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RADIOLYTIC DEGRADATION OF AQUEOUS CYTOSINE: ENHANCEMENT BY A SECOND ORGANIC SOLUTE

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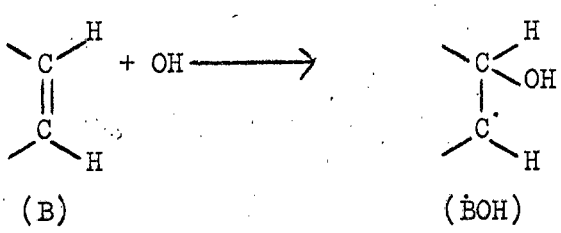
ABSTRACT

The resistance of the pyrimidine bases to the action of ionizing radiation in oxygen-free aqueous solution is interpreted in terms of a self-protective reconstitution reaction. The proposed reaction scheme is supported by the finding that certain organic solutes are as effective as molecular oxygen in increasing the yield for base destruction. A re-assesment of certain proposals regarding the effects of oxygen on radiobiological changes is suggested.

The purine and pyrimidine moieties represent major loci of chemical change in the radiation-induced degradation of nucleic acids in aqueous solution. (1) Studies of the radiation chemistry of the individual base-water systems have provided considerable information on the nature of some of the important reactions. (2-9) Of particular interest has been the finding that the presence of oxygen during irradiation leads to a two to threefold increase in the amount of base destroyed. (6-8) The available data suggest that some type of reconstitution reaction or back reaction is involved in the radiolysis of the oxygen-free solutions. (8,9) In the present report we offer a formulation of this reconstitution reaction and show that certain added organic solutes are as effective as molecular oxygen in increasing the degradation of cytosine in irradiated aqueous solution.

The actions of ionizing radiation on solutes in dilute aqueous solution are initiated by the radiation induced step $H_2O \rightsquigarrow H_2O^+ + e^-$ which is followed within 10^{-11} seconds by the reactions $H_2O^+ + H_2O \longrightarrow H_3O^+ + OH$, $e^- + H_2O \longrightarrow e^-_{aq}$ where e^-_{aq} represents the hydrated electron. (10) Subsequent reactions of OH and e^-_{aq} with added solutes determine the radiation chemistry of the system. For γ-rays, the yields of those species per 100 eV absorbed energy is given by $G_{e^-_{aq}} \simeq 2.9$, $G_{OH} \simeq 2.4$. (11)

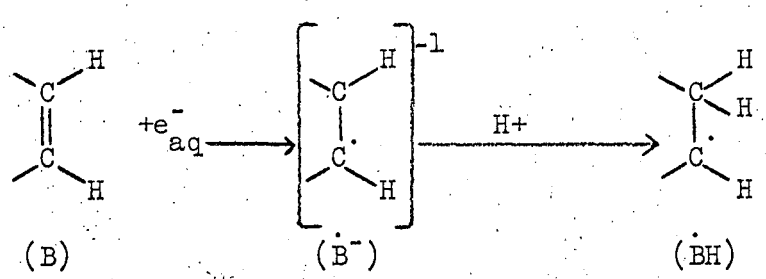
Scholes and Weiss (2) and Eckert and Monier (3) have shown that the OH radical reacts rapidly with the pyrimidine bases by addition at the 5,6 double bond to give the hydroxyhydropyrimidyl radical:



(1)

The production of glycols, hydroxyhydroperoxides and related derivatives of the pyrimidine bases in oxygen-flushed solutions (1,2) can be interpreted in terms of reaction 1 as the principal path for OH removal. The 100 eV yield for base destruction , G(-B), in 10^{-3} M solutions of thymine, uracil and cytosine under γ-rays approximates the primary yield of OH radicals viz. G ≈ 2.4. The hydrated electron e^-_{aq} reacts preferentially with molecular oxygen to form O_2^- which is unreactive toward the pyrimidine bases.

In the absence of oxygen, the G value for destruction of the pyrimidine and purine bases has been found to be about one-third that observed in oxygenated, neutral solution. Latarjet et al. (8) report G(-B) ≈ 0.8 for oxygen-free 10^{-3} M thymine solutions under γ-rays and the datum of Ponnamperuma et al. (6) gives G(-B) = 0.95 for aqueous cytosine under similar irradiation conditions. Now, as we have already noted, the evidence is that the OH radical is quantitatively scavenged via reaction 1 at relatively low concentrations of the pyrimidine bases. And, Hart et al. (10) have established by physical methods that the reactions of e^-_{aq} with the pyrimidines are among the fastest of known radical-molecule reactions. It would appear then that some type of reconstitution reaction is acting to reduce the G-value for base destruction in the oxygen-free system. If, as seems likely, the hydrated electron, e^-_{aq} also adds to the labile 5,6 position

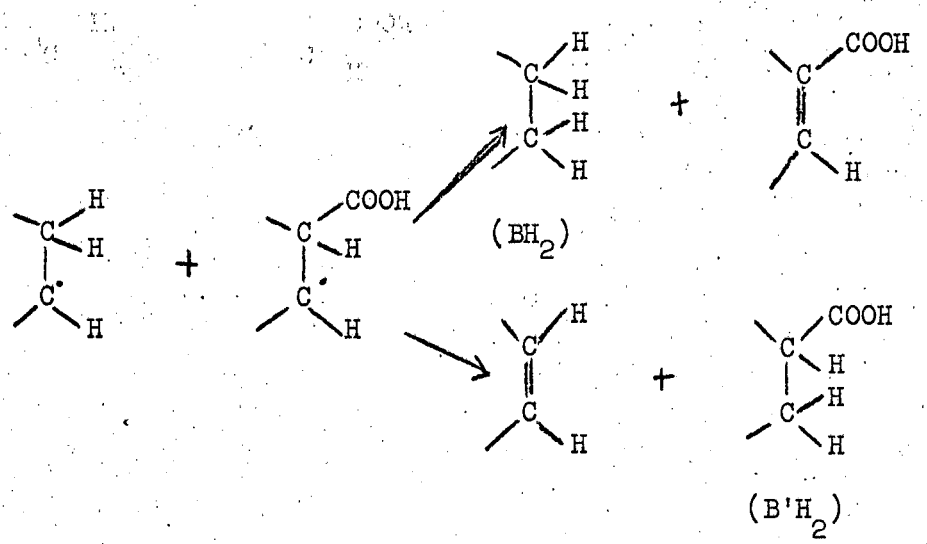


(2)

then the reconstitution reaction may be interpreted in terms of water regeneration via; $\dot{B}H + \dot{B}OH \longrightarrow 2B + H_2O$ or, $\dot{B}H + \dot{B}OH \longrightarrow B + BH_2O$ followed by $BH_2O \longrightarrow B + H_2O$ or both. On this basis it follows that addition of a second organic solute that is preferentially reactive towards OH via $RH + OH \longrightarrow \dot{R} + H_2O$, would lead on replacement of OH by $\dot{R}$ in reaction 1 to an enhancement in G(-B) since the possibility for self-protection through water elimination would be excluded. This increase in G(-B) would correspond to an increase in the yield of products saturated at the 5,6 position.

Cytosine was chosen for study here because the dihydrocytosine derivatives obtained on saturation of the 5,6 double bond hydrolyze readily to give the corresponding 5,6 dihydrouracil derivatives and ammonia (12) a product conveniently followed analytically. Sodium formate and ethanol were used as second solutes; each of these compounds is relatively inert towards e^-_{aq} and at the same time is extremely reactive towards OH via $HCOO^- + OH \longrightarrow \dot{C}OO^- + H_2O$ and $CH_3CH_2OH + OH \longrightarrow CH_3\dot{C}HOH + H_2O$. (10,11) The effects of added formate and ethanol on ammonia yields in the γ-radiolysis of oxygen-free .06 M solutions of cytosine at pH ~ 7 are shown in Fig. 1. We see that $G(NH_3)$ increases abruptly with increasing solute concentration and reaches a limiting value of approximately 2.9 at the higher formate concentrations.

Now, if our interpretation of this enhancement is correct, the hydrated electron e^-_{aq} is removed via reaction 2 and the OH radical is converted in the presence of formate to the $\dot{C}OO^-$ radical which in turn is removed via the analogue of reaction 1. The radical products of these reactions then undergo disproportionation via reaction 3 or reaction 4 or both.



(3)

(4)

where BH_2 and $B'H_2$ represent labile 5,6-dihydrocytosine derivatives that undergo rapid hydrolysis (12) to give ammonia and, respectively, hydrouracil (5,6-dihydrouracil) and a hydrouracil carboxylic acid. To confirm the formation of the hydrouracil nucleus, the irradiated cytosine-formate solutions were passed through Dowex 50 in the acid form to quantitatively remove cytosine. (13) A series of control runs with authentic materials showed that neither hydrouracil or hydrouracil-6-carboxylic acid are retained by Dowex 50. The water effluent was freeze-dried, redissolved in water at pH ~ 7 and then read spectrophotometrically over the range 210 mμ to 350 mμ against an unirradiated sample that had undergone the identical treatment. No characteristic absorption maxima were found under these conditions. However, addition of sodium hydroxide to a concentration of 0.1 N resulted in the immediate development of the characteristic absorption maximum at 230 mμ that is exhibited by hydrouracil and its derivatives in alkaline solution at pH values above 12 to 13. (14) The differential absorption curve of the irradiation products is compared in Fig. 2 with absorption spectra for authentic dihydrouracil and dihydrouracil-6-carboxylic acid. Since the

spectra of these two substances are essentially identical other methods had to be employed to distinguish their relative contribution to the absorption given by the product material. Advantage was taken of the fact that the effect of base on the optical absorption of hydrouracil and its derivatives is associated with the production of enol forms which are unstable and are rapidly hydrolyzed with characteristic first-order rates to give the corresponding ureido acid. Batt et al. (14) have reported half-times of 5.0 minutes and 11.1 minutes, respectively, for the hydrolysis of hydrouracil and hydrouracil-6-carboxylic in 0.1 N sodium hydroxide. The irradiation products whose initial absorption spectra in 0.1 N sodium hydroxide is shown in Fig. 2 showed a decrease in optical density at λ_{max} = 230 mμ corresponding to a half-time of ~12.1 minutes over a minimum of three half-time periods. Reaction 4 seems to occur preferentially over reaction 3. Preliminary spectrophotometric determinations of $G(B'H_2)$ based on $\epsilon_{max} \simeq 8 \times 10^3$ (14) gave values considerably below that anticipated on the basis of the indicated mechanism. Apparently $B'H_2$ undergoes a slow hydrolytic dark-reaction to form the corresponding ureido acid. However, the ureido acids revert to the original hydrouracil derivatives in the presence of dilute mineral acid. (14) Treatment of the irradiated cytosine solutions with 0.1 N hydrochloric acid for several hours prior to analysis gave $G(B'H_2) \simeq 2.4$ in good agreement with the reaction mechanisms described here. (16)

These findings would appear to cast some doubt on recent proposals regarding the nature and locus of the primary radiobiological lesion. Various authors have commented on the fact that the pronounced enhancement by oxygen of the radiation-induced degradation of the

pyrimidines and purines in aqueous solution is closely paralleled by the two to threefold increase in radiobiological damage effected by oxygen. And, it has been suggested (16) that this similarity in oxygen response is a strong argument in favor of a common radiation chemical process. However, we have found here that radicals of certain organic substances are as effective as oxygen in increasing the degradation of cytosine in irradiated aqueous solution. The possibility that organic radicals in anoxic biological systems under irradiation are equally effective in the enhancement of cytosine degradation cannot be dismissed. Hence, the interpretation of the oxygen-effects in radiobiology in terms of the radiation chemistry of pure base-water systems may prove to be misleading. (17)

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17. This work was performed under the auspices of the U. S. Atomic Energy Commission.

LEGENDS FOR FIGURES

Figure 1. Effect of second-solute concentrations on $G(NH_3)$ in the γ-radiolysis of .05 M cytosine, evacuated, pH 7 ± .3. Cytosine (Calbiochem., A grade, Lot 35887) was purified by fractional recrystallization from cold water. Solutions were irradiated in evacuated pyrex ampoules under Co^{60} γ-rays at a dose rate of 1.2×10^{18} eV/gm/min for a total dose of 6.5×10^{18} eV/gm. Ammonia was separated by the Conway diffusion method (15) following addition of 1 ml of sample to 9 ml of 10 N potassium carbonate. The diffusates were assayed by means of the Nessler reaction.

Figure 2. Absorption spectrum in 0.1 N sodium hydroxide of the dihydrouracil derivatives formed in the γ-radiolysis of .05 M cytosine is given by dashed line. The solid curve represents the normalized spectra of authentic dihydrouracil and dihydrouracil-6-carboxylic acid which are practically indistinguishable with the Beckman DB spectrophotometer.

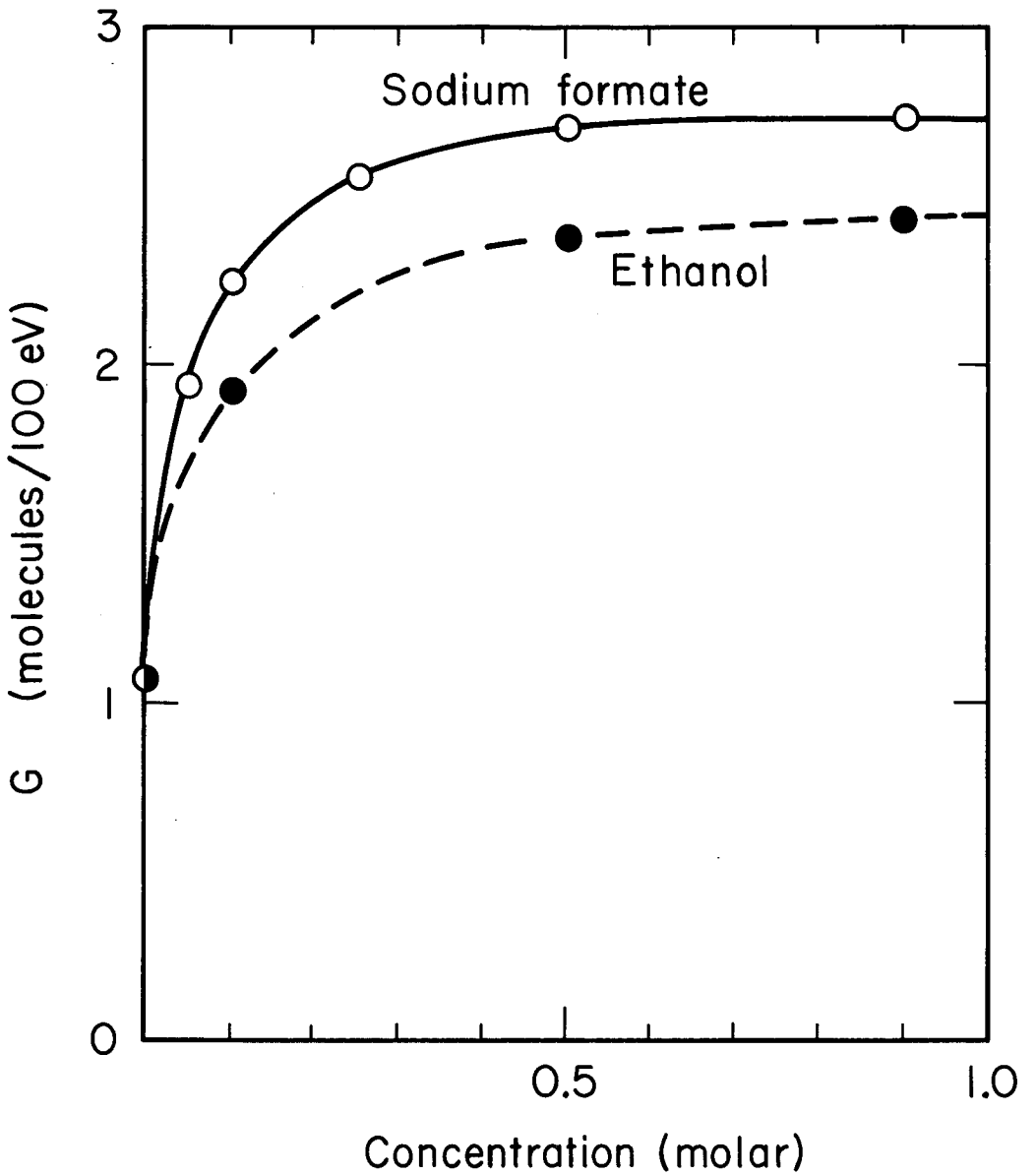

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Fig. 1

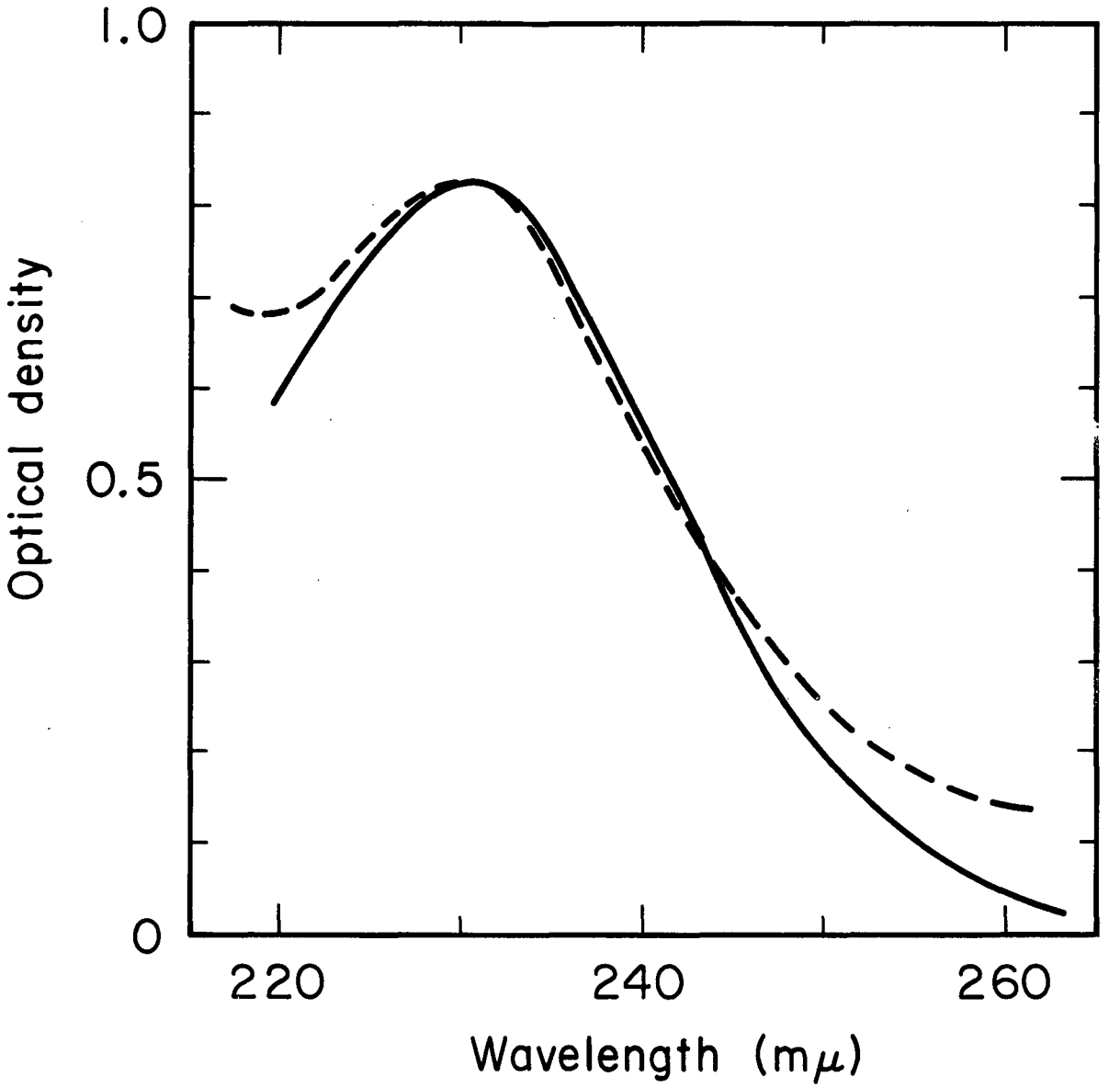

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Fig. 2