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Title: HYDROPEROXIDE FORMATION IN THE RADIOLYSIS OF AQUEOUS DNA AND THE PYRIMIDINE BASES

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The formation of cytosine hydroperoxide was detected in aqueous cytosine solutions irradiated with Co-60 γ-rays at pH 2. The nucleotide of cytosine, cytidylic acid, forms a hydroperoxide which was detected in neutral solution. The stability of the hydroperoxides of cytosine, thymine, and their nucleotides was found to be a function of pH, with the decomposition rate increasing with increasing pH.

Two hydroperoxides, differing in their thermal stabilities, were found in irradiated aqueous DNA. Through their decay kinetics and energetics, the fast-decaying hydroperoxide was identified with the cytosine component and the slower-decaying one with the thymine component of the DNA.

The hydroperoxide formation in cytosine and DNA was studied as a function of dose, oxygen concentration, DNA structure, and initial cytosine and DNA concentration.

The thermal decomposition of the thymine hydroperoxide in DNA was found to be influenced by the presence of added solutes. By studying the rate of decomposition at 80°C in the presence of added electrolytes, all having an ionic strength of 0.01, the change in rate constant was shown to be a specific effect of the solute rather than an ionic strength effect. Urea, a non-electrolyte, caused a similar change in the rate constant.

Hydroperoxide Formation in the Radiolysis of Aqueous DNA and the Pyrimidine Bases

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INTRODUCTION

Radiation chemistry is conventionally regarded as the study of the chemical effects produced by the absorption of ionizing radiation in matter. The term ionizing radiation usually includes the radiation from radioactive nuclei (α-, β -, and γ- rays), high energy charged particles such as electrons, protons, and deuterons, and electromagnetic radiation of very short wavelength (hard and soft X-rays). However, it has been shown by a number of investigations that ionization can also be caused by ultraviolet light. For example, Terenin and Vilessov (72) have reported ionization in aromatic amines, amino acids and nitrogenous bases caused by vacuum ultraviolet radiation and Grossweiner and Joschek (31), using flash photolysis techniques, have observed ionization in aqueous solutions of various organic solutes with near u. v. radiation. Radiation chemistry should be distinguished from "radiochemistry", a term which is generally used to designate chemical studies employing radioactive isotopes in which the isotope is not used as a source of radiation.

The study of the chemical effects produced in matter by electromagnetic radiation of wavelengths longer than X-rays has conventionally been classed as photochemistry. As just mentioned,

there is a region in the far ultraviolet where it may be difficult to separate photochemistry and radiation chemistry. In general though, there are several important differences between the two. In photochemistry the main primary process due to absorption of the radiation is the production of electronically excited states. These excited states may undergo a variety of decay mechanisms such as dissociation into free radicals, intersystem crossing, internal conversion, return to the ground state through radiative emission of fluorescence or phosphorescence and loss of energy through collisions and energy transfer. For the usual experimental conditions used, one photon interacts with only one molecule[1], and it is possible to use monochromatic light to produce a single well-defined excited state in one of the reactants of the system. At low absorbed doses, these excited molecules are produced in a random distribution throughout the medium, although at higher doses, a density gradient may occur across the irradiation chamber. In contrast, in radiation chemistry the energy of the incident photon or particle is so large that in its passage through the medium it ionizes, and excites as well, a large number of molecules. In addition, the secondary electrons produced by the ionization may have sufficient energy to cause further ionization and excitation in the medium. These high energy particles are

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[1] At laser intensities, two photons may interact simultaneously with one molecule.

not selective and the molecule may be raised to any one of its possible ionized or excited states, the nature of which may not be known. As a result, the number of possible primary species is greatly increased and one must consider chemical consequences arising not only from excited molecules, but also from reactions and energy transfers of ionic species. Furthermore, since the ionizing radiation produces molecules which may be in more highly excited states than those formed by light absorption, the reactions observed in photochemistry are not necessarily the only ones which might result from excited molecules formed by radiolysis. In radiation chemistry the primary species are not produced in a random distribution in the medium, even at low doses, but lie initially along the track of the radiation. This initial spatial distribution of the primary species varies for different radiations according to the L. E. T. of the radiation and is probably the main factor in the differences in the chemical effects observed by using various radiations. Because of the complexity of the primary process in radiation chemistry, the elucidation of many mechanisms of reactions has often been very slow.

The Interaction of High Energy Radiation with Matter

Charged particles lose energy by inelastic collisions with electrons in the absorbing material, causing excitation and ionization, and by emission of electromagnetic radiation. At low energies they

may also undergo elastic collisions. The rate of energy loss, or linear energy transfer (L. E. T.) per unit path length is directly proportional to its mass and inversely proportional to its velocity.[2] As a particle penetrates a medium, its velocity becomes less and its L. E. T. increases. An α-particle will cause a much greater ion density along its track than will an electron of equal energy. Thus, for charged particles, the process is one of a continual energy loss through a large number of interactions, with the track becoming more dense as the particle proceeds.

In contrast, when electromagnetic radiation passes through a a medium, some of the photons may not undergo any interaction, and the overall effect is a reduction of intensity of the incident beam. This reduction is given by the expression

$$I = I_0 e^{-\mu x}$$

where I_0 is the incident intensity, x the thickness of the absorber, and μ the total linear absorption coefficient of the material.

High energy electromagnetic radiation interacts with matter by three basic mechanisms. These are the photoelectric effect, the Compton effect and pair-production. The photoelectric effect is the

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[2] In actual practice, only a mean value is usually calculated for the L. E. T. as the energy lost by the primary particle in the track may not be absorbed locally. Instead, it may be partially transferred to secondary electromagnetic radiation.

transfer of the entire energy of the incident photon to a single electron, which then is ejected with energy equal to the difference between the energy of the photon and the binding energy of the electron. This mechanism is most probable for high atomic number materials and for low photon energies. The Compton effect involves the transfer of only part of the photon energy to the electron, with the photon of decreased energy continuing on. Compton interactions predominate for photons of intermediate energies ($\sim$ 1 to 5 Mev). Pair-production, which has a threshold energy of 1.02 Mev, results when a photon interacts in the vicinity of an atomic nucleus or an electron and is converted to an electron-positron pair. Thus, the overall result of the absorption of electromagnetic radiation in a medium is the production of energetic electrons which then interact in the manner described for charged particles. For Co-60 γ-rays (1.17 and 1.33 Mev), the Compton effect is the most important mode of interaction.

The Action of Ionizing Radiation on Water and Aqueous Solutions

The radiation chemistry of water and aqueous solutions has been studied extensively. This interest in water is due, in part, to its relative simplicity and the availability of a wide variety of aqueous systems on which there exists a large quantity of related information

from other physical and chemical studies, and, in part, because of the importance of aqueous systems in radiobiology and in reactor technology.

In early work it was observed that both oxidation and reduction occurred in irradiated aqueous solutions, and in 1914 it was suggested by Debierne (19) that this chemical action might be due to free radicals formed in the water by the radiation. In 1944 Weiss (73) identified these properties with the H and OH radicals.

By the mechanism for the interaction of radiation with matter described previously, the primary step is the ionization of the water.

$$H_2O \longrightarrow H_2O^+ + e^-$$

For an α-particle, the distance between spurs[3] will be about 10 Å, for electron about 5000 Å and for γ-photons around 10^4 Å Electrons with sufficient energy will cause further ionization and excitation until reaching thermal energies. The excited molecules formed in this manner are considered to return to the ground state in a non-radiative process or dissociate into H and OH radicals which recombine in the water cage to give water. It should be pointed out that as yet, no concrete evidence has been found for the existence of excited

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[3] The spur is an important concept in radiation chemistry. The term is used to designate a small cluster of excited and ionized species.

water molecules. Recently Dainton (15) suggested that the observed increase in the radical yields under acid and alkaline conditions might be due to the action of excited molecules.

Two main models have been postulated to explain the fate of the ionized species, H_2O^+ and e^-. The Samuel-Magee theory (62) proposes that the electron travels about 20 °A from the parent ion before being thermalized and that it is then recaptured by the positive ion since it will not have escaped the electrostatic field of the parent ion. This recapture gives a highly excited water molecule which immediately dissociates into radicals.

$$H_2O^+ + e^- \longrightarrow H_2O^{**} \longrightarrow H + OH$$

These radicals are presumed to be formed with sufficient energy to escape the water cage, giving a similar distribution for both radicals.

Lea (46) calculated that the electron would travel about 150 °A from the parent before being thermalized and thus would escape the electrostatic field of the positive ion. Both ions then undergo hydrolysis, with the electron forming the unstable species H_2O^-.

$$H_2O \longrightarrow H_2O^+ + e^-$$

$$e^- + H_2O \longrightarrow H_2O^-$$

$$H_2O^- + H_2O \longrightarrow OH^- + H_2O + H$$

$$H_2O^+ + H_2O \longrightarrow H_3O^+ + OH$$

overall

$$H_2O \longrightarrow H + OH$$

This model then gives a different distribution for the radicals with the H being formed at some distance from the track. Both of these models lead to the same stable end-products and, at present, with the models not resolved, features of both are accepted.

Magee and Burton (49) pointed out that the reaction

$$e^- + H_2O \longrightarrow H + OH^-$$

is endothermic and could only happen in liquid water where the solvation energy from the OH^- was available. As an alternative they suggested that this reaction might not occur, but instead the electron might remain in the solvated form e_{aq}^-. Since that time, a large quantity of evidence as to the nature of the reducing species has been accumulated.

It was noticed that the results obtained in acid solution often differed from those obtained in neutral solution. Anderson and Hart (2) found that the reducing radical reacts about five times faster with oxygen and hydrogen peroxide in neutral solution than in acid solution and concluded that the reducing species was not the same in both instances. Barr and Allen (4) found that the reducing species formed

when neutral aqueous solutions containing hydrogen, oxygen, and hydrogen peroxide were irradiated reacts much faster with oxygen than with hydrogen peroxide, while in solutions containing only oxygen and hydrogen peroxide, the reducing species reacts with both at comparable rates. Thus, it was concluded that there were two types of reducing species, and they suggested that the one existing in acid solution was atomic hydrogen and the other was the solvated electron.

$$e_{aq}^{-} \overset{H^{+}}{\rightleftharpoons} H$$

The existance of the solvated electron is now well established. It has been shown that the major reducing species in irradiated neutral water has a unit negative charge (14, 16). The absorption spectrum of the transient hydrated electron has been observed in the pulsed radiolysis of aqueous solutions (6, 32, 43). In addition, the rate constants for the reaction of e_{aq}^{-} with a large number of species have been measured using pulse radiolysis techniques (55). Weiss (74) has suggested that the solvated electron is similar to the "polaron" which is known from studies of the electron in polar crystals. The electron should not be regarded as being attached to a particular water molecule, but rather as belonging to a large number of water molecules.

Dainton, et al. (16) showed that the reducing species in acid solution was uncharged at pH 2. Comparison of the rate constants

of the reducing species in acid solution with rate constants for hydrogen atom reaction has shown good agreement (13). Absolute evidence for the identification of the oxidizing species as OH has not been found, but there is a large amount of evidence which supports this conclusion. The oxidizing species has been shown to be uncharged (36), electron spin resonance studies have detected the OH in irradiated ice (54) and studies with systems such as Fentons reagent have indicated the presence of the OH radical.

It was found that both hydrogen gas and hydrogen peroxide are formed in irradiated aqueous solutions and that their yields are largely independent of solute, solute concentration, and pH. These products are considered to arise from reaction between primary radicals in the spurs.

$$H + OH \longrightarrow H_2O$$

$$2H \longrightarrow H_2$$

$$2OH \longrightarrow H_2O_2$$

Armstrong (3) suggested that the reaction

$$2\, e_{aq}^- \longrightarrow H_2 + 2OH^-$$

might also occur and this has been confirmed by studies using pulse radiolysis (21). The H_2 and H_2O_2 formed in this manner are called "molecular products". Therefore the overall decomposition

of water by ionizing radiation can be represented by the equation

$$H_2O \longrightarrow H_2,\ H_2O_2,\ H,\ OH,\ e^-_{aq},\ H_3O^+_{aq},\ OH^-_{aq}$$

In pure water no overall decomposition is observed. This can be explained by reaction of the molecular and radical products.

$$H + H_2O_2 \longrightarrow H_2O + OH$$

$$OH + H_2 \longrightarrow H_2O + H$$

$$e^-_{aq} + H_2O_2 \longrightarrow OH^- + OH$$

$$H + OH \longrightarrow H_2O$$

However, in the presence of a solute, one or more of the radical products may be removed and an overall decomposition will be observed. By using specific radical scavengers, the yields of the radical and molecular products can be obtained.

Yields in radiation chemistry are usually given as G values. For a species X, G(X) is the number of molecules of product X formed per 100 ev of energy absorbed by the system. The primary yields of the radical and molecular products are designated with a subscript as G_x. The amount of energy absorbed is usually obtained by comparison with a reference system for which a G value is accurately known. For solutions, the most common reference is the Fricke Dosimeter, which involves the oxidation of an aerated acid

solution of ferrous ammonium sulfate to the ferric salt. The $G(Fe^{+3})$ for Co-60 γ-rays has been measured as 15.8 ± 0.3 (45). Table I gives the radical and molecular product yields in irradiated aqueous solutions for several different radiations. Table II shows the pH dependence of the radical and molecular product yields in water irradiated with X- and γ-rays.

Table I. Typical Radical and Molecular Product Yields in Irradiated Aqueous Solutions.

Radiation Source	pH	$G(-H_2O)$	G_{H_2}	$G_{H_2O_2}$	G_H	G_{OH}	Reference
$B^{10}(n, \alpha)Li^7$ recoil nuclei	0.5	3.55	1.65	1.55	0.25	0.45	(47)
5.3 Mev α	0.5	3.65	1.57	1.45	0.60	0.50	(48)
5.5 kev electrons	1	4.1	0.6	1.0	2.9	2.1	(10)
γ-rays and fast electrons	0.5	4.5	0.4	0.80	3.70	2.90	(35)

Table II. pH Dependence of Radical and Molecular Yields in X- and γ-Ray Irradiated Water*

pH	G_{H_2}	$G_{H_2O_2}$	G_H	$G_{e_{aq}^-}$	G_{OH}
0.5	0.40	0.80	3.70		2.90
2 to 4	0.5		0.55	2.65	
5	0.55	0.8	1.9	0.9	2.2
7	0.42	0.71	2.80		2.22
			0.6	2.3	
			0	2.85	
8	0.6	0.65	0.9	1.7	2.5
13	0.7	0	0.15	2.5	4.1
	0.45	0.6	3.5		3.2

*Table taken from Spinks and Woods (70, p. 259).

Radiation Chemistry of Aqueous Nucleic Acids and Related Compounds

Most of the quantitative results for radical and molecular yields have come from the study of inorganic systems where the primary products[4] and the radiolysis mechanism were known, or readily discovered, and the radical and molecular yields could then be calculated from the mechanism. The situation is entirely different for complicated organic systems such as the nucleic acids. Here the primary products are not easily surmised and one of the major problems is to first identify these primary products. Then values for the radical and molecular yields can be assumed from the inorganic work and, on this basis, one can try to work out the mechanism.

In the presence of an organic solute, different behavior is exhibited by the reducing species. Only the hydrogen atoms will lead to the production of molecular hydrogen.

$$RH + H \longrightarrow R\cdot + H_2$$

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[4] A primary product is the initial stable product formed by reaction of a primary radical. These primary products may sometimes undergo further reaction with radicals and form secondary products. Hence, it is important to determine initial G values from the dependence of G on total dose.

The e_{aq}^- will react preferentially with unsaturated bonds or with carbonyl compounds which may have been produced during the irradiation. However, for solutions containing oxygen, this difference disappears because both reducing species react with the oxygen to form the hydroperoxy radical or its anion.

$$H + O_2 \longrightarrow HO_2$$

$$e_{aq}^- + O_2 \longrightarrow O_2^-$$

$$H^+ + O_2^- \longrightarrow HO_2 (pK = 4.5)$$

In general organic solutes do not react with hydroperoxy radicals and so the only attacking species will be the OH radical (63).

Because of the discovery that the DNA molecule contains the chemical code that directs cell functions, a great deal of interest in the biological significance of radiation damage to nucleic acids and their related compounds has arisen in recent years. For example, Latarjet, Ekert, and Demerserman (44) have suggested that the peroxidation which occurs in irradiated nucleic acids may cause errors in base pairing due to changes localized on a particular base or to changes in secondary chain structure due to bond breakage. It has been shown that the effects caused by irradiation of the nucleic acids and polynucleotides are basically chemical effects (66) and, in the case of aqueous solutions in particular, are due to the reactive

species formed in the water by the radiation. Some care must be taken in drawing conclusions from radiation damage in dilute solutions and applying them to DNA in the biological environment. It was shown by Hutchinson (38) that the radiation damage to an enzyme molecule is increased two to five times when water is present. However, the chemical changes and bond breakage which occur in aqueous solutions are certainly of very great importance in helping to understand and interpret the effect of radiation on biological systems and thus on genetic effects.

Early work showed that the radiolysis produced an increase in the number of titratable acid groups, liberation of inorganic phosphate, formation of free purine bases, deamination and ring fission and labile phosphate esters (65). Only the work relevant to this thesis will be summarized here.

Pyrimidines and Purines

Because of the probability of genetic effects caused by radiation damage to the bases in DNA, many investigations have centered on the radiation chemistry of the pyrimidine and purine bases themselves.

Studies on aqueous solutions of thymine, uracil and cytosine showed that a decrease in the ultraviolet absorption at 260 mμ occurred upon irradiation with hard and soft X-rays (5, 68). The

indication is that the radiation-produced radicals attack the 4:5 bond causing loss of the chromophoric character of the pyrimidine ring. Initial G values for the measured base loss are tabulated in Table III. The thymine destruction shows a strong dependence upon thymine concentration for irradiation with 200 kev X-rays, but relatively little dependence with Co-60 γ-rays. Changes in pH seem to have little effect. Scholes et al. (65) reported that cytosine in neutral solution underwent a rapid post-irradiation decrease in optical density, but details were not given and no explanation was offered for the decrease.

Table III. Initial G Values for the Destruction of the Pyrimidine Bases in Oxygenated Aqueous Solution

Base	Concentration (M)	Radiation Source	pH	G (-Base)	Ref.
cytosine	2×10^{-4}	200 kev X-rays	5.2	2.05 - 2.28	(68)
uracil	2×10^{-4}	"	5.2	1.88 - 1.93	(68)
thymine	2×10^{-5}	"	5.2	1.23	(65)
	2×10^{-4}	"	5.2	1.89	(65)
	2×10^{-3}	"	5.2	2.76	(65)
	8×10^{-5}	Co-60 γ-rays	5.0 - 5.5	2.53 $\pm$ 0.05	(64)
			2.0 - 2.2	2.50 $\pm$ 0.05	(64)
	2×10^{-4}	"	5.0 - 5.5	2.57 $\pm$ 0.05	(64)
			2.0 - 2.2	2.65 $\pm$ 0.05	(64)
	2×10^{-3}	"	2.0 - 2.2	2.70 $\pm$ 0.1	(64)

Irradiation of thymine and uracil in the presence of oxygen has been shown to give pyrimidine hydroperoxides by saturating the 4:5 ethylenic bond (69, 18). Complete identification has been done in the case of thymine. Ekert and Monier (24) synthesized the cis- and trans-isomers of both 4-hydroxy-5-hydroperoxy thymine and 4-hydroperoxy-5-hydroxy thymine and showed that the radiation product from thymine was a mixture of the cis- and trans- isomers of the 4-hydroxy-5-hydroperoxy thymine. The hydroperoxides have also been characterized by their rate contants for their reaction with iodide ion (63) and this method has indicated the presence of two isomers for the hydroperoxides of uracil and dimethyluracil. It was stated by Scholes (65) that only very small amounts of hydroperoxide were detected when neutral solutions of cytosine were irradiated, although its presence was found for solutions of low pH. No actual figures or results were presented. Ekert and Monier (24) separated radiolysis products from oxygenated solutions of cytosine and postulated their origin from a hydroperoxide, but they were unable to detect the hydroperoxide itself.

Because of its relative stability, thymine hydroperoxide has been well investigated. The yield was found to depend both on concentration of thymine and on pH. G values for oxygenated thymine solutions irradiated with 200 kev X-rays reported by Scholes (65) are shown in Table IV.

Table IV. Dependence of Thymine Hydroperoxide Yield on Concentration and on pH.

	G(Hydroperoxide)	$G(H_2O_2)$
Thymine, pH = 5.3 (moles/liter)		
3.2×10^{-2}	1.73	1.86
3.2×10^{-3}	1.31	1.71
3.2×10^{-4}	1.12	1.73
3.2×10^{-5}	0.71	1.78
pH (Thymine = 2×10^{-4} M)		
8.00	1.02	1.81
5.08	1.05	1.73
3.05	0.58	1.79
1.95	0.33	2.35

Comparison of these G values with the G values for the base destruction of thymine shows that the hydroperoxide formation accounts for about half the disappearance of the pyrimidine molecule. It has been suggested that other consequences of pyrimidine irradiation are deamination and formation of glycols (63), but no other products have actually been identified and there is insufficient information available to establish a quantitative mechanism.

The amount of base destruction in neutral solution upon irradiation of the purines is less than that for the pyrimidines, with G values

of 1.00 to 1.13 for 2×10^{-4}M solutions of oxygenated adenine and 1.36 to 1.40 for xanthine (65). A back reaction leading to the reformation of the purines has been postulated to explain this and some evidence supporting this suggestion has recently been reported (41). No hydroperoxides have been detected under any pH conditions.

The addition of the sugar and phosphate components in the nucleosides and nucleotides has been found to reduce the amount of base destruction in the order free base > nucleoside > nucleotide (66). Hydroperoxides are formed on the pyrimidine bases.

Nucleic Acids

G values have been reported for the destruction of the individual bases in DNA upon irradiation and are summarized in Table V. These values were obtained by separating the liberated bases by paper chromatography and measuring the ultraviolet absorption of the eluates. Collyns, Okada, and Wheeler (11) reported that measurements of the optical density at 260 mμ at pH 2.5 could be used as a measure of the extent of total base destruction in DNA. They based this on the fact that the G value for the total base destruction obtained in this way was comparable to that obtained by adding the G values for the destruction of the individual bases determined by the paper chromatography separation.

Table V. Initial yields for Purine and Pyrimidine Base Destruction in Oxygenated Aqueous DNA (calf thymus) Solutions* Using 200 kev X-rays.

DNA (wt. %)	G(-Base)			
	Thymine	Cytosine	Adenine	Guanine
0.005	0.33	0.23	0.25	0.18
0.20	0.64	0.38	0.39	0.26

*Values taken from Scholes et al. (65).

In 1957 Daniels et al. (18) reported the detection of hydroperoxides ($G \sim 1.0$) in 0.1 percent oxygenated DNA solutions irradiated with 200 kev X-rays. They observed the post-irradiation decay of both the hydroperoxide and the hydrogen peroxide formed and found a decay curve for the hydroperoxide which indicated the presence of two concurrent first order processes with the slower component accounting for about two-thirds of the total. They estimated rate constants of $\sim 4 \times 10^{-4}$ min^{-1} for the slow component and $\sim 1 \times 10^{-2}$ min^{-1} for the fast one. Scholes, Ward, and Weiss (65) give G values for a DNA hydroperoxide formed at several different concentrations.

DNA (wt %)	G(hydroperoxide)
0.005	0.17
0.025	0.30
0.10	0.55

They reported that thymidylic acid formed the only hydroperoxide

stable in neutral solutions and, thus, concluded that the stable hydroperoxide in irradiated DNA must be associated with the thymine molecule. In addition, they found that it was completely precipitated when the irradiated solution was treated with trichloracetic acid, indicating that the thymine hydroperoxide remained attached to the DNA. No further investigation of the second hydroperoxide has been reported.

Irradiation of undenatured DNA leads to an increase in the optical density at 260 mμ. Numerous investigations utilizing ultraviolet absorption measurements and titration studies have shown that an important consequence of the chemical action of radiation on aqueous DNA is the breakage of hydrogen bonds (11, 12, 58) with G values from 12 to 38 reported. Butler (8) reported that the change in optical density with irradiation is dependent upon salt concentration and concluded there was less hydrogen bond breakage in NaCl solutions than in water. It was suggested (58, 65) that this loss of hydrogen bonding was the result of the internucleotide bond breakage. Although hydrogen bond breakage is not involved with the primary reaction mechanism for hydroperoxide formation, it may be important in so far as DNA structure may affect the yields or subsequent behavior in solution.

As this brief summary indicates, the determination of the primary radiation products of DNA is very far from complete and

much investigation remains to be done before its radiation chemistry is well understood. In this context, the clarification of the situation concerning the fast-decaying hydroperoxide in DNA seemed of importance.

Aims of the Present Study

The primary aim of this work was to investigate further the hydroperoxide formation in DNA. Before this study commenced, the situation was uncertain both with regard to the number of different hydroperoxides formed or to their identity. As mentioned previously, an increased understanding of the primary processes occurring upon irradiation of DNA is of importance in light of possible genetic effects.

Because of the well known instability of peroxides and the complexity of the DNA reaction mixture, identification of the hydroperoxides by conventional separation techniques such as chromotography did not seem feasible. Therefore, it was decided to characterize the hydroperoxides through their kinetics and energetics. As kinetic rate constants do not necessarily provide a unique identification, a knowledge of the energetics was deemed desirable.

Since hydroperoxide formation has only been reported for the pyrimidines and their related nucleotides, and not for the purines, it seemed likely that this second hydroperoxide in DNA might be associated with the cytosine component. However, as no concrete

evidence for the existence of cytosine hydroperoxide has been reported, the investigation of its formation and stability in aqueous solution was a necessary part of this research.

EXPERIMENTAL METHODS

Apparatus and Materials

Cobalt Source

The ionizing radiations used in all experiments were Co-60 γ-rays (1.17 and 1.33 Mev). The irradiator contains a 3600 curie source which is separated from the sample chamber by a lead-concrete shield. The source consists of 12 rods, each containing approximately 300 curies of Co-60 encased in stainless steel capsules. When the shield is drawn back, the rods, which are in a circular arrangement, can be moved upward mechanically to surround the stainless steel irradiation chamber. The irradiation chamber is a cylinder 10-1/4" in diameter and 5" high with a 5" x 5" circular well in the bottom.

Irradiation Vessels

Solutions were irradiated in specially designed cylindrical glass cells terminating at one end in 1-1/2" of capillary tubing and at the other end in a capped ground glass joint. A diagram of an irradiation cell is shown in Figure 1a. Each cell contained 28 ± 1 ml of solution. Initially the cells were cleaned chemically, filled with triply distilled water (see below), and irradiated for eight hours to remove any

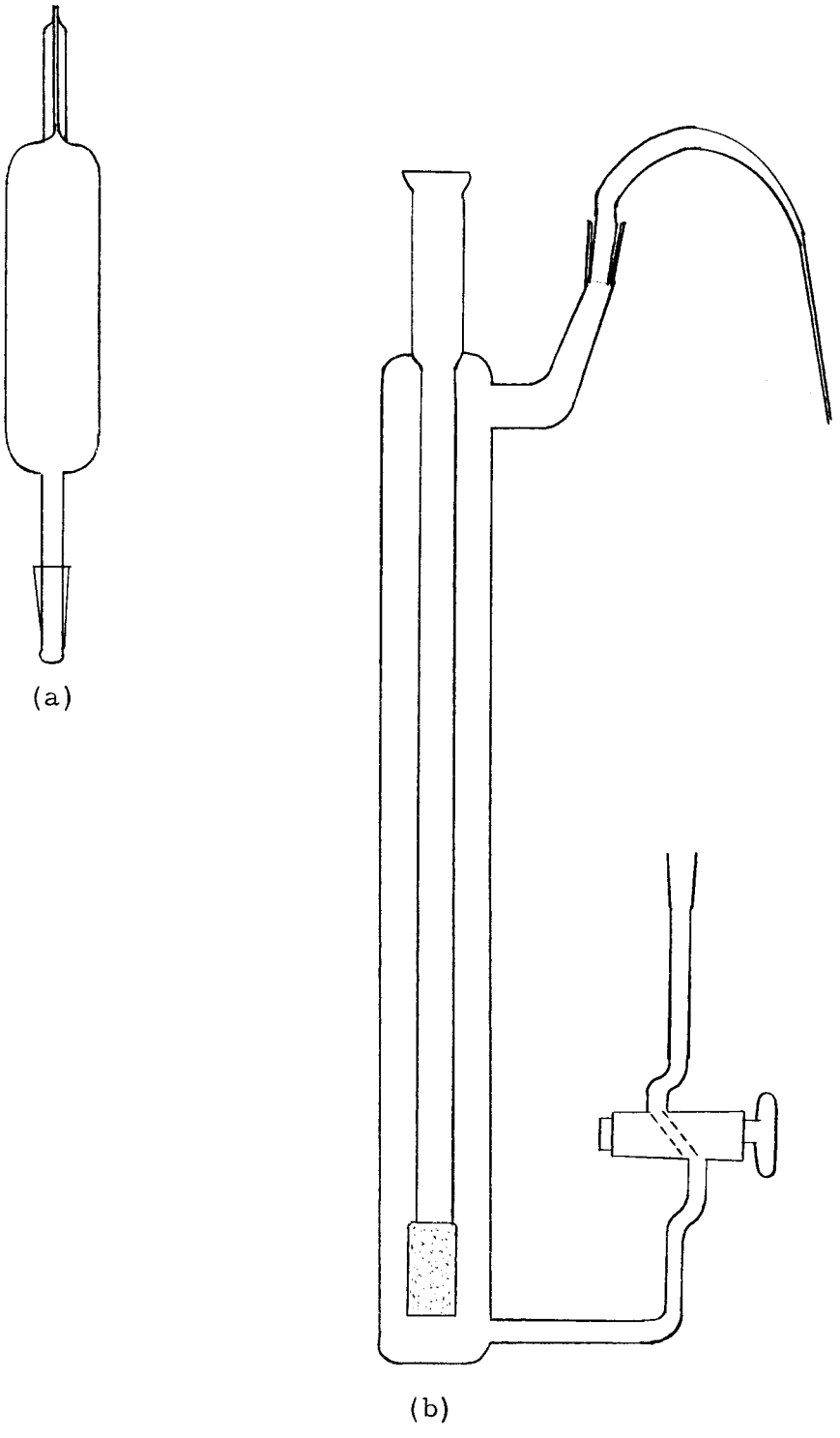

Figure 1. (a) Irradiation cell. (b) Gas bubbler for oxygen saturating solutions and filling cells.

radical scavengers adhering to the glass. During regular use, the cells were cleaned by connecting to an aspirator and drawing through triply distilled water. In order to fill the cell with the solution to be irradiated, it was fitted into a ground glass joint on the gas bubbling apparatus (see Figure 1b) and the solution was forced into the cell by the gas pressure.

When being irradiated, the cells were placed on a stand which fit into the 5" x 5" well in the Co-60 irradiator. There were four different cell positions on the stand, and each cell was numbered and always placed in the same stand position for irradiation. This was necessary to obtain dose reproducibility since the sample chamber could not be rotated and, therefore, a uniform flux from all directions was not assured.

Dosimetry

The dose rate of the irradiator was determined by using the Fricke Dosimeter, which consists of an aerated 10^{-3}M solution of ferrous ammonium sulfate, 10^{-3}M in sodium chloride and 0.8 N in sulfuric acid. The cells were filled with the ferrous solution, irradiated, and the concentration of ferric ions determined by measuring the optical density at 304 mμ. This procedure was repeated for several doses and a linear plot of $[Fe^{+3}]$ against time of irradiation made. The dose rate was then calculated from the slope of the

graph and the known G value (15.8 ± 0.3) for the Fricke solution (45). The dose rate was determined for each of the cells in their respective positions. Since the rods had to be raised by mechanical means, it was necessary to use precise timing for this procedure in order to obtain a reproducible dose. This was accomplished by cranking the rods up and down to a metronome set at 120 beats per minute. The same number of turns (one to a beat) was made each time, thus making the procedure completely reproducible.

The decay of the source with time was calculated from the half-life of Co-60. The dose rates obtained for the four positions are given below.

Position	Dose for Rod Advance-Withdrawal (ev/l)	Dose Rate (ev/l min)
1	9.5×10^{19}	3.13×10^{20}
2	3.39×10^{19}	1.85×10^{20}
3	2.81×10^{19}	1.03×10^{20}
4	1.75×10^{19}	0.58×10^{20}

Oxygenation of Solutions

Most of the solutions used for the experiments were saturated with oxygen before irradiation. Bottled compressed oxygen was first bubbled through concentrated sulfuric acid to remove any organic impurities, then passed through triply distilled water (see below) to remove any traces of the sulfuric acid and finally bubbled for at least

15 minutes through the solution to be irradiated. The entire glass bubbling apparatus was connected by grease-free ground glass ball joints. Cells were filled directly from the gas bubbler to avoid any oxygen escape.

Cleaning of Glassware

All of the glassware was initially cleaned with acid dichromate cleaning solution to remove any organic matter and then rinsed with triply distilled water (see below). During regular use the glassware used with organic solutions was periodically cleaned with the dichromate solution. All other glassware was kept clean by rinsing in tap distilled water followed by several rinses with triply distilled water.

Materials Used

1. The distilled tap water was first redistilled from a solution of alkaline permanganate and then from a solution of acid dichromate. This procedure was to remove any organic matter which might act as radical scavengers. This triply distilled water was used for making all solutions and for the final rinsing of glassware.

2. The cytosine, thymine, and thymidine-3', 5'-diphosphate sodium salt were Calbiochem, A grade. The cytidylic acid, deoxycytidine-5' - monophosphate, and thymidine-5'-monophosphate disodium salt were from Schwarz Bioresearch Inc. The DNA used

was highly polymerized Deoxyribosenucleic acid, Na salt (ex salmon sperm) A grade from Calbiochem.

3. All other chemicals were reagent grade and were used without further purification. Stock solutions of potassium acid phthalate (20 gm per liter), potassium iodide (66 gm KI, 0. 2 gm NaOH per liter), and potassium iodide with molybdate catalyst (0. 2 gm ammonium molybdate per liter) were made up and stored in polyethylene bottles to avoid decomposition of the solutions.

Thermostating of Solutions

The constant temperature bath consisted of a 8"x 10" cylindrical glass jar completely covered by 1" thick fiber glass insulation. The heating or cooling liquid was pumped by a Haake circulator, Model F, through a copper coil (6" diameter and 10" high) placed inside the glass jar. Holes cut in the lucite lid of the bath permitted the insertion of a stirrer, a monitoring thermometer, and a flask containing the sample. The temperature was controlled by a rotating contact magnet thermometer in the circulator. A stirrer attached to a variable speed reversible Heller motor, model 2T 60-900 was used to agitate the water in the bath. This arrangement permitted the temperature of the water bath to be controlled to an accuracy of ± 0.1°C between 12°C and 100° C.

The cell compartment of the Gilford spectrometer was also thermostated. Thermospacers containing coils for circulation of a

fluid were placed on each side of the cell compartment, and could be connected to either the Haake circulator or a Radiometer Water Thermostat, type V TS13. The temperature of the cell compartment was monitored by a platinum resistance temperature probe imbedded in the cell compartment. This probe was connected through a separate channel in the Gilford recorder and could be automatically recorded on the chart paper.

Methods of Analysis

Peroxide Measurement

Hydrogen peroxide and organic hydroperoxides will oxidize I^-. The rate of the reaction under controlled iodide ion concentration at pH = 4.0 is characteristic for a particular peroxide. Rate constants for the reaction of several different peroxides with I^- at pH = 4.0 and 25°C are given below (65).

Compound	Rate Constant ($M^{-1} sec^{-1}$)
4-hydroxy-5-hydroperoxy thymine	1.60
4-hydroperoxy-5-hydroxy thymine	7.53
hydroperoxide from irradiated thymine solutions	1.59
hydroperoxide from irradiated uracil solutions	1.57 and 6.08
hydrogen peroxide	1.13×10^{-2}

Since the rate constant for hydrogen peroxide is 100 times slower than those for the organic hydroperoxides, it becomes possible to distinguish the hydrogen peroxide from the hydroperoxides kinetically. Upon addition of iodide ion, the organic hydroperoxides react very quickly, while the hydrogen peroxide reaction is slow enough that its progress can be followed spectrophotometrically. In the presence of sufficient iodide the reaction becomes pseudo-first-order, thus simplifying the kinetic treatment. By plotting the logarithm of the concentration against time and extrapolating to zero time, the amount of hydrogen peroxide present initially is obtained. Reaction in the presence of molybdate catalyst gives the total peroxide present and by subtraction, the concentration of the organic hydroperoxide is determined.

Because the determination requires extrapolation to zero time, obtaining accuracy and reproducibility required very critical timing. The following procedure was used. Ten ml of KHP and 10 ml of KI (no catalyst) were pipetted into a 25 ml volumetric flask, and 10 ml KHP and 10 ml KI with molybdate catalyst pipetted in a second flask. Then, as quickly as possible, since the catalysed KI autoxidises slowly at pH 4, either 3 or 5 ml of the solution to be analyzed was added to the flask containing the catalyst. Exactly one minute later, 3 or 5 ml of the irradiated solution was pipetted into the flask containing no catalyst and zero time was taken to be the time when this

solution was first released from the pipette. A stopwatch reading in seconds was used for all timing. The reaction was followed at 352 mμ in the thermostated (25° C) cell compartment of a Gilford recording spectrophotometer, model 2000. Cell positions were automatically changed and the optical density automatically recorded on a chart recorder to a preset timing sequence. A sample spectrum from the recorder is shown in Figure 2. The chart recorder was calibrated for full scale readings by means of an internal standard in the Gilford. The extinction coefficient of I_3^- at 352 mμ, determined by using solutions of hydrogen peroxide standardized against ceric, was 25,500 $M^{-1} cm^{-1}$. The concentration of peroxide in the original sample was calculated using the relation

concentration (μ moles/liter) = absorbance x 40.8 x dilution.

Example calculation: Figure 3 shows a plot of log $(A_\infty - A)$ versus time for a sample of irradiated DNA measured at 31 minutes after irradiation. (A is used to denote absorbance.) Extrapolating to zero time gives

$$A_{H_2O_2} = .204$$

$$[H_2O_2] = .204 \times 40.8 \times 23/3 = 6.38 \times 10^{-6} M$$

$$A_{\text{total peroxide}} = A_\infty = .310$$

Figure 2. Gilford spectrum for DNA.

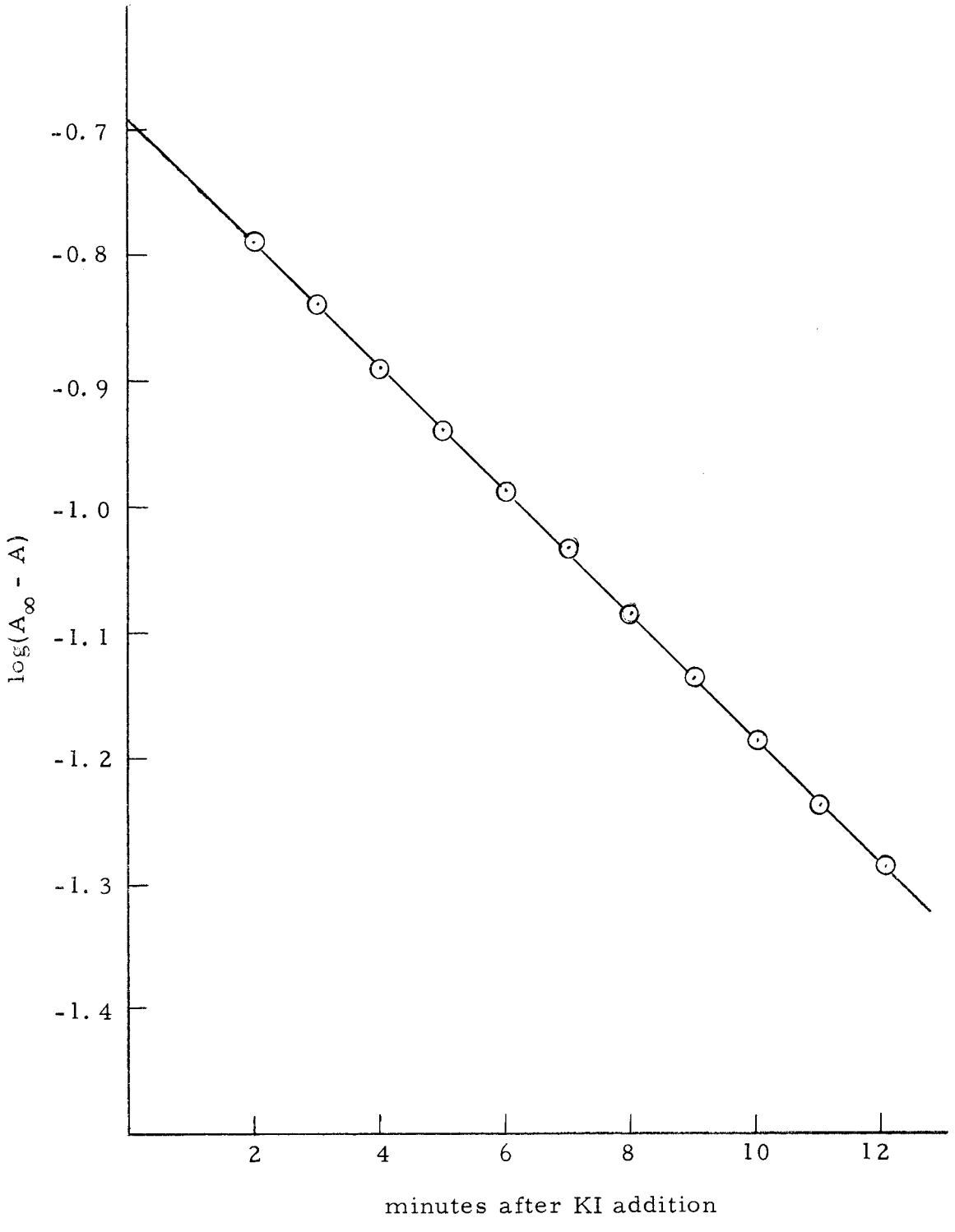

Figure 3. First order kinetic plot for a 0.1 percent DNA solution measured 31 minutes after irradiation.

$$A_{\text{hydroperoxide}} = A_{\infty} - A_{H_2O_2} = .310 - .204 = .106$$

$$[\text{hydroperoxide}] = .106 \times 40.8 \times 23/3 = 3.32 \times 10^{-6} M.$$

Measurement of DNA Composition and Concentration

The determination of the concentration, A-T fraction, and fraction remaining in native form for a sample of DNA was made by the spectral method of Hirschman and Felsenfeld (34). Optical measurements were done on a Zeiss PMQ 11 spectrophotometer. The DNA sample was heated to 100°C for approximately ten minutes. The absorbance at 250, 260, 270, and 280 mμ was read and corrected for thermal expansion. The solution was then cooled and the absorbance at the four wavelengths measured again. The concentration C of DNA in the sample and the A-T fraction ϕ can then be calculated from the high temperature spectrum using the formulas:

$$C = \mu_2 L_1 + \mu_3 L_2$$

$$C\phi = \mu_2 L_3 + \mu_3 L_1$$

where

$$\mu_2 = \Sigma A_i B_i$$

and

$$\mu_3 = \Sigma A_i \gamma_i .$$

A_i is the absorbance at wavelength i, and B_i, γ_i, L_1, L_2, and L_3 are

parameters determined by Hirschman and Felsenfeld. To get the extent of denaturation of the sample, the concentration of DNA is calculated using the hyperchromicity spectrum (difference between high temperature and re-cooled spectra). The fraction of bases remaining in helical form is then just the ratio of the concentration obtained from the hyperchromicity spectrum to the concentration obtained from the high temperature spectrum.

The amount of base destruction upon irradiation was determined by measuring the optical density (at natural pH) at 260 mμ as a function of dose and calculating the G value from the slope where the optical density became a linear function of dose. Absorbance measurements were made using 7.0×10^{-4} M DNA as a reference solution.

Measurement of DNA Melting Curves

The melting temperature, T_M, for both irradiated and non-irradiated samples of DNA was measured in the thermostated cell compartment of the Gilford. The sample to be measured was diluted to a suitable concentration, usually 1 ml to 25 ml, and the absorbance at 260 mμ and 25°C measured. The temperature was then raised and the sample allowed to equilibrate for approximately ten minutes after the cell compartment reached the desired temperature. A plot was made of the absorbance relative to the absorbance at

25° C versus the temperature and the T_M taken as the temperature at which the absorbance was midway between the constant value at low temperature and the constant value reached at high temperatures.

Measurement of Cytosine Destruction

The loss of cytosine upon irradiation was determined spectrophotometrically at 275 mμ in the Zeiss PMQ11 spectrophotometer. The measured extinction coefficient at pH 2 was 9750.

RESULTS

Characterization by Kinetics and Energetics

Monomer Components[5]

Oxygen saturated solutions of cytosine (3×10^{-2}M) were irradiated with doses up to 7.9×10^{21} ev/liter and analyzed with iodide reagent for hydroperoxide formation. No hydroperoxide could be detected in alkaline or neutral solutions, but at pH 2 a stable hydroperoxide was found. By lowering the iodide concentration to a point where its reaction with the hydroperoxide became slow enough to follow spectrophotometrically, it was possible to measure the pseudo-first-order rate constant for reaction of the hydroperoxide with iodide. By using several iodide concentrations (summarized in Table VI), a linear plot of the rate constant as a function of iodide concentration was obtained (Figure 4) and the second-order rate constant calculated to be 1.7 $M^{-1}sec^{-1}$. This value is in the same range as those reported by Scholes and Weiss (65) for the hydroperoxides of thymine (1.54 $M^{-1}sec^{-1}$), uracil (1.57 and 6.08 $M^{-1}sec^{-1}$) and dimethyluracil (3.83 and 12.6 $M^{-1}sec^{-1}$).

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[5]Cytosine, thymine (and adenine and guanine) may be regarded as the monomer components of the co-polymer DNA.

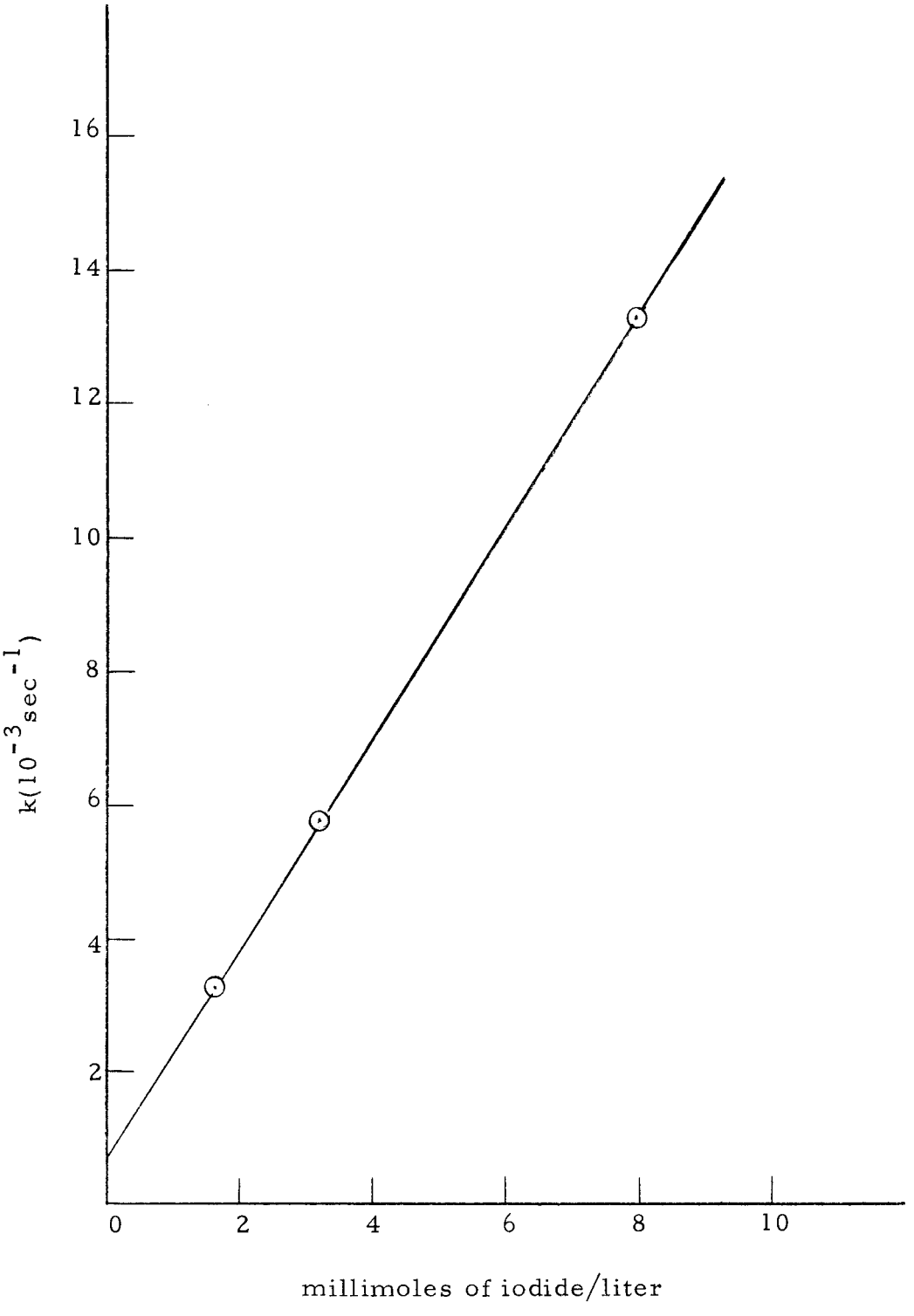


Figure 4. Dependence of first-order rate constant for cytosine hydroperoxide decomposition upon iodide concentration.

Table VI. Dependence of First-Order Rate Constant upon Iodide Concentration

$[I^-]$ (M)	$k(sec^{-1})$
1.59×10^{-3}	3.36×10^{-3}
3.17×10^{-3}	5.82×10^{-3}
7.96×10^{-3}	1.33×10^{-2}

The fact that no hydroperoxide was found in neutral solution could be due to either a very rapid post-irradiation decay or a change in mechanism of formation, or both. In an attempt to investigate this point, the thermal decay of the cytosine hydroperoxide at 25° C was measured over a pH range of 1.0 to 4.4. The decomposition followed first-order kinetics and the rate constant exhibited a strong dependence upon pH, increasing very rapidly around pH 4 (see Figure 5 and Table VII). Decomposition rates above pH 4.4 could not be determined because they were too fast to be accurately measured by the method of analysis being used. Consequently, a change in mechanism in neutral solution cannot be ruled out as a possibility. The optical density measurements showed that the decomposition of the hydroperoxide was paralleled by an increase in the hydrogen peroxide concentration, indicating the process to be a hydrolysis.

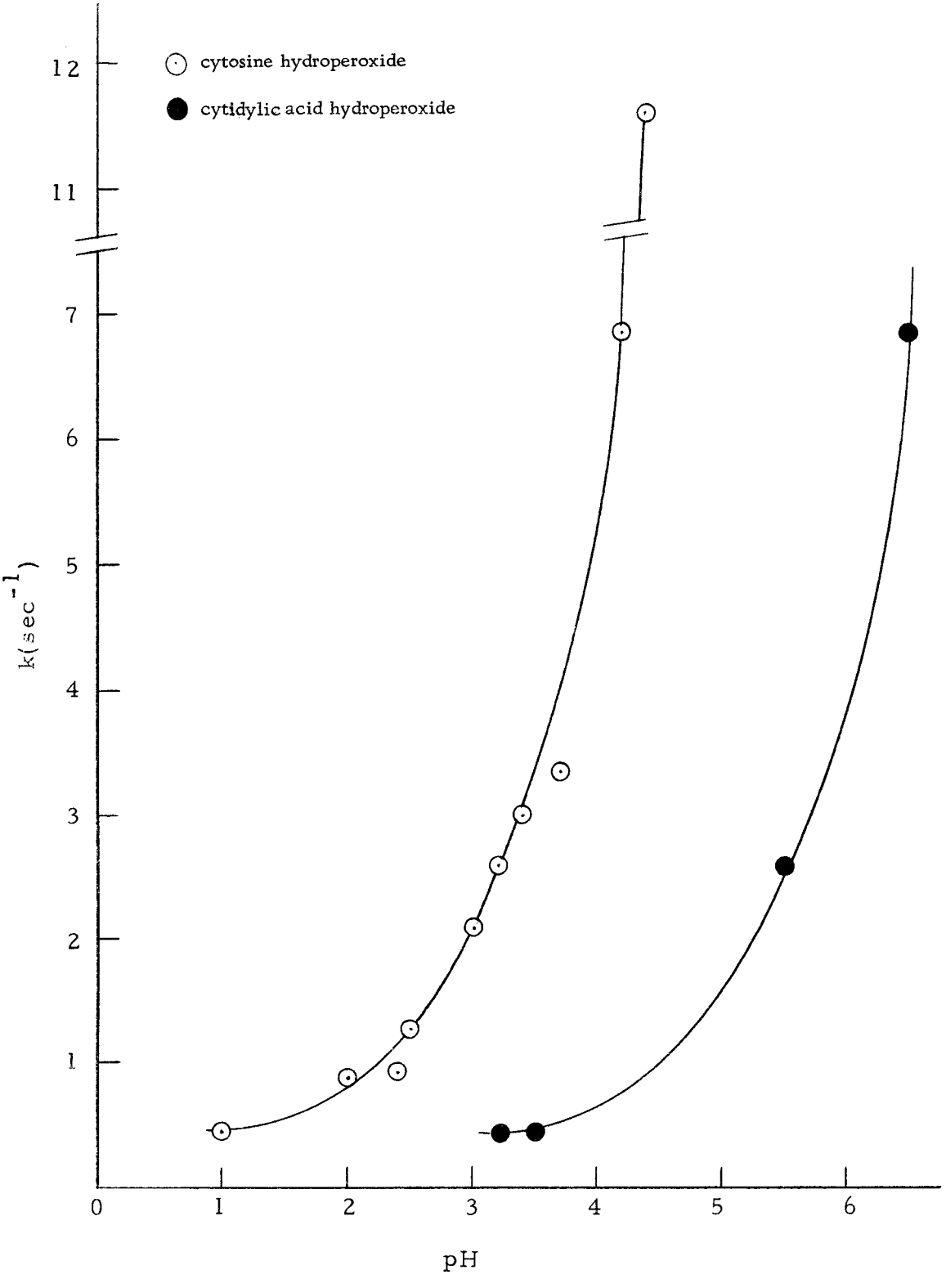


Figure 5. Rate of thermal decay at 25°C of cytosine and cytidylic acid hydroperoxides as a function of pH.

Table VII. Dependence of Cytosine Hydroperoxide Decomposition at 25°C upon pH

pH	$k(sec^{-1})$
1.0	$4.5 \pm 0.5 \times 10^{-5}$
2.0	$8.7 \pm 0.5 \times 10^{-5}$
2.4	$9.12 \pm 0.24 \times 10^{-5}$
2.5	$1.28 \pm 0.11 \times 10^{-4}$
3.0	$2.1 \pm 0.5 \times 10^{-4}$
3.2	$2.61 \pm 0.5 \times 10^{-4}$
3.4	$2.99 \pm 0.5 \times 10^{-4}$
3.7	$3.35 \pm 0.25 \times 10^{-4}$
4.19	$6.85 \pm 0.52 \times 10^{-3}$
4.39	1.16×10^{-3}

The investigation of cytosine hydroperoxide showed that the thermal decomposition in the pH region of aqueous DNA (pH$\sim$6) was much too rapid to be the fast hydroperoxide detected by Daniels et al. (18), who gave $k \sim 2 \times 10^{-4} sec^{-1}$. Because the effect of substitution at N_1 in forming nucleosides and nucleotides might greatly influence the kinetics, hydroperoxide formation was investigated in irradiated 3×10^{-3}M solutions of cytidylic acid, deoxycytidine-5'-monophosphate, and the related thymine group, thymine, thymidine-3', 5'-diphosphate, and thymidine-5'-monophosphate. The hydroperoxides of these compounds were also found to show a strong pH effect on the rate of thermal decay (see Table VIII and Figure 6). A variance of the rate constant was noted for different buffering solutions. For

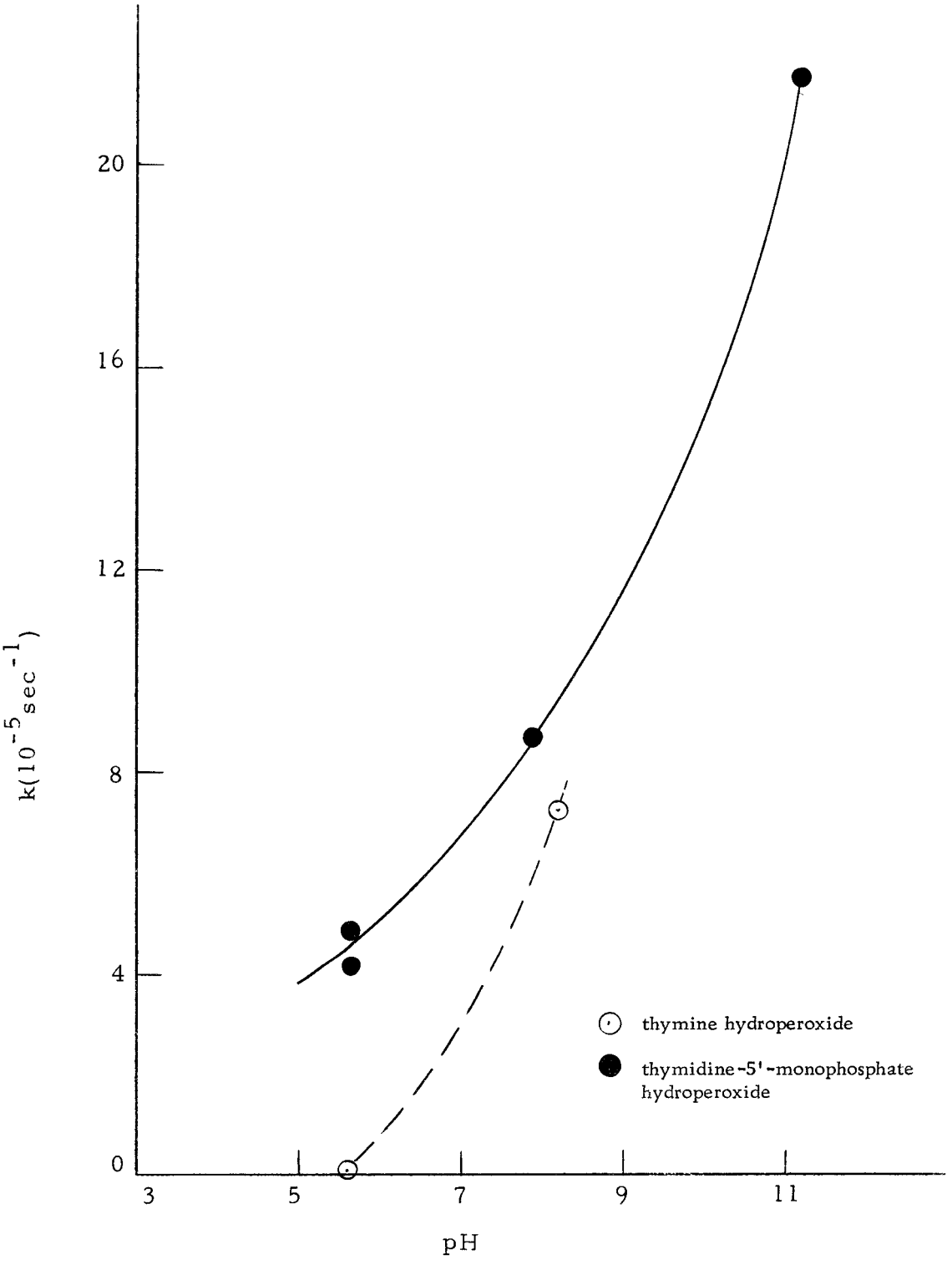


Figure 6. Rate of thermal decay at 25° C of thymine and thymidine-5'-monophosphate hydroperoxide as a function of pH.

Table VIII. Dependence of Hydroperoxide Decomposition at 25°C upon pH

Hydroperoxide from	pH	Buffering agent used	$k(sec^{-1})$
Cytidylic acid (2', 3')	3.23	---	4.27×10^{-5}
	3.52	---	4.37×10^{-5}
	5.55	NaOH	2.59×10^{-4}
	6.5	$NaH_2PO_4 + Na_2HPO_4$	6.85×10^{-4}
Deoxycytidine-5'-monophosphate	3.69	---	9.20×10^{-5}
Thymine	5.6	---	1.34×10^{-6}
	5.63	KHP, phosphate	8.40×10^{-7}
	8.2	NaOH, phosphate	7.25×10^{-5}
Thymidine-5'-monophosphate	5.5	H_2SO_4, NaOH	1.07×10^{-4}
	5.66	KHP	4.09×10^{-5}
	5.64	NaOH, phosophate	4.95×10^{-5}
	7.9	---	8.57×10^{-5}
	11.1	NaOH	2.18×10^{-4}
Thymidine-3', 5'-diphosphate	8.24	---	5.05×10^{-5}

pH greater than 8, the hydrogen peroxide concentration also decreased with time.

The temperature dependence of the rate constant for thermal decay was measured for 3×10^{-3}M solutions of cytidylic acid and thymine. Values for k at different temperatures are shown in Table IX. The pH of 5.5 was chosen to correspond to the natural pH of DNA solutions used.

Table IX. Temperature Dependence of the Thermal Decomposition of Hydroperoxides of Cytidylic Acid and Thymine (3×10^{-3}M, pH 5.5)

Hydroperoxide	Temp. °C	k(sec^{-1})
Cytidylic acid	25.0 ± 0.1	2.59×10^{-4}
	35.1 ± 0.1	4.18×10^{-4}
	47.1 ± 0.1	7.6×10^{-4}
Thymine	25.0 ± 0.1	5.1×10^{-7}
	54.1 ± 0.1	1.16×10^{-5}
	73.2 ± 0.1	8.75×10^{-5}

An Arrhenius plot of log k against 1/T was linear for both hydroperoxides (see Figure 7) in the temperature range used and gave an activation energy $\Delta E^{\ddagger}$ of 10.3 kcal for the cytidylic acid hydroperoxide and 22.3 kcal for the thymine hydroperoxide.

Deoxyribosenucleic Acid

Oxygenated solutions of 0.1 percent DNA in both water and

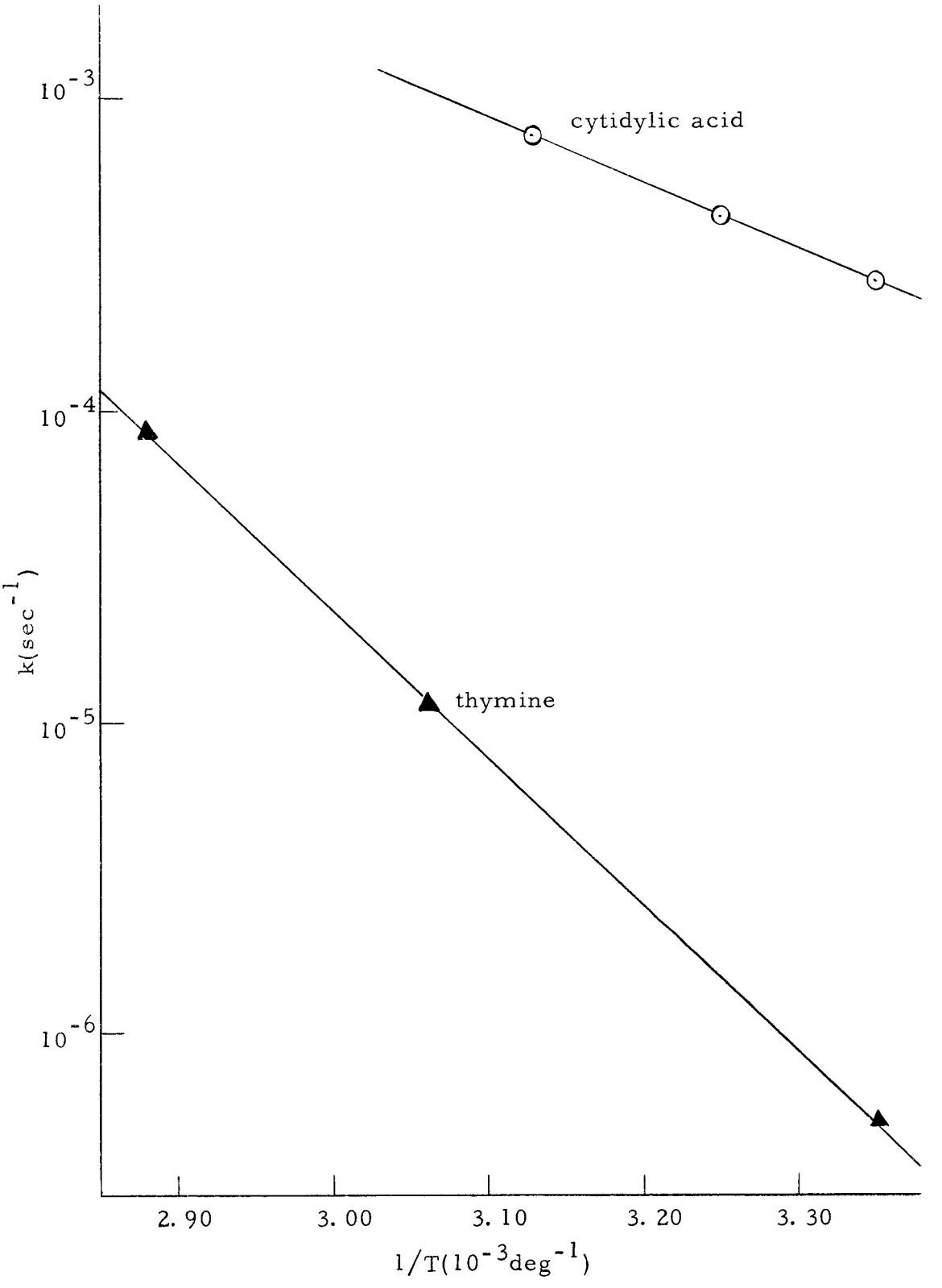


Figure 7. Arrhenius plot of rate constant for hydroperoxides of cytidylic acid and thymine.

10^{-2}M NaCl were irriadiated and the thermal decay of the hydroperoxide measured at 25°C. The first-order kinetic plot of log $(A_\infty - A)$ against time is shown in Figure 8. The curvature indicates two concurrent first-order processes are occurring. The processes were separated by extrapolating the slower component back to zero time and subtracting the slow component from the total. This gave two first-order rate constants which were of the magnitude estimated by Daniels et al. (18). The temperature dependence of the two rate constants is given in Table X and in the Arrhenius plot shown in Figure 9.

Table X. Temperature Dependence of DNA Hydroperoxide Decomposition (0.1% DNA, dose of 5.66×10^{21} ev/liter)

DNA Solution	Temp. °C	$k_{fast}(sec^{-1})$	$k_{slow}(sec^{-1})$
0.1% in H_2O	13.8 ± .2	1.44×10^{-4}	1.58×10^{-6}
	24.9 ± .1	2.98×10^{-4}	4.80×10^{-6}
	34.0 ± .1	4.02×10^{-4}	9.15×10^{-6}
	53.2 ± .1		4.35×10^{-5}
	63.7 ± .1		1.20×10^{-4}
	73.0 ± .1		2.51×10^{-4}
	80.0 ± .1		4.99×10^{-4}
0.1% in 10^{-2}M NaCl	25.0 ± .1	2.83×10^{-4}	2.95×10^{-6}
	38.2 ± .1		9.36×10^{-6}
	53.1 ± .1		6.6×10^{-5}
	65.0 ± .1		2.56×10^{-4}
	74.0 ± .1		4.97×10^{-4}
	80.0 ± .1		7.99×10^{-4}

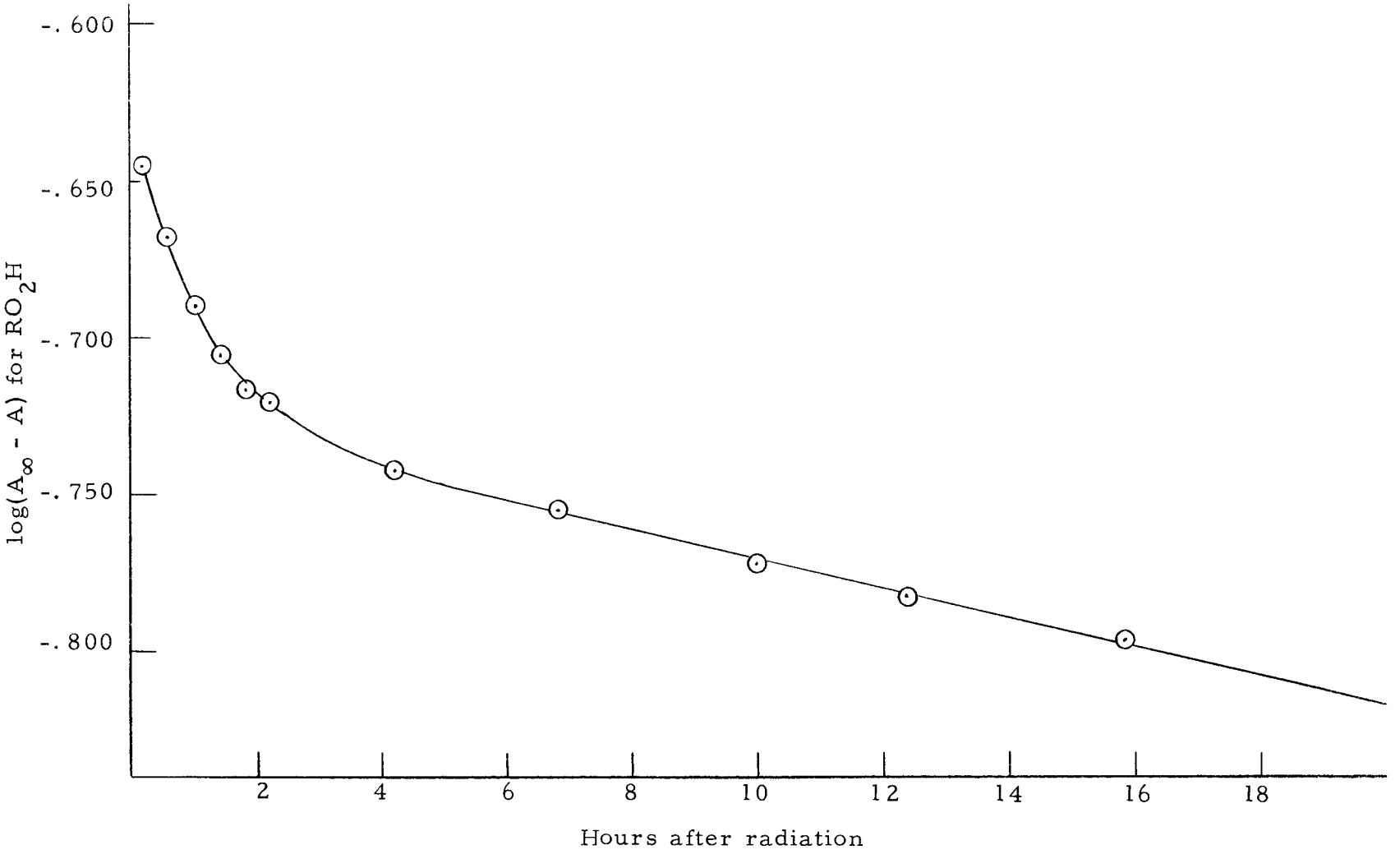


Figure 8. Thermal decomposition at 25°C of total hydroperoxide from 0.1 percent DNA in 10^{-2}M NaCl.

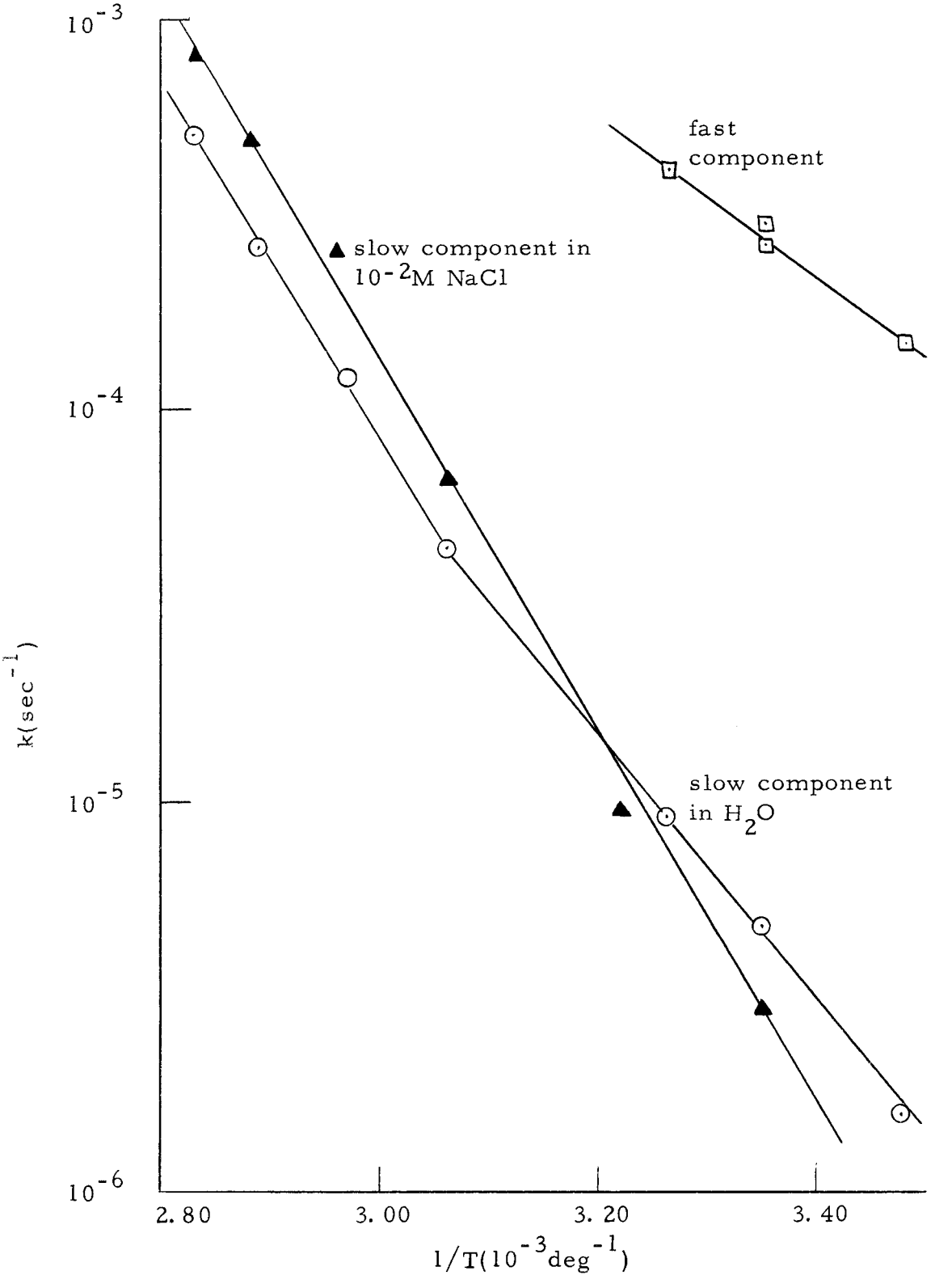


Figure 9. Arrhenius plot of rate constant for DNA hydroperoxide.

The data in Table X shows that there is a difference in the rate constant for the slow component in water and in 10^{-2} M NaCl. It was not possible to obtain the required data to determine whether this is also the case for the fast component. The data at 25°C indicates that there may be a small difference.

The Arrhenius plot (Figure 9) of log k against 1/T gave an activation energy $\Delta E^{\ddagger}$ of 10.2 kcal for the fast component. The situation for the slow component is more complicated because, a priori, the data can be analyzed in two ways. The data for DNA in 10^{-2}M NaCl gives a linear plot with $\Delta E^{\ddagger}$ of 22.1 kcal. However, the results for the DNA in water do not lie in a single straight line. One can assume two different processes, with the transition from the one at low temperatures to the one at high temperatures occurring quite sharply. On this basis, the results can be separated into two straight lines, giving $\Delta E^{\ddagger}$ of 21.5 kcal at high temperatures and 15.2 kcal at low temperatures. An alternate possibility for treating the data is to assume that two processes are occurring simultaneously, with one dominating at high temperature. Then the curve can be separated into two distinct processes by extrapolation and subtraction. When this was done by extrapolating the high temperature line back to low temperatures, an activation energy of 12.9 kcal was obtained for the process at low temperature.

Values for the entropy of activation $\Delta S^{\ddagger}$ were calculated using

the relation

$$\frac{\Delta H^{\ddagger} - \Delta F^{\ddagger}}{T} = \Delta S^{\ddagger}$$

where

$$k_{rate} = (kT/h)e^{-\Delta F^{\ddagger}/RT} .$$

The values obtained for $\Delta H^{\ddagger}$, $\Delta F^{\ddagger}$, and $\Delta S^{\ddagger}$ are summarized in Table XI. Averaging the calculated values gives a $\Delta S^{\ddagger}$ of - 43. 2 ± 0. 2 e. u. for the fast component, and - 12. 6 ± 0. 3 e. u. for the slow component in 10^{-2}M NaCl. For the slow component in water at high temperatures, $\Delta S^{\ddagger}$ is - 15. 3 ± 0. 2 e. u. and at low temperatures either - 34. 5 ± 0. 1 or - 43. 0 ± 0. 1 e. u.

By comparing the values of k and $\Delta E^{\ddagger}$ obtained for the hyperperoxides of the monomer components and those found in aqueous DNA, it can be seen that the values obtained for cytidylic acid are in very close agreement with the fast-decaying hydroperoxide of the DNA. Thus, the fast hydroperoxide can be identified with reasonable certainty with the cytosine component of the nucleic acid. Henceforth in this paper it will be designated as CO_2H.

Unambiguous identification of the slow component is not so easily obtained. A value for k comparable to that for the slow component in DNA was not found among the monomers. Thymine hydroperoxide decayed too slowly by a factor of five and the thymidine-5-monophosphate too rapidly by a factor of ten. Since no good value

Table XI. Thermodynamic Functions for the Thermal Decomposition of the DNA Hydroperoxides

DNA hydroperoxide	Temp. °K	$\Delta E^{\ddagger}$ (kcal/mole)	$\Delta H^{\ddagger}$ (kcal/mole)	$\Delta F^{\ddagger}$ (kcal/mole)	$\Delta S^{\ddagger}$ (e. u.)
fast component	287.0	10.1_5	9.5_8	21.9_4	-43.0_7
	298.1		9.5_5	22.4_1	-43.1_5
	307.2		9.5_4	22.8_9	-43.4_6
slow component in 10^{-2} M NaCl	298.2	22.1_1	21.5_1	25.1_4	-12.1_7
	311.4		21.4_9	25.5_6	-13.0_7
	326.3		21.4_6	25.5_4	-12.5_0
	338.2		21.4_3	25.8_2	-12.2_7
	347.2		21.4_2	25.8_2	-12.6_7
	353.2		21.4_0	25.9_4	-12.7_4
slow component in water	287.0	15.2_1 [a]	14.6_4	24.5_5	-34.5_3
	298.1		14.6_1	24.8_5	-34.3_5
	307.2		14.6_0	25.2_2	-34.5_7
	326.4		14.5_6	25.8_2	-34.5_0
	326.4	21.5_2	20.8_7	25.8_2	-15.1_7
	336.9		20.8_5	25.9_9	-15.2_6
	346.2		20.8_3	26.1_9	-15.4_8
	353.2		20.8_1	26.2_7	-15.4_6
	287.0	12.9_3 [b]	12.3_6	24.6_8	-42.9_3
	298.1		12.3_3	25.1_1	-42.8_8
	307.2		12.3_2	25.5_7	-43.1_4

[a] Assuming two different processes, each in different temperature range.

[b] Assuming two simultaneous processes at lower temperature.

for k in the pH range 5.5 - 6.5 was obtained for thymidine-3,5-diphosphate, this monomer cannot be ruled out as a possibility. Cost of obtaining the compound prohibited further investigation. However, comparison of the activation energies would seem to resolve this. The $\Delta E^{\ddagger}$ of 22.3 kcal obtained for thymine hydroperoxide is in very good agreement with the activation energy of the slow component in DNA both in sodium chloride and in water at high temperatures. In light of this, and the fact that no isomers of cytosine hydroperoxide were found in neutral solution, it seems justified to assert that the slow hydroperoxide in DNA can be identified with the thymine component. Further references to this hydroperoxide will be as TO_2H.

Aspects of Radiation Chemistry

Cytosine

G values were determined for the hydroperoxide formation in acid solutions of cytosine since this is the pH range where the hydroperoxide is stable. The dependence of the G values upon oxygen and cytosine concentration are summarized in Table XII. Values designated by an * are initial G's determined by plotting the concentration formed as a function of dose and taking the G value as the slope where the relationship is linear. In other words, initial G values

are independent of dose.

Table XII. Efficiency of Cytosine Hydroperoxide Formation upon Co-60 γ-Radiolysis of Aqueous Cytosine (pH 2, dose 3.23×10^{20} ev/l min)

Cytosine (M)	O_2	$G(H_2O_2)$	$G(CO_2H)$	G(-C)
3×10^{-2}	air equib.	1.22	0.11	
	1 atm.	2.88*	0.58*	3.2*
3×10^{-3}	air equil.	1.28	.10	
	1 atm.	2.58*	.52	3.3*
6×10^{-4}	air equil.			3.2*
	1 atm.			3.2*
1.2×10^{-4}				2.9*
6×10^{-5}				2.8*

The cytosine hydroperoxide formation seems to be quite dependent upon oxygen concentration, but rather insensitive to the cytosine concentration. The magnitude of the $G(CO_2H)$ indicates that the hydroperoxide is an important primary product of the radiolysis. The G value of 3.2 obtained for the loss of cytosine is higher than that of 2.05 - 2.28 reported by Scholes (68) for 200 kev X-rays, but his value was measured at pH 5.2 as compared to the pH 2 used here. The loss of cytosine was found to be independent of oxygen concentration. The other primary products of the radiation were not identified and this important work still remains to be done.

Other Monomer Components

Since the radiation chemistry of thymine is reasonably well summarized in the literature (65) no further investigation was made by this researcher.

The magnitude of hydroperoxide formation is reduced in the presence of sugar components. The measured G for hydroperoxide formation in oxygen-saturated cytidylic acid solution (3×10^{-3}M) at pH 5.5 was 0.17 and for thymidine-5-monophosphate was 0.36.

DNA

The hydroperoxide formation in DNA was studied as a function of dose, DNA concentration, and DNA structure. Table XIII summarizes the results. All values are initial G values. Figure 10 shows the dose dependence of the total hydroperoxide formation for three different DNA concentrations. Figures 11 and 12 show the dose dependence of the TO_2H and CO_2H formation in 0.1 percent DNA in water and in 10^{-2}M NaCl. The total hydroperoxide formation and the loss of DNA increase with increasing DNA concentration while the amount of hydrogen peroxide formed decreases. The total hydroperoxide formation is approximately twice that reported by Scholes, Ward, and Weiss (65) for 200 kev X-rays. This difference cannot be completely accounted for, even assuming that they failed to measure

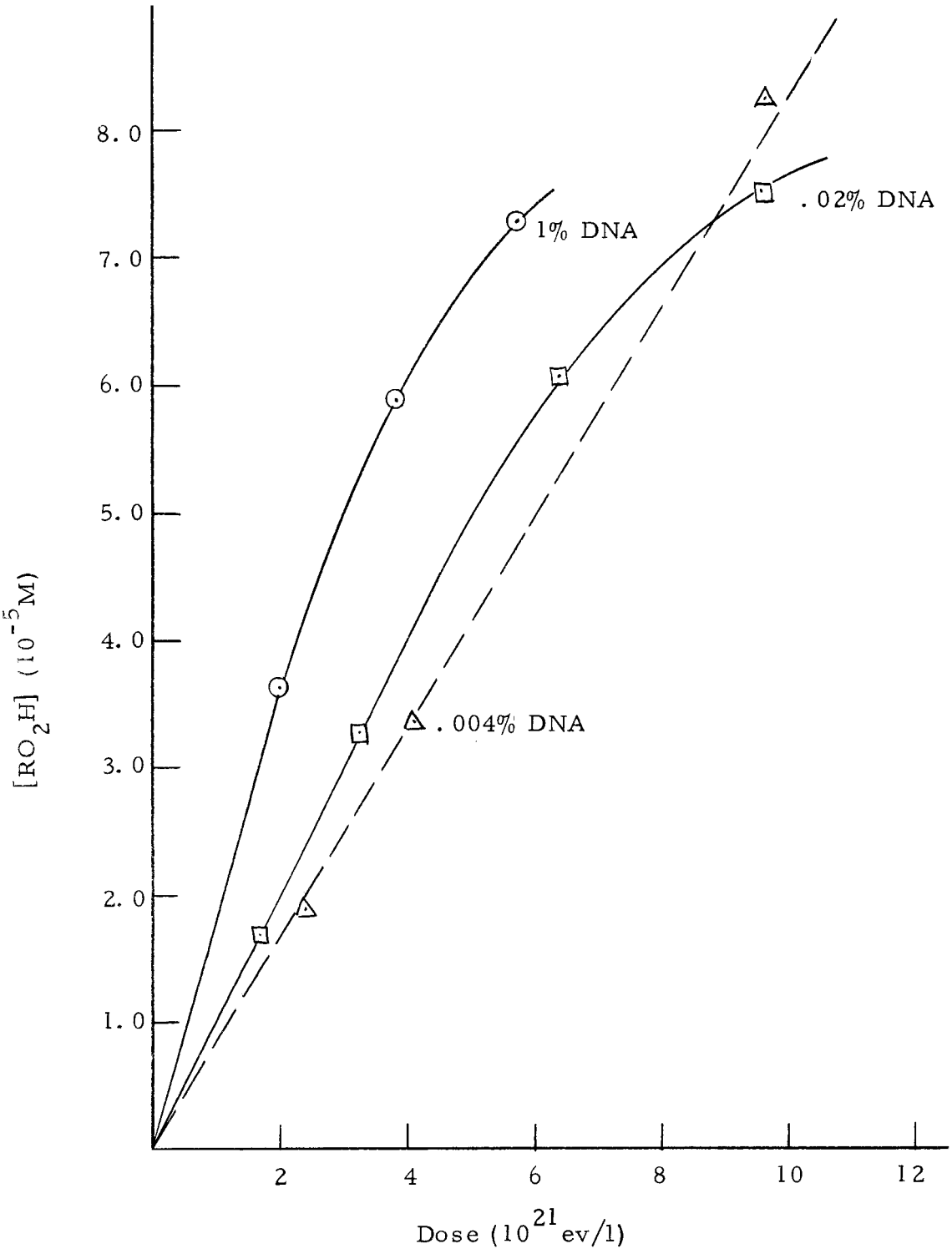


Figure 10. Dependence of DNA total hydroperoxide formation upon dose. (For .004% DNA scale is 10^{-6}M, 10^{20} ev/l.)

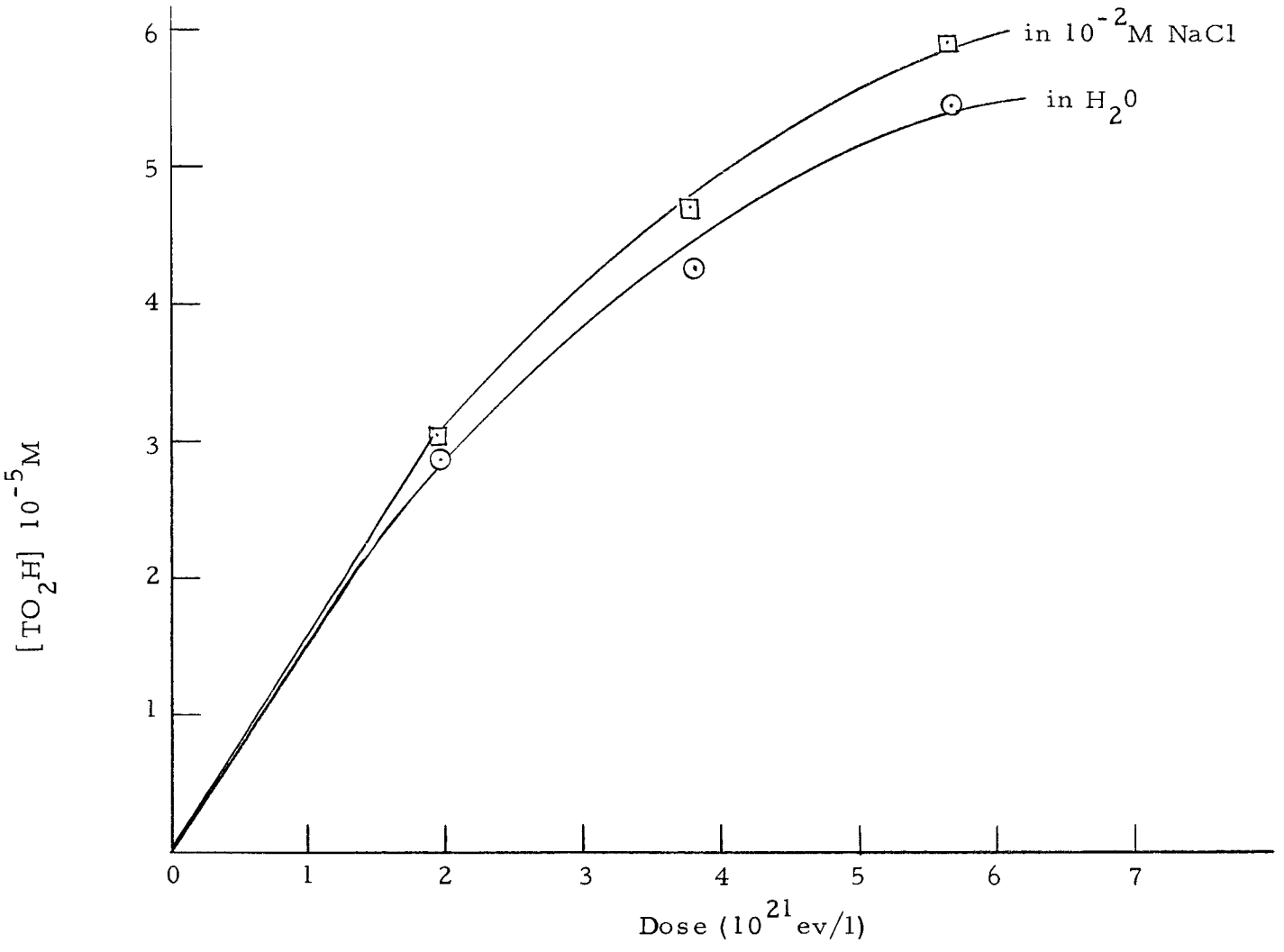


Figure 11. Dependence of TO_2H formation in 0.1% DNA upon dose.

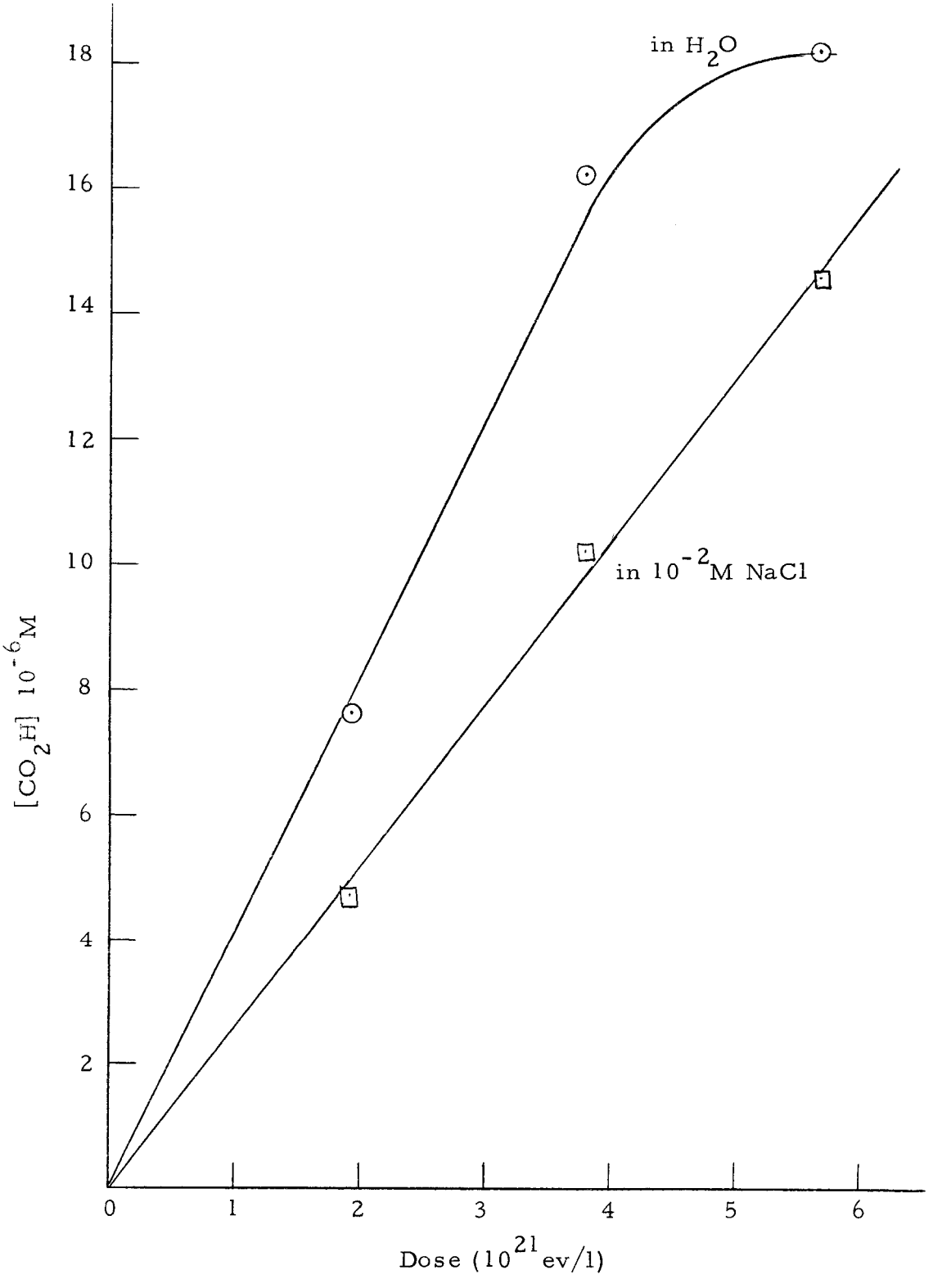


Figure 12. Dependence of CO_2H formation in 0. 1 percent DNA dose.

the fast hydroperoxide.

Table XIII. Efficiency of DNA Hydroperoxide Formation upon Co-60 γ-Radiolysis at 25° C

[DNA] (wt%)	$G(TO_2H)$	$G(CO_2H)$	$G(BO_2H)^*$	$G(H_2O_2)$	G(-DNA)
0.1% in water	0.89	0.25	1.14	1.98	1.58
0.1% in 10^{-2}M NaCl	0.95	0.16	1.11	1.80	
0.02% in water			0.61	2.18	1.48
0.004% in water			0.52	2.52	1.45

*B denotes base = T + C.

Factors Affecting the Decay of the Hydroperoxides in DNA

Structure of the DNA

The presently accepted model for DNA structure in its native state is that of two antiparallel chains interwoven in a double helix of planar bases stacked about a common axis. The denaturation of DNA comes as a result of the collapse of this uniquely ordered structure. Presumably, complete denaturation would result in two separated complementary strands of the helix. Partial denaturation indicates a DNA structure with some helical regions and some separated regions. A large variety of biological and physical methods have been used to study this denaturation process and a fairly comprehensive review on this subject has been written by Marmur, Rownd,

and Schildkraut (53). One of the common methods employs spectrophotometry and the hyperchromic effect. For this present investigation the optical method described by Hirschman and Felsenfeld (34) and the measurement of melting curves and T_M used by Marmur and Doty (52) have been used. The T_M is that temperature at which half of the increase in optical density observed upon addition of acid, base, or heat has been reached.

Figure 13 shows melting curves measured for 0.1 percent DNA solutions in 10^{-2}M NaCl. Even after 30 minutes irradiation there is still an easily measurable amount of hyperchromicity left. The data shows that the T_M has shifted from $\sim$ 64°C for the unirradiated solution to $\sim$ 49°C for a solution irradiated for 30 minutes (dose 5.66×10^{21} ev/l). Calculations employing the Hirschman and Felsenfeld method indicated that the unirradiated solution was more than 60 percent in the helical form and that after being irradiated for 30 minutes, more than 25 percent remained in the helical form. In order to determine whether the rate of thermal decomposition of the DNA hydroperoxides depended on the amount of structure of the DNA remaining in the helical form, a solution of 0.1 percent DNA in 10^{-2}M NaCl was deliberately denatured by heating to 100°C before irradiation. Optical density measurements after irradiation showed no increase upon further heating, indicating complete denaturation. The rate constants for the decomposition of the DNA hydroperoxides

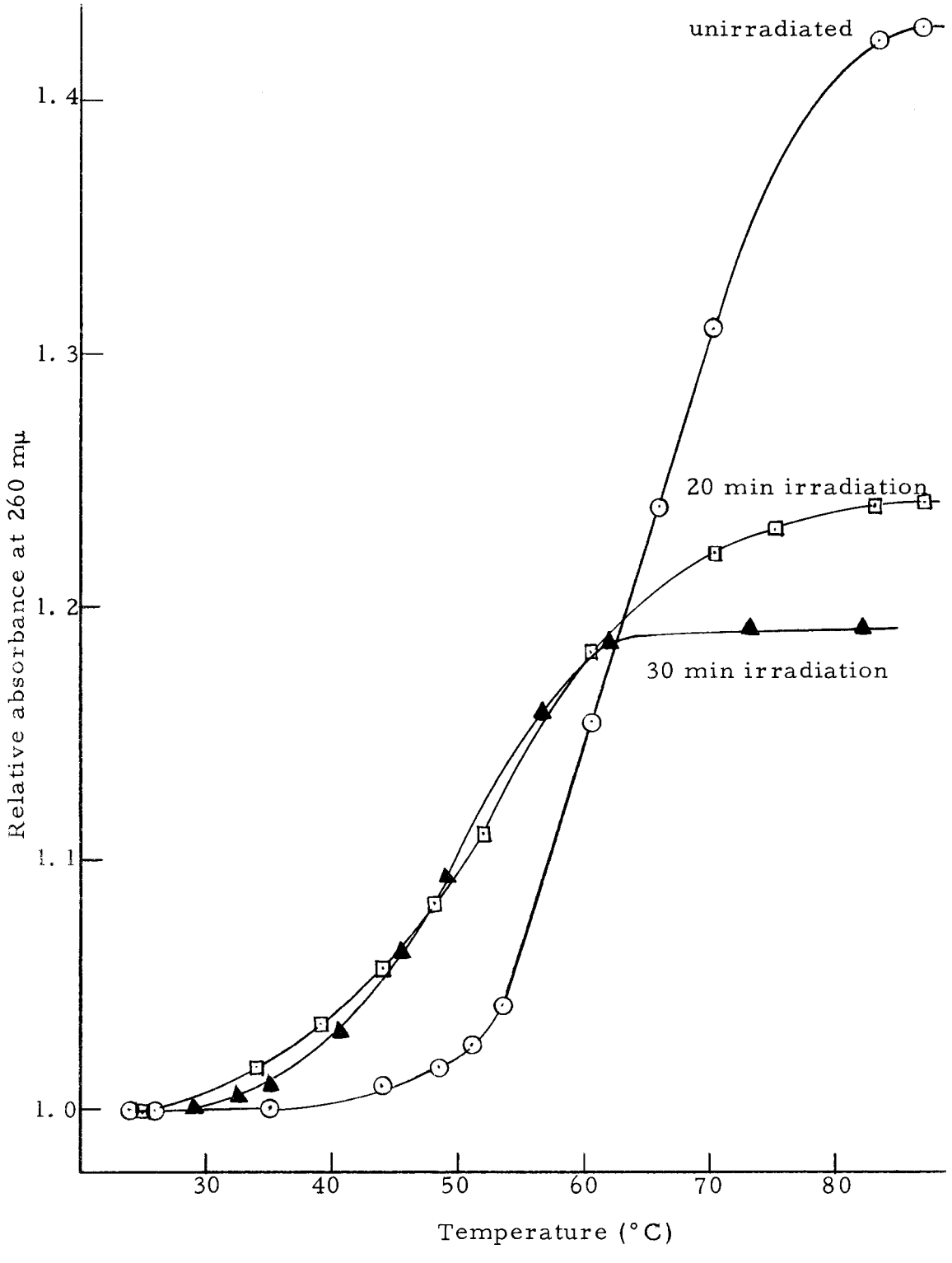


Figure 13. Melting curves for 0. 1 percent DNA in 10^{-2}M NaCl.

at 25°C were then measured and compared with those obtained for solutions with more than 25 percent of the structure still intact. No significant difference was observed in either the rate constant for TO_2H or that for CO_2H. In addition, the melting curves show that the amount of intact DNA structure remaining depends upon dose. However, no significant change in rate constants was observed with increasing dose. Thus the data indicates fairly conclusively that for the DNA in sodium chloride, the rate of decay of the DNA hydroperoxides are independent of the DNA helical structure itself.

When preparing the DNA solutions in water no particular care was taken to keep the solutions cold in order to preserve structure. Attempts to measure melting curves for these aqueous solutions containing no added electrolyte yielded no increase in optical density and thus indicated practically complete denaturation had occurred. However, in order to decrease the optical density at 260 mμ to an amount which could be read in the spectrophotometers, the 0.1 percent DNA solutions had to be diluted from 1 to 25 ml. Therefore, it is very possible that any native structure present in the 0.1 percent solutions used for rate constant measurements may have been destroyed by this further dilution.

Ionic Effects

To account for the difference between the rate of decay of the

TO_2H in water and in 10^{-2}M NaCl, the possibility of a strong ionic strength effect was considered. In order to investigate this point, solutions of different electrolytes, all having the same ionic strength of 0.01, were added to the irradiated solution and the rate of decomposition at 80°C measured. Experiments with 10^{-2}M NaCl showed that the rate constant at 80°C was not dependent on whether the electrolyte was added before or after the irradiation. Therefore, for experimental convenience, the electrolyte in these measurements was added after irradiation and before equilibrating at 80°C. The rate constants for the different electrolytes are summarized in Table XIV. There is a change of nearly 900 percent in the magnitude of the observed rate constants.

Table XIV. Influence of Inert Solutes (10^{-2}M) on Hydroperoxide Decay Kinetics at 80°C

Solute	$k(sec^{-1})$
No salt added $[Na^+]$ 10^{-3}M*	4.99×10^{-4}
NaF	4.0×10^{-4}
NaCl	7.99×10^{-4}
$NaClO_4$	6.85×10^{-4}
$NaNO_3$	7.59×10^{-4}
Na_2SO_4 (10^{-2}N)	6.08×10^{-4}
NH_4F	4.0×10^{-4}
NH_4Cl	6.22×10^{-4}
LiCl	6.1×10^{-4}
CsCl	$> 9 \times 10^{-4}$
Urea	1.4×10^{-3}

*The sodium salt of DNA was used; this concentration represents the maximum which could be available to the solution, neglecting counter-ion binding effects.

This large range of rate constants, observed using different elctrolytes of the same ionic strength, indicates that a strong specific ion effect is occurring in addition to whatever simple ionic strength effect may be present. As the discussion later in the thesis indicates, this may be due to an effect of the ions on the water structure. In order to test whether the observed rate change upon addition of electrolytes was due to the electrostatic effect of the ions, the non-electrolyte urea (10^{-2}M) was added to the irradiated solution. The observed increase in the rate constant (see Table XIV) indicates that the effect is not primarily electrostatic in nature.

DISCUSSION

In this section, the implications of the experimental results are considered in a discussion of the following points:

1. The significance of the activation energies and entropies in relation to hydroperoxide decomposition mechanism and the possible effects of the monomer structure on the decomposition rate of the hydroperoxide.
2. Certain aspects of the radiation chemistry of cytosine with respect to the mechanism of the hydroperoxide formation.
3. The radiation chemistry of DNA.
4. The effect of added solutes on DNA and water structure and the relationship of this structure to the rate of decomposition of the DNA thymine hydroperoxide.

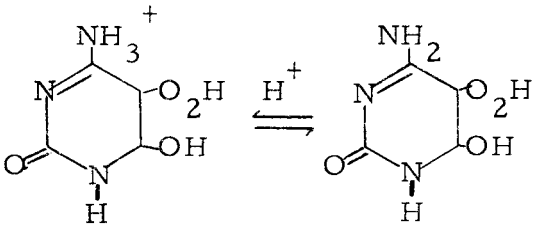
Contributions to Hydroperoxide Decomposition Mechanism

The experimental results for the hydroperoxides of cytosine and cytidylic acid indicated that their thermal decomposition was a hydrolysis reaction. The low activation energies (15 to 22 kcal) and entropies (-34 to -13 e. u.) found for the hydroperoxide decompositions suggest a non-radical polar type mechanism for the hydrolysis. The dissociation energy of the oxygen-oxygen single bond is about 35 kcal.

However, in an S_N2 type reaction, a new bond forms simultaneously with the breaking of the old bond, and therefore, lower activation energies can be accommodated. The negative entropy of activation indicates a bimolecular rate-determining step which has a specific orientation in the activated complex of the transition state. This aspect will be discussed further in connection with the DNA. Edwards (23, p. 71), has pointed out that for all cases in which quantitative data have been obtained for non-radical peroxide reactions, the values for the entropy of activation are negative and lie in the range from -10 to -35 e. u. This is precisely the range found for the activation entropies here.

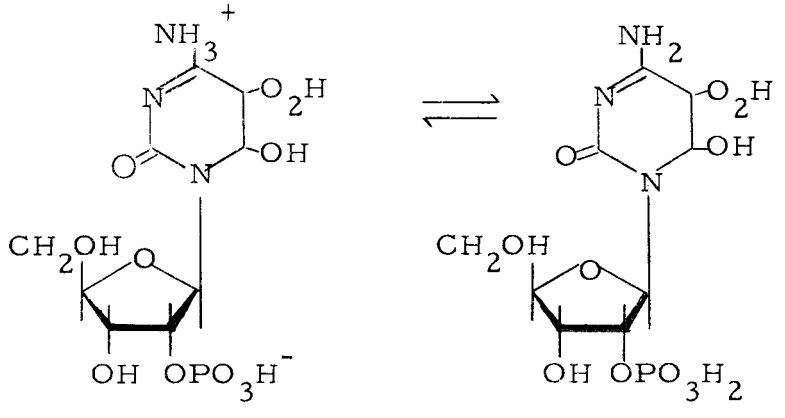
The strong dependence of the hydroperoxide stability upon pH can be discussed in terms of structure. For the hydroperoxides of both cytosine and cytidylic acid, the pH region of very rapid increase in the decomposition rate is close to the pK of the monomer base. The pK of cytosine is 4. 6, and for cytidylic acid is 4. 3 (40, p. 135, 137). Therefore, it seems reasonable to assume that an acid-base equilibrium is involved.

The structure of the hydroperoxide formed in irradiated thymine solutions has been shown to be that of 4-hydroxy-5-hydroperoxy thymine (24). Assuming the same orientation at the 4 and 5 carbons for cytosine hydroperoxide, the following equilibrium can be written:



As the acid form is the more stable of the two with regard to hydroperoxide decomposition, the implication is that the extra proton on the amino group destabilizes the transition state, perhaps through hydrogen bonding.

With cytidylic acid, the effect of the added sugar and phosphate components must be considered. Jordon (39) has calculated that in aqueous solution, cytidylic acid exists almost entirely in the zwitterion form with a cationic amino and the anionic phosphate. The following equilibrium can be written:



The consequence of the introduction of the phosphate group is that the acid strength of the amino has been weakened. Therefore, the protonated form will exist at higher pH values than it will in cytosine. In comparison with cytosine, where the unprotonated structure leads

to a faster hydroperoxide decomposition at a given pH, the nucleotide hydroperoxide should be the more stable of the two, as is observed.

In the case of cytosine, assuming that the hydroperoxide undergoes this acid-base ionization with the acid ($H^+ - CO_2H$) and neutral (CO_2H) forms hydrolyzing at different rates, the following mechanism can be written:

$$H^+ - CO_2H + H_2O \xrightarrow{k_1} H^+ - COH + H_2O_2$$

$$K \downarrow\uparrow H^+$$

$$CO_2H + H_2O \xrightarrow{k_2} C - OH + H_2O_2$$

The fact that the hydroperoxide is more stable in acid solution than in neutral eliminates the possibility of acid catalysis. Base catalysis was not considered, as all the observed rate constants were measured in solutions of pH less than 4.4 where the hydroxyl ion concentration is very small. The above mechanism leads to the relation

$$\frac{1}{k_{obs} - k_1} = \frac{1}{k_2 - k_1} + \frac{[H^+]}{(k_2 - k_1)K}$$

A plot of $\frac{1}{k_{obs} - k_1}$ against $[H^+]$ should give a straight line. By treating k_1 as an adjustable parameter, a good fit was obtained for $k_1 = 0.73 \times 10^{-4} sec^{-1}$ (see Figure 14). This gave $k_2 = 7 \times 10^{-4} sec^{-1}$

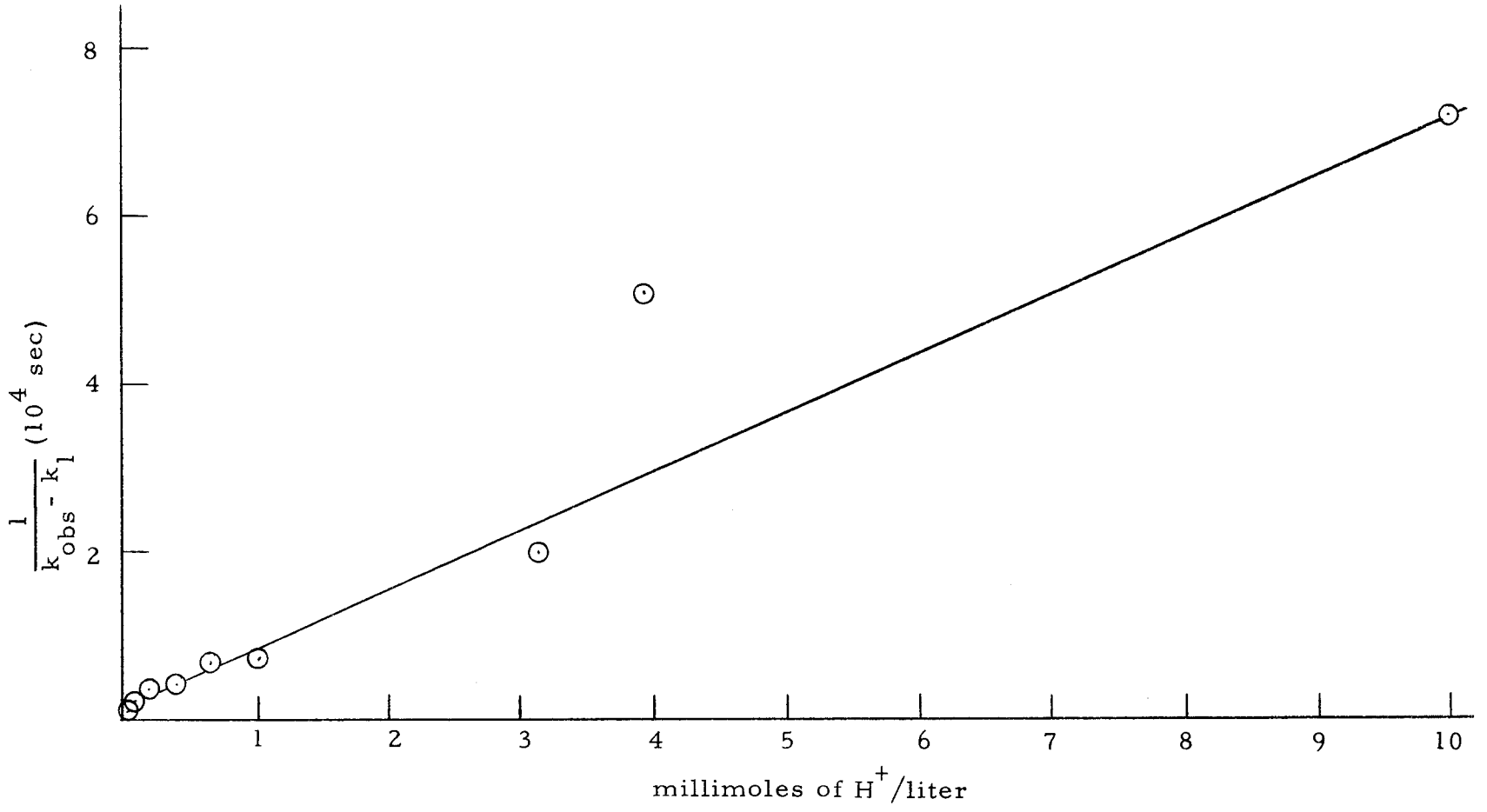
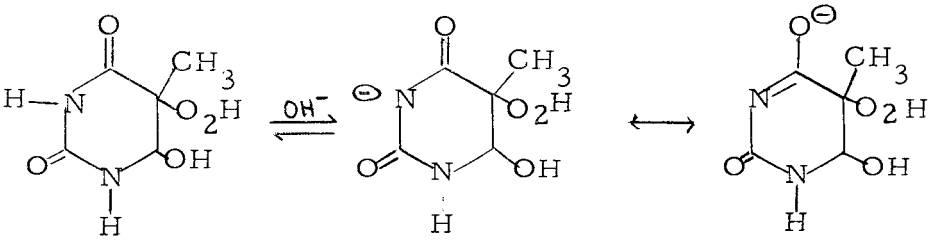


Figure 14. Functional plot for decay mechanism of cytosine hydroperoxide.

and a pK = 3.7 for the hydroperoxide.

Accordingly, the observed rate constant for the cytosine hydroperoxide decomposition should have a limiting value of $k_2 = 7 \times 10^{-4}$ sec^{-1}. The observed rate constant at pH 4.4 exceeds this amount. Clearly then, this mechanism is inadequate for higher pH ranges, with either a different or more complex reaction occurring.

In the higher pH regions, the experimental results do not show conclusively that the thymine hydroperoxide decomposition is simply a hydrolysis. The overall decrease in the total peroxide may be due to a hydroperoxide decomposition not leading to hydrogen peroxide, or it may be just due to a greater instability of the hydrogen peroxide itself at high pH. In basic solution, the following equilibrium exists for thymine:



The observed increase in hydroperoxide decomposition with increasing pH is in agreement with the cytosine case, where an electron donating substituent on the ring increases the decomposition rate. Without further data, it is difficult to suggest a reason for the large difference in rate constants for the hydroperoxide decompositions of thymine and thymidylic acid at pH ~ 5.6.

At 25°C there is a factor of approximately 100 between the rate constants for the thermal decomposition for CO_2H and TO_2H in both the monomers and in DNA, with the CO_2H being the faster. This difference can perhaps be explained in terms of structure. The methyl in the thymine hydroperoxide is a rather bulky group and may provide sufficient steric hindrance to the incoming water to slow the rate by a factor of 100. However, without further investigation, the difference in the inductive effects of the NH_2 and the C = 0 substituents on the ring cannot be ruled out as a major factor. One way of investigating this effect would be to compare the decomposition rates of cytosine hydroperoxide and 5-methyl cytosine hydroperoxide.

Aspects of the Radiation Chemistry of Cytosine

A proposed mechanism must account for both the nature of the stable primary products formed and their stoichiometry and should be clearly related to the initial reactive species. The γ-radiolysis of aqueous solutions produces as primary reactive species H, OH, and e_{aq}^-, but in the present of oxygen, both the H and e_{aq}^- will react very rapidly to give HO_2 (pK = 4.5). The rate constants for reaction with oxygen are $1.88 \pm 0.2 \times 10^{10} M^{-1} sec^{-1}$ for the e_{aq}^- and $2.6 \times 10^{10} M^{-1} sec^{-1}$ for H (54). For aqueous solutions less than 0.1 M, interaction of the radiation with the solute will be negligible and thus, for the solutions used in this investigation, the

formation of the primary products must come from reaction of the OH and HO_2 (or O_2^-) radicals.

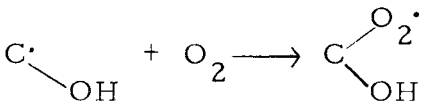
The primary purpose of this work was not the radiation chemistry of cytosine. However, some observations were made for the purpose of aiding the discussion of effects in DNA. Since the identification of all the primary products formed in irradiated cytosine solutions was not made, a complete mechanism cannot be proposed. The following discussion is based on the measured yields of cytosine loss $G(-C)$, hydroperoxide formation, $G(CO_2H)$, and the hydrogen peroxide formation, $G(H_2O_2)$. (See Appendix 1 for explanation of symbolism used in this section.)

The OH radical is known to add rapidly to aromatic rings. The rate constant for the reaction of OH with benzene at pH 3 has been measured as $3.3 \pm 0.8 \times 10^9\ M^{-1}Sec^{-1}$ (22), and for thymine as $3.1 \times 10^9 M^{-1} sec^{-1}$ (64).

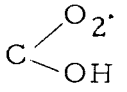
Accordingly, one can write as an initial step:

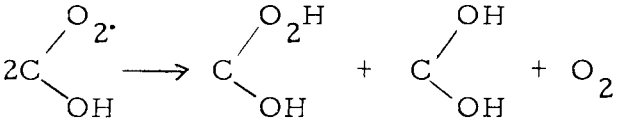
$$C + OH \longrightarrow C\!\!\begin{array}{l}\diagdown \\ \diagup\ OH\end{array} \qquad \text{(a)}$$

The reaction for this type of radical with oxygen is also known and, in cases where the rate constant has been measured, is very fast. For addition of O_2 to the $C_6H_6OH\cdot$ radical, $k = 5.0 \pm 0.6 \times 10^8 M^{-1} sec^{-1}$ (22). Therefore, in oxygen saturated solutions, one would expect the reaction



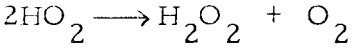
(b)

The formation of the cytosine hydroperoxide could occur by reaction of two 



(c)

A mechanism of this type has been suggested by Daniels and Grimison (17) for the formation of hydroperoxide in the 1849° A photolysis of dilute thymine solutions and is strongly supported by the experimental evidence. The reaction of two hydroperoxy radicals to give hydrogen peroxide is well known, with a measured rate constant of $5 \times 10^{6} M^{-1} sec^{-1}$ (76).



(d)

The initial yields for the radicals and molecular hydrogen peroxides at pH 2 can be taken as $G_{OH} = 2.5$,

$$G_H + G_{eq}^- = 3.2 = G(HO_2),$$

and

$$G_{H_2O_2} = 0.8.$$

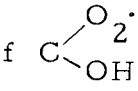
If reaction (d) were the only mode of hydrogen peroxide formation,

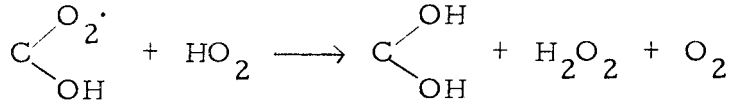
the maximum $G(H_2O_2)$ would be given by the stoichiometric relation

$$G(H_2O_2) = G_{H_2O_2} + 1/2\, G(HO_2)$$

$$= 0.8 + 1.6 = 2.4$$

But the measured $G(H_2O_2)$ in both 3×10^{-2}M and 3×10^{-3}M cytosine solutions exceeds the amount, indicating an additional mechanism for hydrogen peroxide formation.

In 3×10^{-2}M cytosine, $G(CO_2H) = 0.58$. Clearly then, the number of  radicals disappearing via reaction (c) cannot exceed 1.2. For the disappearance of the rest, a radical cross reaction can be written:

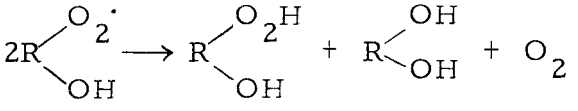
 (e)

The reaction sequence (a) through (c) can then account for the observed yields of cytosine hydroperoxide and hydrogen peroxide. However, this mechanism is still inadequate for two reasons. Inclusion of reaction (e) implies a competition between the formation of CO_2H and H_2O_2 contrary to the experimental results, which show that $G(H_2O_2)$ increases as $G(CO_2H)$ increases. In addition, the only mode of disappearance of cytosine is by OH radical attack, giving a maximum $G(-C) = G(OH) = 2.5$. The observed $G(-C)$ exceeds this at all concentrations, giving a limiting value of $G(-C) = 3.2$.

Therefore, the conclusion must be made that the reducing species also attack the cytosine molecule, giving a radical such as $C^{\cdot}\!\!\diagdown\!\!H$ or $C^{\cdot}\!\!\diagdown\!\!O_2H^{\cdot}$. Further identification of primary products needs to be made before a reaction mechanism involving these radicals can be proposed.

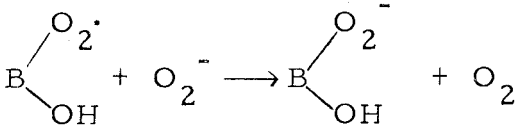
Radiation Chemistry of DNA

When proposing a mechanism for the formation of the hydroperoxides in DNA, there are several features which must be considered. First, the G values for peroxide formation clearly show that as the amount of hydroperoxide formed increases, the hydrogen peroxide formation decreases; i. e., these two processes seem to be in competition. This must be contrasted with the cytosine case above. Second, the reaction which was proposed for the formation of cytosine hydroperoxide

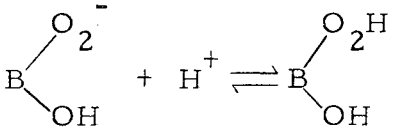


does not seem very likely in DNA. Since DNA structure is that of a long chain, there would be some difficulty encountered in getting the two radical groups together for reaction.

At pH 5-6, the reducing species will be predominantly O_2^-. The following mechanism is suggested where B (for base) is used to denote either the cytosine or thymine component.[6]



(f)



(g)

$$O_2^- + O_2^- \xrightarrow{2H_2O} H_2O_2 + O_2 + 2OH^- \qquad \text{(h)}$$

The rate constant for reaction (h) at 25°C has been measured as $3.4 \times 10^7 M^{-1} sec^{-1}$ (20). Reactions (f) and (g) and (h) show a competition for the O_2^- which accounts for the observed increase of hydroperoxide formed with a corresponding decrease in hydrogen peroxide.

At pH 5-7, the primary radical and molecular yields can be taken as $G_{(H + eq^-)} = 2.8$, $G_{OH} = 2.2$ and $G_{H_2O_2} = 0.75$. Scholes, et al. (65) concluded that in DNA solutions irradiated with 200 kev

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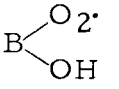
[6] The same symbolism is used in this section as in the discussion on cytosine (see Appendix 1) with the exception that there is a sugar component on the $-N_2-$.

X-rays, 73-80 percent of the OH radicals react with the bases and about 20-27 percent with the sugar components. Assuming that Scholes values are correct, the number of OH radicals attacking the bases would be 1.61 to 1.76. The yields for base destruction (Table V) measured by Scholes (65) for 0.2 percent DNA solutions show 61 percent of the total measured base destruction from 200 kev X-rays is due to destruction of the pyrimidine bases. Assuming that this total base destruction is due only to OH radical attack, this means that only 0.98 to 1.07 of the OH radicals could attack the pyrimidine bases, thymine and cytosine. In irradiated 0.1 percent DNA solutions, the measured yield for total hydroperoxide formation $G(BO_2H)$ was 1.14. This implies that all the  radicals formed proceed to the hydroperoxide via reactions (f) and (g). The number of O_2^- available for hydrogen peroxide formation by reaction (h) would then be

$$G(O_2^-) - 1.14 = 2.8 - 1.14 = 1.66,$$

giving a maximum yield for hydrogen peroxide formation $G(H_2O_2)$ of

$$0.83 + G_{H_2O_2} = 0.83 + 0.75 = 1.58.$$

Clearly, H_2O_2 must be being formed by an additional mechanism since the measured hydrogen peroxide yield for DNA concentrations from 0.004 to 0.1 percent exceeds this amount. If all the radicals

$B\begin{smallmatrix}O_2\cdot\\OH\end{smallmatrix}$ formed by the pyrimidine proceed via reaction (f), then one is forced to the conclusion that the radicals formed by OH attack on the purine bases or the sugars are leading to hydrogen peroxide formation. If one assumes that hydrogen peroxide formation results from a purine reaction, then the following relation can be written.

$$\text{Purine - OH}\cdot + O_2^- \longrightarrow \text{products} + H_2O_2 \qquad \text{(i)}$$

Inclusion of this reaction gives the stoichiometric relationship

$$G(H_2O_2) = G_{H_2O_2} + 1/2\ G(\text{-total bases}) - G(BO_2H) + 1/2G(O_2^-)$$

$$= 2.15 + 1/2\ G(\text{-total base}) - G(BO_2H)$$

A comparison can be made between the values for $G(H_2O_2)$ calculated from this expression and those experimentally measured.

DNA (wt %)	$G(H_2O_2)$ calculated	$G(H_2O_2)$ measured
0.1	2.00	1.98
0.02	2.28	2.18
0.004	2.36	2.52

Considering the difficulty in accurately measuring the initial G values at low DNA concentrations, the calculated G values for hydrogen peroxide formation are in good agreement with the experimental values.

The relationship between the amount of CO_2H and TO_2H can be

correlated with the DNA base composition. The measured G for the hydroperoxide formed in 3×10^{-3}M cytidylic acid at pH 5. 3 was 0. 17 and that for thymidylic acid was 0. 36. The A-T/G-C ratio for salmon sperm DNA is 1. 43 (50, p. 138). Assuming that the efficiency of hydroperoxide formation is the same in DNA as in the monomers, the following expressions can be written.

$$\frac{G(TO_2H)_{DNA}}{G(CO_2H)_{DNA}} = \frac{G(TO_2H)_{monomer}}{G(CO_2H)_{monomer}} \times \frac{A - T}{G - C} = \frac{0.37 \times 1.43}{0.17}$$

$$G(TO_2H)_{DNA} = G(BO_2H)_{DNA} \times \frac{0.36(1.43)}{0.17 + 0.36(1.43)}$$

$$G(CO_2H)_{DNA} = G(BO_2H)_{DNA} \times \frac{0.17}{0.17 + 0.36(1.43)}$$

Using $G(BO_2H) = 1.14$ for 0. 1 percent DNA, one finds:

	calculated	measured
$G(TO_2H)$	0. 86	0. 89
$G(CO_2H)$	0. 28	0. 25
$\frac{G(TO_2H)}{G(CO_2H)}$	3. 03	3. 55

Another way of calculating the expected ratio of $G(TO_2H)/G(CO_2H)$ in DNA is to use the efficiency of hydroperoxide formation in the pyrimidines themselves. The measured yields for hydroperoxide formation and base loss in 3×10^{-3}M solutions of cytosine and thymine are

G(cytosine hydroperoxide) = 0. 52,

G(thymine hydroperoside) = 1. 21,

and G(-cytosine) = 3. 2 to 3. 3.

Taking Scholes value for thymine loss (65),

G(thymine) = 2. 76.

Then

$$\frac{G(TO_2H)_{DNA}}{G(CO_2H)_{DNA}} = \frac{\text{fraction thymine forming hydroperoxide}}{\text{fraction cytosine forming hydroperoxide}} \times \frac{A - T}{G - C}$$

$$= \frac{1.21/2.76}{0.52/3.3} \times 1.43 = 3.84$$

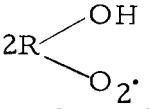
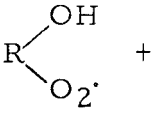
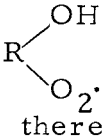
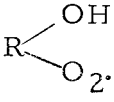
These calculated values must be regarded as being in agreement with experiment. Therefore, the ratio of TO_2H/CO_2H formed in DNA would seem to be a simple function of the DNA base composition. Unfortunately, other samples of DNA to extend this work were not available.

The implications here are the following:

1. The chemical products can be accounted for in terms of radical reactions and consideration of excitation mechanisms, electron hole migration, etc., is not necessary.

2. Reactive units react individually and independently. Denatured DNA consists of a long chain whose backbone is composed of large numbers (in excess of 10^4) of 2-deoxyribose sugar residues joined by 3', 5'-phosphodiester linkages. Each 2-deoxyribose is

linked to one of the four bases thymine, cytosine, adenine, or guanine. Upon irradiation at the γ-intensities and doses employed here, the probability of an OH attack on any two adjacent bases is very very small. In addition, as shown by the above calculations, the efficiency of hydroperoxide formation in DNA seems to be the same as that for the individual monomers, indicating that once the base has been attacked by the OH radical, it is not influenced in further reactions by the presence of the neighboring bases.

3. The change of mechanism from  in the monomers to  for hydroperoxide formation in DNA suggests that it would be interesting to work with oligonucleotides to determine the range in which the  essentially becomes immobilized. As mentioned above, there would be difficulty in getting together for reaction two  radical groups which are widely separated on the chain. However, there should be a region of nucleotide chain length where some diffusion is still possible and where the cross-over from the predominance of one mechanism over the other occurs.

Factors Affecting the Decay of the Hydroperoxides in DNA

The results obtained for irradiated DNA solutions in 10^{-2}M NaCl clearly showed that the rate constant for thermal decay of the TO_2H was not dependent upon the DNA helix-coil structure, since in passing from 25°C to 80°C one goes through the 'melting' region without changing the characteristics of the kinetics. Therefore, it seems unlikely that the rather sharp change in the activation energy for the TO_2H decay in water can be accounted for in terms of the DNA helix-coil transition. Rather, it seems probable that it is due to a change in the structure of the water.

Several theories to describe the structure of water have been put forth in the last few years. All of them maintain the existance of a hydrogen-bonded network of molecules, but differ on the extent and structural detail of this network, and most of them assume the presence of two distinct states for the water molecules. The theories can be divided into two main classes. The first group consists of models in which the majority of molecules have an essentially crystalline or lattice structure of tetrahedrally hydrogen-bonded molecules with interstitial spaces large enough to contain free non-associated molecules which have been displaced from the lattice sites. Samoilov (61) and Forslind (28) have suggested that this lattice structure is ice-like while Pauling and Marsh (57) and Frank

and Quist (29) have suggested that it is similar to that of gas hydrates. The second group of models emphasize the limited extent of the hydrogen-bonded networks. They suggest that the hydrogen-bonded networks form flickering clusters with regions of non-hydrogen bonded molecules in between. The main proponents of this type of model have been Frank and Wen (30) and Némethy and Scheraga (56). Recently Kavanau has published a detailed review of the various models along with their experimental and theoretical arguments (42). Various theoretical arguments and bits of experimental evidence have been put forth both for and against all of these various models, and as yet, the situation is far from being resolved. There is not only controversy over the nature of the different water species, but indeed, over whether several different species even exist. Evidence from the Raman and infrared OH vibrational bands have not indicated the presence of any water molecules having different numbers of hydrogen bonds from others (25).

Experimental evidence such as changes in intensity of bands in the Raman and absorption spectra, changes in magnetic resonance of protons, and changes in density, heat capacity, viscosity, dielectric constant, dielectric relaxation time, and temperature of maximum density indicate that ions have an effect on water structure that is more than that due to simple dielectric polarization, binding and compression of nearest-neighbor molecules. Frank and Wen (30) have defined three concentric regions around an ion in water. Region A consists of strongly oriented polarized water molecules which are

essentially immobilized. Region B is one of disorder in which the water structure has been broken by the electrostatic field of the ion. The outer region contains water having the 'normal' or undisturbed water structure, whatever that may be. The size and charge of the ions determine the extent of these regions, with region B being the most sensitive to changes. Region B is large for small or highly charged ions and therefore, these ions are said to be net "structure breakers". Certain small ions such as Li^+ and F^- are exceptions in that they can integrate into the tetrahedrally bonded water structure and thus eliminate region B. For larger ions, the electrostatic field is weaker and region B is smaller. Region A is still present and leads to the classification of these ions as "structure makers". The overall net effect of structure making or breaking depends on the balance of the two regions.

In order to relate the effect of ions on the water structure to a discussion of the observed DNA properties, it is necessary first to discuss the interaction of water with the DNA itself. Falk, Hartman, and Lord (26, 27) have investigated the hydration of DNA solid fibers by both spectroscopic (infrared and ultraviolet) and gravimetric methods. They reported that the primary hydration sites are the phosphate groups. The water adsorption occurs in layers around the phosphate groups with a drop of approximately 2 kcal/mole in the adsorption energy between the first and second layer. At 65 percent

relative humidity, the hydration of the phosphate groups is complete with a total of about six water molecules/nucleotide adsorbed. It is also possible that some of this water adsorption occurs on the oxygen of the phosphate-sugar linkage and on the C-O-C of the ribose. Above 65 percent relative humidity hydration occurs at the carbonyls and ring nitrogens of the bases. By 80 percent relative humidity the water molecules have completely filled the DNA groove and further hydration causes swelling of the helix. They conclude that at 92 percent relative humidity there are about 20 water molecules per nucleotide and suggest that this adsorbed water may be responsible for the stability of the DNA helix. Pouyet, Jacob, and Duane (59), in an investigation utilizing sedimentation velocity measurements calculated that for the approximately 18 molecules/two nucleotides adsorbed on the bases themselves in native DNA, 11.5 adsorbed preferentially on the adenine-thymine pair and seven on the guanine-cytosine pair. They also reported that preferential hydration was a property of the DNA helix and was very much weaker for denatured DNA solutions. However, Hearst (33), using density measurements on DNA electrolyte solutions, found no correlation between the net hydration of DNA and the helix stability.

Pouyet et al. (59) found that the number of water molecules preferentially linked per nucleotide in aqueous DNA solutions was strongly dependent upon the presence of salt, with the main effect

being due to the cation. They proposed that in the concentration range $0 < m < 3$ the effect on the preferential hydration was due to interactions between the phosphate groups and the cations with the outer hydration layer being displaced and only above $m = 3$ did penetration of the ions into the internal shell around the bases occur. It should be remembered that the added solutes used in the present research were 10^{-2}M. However, for denatured DNA one would expect the bases to be affected at much lower concentrations. The specific effects of different cations were attributed to differences in their hydration and polarizability. They calculated, on the basis of ion-permanent dipole interactions, that for interactions with the bases, there would be a preferential adsorption of ions on the guanine-cytosine pair over the adenine-thymine pair.

There are two things which need to be explained about the DNA TO_2H thermal decay. First, why is there a difference between the rate of decay in water and in NaCl and second, why is there a difference in the activation energy at low and high temperatures in water and no difference in NaCl? In accordance with the evidence for DNA preferential hydration just discussed, in an aqueous solution of DNA which is to a large extent denatured, there will be preferential hydration at the phosphate groups and most probably at the bases themselves, although these would be much weaker as they would result from induced dipole interactions rather than charge-dipole

interactions as in the case of the phosphate groups. However, in the presence of 10^{-2}M NaCl some of these water molecules will be replaced by the sodium ions. Pouyet et al. (59) found a preferential hydration of only eight water molecules remaining in native DNA solutions in the presence of sodium ions of this concentration. The low entropies of activation have indicated a highly ordered transition state. If the assumption is made that the water surrounding the DNA, being ordered by the preferential hydration of the DNA chain, has the desired orientation for the attack on the oxygen-oxygen bond of the hydroperoxide, then one would expect a faster rate of decomposition of the hydroperoxide in water than in 10^{-2}M NaCl, where the net preferential hydration has been lowered and the preferred water orientation lost. This is what is observed in the lower temperature range. The strength of the interaction between the DNA phosphate groups and the adsorbed water molecules will certainly decrease with increasing temperature and increasing molecular motion. Burns and Dunford (7), using data on acoustic absorbance, specific volume, isothermal compressibility, and heat capacity have found a marked difference in the behavior of water above and below 60°C and have concluded that in general, several sets of experimental parameters show that the bulky structure of the water at low temperatures disappears by 60°C. Therefore, the change in activation energy around 50°C for the TO_2H decomposition in water can be ascribed to a

breakdown of the preferential hydration of the DNA chain, giving a behavior similar to that found in the NaCl. With the precisely oriented water network near the DNA broken up, the attacking water will be the free monomer of the bulk solvent. The observed change in the activation energy for the TO_2H decomposition in water was 6.3 kcal/mole, using 15.2 kcal as the activation energy at room temperatures. Marchi and Eryring (51), using significant structure theory, have calculated a $\Delta H°$ of 6.9 kcal/mole for the transition in water from ice-like structure to free monomer. Stevenson (71), utilizing data on the vibrational absorption spectrum of water monomer in CCl_4 solution, has calculated a ΔH of 5.5 kcal/mole and from data on the vacuum ultraviolet absorption spectrum of liquid water, a ΔH of 6.34 ± 0.33 kcal/mole. The agreement of the experimental change in activation energy for the TO_2H decomposition with the values reported for the change in water structure from network to free monomer supports the suggestion that the effect observed in the DNA in water is due to a change in the water structure. Marchi and Eyring calculated a value of 17.3 e.u. for $\Delta S°$ for the transition while Stevenson found $\Delta S° = 6.2$ e.u. The experimental change observed for the TO_2H was 19.2 e.u., which, though higher, is still in the same range as the values of Marchi and Eyring.

The change in the rate of decomposition at 80°C with different electrolyte solutions can now be explained in terms of ionic effects

on the bulk water. There is not complete agreement in the literature about which ions should be classed as water structure breakers and which should be called structure formers. For example, Kavanau (47) gives Li^+, Na^+, and F^- as net structure makers and NH_4^+, Cs^+, Cl^-, NO_3^-, and ClO_4^- as breakers. Choppin and Buijs (9), on the basis of infrared studies find that F^- makes structure while Na^+, Li^+, ClO_4^-, NO_3^-, and Cl^- break structure. However, those ions generally classed as structure formers have lower entropies of hydration than the structure breakers. Figure 15 shows a plot of the rate constant at 80°C against the net hydration entropy for some of the electrolyte solutions used. The hydration entropies for the salts were obtained by adding together the individual hydration entropies of the ions. The values taken for the individual ions are those reported by Hunt (37, p. 16) and are based on $\bar{S}°$ of H^+ equal to zero and a standard state of m = 1. Hydration entropies were not available for ions such as nitrate, sulfate, and perchlorate. The plot shows a clear correlation between net hydration entropy of the ions and the TO_2H decomposition rate constant. The fact that the activation energies of the TO_2H decomposition at high temperatures are essentially the same indicates that a limiting value for $\Delta E^{\ddagger}$ has been reached. Therefore, the change in the rate constant at 80°C with different electrolytes must be regarded as being due to an entropy effect on the bulk water due to ion hydrations. The effect of the

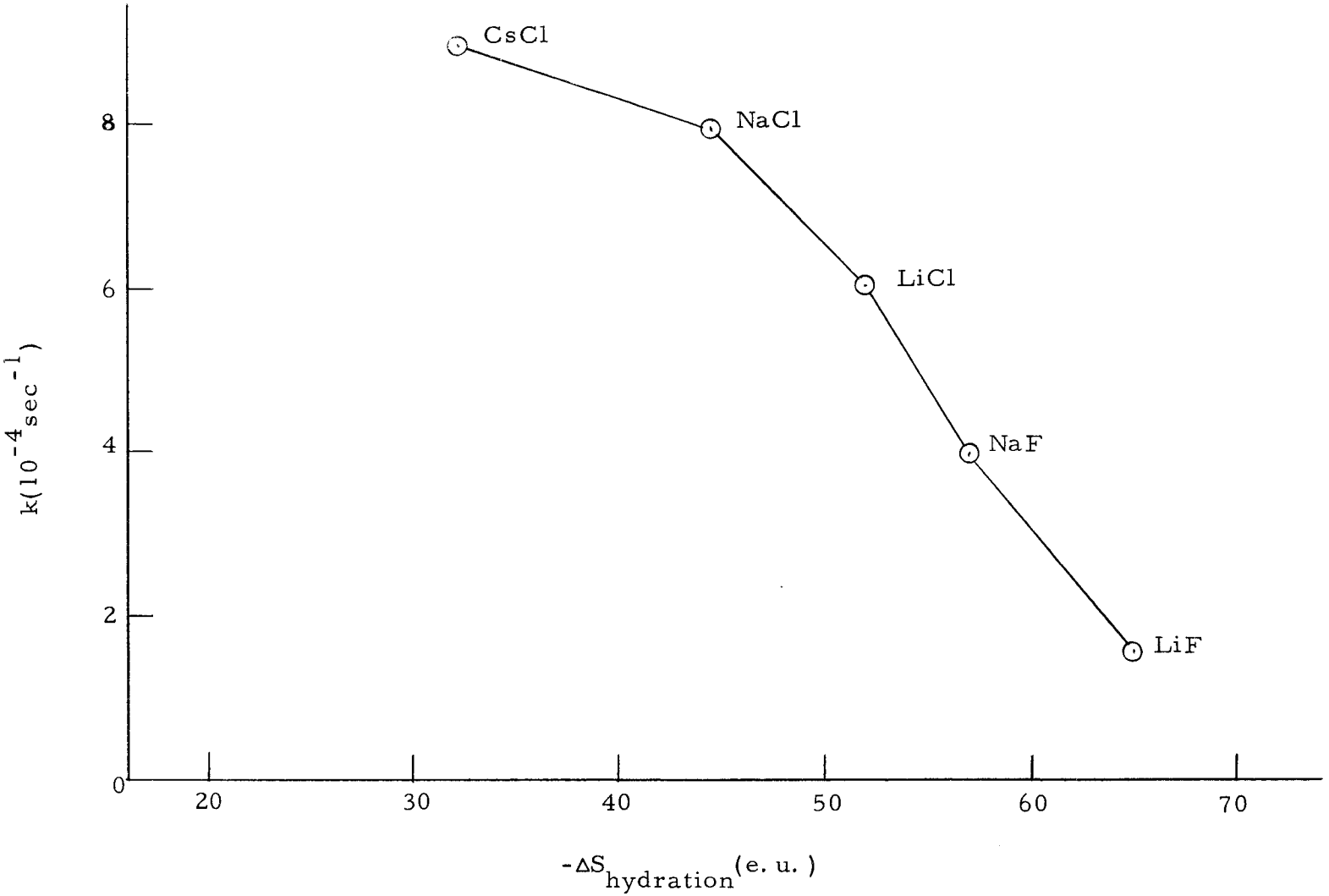


Figure 15. Variation of TO_2H decomposition rate with electrolyte entropy of hydration at 80° C.

non-electrolyte urea in increasing the decomposition rate supports the view that this change is an entropy effect. Abu-Hamdiyyah (1) reported that urea participates in the formation of water-urea clusters with the result of increasing the entropy of the water.

To summarize, the difference in the behavior of the thermal decomposition of the TO_2H can be explained in terms of two different effects on water structure. At low temperatures, the presence of the cation destroys the orientation of the preferentially hydrated water surrounding the DNA chain, causing a higher activation energy for the TO_2H hydrolysis. At high temperatures, where the preferentially hydrated water is lost in both water and sodium chloride, the difference in rate constants is due to an entropy effect of the added solute on the bulk water.

The water structure at room temperature may also provide an explanation for the difference in the relative amounts of CO_2H and TO_2H formed upon irradiation in water and in sodium chloride. Pullman and Pullman (60), using molecular orbital methods, have calculated that in DNA the guanine-cytosine pair are both better electron donors and better electron acceptors than the adenine-thymine pair. Consequently hydrogen bonding is stronger in the G-C pair than in the A-T pair. All solutions were irradiated at room temperature where, in the solutions containing NaCl, considerable helical structure remained. Therefore, the preferentially

hydrated water in the helix may have been more strongly bound to the cytosine than to the thymine, providing greater shielding against OH radical attack on the cytosine. For the regions where the helical structure has been lost, the difference may be due to the effect of the sodium ion. Utilizing Pouyet's (59) calculation that a stronger interaction exists between ions and the G-C pair than for the A-T pair, one can say that a greater ion atmosphere around the cytosine component retards the OH attack, thus giving less CO_2H in the presence of NaCl than in water.

Summary

Hydroperoxide formation in irradiated cytosine solutions was detected only in acid solution. However, its nucleotide form, cytidylic acid, gives a more stable hydroperoxide at the pH of aqueous DNA. The yield of hydroperoxide is reduced in the presence of the sugar and phosphate components.

The formation of two hydroperoxides in irradiated aqueous DNA was confirmed, with the fast-decaying one being identified through its decay kinetics and energetics with the cytosine component and the more stable one with the thymine component. The yield of the thymine hydroperoxide in DNA is about three and one-half times that of the cytosine hydroperoxide. The relationship between the hydroperoxide and hydrogen peroxide yields indicated a change in

mechanism of hydroperoxide formation in going from the monomer base to the DNA, but the efficiency of hydroperoxide formation seemed to be the same in the DNA as in the monomers.

The kinetics for the thermal decay of the slow hydroperoxide in DNA was found to be influenced by the presence of added solutes, with the effect interpreted as being due to a change of the DNA preferential hydration at low temperatures and to a change in the entropy of the bulk water structure at high temperature.

BIBLIOGRAPHY

1. Abu-Hamdiyyah, M. The effect of urea on the structure of water and hydrophobic bonding. Journal of Physical Chemistry 69: 2720-2725. 1965.

2. Anderson, A. R. and E. J. Hart. Hydrogen yields in the radiolysis of aqueous hydrogen peroxide. Journal of Physical Chemistry 65:804-810. 1961.

3. Armstrong, D. et al. Primary products in the irradiation of aqueous soltuions with x or gamma rays. In: Proceedings of the Second United Nations International Conference on the Peaceful Uses of Atomic Energy, Geneva, 1958. Vol. 29. Geneva, United Nations, 1958. p. 80-90.

4. Barr, N. F. and A. O. Allen. Hydrogen atoms in the radiolysis of water. Journal of Physical Chemistry 63:928-931. 1959.

5. Barron, E. S. G., P. Johnson and A. Cobure. Effect of x-irradiation on the absorption spectrum of purines and pyrimidines. Radiation Research 1:410-425. 1954.

6. Boag, J. W. and E. J. Hart. Absorption spectra in irradiated water and some solutions. Nature 197:45-47. 1963.

7. Burns, N. M. and H. B. Dunford. On the effect of temperature on the structure of water. In: Abstracts of the 153d Meeting, American Chemical Society. 1967. p. R 184.

8. Butler, J. A. V. Changes induced in nucleic acids by ionizing radiations and chemicals. Radiation Research, Supplement 1:403-416. 1959.

9. Choppin, G. R. and K. J. Buijs. Near-infrared studies of the structure of water. II. Ionic solutions. Journal of Chemical Physics 39:2042-2050. 1963.

10. Collinson, E., F. S. Dainton and J. Krah. Effects of linear energy transfer on the radiolysis of water and heavy water. Nature 187:475-477. 1960.

11. Collyns, B. et al. Chain scission and hydrogen bond breakage on irradiation of DNA. Radiation Research 3:526-536. 1965.

12. Cox, R. A. et al. Effects of gamma-rays on solutions of sodium deoxyribonucleate. Nature 176:919-921. 1965.

13. Czapski, G., J. Jortner and G. Stein. The role of hydrogen atoms in the decomposition of hydrogen peroxide and in the radiation chemistry of water. Journal of Physical Chemistry 65:964-966. 1961.

14. Czapski, G. and H. A. Schwarz. The nature of the reducing radical in water radiolysis. Journal of Physical Chemistry 66:471-474. 1962.

15. Dainton, F. S. and W. S. Watt. The effect of pH on the radical yields in the γ-radiolysis of aqueous systems. Nature 195:1924. 1962.

16. Dainton, F. S. et al. Evidence for the unit charge on the "hydrogen atom" formed by the action of ionizing radiation on aqueous systems. Proceedings of the Chemical Society, 1962, p. 140-141.

17. Daniels, M. and A. Grimison. Photolysis of the aqueous thymine system. I. Excitation at 1849 °A. Biochimica et Biophysica Acta 1967. (In press)

18. Daniels, M. et al. Chemical action of ionizing radiations. Part XVII. Degradation of deoxyribonucleic acid in aqueous solution by irradiation with x-rays (200 kev). Journal of the Chemical Society, 1957, p. 226-234.

19. Debierne, A. Recherches sur les gaz produits par les substances radioactives. Décomposition de l'eau. Annales de Physique (Paris) 2:97-127. 1914.

20. Dorfman, L. M. and M. S. Mathison. Pulse radiolysis. Progress in Reaction Kinetics 3:237-301. 1965.

21. Dorfman, L. M. and I. A. Taub. Pulse radiolysis studies. III. Elementary reactions in aqueous ethanol solution. Journal of the American Chemical Society 85:2370-2374. 1963.

22. Dorfman, L. M., I. A. Taub and D. A. Harter. Rate constants for the reaction of the hydroxyl radical with aromatic molecules. Journal of Chemical Physics 41:2954-2955. 1964.

23. Edwards, J. O. Nucleophilic displacement on oxygen in peroxides. In: Peroxide reaction mechanisms, ed. by J. O. Edwards. New York, Interscience, 1962. p. 67-106.

24. Ekert, B. and R. Monier. Structure of thymine hydroperoxide produced by x-irradiation. Nature 184:58-59. 1959.

25. Falk, M. and T. A. Ford. Infrared spectrum and structure of liquid water. Canadian Journal of Chemistry 44:1699-1707. 1966.

26. Falk, M., K. A. Hartman, Jr. and R. C. Lord. Hydration of deoxyribonucleic acid. I. A gravemetric study. Journal of the American Chemical Society 84:3843-3846. 1962.

27. Falk, M., K. A. Hartman, Jr. and R. C. Lord. Hydration of deoxyribonucleic acid. II. An infrared study. III. A spectroscopic study of the effect of hydration on the structure of deoxyrobonucleic acid. Journal of the American Chemical Society 85:387-394. 1963.

28. Forslind, E. A theory of water. Acta Polytechnica 115:9-43. 1952.

29. Frank, H. S. and A. S. Quist. Pauling's model and the thermodynamic properties of water. Journal of Chemical Physics 34: 604-611. 1961.

30. Frank, H. S. and W. Wen. III. Ion solvent interaction. Structural aspects of ion-solvent interaction in aqueous solutions: a suggested picture of water structure. Discussions of the Faraday Society 24:133-140. 1957.

31. Grossweiner, L. I. and H. I. Joschek. Optical generation of hydrated electrons from aromatic compounds. In: Solvated electron, ed. by R. F. Gould. Washington, D. C., American Chemical Society, 1965. p. 278-288. (Advances in chemistry series, no. 50)

32. Hart, E. J. and J. W. Boag. Absorption spectrum of the hydrated electron in water and in aqueous solutions. Journal of the American Chemical Society 84:4090-4095. 1962.

33. Hearst, J. E. Determination of the dominant factors which influence the net hydration of native sodium deoxyribonucleate. Biopolymers 3:57-68. 1965.

34. Hirschman, S. Z. and G. Felsenfeld. Determination of DNA composition and concentrations by spectral analysis. Journal of Molecular Biology 16:347-358. 1966.

35. Hochanadel, C. J. and S. C. Lind. Radiation chemistry. Annual Review of Physical Chemistry 7:83-106. 1956.

36. Hummel, A. and A. O. Allen. Radiation chemistry of aqueous solutions of ethanol and the nature of the oxidizing radical OH. Radiation Research 17:302-311. 1962.

37. Hunt, J. P. Metal ions in aqueous solution. New York, W. A. Benjamin Inc., 1963. 124 p.

38. Hutchinson, F. Sufhydryl groups and the oxygen effect on irradiated dilute solutions of enzymes and nucleic acids. Radiation Research 14:721-731. 1960.

39. Jordon, D. O. Physiochemical properties of the nucleic acids. Progress in Biophysics 2:51-89. 1951.

40. Jordon, D. O. The chemistry of nucleic acids. London, Butterworths, 1960. 358 p.

41. Kamal, A. and W. M. Garrison. Radiolytic degradation of aqueous cytosine: enhancement by a second organic solute. Nature 206:1315-1317. 1965.

42. Kavanau, J. L. Water and solute-water interactions. San Francisco, Holden-Day, 1964. 101 p.

43. Keene, J. P. Optical absorptions in irradiated water. Nature 197:47-48. 1963.

44. Latarjet, R., B. Ekert and P. Demerseman. Peroxidation of nucleic acids by radiation: biological implications. Radiation Research, Supplement 3:247-256. 1963.

45. Lazo, R. M., H. A. Dewhurst and M. Burton. The ferrous sulfate radiation dosimeter: a calorimetric calibration with gamma rays. Journal of Chemical Physics 22:1370-1375. 1954.

46. Lea, D. Actions of radiations on living cells. Cambridge, Cambridge University Press, 1946. 402 p.

47. Lefort, M. Radiation chemistry. Annual Review of Physical Chemistry 9:123-151. 1958.

48. Lefort, M. and X. Tarrago. Radiolysis of water by particles of high linear energy transfer. The primary chemical yields in aqueous acid solutions. Journal of Physical Chemistry 63:833-836. 1959.

49. Magee, J. L. and M. Burton. Elementary processes in radiation chemistry. II. Negative ion formation by electron capture in neutral molecules. Journal of the American Chemical Society 73:523-532. 1951.

50. Mahler, H. R. and E. H. Cordes. Biological chemistry. New York, Harper and Row, 1966. 872 p.

51. Marchi, R. P. and H. Eyring. Application of significant structure theory to water. Journal of Physical Chemistry 68:221-228. 1964.

52. Marmur, J. and P. Doty. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. Journal of Molecular Biology 5:109-118. 1962.

53. Marmur, J., R. Rownd and C. L. Schildkraut. Denaturation and renaturation of deoxyribonucleic acid. Progress in Nucleic Acid Research 1:231-300. 1963.

54. Mathison, M. S. The formation and detection of intermediates in water radilysis. Radiation Research, Supplement 4:1-13. 1964.

55. Mathison, M. S. The hydrated electron in radiation chemistry. In: Solvated electron, ed. by R. F. Gould. Washington, D. C., American Chemical Society, 1965. p. 45-54. (Advances in chemistry series, no. 50)

56. Némethy, G. and H. A. Scheraga. Structure of water and hydrophobic bonding in proteins. I. A model for the thermodynamic properties of liquid water. Journal of Chemical Physics 36: 3382-3400. 1962.

57. Pauling, L. and R. E. Marsh. The structure of chlorine hydrate. Proceedings of the National Academy of Sciences 38:112-118. 1952.

58. Peacocke, A. R. and B. N. Preston. The action of γ-rays on sodium deoxyribonucleate in solution. Proceedings of the Royal Society of London, Ser. B, 153:103-110. 1960.

59. Pouyet, G., M. Jacob and M. Daune. Hydration préférentielle de l'acide désoxyribonucléique. Journal of Molecular Biology 13:817-832. 1965.

60. Pullman, B. and A. Pullman. The electronic structure of the purine-pyrimidine pairs of DNA. Biochimica et Biophysica Acta 36:343-350. 1959.

61. Samoilov, O. Ya. Structure of aqueous electrolyte solutions and the hydration of ions, tr. by D. J. G. Ives. New York, Consultants Bureau, 1965. 185 p.

62. Samuel, A. H. and J. L. Magee. Theory of radiation chemistry. II. Track effects in radiolysis of water. Journal of Chemical Physics 21:1080-1087. 1953.

63. Scholes, G. The radiation chemistry of aqueous solutions of nucleic acids and nucleoproteins. Progress in Biophysics 13: 59-104. 1963.

64. Scholes, G., P. Shaw and R. L. Willson. Pulse radiolysis studies of aqueous solutions of nucleic acid and related substances. In: Pulse radiolysis, ed. by J. A. Swallow and J. H. Baxendale. London, Academic Press, 1965. p. 151-164.

65. Scholes, G., J. F. Ward and J. Weiss. Mechanisms of the radiation-induced degradation of necleic acids. Journal of Molecular Biology 2:379-391. 1960.

66. Scholes, G. and J. Weiss. Chemical action of x-rays on nucleic acids and related substances in aqueous systems. Experimental Cell Research, Supplement 2:219-241. 1952.

67. Scholes, G. and J. Weiss. Oxygen effects and formation of peroxides in aqueous solution. Radiation Research, Supplement 1:177-189. 1959.

68. Scholes, G. and J. Weiss. Organic hydroxy-hydroperoxides: a class of hydroperoxides formed under the influence of ionizing radiations. Nature 185:305-306. 1960.

69. Scholes, G., J. Weiss and C. M. Wheeler. Formation of hydroperoxides from necleic acids by irradiation with x-rays in aqueous systems. Nature 178:157. 1956.

70. Spinks, J. W. T. and R. T. Woods. An introduction to radiation chemistry. New York, John Wiley and Sons, 1964. 477 p.

71. Stevenson, D. P. On the monomer concentration in liquid water. Journal of Physical Chemistry 69:2145-2152. 1965.

72. Terenin, A. and F. Vilessov. Photoionization and photodissociation of aromatic molecules by vacuum ultraviolet radiation. Advances in Photochemistry 2:385-421. 1964.

73. Weiss, J. Radiochemistry of aqueous solutions. Nature 153: 748-750. 1944.

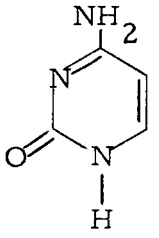
74. Weiss, J. Primary processes in the action of ionizing radiations on water: formation and reactivity of self-trapped electrons ('polarons'). Nature 186:751-752. 1960.

APPENDIX

APPENDIX I

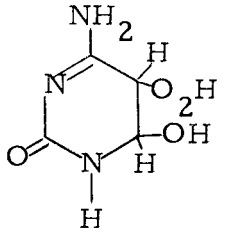
The symbols used in this section are as follows:

Cytosine



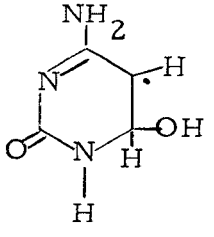
Symbolized as C

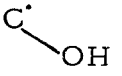
Cytosine Hydroperoxide

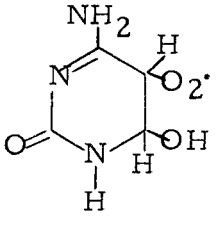


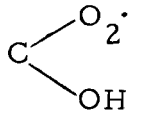
Symbolized as CO_2H

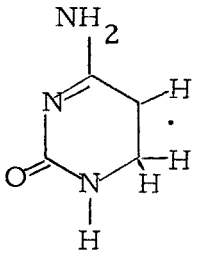
Intermediate species



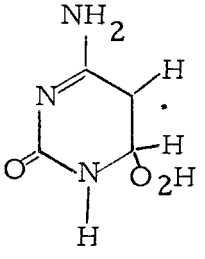
Symbolized as 



Symbolized as 



Symbolized as C•—H



Symbolized as C•—O_2H