



Free-Radicals: Chemistry and Biology

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



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Free Radicals: Historical Aspects

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From Hypothesis to Reactivity

1900	GOMBERG	$\text{Ph}_3\text{C}\cdot$ Radicals: $\text{Ph}_3\text{C-X} \rightarrow \text{Ph}_3\text{C}\cdot \rightarrow \text{Ph}_3\text{C-CPh}_3$
1927	PANETH	C-centered radicals $\text{R-X} + \text{M} \rightarrow \text{RM}^+ \text{X}^- \rightarrow \text{R}\cdot + \text{MX}$
1934	RICE	Chain mechanism hypothesis.
1937	KARASH, HEY, WATERS	Radicals as intermediates of known reactions (anti-Markovnikov addition to olefins, arylation ...)
1940-45		Radical polymerizations (rubber substitutes), auto-oxidation processes, radical reactivity.
1945-1960		E.S.R. spectrometers (electron spin resonance) (10 nM sensitivity), Structure of radicals.
1957	WALLING	Radical reactivity framework

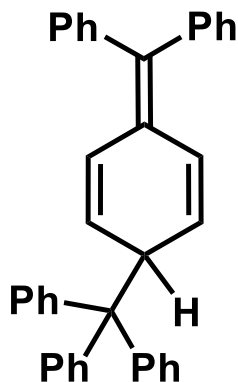
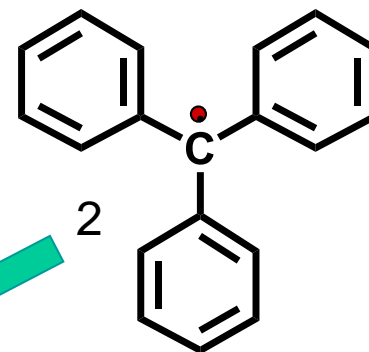
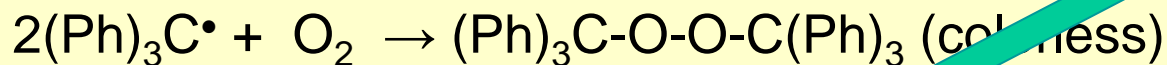
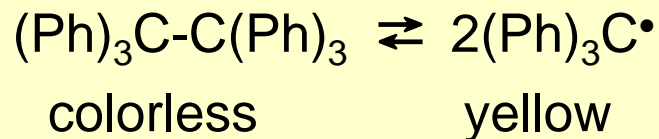
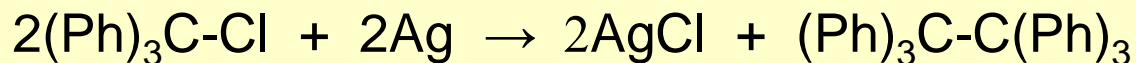


Structure – Synthetic Applications - Biology

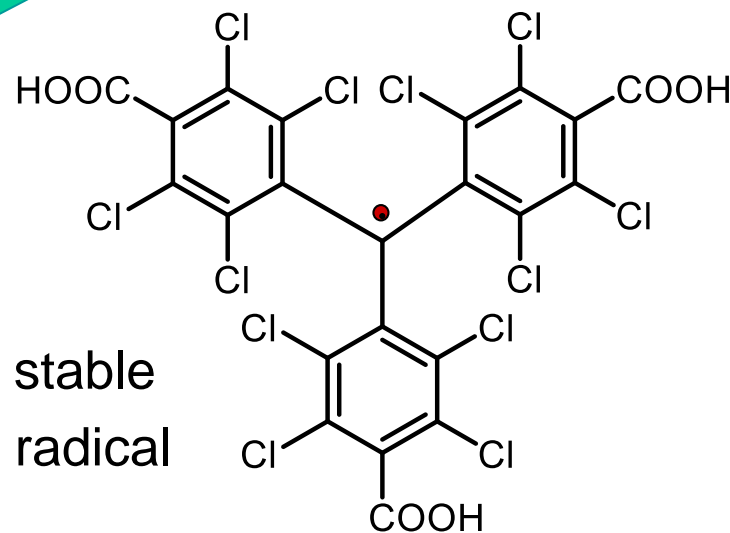
- 1960-70 **INGOLD**
- Fast kinetic techniques (stop-flow, pulse radiolysis and photolysis, e.s.r., polarography).
 - Biological issues. Radical reactivity
- 1970 **CLOSS**
- CIDNP (NMR applied to radical reactions)
- 1975 **FUKUI-MARCUS**
- Frontier orbital theory
 - Electron-Transfer Processes
- 1970-80 **MINISCI-KOCHI**
BARTON
- Polarity of free radicals
 - Inter/intramolecular regio- and stereoselectivity
 - New selective radical sources.
 - Synthetic potentiality in complex reactions
- 1980-99
- Synthetic applications - Enantioselectivity
 - Combustions and environmental issues
 - Metal catalysis
 - Design of new materials.
- 1980-2007
- Role in Biology and Medicine.



The Gomberg Hypothesis (1900) of Triphenylmethyl Radical



Structure of
Trityl radical dimer



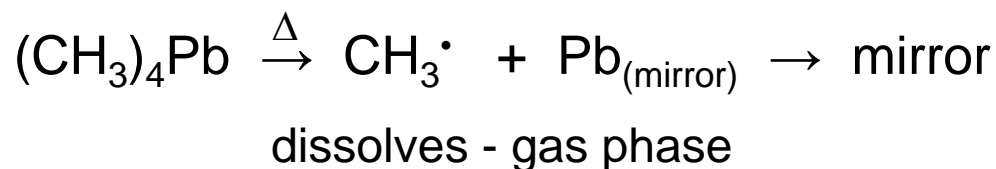
Gomberg, M. *J. Amer. Chem. Soc.*, **1900**, 22, 757, Gomberg, M.
Berichte, **1900**, 32, 3150; Gomberg, M. *Chemical Reviews*, **1924**, 1, 1



Radical Intermediates in Gases and Liquids

- 1st Studies

1. Decomposition of Organometallics in gas phase



Paneth, F.; Hofeditz, *Berichte*, **1929**, 62, 1335

2. Autoxidation of benzaldehyde



Bäckström, H. L. J. *J. Amer. Chem. Soc.* **1927**, 49, 1460

3. Vinyl polymerization

Flory's paper on the kinetics of vinyl polymerization - treated as a free radical chain reaction - outlined the framework for a lot of work to follow.

Hey, D. H; Waters, W. A., *Chem. Rev.* **1937**, 21, 169

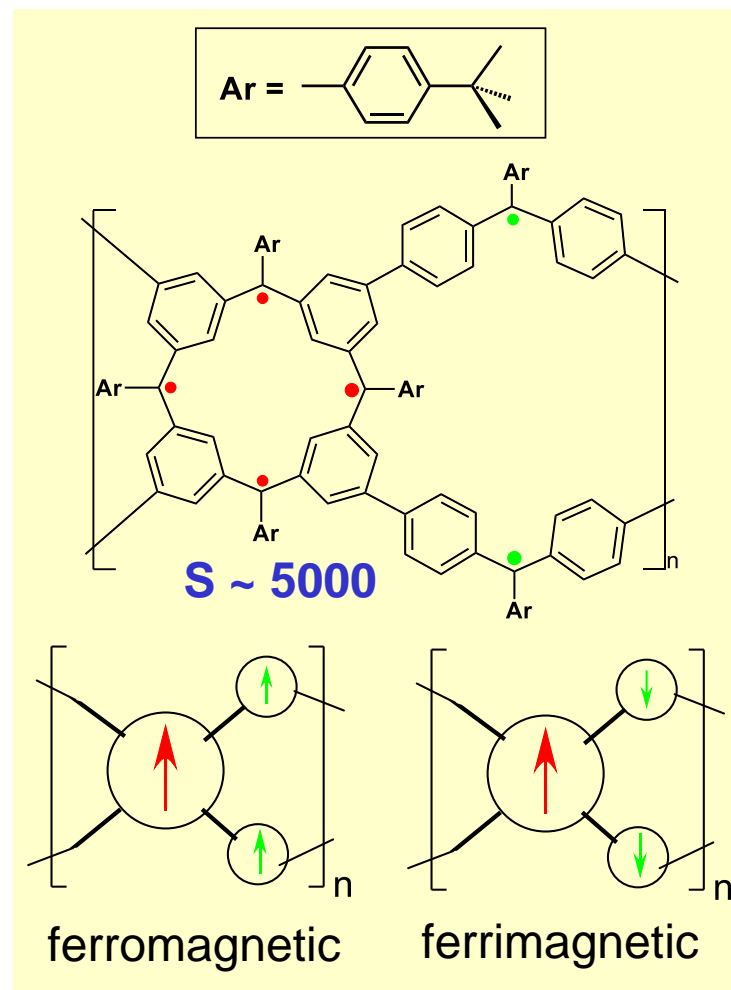
Flory, P. J. *J. Amer. Chem. Soc.* **1937**, 59, 241



Polyradicals and Organic Magnetic Materials

Nebraska Chemists
Create First **Plastic Magnets**
Lincoln - Nov. 25, 2001

"There are already known organic magnets, but they are based on crystals of small molecules,"
"What is unique about this research is this is the first organic polymer that can be said to be magnetic."



S. Rajca et al.



History of Oxygen Molecule (O₂)

- XV° **Leonardo**: One of air components sustains combustion
- 1772 **Priestley-Scheele**: Discovered Oxygen
- 1775 **Lavoisier**: Oxygen as element – Name: acid generator
- 1811 **Avogadro**: Oxygen is a Diatomic Molecule
- 1848 **Faraday**: Oxygen is a Paramagnetic Molecule
- 1928 **Mullikan**: Oxygen is a Triplet Molecule
- 1934 **Herzberg**: Observation of Singlet Oxygen
- 1941 **Ruben-Kamen**: Oxygen in photosynthesis from water
- 1964 **Foote & Wexler**: Rediscovered Singlet Oxygen



Hydroxyl Radical and The Fenton Reaction

- Hydroxyl radicals (HO^\bullet) are between the most oxidant and reactive radical specie.
- The impact of Ferrous salts on H_2O_2 reduction and its exceptional oxidant power were discovered over 100 years ago by Fenton*.
- The Fenton reaction in form indicated below, including the hydroxyl radical, was suggested over 75 years ago by Haber and Weiss.**



- In 1950-60 the hydroxyl radical was investigated by radiolysis of water and its reduction and oxidation redox potentials measured:



- In 1970 the role of hydroxyl radical in troposphere was identified

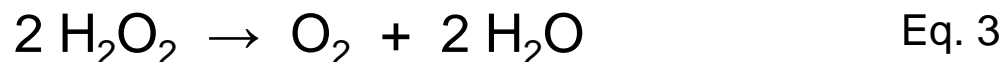
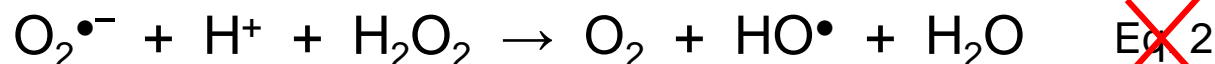
* H.J.H. Fenton. *J. Chem. Soc.* **1894**, 65, 889.

** F. Haber and J.J. Weiss. *Proc. Roy. Soc. London, Ser. A.* **1934**, 147, 332



The Not-Existing Weiss Reaction

Proposed chain reaction for the Hydrogen Peroxide decomposition :



The reaction 2 was proposed by Weiss in 1937 after the discovery in 1934 by Neumann of potassium superoxide* (KO_2), containing the radical anion $\text{O}_2^{\bullet-}$. Despite some results which indicate that the reaction is very slow ($< 2 \text{ M}^{-1}\text{s}^{-1}$), it was reconsidered in 1969 in a biological context, but now is clear that the disproportionation of $\text{O}_2^{\bullet-}$ is faster and Fenton reaction is the source of HO^\bullet .



(The venerable name superoxide is still allowed, the systematic names dioxide(\bullet 1-), or, derived from oxidane, dioxidanidyl, are recommended by IUPAC. Similarly, HO_2^\bullet is named hydridodioxygen(\bullet) or dioxidanyl, but hydrogen dioxide is also a systematic and acceptable name).

McCord JM, Fridovich I. *J Biol Chem* **1969**; 244, 6049–6055.

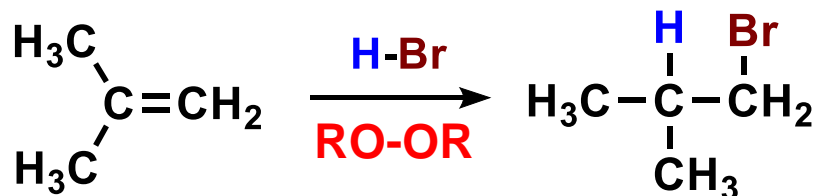


Fenton Reaction: A Modern View

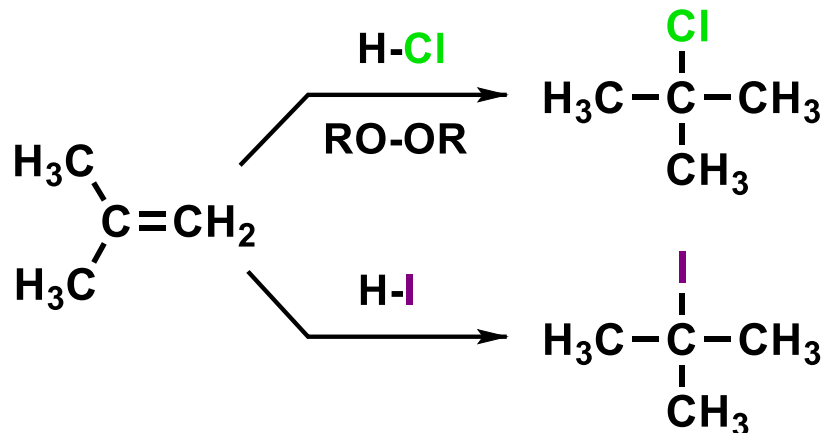
Number	Reaction	Rate constant (Lmol ⁻¹ s ⁻¹)
1	$\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \cdot\text{OH} + \text{OH}^-$	63
2	$\text{Fe}^{2+} + \cdot\text{OH} \rightarrow \text{Fe}^{3+} + \text{OH}^-$	3×10^8
3	$\text{H}_2\text{O}_2 + \cdot\text{OH} \rightarrow \text{HO}_2\cdot + \text{H}_2\text{O}$	3.3×10^7
4	$\text{Fe}^{2+} + \text{HO}_2\cdot \rightarrow \text{Fe}^{\text{III}}(\text{HO}_2)^{2+}$	1.2×10^6
5	$\text{HO}_2\cdot \rightarrow \text{O}_2\cdot^- + \text{H}^+$	$1.58 \times 10^5 \text{s}^{-1}$
6	$\text{O}_2\cdot^- + \text{H}^+ \rightarrow \text{HO}_2\cdot$	1×10^{10}
7	$\text{Fe}^{2+} + \text{O}_2\cdot^- + \text{H}^+ \rightarrow \text{Fe}(\text{HO}_2)^{2+}$	1×10^7
8	$\text{Fe}^{3+} + \text{H}_2\text{O} \rightleftharpoons \text{FeOH}^{2+} + \text{H}^+$	$K = 2.9 \times 10^{-3} \text{M}$
9	$\text{Fe}^{3+} + \text{H}_2\text{O} \rightleftharpoons \text{Fe}(\text{OH})_2^+ + 2\text{H}^+$	$K = 7.62 \times 10^{-6} \text{M}^2$
10	$2\text{Fe}^{3+} + 2\text{HO}_2 \rightleftharpoons \text{Fe}_2(\text{OH})_2^{4+} + 2\text{H}^+$	$K = 8 \times 10^{-4} \text{M}$
11	$\text{Fe}^{3+} + \text{H}_2\text{O}_2 \rightleftharpoons \text{Fe}(\text{HO}_2)^{2+} + \text{H}^+$	$K = 3.1 \times 10^{-3} (^{\dagger})$
12	$\text{FeOH}^{2+} + \text{H}_2\text{O}_2 \rightleftharpoons \text{Fe}(\text{OH})(\text{HO}_2)^+ + \text{H}^+$	$K = 2.0 \times 10^{-4}$
13	$\text{Fe}(\text{HO}_2)^{2+} \rightarrow \text{Fe}^{2+} + \text{HO}_2\cdot$	$2.7 \times 10^{-3} \text{s}^{-1}$
14	$\text{Fe}(\text{OH})(\text{HO}_2)^+ \rightarrow \text{Fe}^{2+} + \text{HO}_2\cdot + \text{OH}^-$	$2.7 \times 10^{-3} \text{s}^{-1}$
15	$\text{Fe}(\text{III})^{(\dagger)} + \text{HO}_2\cdot \rightarrow \text{Fe}^{2+} + \text{O}_2 + \text{H}^+$	$< 2 \times 10^3$
16	$\text{Fe}(\text{III}) + \text{O}_2\cdot^- \rightarrow \text{Fe}^{2+} + \text{O}_2$	5×10^7
17	$\text{HO}_2\cdot + \text{HO}_2\cdot \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$	8.3×10^5
18	$\text{HO}_2\cdot + \text{O}_2\cdot^- + \text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$	9.7×10^7
19	$\cdot\text{OH} + \text{HO}_2\cdot \rightarrow \text{H}_2\text{O} + \text{O}_2$	7.1×10^9
20	$\cdot\text{OH} + \text{O}_2\cdot^- \rightarrow \text{OH}^- + \text{O}_2$	1×10^{10}
21	$\cdot\text{OH} + \cdot\text{OH} \rightarrow \text{H}_2\text{O}_2$	5.2×10^9
22	$\text{H}^+ + \text{SO}_4^{2-} \rightleftharpoons \text{HSO}_4^-$	$K = 1 \times 10^{-2} \text{M}^{-1}$
23	$\text{HSO}_4^- + \cdot\text{OH} \rightarrow \text{H}_2\text{O} + \text{SO}_4^{\cdot-}$	1.7×10^6
24	$\text{SO}_4^{\cdot-} + \text{H}_2\text{O}_2 \rightarrow \text{HSO}_4^- + \text{HO}_2\cdot$	1.2×10^7
25	$\text{SO}_4^{\cdot-} + \text{HO}_2\cdot \rightarrow \text{HSO}_4^- + \text{O}_2$	3.5×10^9
26	$\text{SO}_4^{\cdot-} + \text{Fe}^{2+} \rightarrow \text{SO}_4^{2-} + \text{Fe}^{3+}$	9.9×10^8



The Question of HBr Addition to Olefins



- Sometimes Markovnikov, sometimes not
- Always anti-Markovnikov



Always Markovnikov,

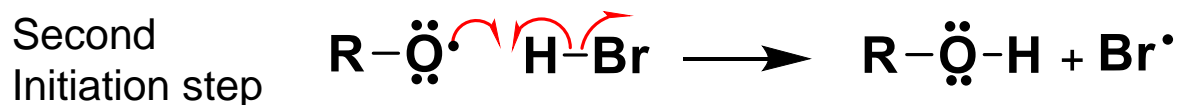
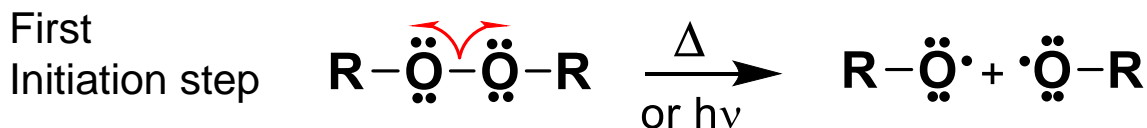
Always Markovnikov,

- How can the anomalous HBr outcome be explained ?

Kharasch M.S.; Engelmann, H.; Mayo, F.R. *J. Org. Chem.* **1937**, 2, 288.



First Step: Initiation

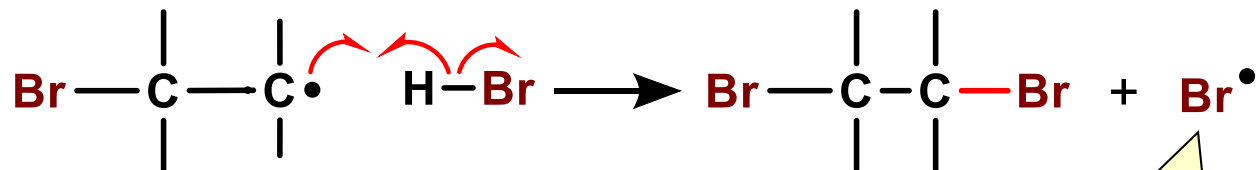
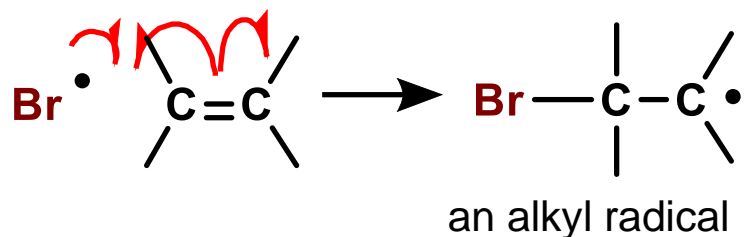


- The first step in the process is the formation of the radicals
- This is usually called: the initiation steps
- Question: why is in the second step the 'H' abstracted and not the 'Br'? (Hint: BDE of HO-Br is 234 kJ·mol⁻¹)



The Propagation Steps

Two propagation, or chain-carrying steps

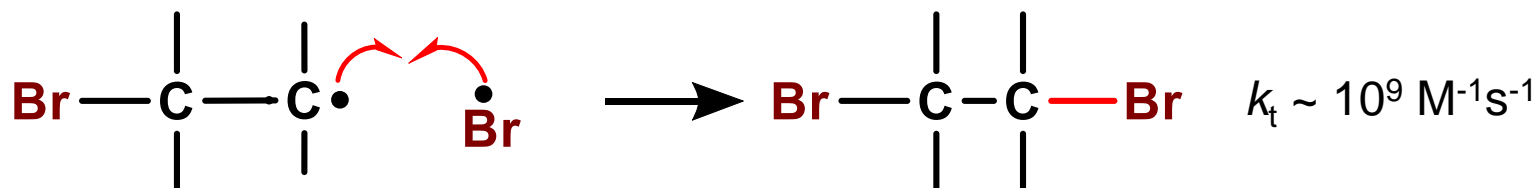
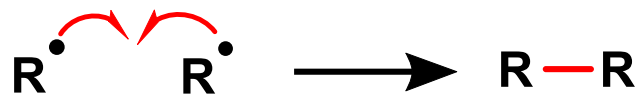


Recycle to the first propagation step and start all over again

- The bromide radical adds to the double bond to give an alkyl radical; this can react with a new molecule of HBr and the cycle continues.

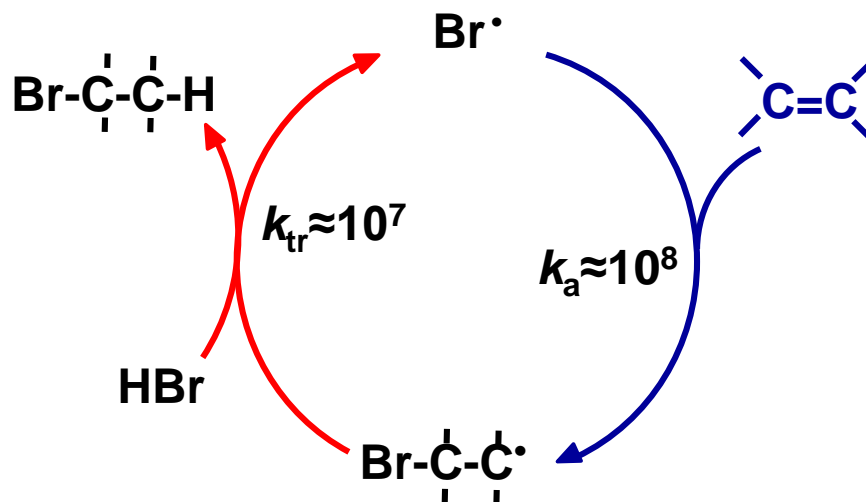


Termination Steps



- How can we avoid the occurrence of undesired termination steps?

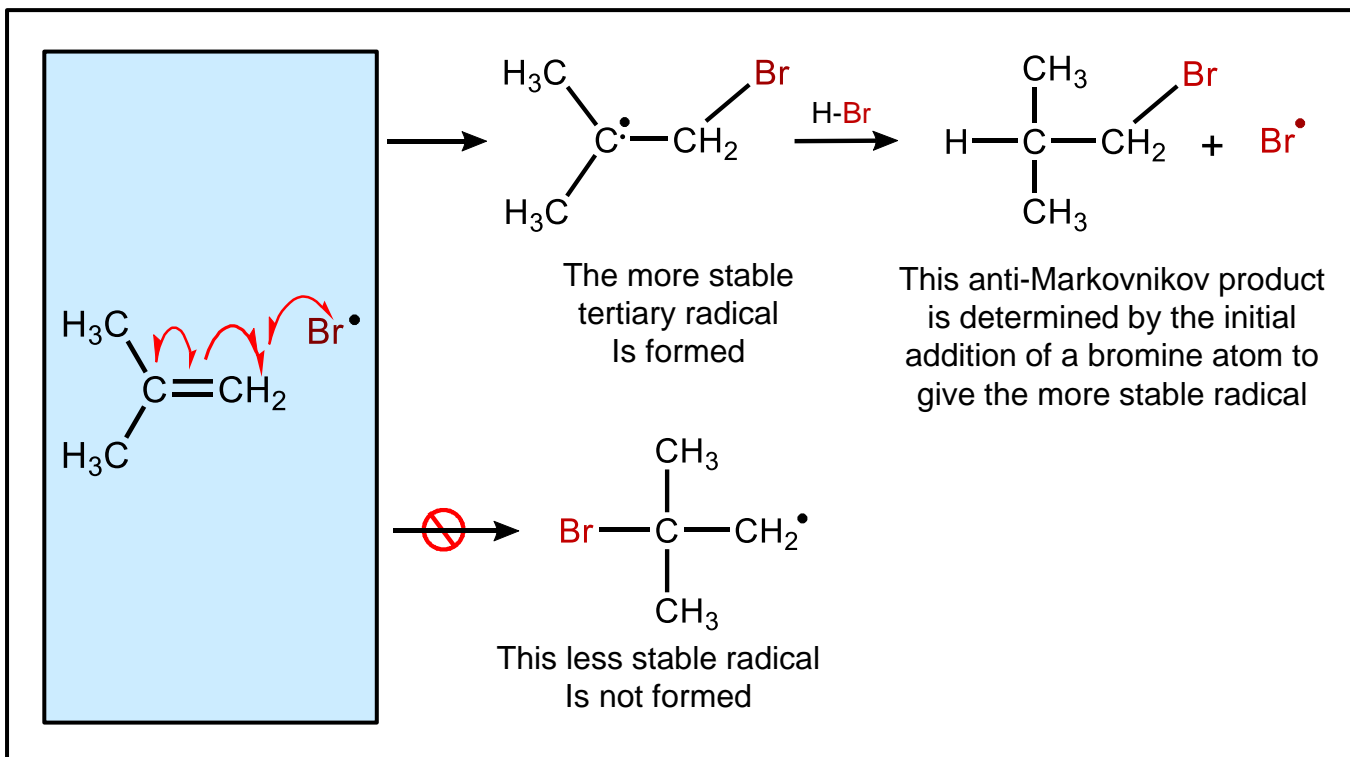
- Efficient chain ($k_{tr} \uparrow$)
- Not too much fast initiation
- High [HBr] and alkene





The Result: anti-Markovnikov

Radical addition



- In the radical reaction, the less hindered carbon atom is attacked and/or the most stable radical will be formed, which explains the opposite (anti-Markovnikov) mode of addition.



R. G. W. Norrish and Flash Photolysis

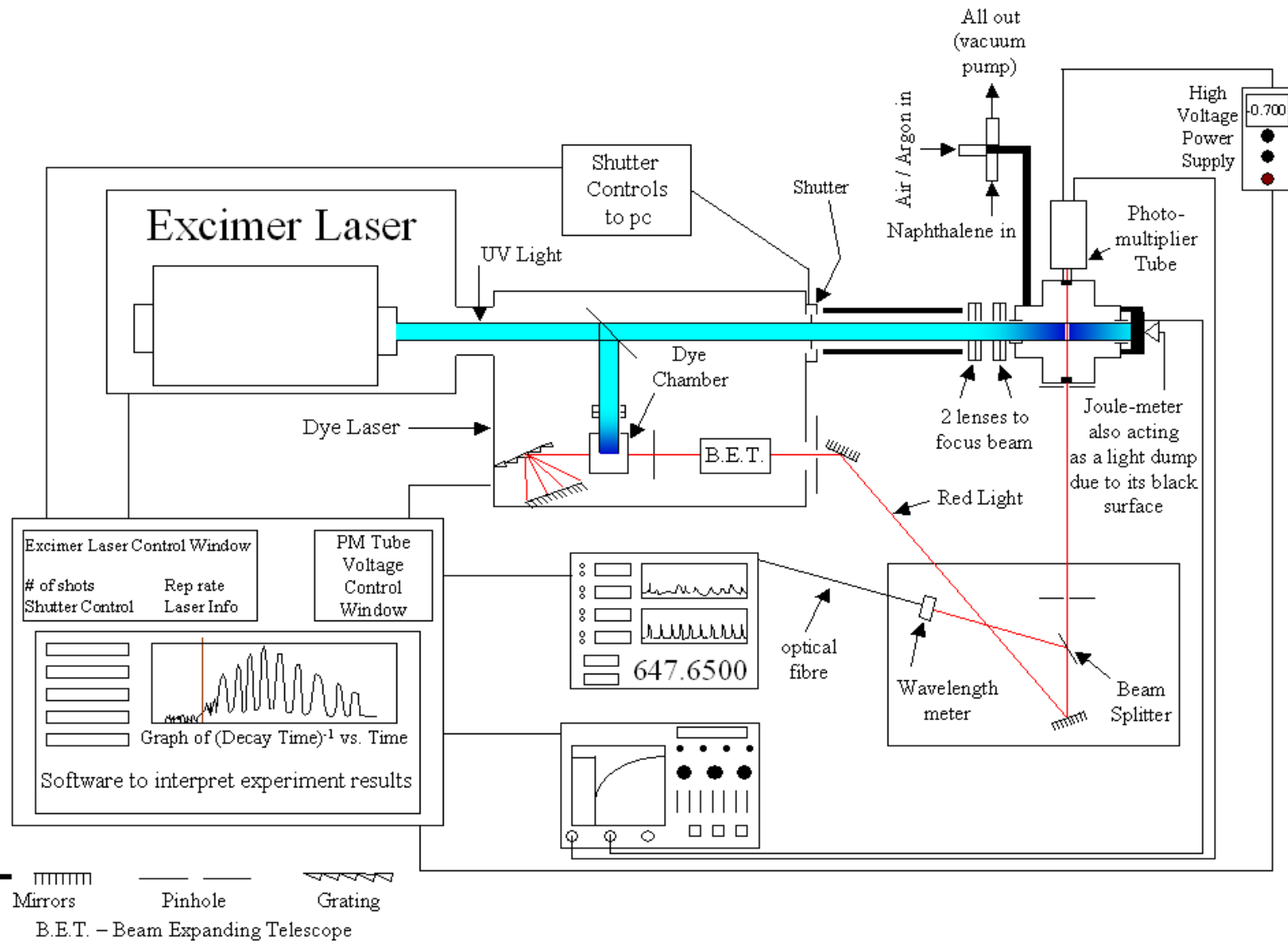
- Professor of chemistry at Cambridge; established, with W. A. Noyes and a few other physical chemists who worked in the gas phase, the basic reactions of ketone chemistry.
- Seminal Norrish papers were published in the 30's in Transactions of the Faraday Society.
- Norrish, and his student George Porter, shared the 1967 Nobel Prize in chemistry for the development of *Flash Photolysis*. This technique has become absolutely crucial in mechanistic studies in the field. It is sometimes referred to as the *spectroscopy of transient intermediates*.
- Karl Ziegler (Ziegler/Natta) was the first to measure the absorption spectrum of a free radical when he studied the dissociation of hexaarylethanes (Ziegler, K; Ewald, L, *Ann.* 1929, 473, 169; Picard, J. *Ann.* 1911, 381, 347).

Norrish, R. G. W.; Appleyard, M. E. S. *J. Chem. Soc.* **1934**, 874.

Norrish, R. G. W., Porter, G. *Nature*, **1949**, 164, 658

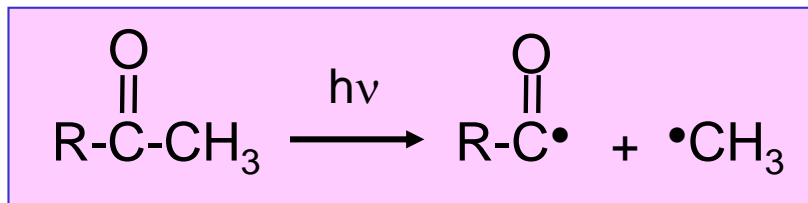


Flash Photolysis Equipment



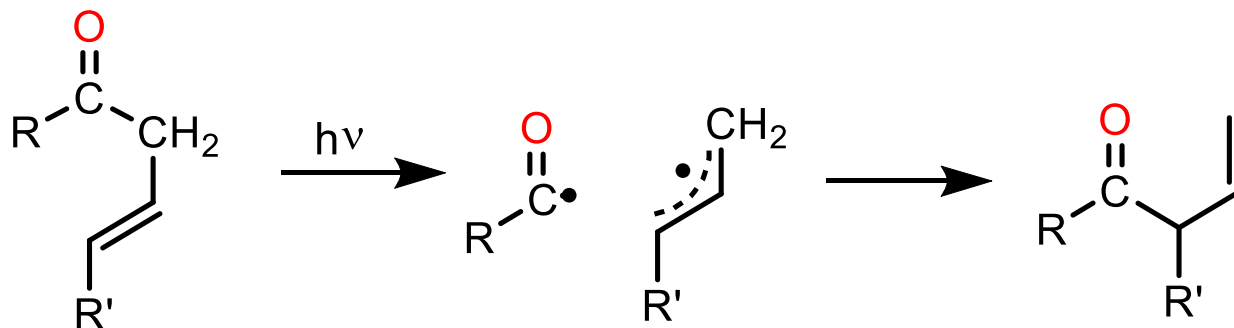


Evidence for Radicals in the Norrish Type I Decarbonylation of Ketones



Rate constants as
a function of R, s⁻¹

Me	10 ³
Et	10 ⁶
<i>iso</i> -Pr	10 ⁸
<i>tert</i> -But	10 ⁹
CH ₂ -Ph	10 ¹⁰



Gilbert, A.; Baggott, J. *Essentials of Molecular Photochemistry*, 1991, CRS Press, Boca Raton, FL.



Rate Coefficients and Quantum Yields for Homolytic Cleavage of Ketones

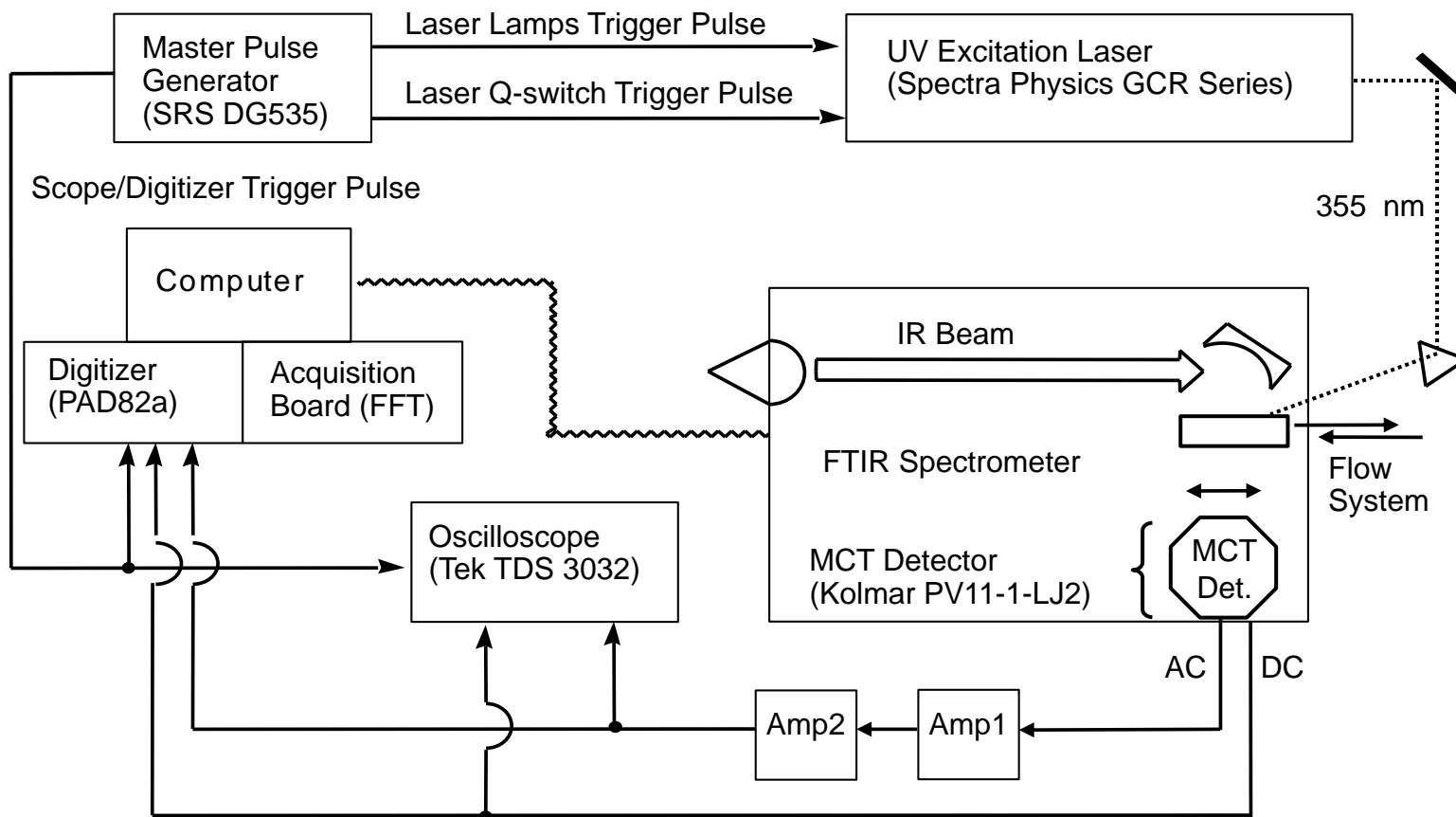
ketone	ϕ_s	ϕ_T	$k(S_1)$	$k(T_1)$
MeCO-Bu ^t	0.18	0.33	$<10^8$	$>10^9$
cyclobutanone	0.3		$>10^9$	
cyclopentanone		0.2	$<10^8$	2×10^8
cyclohexanone		0.2	$<10^8$	2×10^7
PhCOCH ₂ Ph		0.4		2×10^6
Phenyl- <i>t</i> -Butylketone		0.3		1×10^7
dibenzylketone		0.7		10^{10}
PhCOCH ₂ Ph		0.4		10^6

Triplet reactions and rate and quantum yield increase with radical stability.



A Nanosecond Spectrometer

Built to observe changes in the infrared spectrum following a UV or visible pulse.



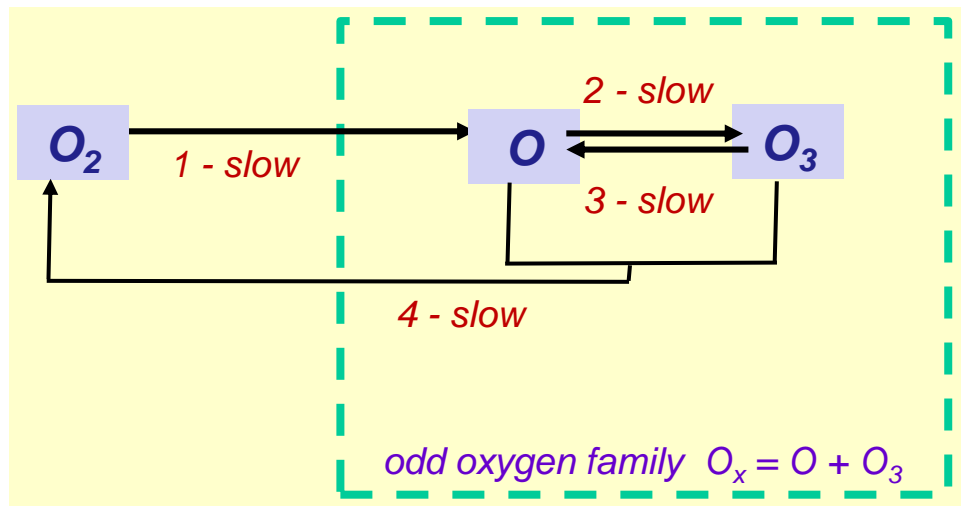
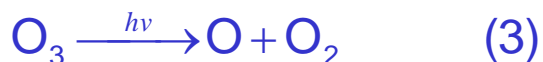


Radicals in Atmosphere Chemistry and Environment

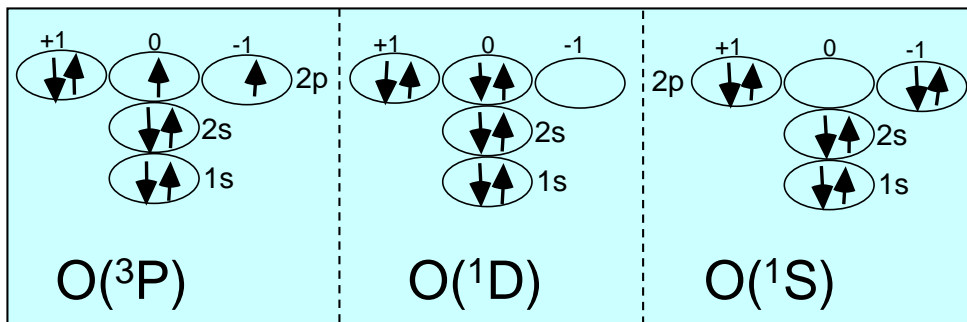
Stratospheric ozone

“Chapman” reactions (1930)

oxygen-only chemistry



Initiation:



The three states at lower energy of oxygen atom

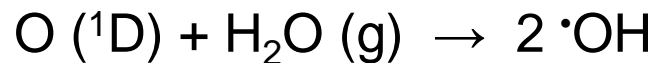


Radicals in Atmosphere Chemistry and Environment (2)

The atmosphere is an oxidizing environment: Some species such as the hydroxyl radical ($\cdot\text{OH}$) has very low concentration in the atmosphere (peak ~ 1 ppt, 10^6 cm^{-3} , $\tau = \sim 1$ sec) but is strong enough to react almost with all organic species. This species is called “Mother Nature’s vacuum cleaner”.

Generation of Hydroxyl radicals in Atmosphere

1) H-abstraction of O atom (generated by O_3 photolysis) from water

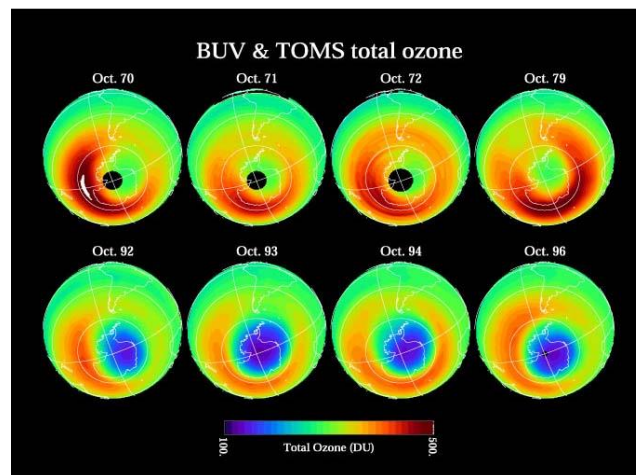
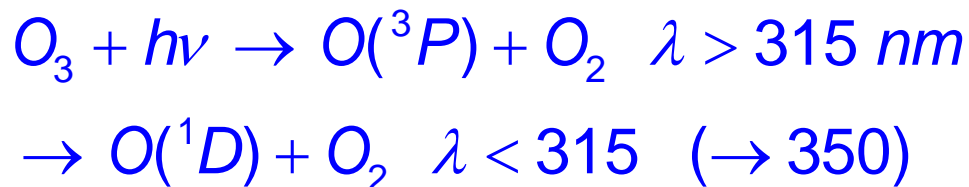


2) Photolysis of hydrogen peroxide (less important)





Environmental Issues: Basics of Tropospheric Photochemistry...



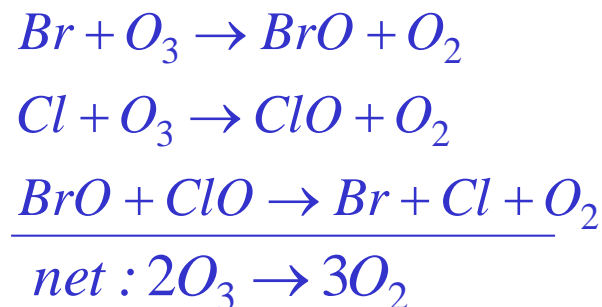
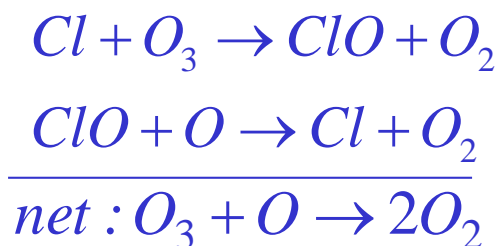


Stratospheric Catalytic Ozone Destruction

Stratospheric halogen sources:

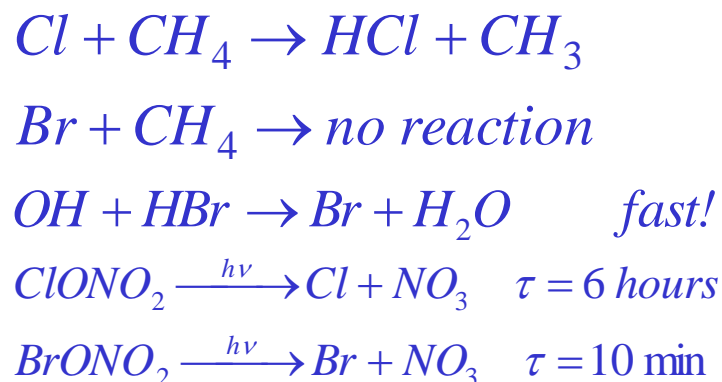
natural – CH₃Cl, CH₃Br ... short-lived CH_xX_y species, like CH₃I, CH₂Br₂, CHBr₃
 man made – CFC's -11 (CFCl₃), -12 (CF₂Cl₂)...

Catalytic O₃ destruction, e.g.:



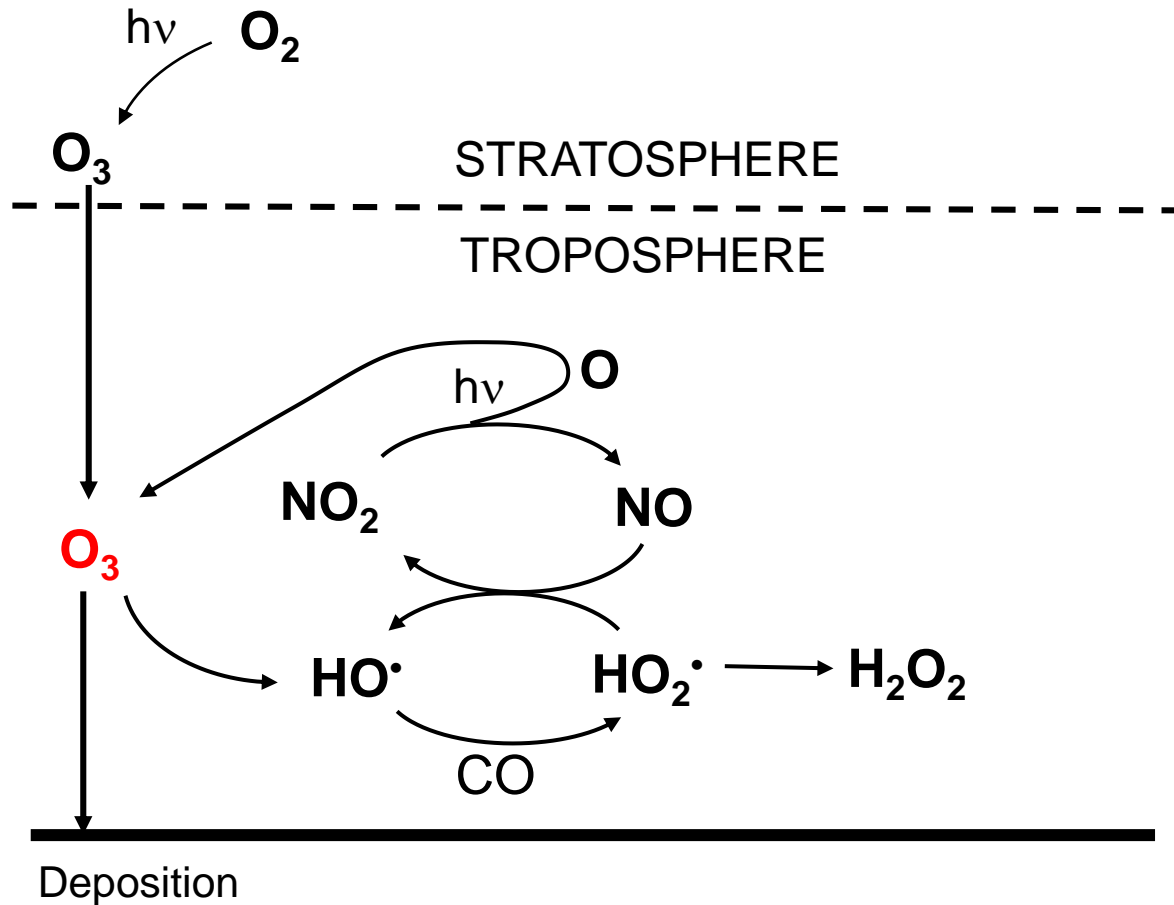
Br• is 40-100× more effective than Cl•:

The net result is that much less bromine is tied up in reservoir species and more is available for the ozone-destroying cycles.



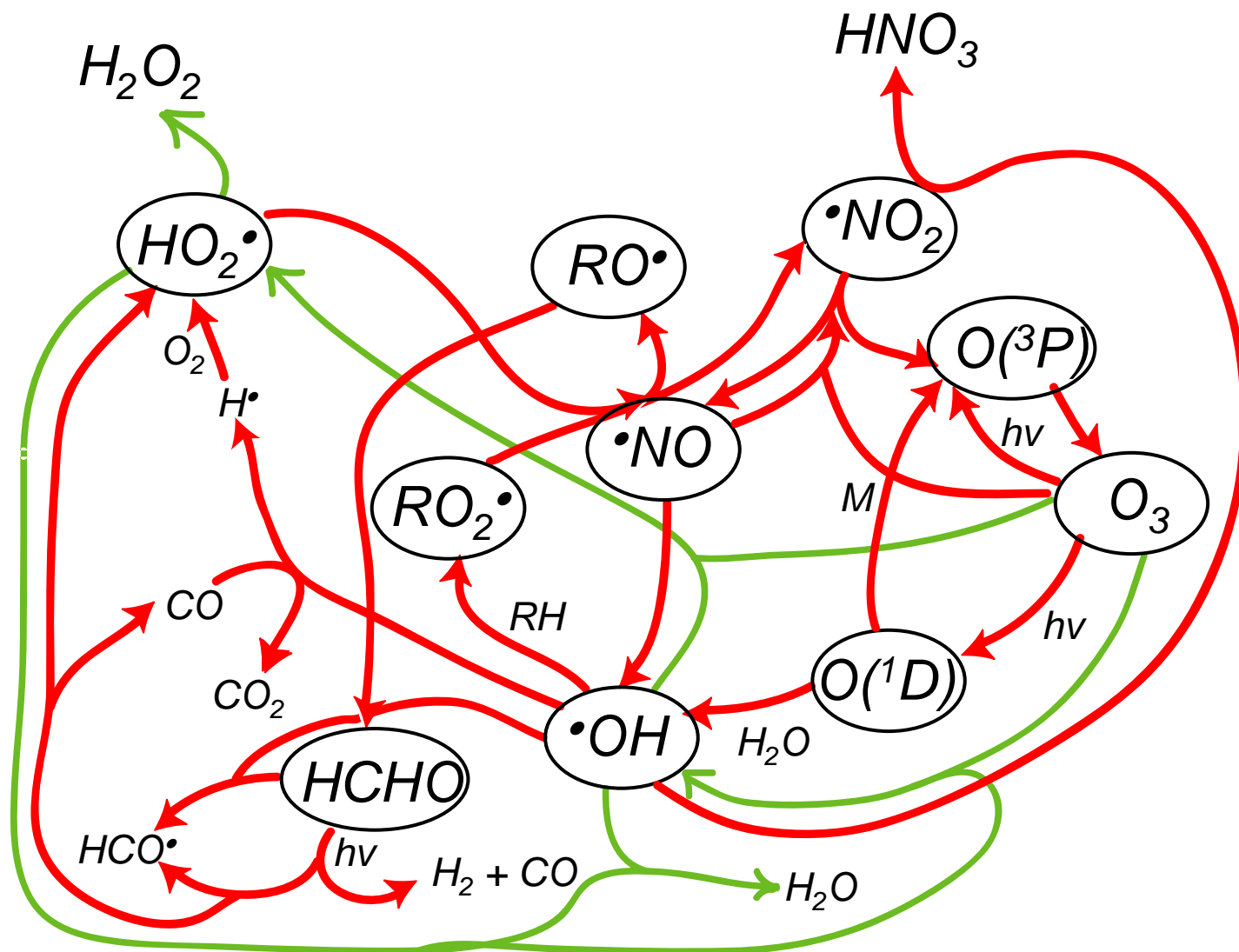


Mechanism for O_3 - HO_x - NO_x -CO Chemistry in the Troposphere





Tropospheric Photochemistry (simplified)





History of Radicals in Biology

Before 1950s: “golden age” of chemistry

Many free radicals have been discovered and described

Haber Weiss (1934): Superoxide + Hydrogen peroxide \rightarrow Hydroxyl radical (HO \cdot)

Baeyer Villiger (1901): discovered peroxyxynitrite (ROONO)

Early 1950s: presence of radicals in biological materials

Commoner et al. (1954) *Nature* 174: 689-691

1956: Radicals may be by-products of enzymatic reactions *in vivo*?

Harman (1956): radicals are “pandora’s box” of evils (aging, mutations, cancer,)

1969: Discovery of superoxide dismutase (SOD)

McCord & Fridovich *JBC* **1969**, 244, 6049-6055.

Free radicals must be important for biology if the body has a defense enzyme(s)!

Intensive investigations on the role of oxidants in disease begun

Since late 70s: Radicals are important for normal biology

Mittal, Murad (1977): superoxide anion stimulates formation of cGMP

Ignarro, Kadowitz (1985); Moncada (1987): \cdot NO is important in circulation

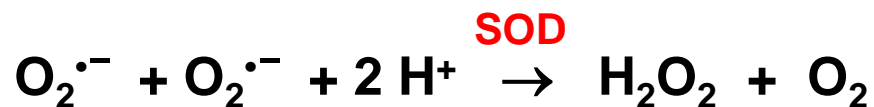


Role of Radical Species in Biochemistry and Medicine

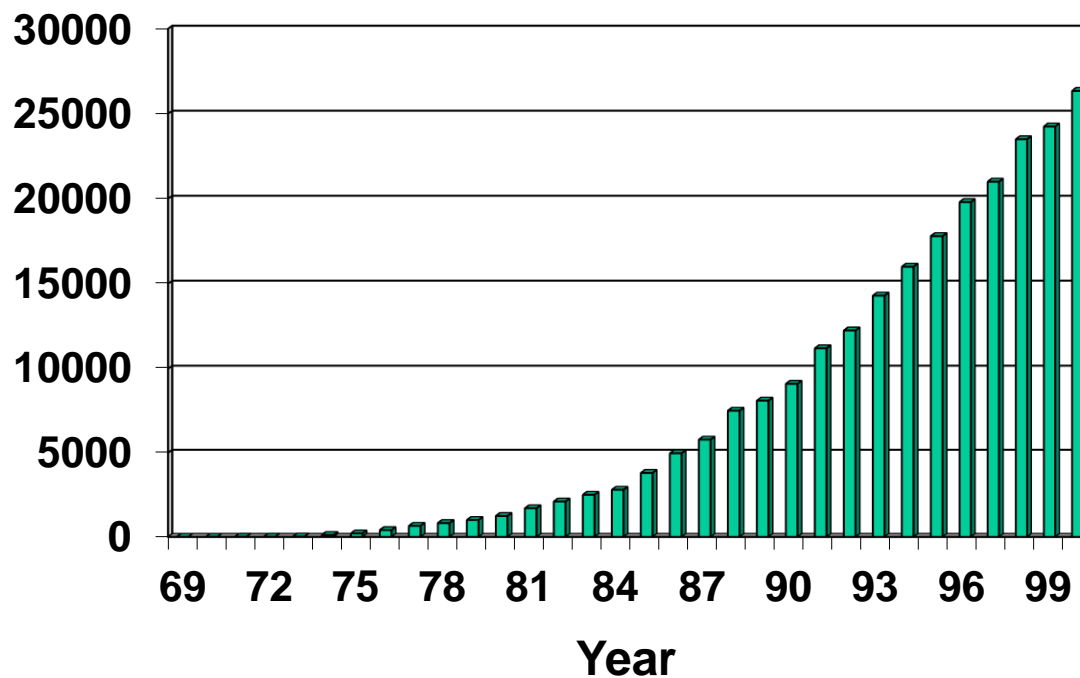
- The existence of free radicals, as chemical entities, was inferred 100 years ago but not universally accepted for nearly 40 years. The existence and importance of free radicals in biological systems was not recognized until the mid-1950's, by a small number of visionary scientists who can be credited with founding the field of reactive oxygen biochemistry.
- For most of the remaining 20th century, reactive oxygen species (ROS) were considered a type of biochemical “rusting agent” that caused tissue damage and disease.
- In 21st century, reactive oxygen biochemistry is maturing as a discipline and establishing its importance among the biomedical sciences. It is now recognized that virtually every disease state involves some degree of oxidative stress.
- Moreover, we are now beginning to recognize that ROS are produced in a well-regulated manner to help maintain homeostasis on the cellular level in normal, healthy tissue.
- Emerging technologies, particularly proteomic technologies are discussed in scientific community that will facilitate further evolution in the field of free radical biochemistry



Superoxo Dismutase (SOD)



Cumulative Publications



McCord & Fridovich *JBC* 1969, 244, 6049-6055.



Toxicology: Carbon Tetrachloride

reductive degradation - initial steps



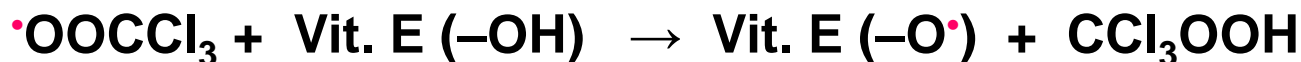
reduction by many other reducing species, e.g., **enzymatically provided electrons** or reducing radicals

biological significance



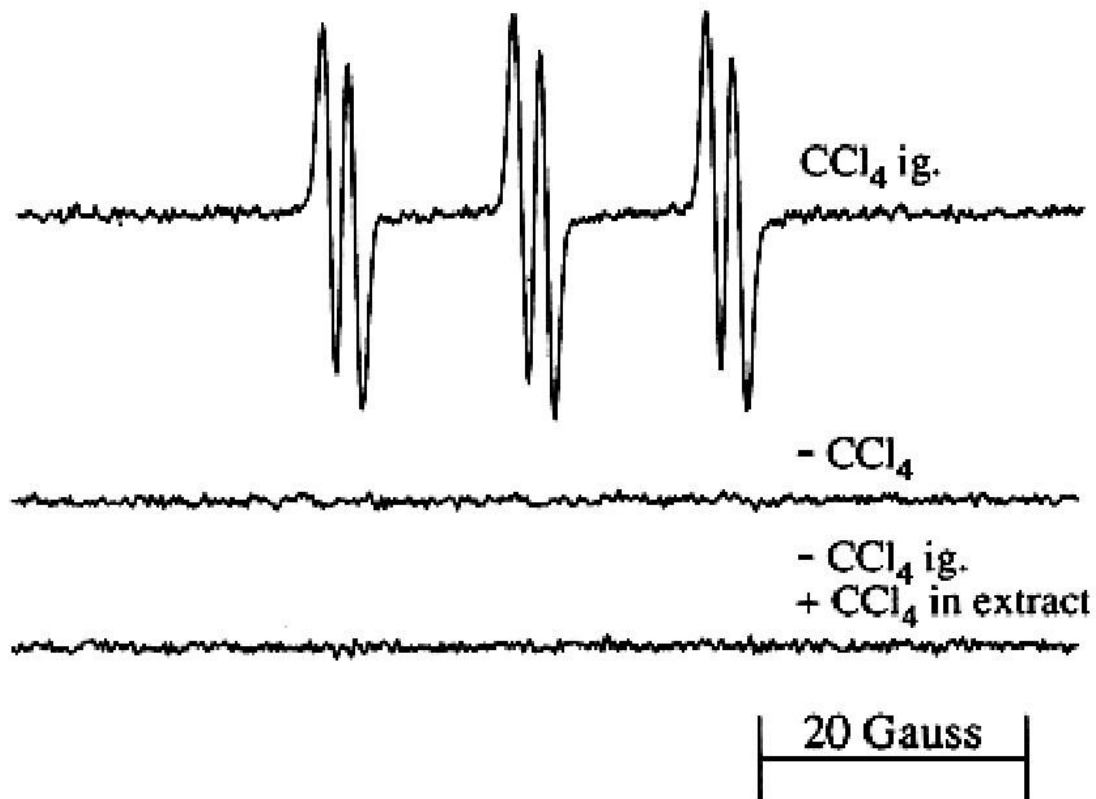
$\cdot\text{OOCCl}_3$ initiates lipid peroxidation

It readily depletes, e.g., antioxidants





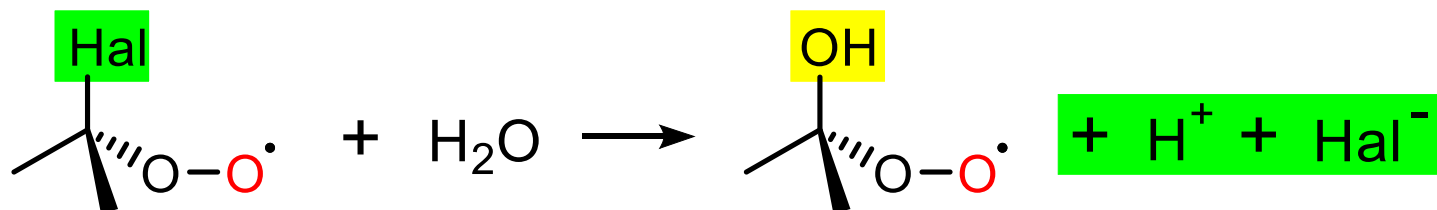
Spin Trapping in Vivo of the Trichloromethyl Radical Metabolite of CCl_4



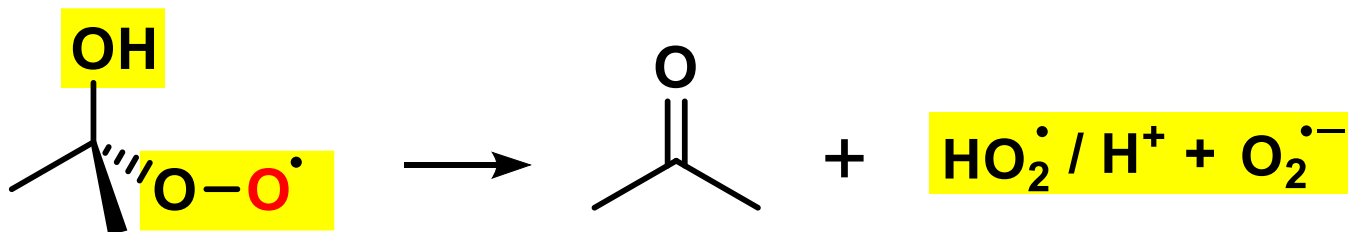
Hanna, P.M., Kadiiska, M.B., Jordan, S.J., and Mason, R.P., *Chem. Res. Toxicol.*, **1993**, 6, 711-717.



α -Halogenated Peroxyl Radicals

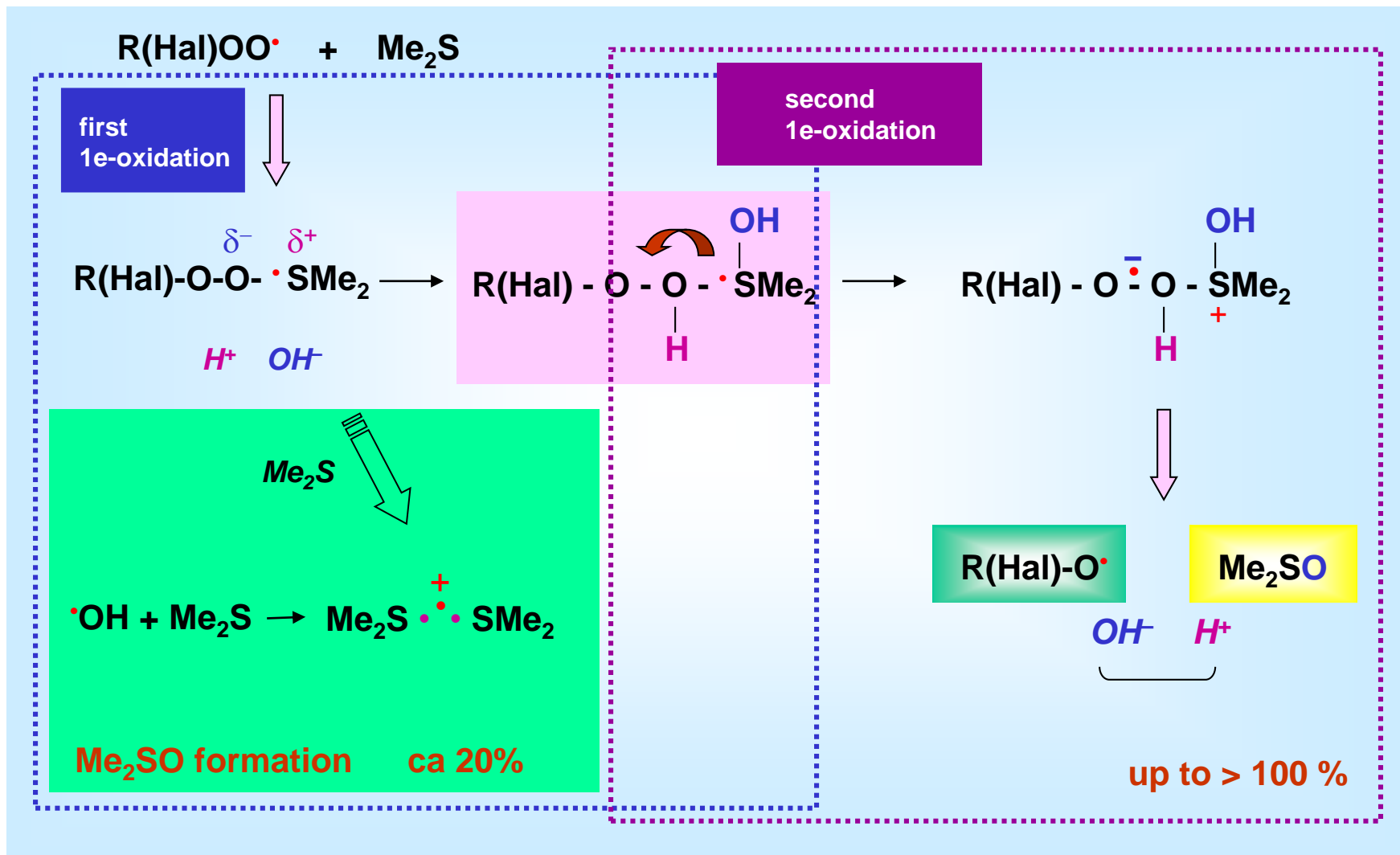


Hal = F, Cl, Br, I





Oxidation of Organic Sulfides





Toxicology: Ethanol Toxicity - Hydroxyethyl Radical Formation in Liver Microsomes

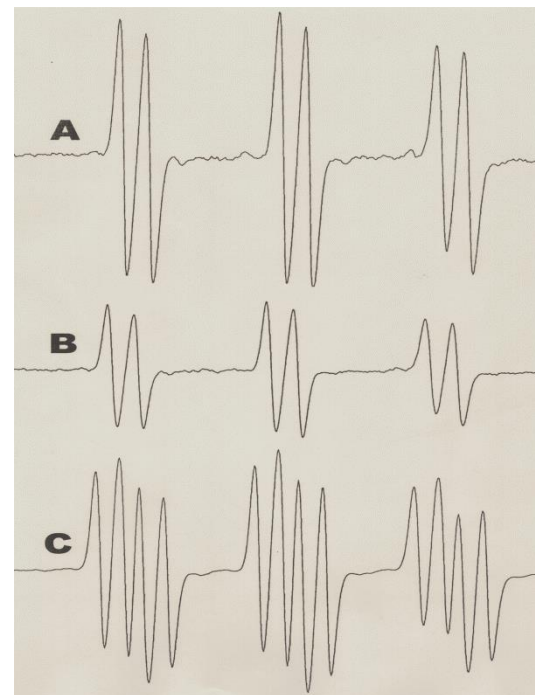
Spin trapping experiments have demonstrated that hydroxyethyl radical formation by liver microsomes requires a source of reactive oxygen intermediates¹⁻⁴.

¹Albano E, Tomasi A, Goria-Gatti L and Dianzani MU (1988), *Chem.-Biol. Interact.* **65**: 223-234.

²Reinke LA, Rau JM and McCay PB (1990), *Free Rad. Res.* **9**: 205-211.

³Knecht KT, Thurman RG and Mason RP (1993). *Arch. Biochem. Biophys.* **303**:339-348.

⁴Rashba-Step J, Turro NJ and Cederbaum AI (1993), *Arch. Biochem. Biophys.* **300**: 401-408.



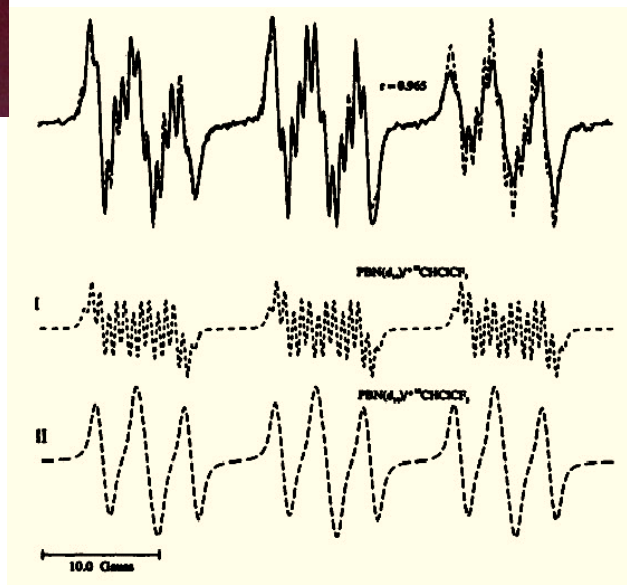
- A. Rat liver microsomes + ethanol, POBN, & NADPH.
- B. As A, but + catalase
- C. As A, but with 1-¹³C-ethanol. The 12-line EPR spectrum identifies the 1-hydroxyethyl radical.



Free Radical Metabolism of Halothane in Vivo: Radical Adducts Detected in Bile



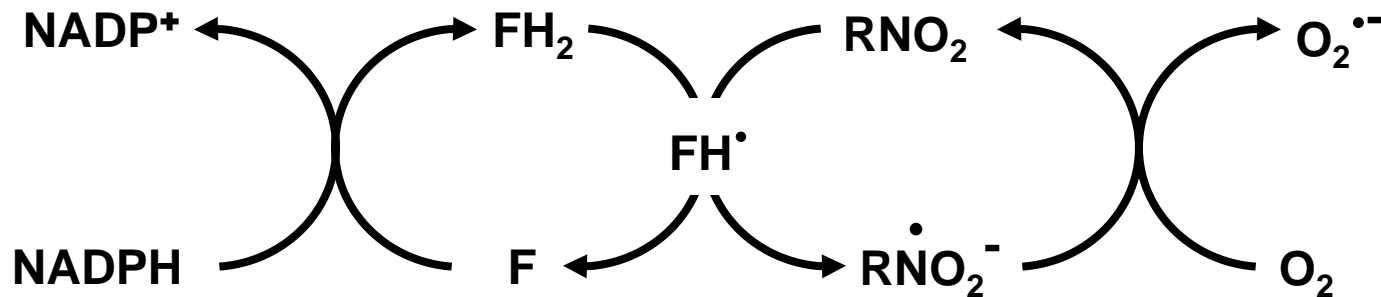
Bile samples collected every 20 min or 2 h in tube containing DP and BC



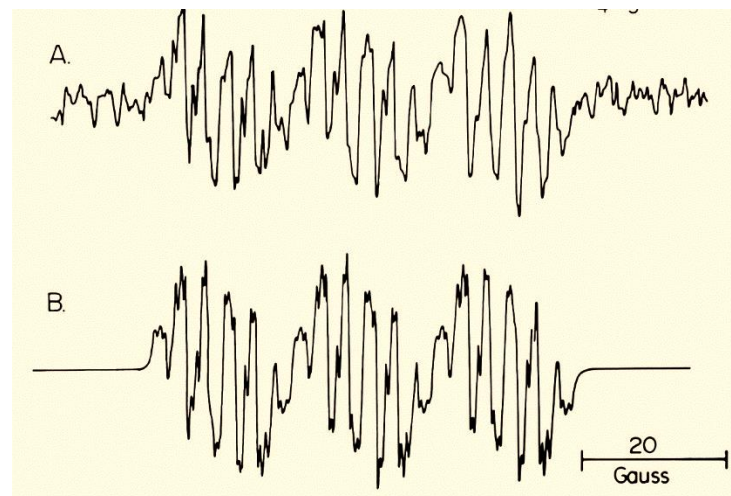
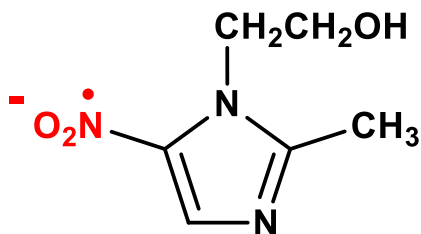
Knecht, K.T., DeGray, J.A., and Mason, R.P., *Mol. Pharmacol.* 41: 943-949, 1992



Metronidazole Anion Radical



ESR Spectrum of
Metronidazole Anion Radical
and Computer Simulation

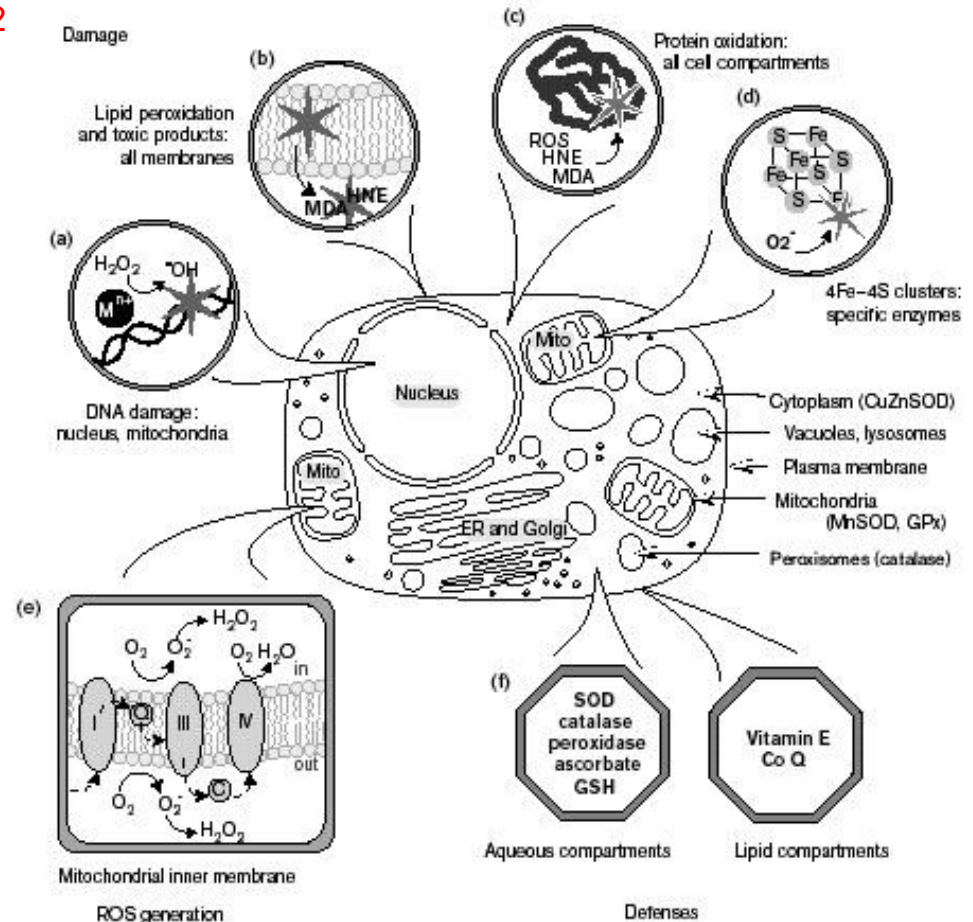


Perez-Reyes, E., Kalyanaraman, B., and Mason, R.P., *Mol. Pharmacol.* 17:239-244, 1980



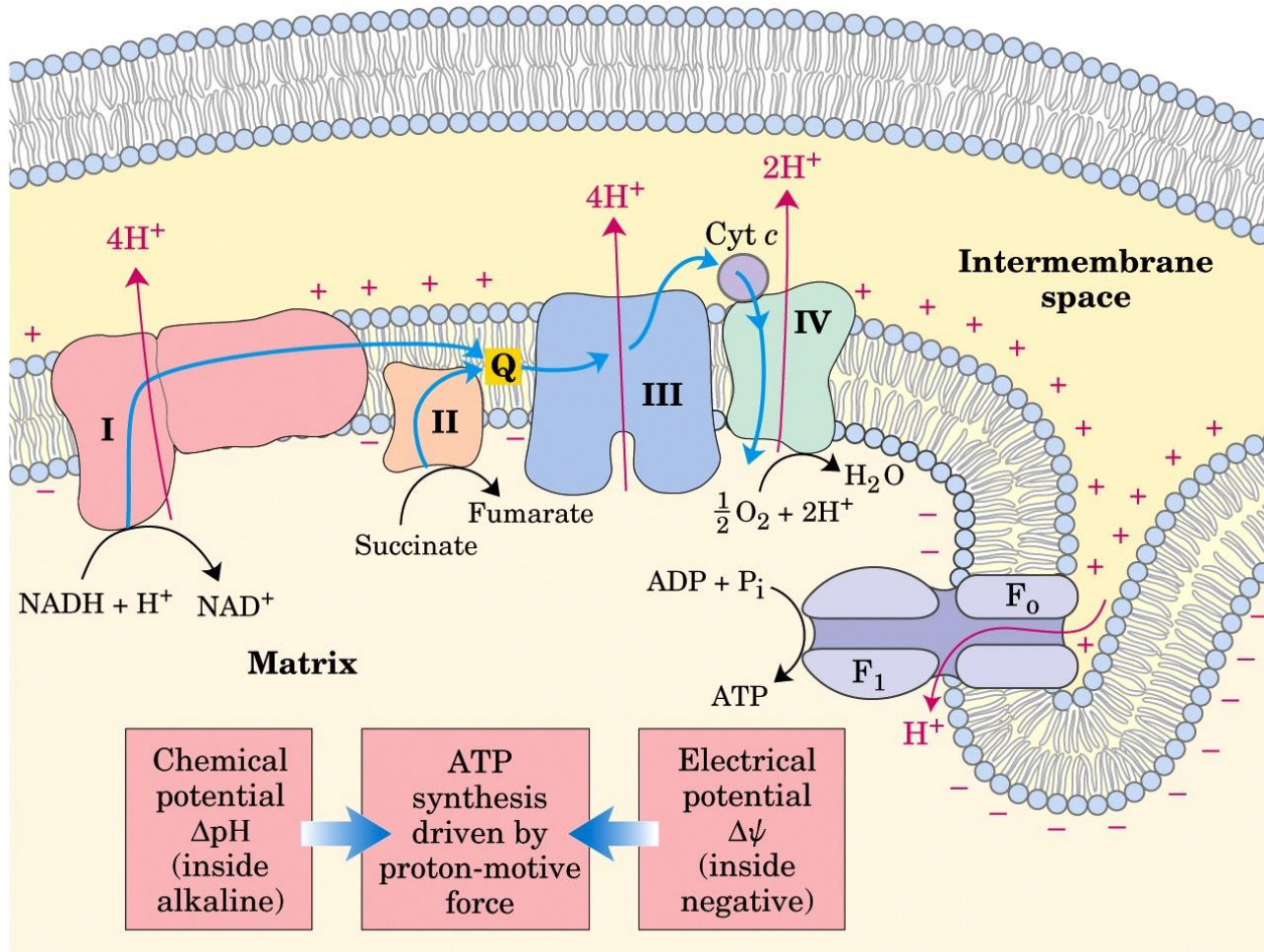
Oxygen Reactions in Oxidative Degradation and Oxidative Stress

- Air contains 21% dioxygen, O_2
- Animals, plants, and aerobic bacteria require dioxygen for efficient production of energy.
- The earliest forms of life were anaerobic.
- Dioxygen is toxic to all forms of life, whether anaerobic, aerotolerant, or aerobic.
- Life coexists with dioxygen by use of antioxidant defense systems or by repairing or replacing the components damaged by oxidative stress.





Oxygen Reactions in Respiration





Peroxinitric Acid and Peroxynitrate Anion

1901 - Bayer – mention of Peroxynitric acid

1907 - Rashing – Mixture of nitrous acid and hydrogen peroxide had unusual strong oxidizing properties.

1929 – Gleu and Roell - Correct formula as ON-OOH

1952 - Halfpenny and Robinson suggest homolytic decomposition of peroxynitric

1954 - Taube from isotopic labeling studies concluded that the decomposition is homolytic (but, in 1968, Hughes disagree)

Before 1990 – 40 partial work on the subject

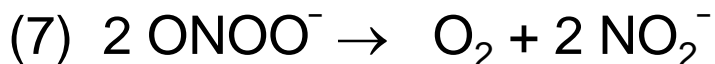
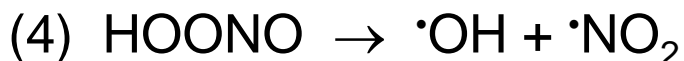
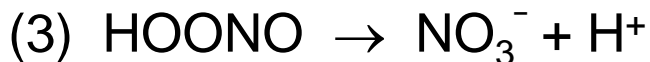
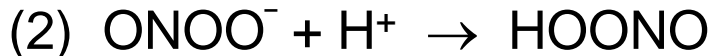
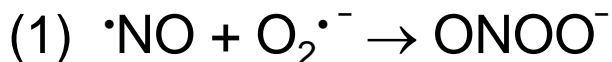
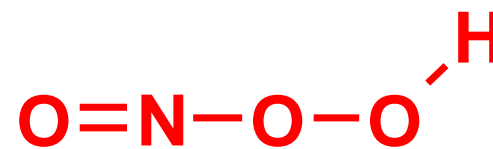
1990 – Beckman proposed for the first time that peroxynitrite can be produced *in vivo* by the reaction of NO with superoxide (the rate constant of this reaction was recently measured $1.6 \times 10^{10} \text{ M}^{-1}\text{s}^{-1}$)

1991-2007 – A lot of work on chemistry and biochemistry of the compound and its role as oxidant and signaling agent.



Chemistry of Peroxynitric Acid

- $pK_a = 6.8$ (phosphate buffer)
- Decomposition catalyzed by transition metal salts
- The anion is stable for week at 5°C
- Isomerizes to nitrate anion
- Decompose into $\cdot\text{OH}$ and $\cdot\text{NO}_2$



$$k_1 = 1.6 \times 10^{10} \text{ M}^{-1}\text{s}^{-1} \quad k_{-1} = 1.7 \times 10^{-2} \text{ s}^{-1}$$

$$k_2 = 1 \times 10^{10} \text{ M}^{-1}\text{s}^{-1} \quad k_{-2} = 1.6 \times 10^3 \text{ s}^{-1}$$

$$k_3 = 1.2 \text{ s}^{-1}$$

$$k_4 = 1 \times 10^{-2} \text{ s}^{-1} \quad k_{-4} = 4.5 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$$

$$k_5 = 2.5 \times 10^5 \text{ M}^{-1}\text{s}^{-1} \quad k_{-5} = 25 \text{ s}^{-1}$$

$$k_6 = 0.25 \text{ s}^{-1}$$

$$k_7 = 2 \times 10^{-4} \text{ M}^{-1}\text{s}^{-1}$$