Pulse Radiolysis of Nucleic Acid Constituents and Related Compounds. III. Optical Spectra and Reactivity of Organic Free Radicals Formed by Reaction of Hydroxyl Free Radicals with Pyrimidine Bases[1]

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Abstract: Dilute N_2O-saturated aqueous solutions of uracil (U), thymine (T), cytosine (C), and 5-methylcytosine (MC) were irradiated with submicrosecond pulses of 10-MeV electrons, and changes in optical absorbance in the wavelength range 300–600 nm were monitored for periods up to 1700 μsec. Radicals formed at pH 7 by reaction of OH· with pyrimidine bases have spectra with the following maxima: U, 375, T, 375, C, 335, 440, MC, 325, 485 nm. These spectra decay for 50–80 μsec without significant change in shape. Spectra of radicals formed at pH 11.4 and ~12.5 differ from pH 7 spectra. Compounds with a 5-methyl group give spectra at pH ~12.5 with a pronounced absorption maximum near 525–550 nm which is absent from spectra of 5-hydro compounds. At the higher pH's spectra undergo changes in shape at rates which are much faster than radical decay. These reactions are pseudo-first-order and their rates depend on pH. They are attributed to reactions between radicals and OH^- ion. Four general kinds of organic free radicals are found: R_1· (neutral radicals formed by addition of OH· to a base in neutral solutions); R_2· (singly charged anionic radicals obtained by addition of OH· to pyrimidine base anions in alkaline solutions or by reaction of R_1· with OH^-); R_3· (doubly charged anionic radicals obtained by addition of $O\cdot^-$ to pyrimidine base anions in strongly alkaline solutions, or reaction of R_2· with OH^-); and R_4· (substituted methyl radicals, RCH_2·, formed by reaction of $O\cdot^-$ with the methyl group of thymine or 5-methylcytosine anions). Specific structures are suggested for the radicals for each of the pyrimidine bases, and spectra are tentatively assigned to these structures. Long-term radical decay is frequently obscured by formation of intermediate and permanent absorbing products. Available data are consistent with decay by second-order processes with $2k \sim 2 \times 10^9\ M^{-1}\ \text{sec}^{-1}$ at pH 7, and somewhat less at higher pH.

In previous papers[4] we have pointed out the importance of studies of the radiation chemistry of nucleic acid constituents to radiobiology, and the contribution which such studies make to the chemistry of heterocyclic free radicals. We have also discussed the structure and reactivity of free radicals formed by hydrated electron (e_{aq}^-), hydrogen atom (H·), and hydroxyl free radical (OH·) attack on thymine, and have investigated an extremely short-lived transient absorbance in irradiated cytosine solutions. This transient was shown to be caused by a rapid reaction between OH^- ion and the free radical formed by addition of OH· to cytosine. In this paper we report further pulse radiolysis studies of the reaction of OH· with pyrimidine bases. This reaction is the predominant initial step in radiation-induced pyrimidine base destruction in air-saturated aqueous media,[5] and possibly in biological media as well. It is readily studied in N_2O-saturated solutions. N_2O converts e_{aq}^- to OH·, approximately doubling the yield of OH· resulting from radiolysis of water. The bases (uracil (U), thymine (T), cytosine (C), and 5-methylcytosine (MC)) were selected to permit determination of whether the substituents on the C-4 and C-5 positions of the pyrimidine ring alter the spectra and reactivity of the radicals. Experiments were carried out in alkaline as well as neutral solutions because steady-state work has shown that the radiation chemistry of pyrimidine bases changes as the pH during irradiation is increased from 7 to 14. Yields for disappearance of the 5,6 double bond of the 5-methyl compounds (T and MC) drop from about 2.6 at pH 7 to approximately 0 at pH 14. With the 5-hydro compounds (U and C) they show much less variation.[6] These changes are attributable in part at least to differences in base structure and to ionization of the bases and OH· as acids. Choice of the particular pH values reported on here was based on earlier pulse radiolysis experiments which showed that the spectra of radicals formed by thymine in N_2O-saturated solution at pH 11.4 and 12.4 are different.[4c]

It is convenient to consider the results presented below as having three kinetic components.

(a) Radical Formation. This stage is complete in at most 2 μsec, and need not be considered further.

(b) Adjustment of the Initial Radicals to Conditions in the Solution. These reactions give changes in radical structure without reducing the number of radicals.

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(2) University of California, Los Angeles, Calif.

(3) Gulf General Atomic, Inc.

(4) (a) L. S. Myers, Jr., and L. M. Theard, *J. Amer. Chem. Soc.*, **92**, 2868 (1970); (b) L. S. Myers, Jr., A. Warnick, M. L. Hollis, J. D. Zimbrick, L. M. Theard, and F. C. Peterson, *ibid.*, **92**, 2871 (1970); (c) L. S. Myers, Jr., M. L. Hollis, and L. M. Theard, "Radiation Chemistry," Advances in Chemistry Series, No. 81, American Chemical Society, Washington, D. C., 1968, pp 345 ff.

(5) (a) J. J. Weiss, *Progr. Nucl. Acid Res. Mol. Biol.*, **3**, 103 (1964); (b) G. Scholes, *Progr. Biophys. Biophys. Chem.*, **13**, 59 (1963).

(6) (a) L. S. Myers, Jr., J. F. Ward, W. T. Tsukamoto, D. E. Holmes, and J. R. Julca, *Science*, **148**, 1234 (1965); (b) L. S. Myers, Jr., J. F. Ward, W. T. Tsukamoto, and D. E. Holmes, *Nature*, **208**, 1086 (1965); (c) G. Scholes, J. F. Ward, and J. Weiss, *J. Mol. Biol.*, **2**, 379 (1960); (d) N. S. Ranadive, K. S. Korganoker, and M. B. Sahasrabudhe, *Proc. Int. Conf. Peaceful Uses At. Energy, 2nd, 1955*, 299 (1956).

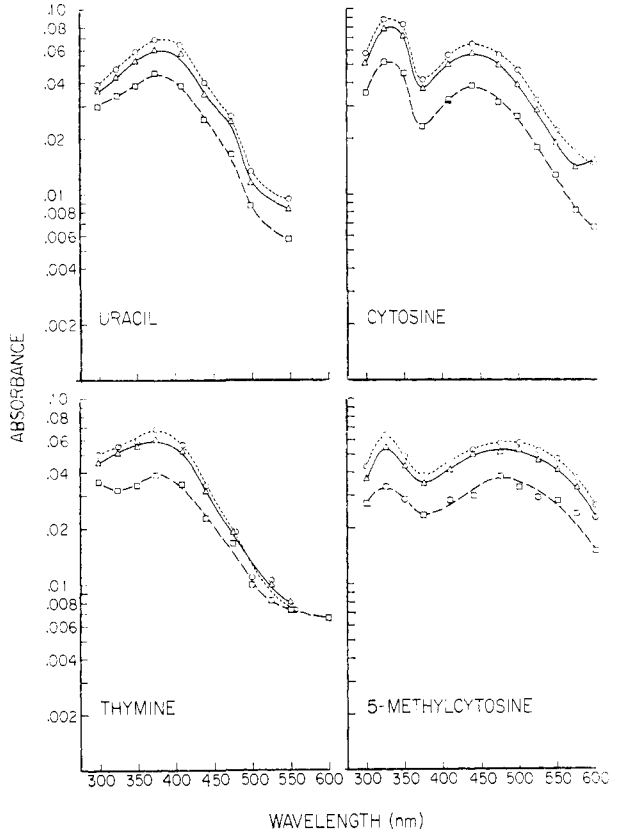


Figure 1. Absorption spectra of radicals formed by reaction of OH· with pyrimidine bases in N_2O-saturated 5×10^{-4} M aqueous solutions, pH 7. Time after the pulse in microseconds: ····○····, 4; —△—, 14; – –□– –, 70 (50 for uracil).

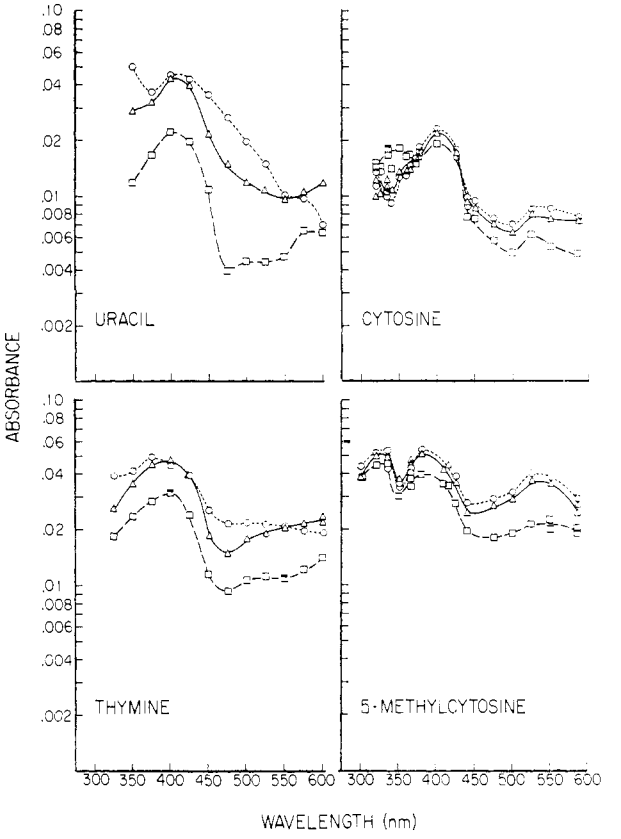


Figure 2. Absorption spectra of radicals formed by reaction of OH· with pyrimidine bases in N_2O-saturated 5×10^{-4} M aqueous solution, pH 11.4. Time after the pulse in microseconds: ····○····, 4; —△—, 14; – –□– –, 70 for C and MC, 80 for U and T.

They may be intramolecular, such as dissociation of a thermally unstable radical, or may involve reaction with a component of the solvent such as H^+, OH^-, or water. They may be very fast because of a large concentration of reactant in spite of a moderately small rate constant, and are frequently pseudo-first-order. An excellent example is the above-mentioned reaction between OH^- and the cytosine–OH· adduct radical. This reaction has a half-life of about 0.5 μsec at pH 11.7 and it causes large changes in the absorption spectrum but no radical decay.

(c) **Radical Decay**. These reactions involve radical combination reactions which reduce the number of radicals. There are at least two reports[4a,7b] that radicals of the kind we are investigating react by radical–radical reactions with a second-order rate constant of about 2×10^9 M^{-1}sec^{-1} ("normal" decay). The present work results in a similar value. This value gives a first half-life of about 100 μsec for radical decay after a 1000-rad pulse ($\sim 5 \times 10^{-6}$ M radicals). Radical decay reactions do not result in a change in shape of the spectrum unless an absorbing (colored) intermediate or final product is formed.

It follows that large changes in shape of spectra of radicals in times appreciably less than 100 μsec are very likely the result of reactions described in paragraph b. Much of the emphasis in this work is on this kind of reaction.

Several pulse radiolysis studies related to this work have appeared recently.[7] Earlier work is cited in references 4c and 7a.

Experimental Section

The procedures used in this work have been described previously.[4a] N_2O-saturated aqueous solutions of pyrimidine bases (5×10^{-4} M) were exposed to short pulses of 10-MeV electrons, and changes in optical absorbance were determined from photographs of oscilloscope tracings showing the output of a photomultiplier tube as a function of time. Commercially available compounds (thymine was recrystallized) and triply distilled water were used. The pH was adjusted with dilute NaOH and H_2SO_4 solutions. Fresh solution was put into the sample cell for each pulse. Radiation exposure conditions are given in Table I. The radiation dose was determined for each pulse by collecting the electrons on an aluminum block and reading the charge on an electrometer calibrated by comparison with hydrated electron absorbance. Justi-

Table I. Radiation Exposure Conditions

Compound	pH	Pulse length (nsec)	Dose/pulse (10^3 rads)
All bases	7	4	1.0–1.3
Thymine	11.4, 12.4	100	0.9–1.4
Uracil	11.4, 12.4	500	1.2–1.5
Cytosine, 5-Methylcytosine[a]	11.4, 12.6	10	0.9–1.3
5-Methylcytosine[b]	12.6	4	1.2–2.2

[a] Spectra. [b] Rate data.

(7) (a) G. Scholes and R. L. Willson, *Trans. Faraday Soc.*, **63**, 2983 (1967); (b) R. M. Danziger, E. Hayon, and M. E. Langmuir, *J. Phys. Chem.*, **72**, 3482 (1968); (c) C. I. Greenstock, M. Ng, and J. W. Hunt, "Radiation Chemistry," Advances in Chemistry Series, No. 81, American Chemical Society, Washington, D. C., 1968, p 397; (d) J. F. Ward and I. Kuo, *ibid.*, p 368; (e) G. Scholes, R. L. Willson, and M. Ebert, *Chem. Commun.*, 17 (1969).

fication for the assumption that reactions of OH· account for ~90% of the organic radicals formed, and that the remaining 10% formed by reactions of H· do not distort the results greatly is given in ref 4b.

Steady-state experiments were done by exposing 10-ml volumes of 5×10^{-4} *M* solutions, saturated with N_2O, to ^{60}Co γ rays (40,000 rads) and determining absorbance with a recording spectrophotometer (1-cm cell). Samples were exposed to air during the latter step, but this was long after radiation-produced radicals would have reacted.

Absorption spectra are plotted on semilogarithmic coordinates to make it easy to recognize changes in spectral shape (and radical structure) in the presence of radical decay. If only one kind of radical is present, and it decays to transparent products, the entire spectrum moves downward on the graph without change of form. Any change in form indicates a change in composition. Errors in measurements of low absorbances appear exaggerated, and intense absorption maxima appear deemphasized as compared with the usual appearance of spectra on linear coordinates.

Results

Absorption spectra of radicals present at various times after pulse radiolysis of N_2O-saturated aqueous solutions of pyrimidine bases, pH 7, 11.4, and 12.4 (U and T) or 12.6 (C and MC), are given in Figures 1, 2, and 3, and the wavelengths of maxima are tabulated in Table II. The time for the earliest spectra, 4 μsec after the pulse, was chosen to avoid the short-lived transient absorbance in cytosine solutions at pH 11.4.[4b] The 14-μsec time was selected because several of the other systems undergo fairly rapid changes which are nearly complete at the end of this period, and the spectra appear fairly well stabilized. The latest spectra are for the longest times at which absorbances could be measured on the same oscillograms used to obtain some of the earlier points. The times are long enough for spectral changes caused by reactions other than radical decay to be obvious.

Table II. Wavelengths of Absorption Maxima of Pyrimidine Bases and of Radicals Formed by their Reactions with Hydroxyl Free Radical at pH 7, 11.4, and ~12.5

Compound	Time after pulse, μsec	Wavelength of maxima (nm) pH 7	pH 11.4	pH ~12.5
Uracil	Unirradiated	257	283	283
Radicals from U	4	375	<350	<325
			400	420
				>600
	80		400	
			>525	
Thymine	Unirradiated	265	288	288
Radicals from T	4	375	<325	<325
			375	390
				560
	80		400	
			>550	
Cytosine	Unirradiated	265	270	279
Radicals from C	4	335	<325	<300
		440	400	385
			>500	550
	70		350	
			400	
			>500	
5-Methylcytosine	Unirradiated	272	275	285
Radicals from MC	4	325	330	220
		485	380	380
			525	525
	70		330	
			360	
			535	

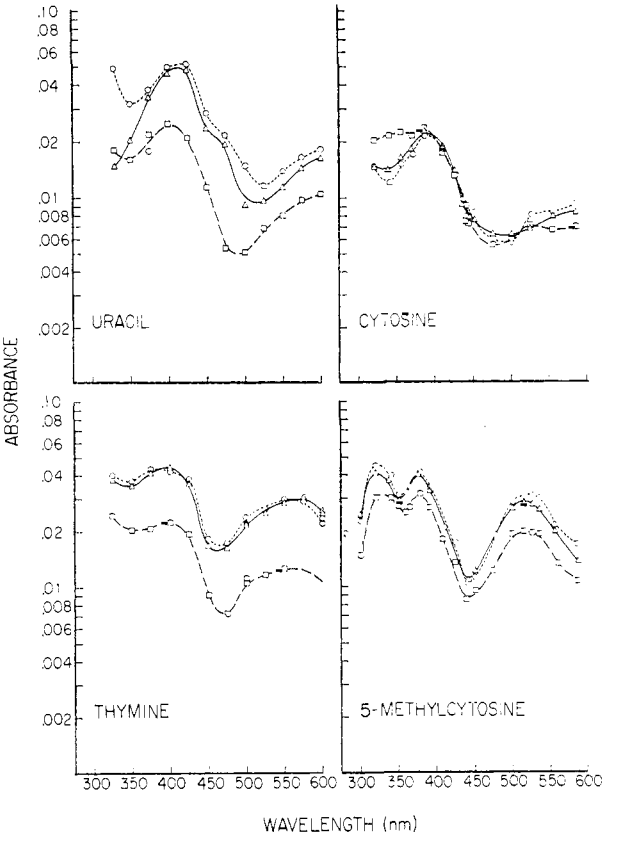


Figure 3. Absorption spectra of radicals formed by reaction of O· and OH· with pyrimidine bases in N_2O-saturated 5×10^{-4} *M* aqueous solution, pH 12.4 or 12.6. Time after pulse in microseconds: ····O····, 4; —△—, 14; – -□- –, 60 for C, 70 for MC, 80 for U and T.

Comparison of the spectra show the following: At pH 7 (Figure 1 and Table II) (1) the substituent on C-5 (H or CH_3) has little effect on the chromophoric group of the radicals (*cf.* U *vs.* T, C *vs.* MC). (2) The substituent on C-4 (=O or $-NH_2$) has a large effect on the chromophoric group of the radicals. Replacement of an oxo by an amino group, although it has little effect on spectra of the parent compounds (λ_{max} T = 265, MC = 272 nm), shifts the spectra of the radicals about 100 nm toward the red. (3) The radicals decay, except for T, with little change in spectral shape. Even with T the shape change is small compared with some of the changes observed at higher pHs. (4) The fractional decays of the absorption maxima are about the same with all four compounds, and are consistent with half-lives of the order of 100 μsec. This and the previous observation are consistent with decay by second-order radical–radical reactions, and little reaction with the solvent.

At pH 12.5 (Figure 3 and Table II) (1) all spectra are different from the corresponding spectra obtained at pH 7. (2) The substituent on C-5 (H or CH_3), in contrast to the result at pH 7, has a dominant affect on the spectrum. The compounds with a 5-methyl group give spectra with a pronounced maximum at 525–550 nm. (3) The change from a 4-oxo to a 4-amino group has relatively little affect on the chromophoric group. (4) Radicals from T and MC decay

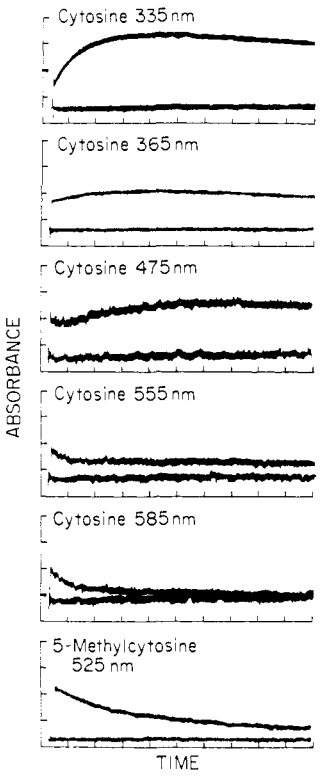


Figure 4. Oscillograms obtained on pulse radiolysis of cytosine and 5-methylcytosine solutions, pH 12.6. The horizontal scale is 100 μsec/div for cytosine, 50 μsec/div for 5-methylcytosine.

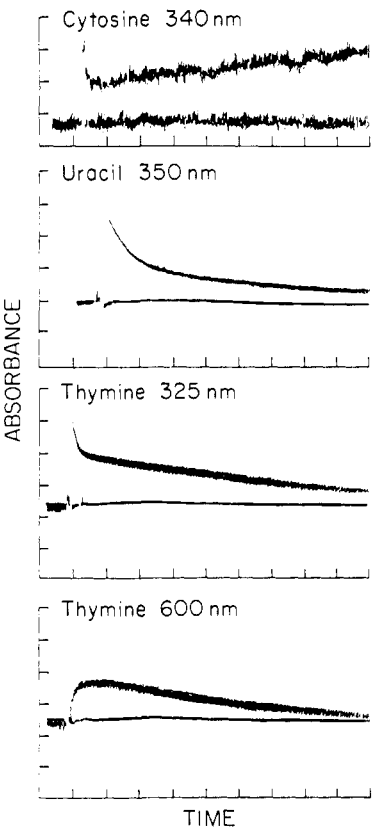


Figure 5. Oscillograms showing rapid changes in absorbance during the first few microseconds after a pulse of about 1000 rads, pH 11.4. The time scale for C is 10 μsec/div, for U and T 20 μsec/div.

with little change in spectral shape, but those of U and C show significant changes. Secondary reactions giving absorbing products must be occurring and they are more prominent in 5-hydro than 5-methyl compounds. (5) The fractional decays of U and T radicals are about the same, and consistent with half-lives of the order of 100 μsec, but the decay of absorbance in solutions of the amino compounds, MC, and particularly C, is much slower. Moreover, the spectrum obtained with C grows at short wavelengths. This is illustrated in the oscillograms of Figure 4 which cover a period of about 950 μsec for C, and 475 for MC.

At pH 11.4 (1) the 4-μsec spectra, except for the one given by C, are different from the ones obtained at pH 7 or ~12.5. (2) Large rapid decreases in absorbance during the first 14-μsec period occur in the U and T spectra at short wavelengths and at ~475 nm. These indicate reactions with the solvent or intramolecular reactions. The decreases are illustrated for specific wavelengths in the oscillograms (Figure 5). The rapid decay shown for cytosine is complete within 4 μsec and hence does not show in the spectra of Figure 2. The early thymine and uracil absorbance decays more slowly, but still faster than "normal" decay due to radical–radical reactions. (3) Because of the rapid decays the 4-μsec spectra at pH 11.4 are not the spectra of the initial products formed by the pyrimidine base + OH· reaction, but contain large components from secondary products. This is not true of the spectra at pH 7 and ~12.5. Unless reactions occur with a half-life shorter than the system rise time (300 nsec) these latter spectra can be attributed to the initial products.

Table III gives information from steady-state experiments about the formation of stable products which may interfere with observation of radical decay in pulse radiolysis. Absorbances per 1000 rads were computed from measurements at 40,000 rads assuming a linear relationship between dose and absorbance. This procedure results in an inconsequential underestimate of the absorbance. Many of the values at wavelengths of 350 nm and below are comparable to the absorbances

Table III. Absorbances of Stable Irradiation Products in N_2O-Saturated Aqueous Solutions of Pyrimidine Bases

Compound	Wavelength (nm)	Absorbance (1000 rads, 8-cm light path) pH 7.0	pH ~12.5
Uracil	300	0.020	Very high
	325	0.009	0.006
	350	0.002	0.001
	375	0.001	<0.001
	400	<0.001	<0.001
Thymine	300	0.005	Very high
	325	<0.001	0.024
	350	<0.001	<0.001
	375	<0.001	<0.001
	400	<0.001	<0.001
Cytosine	300	0.028	Very high
	325	0.025	0.027
	350	0.012	0.012
	375	0.006	0.009
	400	0.003	0.006
5-Methylcytosine	300	0.074	Very high
	325	0.010	0.014
	350	0.002	0.004
	375	<0.001	0.002
	400	<0.001	0.001

Table IV. Decay of Transient Absorbance, pH 7

Compd	λ (nm)	Dose[a]	Absorbance[b] Initial	At 1600 μsec	$2k/\epsilon$ (10^6 sec^{-1})	$2k$ (10^9 M^{-1} sec^{-1})	Duration[c] (μsec)
Uracil	408	1170	0.074	0.006	1.45	2.1	600
Thymine	375	1080	0.075	0.002	1.52	2.2	>1200
Cytosine	325	1130	0.093	0.024	See text		None
	375	1080	0.045	0.007	See text		None
	475	1120	0.066	0.002	2.00	2.5	>500
	550	1080	0.028	0.002	4.14	2.2	>500
5-Methylcytosine	325	1200	0.076	0.002	1.56	2.2	>800
	375	1170	0.047	0.002	1.36	1.2	>800
	475	1180	0.069	0.002	2.05	2.3	>600
	575	1250	0.048	0.005	1.52	1.2	200

[a] Rads per pulse. [b] 8-cm light path. [c] Duration of linear relationship between 1/OD and time.

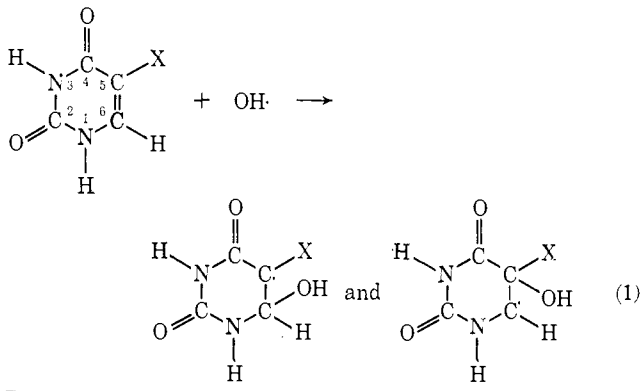
in the spectra, Figures 1, 2, and 3. The entries "very high" at pH ~12.5 mean absorbances of 0.2–0.4. They are difficult to determine accurately because the absorption spectra of both control and irradiated solutions rise abruptly at wavelengths near 300 nm.

Second-order rate constants for decay of absorbance over periods up to 1600 μsec at pH 7 are given in Table IV for selected wavelengths. Values of $2k$ assume an initial yield of radicals of 5.8×10^{-6} M per 1000 rads. Absorbance decay at the higher pH's is generally not second order.

Discussion

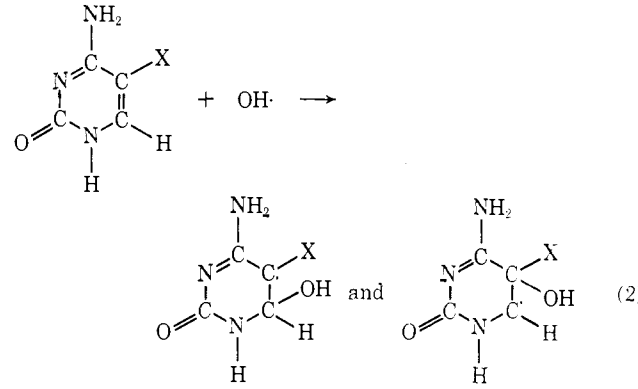
Radical Formation and Stabilization. In this section we suggest (a) reactions for formation of radicals, (b) tentative assignments of spectra to specific radical structures, and (c) reactions for the interactions between radicals and the solvent. These are based in part on existing information on the steady-state radiation chemistry of pyrimidine bases.

At pH 7, hydroxyl free radicals and the pyrimidine bases used in this work are uncharged. Product analyses,[8] results of epr measurements on radicals generated chemically in solution,[9] and general considerations about radical reactivity give convincing evidence that the spectra of Figure 1 can be attributed to radicals formed by addition of OH· to the 5,6-carbon–carbon double bond of the bases. This reaction with uracil (X = H) or thymine (X = CH_3) gives neutral radical products such as the 5,6-dihydrouracil-5-yl and 5,6-dihydrouracil-6-yl radicals



(1)

Analogous addition reactions occur with cytosine (X = H) and 5-methylcytosine (X = CH_3)



(2)

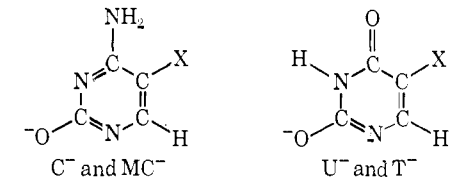
These products are referred to as type R_1· radicals in the subsequent discussion. The proportions of 5-yl and 6-yl radicals are unknown and probably are different with the different compounds. Reactions of OH· with the amino, methyl, or oxo groups are at most minor reactions. The decay of the spectra (Figure 1) without significant change in shape for 50–70 μsec indicates that at pH 7 R_1· radicals do not undergo reaction with the solvent during the period considered.

In the alkaline solutions both the pyrimidine bases and the hydroxyl free radical are partially ionized (Table V).

Table V. Fraction of Reactants in Anionic Form

Substance	pK	Fraction ionized pH 11.4	pH 12.4	pH 12.6
C	12.2	0.14		0.70
MC	12.4	0.09		0.61
U	9.5	~1.0	~1.0	
T	9.8	~1.0	~1.0	
OH·	11.9	0.24	0.76	0.83

Structures of the anions (X = H for C^- and U^-, CH_3 for MC^- and T^-) follow



C^- and MC^- U^- and T^-

Relatively little is known about the products of reactions involving the anionic species, and therefore definite

(8) M. N. Khattack and J. H. Green, *Intern. J. Rad. Biol.*, **11**, 131, 137, 577 (1966). These authors refer to earlier papers.

(9) (a) M. G. Ormerod and B. B. Singh, *ibid.*, **10**, 533 (1966); (b) J. F. Ward, A. A. Al-Thannon, and L. S. Myers, Jr., unpublished results; (c) Cl. Nicolau, M. McMillen, and R. O. C. Norman, *Biochem. Biophys. Acta*, **174**, 413 (1969).

assignment of the spectra observed in alkaline solutions (Figures 2 and 3) to specific radical structures cannot be as well supported as at pH 7. The assignments suggested below are based largely on evidence that in very strongly alkaline air-saturated solutions (pH 14), in which the initial reactants are $O\cdot^-$ and the anions of the pyrimidine bases (a) radiolysis of T gives significant yields of hydroxymethyluracil (the 5-CH_3 group is oxidized), (b) radiolysis of MC and T gives small chromophore disappearance yields, (conjugation of the 5,6 double bond with other unsaturated groups is not changed), and (c) radiolysis of U and C gives chromophore disappearance yields which are about the same as those observed at neutral pH (the 5,6 double bond is saturated).[6] The results are much more complex in alkaline than in neutral solutions, and are different for each compound. Accordingly each compound is discussed separately below.

Cytosine. At pH 12.6 (Figure 3) the spectrum is different from the one at pH 7, showing that a different radical, $R_2\cdot$, is formed. Decay occurs only at long wavelengths. Apparent lack of decay at other wavelengths is caused by a growing absorbance of a permanent product (Table III and Figure 4). Growth is shown in the spectra at wavelengths of 325–400, and in the oscillograms at all but the longest wavelengths for nearly 500 μsec. There are no rapid changes in absorbance indicative of reactions of $R_2\cdot$ with the solvent.

At pH 11.4 (Figure 2) the 4-μsec spectrum is similar to the spectrum obtained at pH 12.6, showing that $R_2\cdot$ is present. Earlier pH 11.4 spectra however, as discussed in a previous paper,[4b] show that the 4-μsec spectrum is not the spectrum of the initial product(s). Instead, the initial (1 μsec) spectrum is the spectrum of a mixture of $R_1\cdot$ and $R_2\cdot$ radicals formed in nearly equal amounts. The shape of the early spectrum changes to that of the 4-μsec spectrum with a half-life of about 1 μsec by a reaction of $R_1\cdot$ radicals with OH^- ion. The proportion of $R_2\cdot$ formed initially was found to increase as the pH is increased from 10.8 to 11.8.

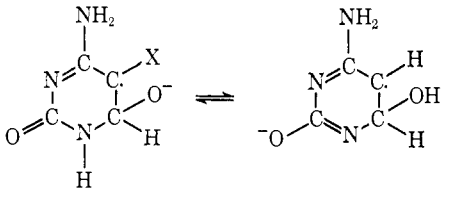
A scheme for radical formation and reaction with the solvent consistent with these observations, the data of Table V, and the chemistry referred to follows: (a) at pH 7 formation of $R_1\cdot$ by reaction 2 above; (b) at pH 12.6 formation of $R_2\cdot$ by

$$C^- \text{ (or C)} + OH\cdot \text{ (or } O\cdot^-) \longrightarrow R_2\cdot \qquad (3)$$

and (c) at pH 11.4 formation of $R_1\cdot$ and $R_2\cdot$ by reactions 2 and 3, followed by reaction 4

$$R_1\cdot + OH^- \longrightarrow R_2\cdot + H_2O \qquad (4)$$

The structure of $R_1\cdot$ is discussed above. The structure of $R_2\cdot$ is believed to be the pair of tautomeric singly charged anion radicals, 5-yl (shown below) or 6-yl, formed by addition of $O\cdot^-$ to C, or of $OH\cdot$ to C^- (reaction 3), or by reaction 4. The ratio of the tautomers at equilibrium is unknown.



(5)

The relative proportions of reactions 2 and 3 will vary with the pH, and will depend on both the degree of dissociation of the reactants at the particular pH, and the rate constants for the reactions. The rate of reaction 4 varies with the pH.

Suggested assignments of spectra to these radical structures are included in Table VI.

Table VI. Assignment of Spectra to Radicals Formed by Reaction of $OH\cdot$ or $O\cdot^-$ with Pyrimidine Bases

Spectrum Figure	pH	Compd	Time after pulse (μsec)	Radical	Type
1	7	U, C, MC, T	0–70	$R_1\cdot$	Neutral $OH\cdot$ adduct
2	11.4	C	4	$R_2\cdot$	Single negative charge
3	12.6	C	4	$R_2\cdot$	Single negative charge
2	11.4	U, T	<4	$R_2\cdot$	Single negative charge
2	11.4	U	80	$R_3\cdot$	Double negative charge
3	12.4	U	0–80	$R_3\cdot$	Double negative charge
2	11.4	T	80	$T_3\cdot$	Double negative charge
3	12.4	T	0–80	$R_4\cdot$	$R\text{–}CH_2\cdot$
3	12.6	MC	0–80	$R_4\cdot$	$R\text{–}CH_2\cdot$

Uracil. At pH 12.4 the 4-μsec spectrum differs from the pH 7 spectrum, and a rapid decay at 325 nm indicates that the initial radical reacts with the solvent.

At pH 11.4 the spectrum at 4 μsec after the pulse differs from either the pH 7 or 12.4 spectrum. From inspection of the oscillograms (*cf.* Figure 5, U), although decay is too rapid for quantitative readings, it is obvious that the initial pH 11.4 spectrum would differ more than appears from the 4-μsec spectrum shown. Thus a different radical is formed initially at each of the pH's investigated.

A rapid decay at short wavelengths and at ~500 nm changes the shape of the spectrum at pH 11.4 so that at 80 μsec after the pulse the spectrum is almost identical with the 80-μsec spectrum at pH 12.4. The rapid decay at short wavelengths follows pseudo-first-order kinetics and the half-life is inversely proportional to OH^- ion concentration over a 10-fold concentration range (Table VII). The slight decrease in rate constant at high pH

Table VII. Decay of Short-Lived Transient in Uracil Solutions

pH	Wavelength (nm)	$t_{1/2}$ (μsec)	k (10^7 M^{-1} sec^{-1})
11.6	330	9.4	1.8
11.9	330	4.9	1.8
12.2	330	3.2	1.4
12.4	330	2.9	1.2
	340	1.8	1.5

may be caused by an increasing rate of growth of a permanent product. The difference in values at 330 and 340 nm is consistent with this assumption. The kinetic behavior of this transient is similar to that of the short-lived transient absorbance discussed pre-

viously in detail for cytosine.[4b] However, the spectral changes are different, in that with uracil the initial spectrum at pH 11.4 is not like the spectrum at pH 7. A reaction scheme consistent with the above follows.

At pH 7

$$U + OH\cdot \longrightarrow R_1\cdot \quad (1)$$

At pH 11.4

$$U^- + OH\cdot \longrightarrow R_2\cdot \quad (6)$$

$$R_2\cdot + OH^- \longrightarrow R_3\cdot \quad (7)$$

At pH 12.4

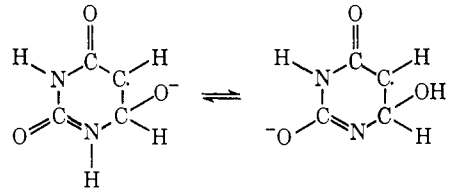
$$U^- + OH\cdot \longrightarrow R_2\cdot$$

$$R_2\cdot + OH^- \longrightarrow R\cdot_3$$

$$U^- + O\cdot^- \longrightarrow R_3\cdot \quad (8)$$

This scheme differs from the scheme for cytosine in two respects: (a) at pH 11.4 U is in the anionic form, reaction 1 does not occur, and no $R_1\cdot$ can be formed; (b) the radical undergoing reaction with OH^- ion, $R_2\cdot$, is analogous to the product formed when the cytosine radical, $R_1\cdot$ reacts with OH^-, and the product, $R_3\cdot$, is a species with no analog in cytosine solutions.

The structure of $R_2\cdot$ is believed to be analogous to that of the $R_2\cdot$ radical proposed for cytosine: a pair of tautomeric singly charged anion radicals. The $R_3\cdot$



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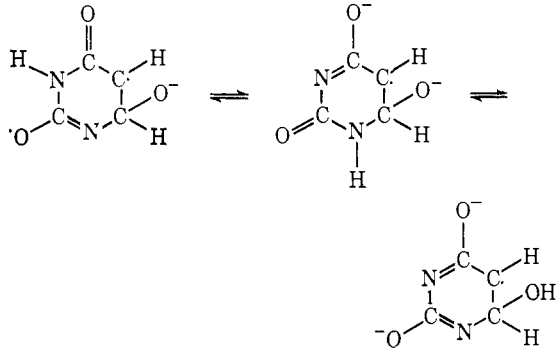
radical is believed to be a doubly charged anion radical which has three possible tautomeric structures. Cor-



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responding 6-yl forms are also plausible. A second stage of ionization of the uracil–OH adduct radical is expected. Uracil itself undergoes ionization to a doubly charged anion with a pK of about 13. Addition of OH· to uracil should lower the pK.

As with cytosine, at intermediate pHs between 7 and 12.4 the proportions of reactions 1, 6, and 8, and the rate of reaction 7 will depend on pH.

See Table VI for assignment of spectra.

Thymine. At pH 12.4 the spectrum is different from any other spectrum given by U or T. Decay is "normal" and no changes in spectral shape occur.

At pH 11.4 the early spectrum undergoes rapid decay at short wavelengths and at ~450 nm to give a spectrum very much like the 80-μsec spectra given by uracil at pH 11.4 and 12.4.

A scheme for radical formation and reaction with the solvent consistent with these observation follows.

At pH 7

$$T + OH\cdot \longrightarrow R_1\cdot \quad (1)$$

At pH 11.4

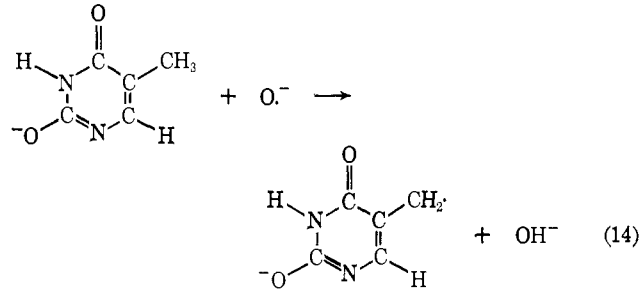
$$T^- + OH\cdot \longrightarrow R_2\cdot \quad (11)$$

$$R_2\cdot + OH^- \longrightarrow R_3\cdot \quad (12)$$

At pH 12.4

$$T^- + O\cdot^- \longrightarrow R_4\cdot \quad (13)$$

The reactions for formations of radicals from T are, with one exception, analogous to reactions of U. The ionization of thymine and uracil are similar, and radicals $R_1\cdot$, $R_2\cdot$, and $R_3\cdot$ have the same structure except for the 5-CH_3 group on the thymine radical analogs. The $T^- + O\cdot^-$ reaction analogous to 8 is probably slow because of charge repulsion, and might not compete successfully with the following alternative reaction at pH 12.4.



(14)

This reaction, which is impossible with uracil, results in formation of a substituted methyl radical, $RCH_2\cdot$; and accounts for formation of 5-hydroxymethyluracil during irradiation of alkaline thymine solutions. Presence of a maximum at ~550 nm in the spectrum of the two 5-methyl compounds (T and MC) but not the 5-hydro compounds is strong evidence that different types of radicals are formed because 5-hydro and 5-methyl groups are not ordinarily important parts of a chromophoric group.

See Table VI for assignment of spectra.

5-Methylcytosine. At pH 12.6 the spectrum differs from the spectrum given by cytosine in the same way that the uracil and thymine radical spectra differ.

At pH 11.4 the 4-μsec spectrum, unlike spectra of the other radicals, does not undergo rapid changes. It is not like the spectrum obtained at pH 7, and at no time through 70 μsec does it resemble the entire pH 12.6 spectrum. It does have the same absorption maxima as are observed at pH 12.6, however, and the broad long-wavelength maximum of the pH 7 spectrum, which suggests that the absorbing species include both those present at pH 7 and at pH 12.6. The spectrum is not a simple combination in any proportion of the pH 7 and 12.6 spectra because the absorbance at 375 nm is too high. It seems that two absorbing species whose proportions depend on pH are present. Any reaction with OH^- is either very fast, within the rise time of the pulse radiolysis equipment, or slow. The flattening of the spectrum at long wavelengths (*cf.* T spectrum at pH 11.4) may be due to this reaction, producing an $R_2\cdot$ type radical.

The following relatively complex scheme appears to fit the observations

At pH 7

$$MC + OH\cdot \longrightarrow R_1\cdot \quad (2)$$

At pH 11.4

$$MC + OH \longrightarrow R_1\cdot$$

$$R_1\cdot + OH^- \longrightarrow R_2\cdot \text{ (slow)} \quad (15)$$

$$MC\cdot^- + OH\cdot \longrightarrow R_2\cdot \quad (16)$$

$$MC + O\cdot^- \longrightarrow R_2\cdot \quad (17)$$

$$MC^- + O\cdot^- \longrightarrow R_4\cdot \text{ (small yield)} \quad (18)$$

At pH 12.6

$$MC^- + OH\cdot \longrightarrow R_2\cdot$$

$$MC + O^- \longrightarrow R_2\cdot$$

$$MC^- + O^- \longrightarrow R_4\cdot \text{ (large yield)}$$

The structures of the radicals are analogous to those proposed for thymine. Spectra obtained with MC are assigned to $R_1\cdot$ and $R_4\cdot$ radicals (Table VI) but reactions at pH 11.4 are not understood well enough to merit assignments to $R_2\cdot$ and $R_3\cdot$.

Radical Decay. The question of the ultimate decay of radicals has not been investigated in detail. The limited evidence which we have is consistent with decay by expected radical–radical processes with rate constants in neutral solutions of $2k \approx 2 \times 10^9\ M^{-1}\ \text{sec}^{-1}$. In alkaline solutions the decay is somewhat slower in keeping with the formation of negatively charged ion radicals. Observation of long-term decay is frequently interfered with by secondary products which absorb significantly. The spectra of these products have maxima at $\lambda < 300$ nm. Their presence causes absorbance to decrease more slowly than radical concentration, or even to increase. Observations with the different compounds follow.

Absorbances observed with cytosine, pH 7, at 550 and 475 nm give linear plots of $1/A$ *vs.* t (A = absorbance) until they approach zero, and a value of $2.2 - 2.5 \times 10^9\ M^{-1}\ \text{sec}^{-1}$ for the second-order rate constant, $2k$ (Table V). At shorter wavelengths growth of a permanent product causes absorbance decay to stop when it reaches values very close to those in Table III (*cf.* Table IV, values of absorbance at 1600 μsec). Attempted correction for a daughter overcorrects the decay data, suggesting that the product is a tertiary product.

With uracil at pH 7, decay at 408 nm (near the absorption maximum) gives a linear $1/A$ plot *vs.* time for 600 μsec (Table IV). The second-order rate constant, $2k$, equals $2.1 \times 10^9\ M^{-1}\ \text{sec}^{-1}$, which is similar to previously referred to values. At times longer than 600 μsec, when the absorbance is only 11% of the initial, the rate of decay at 408 nm decreases and a long-lived product is observed which at 1600 μsec is 8% of the initial. The decay data do not agree with requirements for either a parent–daughter relation, or two absorbing products formed initially. The absorbance at 1600 μsec is larger than that of the permanent product in irradiated U solutions (Table III). We suggest that it is caused by a tertiary intermediate product of radical decay. Formation of such a product is much slower than formation of an immediate daughter.

At pH 11.4 and 12.4 decay kinetics are obviously complex and have not been established. Growth occurs at 325 nm (pH 12.4) for at least 100 μsec. In 66 μsec the absorbance increases 0.003, and is of the order of magnitude given in Table III for a permanent product. The apparent fractional decays as judged from average changes in spectra are consistent with second-order rate constants of the order of $10^9\ M^{-1}\ \text{sec}^{-1}$.

With thymine at pH 7 decay of absorbance at λ_{max} is uncomplicated and plots of $1/A$ *vs.* t are linear for 1200 μsec (Table IV).

With 5-methylcytosine at pH 7, the maxima give linear $1/A$ *vs.* t plots until absorbance disappears, and "normal" rate constants. Decay is slower at pH 12.6.