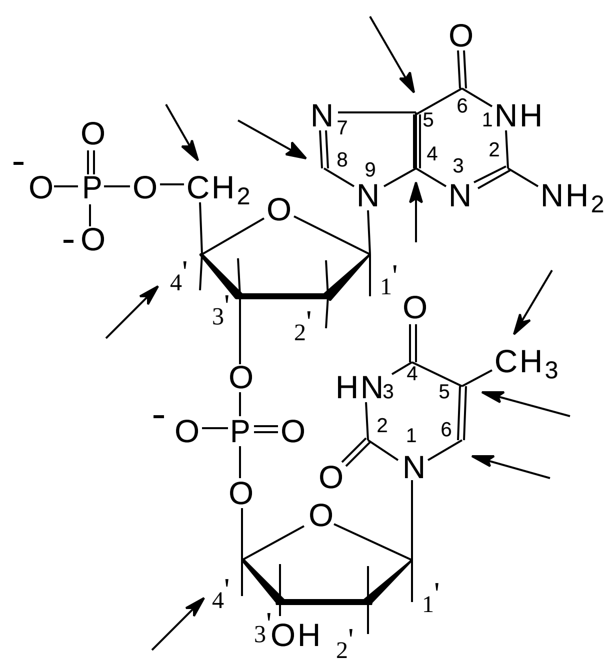
A. DNA damage: Hot spots for free radical attack

The normal human excretes approximately 100 nmol of thymine glycol, thymidine glycol and hydroxymethyluracil every day. From this it can be calculated that on average there is a minimum of 10^3 oxidative hits per day on the DNA within each of the $6x10^{13}$ cells in the body. Because these are not the only possible oxidation products of DNA the total number of oxidative hits on DNA must be much higher [1]. A recent estimate puts it at 1.5 x 10^5 oxidative hits/DNA in a human cell each day. That would be 10^{19} total hits in a human each day [2].

Histones associated with nucleosomal DNA appear to offer protection against oxidative DNA damage [3].

Mitochondrial DNA has higher oxidative damage than nDNA (*e.g.* rat liver: 8-OHdG 1/130,000 dG nDNA; 1/8000 mitDNA [4]). Reasons could be:
 the lack of histones,
 the proximity of mtDNA to ROS generated during electron transport,
 proximity to the mitochondrial membrane (lipid peroxidation) or
 inefficient repair (damage accumulation) [5].

Hot spots for free radical attack:



B. Damage by ROS

$O_2^{\bullet -}$ and H_2O_2 are not reactive enough but they can form **$HO^{\bullet}$**:

$O_2^{\bullet -} + HOCl \rightarrow \mathbf{HO^{\bullet}} + Cl^{-} + O_2$ [6]

$O_2^{\bullet -} + NO^{\bullet} \rightarrow ONOO^{-} + H+ \rightarrow ONOOH \rightarrow$ "$NO_2^{\bullet} + \mathbf{HO^{\bullet}}$" [7]

$2\ O_2^{\bullet -} + 2H^{+} \rightarrow H_2O_2 + O_2$ [8]

$[4Fe\text{-}4S]^{2+} + 2O_2^{\bullet -} + 2H^{+} \rightarrow [3Fe\text{-}4S] + H_2O_2 + Fe^{2+}$ [8]

$Fe^{2+} + H^{+} + H_2O_2 \rightarrow \mathbf{HO^{\bullet}} + Fe^{3+} + H_2O$

$O_2^{\bullet -} + Fe^{3+} \rightarrow Fe^{2+} + O_2$ [8]

$H_2O_2 + Fe(II)DNA \rightarrow \mathbf{HO^{\bullet}} + Fe(III)DNA + OH^{-}$

$HO^{\bullet}$ is right next to the DNA!!!!!!!

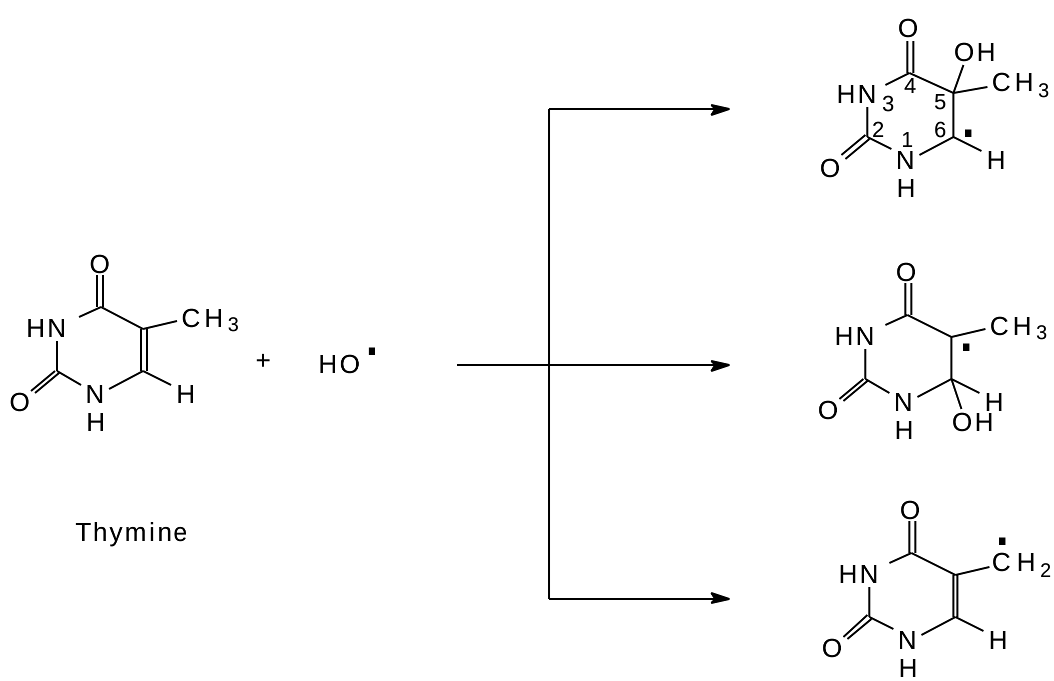
Don't forget about Fe(IV) & Fe(V):

$Fe^{2+} + H_2O_2 \rightarrow FeO^{2+} + H_2O$ — FeO^{2+} = ferryl iron

$Fe^{3+} + O_2^{\bullet -} \rightarrow [Fe^{3+}\text{-}O_2^{\bullet -} \leftrightarrow Fe^{2+}\text{-}O_2] \leftarrow Fe^{2+} + O_2$ — $Fe^{3+}\ O_2^{\bullet -}$ = perferryl

1. $HO^{\bullet}$ Attack on Pyrimidines [9]

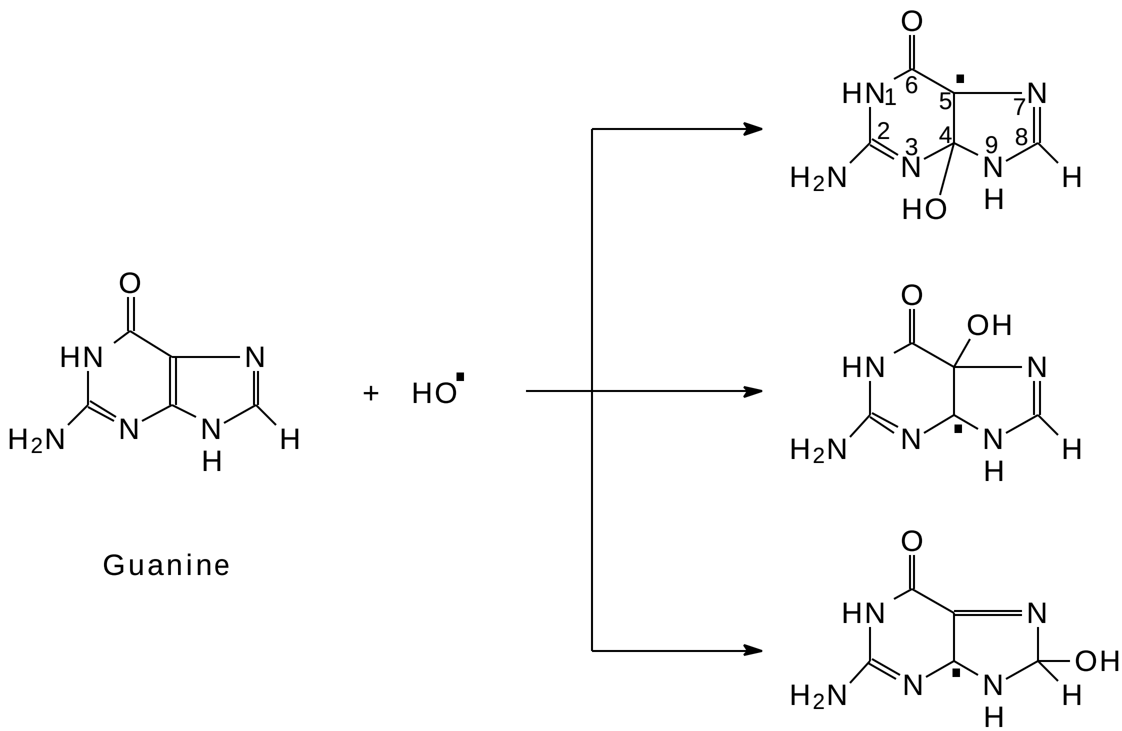
Radicals formed



Thymine

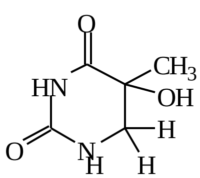
2. $HO^{\bullet}$ Attack on Purines [9]

Radicals formed

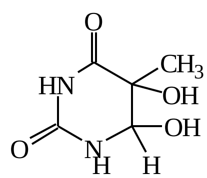


Guanine

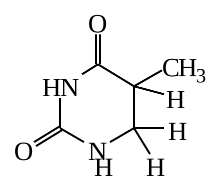
3. Base damage: (shown is a subset of oxidative base products from more than 20 products known) [10]



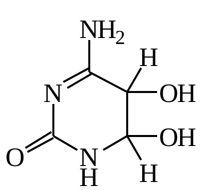
5-Hydroxy-6-hydrothymine



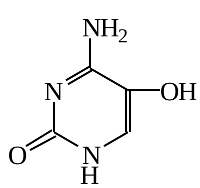
Thymine glycol (cis and trans)



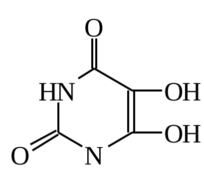
5,6-Dihydrothymine



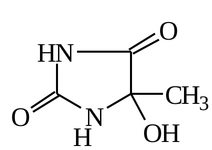
Cytosine glycol



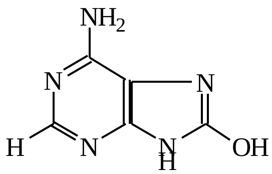
5-Hydroxycytosine



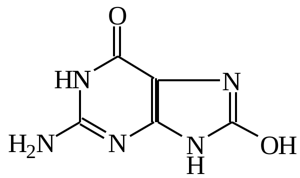
5,6-hydroxy uracil



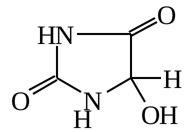
5-hydroxy-5-methylhydantoin



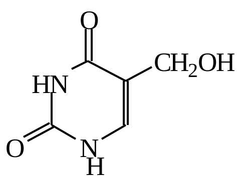
8-Hydroxyadenine



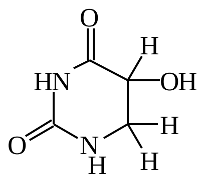
8-Hydroxyguanine



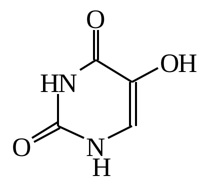
5-hydroxy
hydantoin



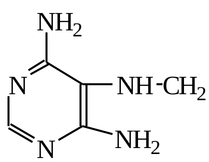
5-hydroxymethyluracil



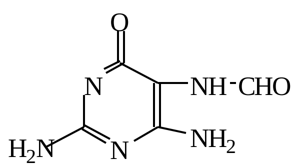
5-hydroxy-6-
hydrouracil



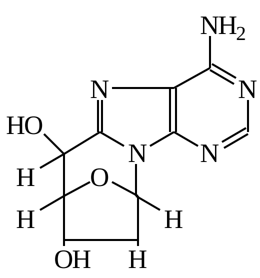
5-hydroxyuracil



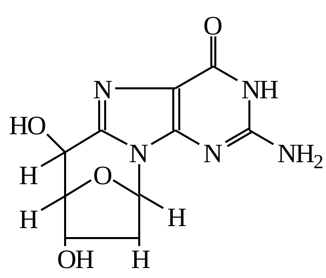
4,6-Diamino-5-
formamidopyrimidine



2,6-Diamino-4-hydroxy-
5-formamidopyrimidine

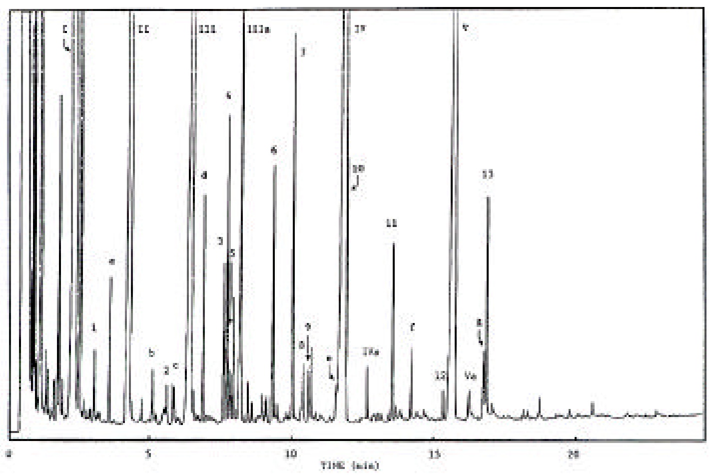


8,5'-Cyclo-2'-deoxyadenosine

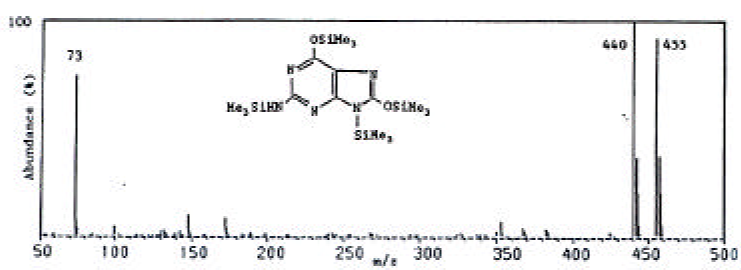


8,5'-Cyclo-2'-deoxyguanosine

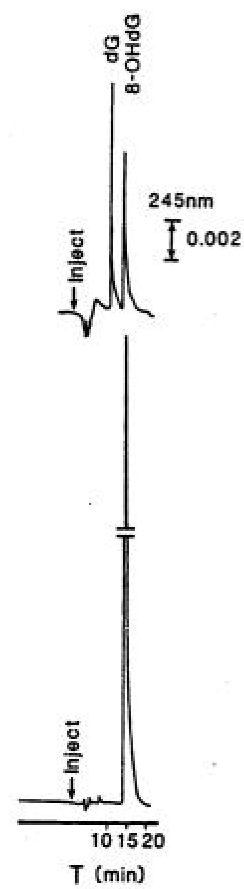
4. Detection of the damaged bases: GC/MS and HPLC/EC



Gas chromatogram of a DNA sample, that was γ-irradiated in aqueous solution, followed by hydrolysis with formic acid and trimethylation. 28 peaks were identified with more than 10 being oxidative products of bases [10].



Electron-Ionization Mass spectrum of the Me_3Si derivative of 8-hydroxyguanine peak 13 in the gas chromatogram above [10].



Comparison of optical and electrochemical detection of 2’-deoxyguanosine and 8-hydroxy-2’-deoxyguanosine [11].

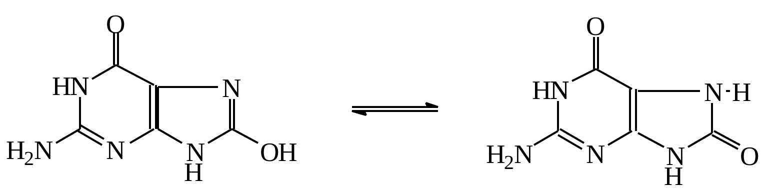
5. Be aware:

GC/MS can overestimate base damage products due to artificial generation during derivatization;
HPLC can underestimate the amount of 8-OHdG if the enzymatic hydrolysis is not complete; and of course
DNA damage can be overestimated if oxidatively damaged during its isolation.

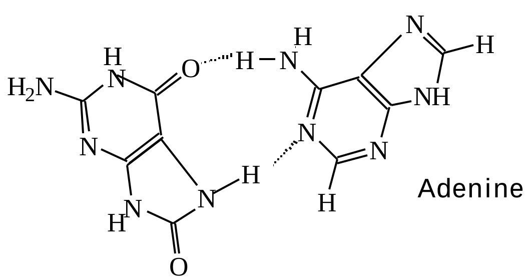
8-oxoG content in urine can be affected by diet or oxidative damage to RNA [5];
8-oxodG content can be affected by oxidized guanine from the dGTP precursor pool [5];
8-oxo-dG is a transient product and can compete with dG in oxidation. Oxidized purine bases in general can be futher oxidized at a much faster rate than the initial oxidation of their parent compounds [5].

6. Misreading of 8-OHdGua

8-hydroxyguanine



8-OH-Gua (enol) 8-oxo-Gua (keto)



8-oxo-Gua

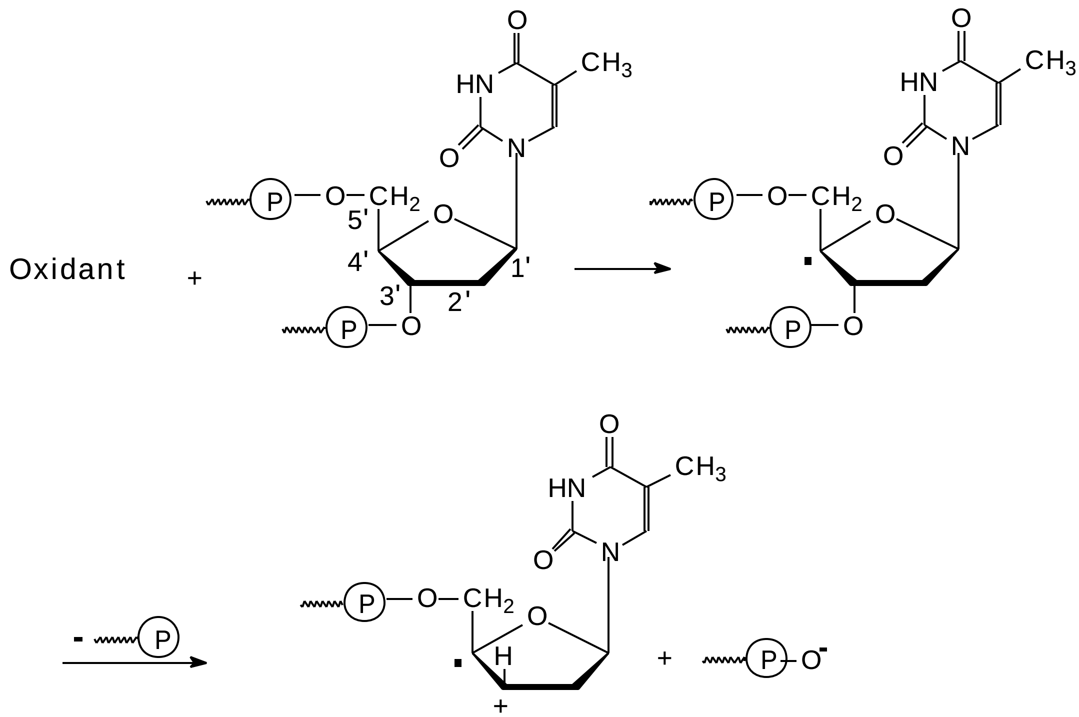
Basepairs

A—T

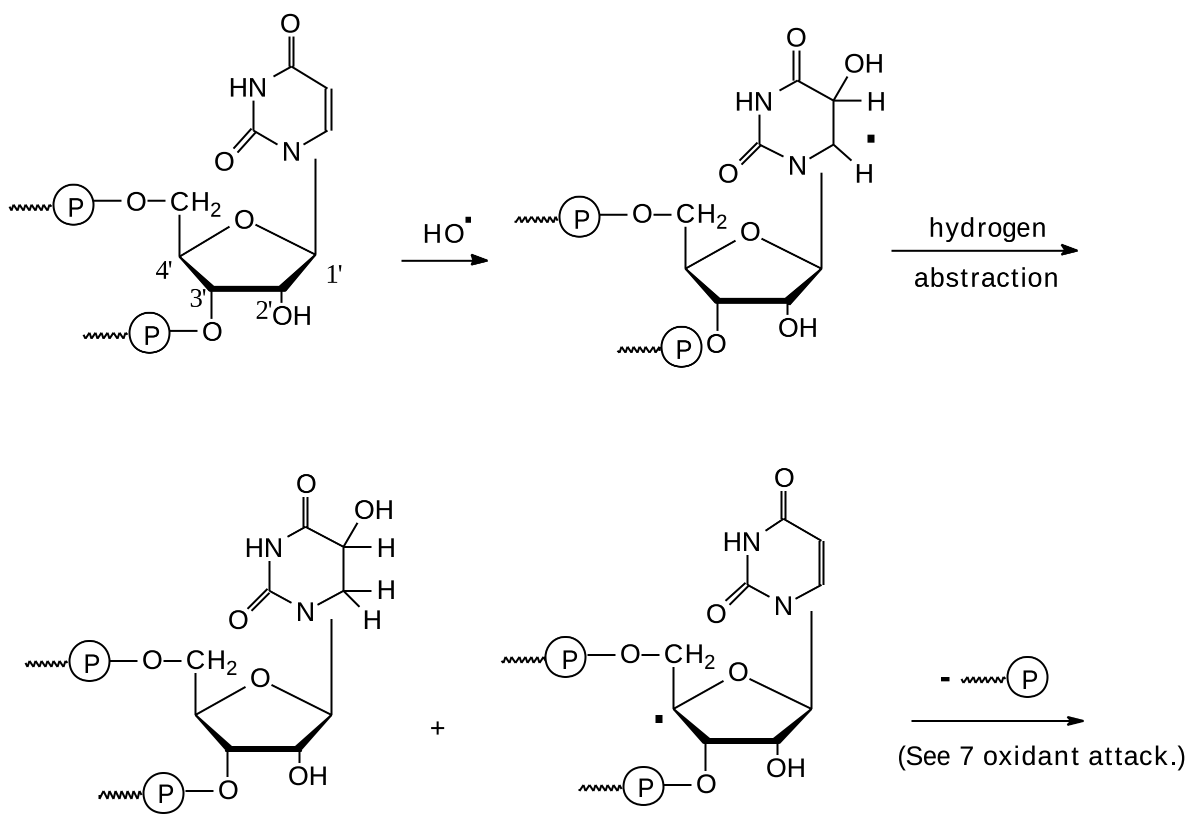
G—C

8 OHG—A

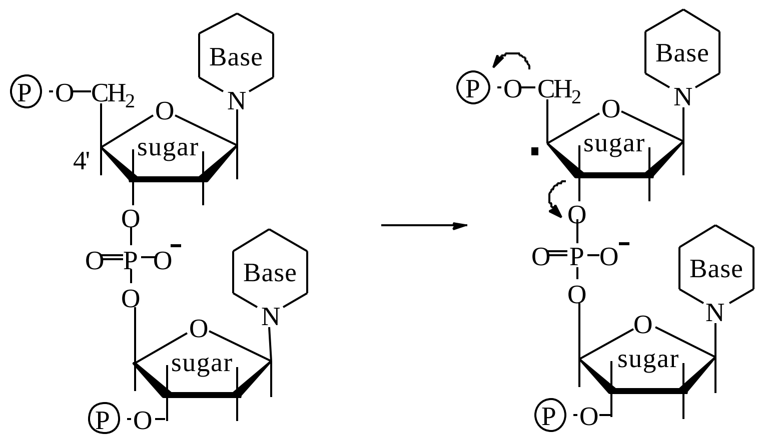
7. Oxidant Attack on Sugar:



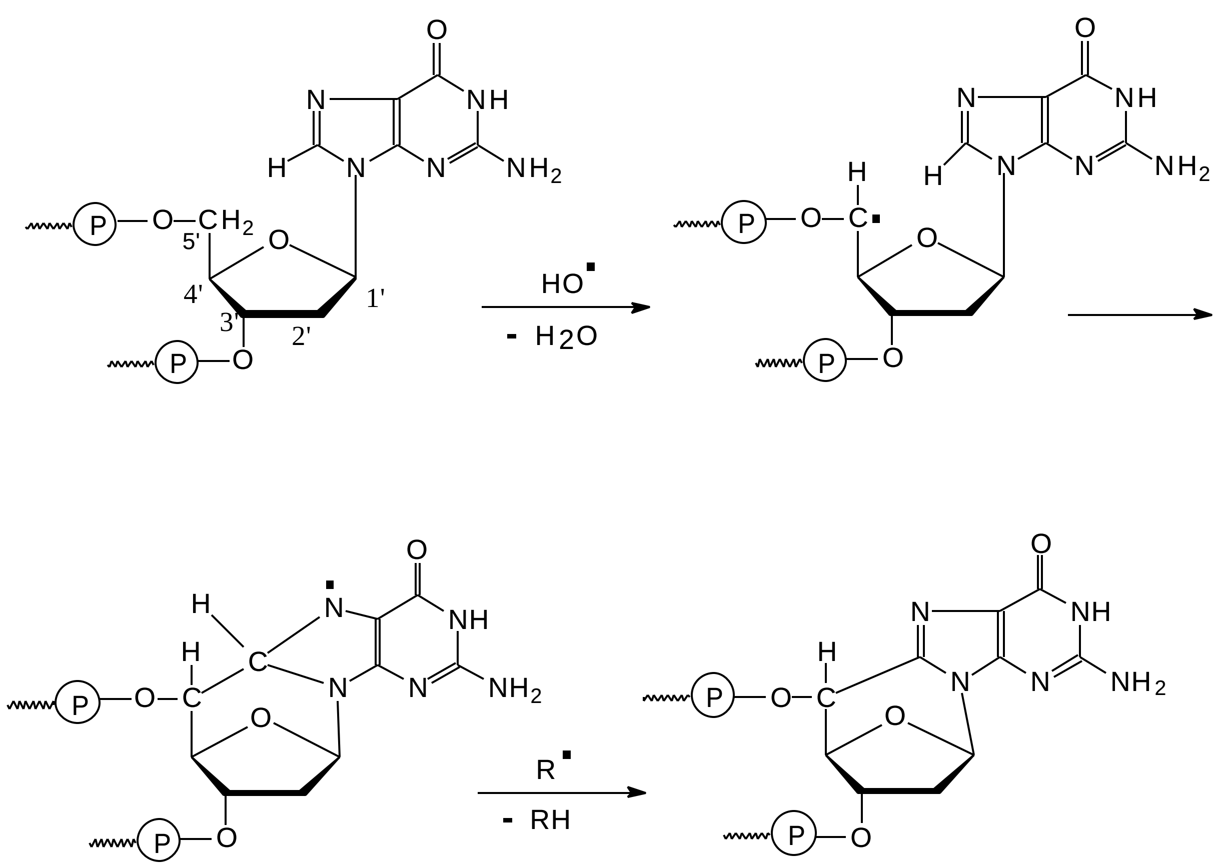
8. Transfer of Damage from a Base to a Sugar [9]



9. Strand breaks: DNA single strand breaks result because of the collapse of the sugar !!



10. HO• Attack on 5' carbon [9]

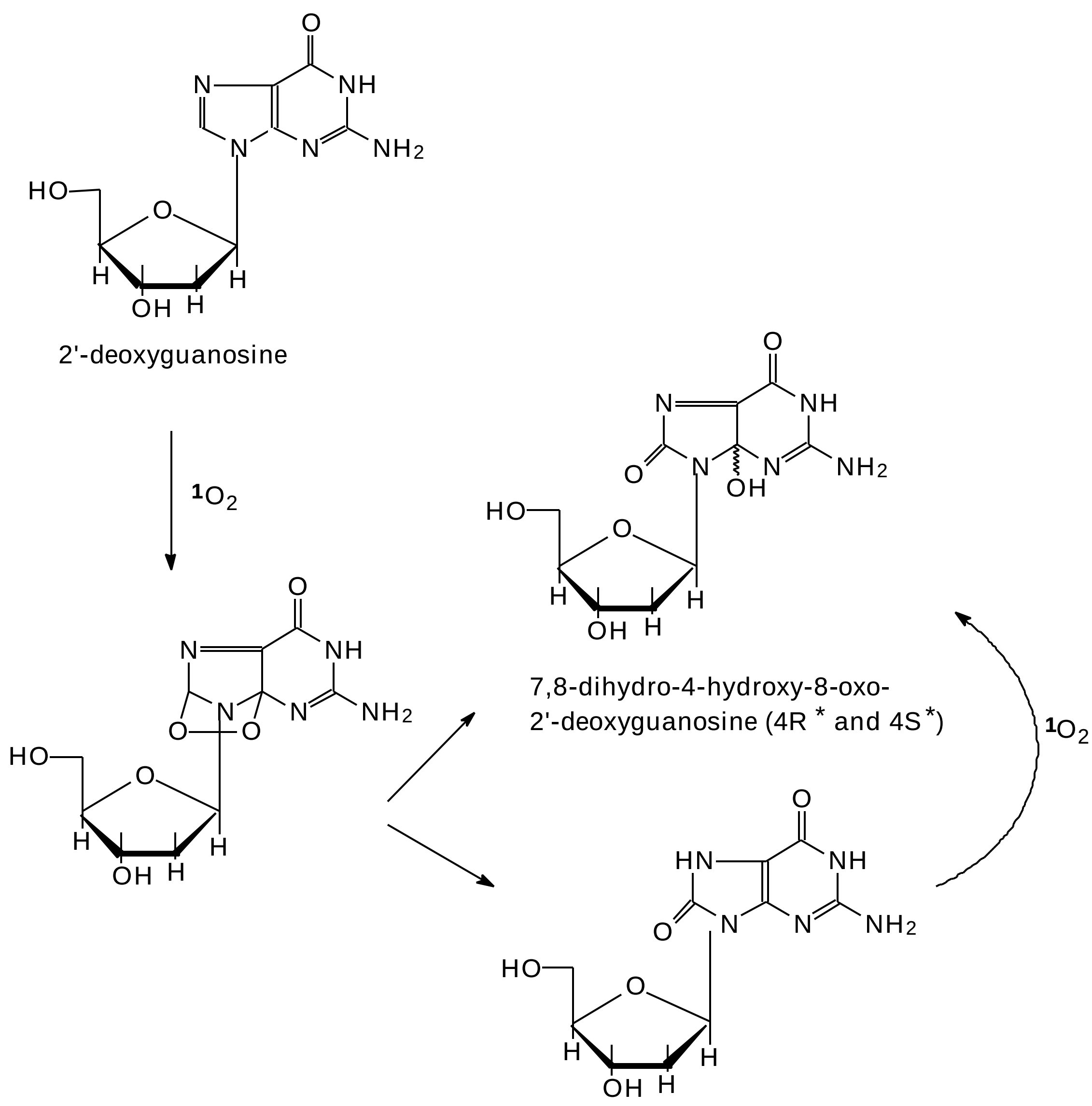


11. Detection of strand breaks

Single cell electrophoresis (comet assay),
alkaline elution or
fluorometric analysis of DNA unwinding (FADU)

12. Singlet Oxygen Attack [12]

$^1O_2$ reacts with dG to form 8OHdG [13]

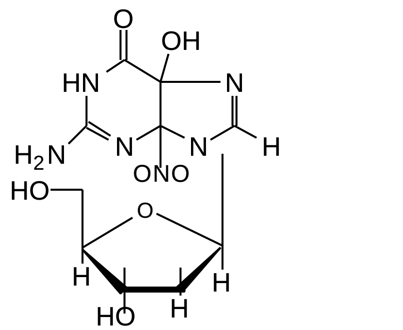


C. Damage by RNS:

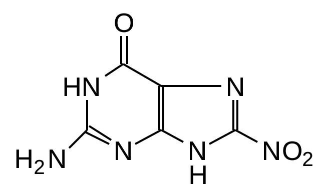
Peroxynitrite is a strong oxidant formed by reaction of nitric oxide with superoxide:

$^{\bullet}NO + O_2^{\bullet -} \rightarrow ONOO^- + H^+ \rightarrow ONOOH \rightarrow$ "$^{\bullet}NO_2 + HO^{\bullet}$" [7]

Peroxynitrite can react with 2'-deoxyguanosine to form 4,5-dihydro-5-hydroxy-4-(nitrosooxy)-2"-deoxyguanosine (nox-dG) or 8-nitroguanine [14]

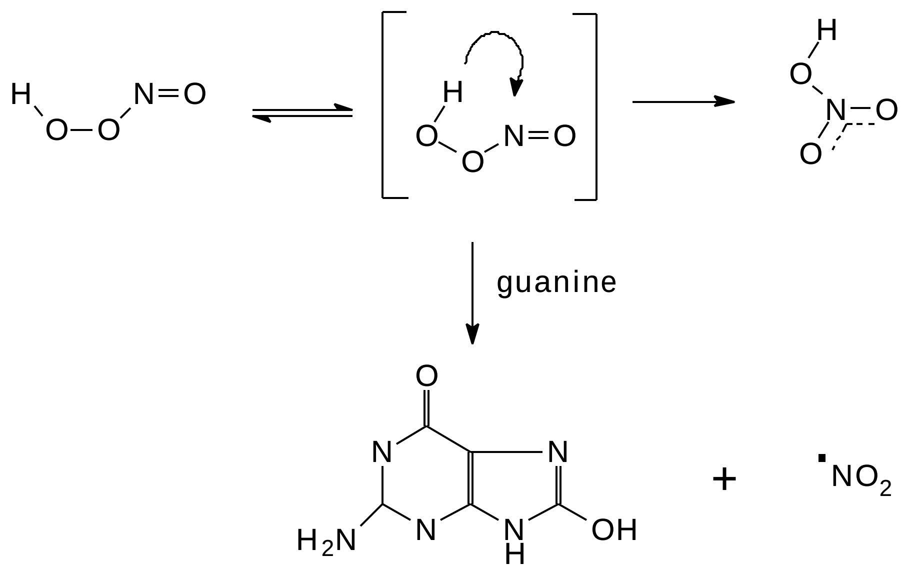


4,5-dihydro-5-hydroxy-4-(nitrosooxy)-2'-deoxyguanosine



8-nitroguanine

Proposed mechanism of the formation of 8OHdG by peroxynitrite [15]:



8-Hydroxy-2'-deoxyguanine

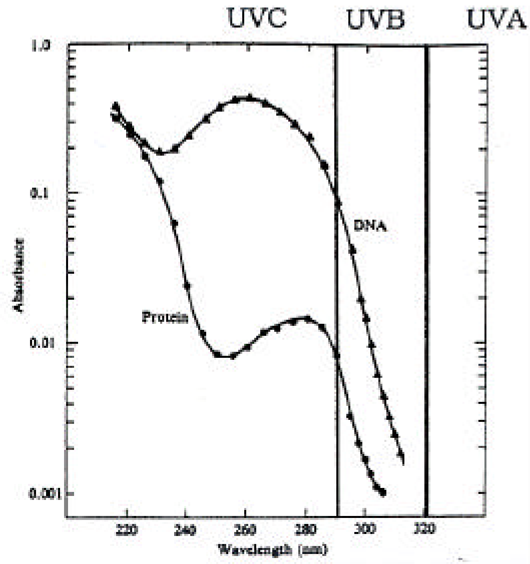
Detection: HPLC/UV or ECD

Peroxynitrite oxidizes 8OHdG faster than it oxidizes dG itself [16].

D. Damage by UV light:

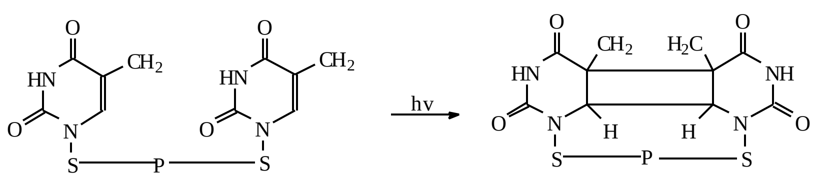
1. DNA absorption spectrum

Absorption spectra of DNA (calf thymus) and a protein (bovine serum albumin) at equal concentration (≈20 µg.mL) [21].



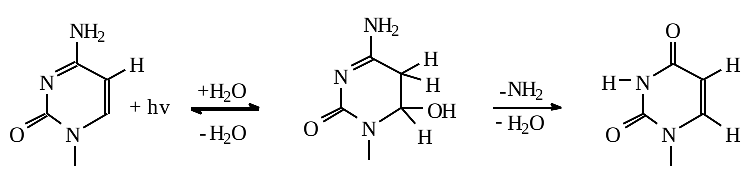
2. Photoproducts of DNA [1]:

dimers:



pyrimidine dimers

hydrates:

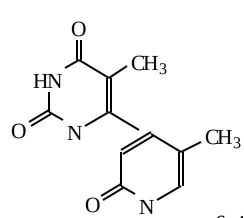


cytosine

6-hydroxy-5,6-dihydrocytosine

uracil

adducts:

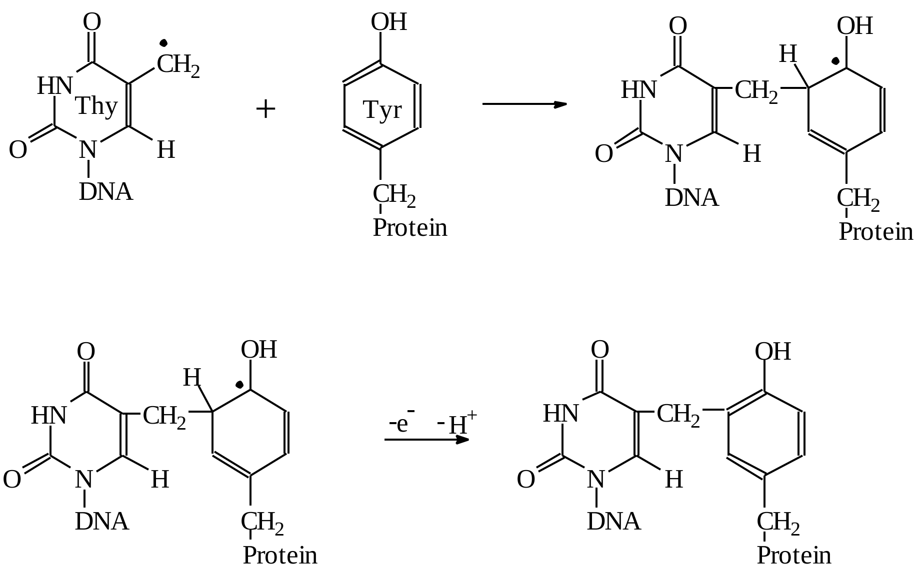


6-4'[pyrimidine-2'-one] thymine

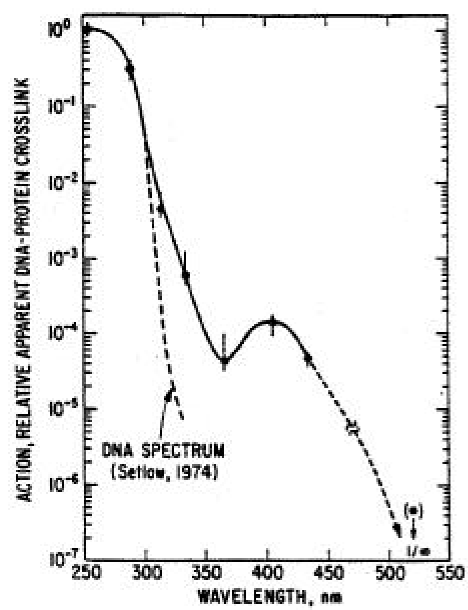
3. Photosensitization

$$UVA + Sensitizer \longrightarrow Sen^* \rightarrow \begin{cases} {}^1O_2 \\ O_2^{\bullet -} \longrightarrow H_2O_2 \longrightarrow HO^\bullet \end{cases}$$

4. DNA-Protein-Crosslinking (DPC): produced by UV light or $HO^\bullet$ Attack



5. Action spectrum of DNA-Protein-Crosslinking



Action spectrum for the relative induction of DNA-protein crosslinks by UV and visible radiations [17].

6. Detection of DNA-Protein-Crosslinking:

GC/MS,
alkaline elution,
K-SDS assay [18],
FADU (fluorometric analysis of DNA unwinding): DPCs decrease the rate at which DNA unwinds and thereby diminishes the extent of strand breakage measured by standard FADU [19]

E. Intriguing news

Oxidation of DNA might be possible by long distance electron transport along the Π stack of the DNA double helix. [20].

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