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This Week's Citation Classic

McCord J M & Fridovich I. Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein). *J. Biol. Chem.* **244**:6049-55, 1969.
[Dept. Biochemistry, Duke Univ. Medical Center, Durham, NC]

The paper characterizes a new enzymic activity, superoxide dismutase, and ascribes this activity to a copper-containing protein of heretofore unknown function. The purification of the enzyme from bovine erythrocytes is described, as is a 'standard assay' for superoxide dismutase activity. [The *SCI*® indicates that this paper has been cited over 1,200 times since 1969.]

Joe M. McCord
Department of Biochemistry
College of Medicine
University of South Alabama
Mobile, AL 36688

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"In June 1967, I entered Irwin Fridovich's lab as a graduate student at Duke. For a decade Fridovich and Handler had studied xanthine oxidase, observing that its action on hypoxanthine initiated the free radical chain oxidation of sulfite, suggesting an intermediate in the enzymic reaction was a free radical. The enzyme reduced cytochrome *c* only in the presence of oxygen or an electron carrier such as methylene blue. Furthermore, preparations of myoglobin or carbonic anhydrase competitively inhibited the aerobic reduction of cytochrome *c*. The hypothesis was that xanthine oxidase passed electrons to cytochrome *c* only if O_2 were bound at the active site as an electron-conducting 'bridge,' with the free radical O_2^-an enzyme-bound intermediate. This site could be covered by the binding of myoglobin or carbonic anhydrase to xanthine oxidase.

"My project was to demonstrate by physical methods the binding of carbonic anhydrase to xanthine oxidase. Ten months were devoted to ever more elaborate experiments designed to show that which did not exist. As frustration mounted, I began to rethink the problem, finally suspecting that the negative data were telling me something.

"The breakthrough came on April 2,1968. I was studying the methylene blue mediated reduction of cytochrome *c* by xanthine oxidase–a process understood to involve reduced methylene blue in free solution. The process showed saturation by cytochrome, but kinetic constants differed. At low cytochrome concentrations the reduced dye autoxidized; at higher cytochrome concentrations saturation occurred. That evening it occurred to me that oxygen could carry electrons in an analogous manner, through free solution. Saturation would be seen because at low cytochrome concentration the superoxide radicals could dismute:$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$ .If this were true, more cytochrome should be required for saturation at higher rates of O_2^- production. Because dismutation is second order in O_2^-, the K_m for cytochrome should be a power function of xanthine oxidase concentration! Kinetic experiments the next day confirmed the prediction. Oxygen carried electrons through solution as O_2^-, a free radical. How, then, did the inhibitory proteins work? From stoichiometric considerations, they clearly had to remove O_2^- from solution *catalytically*: they were *superoxide dismutases*. It quickly became clear that myoglobin and carbonic anhydrase were not themselves the culprits –both preps contained superoxide dismutase as a minor impurity.

"The first publication,[1] rarely cited, is the important one. The present publication was useful because it defined the new enzymic activity and described its isolation and assay, rendering it available as a highly specific tool for those investigating oxygen metabolism and toxicity. Two international conferences have yielded proceedings of broad scope."[2-4]

1. **McCord J M & Fridovich I.** The reduction of cytochrome *c* by milk xanthine oxidase. *J. Biol. Chem.* **243**:5753-60, 1968.
[The *SCI*® indicates that this paper has been cited over 180 times since 1968.]
2. **Michelson A M, McCord J M & Fridovich I**, eds. *Superoxide and superoxide dismutases*. New York: Academic Press, 1977. 568 p.
3. **Bannister J V & Hill H A O**, eds. *Chemical and biochemical aspects of superoxide and superoxide dismutase.*New York: Elsevier/North Holland. In press, 1981.
4. **Bannister W H & Bannister J V,** eds. *Biological and clinical aspects of superoxide and superoxide dismutase.*New York: Elsevier/North Holland. In press, 1981.