12B. Biological oxidations. Effects of free radicals to organisms. Lipoperoxidactions,antioxidants.

Oxidative stress

- **Oxidative stress appears during large accumulation of arising reactive forms of *oxygen/nitrogen*, when the organism is unable to dispose of them.**
- **Oxidative stress harms the cells (especially cell membranes), proteins, enzymes, genetic material and contributes to development of infectious and degenerative diseases.**
- **Is supposed to participate in development of atherosclerosis, diabetes, tumour diseases, degenerative nervous diseases, aging, …**
- **Doesn´t have only undesirable effect – under the supervision of white blood cells, it serves for killing of bacteria, parasites, viruses, tumour cells…**

TEST

Free radicals

Any molecule/atom capable of independant existence with 1/more unpaired electrons

Atom: proton, neutron, electron shell (orbital)

Radical: contains free unpaired electron in outer orbital (it can be atom or molecule, neutral or ion)

-homolytic cleavage of covalent bond (energetically difficult, not often in biolog. systems)

- by reduction, oxidation

- majority of biomolecules are not radicals

Radical reactions

Radical: effort for pairing of electrons, mostly significant reactivity

Generally three stages

- initiation
- propagation
- termination

Reactive forms of oxygen and nitrogen (ROS, RNS)

- Overall term for free radicals and some non-radical compounds (RONS)
- Significant physiological functions in organism
- Toxical under certain conditions
- Transformations catalysed by ionts of transition metals
- **Fenton´s reaction**: H2oO2 + Fe2+ …. HO. + HO- + Fe3+
- Regeneration Fe2+: O2-. …
- Haber-Weiss reaction
- Transition metals: first row of d-elements has unpaired electrons which can be considered as free radicals, except Zn
- The most significant: **Fe, Cu, Mn and Zn**
- **In organism bound in depot forms, inactive, transferin, feritin, ceruloplasmin**

TEST

ROS (reactive oxygen species)

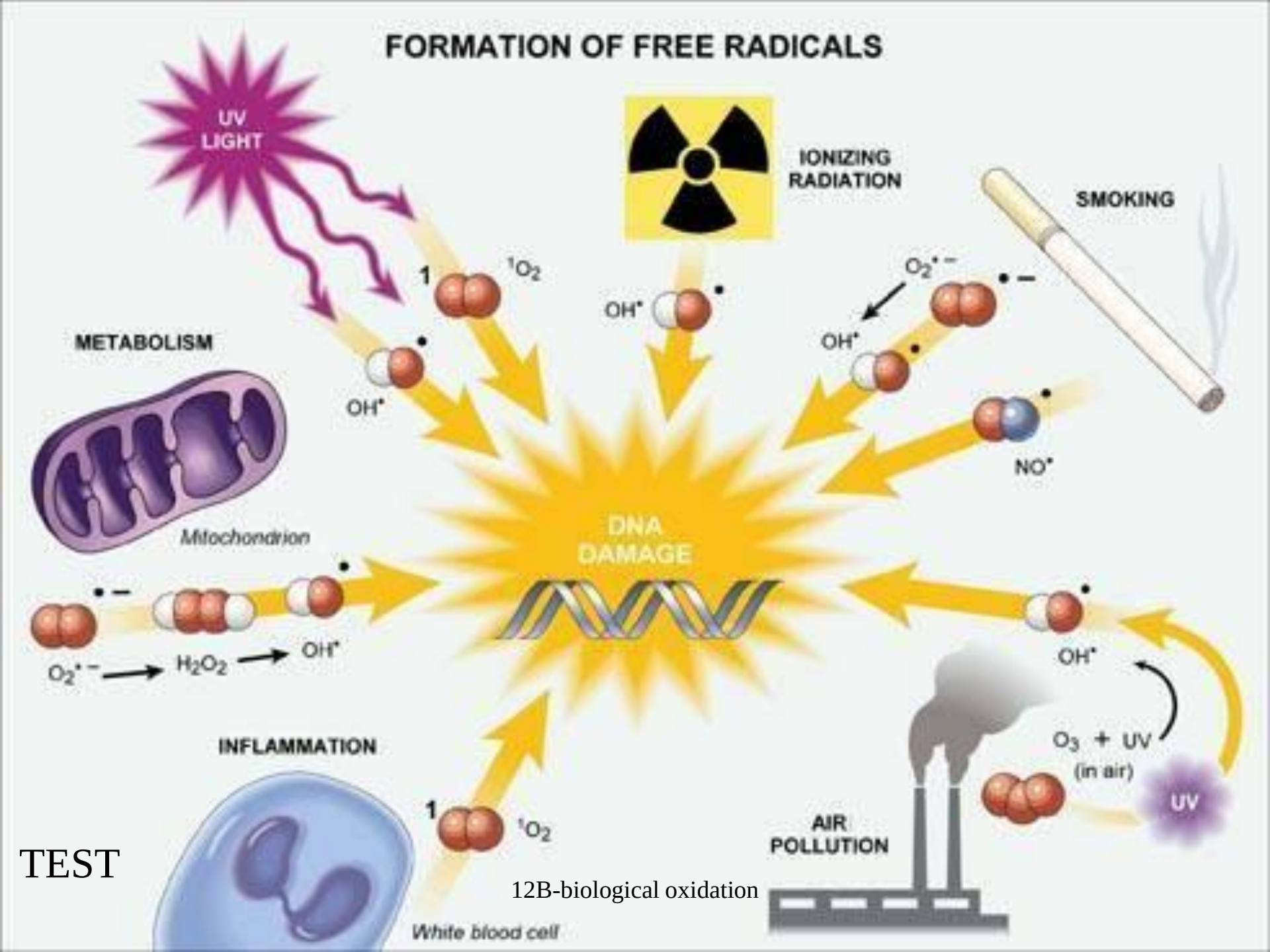
free radicals	are not free radicals
superoxid, $O_2^{\cdot -}$	hydrogen peroxide, H_2O_2 (Fenton´s reaction)
hydroxyl radical, $OH^{\cdot}$	hypochlorus acid, HClO
peroxyl, $ROO^{\cdot}$	ozone, O_3
alkoxyl, $RO^{\cdot}$	singlet oxygen, $^1O_2$
hydroperoxyl, $HO_2^{\cdot}$	

TEST

RNS (reactive nitrogen species)

free radicals	are not free radicals
nitric oxide, $NO\cdot$	nitrosyl, NO^+
nitrogen dioxide, $NO_2\cdot$	nitrous acid, HONO
	dinitrogen oxide, N_2O_3
	dinitrogen tetroxide, N_2O_4
	peroxynitrite, $ONOO^-$
	alkylperoxinitrite, ROONO
	hypochlorous acid, HOCl
	hypochlorite ClO^-

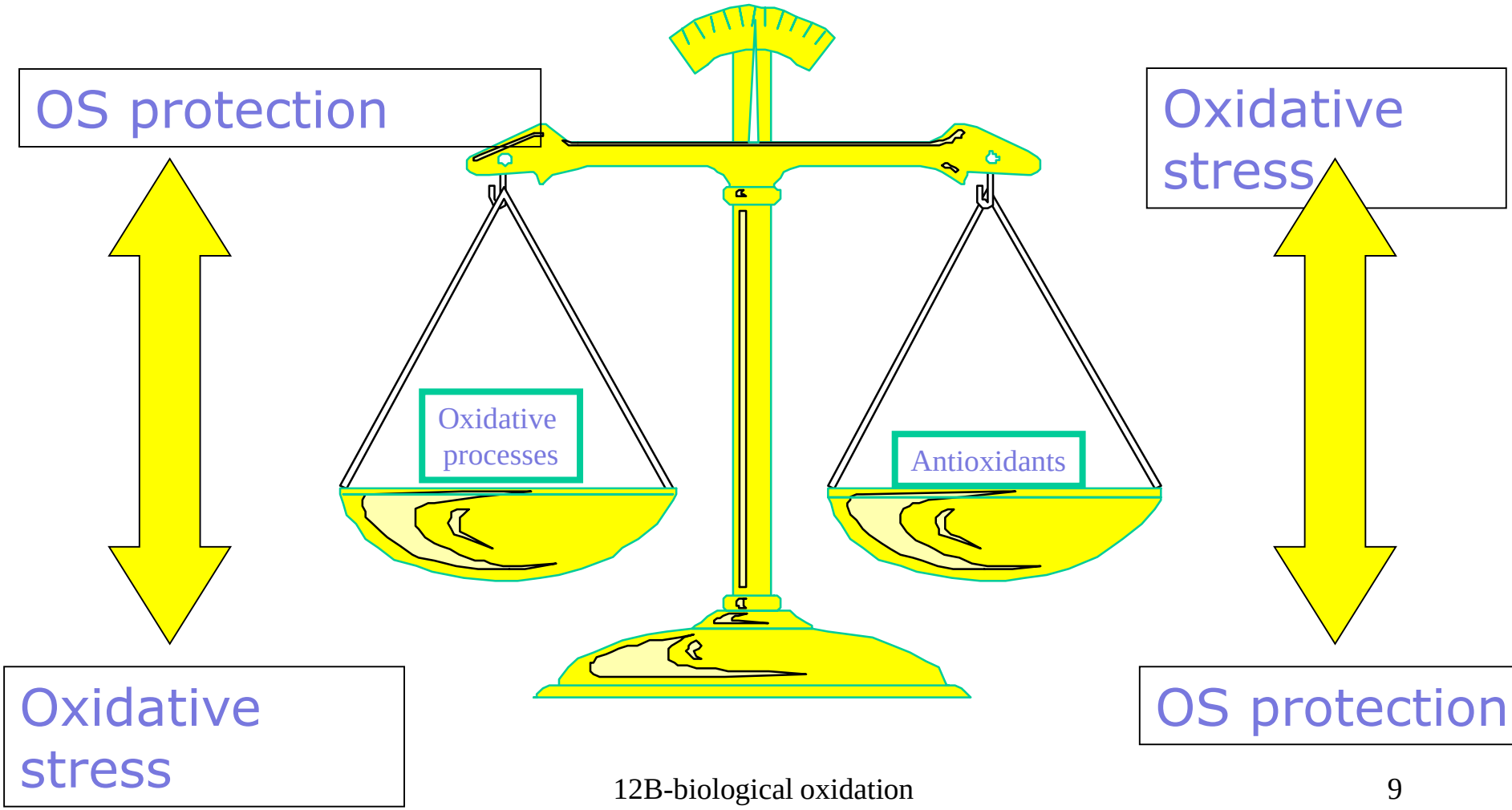
TEST


FORMATION OF FREE RADICALS
UV LIGHT
IONIZING RADIATION
SMOKING
METABOLISM
Mitochondrion
DNA DAMAGE
INFLAMMATION
White blood cell
AIR POLLUTION
$^1O_2$
OH•
$O_2^{\bullet -}$
NO•
H_2O_2
O_3 + UV
(in air)
UV

TEST

Oxidative stress

- antioxidative processes can´t manage the elimination of excessive free radicals



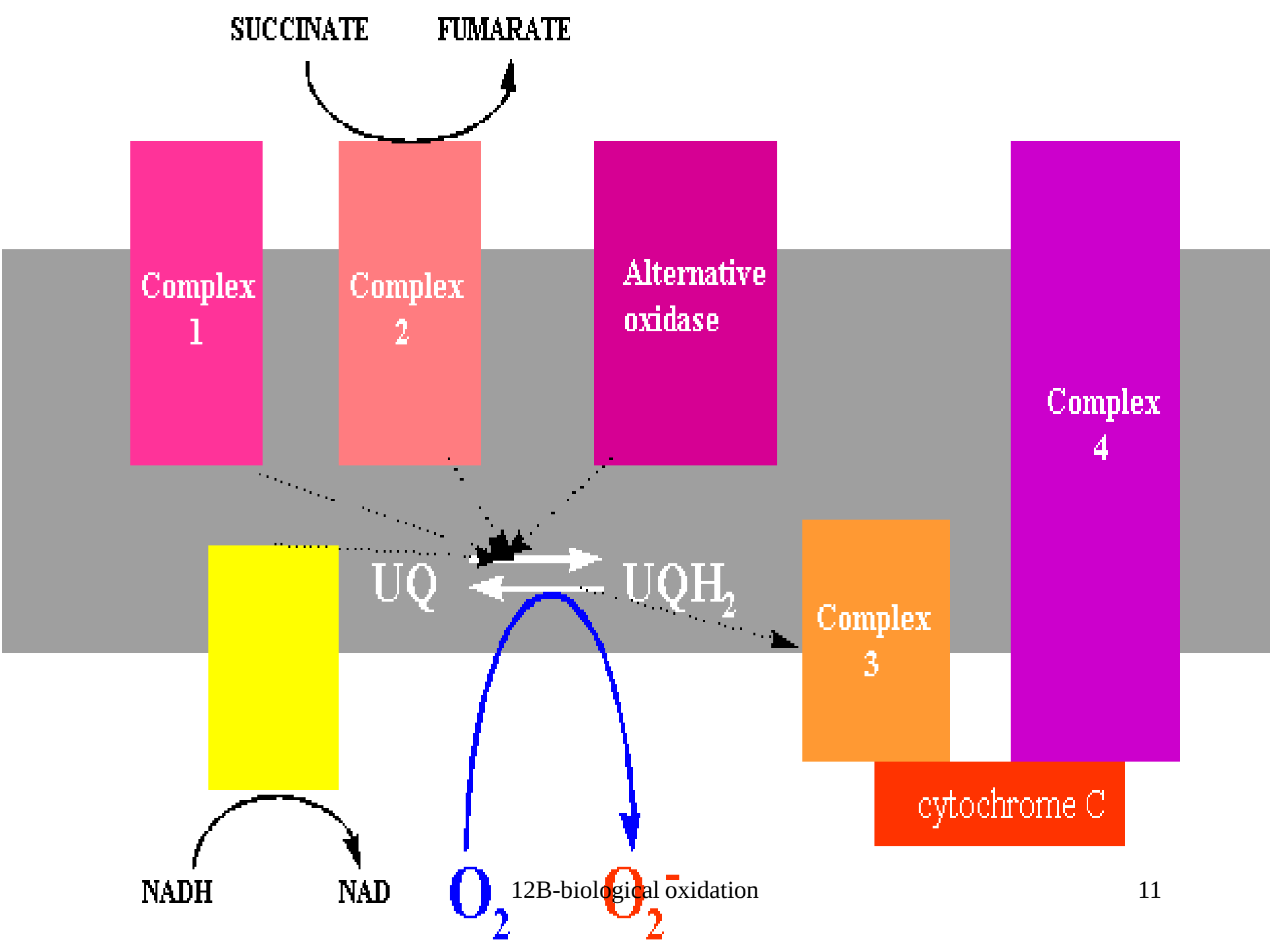
Where do free radicals come from?

Main ROS producers: membrane bound enzymes alternatively coenzymes with flavine structure, hem coenzymes, enzymes with Cu in active center

1. *Mitochondrial respiratory chain*: especially superoxide, subsequently H_2O_2

- about 1- 4% O_2 entering the respiratory chain (**especially complexes I and III**)

TEST


SUCCINATE
FUMARATE
Complex 1
Complex 2
Alternative oxidase
Complex 4
UQ
UQH2
Complex 3
cytochrome C
NADH
NAD
O2
O2-

Where do free radicals come from?

2. *endoplasmatic reticulum*

formation of superoxide (cytochrom P- 450)

3. *specialized cells* (leukocytes, macrophages)

production of superoxide by NADP-oxidase

4. *oxidation of hemoglobine to methemoglobin*

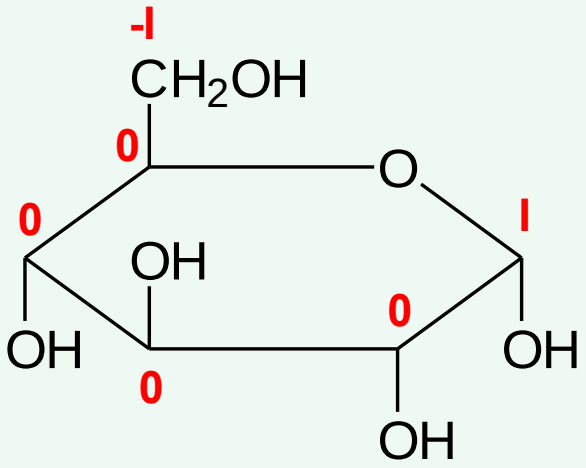
(erythrocyt is „charged“ by antioxidants)

TEST

Respiratory chain
~
Reactive forms of oxygen

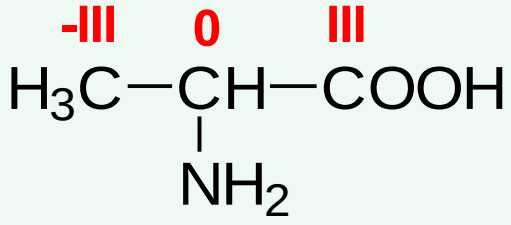
Nutrients are reduced forms of carbon

because of prevailing low oxidation numbers of carbon



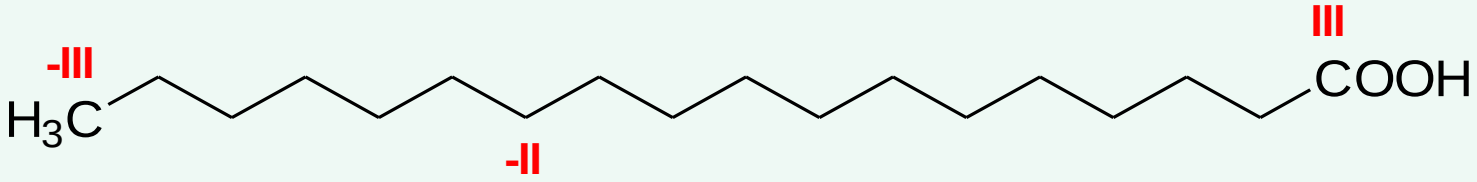
glucose: 6,7 % H

average ox.n. C = 0,0



alanine: 7,9 % H

average ox. n. C = 0,0



stearic acid: 12,8 % H

average ox. n. C = -1,8 $\Rightarrow$ carbon is the most reduced

Two ways of ATP formation in cell

95 % of ATP is formed by **aerobic phosphorylation (in presence of O_2):**

ADP + P_i + H^+ gradient energy → ATP

5 % is formed by **substrate phosphorylation:**

ADP + macroergic phosphate ⇄ ATP + second product

higher / comparable energetic contain as ATP

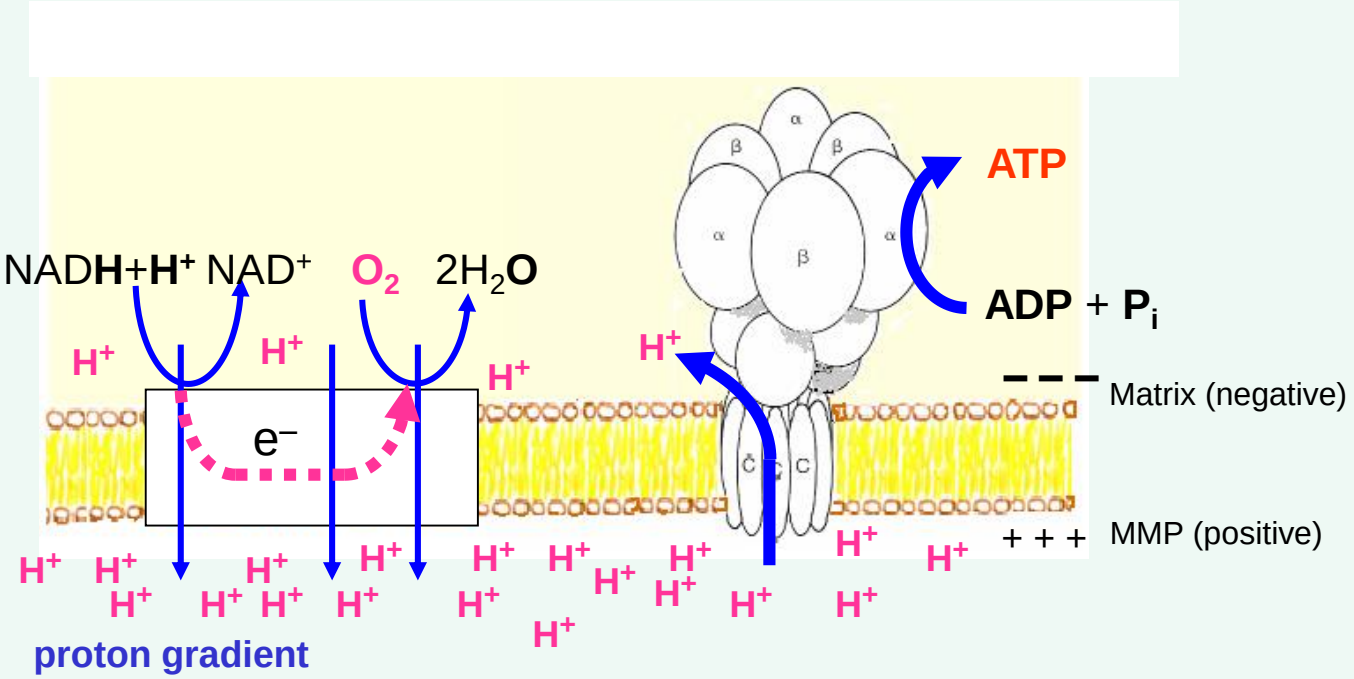
ADP + 1,3-bisPglycerate ⇄ ATP + 3-P-glycerate (glycolysis)

ADP + phosphoenolpyruvate → ATP + pyruvate (glycolysis)

GDP + [succinyl-CoA + P_i ⇄ succinylphosphate] ⇄ GTP + succinate + CoA (citrate cycle)

ADP + creatine-P ⇄ ATP + creatine (muscle, direction of reaction is influenced by current concentration)

RC is system of redox processes in inner mitochondrial membrane, begining with NADH oxidation and ending with O_2 reduction to water.



Transfer of electrons in inner mitochondrial membrane is connected to transfer of protons through membrane to intermembrane space. Proton gradient is used to ATP synthesis.

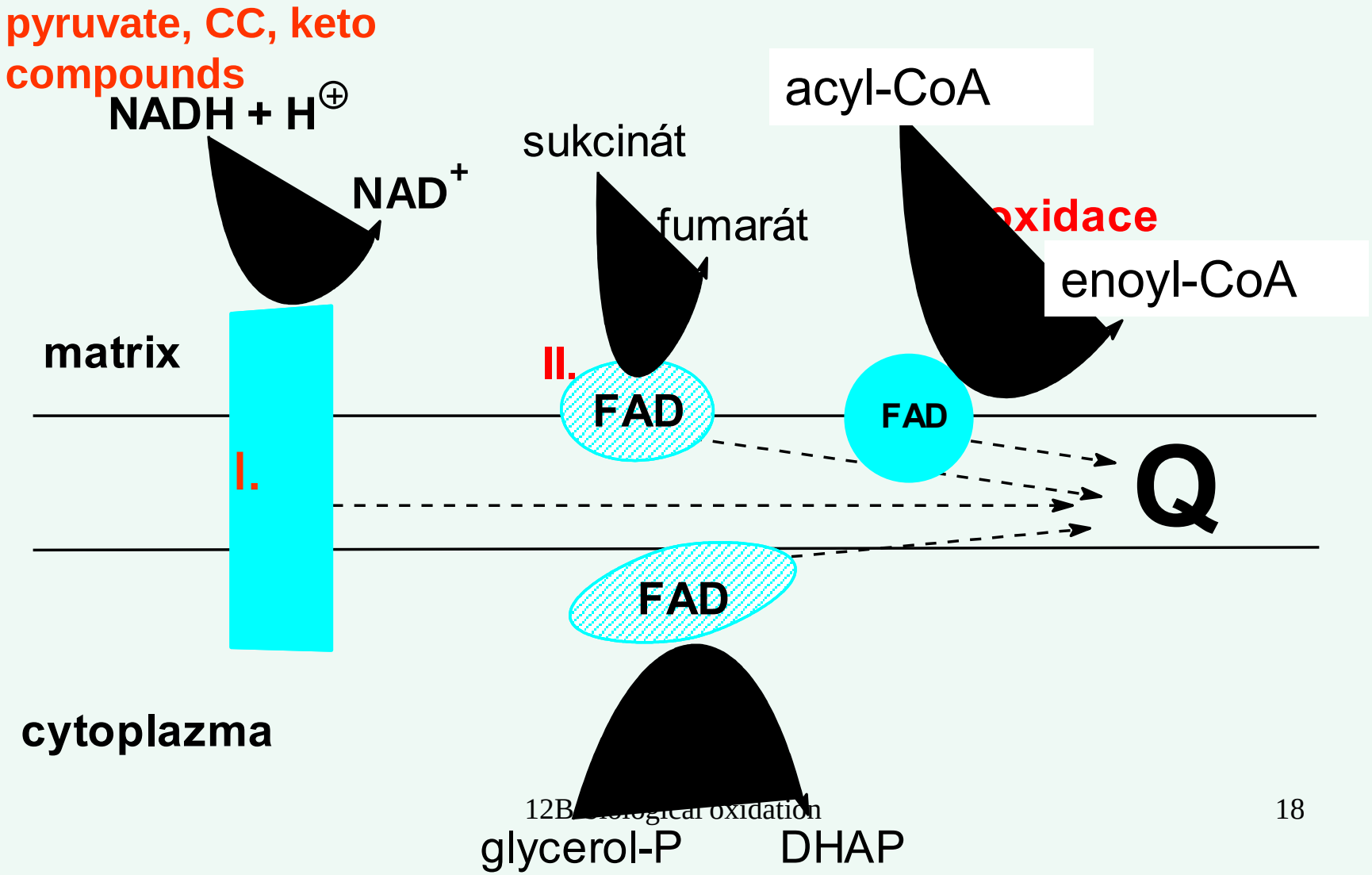
Components of respiratory chain

- substrates ($NADH+H^+$, $FADH_2$)
- enzyme complexes (I – IV)
- cofactores bound to enzymes of complexes (FMN, FAD, Fe-S, hem)
- individual components between complexes (ubiquinone, cytochrome *c*)

Distinguish:

hem (cyclic tetrapyrrole chelating Fe ion) × cytochrome (hem protein)

Collecting points for reduction equivalents



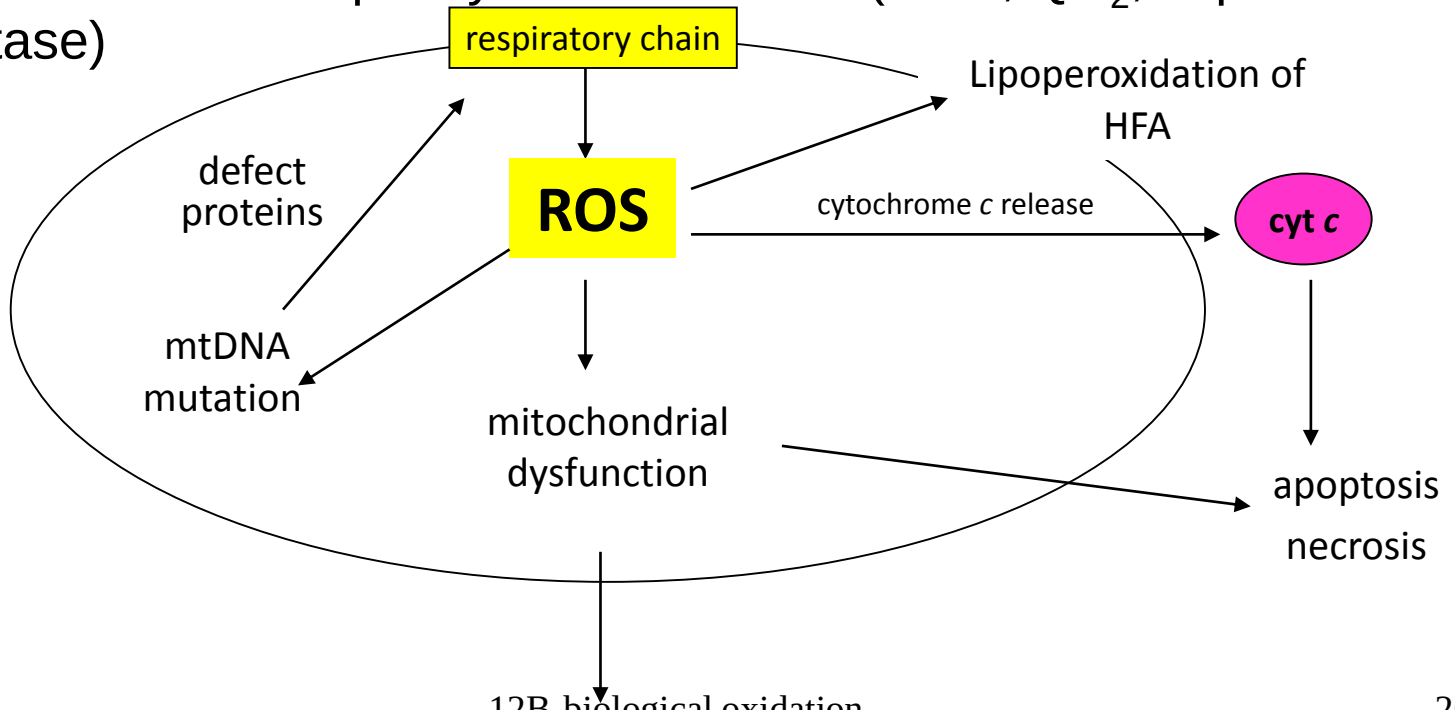
Enzyme complexes in RC

	Name	Cofactors	Oxidation	Reduction
I.	NADH-Q oxidoreductase*	FMN, Fe-S	$NADH \rightarrow NAD^+$	$Q \rightarrow QH_2$
II.	succinate-Q reductase	FAD, Fe-S, cyt *b*	$FADH_2 \rightarrow FAD$	$Q \rightarrow QH_2$
III.	Q-cytochrome-*c*-reductase	Fe-S, cyt *b*, c_1	$QH_2 \rightarrow Q$	cyt $c_{ox} \rightarrow$ cyt c_{red}
IV.	cytochrome-*c*-oxidase	cyt *a*, a_3, Cu	cyt $c_{red} \rightarrow$ cyt c_{ox}	$O_2 \rightarrow 2\ H_2O$

* also called NADH dehydrogenase

Mitochondria and oxidative stress

- about 98 % of O_2 is consumed in RC (cytochrome-*c*-oxidase)
- except of water, reactive forms of oxygen (ROS, reactive oxygen species) are formed
- complexes I and III are main sources of ROS (formation of superoxide)
- production of superoxide increases if flow of electrons in RC slows down or turns around
- mitochondria contain plenty of antioxidants (GSH, QH_2, superoxide dismutase)



Mitochondria and apoptosis

- apoptosis is regulated process of cell extinction with minimal response to surrounding tissue
- apoptosis is important for natural tissue regeneration
- regulatory apoptotic proteins belong to Bcl-2 family (B-cell lymphoma 2),
- Some of them are anti-apoptotic (Bcl-xl), others pro-apoptotic (Bax, Bak)
- Bax and Bak proteins oligomerize to form a pore in outer mitochondrial membrane
- cytochrome c is released to cytosole, binds to inactive caspases and other proapoptotic factors –**apoptosom** is formed – that triggers executive stages of apoptose (caspase cascade)

Reactive forms of oxygen in organism

Radicals	Neutral, aniont, cationts
Superoxide $\cdot O_2^-$	Hydrogen peroxide HOOH
Hydroxyl radical $\cdot OH$	Hydroperoxides* ROOH
Peroxyl radical* $ROO\cdot$	Hypochlorous acid HClO
Alkoxyl radical $RO\cdot$	Singlet oxygen $^1O_2$
Hydroperoxyl radical $HOO\cdot$	Peroxynitrite $ONOO^-$
Nitric oxide $NO\cdot$	Nitronium NO_2^+

* Derivates of phospholipides during lipoperoxidation: PUFA-OO·, PUFA-OOH

Hydroxyl radical HO·

- **The most reactive** free radical, reacts immediately with molecules in place of formation
- Reacts with all the molecules in living organisms
- Extremly strong oxidation agent
- Formation: Fenton´s reaction, homolytic cleavage of O-O bond in H2O2, ionizing radiation, in reaction of HOCl with O2-., ultrasound, in lithotripsia and lyophilisation

Strongly reactive hydroxyl radical •OH is formed in Fenton´s reaction

$$H_2O_2 + Fe^{2+} \rightarrow \bullet OH + OH^- + Fe^{3+}$$

or from hydrogen peroxide and superoxide, catalyzed by Fe^{2+} ions:

$$H_2O_2 + \bullet O_2^- \rightarrow \bullet OH + O_2 + OH^-$$

Superoxide anion-radical $\cdot O_2^-$

- is formed by <u>one electron</u> reduction of dioxygen
- relatively little reactive
- acts as oxidation and reduction agent (reduction of cytoch. C x oxidation of ascorbate)
- dirrect damage of biomolecules highly selective
- indirrect facilitates HO formation.
- formation of peroxynitrile after reaction with NO.

$$O_2 + e^- \rightarrow \cdot O_2^-$$

[this is not a reaction, only one redox pair]

Superoxide formation in organism

- **so called respiratory inflammation** (NADPH oxidase, phagocytizing leukocytes)

  $2\ O_2 + \ NADPH \ \rightarrow \ 2\ \cdot O_2^- + \ NADP^+ + H^+$

- **spontaneous oxidation of hemoproteins**

  $hem\text{-}Fe^{2+} \ + \ O_2 \ \rightarrow \ hem\text{-}Fe^{3+} \ + \ \cdot O_2^-$

[these are the reactions, combination of two redox pairs]

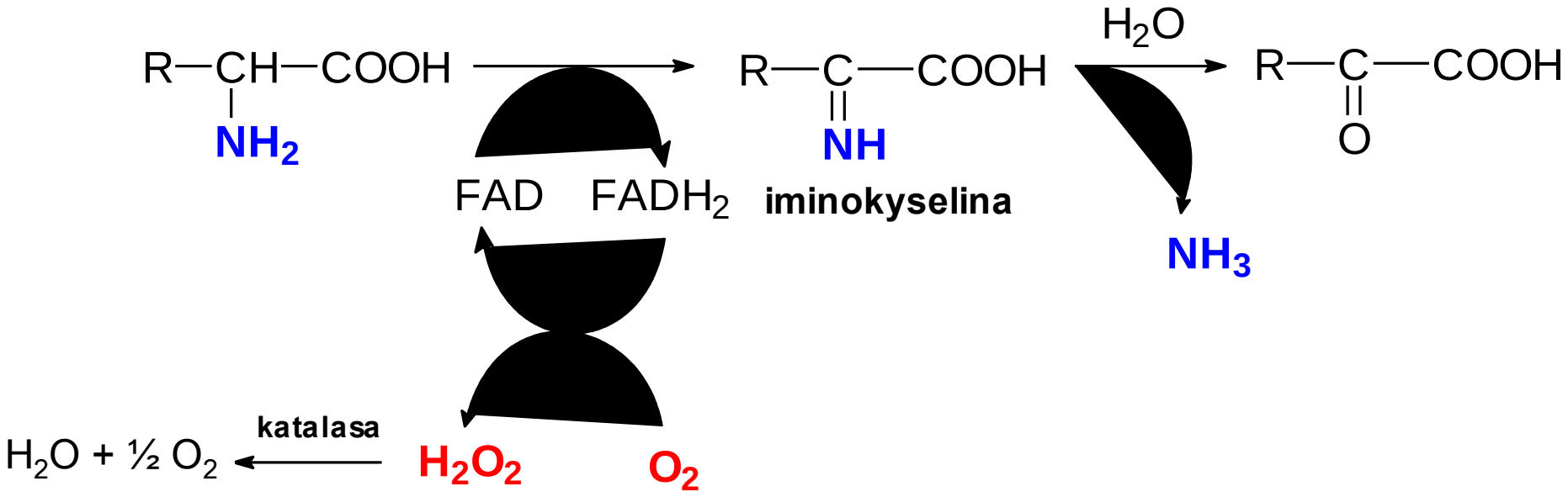
Singlet oxygen $^1O_2$

- Excitated state of triplet dioxygen, molecular O2 with paired spins, more reactive than common O2,
- Formed in photochemical reactions, also after light absorption by some pigments (porphyrins)
- Causes biologic damage (damage of retina, porphyria)
- Treatment of neonatal hepatitis, psoriasis
- Interaction with other molekules $^3O_2 \rightarrow {}^1O_2$
- Chemical reactions (formation of hydroperoxides, endoperoxides from compounds with one or more double bonds/conjugated systems
- Formation of carbonyl compounds from tryptophane
- Transfer of excitation energy (quenching)

Hydrogen peroxide H_2O_2

- *in vitro* relatively unstable compound, easily decomposed to water and oxygene
- In organism is formed in AA/amines deamination
- also in xanthinoxidase reaction
- two electron reduction of O_2
- can oxidize -SH groups of enzymes, produce hydroxyl radical, …
- Little reactive, toxic in high concentrations

Oxidative deamination of aminoacids provides ammonia, oxoacid and hydrogen peroxide



Xanthinoxidase produces hydrogen peroxide

hypoxanthin + O_2 + H_2O $\rightarrow$ xanthin + **H_2O_2**

xanthin + O_2 + H_2O $\rightarrow$ uric acid + **H_2O_2**

Majority of tissues, mainly livers

Compare: reduction of dioxygen

Reduction type	Partial reaction (redox pair)
Four electron	$O_2 + 4\ e^- + 4\ H^+ \rightarrow 2\ H_2O$
One electron	$O_2 + e^- \rightarrow \cdot O_2^-$
Two uelectron	$O_2 + 2\ e^- + 2\ H^+ \rightarrow H_2O_2$

Hypochlorous acid HClO

- Formed in neutrophilic granulocytes from hydrogen peroxide and chloride anion
- Reaction is catalyzed by myeloperoxidase
- HClO has **strong oxidative** and bactericidal effects

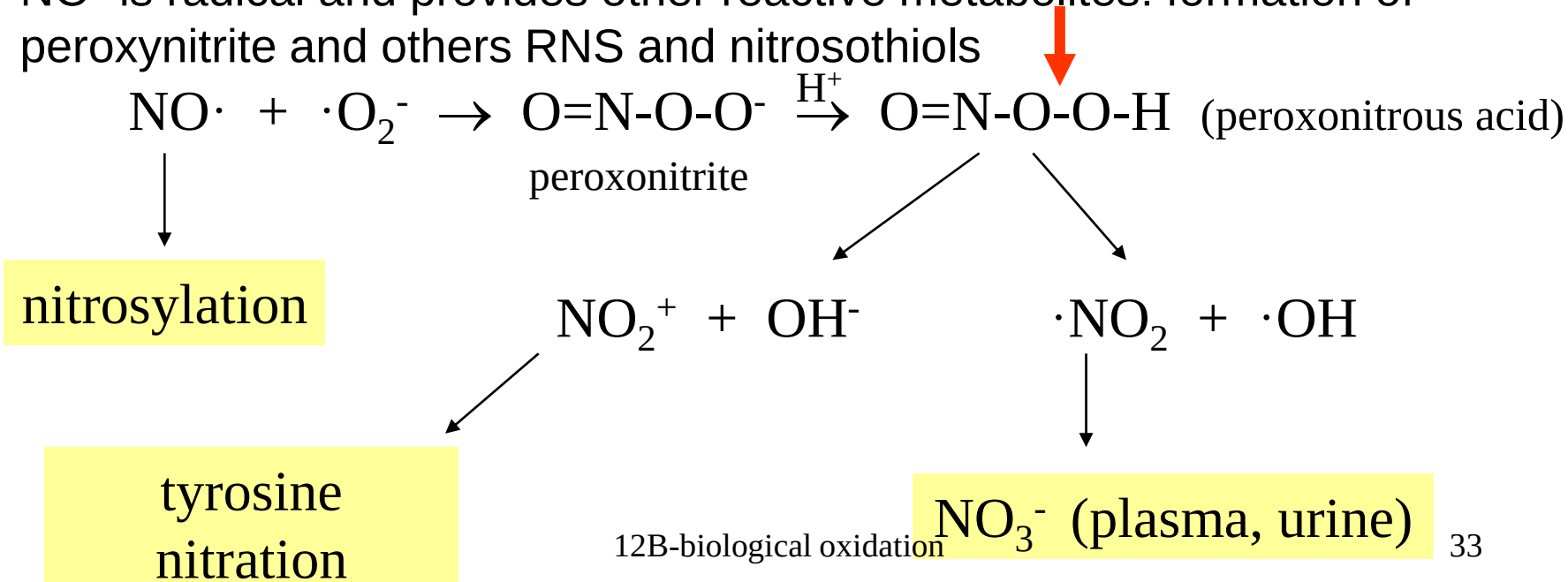
$$H_2O_2 + Cl^- + H^+ \rightarrow HClO + H_2O$$

- Damage of biomolecules:
- Damage of proteins (transforms Met to Met sulphoxide, chloration of Tyr to form 3-chlortyrosine, damage of -SH group of membrane proteins
- Chloration of DNA bases (especially pyrimidines)
- Oxidation of thiols, ascorbate and NADPH

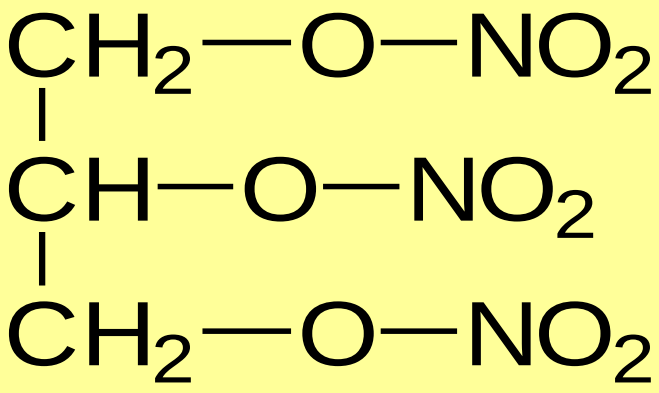
Nitric oxide NO· formation from arginine

has 1free electron, free radical

- Free diffusion between cells 1-10s, in blood catched by erythrocytes, produced by NO synthase: nNOS, eNOS, iNOS
- Exogenous sources: medicaments, vasodilatatie
- Phys. function (vasodilatation, neurotransmitter, macrophages-bactericidal effect
- NO· binds to guanylatecyclase ⇒ cGMP ⇒ relaxation of smooth muscles (especially vessels) and other effects…
- NO· is radical and provides other reactive metabolites: formation of peroxynitrite and others RNS and nitrosothiols



NO releasing compounds

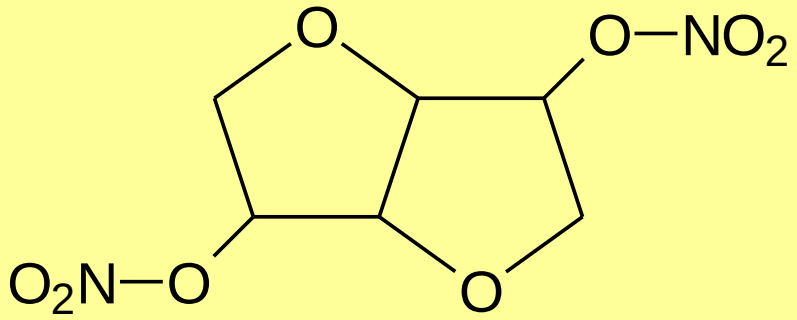


glycerol trinitrate (glyceroli trinitras)

yellowish oily liquid

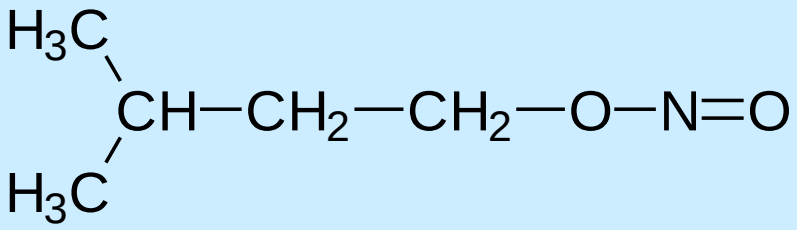
classic medicament, fast action

sublingual tablet, spray, plaster



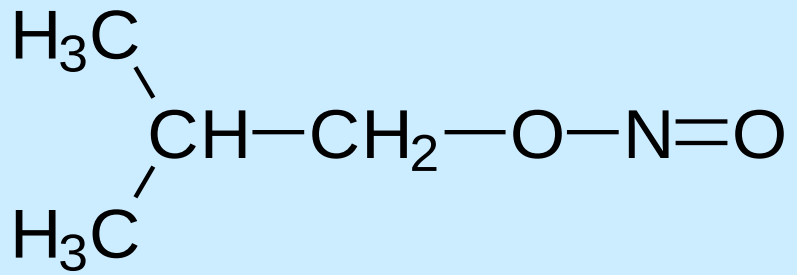
isosorbide dinitrate (isosorbidi dinitras)

more advantageous pharmacokinetic properties



amyl-nitrite (amylis nitris)

volatile liquid, inhalation use



isobutyl-nitrite

volatile liquid, new drug

poppers, rush, liquid aroma …

$Na_2[Fe(CN)_5NO]$

natrium nitroprusside (natrii nitroprussias)

disodium pentacyanonitrosylferrate

ruby red crystals

extremly efficiant, i.v. infusion

Peroxynitrite ONOO-

- Strong cytotoxic oxidation agents
- Toxic effects:
- Deplexation of –SH groups and other antioxidants
- Oxidation of lipids
- DNA breaks, nitration and deamination of DNA bases (G)
- Nitration of aromatic AA (Tyr, Phe, Trp) 3-nitrotyrosine (inaktivation of enzymes, interference with signal transduction)
- Oxidation of Met to sulphoxide

$$NO\cdot + \cdot O_2^- \rightarrow O{=}N\text{-}O\text{-}O^-$$

peroxonitrite

↓

nitrosylation

Sulphurous radicals

- In vivo –SH –antioxidants, but thiols can be source of free radicals as well
- Formation of thiol radicals GS.
- Formation of potential cytotoxic radicals

Exogenous causes of free radicals formation

- Ultraviolet or ionizing radiation (UV light, γ radiation, X- ray)
- Smoking
- Air pollution
- Intoxication (PCB, CL4, chloroform, alcohol
- Food (thermal processing, crushing, light influence)

TEST

Endogenous causes of free radicals formation

- Reaction catalyzed by XOD (injuries, necrosis)
- Decay of phagocytes and macrophages (inflammations, sepses, burns)
- Synthesis of prostaglandins
- Hyperglycemia
- Reperfusion after previous ischemia (oxygen debt)

Function of free radicals in healthy orgamnism

I Tool of oxidases and oxygenases

- *cytochromoxidase* (toxic intermediates, H_2O_2 and superoxides, bount to enzymes)

(mitochondrial respirátory chain)

- *monoxygenases* (oxygenases with mixed function) - activate O_2 in liver ER or in gland mitochondria; hydroxylation

(cytochrome P450, oxidation of wide range of substrates using O2, liver P450- metabolism of xenobiotics)

I Tool of oxidases and oxygenases

- **Xanthinoxidase (XOD)** –oxidation of xanthinu to uric acid
- **Proline and lysinehydroxylases** (hydroxylate Pro and Lys in colagen synthesis)
- **Tyrosinehydroxylase** (hydroxylates Tyr, begining of synthesis of dopamine, adrenaline, noradrenaline)

Synthesis of thyroid gland hormons

-Thyreoperoxidase

Oxidation of I- to I2 by hydrogen peroxide ---mono and di iodotyrosine, thyroxine T4, triiodothyronine T3

Ovum fertilization

Sperm

Disruption of ovum membrane during penetration –O2- production.

Ovum

Prevention of other sperms penetration – production of H2O2 ..formation of transverse bonds in membrane

Function of free radicals in healthy organism II

ROS and RNS against bacteria, phagocytosis

Form of protection against extraneous particles and microorganisms

Macrophages, neutrophils, NK cells

Enzymes participating in disabiling of absorbed microorganisms in phygocyte

- enzyme complex *NADPH-oxidase* of leukocytes and macrophages (respiratory inflammation)
- *myeloperoxidase* – catalysis of reaction

$H_2O_2 + Cl^- + H^+ = HClO + H_2O$

- *iNOS: NADPH* dependent *(Arg-Citrulin..NO)*

Function of free radicals in healthy organism III

- signal molecules
  primary messenger ⇒ secondary messenger ⇒ info net
- *redox state of cell influences function of that net*
- *redox state:* capacity of antioxidative system, accesibility of reduction equivalents, intensity of oxidation load (RONS)

⇒ROS: *secondary* messengers

⇒NO (neurotransmitter, vascular endotel-relaxation vascular walls, NO in phagocytizing cells)

Immune protection *vs.* regulation

massive production of ROS as a tool of immune *protection*

X

Induction of changes in low ROS levels, which are probably *regulatory mechanism*

Positive effects of oxygen radicals

- **Intermediates of** oxidase and oxygenase reactions (cyt P-450), during reactions radicals are bound to enzyme so they don´t harm surrounding tissues
- **bactericidal effect** of phagocytes, respiratory inflammation (NADPH-oxidase)
- **signal molecules** (primary messengers), proven in NO· so far, some other radicals are supposed to have similar effects

Oxidative stress

When balance between formation and elimination of RONS is broken,

oxidative stress happens

balance can be broken on **both** sides!!

Causes of oxidative stress formation:

-excessive formation of RONS,

- insufficient activity of antioxidative defense system,
- combination

Damage of lipids - attack to unsaturated FA

Peroxidation of lipids

- Chain reaction
- **enzyme peroxidations of lipids** (synthesis of prostanoids, leukotrienes, active center of hydro- and endoperoxidases (COX and lipoxygenases)
- **non-enzyme peroxidations of lipids** – patological process, intermediates of lipoperoxidation, binding to proteins, influence of fluidity

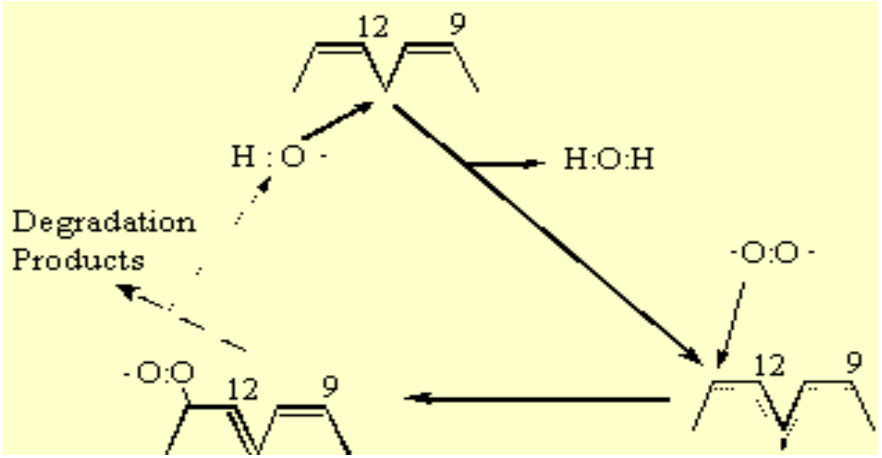
Damage

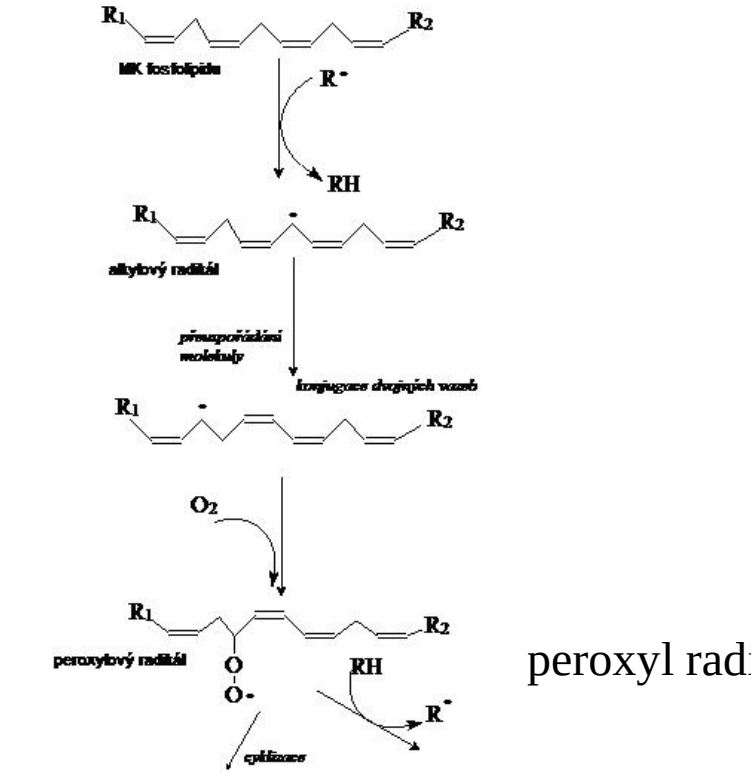
- loss of multiple bonds
- Formation of reactive metabolits (aldehydes)

Effect

- change of fluidity, permeability of membranes
- effect to membrane bound enzymes

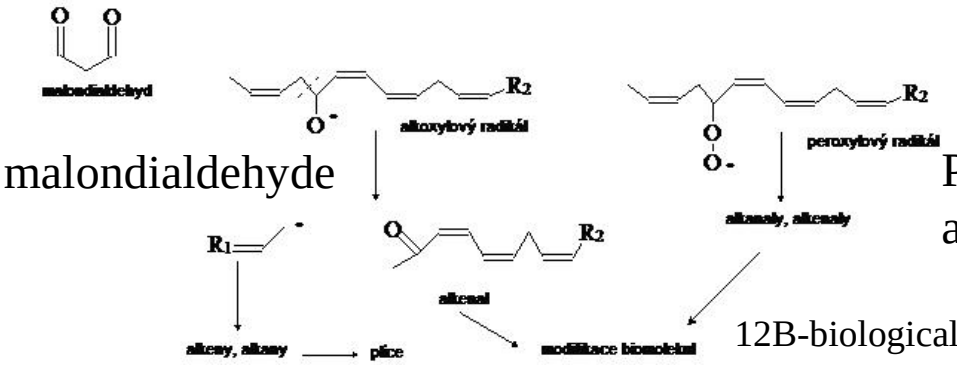
Peroxidation of linoleic acid





peroxyl radical

hydroperoxide



malondialdehyde

Peroxide radical– alkanals, alkenes….modification, lungs

Damage of proteins

Dirrect damage of proteins by RONS influence
-oxidation, hydroxylation, nitration, chloration AA, no chain reaction
Indirrect damage of proteins by products of lipid peroxidation
-alkoxyl and peroxyl radicals
Malondialdehyde and 4-hydroxynonenal, formation of transverse bonds between neighbouring chains, formation of carmobyne compounds

Damage

- agregation and networking,
- fragmentation and cleavage
- reaction with hem Fe
- modification of functional groups

Consequence

- changes in ion transport
- changes in aktivity of enzymes
- proteolysis, activstion of proteases and phospholipases by Ca21 accumulation in cytosol
- formation of new antigen determinants with subsequent autoimmune reactions

DNA damage

- **Hydroxylation of purine bases**

- 8-hydroxyadenin, 8-hydroxyG, 8-oxoG, FapyG, FapyA

- **Hydroxylation of pyrimidine bases**

-thyminglycol, uracilglycol,…

- **Hydroxylation of carbohydrate residues**

-oxidation and fragmentation – releasing of bases, interruption of DNA chain and malondialdehyde formation

Damage

- cleavage of carbohydrate cycle
- modification of bases
- breaks of chain

Consequence

- mutation
- translation errors
- Inhibition of proteosynthesis
- missmatching

TEST

Damage of biomolecules

Compound	Damage	Consequences
Lipids	- oxidation of PUFA (loss of double bonds) -formation of reactive compounds (aldehydes and ROO·) -oxidation of cholesterol	- change in membrane permeability - damage of membr. enzymes, proteins - change in membrane fluidity
Proteins	- modification of -SH and phenyl (arom.) AA - Formation of transverse bonds between chains-networking and agregation - fragmentation + cleavage	changes in ion transport Ca^{2+} enter to cytosol changes in aktivity of enzymes formation of new antigen determinants activation of proteases and phospholipases
DNA	modification and cleavage of deoxyribose modification of bases breaks of chain formation of transverse bond between DNA a protein chains	mutations missmatches translation errors inhibition of proteosynthesis

How can we quantify oxidative stress?

Detection of free radicals

- quite difficult because of phys. chem. properties

Measuring of oxidative stress products

- simplier, wide range of oxidative stress markers

Markers of oxidative stress

Appraisal of lipoperoxidation:

malondialdehyde (MDA), conjugated dienes, isoprostans

Appraisal of protein damage:

protein hydroperoxides

appraisal of DNA damage:

determination of modificated nucleosides (8-oxoG)

Determination of antioxidants

ascorbate

tocoferol

SOD

GSHPx

glutathion

Diseases connected to oxidative stress

Neurologic

Alzheimer disease

Parkinson disease

Endocrine

Diabetes

Gastrointestinal

Acute pankreatitis

Vascular

Aterosclerosis

Other

Obesity

Organe transplantation, cancer

Antioxidative protective system

Three types of protection

- *inhibition* of excessive RONS production
- *capture* and elimination of radicals (catcher)
- *reparative* mechanisms of damaged biomolecules

TEST

Summary of FR antioxidants and catchers

1. Endogenous antioxidants

- *enzyme* (cytochrome c, SOD, GSHPx, catalase)
- *non-enzyme*
  - membrane (α -tocoferol, β-carotene, coenzyme Q_{10})
  - non-membrane (ascorbate, urates, transferine, bilirubin)

2. Exogenous antioxidants

- *inhibitors of FR formation* (regulation of enzyme activity)
- *scavengers of formed FR* (enzymes,non-enzymes)
- *trace elements* (Se, Zn)

TEST

Antioxidative systems of organism

1. Enzymes (endogenous)

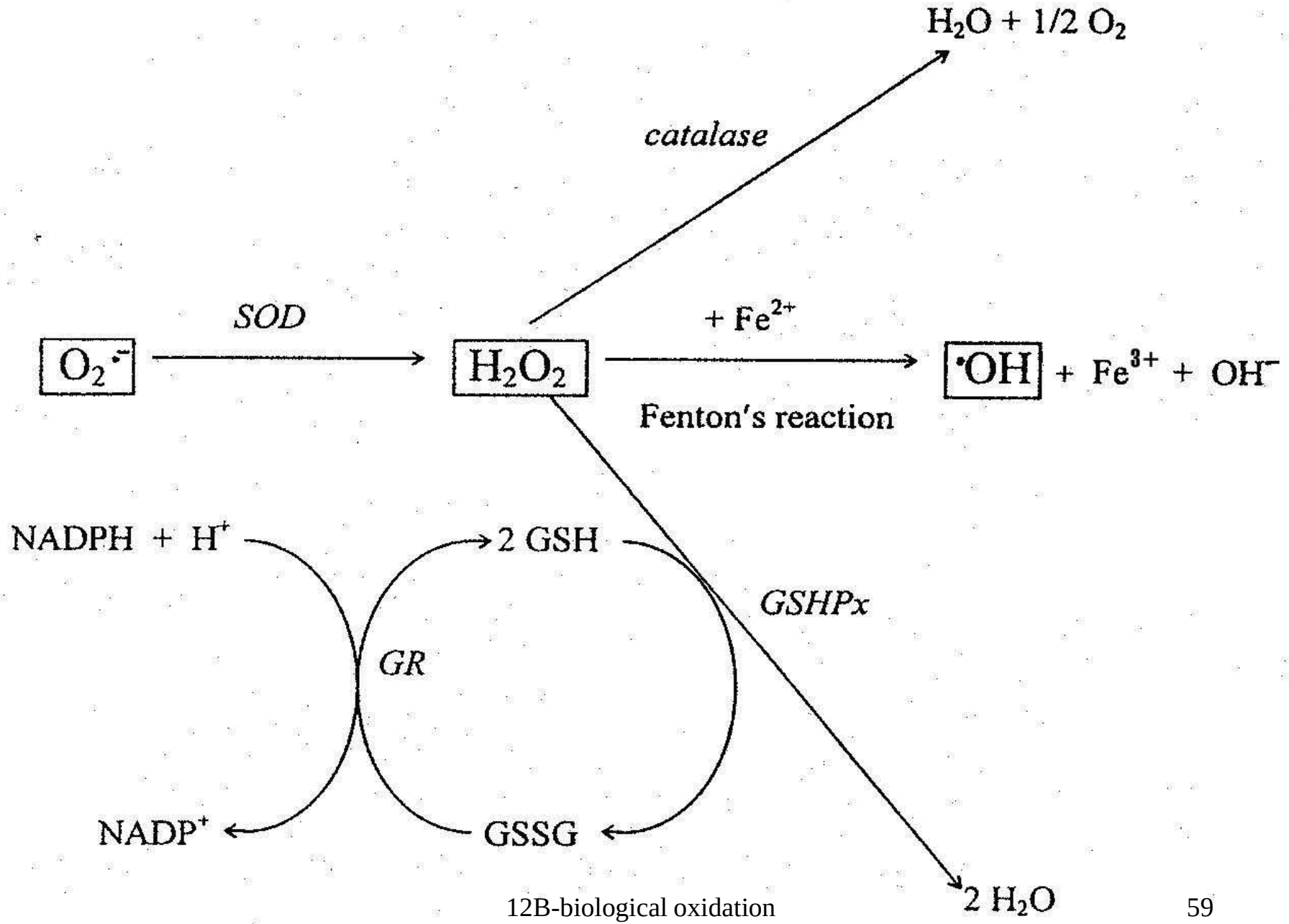
superoxide dismutase, catalase, glutathionperoxidase

2. high molecular weight antioxidants (endogenous)

transferrin, ferritin, ceruloplasmin,… bind free metal ions

3. low molecular weight antioxidants (exogenous, endogenous)

- reducing compounds with phenol -OH (tocoferol, flavonoids, urate)
- reducing compounds with enolo -OH (ascorbate)
- reducing compounds with -SH group (glutathion, dihydrolipoate)
- compounds with extensive systém of konjugated double bonds (carotenoides)


$H_2O + 1/2\ O_2$
catalase
SOD
$+ Fe^{2+}$
$O_2^{\bullet -}$
H_2O_2
$^{\bullet}OH + Fe^{3+} + OH^-$
Fenton's reaction
NADPH + H^+
2 GSH
GSHPx
GR
$NADP^+$
GSSG
$2\ H_2O$

Superoxide dismutase

- present in every cell, phylogenetically very old enzyme
- catalyse dismutation of superoxide

  $2 \cdot O_2^- + 2\ H^+ \rightarrow O_2 + H_2O_2$

- oxidation numbers of oxygen in reaction: $(-½) \rightarrow (0) + (-I)$

two isoforms: SOD1 (Cu, Zn, cytosol), *dimer, Cu = redox center*
cytosol, intermitochondrial space, hepatocyte, brain, erytrocyte, high permeability, catalysis at pH 4,5-9,5

- SOD2 (Mn, mitochondria), tetramer, mitochondrial matrix, lower stability than Cu, Zn – SOD, phylogenetically younger

Elimination of H_2O_2 in organism

- **catalase** present in erytrocytes

disproportionation H_2O_2, $H_2O_2 \rightarrow ½\ O_2 + H_2O$, at high levels of H2O2

detoxication of alkylperoxides : $H_2O_2 + ROOH \rightarrow O_2 + H_2O +$ ROH

- **glutathion peroxidase** (elimination of interacellular hydroperoxides)
- contains selenocystein, second substrate - glutathion (G-SH) reduces H_2O_2 and hydroperoxides of phospholipides (ROOH)

detoxication of hydrogen peroxide, GSSG reduces to GSH using glutathionreductase

$$2\ G\text{-}SH + H\text{-}O\text{-}O\text{-}H \rightarrow G\text{-}S\text{-}S\text{-}G + 2\ H_2O$$

$$2\ G\text{-}SH + R\text{-}O\text{-}O\text{-}H \rightarrow G\text{-}S\text{-}S\text{-}G + R\text{-}OH + H_2O$$

harmless derivate

Glutathionperoxidases

eliminate intracellular hydroperoxides and H_2O_2

$2\ GSH + ROOH \rightarrow GSSH + H_2O + ROH$

- *cytosol GSH – glutathion peroxidase* (EC 1.11.1.9, cGPx)
- *extracellular GSH – glutathion peroxidase* (eGSHPx)
- *phospholipidhydroperoxide GSH - peroxidase (EC 1.11.1.12, PHGPx)*

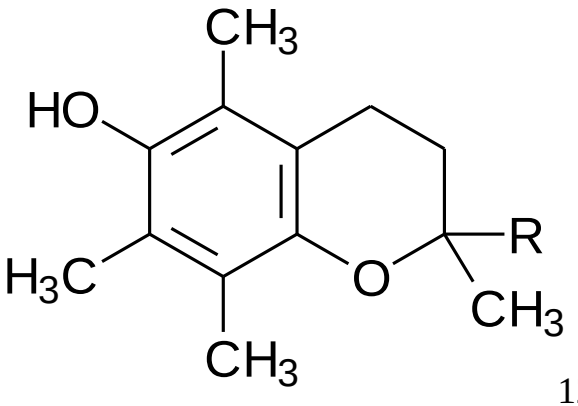
Low molecular weight antioxidants

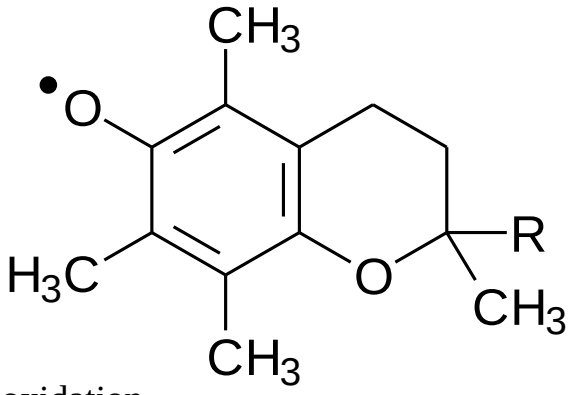
Lipophilic	Hydrophilic
Tocoferol	L-ascorbate
Carotenes	Flavonoids
- Lycopene	Dihydrolipoate[a]
- Lutein	Glutathion[a]
Ubiquinol[a]	Urci acid [a]

[a] Endogenous compounds.

Tocoferol

- Lipophilic antioxidant of cell membranes and lipoproteins
- Reduces peroxyl radicals of phospholipids to hydroperoxides, which are further reduced by GSH, tocoferol oxidises to stable radical
- PUFA-O-O· + Toc-OH → PUFA-O-O-H + Toc-O·
- Toc-O· partially reduces to Toc-OH by ascorbate to GSH (phare interface)
- Toc-O· + ascorbate → Toc-OH + semidehydroascorbate

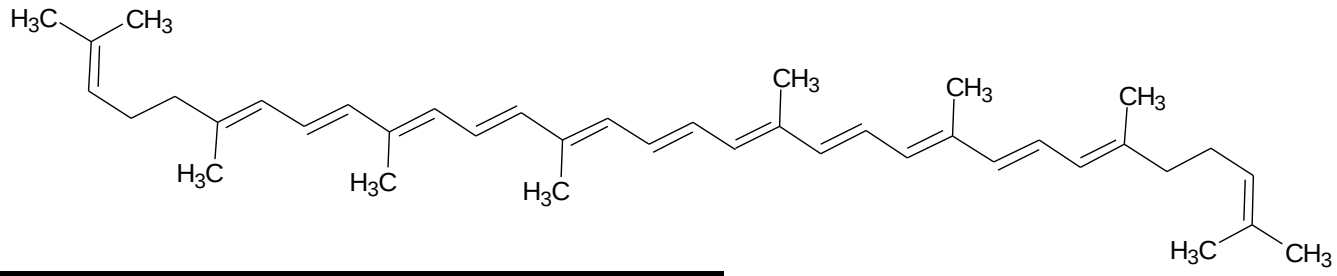




Carotenoides

- Carotenoides are polyisoprenoid carbohydrates (tetraterpens)
- Eliminate peroxyl radicals while changing themselves to stable carotene radical
- Are able to quench (deexcitate) singlet oxygen
- Sources in food: leaf vegetable, yellow, orange and red coloured vegetable and fruit
- The most efficient antioxidant is **lycopene**, present in some food, **mainly tomatos** and their products (ketchup, puree) – je thermally stable
- High intake of lycopene in the Mediterranean

Contain of lycopene in food (mg/100 g)

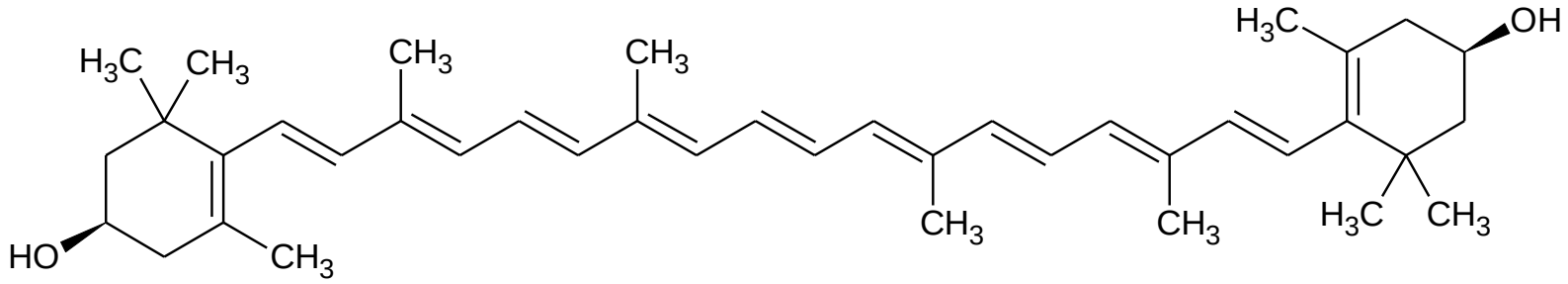


Tomato puree	10-150
Ketchup	10-14
Tomato juice/sauce	5-12
Melon	2-7
Papaya fresh	2-5
Tomatos fresh	1-4
Apricot compote	~ 0,06
Apricot fresh	~ 0,01

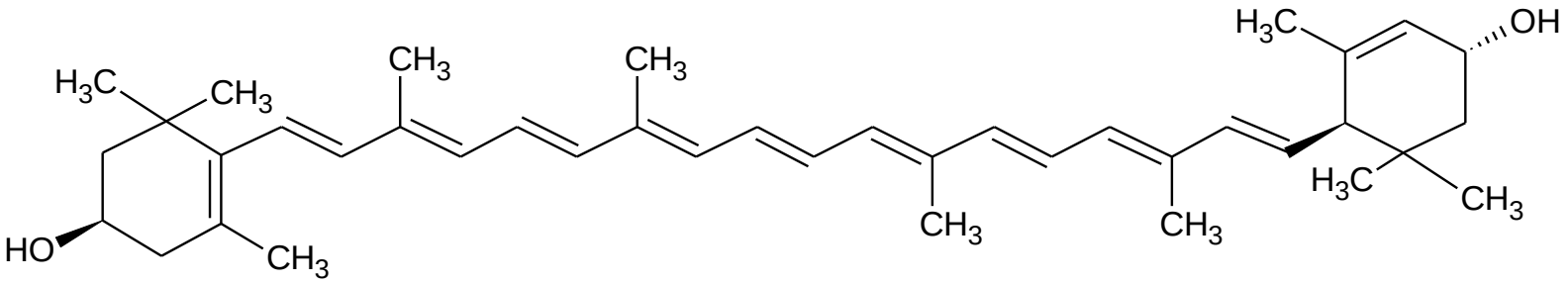
For lycopene effective release and absorbtion is suitable to cook tomatos and consume with oil

Zeaxanthine and luteine

- belong to xanthophyles, oxygenic derivates of carotenoides
- differs in double bond location and number of chiral centers
- present especially in green leaf vegetable
- present in yellow spot (macula lutea) and protects it from degeneration



zeaxanthine (two chiral centers)



luteine (three chiral centers)

Ubiquinol (QH_2)

- Present in every membrane
- Endogenous synthesis of interstinal mikroflóra from tyrosine and farnesyl diphosphate (turn in cholesterol biosynthesis)
- Exogenous sources: sprout oil, liver, meat
- Reduced form of QH_2 helps in tocoferol regeneration
- $Toc\text{-}O\cdot + QH_2 \rightarrow Toc\text{-}OH + \cdot QH$

L-Ascorbate (vitamine C)

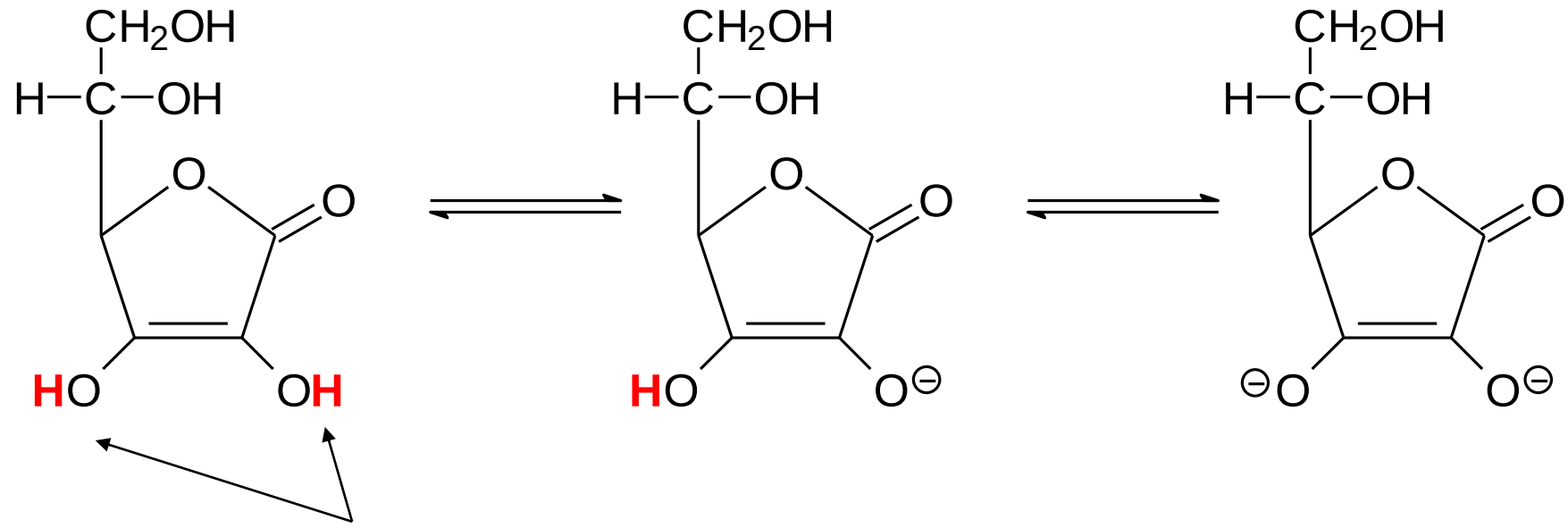
- Cofactor of proline hydroxylation (colagene synthesis)
- Cofactor (reductant) of dopamine to noradrenaline hydroxylation
- Strong reduction agent ($Fe^{3+} \rightarrow Fe^{2+}$, $Cu^{2+} \rightarrow Cu^{+}$)
- Facilitates Fe absorbtion from food
- Reduces radicals $\cdot OH$, $\cdot O_2^-$, $HO_2\cdot$, $ROO\cdot$,...
- Regenerates tocoferol radical
- Eliminates to oxalate !!
- Excessive ascorbate has prooxidative effects:

  Fe^{2+} a Cu^{+} catalyse formation of hydroxyl radical

  ascorbate + $O_2 \rightarrow \cdot O_2^-$ + $\cdot$monodehydroascorbate

L-Ascorbic is diprotic acid

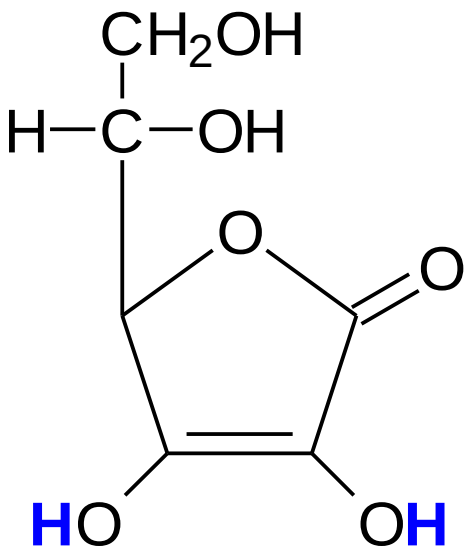
$pK_{A1} = 4,2$ $pK_{A2} = 11,6$



two enol hydroxyls

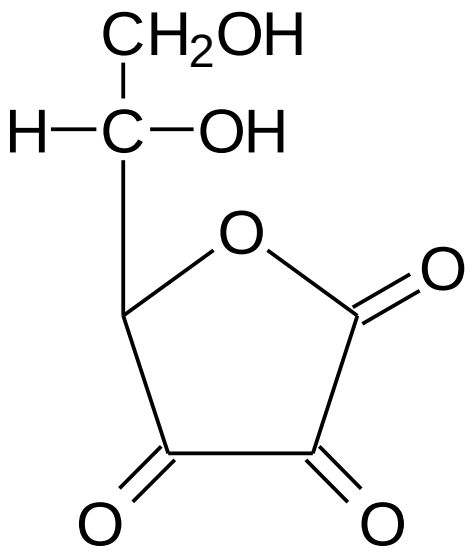
Two conjugated pairs:
ascorbic acid / hydrogenascorbate
hydrogenascorbate / ascorbate

L-Ascorbic acid has reduction effects



ascorbic acid

(reduced form)



dehydroascorbic acid

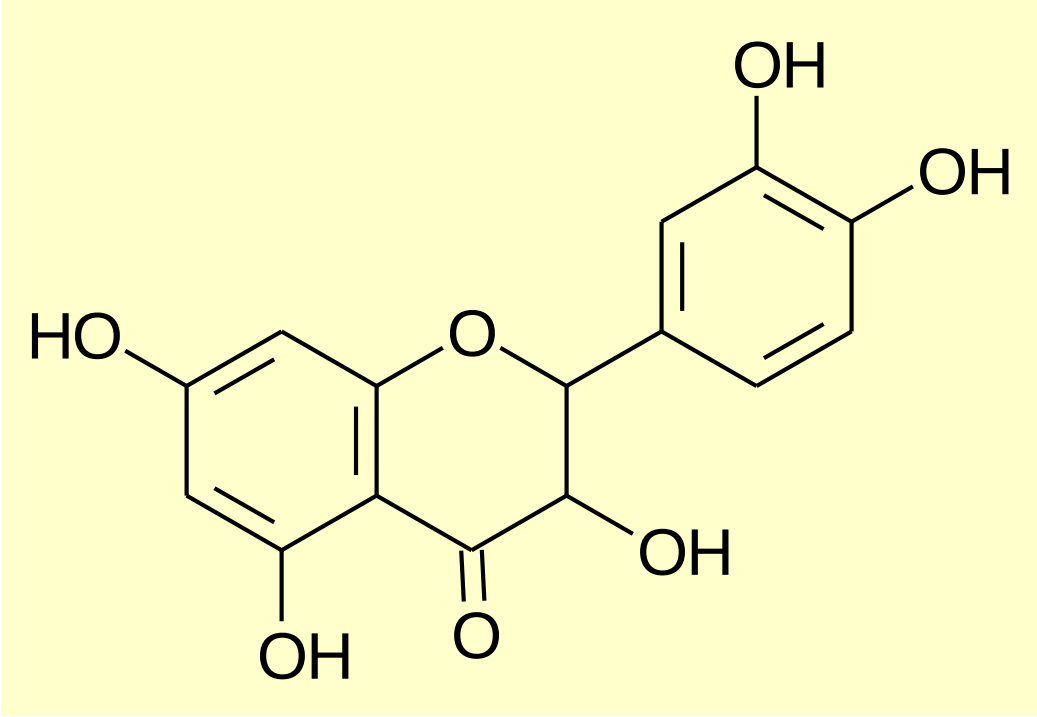
(oxidized form)

Flavonoids and other polyphenols

- Ubiquitary spread in plants, most common reduction compounds in our food
- Total intake is about 1 g (much higher than vitamines)
- Derivates of chromane (benzopyrane) containe many phenol hydroxyls
- Main representative is **quercetin**
- reduce free radicals while are transformed to little reactive fenoxyl radicals
- Chelatate metal ions (Fe^{2+}, Cu^{+}) preventing them from participating in Fenton´s reaction

Main sources of flavonoids and other polyphenols

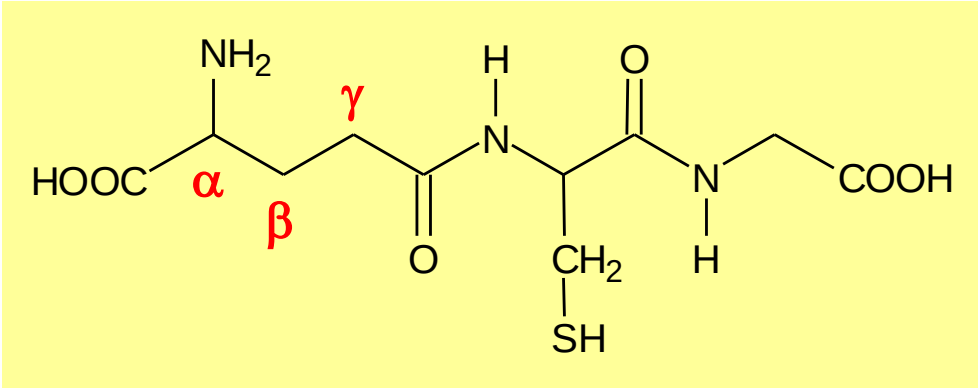
- vegetable (especially onion)
- fruit (apples, citruses, grapes)
- green, black tea
- cocoa, chocolate
- olive oil (Extra Virgin)
- red wine



quercetin

Glutathion (GSH)

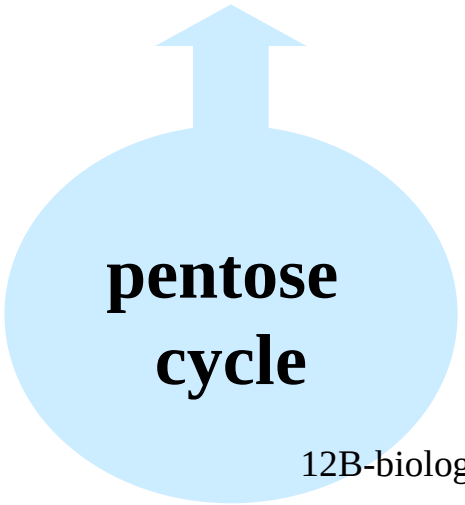
- tripeptid
- γ-glutamylcysteinylglycine
- produced in every cell
- reduction agent (-SH)



- reduced H_2O_2 and ROOH (glutathion peroxidase)
- reduces different oxygen radicals
- regenerates -SH groups of proteins and coenzyme A
- participates in tocoferol and ascorbate regeneration

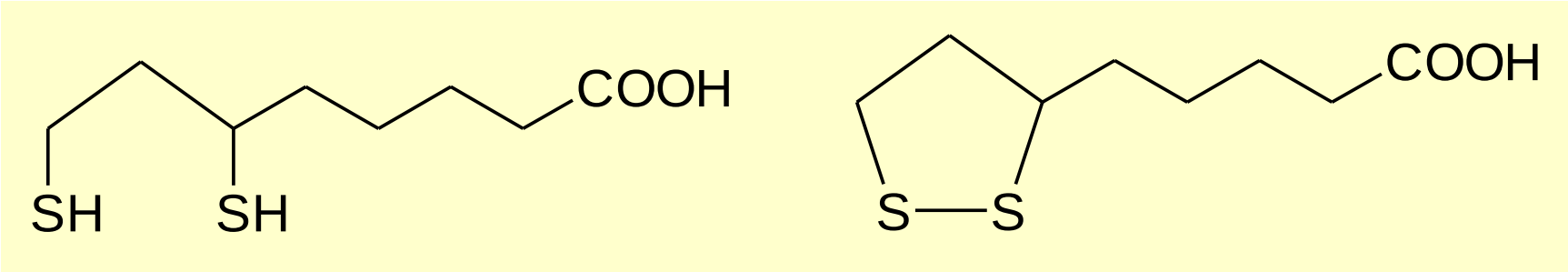
Regeneration of GSH reduced form

- fluent regeneration of glutathion (GSH) reduced form must be ensured
- **Glutathion reductase,** important in erytrocytes
- $GSSG + NADPH + H^+ \rightarrow 2\ GSH + NADP^+$



Dihydrolipoate

- cofactor of oxidative decarboxylation of pyruvate and 2-oxoglutarate
- reduces many radicals (mechanism is unknown)
- participates in tocoferol regeneration
- terapeutic using (acidum thiocticum) – diabetic neuropathy



dihydrolipoate
(reduced form)

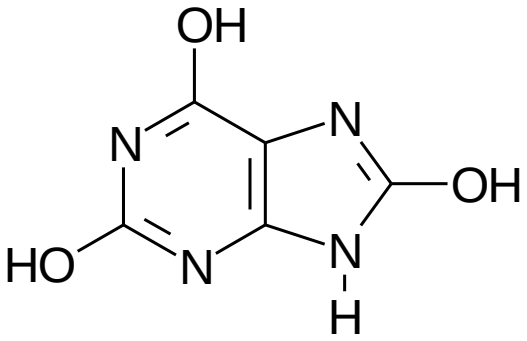
lipoate
(oxidized form)

Uric acid

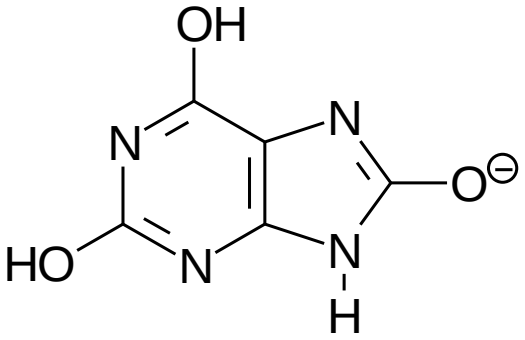
- Final catabolit of purine bases, diprotic acid
- In tubules resorbes from 90 %
- **The most common antioxidant of blood plasma (150-400 µmol/l)**
- Significant reduction effects, reduces RO· radicals
- Binds Fe and Cu cations

Lactim form of uric acid is diprotic acid

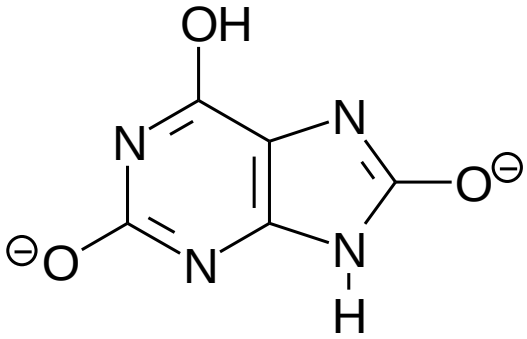
$pK_{A1} = 5,4$ $pK_{A2} = 10,3$



uric acid



hydrogenurate



urate

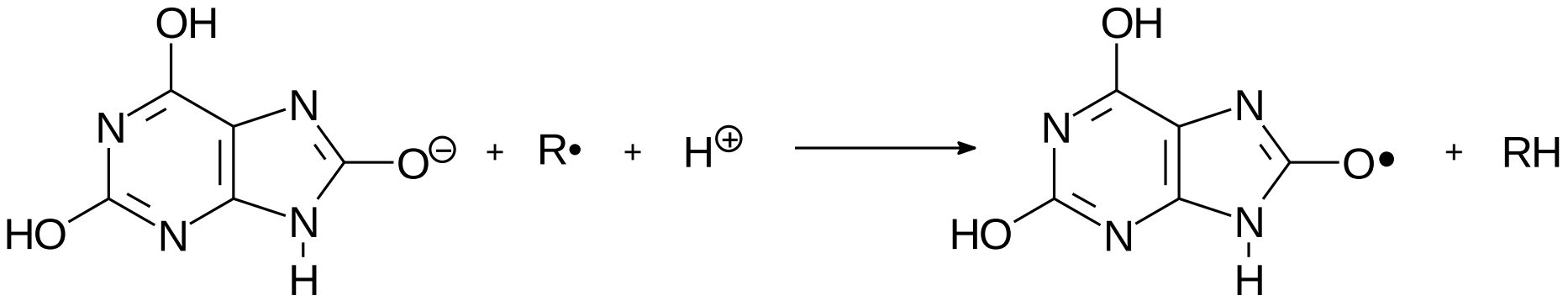
2,6,8-trihydroxypurine

Reduction effects of uric acid

Compare concentrations in blood plasma:

Ascorbate: 10 - 100 µmol/l

Urate: 200 - 420 µmol/l



hydrogenurate (reduced form)

radical (oxidized form)

hydrogenurate cleaves one electron

R· is for example ·OH, superoxide,…

different transformations

High molecular weight antioxidants

- Bind ionts of transition metals, change their oxidation state and prevent their participation in radical reactions
- Ferooxidase activity- mobilization of Fe intracellular reserves
- **Transport proteins** (transferin, lactoferin, ceruloplasmin)
- **Reserve proteins** (feritin, hemosiderine, neuromelanine), haptoglobine, hemopexine
- **Proteins containing large amount of thiol groups** (metalothioneins, albumine)

Trace elements affecting FR

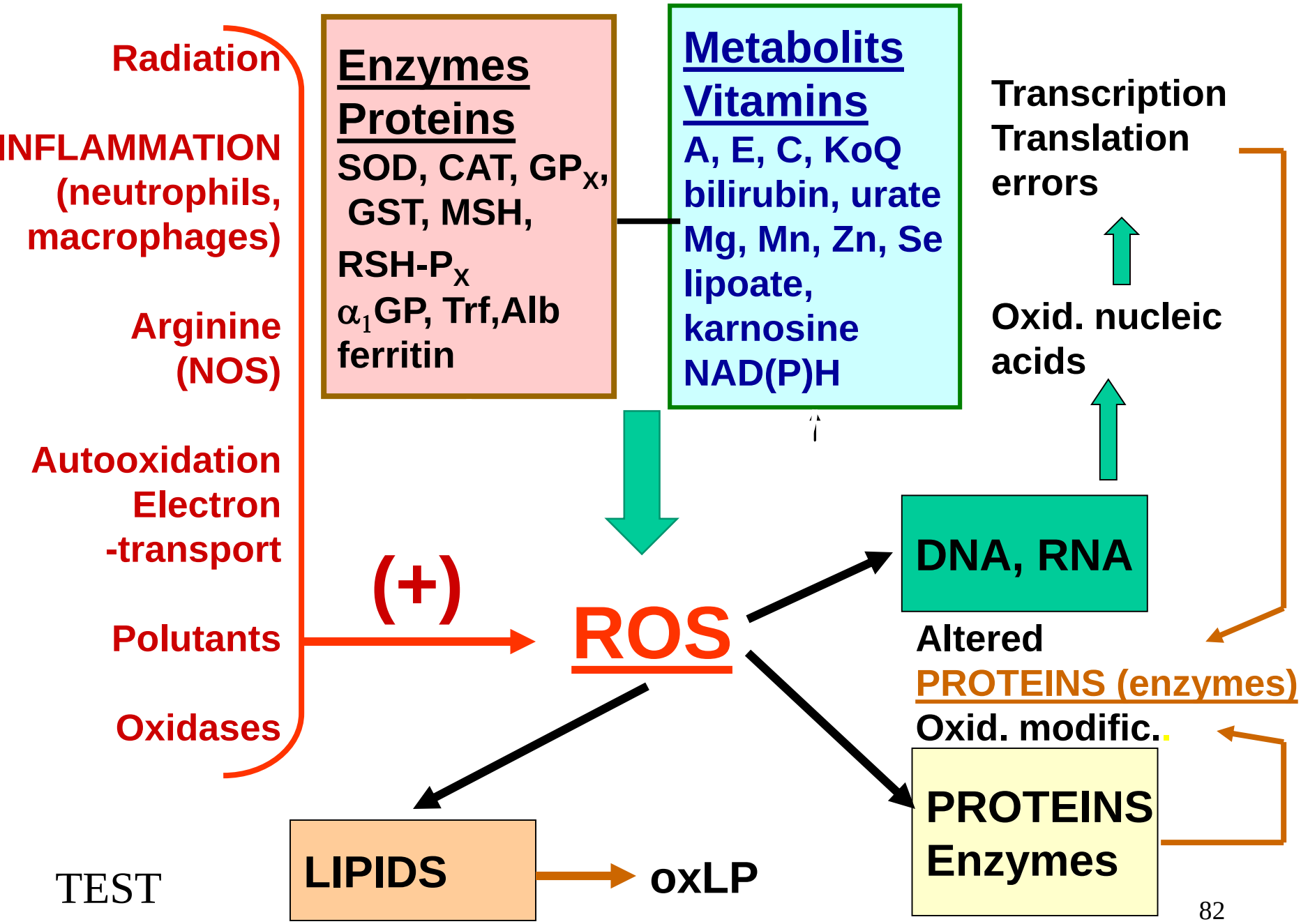
Selenium

affects vit. E resorption, part of selenoproteins

⇓ Se = insufficient immune response, hemolysis of erythrocytes, synthesis of methemoglobine

Zinc

stabilization of cell membranes, amplification of immune response, Fe antagonist


Radiation
INFLAMMATION (neutrophils, macrophages)
Arginine (NOS)
Autooxidation Electron -transport
Polutants
Oxidases
Enzymes Proteins
SOD, CAT, GPX, GST, MSH,
RSH-PX
α1GP, Trf,Alb
ferritin
Metabolits Vitamins
A, E, C, KoQ
bilirubin, urate
Mg, Mn, Zn, Se
lipoate,
karnosine
NAD(P)H
Transcription Translation errors
Oxid. nucleic acids
DNA, RNA
(+)
ROS
Altered PROTEINS (enzymes)
Oxid. modific.
PROTEINS Enzymes
LIPIDS
oxLP

TEST