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PROGRESS REPORT

 TO

THE UNITED STATES ATOMIC ENERGY COMMISSION

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BIOLOGICAL EFFECTS OF RADIATION AND RELATED BIOCHEMICAL AND PHYSICAL STUDIES

Proposal No. 5: Chemical and Biological Studies on Nucleic Acids and Derivatives

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Abstract

These studies attempt to elucidate the chemistry of purine N-oxides in the excited state, to evaluate their potential as sources of radicals, to consider possible routes of their natural formation, and to assess the importance of this to oncogenesis. The stable free-radical photochemically induced in solid purine N-oxide derivatives has been characterized as an amidogen radical delocalized through both rings. It has been determined that photochemical deoxygenation in solution proceeds from the N-oxide or N-hydroxy anion species. A two step rearrangement of a purine 1-oxide to a purine 3-oxide has been characterized.

A fetal component (antigen) present in a mouse tumor has been partially characterized. It was not detectable when the tumor cells were cultivated in monolayers, but reappeared when the cells were implanted in mice. Living mouse and rat sperm have been shown to penetrate normal diploid cells when mixed in culture, and induced abnormalities in morphology and growth in the recipient cells.

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I. Title: "Chemical and Biological Studies on Nucleic Acid Derivatives"

II. Objective: The purpose of these studies is to elucidate the chemistry of purine N-oxides in the excited state, to evaluate their potential as sources of radicals and to consider possible routes of their natural formation, and to assess the importance of this to oncogenesis. These are a fundamental adjunct to, and are correlated with, a larger program that seeks to determine the chemical events that lead to oncogenesis by some purine N-oxides.

III. Material and Methods: Samples are irradiated in degassed solutions (N_2) that have been adjusted to appropriate pH values, based on known pK_a values. Irradiations are carried out either in an immersion apparatus with a 450W Hanovia high-pressure mercury lamp with a Corex filter or in a Rayonet Photochemical Reactor equipped with 254 or 300 nm lamps and a Merry-Go-Around apparatus. Aliquots are withdrawn periodically and the photoproducts are analyzed by ion-exchange chromatography. For identification the solutions are concentrated in vacuo to a small volume when the reactions are complete and the products are separated by chromatography, usually with Bio-Rad AG-50, X8 (H^+) or with silica thin layer plates. Yields of reaction products are calculated from their known ε_{max} values. The structure of unknown products is established by appropriate techniques, including UV, IR, NMR and mass spectral and elemental analyses, and by total synthesis when the compound has not been reported previously.

IV. Results:

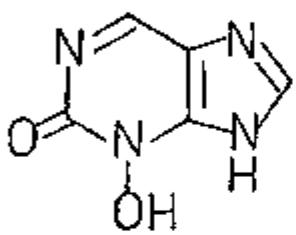
1) Work completed and published. "Purine N-Oxides. LVI. Photoisomerization of 1-Hydroxy- to 3-Hydroxyxanthine. Photochemistry of Related 1-Hydroxypurines." [1]

UV irradiation of solutions of 1-hydroxyxanthine causes extensive photoreduction and some photoisomerization to 3-hydroxyxanthine. This rearrangement from N-1 to N-3 occurs in either the neutral species of 1-hydroxyxanthine or its anion. Two structurally related purines, 1-hydroxyguanine and 1-hydroxyisoguanine, showed no evidence of comparable photoisomerization of the N-hydroxyls. The former undergoes photoreduction only, regardless of the ionic state. Irradiation of the cation of 1-hydroxyisoguanine yielded isoguanine and its 8-hydroxy derivative, while irradiation of the anion induced photoreduction and ring opening to two imidazoles, 4(5)-amino- and 4(5)-ureidoimidazole-5(4)-carboxamides.

2) Work nearly completed and manuscripts in preparation.

a) Purine N-Oxides. LX. T.-C. Lee, F.L. Lam and G.B. Brown. 3-Hydroxy-2-oxo-2,3-dihydropurine, 1, was prepared

from 5-aminocytosine 3-oxide.[2/] Like 3-hydroxyxanthine it reacted with acetic anhydride to form the acetyl ester, which undergoes 8-substitution reactions. Xanthine oxidase was found to oxidize C-8 preferentially to give mainly 3,8-dihydroxy-2-oxo-2,3-dihydropurine and a trace of 3-hydroxyxanthine. In our last Progress Report we reported that UV irradiation (300 nm) of the neutral molecule (at pH 3.0) produced photoreduction to 2 (21%) and a small amount of 2,8-dioxopurine, 3 (1%). No 8-substitution product has been observed previously from irradiations of 1-, and 3-hydroxyxanthines under similar conditions. Irradiation of the anion also gave 7% of 2 and a trace of 3. The major product from the anion gave a positive Pauly test suggesting an imidazole structure. Data from the mass spectra of this unstable product were not sufficient to assign its structure.



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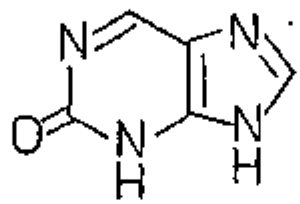

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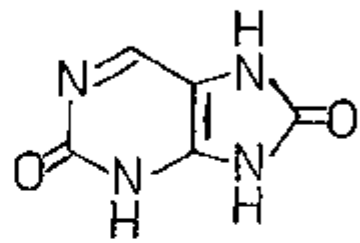

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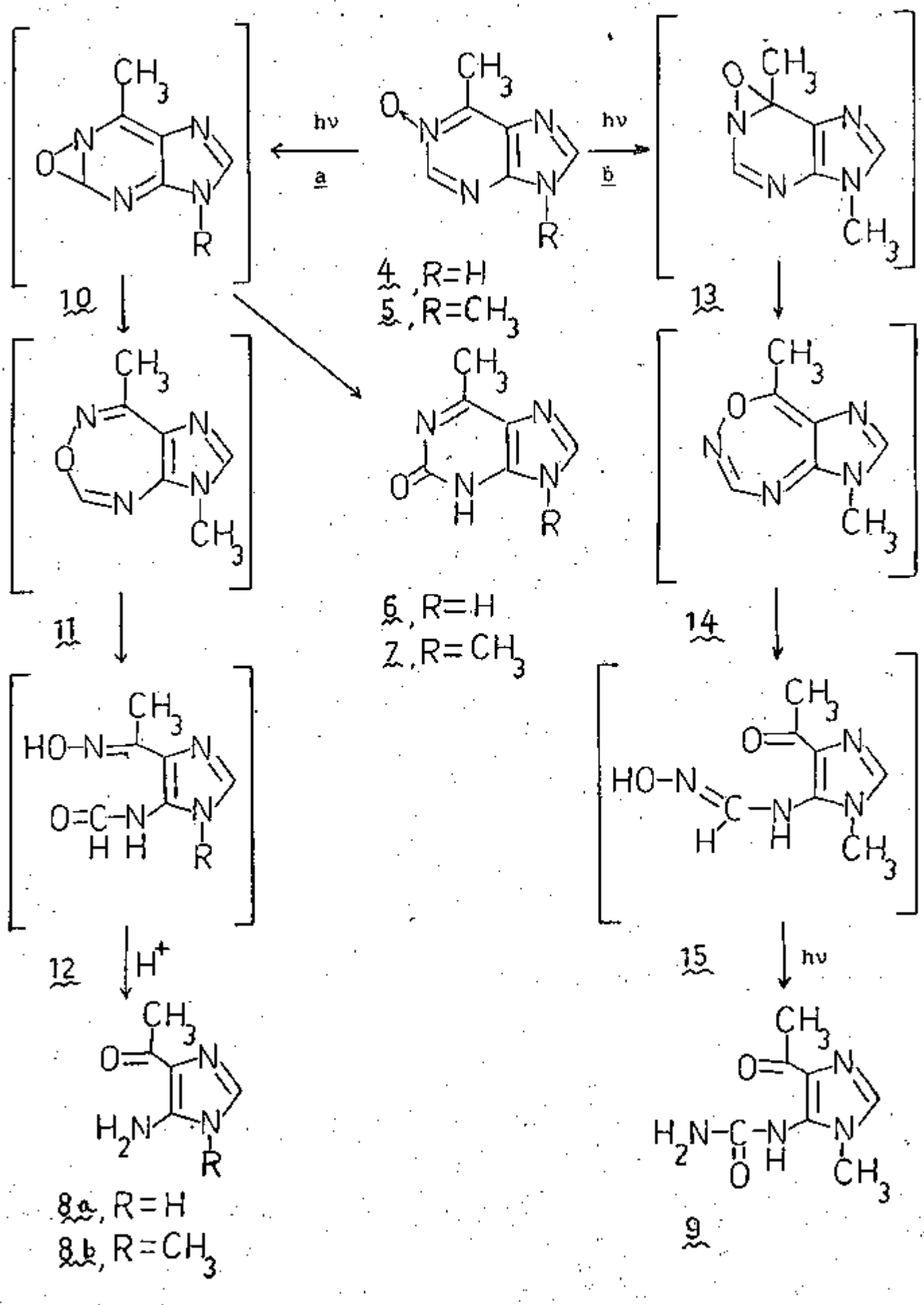
b) Photoreactions of 6-methyl- and 6,9-dimethylpurine-1-oxides. In our last Progress Report we discussed the results of UV irradiations of 6-methylpurine 1-oxide, 4, and 6,9-dimethylpurine 1-oxide, 5. Photoreduction and photorearrangement predominated, but some imidazoles, resulting from ring opening, were also observed.

The photoinduced migration from N to C of the oxygen of purine N-oxides is thought to proceed via a transient oxazirane intermediate.3/ Path a (Scheme I) illustrates the presumed oxazirane precursor, 10, of the 2-hydroxy-6-methylpurines, 6, and the oxadiazepine, 11, that would result from ring expansion of 10. Opening of the ring expanded intermediates, such as 11, has been proposed to explain the formation of some photoproducts of N-oxides. Hydrolysis of 11 would account for the formation of the amino-acetylimidazoles, 8, observed from irradiations of these compounds in acid.

One photolysis product from 5, an unknown discussed in our last Progress Report, has now been identified as 1-methyl-4-acetyl-5-ureidoimidazole, 9. It is detected only from irradiations in alcohol solutions. The formation of 9 may be reasonably explained by formation of the isomeric oxazirane, 13 (path b). Ring expansion

SCHEME I



of 13 to the imidazoloxadiazepine, 14, and hydrolysis of 14, would yield 15. Conversion of 15 to 9 could conceivably be accomplished by photorearrangement of the formamidoxime to the ureide of 9. While photoisomerization of oximes to the corresponding amides is known,[4,5] the corresponding rearrangement of formamidoximes to ureas has not been described. To test whether such a rearrangement was possible, N-phenylformamidoxime[6] was irradiated in alcohol solution. It was rapidly converted to several products but did not yield phenylurea. This suggests that formation of 9 is not preceded by hydrolysis to 15, but instead proceeds by direct photolysis of 14 to 9.

Quenching Study. Paramagnetic transition metal salts, such as Cu^{++}, Ni^{++}, Co^{++} and Cr^{+++}, strongly quench the triplet excited state.[8,9] Direct photolysis of 5 in aqueous solution (pH 3, 6 or 9) yielded equal amounts of deoxygenation and rearrangement (~10%). A study of the effect of paramagnetic, inorganic triplet quenchers on the photochemistry of 5 indicates that a triplet excited state is involved in photodeoxygenation. This conclusion is supported by the absence of any dimunition of photoreduction by a weak triplet quencher, Mn^{+2} (Table I).

Table I

Quenching Study of 6,9-Dimethylpurine in Aqueous Solution[a,b]

Expt. No.	Time (min)	Quencher (M)	6,9-Dimethylpurine, %	7,%	8,%	5,%
1	60	None	11	10	0	4
2	60	Ni^{++}[c] (0.0203)	0	11	37	8
3	60	Ni^{++} (0.0041)	0	13	25	5
4	60	Cu^{++}[d] (0.0175)	0	11	28	7
5	60	Cu^{++} (0.0035)	0	13	38	10
6	60	Mn^{++}[e] (0.0200)	9	10	0	3

[a] Carried out in Rayonet Photochemical Reactor equipped with 3000 Å lamps with a Merry-Go-Around Apparatus.
[b] ~2.2×10^{-3} M of 2.
[c] $NiSO_4 \cdot 6H_2O$.
[d] $CuCl_2 \cdot 2H_2O$.
[e] $MuCl_2 \cdot 4H_2O$.

c) 6,7-Dimethylpurine. Methylation of 6-methylpurine with dimethylsulfate in methanolic potassium hydroxide was reported to yield 6,9-dimethylpurine and 3,6-dimethylpurine.[10] This procedure was employed to prepare a sample of the former for the studies discussed in the preceding section. A third product, identified as the previously unknown 6,7-dimethylpurine (5%) was also isolated from the reaction mixture. The product was assigned the structure 6,7-dimethylpurine, rather than the isomeric

1,6-derivative, by the resemblance of its UV absorption to that of 7-methylpurine (267 nm) and the difference from that of 1-methylpurine (275 nm) and by the closeness of its pK of protonation, 2.68 ± 0.06 to those of 6-methylpurine, 2.6,[11] and 6,9-dimethylpurine, 3.2[10] in contrast to those of 3,6-dimethylpurine, 4.8[10] and 1-ethylpurine, 5.08.[12] Further confirmation was provided by examination of the difference in chemical shifts in CD_3SOCD_3 between the 2- and 8-protons of the three dimethylpurines. The 1- and 3-alkyl derivatives of purines show larger Δδ values than the 7- and 9-alkyl derivatives.[13] 6,7-Dimethylpurine showed a small Δδ comparable to those of 6-methyl- and 6,9-dimethylpurine and considerably smaller than the Δδ for 3,6-dimethylpurine.

Mass Spectra of Purine N-oxides. (In collaboration with Dr. Frank Field, et al. at the Rockefeller University). The compounds prepared for the photochemical studies have also proved useful for mass spectral studies. Although the mass spectra of some purines have been reported,[14,15] no such studies of purine N-oxides have been published. The major fragments (m/e) from all N-hydroxyxanthines and N-hydroxypteridines are M-16, rather than the M-17 peak expected for loss of OH. In contrast, 6-substituted purine 1-oxides are found to have two major molecular ions, M-16 and M-17 (Table II).

Table II

Mass Spectra of 6-Substituted Purine N-oxides

Compound	M,% Abundance	M-16,% Abundance	M-17,% Abundance
6-Methylpurine 1-oxide	150(89.6)	134(73.0)	133(44.2)
6,9-Dimethylpurine-1-N-oxide	164(100)	148(41.7)	147(36.1)
6-Methylpurine-3-N-oxide	150(45.3)	134(100)	133(2.5)
6-Methoxypurine-3-N-oxide	166(18.0)	150(41.2)	149(23.7)
6-Methoxy-7-methylpurine-3-N-oxide	180(10.8)	164(25.7)	163(9.5)

The conditions for facile deuterium exchange of the methyl group at the 6-position of 6-methylpurine 1-oxides permitted us to make deuterium labeled derivatives. Further study of the metastable ions and fragmentation patterns from the deuterium compounds are underway to establish the mechanism of the fragmentation. Samples of collidine N-oxide and 2,6-dimethylquinoline N-oxide are also being prepared for use in these studies.

3) Work in progress.

a) Photochemical reaction of 7-[16] and 9-[17]hydroxyxanthines. The photochemical reactivity of 7- and 9-hydroxyxanthines were examined as part of the studies on the general chemical reactivities of these derivatives. The results are summarized in Table III.

Table III

Photolysis of 7- and 9-Hydroxyxanthines

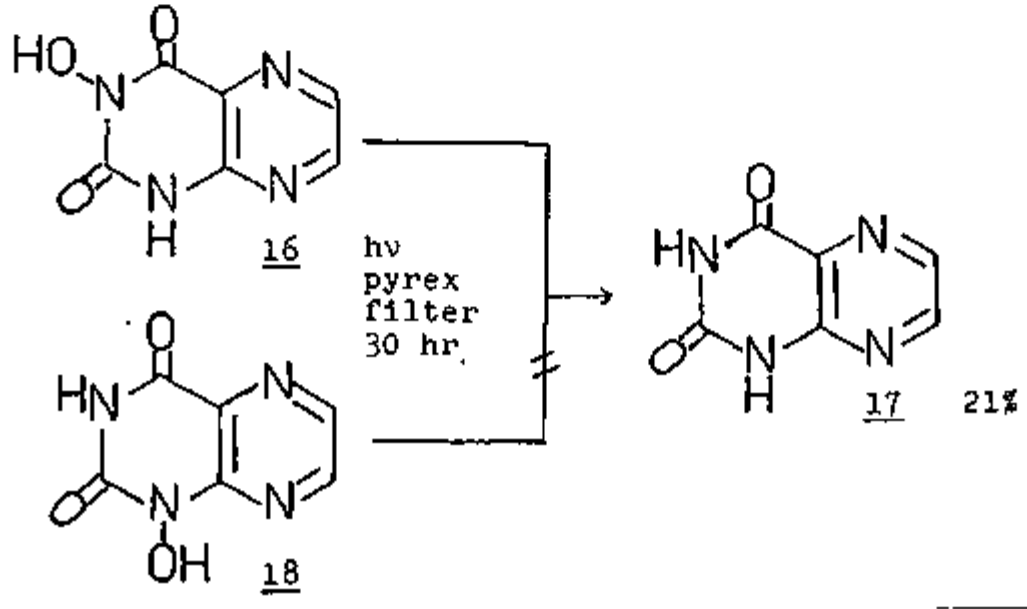
Starting Material	pH	Products[a] Uric Acid,%	Xanthine,%	Unreacted Starting Material,%
7-Hydroxyxanthine[b]	3.0	20	21	45
	10.0	23	20	37
9-Hydroxyxanthine[c]	3.6	0.2	27	0
	10.0	10	27	0

[a] 254 nm lamps, [b] pK_a 5.04 and 9.64, [c] pK_a 5.06 and 8.41

7-Hydroxyxanthine is readily esterified and its ester undergoes reactions identical to those of 3-hydroxyxanthine.[16] It is also an oncogen (unpublished). In contrast, 9-hydroxyxanthine is not easily esterified and undergoes rearrangement to uric acid only under vigorous conditions.[17] These differences were also manifested in their photochemical reactivities. 7-Hydroxyxanthine undergoes rearrangement and reduction to the same extent at both pH 3 and 10. The first ionization of 7-hydroxyxanthine is from the 7-hydroxy group, and the similarity of results at pH's above and below this ionization suggests that the excited state pK is near or below pH 3.

The first ionization of 9-hydroxyxanthine, however, was deduced to occur from both the N_3-H and the N_9-OH. Even as the dianion at pH 10 it yielded only 10% rearrangement and this was almost eliminated at pH 3. It is possible that in this compound the excited state pK would be associated with ionization at N-3, the usual position for the first ionization for xanthine,[18] rather than at the N_9-OH.

b) Photochemistry of 1- and 3-hydroxypteridines. 3-Hydroxypteridine, 16,[19] (3-hydroxylumazine) a structural analog of 1-hydroxyxanthine (kindly supplied by Dr. L. Bauer) was irradiated to examine its ability to undergo N-hydroxyl isomerization. To our surprise only the photoreduction product, pteridine, 17, was obtained at both pH 3 and 10. Under the same conditions the anticipated rearrangement product, 1-hydroxypteridine, 18, was unchanged. These results indicate that even the presence



of two adjacent carbonyl groups is not sufficient criterion for an allylic photoisomerization of N-hydroxyl groups.

c) 8-Substitution reaction. In our previous Progress Report, we mentioned that the irradiation of 1-hydroxyxanthine in 3 N TFA yielded a small amount of a product, tentatively identified as 8-trifluoromethylxanthine. A sample of this compound, previously unreported, has now been prepared, by deamination of 8-CF_3-guanine, for structure confirmation, determination of its extinction coefficient and quantitation of the formation of the product. Both 1- and 3-hydroxyxanthine yield 8-CF_3-xanthine in 1-2% yield and 3-hydroxyguanine produces 8-CF_3-guanine in a comparable yield. Xanthine under similar photolysis conditions (254 nm, N_2) does not produce 8-CF_3-xanthine and prolonged irradiation (24 hr) of it results in complete destruction of the purine chromophore.

d) Reaction of photoinduced radicals with organic solvents and correlations with photoreactions in solution. The isolation of 8-CF_3-xanthine from irradiation of 3-hydroxyxanthine in CF_3CO_2H suggested that this mode of reactivity might be exploited to elucidate the degree of radical participation in the photoreactions of 3-hydroxyxanthine and in the reactions of 3-acetoxyxanthine in solution. A sample of solid 3-hydroxyxanthine, irradiated as a suspension in ethyl acetate as described[20] to a maximum radical content, was allowed to react in CF_3CO_2H. The solvent was removed and the residue was chromatographed. Unreacted 3-hydroxyxanthine and a trace of a product, tentatively identified as uric acid, were obtained but no 8-CF_3-xanthine could be detected. Reaction of the irradiated 3-hydroxyxanthine in refluxing CF_3CO_2H gave uric acid in sufficient quantity for spectral identification, and 3-hydroxyxanthine and xanthine, but again no 8-CF_3-xanthine. The uric acid may well arise by esterification and rearrangement of the ester.

Because 3-acetoxyxanthine does not undergo spontaneous reduction in strong acid,[21] its reactions with trifluoroacetate were examined in 5 M potassium trifluoroacetate adjusted to ca. pH 3. In this system, neither 3-acetoxyxanthine nor the radical photoinduced in 3-hydroxyxanthine gave 8-CF_3-xanthine.

In the preceding proposal it was reported that the radical photoinduced in 3-hydroxyxanthine reacts in refluxing CH_3OH to yield 8-OCH_3-xanthine, in addition to 3-hydroxyxanthine and xanthine. It was noted that formation of this product is unusual, since no uric acid is produced from the reaction of the radical in water, and the photo-initiated addition of alcohols to purines occurs on the carbon of CH_3OH to yield 8-CH_2OH-purines not 8-OCH_3-derivatives.[22-24] The product from the reaction of the radical with CH_3OH has been carefully distinguished from 8-CH_2OH-xanthine. The extent of this 8-substitution by irradiated solid 3-hydroxyxanthine was examined with other nucleophilic solvents, including isopropanol, pyridine, and mercaptoethanol. With the first two only the products usually obtained, xanthine and unreacted 3-hydroxyxanthine were observed. With mercaptoethanol some minor components were detected by column chromatography, but these did not manifest UV absorption at long wavelengths, suggesting that substitution did not occur at

C-8 on the sulfur. There was also much more reduction to xanthine. In a control experiment, reacting un-irradiated 3-hydroxyxanthine with refluxing mercaptoethanol, there was also substantial reduction and some minor products. The similarity of the two sets of data indicate that the reactions are not associated with the radical.

In 12 N HCl the radical gave a small amount of an unidentified new product (pH 1, 277 nm; pH 12, 296 nm) that is eluted by water from AG-50 (H^+), but which does not agree with spectral values for either uric acid or 8-chloroxanthine.

When the radical is placed in either methanolic HCl or concentrated H_2SO_4 a gas is evolved. That evolved from H_2SO_4 has been identified by mass spectrometry as CO_2.

A study of the reaction of photoinduced radicals with CH_3OH was extended to other N-hydroxypurines. 3-Hydroxy-1-methylxanthine, irradiated as a solid and allowed to react with CH_3OH, gave 1-methyl uric acid (spectral identification), 1-CH_3-3-hydroxyxanthine and 1-CH_3-xanthine. 1,7-Dimethyl-3-hydroxyxanthine, under the same conditions, gave no starting material and no 1,7-dimethylxanthine, but yielded several unidentified products.

In a complementary study, 3-hydroxyxanthine was irradiated (254 nm) in CH_3OH both in the presence of O_2 and under N. Xanthine was the major product and no 8-OCH_3-xanthine was detected in either instance. When 1,7-dimethyl-3-hydroxyxanthine was irradiated in CH_3OH solution under N_2 and the sample was chromatographed over AG-50 (H^+) after removing the solvent, two components were observed. The first, eluted with water, manifested only end absorption in the UV and is apparently a product(s) resulting from decomposition. The second was an unknown with discrete UV absorption. No starting material and no 1,7-dimethylxanthine were present. These results parallel those from the irradiation of the solid, followed by reaction with CH_3OH. A larger sample (100 mg) of 1,7-dimethyl--3-hydroxyxanthine was irradiated in CH_3OH solution. Four products, two minor and two major, could be separated by tlc. Studies on structural characterization of the major products are in progress.

e) Comparison of the Redox ability of the photoinduced radical to that of 3-acetoxyxanthine. The reactivity of the photoinduced radical of 3-hydroxyxanthine with KI was examined to determine whether this radical could act as an oxidizing agent comparable to 3-acetoxyxanthine.[21,25] The study was complicated in aqueous solutions of the radical by the presence of UV absorption near the wavelength (350 nm) used to monitor the formation of I_3^-.[25] To circumvent this difficulty samples of equal weight (1.0 mg, 6 X 10^{-3} mmole) of irradiated 3-hydroxyxanthine were dissolved in equal volumes (2.7 ml) of 0.01 M phosphate buffer with one buffer containing 10^{-3} M KI. The sample without KI was used as a reference solution in measuring the UV absorption at 350 nm. This O.D. was 0.1. If it is assumed (a) that only one radical species (amidyl) is present, (b) that it accounts for 10% of the weight (12 ± 5% radical determined by ESR) and (c) that it requires two equivalents of this species per mole of I_2 formed, then 3 X 10^{-4} mmole of I_2 should be formed. This should manifest an O.D. of 1.1 (based on ε = 1 X 10^4 for I_3^- at 350 nm).[25] The observed O.D. of 0.1 probably represents the experimental error of the system, rather than a 9% conversion

of I^- to I_2 by the photoinduced radical.

f) Attempts to simulate photoreactions with Radical Systems. Two experiments were undertaken to determine whether the effects manifested by irradiation of 3-hydroxyxanthine in CF_3COOH solution or by irradiation of the solid and reaction with CH_3OH to yield 8-CH_2OH-xanthine could be simulated by a radical generating system. Xanthine was allowed to react in CF_3CO_2H under reflux with excess potassium persulfate. Persulfate has been reported to react with amides to yield amidyl radicals,[26] hence this system might react in a similar manner to amidyl radicals formed by UV irradiation in solution. No reaction products, 8-CF_3-xanthine in particular, could be detected by ion-exchange chromatography, but the presence of radical species in solution was not documented. Persulfate has also been reported to react with CH_3OH to yield initially the radical cation of CH_3OH ($CH_3{}^+OH$), followed by hydrogen abstraction by this species to produce $\cdot CH_2OH$.[27] The reaction of xanthine with potassium persulfate in refluxing CH_3OH, however, yielded no detectable reaction products. From these experiments persulfate does not appear to be a useful model for simulating reactions of the photoinduced radical or photoexcited species from 3-hydroxyxanthine in solution.

g) Structure proof of the solid state radical of 3-hydroxyxanthine. 7-Butyl-1-methyl-3-hydroxyxanthine, prepared from the 7-butyl-1-methylguanine discussed in our previous proposal, has now been synthesized in a small quantity. A synthesis of a larger sample is underway and we plan ESR and radical trapping studies with this compound in collaboration with Dr. Chester Alexander, University of Alabama.

V. Conclusion: We have shown earlier that photoreduction of N-hydroxypurines occurs from the triplet excited state.The present report presents evidence for a similar involvement of the triplet of N-oxidized purines constrained in the N-oxide form. Rearrangement of the oxygen to the adjacent carbon was previously shown to be associated with the enolate anion of N-hydroxypurines. This process must proceed from the excited singlet both in the enolate anion of N-hydroxypurines and, as the present studies show, in purine N-oxides.

We have presented evidence for parallel migrations of the N-oxide oxygen to both adjacent carbon atoms. One is associated with formation of the 2-carbonyl derivative, and can also yield one set of ring-opened products, while migration in the opposite direction leads to ureido-imidazole derivatives.

An unusual photo-isomerization of 1- to 3-hydroxyxanthine has been elucidated. The rearrangement occurs in both the non-ionized and ionized form of 1-hydroxyxanthine and is apparently unique to that compound. The observation of the photo-isomerization, even under acidic conditions that should preclude the presence of the anion, suggests that the enolate anion does not participate in this reaction. A photoenol containing a nitrone group was proposed as an intermediate.

Some unusual and unexpected formation of 8-trifluoromethyl-substituted purines has been documented from irradiations of N-hydroxypurines in CF_3COOH. This is presumed to be a radical substitution reaction, but efforts to enhance or to simulate the reaction with radical systems have thus far not been successful.

Some reactions of the radical photoinduced in solid 3-hydroxyxanthine are unusual and are not paralleled by similar reactions when 3-hydroxyxanthine is irradiated in solution in organic solvents. We did not find that this radical behaved as an oxidizing agent toward iodide ion in solution. These differences in reactivity suggest that photoreduction in solution, reduction of the radiation-induced radical of 3-hydroxyxanthine in solution and spontaneous reduction of 3-acetoxyxanthine in solution may not involve a common radical species, although each manifests some properties of a radical reaction and one does involve a demonstrable radical.

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Studies of the origin, nature and significance of gene products (antigens) which appear in tumors but not in the visceral tissues of normal adults have continued. We have also studied the effect of sperm on recipient somatic cells to learn more about the resulting appearance of embryo-related antigens on these cells. These studies were extended to cells isolated from normal rat liver.

We have isolated and partially characterized a fetal component present in a mouse tumor. Although present in aqueous extracts of 15 to 19 day mouse embryos, this component was not detectable as such in normal adult mouse tissue. It did not appear to be related to any known mouse virus. This component migrated with a β-2 mobility in agarose immunoelectrophoresis in a buffer of ionic strength 0.075 at pH 8.6. It could be eluted by Sephadex G-200 dextran column chromatography in a position similar to ovalbumin, and it showed a sedimentation coefficient in sucrose density gradients of about 3.5S. The fetal component was not detectable when the tumor was cultivated as monolayers in cell culture. Extracts of the cultured tumor cells and the concentrated growth media derived from the cultures no longer gave an immunoprecipitin reaction with specific antisera prepared against the fetal component. On reimplantation of the cultured cells into mice, the fetal component reappeared as early as seven days in the tumor which had developed, and was detected circulating in the animal blood after about three weeks. Various growth media were used in efforts to initiate or promote synthesis of this fetal component in vitro. These efforts have thus far been unsuccessful. This fetal component was also detected in extracts of various tumors of mice and in their sera; these tumors did not fall into any one single class. A cross-reacting skin component was isolated from adult mice. It absorbed out the anti-fetal component antibody from the specific antisera and differed from the true fetal antigen by having a lower molecular size and showing no migration in immunoelectrophoresis at pH 8.6.

Living mouse and rat sperm readily penetrated various normal diploid cells when simply admixed in culture. The ratio of sperm to cells employed was about 5:1. The entry, which could be observed in the living state, was also followed by scanning and transmission electron microscopy, by light microscopy on fixed and stained specimens, and by autoradiography using sperm the DNA of which was previously labeled in vivo with ^{3}H-thymidine. Labeled DNA passed directly from mouse sperm heads to nuclei of Chinese hamster cells after penetration. Living as well as heat-killed mouse and rat sperm were taken up by normal mouse and hamster cells and by cells cultured from the liver of normal and genetically diseased rats. Abnormalities in the morphology and growth of recipient cells resulted within 2 days. Bi- and multi-nucleated cells, arising from endomitosis rather than by cell-cell fusion, yielded permanently transformed colonies with growth and morphology characteristics similar to those resulting from parallel treatment

with carcinogens which ultimately lead to malignant conversion. These and related studies have raised the possibility that some tumors of the prostate or cervix might have a sperm vector.

Cultures of cells which have been carried continuously for more than a year were established from the livers of a normal Sprague Dawley and from a Gunn rat. The latter is a mutant with a genetic deficiency in the liver enzyme bilirubin uridine diphosphate glucuronyl transferase. The epithelioid cells from both strains show a predominant chromosome number 2n = 42, excrete serum albumin and α_2 and β serum globulins as observed by immunodiffusion and immunoelectrophoresis, and contain arylhydrocarbon hydroxylase; catalase activity was found in early culture passages. Benzpyrene or methylazoxymethanol acetate treatment changed the typical growth pattern consisting of flat regularly shaped cells to one which had lost population density inhibition and showed irregular piling foci and abnormal karyotypes. These cells which grow in soft agar, like transformed cells, are being examined for tumorigenesis in vivo. The liver cells from the Gunn rat serve as marker cells for ongoing studies on genetic repair and the relationship between chromosomal properties and the state of the genetic disease.

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* AEC support is acknowledged.

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