

Oxygen 2000

Sunrise Free Radical School

**November 17-20, 2000
San Diego, CA**

Facts and Fiction in Free Radical Biochemistry: The Haber-Weiss Cycle

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SD/title

Fenton

H. J. H. Fenton described the oxidation of tartaric acid by the addition of iron(II) and HOOH or HOCl acid for the first time in 1876 (1), found that iron acts catalytically (2) and determined the reaction product of tartaric acid, dihydroxymaleic acid, in 1896 (3).

He did not concern himself very much with the mechanism.

A biography was published in 1993 (4).

- (1) Fenton, H.J.H. (1876) On a new reaction of tartaric acid. *Chem. News*, **33**, 190-190.
- (2) Fenton, H.J.H. (1894) The oxidation of tartaric acid in presence of iron. *J. Chem. Soc., Proc.*, **10**, 157-158.
- (3) Fenton, H.J.H. (1896) Constitution of a new dibasic acid, resulting from the oxidation of tartaric acid. *J. Chem. Soc., Trans.*, **69**, 546-562.
- (4) Koppenol, W.H. (1993) The centennial of the Fenton reaction. *Free Radical Biol. Med.*, **15**, 645-651.

Manchot

Manchot and coworkers wrote several papers between 1901 and 1928 on the stoichiometry of the reaction of hydrogen peroxide with ferrous ions.

When **iron is present in excess** over hydrogen peroxide the stoichiometry is simple: two iron(II) are oxidized per hydrogen peroxide.

When **hydrogen peroxide is present in excess** oxygen evolution is found and more than two iron(II) are oxidized.

Manchot and Lehman postulated that Fe_2O_5 was formed, which was reduced to Fe(III) either by excess iron(II), or by excess hydrogen peroxide. The latter reaction generates dioxygen. They were aware that under certain conditions even more hydrogen peroxide per iron(II) could be consumed than could be accounted for by Fe_2O_5 , and proposed that iron(II) was regenerated, noting that hydrogen peroxide could also act as a reducing agent (1)!

(1) Manchot, W. and Lehman, G. (1928) Über die Einwirkung von Hydroperoxyd auf Ferrosalz. *Liebig's Ann. Chemie*, **460**, 179-201.

SD/Manchot

Haber and Willstätter

The chain reactions 2 and 3



were originally proposed by Haber and Willstätter in a paper on radical reactions in organic chemistry and biochemistry, and specifically to explain the action of catalase(1). The opinion of Haber and Willstätter was that enzymes initiated radical reactions, after which the chain reactions consumed the substrate. Iron just acted in the same way.

Superoxide, hydrogen peroxide and the hydroxyl radical were proposed in the mid-twenties as chain carriers in combustion processes; yet Pauling claims to have "discovered" superoxide in the thirties (2).

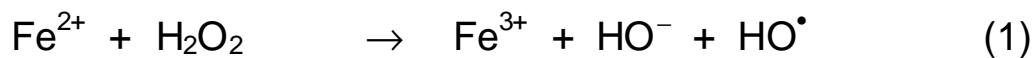
- (1) Haber, F. and Willstätter, R. (1931) Unpaarigkeit und Radikalketten im Reaktion-Mechanismus organischer und enzymatischer Vorgänge. *Chem. Ber.*, **64**, 2844-2856.
- (2) Pauling, L. (1979) The discovery of the superoxide radical. *Trends Biochem. Sci.*, **4**, N270-N271.

SD/HabWill

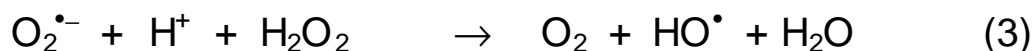
Haber and Weiss

Haber and Weiss (1) dismissed Fe_2O_5 and showed that even more hydrogen peroxide per iron(II) was consumed if the mixing of hydrogen peroxide with iron(II) was improved: they let one solution flow into the other from a rotating buret that was modified to have a horizontal tip. The interesting concept in their paper was that hydrogen peroxide was consumed by a *chain reaction*.

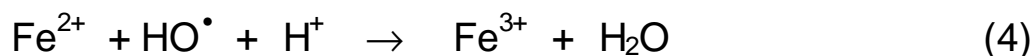
The interesting concept in their paper was that hydrogen peroxide was consumed by a chain reaction. The Fenton reaction initiates the chain, Reaction 1,



which is then followed by chain Reactions 2 and 3,



while chain termination is caused by Reaction 4:



- (1) Haber, F. und Weiss, J. (1932) Über die Katalyse des Hydroperoxydes. *Naturwiss.*, **51**, 948-950.

SD/HW1

Point 1

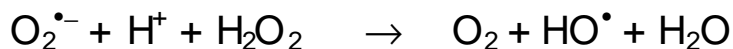
What is known as the Haber-Weiss cycle should be named the Haber-Willstätter cycle. The major reason why the cycle consisting of Reactions 2 and 4 are misnamed is that the original papers were written in German; however the often-quoted paper by Haber and Weiss (1) does give credit to that paper by Haber and Willstätter (2).

In these papers no reference to Fenton was made.

SD/point1

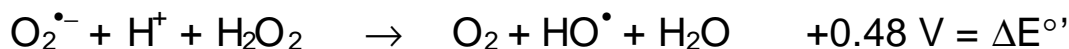
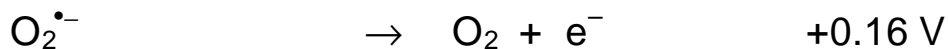
- (1) Haber, F. and Weiss, J. (1934) The catalytic decomposition of hydrogen peroxide by iron salts. *Proc. Roy. Soc.*, **147**, 332-351.
- (2) Haber, F. und Willstätter, R. (1931) Unpaarigkeit und Radikalketten im Reaktion-Mechanismus organischer und enzymatischer Vorgänge. *Chem. Ber.*, **64**, 2844-2856.

Thermodynamics



$$E^{\circ'}(\text{O}_2/\text{O}_2^{\bullet-}) = -0.16 \text{ V (1 } m \text{ O}_2\text{)},$$

$$E^{\circ'}(\text{HO}^{\bullet}/\text{H}_2\text{O}) = 0.32 \text{ V (pH7) (1)}$$



$$\Delta G^{\circ'} = -nF\Delta E^{\circ'}$$

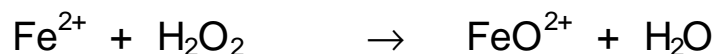
$$= -11.1 \text{ kcal/mol or } -46.3 \text{ kJ/mol}$$

- (1) Koppenol, W.H. (1989) Generation and thermodynamic properties of oxyradicals. In *Focus on Membrane Lipid Oxidation, Vol. I* (Vigo-Pelfrey, C., Ed.) pp. 1-13, CRC Press, Boca Raton.

thermodynamics

Criticisms

During the thirties the suggestion was made that the reaction of ferrous ion with hydrogen peroxide could yield oxoiron(IV) (1):



and by the late forties the mechanism for the decomposition of hydrogen peroxide by Haber and Weiss had been criticised by George (2), Abel (3), and Medalia and Kolthoff (4).

- (1) Bray, W.C. and Gorin, M.H. (1932) Ferryl ion, a compound of tetravalent iron. *J. Am. Chem. Soc.*, **54**, 2124-2125.
- (2) George, P. (1947) Some experiments on the reactions of potassium superoxide in aqueous solutions. *Disc. Faraday Soc.* **2**, 196-205.
- (3) Abel, E. (1948) Über die katalytische Zersetzung von Wasserstoffsperoxyd durch Eisensalze. *Österreichische Chemiker-Zeitung*, **49**, 79-80.
- (4) Medalia, A.I. and Kolthoff, I.M. (1949) Redox recipes. I. Reaction between ferrous iron and peroxides. General considerations. *J. Polymer Sci.*, **4**, 377-398.

SD/Criticisms

George

P. George mixed solid potassium superoxide with water (acidic and alkaline), hydrogen peroxide, acidic iron(II) solutions and a "strong" catalase solution and measured the amount of dioxygen formed (1).

He showed that the potassium superoxide in water yielded dioxygen and hydrogen peroxide, and that the amount of dioxygen was the same whether or not hydrogen peroxide was present.

This amount of dioxygen corresponded to the amount of potassium superoxide added to the solution.

- (1) George, P. (1947) Some experiments on the reactions of potassium superoxide in aqueous solutions. *Disc. Faraday Soc.*2, 196-205.

SD/George1

George (continued)

Had superoxide reacted with hydrogen peroxide, as in Reaction 2, and started the chain consisting of Reactions 1 and 2,



then the amount of dioxygen would have corresponded to the amount of hydrogen peroxide present. *However, he found [an](#) amount of dioxygen that corresponded to the dismutation of superoxide.* Thus, George concluded:

"that under the these experimental conditions the chain - propagation reaction, proposed by Haber and Weiss for the evolution of oxygen in the catalytic decomposition of hydrogen peroxide, does not occur".

SD/George2

Weiss

The response of Weiss can be found in the same discussion (1):

"I do not think that the experiments of Dr. George have any bearing on the mechanism of the hydrogen peroxide reaction. It is quite clear from his paper that the experiments were carried out in a *heterogeneous* system—*solid KO₂ in H₂O₂ solutions.*"

It was argued that the high concentration of superoxide close to the dissolving potassium superoxide favoured the dismutation over the reaction of superoxide with hydrogen peroxide; superoxide would not have time to diffuse away of the potassium superoxide particle.

(1) Weiss, J. (1947) *Disc. Faraday Soc.* **2**, 217-218.

SD/weiss1

George

P. George modified his criticism as follows:

"The experiments with KO_2 indicate that reaction (2) (*i.e.* the reaction of superoxide with hydrogen peroxide) is not very efficient."

He then addressed the issue of diffusion and presented results from experiments in which potassium superoxide was dissolved in concentrated hydrogen peroxide (85 and 96.5%) and showed:

"that with this very concentrated hydrogen peroxide no more oxygen is evolved than is observed with water alone" (1).

George, P., (1947) *Disc. Faraday Soc.* **2**, 219-221.

SD/George3

Admission

In a paper from Weiss and Humphrey from 1949 (1) one finds the following comment on Reaction (1):



"which, in the presence of ferric ions, largely replaces" (the reduction of hydrogen peroxide by superoxide).

The next three pages in that issue of *Nature* contains an article by Barb, Baxendale, George and Hargrave (2) in which the authors concluded that under conditions of excess hydrogen peroxide:

- (a) "oxygen is not evolved as the result of a reaction between HO_2^{\bullet} and hydrogen peroxide,"
 - (b) "the oxygen is evolved in a reaction involving ferric ... ions,"
- and
- (c) "ferrous ions are regenerated by the oxygen evolution reaction."

- (1) Weiss, J. and Humphrey, C.W. (1949) Reaction between hydrogen peroxide and iron salts. *Nature*, **163**, 691
- (2) Barb, W.G., Baxendale, J.H., George, P., and Hargrave, K.R. (1949) Reactions of ferrous and ferric ions with hydrogen peroxide. *Nature*, **163**, 692-694.

SD/Admission

Point 2

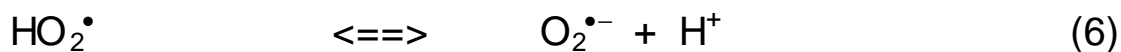
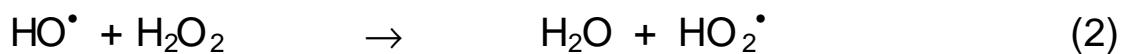
Thus, already in 1949 George and coworkers had shown that the reaction of superoxide with hydrogen peroxide is too slow in comparison to the dismutation reaction of superoxide to be important (1), and Weiss appeared to have accepted that conclusion (2).

- (1) Barb, W.G., Baxendale, J.H., George, P., and Hargrave, K.R. (1949) Reactions of ferrous and ferric ions with hydrogen peroxide. *Nature*, **163**, 692-694.
- (2) Weiss, J. and Humphrey, C.W. (1949) Reaction between hydrogen peroxide and iron salts. *Nature*, **163**, 691.

Point 2

Mechanism

The necessary reactions are



A more extensive description followed in 1951 (1,2).

- (1) Barb, W.G., Baxendale, J.H., George, P., and Hargrave, K.R. (1951) Reactions of ferrous and ferric ions with hydrogen peroxide. Part I. The ferrous ion reaction. *Trans. Faraday Soc.*, **47**, 462-500.
- (2) Barb, W.G., Baxendale, J.H., George, P., and Hargrave, K.R. (1951) Reactions of ferrous and ferric ions with hydrogen peroxide. II. The ferric ion reaction. *Trans. Faraday Soc.*, **47**, 591-616.

SD/mechanism

Corroboration

The work involved in establishing a reaction mechanism was far more complicated than it is now. Reactions were initiated, quenched at a particular time and the products determined. As a result it was not always possible to determine a rate constant. It should also be noted that at the time of these investigations the pK_a of hydrogen dioxide was not precisely known. A re-examination by pulse radiolysis of the rate constants of iron(II) and (III) with hydrogen dioxide and superoxide in 1985 (1) corroborates the mechanism proposed by Barb *et al.* (2,3).

- (1) Rush, J.D. and Bielski, B.H.J. (1985) Pulse radiolytic studies of the reactions of hydrodioxyl/superoxide with Fe(II)/Fe(III) ions. The reactivity of hydrodioxyl/superoxide with ferric ions and its implication on the occurrence of the Haber-Weiss reaction. *J. Phys. Chem.*, **89**, 5062-5066.
- (2) Barb, W.G., Baxendale, J.H., George, P., and Hargrave, K.R. (1951) Reactions of ferrous and ferric ions with hydrogen peroxide. Part I. The ferrous ion reaction. *Trans. Faraday Soc.*, **47**, 462-500.
- (3) Barb, W.G., Baxendale, J.H., George, P., and Hargrave, K.R. (1951) Reactions of ferrous and ferric ions with hydrogen peroxide. II. The ferric ion reaction. *Trans. Faraday Soc.*, **47**, 591-616.

SD/Corroboration

1st Rediscovery

In 1970 the reaction of superoxide with hydrogen peroxide is revived (1), and many groups determine a rate constant for this reaction (Table 1).

- (1) Beauchamp, C. and Fridovich, I. (1970) A mechanism for the production of ethylene from methional. *J. Biol. Chem.*, **245**, 4641-4646.

Rediscovery1

Table 1. Measured bimolecular rate constants for the reaction of $O_2^{\cdot-}$ and HO_2^{\cdot} with H_2O_2

Authors	k ($M^{-1}s^{-1}$)	
	$O_2^{\cdot-} + H_2O_2$	$HO_2^{\cdot} + H_2O_2$
Ferradini and Seide (1)		0.20 ± 0.01
Rigo <i>et al.</i> (2)	$\leq 5 \times 10^{-3}$	
Ferradini <i>et al.</i> (3)	2.25 ± 0.20	
Koppenol <i>et al.</i> (4)		3.0 ± 0.6
Melhuish and Sutton(5)	< 0.3	
Weinstein and Bielski(6)	0.13 ± 0.07	0.05 ± 0.09

- (1) Ferradini, C. and Seide, C. (1969) Radiolyse de solutions acides et aérées de peroxyde d'hydrogène. *Int. J. Radiat. Phys. Chem.*, **1**, 219-224.
- (2) Rigo, A., Stevanato, R., Finazzi-Agro, A., and Rotilio, G. (1977) An attempt to evaluate the rate of the Haber-Weiss reaction by using hydroxyl radical scavengers. *FEBS Lett.*, **80**, 130-132.
- (3) Ferradini, C., Foos, J., Houée, C., and Pucheault, J. (1978) The reaction between superoxide anion and hydrogen peroxide. *Photochem. Photobiol.*, **28**, 697-700.
- (4) Koppenol, W.H., Butler, J., and van Leeuwen, J.W. (1978) The Haber-Weiss cycle. *Photochem. Photobiol.*, **28**, 655-660.
- (5) Melhuish, W.H. and Sutton, H.C. (1978) Study of the Haber-Weiss reaction using a sensitive method for detection of OH radicals. *J. Chem. Soc., Chem. Commun.*, 970-971.
- (6) Weinstein, J. and Bielski, B.H.J. (1979) Kinetics of the interaction of HO_2 and $O_2^{\cdot-}$ radicals with hydrogen peroxide; the Haber-Weiss reaction. *J. Am. Chem. Soc.*, **101**, 58-62.

Dogma

During the late seventies the reduction of an iron(III)complex by superoxide, and the subsequent oxidation of this iron(II)complex by hydrogen peroxide became known as the "Fenton-catalysed Haber-Weiss reaction." Later it was realized and accepted that other compounds (ascorbate, for instance) could reduce the iron(III)complex.

Due to the belief in the "Fenton-catalysed Haber-Weiss reaction" one finds in the biochemical and biomedical literature many papers where tissues and organs are protected by scavengers against the evil hydroxyl radical. Scavenging would require concentrations that are unphysiological, and is therefore kinetically not possible.

Critical remarks:

1. A reaction that does not take place should not be named after the wrong person.
2. The physiological iron complex is unknown; consequently the rate constants for the reduction by superoxide (or ascorbate) and oxidation by hydrogen peroxide are unknown.
3. The evidence for the Fenton reaction *in vivo* is not convincing. That is not to say that excess iron is not toxic.

Dogma

Table 2. Rate constants and activation enthalpies for the Fenton reaction with the physiologically relevant ligands atp and citrate (1)(2).

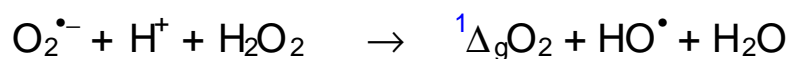
Reaction	k, M ⁻¹ s ⁻¹		ΔH [‡] , kcal/mol
	25°C	37°C	
Fe²⁺atp + ROOH			
R = H	6.7 × 10 ³	1.6 × 10 ⁴	11.6
R = t-butyl	1.3 × 10 ³	2.7 × 10 ³	10.6
R = cumyl	3.1 × 10 ³	6.5 × 10 ³	12.4
Fe²⁺citrate + ROOH			
R = H	4.9 × 10 ³	n.d.	n.d.
R = t-butyl	1.8 × 10 ³	3.4 × 10 ³	8.9
R = cumyl	2.2 × 10 ³	4.2 × 10 ³	9.4

- (1) Rush, J.D. and Koppenol, W.H. (1990) Reactions of Fe(II)-ATP and Fe(II)-citrate complexes with *tert*-butyl hydroperoxide and cumylhydroperoxide. *FEBS Lett.*, **275**, 114-116.
- (2) Rush, J.D., Maskos, Z., and Koppenol, W.H. (1990) Reactions of iron(II)nucleotide complexes with hydrogen peroxide. *FEBS Lett.*, **261**, 121-123.

Table 2

2nd Rediscovery

In 1994 the reaction of superoxide with hydrogen peroxide surfaced again (1), but this time with a twist: the oxygen is in the singlet ($^1\Delta_g$) state, which requires a positive Gibbs energy change.



$$\Delta G^\circ = +11.5 \text{ kcal/mol or } +48.1 \text{ kJ/mol}$$

This time there were no rebuttals.

- (1) Khan, A.U. and Kasha, M. (1994) Singlet molecular oxygen in the Haber-Weiss reaction. *Proc.Natl.Acad.Sci.USA*, **91**, 12365-12367.

Rediscovery2

3rd Rediscovery

Given the periodicity of the Haber-Weiss cycle, the third rediscovery is expected by 2018.

end

rediscovery3