.8 in an air-saturated solution of pH 1, containing 0.01 M NaCl (45). If these results are correct, clearly the composition of the solvent has a critical effect on the radiolysis process, and significant comparisons can be made only if

the medium is kept constant, or if the effects of pertinent variables have been determined and the appropriate adjustments made.

The results obtained at or near neutrality are especially relevant. According to Barszcz and Shugar (42), $\underline{G}$(-cyt) is 3-3.8 in 0.01 M phosphate of pH 7.4 and the value is little different in absence of buffer (pH ~ 6.5). Ward and Kuo (45) found a value of 2.8 at pH 3.7. Finally, Scholes *et al.* (34) have reported the lower value of 2.2 at pH 5.2; according to their results, the decrease in absorbance corresponded to the amount of cytosine decomposition determined by chromatography, which implies that the radiolysis products have no appreciable absorbance. It will be seen that the results of the present work do not agree with that inference.

Ekert and Monier (48) investigated the products of the radiolysis. After exposing initially 1 mM cytosine under air to 150 krad, they found the following compounds: uracil glycol ($\underline{G}$ = 0.20), cytosine glycol, and 5-hydroxycytosine ($\underline{G}$ = 0.34); these products do not, of course, account for all the cytosine decomposed.

Quite different results were obtained by Khattak and Green (50), in absence of oxygen. Their value of $\underline{G}$(-cyt) was 1.8, and the products were 5-hydroxycytosine (26%), uracil (17%), 5,6-dihydroxycytosine (8%) plus a trace of its dihydro derivative, and hydroxydihydrocytosine (6%), for a total recovery of 56%.

A third study was done by Holian and Garrison (46). In their experiments, the pH was 3-4, oxygen was removed, but Cu(II) was added to scavenge aqueous electrons. They report a higher $\underline{G}$(-cyt) value, 2.7, and quantitative recovery of the products: cytosine glycols (84%) and 5-hydroxycytosine (16%). In a later paper (53), these authors report

that, at pH 7, the yield of dihydrocytosine derivatives is less than 0.9.

In evaluating the above results, one should take due notice of those obtained more recently with thymine, which clearly show that the identification of radiolysis products is an extremely complex question, fraught with uncertainties; for details, one should consult the cited review (60), as well as the original references. Since the products are so numerous--more than a dozen have been identified (60)--the adequacy of the procedures employed for analysis must be regarded with due skepticism. It is also important to keep in mind that the accumulation of a given product depends upon its susceptibility to radiolysis, relative to that of the starting material and of the other products; in general, the radiosensitivities will be different and the relative yields will then vary continuously with the fraction decomposed. At any rate, the results so far reported are not in good agreement.

The above difficulties were taken into account in designing the present investigation. Its principal purpose has been to determine the value of $\underline{G}$(-cyt) in a medium of well-defined composition, and we have deliberately avoided, in so far as possible, the question of what products are formed. This approach is justified by the fact that biological activity is, in general, critically dependent on the chemical structure. In many cases the activity is obliterated, or radically changed, by <u>any</u> chemical change, so that it makes little difference whether the original product is converted into a product P_1, or P_2, or a complex mixture $P_1 \ldots . P_i$.

Cytosine has an intense, characteristic spectrum in the ultraviolet, and measurement of the changes in that spectrum constitutes a convenient way of following the radiolysis. To interpret these changes

properly it is, however, necessary to establish whether the spectra of the products overlap that of cytosine. The next section of the paper presents a theoretical treatment of the relationship between absorbance and radiolytic yield which is then applied to the interpretation of the experimental results.

Spectral Changes Due to Radiolysis

Let a substance S in solution be converted by radiation into a mixture of products $P_1 \ldots P_i$:

$$S + \ldots \qquad \upsilon_1 P_1 + \ldots \upsilon_i P_i \tag{1}$$

υ_i represents the amount of substance P_i, measured in moles, formed by the radiolysis of one mole of S, i.e., $\upsilon_i = \underline{G}_i / \underline{G}(-S)$; υ_i is not a conventional stoichiometric coefficient, because, in general, it is not integral and, furthermore, it may vary with the dose $\underline{D}$.

Let us first assume that the υ_i do not change with dose, i.e., $\underline{G}$ remains constant. It is convenient to define an "equivalent absorption coefficient" for the products by the expression:

$$\varepsilon_{\Sigma}^{\lambda} = \sum_i \upsilon_i \varepsilon_i^{\lambda} \tag{2}$$

where ε_i^{λ} is the molar absorption coefficient of the ith product at wavelength λ. If $\varepsilon_{\Sigma}^{\lambda}$ is zero (or small enough to be negligible relative to the absorption coefficient of S_1 ε_S^{λ}) the absorbance remaining after a dose $\underline{D}$ is given by:

$$\underline{A}_D^{\lambda} = [S]\varepsilon_S^{\lambda} = ([S]_o - \underline{G}(-S)\underline{E}_v)\varepsilon_S^{\lambda} \tag{3}$$

where $\underline{E}_v$ is the energy absorbed per unit volume.

It follows from (3) and the assumptions stated above that $\underline{A}_D^\lambda$ decreases linearly with dose, and that the slope of the line equals $\underline{G}(-S)$:

$$\underline{G}(-S) = (\underline{A}_o^\lambda - \underline{A}_D^\lambda)/\underline{E}_v\varepsilon_S^\lambda \tag{4}$$

If ε_Σ is greater than zero, then equation (3) becomes:

$$\underline{A}_D^\lambda = [S]_D\varepsilon_S^\lambda + ([S]_o - [S]_D)\varepsilon_\Sigma^\lambda = A_o^\lambda - \underline{G}(-S)\underline{E}_v(\varepsilon_S^\lambda - \varepsilon_\Sigma^\lambda) \tag{5}$$

$\underline{A}_D^\lambda$ is still a linear function of dose, but $\underline{G}(-S)$ is now measured by:

$$\underline{G}(-S) = (\underline{A}_o^\lambda - \underline{A}_D^\lambda)/\underline{E}_v(\varepsilon_S^\lambda - \varepsilon_\Sigma^\lambda) > (\underline{A}_o^\lambda - \underline{A}_D^\lambda)/\underline{E}_v\varepsilon_S^\lambda \tag{6}$$

Next, consider the absorbances at two different wavelengths, $\lambda = \alpha,\beta$. In general, the fractional change in absorbance will vary with wavelength. However, it may be observed in some cases that the fractional changes after a given $\underline{D}$ are the same, i.e.

$$(\underline{A}_o^\alpha - \underline{A}_D^\alpha)/\underline{A}_o^\alpha = (\underline{A}_o^\beta - A_D^\beta)/\underline{A}_o^\beta \tag{7}$$

If so, with the appropriate substitutions one gets:

$$\underline{E}_v\underline{G}(-S)(\varepsilon_S^\alpha - \varepsilon_\Sigma^\alpha)/[S]_o\varepsilon_S^\alpha = \underline{E}_v\underline{G}(-S)(\varepsilon_S^\beta - \varepsilon_\Sigma^\beta)/[S]_o\varepsilon_S^\beta \tag{8}$$

and it follows that the following condition must hold:

$$\varepsilon_\Sigma^\alpha/\varepsilon_S^\alpha = \varepsilon_\Sigma^\beta/\varepsilon_S^\beta \tag{9}$$

This condition will of course be fulfilled if $\varepsilon_\Sigma^\alpha = \varepsilon_\Sigma^\beta = 0$. The alternative possibility, that $\varepsilon_\Sigma^\lambda$ be > 0 and proportional to ε_S^λ at <u>all</u> the

values of λ being considered, is extremely unlikely; it can almost certainly be discounted if, in the range considered, ε_S shows a well-defined maximum or other characteristic inflection(s). In other words, if it is found empirically that equation (7) holds for arbitrary α and β, one may conclude with near certainty that $\varepsilon_\Sigma^\lambda$ is negligible.

The foregoing argument can be generalized to the case when $\underline{G}$ is not constant with dose. In that case, of course, $\varepsilon_\Sigma \equiv f(\underline{D})$ and $\underline{A}_{\underline{D}}^\lambda$ will not be a linear function of $\underline{D}$. However, ε_Σ will have an definite value at any given dose, and equations (5-9) will hold for all λ <u>at that dose</u>.

Experimental Section

Materials

Two samples of cytosine were investigated, and both gave the same results; one sample, cytosine.H_2O, was from Schwarz Bioresearch, the other, cytosine.$\frac{1}{2}H_2O$, from Sigma Chemical. The sodium phosphates, of analytical-reagent grade, were obtained from Fisher Scientific.

All solutions were prepared with distilled water that had been passed through a deionizing resin, redistilled from alkaline permanganate and finally distilled a third time. All irradiated solutions contained 0.02 M phosphate, pH 7 ± 0.05 (Beckman Model SS-2 pH meter); they were made up by diluting a 0.2 M stock solution, containing 10.76 g $NaH_2PO_4 \cdot H_2O$ and 17.24 g Na_2HPO_4 per liter.

Methods

The irradiated samples had a volume of 9 ml and were contained in 10 ml glass vials. They were irradiated in a Gammacell-200 cobalt-60 irradiator, equipped with the gas-inlet and sample-holder accessories (Atomic Energy of Canada). To minimize possible post-irradiation changes, the samples were treated and examined as soon as possible,

usually within 10-20 minutes. The measurements used to calculate $\underline{G}$(-cyt) were corrected, if necessary, by extrapolation to zero time.

The dose-rate, measured with the Fricke dosimeter, was between 2700 and 2000 rad min^{-1}. The calculation was based on $\varepsilon(Fe^{3+}$, 304 nm) 2195 M^{-1} cm^{-1}, $\underline{G}(Fe^{3+})$ 15.6 (75).

Oxygen Saturation or Removal

Oxygen was passed first into a pressure-regulating and ballast flask (76), then through the Gammacell inlet tube, and finally through a thin glass capillary into the solution being irradiated. The ballast flask was a 500-ml Erlenmeyer that contained about 100 ml of water. The flask was fitted with an inlet and an outlet tube, as well as a gauge tube; the latter was placed vertically through the stopper so its lower end would dip below the surface of the water. Excess pressure in the flask was then indicated by the level of the water in the gauge tube. In this way the pressure could easily be adjusted to give the desired rate of flow through the solution being irradiated, 1-2 ml s^{-1}.

To remove oxygen, helium was passed through the solution prior to irradiation. In this case, the sample vial was closed with a polyethylene cap, into which there has been drilled two small holes. The gas flow was regulated as described above, and it was maintained for 15 min at 0.3 ml s^{-1}. Then the capillary was withdrawn, the holes were immediately covered with Parafilm, and the vial was placed in the Gammacell.

Quantitative Absorbance Measurements

Cytosine solutions, initially 0.1 mM, were treated as follows; a

3.00 ml aliquot was added to 3.00 ml of HCl, of such concentration that the final pH was 3.0. In a similar way, a 3.00 ml aliquot of irradiated solution was adjusted to pH 11.0 by mixing it with 3.0 of NaOH. Cytosine solutions of 1 and 10 mM concentration were first diluted 10 or 100 times, respectively, with pH-7 buffer, and then treated as described above.

At pH 3 and 7, the spectra above 200 nm were determined both for the irradiated samples and the controls with a recording spectrophotometer (Cary Model 14); measurements at selected wavelengths, used in calculation of $\underline{G}$, were made with a single-beam spectrophotometer (Beckman Model DU, fitted with a Gilford Model 22 photometer and lightsource). At pH 11, measurements were made at selected wavelengths, as needed. All measurements were made in 1-cm quartz cells.

Results

Spectrum of Cytosine

Cytosine is a weak acid, with pK of approximately 4.5 and 12.2 (60,77). At pH 3, therefore, cytosine exists as the unionized base, at pH 7 and 11 as the anion; the first ionization causes a shift in the maximum and a decrease in its intensity. The following values were obtained in the present work (wavelengths are in nanometers, the molar absorption coefficients in M^{-1} cm^{-1}): in pH-7 phosphate, maximum 266 and 6400, minimum 247 and 4700; in HCl, pH 3, 275 and 10,000, 238 and 1400; in NaOH, pH 11, 268 and 6300, 249 and 4600. The values are in satisfactory agreement with those reported in the literature (77).

Spectra of Irradiated Solutions at pH 7

Fig. 6 shows the family of curves obtained by irradiating initially 0.1 mM cytosine at pH 7 and measuring the spectrum without changing the pH. Although the absorbance at the maximum, 266 nm, decreases rapidly with dose, for curves B-E the absorbance below 250 nm increases with dose, and that around 252 nm remains nearly constant. Qualitatively this shows that $\varepsilon_{\Sigma}^{252}$ is nearly equal to ε_{cyt}^{252} (ca. 4900), and that $\varepsilon_{\Sigma}^{\lambda} > \varepsilon_{cyt}^{\lambda}$ in the range λ = 250-225 nm.

A plot of the absorbance at the maximum vs. dose, i.e., $A_{-D}^{266}/A_{-o}^{266}$ is shown in Fig. 7, curve B. The slope of this line is not equal to $\underline{G}$ because $\varepsilon_{\Sigma}^{266} > 0$, as we shall see more clearly below. But it is interesting to note that the apparent value of $\underline{G}$ calculated from the slope of the line is about 2.3, in good agreement with the value reported by Scholes et al. (34) that was mentioned in the Introduction. In other words, our experimental data are in excellent agreement with the earlier paper, but we differ in the interpretation. It is obvious from inspection Fig. 6 that plots of $A_{-D}^{\lambda}/A_{-o}^{\lambda}$ at λ = 266-252 will have a rapidly decreasing slope, due to the fact that $\varepsilon_{\Sigma}^{\lambda}$ is rising rapidly relative to $\varepsilon_{cyt}^{\lambda}$ [cf. equation (5)].

The products responsible for the absorbance at 220-250 nm are not completely stable. Fig. 8 shows the post-irradiation changes taking place in a representative case at three selected wavelengths.

Spectra of Irradiated Solutions at pH 3

Fig. 9 shows the family of curves obtained when the solutions which had been irradiated at pH 7 were adjusted to pH 3 prior to measuring the spectrum.

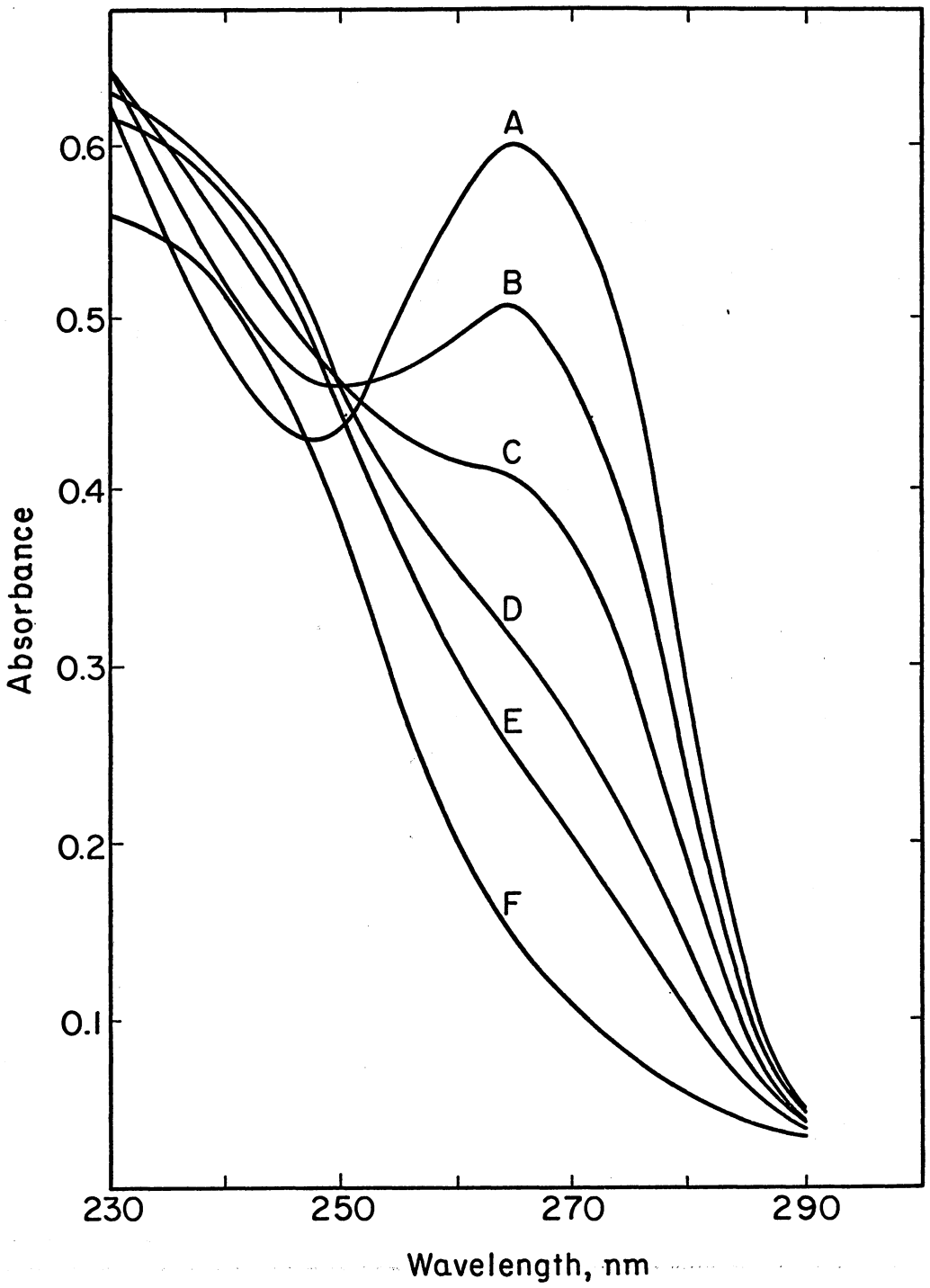

Figure 6. Spectra of Cytosine Solutions, Initially 0.1 mM, After Exposure to Increasing Doses, Measured at pH 7. Doses in krads: A, zero; B, 6.7; C, 13.4; D, 20.1; E, 26.8; F, 40.2.

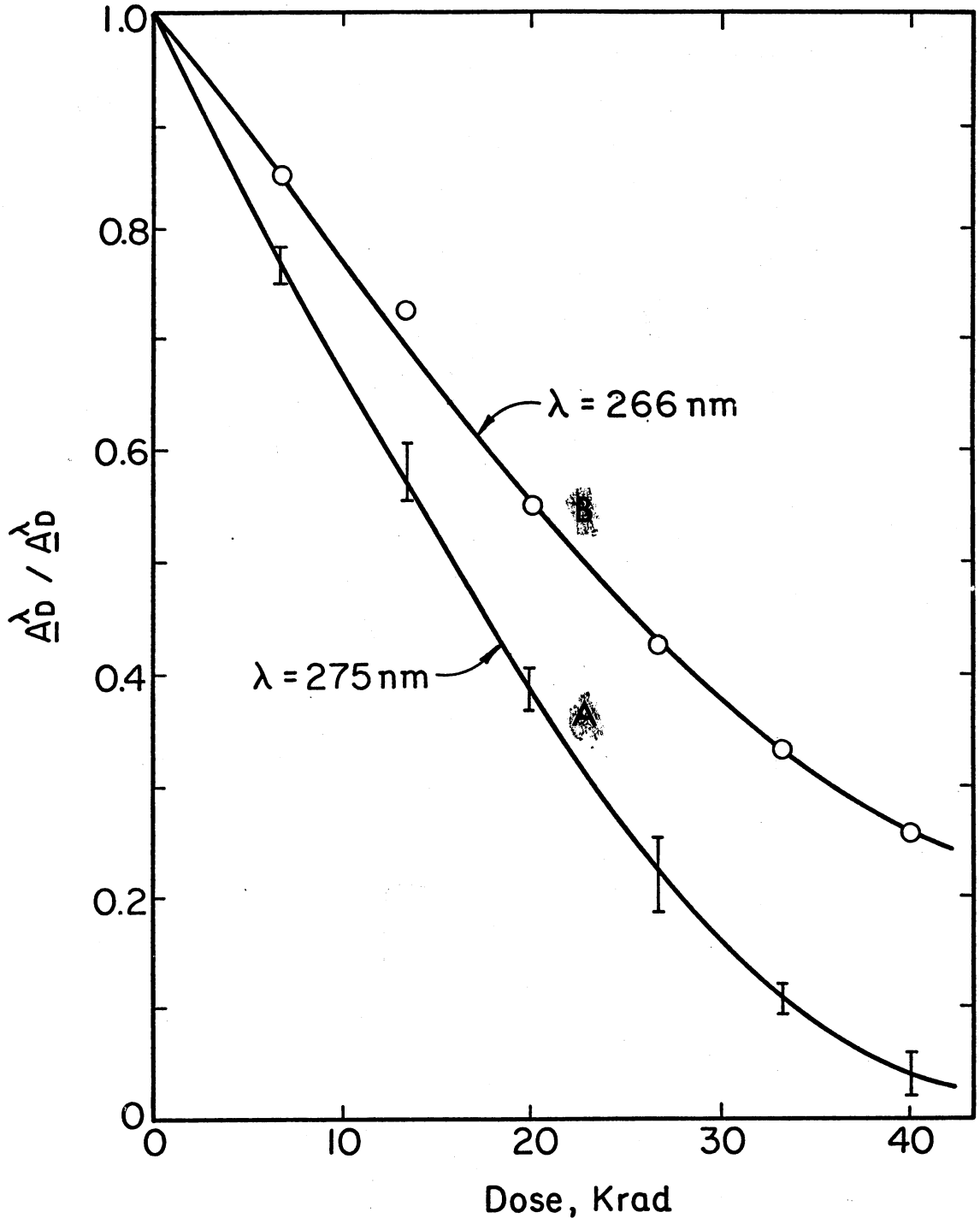

Figure 7. Fractional Absorbance as a Function of Dose.
Curve A: $A_D^{275}/A_o^{275} = [cyt]/[cyt]_o$, at pH 3
Curve B: A_D^{266}/A_o^{266}, at pH 7

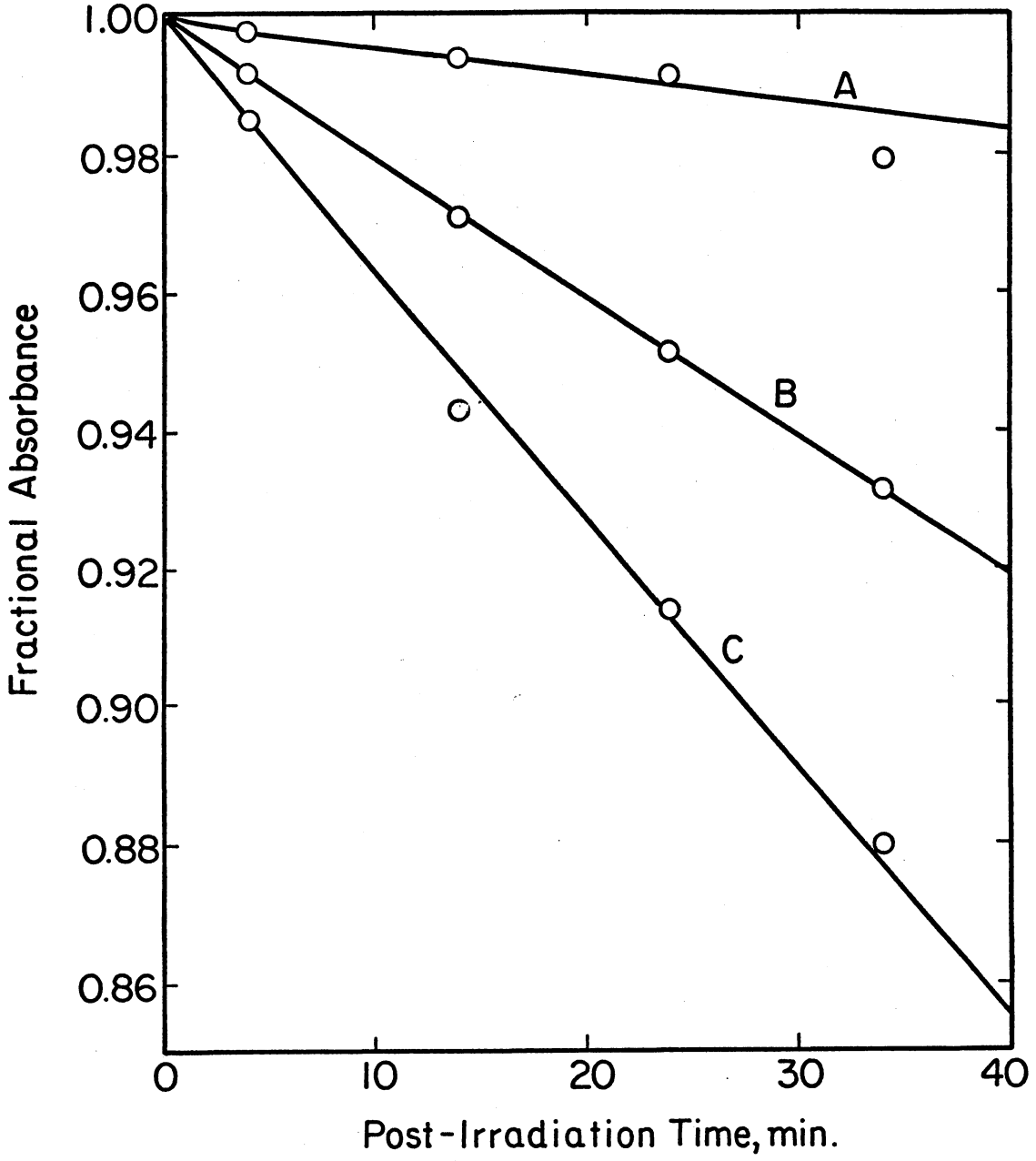

Figure 8. Changes in Fractional Absorbance Occurring After Irradiation in Initially 0.1 mM in Cytosine After Exposure to 21 krads: A, at 220 nm; B, 232.5 nm; C, 248 nm

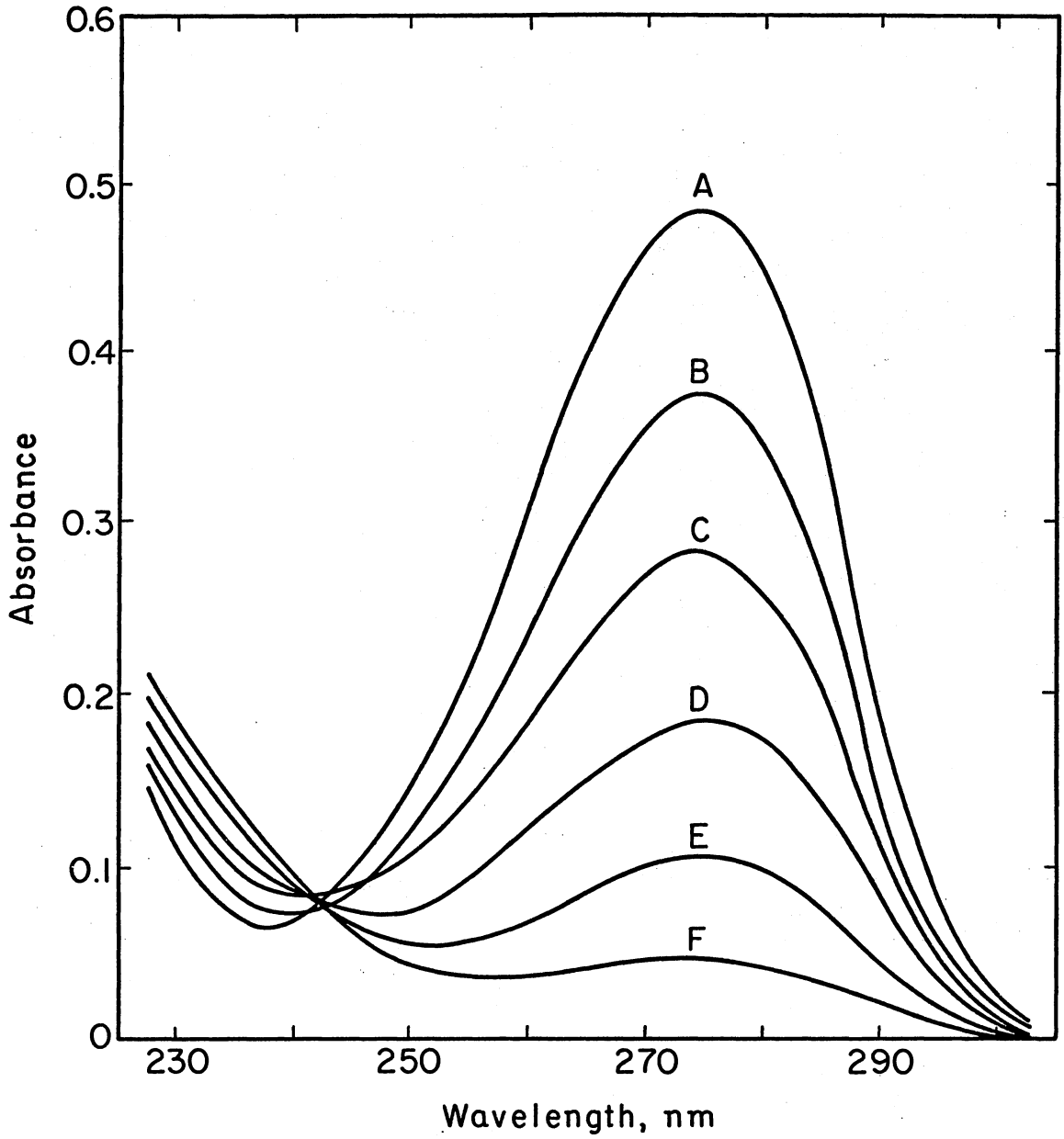

Figure 9. Spectra of Cytosine Solutions, Initially 0.1 mM, After Exposure to Increasing Doses, Measured at pH 3. Doses in krads: A, zero; B, 6.7; C, 13.4; D, 20.1; E, 26.8; F, 40.2

Numerical values of the ratios $\underline{A}_D^{\lambda}/\underline{A}_o^{\lambda}$ at 275 nm and at two other wavelengths, respectively 8 nm above and 9 nm below the maximum, are given in Table IV. It may be seen that these values remain sensibly constant, in the interval $\underline{A}_D^{\lambda}/\underline{A}_o^{\lambda}$ = 1 to 0.1.

The significance of these results has been pointed out in a preceding section--one may conclude from them that $\varepsilon_{\Sigma}^{275}$ (at pH 3) is negligible in comparison with $\varepsilon_{cyt}^{\lambda}$ in the range λ = 264-283.

Values of $\underline{A}_D^{275}/\underline{A}_o^{275}$ are plotted in Fig. 7, curve A. The initial slope of this line is some 30% greater than that of curve B, and this measures the error made by neglecting $\varepsilon_{\Sigma}^{266}$. Its value can be calculated by solving equations (6) and (4) simultaneously, and the result is ~1900.

Calculation of G(-cyt)

Although curve A in Fig. 7 is nearly straight to about 90% decomposition, the deviation of the best-fitting curve from linearity is appreciable. Since it has been demonstrated by the data in Table IV that equation (4) applies in initially 0.1 mM cytosine solutions, $\underline{G}$(-cyt) must be decreasing appreciably with dose.

The values of $\underline{G}$(-cyt) are shown in Fig. 10. The points in this figure represent the average of 4-6 determinations, and the bars indicate the extreme deviations. The line represents the least-square fit of the data. It corresponds to the expression:

$$\underline{G}(-cyt) = G_o(-cyt) + b\underline{D} = 3.35 - 0.024\ \underline{D}^* \ . \qquad (10)$$

where $\underline{G}$ is in the usual units[3] and $\underline{D}^*$ is the dose in krads.

Measurements were also made in 1 and 10 mM cytosine. The results

TABLE IV

RATIO OF THE ABSORBANCES $A^{\lambda}_{-D}/A^{\lambda}_{-O}$, AT pH 3

Wavelength nm	Dose in krad				
	6.7	13.4	20.1	26.8	33.5
264	.761	.582	.381	.230	.113
275	.770	.581	.385	.224	.101
283	.778	.589	.392	.231	.104

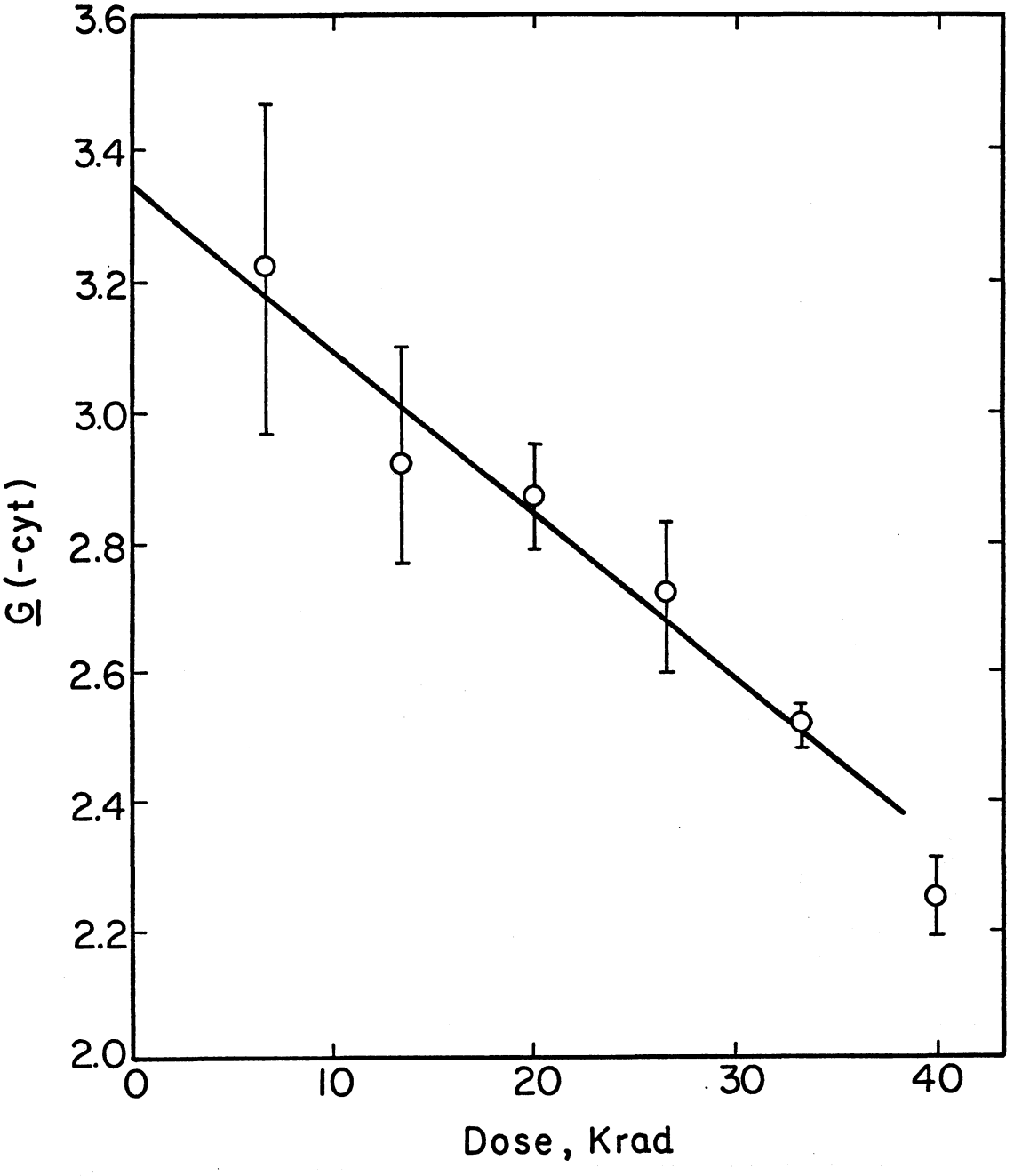

Figure 10. Decomposition Yield of Cytosine, for Initially 0.1 mM Cytosine, as a Function of Dose

are summarized in Table V; i.e., the values of $\underline{G}_o$(-cyt) are the same within experimental error and average 3.3 ± 0.01. Equation (10) adequately represents $\underline{G}$ in the range investigated, to 40% decomposition--radiolysis of the more concentrated solutions took proportionally larger doses, of course, and the irradiation was not continued beyond that point.

One may ascribe the decrease of $\underline{G}$ with dose to competition by the radiolysis products for the solvent radicals which are reacting with the cytosine. It may be estimated that the effective equivalent reactivity of the products is about one-fifth that of cytosine; that is why the cytosine decreases nearly in proportion to dose to beyond 50-60% decomposition.

Radiolysis in Deaerated Solutions; Effect of Oxygen Depletion

The solubility in water of oxygen at 1 atm pressure and 20°C is 1.3 mM; in air-equilibrated solutions $[O_2]$ is 0.27 mM (58). In our experiments the solvent contained 0.02 M phosphate and the temperature was 22-26°C; the effect of these factors on the solubility may be appreciable but cannot be large.

The results shown in Fig. 11 show that the removal of oxygen from solution has a very great influence on the course of the radiolysis. Note that curve C was obtained after exposure to a greater dose than that represented by curve F in Fig. 6, but that in the former case $\underline{A}_D^{275}/\underline{A}_o^{275}$ is still about 0.6. Also note that curves B and C intersect A at about 295 nm; this means that ε_Σ^{295}, instead of being negligible, is equal to ε_{cyt}^{295}, ca. 1800.

TABLE V

VALUES OF $\underline{G}_o$(cyt) AND $\underline{G}$(cyt)

Initial cytosine concentration mM	Oxygen concentration	$\underline{G}_o$(-cyt)	$\underline{G}$(-cyt) fraction 0.25	$\underline{G}$(-cyt) decomposed 0.40
0.1	Satd. by bubbling	3.35	3.16	3.03
1.0	" " "	3.30	3.10	2.97
10	" " "	3.23	3.17	3.12
1.0	Initially equilibrated with air	3.0	2.7	2.1

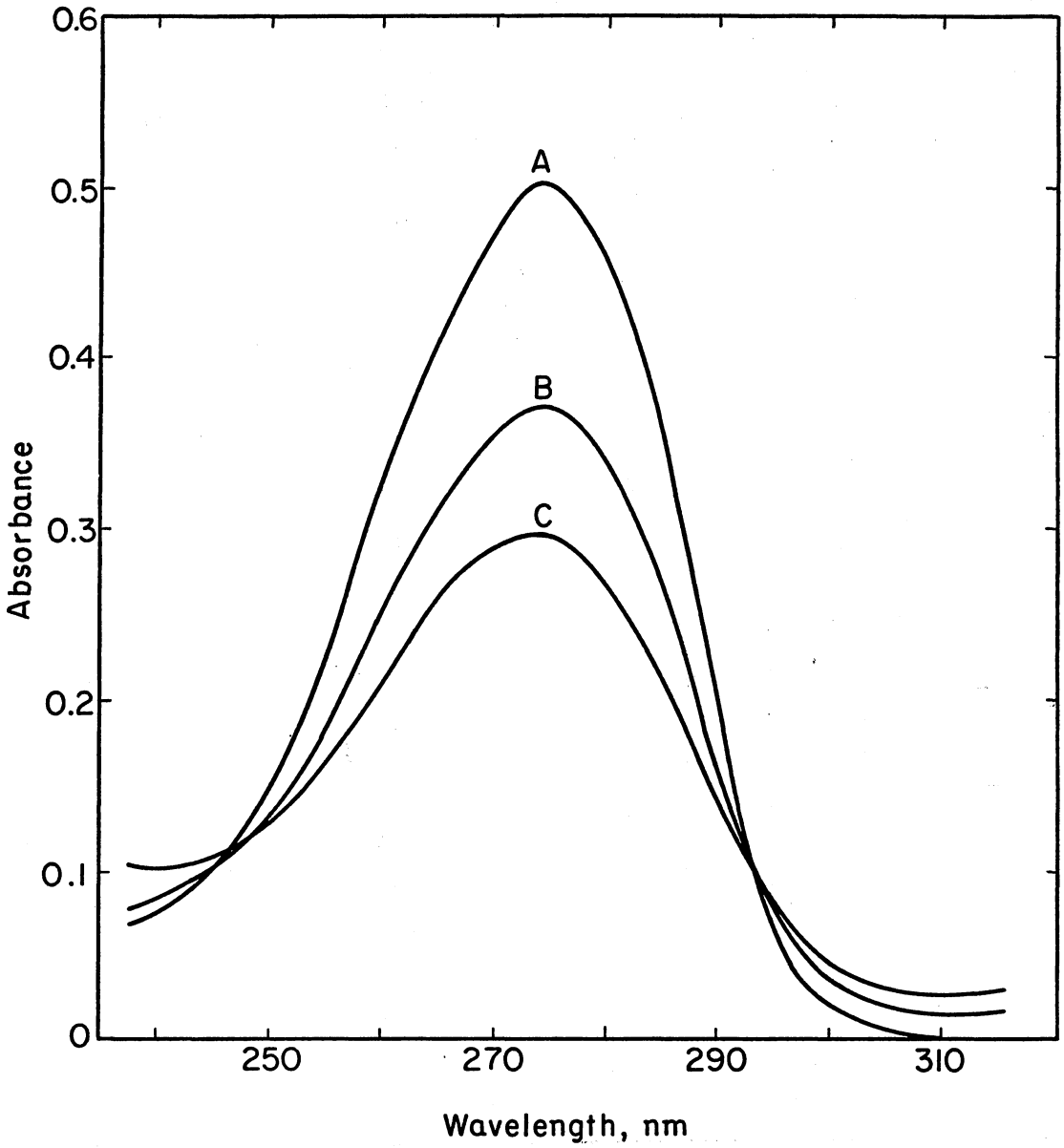

Figure 11. Spectra of Cytosine Solution, Initially 0.1 mM, After Exposure to Varying Doses in Deaerated, Helium-saturated Solution, Measured at pH 3. Doses in krads: A, zero; B, 26.8; C, 53.6

These results indicate that the products obtained in absence of oxygen are qualitatively different from those obtained in oxygen-saturated solutions. This deduction can be made conclusively, without any independent knowledge of the identity of the products.

The rate of change of $\underline{A}^{\lambda}$ with dose is greatest at 275 nm, and from this it can be deduced that the ratio $\varepsilon_{\Sigma}^{275}/\varepsilon_{cyt}^{275}$ is smaller at that wavelength than at other values of λ--this should be expected, since ε_{cyt}^{275} is maximal. But the absolute value of $\varepsilon_{\Sigma}^{\lambda}$ cannot be obtained from the present data, and hence $\underline{G}$(-cyt) cannot be calculated. We may however reasonably surmise that $\varepsilon_{\Sigma}^{275} > 0$. From equation (6) we then obtain the inequality $\underline{G}$(-cyt) > 1.4.

If oxygen is present, but in limited quantity, we may expect results which are intermediate between those described above; the ratio cytosine/oxygen would of course be of determining importance. From the data given above it may be seem that for 0.1 mM cytosine the ratio is initially > 10 in oxygen-saturated solutions, and about 3 in air-equilibrated ones. The last line of Table V gives the results obtained in an air-equilibrated solution; $\underline{G}_{O}$ is only slightly smaller than in oxygen-saturated solutions, but $(d\underline{A}_{D}^{275}/d\underline{D})$ is substantially smaller.

In 1 and 10 mM cytosine solutions, the effect of oxygen depletion was of course much more marked. After irradiation for 10-20 minutes, dissolved oxygen was nearly exhausted, and the results approximated those obtained in deaerated solutions. The exact course of the radiolysis would of course depend on how rapidly oxygen was replenished by diffusion from the surface--likely the results would vary, to some extent, with the dose rate and the shape of the vessel.

Discussion

Applications of Spectrophotometry to the Study of Radiolytic Reactions

The present study, as well as those cited previously, have demonstrated that the radiolysis of cytosine is a complex process; many products are formed and, moreover, their yields and even their identity depend critically on the composition of the system and other factors. Cytosine is by no means extreme in these respects, and many other biological substances must be even more complicated.

To minimize these difficulties it is important to find methods of study that are relatively easy to execute and that can provide a large number of fairly precise data. Spectrophotometry is such a technique.

Obviously it has limitations--it cannot be used if neither the starting materials nor the products have a measurable absorbance, and such cases do exist. But they are not common, and there is a large scope for applications outside that domain.

In the preceding sections of the paper we have demonstrated how $\underline{G}(-S)$ can be calculated directly from absorbance measurements if $\varepsilon_S^\lambda >> \varepsilon_\Sigma^\lambda$. Nothing else need then be known about the identity and properties of the products.

Even more information can be obtained concerning the radiolysis process if at some other wavelengths $\varepsilon_\Sigma^\lambda$ becomes comparable to, or greater than, ε_S^λ. This will be illustrated in the third part of this discussion.

If the absorbance of products overlaps that of the initial substance at all accessible wavelengths, then the value of $\underline{G}(-S)$ cannot be

obtained by spectrophotometry alone. It may, however, still be possible to use the technique to advantage in conjunction with other information; examples will be given in future papers.

G(-cyt) and Some Aspects of the Radiolysis Mechanism

The simplicity of the procedure employed for the determination of G(-cyt) and the relatively good precision attained inspires confidence in the accuracy of the result, 3.3 ± 0.1. It appears that the value is affected little or none by moderate lowering of the pH and by the phosphate buffer, while oxygen concentration has a drastic effect. The solvent medium employed in this work has been completely specified with respect to these variables.

Let us now consider the result in the light of the mechanisms which are thought to occur in dilute aqueous solutions upon irradiation. It is generally agreed that the radiolysis of the water, if formulated in accordance with equation (1), may be represented as follows: (9).

$$H_2O \xrightarrow{\sim\sim} 0.284\ (H_2O\cdot)^- + 0.284\ (OH\cdot) + 0.284\ H_3O^+ + 0.053\ (H\cdot) + 0.074\ H_2O_2 + 0.047\ H_2 \qquad (11)$$

Cytosine reacts very rapidly with (OH·), the specific rate constant being $k_{12} = (2.5\text{-}4 \times 10^9\ M^{-1}s^{-1})$ (60):

$$\text{cyt} + (OH\cdot) \rightarrow (\text{cyt OH}\cdot) \qquad (12)$$

It may therefore be expected that all (OH·) will react in this way, even in the most dilute solutions studied. Cytosine also reacts very rapidly with the hydrated electron ($k_{13} = 7\text{-}13 \times 10^9$): (60):

$$\text{cyt} + (H_2O\cdot)^- \rightarrow (\text{cyt}\cdot)^- + H_2O \qquad (13)$$

and somewhat more slowly with (H·) ($k_{-14} = 10^8$ at pH 1) (5):

$$\text{cyt} + (H\cdot) \rightarrow (\text{cyt H}\cdot) \qquad (14)$$

In oxygen-saturated solution, the situation is however complicated by the fact that $(H_2O\cdot)^-$ and (H·) also react very rapidly with the oxygen (5):

$$(H_2O\cdot)^- + O_2 \rightarrow (O_2\cdot)^- + H_2O \qquad k_{-15} = 2 \times 10^{10} \qquad (15)$$

$$(H\cdot) + O_2 \rightarrow (HO_2\cdot) \qquad k_{-16} = 2 \times 10^{10} \qquad (16)$$

On the basis of the above rates, it may be inferred that (H·) will be completely consumed by reaction with the oxygen. Reaction (13) should predominate over (15) in 10 mM cytosine solution, but the reverse should be true in 0.1 mM solution. In point of fact, $\underline{G}$(-cyt) is not substantially different in the three cases. This suggests that reaction with the hydrated electron does not cause destruction of the cytosine--following (13), the electron would be transferred to oxygen:

$$(\text{cyt}\cdot)^- + O_2 \rightarrow \text{cyt} + (O_2\cdot)^- . \qquad (17)$$

(CytOH·) can react rapidly with oxygen:

$$(\text{CytOH}\cdot) + O_2 \rightarrow [\text{Cyt(OH)}(O_2\cdot)] \qquad (18)$$

Willson (54) has estimated that the rate constant for this reaction is $k_{-18} = 2 \times 10^9$. Then (cyt(OH)O_2·) and (O_2·) will be the predominant intermediates present during the irradiation.

We may then expect these intermediates to react in three ways.

($O_2\cdot$) may disproportionate--this is a well known reaction (k_{-19} = 1.6 x 10^7)(5):

$$2(O_2\cdot)^- + 2\ H_2O \rightarrow O_2 + 2\ OH^- + H_2O_2 \qquad (19)$$

Secondly, the two radicals may react one with the other:

$$[cyt(OH)O_2\cdot] + (O_2\cdot)^- + H_2O \rightarrow O_2 + cyt(OH)O_2H - OH^- \qquad (20)$$

This reaction is, of course, analogous to (19). Finally, there could be disproportionation of the cytosineperoxyl radical. Little is known about such reactions but likely the molecule would be fragmented, giving urea, ammonia, and other small products:

$$2(C_4H_6N_3O_4\cdot) + \upsilon_{H_2O}H_2O \rightarrow (CH_4N_2O) + NH_3 + \ldots \qquad (21)$$

It is pertinent to consider, in this connection, the work of Daniels and Schweibert (44), although this was done largely at pH 2. At that pH, cytosine is in a different state of ionization, and $(H_2O\cdot)^-$ is undoubtedly consumed by reaction with H_3O^+:

$$(H_2O\cdot)^- + H_3O^+ \rightarrow (H\cdot) + 2\ H_2O \qquad k_{-21} = 2.3 \times 10^{10} \qquad (21)$$

$\underline{G}$(-cyt) was nevertheless found to be 3.2-3.3, about the same as in the present work at pH 7, and this supports the inference that $(H_2O\cdot)^-$ does not cause the destruction of cytosine.

The cited authors obtained evidence for the formation of a peroxide, as represented in equation (20). This peroxide was rapidly converted to a non-peroxide product and H_2O_2, and the rate of this reaction increased with pH so that no appreciable amount of peroxide would accumulate at pH 7. Although the non-peroxide product was not directly iden-

tified, it is plausible that this reaction pathway would lead to the cytosine and/or uracil glycols isolated by Ekert and Monier (48).

From the stoichiometric points of view, if every radical formed in reaction (11) reacted with cytosine, and if each of the resulting products would in turn be converted, by some mechanism or another, into a derivative product, $\underline{G}$(-cyt) could be as high as 5.9. On the other hand, if all of the cytosine were decomposed via reaction (20), the yield would be equal to $\underline{G}(OH\cdot)$ i.e., 2.7. The most plausible way to rationalize the somewhat higher yield which has in fact been found is to postulate that a fraction of the $(O_2H\cdot)$ radicals reacts directly with cytosine, presumably to form the radical:

$$(cyt) + (O_2H\cdot) \rightarrow (cyt\,O_2H\cdot) \qquad (22)$$

which might then react by reactions similar to (20). Stoichiometric balance would not be violated, in the first place because $(O_2H\cdot)$ is produced in greater amounts than $(OH\cdot)$, and also because reaction (21) provides an alternative pathway for the reaction of cytosineperoxyl radicals.

Further Characterization of the Products

As we have already noted, if at some wavelength α, $\varepsilon_{\Sigma}^{\alpha} = 0$, while at some other wavelength β, $\varepsilon_{\Sigma}^{\beta} > 0$, the value of the latter can be calculated by solving equations (4) and (5) simultaneously. It may then be instructive to compare the results with what is known about the products from other, independent measurements.

Table VI reports selected spectral data for substances that have been identified as products in the radiolysis of cytosine, or that may

TABLE VI

MOLAR ABSORPTION COEFFICIENTS OF SOME PYRIMIDINE DERIVATIVES

Compound	pH	Wavelength, nm	ε $M^{-1}cm^{-1}$	Reference Comments
Cytosine	11	298	300	This work
		315	~0	
5,6-Dihydro-cytosine	8	239 (max)	11,300	(78)
Photohydrated 3-cytidylic acid	8.4	240 (max)	13,000	(79)
	1.9	240	2,000	
Uracil glycol	10	220 (max)		(80)
Uracil	11	285 (max)	5,900	This work
		298	1,300	This work
		315	0	This work
5-Hydroxy-cytosine	11	221 (max)		(48)
		273 (min)		(48)
		298	3,000[a]	(48)
		317.5 (max)	4,200	(48)

[a]Estimated

plausibly be products.

We see from this table that at pH 11 ε^{λ} for 5-hydroxycytosine and uracil are much larger than $\varepsilon^{\lambda}_{cyt}$ in the region λ = 285-320 nm. In point of fact, the irradiated solutions, after adjustment to pH 11, showed negligible absorption at 317.5 nm, and we may deduce from this that the yield of 5-hydroxycytosine was less than 3% (this would give $\underline{A} \doteq 0.010$ in 5×10^{-4} M solution). Similarly the low absorbance found at 290 nm allows us to exclude the formation of uracil.

According to previous reports, 5-hydroxycytosine is a product in the absence of oxygen ($\underline{G} \sim 0.4$) (46,50) Ekert and Monier (48) found it in solutions which had been irradiated "under air", but in the conditions they employed (1 mM cytosine, 24 krad min^{-1}) the dissolved oxygen would soon have been exhausted.

Dihydrocytosine derivatives in neutral solution have a maximum around 240 nm, and ε^{max} is of the order of 10,000; also we see that the spectrum of hydrated cytidylic acid changes like that of cytosine on lowering the pH. Our results are consistent with the formation of dihydrocytosine derivatives with a $\underline{G}$ value of about 2, provided further that the rest of the products, formed with a $\underline{G}$ value of about 1, have little or no absorbance at 240 nm and above.

This is a reasonable hypothesis. It is pertinent to note, in this connection, that in the case of thymine, which has been more thoroughly studied, some 30% of the base is indeed converted into urea and urea derivatives, which would have the requisite spectral characteristics (60).

We have seen that the radiolysis products are much less reactive than cytosine toward the radicals causing cytosine destruction. This

is consistent with the conclusions stated above, even though there is not much pertinent data available. The reaction of (OH·) with dihydrouracil is some five times slower than that with uracil (80), while urea and derivatives would doubtless react more slowly ($\underline{k}$ for acetamide is 10^7-10^8) (81).

The foregoing interpretation is necessarily quite tentative, because it is based on very incomplete knowledge of the actual products and their properties. However, it provides a fairly complete picture of the radiolysis process, which is consistent with the available data.

CHAPTER V

RADIOLYSIS OF CYTOSINE IN HELIUM-SATURATED SOLUTIONS

(To be Submitted for Publication By:

G. Gorin and N. Ohno)

(Abstract)

Cytosine solution, 1.0×10^{-4} M, in 0.02 M phosphate buffer of pH 7, was irradiated with Co^{60} gamma-rays. $\underline{G}_o$(-cyt), is 1.8 molecules/(100 eV), and it decreases with dose. Up to 35% decomposition $\underline{G}$(-cyt) is described by the expression ($\underline{D}$* = dose in krad): $\underline{G}$(-cyt) = 1.8 - 0.02 D*. The spectrum changes as the radiolysis advances, but up to 35% decomposition the absorbance remains constant at 246 and 294 nm (isosbestic points). The spectrum of the irradiated solutions was also measured at other wave lengths, 228-350 nm, and at pH 3 and 11 as well as at 7. The spectral data are consistent with the finding by other investigators that 5,6-dihydrocytosine derivatives, 5-hydroxycytosine, and uracil are the products of radiolysis. The $\underline{G}_o$ values for the formation of the aforementioned products are 0.8, 0.4, and 0.1, respectively. A radiolysis mechanism is proposed. It is postulated that regeneration of cytosine is responsible for the low value of $\underline{G}_o$(-cyt). The reactivity of the products toward water radicals is much higher than that in oxygen-saturated system.

Introduction

Several studies have been done on the radiolysis of cytosine solutions in the presence of radical scavengers, e.g., O_2, N_2O, and Cu^{+2}, but only a few papers (50,51,53) have reported the radiolysis in the absence of such scavengers.

According to Khattak and Green (50), the value of $\underline{G}_o$(-cyt) in the latter system is 1.8. This value is one-third of the $\underline{G}$ value for the radicals derived from water, i.e., $\underline{G}(Z\cdot) = \underline{G}(OH\cdot) + \underline{G}(H_2O\cdot)^- + \underline{G}(H\cdot) \cong 6$. It has been postulated that the low value may be due to regeneration of cytosine through disproportionation of cytosine radicals (26,27). However, direct evidence for this hypothesis has not yet been provided.

Khattak and Green identified 5-hydroxycytosine ($\underline{G}$ = 0.46), uracil ($\underline{G}$ = 0.3), and 6-hydroxycytosine (a trace amount) among the products. Holian and Garrison (53) reported the $\underline{G}$ value for the formation of 5,6-dihydrocytosine derivatives to be about 0.9. This value is much higher than that obtained by Khattak and Green, who found only a trace of cytosine glycol. The formation of uracil had been reported earlier by Ponnamperuma *et al.* (51).

The present paper reports: the value of $\underline{G}_o$(-cyt); the dependence of $\underline{G}$(-cyt) on the dose; a maximum value for $\underline{G}_o$(5-hydroxycytosine); $\underline{G}_o$ values for 5,6-dihydrocytosine derivatives and uracil; the development of isosbestic points; and a reaction scheme.

Methods

The chemicals, buffer solutions, and procedures for irradiation and quantitative measurements were as described in the preceding chapter. Uracil was purchased from Sigma Chemical Company. The initial

concentration of cytosine was 1.0×10^{-4} M in all cases and the dose rate was 2750 rad $minute^{-1}$.

To remove oxygen, helium was passed through the solution prior to irradiation. In this case, the sample vial was closed with a polyethylene cap, into which there has been drilled two small holes. The gas flow was regulated as described above, and it was maintained for 15 min at 0.3 ml s^{-1}. Then the capillary was withdrawn, the holes were immediately covered with Parafilm, the vial was placed in the Gamma-cell and irradiated forthwith. (According to Czapski (82), bubbling N_2 for 5 minutes at the rate of 1 ml/sec reduces the concentration of O_2 in 100 ml of water from 10^{-4} to 10^{-7} M. In our experiment, 15 minutes gave consistent radiolysis rates, reproducible to ± 3%).

The spectral data for uracil were obtained the same manner as those for cytosine, described in the preceding chapter.

Results

Fig. 12 shows the spectra obtained when a solution of cytosine, initially 0.1 mM in phosphate buffer of pH 7, was irradiated with increasing doses, and then the pH of an aliquot portion was adjusted to 3. It should be noted that while the absorbance at the maximum, 275 nm, decreased, the absorbance at two other wave lengths remained nearly constant (isosbestic points). Fig. 13, curve A, shows the change in absorbance taking place at 275 nm as a function of dose. Table VII the location of the isosbestic points and the absorbances at those points.

Another aliquot portions of the irradiated solutions was examined without changing the pH, i.e., at pH 7. Fig. 14 shows the results.

TABLE VII

ISOSBESTIC POINTS OF IRRADIATED SOLUTIONS

pH	Isosbestic points nm	Absorbance at the isosbestic point
3	246	0.24
3	294	0.18
11	285	0.24
7	248	0.45
7	286	0.10

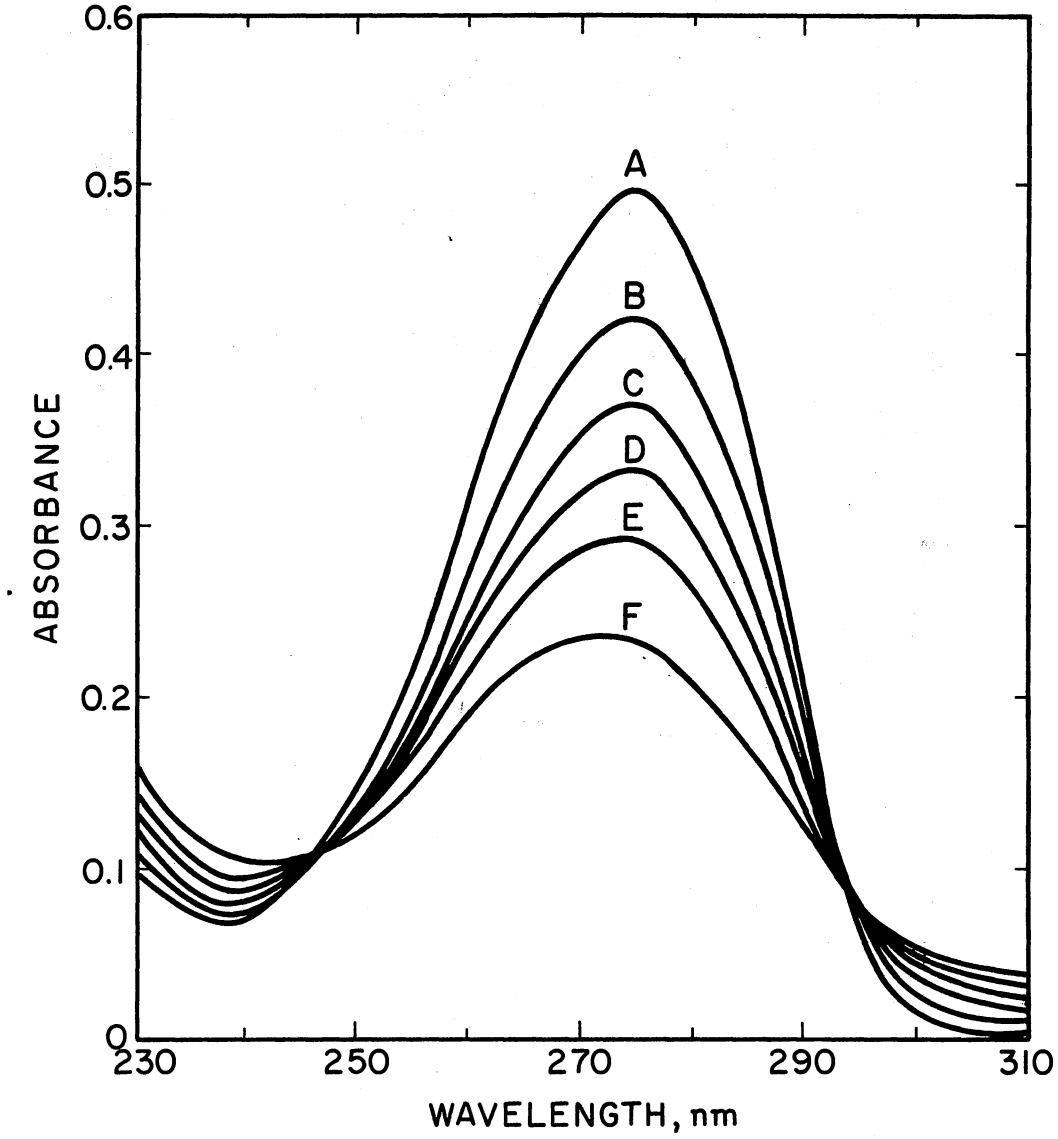

Figure 12. Spectra of Irradiated Cytosine Solutions, Initially 0.1 mM, Measured at pH 3, Dose in krad: A, 0; B, 13.8; C, 27.6; D, 41.4; E, 55.2; F, 69.0

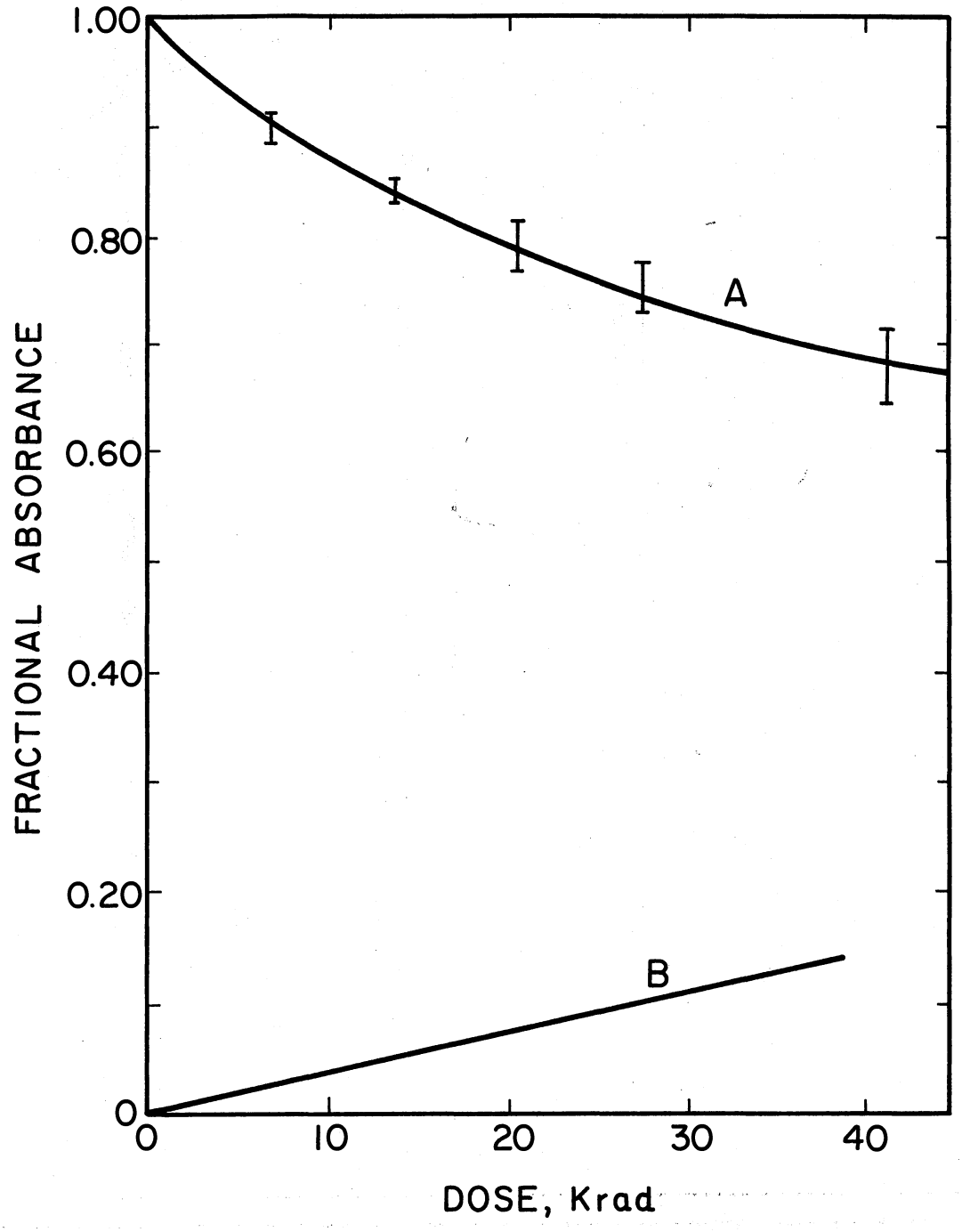

Figure 13. Fractional Absorbance: A, Fractional Absorbance Decrease at 275 nm and pH 3; C, Fractional Absorbance Increase of 5-hydroxycytosine, at 315 nm and pH 11, here 0.42 Absorbance is Taken as 1.0

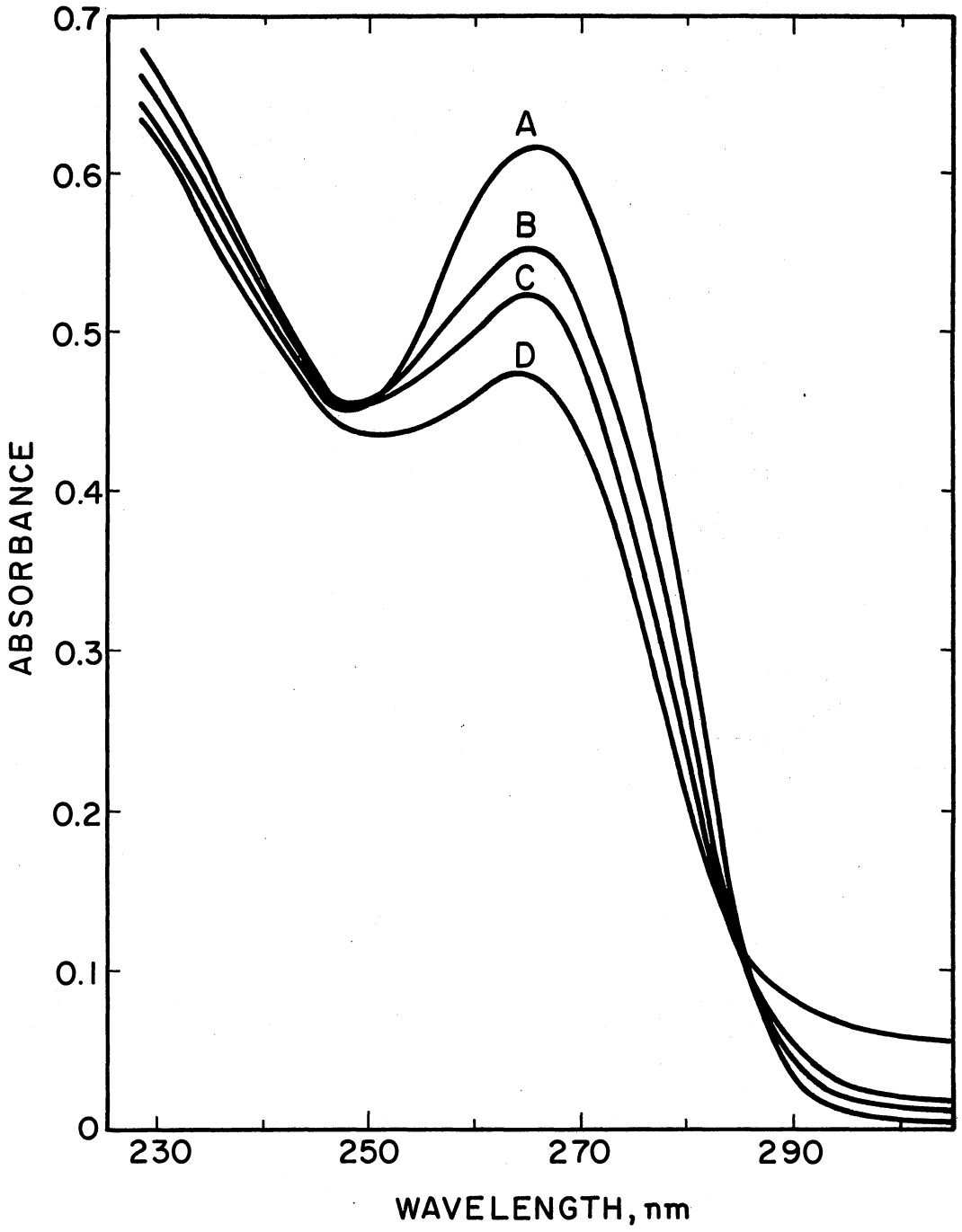

Figure 14. Spectra of Irradiated Cytosine Solutions, Initially 0.1 mM, Measured at pH 7, Dose in krad: A, 0; B, 13.8; C, 34.3; D, 55.2

Again, a decrease in absorbance occurrs at the maximum, 266 nm, while the absorbance at 286 nm remains constant. Curves B and C cross A at the same point, 248 nm, but at a higher dose the absorbance at this wave length decreases slightly.

Finally, a third aliquot portion was adjusted to pH 11 before measuring the spectrum; in this case, only one isosbestic point is found. The change in absorbance taking place at 315 nm as a function of dose is shown by curve B, Fig. 13.

Discussion

An interpretation of the occurrence of isosbestic points in irradiated solutions has been presented by Raff and Gorin (83). They have pointed out that the phenomenon will occur only if: (1) products $P_1 \ldots\ldots P_i$ are produced in a constant ratio; and (2) the weighted sum of the absorbance of the products equals that of the original solute at some wave length(s).

Constancy of absorbance at a single wave length might possibly be due to fortuitous combination of factors. However, in the present case <u>two</u> isosbestic points are observed at pH 3 and 7, respectively, while one point is found at pH 11.

These findings exclude beyond reasonable doubt all explanations but the one proposed above. Since the spectrum of cytosine changes with pH, and those of the products do also, one should not expect the positions of the isosbestic points and/or the magnitudes of the absorbance to be the same at the three pH values. The significant point is that one or more isosbestic points <u>exist</u> at a given pH.

If nothing were known about the products, something about them

could be deduced from the position of the isosbestic points and the magnitude of the absorbance. However, some products have been identified by previous investigators and the results can be interpreted more easily by taking their findings as basis for discussion.

The formation of uracil and of 5-hydroxycytosine have been reported by Ponnamperuma (51) and by Khattack and Green (50). These products were formed in de-aerated solutions in the absence of radical scavengers such as Cu^{+2} and N_2O. Table VIII lists the spectral characteristic of these two compounds along with those of cytosine at the three pH values.

Formation of 5-Hydroxycytosine and Uracil

Fortunately, 5-hydroxycytosine reportedly has a maximum at 317.5 nm and pH 11, where cytosine and uracil do not absorb. Curve B in Fig. 13 shows the fractional absorbance increase of the product around 315 nm, calculated by taking the absorption coefficient of 5-hydroxycytosine as 4200 M^{-1} cm^{-1} and assuming that the absorbance is due entirely to that product. The increase is linear, i.e., the product responsible for the absorbance is accumulating approximately in proportion to the dose, in the range indicated. The G_{-o} value can be estimated to be 0.4 molecules/(100 eV).

At 298 nm and pH 11 the absorption coefficient of uracil is 2600 M^{-1} cm^{-1}, that of 5-hydroxycytosine is estimated to be 3000 M^{-1} cm^{-1}, and that of cytosine is only 300 M^{-1} cm^{-1}. From these data the value of G_{-o}(uracil) can be estimated to be 0.1.

At pH 3, the spectra of both cytosine and uracil are quite different from those of the compounds at pH 11. Therefore the isosbestic point at pH 11 is shifted at a different wave length and the magnitude of

TABLE VIII

ABSORPTION COEFFICIENTS OF URACIL, 5-HYDROXYCYTOSINE, CYTOSINE

Compounds	Max. nm	Min.	e_{max} ($M^{-1}cm^{-1}$)	nm	e	pH
Uracil[a]	258		7900	275	3600	3
	258		7900	228	1800	7
	285		5900	298	1300	11
				315	0	11
5-Hydorxy-cytosine	215	250				1
	300		5380[b]			1
	221	273		298	3000[c]	11
	317.5		4200	315	4200[c]	11
				228	8000[c]	7
				275	3000	3
Cytosine[a]				228	7100	7
				298	300	11
				315	0	11

[a]The data for uracil and cytosine were measured.

[b]Ekert and Monier (48).

[c]Estimated from Ekert and Monier.

the absorbance is also changed. Table VIII shows this observation.

Estimation of G(-cyt)

Although Fig. 12 shows that the absorbance of cytosine solution at 275 nm and pH 3 decreases sharply with the dose, we can also see that the absorbance remains constant at 294 nm. Therefore, it cannot be assumed that the absorbance of the products at 275 nm would be negligible.

If we take the yields for 5-hydroxycytosine and uracil estimated in the previous section, we can calculate that their effective absorption coefficient at 275 nm and pH 3 would be 1600 M^{-1} cm^{-1}. On this basis, G(-cyt) can be calculated, and the results are shown in Fig. 15 line A.

The bars represent experimental points and their estimated uncertainties. The line drawn through the points represents the best least-square fit, and corresponds to the expression (D^* = dose in krads):

$$G(-cyt) = 1.8 - 0.02\ D^*$$

The dose D must be within 0 and 25 krads; beyond that point higher terms have to be added to the equation.

In this equation, the ratio of the second term to the first is greater than in the equation found for the oxygen saturated system. This indicates that the radiolysis products are more "radiosensitive" than those obtained in the former case, i.e. they compete more strongly with cytosine for the radiation-produced species that are causing decomposition.

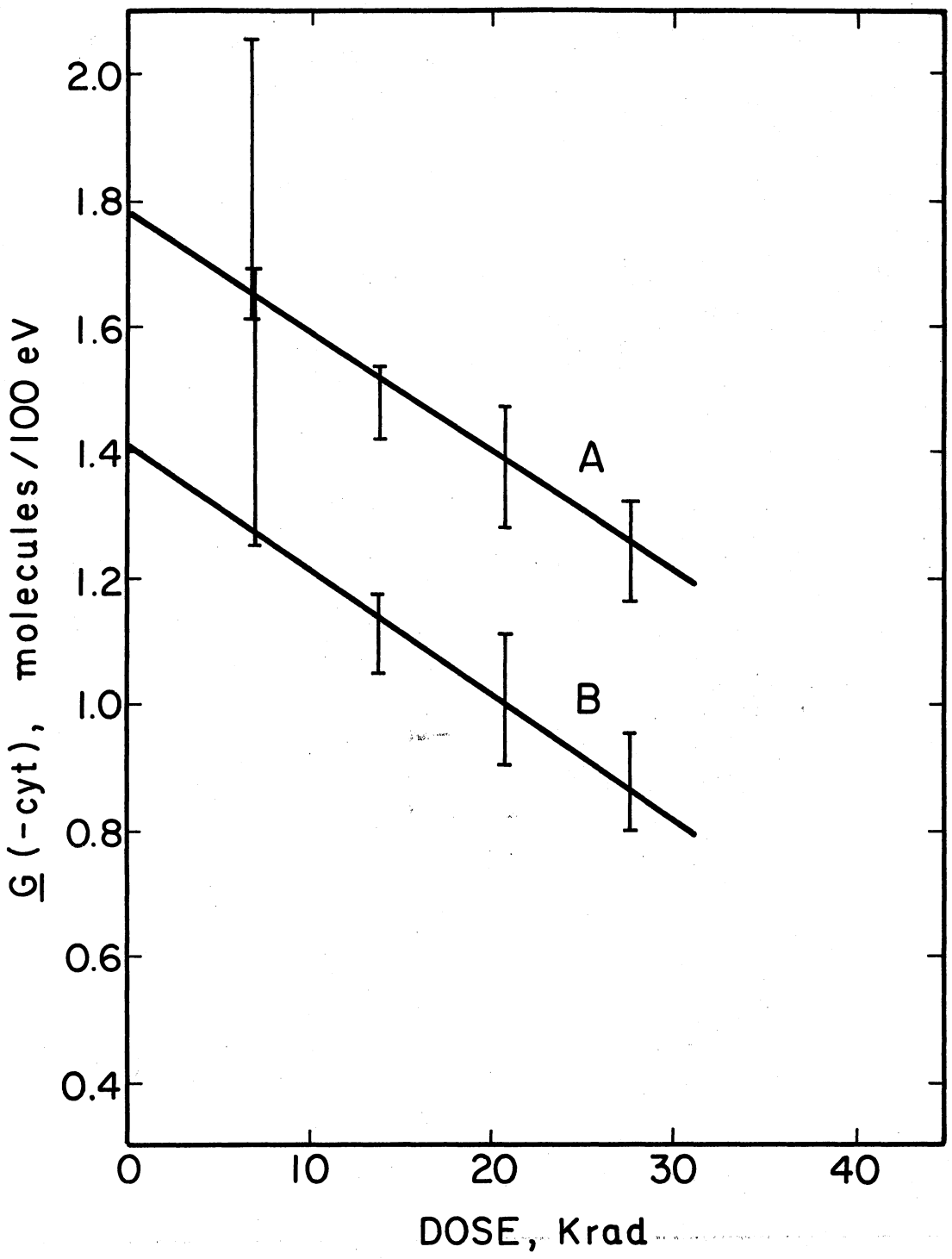

Figure 15. G(-cytosine) vs. Dose: A, Calculated Value; B, Apparent Value. Both are measured at 275 nm and pH 3

Formation of 5,6-Dihydrocytosine Derivatives

The curves obtained at pH 7 and represented in Fig. 14 show that the change of absorbance with dose below 260 nm was small. In the region 245-228 nm the absorbance increases only slightly, showing that the absorbance of the products is slightly larger than that of cytosine. Note that the absorbance at 228 nm remains quite high compared to that as other wave lengths. Although 5-hydroxycytosine and uracil absorb as this wave length, the sum of their $\underline{G}$ values, 0.4 + 0.1, is less than one third of the cytosine being decomposed. Therefore these products cannot fully account for the observed absorbance and some other product(s) must be present in addition.

The formation of 5,6-dihydrocytosine derivatives can be inferred from the spectral changes at pH 3, 7, and 11, by the same reasoning that has been employed in the preceding section. At 228 nm and pH 7, the absorption coefficients of 5,6-dihydrocytosine derivatives, 5-hydroxycytosine, and uracil are estimated to be 10,000, 8,000, and 1,800 M^{-1} cm^{-1}, respectively. Then, the $\underline{G}_o$(5,6-dihydrocytosine derivatives) can be calculated to be 0.8.

Table IX lists the $\underline{G}_o$ value for the dihydrocytosine derivatives, 5-hydroxycytosine, and uracil. The sum of the values is 1.3, about 70% of the value of $\underline{G}_o$(-cyt).

Simplified Mechanism of Radiolysis

Let us now propose a simplified scheme as follows:

$$3\ H_2O \xrightarrow{(1/2)G\ dD/dt} (H_2O\cdot)^- + (OH\cdot) - H_3O^+ \qquad (1)$$

$$cyt + OH\cdot \rightarrow (cyt.\ OH\cdot) \qquad (2)$$

TABLE IX

VALUES OF $G_o(P)$

Products	$G_o(P)$
5,6-dihydrocytosine derivatives	0.8
5-hydroxycytosine	0.4
Uracil	0.1
Total	1.3

$$\text{cyt.} + (H_2O\cdot) \rightarrow (\text{cyt.})^- + H_2O \quad (3)$$

$$(\text{cyt. OH}\cdot) + (\text{cyt.})^- \rightarrow \text{cyt.} + U \quad (4)$$

$$U + (OH\cdot) \rightarrow (UOH\cdot) \quad (5)$$

$$U + (H_2O\cdot)^- \rightarrow (U\cdot)^- + H_2O \quad (6)$$

$$(UOH\cdot) + (U\cdot)^- \rightarrow 2W \quad (7)$$

Here, reaction (4) represents a variety of recombinations of cytosine radicals, some which regenerate cytosine, for example:

$$(\text{cyt.OH}\cdot) + (\text{cyt.})^- \xrightarrow{H_3O^+} 2\ \text{cyt} + 2\ H_2O$$

The occurrence of such reactions can explain why $\underline{G}(-\text{cyt})$ is less than the yield of $(OH\cdot)$ and $(H_2O\cdot)^-$.

As has already been pointed out, from the dependence of $\underline{G}(-\text{cyt})$ on dose it may be inferred that the products formed in the absence of oxygen compete more strongly for the aforementioned radicals than the products formed in oxygen-saturated solutions. To some extent this may be ascribed to the presence of 5-hydroxycytosine and uracil among the products; the latter reacts rapidly with $(OH\cdot)$ and $(H_2O\cdot)^-$

$$\text{uracil} + (H_2O\cdot)^- \qquad k = 15 \times 10^9\ M^{-1}\ cm^{-1}$$

$$\text{uracil} + (OH\cdot) \qquad k = 4.5 \times 10^9\ M^{-1}\ cm^{-1}$$

and it may be reasonably supposed that the former would also, although data for it are not available.

A more definitive interpretation must, however, await fuller characterization of the radiolysis products.

CHAPTER VI

FRICKE DOSIMETER FOR THE DETERMINATION OF DOSE RATE

The Fricke dosimeter (84) is a chemical dosimeter for ionizing radiation. It is based on the oxidation of ferrous sulfate to ferric sulfate in aqueous acidic solutions. The radiation induced chemical effect is "indirect", i.e., caused by reactions between ferous ions and the reactive species, which are produced directly and indirectly from the actions of radiation on water.

The value of $\underline{G}(Fe^{+3})$ is 15.6 molecules/(100 eV) (9). This high yield makes the dosimeter useful in radiation chemistry. The dosimeter is excellent for a wide dose range, 4000 - 50,000 rads (9). The yield is linearly porportional to absorbed dose over the dose range, specified above and nearly independent of the type of radiation (Co^{60} gamma-rays, X-rays, and 1 - MeV electrons), dose rate, and temperature (~ $20^{o}C$). The determinations of $\underline{G}$(-solute) and $\underline{G}$(product) require only a simple calculation procedure which is easy and convenient for routine use. It is the most commonly used dosimeter for radiolysis of aqueous solutions. The dosimeter solution consists of 1.0×10^{-3} M $Fe(NH_4)-(SO_4)_2$, 1.0×10^{-3} M NaCl, and 4.0×10^{-1} M H_2SO_4. The presence of chloride ion inhibits the oxydation of ferrous ions by organic impurities. The water should be pure (triply distilled).

The reaction scheme for the radiolysis is as follows:

$$3\ H_2O \xrightarrow{(\frac{1}{2})\ G(Z\cdot)(dD/dt)} (H_2O\cdot)^- + (OH\cdot) + H_3O^+$$

$$(H_2O\cdot)^- + H_3O^+ \rightarrow (H\cdot) + 2\ H_2O$$

$$O_2 + (H\cdot) \rightarrow (HO_2\cdot)$$

$$Fe^{+2} + (OH\cdot) \rightarrow Fe^{+3} + OH^-$$

$$Fe^{+2} + (HO_2\cdot) \rightarrow Fe^{+3} + HO_2^-$$

$$HO_2^- + H_3O^+ \rightarrow H_2O_2 + H_2O$$

$$Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + (OH\cdot) + OH^-$$

$$4\ Fe^{+2} + O_2 + 2\ H_3O^+ \rightarrow 4\ Fe^{+3} + 2\ OH^- + 2\ H_2O$$

In the presence of O_2, it should be noted that three Fe^{+2} ions in acidic medium are oxidized for each $(H_2O\cdot)^-$ and $(H\cdot)$ produced, one Fe^{+2} per $(OH\cdot)$, and two Fe^{+2} per H_2O_2 molecule.

The Fe^{+3} ion has maximum absorbance, $\varepsilon_{max} = 2193\ M^{-1}\ cm^{-1}$, at 304 nm (25°C). The Fe^{+2} ion does not absorb at this wavelength. Thus, the spectrophotometric method is the simplest and most sensitive way to determine the concentration (Fe^{+3}). The calculation procedure is described below.

The mean absorbed does $\underline{D}$ in the volume occupied by the dosimeter solution is derived as follows:

$$\underline{D}\ (\text{in rads}) = \underline{E}/\underline{W} = (1.602 \times 10^{-12}\ (\underline{P}/\underline{G}))\ (1/\underline{Vd}) \qquad (1)$$

where

$\underline{E}$ = absorbed energy in ergs

$\underline{W}$ = weight of the dosimeter solution in gram

$\underline{P}$ = number of molecules of product formed

$\underline{G}$ = number of molecules of product formed/100 eV

1 eV = 1.602×10^{-12} ergs

1 rad = energy absorption of 100 ergs/gram

$\underline{V}$ = volume of the dosimeter solution in ml

$\underline{d}$ = density of the dosimeter solution in gram/ml

In the ferrous sulfate dosimeter, the concentration of Fe^{+3} ions formed by irradiation is given, in a 1-cm cell, by:

$$\underline{C}_{Fe^{+3}} \text{ (moles/liter)} = \Delta\underline{A}/(\varepsilon_{max} \times 1 \text{ cm}) \qquad (2)$$

where ΔA is equal to $(A_{Fe^{+3}} - A_{Fe^{+2}})$, the absorbances of the irradiated and unirradiated (blank) dosimeter, respectively. Then, the number of Fe^{+3} ions formed is given by:

$$\underline{P} = \underline{C} \times \underline{V} \times 6.02 \times 10^{23} \text{ molecules/mole}$$

$$= 6.02 \times 10^{20} \times \underline{C} \times \underline{V} \qquad (3)$$

Substitution of equations (2) and (3) into equation (1) gives $\underline{D}$ in the following expression:

$$\underline{D} = 1.60 \times 10^{-12} \ (\underline{P}/\underline{G})(1/\underline{Vd})$$

$$= 1.60 \times 10^{-12} \ (6.02 \times 10^{20} \times (\Delta\underline{A}/\varepsilon_{max}) \times V) \ (1/\underline{G})(1/\underline{Vd}) \qquad (4)$$

The value of $\underline{G}(Fe^{+3})$ is 15.6: the dosimeter density, $\underline{d}$ = 1.024 gram/ml;

the absorption-coefficient of Fe^{+3} at 304 nm, ε_{max} = 2193 M^{-1} cm^{-1}. Therefore, equation (4) reduces:

$$\underline{D} = 2.84 \times 10^{4} \times \Delta\underline{A}/min.$$

The dose rates used in the irradiations of dioxygenated and oxygenated 1.0×10^{-4} M cytosine solutions were 2750 and 2680 rad $min.^{-1}$, respectively. The irradiation vessel used in the dosimetry was a 10 ml glass vial. Seven vials were placed in the cylindrical irradiation chamber of the gamma cell. An average deviation of the mean absorbance value was ±1.5%. For the dose rate determination, the above procedure was repeated three times. The average results agreed within ± 0.4%.

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