

# This Week's Citation Classic

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Cleland W W. Dithiothreitol, a new protective reagent for SH groups.

*Biochemistry* 3:480-2, 1964.

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Because of its low redox potential, dithiothreitol (and its isomer, dithioerythritol) is capable of maintaining monothiols completely in the reduced state and of reducing disulfides quantitatively. It proved much superior to the thiols then used as protective reagents for sulfhydryl groups. [The SC<sup>1</sup>® indicates that this paper has been cited over 810 times since 1964.]

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"When I was hired as an assistant professor at Wisconsin in 1959, they thought they were hiring a lipid biochemist. My interest in enzyme kinetics (which is now the major thrust of research in my lab) developed after I came to Wisconsin, and the theoretical work I published in *Biochimica et Biophysica Acta* in 1963 was an earlier Citation Classic.<sup>1</sup> But for eight years I had a National Institutes of Health grant to study the substrate specificity for acyl-CoA thioesters in the acylation of glycerophosphate to phosphatidic acids. We prepared acyl-CoA's by chemical acylation of CoA, which in those days was only 75 percent reduced. Since it cost \$600 per gram, we wanted some way to reduce the rest and keep it reduced.

"Thiols then, as now, were most readily kept reduced by having an excess of another thiol present, since thiols readily exchange with disulfides. However, the equilibrium constant for thiol-disulfide interchange is near unity. It occurred to me that if one used a dithiol of suitable chain length, that the mixed disulfide of the dithiol and CoA would cyclize internally to give a cyclic disulfide and reduced CoA. Be-

cause the reaction produced two products from one reactant, it should go to completion. A cyclic disulfide with a six-membered ring seemed the best bet, so I bought a bottle of 1,4-butanedithiol. Opening the bottle convinced me instantly that this was not the reagent of choice (it smells like skunk oil, and the deep freeze in which this bottle was stored still reeks of the stuff 20 years later). I figured a couple of hydroxyl groups would cut down on the stench (as well as make the compound water soluble), and a trip to *Chemical Abstracts* showed that indeed 1,4-dithiothreitol and the erythro isomer had been made as part of a search for a better antiarsenical than 1,2-dithioglycerol.<sup>2</sup> I made the compounds, and to my delight they worked exactly as expected, and had a bad smell only at very close range.

"With the publication of the cited article, I began to receive requests for the compounds, and it was rapidly clear that I either had to start a pilot plant run, or convince a commercial vendor to make the stuff. Calbiochem was the first to market dithiothreitol (calling it 'Cleland's Reagent'), and they have continued to advertise the virtues of the compounds, and have distributed bibliographies on their use.<sup>3</sup>

"This paper has been highly cited for the following reason. In the 18 years since this paper was published, these dithiols have become the reagents of choice for keeping thiols, and the thiol groups of enzymes, in the reduced state. CoA became available commercially in the fully reduced state, and the yields of many enzyme isolations improved drastically. It is very satisfying to know that a pair of compounds designed on paper to accomplish a specific purpose did exactly what they were supposed to do; not all of one's ideas are so successful!"

1. Cleland W W. The kinetics of enzyme-catalyzed reactions with two or more substrates or products. I. Nomenclature and rate equations. *Biochim. Biophys. Acta* 67:104-37, 1963. [Citation Classic. *Current Contents* (28):8, 11 July 1977.]
2. Evans R M, Fraser J B & Owen L N. Dithiols. Part III. Derivatives of polyhedric alcohols. *J. Chem. Soc.* 1949: 248-55, 1949.
3. Cleland's reagent: a current bibliography prefaced with Professor Cleland's original paper describing dithiothreitol, a new protective reagent for SH groups. La Jolla, CA: Calbiochem-Behring Corp., 1979. 27 p.