One-Electron Redox Reactions of Water-Soluble Vitamins. II. Pterin and Folic Acid

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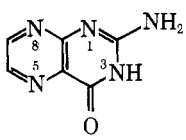
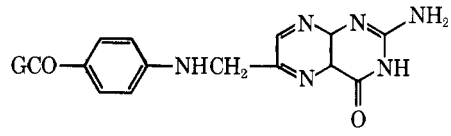
Using the fast reaction technique of pulse radiolysis and kinetic absorption spectrophotometry, the one-electron reductions of pterin (Pt), 3-methylpterin (3-Me-Pt), and folic acid (FH) were studied in aqueous solutions, over the pH range 0–14. The hydrated electron and the acetone ketyl radical were used as reducing agents. The reaction rate constants of e_{aq}^- with these compounds were $1–3 \times 10^{10}$ M^{-1} s^{-1}. The reaction rate constants of the $(CH_3)_2\dot{C}OH$ radical with these compounds were markedly dependent on pH and the dissociation constants of the molecules. The intermediates formed from the one-electron reduction of these pterins have characteristic absorption spectra in the uv and visible regions. These spectra are dependent upon pH and from the change in absorbance with pH at fixed wavelengths, the ionization constants of the free radicals were derived. For pterin, for example, the $\cdot PtH_4^{2+}$, $\cdot PtH_3^+$, $\cdot PtH_2$, $\cdot PtH^-$, and $\cdot Pt^{2-}$ species are postulated with pK_a (radical) values of 2.3, 6.6, 8.1, and 10.3, respectively. Furthermore, over certain pH ranges, the spectra of some of the intermediates change with time. This change was found to be due to protonation reactions by H^+, pterin, and $H_2PO_4^-$, with rate constants of $\sim 2.1 \times 10^{10}$, 7.2×10^8, and 3.1×10^8 M^{-1} s^{-1}, respectively. The N_3 proton in pterin was shown to be involved in the protonation reaction of the intermediates, since 3-methylpterin could not protonate the initial transient species formed. Schemes of reactions are suggested to interpret the nature of the transient species observed. These results are discussed and compared to recent work on the one-electron reduction of aromatic nitrogen heterocyclic compounds.

Pteridines, in particular the 2-amino-4-hydroxy derivatives (pterin), are widely distributed in biological systems. Folic acid,[2–4] a vitamin of the B group, is converted in vivo



pterin



folic acid [G = NHCH(COO⁻)CH_2CH_2COOH]

through a series of enzymic transformations into a coenzyme form. The folic acid coenzymes catalyze reactions concerned with the metabolism of nucleic acids and proteins. A deficiency of folic acid leads to the failure to synthesize the purines and thymine required for DNA synthesis.

In aqueous solutions the folate ion exists[5] in an unfolded extended conformation. However, at high concentrations and temperature (not the conditions used in this work) folate ions are involved in intermolecular association consisting of a vertical stacking interaction.

Pterin and folic acid can easily be reduced by a variety of reducing agents to the corresponding dihydro and tetrahydro derivatives in the pyrazine ring of the molecule. The coenzyme form of folic acid is the 5,6,7,8-tetrahydrofolic acid. Free-radical intermediates have been suggested[6–8] both in the chemical oxidation of reduced pterins by air, H_2O_2, or Fe^{3+} and in the enzymic oxidation of reduced pterin by phenylalanine hydroxylase.

No systematic investigations appear to have been carried out on the nature of the radical intermediates produced from the one-electron reduction of pterin and folic acid (or the one-electron oxidation of the reduced pterins). Presented below are the results obtained on the one-electron reduction in water of pterin, 3-methylpterin, and folic acid by hydrated electrons, e_{aq}^-, the acetone ketyl radical, $(CH_3)_2\dot{C}OH$, and other donor radicals. The fast reaction technique of pulse radiolysis and kinetic absorption spectrophotometry was used to monitor and study the free-radical intermediates produced.

Experimental Section

The pulse radiolysis experimental set-up and conditions used have been described elsewhere.[9–11]

The radiation chemistry of water produces e_{aq}^-, hydroxyl radicals, and H atoms

$$H_2O \longrightarrow e_{aq}^- (2.8), OH (2.8), \text{and } H (0.6)$$

where the numbers in parentheses are the G values (yields of radicals produced per 100 eV of energy absorbed). In part I of this series[11] details and explanations have been given for the choice of the experimental conditions used. Briefly, one-electron reduction by e_{aq}^- was carried out in aqueous solutions containing ~ 1.0 M *tert*-butyl alcohol and saturated with argon. The β alcohol radical produced[9] from this alcohol was found not to interfere with the observations reported below. One-electron reduction by electron transfer from the $(CH_3)_2\dot{C}OH$ radical was carried out in 1–2 M isopropyl alcohol in solutions saturated with N_2O gas.

$$OH + (CH_3)_2CHOH \rightarrow (CH_3)_2\dot{C}OH + H_2O \quad (1)$$

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

$$e_{aq}^- + H^+ \rightarrow H \quad (3)$$

$$H + (CH_3)_2CHOH \rightarrow H_2 + (CH_3)_2\dot{C}OH \quad (4)$$

In acid solutions, e_{aq}^- are converted to H atoms which generate $(CH_3)_2\dot{C}OH$ radicals, reactions 3 and 4. Similar reactions were employed to generate other donor radicals in water.

All the chemicals used were the highest purity research grade available commercially, and were obtained from Calbiochem. Aldrich, and Sigma Chemicals. The reagents used were obtained from Baker & Adamson, Mallinckrodt, and Eastman Chemicals. Solutions were buffered with $HClO_4$, KOH, and various amounts of phosphates and tetraborate. The concentrations of buffer and substrate used were strictly controlled, since these affected the protonation reactions of the free-radical intermediates produced.

The stability in acid and basic solutions of the compounds used was checked in every case. The transient optical absorption spectra presented below were corrected for depletion of the substrate at the appropriate wavelengths and pH. The extinction coefficients derived were based on the G values for e_{aq}^- and OH, and the KCNS radiation dosimetry.[9]

Results and Discussion

The reaction rate constants of e_{aq}^- with pterin (PtH), 3-methylpterin (3-Me-Pt), and folic acid (FH) in aqueous solutions were determined by monitoring the decay kinetics of e_{aq}^- at 700 nm. From the pseudo-first-order decays, the second-order rate constants were determined and are given in Table I. These rates are close to being diffusion controlled as was found for pyrimidines,[12,13] pyrazine,[13] and various aromatic diazines.[13]

Table I. Reaction Rate Constants of e_{aq}^- with Pterin, Folic Acid, and Related Compounds in Water[a]

Registry no.	Compd	pK_a	pH	Ionic form	$k(e_{aq}^- + S)$, M^{-1} s^{-1} [b]
2236-60-4	Pterin, PtH	2.27, 7.92	6.5	PtH	2.5×10^{10}
			11.5	Pt^-	1.9×10^{10}
941-90-2	3-Methylpterin, 3-Me-Pt	2.27	7.7	3-Me-Pt	2.9×10^{10}
59-30-3	Folic acid, FH	~2.3, 8.26	6.0	FH	2.2×10^{10}
			12.0	F^-	1.1×10^{10}
91-18-9	Pteridine, Pt	4.1	6.0	Pt	3.0×10^{10} [c]

[a] Determined in presence of ~0.5 M *t*-BuOH by monitoring decay kinetics of e_{aq}^- at 700 nm. [b] Values to ±10%. [c] At pH 6.0, 78% present in water as pteridine.

Table II. Rate Constants of Electron Transfer Processes to Pterin and Folic Acid by Various Electron Donors in Water

System	pH	Ionic form	Electron donor[a]	k(electron transfer), M^{-1} s^{-1} [f]
Pterin, PtH	0.8	PtH_2^+	$\dot{C}H_2OH$[b]	9.0×10^7
	7.0	PtH	$\dot{C}H_2OH$[e]	$\ll 10^7$
	13.0	Pt^-	$\dot{C}H_2O^-$	6.0×10^8
	7.0	PtH	$CH_3\dot{C}HOH$[b]	3.7×10^7
	13.0	Pt^-	$CH_3\dot{C}HO^-$	1.2×10^9
	0.8	PtH_2^+	$(CH_3)_2\dot{C}OH$	2.0×10^9
	7.0	PtH	$(CH_3)_2\dot{C}OH$	4.5×10^8
	9.4	Pt^-	$(CH_3)_2\dot{C}OH$[e]	$\ll 10^7$
	13.0	Pt^-	$(CH_3)_2\dot{C}O^-$	1.5×10^9
	7.0	PtH	$\cdot CO_2^-$	4.6×10^8
	9.5–13.0	Pt^-	$\cdot CO_2^-$ [e]	$\ll 10^7$
3-Methylpterin, 3-Me-Pt	0.8	3-Me-PtH^+	$\dot{C}H_2OH$[c]	6.0×10^7
	6.3	3-Me-Pt	$CH_3\dot{C}HOH$[d]	3.2×10^7
	0.8	3-Me-PtH^+	$(CH_3)_2\dot{C}OH$	1.9×10^9
	7.0	3-Me-Pt	$(CH_3)_2\dot{C}OH$	2.9×10^8
Folic acid, FH	~0.5	FH_2^+	$(CH_3)_2\dot{C}OH$	1.1×10^9
	6.0	FH	$(CH_3)_2\dot{C}OH$	4.0×10^8
Pteridine, Pt	6.0	Pt	$\dot{C}H_2OH$	3.6×10^8

[a] ~100% electron transfer was found, unless stated otherwise. [b] ~40% electron transfer. [c] ~45% electron transfer. [d] ~55% electron transfer. [e] No electron transfer observed under experimental conditions used. [f] The k values where partial electron transfer occurs were calculated as described in ref 14.

Pterin and folic acid undergo keto–enol tautomerism with a consequent small reduction in alkaline solution of the reaction rate constants with e_{aq}^- (Table I). This reduction in the rate is less than that observed[12] for the enol form of the pyrimidines, and may simply be due to a lower encounter rate for like-charged reactants. These results suggest that the pyrazine ring in pterin and folic acid can be effectively reduced by e_{aq}^-.

Pteridine is unstable in aqueous solution and undergoes partial covalent hydration (~22% at the pH of the experiment). The rate constant given in Table I is the overall value for the mixture of the anhydrous and hydrated forms.

The reaction rate constants of $(CH_3)_2\dot{C}OH$ (and other organic radicals) with pterins were determined by following the formation kinetics of the radical intermediates produced from this electron transfer reaction at the appropriate wavelengths. The rate constants were found, Table II, to be dependent on the state of protonation and the nature of the substituents in pterin.

The efficiencies for the one-electron reduction of substrates by $(CH_3)_2\dot{C}OH$ and the rate constants for this reaction have recently been interpreted, in a number of systems, on the basis of the redox potentials of the substrates and the kinetic potential[14] of the $(CH_3)_2\dot{C}OH$ radical, $E_k^{01} = -0.82$ V. The E^{01} values in the literature for pterin (PtH) are −0.39 and −0.57 V for the PtH and Pt^- forms, respectively.[15] These values have to be more negative (~−0.8 and ~−1.0 V for PtH and Pt^-, respectively), if one is to interpret the data in Table II on the basis of the redox potentials of the donors and acceptors.

Pterin. The one-electron reduction of pterin (PtH, pK_a^1 = 2.27, pK_a^2 = 7.92) by e_{aq}^- in 2.5×10^{-4} M aqueous solutions forms short-lived free radicals which absorb in the visible and uv regions of the spectrum, Figure 1. At pH 5.2, a transient species (T_1) with maxima[16] at ~355 and ~440 nm is produced immediately after the 30-ns electron pulse. This transient absorption changes with time and at ~5 μs after the pulse a species (T_2) with λ_{max} ~ 258, 328, and 448 nm is observed. It can be noted that for the T_2 transient the band in the visible region is red shifted and that in the uv region is blue shifted, with respect to the corresponding bands of the T_1 transient. Similar changes are found on reaction of e_{aq}^- with pterin at pH 7.2, Figure 1 (b) and Table III. At pH 9.2 and 11.7 only one transient absorption is observed at each pH, Figure 1 (c), at the time resolution (τ ~0.2 μs) available.

In neutral solutions at pH 7.2, the transient species formed by electron transfer from the $(CH_3)_2\dot{C}OH$ radical were found to be identical with the T_2 species formed from the reaction of e_{aq}^- with pterin. In acid solutions, e_{aq}^- react very fast with protons, reaction 3. Therefore, one-electron reduction of pterin at pH 0.35 was brought about by the reaction of $(CH_3)_2\dot{C}OH$ with PtH_2^+. Figure 2 and Table III give the results.

The changes in the optical absorption of the transient species T_1 and T_2 with pH have been titrated at fixed wavelengths. The titration curves are shown in Figure 3. Ionization constants for the T_1 radicals of ~6.5 and ~8.4, and pK_a values of 2.3, 6.6, 8.1, and 10.3 for the T_2 radicals (see Table III), were found.

The time dependence for the change from the T_1 to the T_2 species (whenever both species were observed under the experimental conditions used) was found to depend on the presence and concentration of proton donors, HA:

Table III. Absorption Maxima, Extinction Coefficients, Ionization Constants, and Decay Kinetics of the Radicals Produced by Reaction of e_{aq}^- with Pterin and Folic Acid in Water

Substrate[a]	pH	Ionic form	Transient	λ_{max}, nm	ϵ, mM^{-1} cm^{-1}	$2k$, M^{-1} s^{-1}	pK_a (radical) T_1	pK_a (radical) T_2 (or T)
Pterin, PtH	0.35	PtH_2^+	T[b]	260, 320, 350, 450	22.0, 4.0, 4.2, 4.4	1.1×10^8 [b]		2.3
(2.27, 7.92)	5.2	PtH	T_1	~355, ~440	4.3, 1.6		~6.5	
			T_2	~258, 328, 448	19.5, 4.7, 3.5	5.4×10^8 [b]		6.6
	7.2	PtH + Pt^-	T_1	~355, ~440	4.3, 1.4		~8.4	
			T_2	255, 325, 460	21.4, 4.6, 2.1	1.8×10^8 [b]		8.1
	9.2	Pt^-	T	~252, 355, 480	22.0, 5.5, 2.3	1.0×10^8		10.3
	11.7	Pt^-	T	~252, 358, 435	14.0, 4.8, 1.6	5.5×10^7 [c]		
3-Methyl-	0.35	3-Me-PtH^+	T[b]	335, 460	3.9, 3.5	6.1×10^7 [b]		3.1
pterin,	5.2	3-Me-Pt	T_1	~372	5.4		~7.6	
3-Me-Pt			T_2	~457, <355	3.0	1.6×10^8 [b]		7.7
(2.27)	8.3, 9.2	3-Me-Pt	T_2	365	5.4	6.3×10^7 [b]		
Folic acid,	0.5	FH_2^+	T[b]	≤260, 465	28.0, 4.2	8.1×10^6 [b]		~1.0
FH	5.2	FH	T_1	~278, 360, ~440	25.0, 6.2, 1.9		~6.5	
(~1.6,[d] 8.26)			T_2	~278, 360, 460	25.0, 5.5, 3.7	4.4×10^7 [b]		~6.6
	7.2	FH	T_1	280, 355	22.0, 6.4			
			T_2	350, 435	5.6, 2.5	1.1×10^8 [b]		~8.0
	9.4	F^-	T_1	~258, ~290, 365	23.0, 23.0, 7.7		~8.4	
			T_2	~258, ~290, 360	23.0, 23.0, 7.0	5.7×10^7		~10.3
	11.9	F^-	T	255, 290, 365, ~440	21.5, 22.5, 7.2, 2.6	3.0×10^7 [b]		

[a] Values in parentheses are pK_a of the substrate. [b] Transient produced by electron transfer from $(CH_3)_2\dot{C}OH$. [c] At pH 13.0 by electron transfer from $(CH_3)_2\dot{C}O^-$. [d] Spectrophotometrically determined in this work.

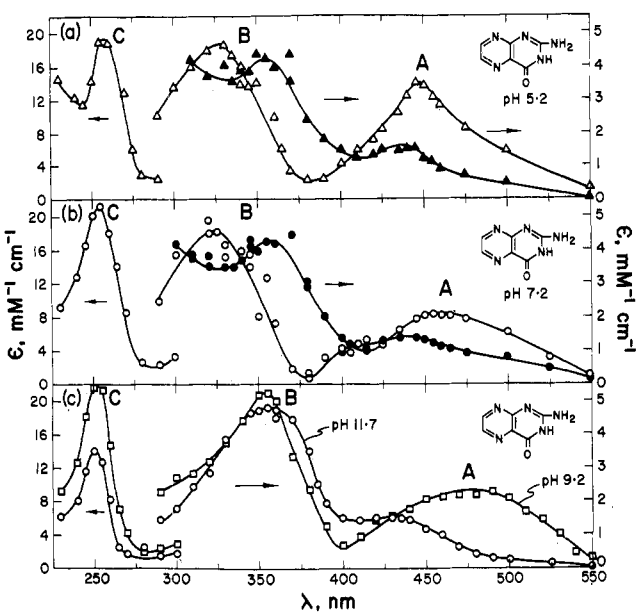


Figure 1. Absorption spectra of transient species (T_1 and T_2) produced from the one-electron reduction of pterin by e_{aq}^- in water. Bands A and B were determined in solutions containing 2.5×10^{-4} M pterin (~5×10^{-5} M used for determining bands C), 0.5 M *t*-BuOH, 2 mM buffer (see text), and argon (1 atm). Dark symbols represent initial transient T_1 (absorbance read ~0.5 μs after the pulse) and open symbols are for transient T_2 (absorbance read at ~5 μs after the pulse): (a) at pH 5.2, (b) at pH 7.2, and (c) at pH 9.2, □, and pH 11.7, ○. Total dose ~4.0 krad/pulse.

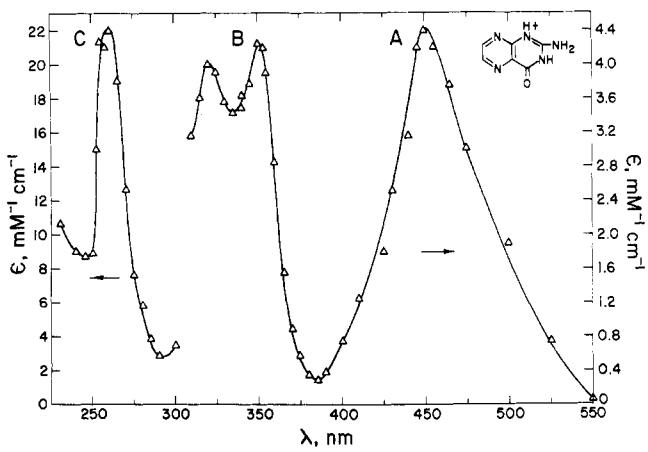


Figure 2. Absorption spectra of transient species produced from the one-electron reduction of pterin by $(CH_3)_2\dot{C}OH$ radicals at pH 0.35. Aqueous solutions contained 2.5×10^{-4} M pterin (~5×10^{-5} M for band C), 1.0 M isopropyl alcohol and argon (1 atm). Total dose ~1.3 krad/pulse.

$$T_1 \xrightarrow{HA} T_2 \qquad (5)$$

The presence of H^+, $H_2PO_4^-$ buffer, and pterin itself was found to accelerate reaction 5. Figure 4 shows the results for protonation of T_1 by pterin, and Table IV the rate constants for various proton donors. The $k(T_1 + HA \rightarrow T_2 + A^-)$ rate constants in solutions containing more than one proton donor were determined by multilinear regression analysis, using a Hewlett-Packard calculator Model 9830. Protonation of T_1 radicals by H^+ gave k_5 ~2.1×10^{10} M^{-1} s^{-1}, while for $H_2PO_4^-$ $k_5 = 3.1 \times 10^8$ M^{-1} s^{-1}.

Evidence that the $>N_3H$ group in pterin itself can act as the proton donor can be seen from the absence of protonation when 3-methylpterin was used instead (see Figure 4 and Table IV), under otherwise identical experimental conditions. This is probably the first time that it has been clearly demonstrated that a substrate can act as a proton donor to a radical species. Similar results[17] have been obtained for the protonation of the corresponding T_1 radical from lumazine.

Based on the above results (and the results given below for 3-methylpterin) the following scheme of reactions is tentatively suggested for a complex molecule such as pterin (and, below, for 3-Me-Pt and FH) with at least four different reactive sites for reduction. Other schemes consistent with the experimental facts can also be written. The one given below was chosen on the basis that pyrazines are known[13] to be readily reduced, and the reduced forms of pterins are in the pyrazine ring.

Based on ESR data, Ehrenberg et al.[6] have inferred that the free-radical species produced by reduction of pterins in

Table IV. Rate Constants for Protonation of Electron Adducts (T_1 Transients) of Pterin and Folic Acid by Various Proton Donors in Water

System	λ monitored, nm	Proton donor, HA	$k(T_1 + HA)$, $M^{-1} s^{-1}$ [a]
Pterin, PtH	455	H_3O^+	$\sim 2.1 \times 10^{10}$
	455	$H_2PO_4^-$	3.1×10^8
	455	PtH	7.2×10^8
	460	H_2O	$\sim 3.0 \times 10^3$
3-Methyl-pterin, 3-Me-Pt	460	H_3O^+	2.2×10^{10}
	460	$H_2PO_4^-$	3.8×10^8
	460	3-Me-Pt	0
	460	H_2O	$\sim 3.0 \times 10^3$
Folic acid, FH	465	H_3O^+	5.9×10^{10}
	465	$H_2PO_4^-$	1.0×10^8
	465	FH	1.5×10^8
	465	H_2O	$\sim 4.0 \times 10^3$

[a] Rate for the first (lowest pH) T_1 species, determined by multilinear regression analysis of observed protonation rates at different concentrations of H_3O^+, $H_2PO_4^-$, and substrate.

acidic media is a pyrazyl dihydro radical cation ($\cdot PtH_3^+$). In the time scale employed for ESR experiments, only longer lived species, e.g., the T_2 species, would be present. Hence, we have postulated the T_2 species to be primarily pyrazyl radicals. The scheme presented below takes into consideration the possibility that the initial site for the one-electron reduction of pterins may be in the pyrimidine part of the molecule, giving the T_1 species, as was previously shown[18] for the one-electron reduction of pyrimidines.

Ionization Constants of the T_2 Species. The first protonation of pyrazine occurs at $pK_a = 0.65$, while in pterin the fusion of the isocytosine ring shifts it to a $pK_a = -3$. The dihydro cation radical of pyrazine was found[13] to have a $pK_a = 10.5$, and the base-weakening effect of the isocytosine ring can be expected to shift the pK_a of the pyrazyl radical in pterin to lower pH values. The $pK_a = 6.6$ for the $\cdot PtH_3^+$ radical is attributed to this step. The pK_a (radical) values of 2.3 and 8.1 are close to those of the parent molecule, and are therefore attributed to the same ionization steps as in pterin.

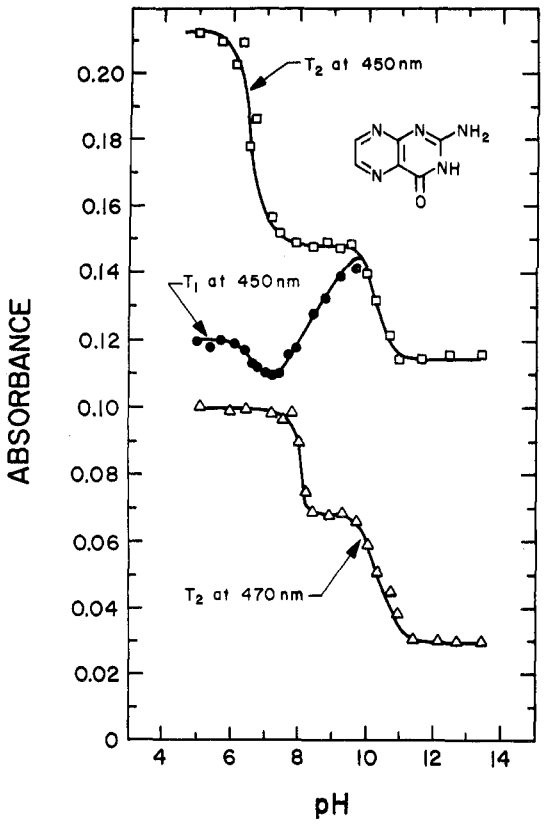
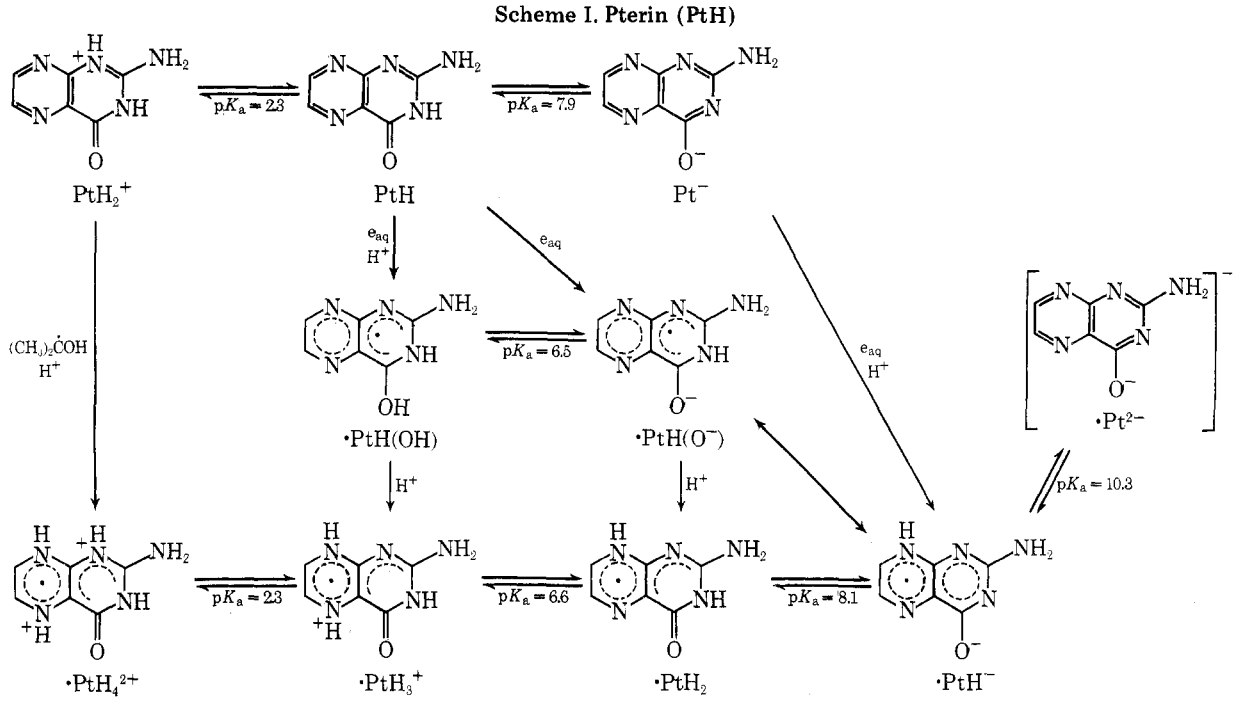


Figure 3. Dependence upon pH of the absorbance of the transient species T_1 and T_2 monitored at 450 and 470 nm. Solutions contained 2.5×10^{-4} M pterin, 0.5 M *t*-BuOH, and argon (1 atm). Total dose ~15 krad/pulse.

The $pK_a = 10.3$ for the $\cdot PtH^-$ radical to give species $\cdot Pt^{2-}$ is tentatively suggested, and is consistent with the absence of a pK_a (radical) = 10.3 in the case of 3-methylpterin; see below.

Ionization Constants of the T_1 Species. In the pH range 5.2–7.2, the addition of an electron to PtH is postulated to occur in the pyrimidine ring to give the ketyl radical and radical anion (see Scheme I). The pK_a ~6.5 is close

Scheme I. Pterin (PtH)



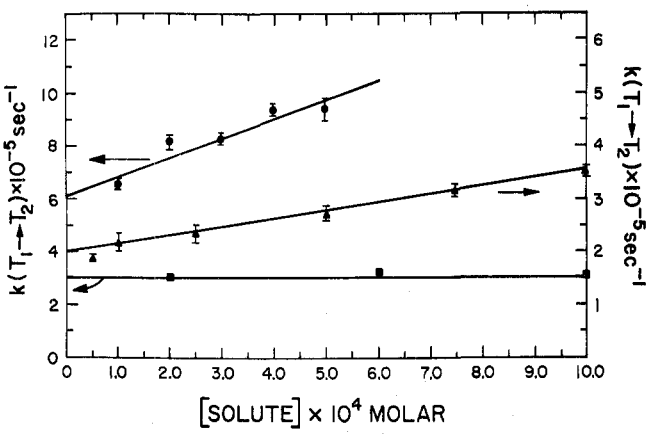


Figure 4. Dependence of the rate of protonation of T_1 species to form T_2 species upon the concentration of (a) pterin, ●, 0.5 M *t*-BuOH, 1 mM $H_2PO_4^-$, pH 6.3; (b) 3-methylpterin, ■, 0.5 M *t*-BuOH, 0.2 mM $H_2PO_4^-$, pH 5.1; and (c) folic acid, ▲, 1.0 M *t*-BuOH, 1 mM $H_2PO_4^-$, pH 6.3.

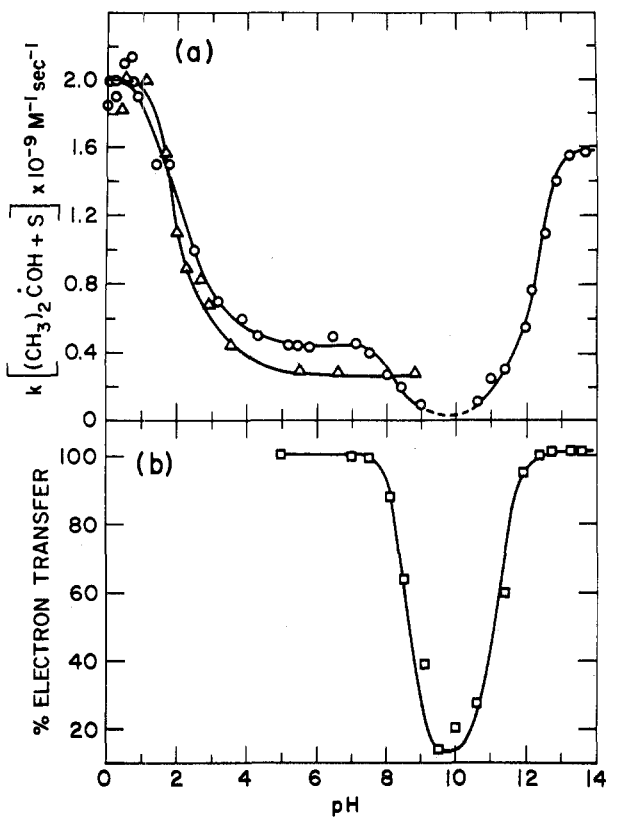


Figure 5. Dependence upon pH of the electron transfer reaction of $(CH_3)_2\dot{C}OH$ radicals to pterin and 3-methylpterin in water: (a) rate constants for electron transfer to pterin, ○, and 3-methyl pterin, △; (b) percentage efficiency for electron transfer to pterin. Solutions contained 2.5×10^{-4} M solutes, 1.0 M isopropyl alcohol, argon (1 atm), and total dose ~2 krad/pulse.

to those of similar radicals derived from various pyrimidines[18] and guanine.[19]

Reactivity toward $(CH_3)_2\dot{C}OH$ Radicals. Figure 5 (a) shows that the rate constant for the reaction of $(CH_3)_2\dot{C}OH$ radicals with pterin is strongly dependent on pH and the state of protonation of pterins and the acetone ketyl radicals. Apparent pK_a values are observed in Figure 5 (a) which reflect the pK_a = 2.3 and 7.9 for PtH_2^+ and PtH, and $pK_a \sim 12.2$ the ionization constant of the $(CH_3)_2\dot{C}OH$ radical.[20] Figure 5 (b) demonstrates that the percentage efficiency for electron transfer from $(CH_3)_2\dot{C}OH$ radicals to the Pt^- form of pterin is almost zero, indicating that the

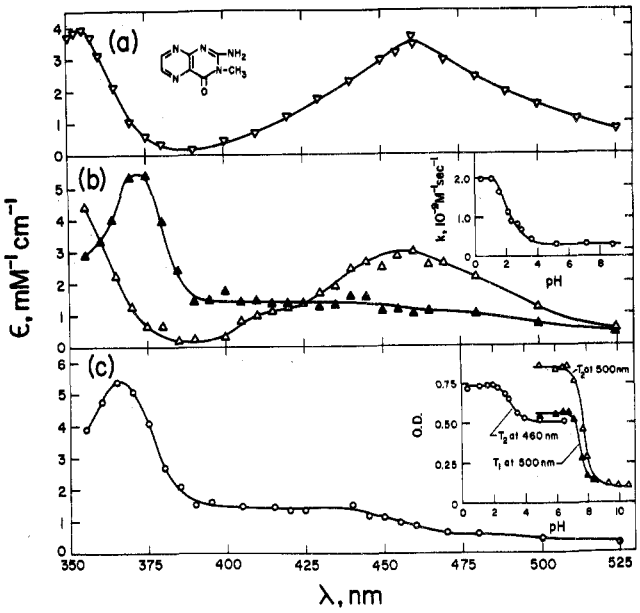


Figure 6. Absorption spectra of transient species (T_1 and T_2) produced from the one-electron reduction of 3-methylpterin in water. Solutions contained 5.0×10^{-4} M 3-Me-pterin, 0.5 M *t*-BuOH (or 1.0 M isopropyl alcohol), 2 mM buffer, and argon (1 atm). Dark symbols represent initial transient T_1 (absorbance read ~0.5 μs after the pulse) and open symbols represent T_2 transients (absorbance read at ~5 μs after the pulse). (a) reduction by $(CH_3)_2\dot{C}OH$ radicals, pH 0.35, 1.2 krad/pulse; (b) reduction by e_{aq}^- at pH 5.2 (~4 krad/pulse). Insert, dependence upon pH of the rate of electron transfer from $(CH_3)_2\dot{C}OH$ radicals to 3-Me-Pt, N_2O-saturated solutions (1.2 krad/pulse); (c) reduction by e_{aq}^- at pH 8.3 (~4 krad/pulse). Insert, dependence upon pH of the absorbance of T_2 at 460 nm, produced by electron transfer from $(CH_3)_2\dot{C}OH$ (1.2 krad/pulse); and of T_1 and T_2 transients at 500 nm, produced from reaction with e_{aq}^- (~6 krad/pulse).

redox potential of Pt^- is much more negative than the kinetic potential[14] $E_k^{01} = -0.82$ V of $(CH_3)_2\dot{C}OH$. The kinetic potentials of the $(CH_3)_2\dot{C}O^-$ and $CH_3\dot{C}HO^-$ radicals are <−1.0 V and explain the 100% transfer from these radicals to Pt^-.

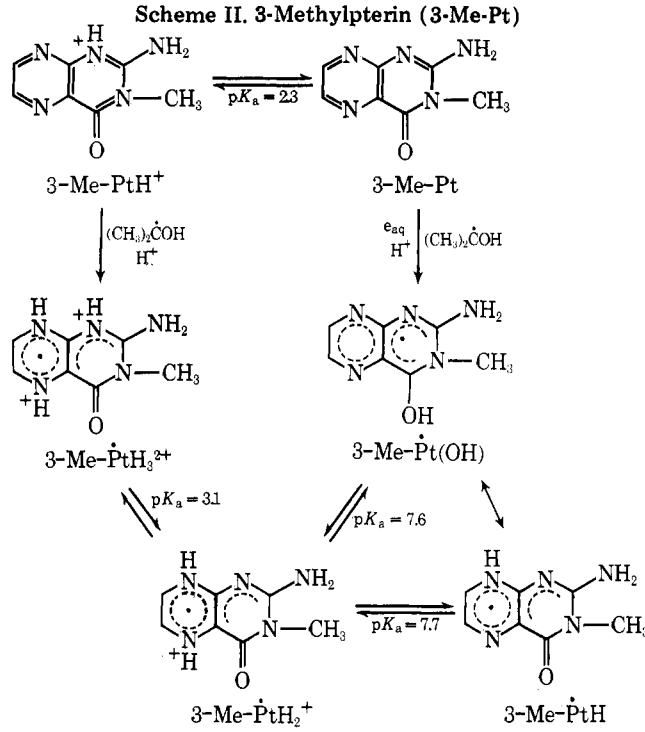
3-Methylpterin. 3-Methylpterin (pK_a = 2.3) was found to be unstable at pH >11.0. One-electron reduction of this compound was brought about by e_{aq}^- and acetone ketyl radicals. The $(CH_3)_2\dot{C}OH$ radicals were found to reduce 3-Me-Pt and 3-Me-PtH^+ with 100% efficiency, and reaction rate constants of 2.9×10^8 and 1.9×10^9 M^{-1} s^{-1}, respectively (see Tables I and II), were obtained.

The absorption spectra of the free-radical intermediates produced at pH 0.35, 5.2, and 8.3 are shown in Figure 6. At pH 5.2, an initial (T_1) transient species is observed whose spectrum changes with time to give a second (T_2) transient species. The change from the T_1 to the T_2 species is accelerated in the presence of good proton donors. Table IV shows the rate constants for this protonation reaction by H_3O^+ and $H_2PO_4^-$. No protonation by 3-Me-Pt itself occurred, supporting the statement made above for pterin that the N_3H hydrogen in pterin is a proton donor.

The changes with pH for the absorbances of these radicals at fixed wavelengths are shown in Figure 6 and Table III. pK_a values of ~7.6 for the T_1 species and 3.1 and 7.7 for the T_2 species have been observed.

Scheme II shows the reactions suggested to occur in this system. The initial (T_1) species observed at pH 5.2 is ascribed to a ketyl radical—3-Me-$\dot{P}t$(OH)—which on protonation to give the T_2 species forms predominantly a pyrazyl type of radical, 3-Me-$\dot{P}tH_2^+$. Since this molecule cannot undergo keto–enol tautomerism, ionization of the above radical is suggested to form 3-Me-$\dot{P}tH$. Further ionization

Scheme II. 3-Methylpterin (3-Me-Pt)



of the latter radical presumably occurs at pH > 11.0, a pH region which could not be examined owing to the instability of 3-Me-Pt itself.

Folic Acid. The reactivity of folic acid toward the one-electron reducing agents is quite similar to that of pterin; see Tables I and II. The spectral and physicochemical properties of the free-radical intermediates produced are

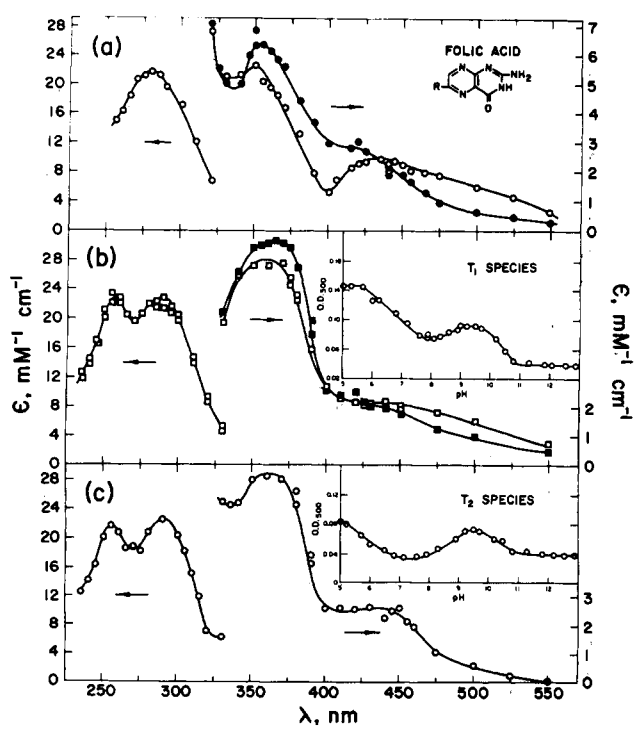


Figure 7. Absorption spectra of transient species (T_1 and T_2) produced from the one-electron reduction of folic acid by e_{aq}^- in water. Solutions contained 2.0×10^{-4} M folic acid (5.0×10^{-5} M at $\lambda <$ 320 nm), 0.5 M *t*-BuOH, 2 mM buffer, and argon (1 atm). Dark symbols represent T_1 species (absorbance read at ~0.5 μs) and open symbols represent T_2 species (absorbance read at ~5 μs): (a) at pH 7.2; (b) at pH 9.4, insert, change in absorbance of T_1 species with pH; and (c) at pH 11.9, insert, change in absorbance of T_2 species with pH. Total dose ~4 krad/pulse.

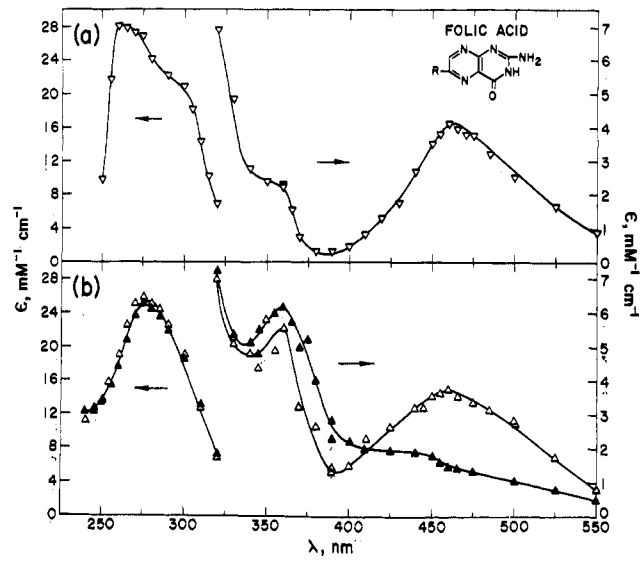


Figure 8. Absorption spectra of transient species (T_1 and T_2) produced from the one-electron reduction of folic acid in water. Solutions contained 2.0×10^{-4} M folic acid (5×10^{-5} M at $\lambda < 360$ nm). (a) 1.0 M isopropyl alcohol, pH 0.5, argon (1 atm), and 1.5 krad/pulse. Dark symbols represent T_1 species (absorbance read at ~0.5 μs) and open symbols represent T_2 species (absorbance read at ~5 μs).

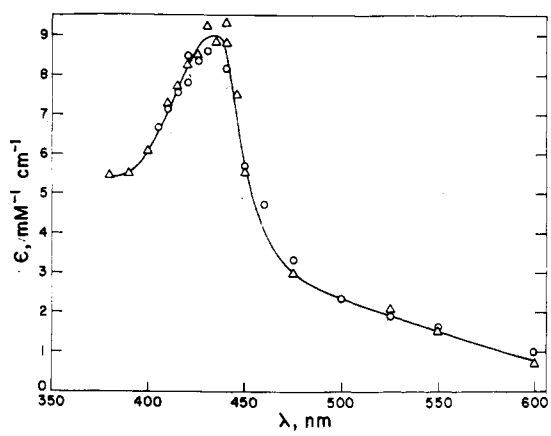


Figure 9. Absorbance spectra of transient species produced from the reaction of OH radicals with folic acid (10^{-4} M, N_2O-saturated aqueous solution) at pH 5.1, △, and pH 10.5, ○. Total dose ~7 krad/pulse.

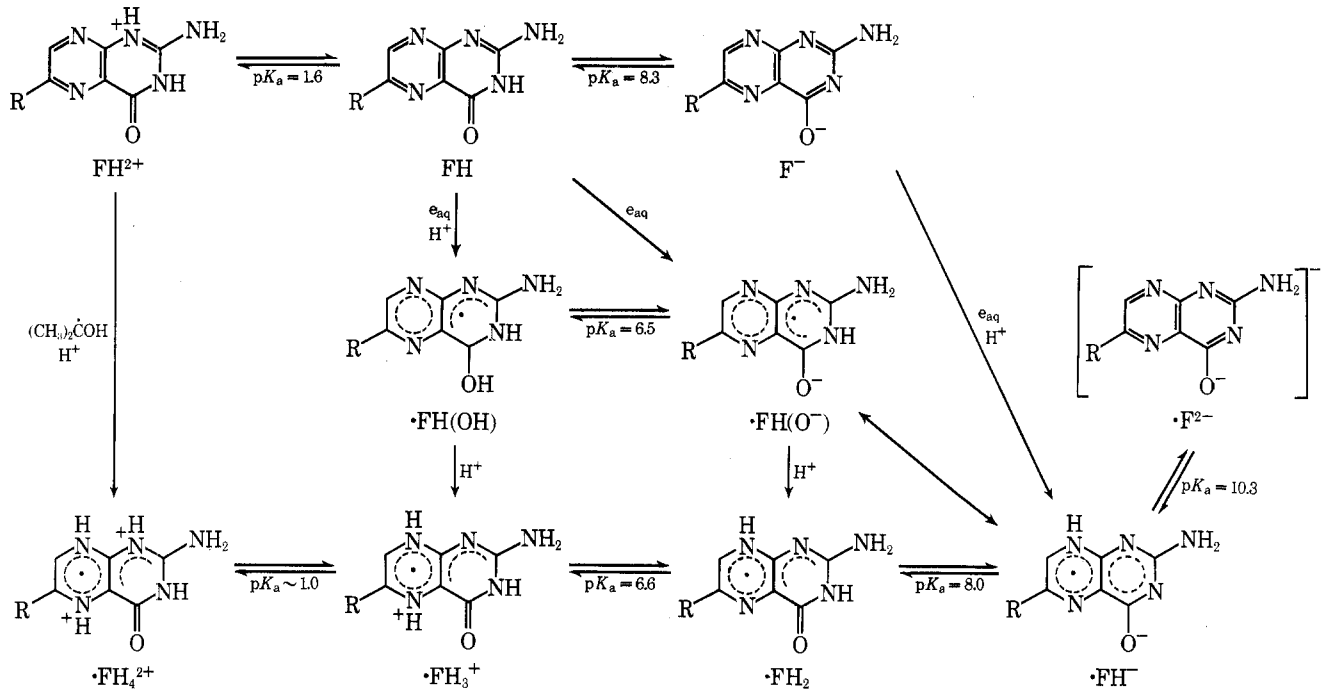
also similar to those observed from pterin (Figures 7 and 8 and Table III). This is not surprising since the side chain at the C_6 position is not expected to have a strong influence on the reduction or spectral characteristics of the transient species formed.

At pH 5.2, 7.2, and 9.4, the absorption spectra of the initial (T_1) transient species formed change with time. Protonation of T_1 gives rise to the T_2 species formed at a later time after the pulse. Table IV shows the rate constants for the protonation of the T_1 species formed at pH 5.2 by H_3O^+, $H_2PO_4^-$, and folic acid itself. These rate constants are somewhat different from those observed for pterin. No explanation is presently available.

The scheme of reactions tentatively suggested for folic acid is shown in Scheme III. The nature of the radical intermediates and their ionization constants are similar to those for pterin.

Reaction with OH Radicals. The reaction of hydroxyl radicals with aromatic compounds is less specific than that of e_{aq}^-—addition to the ring can occur at various positions. The reaction rate constants of OH radicals with folic acid

Scheme III. Folic Acid (FH)



at pH 5.1 and 10.5 were determined by monitoring the formation kinetics of the transient species produced from this reaction, Figure 9. The k (OH + FH) = 9.6×10^9 M^{-1} s^{-1} at pH 5.1 and k (OH + F^-) = 1.2×10^{10} M^{-1} s^{-1} at pH 10.5. No change was observed in the absorption spectra of this intermediate from pH 5.1 to 10.5 (Figure 9). The OH radicals presumably add at the pyrimidine, pyrazine, and *p*-aminobenzoic acid rings. In addition some abstraction of an H atom from the glutamic acid side chain may occur. The radicals decay by second-order kinetics with $2k = 2.9 \times 10^8$ M^{-1} s^{-1} at pH 5.1 and $2k = 6.0 \times 10^7$ M^{-1} s^{-1} at pH 10.5.

Conclusions

The nature of the free-radical intermediates formed from the one-electron reduction of pterin, 3-methylpterin, and folic acid in water at different pH values have been suggested. Schemes of reactions have been proposed.

These intermediates decay by second-order kinetics, with rate constants that range from $\sim 10^7$ to $\sim 5 \times 10^8$ M^{-1} s^{-1}; see Table III. Disproportionation reactions of these free radicals presumably occur with the formation of the corresponding dihydropyrazine derivatives. No transient spectra were observed from the decay of the T_2 species (or T species when no T_1 species were observed).

The intermediates formed appear to be good reducing agents. For example, the $\cdot PtH^-$ and $\cdot Pt^{2-}$ radicals have been found to reduce anthraquinone 2,6-disulfonate (E^{01} = −0.184 V) with efficient formation of the $\cdot AQ^-$ radical anion, with $k \sim 10^9$ M^{-1} s^{-1} for the electron transfer process.

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