This Week's Citation Classic®

Halliwell B. Superoxide-dependent formation of hydroxyl radicals in the presence of iron chelates. *FEBS Lett.* 92:321-6, 1978; and,
Hailiweil B & Gutteridge J M C. Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem. J.* 219:1-14, 1984.
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Paper 1 demonstrated, using aromatic hydroxylation(the iron dependent formation of hydroxyl radicals from superoxide (O_2) and hydrogen peroxide, illustrating one mechanism of O_2 toxicity. Paper 2 was the first review to emphasize the key role of transition metals in oxidative damage. [The *SCI*® indicates that these papers have been cited in more than 410 and 835 publications, respectively.]

Transition Metal Ions and Oxidative Damage

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After completing a biochemistry degree at Oxford University In 1971,1 transferred to botany for a PhD under Vernon Butt and Bob Whattoy. I found that some of the glyoxyfate **decarboxylase activity of organelles** from spinach **leaves (the "rats" of plant biochemists) is partly due to formation of hydrogen peroxide (H_2O_2), which directly oxidizes glyoxylate. I also observed that illuminated chloroplasts reduce cytochrome c, a reaction which, to my surprise,** was **inhibited by catalase.**

In 1972, I read the first paper[1] describing superoxide dismutase (SOD), an enzyme specific for the superoxide radical (O_2). Rebecca Gershman and Daniel Gilbert had proposed that oxygen toxicity involves free radicals, and the work of J.M.McCord and I. Fridovich[1] alerted me **to this concept They showed that SOD** contaminates **some commercial proteins. I discovered[2] that commercial catalase can** contain **SOD.** This **explained my observations with chloroplasts: they produce O_2^- which reduces cytochrome c.**

In 1974,I moved to King's College. The heed of biochemistry (Henry Arnstein) encouraged me to pursue research into **this** "unfamiliar" **field (no worries then about "accountability," "targeting," or "relevance"). My first PhD student (Christine H. Foyer) and I described the "ascorbate-glutathtone cycle" by which chloroplasts detoxify H_2O_2.[3] SOD is a key biological antioxidant,but O_2^- has limitsd toxicity.Friclovich proposed that O_2^- reacts with H_2O_2 to form highly damaging hydroxyl ($^{\cdot}OH$) radicals. Although this reaction is far too slow, its possible catalysis by transition metals was debated at the 1976 SOD meeting In France. Evidence consistent with iron catalysis of $^{\cdot}OH$ formation from O_2^- and H_2O_2 appeared in 1976,[4] and a direct demonstra-**tionIn 1978.[5] **My 1978 *FEBS Letters* paper directly demonstrated $^{\cdot}OH$ formation using a new type of assay and explained prior results of Buettner et ai.** (see **accompanying commentary). Later I found that DETAPAC slows reaction of O_2^- with iron, but Gerald Cohen showed that ferrous-DETAPAC still forms $^{\cdot}OH$ from H_2O_2. Hence we soon gave up using DETAPAC Later in 1978, I reported that bathophenanthroline** sutfonate* inhibits **$^{\cdot}OH$ generation. John M.C. Gutteridge and I found that desferrioxamine[7] is even better and proposed[7] that iron chelation is one strategy to inhibit radical damage in vivo.**

We realized that the amount and location of iron ions in vivo is an important factor controlling oxidative **damage, and safe** binding **of** iron **is an** antioxidant **defense. G. Czapski et al.[8] found that copper ions mediate site-specific damage by generating $^{\cdot}OH$. Our *Biochemical Journal* review was the first to emphasize the relation of transition metals to free** radicals **and human disease. As our concepts developed and extended, we published further reviews[9] and a book.[10]**

1. McCord J M & Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprcin (hemocuprcin). *J. Biol. Chem.* 244:6049-55,1969. (Cited 3,275 times.) [See also: **McCord J M.** Citation Classic. (Barrett J T, ed.) *Contemporary classics in the life sciences. Volume 2: the molecules of life.* Philadelphia: ISI Press, 1986. p. 189.]
2. **Halliwell B.** Superoxide dismutase: a contaminant of bovine catalase. *Biochem. J.* 135:379-81,1973.
3. **Foyer C H & Hailiweil B.** The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. *Planta* 133:21-5,1976. (Cited 155 times.)
4. **Fong K L, McCay P B, Poyer J L, Misra H P & Keele B B.** Evidence for superoxide-dependent reduction of Fe^{3+} and its role in enzyme-generated hydroxyl radical formation. *Chem. Biol. Interne.* 15:77-89, 1976. (Cited 135 times.)
5. **McCord** J M & **Day** E D. Superoxide-dependent production of hydroxyl radical catalyzed by iron-EDTA complex. *FEBS Lett* 86:139-42,1978. (Cited 545 times.)
6. **Halliweil B.** Superoxide-dependent formation of hydroxyl radicals in the presence of iron salts. *FEBS Lett.* 96:238-42, 1978. (Cited 180 times.)
7. Gutteridge J M C, **Richmond R & Halliwell B.** Inhibition of iron-catalyzed hydroxyl radical formation and of lipid peroxidation by desferrioxamine. *Biochem. J.* 184:469-72,1979. (Cited 290 times.)
8. **Samuni A, Chevion M & Czapski** G. Unusual copper-induced sensitization of the biological damage due to superoxide radicals. J.. *Biol. Chem.* 256:12632-5,1981.
9. **Halliwell B & Gutteridge J M C.** Oxygen free radicals and iron in relation to biology and medicine. Some problems and concepts. *Arch. Biochem. Biophys.* 246:501-14, 1986. (Cited 295 times.)
10. , *Free radicals in biology and medicine.* Oxford, England: Clarendon Press, 1985.

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