exerted on the triplet autoionization. The former reflects a balance of the effects on autoionization and deactivation of the excited-singlet and triplet states. If this picture is correct, then reaction 2 is not a one-stage process but proceeds through a higher excited triplet T_2 which then undergoes autoionization with some activation energy. Apparently by coincidence this activation energy is almost balanced by the activation energies of the deactivating processes, and therefore the overall photoionization of phenols in neutral solutions appears to be little affected by temperature.

For tyrosine $E(T_1) = 3.5$ eV[18] and the triplet–triplet transition displays a broad ill-defined band in the region 300–700 nm,[19] where tyrosine (S_0) hardly absorbs. Therefore most molecules in the T_2 state are produced with $E < 7.5$ eV above ground state. The ionization potential of phenol in the gas phase is 8.5 eV.[20] The effective IP in water is not known; a reduction of ~1 eV due to relatively fast polarization effects is in accordance with the foregoing discussion.

In the case of the phenolate anions, fast intersystem crossing (no fluorescence was observed[15]), low ionization potentials (the electron affinity of PhO^- is ~1.2 eV[21]), and relatively high triplet energies—these three factors account for the reaction proceeding by thermal ionization of their triplets. This combination of factors is not common, *e.g.*, at 337 nm β-naphtholate undergoes direct ionization from its S_1 state,[22] which is longer lived than the S_1 state of phenolate.

Acknowledgment. We are indebted to Professors M. Ottolenghi and U. K. Klaning for some valuable discussions and to Mrs. L. Dogliotti for carrying out some of the flash photolysis experiments. Partial financial support received from the Army Research Office, Durham, is gratefully acknowledged.

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Addition of Hydroxyl Radicals to Pyrimidine Bases and Electron Transfer Reactions of Intermediates to Quinones

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Abstract: The transient optical absorption spectra of the radicals produced from the reaction of hydroxyl radicals with pyrimidine bases in aqueous solution have been determined using the technique of pulse radiolysis. Uracil, thymine, cytosine, 1- and 3-methyluracil, 1,3-dimethyluracil, and dihydrouracil have been studied as a function of pH in order to examine the role of the tautomeric forms of the pyrimidine bases with regard to the site(s) of OH radical addition. The transient spectra obtained at pH ~11.0 in solutions of uracil, thymine, and 1- and 3-methyluracil were different from those observed in neutral solution. Examination of the change in initial absorbance at a fixed wavelength with pH revealed a "titration-type" curve from which a pK was derived. In all cases, the pK value obtained was identical with the pK_a^1 of the pyrimidine base used, indicating that the changes in the spectra are due to the tautomerization of the molecule and not to the acid–base properties of the OH-radical adducts to the pyrimidines. The transient spectrum of the radical produced from OH addition to 1,3-dimethyluracil was identical in neutral and alkaline solutions, supporting the above conclusion. With cytosine, the pK ~ 4.5 ± 0.1 obtained corresponded to the $pK_a^1 \sim 4.6$ for the proton dissociation of N_3. The observed red shift of the transient spectra in alkaline solution, compared to neutral solution, is probably due to the increased resonance energies of the lactim form *vis-à-vis* the lactam form of the pyrimidines radicals. From the decay kinetics of the intermediates, it is suggested that substitution at the N_1 position reduces the extent of ring opening which results from radical addition across the 5,6 double bond. The intermediates produced were found to react with menaquinone (vitamin K_3, $E^\circ = 0.42$ V) leading to the formation of the semiquinone radical anion MQ^-. The reaction rate constants for electron transfer to MQ were found to be relatively high, ~1–5 × 10^9 M^{-1} sec^{-1}, for the large number of bases examined. The efficiency of electron transfer, (*i.e.*, percentage) was found to be markedly dependent upon the nature of the pyrimidine and nucleotide examined, as well as on the tautomeric form. Increased efficiency of electron transfer occurs when the odd electron is resonating with ionic tautomeric forms of the pyrimidine radicals. Various explanations are offered for these results. The dissociation constant of the semiquinone radical of menaquinone was found to be 4.5 ± 0.1.

The study of the effect of high energy radiations on biochemicals has become a subject of increasing interest in recent years. The reactions of hydroxyl radicals, the main oxidizing species produced from the radiation chemistry of water, with various pyrimidine bases have been investigated using different fast-reaction techniques in order to establish the site(s) of attack on the pyrimidines and the effect of molecular

(1) (a) Pioneering Research Laboratory; (b) Radiation Biology Laboratory.

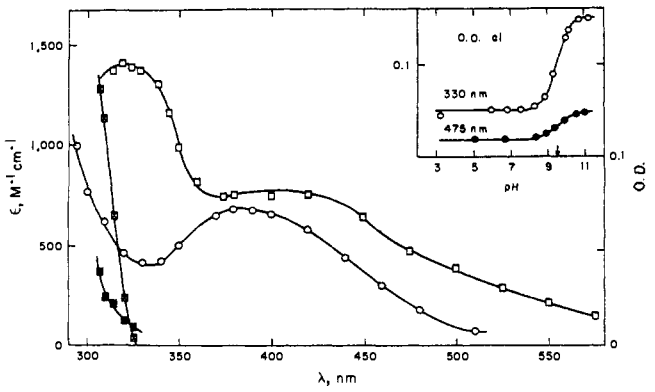

Figure 1. Absorption spectra of transients produced from the reaction of OH radicals with 1 mM uracil, N_2O (1 atm), at pH 3.15 (○) and pH 10.7 (□), read at ~0.1 μsec after pulse. Absorbance at pH 10.7 read at 100 μsec (■) and 10 msec (⊠) after the pulse, plotted as OD. Total dose ~8.6 krads/pulse. Insert: OD at 330 and 475 nm *vs.* pH.

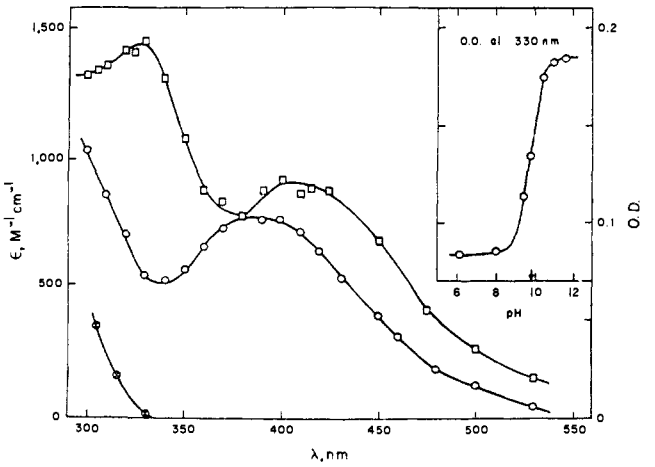

Figure 2. Absorption spectra of transients produced from the reaction of OH radicals with 1 mM 1-methyluracil, N_2O (1 atm), at pH 4.3 (○) and pH 10.9 (□), read at ~0.1 μsec after pulse. Absorption of permanent product at pH 4.3 (⊗). Total dose ~10.5 krads/pulse. Insert: OD at 330 nm *vs.* pH.

structure on these reactions. Pulse radiolysis[2-7] and esr[8-13] studies of the intermediates produced have been carried out. In the pulse radiolysis work the intermediates were usually observed at a time resolution of $\leqslant$0.5 μsec, while most of the esr work was carried out under steady-state conditions, except for the recent investigation[13] where the esr spectra of the radicals were determined at ~0.3 μsec after the electron pulse. In some of the esr studies, the OH radicals were produced by the photolysis of H_2O_2[11] or by the reaction of Ti^{3+} with H_2O_2.[8-10]

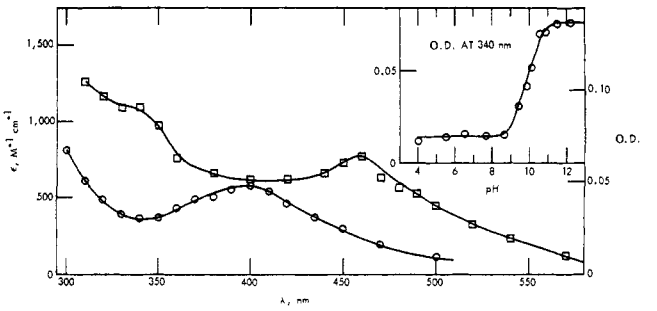

Figure 3. Absorption spectra of transients produced from the reaction of OH radicals with 1 mM 3-methyluracil, N_2O (1 atm), at pH 4.0 (○) and pH 11.0 (□), read at ~0.1 μsec after pulse. Insert: OD at 340 nm *vs.* pH.

All the results obtained with pyrimidines have been interpreted on the basis of addition of the OH radical across the 5,6 carbon–carbon double bond, at position 5 and (to a small extent) at position 6. Different transient optical absorption spectra were observed[2-7] in the pulse radiolysis of air-free aqueous solutions of uracil, thymine, and cytosine on varying the pH from ~6 to ~11–13, but no indication was provided as to whether this change was due to the tautomerization of these molecules or to the acid–base properties of the free-radical intermediates produced. Furthermore, some of the esr work was carried out in acid solutions only,[8-10] and in some cases[11,12] no radicals could be observed in either neutral or alkaline solutions.

This pulse radiolysis work was initiated with the object of studying systematically the effect of pH and the role of the tautomers of the pyrimidine bases with regard to OH-radical addition reactions. The importance of such an investigation lies in the possibility that mutations may arise from mispairing of the bases in DNA due to the occasional appearance of rare tautomers. The following pyrimidines were examined: uracil, thymine, cytosine, 1- and 3-methyluracil, 1,3-dimethyluracil, and dihydrouracil. In addition, following the recent interesting observation[14] that some of the intermediates produced from the reaction of OH radicals with various biochemicals transfer effectively an electron to quinones to form the semiquinone radical anions, the efficiency and rates of such electron transfers from the OH adducts to pyrimidines have been studied in detail.

Experimental Section

Since pulses of ~2.3-MeV electrons and ~30-nsec duration were used, as provided by the Febetron (Field Emission Corp.) 705 pulsed radiation source. Experimental details regarding the dosimetry, pulsed Xenon lamp monitoring light, and electronic circuitry have been described elsewhere.[15-17] Fresh solutions were used for each pulse, and photolysis of the pyrimidines and particularly menaquinone by the monitoring light was minimized by using a synchro-

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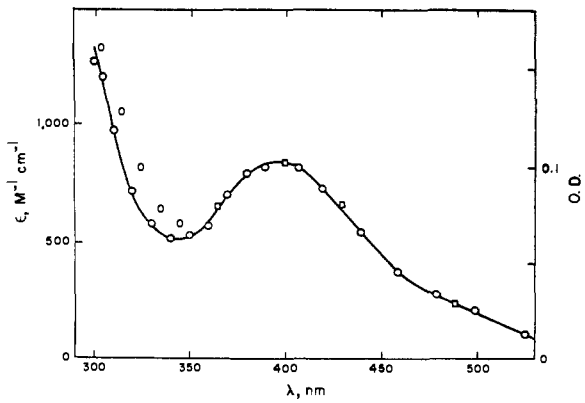

Figure 4. Absorption spectra of transients produced from the reaction of OH radicals with 1 m*M* 1,3-dimethyluracil, N_2O (1 atm), at pH 3.7 (○) and pH 10.7 (□). Total dose ~10 krads/pulse.

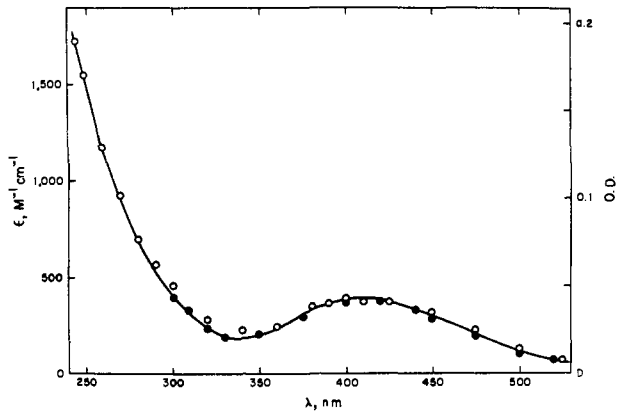

Figure 5. Absorption spectra of transients produced from the reaction of OH radicals with 2 m*M* dihydrouracil, N_2O (1 atm), at pH 6.8 (○), and of H atoms with 8 m*M* uracil at pH 1.0 (●), in the presence of 1.0 *M* *tert*-butyl alcohol. Total dose ~8.8 krads/pulse.

nized shutter (open for ~2.5-msec) and appropriate cut-off filters. The optical path of the cells was 2 cm.

Most of the pyrimidines used were supplied by Cyclochemicals, the nucleosides and menaquinone were supplied by Calbiochem, and 3-methylcytidine was supplied by Schwarz-Mann. The pH of the solutions was adjusted using perchloric acid, potassium hydroxide, sodium tetraborate (1–3 m*M*), and potassium phosphates (~3 m*M*). Solutions were prepared fresh just prior to radiolysis and were made alkaline in an oxygen-free medium.

Based on the published[18] rate constants for the reactions of e_{aq}^- and OH radicals with the pyrimidines used in this work, ~1 m*M* concentrations were used in order to convert >95% of e_{aq}^- into OH radicals by N_2O, and to scavenge all the hydroxyl radicals produced at the doses employed.

Extinction coefficients were derived taking $G(e_{aq}^-) = G(OH) = 2.8$, using a KCNS solution as a dosimeter. It should be pointed out that the extinction coefficients given in Table I and Figures 1–7 were calculated assuming that the OH radicals produce only *one* radical. This is probably not the case with most pyrimidines studied, and these values might be low by a factor of 2–3. Furthermore, the second-order decay of the intermediates might consequently be low by a factor of 2–3.

Results

Transient Spectra of OH Radical Adducts to Pyrimidines. Owing to the optical absorption of pyrimidines in aqueous solution, the transient spectra of the radicals produced in the pulse radiolysis of N_2O-saturated solutions could not be determined below ~300 nm

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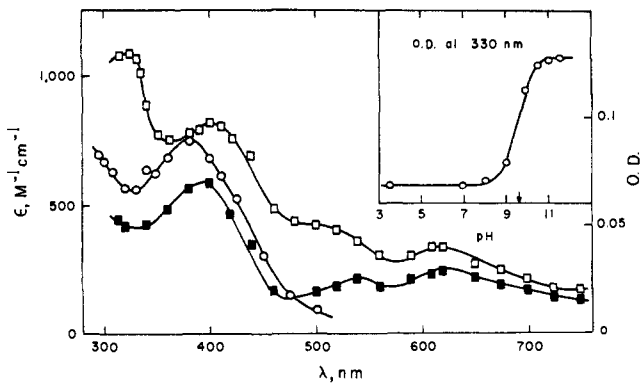

Figure 6. Absorption spectra of transients produced from the reaction of OH radicals with 1 m*M* thymine in aqueous solution, N_2O (1 atm), at pH 3.5 (○) and pH 11.1 (□), read at ~0.1 μsec after pulse. Absorbance at pH 11.1 (■) read at ~25 μsec after pulse. Total dose ~10 krads/pulse. Insert: OD at 330 nm *vs.* pH.

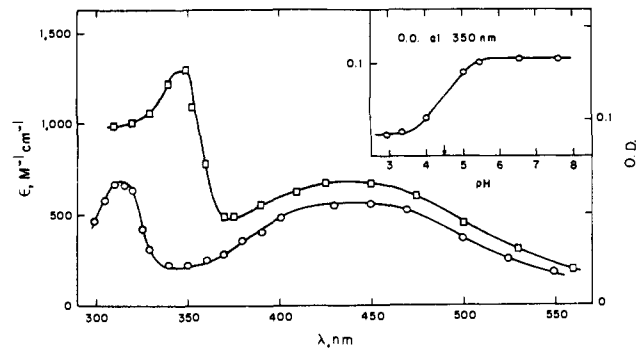

Figure 7. Absorbance spectra of transients produced from the reaction of OH radicals with 1 m*M* cytosine, N_2O (1 atm) at pH 3.05 (○) and pH 6.5 (□), read at ~0.1 μsec after pulse. Total dose ~7.8 krads/pulse. Insert: OD at 350 nm *vs.* pH.

in neutral solutions, and ~310 nm in alkaline solutions beyond the pK_a^1 of the pyrimidines. In all cases, the spectra were corrected for the change in transmission in the 300-nm region due to the disappearance of the pyrimidine molecules.

Uracil and Derivatives. The optical spectrum of the OH-radical adduct to uracil at pH 3.2 is shown in Figure 1. This spectrum is qualitatively similar to the one previously reported,[2] but the second band below ~330 nm was not observed in other studies.[4–6] The radicals decay by second-order kinetics (Table I). The reaction of OH radicals with the first tautomeric (monoanion) form of uracil ($pK_a^1 = 9.5$ and $pK_a^2 \sim 13.0$) was examined at pH 10.7 in order to avoid the formation of O^- radicals [pK ($OH \rightleftharpoons O^- + H^+$) ~ 11.9]. The 380-nm band appears to be shifted to ~400 nm, and another band with λ_{max} ~320 nm is observed (Figure 1), which decays by first-order kinetics with $k = 5.6 \times 10^4$ sec^{-1}. This probably indicates a ring opening of the uracil molecules, in agreement with the esr spectra obtained by Neta[12] under similar conditions. In addition, new long-lived intermediates are produced in alkaline solutions which start absorbing below about 330 nm (see Figure 1). Examination of the change in initial absorbance at 330 nm and at 475 nm with pH reveals a "titration-type" curve from which a $pK \sim 9.55 \pm 0.1$ can be derived (see insert, Figure 1). These results indicate that (a) the change with pH is apparently not due to the acid–base prop-

Table I. Absorption Maxima, Extinction Coefficients, and Decay and Dissociation Constants of Intermediates Produced from the Reaction of OH Radicals with Pyrimidine Bases in Aqueous Solution

Pyrimidine	Radical	pH	λ_{max}, nm	ϵ, M^{-1} cm^{-1} [a]	$2k/\epsilon$	$2k$, M^{-1} sec^{-1} [a]	pK_a^S	pK_a^R
Thymine	·TOH	6.7	<300	>700				
			380	750	9.5×10^5	7.1×10^8	9.9	9.6 ± 0.1
		11.0, 11.85	320	1100	1.6×10^5 sec^{-1} [b]		>13.0	
			400	800	3.8×10^5	3.0×10^8		
			620	330				
		11.85	390, 540, 620 [c]					
Uracil	·UOH	3.15	<295	>1000				
			380	700	1.0×10^6	7.0×10^8	9.5	9.55 ± 0.1
		11.0	320	1400	5.6×10^4 sec^{-1} [b]		13.0	
			400	800				
Dihydrouracil	·UH [d]	6.7	<245	>1700				
			410	400	2.3×10^6	9.2×10^8		
1-Methyluracil	1-MeUOH	4.3	<300	>1000		1.2×10^9		
			390	750	1.1×10^6	8.3×10^8	9.8	9.8 ± 0.1
		11.0	325	1400	3.4×10^5	4.8×10^8		
			410	900	4.3×10^5	3.9×10^8		
3-Methyluracil	3-MeUOH	4.0	<300	>800				
			400	650	1.6×10^6	1.1×10^9	9.9	9.9 ± 0.1
		11.0	460	770	6.5×10^4 sec^{-1}			
1,3-Dimethyl-uracil	1,3-DiMe-UOH	3.7, 11.0	<300	>1200		1.0×10^9		
			400	850	9.5×10^5	8.1×10^8		
Cytosine	·COH	3.1	312	650	3.7×10^6	2.4×10^9		
			440	550	4.1×10^5	2.2×10^8	4.6, 12.2	4.5 ± 0.1
		6.6	345	1300	5.0×10^5	6.5×10^8		
			440	650	9.3×10^5	6.0×10^8		
Cytosine	·CH [e]	1.0	450	500				

[a] Values to ±15%, and ϵ calculated assuming OH radicals produce one radical only; see text. [b] Transient decays by first-order process. [c] Slow decaying transient. [d] Radical produced from reaction of OH with dihydrouracil. [e] Produced from reaction of H atoms with cytosine at pH 1.0 in the presence of 1.0 *M* *t*-BuOH.

erties of the OH-adduct radicals, and (b) the change is due to tautomerization of the molecule leading to increased resonance of the radical and/or to a different site of attack by OH radicals. The increased resonance energies of the lactim form of uracil compared to the lactam form have been shown.[19]

The transient spectra produced from 1-methyluracil at pH 4.3 and 10.9 (Figure 2) are similar to those obtained from uracil. From the change in absorbance with pH, one derives a pK = 9.8 ± 0.1, in good agreement with pK_a^1 = 9.8 of the molecule. In both neutral and alkaline solutions, however, the radicals decay by second-order kinetics.

In neutral solution, the OH-radical adduct to 3-methyluracil is somewhat similar to that observed with uracil and 1-methyluracil. However, at pH 11.0 a band with λ_{max} ~460 nm is produced (Figure 3) which is significantly red-shifted compared to that formed with the other two pyrimidines. Furthermore, the transient decays by first-order kinetics, with $k = 6.5 \times 10^4$ sec^{-1}, quite similar to the decay of the transient produced in alkaline solutions of uracil. These results would seem to indicate that substitution at the N_1 position in uracil (the position in nucleotides and nucleosides) reduces the extent of ring opening which results from radical addition at the 5,6 double bond. The change in OD at 340 nm with pH (Figure 3) gives a pK = 9.9 ± 0.1 in agreement with the pK_a^1 = 9.9 of 3-methyluracil.

The addition of OH radicals to 1,3-dimethyluracil gives a transient spectrum which is independent of pH in the range 3.7–10.7, Figure 4 (the insignificant discrepancy results from the presence of a small amount of an isomer produced on synthesis, according to Cyclochemicals). The spectrum is similar to that produced in neutral solutions of uracil and 1-methyluracil. The transient decays by second-order kinetics at pH 3.7 and 11.0, supporting the suggestion made above that ionization of the N_1 position in uracil and in 3-methyluracil leads to ring rupture.

The reaction of OH radicals with dihydrouracil at pH 6.8, and the addition of H atoms to uracil at pH 1.0 give rise to identical transient spectra (Figure 5). These are also fairly similar to the spectra obtained by OH addition to all the uracil substituents in neutral solution.

Thymine. The transient spectrum produced from the reaction of OH radicals with thymine at pH 3.5 (Figure 6) is not too different from that observed from uracil at pH 3.2. The transient species also decays by second-order kinetics (Table I). At pH 11.1, however, in addition to the bands with maxima at ~320 and ~400 nm, other weaker bands were found which absorb up to 750 nm and beyond (see Figure 6). The 320-nm band decays by a first-order process, $k = 1.6 \times 10^5$ sec^{-1}, and the 400-nm band by second order. The spectrum read at 25 μsec after the electron pulse shows that the various absorption bands formed in alkaline solutions decay at different rates (Figure 6).

The change in absorbance at 330 nm with pH again shows a pK ~9.6 ± 0.1, close to the pK_a^1 = 9.9 of thymine. This result (insert, Figure 6) indicates that the changes are due to the proton ionization of thymine and not to any acid–base properties of the OH-adduct radical.

Cytosine. Cytosine has pK_a^1 ~ 4.6 and pK_a^2 ~ 12.2.

(19) B. Pullman and A. Pullman, "Quantum Biochemistry," Interscience, New York, N. Y., 1963, p 208.

Using the nmr technique the N_3 position has been shown[20] to be the site of proton dissociation of pK_a^1. In neutral solution, ~15% of the NH_2 group in cytosine is present[21] as the imino tautomer. On reaction of OH radicals with cytosine at pH 3.1, bands with maxima at ~312 and ~440 nm were observed which decayed by second-order kinetics (Figure 7). At pH 6.6, the species absorbing in the uv has a maximum at ~345 nm, while the 440-nm band remains essentially the same. On monitoring the OD at 350 nm with change in pH (insert Figure 7), a pK value of 4.5 ± 0.1 is derived which is similar to the pK_a^1 of cytosine.

Electron-Transfer Processes to Menaquinone. The role and importance of electron-transfer reactions and the involvement of quinones in biochemistry are well known.[22,23] In a preliminary investigation[14] it was found that the OH adducts to some pyrimidines bases (and other biochemicals) can react with quinones to produce the semiquinone radical anion. Similar electron-transfer processes to *N*-ethylmaleimide, a weaker oxidizing agent, were not observed.[24] A systematic study of such electron-transfer processes to quinones has now been carried out.

Menaquinone (vitamin K_3) has been used as the model quinone because of its relative solubility in water and its high redox potential, $E^\circ = 0.42$ V. The optical spectrum of the semiquinone radical anion MQ^- was obtained from the pulse radiolysis of 5×10^{-5} M MQ, 0.1 M formate, N_2O (1 atm) at pH 6.9 (see Figure 8). The following reactions take place.

$$OH + HCO_2^- \longrightarrow CO_2^- + H_2O \quad (1)$$

$$e_{aq}^- + N_2O \longrightarrow N_2 + OH + OH^- \quad (2)$$

$$CO_2^- + MQ \longrightarrow MQ^- + CO_2 \quad (3)$$

Reaction 3 is ~100% efficient and $k_3 = 5.4 \times 10^9$ M^{-1} sec^{-1}.[14] The rate constants of reactions 4 and 5 were determined to ascertain the experimental conditions used, where $k_4 = 5.4 \times 10^{10}$ M^{-1} sec^{-1} and $k_5 = 5.5 \times 10^9$ M^{-1} sec^{-1}. The spectrum of the semiquinone radical was derived from the pulse radiolysis of 5×10^{-5} M MQ, 0.5 M isopropyl alcohol, N_2O (1 atm) at pH 2.9. The $(CH_3)_2\dot{C}OH$ radicals produced transfer to MQ to form MQH·

$$e_{aq}^- + MQ \longrightarrow MQ^- \quad (4)$$

$$OH + MQ \longrightarrow \text{adduct} \quad (5)$$

$$OH + (CH_{32})CHOH \longrightarrow (CH_3)_2\dot{C}OH + H_2O \quad (6)$$

$$(CH_3)_2\dot{C}OH + MQ \longrightarrow CH_3COCH_3 + MQH\cdot \quad (7)$$

with $k_7 = 6.2 \times 10^9$ M^{-1} sec^{-1} (ref 14). By following the change in absorbance at 400 nm with pH, the dissociation constant was obtained (Figure 8).

$$MQH\cdot \rightleftharpoons MQ^- + H^+$$
$$pK_a = 4.5 \pm 0.1$$

The spectrum of MQ^- has maxima at 395 and 300 nm with ϵ values of 1.2×10^4 and 1.25×10^4 M^{-1} cm^{-1}, respectively, and decays with $2k = 5.5 \times 10^8$ M^{-1} sec^{-1}.

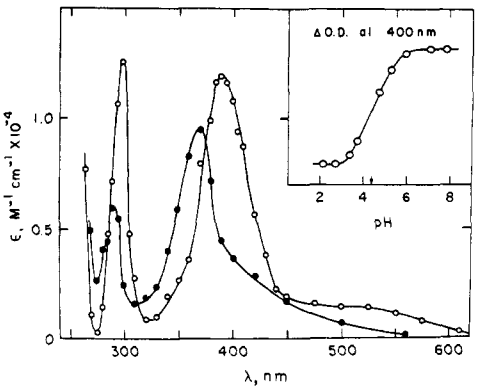

Figure 8. Absorption spectrum of the semiquinone radical (●) and radical anion (○) of menaquinone. Spectra determined from the pulse radiolysis of 5×10^{-5} M menaquinone, N_2O (1 atm), in the presence of 0.5 M isopropyl alcohol at pH 2.9 and 0.1 M HCO_2^- at pH 6.9, respectively. Insert: Absorbance at 400 nm *vs.* pH. Total dose ~1.2 krads/pulse. Spectra corrected for change in transmission due to decomposition of menaquinone.

The spectrum of MQH· has maxima at ~370 and 290 nm with ϵ values of 9.5×10^3 and 6×10^3 M^{-1} cm^{-1}, respectively, and decays much faster with $2k = 3.4 \times 10^9$ M^{-1} sec^{-1}.

The rates of electron transfer from the radicals to menaquinone were determined by measuring the rate of formation of MQ^- at 400 nm or MQH· at 370 nm and using low doses of ~1 krad/pulse. In all cases, the absorbance of the OH adducts to pyrimidines relative to that of MQ^- or MQH· is very small at 400 and 370 nm. Table II presents the efficiency (determined as a percentage and based on 100% transfer from CO_2^- or from e_{aq}^- to MQ) and the rate of formation of MQ^- from the various pyrimidine radicals. These were obtained at two pH values in order to determine the role of the various tautomeric forms on the electron-transfer processes. In almost all cases, increased efficiency of transfer was found on proton ionization of N_1 or N_3.

Discussion

The reaction of hydroxyl radicals with the pyrimidine bases of DNA has been shown from early studies to add to the 5,6 double bond of the molecule. Support for this site of attack can be derived from the difference in the reaction rate constants[4] of k(OH + uracil) = 5.0×10^9 M^{-1} sec^{-1} and k(OH + dihydrouracil) = 8×10^8 M^{-1} sec^{-1} determined at pH ~5–6, and from the formation of uracil glycols[25] as permanent products. What has been considerably debated and is still in question is the extent of addition of OH at the C_5 and/or C_6 positions for the different pyrimidines. Esr studies of aqueous solutions at room temperature have not provided definitive answers since either (a) the experiments were carried out observing steady-state concentrations of the free radicals, and the *initially* produced radicals were not detected in most cases, or (b) in pulsed esr experiments[13] the spectral resolution was not adequate to identify the radicals.

This situation is further complicated by the uncertainty which still exists for the sites of proton ionization from some pyrimidines,[20,21,26] in particular for uracil and thymine.

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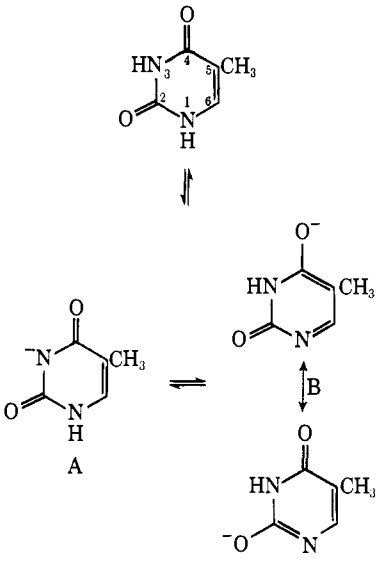
(25) G. Scholes, "Radiation Chemistry of Aqueous Systems," G. Stein, Ed., Weizmann Science Press, Jerusalem, Israel, 1968, p 259.
(26) E. Wittenberg, *Chem. Ber.*, **99**, 2391 (1966).

Table II. Efficiency and Rates of Electron Transfer to Menaquinone from Intermediates Produced by the Reaction of OH Radicals with Pyrimidine Bases[a]

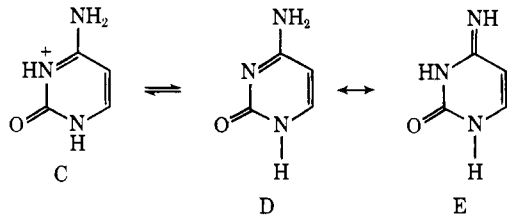
Solute, S	[S], mM	Radical	Low pH[b] pH	%	k, M^{-1} sec^{-1}	High pH[c] pH	%	k, M^{-1} sec^{-1}	pK_a[8]
T	1	·TOH	7	12	3.9×10^9	11	41	5.2×10^9	9.9, >13
TH_2	3	·TH	7	56	7.0×10^9				
TMP	1	Mixed	7	22	5.0×10^9	11	40	1.0×10^9	1.6, 6.5, 10.0
U	1	·UOH	7	17	4.2×10^9	11	62	4.1×10^9	9.5, 13.0
UH_2	3	·UH	7	66	4.6×10^9				
UMP	1	Mixed	7	10	3.1×10^9	11	50	1.0×10^9	6.4, 9.5
1-MeU	1	1-MeUOH	7	19	3.7×10^9	11	55	4.1×10^9	9.8
3-MeU	1	3-MeUOH	7	31	2.7×10^9	11	79	3.5×10^9	9.9
1,3-DiMeU	1	1,3-di-MeUOH	7	8		10.7	11		
6-MeU	1	6-MeUOH	7	69	3.9×10^9	10.5	34	6.8×10^9	9.7
5-NH_2U	2	5-NH_2UOH	7	24					
6-NH_2U	2	6-NH_2UOH	7	14					
C	1	·COH	2.9	14	3.0×10^9	7.0	60	4.9×10^9	4.6, 12.2
CMP	2	Mixed				7.0	37		4.5, 6.3
i-C	1	*i*-COH	3.2	20	3.0×10^9	7.0	70	4.8×10^9	4.0, 9.6
1-MeC	1	1-MeCOH				7.0	50	4.6×10^9	8.7
5-MeC	1	5-MeCOH				7.0	26	2.0×10^9	4.6, 12.4
3-MeCyt	1	3-MeCytOH	6.9	12	2.7×10^9	9.3	14	1.2×10^9	8.2, 10.5
OA	1	·OAOH	6.8	13	6.0×10^9	10.9	50	8.5×10^8	2.8, 9.45, 13.0

[a] Monitored at 400 nm in N_2O (1 atm), using 5×10^{-5} M menaquinone and ~1 krad/pulse. [b] Percentage and rate values to ±15%. [c] Percentage and rate values to ±10%.

It has recently been suggested[26] that the first proton ionization for thymine, for example, produces ionic forms A and B with a 1.1:1 mixture of the monoanions



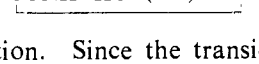
A and B. With cytosine the ionization of the first proton pK_a^1 = 4.6 comes from N_3–H.[21]



The effect of pH on the transient absorption spectra of the OH adducts to pyrimidines and the efficiency of electron transfer from these adducts to quinones (see more below) were carried out with the expectation that it might shed some light on the above-mentioned difficulties. However, only partial success was obtained.

The spectra of the OH adduct to uracil at pH 3.2 and 10.7 (Figure 1) are reminiscent of the spectra of the radicals i and ii and

produced[27] from the reaction of OH with glycine anhydride at pH 6.0 and 11.0, respectively. The OH adduct to C_5 in uracil can be represented as



in neutral solution. Since the transient spectra of 1-MeUOH and UOH are similar, and the efficiency (Table II) of electron transfer to menaquinone from these two radicals is about the same in neutral and in alkaline solutions—but different from 3-MeU—it would appear to be consistent with *N_3–H as the position of proton ionization in both the parent compound*[28] *and the free radical.* The odd electron has various resonating structures, of the type suggested in esr work.[11,12] Thus, like the radicals produced from addition of OH to the different uracils in alkaline solution, the deprotonated radical ii transfers ~90% to MQ, whereas the radical i transfers only ~5%.[14] The sarcosine anhydride radical

does not transfer an electron to MQ (≤5%) in either neutral or alkaline solutions.

It is interesting to note that with 3-methyluracil the ionic forms F and G have been suggested[28] to be present. These could account for the difference in the transient spectra (Figure 3) and the increased efficiency of electron transfer to MQ (see Table II) at both pH 7.0 and 11.0. The radicals produced by OH addition to F and G can be represented as RCOH and RCO$^-$ radicals, and electron transfer from such radicals to menaquinone has been shown[14,24] to be very efficient.

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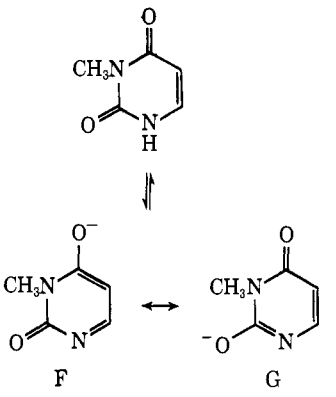

F G

In support of the proton ionization reactions of N_1 and N_3 given above, the OH adduct spectrum of 1,3-dimethyluracil remains the same in alkaline solutions (Figure 4), and the efficiency of electron transfer to MQ is $\leqslant 10\%$ (Table II). This shows that substantial electron transfer occurs only when the odd electron is resonating with the ionic tautomeric forms of the pyrimidines. Further support can also be obtained from the observation that the sarcosine anhydride radical does not transfer to MQ.

It is tempting to suggest that the structured spectrum of the thymine–OH radical in alkaline solutions is connected with the various ionic forms A, B_1, and B_2 suggested[26] to be present, and with a change in the proportion of radical addition at C_5 and C_6 positions. This might also account for the lower efficiency of transfer of $\dot{T}$–OH to MQ, compared to uracil, 1-MeU, and 3-MeU.

The rate constant for reaction of OH radicals with cytosine increases by only $\sim 50\%$ between pH 2.0 and 7.0 ($pK_a^1 \sim 4.6$), presumably indicating that the main site of addition is at C_5–C_6 and less at N_3–C_4 position, assuming similar rates of addition to both double bonds. The change in the transient spectra (Figure 7) and the increase in the efficiency of transfer to MQ (Table II) between pH 3 and 7.0 could be due to a stronger resonance of the odd electron in $\dot{C}$–OH with the amino group at pH 7.0. Radicals of the type $NH_2\dot{C}$–, as in $NH_2\dot{C}HCOO^-$ and $NH_2\dot{C}HCONH_2$, have been found[14,29] to transfer efficiently to MQ. The OH adduct to 3-methylcytidine transfers only $\sim 12\%$ at pH 6.9 and 9.3, compared with $\sim 60\%$ for cytosine at pH 7.0 (Table II). This result suggests that the imino form of 3-methylcytidine[21] is ineffective in the electron-transfer process to MQ.

(29) P. S. Rao and E. Hayon, *Biochem. Biophys. Acta*, in press.

It is worth noting that the efficiency of electron transfer from 1-methylcytosine at pH 7.0 is the same as that from cytosine ($\sim 50\%$). This observation suggests that it is the amino group in cytosine that is involved in the electron-transfer reaction. Just like thymine, 5-methylcytosine is also a poor electron-transfer radical. This is presumably due to the greater inability of a radical with a methylated C_5 position to form various tautomers and, in part, to a change in the proportion of OH radical addition at C_5 and C_6 positions. The electron transfer to MQ from isocytosine, *i*-$\dot{C}$OH, is $\sim 70\%$ at pH 7.0, significantly higher than cytosine.

Electron Transfer to Menaquinone. Some aspects of the nature of the radicals involved in the electron-transfer reaction to MQ have already been discussed above. The following additional points appear to be of some interest. (a) With TMP, UMP, and CMP the percentage of electron transfer is much smaller compared to T, U, C, 1-MeU, and 1-MeC. This is explained as due to the reaction of OH radicals with the sugar moiety, and the new radical formed transfers an electron to MQ less effectively.[14] (b) The radicals produced by abstraction of a hydrogen atom from dihydrothymine and dihydrouracil transfer efficiently to MQ. (c) The 6-MeU̇OH transfers $\sim 70\%$ at pH 7.0 and only $\sim 35\%$ at pH 10.5. In alkaline solution one can conclude (from ref 30) that 6-MeU might exist mainly as form B (with the methyl group in the C_6 position), and electron transfer from such a OH-radical adduct is less efficient than from form A, where the electron is transferred from an ionized nitrogen. (d) The rates of electron transfer to MQ are in the range $1\text{–}6 \times 10^9\ M^{-1}\ \text{sec}^{-1}$, *i.e.*, close to diffusion-controlled reaction.

Similar rate constants and percentages of electron transfer of these radicals to *p*-benzoquinone, a much stronger oxidizing agent, $E^\circ = 0.7$ V, than menaquinone, were also observed.[31] It is hoped that further research in electron transfer processes to oxidizing agents will provide information on the structure and chemistry of these biologically important free radicals. The determination of the nature of the products formed subsequent to these electron-transfer reactions should be carried out. It is expected that the presence of good acceptors with high redox potential will lead to a change in the nature and composition of the products produced from these biological radicals, as well as enhance the "fixation" of radiation damage.

(30) I. Wempen and J. J. Fox, *J. Amer. Chem. Soc.*, **86**, 2474 (1964).
(31) M. Simic and E. Hayon, to be published.