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The pyrimidine and purine moieties represent major sites of chemical degradation in the radiolysis of nucleic acids in aqueous solutions containing molecular oxygen.^{1,2} Radiation chemical studies of representative pyrimidines such as cytosine, uracil and thymine in dilute aqueous solution have shown that OH radicals formed in the radiation decomposition of water add preferentially to the 5,6 carbon-carbon double bond to form the hydroxypyrimidyl radical.^{1,3-5} In oxygenated solution, this radical is scavenged by oxygen and in subsequent steps yields the 5,6 glycol as a major radiolysis product.^{1,3,4}

Reaction stoichiometries in the radiolysis of the purines in oxygenated solution remain to be established.^{2,6} Adenine has received the most attention and is reported by Scholes and Weiss^{1,6} to yield ammonia with $G(\text{NH}_3) = 0.5$ (molecules/100 eV) on radiolysis with γ -rays in dilute aqueous solution; they suggest that such deamination arises as a consequence of OH addition to the central 5,6 double bond.⁷ Miss Conlay⁸ has isolated organic products from the same system and finds 8-hydroxyadenine and 3,4,5-triaminopyrimidine in yields corresponding to $G \approx 0.1$. The latter products provide evidence for OH attack at the 7,8 position of the imidazole ring. Such reaction is in accord with the Pullmans' molecular-orbital calculations⁹ which indicate that the 7,8 bond of the purine nucleus has the highest mobile bond order. However, since the yield

for OH production in the radiation decomposition of water corresponds to $G_{OH} = 2.4$ for γ -rays¹⁰ it follows that no conclusions regarding the major locus of reaction of OH with the purine nucleus can be made on the basis of the reported experimental yields.

Now, if glycol formation at the central 5,6 position is involved in the radiolytic oxidation of the purines, it is clear that such products on mild hydrolysis would liberate a reactive carbonyl function. Uric acid and xanthine for example would yield alloxan whereas the corresponding products from hypoxanthine and adenine would be expected to undergo further hydrolysis with liberation of free mesoxalic acid.¹¹

Oxygen-saturated solutions of uric acid, xanthine, hypoxanthine and adenine (Calbiochem, A grade, chromat. homogeneous) were irradiated with Co^{60} γ -rays at dosages in the range 2.8 to 5.6 10^{18} eV/ml. The irradiated solutions were made 2 N in hydrochloric acid, treated with 2,4-dinitrophenylhydrazine reagent at 70°C for 1 hour, cooled, and extracted with chloroform. The hydrazones were then transferred to filter-paper and chromatographed with a butanol-ammonia solvent system.¹² Control solutions containing known amounts of alloxan and mesoxalic acid were similarly treated and the authentic hydrazone derivatives were chromatographed in parallel with the irradiation products. Appropriate areas of the chromatogram were eluted and assayed spectrophotometrically. The indicated carbonyl products are formed in the yields given in Table I.

In the case of xanthine the evidence is that OH radicals are quantitatively removed at the 5,6 double bond to give $G(\text{alloxan}) = 2.4 \approx G_{OH}$. The carbonyl yields from hypoxanthine and uric acid indicate that although the 5,6 position of these compounds also represents the major locus of OH attack, other competing sites are involved. Adenine, on the other hand, appears to be

essentially unreactive at the 5,6 position. That adenine is, nevertheless, undergoing chemical change is evidenced by the fact that the yield for base destruction under the conditions of Table I (pH 3) corresponds to $G(-B) = 1.9$. This value was obtained through chromatographic isolation (on Dowex 50) of unchanged base from an irradiated solution. Adenine is the only aminopurine of the four purines listed in Table I: The question of whether the amino group is exerting a directing influence or is actually involved chemically as competing reaction locus remains to be elucidated.

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

References and Footnotes

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- (2) G. Hems, *Radiation Res.* 13, 777 (1960).
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- (6) G. Scholes, *Progress in Biophysics* 13, 59 (1963).
- (7) We use here the notation in which the carbon-carbon double bond of both the purine and pyrimidine nuclei is referred to as the 5,6 position.
- (8) J. J. Conlay, *Nature* 197, 555 (1963).
- (9) B. Pullman and A. Pullman, Comparative Effects of Radiation, Ed. by M. Burton et al., (John Wiley and Sons, New York, 1960).
- (10) E. Hayon, *Trans. Faraday Soc.* 61, 734 (1965).
- (11) See, for example, G. A. Howard, Chemistry of the Carbon Compounds, Vol. IV, Ed. by E. H. Rodd, (Elsevier Publishing Co., 1960).
- (12) B. M. Weeks and W. M. Garrison, *J. Phys. Chem.* 69, 4131 (1965).

Table I

Formation of carbonyl products in the γ -radiolysis of purine bases in oxygenated solution.^a

<u>Purine</u>	<u>Conc (mm)</u>	<u>Carbonyl product</u>	<u>Yield (G)</u>
Xanthine	2	alloxan	2.4
Hypoxanthine	2	mesoxalic acid	1.7
Uric acid	0.6 ^b	alloxan	1.2
Adenine	2	mesoxalic acid	~0.1

^aYields of the indicated carbonyl products are essentially independent of pH over the range 3 to 7.

^bSolubility limit.

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