



evropský
sociální
fond v ČR



EVROPSKÁ UNIE



MINISTERSTVO ŠKOLSTVÍ,
MLÁDEŽE A TĚLOVÝCHOVY



OP Vzdělávání
pro konkurenceschopnost

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Tvorba ROS a RNS, jejich technologický a biologický význam II

Důsledky interakce ROS a RNS s biomolekulami

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Ústav chemických léčiv, FaF VFU Brno

Negativní stránky působení ROS a RNS

- **Poškozování struktury proteinů**
 - fragmentace, cross-linking, hydroxylace, nitrace a nebo oxidace aminokyselin
- **Poškozování struktury DNA a RNA**
 - oxidační poškození bazí, hydrolýza řetězců, změny struktury, ovlivnění biologické aktivity
- **Poškozování struktury lipidů**
- **Programovaná smrt buňky - apoptosa**
 - vliv volných radikálů na indukci apoptosy

Poškození struktury proteinů ROS a RNS – poškození polypeptidového řetězce

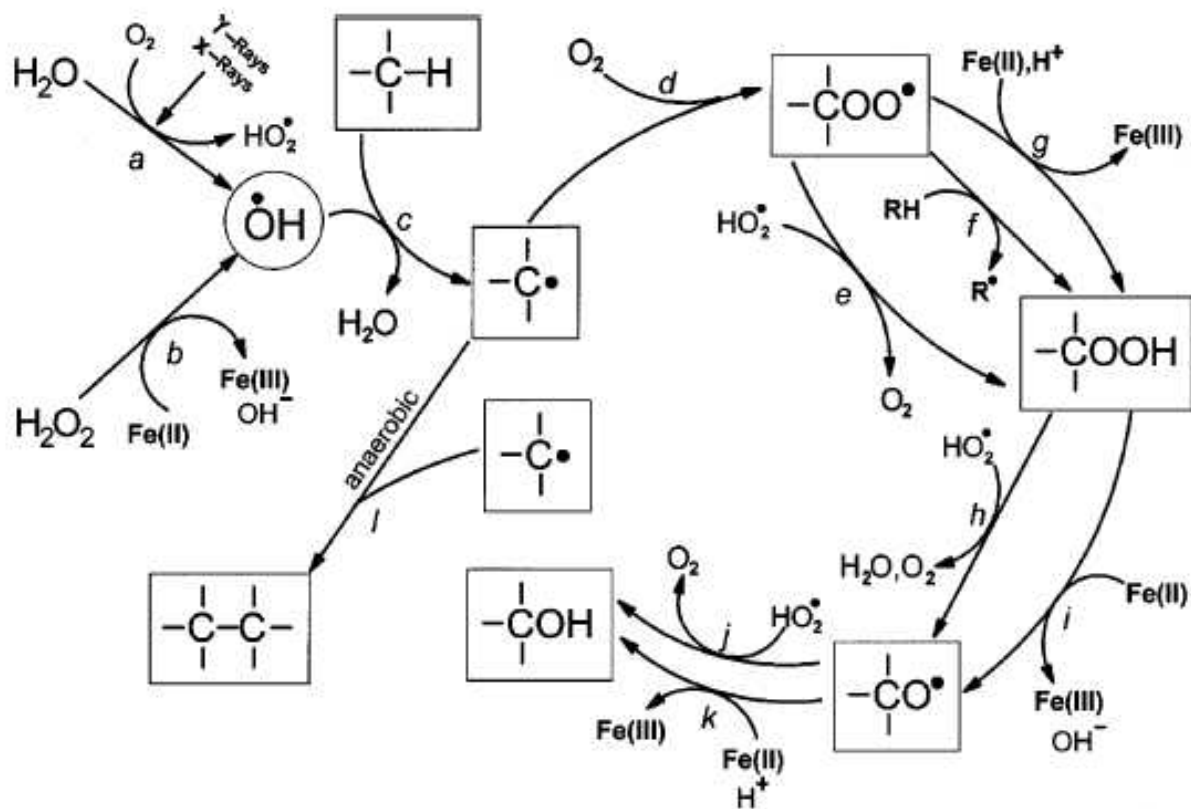
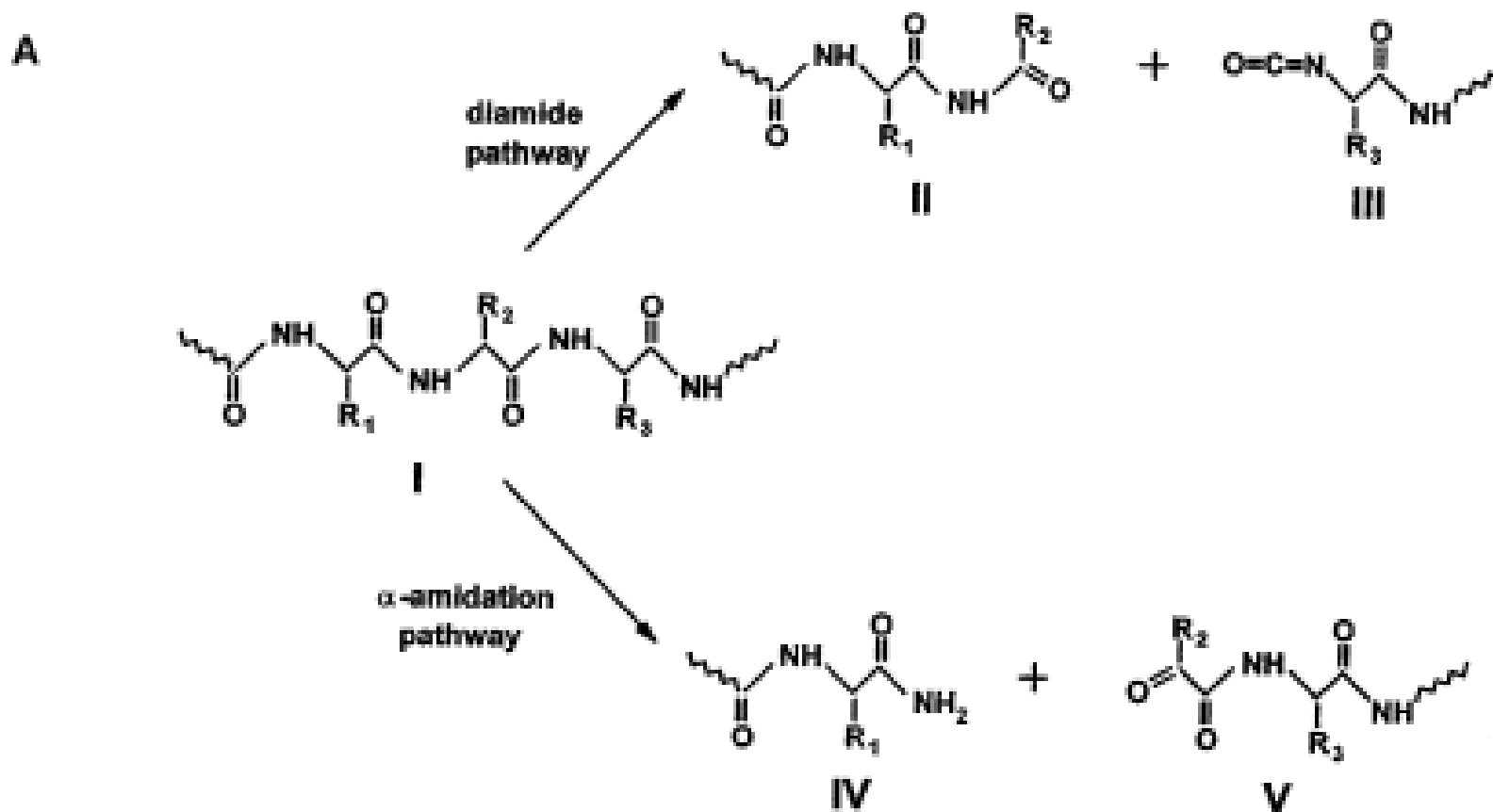


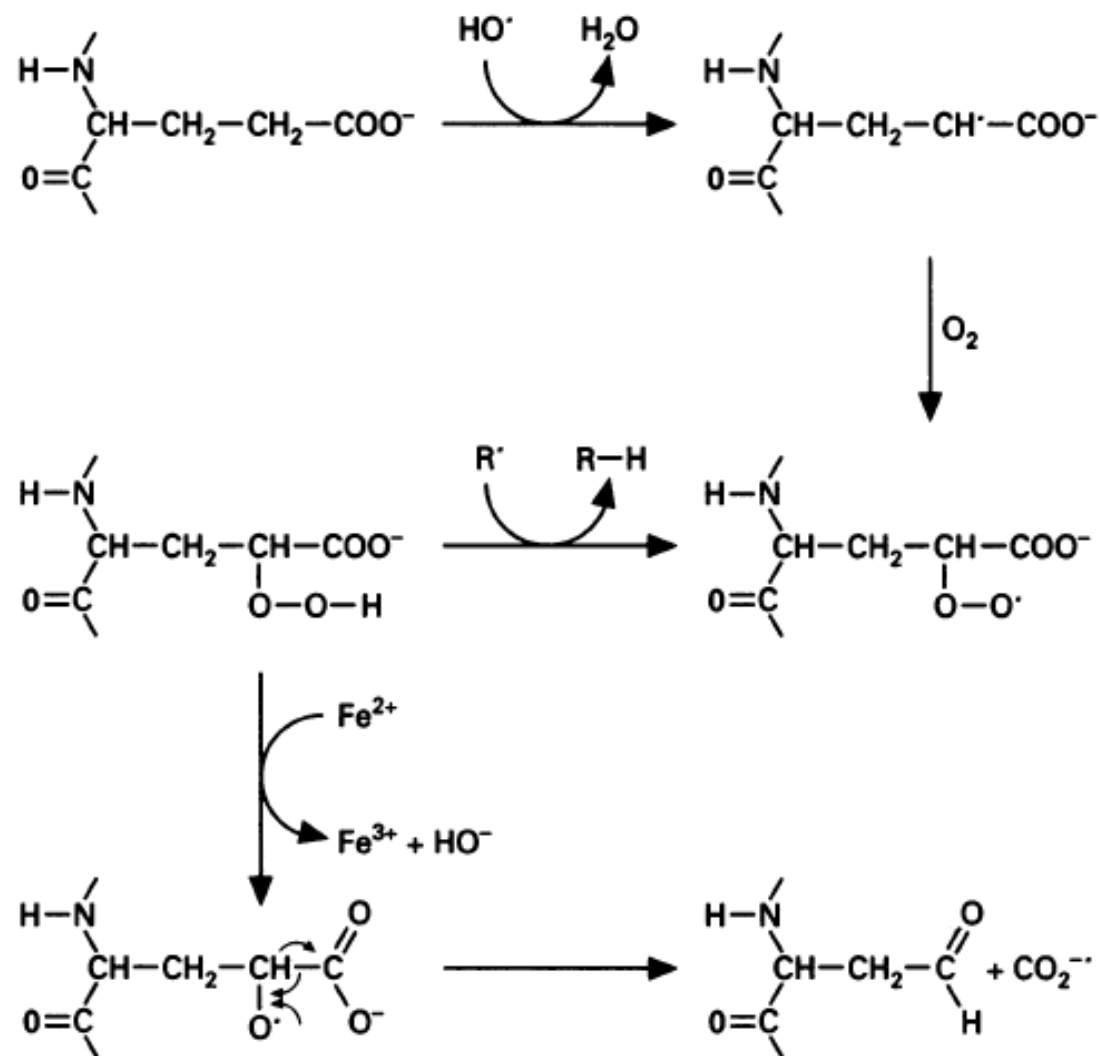
Fig. 1. Free radical-mediated oxidation of the protein polypeptide backbone

Poškozování struktury proteinů ROS a RNS – poškození polypeptidového řetězce

Oxidative cleavage of the polypeptide chain

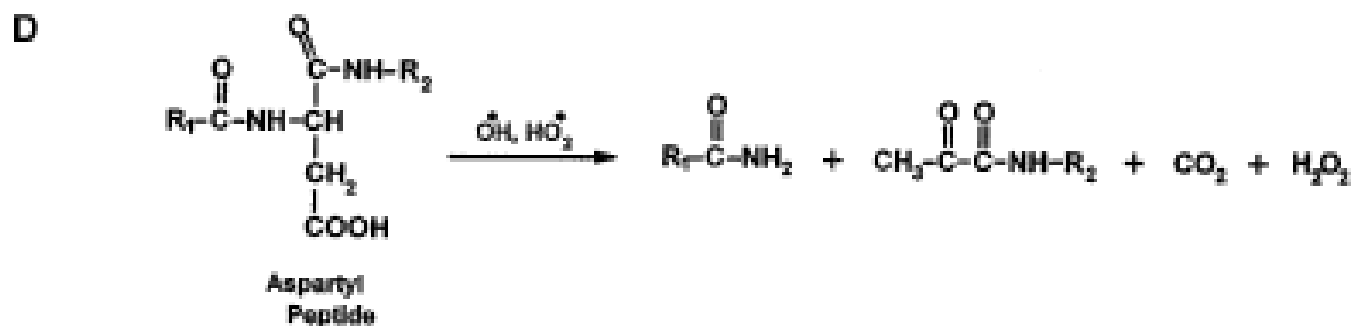
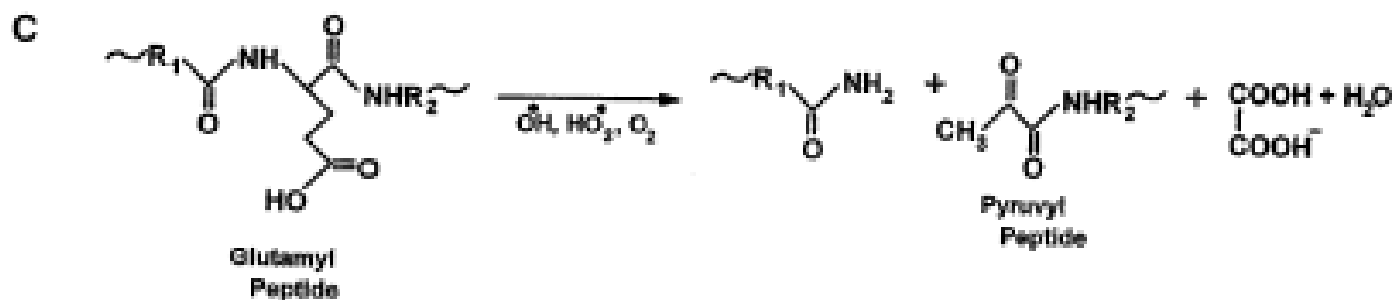
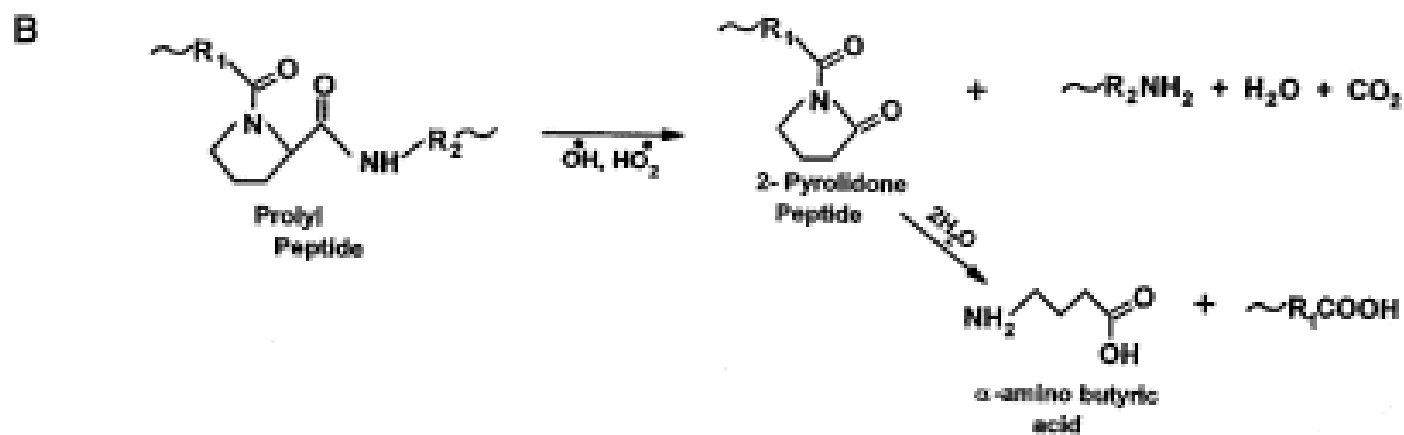


Poškození struktury proteinů ROS a RNS – poškození polypeptidového řetězce



Scheme 1

Poškozování struktury proteinů ROS a RNS – poškození polypeptidového řetězce



Poškození struktury proteinů ROS a RNS – poškození postranních řetězců

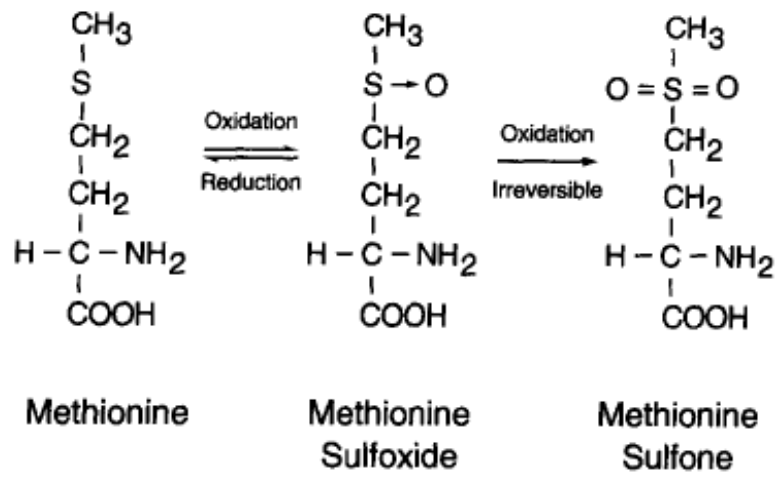
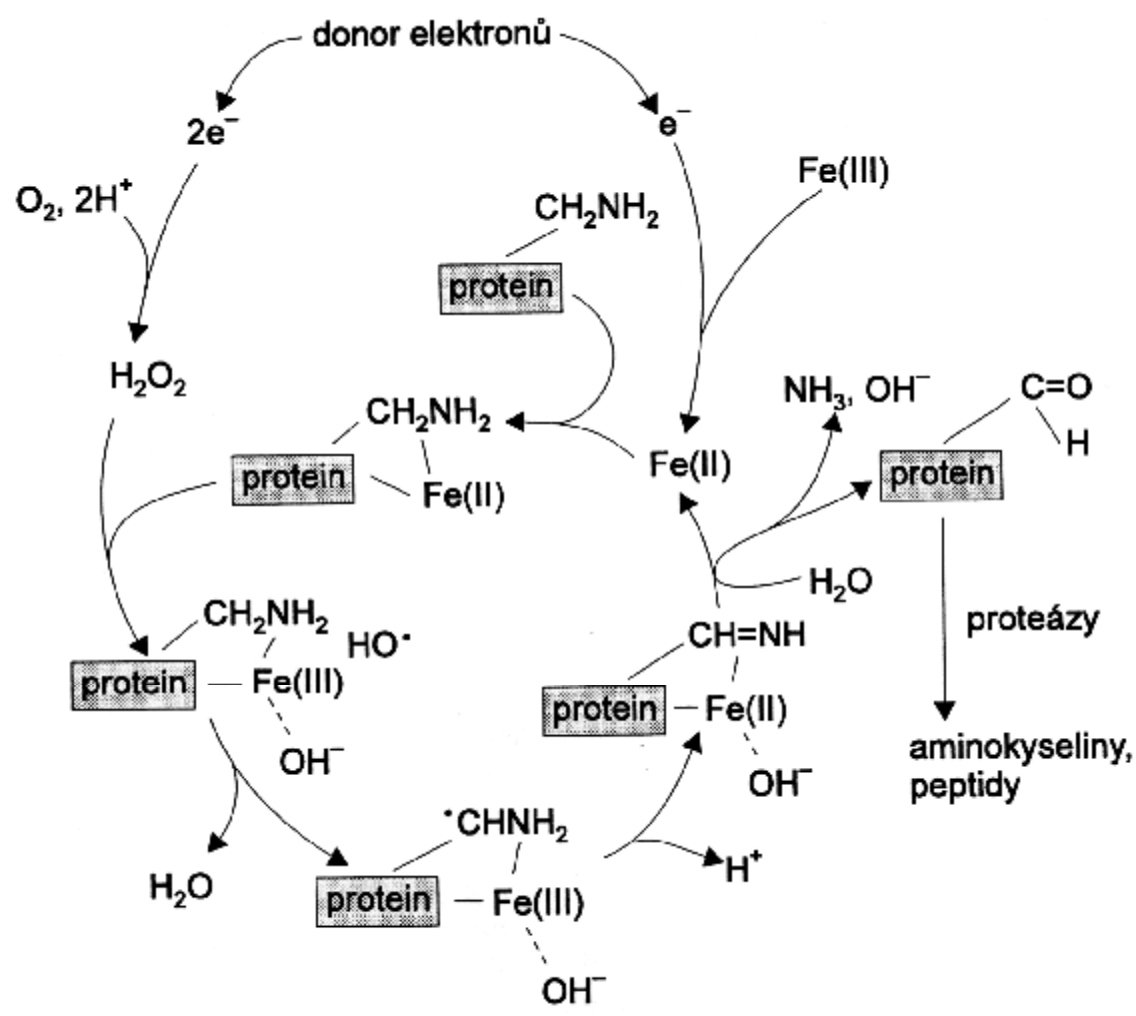


Fig. 1. Structures of methionine and its oxidation products.

Amino acid	Products
Arginine	Glutamic-semialdehyde
Lysine	2-Amino-adipic-semialdehyde
Proline	Glutamic-semialdehyde 2-pyrrolidone 4,5-hydroxyproline pyroglutamic acid
Cysteine	Cysteine disulfides, Sulfenic acid
Threonine	2-amino-3-keto butyric acid
Leucine	3,4,5-hydroxy leucine
Histidine	2-oxo-histidine

Lit.: Stadtmann E.R. et al. *Amino Acids* 25, 207-218 (2003); Vogt W. *Free Radic. Biol. Med.* 18(1), 93-105 (1995).

Poškozování struktury proteinů ROS a RNS – vytváření proteinových karbonylů, Amadoriho produkty

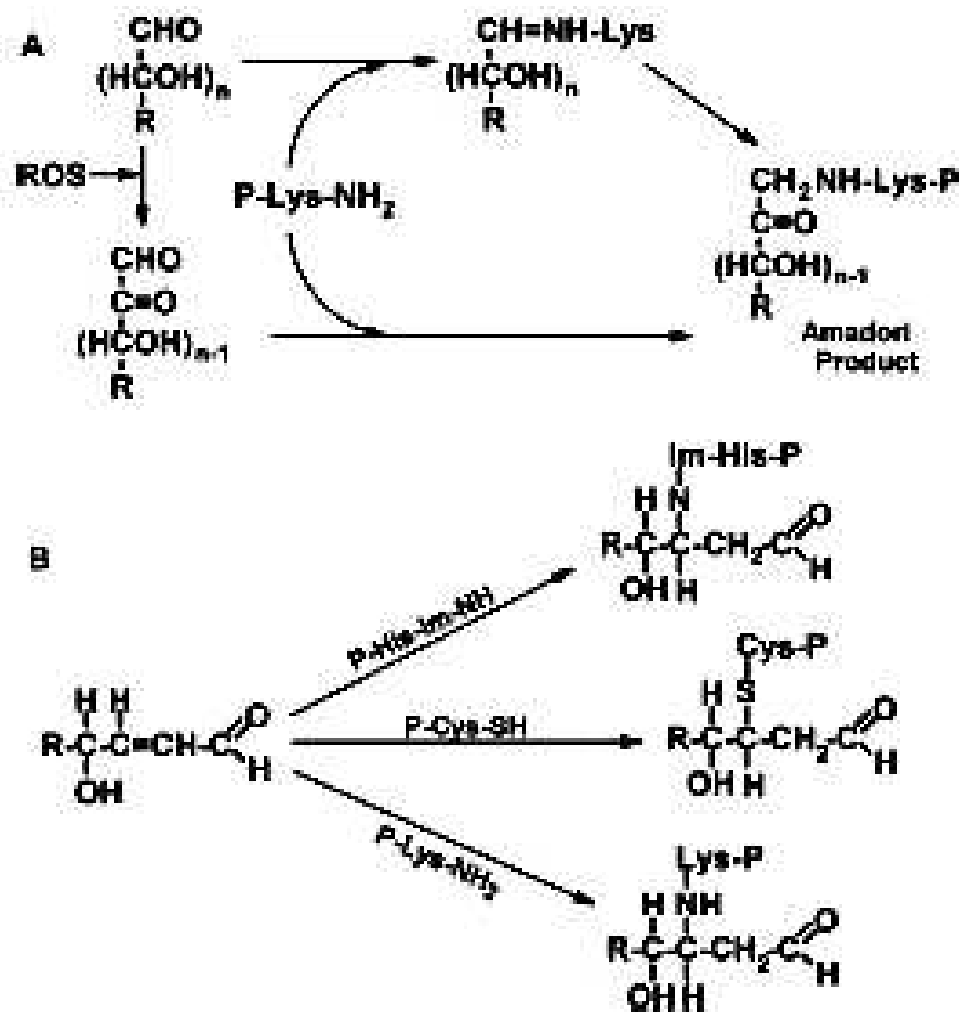


Fig. 4. Generation of carbonyl derivatives of proteins. **A** By glycation/ glycooxidation of lysine amino groups. **B** By reactions of α,β -unsaturated aldehydes with lysine, cysteine, or histidine residues of proteins

Poškozování struktury proteinů ROS a RNS – oxidace aromatických postranních řetězců

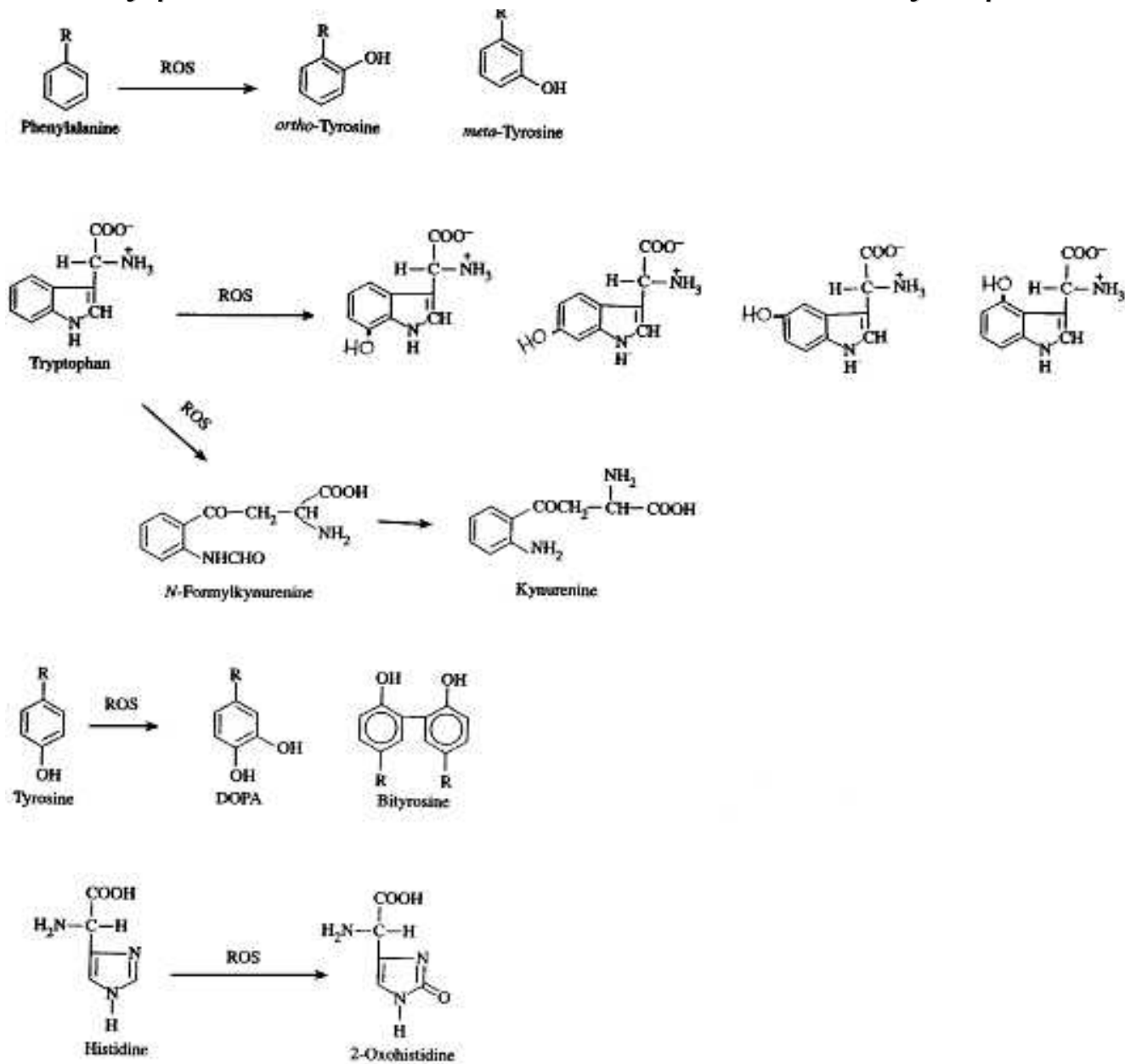


Fig. 5. Oxidation of aromatic amino acid residues

Poškozování struktury proteinů ROS a RNS – cross-links

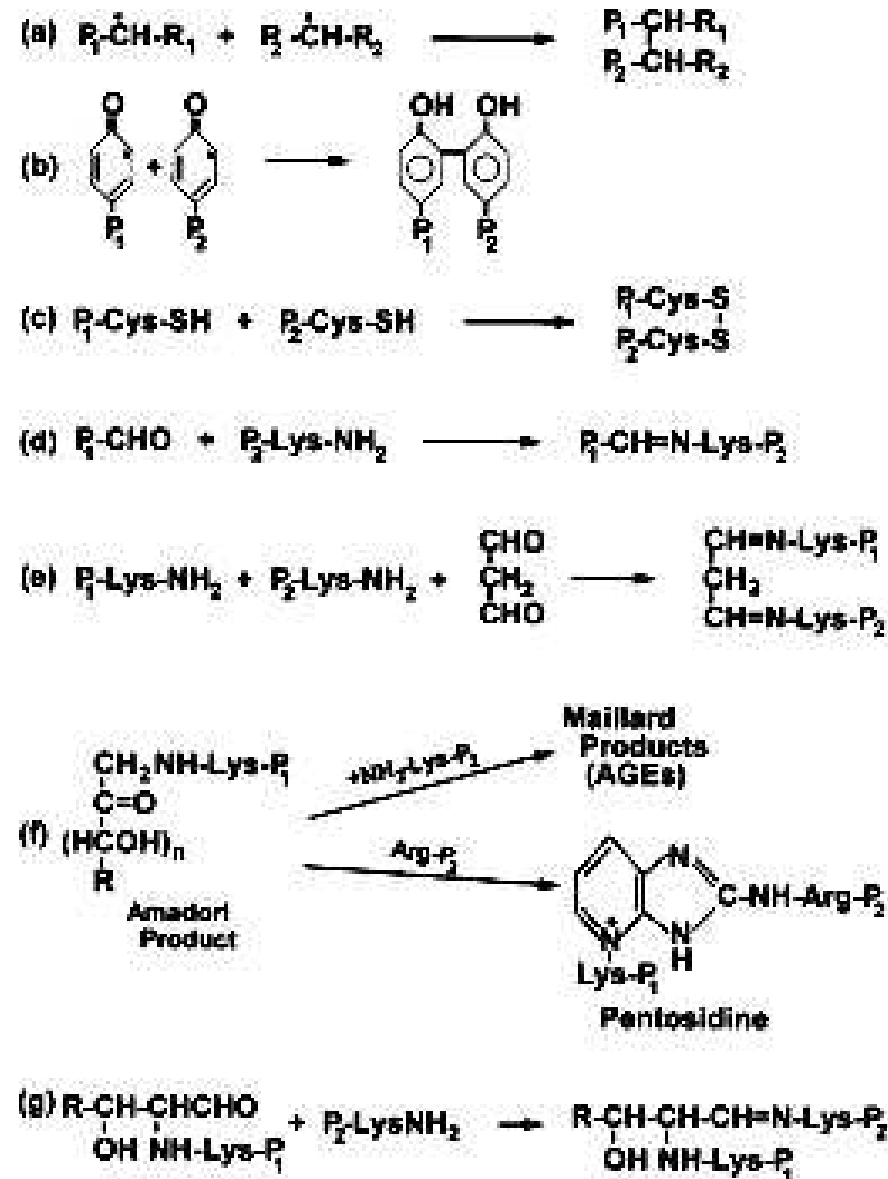
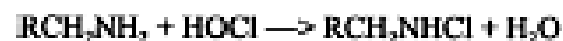
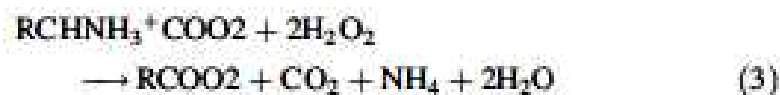
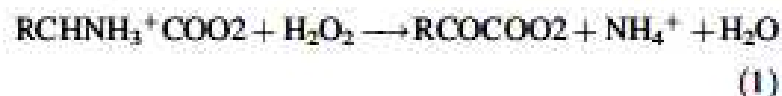


Fig. 6. Formation of protein-protein cross-linked derivatives

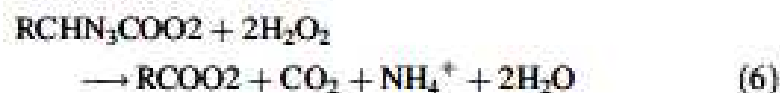
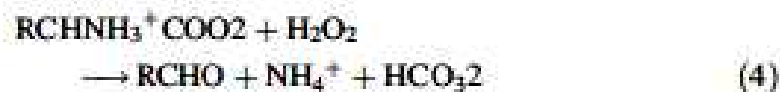
Poškozování struktury proteinů ROS a RNS – další reakce



Pathway A



Pathway B



In addition, some of the amino acid is converted an oxime (reaction 7):



Markery poškození proteinů

Product	Physiological levels	Pathological levels	Source
Dopa	85 pmol/mg of LDL protein (6/10000 tyrosines)	410 pmol/mg of protein (14/10000 tyrosines) in advanced human atherosclerotic plaques	Unpublished work
α - and m -tyrosine	62 and 35 pmol/mg of LDL protein (5 and 3/10000 phenylalanines) respectively	105 and 175 pmol/mg of protein (3.5 and 6/10000 phenylalanines) respectively in plaques	Unpublished work
	5/10000 phenylalanines in human lenses of any age	No increase in atherosclerotic aorta samples compared with normal	[310]
		Unchanged with age	[242]
<i>N</i> -Formylkynurenine; kynurenine	ND	ND	
Dityrosine	0.2 pmol/mg of LDL protein (0.02/10000 tyrosines)	150 pmol/mg of protein (5/10000 tyrosines) in plaques	Unpublished work
		10-fold elevated in aortic lesions in comparison with normal aortic samples; 0.03/10000 tyrosines in lens proteins from old people	[310]
	0.01/10000 tyrosines in human lens proteins from young people		[242]
Dimers of hydroxylated aromatic amino acids	ND	ND	
2-Oxohistidine	ND	ND	

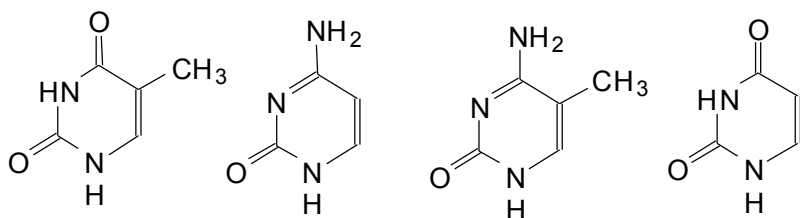
Markery poškození proteinů

Hydro(pero)xyleucine	β -Hydroxyleucine 2: 5 pmol/mg of LDL protein (0.1/10000 leucines)	β -Hydroxyleucine 2: 20 pmol/mg of protein (0.2/10000 leucines)	Unpublished work
Hydro(pero)xyvalines	β -Hydroxyvaline 1: 5 pmol/mg of LDL protein (0.1/10000 valines)	β -Hydroxyvaline 1: 10 pmol/mg of protein (0.1/10000 valines) in plaques	Unpublished work
3-Chlorotyrosine	Normal aorta: 0.8/10000 tyrosines	Atherosclerotic aorta: 4.2/10000 tyrosines	[310]
3-Nitrotyrosine	< 10 pmol/mg of LDL protein (< 1/10000 tyrosines)	< 10 pmol/mg of protein (< 0.3/10000 tyrosines) in plaques 100-fold elevated in aortic lesion LDL compared with normal plasma LDL	Unpublished work* [310]
<i>p</i> -Hydroxyphenylacetaldehyde	ND	ND	
Aminomalonic acid	0.04–0.3/1000 total amino acids in two <i>E. coli</i> strains	0.2/1000 glycines in post-mortem human plaque	[311]
5-Hydroxy-2-aminovaleric acid	0.15 nmol/mg of protein in 100000 g supernatants of young mouse livers	Unchanged in old mouse livers; elevated by hyperoxic exposure	[312]
Protein carbonyls	~ 1 nmol/mg of protein in many physiological tissue samples	≤ 8 nmol/mg of protein in diseased brain samples	[190,191]

* Below UV-HPLC detection limit.

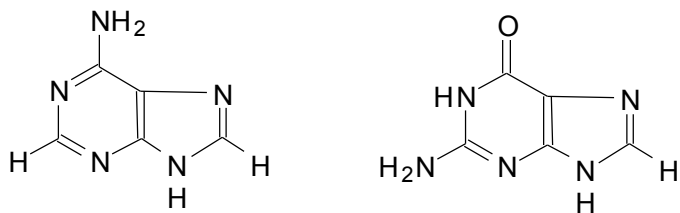
Poškozování struktury DNA – součásti DNA a RNA

Pyrimidines:



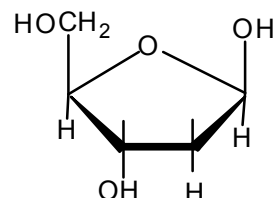
thymine cytosine 5-methylcytosine uracil

Purines

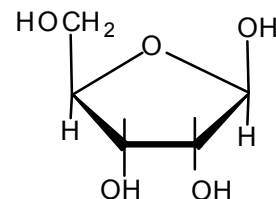


adenine guanine

báze

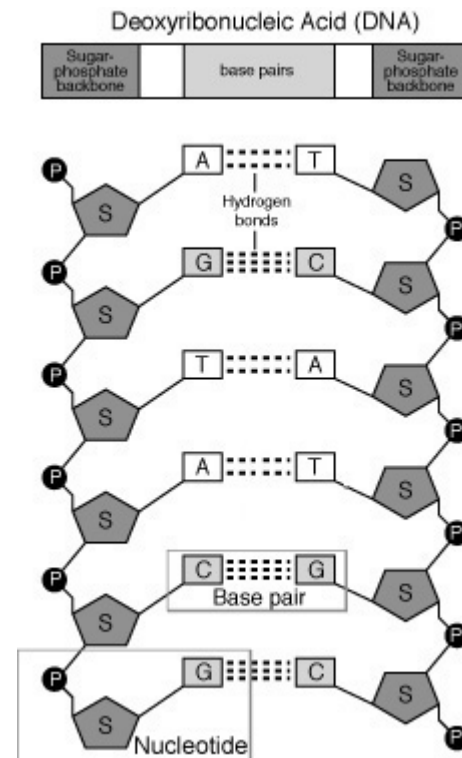


β-D-2-Deoxyribose



β-D-Ribose

cukry



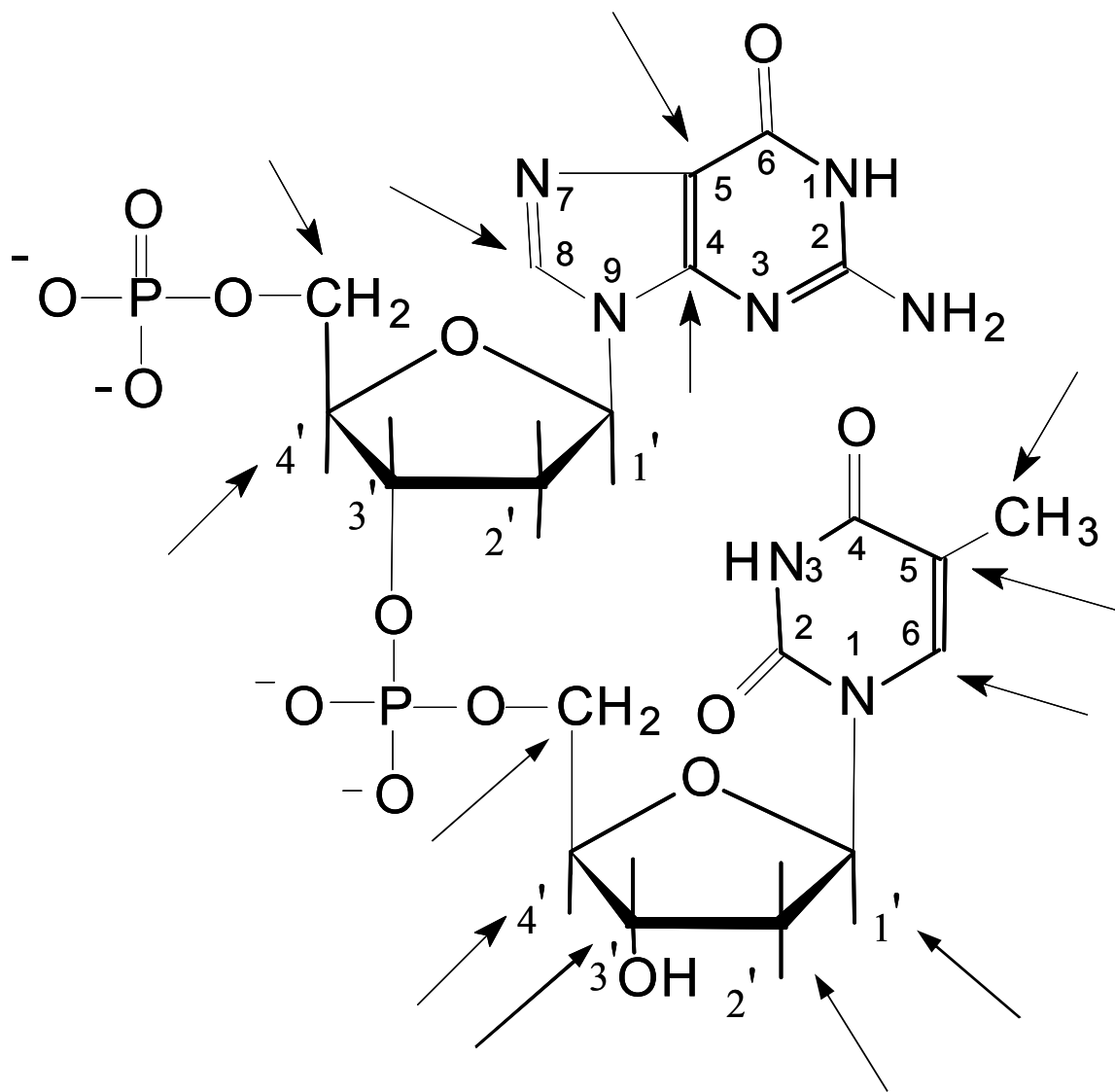
fosfátový skelet

DNA-Structure adapted from:

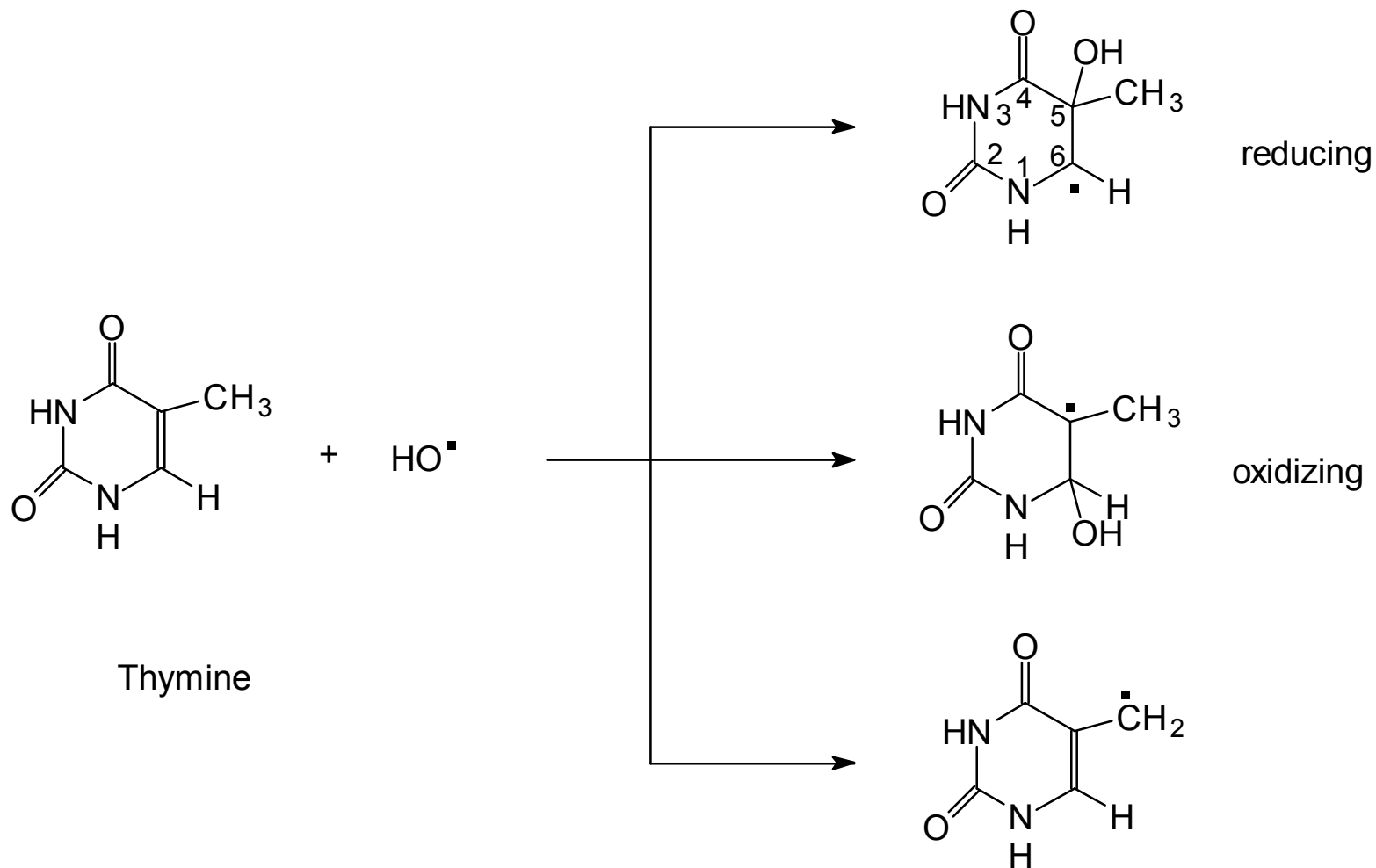
National Human Genome Research Institute (NHGRI); by artist Darryl Leja

<http://www.nhgri.nih.gov/DIR/VIP/>

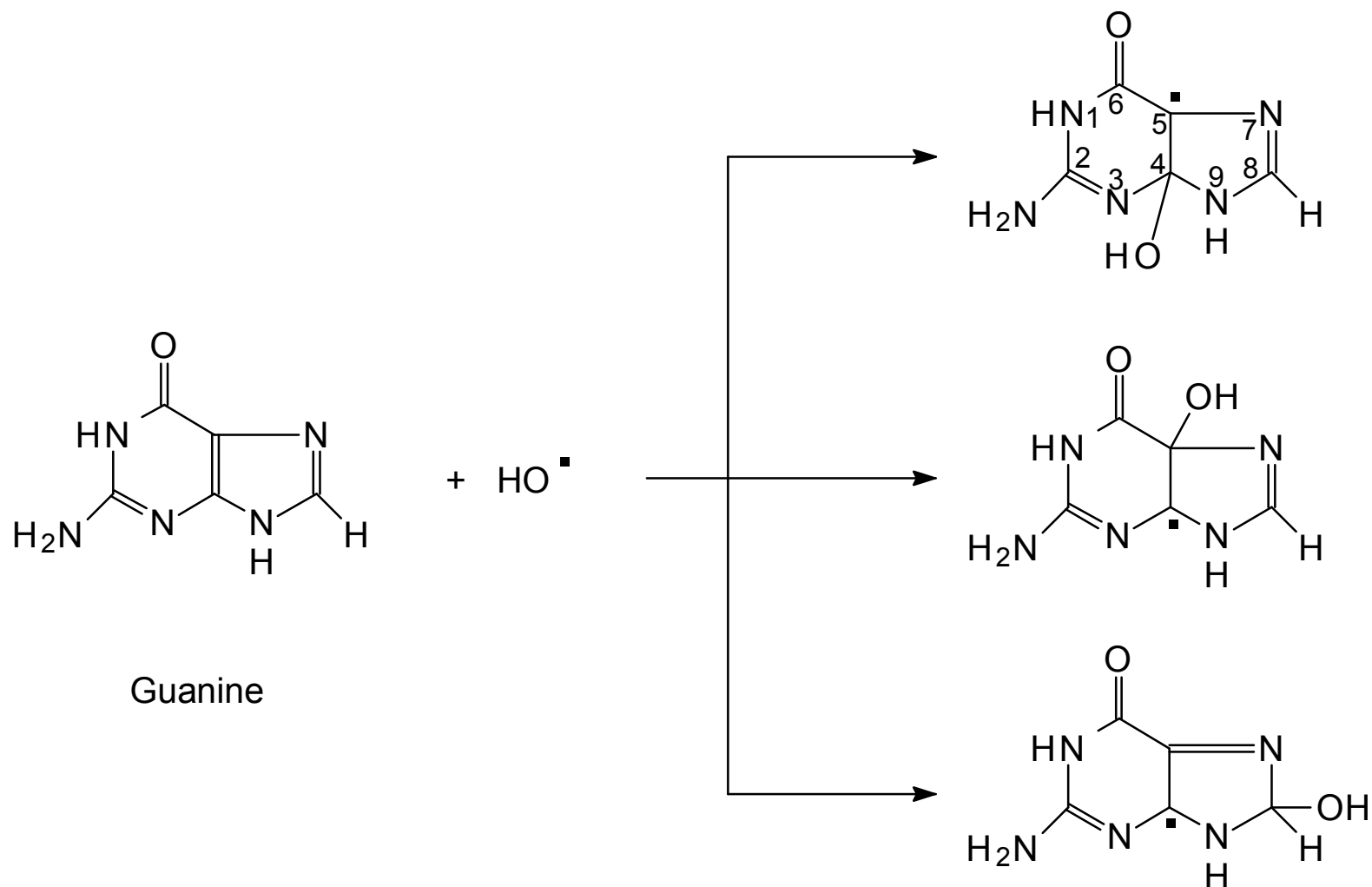
Poškození struktury DNA – obvyklá místa ataku volných radikálů



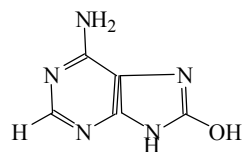
Poškozování struktury DNA – reakce hydroxylového radikálu s pyrimidiny



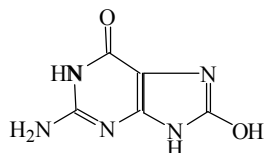
Poškozování struktury DNA – reakce hydroxylového radikálu s puríny



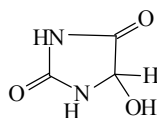
Poškozování struktury DNA – příklady produktů oxidace bazí



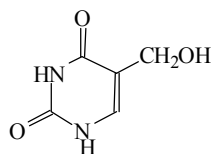
8-hydroxyadenine



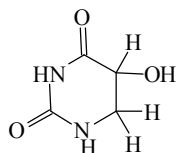
8-hydroxyguanine



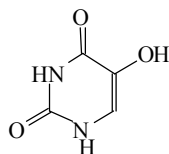
5-hydroxy
hydantoin



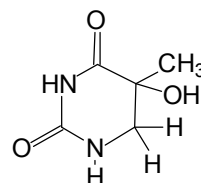
5-hydroxymethyluracil



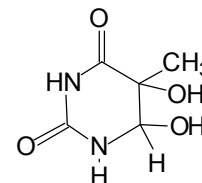
5-hydroxy-6-hydrouracil



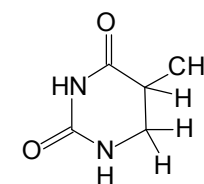
5-hydroxyuracil



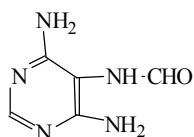
5-hydroxy-6-
hydrothymine



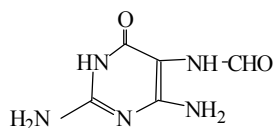
Thymine glycol
(cis and trans)



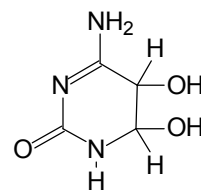
5,6-dihydrothymine



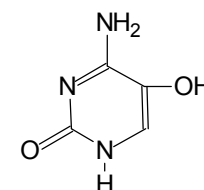
4,6-Diamino-5-
formamidopyrimidine



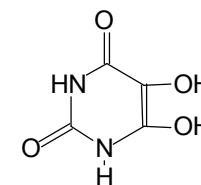
2,6-Diamino-4-hydroxy-
formamidopyrimidine



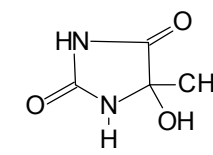
Cytosine glycol



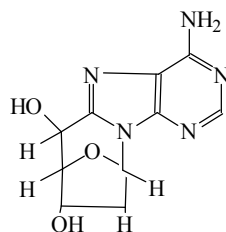
5-hydroxycytosine



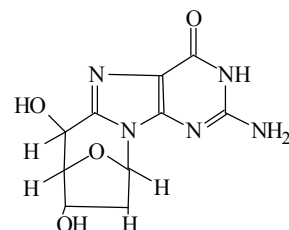
5,6-dihydroxy
uracil



5-hydroxy-5-
methylhydantoin



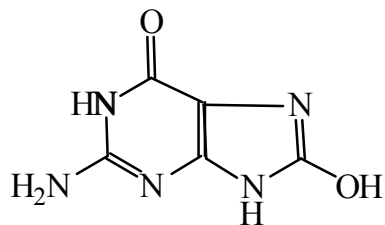
8,5'-cyclo-2'-deoxyadenosine



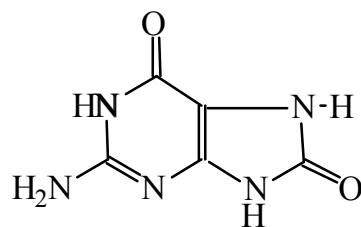
8,5'-cyclo-2'-deoxyguanosine

Poškozování struktury DNA – mutace a bodové změny párování bází

8-hydroxyguanine



8 OH-Gua (enol)



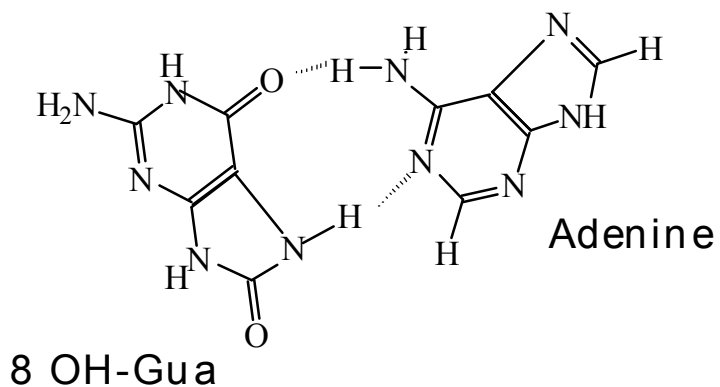
8 oxo-Gua (keto)

Basepairs

A-T

G-C

8 OHdG-A

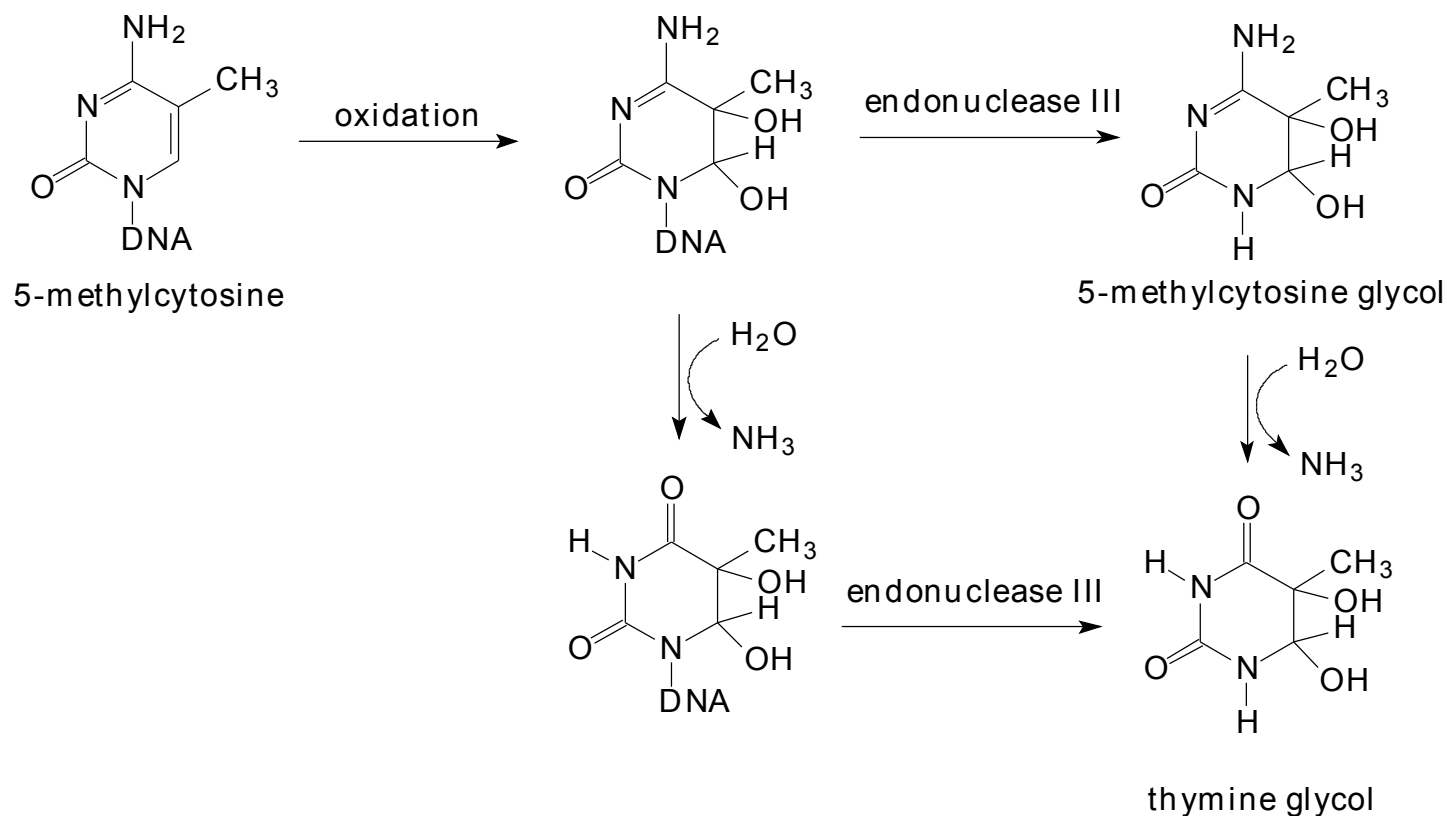


8 OH-Gua

Adenine

**Produkt oxidace guanínu
8-oxodeoxyguanozin vede
k mutacím v párování bází
(GC→AT transversion).**

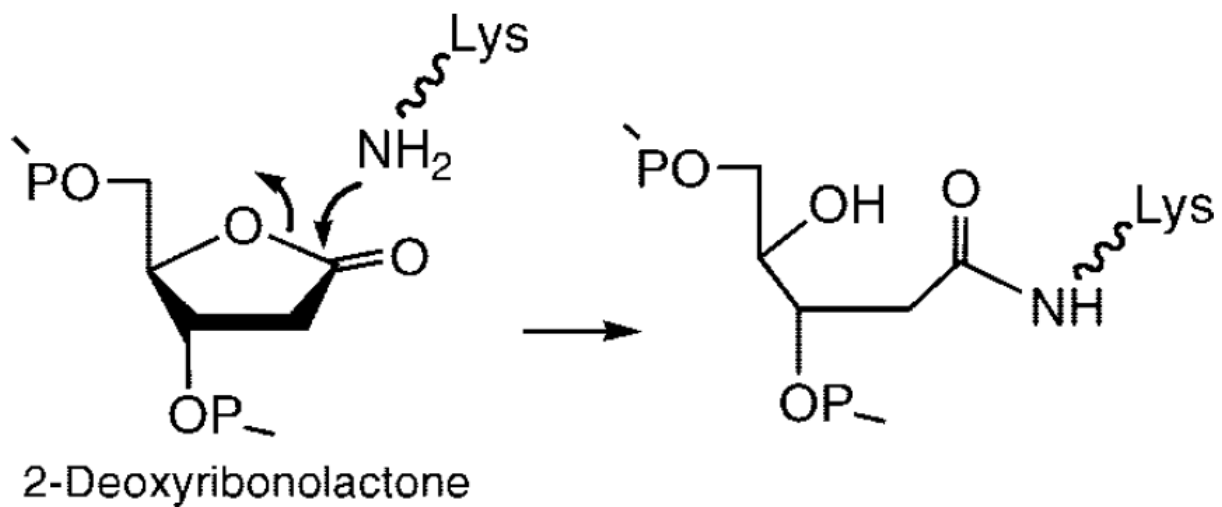
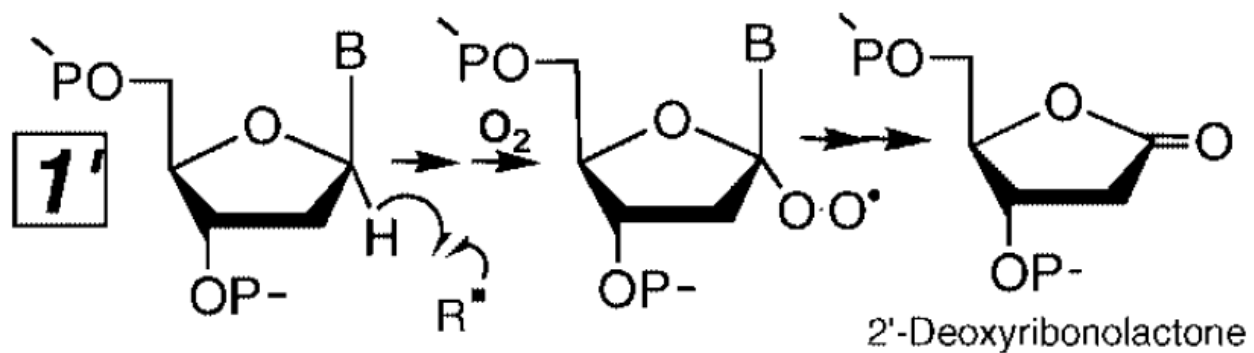
Poškození struktury DNA – mutace a bodové změny párování bází



- Oxidace cytosinu vede ke**
- a) ztrátě amino skupiny,**
 - b) mutaci (GC→AT transition).**

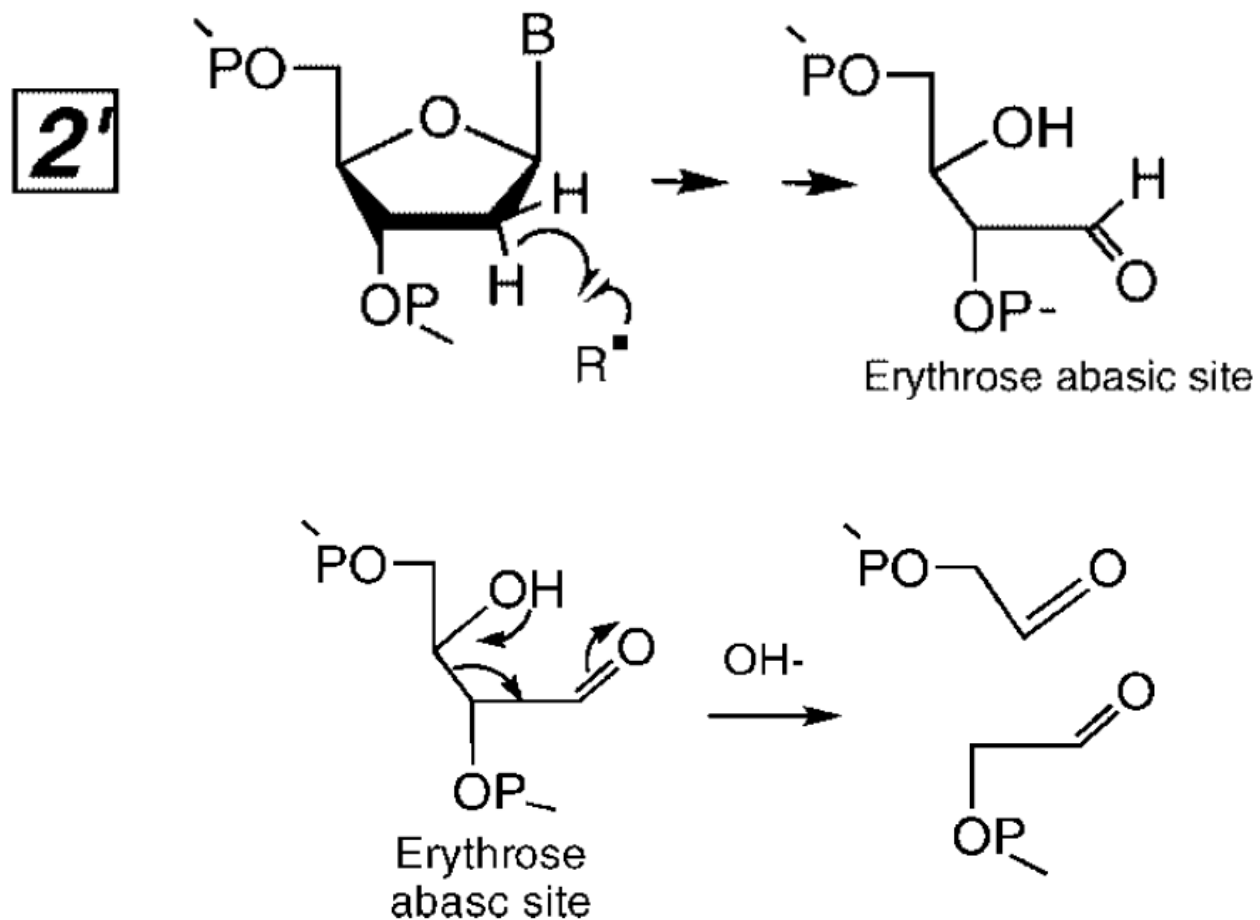
Poškozování struktury DNA – cukerné složky a bází

oxidační poškození deoxyribosy v pozici 1'



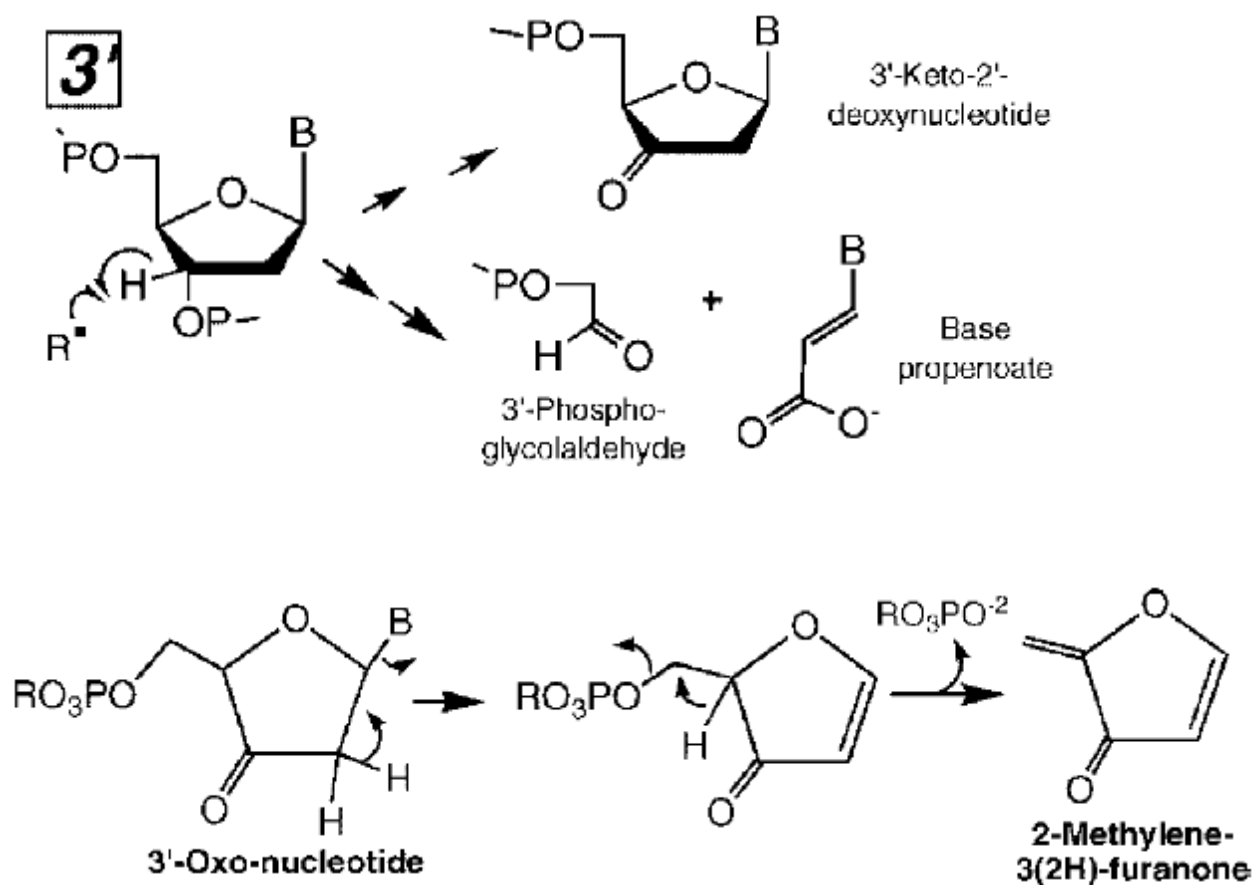
Poškozování struktury DNA – cukerné složky a bází

oxidační poškození deoxyribosy v pozici 2'



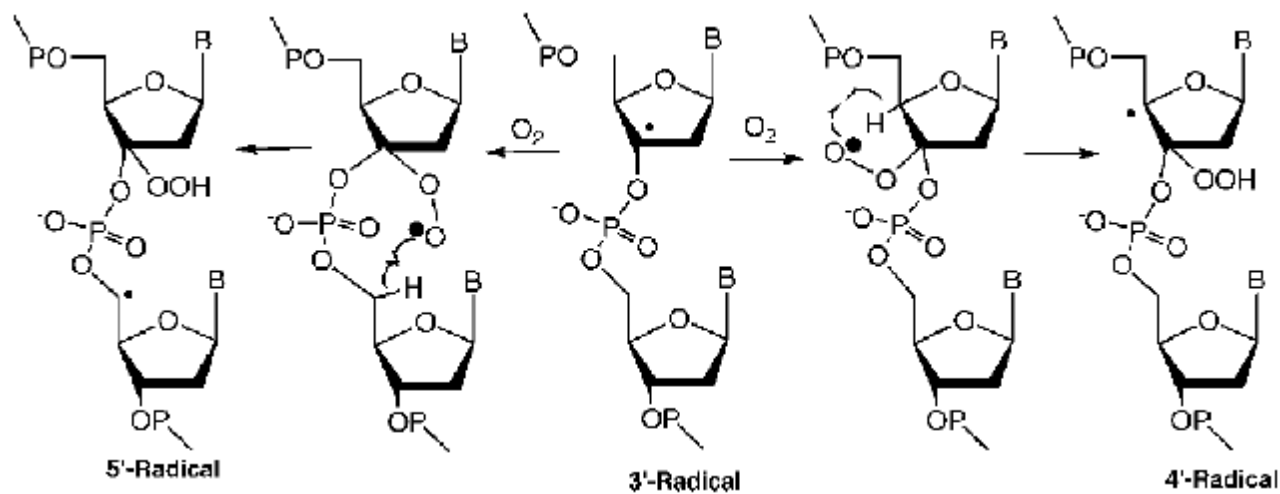
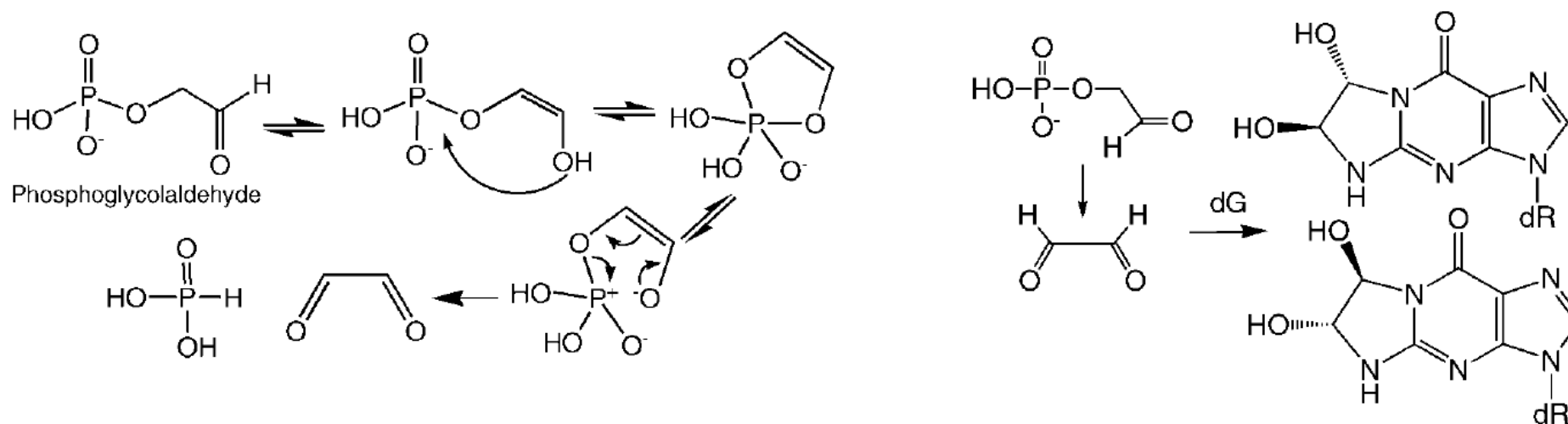
Poškozování struktury DNA – cukerné složky a bází

oxidační poškození deoxyribosy v pozici 3'



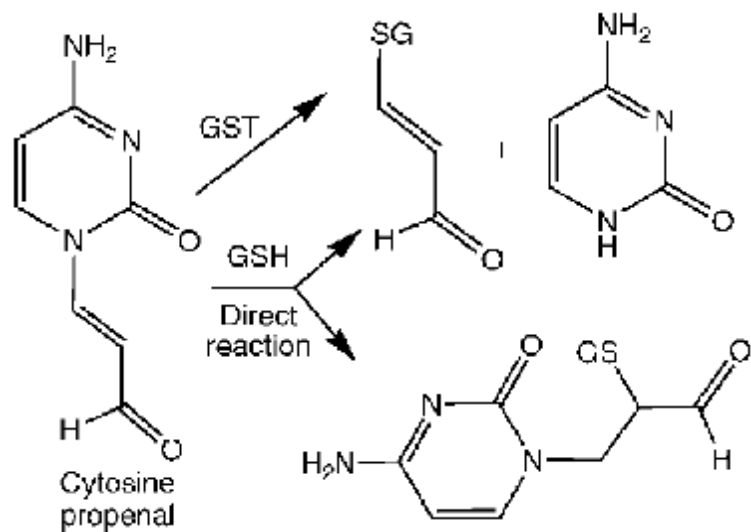
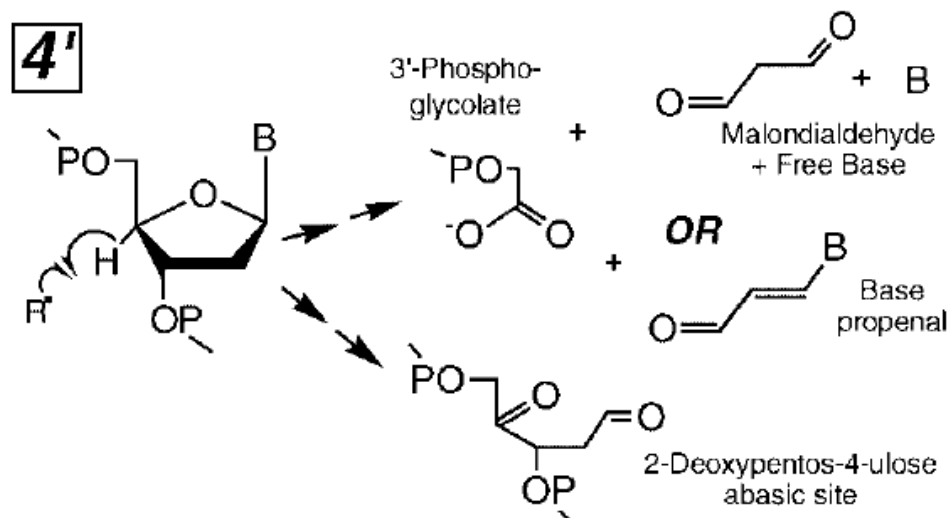
Poškozování struktury DNA – cukerné složky a bází

konsekvence oxidačního poškození deoxyribosy v pozici 3'



Poškozování struktury DNA – cukerné složky a bází

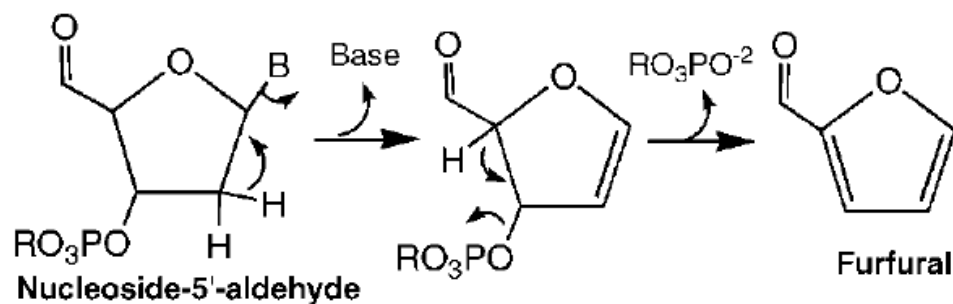
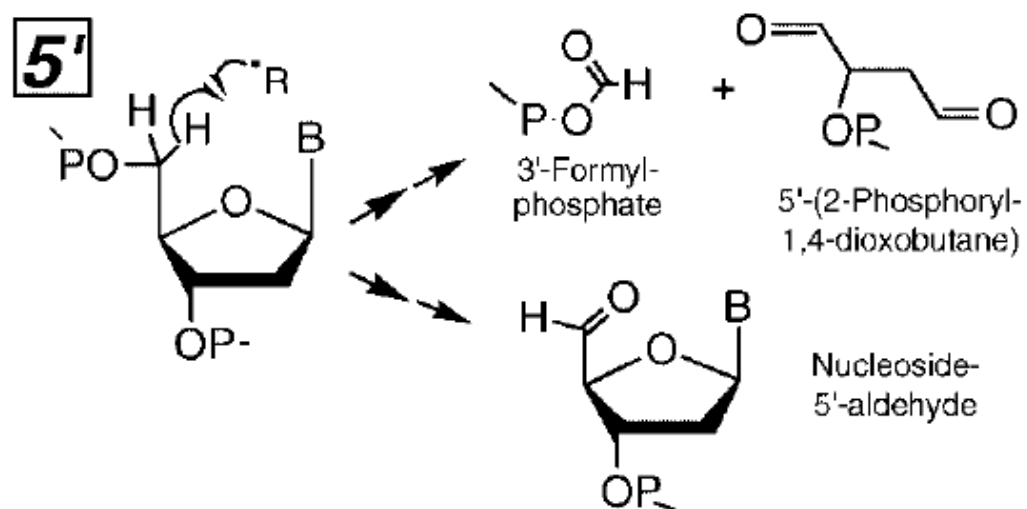
oxidační poškození deoxyribosy v pozici 4'



Metabolická modifikace cytosin-propenalu reakcí s GSH

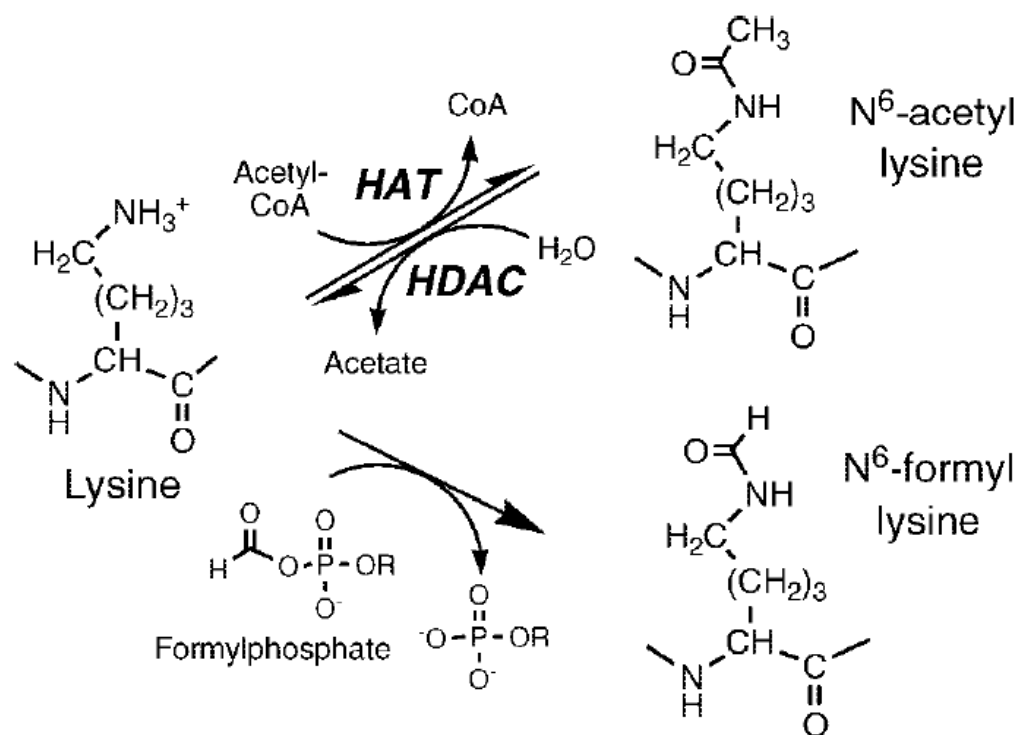
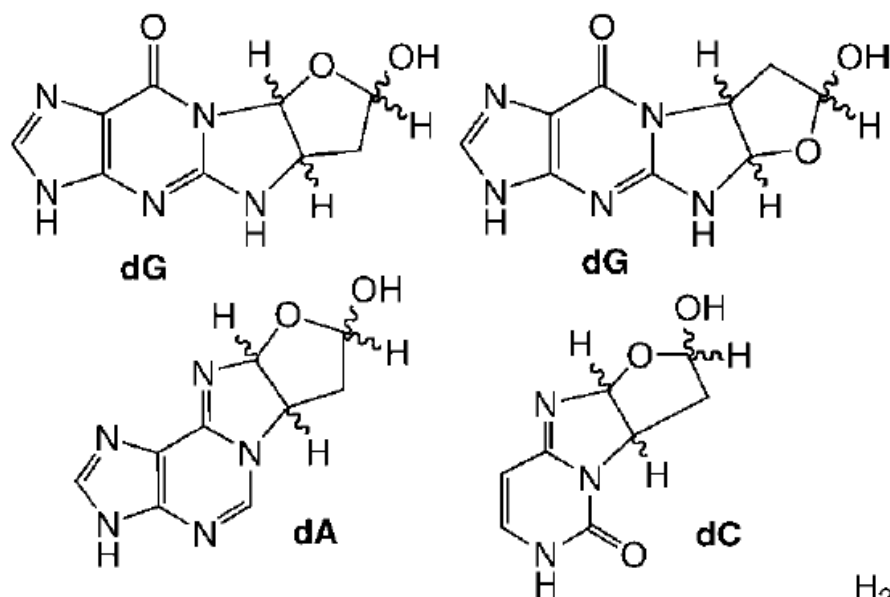
Poškozování struktury DNA – cukerné složky a bází

oxidační poškození deoxyribosy v pozici 5'

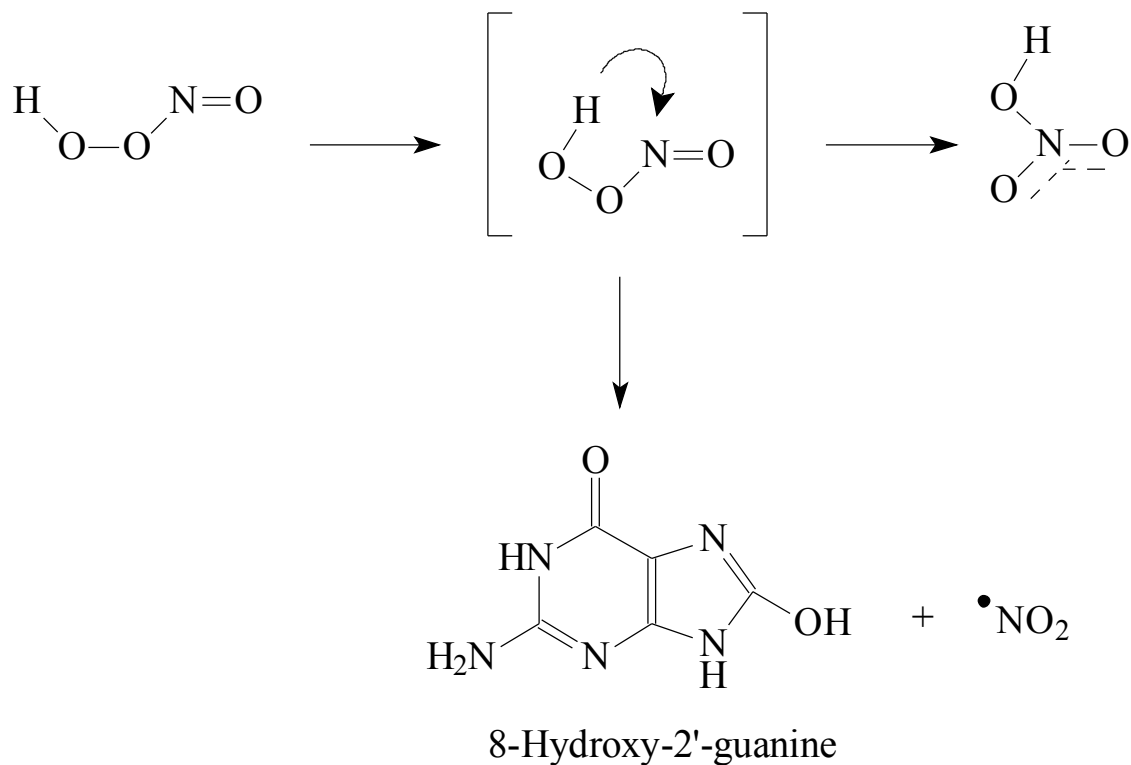
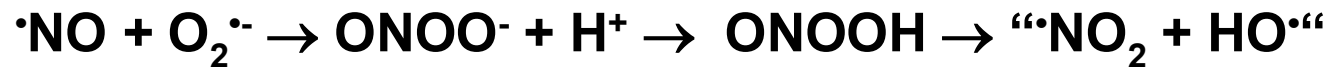


Poškozování struktury DNA – cukerné složky a bází

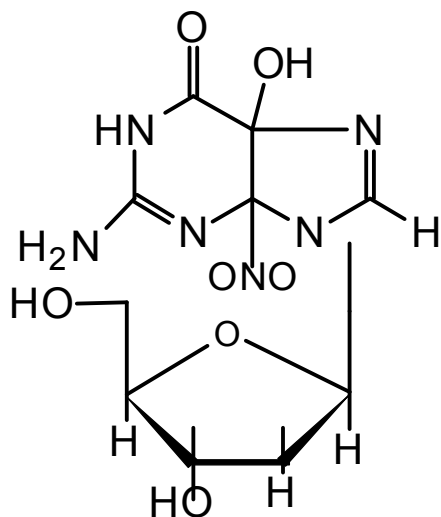
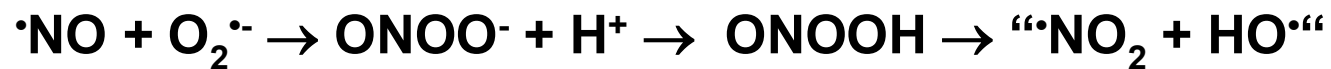
konsekvence oxidačního poškození deoxyribosy v pozici 5'



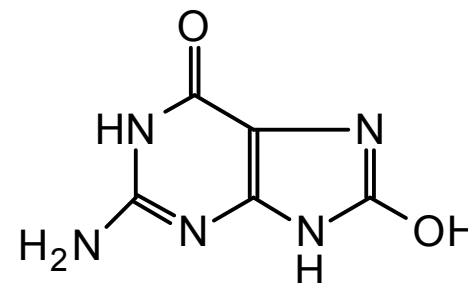
Poškozování struktury DNA – oxidace RNS



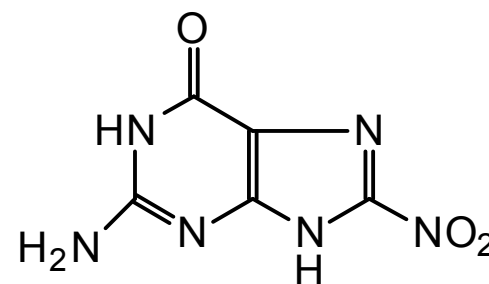
Poškození struktury DNA – oxidace a nitrace RNS



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



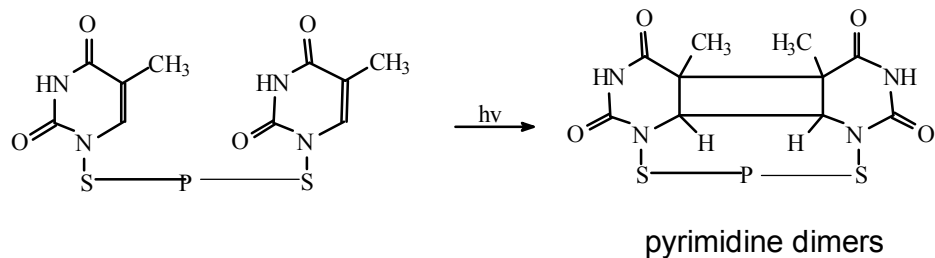
8-hydroxyguanine



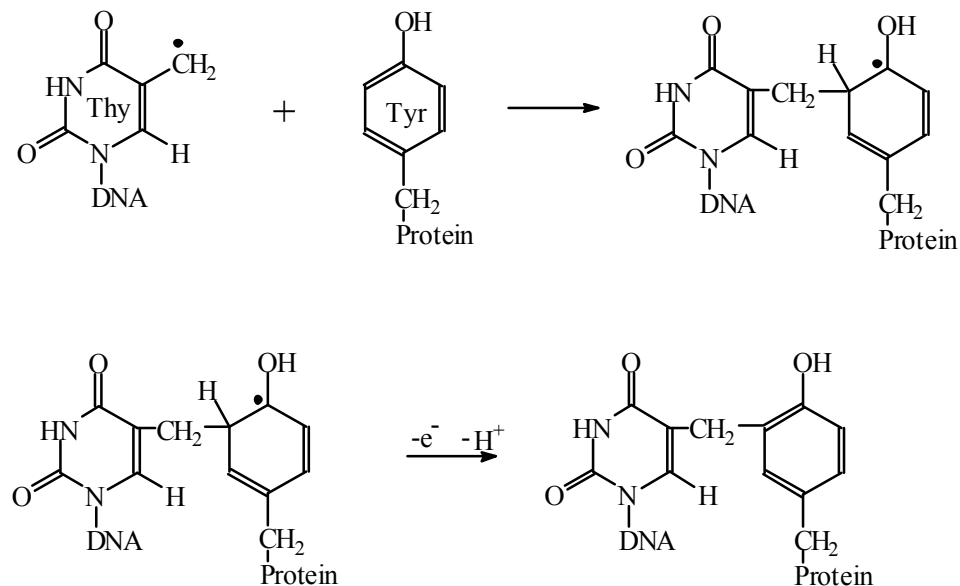
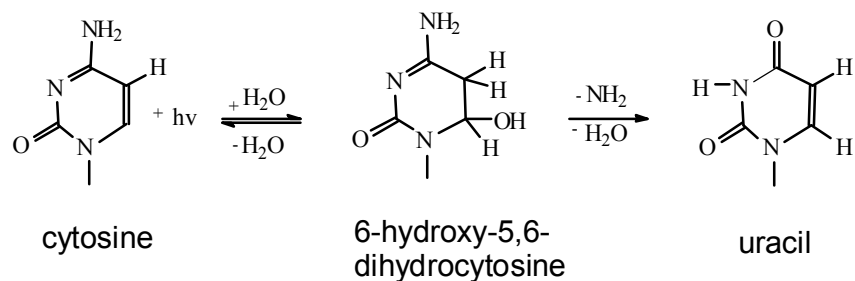
8-nitroguanine

Poškození struktury DNA – UV záření, vytváření dimerů a cross-links

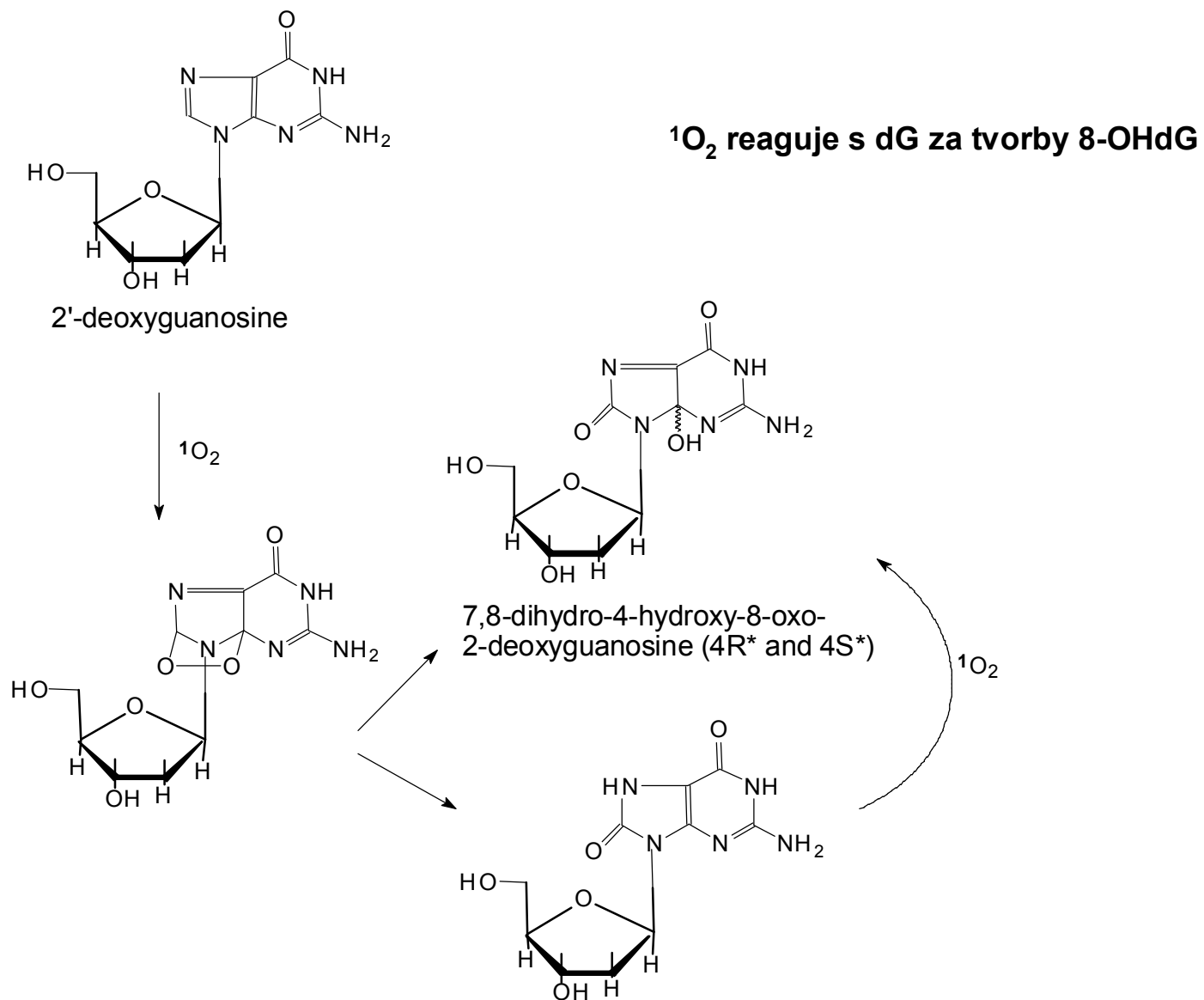
dimers:



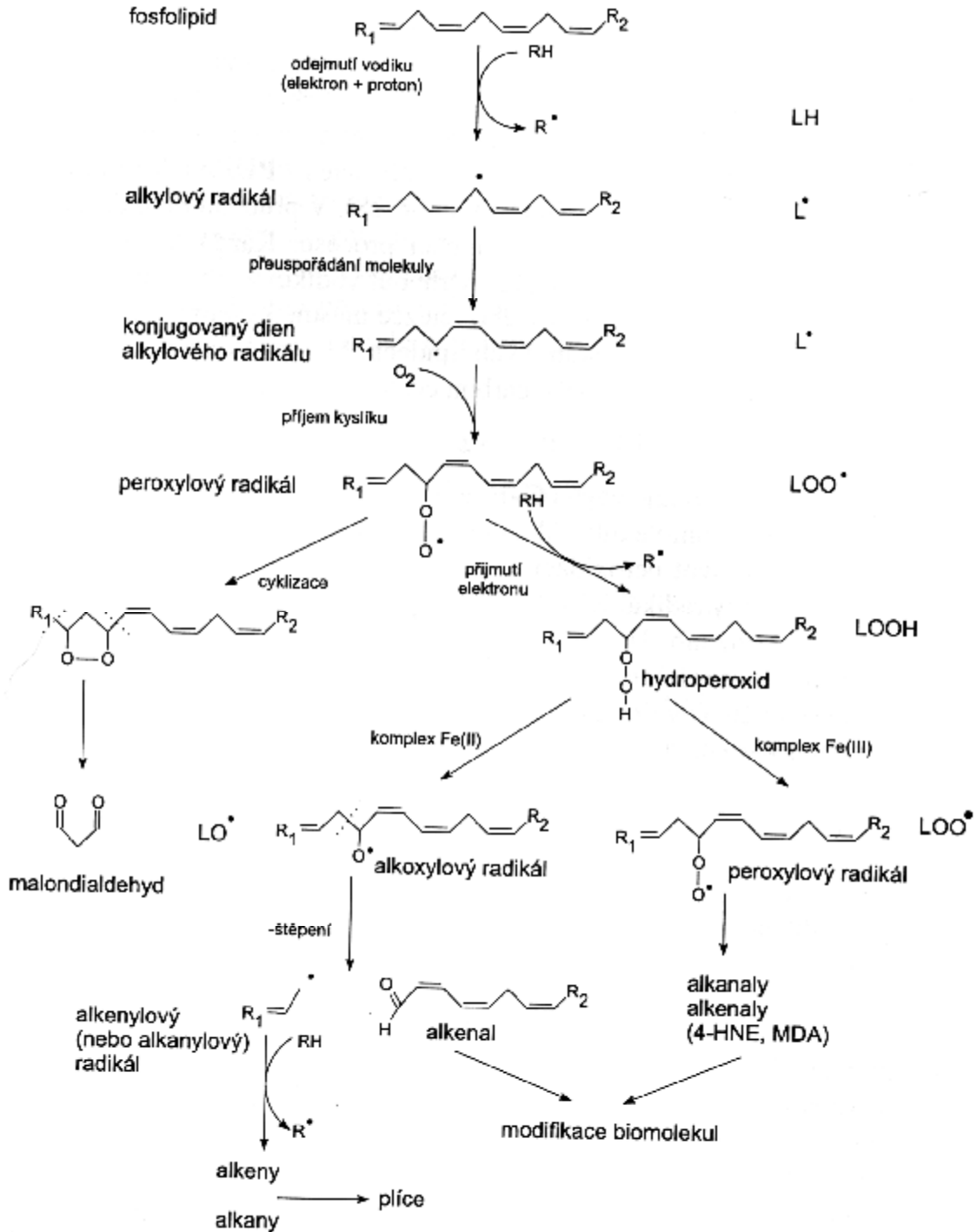
hydrates:



Poškozování struktury DNA – singletový kyslík, oxidace dG



Poškozování struktury lipidů



Lokalizace enzymů způsobujících peroxidaci lipidů ve svalu

LOX: lipoxygenasa

PGHS: prostaglandin H syntasa

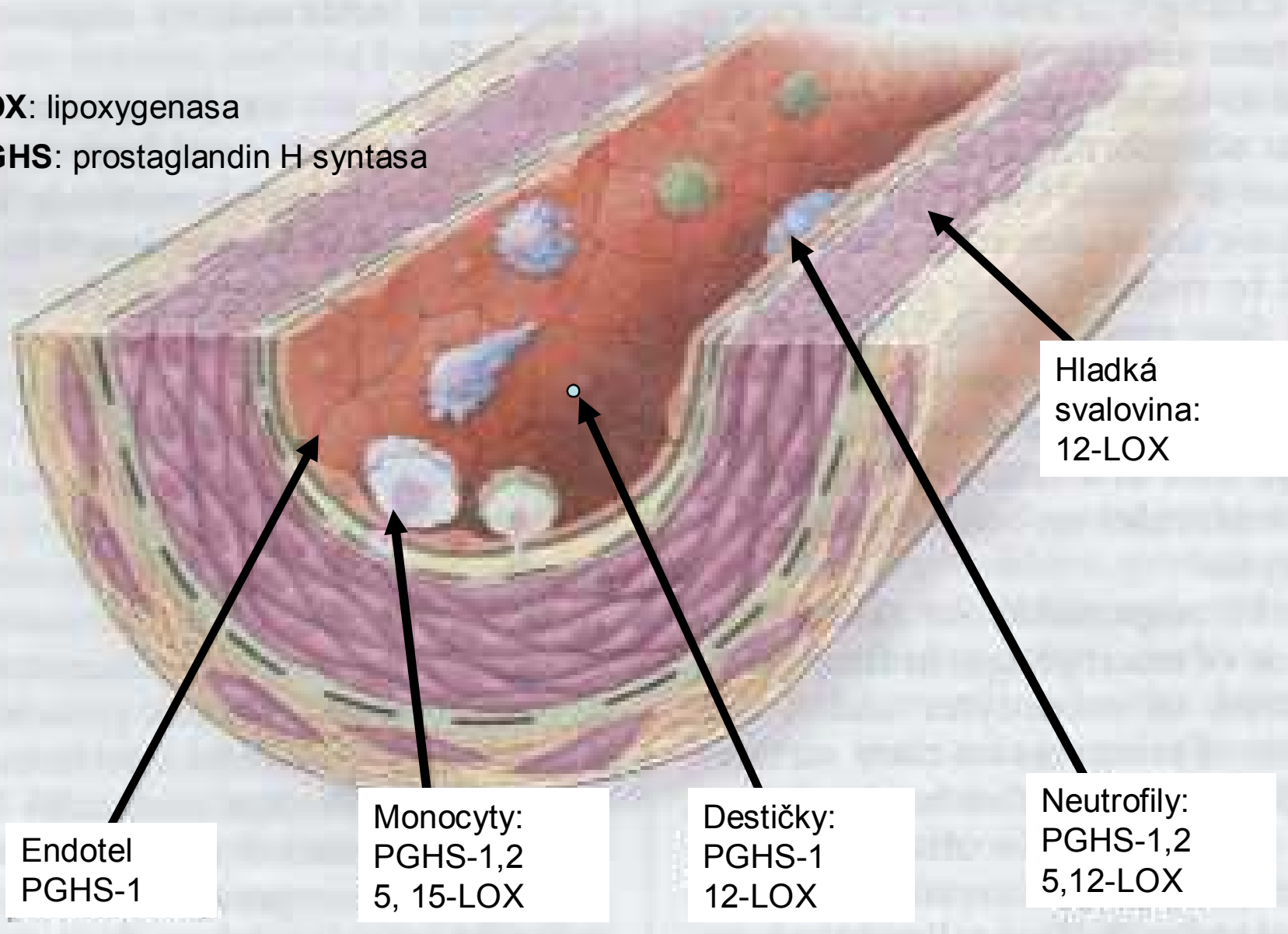


Schéma oxidace lipidů lipoxygenasami

