Reactive Oxygen Species in the Body Antioxidant Defence

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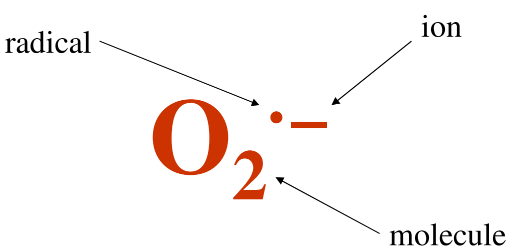
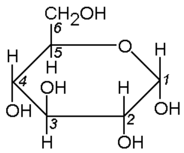
What is a free radical ?

- any chemical species (molecule, atom, ion) capable of independent existence that contains at least one unpaired electron



For what do we need oxygen?

Transfer of electrons (oxidation) from organic substances to oxygen releases huge amount of energy

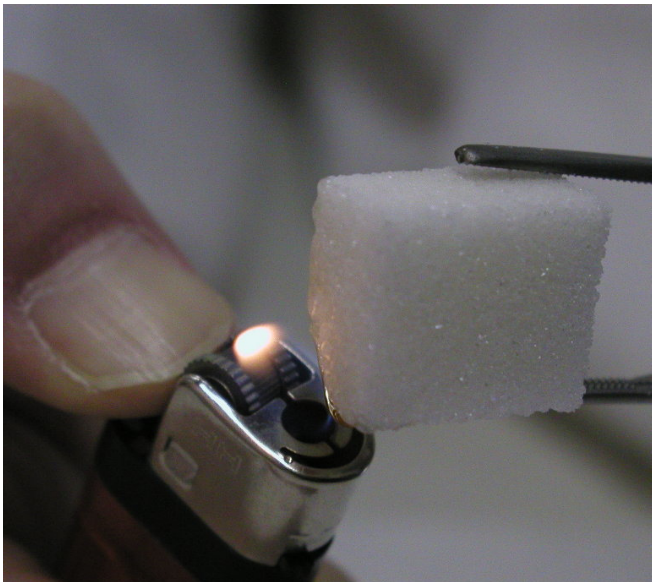
E.g. Glucose: 

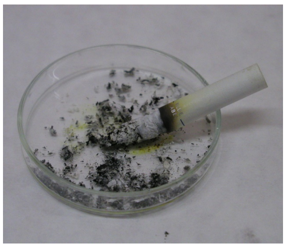
$$C_6H_{12}O_6 \quad + \quad 6\,O_2 \quad \rightarrow \quad 6\,CO_2 \quad + \quad 6\,H_2O$$

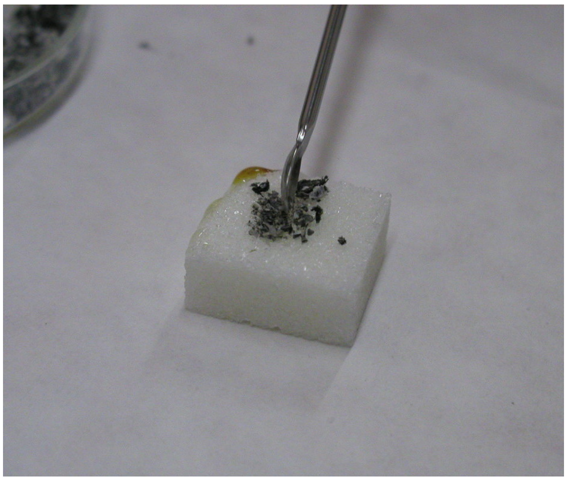
$\Delta G^{o\prime} = -2{,}820$ **kJ/ mol** (180 g of glucose)

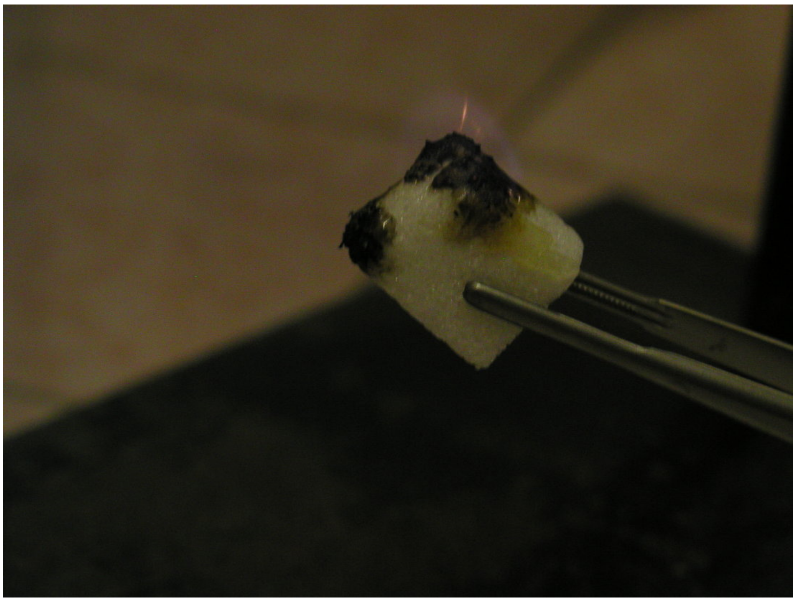
In addition, reactivity of oxygen can be controlled by catalysis with transition metals (iron, copper)

Does sugar burn ?

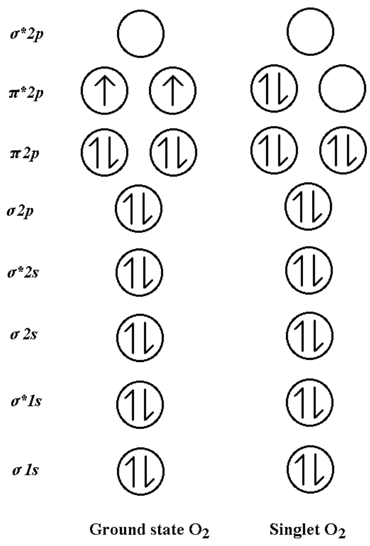




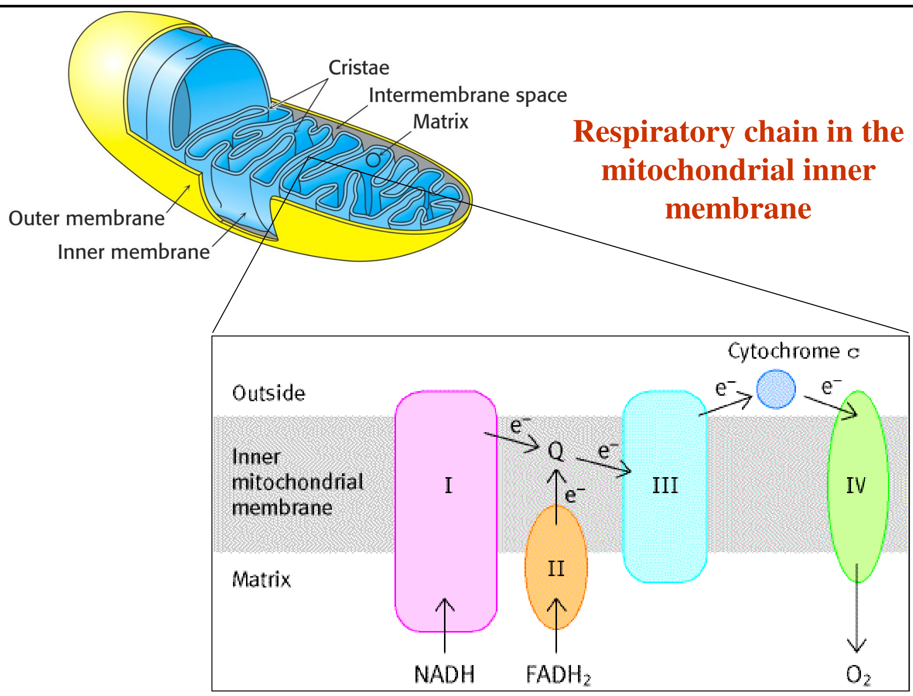




Spin restriction of dioxygen

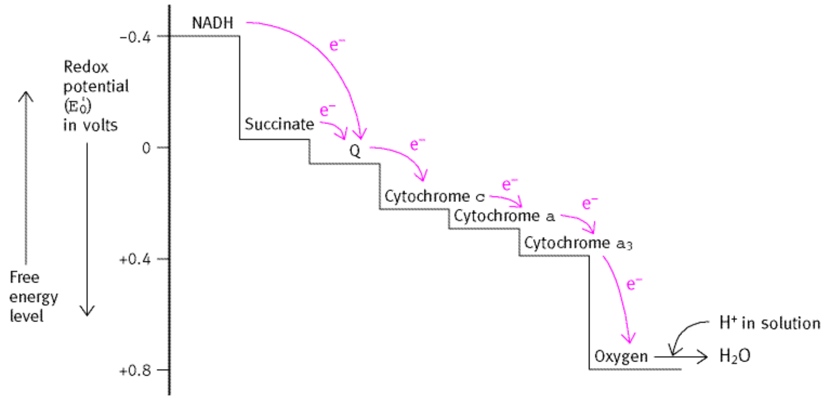


- Normal (triplet) O_2 is a biradical, with high affinity to electrons
- But gain of an electron requires one of the unpaired electrons changes its spin, which is a slow process.
- That's why we do not burn in the air
- Singlet O_2 is an excited, highly reactive species



Respiratory chain in the mitochondrial inner membrane

The respiratory chain:
..electrons flow 'downhill' and terminate at oxygen

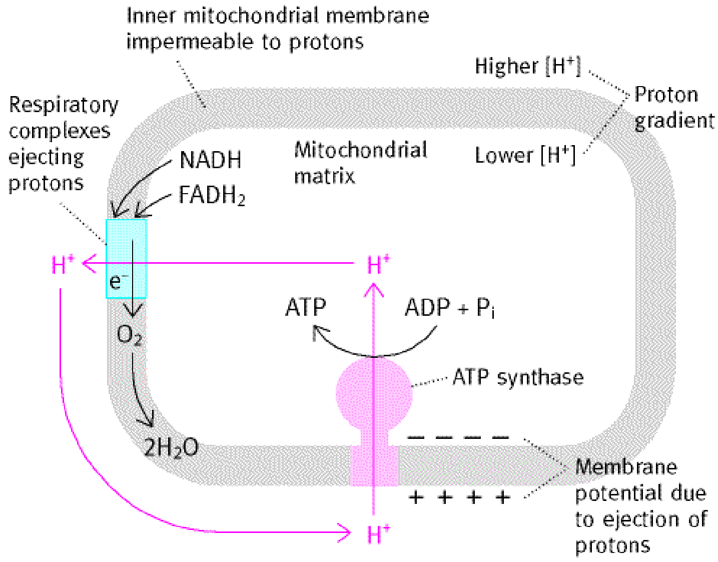


Overall reaction:

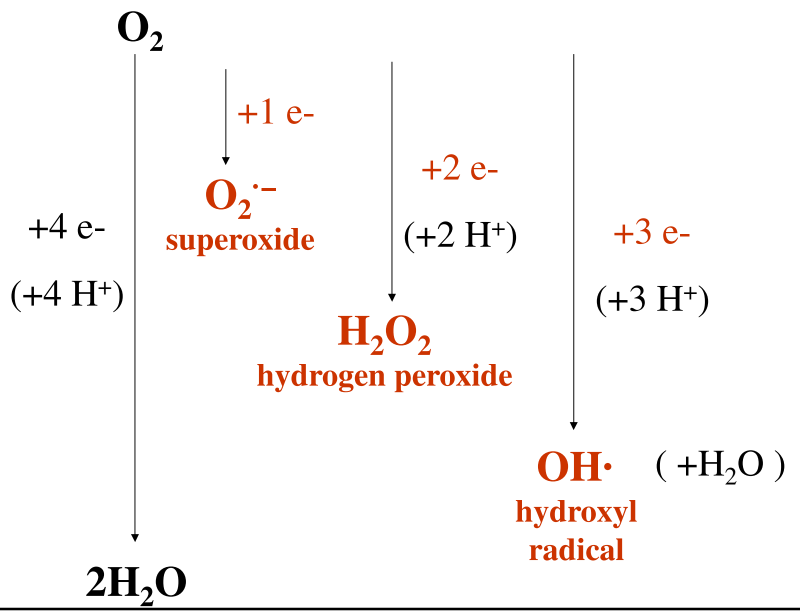
$$\text{NADH} + \text{H}^+ + 1/2\ \text{O}_2 \rightarrow \text{NAD}^+ + \text{H}_2\text{O}$$

$\Delta G^{o\prime} = -219.25$ kJ mol^{-1}

Together with flow of electrons protons are pumped, and the resulting proton gradient powers ATP synthesis



Reactive oxygen species (ROS, 'oxygen radicals') can be derived from intermediates of oxygen reduction to water:

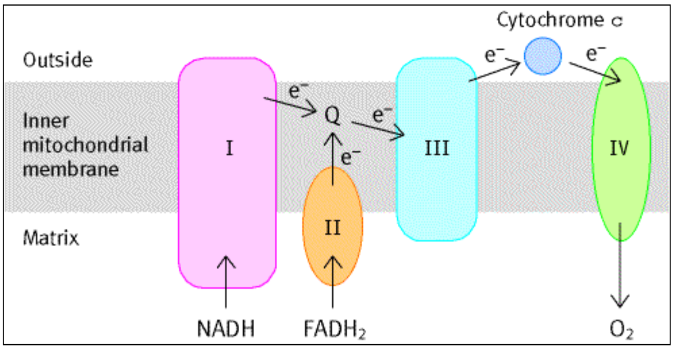


Superoxide

$O_2^{\cdot -}$

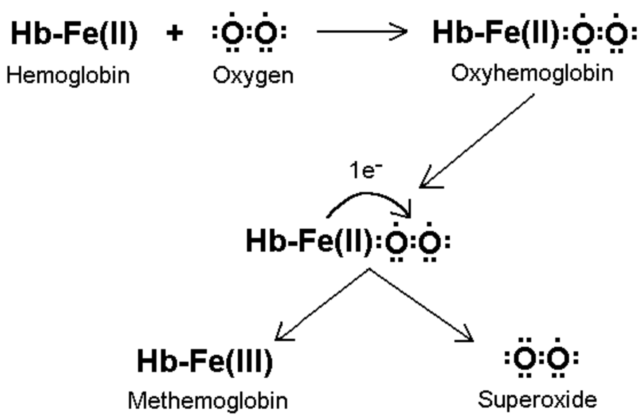
- ***Sources in the body:***
  - Escape of electrons to oxygen
    - Respiratory chain in mitochondria
    - Other similar redox carrier systems, e.g. microsomal cytochrome P450 monooxygenase
  - NAD(P)H Oxidase:
    - Phagocytes ("respiratory burst")
    - Non-phagocytic cells
  - Some enzymes:
    - Xanthine oxidase
    - Cyclooxygenase
    - Lipoxygenase
  - Reaction of FeII-hemoglobin with oxygen
  - Autoxidation (reaction with oxygen) of various compounds (ascorbate, glutathione and other thiols, catecholamines)

Superoxide production in the respiratory chain



- 1-2 % of total body oxygen consumption from in vitro experiments, in vivo surely much less
- Escape of electrons from redox centers of complexes I and III, mostly from semiquinone

Origin of superoxide from oxyhemoglobin



Štípek S et al.: Antioxidanty a volné radikály ve zdraví a nemoci. Grada Publishing, Praha, 2000.

Superoxide

$O_2^{\cdot -}$

- ***Properties:***
  - Moderate reactivity, both oxidising and reducing agent
  - Limited ability to cross biological membranes (only through anion channels or in its protonated form)
  - Releases iron from Fe-S clusters
- ***Fate:***
  - **Dismutation:**

$$O_2^{\cdot -} + O_2^{\cdot -} + 2\,H^+ \rightarrow O_2 + H_2O_2$$

  - **Reaction with nitric oxide:**

$$NO^{\cdot} + O_2^{\cdot -} \rightarrow OONO^{-}$$ (peroxynitrite)

Hydrogen peroxide

H_2O_2

- ***Formation in the body:***
  - Dismutation of superoxide (spontaneous or catalyzed by superoxide dismutase)

$$O_2^{\cdot -} + O_2^{\cdot -} + 2\,H^+ \rightarrow O_2 + H_2O_2$$

  - Directly by action of some enzymes:
    - Xanthine oxidase
    - Monoamine oxidase (MAO)

Hydrogen peroxide

H_2O_2

- ***Properties:***
  - No radical
  - Freely crosses biological membranes
  - Fairly unreactive as such
  - But rapidly reacts with reduced transition metals such as iron, copper (the Fenton reaction)
- ***Fate:***
  - **The Fenton reaction:**

hydroxyl radical

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

  - **Safe decomposition** by glutathione peroxidase, peroxiredoxin or catalase

Hydroxylový radikál

$OH\cdot$

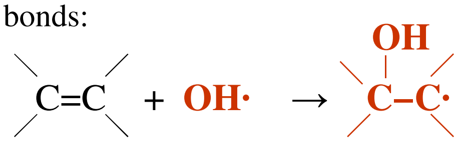
- ***Formation in the body:***
  - Ionising radiation:

$$H_2O \rightarrow H\cdot + OH\cdot$$

  - Fenton reaction:

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

- ***Properties:***
  - Extremely reactive
  - H abstraction: $R\text{-}H + OH\cdot \rightarrow H_2O + R\cdot$
  - Addition to double bonds:



Reactive Oxygen Species (ROS)

- Radicals:
  - Superoxide, $O_2^{\cdot -}$
  - Hydroperoxyl radical, $HO_2^{\cdot}$
  - Hydroxyl radical, OH·
  - Peroxyl radicals, ROO·
  - Alkoxyl radicals, RO·
- Non-radicals:
  - Hydrogen peroxide, H_2O_2
  - Hypochlorous acid, HClO
  - Ozone O_3
  - Singlet oxygen, $^1O_2$

Nitric oxide

NO·

- ***Formation in the body:***
  - **Aerobic**: NO synthase reaction:

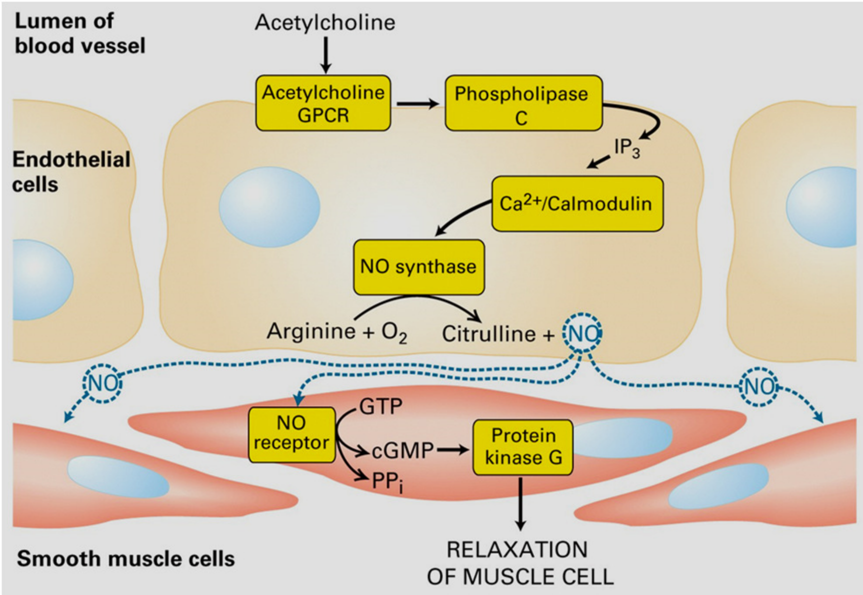
L-Arginine + O_2 + NADPH $\rightarrow$ L-Citrulline + $NADP^+$ + **NO·**

- Three different NO synthases:
  - NOS I (neuronal, constitutive)
  - NOS II (fagocytes, inducible)
  - NOS III (endothelial, constitutive)

- **Hypoxia:** Reduction of nitrite catalysed by hemoglobin and myoglobin:

DeoxyHb(FeII) + NO_2^- + H^+ $\rightarrow$ DeoxyHb(FeIII) + OH^- + **NO·**

NO causes relaxation of smooth muscles in blood vessel wall:



Nitric oxide

NO·

- ***Properties:***
- Gaseous radical
- Reaction with heme iron of guanylate cyclase (…physiological effects)
- Reaction with oxyhemoglobin to nitrate (…inactivation)
- Reaction with sulfhydryl groups on glutathione etc. to nitrosothiol R-S-N=O (…transport/storage)
- Reaction with superoxide to peroxynitrite – then some can form hydroxyl radical (…toxicity):

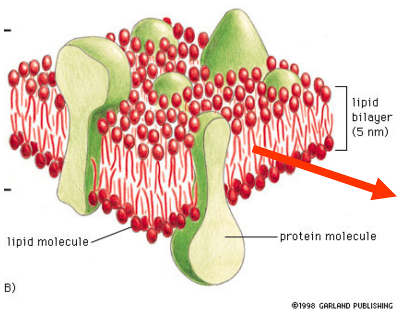
$$NO^{\cdot} + O_2^{\cdot -} \rightarrow OONO^{-}$$ peroxynitrite

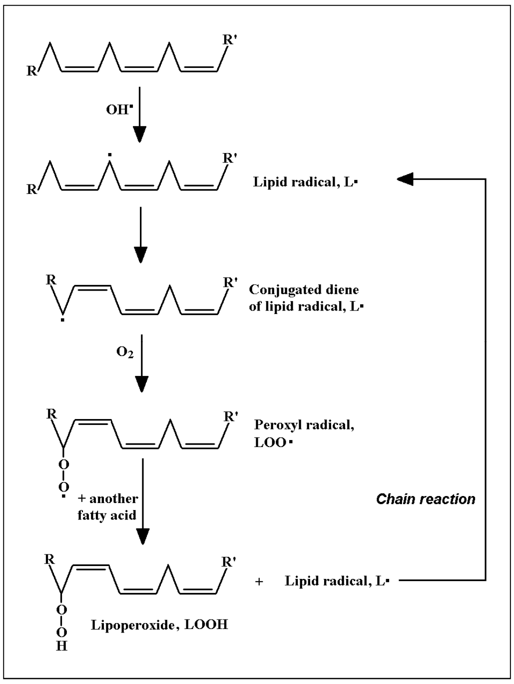
$$OONO^{-} + H^{+} \rightarrow HOONO \rightarrow OH^{\cdot} + NO_2^{\cdot}$$

peroxynitrous acid (HOONO); hydroxyl radical (OH·)

Oxidative damage to biomolecules

- **Lipids:** peroxidation of polyunsaturated fatty acids in membranes
- **Proteins:** oxidation of -SH, carbonylation of $-NH_2$, hydroxylation/nitration of aromatic AA, cross-linking, degradation
- **Nucleic acids:** hydroxylation of bases, single or even double strand breaks ... mutation, cancerogenesis…



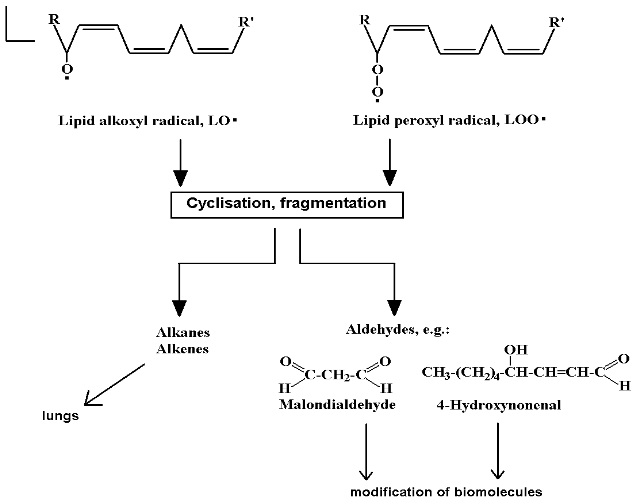


Lipid peroxidation (rancidity)

Further fate of lipid peroxides

$$LOOH + Fe^{2+} \text{ (or } Cu^{+}) \rightarrow LO\bullet + Fe^{3+} \text{(or } Cu^{2+}) + OH^{-}$$

$$LO\bullet + LOOH \rightarrow LOH + LOO\bullet$$



Ionising radiation:

Hydroxyl radical originates from ionisation of water:

$$H_2O + h\nu \rightarrow H\cdot + OH\cdot$$

Reactive oxygen species in the body:

One-electron reduction of oxygen (mitochondria, NADPH oxidase) forms superoxide $O_2^{\cdot -}$

Dismutation of superoxide produces hydrogen peroxide:

$$O_2^{\cdot -} + O_2^{\cdot -} + 2\,H^+ \rightarrow O_2 + H_2O_2$$

Fenton reaction with Fe or Cu converts peroxide to hydroxyl radical:

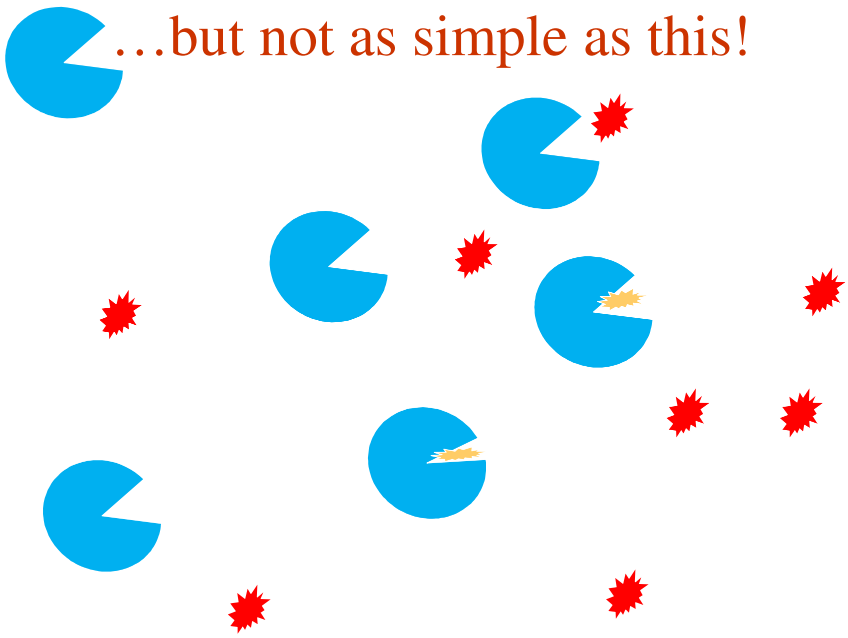
$$H_2O_2 + Fe^{2+} \rightarrow OH^- + OH\cdot + Fe^{3+}$$

Antioxidant defence

- prevention of ROS/RNS formation (regulation of producing enzymes, sequestration of metals)
- scavenging, trapping and quenching of radicals
- reparation systems (phospholipases, proteasome, DNA repair)

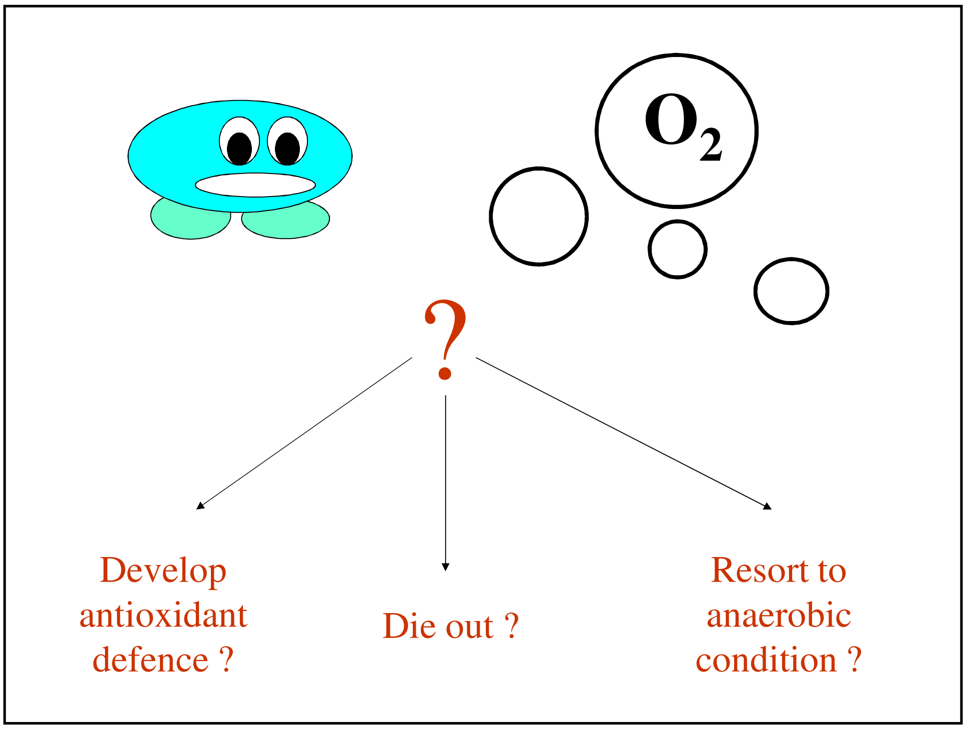
…absolutely necessary for life with oxygen

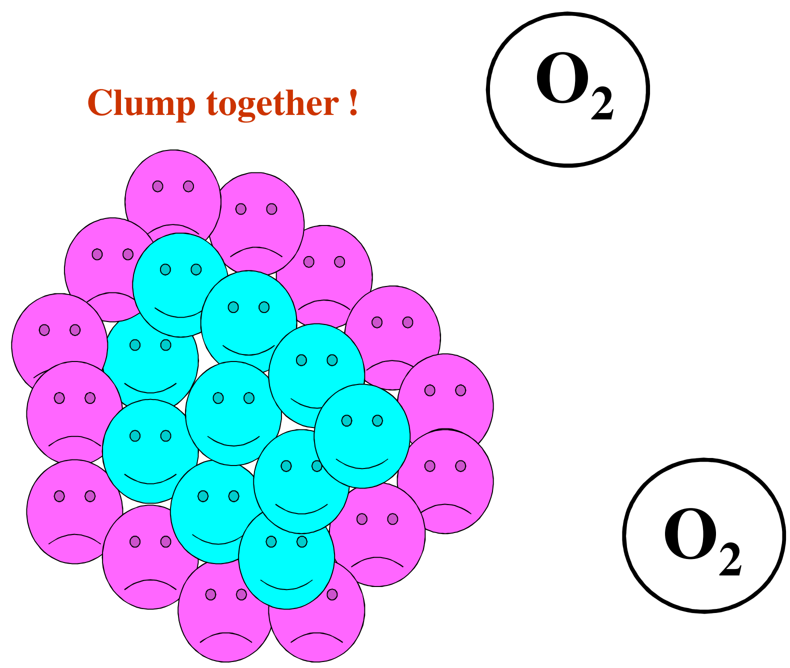
…but not as simple as this!



Antioxidant defence of human body

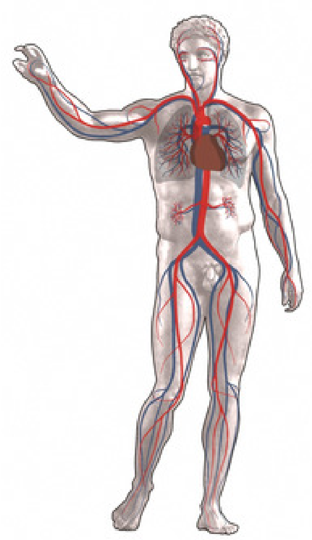
- Anatomy of the body limiting tissue oxygen
- Antioxidant enzymes
- Sequestration of redox active metals
- Antioxidant substrates (scavengers)
- Stress response
- (Repair systems)





Antioxidant defence I

Regulation of tissue O_2



Inhaled air:	160 mmHg O_2
Lung capillaries:	100 mmHg O_2
Arterial blood:	85 mmHg O_2
Arterioles:	70 mmHg O_2
Capillaries:	50 mmHg O_2
Cells:	1-10 mmHg O_2
Mitochondria:	< 0,5 mmHg O_2

Fig: Wikipedia

Antioxidant defence II

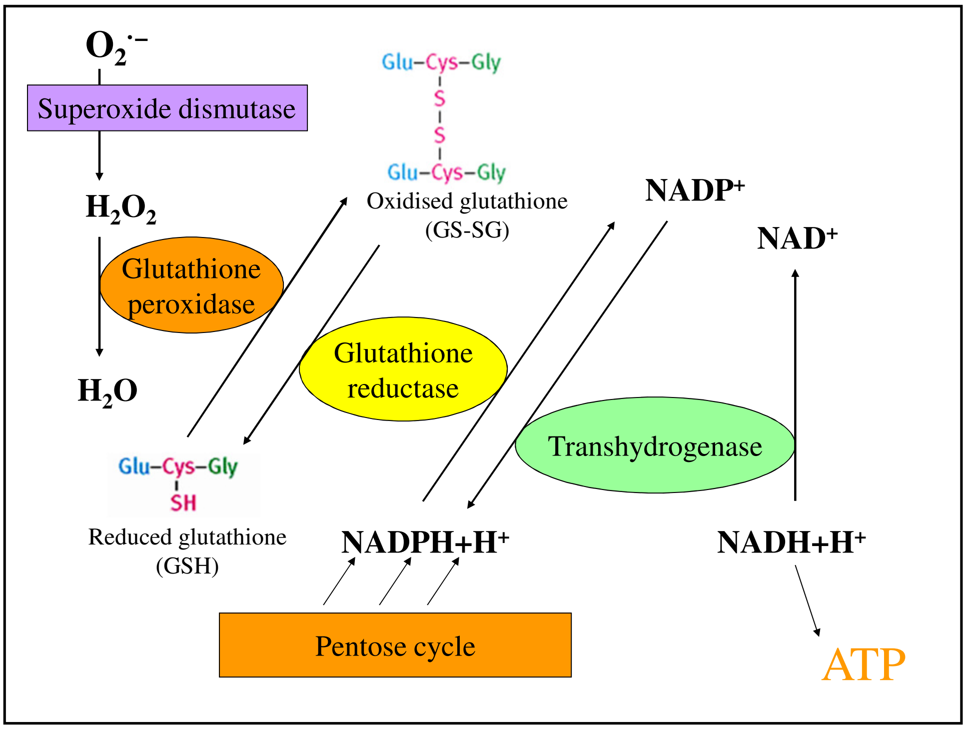
Antioxidant enzymes

- Superoxide dismutase:

  $O_2^{\cdot -} + O_2^{\cdot -} + 2\,H^+ \rightarrow O_2 + H_2O_2$
- Catalase:

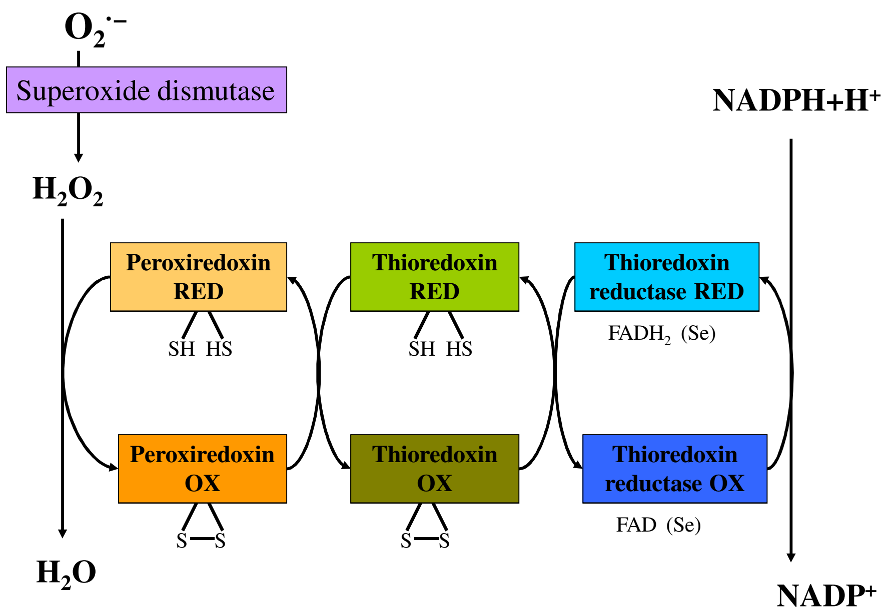
  $2\,H_2O_2 \rightarrow 2\,H_2O + O_2$
- Glutathione peroxidase, peroxiredoxin:

  $H_2O_2 + 2\,R\text{-}SH \rightarrow 2\,H_2O + RS\text{-}SR$



Peroxiredoxin/Thioredoxin

- More recently discovered antioxidant system, more important for removal of hydrogen peroxide than GPX



Antioxidant defence III

Sequestration of metals

- Redox-active transition metals (Fe, Cu) accept/donate one electron easily
  - ... alleviation of spin restriction of dioxygen
  - ... metals are in active centers of all oxygen handling-enzymes
- But, the same properties of Fe, Cu are deleterious if uncontrolled
  - the Fenton oxidant:

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

oxidative damage to biomolecules

Antioxidant defence III

Sequestration of metals

- Iron/copper handling proteins:
  - transferrin: binds 2 atoms Fe^{3+} (transport)
  - lactoferrin: analogous to transferrin, but no Fe release (... only sequestration), leucocytes
  - ferritin: H and L subunits, H is ferroxidase, Fe storage (up to 4500 atoms Fe^{3+})
  - haptoglobin: binds hemoglobin in circulation
  - hemopexin: binds heme in circulation
  - ceruloplasmin: contains Cu, function: ferroxidase (export Fe from the cells)
  - albumin: transport of Cu

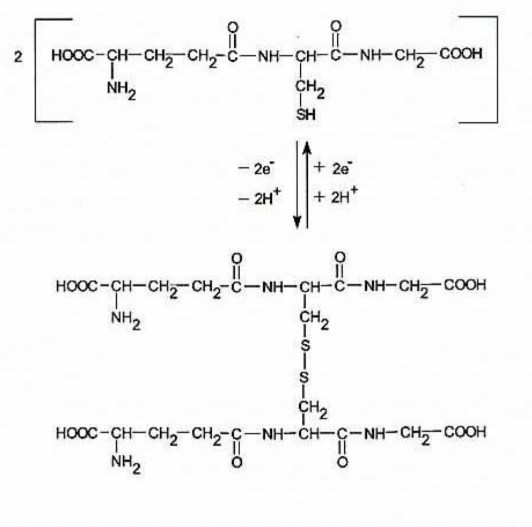
ECT	ICT
Superoxide **Peroxide** **Fe/Cu**	**Superoxide** **Peroxide** **Fe/Cu**
Antioxidant enzymes & glutathione levels very low	**Superoxide dismutase** **Peroxiredoxins** **Glutathione peroxidases** **Catalase**
Sequestration of iron and copper: **- Transferrin, lactoferrin** **- Haptoglobin** **- Hemopexin** **- Ceruloplasmin (ferroxidase)** **- Albumin (binds Cu)**	*Excess iron stored in ferritin, but some redox-active iron present*

Antioxidant defence IV
Low-molecular-weight antioxidant substrates

- ENDOGENOUS:
  - Glutathione
  - Bilirubin
  - Uric acid
  - …
- DIETARY:
  - Ascorbate (Vitamin C)
  - α-Tocopherol (Vitamin E)
  - Carotenoids
  - Plant phenols
  - …

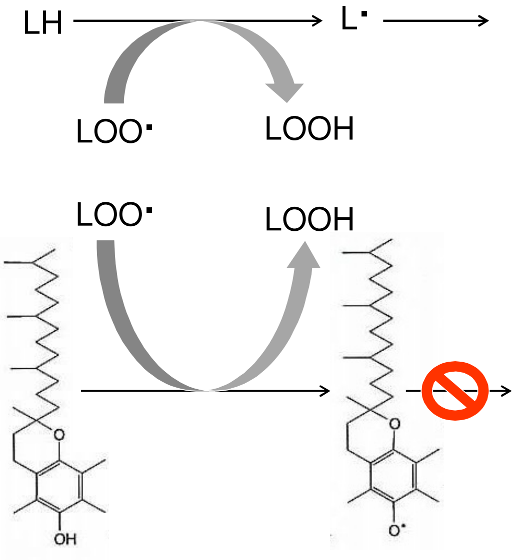
Glutathione (GSH/GSSG)

- tripeptide, in every cell 1-10 mM
- keeps ICT reduced
- substrate for GPX, etc.
- also non-enzymatic reactions with ROS and mixed disulfides with proteins ... products of GSH oxidation are toxic for cell
- in oxidative stress the cell exports GSSG out

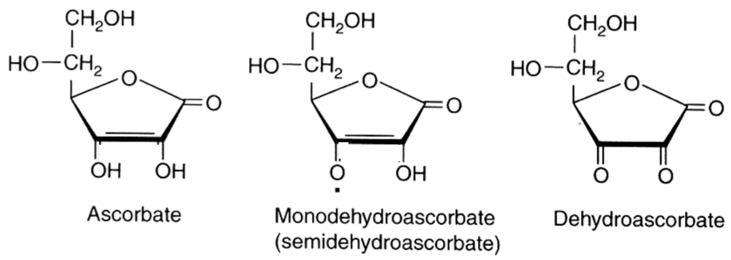


Vitamin E

- group of 8 substances, α-tocopherol most effective
- antioxidant of membranes (lipophilic)
- "**chain-breaking**" ... terminates the chain reaction of lipid peroxidation



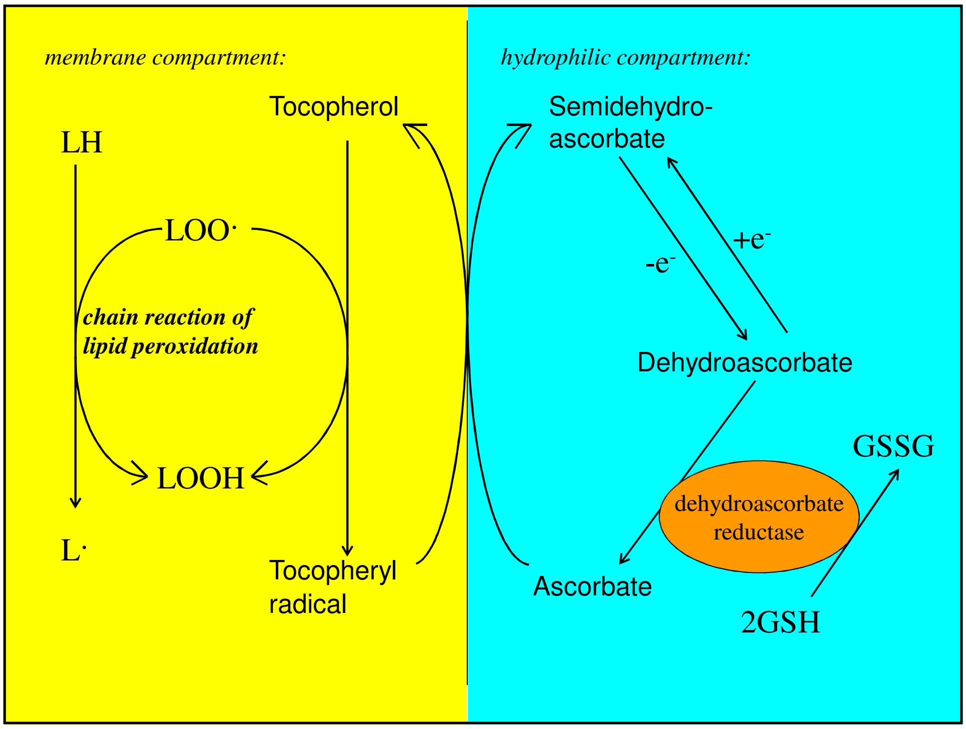
Ascorbate (Vitamin C)



- Redox-active saccharide
- In most animals synthesized from glucuronic acid
- Vitamin for humans, other primates, bats and guinea pigs
- Deficit causes scurvy (scorbut)

Ascorbate in the body:

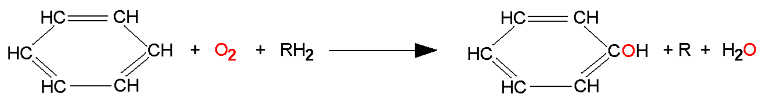
- Main function is pro-oxidant: **cofactor of hydroxylases**
  - Hydroxylation of Pro and Lys in collagen synthesis
  - Synthesis of noradrenaline from dopamine
  - Synthesis of carnitine (… role in oxidation of fat)
  - Activation of hypothalamic peptidic hormones by amidation (CRH, GRH, oxytocin, vasopressin, substance P)
- Reductant for iron: promotes its intestinal absorption
- Potentially dangerous pro-oxidant if iron sequestration impaired (hemochromatosis) (?)
- Daily need 70-100 mg, high doses p.o. excreted by urine (renal threshold cca 200 mg/24 hours)



- **Selenium:**
  - Trace element (daily need 55 µg), possibility of deficiency as well as intoxication
  - Component of several antioxidant enzymes (glutathione peroxidase, thioredoxin reductase) and also e.g. 5‘-deiodase
- **β-carotene** (provitamin A)
  - Antioxidant only in the skin
  - Precursor for biosynthesis of:
    - Retinal … vision
    - Retinoic acid …regulator of gene expression, cellular growth and differentiation

Where RONS are beneficial: I. Active centers of some enzymes

- **Monooxygenases (cytochromes P450):**
  - Detoxication of drugs, synthesis of steroid hormones and bile acids, NO synthases



Mechanism: **oxidation by ferryl intermediate**

Fe^{III} + RH + 2 e^- + O_2 + H^+ → Fe^{III}-OOH^- RH

Fe^{III}-OOH^- RH + H^+ → **Protein$^{\bullet+}$-Fe^{IV}=O** RH + H_2O

Protein$^{\bullet+}$-Fe^{IV}=O RH → Protein-Fe^{IV}-OH R• →
→ Fe^{III} + ROH

Where RONS are beneficial: I. Active centers of some enzymes

- Monooxygenases (cytochromes P450)
- Ribonucleotide reductase (…DNA synthesis)
- Synthesis of prostanoids (enzymatic lipoperoxidation)
- Useful heme peroxidases:
  - Thyreoid peroxidase:

I^- + H_2O_2 + $2H^+$ → I^+ + $2H_2O$, I^+ iodinates thyreoglobulin

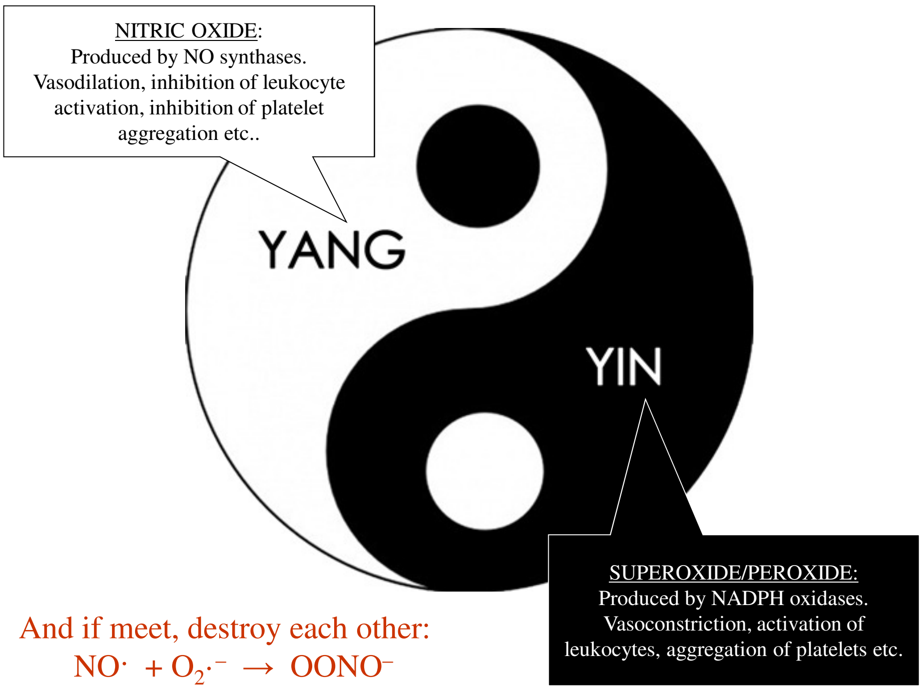
  - Myeloperoxidase of neutrophils
  - Lactoperoxidase in milk, respiratory tract mucus and saliva:

SCN^- + H_2O_2 → $OSCN^-$ + H_2O

(hypothiocyanate $OSCN^-$ is toxic to bacteria)

Where RONS are beneficial: II. Signaling

- E.g. nitric oxide, NO:
  - Smooth muscle relaxation in blood vessels, gastrointestinal tract, corpus cavernosum penis
  - neurotransmitter/ neuromodulator in CNS, function in synaptic plasticity, learning and memory
  - Inhibition of adhesion and aggregation of platelets,
  - Inhibition of adhesion of leukocytes
  - (etc.)



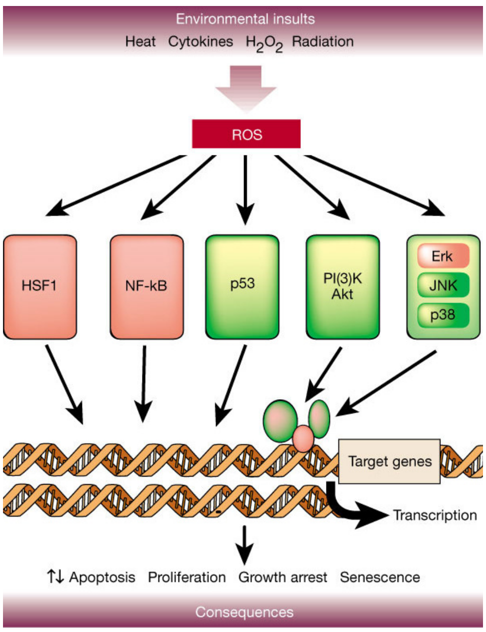
And if meet, destroy each other:

$NO^{\cdot} + O_2{\cdot}^{-} \rightarrow OONO^{-}$

Concept of redox signaling

- H_2O_2 as intracellular signaling molecule
- Redox environment inside the cell – determined mostly by ratios GSH/GSSG and NADH/NAD
- Redox sensors on proteins:
  - critical -SH groups
  - Fe-S centers
- Targets of redox signaling:
  - transcription factors
  - protein kinases and phosphatases

Redox signaling



- Oxidative stress activates certain protein kinases and transcription factors
- Outcome: e.g. stimulation of proliferation, differentiation, cellular senescence (ageing), apoptosis…
- Actions of ROS/antioxidants in the cell can be very specific.

(T. Finkel & N.J. Holbrook, Nature 408 (2000), 239-247)

Antioxidant defence V

Stress response

Oxidation or nitrosylation of sensor -SH

↓

Transcription factors (NFκB, Nrf-2…): activation, nuclear translocation

↓

Induction of gene expression:
- chaperones (heat shock proteins)
- antioxidant enzymes
- metallothionein
- hemoxygenase 1

…→ more resistant to further oxidative stress

Where RONS are beneficial: III. Phagocytosis

- Neutrophils, eosinophils, monocytes, macrophages, microglia
- Often the target particle needs to be marked (coated with opsonins)
- Phagocyte tasks:
  - Recognize
  - Engulf
  - Kill
  - Digest

Neutrophilic granulocyte (polymorphonuclear)

- Segmented nucleus
- Primary (azurophilic) granules: lysozyme, defensins, myelo-peroxidase, proteases
- Specific (secondary) granules: NADPH oxidase, cobalophilin, lactoferrin, lysozyme, collagenase
- Tertiary granules: gelatinase and other enzymes

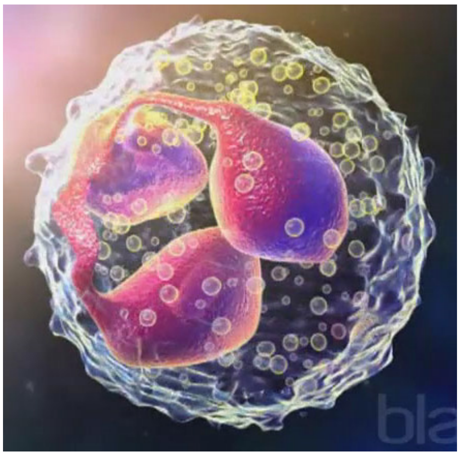


Fig.:http://blausen.com

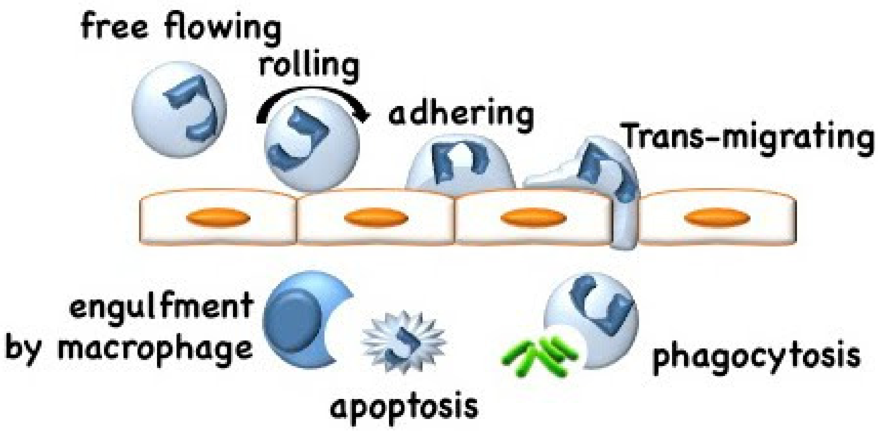


Fig.: http://walchecklab.umn.edu/_/rsrc/1417821135388/research-interests/neutrophil-recruitment

Activation of a phagocytic cell: **Respiratory burst**

- Dramatic increase in consumption of oxygen (for superoxide production, not respiration)
- Dramatic increase in consumption of glucose (for the pentose cycle – produces NADPH for NADPH oxidase)

NADPH oxidase

- Membrane enzyme producing a superoxide flux outwardly or into a phagosome.
- Catalyzes reaction:

  $NADPH + 2O_2 \rightarrow NADP^+ + H^+ + 2O_2{\cdot}^-$
- Latent in resting cell, assembles on the cell membrane from 6 subunits during phagocyte activation.
- Necessary for an ability to kill some kinds of engulfed bacteria (hereditary deficit: chronic granulomatous disease)
- Similar enzymes are present also on the endothelial and many other non-phagocytic cells

Myeloperoxidase

- 2 – 5% of total protein in neutrophils
- Hemoprotein that gives pus its green tinge
- Non-specific peroxidase, in the body catalyzes reaction:

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

- Deficit in humans is common (1 in 2,000-4,000) and is not severe

Hypochlorous acid HClO

- Weak acid ($pK_a = 7.5$; $HClO \leftrightarrow ClO^- + H^+$)
- In acidic medium readily decomposes to chlorine gas:

$$HClO + H^+ + Cl^- \leftrightarrow Cl_2 + H_2O$$

- Strong oxidizing agent:

$$2HClO + 2e^- \rightarrow Cl_2 + 2e^- \rightarrow 2\,Cl^-$$

- Reaction with Fe^{2+} or superoxide generates hydroxyl radical:

$$HClO + Fe^{2+} \rightarrow Cl^- + Fe^{3+} + OH\cdot$$
$$HClO + O_2\cdot^- \rightarrow Cl^- + O_2 + OH\cdot$$

- With organic amines gives chloramines:

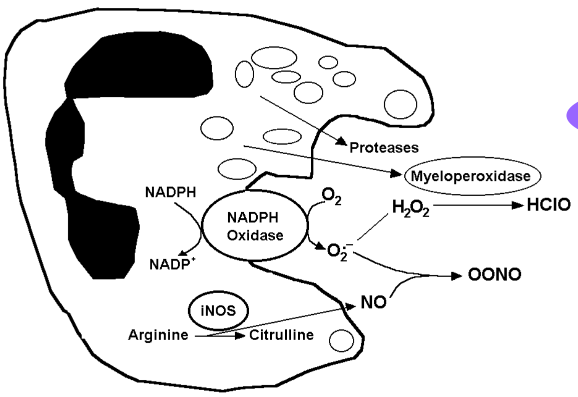
$$R\text{-}NH_2 + HClO \rightarrow R\text{-}NH\text{-}Cl + H_2O$$

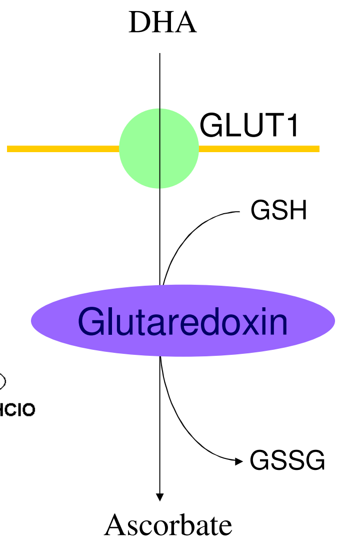
Further neutrophil weapons

- Powerful proteolytic enzymes: elastase, collagenase, cathepsin G, gelatinase etc.
- Lysozyme: cleaves polysaccharide of Gram+ bacterial cell wall
- Lactoferrin: binds iron to make unavailable for bacteria
- Defensins: cationic proteins forming channels in bacterial cell membrane
- NETs (neutrophil extracellular traps): fibers of DNA and serine proteases, catch bacteria extracellularly

RONS generators and other weapons can synergize:
e.g.. HClO inhibits α1-antitrypsin

Activated neutrophiles accumulate dehydroascorbate (DHA)





Protects membrane of the neutrophile from its own ROS ...

Neutrophil ROS in (dental) medicine

- Hydrogen peroxide:

  3 % H_2O_2 is used for wound disinfection, and treatment of acute gum inflammation

- Sodium hypochlorite NaClO (‚Savo‘):

  Root canal treatment (endodontics)

- Chloramine-T:

  General disinfectant in hospitals…