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Department CMIC

Lecture 9 – FR9





Free-Radicals: Chemistry and Biology

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http://iscamap.chem.polimi.it/citterio/education/free-radical-chemistry/

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Lipid Peroxidation

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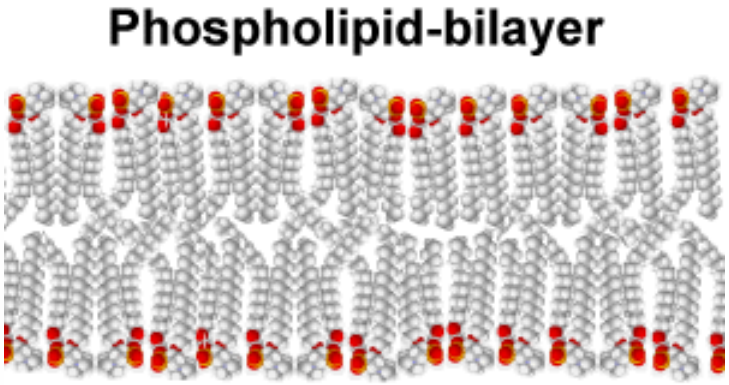
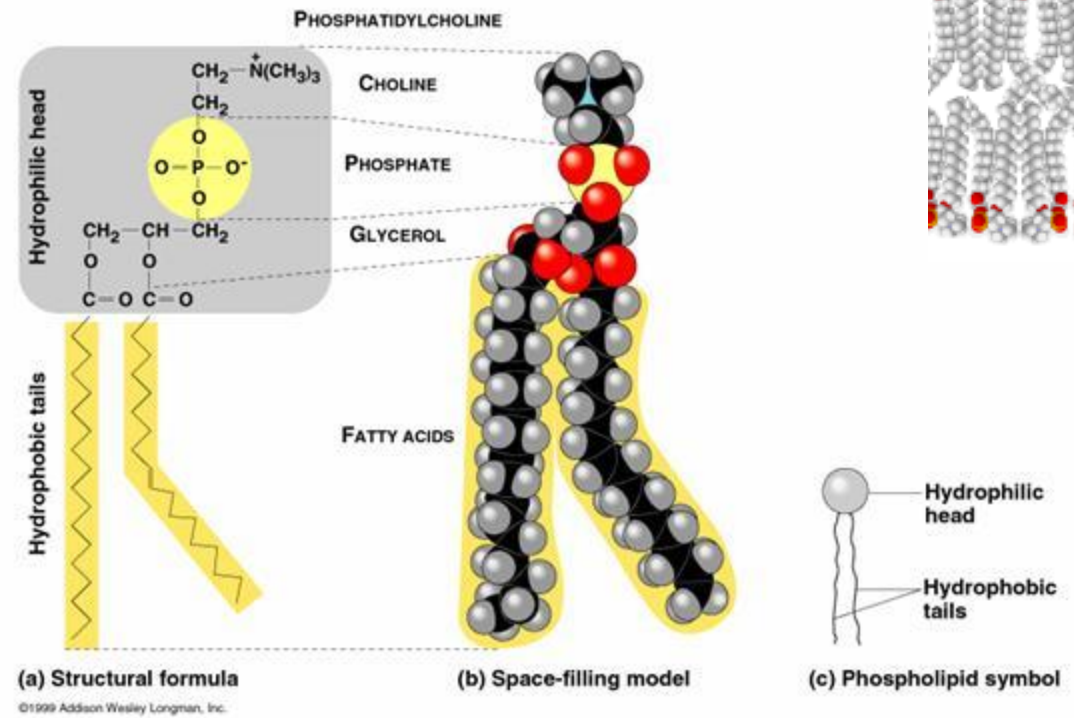
Lipid Structure

Main forms:

1) Triglycerides
2) Phosphoglycerides





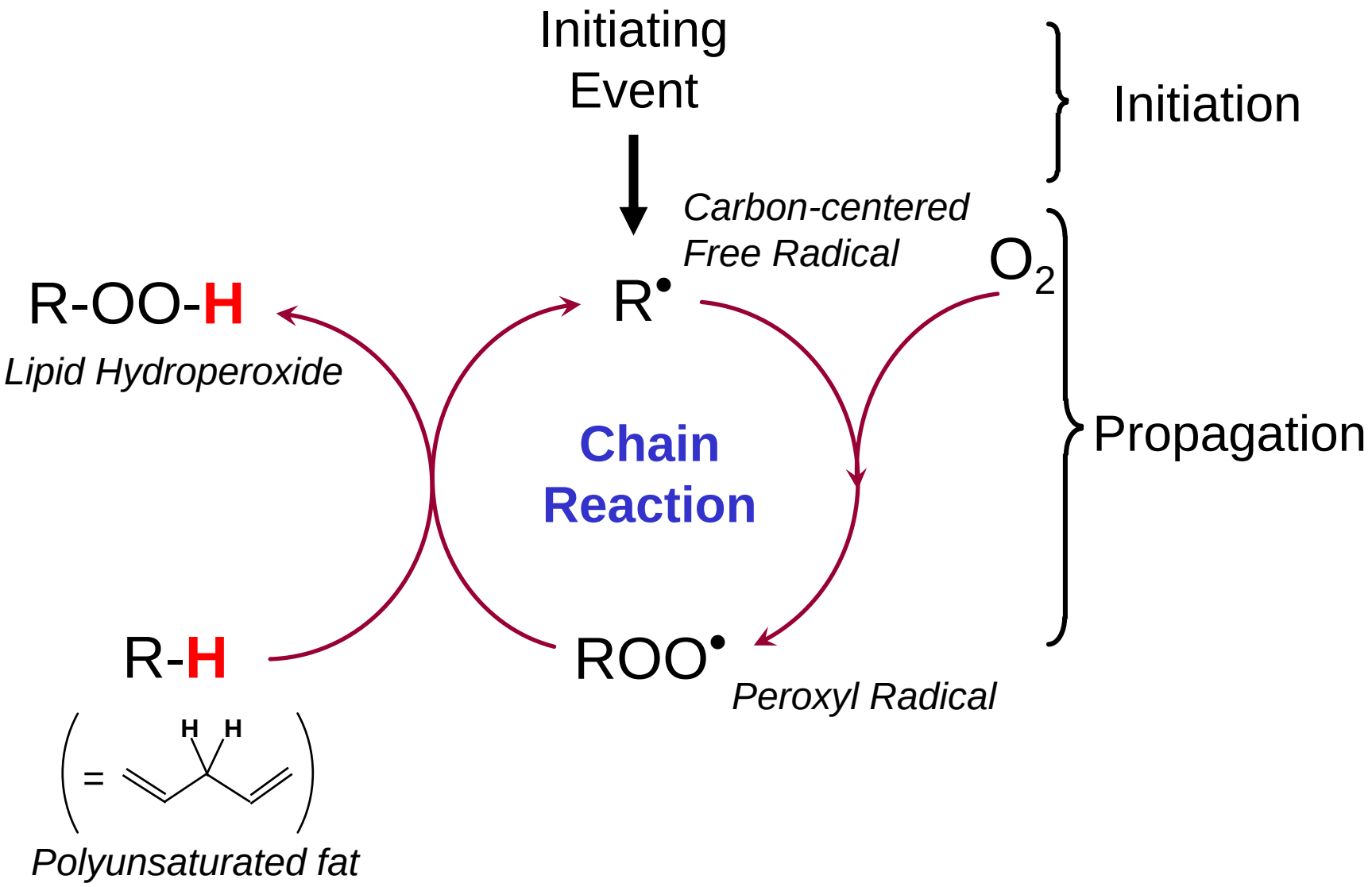
Lipid Peroxidation

Defined as "the oxidative deterioration of polyunsaturated fatty acids" (A.L. Tappel)

Although general peroxidation mechanisms well characterized, highly variable range of products are formed due to:

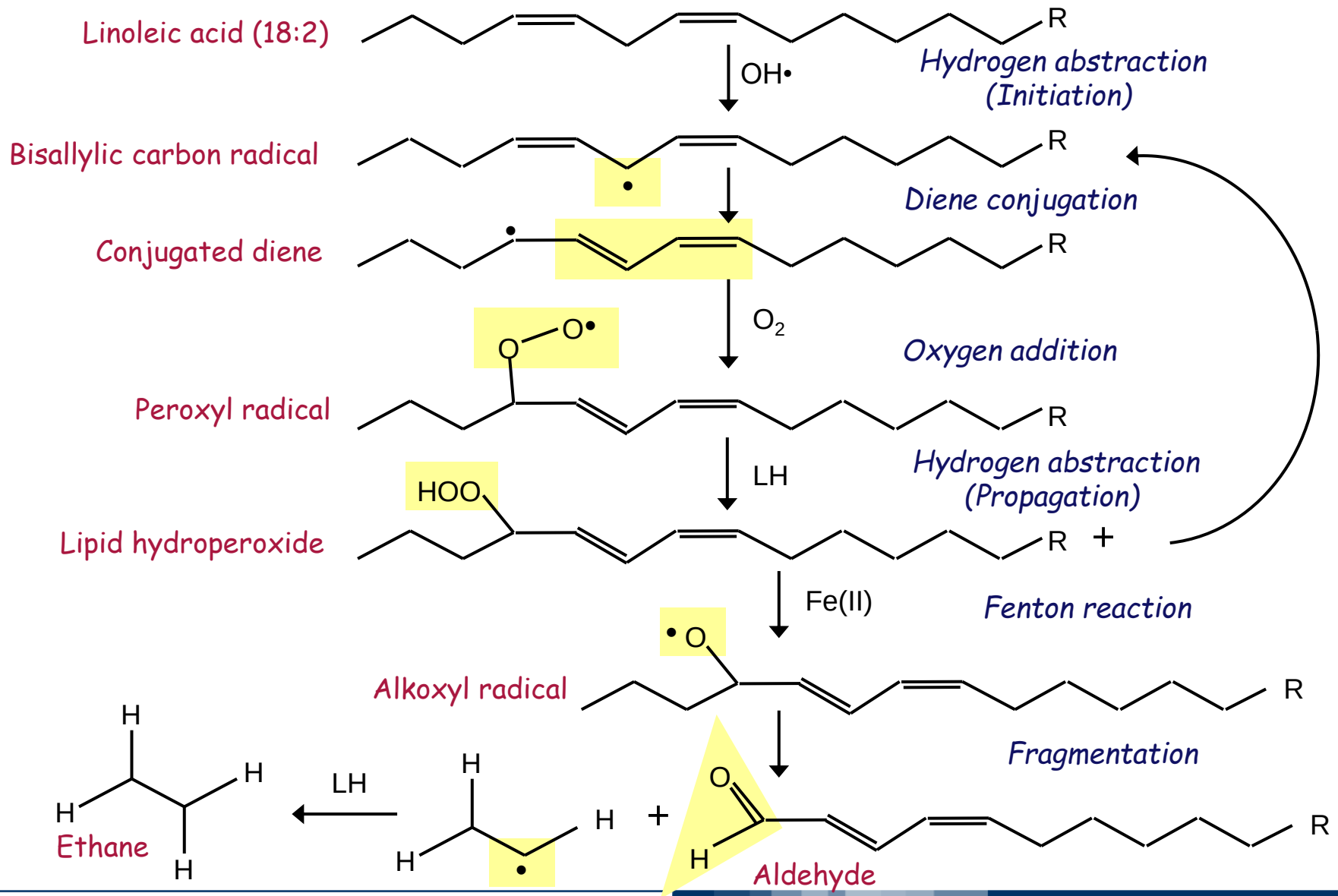
- range of different biological lipid classes (phospholipids, cholesterol esters, triglycerides)
- variable unsaturated fatty acids (16:1, 18:2, 20:4, 22:5); generally, the more unsaturated, the more oxidizable
- non-enzymatic (e.g. hydroxyl radical, metal ion catalyzed) and enzymatic oxidation mechanisms (e.g. lipoxygenases, cyclooxygenases)
- variable end products depending on secondary reactions, effects of antioxidants and repair/turnover

Lipid Peroxidation

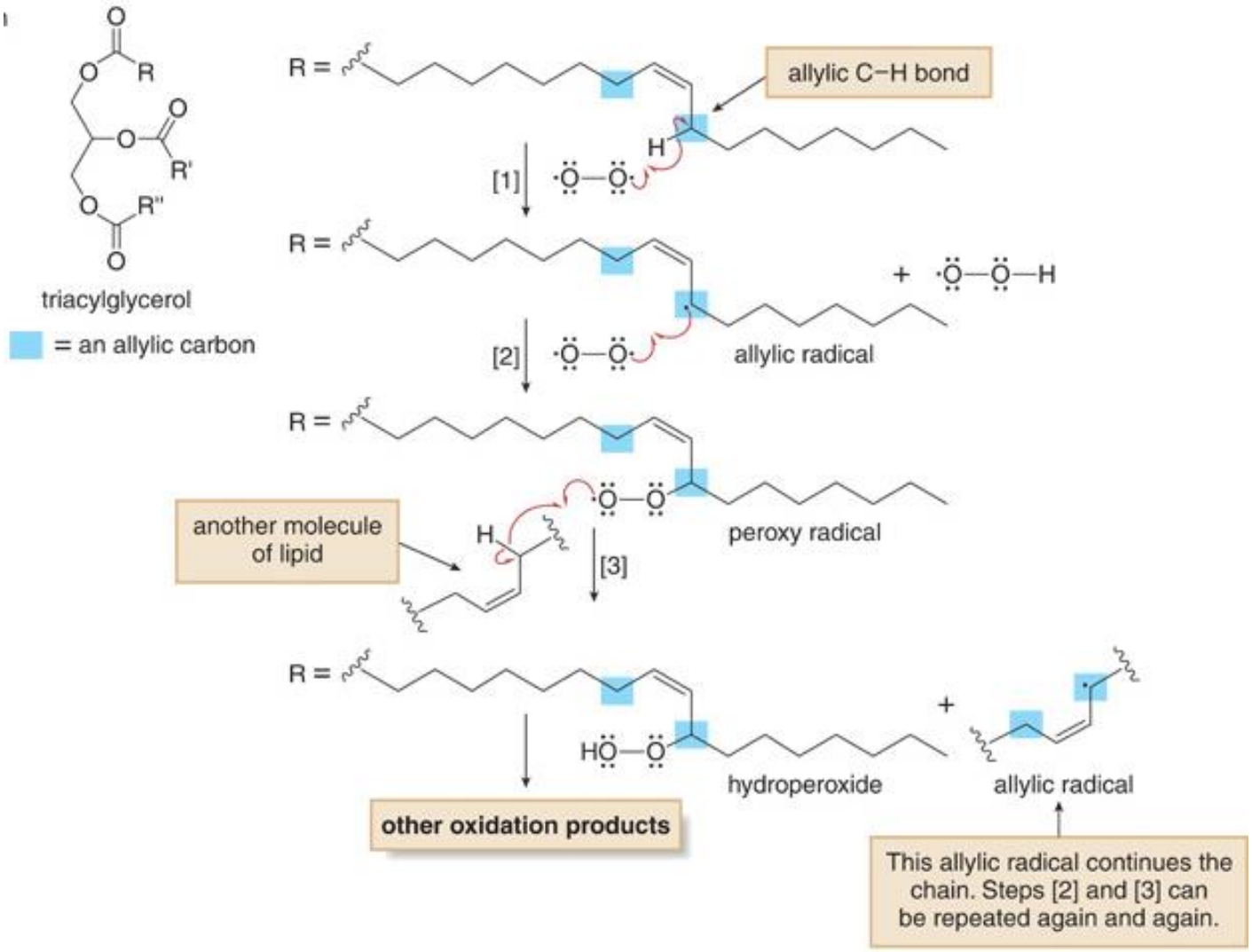


Burton & Traber. *Annu. Rev. Nutr.* 10: 357-382; 1990

Radical-mediated lipid peroxidation: initiation and propagation



The Oxidation of Unsaturated Lipids by O_2



- Oxidation is shown at one allylic carbon only. Reaction at the other labeled allylic carbon is also possible.

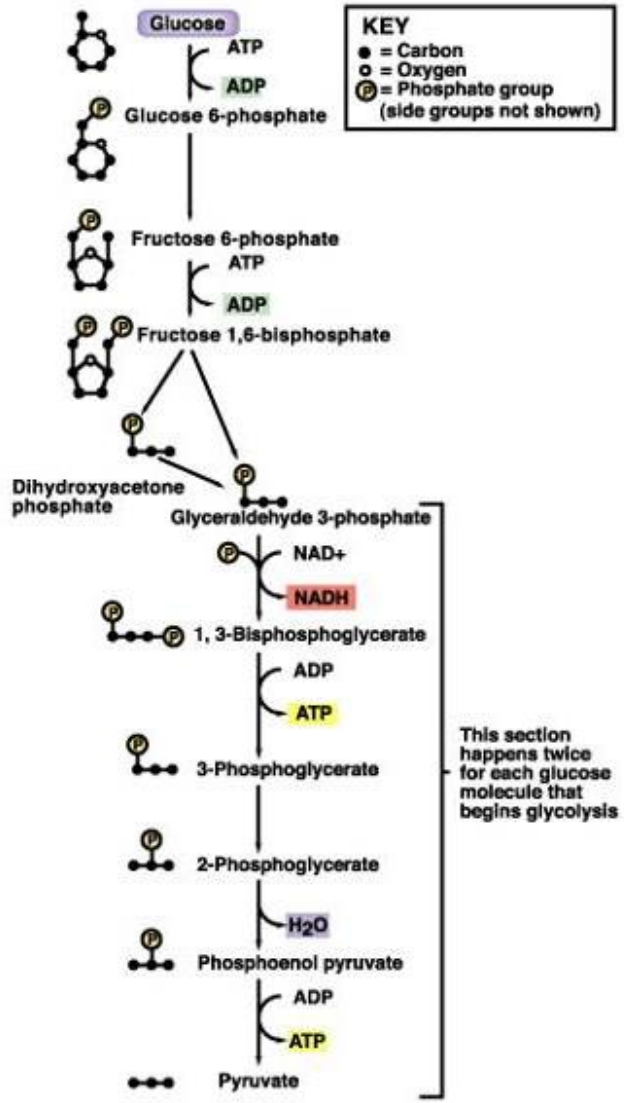
Sources of Free Radicals in Lipid Autoxidation – Exogenous sources

- **Transition Metals and Metal Camplexes**
- **Radiation**
- **Drugs**
  - Nitrofurantoin, adriamycin, methotrexate …
- **Tobacco smoking**
- **Inorganic particles**
  - mineral dust (e.g. asbestos, quartz, silica)
- **Reactive gases**
- **Others**
  - Fever, excess glucocorticoid therapy and hyperthyroidism

Sources of Free Radicals (Endogenous Sources)

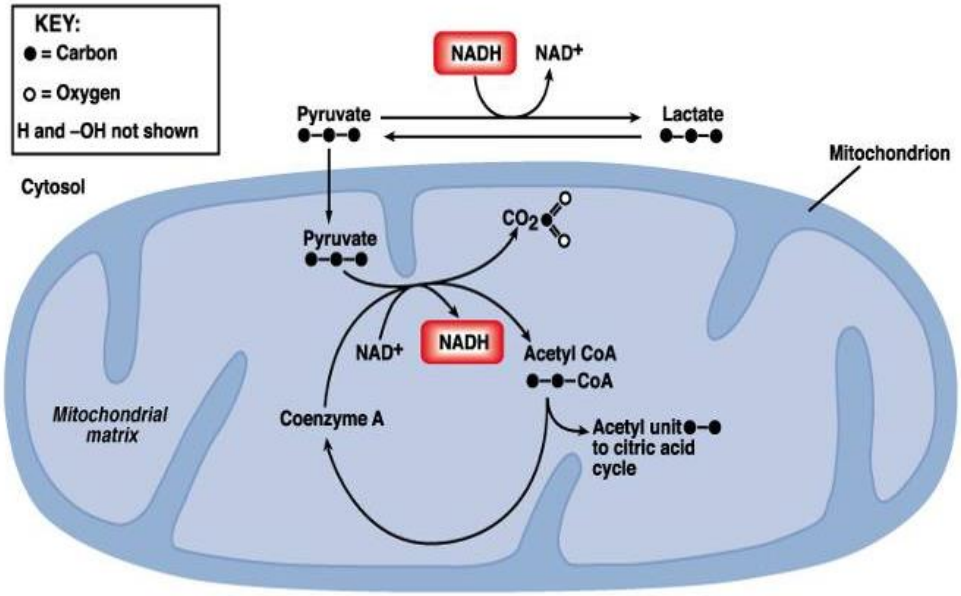
- **Autoxidation**
  - Several biological molecules undergo autoxidation easy, i.e. catecholamines, haemoglobin, myoglobin, reduced cytochrome C thiols and …
- **Enzymatic oxidation**
  - xanthine oxidase (activated in ischemia-reperfusion), prostaglandin synthase, lipoxygenase, aldehyde oxidase…
- **Respiratory burst**
  - a term used to describe the process by which phagocytic cells consume large amounts of oxygen during phagocytosis ; between 70 and 90% of this oxygen consumption can be accounted for in terms of superoxide production
- **Subcellular organelles**
  - Organelles such as mitochondria, chloroplasts, microsomes, peroxisomes and nuclei have been shown to generate $O_2^{\cdot -}$. Mitochondria are the main cellular organelle for cellular oxidation reactions and the main source of reduced oxygen species in the cell.

Electron Shuttle and Mitochondria



Remember that NAD^+ is used in animal cells to shuttle electrons in cellular metabolism

The NAD^+ (nicotinamide adenine dinucleotide) is reduced in 5 separate steps to NADH during **glycolysis**, the **transition reaction** (pyruvate oxidation step)*, and the **Krebs's cycle**.



Sources of free radicals (Endogenous sources)

- **Transition metals ions**
  - Iron and copper play a major role in the generation of free radicals injury and the facilitation of lipid peroxidation.
- **Ischemia reperfusion injury**
  - During ischemia two factors occur, first the production of xanthine and xanthine oxidase are greatly enhanced. Second, there is a loss of both antioxidants superoxide dismutase and glutathione peroxidase.

$$\text{Hypoxanthine} + O_2 + H_2O \xrightarrow{XO} \text{Xanthine} + H_2O_2 + O_2^{\bullet -}$$

$$\text{Xanthine} + O_2 + H_2O \longrightarrow \text{Uric acid} + H_2O_2 + O_2^{\bullet -}$$

Metals in Accelerating Lipid Oxidation

- Formations of alkyl free radical by direct reaction with fats and oils.

$$Fe^{3+} + RH \rightarrow Fe^{2+} + R^{\bullet} + H^{+}$$

- Hydroperoxide decomposition to form peroxy or alkoxy radical.

$$Fe^{3+} + ROOH \rightarrow Fe^{2+} + ROO^{\bullet} + H^{+}$$

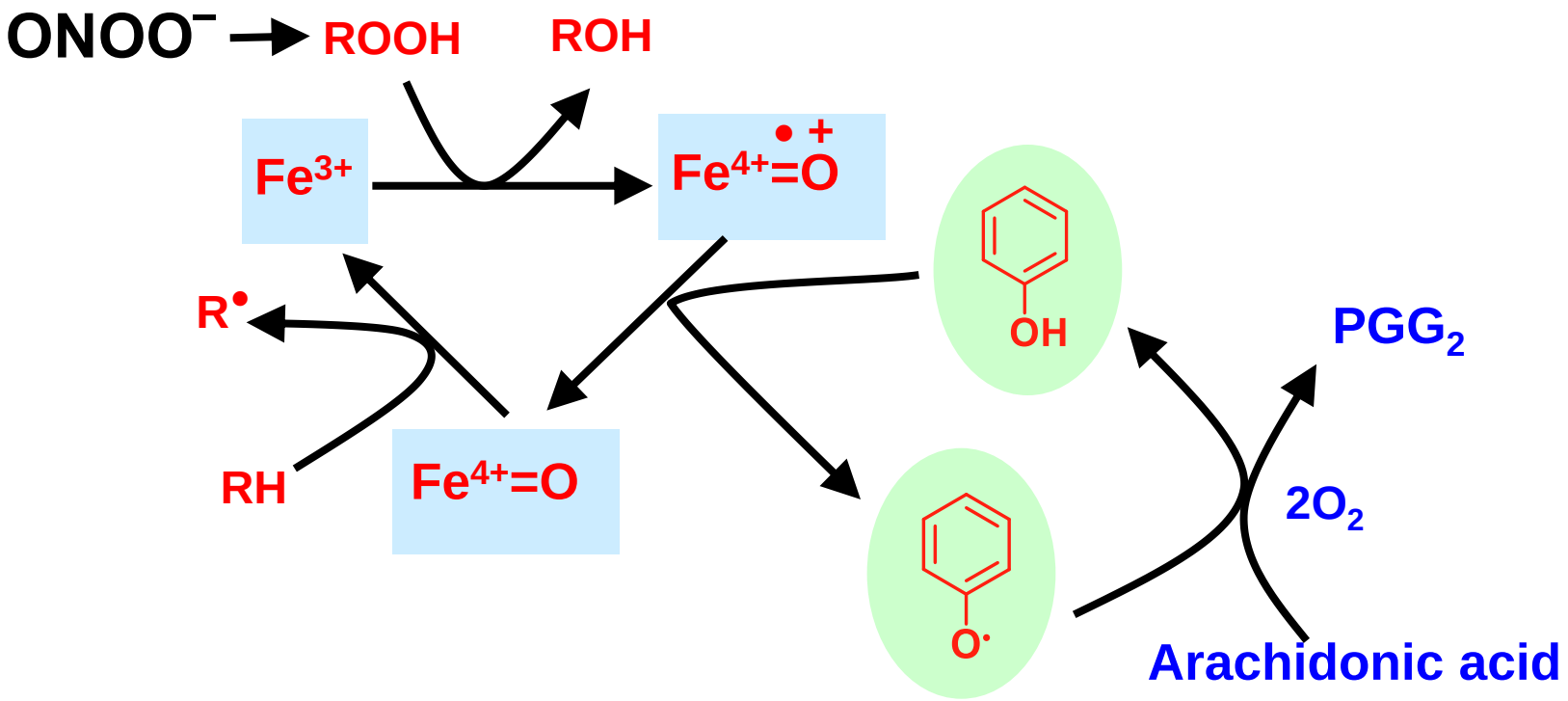
$$Fe^{2+} + ROOH \rightarrow Fe^{3+} + RO^{\bullet} + OH^{-}$$

- Activation of molecular oxygen for singlet oxygen formation.

$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^{\bullet -} \rightarrow {}^1O_2$$

Lipid Metabolism/ Peroxidation

Example: Cyclooxygenase (prostaglandin synthesis)

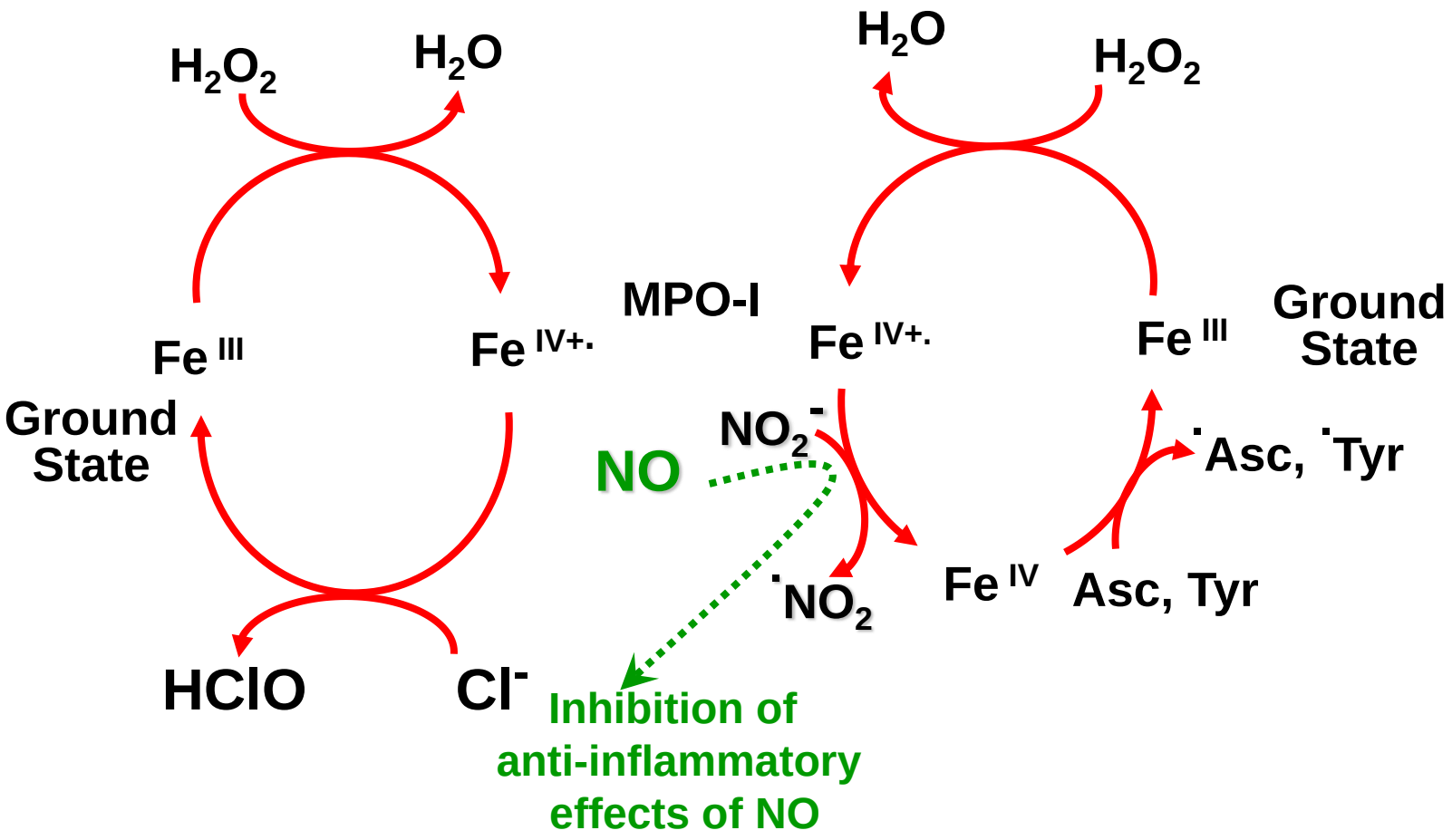


Marnett et al (2000) Curr Opin Chem Biol.5, 545

Lipid Metabolism/ Peroxidation

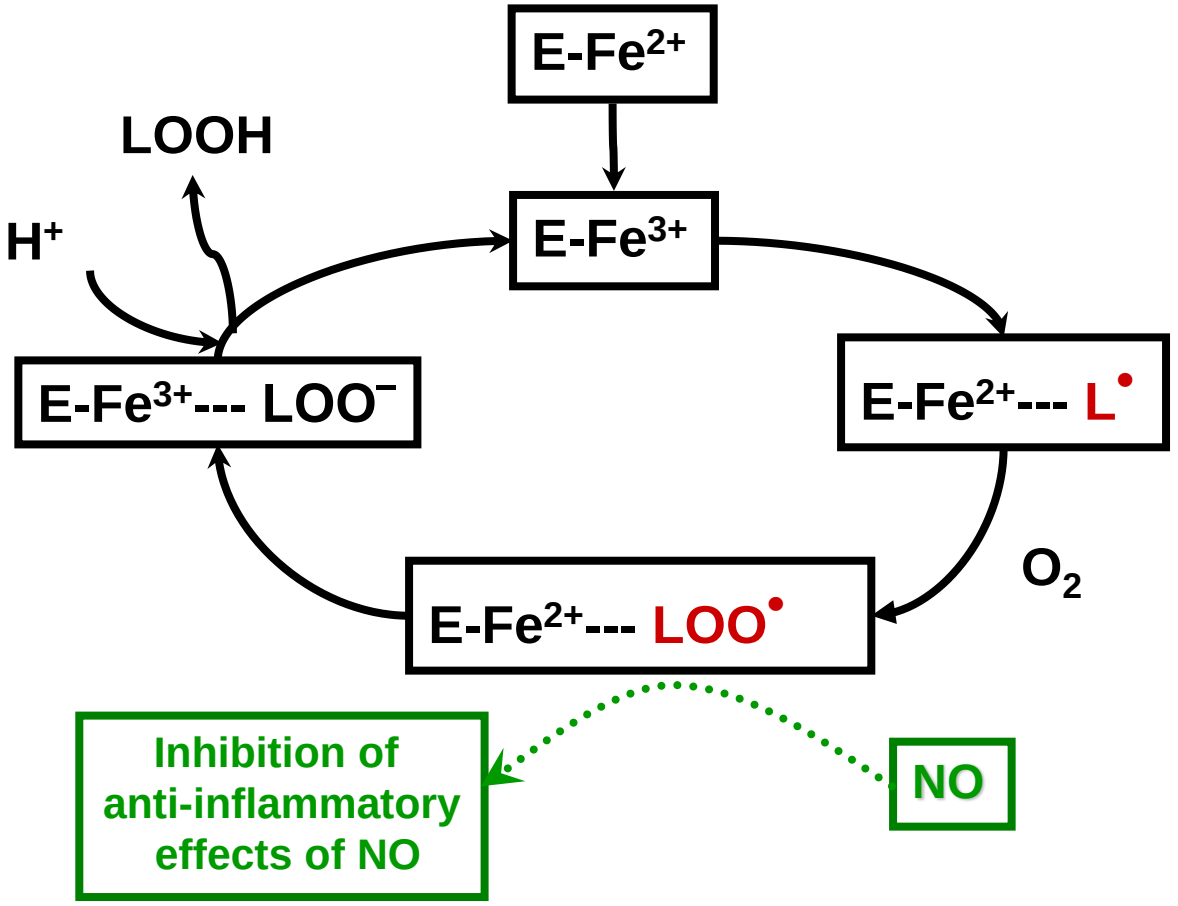
Example: Myeloperoxidase

(Host defence, Inflammation, Cardiovascular disease)



Lipid Metabolism/ Peroxidation

Example: Lipoxygenase (fatty acid metabolism during inflammation e.g. Atherosclerosis)



Minimization of Lipid Oxidation

- If a compound inhibits the formation of free alkyl radicals in the initiation step, or if the chemical compound interrupts the propagation of the free radical chain, the compound can delay the start or slow the chemical reaction rate of lipid oxidation.

- The initiation of free radical formation can be delayed by the use of metal chelating agents, singlet oxygen inhibitors, and peroxide stabilizers.

- The propagation of free radical chain reaction can be minimized by the donation of hydrogen from the antioxidants and the metal chelating agents.

Small Molecules Natural Antioxidants

Vitamins	Alpha tocopherol	Breaks lipid peroxidation Lipid peroxide and $O_2^{\cdot -}$ and $\cdot OH$ scavenger	Fat soluble vitamin
	Beta carotene	Scavenges $\cdot OH$, $O_2^{\cdot -}$ and peroxy radicals Prevents oxidation of vitamin A Binds to transition metals	Fat soluble vitamin
	Ascorbic acid	Directly scavenges $O_2^{\cdot -}$, $\cdot OH$, and H_2O_2 Neutralizes oxidants from stimulated neutrophils Contributes to regeneration of vitamin E	Water soluble vitamin

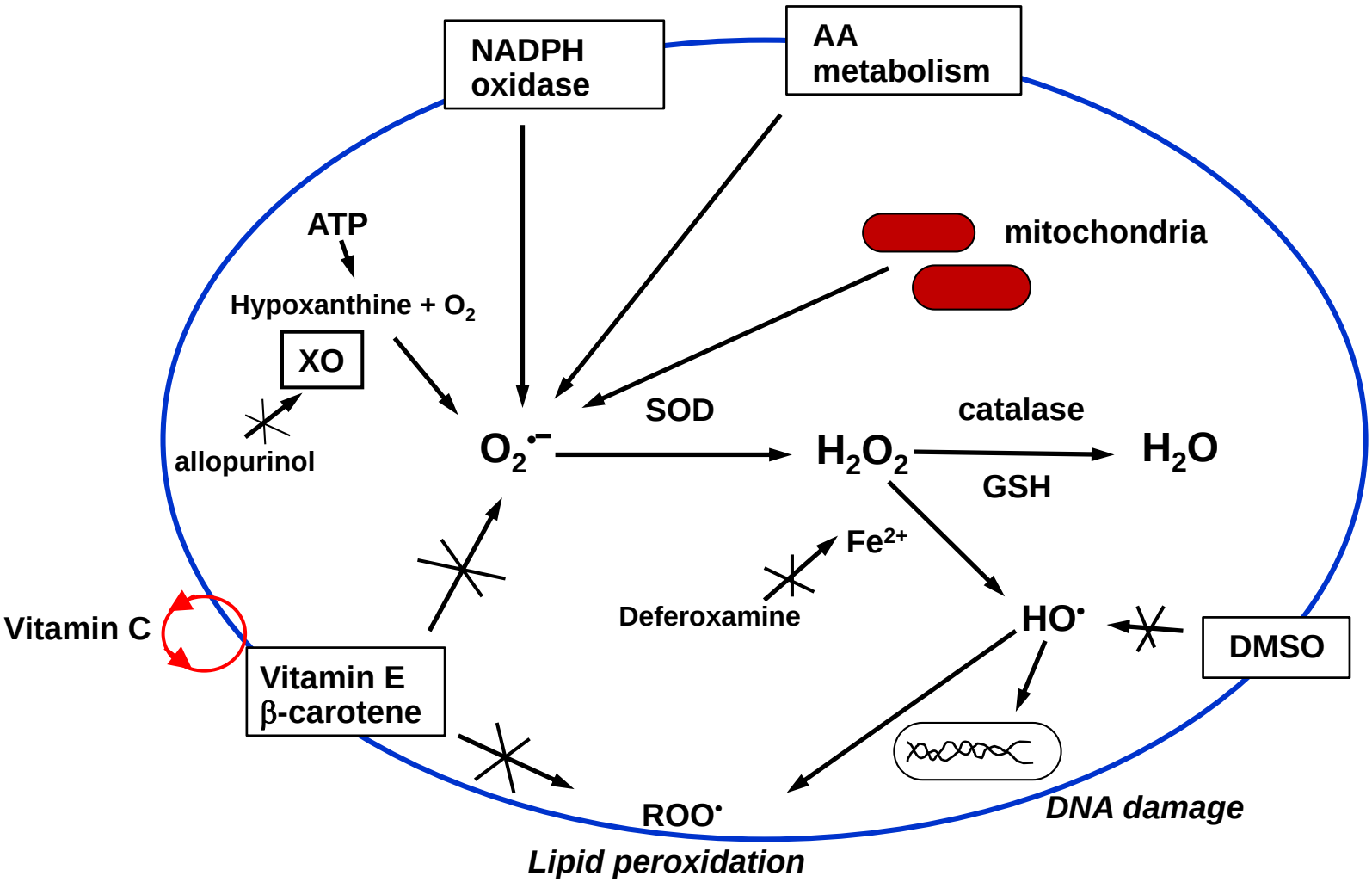
Synergism in Lipid Oxidation

- Synergism occurs when mixtures of antioxidants produce a more pronounced activity than the sum of the activities of the individual antioxidants when used separately.

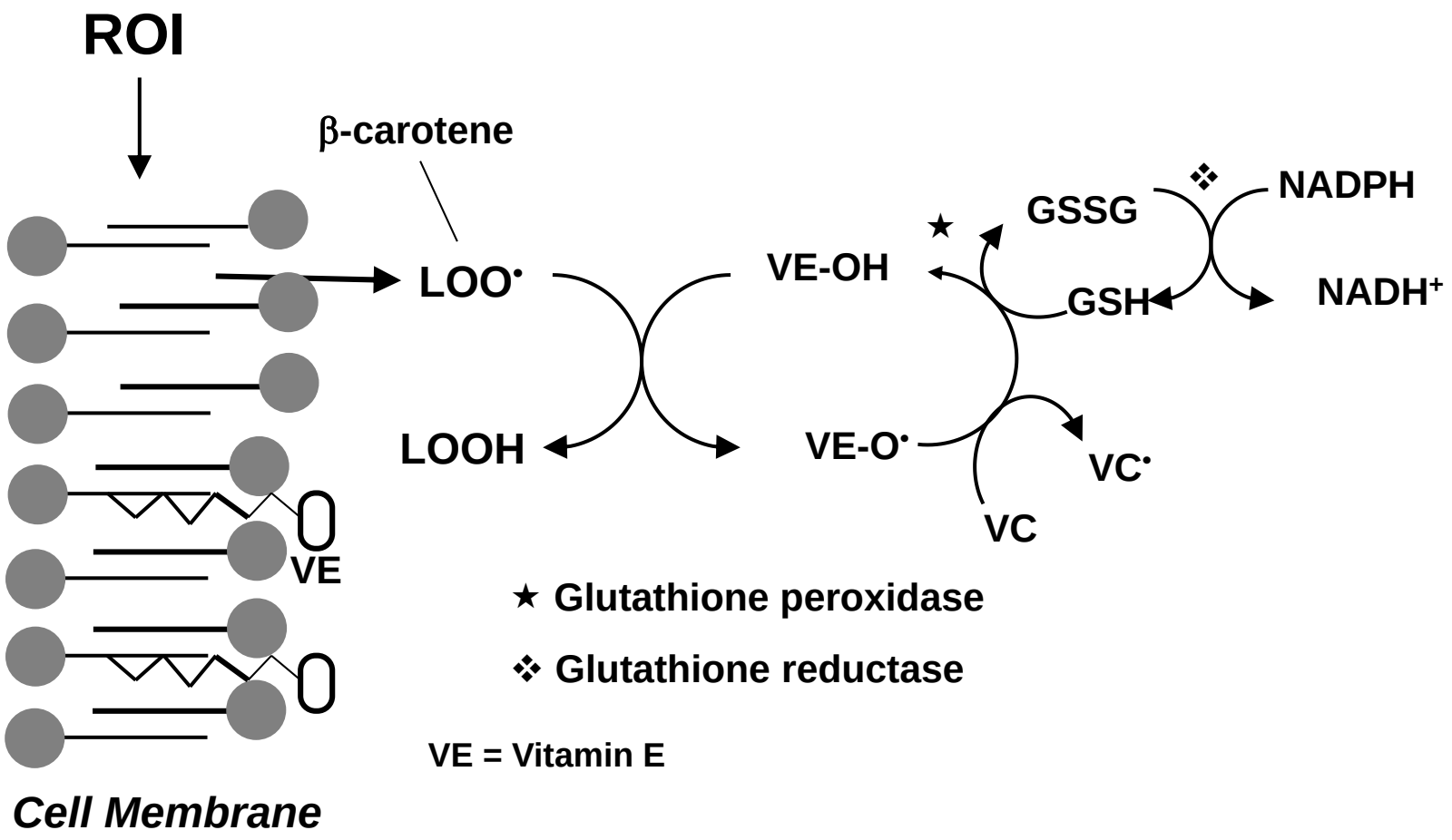
- To have maximum efficiency, primary antioxidants are often used in combination with (1) other phenolic antioxidants, or with (2) various metal chelating agents.

- In principle natural antioxidant are quite effective and apparently can offer the best protection: they are also used in nature in combination. The cost and the availability restrict the practical use of these compounds to food and specialty products.

Simplified Overview of Antioxidants Role in Biological Systems



Cascade Use of Antioxidants



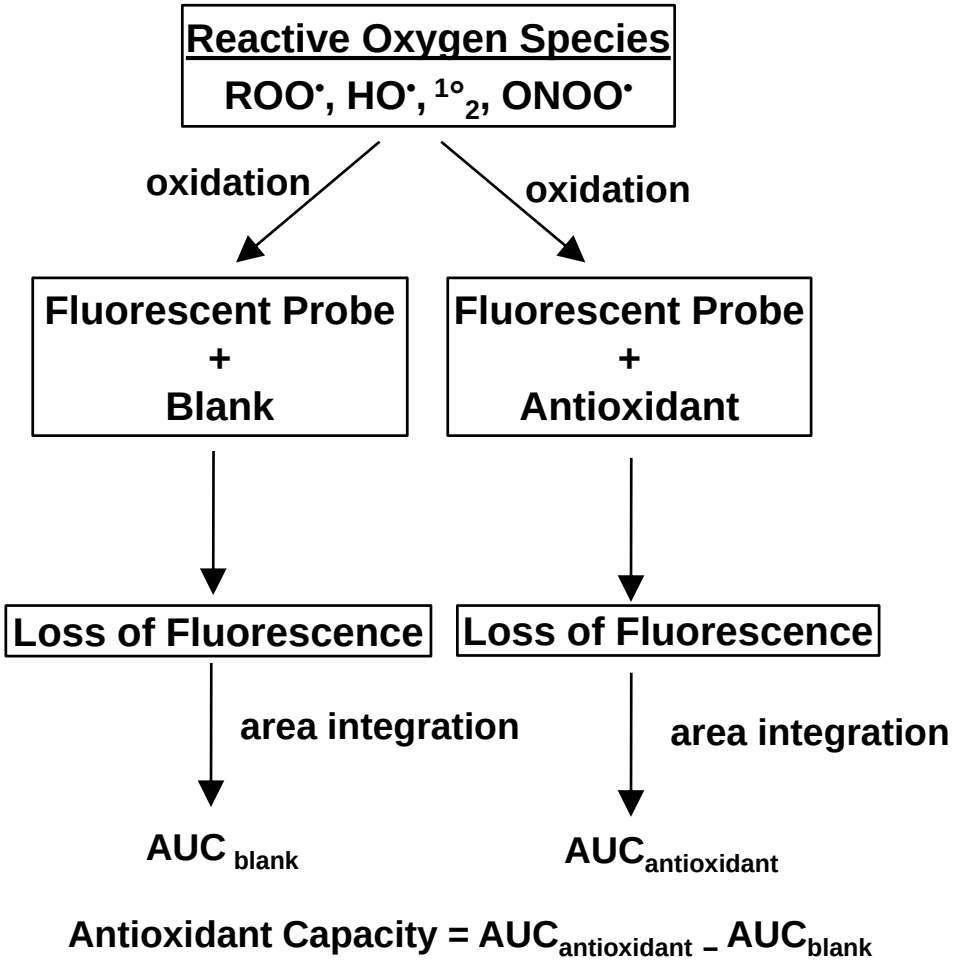
Other less Investigated Antioxidant

- **Glutathione (GSH)**
- **CoQ10**
- **Uric acid**
- **Albumin**
- **Drugs**
  - Xanthine oxidase inhibitors: e.g. allopurinol, folic acid.
  - NADPH inhibitors: e.g. adenosine, calcium channel blockers.
  - Albumin.
  - Inhibitors of iron redox cycling: deferoxamine, apotransferin,
  - ceruloplasmin

Measurements of Antioxidant Activity

- **Antioxidant Biomarkers**
  1. Total antioxidant potential
  2. Superoxide dismutase
  3. Total glutathione
  4. Glutathione peroxidase
  5. Glutathione reductase
  6. GSH/GSSG ratio
  7. Catalase
- **Oxidative Biomarkers**
  1. MDA
  2. Hydrogen peroxide
  3. Total nitric oxide

Total Antioxidant Potential: Principle of the ORAC Assay



$$\text{Antioxidant Capacity} = AUC_{antioxidant} - AUC_{blank}$$

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Lipid Peroxidation: Chemistry

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Autoxidation of Organic Compounds

All organic compounds are thermodynamically instable to oxygen

$$C_nH_{2n+2} + (2n+1)\,O_2 \rightarrow n\,CO_2 + (n+1)\,H_2O \qquad \Delta G° < 0$$

diamagnetic *paramagnetic* *diamagnetic* *diamagnetic*

Mechanism of autoxidation: chain process of radical species

Initiation $X\text{-}Y \rightarrow X + R\text{-}H \rightarrow R + XH$ (r_i)

Propagation $R^{\bullet} + O_2 \rightarrow R\text{-}O\text{-}O^{\bullet}$ (k_p)

$R\text{-}O\text{-}O^{\bullet} + R\text{-}H \rightarrow R\text{-}O\text{-}O\text{-}H + R^{\bullet}$ (k_p)

Termination $2\,RO_2^{\bullet} \rightarrow$ products (k_t)

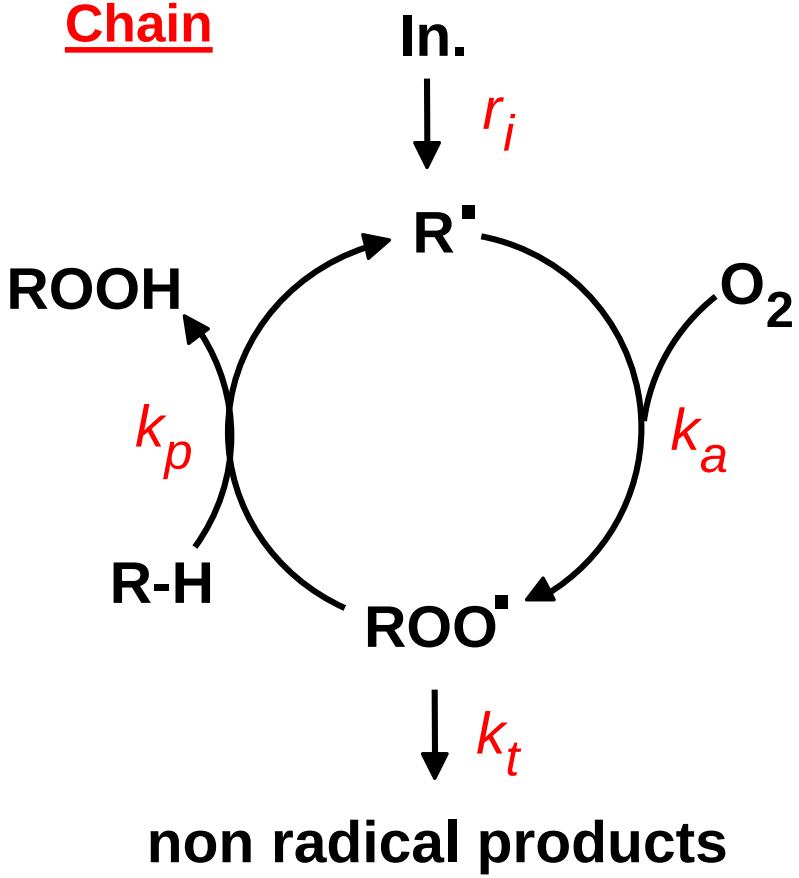
$RO_2^{\bullet} + R^{\bullet} \rightarrow$ products (k'_t)

$R^{\bullet} + R^{\bullet} \rightarrow$ products (k''_t)

$$rate = \frac{d[\mathrm{ROOH}]}{dt} = k_p[\mathrm{RH}]\left(\frac{r_i}{2k_t}\right)^{1/2}$$

Radical Chain and Branching in Autoxidations

Chain



Branching :

$$ROOH \rightarrow RO^{\cdot} + {}^{\cdot}OH \quad (o\ M^{n+})$$

$$RO^{\cdot} + ROOH \rightarrow ROO^{\cdot} + ROH$$

$$RO^{\cdot} + RH \rightarrow ROH + R^{\cdot}$$

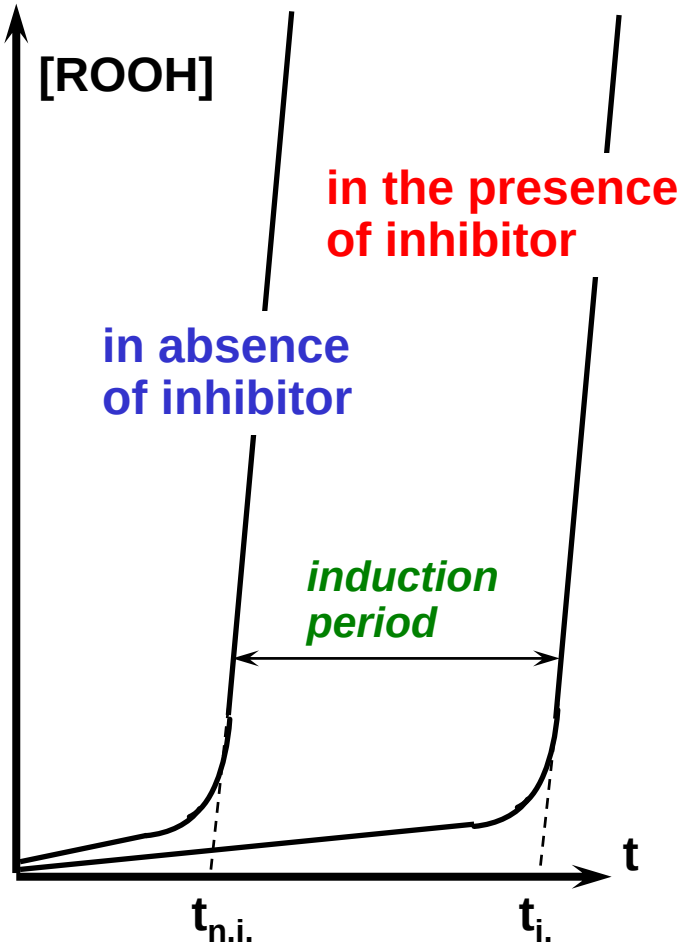
$${}^{\cdot}OH + RH \rightarrow H_2O + R^{\cdot}$$

Autoxidation and Inhibition

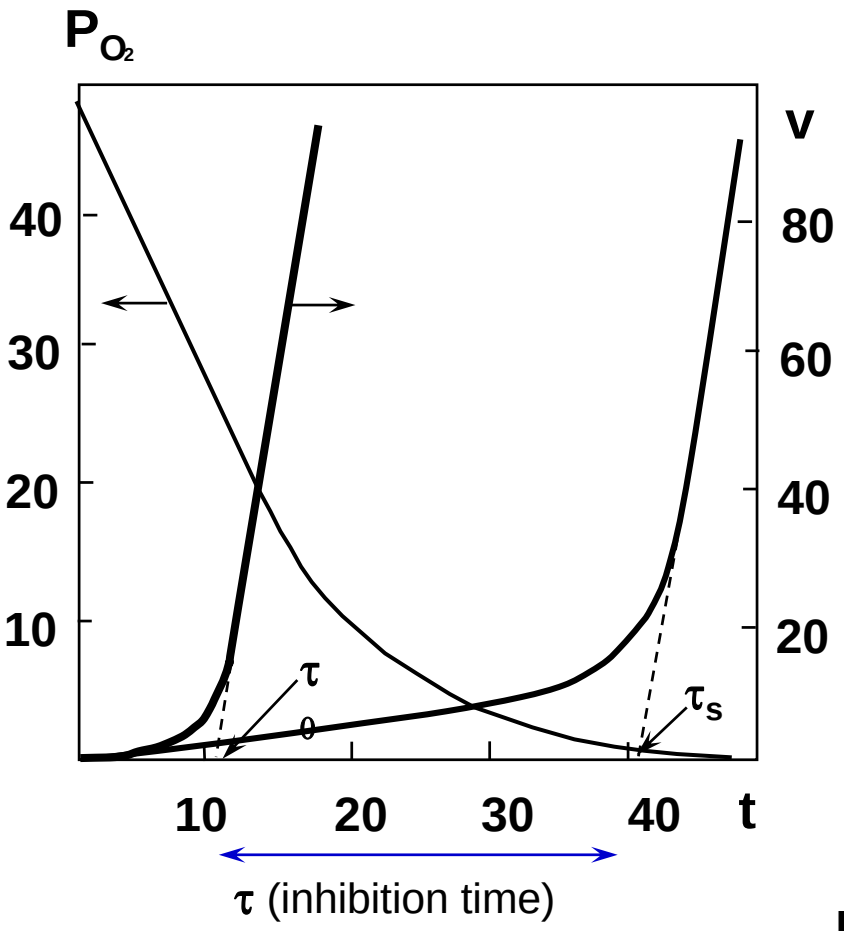
The autoxidation of an organic compound is driven by initiators, i.e. :
a) light b) heat c) metals d) radiations

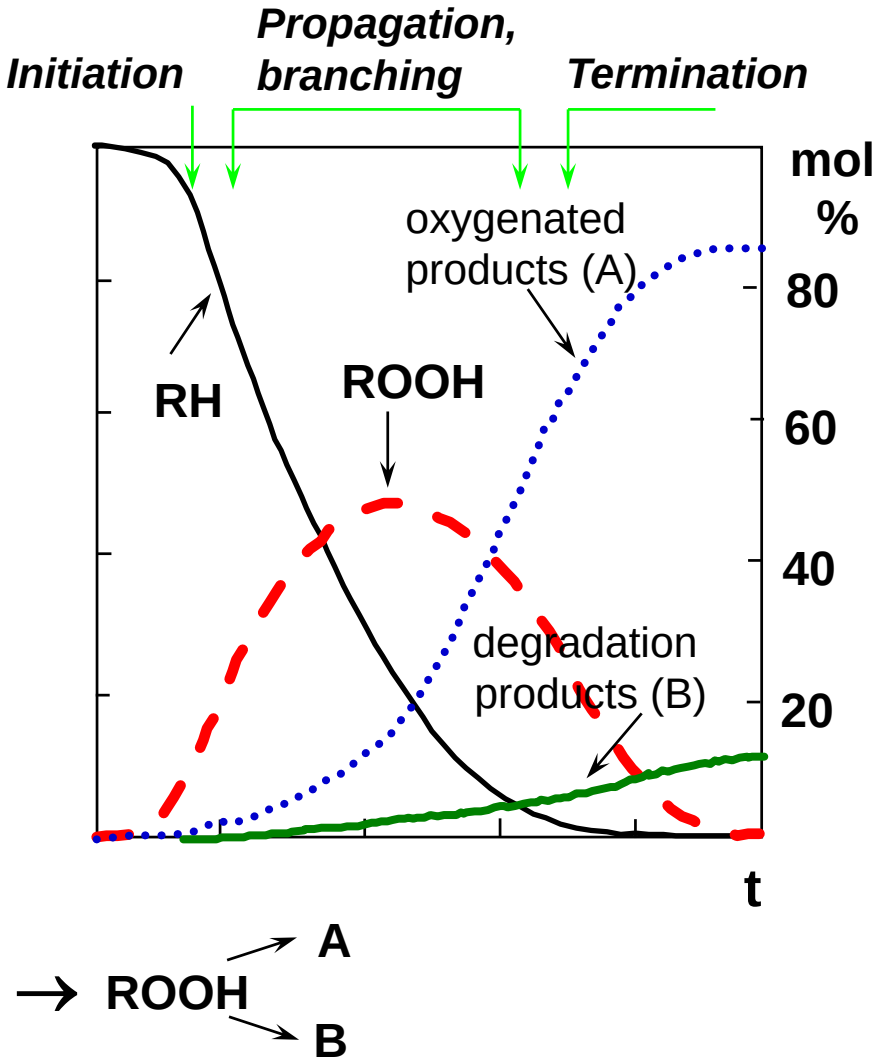
At high temperature the hydroperoxide is unstable and decomposes to radicals. The reaction becomes autocatalytic, fast and all intermediates are unstable toward further oxidations (flames)

At low temperature, after a first stage of growing of hydroperoxide, also this intermediate participate to the initiation (r_i). Under these conditions compounds able to decompose ionically hydroperoxides or forming persistent radicals (able to terminate the chain) does not allow to sustain the catalytic cycle and inhibit the oxidation (antioxidants – i.e. EDTA, thiocarbamates, ALS, Phenols … and retarding agents, i.e. organophosphor compounds and phosphazenes)



Kinetic of Peroxidation





Elementary Lipid Peroxidation

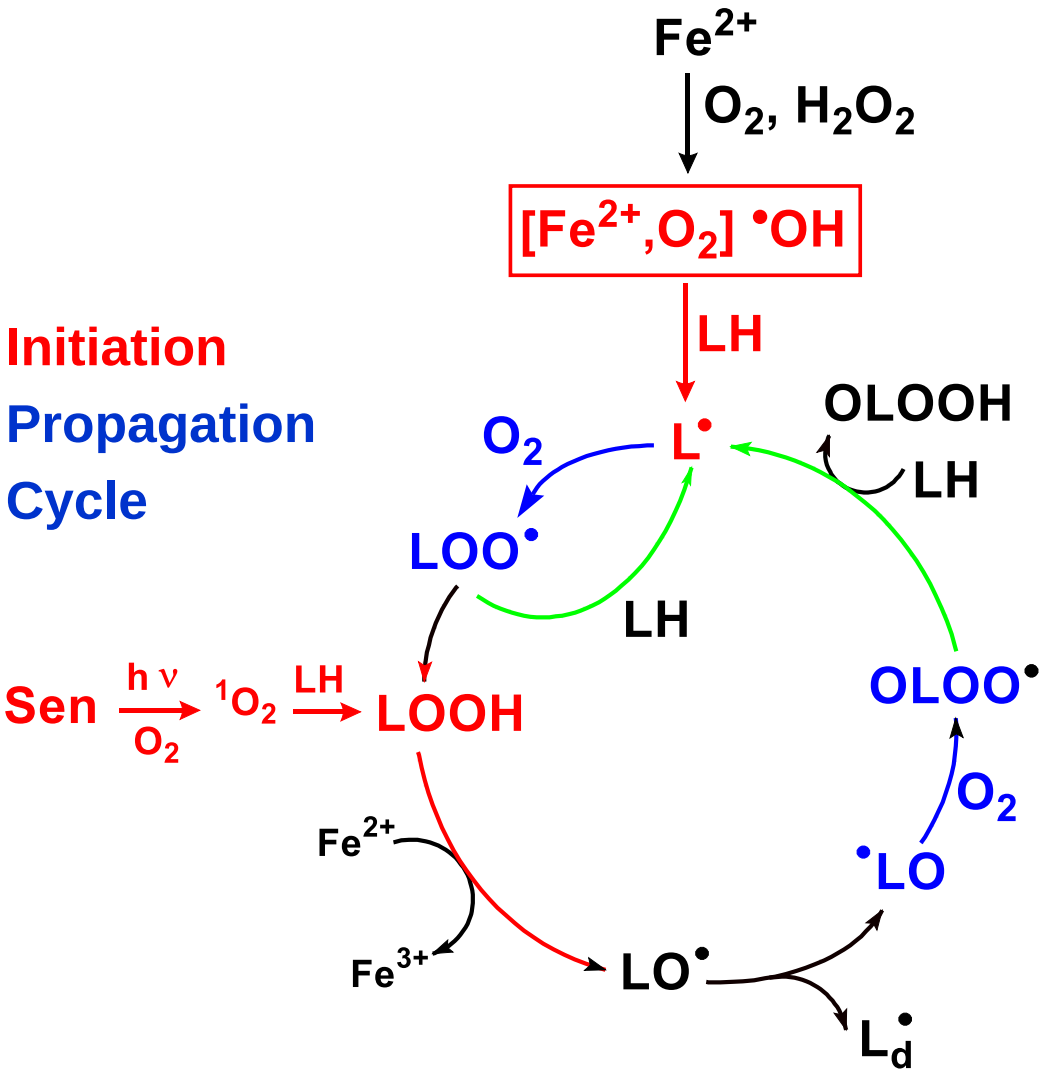
$L\text{-}H + X^{\bullet} \longrightarrow L^{\bullet} + XH$ *Initiation*

$L^{\bullet} + O_2 \longrightarrow LOO^{\bullet}$ *Propagation*

$LOO^{\bullet} + L\text{-}H \longrightarrow L^{\bullet} + LOOH$ *Cycle*

$LOO^{\bullet} + \text{“}R^{\bullet}\text{”} \longrightarrow LOOR$ *Termination*

Lipid Peroxidation: A More Complete View

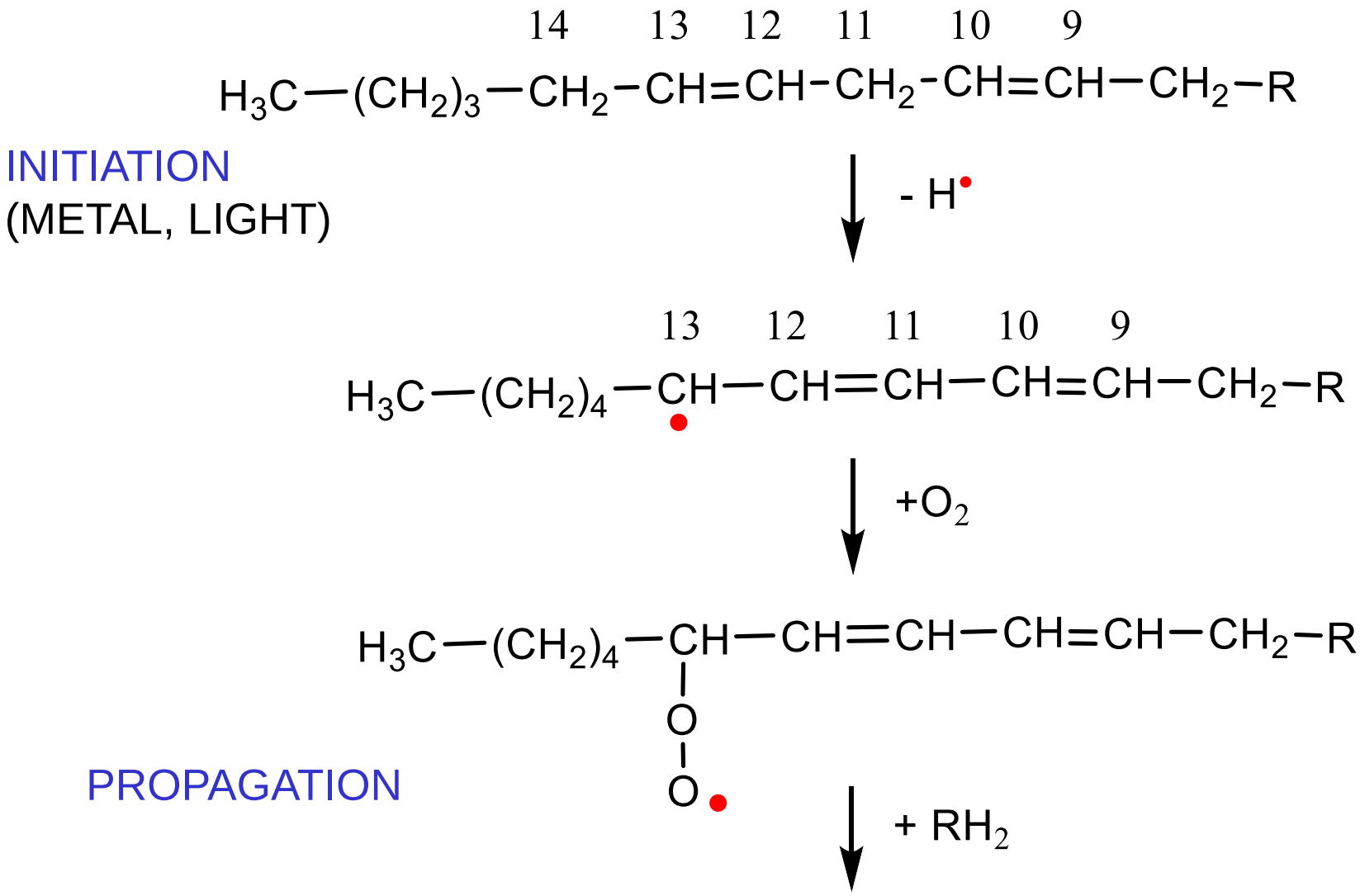


Reaction Rates of Lipid Oxidation

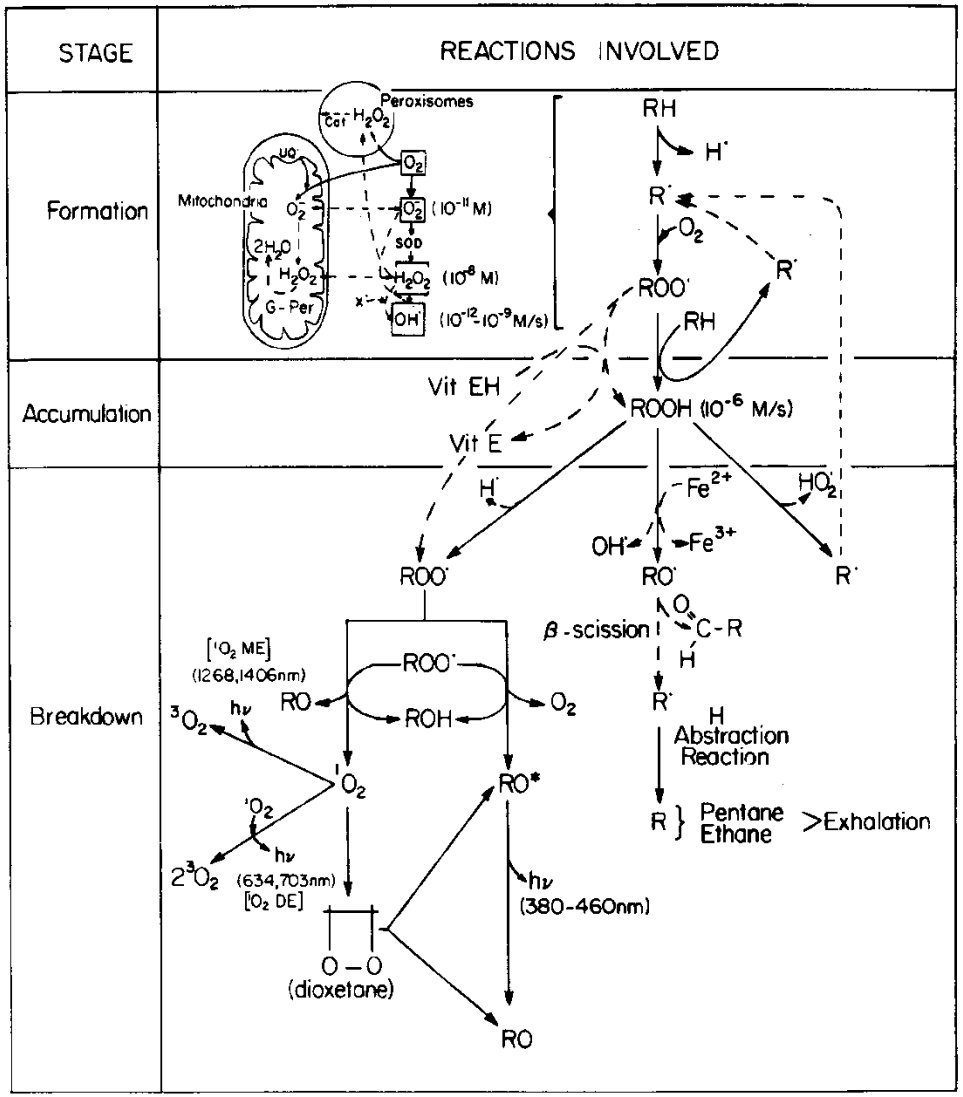
$R^{\bullet} + {}^3O_2$	$k \approx 10^9\ M^{-1}sec^{-1}$
$ROO^{\bullet}$ + Oleic Acid	$k = 1\ M^{-1}sec^{-1}$
$ROO^{\bullet}$ + Linoleic Acid	$k = 60\ M^{-1}sec^{-1}$
$ROO^{\bullet}$ + Linolenic Acid	$k = 120\ M^{-1}sec^{-1}$
$ROO^{\bullet} + ROO^{\bullet}$	$k = 10^5\text{-}10^7\ M^{-1}sec^{-1}$
$R^{\bullet}$ + Antioxidants	$k = 10^7\ M^{-1}sec^{-1}$
$RH + {}^1O_2$	$k = 10^5\ M^{-1}sec^{-1}$

	Oleic Acid	Linoleic Acid	Linolenic Acid
Triplet oxygen	1	27	77
Singlet oxygen	3×10^4	4×10^4	7×10^4

Lipid Autoxidation

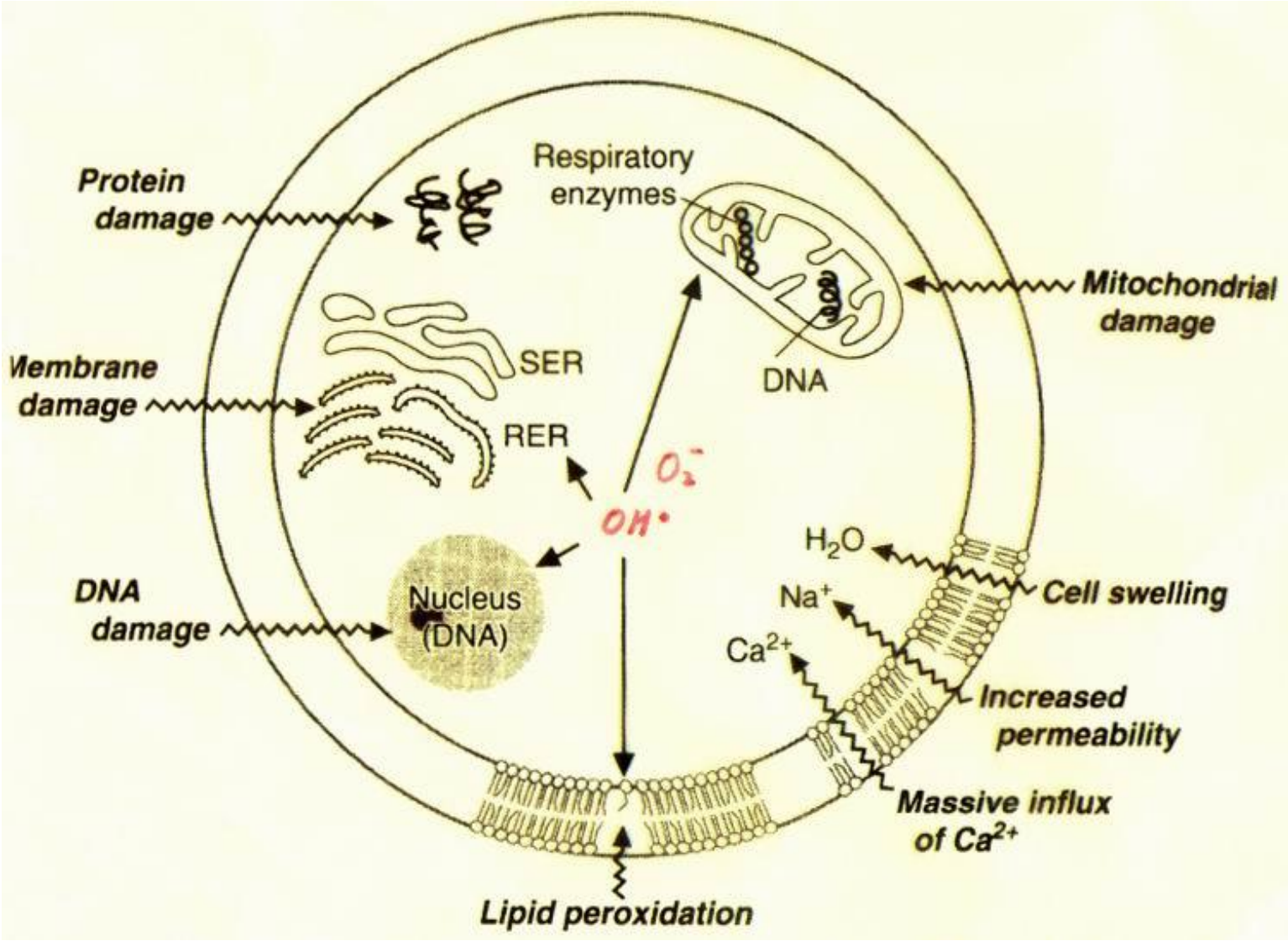


Free Radical Reaction Mechanisms of Lipid Peroxidation

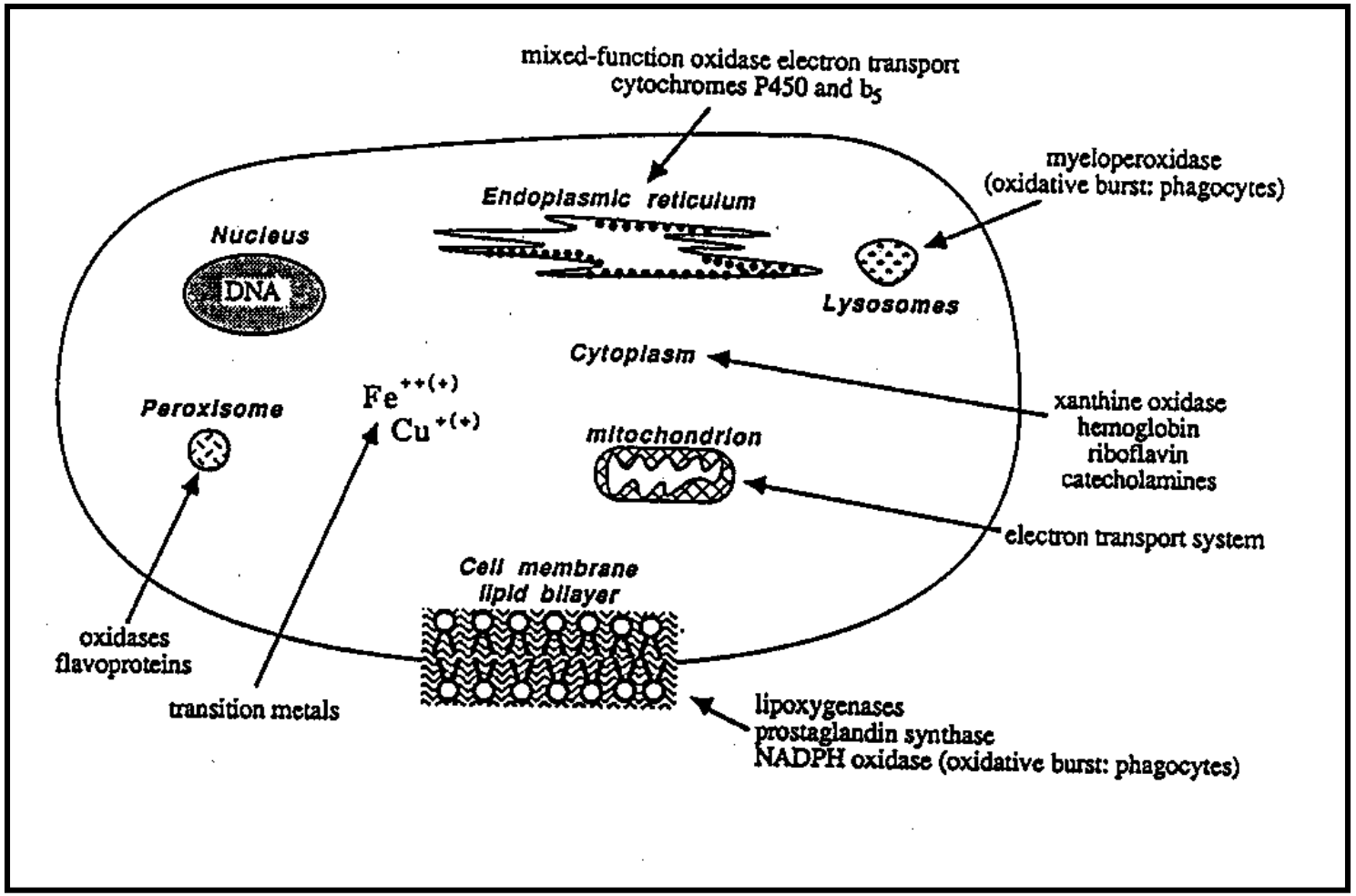


Stages of lipid peroxidation. The first and second stage are the classical reactions for formation and accumulation of lipoperoxides. The third stage, the breakdown of lipoperoxides, focuses particularly on radical reactions leading either to chemiluminescence or to the formation of pentane or ethane. Vitamin E (Vit. EH) stops the chain reaction of lipid peroxidation by reacting with lipid peroxy radicals ($ROO^{\cdot}$). The numbers in brackets express the estimated steady state concentrations or formation rates of oxygen metabolites. G-Per, glutathione peroxidase; Cat, catalase; SOD, superoxide dismutase; ROOH, lipid hydroperoxide; $RO^{\cdot}$, alkoxy radicals; $HO^{\cdot}$, hydroxyl radicals; O_2^{-}, superoxide anion; $^1O_2$ ME, singlet oxygen monomol. emission; $^1O_2$, DE, singlet oxygen dimol. emission.

Eukaryote Cell Structure and Radical Sources-Damages



Cellular Sources of Free Radicals and Lipid Peroxidation Products



Control of Lipid Oxidation

- Application of antioxidants
- Elimination of oxygen by nitrogen flushing or Vacuum packaging
- Elimination of photosensitizers
- Denaturation of lipoxygenase
- Low temperature and dark storage

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Lipid Peroxidation: How to Measure

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Assays of Lipid Peroxidation

Several assays of lipid peroxidation may be useful and accurate for the quantification of oxidant stress.

- Measurement of substrate loss.
- Quantification of lipid peroxidation products.
  - Primary end products
  - Secondary end products

Typical assays are:

- Plasma isoprostanes
- Urinary malonaldehyde
- Exhaled pentane
- Total plasma antioxidant capacity
- Plasma glutathione levels

The Ideal Assay of Lipid Peroxidation

- Assay is accurate, specific, and sensitive index of lipid peroxidation.
- Compounds to be quantified are stable.
- Assay applicable to *in vitro* and *in vivo* studies.
- Assay easy to perform with high throughput.
- Assay economical.

Problems:

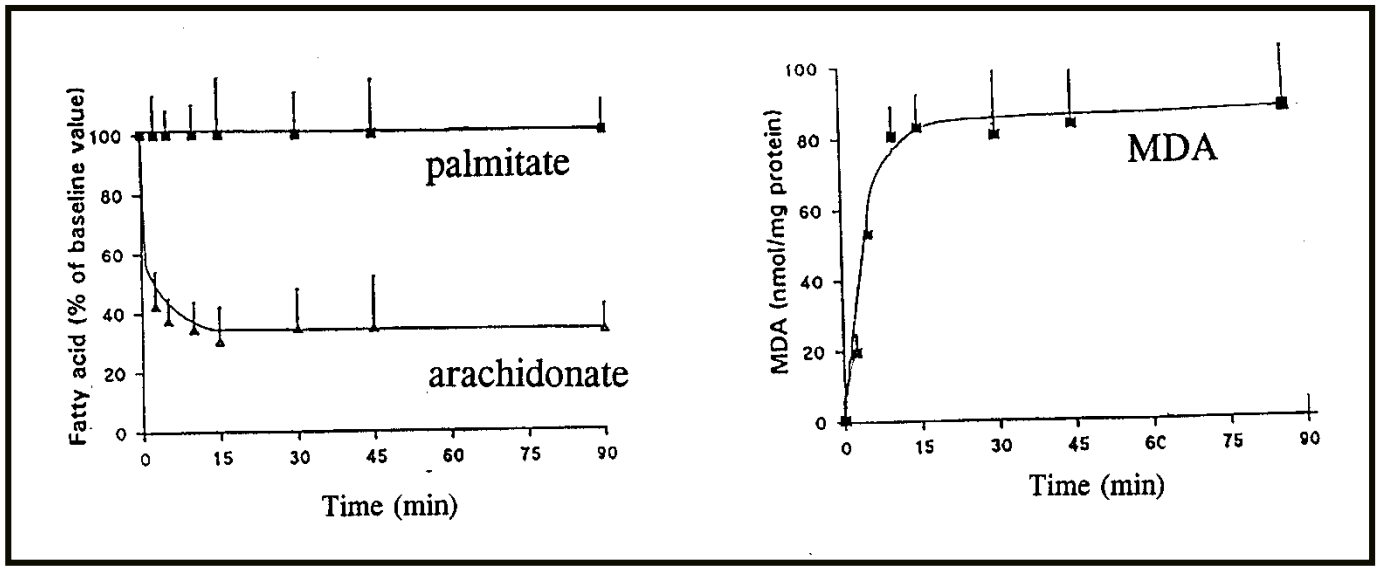
- No assay is ideal.
- Most assays are more accurate when quantifying lipid peroxidation *in vitro* than *in vivo*.
- Little data exist comparing various methods in vivo.
- Few assays accurately provide an integrated assessment of lipid peroxidation in an animal or human as a whole.

Assays of Potential Use to Quantify Lipid Peroxidation *in Vitro* and *in Vivo.*

- Fatty acid analysis
- Conjugated dienes
- Lipid hydroperoxides
- Thiobarbituric acid-reactive substances (TBARS) or malondialdehyde (MDA)
- Alkanes
- F_2-Isoprostanes

Fatty Acid Analysis

- Commonly used to assess fatty acid content in biological fluids or tissues.
- *Method*:
  - Lipid extraction of biological fluid or tissue
  - Transmethylation of fatty acids
  - Separation by GC (or HPLC)
  - Quantification of fatty acid with flame ionization



Disappearance of arachidonic acid is associated with accumulation of peroxidation end products

GC-MS Analysis of Fatty Acids

Advantages

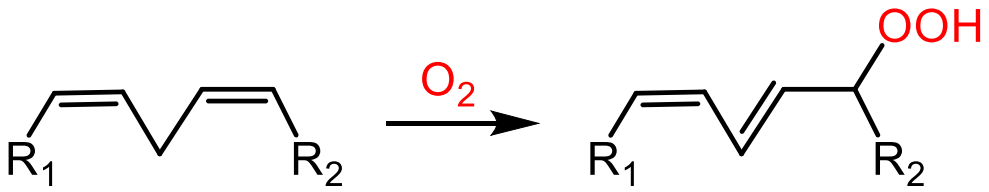
- Easy to perform.
- Equipment readily available.
- Data complement generation of peroxidation products *in vitro* and *in vivo*.

Disadvantages

- Impractical for a number of *in vivo* situations.
- Provides information on disappearance of substrate only.
- May see very little change associated with oxidation *in vivo*.

Conjugated Dienes

- Peroxidation of unsaturated fatty acids results in the formation of conjugated diene structures that absorb light in the wavelength of 230-235 nm which can be quantified spectrometrically.



- Useful for studying oxidation of lipids in vitro.
  - Enhanced assay sensitivity using second derivative spectroscopy, HPLC, or GC/MS.
- Studies have shown increases in animals and humans associated with oxidant stress.
- Inaccurate index of lipid peroxidation when applied to complex biological fluids.
  - A primary diene conjugate from human body fluids is an non-oxygen containing isomer of linoleic acid, octadeca-9-cis-11-trans-dienoic acid.
  - Other compounds-purines, pyrimidines, heme proteins absorb at 234 nm.

Lipid Hydroperoxides

- Primary products of lipid peroxidation.
- Several quantitative methods exist (iodometric, electrochemical, mass spectrometric)
  - Most accurate-chemiluminescence-based HPLC detection.

$$LOOH \longrightarrow LO^{\bullet}$$
$$LO^{\bullet} + \text{isoluminol } (QH^{-}) \rightarrow \text{semiquinone radical } (Q^{\bullet -})$$
$$Q^{\bullet -} + O_2 \rightarrow \text{quinone } (Q) + O_2^{\bullet -}$$
$$Q^{\bullet -} + O_2^{\bullet -} \rightarrow \text{isoluminol endoperoxide} \rightarrow \text{light } (\lambda_{max}\ 430)$$

  - Sensitive (pmol or less) and specific
  - Information regarding which lipid class is oxidized can be obtained.

Advantages: Assays more specific and sensitive than for other peroxidation products.

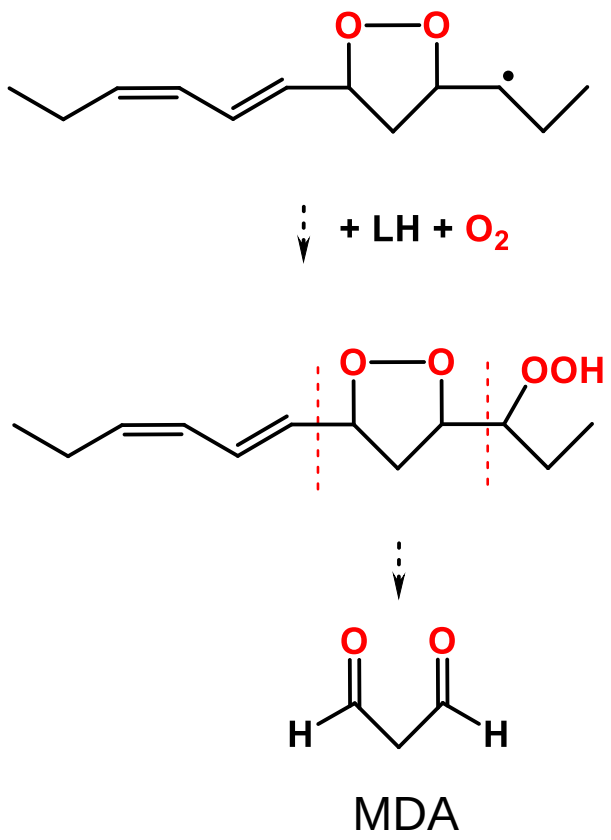
Disadvantages

- Products are unstable.
- *Ex vivo* oxidation a major concern.
- Lack of detectable levels in some fluids and tissues.
- Equipment expensive.

Oxidative Stress Parameter - MDA

Malondialdehyde (MDA) levels:

- polyunsaturated fatty acids, exposed to free radicals, can be oxidized to hydroperoxides which decompose (in the presence of metals) to hydrocarbons and aldehydes
- malondialdehyde (MDA) is a quite reactive intermediate easy to detect owing to its bifuntional nature.

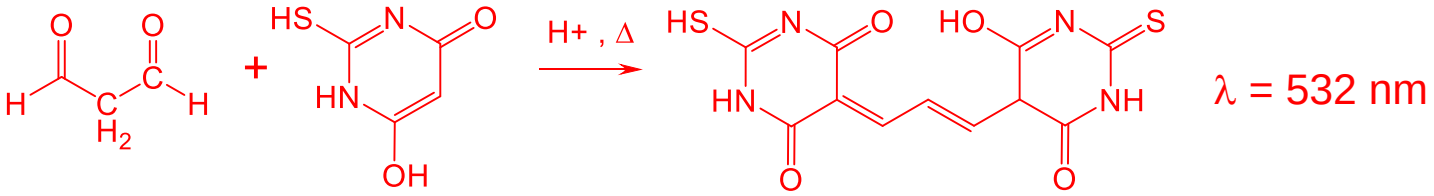


MDA

Most commonly used method to assess lipid peroxidation: measures malondialdehyde (MDA) which is a breakdown product of lipid peroxidation.

Thiobarbituric Acid-Reactive Substances (TBARS)/MDA

- *Method*:
  - Sample to be tested is heated with thiobarbituric acid at low pH and a pink chromogen (believed to be a TBA-MDA adduct) is formed.
  - Quantification-absorbance at 532 nm or fluorescence at 553 nm.



- Quantification of TBARS is an accurate measure of peroxidation in oxidizing systems *in vitro*.
- TBARS quantification in body fluids is inaccurate.
  - Substances other than MDA form chromogens at 532 nm.
  - MDA is formed during the assay procedure.
  - Antioxidants can interfere with the assay.
  - MDA can be derived from the diet.

TBARS/MDA

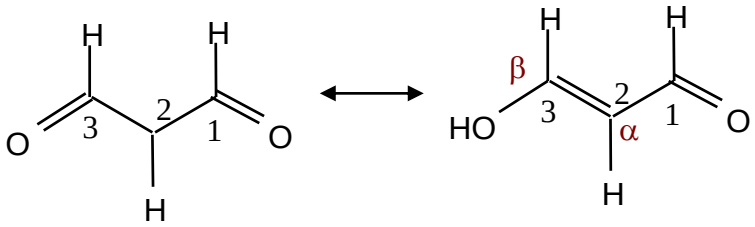
- Assays exist to measure TBARs by HPLC.
- MDA, HNE, and other aldehydes can be quantified by HPLC or GC/MS.
- These assays are generally more specific than TBARs although not necessarily more accurate as an index of lipid peroxidation.

- Levels of TBARS vary widely.
  - Plasma levels
    - Regular assay 4-35 uM.
    - HPLC-coupled 0-0.18 uM.
- TBARS increased in various disorders.
  - Hypercholesterolemia (Chirico *et al.,* Free Rad. Res. Comm. **19:**51, 1993).
    - Controls 0.10 $\pm$ 0.08 uM
    - Hypercholesterolemics 0.61 $\pm$ 0.25 uM

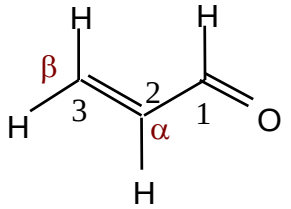
TBARS/MDA and Reactive lipid oxidation products: α,β-unsaturated aldehydes

Summary

- The TBARS assays are important because they are easy to perform and widely available.
- They are a reasonably accurate index of lipid peroxidation in a number of in vitro oxidizing systems.
- They are less reliable as an index of lipid peroxidation in complex biological fluids or in vivo.



Malondialdehyde (β-hydroxy-acrolein)



Acrolein (2-propenal)

Strong electrophiles because of unsaturated carbonyl group, react rapidly with nucleophilic targets (e.g. GSH, protein thiols) (Esterbauer *et al.,* Free Rad. Biol. Med. 1991: 11, 81-128; Uchida, Free Rad. Biol. Med. 2000: 28, 1685-1696)

Alkanes

- Volatile hydrocarbons generated from scission of oxidized lipids.
  - Pentane (n-6 fatty acids)
  - Ethane (n-3 fatty acids)
- *Method of quantification*
  - Collection of gas from an *in vitro* incubation or exhaled air from an animal or human.
  - Concentrating and filtering of samples to remove water and carbon dioxide.
  - Analysis of product by capillary GC.
- *Advantages*
  - Is an integrated assessment of peroxidation in vivo.
- *Disadvantages*
  - Collection of exhaled air for in vivo studies cumbersome and can take several hours to obtain an adequate sample for analysis.
  - Oxygen tension alters alkane formation.
  - Atmospheric contamination of collected gases.
  - Different researchers report a 1000-fold difference in normal levels of pentane generated in humans (4.1-4900 pmol/L).

Formation of Alkanes in Peroxidizing Microsomes

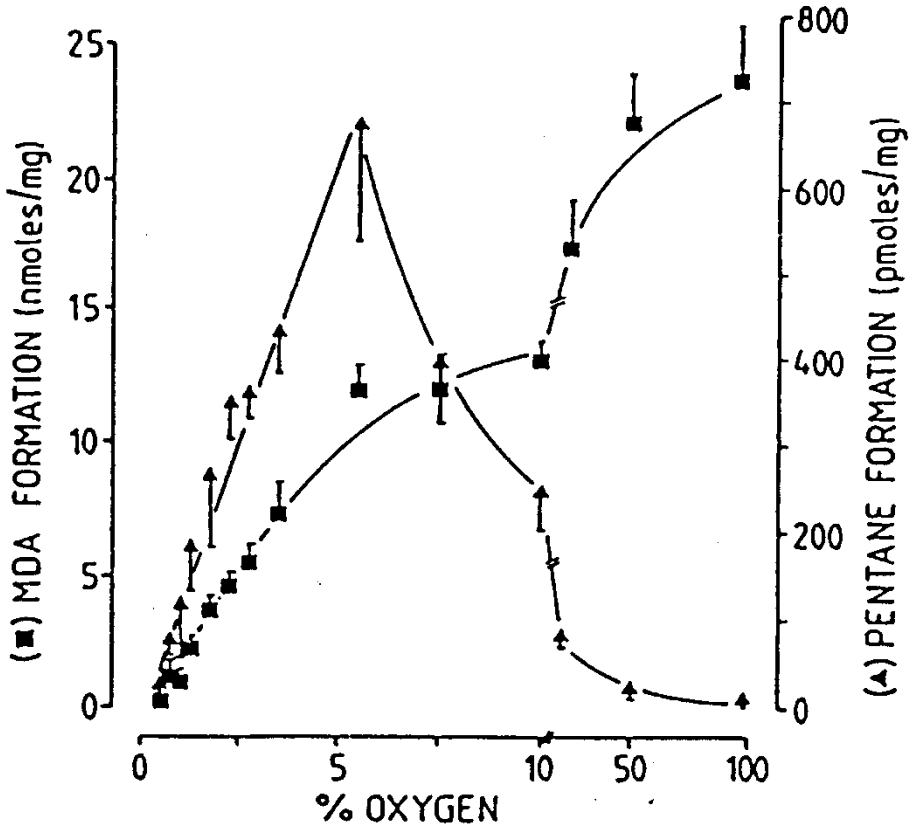


Fig. 2. Oxygen dependence of malondialdehyde (MDA) (■) and pentane (▲) formation in the incubations described in Fig. 1.

Reiter R *et al.* *Biochem. Pharm*. **1987**, *36*, 925-9,.

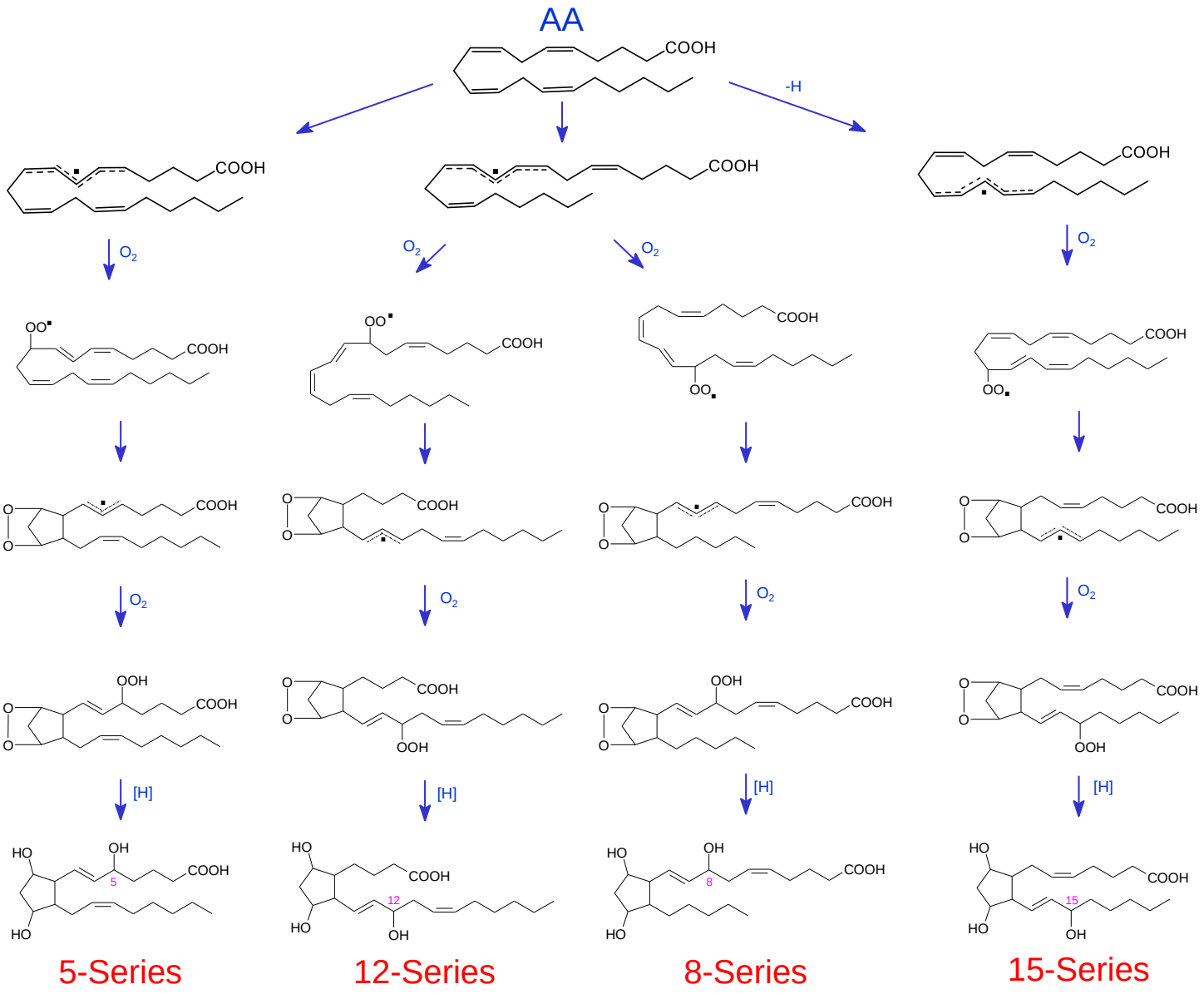
F_2-Isoprostanes Discovered in 1990

They are:

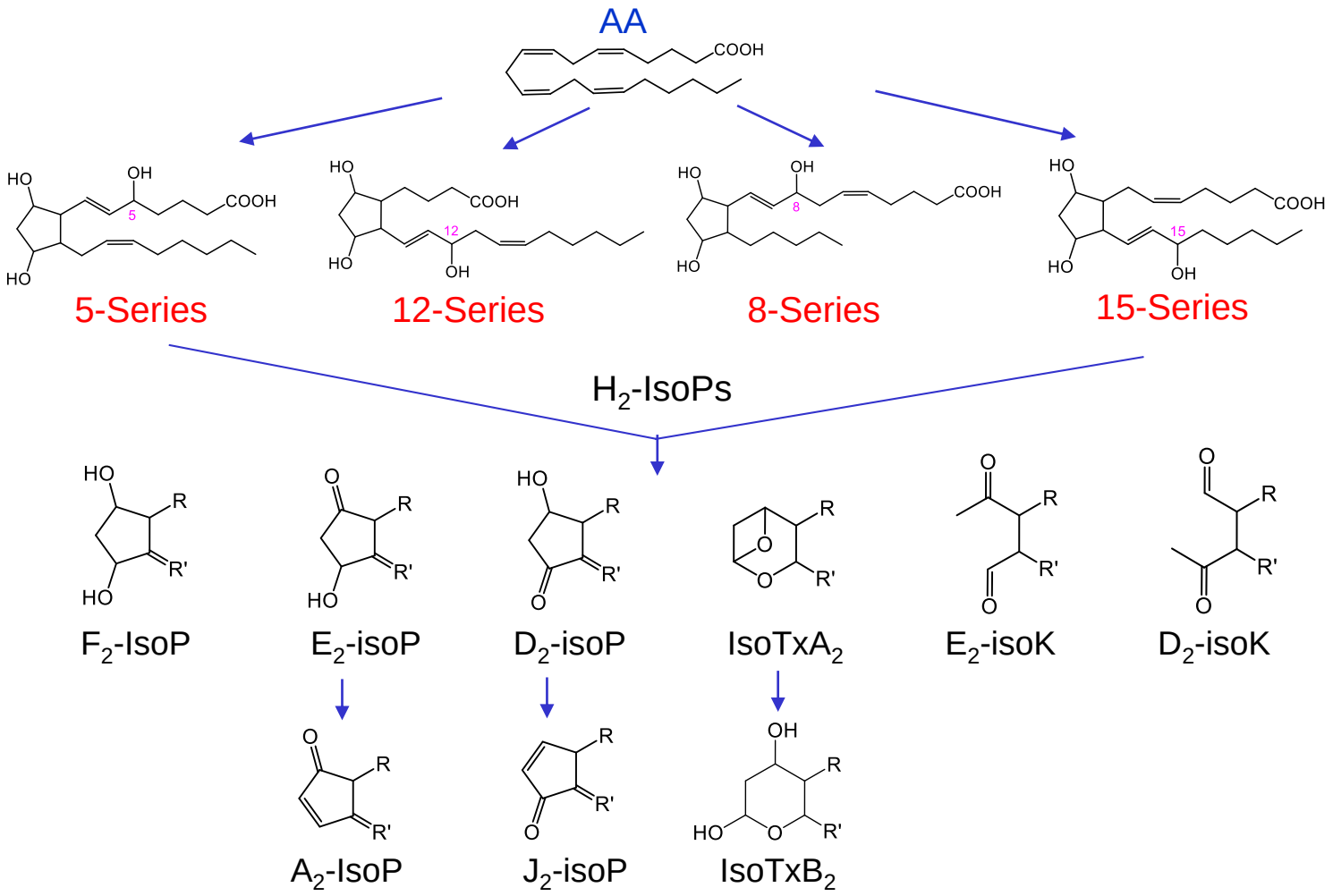
- PGF_2-like compounds produced non-enzymatically by free radical-induced peroxidation of arachidonic acid (AA)
- Produced in abundance *in vivo* in quantities far exceeding cyclooxygenase-derived PGs
- Capable of exerting potent biological actions
- 4 regioiosmers are formed (5-, 8-, 12-, and 15-series), each of which are comprised of 8 racemic diastereomers

- Analysis of F_2-Isoprostanes
  - Measured either free or after liberation from tissue lipids.
  - Purified by Sep-Pak extraction and TLC and derivatized to PFB ester, TMS ethers.
  - Analyzed using stable isotope dilution techniques employing a deuteriated standard by gas chromatography/mass spectrometry.

Pathway of Isoprostane Formation

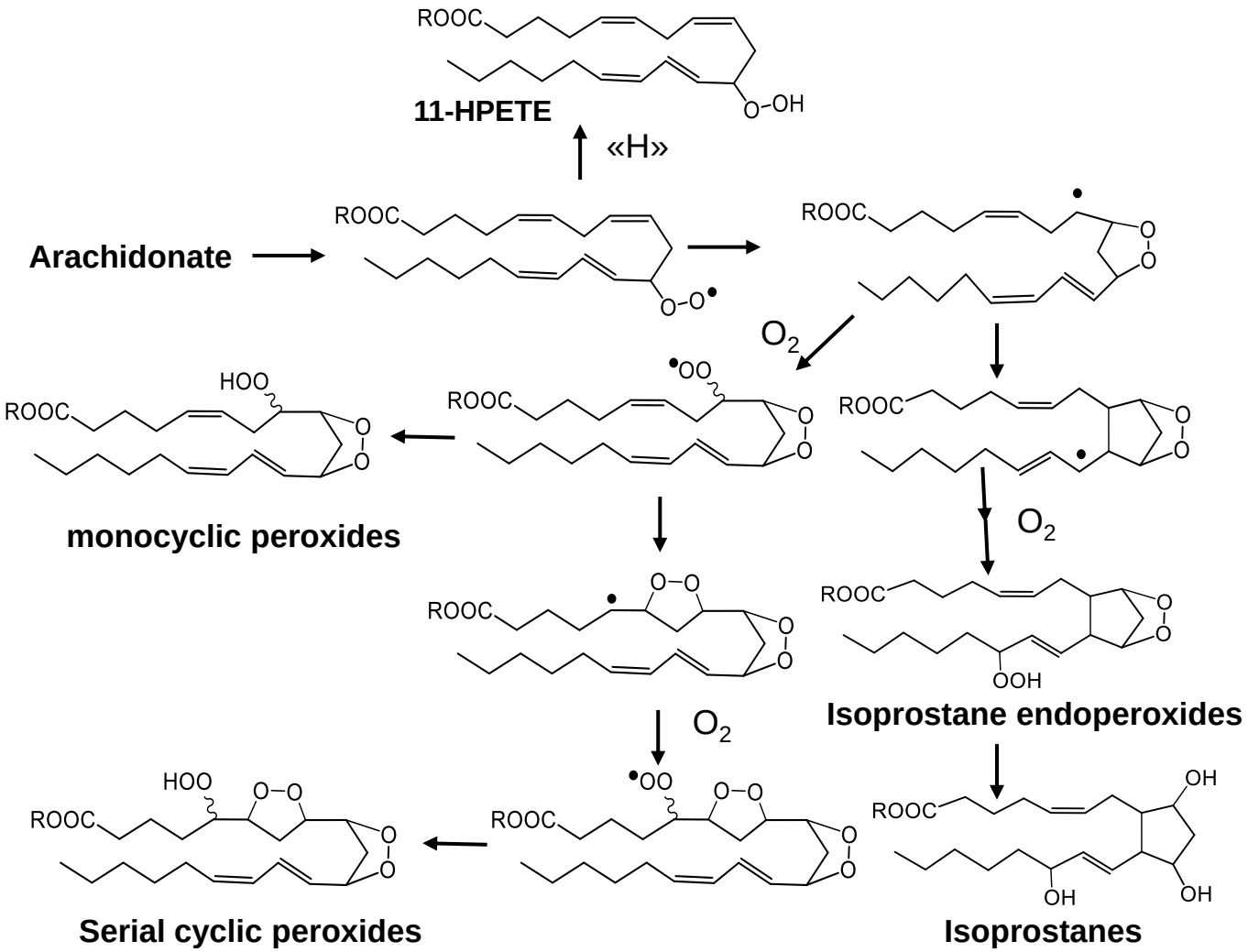


Classes of IsoPs



Isoprostanes comprise four regioisomers with eight stereoisomers. Relatively stable endproducts that are currently viewed as most reliable marker of lipid oxidation *in vivo* (Lawson *et al.*, J. Biol. Chem. 1999: 274, 2444-24444)

Unified Pathway of Arachidonate Oxidation



Attributes of Measuring F_2-IsoPs as a Biomarker of Lipid Peroxidation

- Specific products of lipid peroxidation
- Very stable
- Detectable in all normal biological fluids and tissues
  - Thus allowing the definition of a normal range
- NICI GC/MS assay has a lower limit of detection in the low pg range, a precision of ± 6%, and an accuracy of 96%
- Their formation increases dramatically in animal models of oxidant injury
- Their formation is modulated by antioxidant status
  - Impairment of endogenous antioxidants enhances their formation
  - Administration of antioxidant agents suppresses their formation

An Important Concept Regarding the Formation and Measurement of F_2-IsoPs

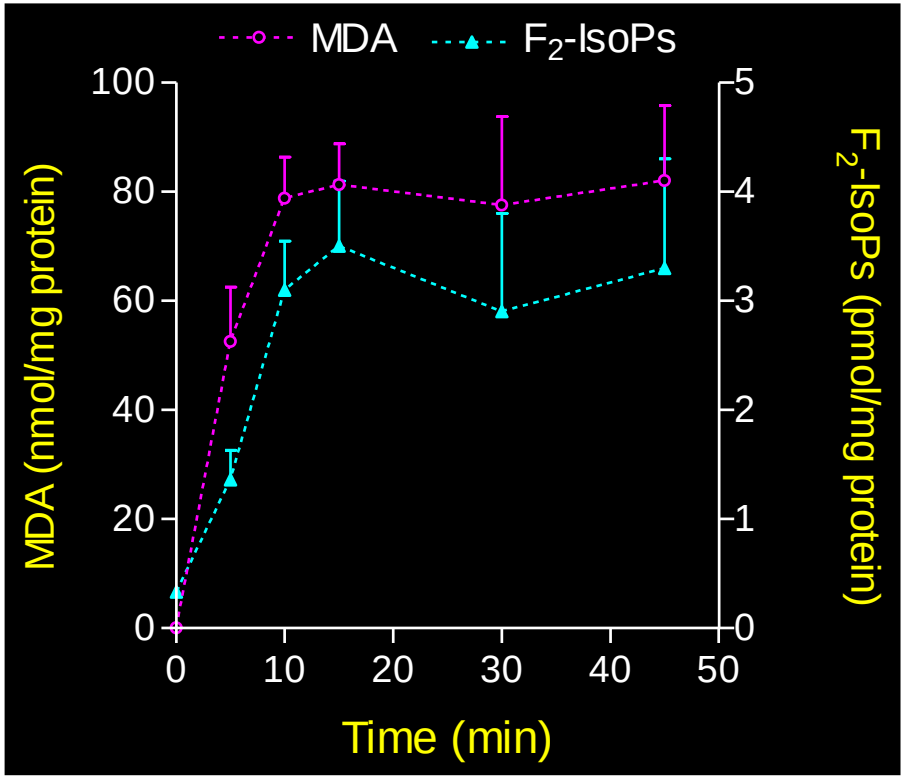
- Initially formed *in situ* on phospholipids (PL) and then released by phospholipase action

- Levels of *free* F_2-IsoPs, *e.g.* in plasma, provides an index of total endogenous production of IsoPs

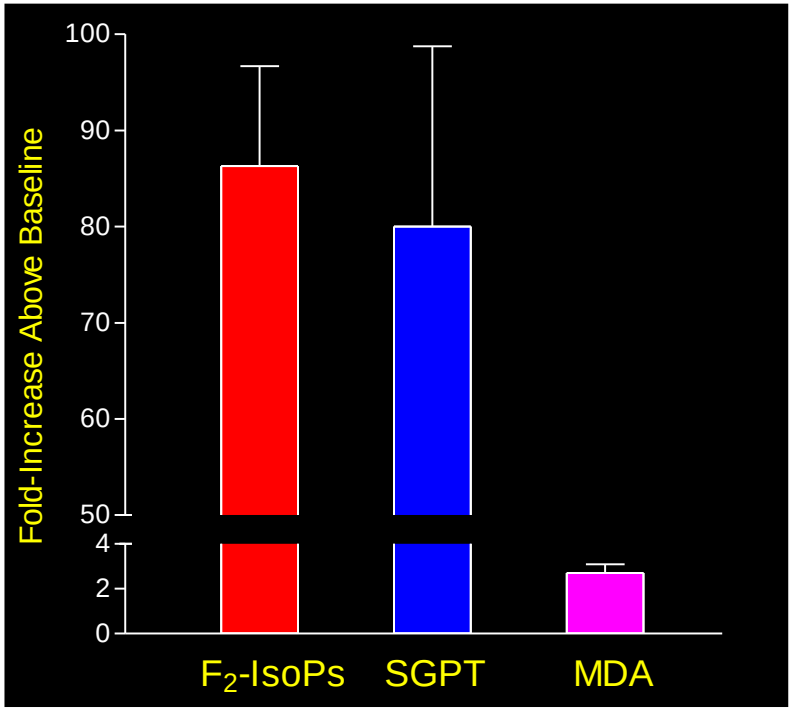
- Levels of *esterified* F_2-IsoPs can localize oxidant injury in key tissues/organs of interest

Comparison of Measurements of F2-IsoPs and MDA

In Vitro



In Vivo



The formation of both *in vitro* correlate well - note there is ~25,000 times more MDA than F_2-IsoPs

However, *in vivo* levels of F_2-IsoPs detected are ~28-fold higher than MDA

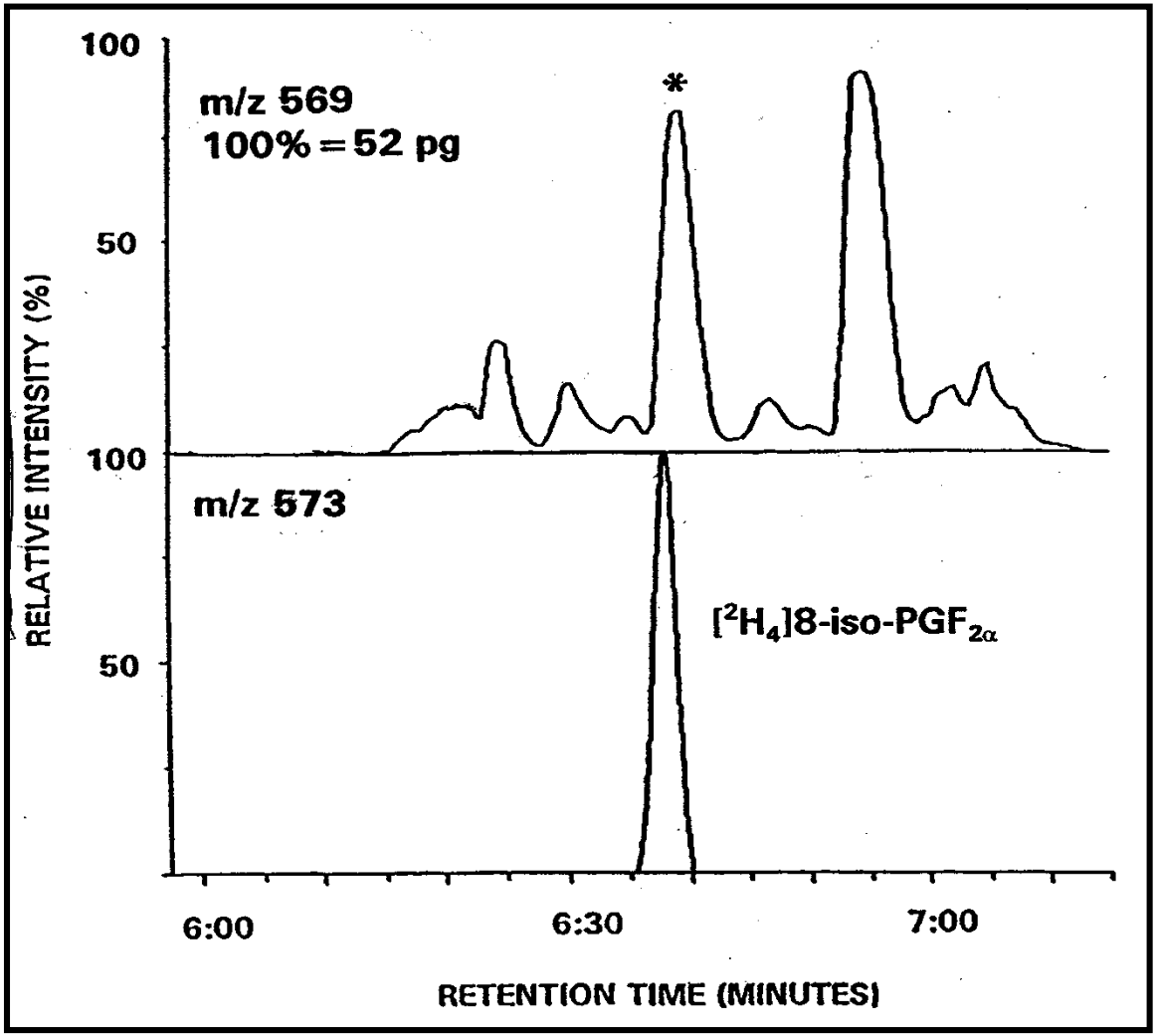
Immunoassay Methods to Quantify IsoPs

- Immunoassays advantageous because they are more economical and less labor intensive.
- Polyclonal antibodies have been made by several investigators and are commercially available.
- Accurate quantification using immunoassays requires initial compound purification.
- Amounts measured by immunoassays often differ from those obtained by mass spectrometry.
- highly specific monoclonal abs has been commercially introduced..

Disadvantages of Isoprostane Quantification to Assess Oxidant Stress

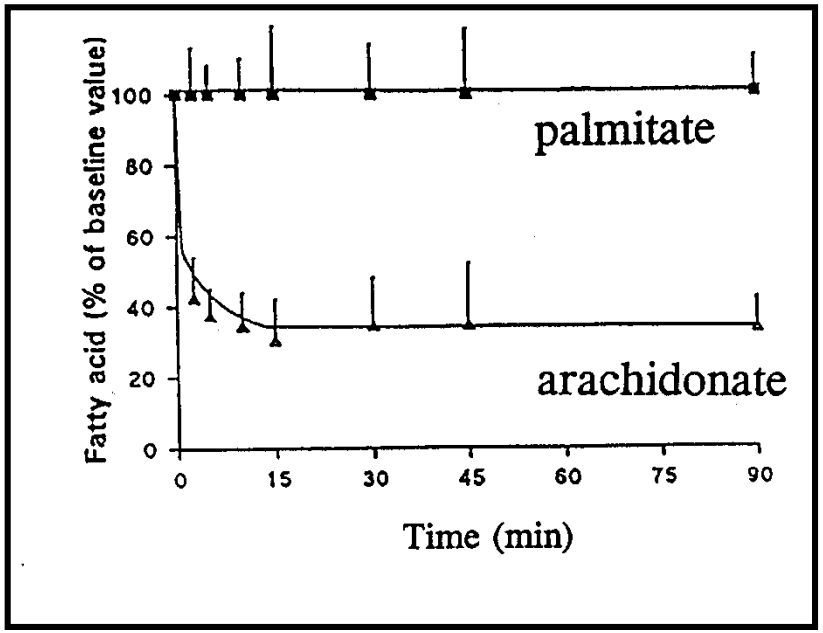
- Samples must either be analyzed immediately or stored at –70^{o} C.
- Increases in IsoPs locally in tissues or fluids aren't detected by measuring systemic oxidant stress.
- F_2-IsoPs represents only one of a myriad of arachidonate oxygenation products.
- Analysis is labor intensive and requires expensive equipment.
- Urinary IsoPs are not a valid measure of systemic oxidant stress since a major source of urinary IsoPs is likely the kidney.

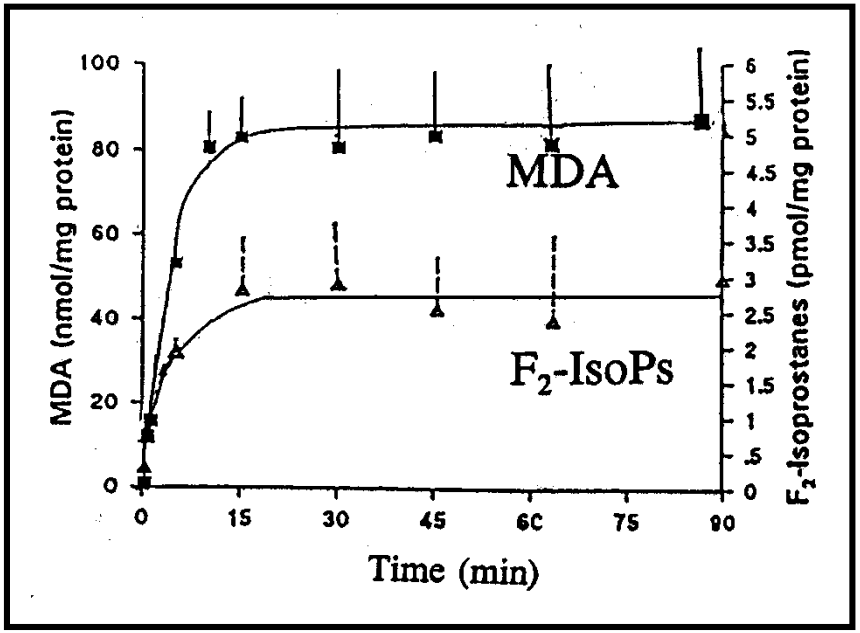
Analysis of F_2-Isoprostanes in Human Plasma



Formation of F_2-Isoprostanes in Peroxidizing Microsomes

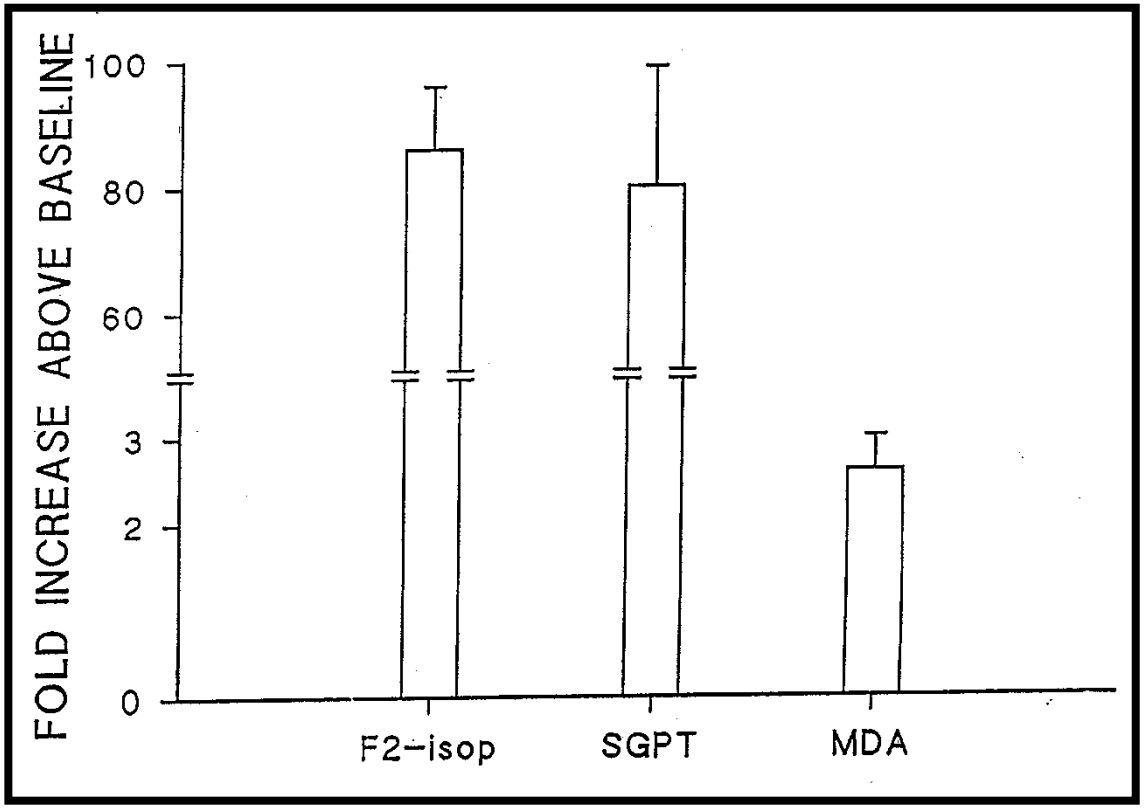
F_2-IsoP formation correlates with disappearance of arachidonate and generation of MDA.



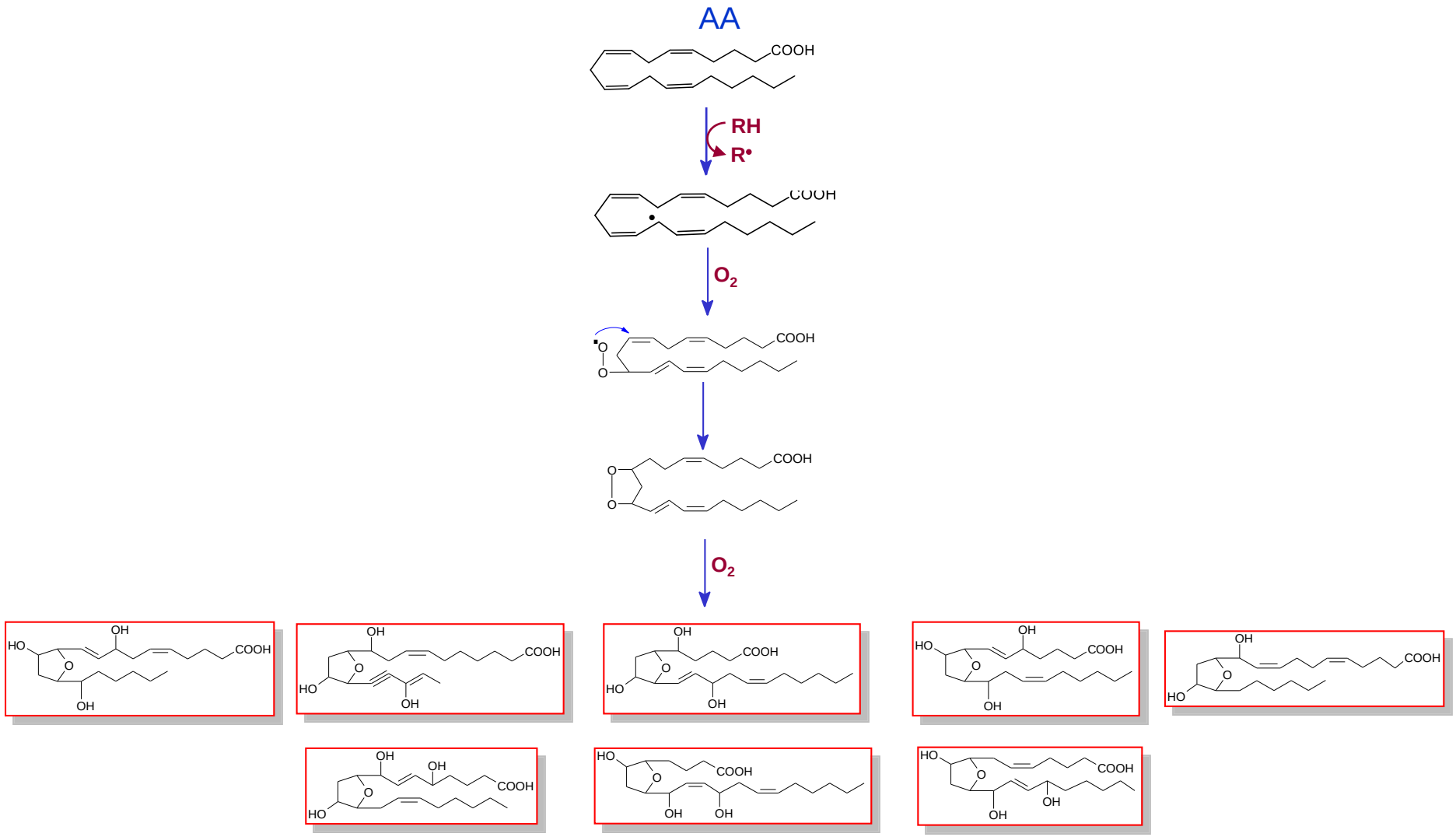


F_2-Isoprostanes Are a Reliable Marker of Lipid Peroxidation in Vivo

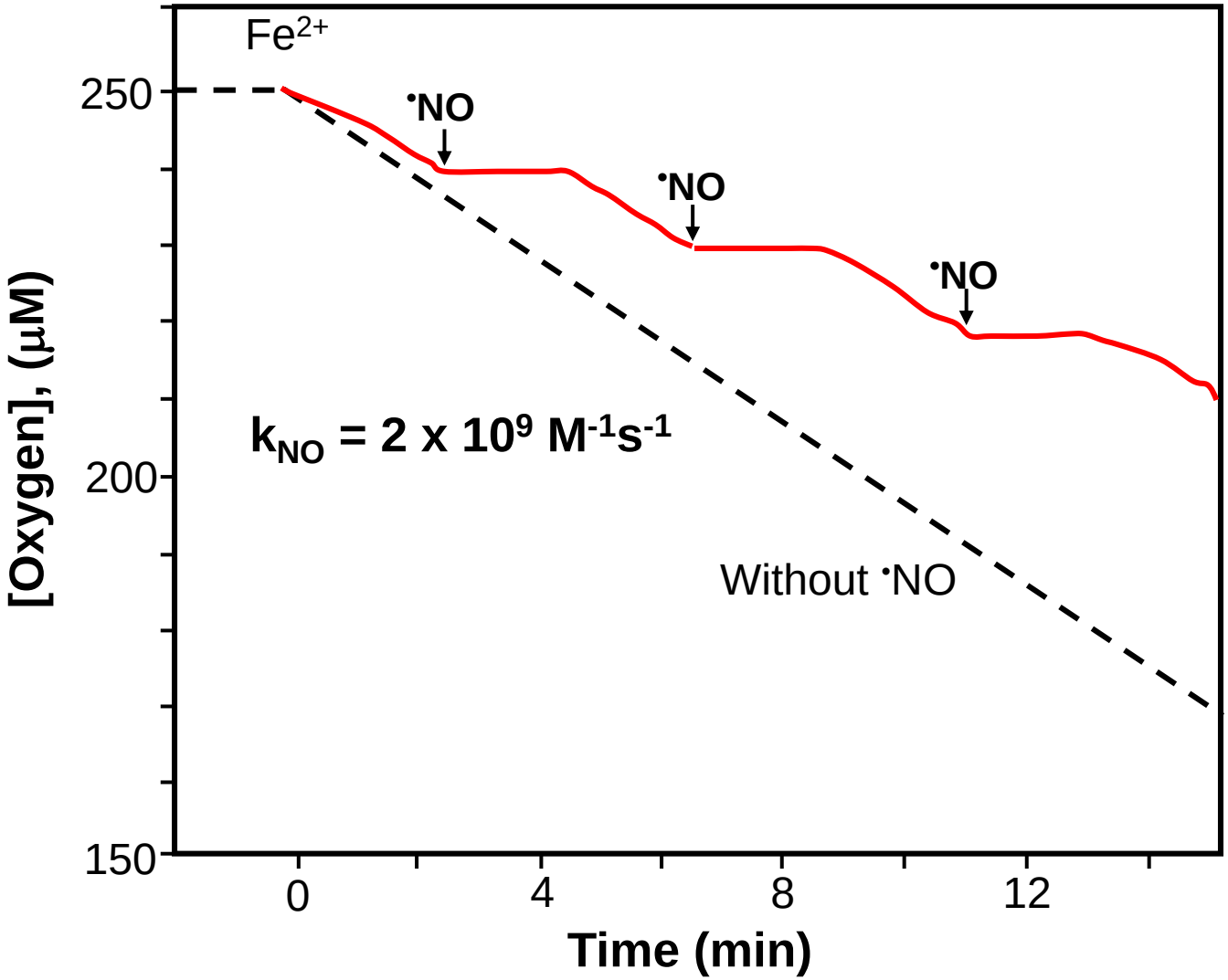
- Comparison of formation of MDA and F_2-IsoPs with hepatic injury in CCl_4-treated rats.



Formation of Isofuran Regioisomers



Oxygen Absorption Determination: •NO Inhibits Iron-Induced Lipid Peroxidation



POLITECNICO DI MILANO



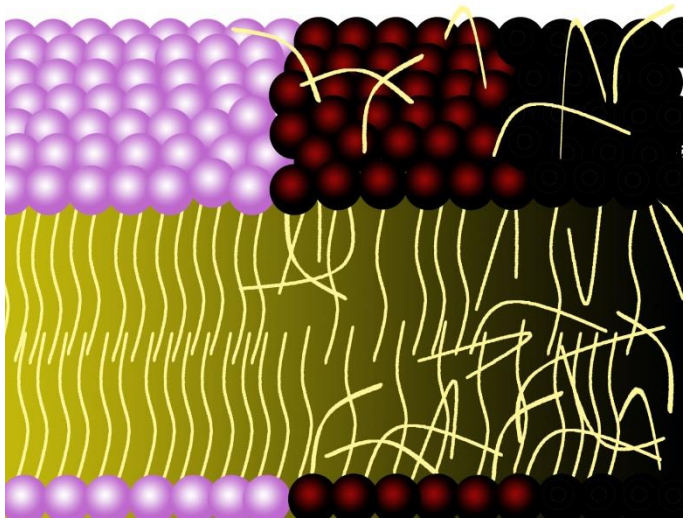


Lipid Peroxidation: Effects

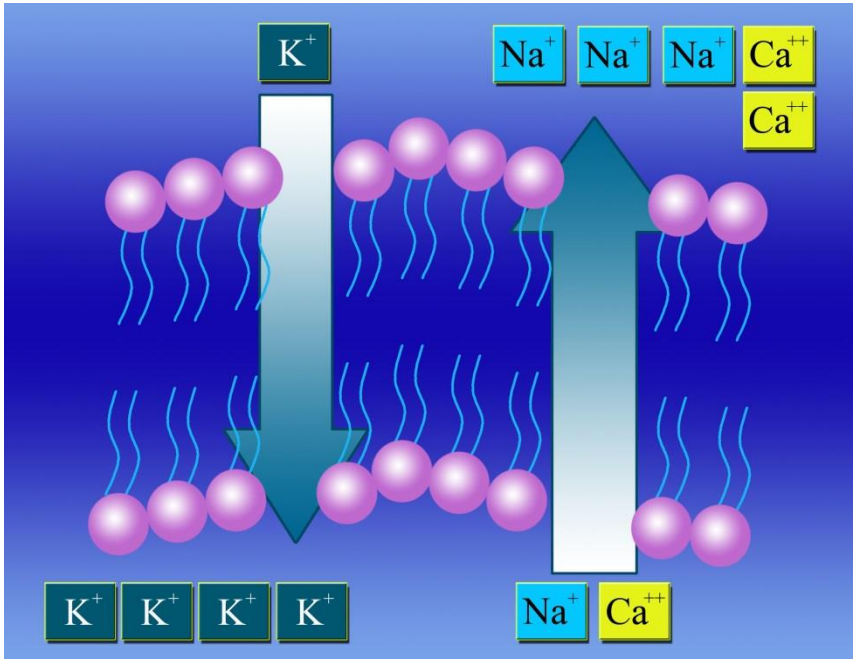
Prof. Attilio Citterio
Dipartimento CMIC “Giulio Natta”

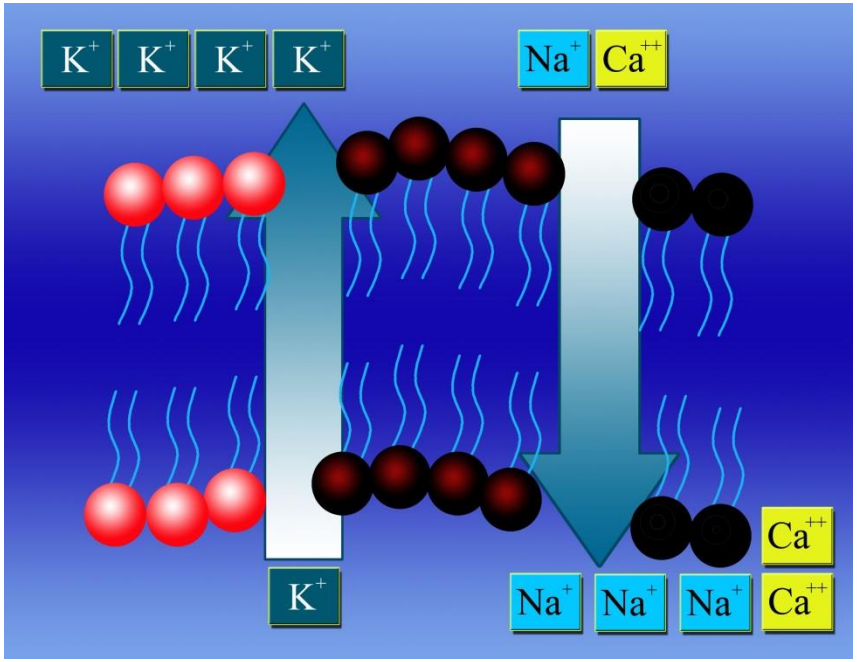
Consequences of Lipid Peroxidation

- **Structural changes in membranes**
  - alter fluidity and channels
  - alter membrane-bound signaling proteins
  - increases ion permeability
- **Lipid peroxidation products form adducts/crosslinks with non lipids**
  - e.g., proteins and DNA
- **Cause direct toxicity of lipid peroxidation products**
  - e.g., 4-hydroxynonenal toxicity
- **Disruptions in membrane-dependent signaling**
- **DNA damage and mutagenesis**



Change of Ion Permeability





Altered ion fluxes

Na^+ influx
K^+ efflux

Cell Swelling

Ca^{++} influx

Activation of calcium-Dependent phospholipases

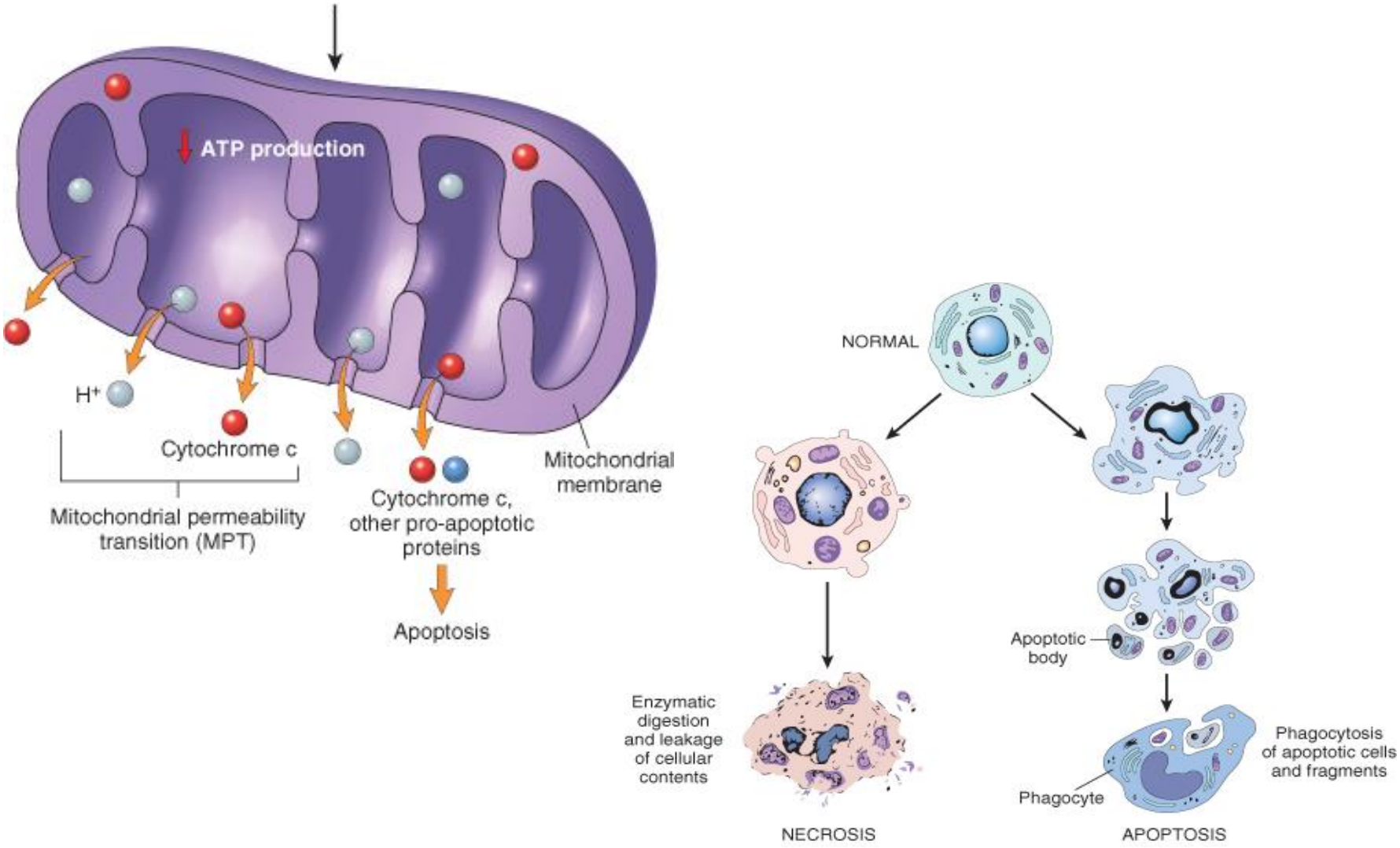
Change in biochemical and physiologic state of cell membrane

Alteration in cytoskeleton and intracellular transport mechanisms

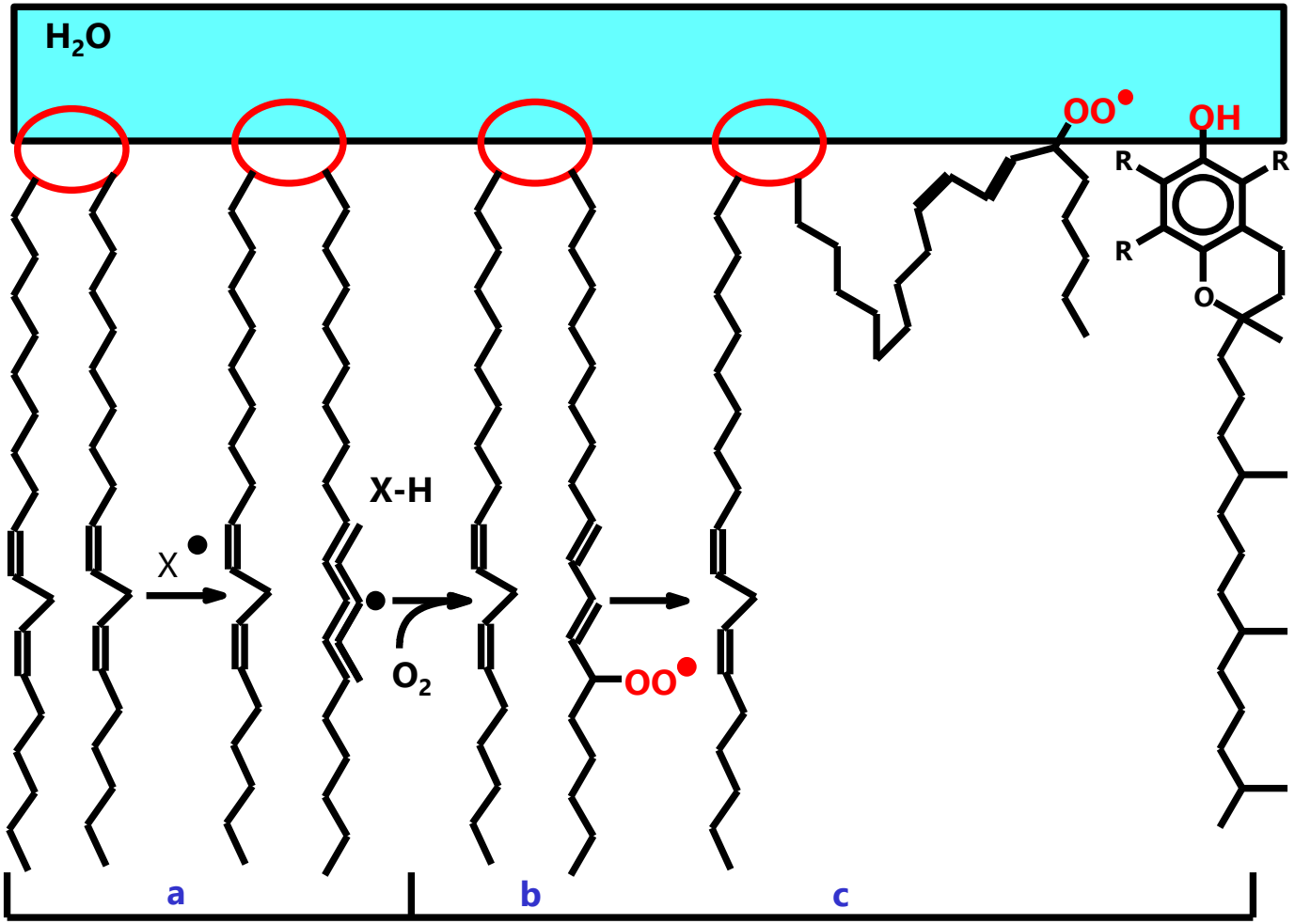
Activation of Ca^{++} dependent Protein kinases

Altered function

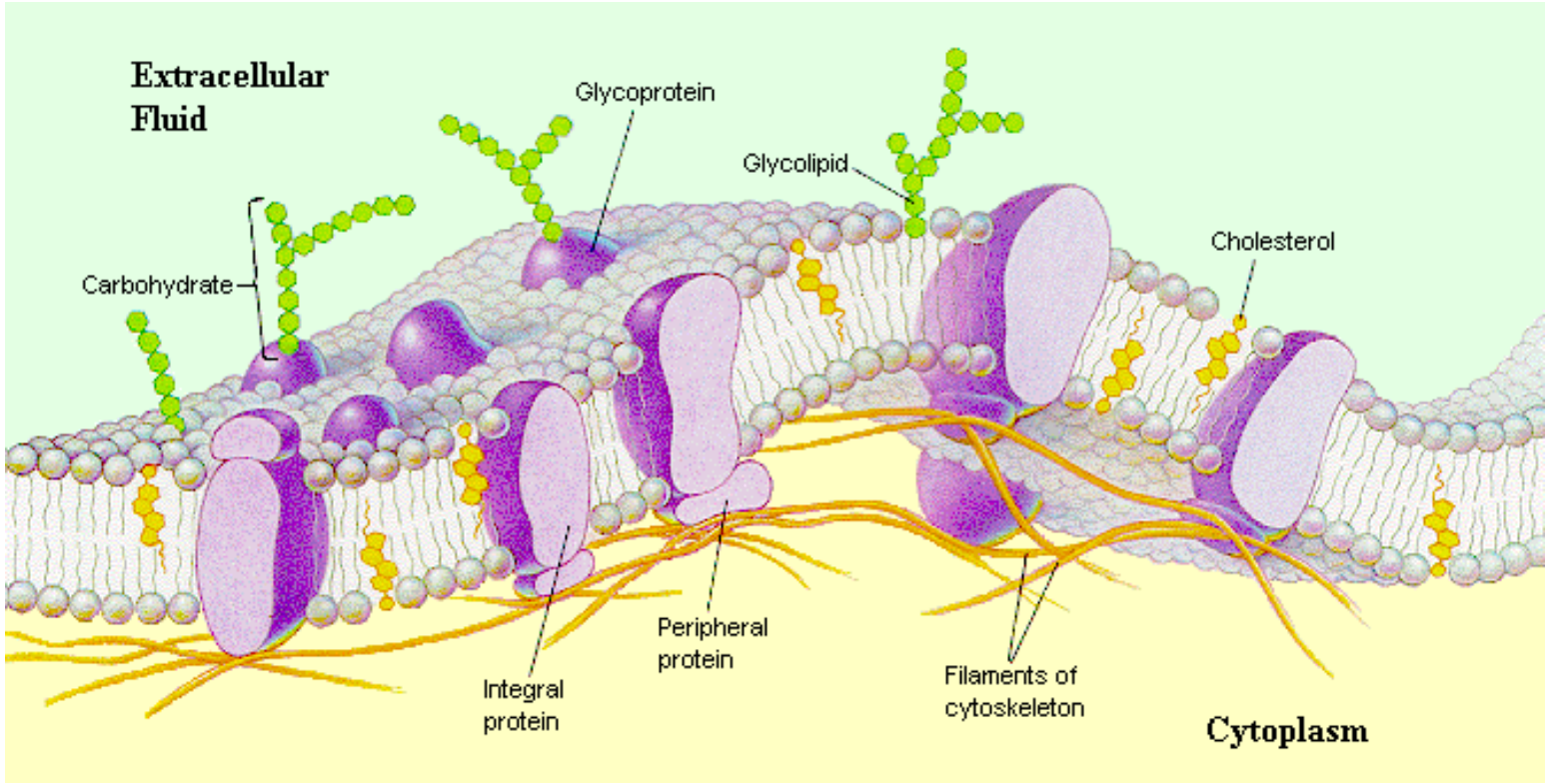
Consequences of Mitochondrial Membrane Lipid Peroxidation



Disruption of Membrane Order by Lipid Peroxidation and Role of Vitamin E

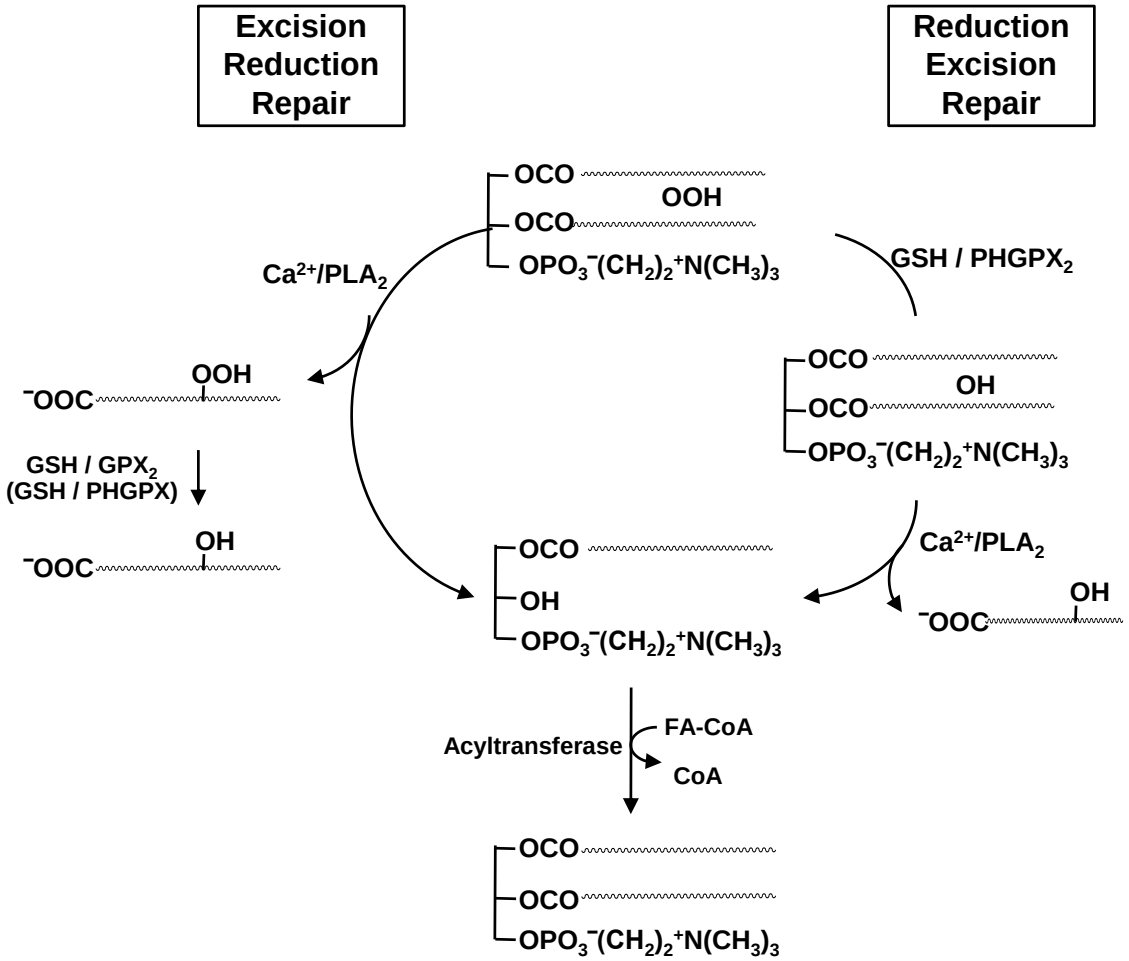


Oxidative Damage to Lipids



- Increase membrane rigidity
- Reduce activity of membrane-bound enzyme
- Alter activity of membrane receptors
- Alter cell permeability

Significance of Lipid Oxidation: Repair of Oxidized Lipids



Ghirotti (1998) *J. Lipid Res.* **39**: 1529-1542

(I)
Lipid-soluble antioxidants (vitamin E) prevent oxidative degradation of lipids and terminate lipid oxidation to form lipid hydroperoxides.

(II)
Phospholipid hydroperoxide can be reduced by phospholipid hydroperoxide GSH peroxidase (PHGPX), or excised by phospholipase A_2 (PLA_2; which preferentially cleaved oxidized fatty acids)

(III)
Cleaved fatty acid hydroperoxide can be reduced by GSH peroxidase (GPX), and reduced phospholipid hydroperoxide can be cleaved by PLA_2.

(IV)
Lyso-phospholipid (lacking one fatty acid chain) can be repaired by acyltransferase