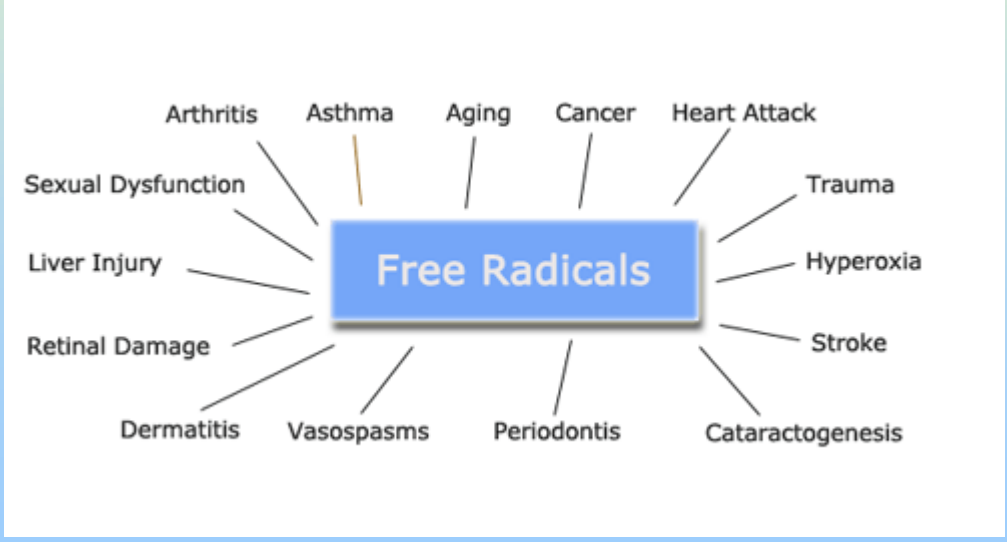
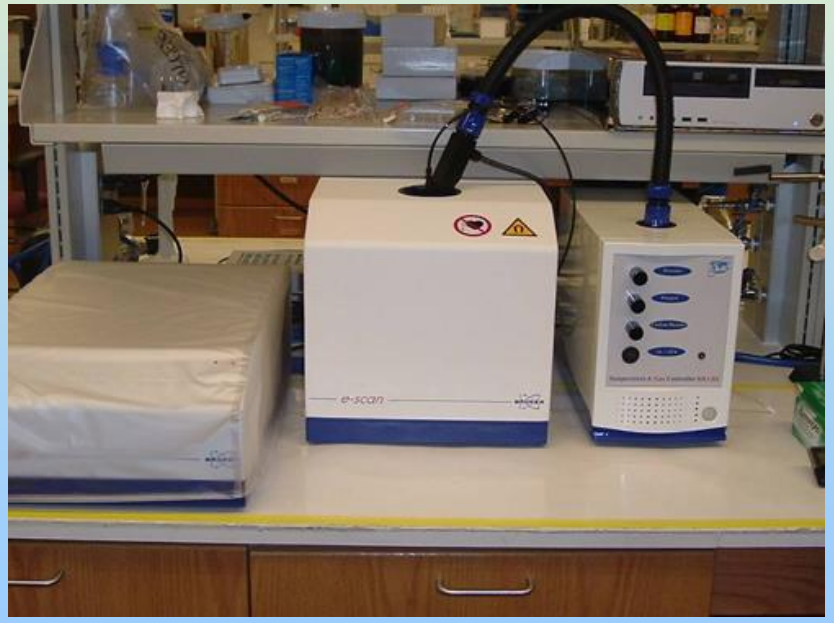
Detection of free radicals and reactive oxygen/nitrogen species



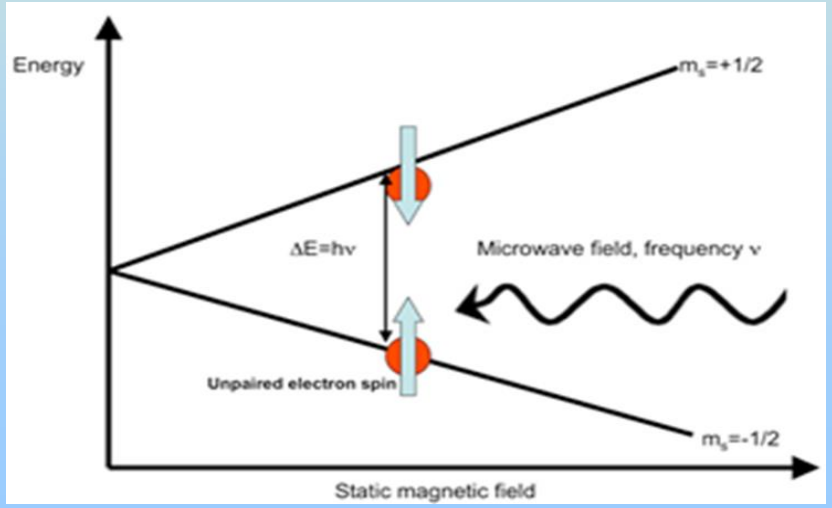
Methods

- Direct assay
- Indirect assay
- Chemical and physical method
- Biochemical methods



Electron spin resonance, electron paramagnetic resonance (ESR, EPR)

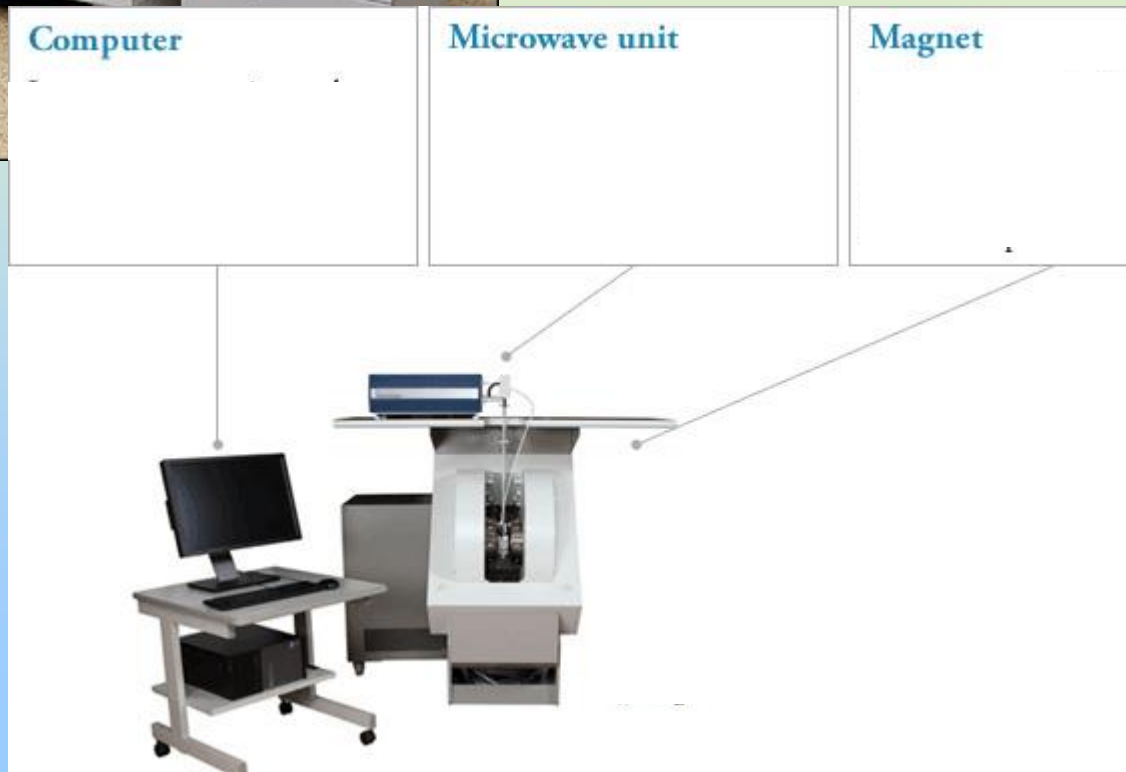
- Method for directly measuring of free radicals
- ESR was developed in the late 1940s
- ESR uses paramagnetism of free radicals
- Sample is given in magnetic field generated by magnets and exposed to microwave radiation
- After absorbing energy, unpaired electrons are excited, they chang spin and may jump from the ground state to a higher energy state



- Unpaired electron can move between the two energy levels by either absorbing or emitting of energy
- Absorption and emision is monitored and converted into a spectrum

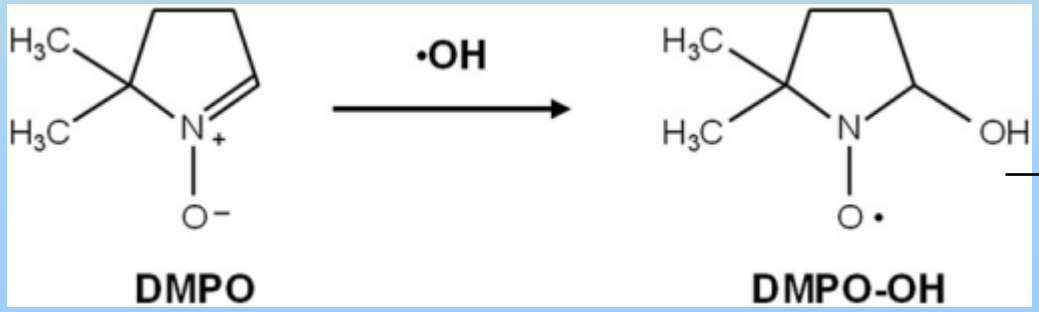
ESR spectrum

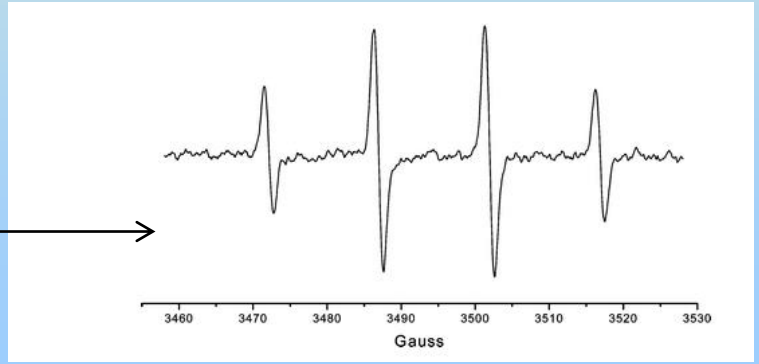
Electron spin resonance spectrometer



Spin trapping

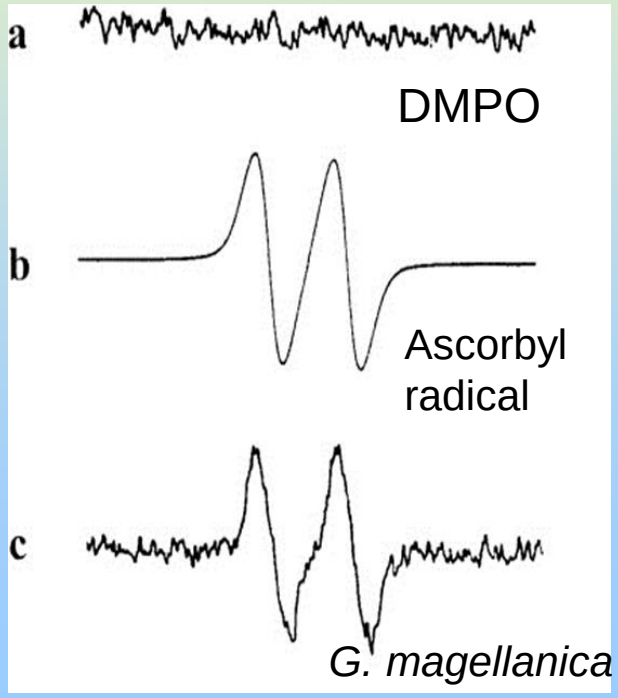
- Spin trapping uses compounds called spin-traps - 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO)
- Spin-trap reacts rapidly with the free radical to form radical-adducts that are more stable and can be detected
- Spin-traps have been used in experimental animals to demostrate the production of free radical *in vivo*
- Toxicity of spin-traps



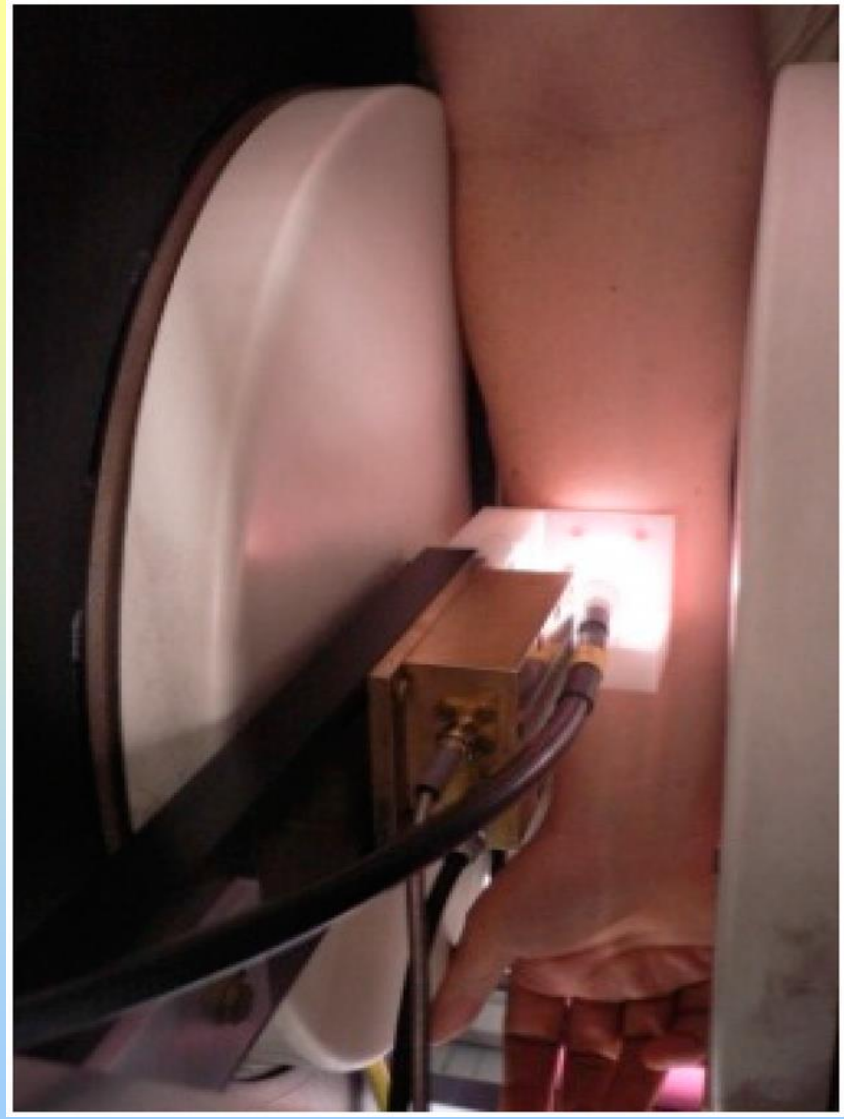


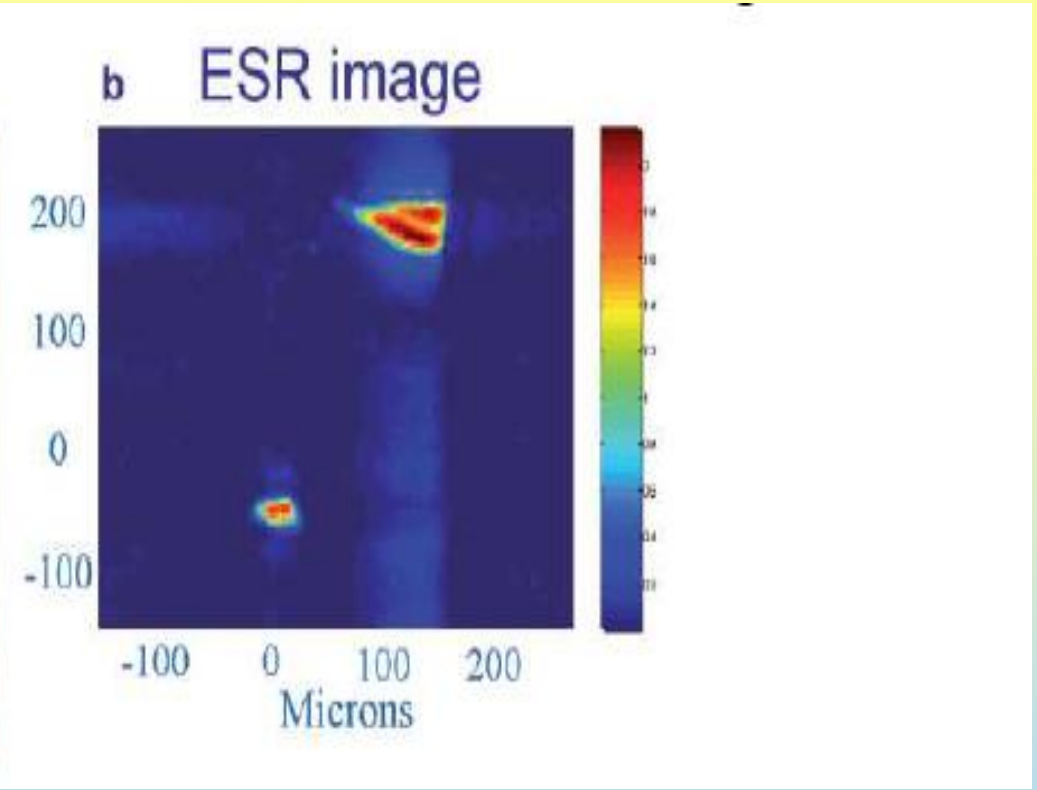
ESR spectrum of the OH adduct of DMPO

Gunera magellanica

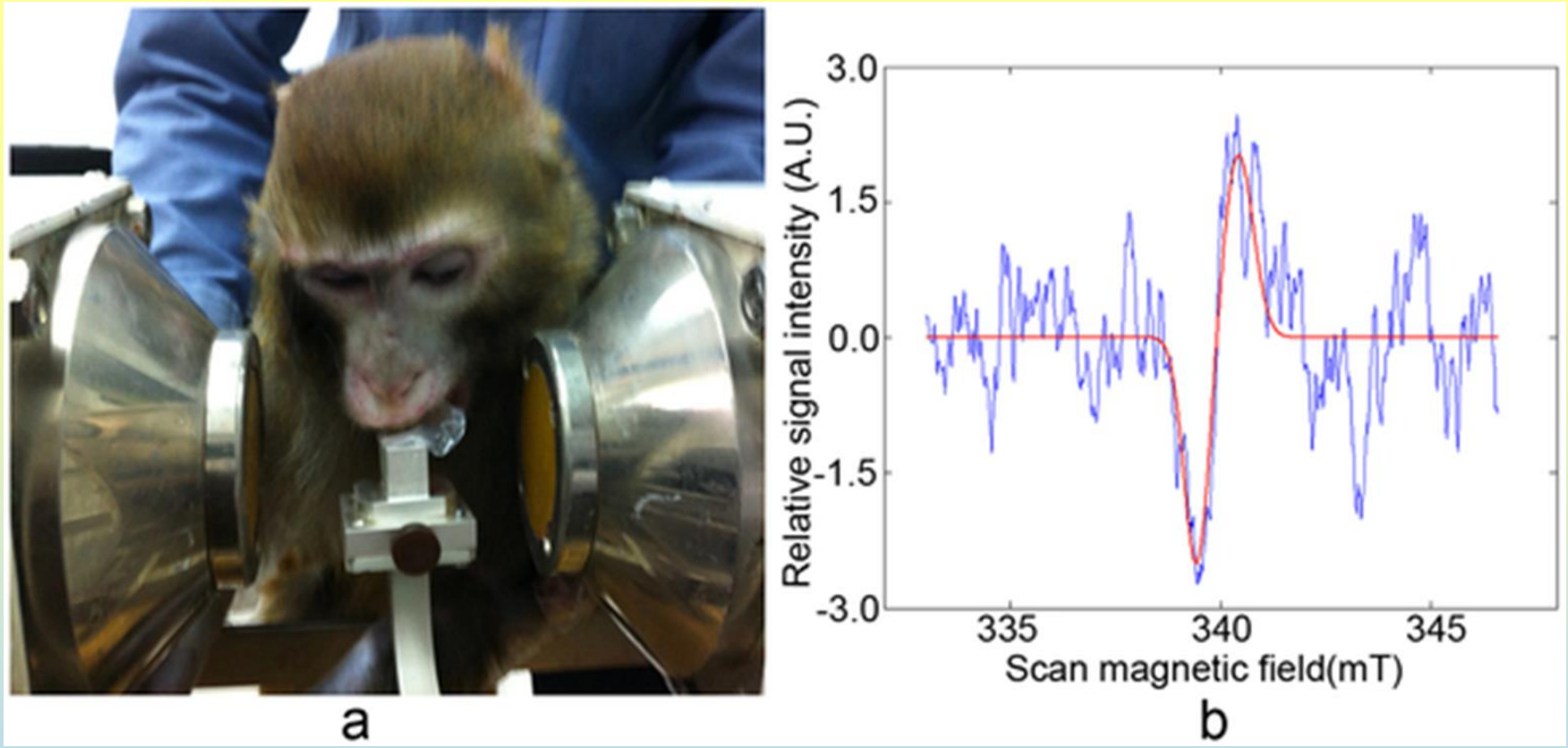


Plant, Cell and Environment (2004), 27, 1415–1423

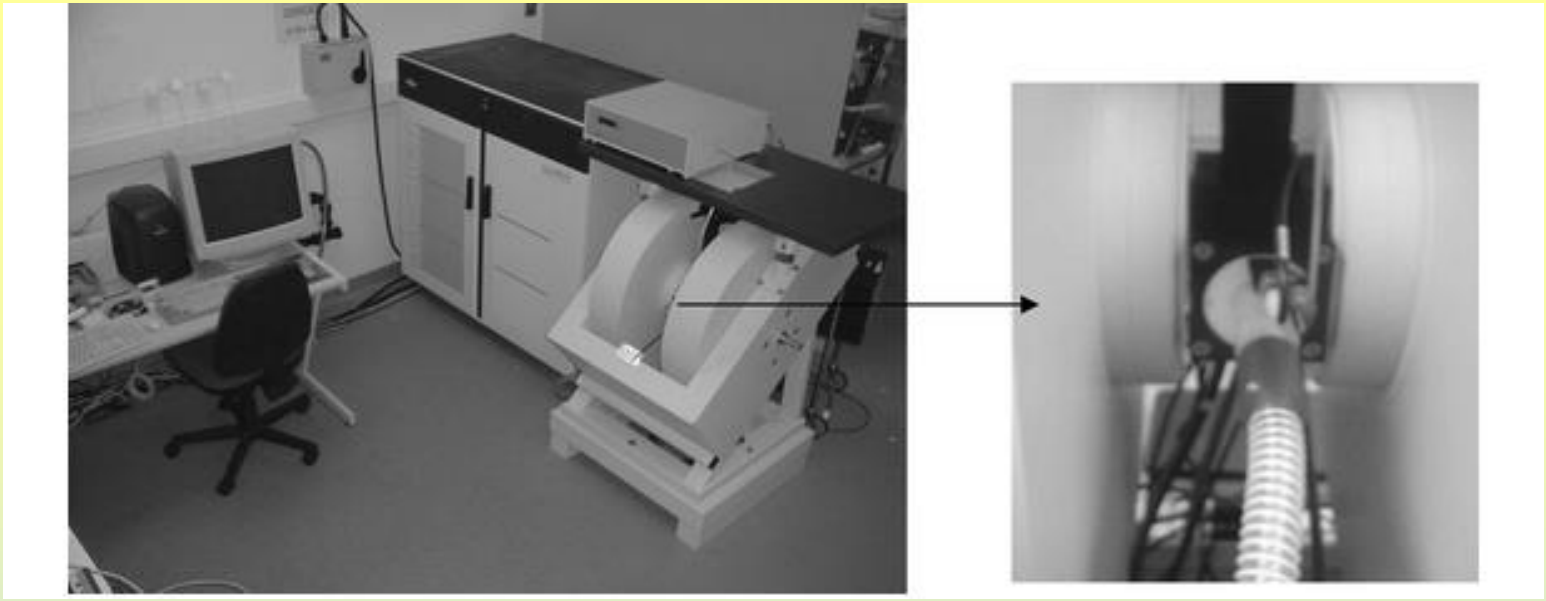


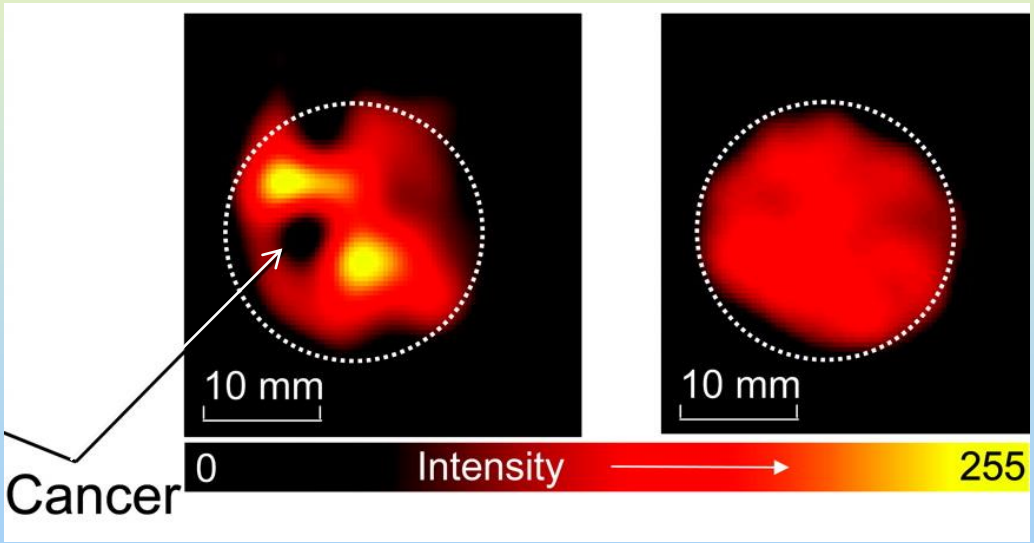


Lohan B.S. et al.: Determination of the Antioxidant Status of the Skin by In Vivo-Electron Paramagnetic Resonance (EPR) Spectroscopy. Cosmetics 2015, *2*, 286-301



Junwang G. et al.: New Developed Cylindrical TM010 Mode EPR Cavity for Xband In Vivo Tooth Dosimetry. PLoS ONE 2014, 9, 1-8





Jackson S. K. at al.: *In vivo* EPR spectroscopy: biomedical and potential diagnostic applications. Faraday Discuss. 2004, 126, 103-117

Mikuni T. at al.: *In vivo* Detection of Gastric Cancer in Rats by Electron Paramagnetic Resonance Imaging. Cancer research 2004, 64, 6495– 6502.

High-Performance Liquid Chromatographic (HPLC) detection of free radicals

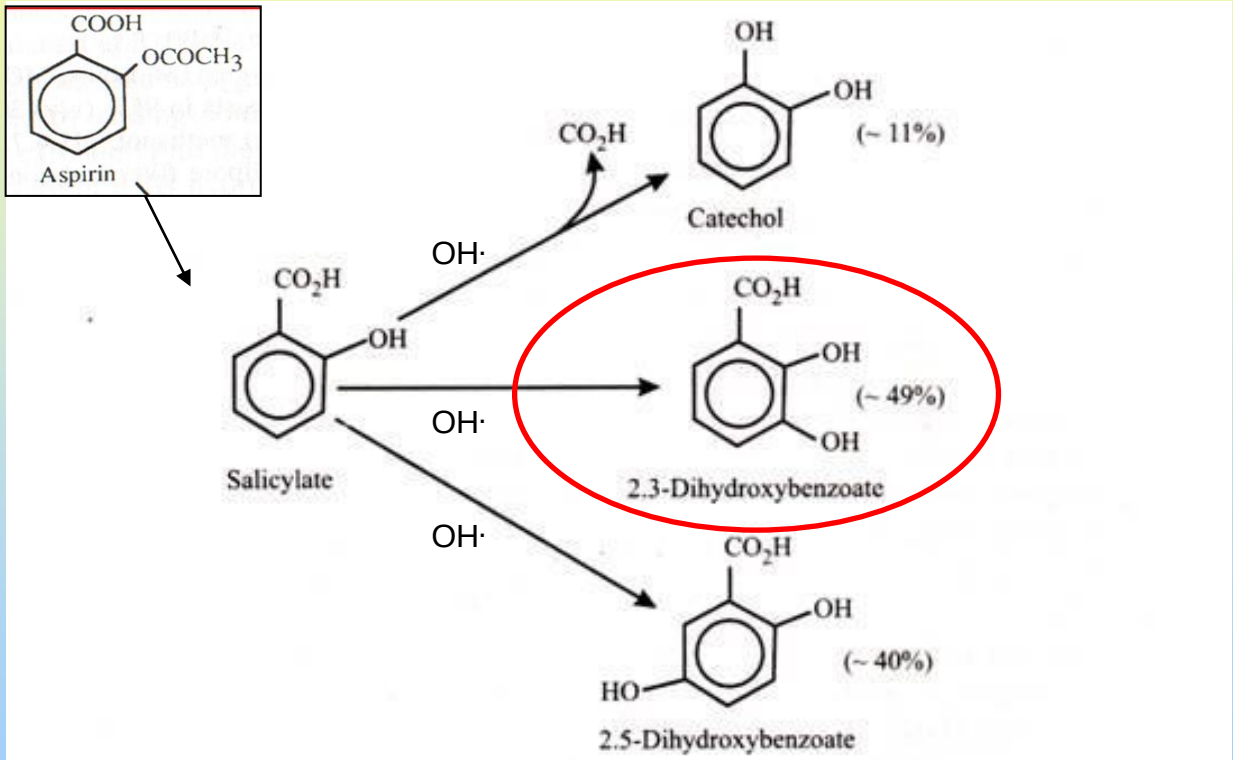
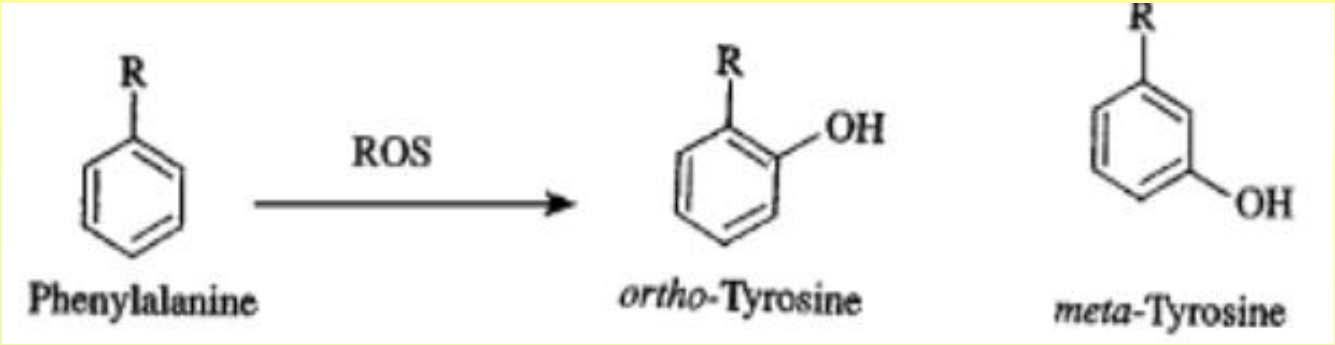
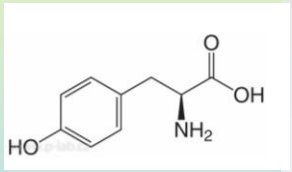


Fig. 5.33 The products of salicylate-OH- interactions (Reprinted with permission from Hallwell, B., and Gutteridge, J. M. C., *Freee Radicals in Biology and Medicine*, Clarendon Press, Oxford, 1989, 55.)



enzym

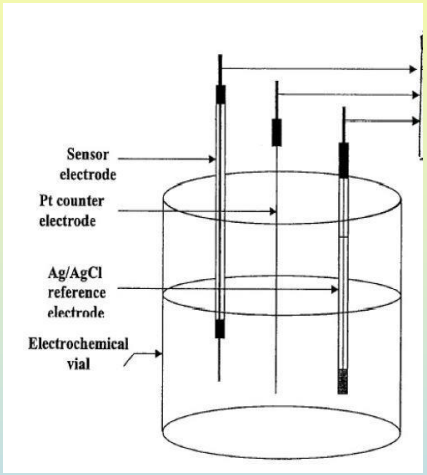


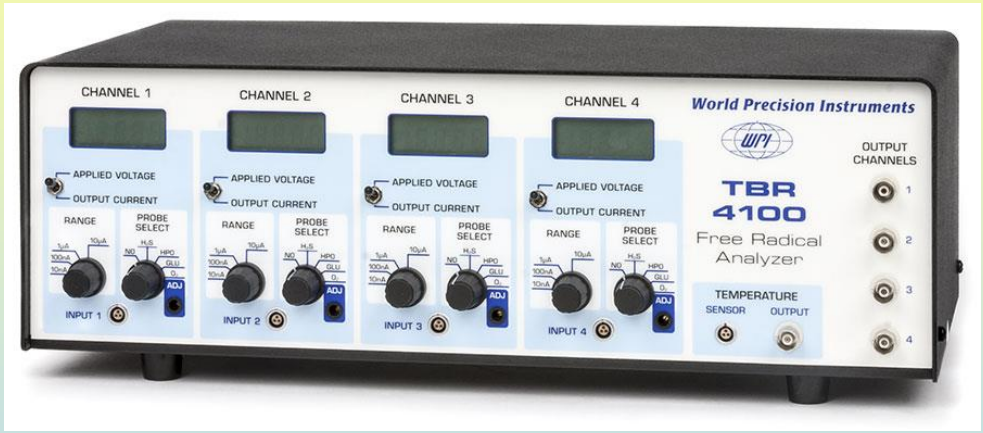
tyrosine

High Performance Liquid Chromatography (HPLC)

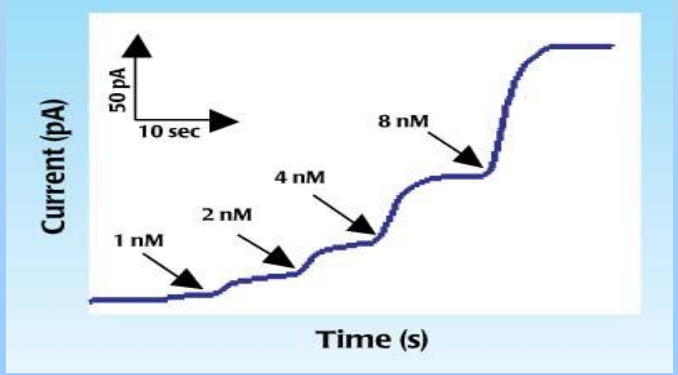
Electrochemical detection of ROS/RNS

- Electrochemical methods study an analyte by measuring the potential or current in a solution by electrodes





- Real-time detection using free radical biosensors for measuring nitric oxide, hydrogen peroxide, hydroxyl radical

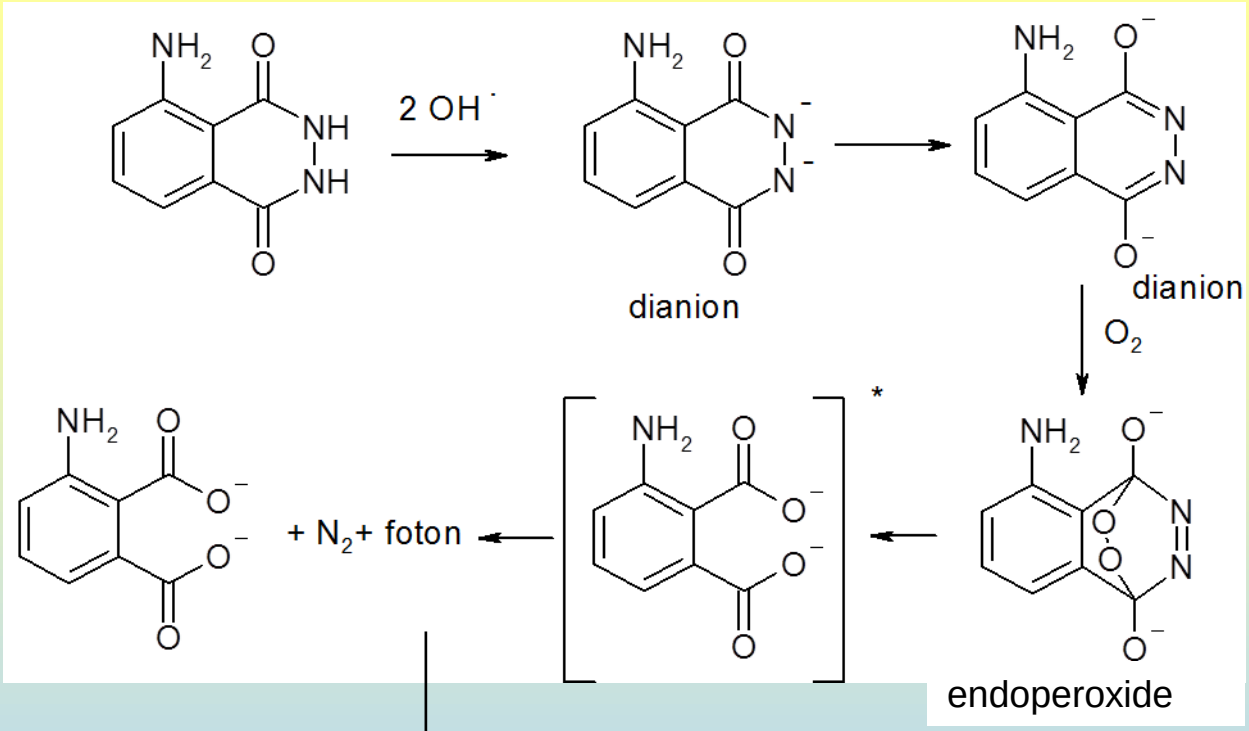


https://www.youtube.com/watch?v=6GzNh0XRfq8

Chemiluminescence measurement for ROS/RNS detection

- Chemiluminescence is the emission of light as the result of a chemical reaction of free radical with specific compounds
- This emitted light can be measured with the help of a luminometer

- Luminol is widely used in free radical detection


2 OH·
dianion
dianion
O_2
endoperoxide
+ N_2+ foton

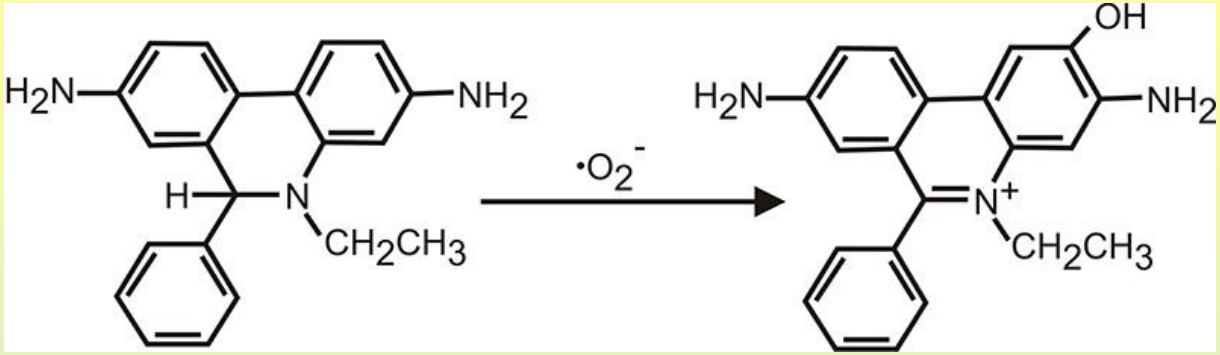
Fluorescence measurement for free radical detection

- Fluorescence is the emission of light by a substance that absorbed light
- In most cases, the emitted light has a longer wavelength, and therefore lower energy, than the absorbed radiation

luminometer

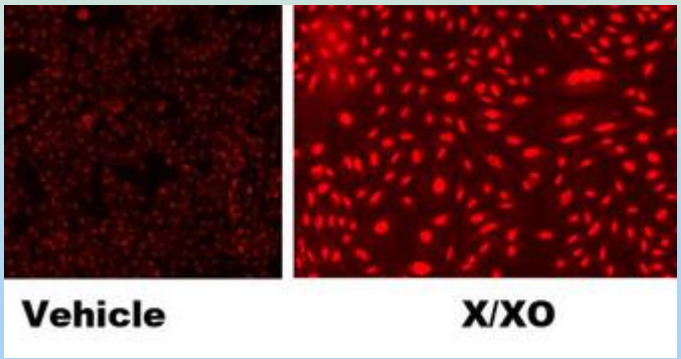
fluorescence microscopy

Fluorescence measurement for free radical detection



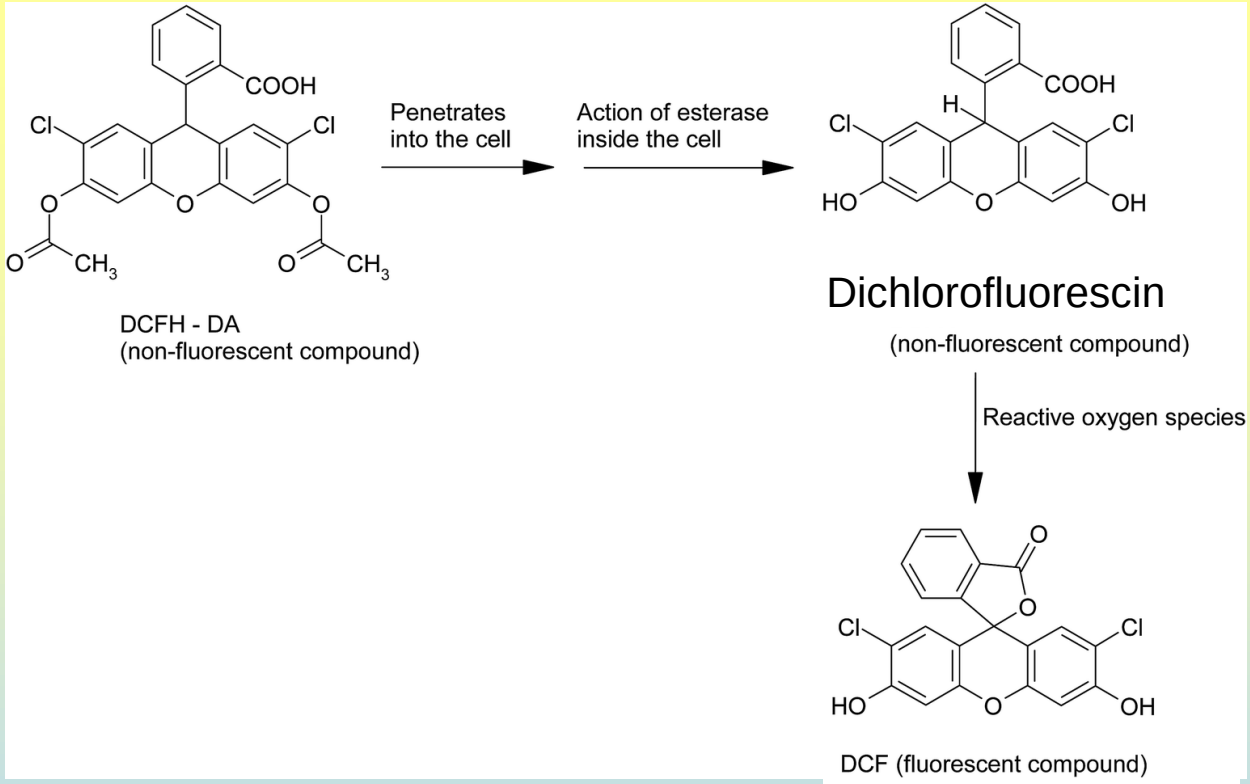
dihydroethidium

2-hydroxyethidium

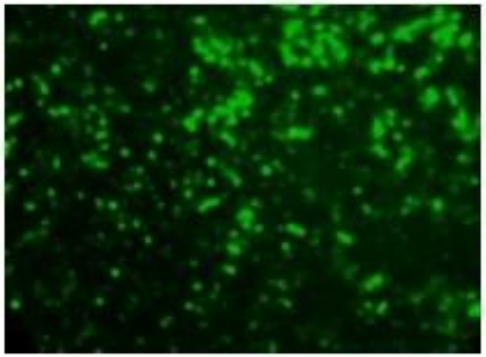


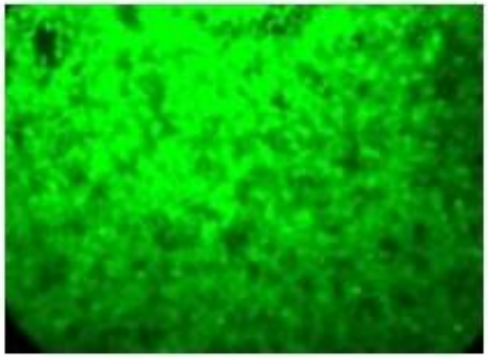
fluorescence microscopy

Free Rad Biol Med (2010), 48, 983-1001



DCFH - DA
(non-fluorescent compound)



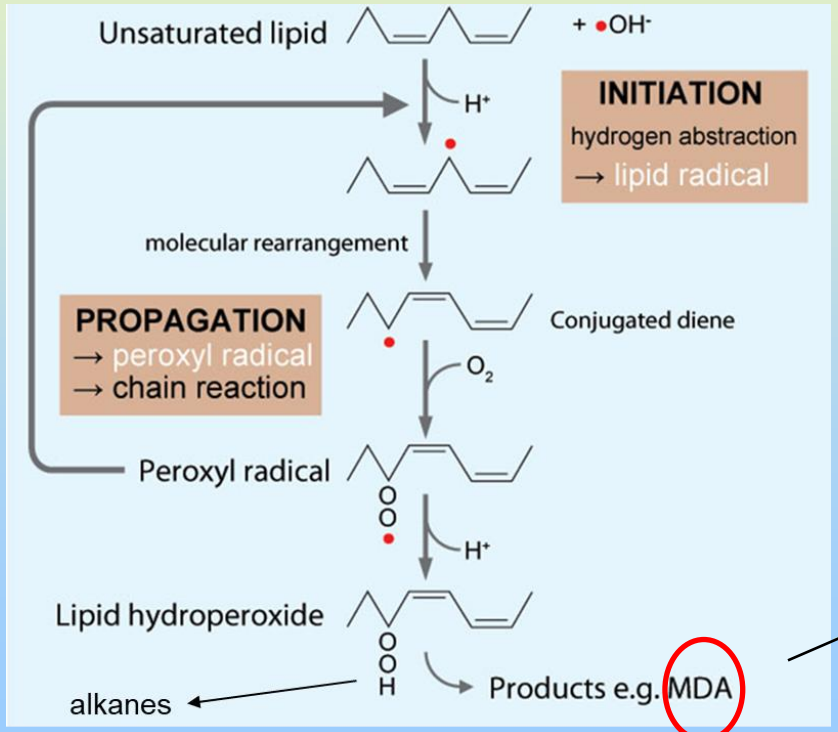


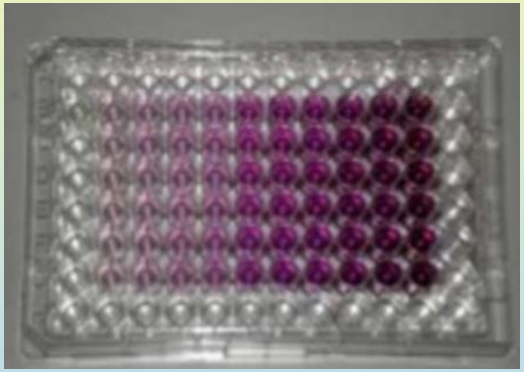
control cells

cells with oxidative stress

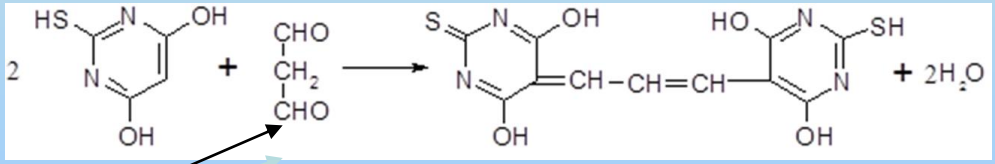
Indirect methods of ROS/RNS detection

- Monitoring of the presence of lipid peroxidation products
  - Detection of malondialdehyde by thiobarbituric acid using spectrophotometer (532 nm wavelength)





thiobarbituric acid



Indirect methods of ROS/RNS detection

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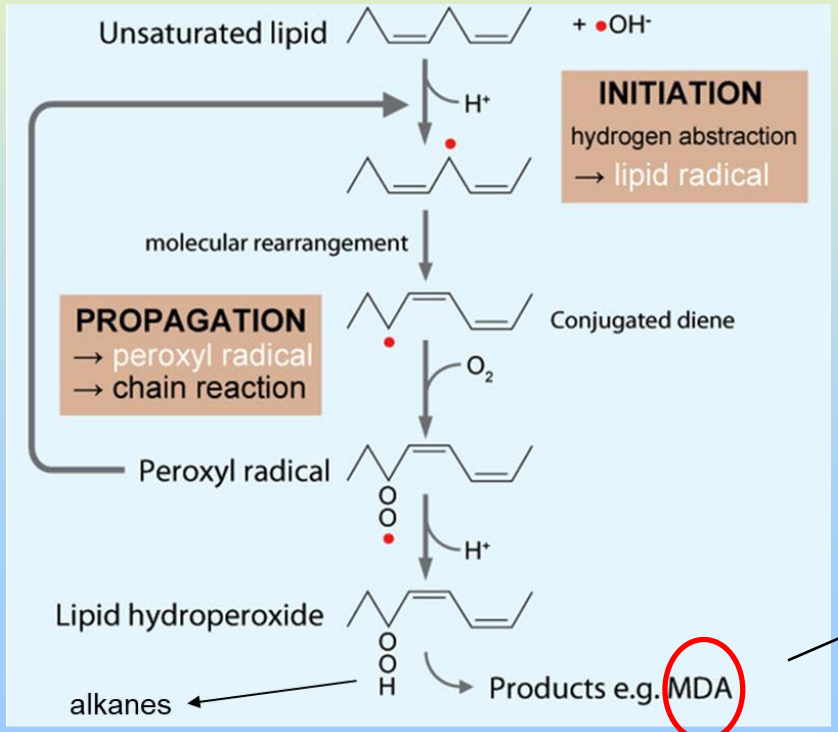
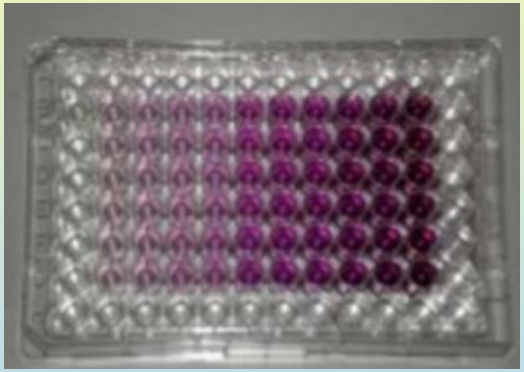
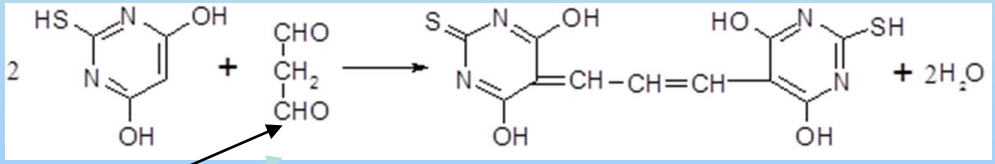
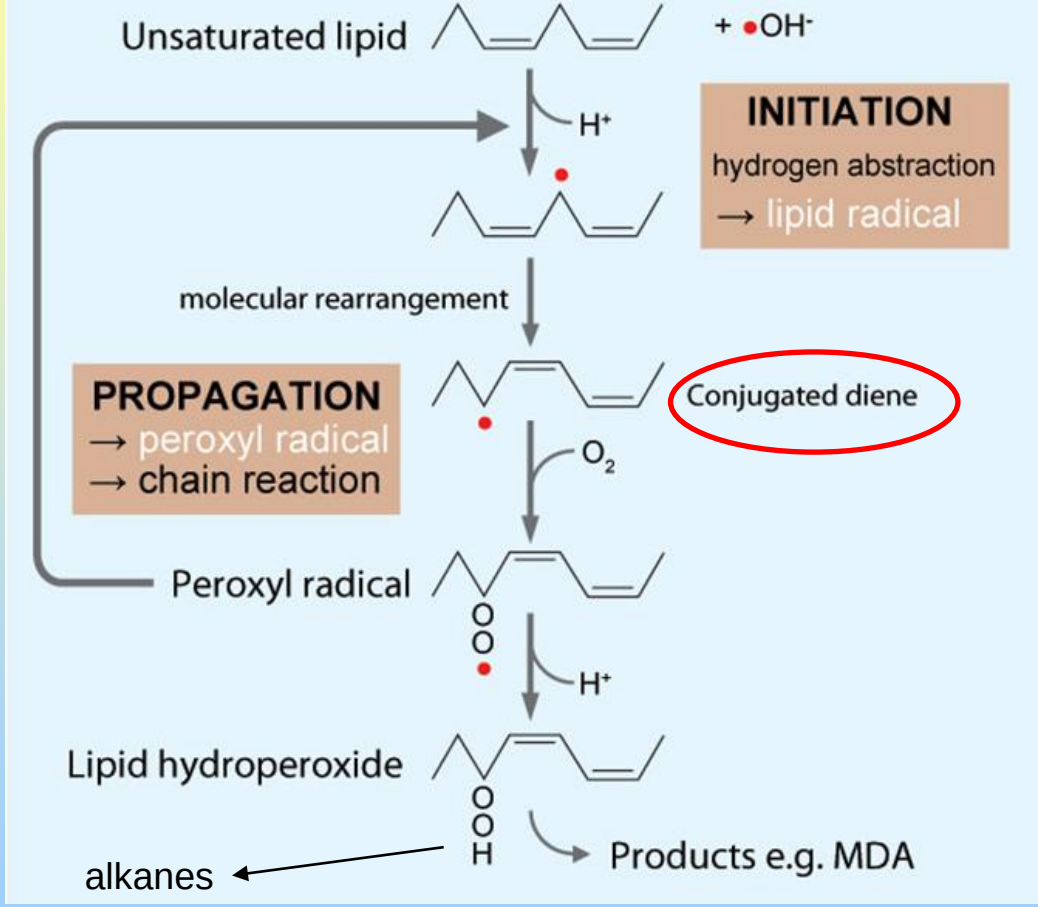




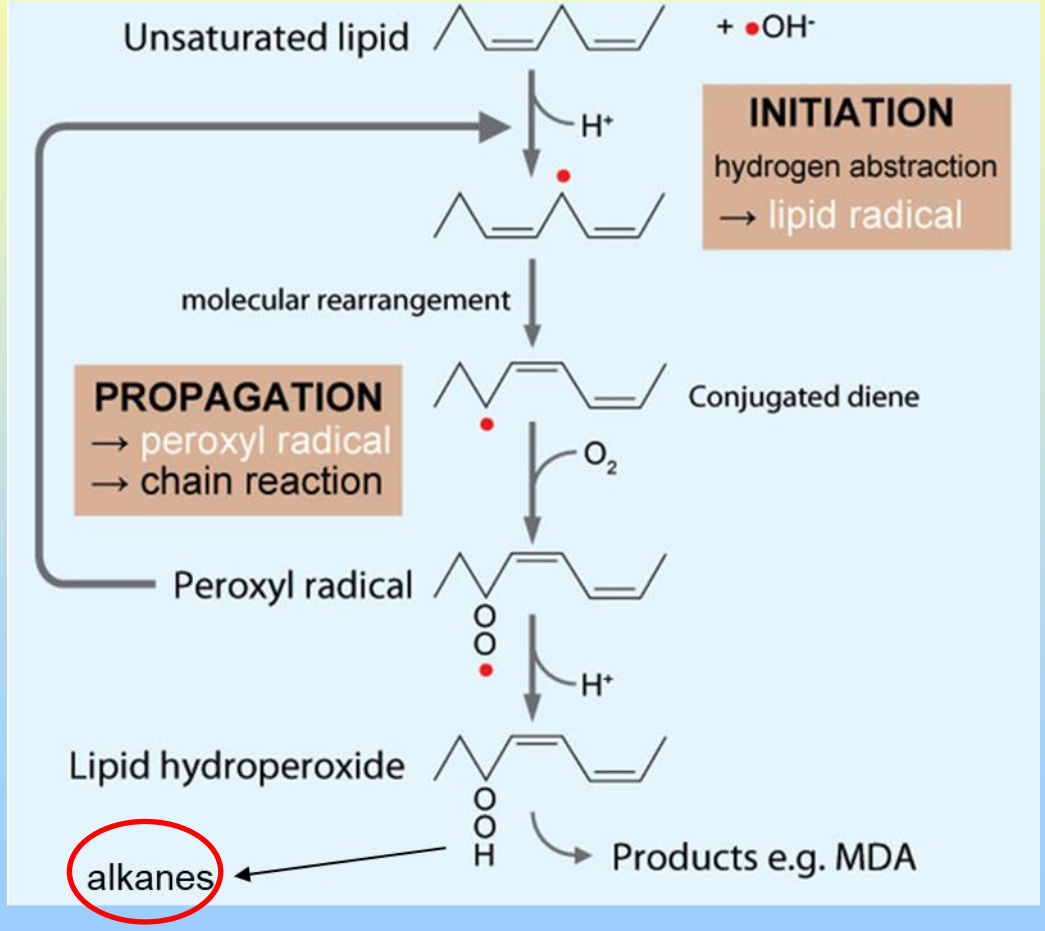
thiobarbituric acid



- Detection of conjugated dienes using spectrophotometer (220-300 nm wavelength)

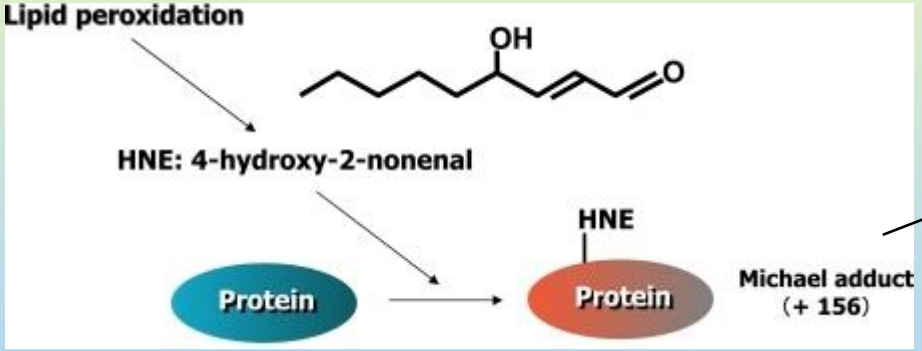


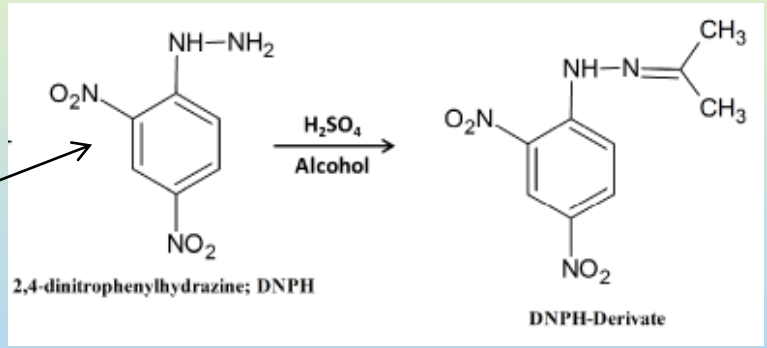
- Breath alkanes (pentane, ethane) as an index of lipid peroxidation (detection by gas chromatography)



- Monitoring of markers for oxidative damage of proteins

  - Detection damaged proteins by 2,4-dinitrophenylhydrazine using spectrophotometer (360–390 nm wavelength)





- Monitoring of markers for oxidative damage of NA

  - Detection of 8-hydroxy-2-deoxyguanosine by HPLC with electrochemical detection as a marker of damaged DNA