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“MECHANISMS FOR RADIATION DAMAGE IN DNA”

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I. Main Research Accomplishments

Overview: Over the last 10 years we have made significant advances impacting our understanding of radiation damage to DNA resulting in about 50 publications in the scientific literature, including three reviews which cover much of our work using electron spin resonance, product analyses and ab initio molecular orbital theory (See Bibliography). The principal objective of our work was the elucidation of the fundamental mechanisms of radiation damage to DNA through the direct and indirect effects. While some initial work on indirect attack of peroxy radicals and protection by thiols were investigated, recently we concentrated on the direct effect of radiation damage on DNA. Our objective was to elucidate the ultimate radiation chemical damage to DNA arising from the direct effect. In this effort we focused on the application of three techniques. ESR (electron spin resonance) spectroscopic measurement of initial radicals formed in DNA and its hydration layer at low temperatures. This technique gives an experimental measure of the initial events of radiation. Ab initio molecular orbital calculations were employed to give highly accurate theoretical predictions of early events such as electron and hole localization sites which serve to test and to clarify the experimental observations. HPLC and GC-mass spectroscopic assays (in joint work with Steven Swarts and Ken Wheeler) of DNA base products formation provide the ultimate chemical outcome of the initial radiation events. The bridge between the early ion radical species (seen by ESR) and the non radical products (observed in product analysis by GC mass spec) is made in ESR studies which follow the chemistry of the early species as they react with water and or other DNA bases. The use of these techniques we believe, has resulted in a new and fundamental understanding of the radiation damage to DNA on a molecular scale. From this work, a working model for DNA damage from the initial ionization event to the eventual formation of molecular base damage products and strand breaks has been formulated. This model provides a focus for our and others future work. Below we describe our results over the past several years which have led to the formulation of

this model.

Approximately 50% of the water in the nucleus is hydration water, i.e., water that is associated with biomolecules in the first few solvation layers. One of the major uncertainties about radiation damage to DNA in the cellular environment is whether this water behaves as bulk water or as an integral part of the DNA. Early workers suggested DNA hydration water actually transfer ionizations as electrons and hole directly into DNA. In our recent work we have thoroughly investigated the effect of DNA hydration water on yields of ion radicals and transfer of charge into the DNA biomolecule (publ. 26, 27, 36, 41, 48). A recent breakthrough is the finding that the hydroxyl radical can be found in the hydration layer at low levels (36,48). It could not be detected previously at low temperatures due to the fact that it produces a different ESR spectrum in the glassy hydration layer than in bulk ice. This discovery has allowed the mapping of the hole transfer-hydroxyl radical yields as a function of the number of water molecules/nucleotide. This work has clarified the dividing line between the direct and indirect effects of radiation. We find that the ESR signal of the hydroxyl radical is not observed in the first 9 waters of hydration per nucleotide in DNA. This suggests that hole transfer in these waters occurs before deprotonation of the water cation to form the hydroxyl radical (48).

Up to our recent work it had been difficult to distinguish the cytosine and thymine anions in DNA since both were doublet spectra of about the same coupling (22). However, we have found that in D₂O the resolution of the spectra improves so that they can be easily distinguished. In addition we found that the cation species, G⁺• and A⁺•, could also be distinguished. In our work on dsDNA we showed through an extensive computer based analysis that the cytosine radical anion (C⁻•) and guanine radical cation (G⁺•) were the dominant ionic species at low temperatures.

We have also found that the radical ion distribution is a function of strandedness of the DNA (23). In single strand DNA we find that there is an increase in the contributions of thymine anion and adenine cation over that found in dsDNA. The strandedness of the DNA is also found to affect the subsequent

chemistry of the ion radicals. For example less 5,6-dihydro-thymin-5-yl ($\text{TH}\cdot$) radical is found in ssDNA than in dsDNA due to a reduced degree of ion transport.

Our recent work confirms that after electron localization on cytosine, the dominant mechanism for electron reaction is transfer to thymine followed by protonation to form $\text{TH}\cdot$ (31). No other anion radical protonation mechanism is found to be competitive due to differences in electron affinities which greatly favor the pyrimidines over the purines and the more rapid reaction of the thymine anion vs. cytosine anion radical. Recent product analyses do suggest at 37°C there is some contribution from protonated cytosine anion radical (42).

Over the past three years we have completed extensive ab initio molecular orbital studies of the ion radicals species involved in DNA radiation damage (24,25,29,30,33,37-39,43,49). In this effort the ionization energies (IE), electron affinities (EA), relative energies, fully optimized structural parameters and spin densities were determined. From calculations of the ionization potentials and electron affinities we predict the initial distribution of charge in irradiated DNA to be thymine anion radical and guanine cation radical as found in ssDNA (24). Calculations for the base pairs provide the explanation for the difference in the radical distribution in ss vs. dsDNA (25). In dsDNA, thymine anion cannot be protonated and stabilized whereas cytosine anion is predicted to be protonated from guanine. The initial ionization localizes the hole at guanine and the electron at thymine, but transfer of a proton from G to C causes the electron to localize on cytosine (28). We have studied the localization of charge on the DNA deoxyribose phosphate backbone which shows the ether oxygen as a site of localization (29). However, this IP is nearly 2 eV above that of the DNA bases which suggests hole transfer to the bases is quite likely. Each of the five possible sugar radicals in a model DNA backbone have been optimized and their relative stability and conformation investigated. The effect of the hydration layer on the electron affinities and ionization potentials has also been a focus of our efforts (30,37). The models used at this time for the bulk solvent are crude, but we have found that discrete

solvation of the ionic species with a single hydration layer allows for good estimations of the effect of solvent. This work is reviewed in publication 43.

In joint effort with Steven Swarts and Ken Wheeler of Wake Forest University, γ -irradiated DNA hydrated to various levels has been analyzed for 14 types of lesions (22,42). These DNA lesions include the release of unaltered bases, and specific DNA base damage. Perhaps the most outstanding finding is that the base damage yields are mainly confined to two products, one from reduction, dihydrothymine, and one from oxidation, 8-hydroxyguanine, properly called 8-oxoguanine; whereas, the base release yields are nonspecific. This product distribution has led us to the overall scheme for the radiation damage to DNA, starting with the initial ionization events assayed by ESR spectroscopy to the final products assayed in this work. In Scheme 1 we give an overview for the oxidative radiation chemical damage to DNA. The numbers on the right give the percentage yields for dry and hydrated DNA (which are similar) and those on the left give the percentage of expected ionizations based on the total valence electrons. Clearly, transfer of the hole from other DNA bases to guanine must occur at an early time and is shown in the figure; however, transfer from the sugar to the base is apparently not as efficient and this nicely explains the near uniform yield of unaltered bases. Scheme I therefore delineates the oxidative radiochemical processes from early processes (ionization) through radical intermediates some seen by ESR, to late processes which yield the damaged bases and base release. A similar scheme for the reductive component in DNA is hoped to result from our future investigations.

We have investigated the effect of binding of polycationic species such as spermine, polylysine and polylysine copolymers on the distribution of charge within the DNA strand and between the DNA strand and the polypeptide. For all polylysine-DNA copolymers we find that the DNA has a disproportionate amount of anion radical likely from electron transfer from the polypeptide. Typically our analyses suggest a 40% increase in anion radical for the DNA polypeptide

Scheme 1

Scheme I. This scheme shows the overall processes which occur after radiation damage to DNA. From the primary ionizations to charge transfer to secondary radical formation and final products. The scheme is a working model is a revised reversion of that published in Radiation Research 145, 304-314 (1996).

complexes. Hole transfer from the polypeptide is apparently not as facile. These results have implications for DNA irradiated with histones. The histones likely provide a protective effect from the direct effect of radiation by transfer of negative charge to DNA. Since it has been well established that the electron does not produce strand breaks, it is clear that increasing the number of anionic radicals on the DNA will likely result in ion recombination and a loss of potential strand break sites.

Some of our most fundamentally important work to DNA radioprotection was performed in our early work on this DOE grant. This work now funded by the NIH is in the area of the interaction of thiyl radicals and oxygen (5,8,9,11,14,17) as well as (DNA) peroxy radicals with thiols (13,18,32). This work has given a wealth of information not known previously which we believe has important implications for radiation damage *in vivo*. Up to our work in this area, the sulfinyl radical, $\text{RSO}\cdot$, was not known to be a product of thiol or carbon based peroxy reaction with RSH . The structure of the thiyl peroxy radical, $\text{RSOO}\cdot$, was uncertain. The conversion of $\text{RSOO}\cdot$ to the sulfonyl radical, $\text{RSO}_2\cdot$, and the subsequent reaction with oxygen to form the sulfonyl peroxy radical, $\text{RSO}_2\text{OO}\cdot$, had not been shown previous to our work. In our recent efforts we have investigated the reactions of thiols with primary ion radicals in γ -irradiated DNA and observed electron transfer from the thiol to the guanine cation radical (32, 35, 40). This is believed to be the major protective effect of thiols from the direct effect of radiation. Our work with DNA-thiols in the presence of oxygen shows the formation of thiol peroxy and sulfoxyl radicals. The formation of these species, instead of the radioprotective disulfide anion, is considered to be the explanation for the chemical oxygen enhancement effect.

Our work with peroxy radicals has also established that the ESR measured O-17 hyperfine couplings give a good estimate of the relative reactivity of the peroxy group and shows that the thiol peroxy radical cannot be an effective hydrogen abstracting agent (16).

Below, we outline some of the main features of our published work performed over the past several years. Original publications and preprints listed under the

publications section should be consulted for details.

a. Relative Abundance and Reactivity of Primary Ion Radicals in γ -Irradiated DNA: Single vs. Double Strand DNA.

Knowledge of the chemical nature and the distribution of the radiation induced damage sites in DNA is important to the understanding of the effect of radiation on biological systems. Until recently most investigations suggested that the thymine anion and guanine cation were the principal species found in γ -irradiated DNA at low temperatures. The fact that the final reaction product found in DNA is mainly the 5,6-dihydrothymine-5-yl radical, $\text{TH}^\bullet$, was a major piece of evidence in support of the thymine anion as the likely anionic primary radical. However, Bernhard, in work with oligonucleotides in aqueous glasses, found that the cytosine anion was the dominant anion radical; he suggested cytosine anion may also be the dominant anion in DNA as well. In previous work, the cytosine anion and thymine anion were difficult to distinguish in both DNA model systems and DNA since the spectra of both are doublets of about equal coupling. In last years effort, we found that the cytosine and thymine anion spectra were considerably more resolved and distinguishable in D_2O (vs. H_2O) matrices and through a series of experiments on polynucleotides, pdAdT and pG-pC, DNA, as well as computer reconstructions we concluded that cytosine anion is the dominant anion in γ -irradiated DNA. Our recent ab initio calculations (see below) and Steenken's measurements of ion radical pK's suggests that the cytosine anion is stabilized by protonation from its complementary base guanine, so that it is better designated as a neutral species, $\text{C}^-(\text{H}^+)^\bullet$. For simplicity we will use the symbol $\text{C}^{\prime-}\bullet$ for both the anion and the protonated species.

In this work an electron spin resonance investigation of the free radical distribution in γ -irradiated frozen samples of single stranded DNA (ssDNA) and double stranded DNA (dsDNA) is reported. Computer analysis of the ESR spectra of γ -irradiated ssDNA in D_2O at 100K suggests a more uniform distribution of the radical ions on

the DNA bases than found for dsDNA, with the approximate ssDNA composition: thymine anion, $T^{\cdot-}$ (30-35%), cytosine anion, $C^{\cdot-}$ (20-28%), guanine cation, $G^{\cdot+}$ (26-28%) and adenine cation, $A^{\cdot+}$ (8-17%) with small amounts of purine anions or pyrimidine cations. Upon annealing γ -irradiated dsDNA, we find that the electron transfers from $C^{\cdot-}$ to T, and the $T^{\cdot-}$ formed protonates at C-6 to produce the 5,6-dihydrothymine-5-yl radical ($TH^{\cdot}$, $TD^{\cdot}$). The rate of deuteration of $T^{\cdot-}$ is shown to be 20-fold less than the rate of protonation. Electron transfer in ssDNA appears to be less facile than in dsDNA. Holes transfer from adenine to guanine in ssDNA over the temperature range 130-190 K, whereas in dsDNA, the transfer of holes from adenine to guanine is nearly complete at 100K. The results suggest that DNA strandedness, which affects DNA conformation, base stacking and cross strand interactions, is important in determining the initial ion radical sites as well as the subsequent ion radical transfer and reactions. A calculation of the ion radical abundances as a function of base transfer, starting from a random distribution in a model DNA strand, suggests base pairing greatly augments hole and electron transfer through the DNA strand and that on average, ion transfer over only ca. 2 bases is sufficient to produce the ion distribution found in ssDNA and dsDNA.

b. Protonation of Nucleobase Anions in γ -irradiated DNA and Model Systems. Which DNA Base is the Ultimate Sink for the Electron?

Reversible and irreversible protonations of the nucleobase anions $C^{\cdot-}$, $T^{\cdot-}$, $G^{\cdot-}$ and $A^{\cdot-}$ have been followed with great interest since they play an important role in the mechanisms of radiation damage to DNA. Cullis et al. have recently reported works on the subject of the stabilization and reaction of primary ion radicals in DNA in which they suggest that the electron migration from $C^{\cdot-}$ to thymine does not occur on annealing and that the $T(C6)H^{\cdot}$ arises only from the original $T^{\cdot-}$ present in irradiated DNA (40kGy) at 77 K. The major support for this contention is the fact that only about 18% of the original signal converts to $T(C6)H^{\cdot}$ in contradiction to the 30% conversion found in our work. In this work we show that the conversion is

dose dependent and varies from 30% at low doses to 13% at high doses as described below.

In this work we attempt to better understand details of the protonations for the nucleobase anions in DNA from investigations of frozen solutions of dGMP•dCMP, dAMP•dTMP, polyG•polyC, poly[dGdC]•poly[dGdC], poly[dAdT]•poly[dAdT] and DNA itself. Analysis of the ESR spectra at a dose of 22 kGy shows that fractional conversion of total radicals to carbon protonated species on annealing is in the order: dAMP•dTMP (43%) > pdAdT = DNA (ca. 23%) > dGMP•dCMP (15%) > polydGdC•polydGdC (6%) > polyG•polyC (3%). Two hydrogen addition radicals make contributions to the polyG•polyC, poly[dGdC]•poly[dGdC] and dGMP•dCMP spectra in H₂O on annealing. They are those formed by protonation at C6 of cytosine anion radical, C(C6)H•, and at C8 of the guanine anion radical, G(C8)H•. Computer analysis reveals that anion protonation reaction in dGMP•dCMP results in mainly C(C6)H•, whereas protonation reaction in polyG•polyC and poly[dGdC]•poly[dGdC] yields mainly G(C8)H•. In dAMP•dTMP and poly[dAdT]•poly[dAdT] as in DNA itself the only DNA base found to undergo an irreversible protonation at a carbon site is thymine resulting in T(C6)H•. The conversion of DNA anion to T(C6)H• is found to be dependent on dose. At low doses (5 kGy), ca. 30% conversion to T(C6)H• is found whereas at high doses (94 kGy), only 13% conversion is found. Results show that at 40 kGy the 18% value reported by Cullis et al. is found in this work as well. The dose dependence is ascribed in part to ion radical recombinations whose probabilities are increased at high doses. A consideration of the rates of protonations of the purine and pyrimidine anion radicals as well as the differences in electron affinities suggest carbon protonation reactions of DNA base anions in irradiated stacked dsDNA at 37°C would be predominantly at thymine and cytosine, whereas in ssDNA all bases could contribute. We suggest electron migration may be expected to be a mechanism for DNA radioprotection.

c. The Influence of Hydration on the Absolute Yields of Primary Ionic Free Radicals in Irradiated DNA at 77K.

1. Total Radical Yields and the Role of Water (26).

In this work, an ESR investigation of the effect of hydration water on the absolute yields (G) of ion radicals in γ -irradiated DNA at 77 K is reported (26). The total DNA radical yield is found to increase by over four fold on addition of the primary hydration layer (20 water molecules/nucleotide) to DNA. At hydrations greater than ca. 20 waters/nucleotide the excess water freezes into a separate and apparently radiologically independent bulk ice phase. Ice formation steals ca. 5 waters from the hydration layer which causes a proportionate (by weight) drop in the total DNA radical ion yield. Hydroxyl radicals ($OD\cdot$) are not observed even at great signal amplification in the primary hydration layer but are found only in the ice phase, which suggests efficient transfer of holes and electrons from the hydration layer to the DNA; as a consequence, DNA and its associated hydration water form the target mass for radiation damage. These results show that water of hydration is critical to radical formation and stabilization in DNA; however, the ice surrounding DNA does not contribute to direct DNA damage and is found to have the same properties as bulk ice. At 18 waters per nucleotide ($\Gamma=18$) about 1/4 of all ion radicals produced by the radiation are trapped at 77 K. The k value for destruction is found to vary only slightly with hydration. Based on the measured G and k values for hydrated DNA a trapped radical ion cluster size is estimated to be about 6 nm. For hydrated DNA the cluster size suggests electron migration distances of about 17 bases (for migration along the DNA strand).

2. Yields of Individual Radicals with Dose (27).

In this work an ESR investigation of the yields of the individual free radicals formed in γ -irradiated frozen DNA as a function of hydration and dose at 77K is performed (27). Analysis of the ESR spectra taken at low hydration shows that the

ion radical composition remains nearly constant with dose, and that few secondary radicals are formed even at high doses (above ca. 50 kGy). For fully hydrated samples the radical composition changes dramatically with dose. Thymine anion radical ($T^{\bullet-}$) is found in abundance at low doses but nearly disappears at higher doses, with a corresponding increase in the N-3 deuterated cytosine anion radical ($C_D^{\bullet-}$). Guanine cation radical ($G^{\bullet+}$) decreases at high doses, with a concomitant increase in secondary radical species ($S^{\bullet}$). Analysis of the dose response data for G values (the yields in $\mu\text{mol/J}$), k values (the destruction constants) and k' values (a new constant that characterizes the change in G with dose) were performed for each of the DNA base free radicals present at 77 K. The G value for each of the base radicals increases with the hydration level. The k values for $C_D^{\bullet-}$ and $G^{\bullet+}$ increase slightly with hydration; however, that of $T^{\bullet-}$ increases substantially. Destruction constants for neutral radicals such as $TH^{\bullet}$ and $C_D^{\bullet}$ are found to be substantially smaller than those for ion radicals and provide an indication of the radical charge state. A negative k' value for $T^{\bullet-}$ and a positive k' value for $C_D^{\bullet-}$ are explained in terms of radiation effects that result in the formation of a deuterated cytosine base, i.e. $C(N3)D^+$, which greatly increases cytosine's electron affinity. The ratio of anion radical to cation radical concentrations is found to be ca. 1.6 and is invariant with hydration. A speculation on the imbalance based on hole-hole combinations in spurs is presented.

3. Electron and Hole Transfer in DNA (23, 26, 27, 28, 34)

Hole and electron migration within DNA and its hydration layer are processes which are important to the understanding of radiation and photolytic processes in DNA (23, 27, 28, 34). The extent of electron migration within DNA is at present a topic of interest and is in dispute in part as a result of an understanding of the various driving forces for electron transfer (34). Results have been reported which suggest migration distances from only a few DNA bases to up to hundreds of bases but each of these may be correct for the systems under investigation. In contrast hole transfer is generally agreed to occur over only a few bases and recently results have

suggested that hole transfer occurs from the inner hydration layer to DNA. Our latest results on electron and hole transfer in DNA have added to the understanding of these processes (34).

Part of the discrepancy in electron transfer distances can be accounted for when it is recognized that electron migration through DNA is not a single process. Migration of an radiation generated "quasi-free" electron before trapping is quite different than the transfer of an electron from one stable site to another by tunneling or activated hopping. Electron transfer reactions must therefore be classified as to the nature of the experiment and their time scale. The type of experimental procedures employed determine those processes that are investigated. For example investigations of the luminescence of DNA (34) after irradiation observe the recombinations of electron and hole which is the dominant mechanism for reaction (ca. 70% by ESR measurements).. Other experiments which employ microwave conductivity measure the dielectric loss due to the mobility of the electron. These experiments suggest migration of electrons on this time scale is confined to the second hydration sheath around DNA and not within the stack bases of the DNA strand. Solvated electrons which react with DNA are far less energetic and likely react in a site specific manner. For example it would be expected that they would preferentially react with pyrimidines not purines and the migration distances measured for solvated electrons are likely to be far smaller than those measured for quasi-free electrons. At low temperatures much longer distances have been reported for a positively charged intercalate but electrostatic interactions but must be included in such a system.

Our ESR experiments at 77K have clearly shown that on electron attachment to DNA, anion radicals are formed chiefly on cytosine (75-85%) (23) . After the initial attachment electron migration to cytosine likely occurs through an activated hopping mechanism which is temporarily quenched by the reversible protonation of cytosine anion at N3 to form a neutral species, C(N3)H•. On annealing to higher temperatures (ca. 190K) migration again ensues and is finally irreversibly quenched

by the protonation of the thymine anion forming the 5,6 dihydro-thymine-5-yl radical, TH• (31). TH• formation accounts the majority of the electrons and therefore transfer of the electron from cytosine to thymine is clearly indicated. The distance of the hopping migration in DNA is especially significant as it will occur after any process that add an electron to DNA. Recent work in our laboratory with bromine substituted DNA bases as electron traps in DNA shows that the initial electron attachment suggests short range travel of about 7 bases; whereas, on annealing the hopping migration extends extends this distance somewhat.

ESR experiments by Graslund and coworkers which measure the rate of formation of TH• at low temperatures in DNA yield an activation energy of 10-15 kcal/mole. Our results show that there is evidence for a lower activation energy for thymine anion radical where electron hopping is not necessary (31). These results suggest to us that the activation energy for electron hopping in DNA is large in comparison to thymine anion protonation. The activation energy for the formation of TH• in DNA (E_t) can be viewed as the sum of two terms: the activation for protonation of the thymine anion radical, E_p plus the energy of electron detrapping from the cytosine trap ("hopping"), E_d , i.e., $E_t = E_p + E_h$. The electron hopping in DNA is depicted in reaction (1) and thymine anion radical protonation in reaction (2).

Our theoretical calculations (25) of the barrier to hopping (reaction 1) suggest a 0.55ev (13 kcal/mole) barrier between the base paired protonated cytosine anion radical ($\cdot CH \cdots G(-H)$) and a base paired thymine anion radical ($\cdot T \cdots A$). This already accounts for the activation energy measured for formation of TH• in DNA, thus the activation energy for protonation of thymine anion radical in DNA (reaction 2) is likely small in comparison to that for hopping (reaction 1). Experimental evidence for this is given by a comparison of the formation of TH• by protonation of thymine anion radical in DNA and in the double stranded polynucleotide, pdAdT (31). It is found that the protonation reaction is nearly complete in pdApdT at

temperatures (160K) where little $\text{TH}^\bullet$ is found in DNA (See publication 31). In DNA this is a result of the electron being trapped on cytosine until annealing to higher temperatures where the hopping rate is increased substantially. However, in pdAdT the electron already resides on thymine and protonation occurs at lower temperatures without the necessity of the activation of electron hopping. The low temperature at which this occurs suggests a activation barrier of only 1-2 kcal/mole.

4. Detection of the Hydroxyl Radical in the DNA hydration layer (36, 48)

DNA in aqueous solution is strongly hydrated by a mass of water approximately equal to the mass of the DNA itself. A variety of investigations have shown that the properties of this hydration layer (about 15–20 waters per nucleotide) are significantly altered from those of bulk water. Low LET irradiation of hydrated DNA deposits approximately an equal amount of energy in this hydration layer as in the DNA itself. Because the radicals expected from the radiation of water ($\cdot\text{OH}$, $\text{e}^{-\bullet}$, $\text{H}^\bullet$) have not been detected in the DNA hydration layer by ESR spectroscopy, even at 4 K, it has been assumed that the radiation damage in the hydration layer is rapidly transferred to the DNA in the form of electrons and holes (reactions 1,2). However, the evidence that reaction 2 occurs has been circumstantial; it is believed that deprotonation of H_2O^+ to form $\cdot\text{OH}$ (reaction 3) occurs on the time scale of a molecular vibration (10^{-14} s) and, as a consequence, may be competitive with hole transfer to the DNA.

In light of the experimental and theoretical ambiguities, the question of whether $\cdot\text{OH}$ is formed in the hydration layer of DNA is still an open one; it is also an important one, since the damage done by $\cdot\text{OH}$ will, in many cases, be different from that done by hole transfer. We have undertaken the search for $\cdot\text{OH}$ in the DNA hydration layer using two lines of investigation: direct ESR detection of $\cdot\text{OH}$ at low

temperature and H_2O_2 product analysis (36). In aqueous glasses, the low field component of the $\cdot\text{OH}$ ESR spectrum at X-band frequencies consists of a broad feature extending over ca. 250 G instead of the well-defined $g_{||}$ component at 2.05 that is found in crystalline ice. The reason for this difference is well understood; in radicals such as $\cdot\text{OH}$, $g_{||}$ is determined by the extent to which hydrogen bonding lifts the degeneracy of the two oxygen p-orbitals which are degenerate in the gas phase. In a glass, there is a large range of hydrogen bonding strengths to $\cdot\text{OH}$ and, therefore, a large range of $g_{||}$'s exists. This leads to the broad low field resonance found in glasses. It has been suggested that the hydration layer in DNA forms a glassy structure, thus, in searching for $\cdot\text{OH}$ in the DNA hydration layer, we have focused on the ESR spectrum of $\cdot\text{OH}$ in a glass as a model for the spectrum expected in DNA.

The second method used as a probe for $\cdot\text{OH}$ in the DNA hydration layer is analysis for its diamagnetic product, H_2O_2 . When H_2O is γ -irradiated in the absence of oxygen, the only source of H_2O_2 is reaction 4.

We have undertaken a study to detect H_2O_2 in the DNA hydration layer through product analysis of anoxic hydrated DNA irradiated at 77 K. H_2O_2 yields have been determined under ambient conditions in dilute aqueous DNA solution; however, in this early work almost all of the H_2O_2 found originated with the bulk water, not with the DNA hydration layer.

Previous ESR reports of γ -irradiated DNA at low temperatures have suggested that hydroxyl radicals are not formed in the first hydration layer of DNA. In this report we show that hydroxyl radicals are produced in low yield in DNA's first hydration layer. Due to the glassy nature of this hydration layer at low temperature the hydroxyl radical gives a broad ESR resonance which is not easily detected. Low field ESR spectra of hydroxyl radicals in an irradiated 6M CsF aqueous glass are shown to be nearly identical to those found in DNA; however, the yields in the aqueous glass ($G = 0.087$ to 0.13 mmole/J) are found to be greater than those in DNA's first hydration layer ($G = 0.035 \pm 0.02$ mmole/J). A large k value for destruction

of the $\cdot\text{OH}$ in DNA's hydration layer limits the yield of $\cdot\text{OH}$ at high doses. The yield of H_2O_2 (which likely results from hydroxyl radical recombinations that occur both during irradiation and upon annealing) is found to be 0.0035 mmole/J in the dose range 65 kGy to 195 kGy. The amount of H_2O_2 formed corresponds to most of that expected from recombination of the $\cdot\text{OH}$ trapped at 77K at the equivalent dose. The yield of trapped $\cdot\text{OH}$ radicals in the first hydration layer is smaller than the number expected for a glassy water system at low temperatures; this has implications for possible hole transfer to DNA.

We have extended this work and established the breakdown between hole transfer and hydroxyl radical formation (48). In this recent work three regimes of radiological behavior for waters of hydration in DNA are found. For the first eight waters/nucleotide (which are glassy), no significant amounts of $\cdot\text{OH}$ are found, suggesting hole transfer to DNA. The second regime of hydration waters comprises up to ca. 14 additional glassy waters/nucleotide ($\Gamma = 22$). In this regime, substantial amounts of glassy $\cdot\text{OH}$ are found, suggesting that only a few holes which escape recombination in spurs charge-transfer to the DNA. In these two glassy regimes no trapped electrons are found, which is in accord with previous work that has reported that all electrons which do not undergo recombination in spurs transfer to DNA. The third regime of hydration water is comprised of bulk (or bulk-like) polycrystalline ice which forms when levels of hydration over 22 waters/nucleotide are reached. These waters form a separate phase from the DNA/glassy water system and neither hole nor substantial electron transfer to the DNA occurs; $\cdot\text{OH}$ in this ice phase is observed with G values that vary slightly with the amount of water in the ice phase, but which are close to the values found for pure ice.

d. Ab Initio Molecular Orbital Calculations on DNA Base Pairs, Their Radical Ions and Related Systems.

Considerable interest has arisen of late in the primary radicals formed in DNA as a result of several reports that the initial ion radical distribution may be dependent on proton transfer reactions between base pairs. Steenken first suggested that

proton transfer reactions may be important in the initial stabilization of DNA ion radicals. Proton transfer from G to C $\cdot$ has been suggested to stabilize the electron on cytosine and account for the dominance of the cytosine anion in DNA.

In this work, *ab initio* molecular orbital calculations of various protonation states of DNA base and DNA base radical ions were performed to aid our understanding of primary DNA radiation processes such as proton transfer between base pairs (24, 25). Each of the structures was optimized at the STO-3G and 3-21G levels of theory, and single point calculations were performed at the 6-31G**/3-21G and 6-31+G(d)//3-21G levels. Each of the protonation states important to proton transfer reactions in base pair radical ions found in irradiated DNA was considered. Calculations for three protonation states for the cytosine reduced radical were performed and the most stable is found to be the N-3 ring protonated species. Calculations for the vertical ionization energies of the individual DNA bases yielded the following order T > C > A > G with the same order found for the adiabatic electron affinities of the bases. The best fit to experiment was found with Koopmans theorem ionization potentials. The energy for proton transfer in the GC and AT base pair radical cation and radical anions were estimated from the energies of the individual species. All proton transfers were found to be energetically unfavorable except for the GC anion radical which is favored by 13 kcal. The results of these calculations combined with previous work lead to a revised model for the initial localization of charge in irradiated DNA. Our results predict that thymine has the highest electron affinity and guanine the lowest ionization potential of the four DNA bases. As a consequence, the electron in irradiated DNA is more likely to be found on thymine and the hole on guanine. Results obtained with base pairs and four base stacked systems again suggest that the electron will localize on thymine. However a simple proton shift from guanine to cytosine also shifts the electron from thymine to cytosine. No such shift from A to T is predicted to be energetically favorable. For the hole, proton shifts are not predicted to alter the hole site due to the fact that guanine hole in the G-C base pair is calculated to be

lower in energy than the adenine hole in the A-T base pair either before or after proton shift within the A-T base pair.

These results therefore suggest that electron attachment will initially favor thymine and subsequent proton transfer reactions between G-C base pairs will shift the electron to cytosine. Previous results in our laboratory have shown that in irradiated double stranded DNA at 100 K, the anion is mainly on cytosine and the hole on guanine. However, very recent results have shown that in single stranded DNA where proton transfer between base pairs is not possible, the thymine anion is found to increase its contribution as expected from the results of this work.

1. Effect of Hydration Water on EAs and IEs (30).

In this work we have performed calculations on DNA base pairs and their ion radicals at the 3-21G level which include waters of hydrations at probable sites of hydration in DNA (30). We have found that the waters of hydration have a small effect on both the ionization potentials and electron affinities. Both the EAs and IEs are slightly increased by the waters of hydration but their relative order is unchanged from the order without waters of hydration, i.e., C>T>A>G. The evidence also shows that the waters of hydration stabilize the anion radicals more than cation radicals.

2. Cation Radical Sites on the DNA Sugar Phosphate Backbone (29).

Up to this time ESR spectroscopy of irradiated DNA at temperatures from 4K to 77K has not shown any radicals other than those on DNA bases. DNA is composed of 55% by weight of sugar phosphate backbone and at least an equal weight to the DNA in hydration water. Thus although they make up less than 25% of the target mass, DNA bases apparently have all the free radicals formed in irradiated DNA. An explanation for this is that ionization in the hydration layer and sugar phosphate backbone transfer spin and charge to the DNA bases. Another explanation is that ionization on the deoxyribose phosphate backbone results in very

broad radical species which are not easily observed by ESR. We find no solid evidence for the latter in our ESR spectra. Since the most damaging effect on radiation is the DNA double strand break it is critical to understand the possible mechanisms of production of radiation lesions on the DNA deoxyribose phosphate backbone. Our calculations (29) show that there are two sites where the cation or hole can localize before subsequent reaction or transfer to the DNA base. These are: 1. localization to the ring ether oxygen in the deoxyribose group or 2. localization to one of the singly bonded oxygens on the phosphate group. The site of localization varies with the backbone structure and conformation and result from the fact the the phosphate and deoxyribose portions of the backbone have nearly the same ionization energy. Most calculations favor the deoxyribose portion of the backbone.

The IE of the sugar phosphate backbone is found to be about 2 eV higher than that of the bases. This explains the transfer of the charge to the bases and the lack of observation of a large fraction of sugar phosphate radicals in DNA. It has become clear in recent pulse radiolysis work that excited DNA base cations may result in DNA strand breaks. It is clear that a DNA base excited state could undergo a charge transfer to the sugar which could then react to form the strand break. Such mechanisms are now under investigation.

3. Scaling of Calculated DNA Base Electron Affinities and Ionization Potentials to Experimental Values (38)

Although experimental gas phase ionizations energies of the DNA bases have been measured by a number of investigators, experimental gas phase electron affinities of the DNA bases have not as yet been reported. A variety of molecular orbital calculations predict the pyrimidines have higher electron affinities than the purines with recent calculations suggesting the following order; T>C>>A>G. Ab initio calculations reported thus far predict negative electron affinities for all the DNA bases which may seem inappropriate for such polar molecular structures. Since the anion radicals of the DNA bases have been observed by ESR spectroscopy in aqueous solution, one might expect positive electron affinities for the DNA bases. However, it is largely the additional energy of solvation of these species (ca. 3 eV) that results in species stable towards dissociation to the aqueous electron and DNA base. As shown in this work, to a high degree of certainty the vertical electron

affinities of all DNA bases are negative and only two of the adiabatic electron affinities are likely to be positive. Although there are no reported EA's for DNA bases in the gas phase, there are many other determinations of gas phase negative electron affinities for similar structures. For example, EA's for benzene, pyridine, pyrimidine, naphthalene, and uracil are found to be all negative (-1.1, -0.7, -0.3, -0.2, and -0.2 (eV)).

The calculation of gas phase electron affinities can be a difficult problem for HF-MO theory. This is even true for small structures for which HF electron affinities are underestimated. In more recent work however, Staley and Strnad have considered a series of hydrocarbons negative ion resonance states, or "negative electron affinities" and have employed HF theory with various basis sets under the Koopmans approximation. While the calculations still underestimate the EA's, they are successfully scaled to experimental electron affinities with excellent fits to experiment found using the D95v basis set.

In this work we perform full calculations of the IE and EA of DNA bases and their ion radicals using the Møller Plesset MP2 technique for electron correlation correction with large basis sets (6-31G* and 6-31+G(d)). In addition Koopmans EA's were calculated at various basis sets, including D95v, 6-31G* and 6-31+G(d). We have compared all these calculations to a variety of experimentally well known vertical EA's and found linear correlation fits to experiment. The best fits for EA are found for the Koopmans values at the D95v level which allows for estimation of the vertical electron affinities of the DNA bases. Calculations at 6-31G* and 6-31+G(d) using both ROHF and ROMP2 theories show a consistent difference between calculated vertical and adiabatic EA's. This allows for a good estimate of DNA base adiabatic EA's, i.e., -0.7; -0.3; 0.2; 0.3; 0.4(eV) from the vertical EA's (-1.23; -0.74; -0.40; -0.32; -0.19 (eV)) for G, A, C, T, and U respectively. While the vertical EAs are all negative the adiabatic values for T, C and U are positive. While EA's must be scaled, we find that Koopmans IP's calculated at the 3-21G level predict vertical IP's of the DNA bases with only a 0.15 eV average absolute deviation from the experimentally reported values and calculations at MP2/6-31+G(d)//6-31G* for the adiabatic ionization potentials of the DNA bases are all within 0.1 eV of experiment.

4. Ab Initio Calculations on the Structure and Relative Stability of Deoxyribose Radicals in a Model DNA Backbone (39).

In this study, we focus on radicals on the sugar moiety of the DNA molecule

for they are known to be precursors in mechanisms leading to strand breakage. There is evidence for a definite role of sugar radicals in the indirect effect of ionizing radiations. Since the C1', C2', C3' and C5' centered radicals have been observed experimentally, theoretical investigation of their structure and relative stability is of importance. In this work, through the use of *ab initio* molecular orbital calculations performed on a DNA fragment composed of one sugar and two phosphate groups, we present the conformations and properties of the five carbon centered radicals which result from hydrogen abstraction (39). The effect of phosphate groups on the sugar radical conformation, energetics and electronic properties are evaluated through a comparison of models with and without phosphate groups. Geometry optimization performed at the ROHF/3-21G level reveals the C1' centered radical is the most energetically favored in all the DNA fragments considered in this study while the C2' radical is the least stable, and maintains a near planar configuration (4_0 T). All energy minima calculated correspond to deoxyribose radicals with a pseudorotation phase angle lying in the South quadrant of the pseudorotation cycle. The phosphate groups significantly affect the puckering mode of the C2' and C3' radicals and energetically destabilize C3' radical relative to the other sugar radicals. Isotropic hyperfine coupling constants significantly differ between models with and without phosphate groups, most particularly in the C3' and C4' radicals. The trend in oxidizing power based on the calculated HOMO energies is predicted to follow the order $\cdot\text{C1}' < \cdot\text{C4}' < \cdot\text{C2}' < \cdot\text{C3}' < \cdot\text{C5}'$. Cytosine attachment to the C1' and C4' deoxyribose radicals does not appear to affect the relative energies nor the isotropic hyperfine couplings of these two species. In both deoxycytidine radicals, the base maintains an anti conformation, which therefore does not disrupt the hydrogen bonding pattern in the base pair.

5. Ab Initio Study of Thiol Aqueous Phase Ionization Energies: Methyl Mercaptan and Cysteamine (37).

As part of an ongoing investigation on radioprotective thiols in this work (begun under the DOE and now funded primarily by the NIH), we employ *ab initio* theory to calculate the ionization energies of two thiols: methyl mercaptan and cysteamine in gas phase and in solution. By comparing these results to the previously calculated ionization energies of the four DNA bases we elucidate the possible mechanisms by which simple thiols protect DNA by electron transfer processes and the influence of hydration on these processes (37). The ionization

energies of two thiol model compounds (methyl mercaptan and cysteamine) are calculated at the ROHF/6-31G* level to aid our understanding of the mechanisms involved in DNA radioprotection. Methyl mercaptan, the thiolate anion and its tri-hydrated form are fully geometry optimized. The resulting gas phase Koopmans ionization energies are 9.68ev, 1.67ev, and 3.63ev respectively. The ionization energy for the solvated methyl thiolate anion, $\text{CH}_3\text{S}^-(\text{aq})$, calculated through the use of the SCRF model ($\epsilon=78$), the Born charge term and a discrete hydration shell, leads to a Koopmans value of 5.6ev. This result is in good agreement with the corrected vertical solution phase ionization energy calculated using the same model (5.4ev), and with experiment ($5.7\pm0.2\text{ev}$). The gas phase ionization energies of cysteamine, its cation, zwitterion and the penta-hydrated form of the latter are reported. We find the Koopmans ionization energy of the anti configuration of the zwitterion to be ca. 6.0ev. Discrete hydration of the negatively charged sulfur increases the ionization energy by ca. 0.55ev per water while hydration of the amine group decreases it by ca. 0.1ev per water. Subjecting the penta-hydrated zwitterion to the SCRF model leads to a Koopmans solution-ionization energy of 6.42ev. These results predict that both the aqueous thiolate anion and the aqueous cysteamine zwitterion will reduce radiation induced DNA base cation radicals via direct electron transfer as found experimentally. Based on the energetics of the solvent-solute interactions, we propose that the electron transfer process between the cysteamine zwitterion and DNA cations will be influenced by the intervening solvent and suggest that displacement of the primary solvation layer upon ion pair formation will decrease the zwitterion's ionization energy, thereby facilitating the electron transfer.

6. Ab Initio Molecular Orbital Calculations of Radicals Formed by $\text{H}\cdot$ and $\cdot\text{OH}$ Addition to the DNA Bases (45)

In this work, we employ *ab initio* molecular orbital theory combined with scaling to known experimental values to determine the electron affinities and ionization potentials of hydrogen and hydroxyl adducts of the DNA bases. We note that OH^- addition to DNA radical cations and H^+ addition to DNA radical anions would result in many of the same base radical intermediates as $\text{OH}\cdot$ and $\text{H}\cdot$ addition. The relative values of electron affinities and ionization potentials are shown to relate to the relative redox properties of the various species. These results

will aid our understanding in radioprotective and repair mechanisms of the radiation induced DNA damaged bases through electron transfer reactions from thiols.

Ab initio molecular orbital calculations of the ionization potentials (IP's) and electron affinities (EA's) of DNA base radicals formed by addition of $H\cdot$ or $OH\cdot$ to DNA bases are presented in this work. IP's and EA's are obtained by calculating the energies of the LUMO and HOMO (Koopmans values) of the cationic and anionic states, respectively. Scaling of the theoretical values to experimentally known ionization potentials and electron affinities of other small radical compounds leads to predicted trends in IP's and EA's of the DNA base adducts. These trends are shown to be in good agreement with experimental redox trends, and aid our understanding of possible electron transfer processes. The present results indicate the $OH\cdot$ and $H\cdot$ adducts of the pyrimidines at C6 are most oxidizing, while the $H\cdot$ adduct of cytosine at N3 is most reducing. The calculated trend in electron affinities in conjunction with experimental observations results in the prediction that only a fraction of the base adducts will be reduced via electron transfer processes from thiols. All five sugar radicals have nearly equal electron affinities; however, their ionization potentials substantially differ. Delocalization effects which result in less electron repulsion in the anionic state are shown to account for differences in EA and IP scales. Conformational distortion of the purine bases upon adduct formation at the C4 and C5 sites is shown to be significant.

7. Structures of Molecular Products Formed from Primary Radical Ions (49).

Primary anion radicals may undergo rapid irreversible protonation reactions, whereas, the cations may undergo irreversible hydroxide ion addition or reversible deprotonation reactions. For each of the DNA base anion and cation radicals likely to undergo such reactions we are optimizing the structures of the product radical species and determining the total energies.

The structures of the isomers of purine hydrates [4(5)-hydroxy-5(4)-hydropurines] have been geometry optimized with ab initio quantum chemical methods at the 6-31G* basis set and with the semi-empirical method PM3. These hydrates which can result from reduction of radical species formed by attack of hydroxyl radical at the 4, 5 double bond in the purines, show significant geometrical distortion when compared to the natural bases. More specifically, the cis isomers adopt a "butterfly" conformation, while in the trans isomers, the pyrimidine and imidazole rings tilt opposite to each other. Our results predict the cis purine hydrate isomers are far more stable than the trans isomers by 10 to 18 kcal/mol at the 6-31G* level, whereas the 4-hydroxy,5-hydropurines are found to be slightly more energetically stable than the 5-hydroxy,4-hydropurines. The "butterfly" conformation of the cis isomers constitutes a bulky lesion which will result in a significant distortion of the DNA helix.

e. Radiation-Induced DNA Damage as a Function of Hydration.

1. Release of Unaltered Bases (22).

In this joint effort with Steven Swarts and Ken Wheeler of Wake Forest University, γ -irradiated DNA hydrated to various levels has been analyzed for several types of lesions (22). These DNA lesions include the release of unaltered bases, specific DNA base damage, and nucleoside damage. These studies are reviewed briefly below.

The release of unaltered bases from irradiated DNA, hydrated between 2 and 33 moles of water per mole of nucleotide (Gamma) was investigated by HPLC. The objective of this study was to elucidate the yield of the four DNA bases as a function of dose, extent of hydration, and the presence or absence of oxygen. The increase in the yield of radiation-induced free bases was not constant with Gamma over the range of hydration examined. For DNA with Gamma between 2 and 15 the yield of

free bases was nearly constant. However for DNA with Gamma > 15 the yield increased rapidly. We suggest that the release of bases originating from irradiation of the hydration water is obtained predominantly by charge transfer from the direct ionization of the first 12-15 water molecules and predominantly by the attack of hydroxyl radicals generated in the outer more loosely bound water molecules.

2. Formation of Specific DNA Base Damage Products (42, 46).

The induction of base damage products in γ -irradiated DNA, hydrated between 2.5 and 32.8 moles of water per mole nucleotide, was investigated using the gas chromatography/mass spectrometry-selected ion monitoring technique. The yields for 14 individual oxidative base damage products were found to be only slightly dependent on the extent of DNA hydration but greatly dependent on the presence of oxygen. Of the base damage products which were assayed in this study, the predominant base damage products found were those derived from guanine (mainly 8-hydroxyguanine and smaller amounts of 2,6-diamino-4-oxo-5-formamidopyrimidine) and thymine (chiefly 5,6-dihydrothymine, with lesser amounts of 5-hydroxy-5,6-dihydrothymine, 5,6-dihydroxy-5,6-dihydrothymine, and 5-hydroxy-5-methylhydantoin). When irradiation of hydrated DNA was performed under oxygen, there was an increase in the combined base damage products derived from cytosine (5-hydroxycytosine, 5-hydroxyuracil, 5,6-dihydroxyuracil, and 5-hydroxyhydantoin) in the DNA. In DNA irradiated at low hydrations ($g=13$), where the direct and quasi-direct effects will have the greatest influence on the induction of damage products, the highest yields were found for 8-hydroxyguanine, 5,6-dihydrothymine, and 2,6-diamino-4-oxo-5-formamidopyrimidine when irradiations were performed under nitrogen. The high yields for 8-hydroxyguanine, 2,6-diamino-4-oxo-5-formamidopyrimidine and 5,6-dihydrothymine compare well with the results of ESR studies which find that the DNA radical precursors which lead to the formation of these nonradical base damage products (e.g. the deprotonated guanine cation radical and the protonated thymine anion) are the major base radicals that are observed in

irradiated DNA. We find (1) the yields of all oxidative products is greater than the yields of reductive products, indicating that there are other reductive products which have yet to be identified, (2) constant yields for the combined guanine and thymine products as well as the release of unaltered bases for $\Gamma = 15-20$ that implies that the addition of hydration water in causing DNA damage is the same as the DNA itself, and (3) the yields of the individual oxidative base damage products can be explained predominantly based on the reactions that form the initial charged radicals in irradiated DNA and on the intermediate reactions that are involved in the transformation of these initial DNA radical to their respective nonradical endproducts. These results are consistent with the proposal that the radiolysis of the inner-most water molecules of the DNA hydration layer constitutes a quasi-direct effect (e.g. are an extension of the direct effect), due to the fact that these water molecules transfer their radiation-induced damage to DNA through the reaction of charges (e.g. holes and electrons).

f. Formation of Sugar Radicals and Adduct Radicals in DNA and DNA models Systems by Secondary Radical Attack (submitted).

In recent efforts we have found that the radical formed by electron attachment to iodoacetamide, $\cdot\text{CH}_2\text{C}(\text{O})\text{NH}_2$, attacks the deoxyribose portion of nucleotides to form sugar radicals as well as adds to the DNA bases. Sugar attack has also been suggested to occur in DNA itself. Attack of this species thus provides a method of production of important sugar radical intermediates in the formation of strand breaks. Whereas the hydroxyl radical undergoes addition reactions to the DNA bases in preference to abstraction from the sugar (about 5 to 1 in DNA), we find the the iodoacetamide radical attacks the sugar group of dAMP in greater proportion to addition, making it more facile to follow subsequent sugar radical processes. We find that this radical species also adds to thymine (at C6) and adenine (at C8) DNA bases in their nucleotides.

We also have preliminary evidence that the $\text{UCH}_2\cdot$ species in dTMP attacks thymine at C-6 to form the adduct species, $\text{UCH}_2\text{T}\cdot$. This has been a controversial point in the literature which we believe that these results help to resolve.

II. Students Trained, Degrees Granted

Undergraduate Students who participated in this program over the past eight years: 18.

Graduate Students who participated in this program over the past eight years: 5.

Ph. D. Degrees Granted:

Anny-Odile Colson, Ph.D. Granted, Spring 1995, Health and Environmental Chemistry. Thesis Title: "Ab Initio Molecular Orbital Calculations of Radiation Induced Radicals and Their Reactions in DNA."

Mengyao Yan, Ph.D. Granted, Spring 92 - Health and Environmental Chemistry, . Thesis Title: "ESR Studies of Free Radical Mechanisms of Radiation Damage and Protection in DNA"

Steven Swarts, Ph.D., Ph.D. Granted, Dec. 87 - in Health and Environmental Chemistry,. Thesis Title: "ESR Study of the Irradiation of Biologically Important Molecules"

III. Bibliography of Publications Associated with this Grant. (1986-1996)

A. Publications 1986-1996

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3. "Low Temperature Autoxidation in Unsaturated Lipids: An Electron Spin Resonance Study," J. Yanez, C. L. Sevilla, D. Becker and M. D. Sevilla, J. Phys. Chem., 91, 487-491 (1987).
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5. "Sulfinyl Radical Formation from the Reaction of Cysteine and Glutathione

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 10. "Radiation Induced Radical Species in DNA" in *Mechanisms of Radiation Interaction with DNA: Potential Implications for Radiation Protection*, DOE/CEC Workshop Report, Jan. 1988, pp.111-121.
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 12. "Study of Lipid Peroxyl Radicals in Urea Clathrate Crystals: Oxygen-17 Couplings and Rotational Averaging," M. D. Sevilla, M. Champagne, and D. Becker, *J. Phys. Chem.*, 93, 2653 (1989).
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 15. "Kinetic Analysis of Low-Temperature Autoxidation of Unsaturated Triglycerides," J. Zhu and M. D. Sevilla, *J. Phys. Chem.*, 94, 1147-1452,

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2. "An ESR Investigation of Radical Intermediates in Cholesterol and Related Compounds: Relation to Solid State Autoxidation," C. L. Sevilla, D. Becker, M. D. Sevilla, presented at 34th Annual Meeting of the Radiation Res. Soc., April 13-17, 1986, Las Vegas.
3. "Ab Initio Studies of Hydrocarbon Peroxyl Radicals," B. H. Besler, P. MacNeille, and M. D. Sevilla, presented at the 19th Annual Midwest Theoretical Chemistry Conference, May 15-17, 1986, Bloomington.
4. "Electron Spin Resonance Investigation of Radical Intermediates in Cholesterol," C. L. Sevilla, D. Becker, and M. D. Sevilla, presented at the 9th International EPR Symposium of the 1986 Rocky Mountain Conference, August 3-7, 1986, Denver.
5. "EPR Spectroscopic Evidence for Spatially Sequential Amide Bond Formation in Plant Polygalactopyranuronides," P. L. Irwin and M. D. Sevilla, presented at the 9th International EPR Symposium of the 1986 Rocky Mountain Conference, August 3-7, 1986, Denver.
6. "Radiation Induced Radical Species in DNA," M. D. Sevilla, presented at the DOE Workshop on Mechanisms of Radiation Interaction with DNA: Potential Implications for Radiation Protection, University of California - San Diego, Jan. 21-22, 1987.
7. "An Electron Spin Resonance Study of the Reaction of Thiyl Radicals with Molecular Oxygen," at the National Meeting of the American Chemical Society, Denver, CO, April 5-10, 1987.
8. "An Electron Spin Resonance Study of the Reaction of Thiyl Radicals with Molecular Oxygen: The Instability of $\text{RSO}\cdot$," presented at the 9th International Congress of Radiation Research, Edinburgh, Scotland, July 19-24, 1987.
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11. "ESR Investigation of the Reactions of Peroxyl Radicals with Thiols," S. Swarts, D. Becker, and M. D. Sevilla, presented at the 195th Meeting of the American Chemical Society (3rd Chemical Congress of North America), Toronto, June 5-11, 1988.
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15. "Reaction of DNA Peroxyl Radicals with Cysteamine: Formation of Sulfoxyl Radicals," Michael D. Sevilla, M. Yan, D. Becker, and S. Gillich, 37th Annual Meeting of the Radiation Research Society, Seattle, Washington, March 18-23, 1989.
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18. "Radiation-Induced DNA Damage as a Function of the Degree of Hydration," S. Swarts, M. D. Sevilla, and K. T. Wheller, UCLA Symposium on Radiation Damage to DNA, Lake Tahoe, CA, Jan., 1990.
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21. "The Influence of Oxygen on the Reaction of Thiols with Radiation Induced DNA Radicals: The Formation of Sulfoxyl Radicals," M. D. Sevilla, S. Gillich, S. Summerfield, D. Becker, and M. Yan, presented at the 38th Meeting of the Radiation Research Society, New Orleans, LA, April 7-12, 1990.
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26. "Relative Abundance and Reactivity of Primary Ion Radicals in Irradiated DNA at Low Temperatures: Single vs. Double Stranded DNA, M.D. Sevilla, D. Becker, M. Yan, S.R. Summerfield and P. Renke, Fortieth Annual Meeting of

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32. "Ab initio Molecular Orbital Calculations on DNA Radical Ions: Effects of Solvation on Electron Affinities and Ionization Potentials of Base Pairs: Anny-Odile Colson, Brent Besler, and Michael D. Sevilla, Eighth American Conference on Theoretical Chemistry, University of Rochester, Rochester, NY, June 28 - July 2, 1993.
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46. "Production of Phosphoryl Radicals in High LET Irradiated DNA," D. Becker, T. LaVere, Y. Razskazovskii and M. D. Sevilla, presented at the 44rd Annual meeting of the Radiation Research Society, Chicago, April 14-17, 1996
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IV. The Present State of Knowledge in This Area of Research.

This is an exciting time in the area of radical and molecular mechanisms of radiation damage to DNA. The initial ionization events leading to radiation damage are becoming increasingly well understood as are the radical reactions that follow and lead to the diamagnetic product formation. A new model for the initial events in the radiation damage to DNA has been formulated and a fundamental understanding of the direct effect of radiation on DNA is progressing from the primary ion radical localization, through the stepwise formation of radical intermediates, to the products formed by the ion radical intermediates. The transition from molecular mechanisms to biological end points (mutagenesis and cell death) are now also beginning to be understood. An understanding of the effect of ionizing radiation on DNA from the initial ionization to biological consequences is the dream of the radiation physicist, chemist and biologist. Recent results by a number of groups have pointed the way toward its fulfillment.

Below we present a brief overview of this area. However, we have recently

written three extensive reviews of radiation damage to DNA each from a different perspective. This first is from an overall perspective ("The Chemical Consequences of Radiation Damage to DNA" D. Becker and M. D. Sevilla, in *Advances in Radiation Biology*, Vol.17, (J. Lett, Ed.) Academic Press (1993)). The second concentrates on the recent contributions of electron spin resonance spectroscopy to this field ("Radiation Damage to DNA" , Michael D. Sevilla and David Becker, in *Royal Society of Chemistry Specialist Review, Electron Spin Resonance*, Vol. 14 (1994)). The third gives a review of the latest theoretical efforts which elucidate radiation damage to DNA ("Elucidation of Primary Radiation Damage in DNA through Application of Ab Initio Molecular Orbital Theory" A.O. Colson and M. D. Sevilla, submitted). These should be consulted for a more complete overview of this area.

Through our efforts and that of other workers (Bernhard et al., Steenken et al., and Hüttermann et al.) we now are confident that at low temperatures the most stable anion is on cytosine in dsDNA , whereas the most stable cation is on guanine in all DNA forms. We have also found that the radical ion distribution is a function of strandedness of the DNA. In single strand DNA we find that there is an increase in thymine anion radical. Modeling of the ion transport in DNA suggest that the base pairing provides a significant increase in electron and hole transport through DNA. Steenken has shown that it is the proton transfer within the GC base pair that stabilizes the anion on cytosine in dsDNA. Our ab initio calculations on electron affinities and ionization potentials of DNA bases and base pairs also confirm the overall model including the energetics of the proton transfer.

Our recent ESR efforts clearly show that at elevated temperatures the DNA anion radicals can migrate to other bases where irreversible reactions occur. For example, these studies show that the thymine anion radical protonates at C6 to form $T(C6)H\cdot$. From product analyses work from Cadet and ourselves it is clear that the guanine cation adds OH^- at C8 to form $G(C8)OH\cdot$. Subsequent reactions of these and other intermediate species account for the diamagnetic base products formed in DNA. For example, reduction of $T(C6)H\cdot$ results in 5,6-dihydrothymine and oxidation of $G(C8)OH\cdot$ results in 8-hydroxyguanine; these are two major diamagnetic products found at low hydration levels (in our joint work with Swarts and Wheeler) for which the direct effect predominates. Our work and that of others (Bernhard et al., Hüttermann et al.) on radical and product formation as a function of hydration has aided our understanding of the contribution of the first few layers of

water to radiation damage to DNA. Our most recent work shows that the first water layer (up to 12 waters/nucleotide) contributes mainly to the direct effect through electron and hole transfer whereas the subsequent layers contribute to the indirect effect. Our recent work which shows that the OH can be found in the glassy hydration layer needs to be further expanded to determine the yield of OH radical as a function of the number of waters/nucleotide.

Ab initio molecular orbital studies of the ion radicals species involved in DNA radiation damage have been quite helpful in confirming and elucidating mechanisms of ion radical localization and reaction in DNA. For example, these calculations correctly predict that the initial localization of charge in irradiated DNA is on thymine anion and guanine cation as found in ssDNA. Calculations for the base pairs (mimicked by results for individual DNA base radicals and their complements in their appropriate protonation states) predict that guanine will protonate the cytosine anion but adenine will not protonate the thymine anion. This provides the explanation for the difference in the radical distribution in ss vs. dsDNA. In dsDNA, thymine can not be protonated and stabilized whereas cytosine can. The ssDNA results are a result of thymine's slightly greater EA whereas in dsDNA the preferential protonation of the cytosine anion yields enough stabilization to overcome the greater EA of thymine and result in localization of the charge on cytosine. Many such insights have arisen from this work and it is planned that such calculations will be an integral part of our upcoming proposed effort. Molecular modeling work by others (Osman, Wallace) have aided our understanding of the effects of DNA lesions on DNA structure and recognition of the lesions by enzymes.

Joint work with Steven Swarts and Ken Wheeler of Wake Forest on yields of products on irradiation of DNA has yielded important new insights to radiation damage to DNA. From the product yields, the overall picture of the radiation damage processes is brought to its ultimate chemical result. Up to this work little information which isolates the direct effect of radiation on DNA was available even though it comprises ca. 40% of the damage. Our investigation of the yield of damage and unaltered bases found for DNA at room temperature are supported by the results of the ESR experiments on irradiated DNA at 77K. The radicals found by ESR are the logical precursors to the products found in the GC-mass spectroscopic analyses. These product analyses need to be continued to address several other fundamental questions. For example the current result show an imbalance favoring oxidative over reductive products. The missing reductive products need to be found. We propose to do this in the project with Ken Wheeler and Steven Swarts described

in the proposal.

For the indirect effect much more information about DNA in solution is available. A number of DNA product analysis studies have been reported by Dizdaroglu et al. and recently Box et al. has reported a series of elegant NMR papers which have nicely shown the products of the indirect effect on dinucleotides. The free radical mechanisms for strand breakage have been elucidated by Schulte-Frohlinde's group at Mulheim in laser pulse photolysis work. These experiments have shown that the guanine cation radical results in strand breaks with about one third the efficiency of a hydroxyl radical attack. However, little is now known of the chemistry of strand break formation from the guanine cation radical. Cadet has pointed out that the protonation state of the guanine cation radical actually affects the products formed. Further work is needed in understanding this mechanism as well as the initial ionization events that occur on very short time scales. Here, the possible formation of sugar radicals by the direct ionization still needs to be elucidated. This is planned in our research effort. Our product analyses studies suggest that hole transfer from the sugar group to the base may not be competitive with deprotonation of the sugar cation to form a carbon centered sugar radical. Here ironically it is the product analyses done at long times that gives our only present information as to the very early processes involving hole transfer.

In summary the hydroxyl radical is known to be the major cause of strand breaks by the indirect effect and the DNA (base and sugar) cation radicals are the major cause from the direct effect. The electron has been shown to be ineffective in the production of strand breaks. Some mechanisms for strand break formation from base and sugar radicals have been elucidated but further work is needed here. The major DNA diamagnetic base products found from radiation damage are consistent with initial free radicals observed in ESR experiments. It has been demonstrated that the indirect effect produces many of the same radicals and diamagnetic products as the direct effect (28).

Often the type of work described above is criticized for not having a direct biomedical application. Yet it is clear that biology as a science is becoming more powerful by its understanding of biological mechanisms at the molecular level. To understand and control radiation damage will require its understanding at this level as well. The ultimate biological effect is a result of complex and intricate cellular mechanisms; however, the immediate effect of radiation can be explained

solely on the physics of the system -ionizations and excitations. The next few microseconds involve the initial chemistry of the system in which biochemical mechanisms have little part. The result of this chemistry can be predicted and the damage which through cellular biochemistry ultimately leads to its biological endpoint elucidated. It is only in this manner that a full understanding of the processes of radiation damage will be obtained.

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