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STUDY OF THE RADIATION EFFECTS
ON
NUCLEIC ACIDS AND RELATED COMPOUNDS

THREE YEAR PROGRESS REPORT

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A. Introduction

Interest in the study of the radiation chemistry and photochemistry of biomacromolecules in aqueous solutions mainly stems from their biological implications. Study of the former was impelled by Muller's discovery (1928) that x-rays cause mutagenic and lethal effects. Study of the latter did not gain momentum until the mid-fifties, after the photoreactivation phenomenon had been explicitly described (Kelner, 1949; Dulbecco, 1949). Yet, photochemical and photobiological studies have made impressive progress during this relatively short interval (Smith and Hanawalt, 1969; Rahn, 1972; Setlow and Setlow, 1972), while the study of radiobiology and radiation chemistry has advanced in a much slower pace (Adams, 1972; Emerson, 1972; Latarjet, 1972; Myers, 1973; Scholes, 1975; Cerutti, 1975; Ward, 1975). The success in photobiology has been mainly attributed to the isolation and characterization of photoproducts of nucleic acid components, particularly the discovery of thymine photodimerization (Wang, 1960, 1961; Beukers and Berends, 1960, 1961). Therefore, the logical analog in this regard would be to isolate and characterize the radiation products of nucleic acid components. Additionally, earlier investigators have revealed the enormous complexity of chemical events in these systems and the possible degradation of nucleic acids by strand breakage (Revell, 1974). Consequently, our research has proceeded along both courses and our first two objectives are:

1) to isolate and characterize radiation products—a chemical study and 2) to determine the action of reactive radiation products on and in biological systems particularly as this action relates to strand breakage—a biological study.

An additional goal is to simulate γ -radiation reactions by exploring various photochemical reaction conditions. Such an approach will facilitate our study of radiation reactions, since the traditional methods have not met with great success by many laboratories. Apparently, the irradiation of nucleic acid components with 313 or 360 nm light in the presence of H_2O_2 (Milas et al., 1937) meets our criteria. This study provides data not only for comparison between photo- and radiation chemistry of nucleic acids but also about the effects of near-UV light on biological systems which could be related to aging, carcinogenesis, and mutagenesis. This constitutes our third objective.

B. Main Research Accomplishments

Substantial progress has been made in all three areas which are summarized below.

B.1. Chemical Study:

In aqueous media, the action of ionizing radiation on nucleic acids or their components is usually indirect and is mediated by the very reactive species produced by the radiolysis of water. These species then react non-selectively with every component of the polymeric nucleic acids including all of the purine and

pyrimidine bases. It is no wonder, therefore, that extremely complex chemical events have been encountered. For example, more than twenty radiation products have been observed for thymine, cytosine, or other bases. Furthermore, some of these radiation products are known to be reactive and therefore unstable. It should be apparent that one would face at least three extremely difficult tasks in any such attempt at chemical isolation, namely:

- a) The actual isolation of a pure product from a large number of closely related radiation products.
- b) The necessary conditions to preserve the reactive products for further chemical and biological studies.
- c) The collection of a sufficient quantity of radiation products from the minute amounts which are isolable from each experiment. The practical limitations here are the volume and the concentration of reaction solutions.

Thus, the isolation of materials in milligram quantities for characterization of a radiation product presents a tedious and formidable task. Obviously, these difficulties have impeded the progress of the study of radiation chemistry in general and consequently the progress of the study of radiobiology. In spite of these plights, decided advances have been made in this laboratory. Our research accomplishments may be summarized as follows:

B.1.a. Isolation and Characterization of Thymine Glycols.

Since the discovery in the late twenties that x rays cause mutagenic effects, the radiation chemistry of nucleic acids has received considerable attention. For some time, two thymine glycols have been indicated as initial radiation products. Although the cis-thymine glycol has been characterized, the actual isolation and identification of the trans-thymine glycol was not previously accomplished. We have successfully purified and identified the trans-isomer and consequently the elucidation of the chemistry of cis- and trans-thymine glycol. This understanding is essential for further study of the radiation reaction of thymine in aerated aqueous solutions as can be seen in B.1.c. (see Appendix I).

B.1.b. Isolation and Characterization of Glycols of 1-Carbamylimidazolidone as Products of Cytosine.

While irradiation of cytosine in aerated aqueous solutions has been reported since 1960, no radiation product has previously been identified. These compounds are the two major products and are the first two ever to be actually isolated and characterized from the irradiation of cytosine. The possible importance of these ring contraction compounds in radiation chemistry and biology should be examined (see Appendix II).

B.1.c. Identification of the Major Hydroperoxy Thymine (T^6OOH).

The major product from γ -radiation of thymine in aerated aqueous solution is shown to be cis-5-hydroxy-6-hydroperoxy-5,6-dihydrothymine by considering its uv, ir, nmr spectral data, the nmr deshielding effect on the OOH proton, and its reduction to cis-thymine glycol. These suggest that the earlier structural assignment (Cadet and Teoule, 1971) was wrong. Furthermore, HOOH oxidation of either cis- or trans-thymine glycol in acidic condition yields >90% of T^6OOH . This result again directly contradicts that reported in the previous paper. Our present findings are readily explained by considering the chemistry of thymine glycols and the reaction of OH radicals with thymine. Also, contrary to the earlier notion that this peroxide should be unstable, it can be rendered sufficiently stable for studying the thymine peroxide interaction with chromosomes, bacterial cells, and nucleic acid components (see Appendix III).

B.1.d. The Attempted Synthesis of the Nucleoside and Nucleotide of T^6OOH .

In our study of the effects of T^6OOH on biological compounds and systems, the findings are extremely interesting and clearly suggest that T^6OOH at very low concentrations has radiomimetic properties (see B.2.). Therefore, it seems highly desirable to expand the study of the effect of T^6OOH . The next

logical choice would be its nucleoside and/or nucleotide since these compounds more closely resemble those of polymeric DNA and understandably have additional properties particularly attractive for the study of biological processes. Earlier, we believed that we had succeeded in the synthesis of the nucleoside of T^6OOH (5-hydroxy-6-hydroperoxy-5,6-dihydrothymidine). However, our various attempts in the separation and purification of the four possible isomers have not been fruitful. The method used for the preparation is essentially the same as that of the synthesis of T^6OOH from its corresponding glycols. Since the latter synthesis gives excellent yields, (see Appendix III), it is reasoned that the method should be applicable to the preparation of T^6OOH nucleoside. Therefore, the purity and the identity of the starting materials, thymidine glycols, are open to question particularly in view of the recent mechanistic understanding of the interconversions of thymine glycols (Appendix III). Since thymidine glycols were prepared according to the reports of Subbaraman et al. (1973) and Cadet and Teoule (1972 b), we have communicated with these two laboratories. Dr. Behrman informed us that their spectral analyses of the cis- and trans-thymidine glycols (also other analogous glycols) were not sufficiently sensitive to allow determination of their purity. Dr. Cadet has visited us recently and will collaborate with us

to study this matter in greater detail. At the time being, we are re-investigating the chemistry of thymidine glycols - a presumably settled matter.

B.1.e. Synthesis and Characterization of 5-Hydroperoxy-methyluracil ($T^{\alpha}OOH$).

It has been shown that radiolysis of thymine and thymidine in aerated aqueous solutions resulted in the formation of $T^{\alpha}OOH$ (Cadet and Teoule, 1971; 1972a).

~~Cadet and Teoule, 1971; 1972~~ Swinehart, et al. (1973) have studied the γ -ray-induced production of $[^3H]H_2O$ from $[^3H]$ -thymine in a single- and double-stranded DNA and have suggested the formation of Thy^{α} as a possible intermediate for $T^{\alpha}OOH$ and other products. The same suggestion was made by Wang and Alcantara (1965) on the photo-oxidation of thymine in aqueous solutions. In view of the possible importance of $T^{\alpha}OOH$ in radio- and photobiology, the study of the effects of $T^{\alpha}OOH$ on biological compounds and systems seems to be warranted; especially in view of the interesting findings that resulted from our study of T^6OOH . In order to gain access to sufficient quantities

of $T^{\alpha}OOH$ for these further studies, improved synthesis of $T^{\alpha}OOH$ was pursued. Now, we are able to obtain $T^{\alpha}OOH$ in over 90% yields by these new methods.

B.2. Biological Study:

Radiobiological effects have been described most frequently in terms of physical and biological changes. This is due to the fact that these changes may be easily perceived or recognized. For instance, the lethal and mutagenic effects of ionizing radiation have been attributed largely to chromosomal aberrations resulting directly or indirectly from the production of strand breaks in DNA. On the other hand, the correlation of radiobiological effects with chemical modifications are infrequent at best. This occurs not only because the chemical changes are imperceptible but also because of the difficulties encountered in γ -radiation of nucleic acids (see p.5). The recent studies carried out by Cerutti and associates, however, may rapidly change this situation (Cerutti, 1975). At the same time, we have launched an investigation of the effect of T^6OOH on nucleic acid components and biological systems. This approach is unique in that it pinpoints the role played in radiobiological effects by a particular compound, in this case the major radiation product of thymine. With the successful synthesis of $T^{\alpha}OOH$, the second major radiation product of thymine, a similar study has been initiated in order to differentiate the effects of these two products on molecular and cellular systems. If quantitative

and qualitative differences were observed, the findings could be related to the known molecular structures and chemical reactivities. Progress has been made in several areas, some in collaboration with other groups.

B.2.a. Effect of T^6OOH on nucleic acid components (by L.W. Parkhill).

This study was undertaken in order to evaluate the effect of T^6OOH existing in a DNA chain or in a DNA precursor pool of the cells. Adenine and uracil were found to be resistant to T^6OOH while guanine, cytosine, and thymine gave rise to many products. There are two interesting findings: 1) cytosine is converted to N(3)-oxide in the presence of T^6OOH which, in turn, reacts to give 85% 5-methyl-5-hydroxyhydantoin and 11% of thymine glycol, and 2) thymine in the presence of T^6OOH yields thymine glycol as the product for both compounds (see Appendix IV).

B.2.b. Effect of T^6OOH and divalent ions of transition elements on C-N glycosidic bonds (by Hahn).

This study was undertaken to investigate the possible mechanism of DNA strand breaks by γ -radiation. It is known (Rhaese and Freese, 1968; Freese, 1971) that $HOOH$ acts on these compounds to yield the bases. Since $HOOH$ becomes inactive in biological systems due to the presence

of catalase and T^6OOH remains active, it would be interesting to investigate such bond cleavages between sugar and base moieties of nucleosides and nucleotides with T^6OOH . When deoxyadenosine, deoxyguanosine, thymidine, or deoxycytidine was treated with T^6OOH in the presence of a catalytic amount of Fe^{++} at room temperature, the corresponding base was detected upon thin layer chromatography. We are in the process of determining the rates of these C-N glycosidic bond cleavages in these nucleosides and in ^{14}C -labeled DNA in order to compare them with the rates of base alterations by γ -radiation.

B.2.c. T^6OOH as a chemical mutagen (by Thomas)

In this study, Prof. Roger M. Herriott and his graduate student, Harvey F. Thomas found that T^6OOH produced streptomycin and viomycin resistant mutants in washed cultures of H. influenzae. The number of mutants of either kind was roughly proportional to the exposure time and to the concentration of T^6OOH . T^6OOH also produces changes in in vitro transforming DNA which lead to an increased number of antibiotic resistant mutants among the recipient H. influenzae. These changes are induced only if the reaction takes place in the presence of divalent ions of transition elements (see Appendix V).

B.2.d. Effect of T^6OOH on chromosomes of cells (by
J.E.T. Kelley and Prof. T. Merz).

This study is mainly concerned with the chromosomal aberration production by T^6OOH . Our early observation was that achromatic gaps were produced in human lymphocyte cultures by treatment with T^6OOH in the first four hours of culture. The frequency of achromatic gaps was about 12-18% at a concentration of 0.1 mM. In general, treatment with a clastogenic agent under these conditions would be expected to produce chromosome aberrations of the deletion and translocation types. Attempts to elicit a significant number of these types of aberrations by using higher concentrations of T^6OOH resulted in a severe depression of mitotic activity. It was decided to try another system which more reproducible cell kinetics.

Chinese hamster V79B-1 cells were treated with T^6OOH and fixed immediately after treatment. The expected aberrations would be achromatic gaps, chromatid deletions, isochromatid deletions, and chromatid exchanges. All of these were observed as given in the following Table.

<u>Conc.</u> T^6OOH	Aberrations*/cell		
	<u>Gaps</u> (Achromatic)	<u>Deletions</u> (Chromatid and Isochromatid x 2)	<u>Exchanges</u> (Chromatid)
0.50 mM	1.03 ± 0.28	0.66 ± 0.23	0.00 ± 0.00
0.75	6.58 ± 0.95	1.22 ± 0.18	0.26 ± 0.07
1.00	12.44 ± 1.67	1.32 ± 0.14	0.16 ± 0.06

*These aberration values are for cells fixed one hour post-treatment. The pre-fixation colcemid period was for two-hours, including the last hour of treatment and the first hour post-treatment. The treatment period was three hours.

To estimate the proportion of late S phase cells present in the metaphase population at this fixation time, pulse-labeling studies were carried out. The results indicate that about 10% of cells were undergoing semiconservative replication with heavy labeled nuclei during the S phase and that about 90% of the cells are presumptively G₂ with unlabeled nuclei.

While deletions and exchanges are generally considered to result from strand breakage, the question of whether achromatic gaps represent strand breaks is a matter of controversy. The values given in the Table are the maximum values obtained. They are given here because they allow a more sensitive estimate of the lower probability types of aberrations, i.e. chromatid exchanges, and isochromatid deletions. These data cannot be taken as evidence that T⁶OOH caused strand break directly, although it indicates such a possibility. Alternatively, it is possible that the strand breakage implicit in these data, result from some abortive repair process. The existence of a repair process subsequent to exposure to exogenously supplied T⁶OOH was tested by assaying for unscheduled DNA synthesis (UDS).

In the UDS assay, the cells were treated with T^6OOH for three hours and were incubated for 90 minutes in medium containing $[^3H]$ -thymidine before fixation. Afterwards, their radioactive grains were counted. The values are given in the following Table.

Treatment with T^6OOH	Labeling Index ⁺	≤ 40 grain cts/nucleus		
		Average $\bar{X} \pm S.E.$	Medium for 150 nuclei	% having ≤ 3 grains cts
0.01 mM	439 (43.9%)	8.720 ± 0.528	6.44	18.00%
0.1	462 (46.2%)	7.180 ± 0.496	5.00	27.33
1.0	89 (8.9%)	7.200 ± 0.608	4.50	44.00
Control	467 (46.7%)	7.087 ± 0.519	5.13	31.33
10 kR*	379 (37.9%)	14.152 ± 0.707	12.9	10.00
Sham	491 (49.1%)	8.213 ± 0.545	5.67	23.33

* Irradiation was done with a Keleket constant potential X-ray machine operated at 250 kvp and 15 ma. The beam filtration was 1 mm Al and the dose rate was 1,000 R/min.

+ Labeling Index is the number of nuclei with grain counts > 40 per 1,000 nuclei. The grain counts upon which these figures are based were not corrected for background. Since the experimental design did not utilize a specific inhibition of semiconservative DNA synthesis, there were many heavily labeled nuclei on the slides for all the treatment with the exception of the 1 mM T^6OOH group. Therefore, all nuclei with grain counts/nucleus > 40 were arbitrarily classified as S phase cells and were not included in the counts.

The values for the control cultures and the x-radiated cultures are an indication that the assay is functioning properly. The values for the low concentration T^6OOH treated cultures do not indicate the presence of a UDS process but with 1 mM T^6OOH treated culture imply a repression of thymidine incorporation. Whether this inhibition applies to both semi-

conservative synthesis and UDS or only to semiconservative incorporation is being investigated.

B.2.e. T^6OOH as a potentially effective synergist of ionizing radiation (by J. Shaeffer and A.M. El-Mahdi)

It is well-known that biological systems under anoxic conditions show resistance to damage from ionizing radiation (Hollaender et al., 1951). In order to overcome the negative "oxygen effect" in cancer radiation therapy, one of the promising techniques is to expose the patients to x-radiation while under increased oxygen pressure (Churchill-Davidson, 1965). As possible carriers of oxygen, a number of transannular peroxides have been evaluated as potential radiation sensitizers in bacteria (Dougherty, 1973). Similarly, T^6OOH is a reactive peroxide and has been shown to be cytotoxic toward H. influenzae and Chinese hamster cells. These properties would make T^6OOH an attractive candidate as a synergistic adjunct of ionizing radiation for experiments using in vivo tumor systems. However, a prerequisite for such experiments is that T^6OOH itself not have lethal toxicity toward experimental animals at relatively high doses. The results showed that toxicity of T^6OOH to healthy (tumor free) and to tumor-bearing mice is rather low. At the 800 mg/Kg/day x 3 days (i.p.) level, all mice have survived the treatment. Therefore, the safety

margin is more than adequate for T^6OOH to be tested as a radiosensitizer (see Appendix VI).

Although the preliminary toxicity study indicates that all mice can tolerate T^6OOH at the 800 mg/Kg/day x 3 days level, the intraperitoneal injection of T^6OOH induces almost immediate (1-2 min) paralysis of the lower half of the animal. The duration is about 10 min and is dose-dependent. A weight loss is also evident during the three day injection period. Pathological examination of appropriate tissues, in particular brain and spinal cord of the central nervous system, are in progress.

B.3. Near-UV Radiation:

Hydrogen peroxide ($HOOH$) in aqueous solutions absorbs light of wavelengths shorter than 400 nm causing its decomposition to yield oxygen and water as final products. The nature of this reaction may best be described by the following generally accepted scheme:

The formation of $\text{HO}\cdot$ radicals by photodissociation of peroxide bond is the key step. $\text{HO}\cdot$, in turn, induces the formation of other oxidative species, $\text{HOO}\cdot$ and O_2 . These are the same species formed from water in the radiolysis of aerated aqueous solutions. Thus, this photolytic reaction condition simulates, at least in part, the radiolysis of compounds in an aerated aqueous solution. Consequently, identical products from reactions of these species should result under both conditions. Once the identity of a radiation product is established, the collection of a sufficient quantity of this particular product becomes a possibility with photolysis rather than an impracticability with radiolysis. In other words, we are taking advantage of the reaction similarity to synthesize a radiation product under a photolytic condition. This approach has enabled us to have a great deal of success in our Chemical Study (see B.1.). Other interesting results should be forthcoming.

In addition, a comparative study of photo- and radiation chemistry of nucleic acids can be deduced from the data obtained from these reactions and from those of direct radiation reactions. Also, it should be pointed out that in biological systems O_2 or HOOH may exist; and, under the influence of the near-UV from the sunlight, similar free radical reactions may occur. It is possible that this study

may yield information to interpret the effects of near-UV light on biological systems. If no efficient protection and repair mechanisms exist, "errors" caused by near-UV will result in damage to or dysfunction of the cells. These "errors" accumulated gradually over a life-span, may be manifested at times as aging, mutagenesis, carcinogenesis, etc. With a complete elucidation of the reaction mechanisms and unambiguous identifications of products of these near-UV reactions, effective means of protection and repair may be designed in order to avoid any harmful consequences.

In this connection, the study of near-UV radiation effects in biological systems has its intrinsic importance in biomedical and environmental research and, therefore, a more intensive study seems highly desirable and should be encouraged. However, these reactions are by no means simple and a thorough investigation would be a formidable task. This can be perceived from the following very brief account.

Irradiation of aqueous solutions of uracil, cytosine, thymine, and thymidine in the presence of equimolar H_2O_2 (~3 mM) with 313 or 360 nm light causes a gradual decrease of the respective characteristic UV absorbances of these solutions. The rates of these decreases are rather slow and the periods of radiation usually are in the range of 10-70 hours. Under these conditions, a large number of photoproducts are formed for each reactant. Among these are the desired

few which are identical to γ -ray radiation products. It is apparent that one would face the same difficult tasks at chemical separation and isolations in these photochemical reactions as those of complex radiation reactions (p. 5). However, there is a distinct advantage in that these photolyses are not limited by the volume and the concentration of reaction solutions. Indeed, by means of these photolytic reactions, the isolation of the desired radiation products for actual identifications and characterizations becomes a probability. Since we are also interested in obtaining data for comparison between photo- and radiation chemistry of nucleic acids and for the effects of near-UV light on biological systems, detailed qualitative and quantitative study were made. The following are lists of photoproducts which have been isolated and identified so far for thymine, cytosine, uracil, and thymidine respectively.

a. Photoproducts of Thymine:

Acetylurea

trans-Thymine glycol

cis-Thymine glycol

Pyruvylurea

5-Hydroxy-5-methylhydantoin

cis-5-Hydroxy-6-hydroperoxy-5,6-dihydrothymine

5-Hydroxy-5-methylbarbituric acid

5-Methylbarbituric acid

Pyruvic acid

Acetol

Formylurea

5,5'-di-5-methylbarbituric acid

b. Photoproducts of Cytosine:

Biuret

1-Carbamylparabanic acid

Urea

Parabanic acid

5-Hydroxy-cytosine

1-Carbamyl-4,5-dihydroxy-imidazolidone-2

Formyl biuret

Cytosine-3-oxide

trans-Uracil glycol

c. Photoproducts of Uracil:

Urea

Oxaluric acid

Parabanic acid

trans-Uracil glycol

cis-Uracil glycol

N-Formyl-N'-glyoxylurea

Formylurea

5-Hydroxyhydantoin

Isodialuric acid

Alloxan

Oxalic acid

5-Hydroxy-6-hydroperoxy-5,6-dihydrouracil

d. Photoproducts of Thymidine

cis-Thymidine glycol (?)

trans-Thymidine glycol (?)

Deoxyribosylformamide

N-Deoxyribosyl-N'-formylurea

N-Deoxyribosyl-N'-acetylurea

N-Deoxyribosyl-N'-pyruvylurea

1-Deoxyribosyl-5-hydroxy-5-methylhydantoin

cis-Thymine glycol

trans-Thymine glycol

Pyruvamide

Deoxyribolactone

Peroxide of Thymidine (?)

For the quantitative aspects, we have studied product analyses, the rates of product decompositions, and the rates of product formations. These data are necessary for the mechanistic understanding of these reactions. The following figures of thymidine irradiation are used to illustrate our approach and similar determinations have been made for other pyrimidines.

C. Future Plans

Certainly, there is an air of enthusiasm about the study of the effects of T^6OOH on biological systems and compounds. This is manifested by the collaborative efforts which have been generated at Hopkins and elsewhere. In order to maintain this valuable teamwork, at least one full-time chemist should be engaged in the preparation of T^6OOH , $T^{\alpha}OOH$, and possibly their nucleosides and nucleotides. At present, the level of support does not provide such an assistant. Notwithstanding, we endeavored to make some progress in our basic study of radiation chemistry during the past year (see B.1.d and B.1.e). It may be perceived that advances in our Biological Studies (see B.2) necessitated continued development in our Chemical Studies (see B.1). Thus, we are requesting a substantial increase in the level of support so that an effective effort can be sustained. Our immediate plans for the future, therefore, are projected on this basis. The proposed plan will be divided into several projects for clarity in our presentation.

Project 1. Chemical Study

This project will deal with four aspects which represent the continuation of our current investigation described in B.1, B.2.a, and B.2.b. These are a) isolation and characterization of various radiation products from pyrimidine and purine basis, and possibly their nucleosides and nucleotides; b) synthesis of various radiation products; c) studies of the effects of T^6OOH , $T^{\alpha}OOH$, etc. on nucleic

acid components, and d) the preparation of various compounds for collaborative work. Dr. Hahn should be assisted by a full-time assistant in this research.

Project 2. Mutagenic Study.

This project will attempt not only to correlate various radiation products such as T^6OOH , $T^{\alpha}OOH$, and their nucleosides with mutagenesis but also to investigate the chemical mechanisms causing such a mutagenic effect. Since the transformation assay we are using is an uncomplicated system, a mechanistic study may be realized. This project is a continuation of B.2.a, B.2.b, and B.2.c. Ms. T. Goldstein will devote full-time to this research. Previously, she worked under the direction of a chemical geneticist, Dr. Roger M. Herriott, and is currently studying a similar project with ultrasound radiation under my supervision.

Project 3. Chromosomal Aberration Study.

Previously, strand breakage and chromosomal aberrations have been investigated intensively as primary events occurring directly or indirectly by radiation energy. The present study is the first to show that chromosomal aberrations may be caused by a reactive radiation product of nucleic acid components. This study would shed a great deal of light on the chemical mechanism of strand breakage. (For personnel see F.2).

Project 4. Radiosensitizer Study.

In this project Drs. James Schaeffer and Anas M. El-Mahdi of the University of Virginia Medical Center, Division of Radiation Oncology will conduct experiments on three aspects concurrently. The first is to continue the pathological examinations of appropriate tissues discussed in B.2.e. Second is to examine the in vivo reproductive capacity of the tumor cells after the in vitro treatment with T^6OOH at low concentrations (10^{-4} - 1 mM); or, in other words, to ascertain the potency of T^6OOH as an anti-neoplastic agent against the Dunn osteosarcoma cells by direct interaction. Third, is to test T^6OOH as a synergistic adjunct of γ -radiation by direct injection of T^6OOH into animals bearing primary tumors on their hind limb. From the view point of the chemical characteristics, T^6OOH seems to be most suited for cancer radiation therapy. This feature should concern potential medical application and should have considerable current interest.

Project 5. Near-UV Radiation Effects.

On the one hand, we can not minimize the importance of the study of the near-UV radiation of purine and pyrimidine bases in our gratifying advances in the γ -radiation study. On the other hand, by its very nature near-UV radiation effects in biological systems may have direct concern in studies of aging, mutagenesis, carcinogenesis, etc. It is obvious to us that an expanded study in this project is warranted especially since a lot of data has been

collected in the past several years. We will continue our research with uracil, cytosine, thymine, thymidine, etc. in the rates of reactions, in the isolation and identification, in the reaction sequences and mechanisms, etc. Dr. K. Acholuno who has had experience in photochemistry of nucleic acids, expressed a great interest in engaging in this study.

D: Relation of Proposed Research to Comparable Work in Progress
Elsewhere.

Radiation chemistry of nucleic acids and related compounds, in fact, deals mainly with free radical reactions of biomacromolecules. These radiation reactions, therefore, must be non-selective and multitudinous. Furthermore, it is troublesome to handle the reactive and unstable radiation products. Because of these predicaments, much of the earlier research is on a stoichiometric level and may yield little information about molecular changes and chemical mechanisms. In recent years, the advent of the pulse radiolysis technique has permitted the in vitro study of the detailed chemical mechanisms between the active species and solutes (i.e. the radiolytic decomposition products of water and purine or pyrimidine derivatives). [These studies are being conducted by Drs. J.F. Ward, L.S. Myers, Jr. at UCLA; Dr. L.P. Theard at San Diego; Dr. J.W. Hunt in Toronto; J.D. Zimbrick at Kansas; E. Hayon at Natick; M. Simic at Houston; etc.] At the same time, advances in methods and instrumentation have aided the characterization of reaction sequences and the identification of radiation products. [These studies are being conducted by Dr. G. Scholes, and associates at Newcastle upon Tyne; Drs. R. Teoule, J. Cadet of Grenoble, France; Dr. Fendler of Texas A and M, College Station, Texas; Dr. G.A. Infante, Catholic University of Puerto Rico.]

In 1971, Cerutti and Hariharen developed the interesting approach of the direct identification and determination of specific

thymine radiation products in DNA in the low dose-range comparable to biological studies. They also demonstrated the post-irradiation removal of radiation modified moieties from γ -irradiated bacteria and mammalian cells (Mattern et al., 1973). Earlier evidence has indicated that such repair mechanisms may be operative (Lett and Sun, 1970; Painter, 1970 and references therein).

The main approach offered in this project is indeed complementary to those of the above and provides information which may not be obtainable by other means. In essence, our strategy is a two-pronged approach; one is to establish the quantitative and qualitative aspects of major radiation products, and the other is to ascertain unambiguously the biological effects of each of these compounds. This research course is a deliberate one, however, it may well be a most effective approach to a long-standing problem.

A study of this nature is necessary in order to define x- or γ -radiation toxicity on a molecule level, to set-up a safety standard, and to design methods of protection against and repair of these changes if they are judged harmful. This information should be useful for the maintenance of proper radiation control and, therefore, the better preservation of public health.

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F. Training of Personnel

Since the inception of this project, the level of support has been mainly for the provision of only one full-time research associate (in the first two years, some funds had to be diverted for the purchase of the Cs^{137} -gammator). Dr. Hahn is in his fifth year with this project and will continue. He was promoted to Assistant Professor last July in recognition of his accomplishment in the study of the radiation chemistry of nucleic acids and related compounds. This position should be helpful for Dr. Hahn in mapping his career. However, it must be noted that at Hopkins as well as at other similar institutions, there is a limit in the number of "real" faculty positions and in the actual physical set-up. Therefore, it is impossible for a member of a research team to become completely independent within the physical confines of a research group. This period is transitional and is generally ended soon enough for such personnel to have a more permanent position. Indeed, research funding during this period is essential for the well-being of such personnel and for the future of educational institutions.

In addition, there are three degree candidates who are writing their theses and should complete the degree requirements soon. All have engaged in the study of certain aspects of the effects of T^6OOH but have varied relationships with our group.

F.1. Mrs. Linda Parkhill (a MS degree candidate at Towson State College with Dr. Kundig) is under my direct supervision. Thesis title: The Effects of Thymine Hydroperoxide on Nucleic Acid Bases.

F.2. Mr. Jonathan E.T. Kelley (a Ph.D. degree candidate in the Department of Radiological Sciences with Dr. Timothy Merz) is in collaboration with us in this study. He developed a great deal of enthusiasm for this project and wishes to continue. With the eminent departure of Dr. Merz, Jonathan has indicated his desire to join our group as a post-doctoral fellow in further research concerning T^6OOH and T^6OOH nucleoside produced chromosomal aberrations and related phenomena. We would like to accommodate Jonathan, however, this is dependent upon the funding level of this contract. Thesis title: An Evaluation of cis-5-Hydroxy-6-hydroperoxy-5,6-dihydrothymine as a Possible Radiochemical Intermediate in Biological Systems.

F.3. Mr. Harvey Thomas (a Ph.D. degree candidate in the Department of Biochemistry) is working under the direction of Dr. Roger M. Herriott. This work has established that T^6OOH is a chemical mutagen. When Mr. Thomas completes his study, we would like to extend this research with the nucleoside and the nucleotide of T^6OOH . Thesis title: Thymine Hydroperoxide as a Chemical Mutagen.

G. Federal Support

Our overall research program is directed toward the understanding of the comparative radiation effects on nucleic acids and their components. At present, our study encompasses radiation chemistry (i.e. ionizing radiation of γ -rays), photochemistry (i.e. non-ionizing radiation of near- and far-UV light), and sonochemistry (possibly ionizing- and non-ionizing-like radiation of sonic waves). We intend to continue this unique research program and to divide our effort equally among these projects with a most capable team of researchers. We seek approximately the same level of support for each area of study. The following is a list of the current status and fundings of our projects.

<u>Project Title</u>	<u>Agency</u>	<u>Level of Funding</u>	<u>Renewal Date</u>
Ultraviolet Irradiation of Nucleic Acids and Related Compounds	ERDA	\$42,000	will be terminated
In Situ Fluorescent Probes for DNA Radiation Damage	ERDA	\$47,998	pending
Studies on Non-dimeric UV Radiation Damage in Cellular DNA	NIH	\$79,614/3 yrs*	05/31/78
This contract	ERDA	\$23,360	08/15/75
Sonochemistry of Nucleic Acids and Related Compounds	PHS-FDA	\$177,455/3 yrs*	09/30/77

* This amount does not include indirect costs as in the case of ERDA contracts.

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