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Buettner G R, Oberley L W & Leuthauser S **W H** C. The effect of iron on the distribution of superoxide and hydroxyl radicals as seen by spin trapping and on the superoxide dismutase assay. *Photochem. Photobiol.* 28:693-5,1978; and, **Buettner G R & Oberiey L W.** Considerations in the spin trapping of superoxide and hydroxyl radical in aqueous systems using 5,5-dimethyl-l-pyrroline-l-oxide. *Biochem. Biophys. Res. Commun.* 83:69-74, 1978. [Radiation Research Laboratory, University of Iowa, Iowa City, IA]

These papers were the first to demonstrate that adventitious iron in buffer solutions drastically alters the course of free radical reactions involving superoxide *and* that cheiating agents are useful tools for modulating these reactions. In addition, they demonstrated many hazards to avoid in oxygen radical ESR spin trapping experiments. (The *SCI*® indicates that these papers have been cited in more than 110 and 165 publications, respectively.]

Iron, Cheiating Agents, and Oxygen Radicals

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In 1976,1 Joined Larry W. Oberiey's group in the Radiation Research Laboratory at Iowa as an NRSA postdoctoral fallow. My PhD thesis work hsd centered on electron spin resonance (ESR) spectroscopy, and they had an ESR spectrometer begging to be ussd. Larry hsd just made the observation that supsroxlds dismutase levels in tumor cells were abnormal.[1] The next logical question was: Is the rate of superoxide production by tumor cells different from normal cell counterparts? Because superoxide is a free radical, It was obvious that ESR spectroscopy offered a possibility for detection—but how? Then a paper[2] on detection of superoxide by ESR spin trapping, as generated by spinach chloroplasts, crossed Larry's desk. This paper was the first application of this new ESR technique to the study of oxygen radical generation in biologic systems. The next problem was to get some of the spin trap, the DMPO.

A short time later, I attended an ESR workshop In J.R. Bolton's laboratory. There I learned more about spin trapping and that AWrich Chemical Co. had just introduced DMPO into its product line. A synthesis was not necessary!

After the DMPO arrived, I naively went into the laboratory one afternoon to collect an ESR spectrum of the DMPO/superoxide spin adduct, using the enzymatic production of superoxide by xanthine/xanthine oxidase. I then planned to attack the original problem, superoxide production by tumor cells. Well, it took several months before the "simple" xanthine oxidase experiment was successful and I never got to the original problem during my fellowship time. These tersely written papers deal with the many problems I encountered in the xanthine oxidase experiment: (1) impurities in the DMPO; (2) the short half-life of the DMPO/superoxide adduct; and (3) the adventitious iron in the buffer* used.

At the 1977 meeting on "Singlet Oxygen and Related Species in Chemistry and Biology,"[3] part of this work was presented as a poster. It was this poster, and our subsequent *Photochemistry and Photobiotogy* paper, which is part of the conference proceedings, that introduced the idea that different cheiating agents could be used to modulate iron catalysis in oxygen radical studies. We showed that EDTA, the common metal cheiating agent employed, actually enhanced the effects of iron while DETAPAC suppressed iron's reactivity. This presentation created quits a stir.

After seeing our poster and the manuscript of our *Photochemistry and Photobiotogy* paper, Barry Halliwell confirmed our results.[4] Because it was over a year from when our manuscript was submitted to the conference organizers and Its subsequent publication, Barry's paper actually appeared in print before our paper.

In the *Photochemistry and Photobiotogy* paper we introduced the acronym DETAPAC for diethylenetriaminepentaacetic acid. We were not aware that DTP A had been in use for this compound. Even though most publications use DTP A in print, "DETAPAC is what is used for verbal communication in nearly every laboratory.

Later it was found that deferrfoximine, a cheiating agent used in the treatment of Iron overload, offered additional advantages in the study of the role of iron in oxygen free radical processes. Many research groups are now using cheiating agents in clinically oriented investigations to determine if they can be used to ameliorate the damage associated with many human health problems, including tissue ischemia-reperfusion injury.[5]

These two papers are cited because they point out problems and offer solutions for those people employing spin trapping. In addition, nearly every researcher in the oxygen radical field has employed cheiating agents to modulate iron catalysis in free radical processes.

1. Oberley L W & Buettner G **R.** Role of superoxide dismutase in cancer a review. *Cancer Res.* 39:1141-9,1979. (Cited 250 times.)
2. **Harbour J R & Botton J R.** Superoxide formation in spinach chloroplasts: election spin resonance detection by spin trapping. *Biochem. Biophys. Res. Commun.* 64:803-7,1975. (Cited 130 times.)
3. Singh A *&* **Petkau** A, eds. Singlet oxygen and related species in chemistry and biology. Proceedings of the International Conference, held in Pinawa, Manitoba, Canada, in 1977 as published in *Photochem. Photobiol* 28:429-933,1978.
4. **Halliwell B.** Superoxide-dependent formation of hydroxyl radicals in the presence of iron chelates. Is it a mechanism for hydroxyl radical production in biochemical systems? *FEBS Lett* 92:321-6, 1978. (Cited 385 times.) {See also: **Halliwell B.** Transition metal ions and oxidative damage. Citation Classic. *Current Contents/Life Sciences* 35(2):9,13 January 1992.]
5. **Aust S D & White B** C Iron chelation prevents tissue injury following ischemia. *Adv. Free Rod. Biol. Med.* 1:1-17, 1985.

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