Impact of the Pioneering Work of Miral Dizdaroglu in Quantifying DNA Base Damage on the Advancements in our Understanding of the Radiation Chemistry of DNA

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Interface Between the Chemistry and Biology

Absorbed dose of radiation, energy delivered, but how does this translate to the biological consequences (lethality, mutations, carcinogenesis

- **Absorbance of energy:** Heterogeneous distribution of energy along tracks (spurs); single and clustered (i.e. DNA double strand breaks) damage

- **Ionizations** of water and biomolecules forming electron-loss and electron-gain centers

- **Primary and secondary free radicals:** water and biomolecules; DNA important target, damage here can lead to deleterious cellular effects (lethality, carcinogenesis)

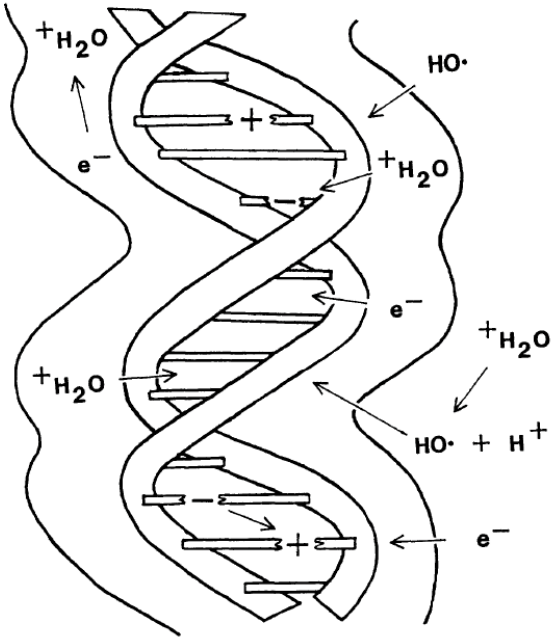
- **Fate of the radicals:** Formation of stable end-products

- **Cellular response:** repair/misrepair

- **Cellular outcomes:** death, mutations, carcinogenesis

The Radiation Chemistry of DNA and its Immediate Environment

- Free radical chemistry at the DNA/water interface
- Reaction pathways of radicals to stable end-products; effect of oxygen bordering hypoxia
- Cellular response to radiation damage (e.g. repair)
- Interventions to increase/decrease damage burden
- Use of hydrated DNA model to explore the radiation chemistry of DNA and its immediate environment
- Base release (HPLC)/DNA base damage products (GC/MS)



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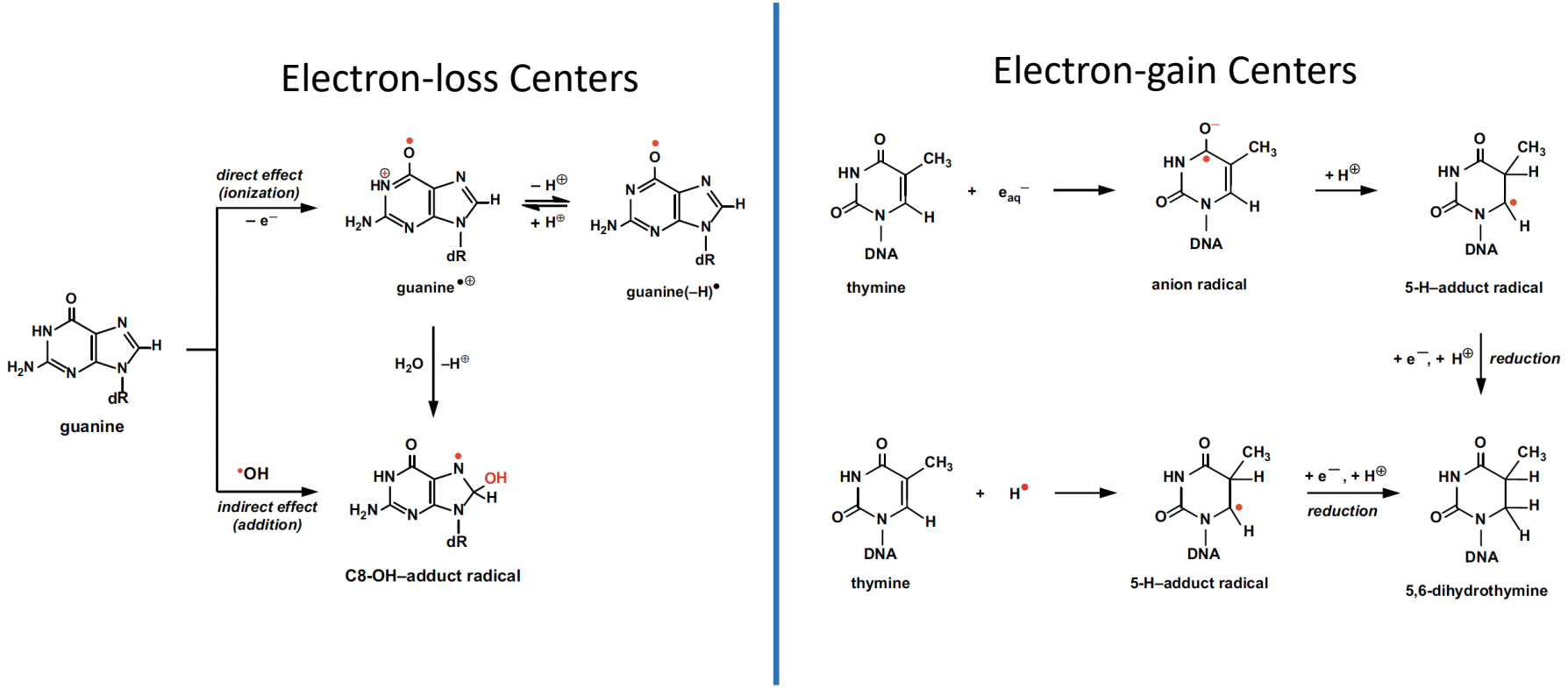
Identifying DNA Damage and Describing Reaction Pathways – Miral Dizdaroglu



Dizdaroglu and Jaruga. Free Radic Res. 2012; 46(4): 382–419

Dizdaroglu et al. Free Radical Research, 2015; Early Online: 1–24

DOI: 10.3109/10715762.2015.1014814

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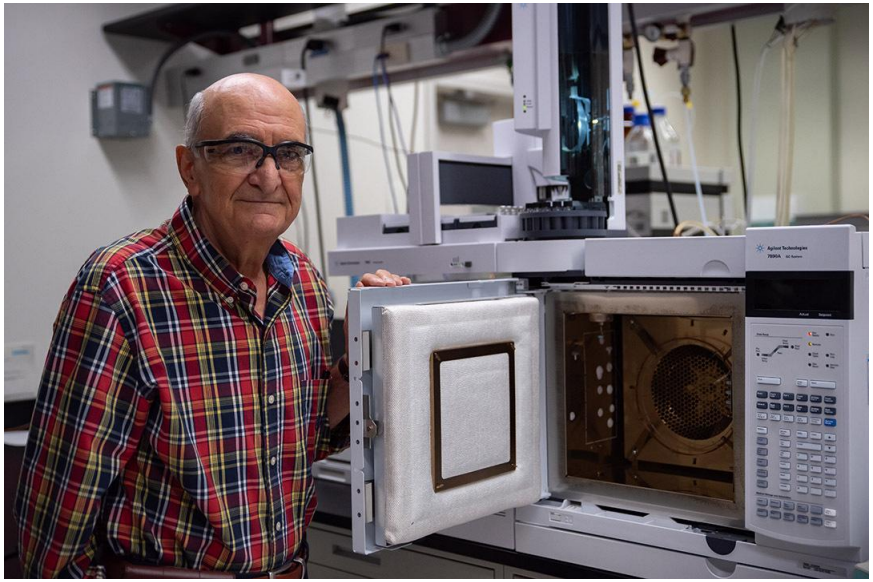
Measurement techniques - Gas Chromatography/Mass Spectrometry (GC/MS)

Use of GC/MS to simultaneously identify and measure many DNA base damage products at once

Excellent resolving power of capillary gas chromatography coupled with sensitive qualitative and quantitative measurement of DNA base damage products

Requires isolation of DNA followed by acid hydrolysis of DNA and then forming the trimethylsilyl derivatives of the free bases to make them volatile for separation on GC column



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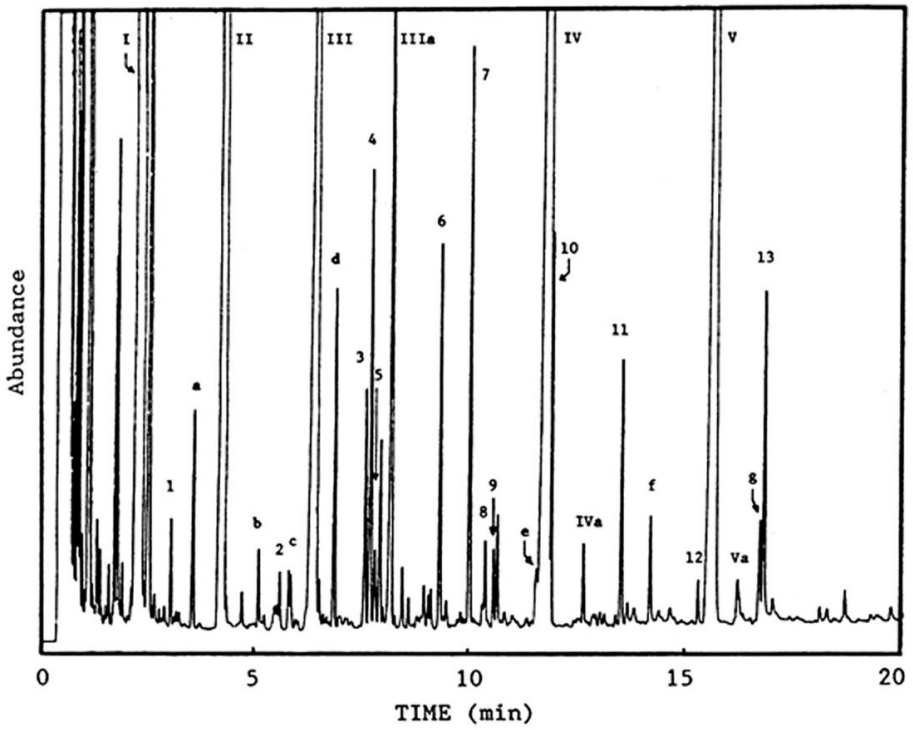
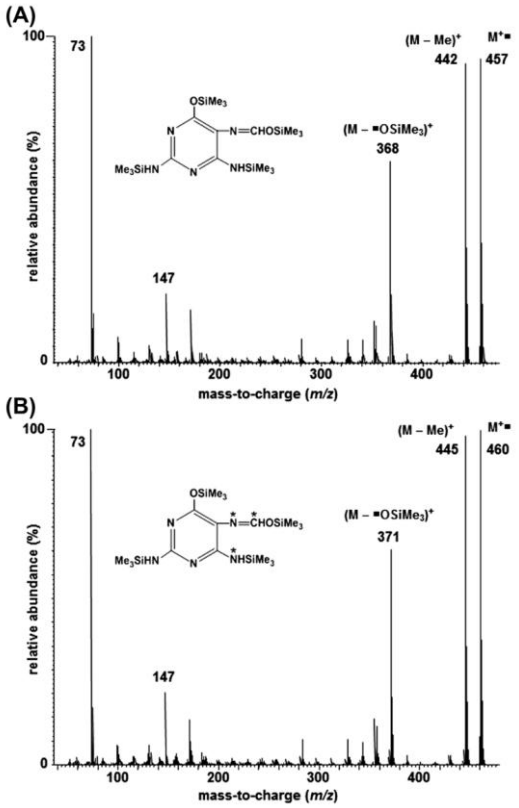
Gas Chromatography/Mass Spectrometry (GC/MS) Assay





Excellent resolving power of the GC capillary column coupled with the superior qualitative and high sensitivity quantitative capability of the mass specrometer

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Challenges of the GC/MS Assay

Acid hydrolysis of DNA – degradation of DNA base damage products – under estimation of base quantities

Trimethylsilyl derivatization – oxidation of unaltered and altered DNA bases – typically results in over estimation of altered base quantities

Swarts etal. Radiat Environ Biophys (1996) 35:41-53.

England et al. Free Radic Res. 1998 Oct;29(4):321-30.

Cadet et al. Free Radic Res. 1998 Dec;29(6):541-50.

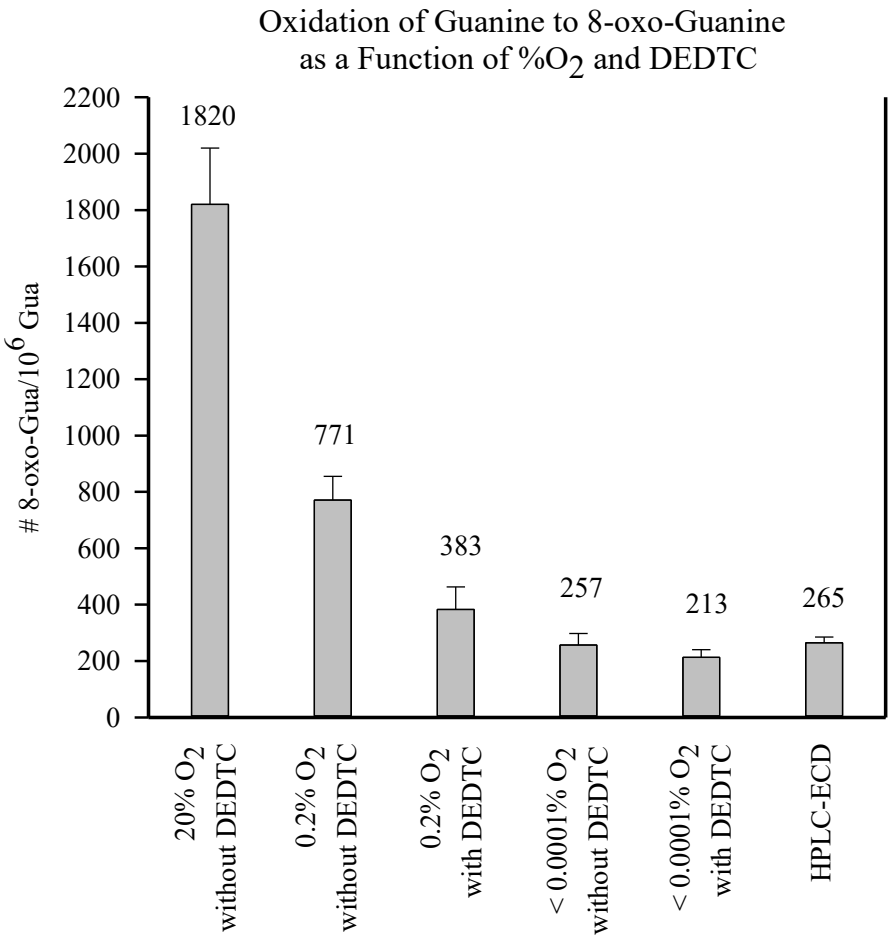
Dizdaroglu M. Free Radic Res. 1998 29(6), 551–563.

Oxidation of Guanine to 8-oxoguanine

Oxidation of Gua to 8-oxo-guanine during derivatization

Oxidation dependent on oxygen presence during derivatization

Elimination of Gua eliminates artifactual production of 8-oxoguanine



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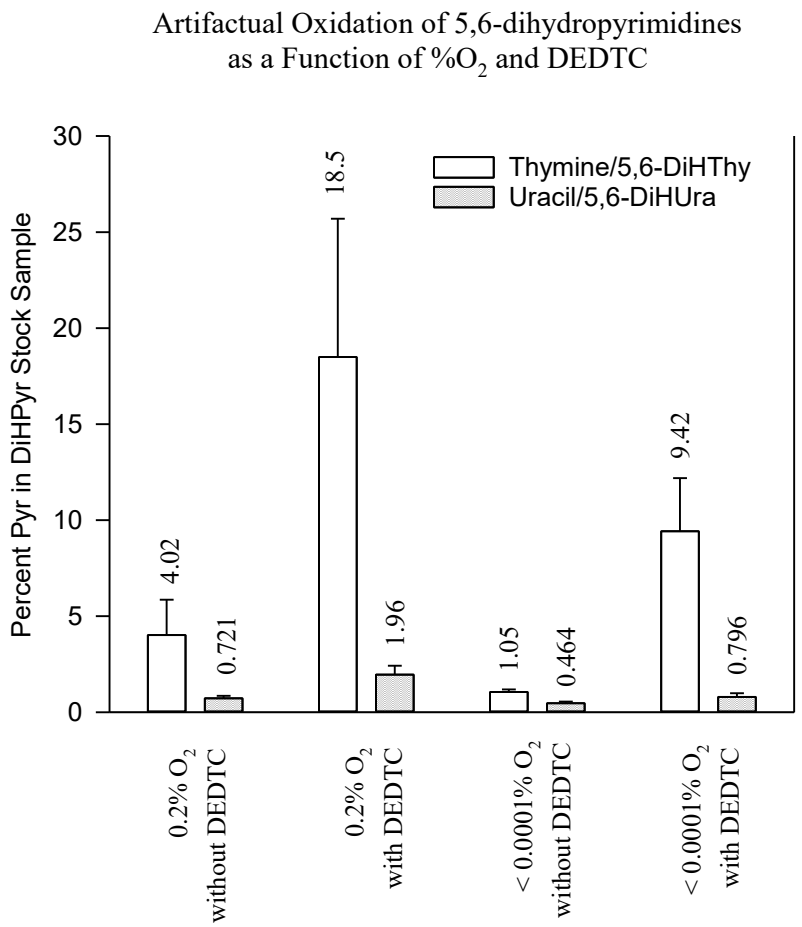
Oxidation of 5,6-diHThy to Thymine

Oxidation of 5,6-diHThy to thymine during derivatization

Oxidation dependent on oxygen presence during derivatization

Elimination of Gua or oxygen eliminates artifactual production of 8-oxoguanine



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Summary GC-MS DNA Base Damage Assay

LIMITING THE ARTIFACTUAL OXIDATION OF NUCLEOBASES in the GC/MS ASSAY

Use of high purity 88% formic acid to avoid recovery losses of bases post-hydrolysis

Optional: Elimination of unaltered bases following acid hydrolysis removes base damage products arising from artifactual oxidation of unaltered bases

Rigorous exclusion of oxygen during derivatization reaction; use ofradical scavengers (diethyldithiocarbamate, sodium salt (DEDTC) or n-butanethiol) can aid in minimizing the artifactual oxidation of nucleobases

Use of silanized glassware

Minimize time between sample derivatization and GC/MS analysis of sample

Must use isotope dilution methods in the calibration and quantification of base damage products by GC/MS

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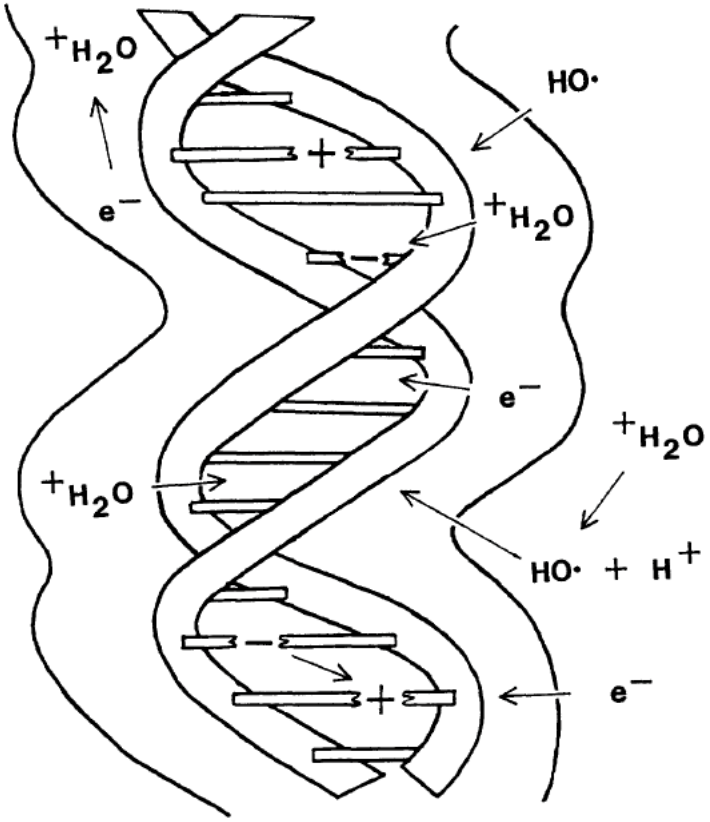
Hydrated DNA – Modeling the DNA/Environment Interface

Description of hydrated DNA model

Direct effect – damage to the DNA through direct ionization of the molecule

Indirect effect – Water radicals reacting with DNA

Hydration water of DNA; first 13-15 waters per nucleotide (Γ) inner most water are in direct contact with DNA; Γ >15 waters per nucleotide second layer of water in primary hydration layer



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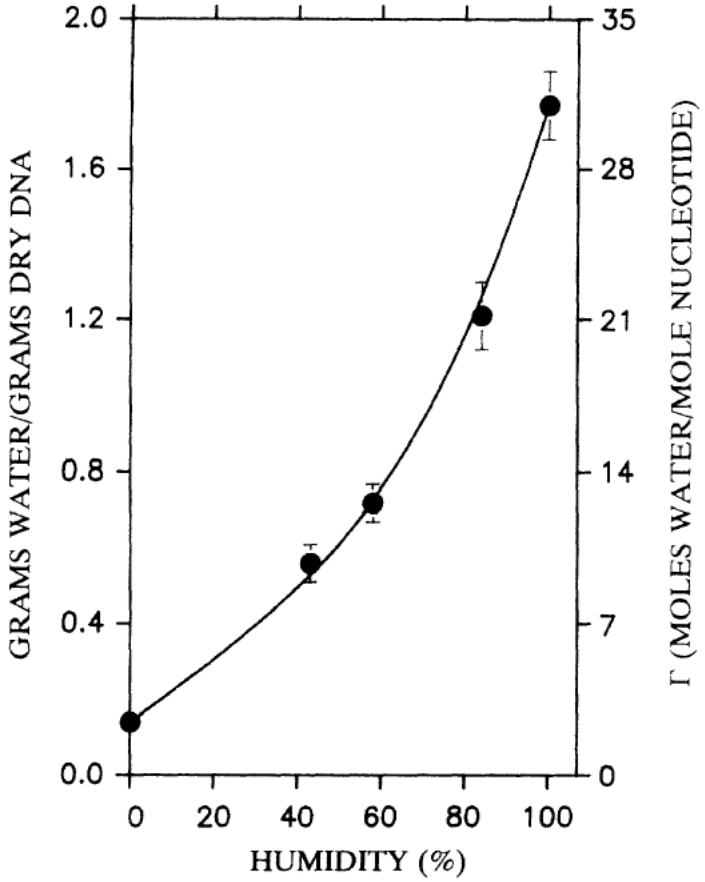
Hydrated DNA – Defining Water Content and Radiation Dose

Hydration of double-stranded salmon sperm DNA as a function of the relative humidity in the hydration chambers.

Hydration is given either as the number of grams of water per gram of dry DNA or as the number of water molecules per nucleotide (Γ). The points represent the mean +/- 1 SEM of at least six independent experiments

Water content (water molecules per nucleotide [Γ]): 2.5, 10, 13, 22, 33

Gamma ray radiation doses: 0, 10, 35, 65, 90 kGy



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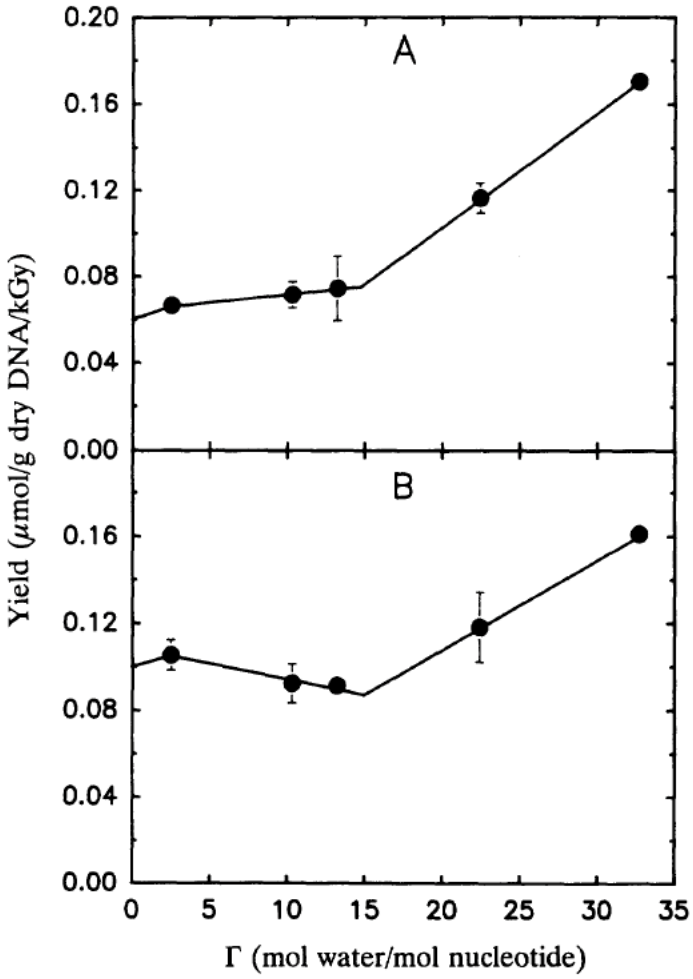
Hydrated DNA Base Damage Summary

The average yield of total released unaltered DNA bases receiving a dose of 90 kGy in the absence (A) and presence (B) of O_2.

The yields were calculated on the basis of the dry weight of DNA (Γ = 0).

The line from the multiple regression fit of Eq. (1) using the G(B) value obtained from Eq (2). The correlation coefficients for the lines in the figure were 0.96 for N_2 and 0.80 for O_2



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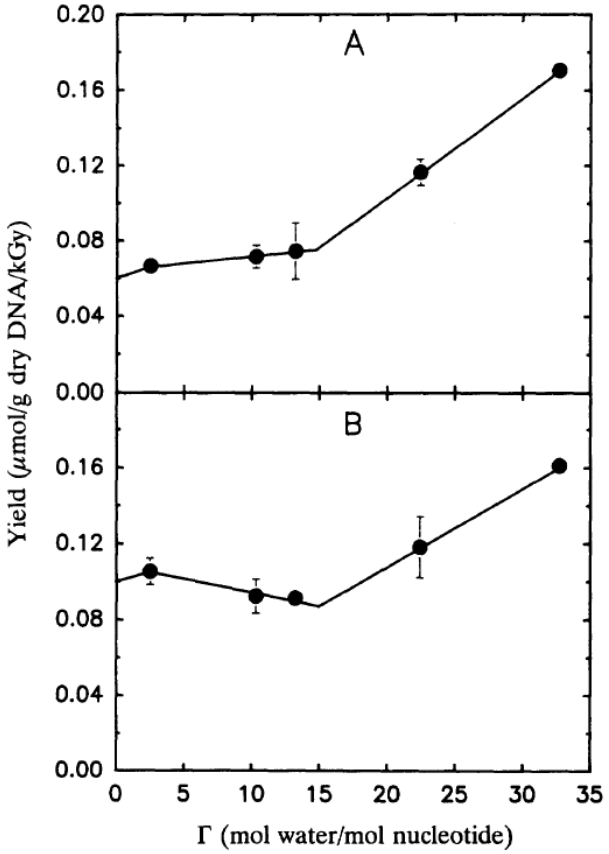
Hydrated DNA – Modeling of Base Release Yields

The yields of base release as a function of DNA water content can be best describe by the model shown in the equation below

$$Y(\text{total}) = G'(\text{C})(X_{\text{C}})(M_{\text{DNA}} + M_{\text{W1}}) + G'(\text{B})(X_{\text{B}})(M_{\text{DNA}} + M_{\text{W1}}) + G'(\text{W2})M_{\text{W2}},$$

TABLE IV
Component *G* Values for Total Base Release from Irradiated DNA[a–c]

Parameter	N_2	O_2
$G(\text{C})$	0.06 ± 0.01^{d}	0.10 ± 0.01
$G(\text{B}) + G(\text{W1})$	0.074 ± 0.01^{c}	0.077 ± 0.01^{c}
$G(\text{W2})$	0.10 ± 0.02	0.09 ± 0.025
Γ_{W1}	14.5^{e}	15.2^{e}



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Summary of Base Release in Hydrated DNA

Results are consistent with the hypothesis that the G-value for the first 12-15 waters molecules of the DNA hydration layer is the same as the G value for the form of DNA to which it is bound (i.e. the pseudo-C or the B form).

The results suggest that the release of bases originating from irradiation of the hydration waters is obtained predominately:

(1) by charge transfer from the direct ionization of the first 12-15 water molecules of the primary hydration layer into the DNA, constituting a **"quasi-direct effect"** that effectively increases the direct effect target (mass of the DNA) by ~75%

(2) by attack of the hydroxyl radicals generated in the outer, more loosely bound water molecules.

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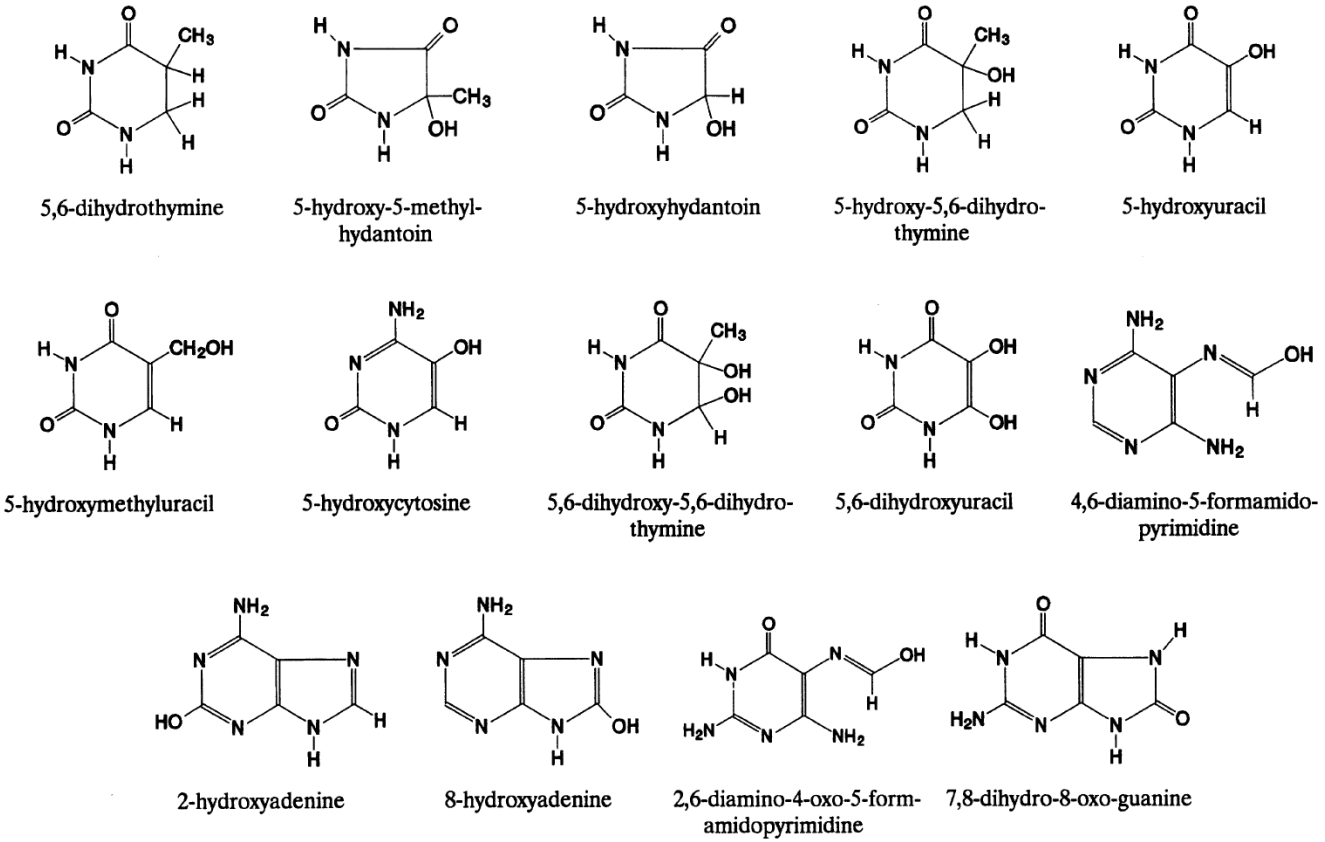
Irradiated Hydrated DNA - DNA Base Damage



DNA base damage products there were surveyed in our GC/MS assay

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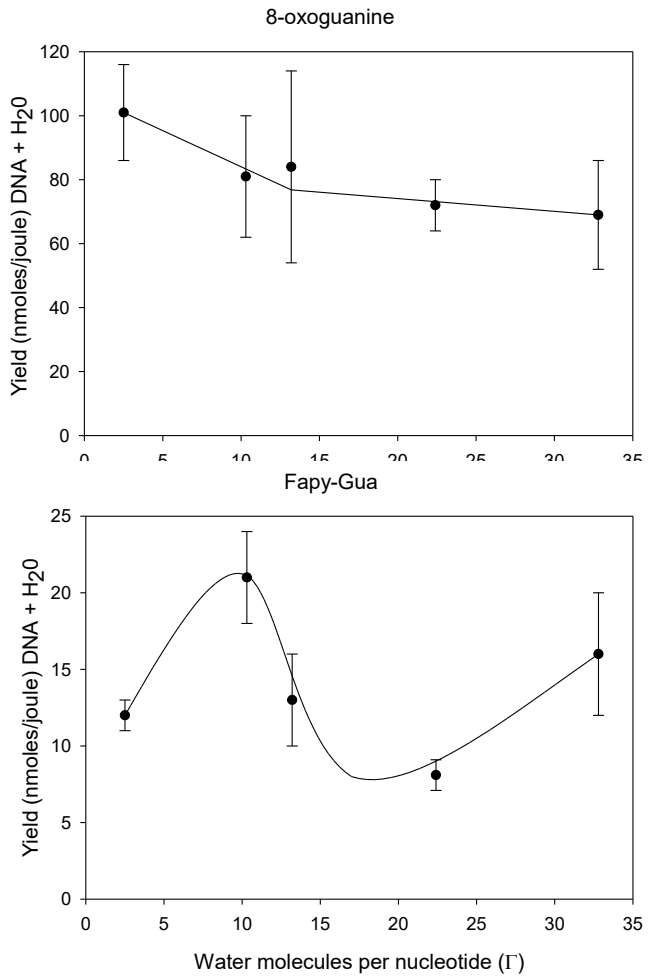
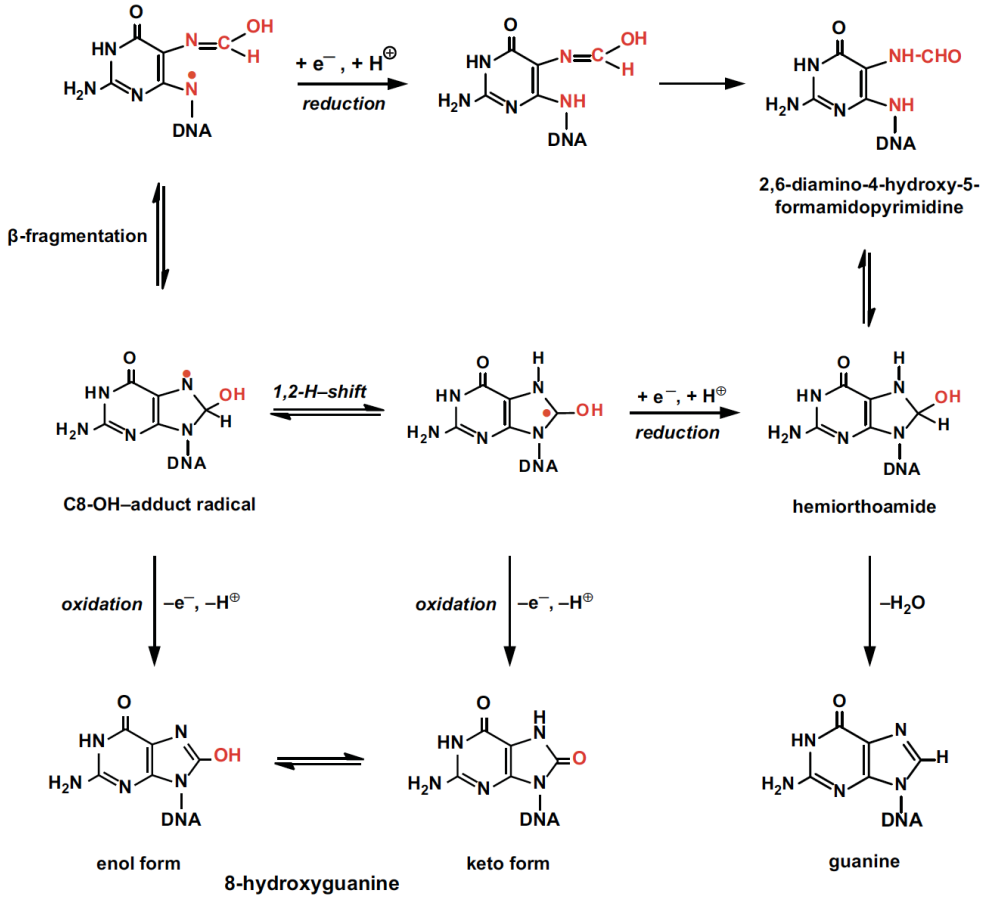
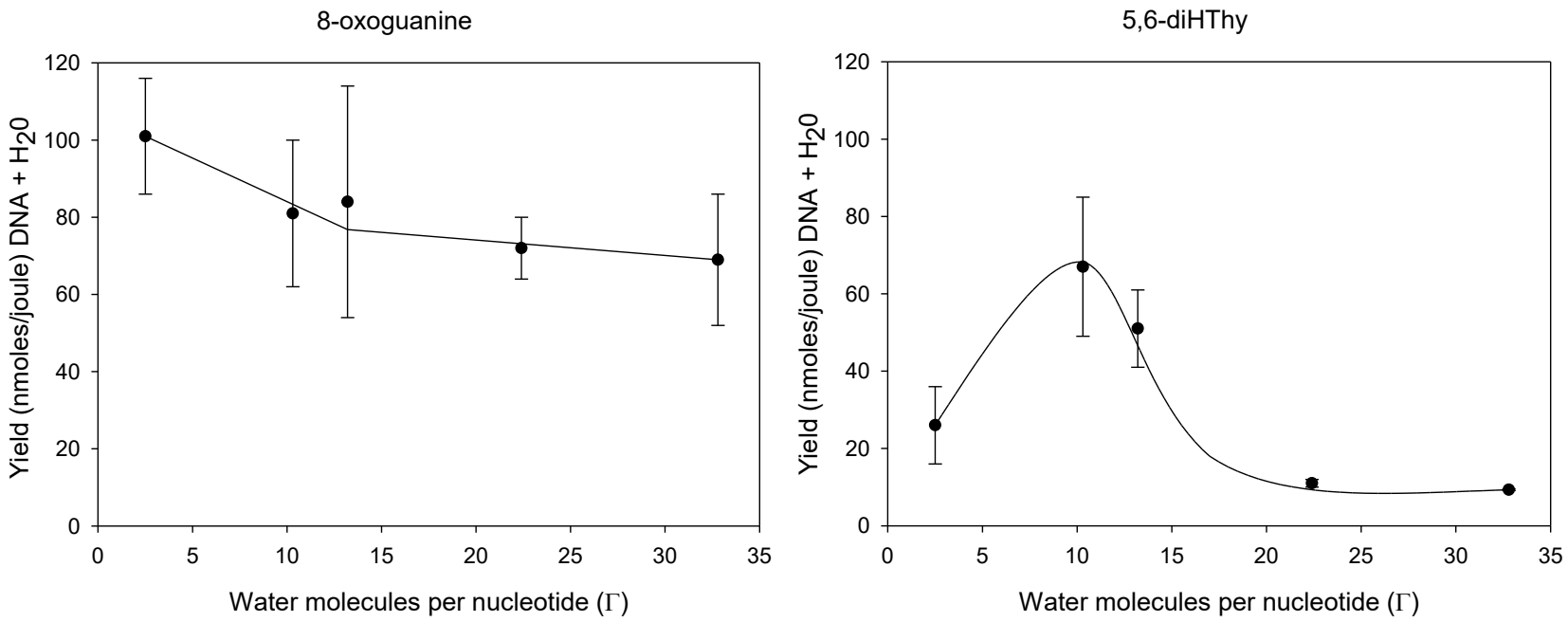
Yield Plots of DNA Base Damage: Comparison of 8-oxoguanine and FAPY-Gua





Yield Plots of DNA Base Damage: Comparison of 8-oxoguanine and 5,6-diHThy



Guanine (8-oxoguanine) and thymine (5,6-diHThy) are the major products of the reaction of electron-loss and electron-gain centers on DNA

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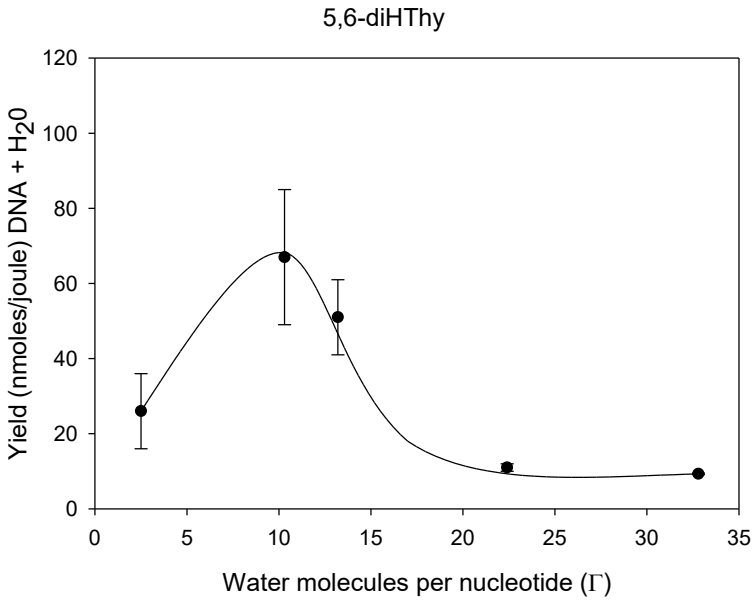
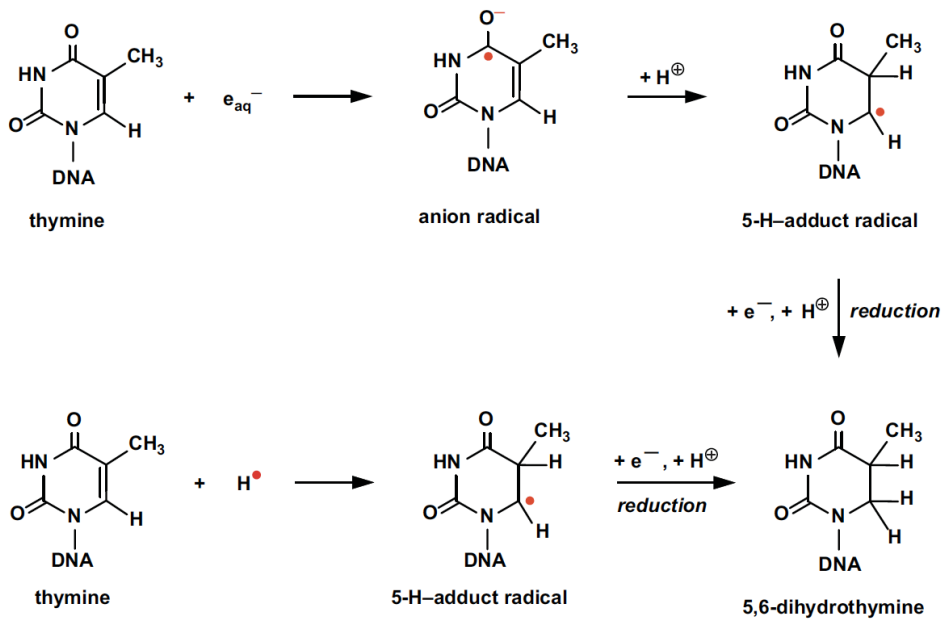
Yield Plots of 5,6-diHThy as Function of DNA Hydration





Yield Plots of DNA Base Damage Products as Function of DNA Hydration

TABLE I
Yields of the Individual End Products in Hydrated DNA γ-Irradiated under Nitrogen

Damage product	Yields (μmol/J × 1000)[a]				
	Γ = 2.5	Γ = 10.3	Γ = 13.2	Γ = 22.4	Γ = 32.8
8-oxo-Gua	101 (15)[b]	81 (19)	84 (30)	72 (8)	69 (17)
FapyGua	12 (1)	21 (3)	13 (3)	8.1 (1.0)	16 (4)
Guanine[c]	12 (1)	7.9 (0.2)	8.0 (0.1)	9.5 (0.9)	9.4 (0.6)
8-OH-Ade[d]	1.4 (0.2)	1.5 (0.3)	1.8 (0.6)	1.3 (0.4)	1.4 (0.5)
FapyAde	0.5 (0.2)	0.8 (0.1)	0.4 (0.1)	1.2 (0.2)	2.2 (1.0)
2-OH-Ade	0.6 (0.1)	0.4 (0.1)	0.5 (0.2)	0.1 (0.1)	0.1 (0.1)
Adenine[c]	21 (1)	13 (1)	14 (2)	16 (2)	17 (1)
5-OH-Ura[e]	1.4 (0.1)	0.7 (0.2)	0.3 (0.2)	1.1 (0.2)	1.6 (1.0)
5-OH-Cyt[e]	0.4 (0.2)	0.1 (0.1)	0.2 (0.1)	0.8 (0.1)	0.7 (0.3)
5,6-diOH-Ura[f]	0.4 (0.2)	0.3 (0.3)	0.4 (0.1)	0.6 (0.5)	0.5 (0.1)
5-OH-Hyd[d]	1.9 (1.3)	0.8 (0.4)	0.4 (0.4)	0.2 (0.2)	0.3 (0.1)
Cytosine[c]	21 (1)	14 (1)	13 (2)	13 (1)	15 (1)
5,6-diHThy	26 (10)	67 (18)	51 (10)	11 (1)	9.3 (0.2)
5-OHMe-Ura	6.1 (0.8)	2.7 (1.1)	2.2 (1.0)	3.5 (0.4)	3.0 (0.5)
5-OH-5,6-diHThy	2.2 (1.2)	1.5 (0.4)	1.3 (0.2)	0.4 (0.1)	0.7 (0.1)
Thy glycol	0.7 (0.3)	0.3 (0.1)	1.1 (0.7)	0.7 (0.1)	1.3 (0.3)
5-OH-5-Me-Hyd[d]	1.7 (0.5)	0.6 (0.5)	0.1 (–)	0.3 (0.3)	0.4 (0.1)
Thymine[c]	19 (1)	12 (1)	13 (1)	17 (1)	20 (2)

[a]The yields are based on the total sample mass and, except for 5,6-diHThy and the release of unaltered bases, are determined from a single dose (90 kGy) due to either the variability or low product yields at doses ≤10 kGy.
[b]Each value represents the mean ± 1 SD of at least three independent experiments.
[c]The yields for the release of unaltered bases were obtained from ref. (*13*).
[d]These base damage products may be formed, either in whole or in part, from the reaction of trace amounts of oxygen with base radicals.
[e]These base damage products are formed from the formic acid-induced deamination/dehydration of Cyt glycol.
[f]This base damage product is the trimethylsilylated form of the acid-induced deamination product of uramil and isouramil.

8-oxoguanine is the major DNA damage lesion followed by total release of unaltered bases. (8-oxoguanine 1.1-2 x BR)

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Yield Plots of DNA Base Damage: Comparison of Damage Classes

TABLE II
Comparison of the Total Yields of Base Damage Products and Base Release in Hydrated DNA γ-Irradiated under Nitrogen[a]

	Water molecules per nucleotide				
	2.5	10.3	13.2	22.4	32.8
Base damage	0.156 (0.031)	0.179 (0.043)	0.157 (0.047)	0.102 (0.014)	0.107 (0.027)
Base release[b]	0.073 (0.003)	0.047 (0.003)	0.048 (0.007)	0.055 (0.006)	0.061 (0.002)
Total[c]	0.229 (0.034)	0.226 (0.047)	0.205 (0.054)	0.157 (0.020)	0.168 (0.029)

[a]The total yields, in μmol/J, were based on total sample mass.
[b]The yields for the release of unaltered bases were obtained from ref. (*13*).

TABLE III
Summary of the Yields of Products that Were Formed from Electron-Loss or Electron-Gain Centers in γ-Irradiated Hydrated DNA[a]

	Water molecules per nucleotide				
	2.5	10.3	13.2	22.4	32.8
Electron-loss centers[b]	0.201 (0.023)	0.158 (0.027)	0.150 (0.044)	0.146 (0.019)	0.158 (0.027)
Electron-gain centers	0.028 (0.011)	0.068 (0.019)	0.052 (0.010)	0.011 (0.001)	0.010 (0.002)

[a]The total yields, in μmol/J, are based on total sample mass.
[b]Sum of the yields of the base damage products derived from electron-loss centers measured in this study and the release of unaltered bases obtained from ref. (*13*).

Base damage is approximately a factor of 2-3 greater than base release;
Have not identified all the major damage products formed from electron-gain centers

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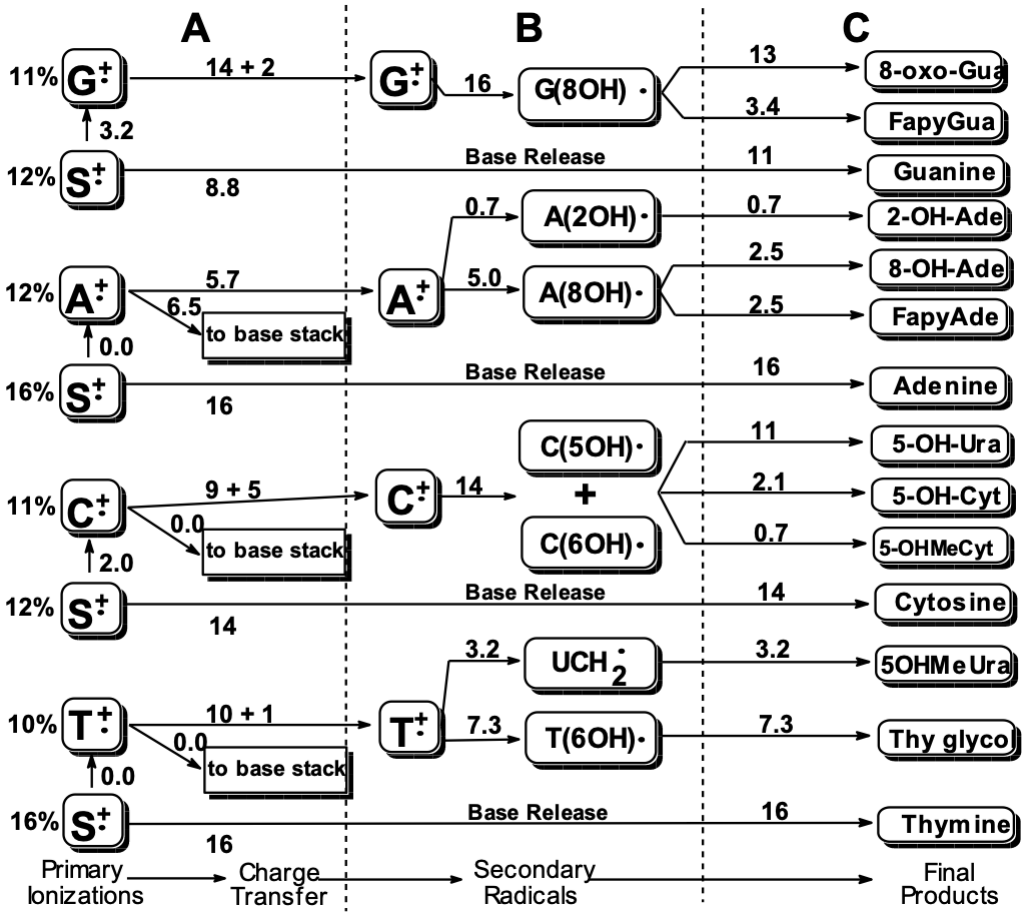
Post-IR: Charge Transfer and Radical Reaction Pathways

(A) Ionization and charge transfer
(B) Formation of secondary radicals by hydroxylation and deprotonation reactions
(C) Late processes (oxidation or reduction) to yield molecular products.

The product yields necessitate that electron-loss on adenine, cytosine and thymine are transferred to guanine, as shown.



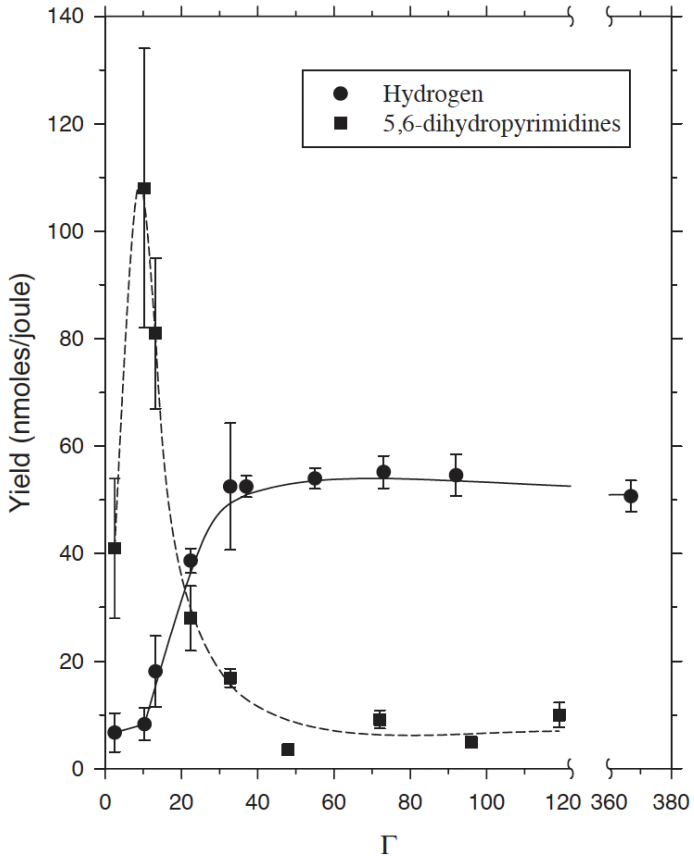
Electron Gain Centers – Formation of Molecular Hydrogen

Molecular hydrogen

Reaction of dry electron with pyrimidines is favored in irradiated DNA when hydrated to $\Gamma \leq 20$

Hydrated electron forms predominantly molecular hydrogen

Molecular hydrogen in combination with the dihydropyrimidines are the predominant electron-gain products



$$2e_{aq}^- + 2H_2O \rightarrow H_2 + 2OH^-$$

Electron Gain Centers – Formation of Molecular Hydrogen

The results are consistent with the results of the release of unaltered bases from irradiated DNA

Both studies support the contention that DNA damage originating from irradiation of the hydration layer it generated predominantly by:

(1) charge transfer from direct ionization of the tightly bound water molecules in the hydration layer (Γ <= 13.2)

(2) the attack of hydroxyl radicals produced in the outer regions of the hydration layer (Γ > 13.2).

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Conclusions

The first 12-15 water molecules of the primary hydration layer constitute a quasi-direct on the DNA, effectively adding to the direct effect target size

Under oxygen free conditions base damage is the major DNA damage class; is approximately a factor of 2-3 greater than strand damage as measured by base release

Charge transfer from sugar to adjoining base reduces damage to the DNA backbone, thereby reducing the likelihood of strand break formation

Guanine is the predominant “sink” for electron-loss centers (“holes”) generated in the sugar and bases (adenine, cytosine and thymine) and transferred into the base stack

The 5,6-dihydropyrimidines and molecular hydrogen are the major base damage products formed from electron-gain centers (dry and aqueous electrons)

Future Directions: Assessing effect of oxygen DNA damage in models with oxygen concentrations bordering the hypoxic region; bound transition metal (i.e. Cu^{+2})

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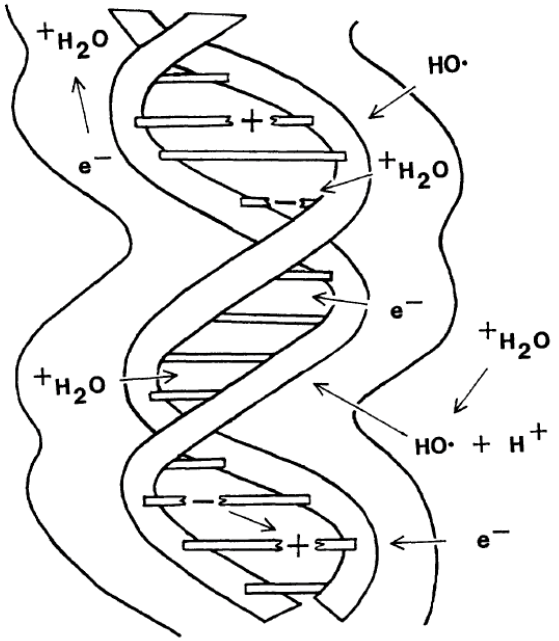

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