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This Week's Citation Classic®

Pryor W A, Stanley J P & Blair E. Autoxidation of polyunsaturated fatty acids: II. A suggested mechanism for the formation of TBA-reactive materials from prostaglandin-like endoperoxides. *Lipids* 11:370-9, 1976.
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The thiobarbituric acid (TBA) colorimetric test is widely used to measure the extent to which polyunsaturated fatty acids (PUFA) have undergone autoxidation. The test is applied in contexts as varied as the rancidity of milk products, PUFA autoxidation *in vitro*, and the occurrence of PUFA autoxidation products in serum or organ homogenates of animals or humans subjected to oxidative stress. Malonaldehyde (MDA) is the principal species that reacts with TBA to give the color that is detected. This publication proposes a mechanism for splitting out a molecule of MDA from the middle of an oxidized PUFA; the mechanism involves a radical-mediated bicyclization to form a prostaglandin-G-like endoperoxide that hydrolyzes to form MDA in the acid medium of the TBA test. [The *SCI*® indicates that this paper has been cited in over 200 publications.]

The Source of Malonaldehyde from Lipid Peroxidations

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Although my 1966 textbook[1] on free radical reaction mechanisms covered organic free radical chemistry and not biochemistry, it found use by some of the pioneers in free radical biology, such as Irwin Fridovich, Denham Harman, and Al Tappel. Thus it happened that, in the late 1960s, I found myself speaking at symposia along with scientists interested in the role of radicals in biological systems, a group that in those pre-Cambrian years immediately following the discovery of superoxide dismutase was remarkably small indeed.

In 1970-1971, armed with a Guggenheim and an NIH Fellowship, I took a sabbatical leave, spending six months with Bill Libby at UCLA and six months with Melvin Calvin at the University of California, Berkeley. Within a week of my arrival at Berkeley, I found myself, with the encouragement and prodding of Lester Packer, out over my head, giving the lectures on free radicals and aging in a popular Berkeley course on gerontology.

During my sabbatical I began to write a research proposal on free radical biology. My criteria for selecting a research area for the proposal were: the field should be important; it should unambiguously involve radicals; and it should have a chemical, as opposed to biological, orientation. For these reasons I decided to write on smog. I suggested a study of the reaction mechanisms of ozone, the most important oxidant in smog, with key biological target molecules that could rationalize the pulmonary pathology that is observed when animals breathe polluted air. It is gratifying to me personally, although certainly extremely sad to acknowledge, that ozone toxicity is, if anything, even more important now than it was then.[2]

Thus, in the early 1970s, research was under way in my laboratory on the ozone-initiated autoxidation of polyunsaturated fatty acids (PUFA). James P. Stanley, a postdoc in the group, began to study the oxidation of PUFA using a number of techniques, including the thiobarbituric acid (TBA) test. Most workers thought that malonaldehyde (MDA) was the principal species produced from PUFA autoxidation that reacts with TBA. However, no one had asked how a three-carbon compound could be split out of the middle of a PUFA containing an 18, 20, or 22-atom chain of carbon atoms. Jim was analyzing oxidized derivatives of PUFA using mass spectroscopy, and he began to obtain evidence for unexpected cyclic products. This was a time of great excitement in the prostaglandin (PG) field, and it was known that MDA could be produced by enzyme-catalyzed oxidation of arachidonic acid. Thus, Jim was led to compare results from a colorimetric test for PG-E derivatives and the TBA test using autoxidized PUFA samples. Both this *Citation Classic* and our earlier communication[3] showed that the development of ultraviolet absorption due to a PG-like derivative and the TBA color occur with a parallel time course. We suggested a mechanism for MDA production that involves a bicyclization to a PG-G-like endoperoxide, which we proposed hydrolyzes to form PG-E-like compounds as well as MDA. Our mechanism requires a PUFA with three or more double bonds to form MDA, explaining this requirement in the TBA test.

This paper has been widely cited for two reasons: It provides an answer to a perplexing chemical conundrum, and the TBA test has, if anything, become even more widely used in recent years. The TBA test is now applied, for example, to the serum of humans in a variety of disease conditions[4] and to oxidation of the low-density lipoprotein particle.[5]

1. **Pryor W A.** *Free radicals.* New York: McGraw-Hill, 1966. 355 p. (Cited 885 times.)
2. **US Congress. Office of Technology Assessment.** *Catching our breath: the next steps for reducing urban ozone.* Washington, DC: US Government Printing Office, July 1989.
3. **Pryor W A & Stanley J P.** A suggested mechanism for the production of malonaldehyde during the autoxidation of polyunsaturated fatty acids. nonenzymatic production of prostaglandin endoperoxides during autoxidation. *J. Org. Chem.* 40:3615-7, 1975. (Cited 120 times.)
4. **Yagi K.** A biochemical approach to atherogenesis. *Trends Biochem. Sci.* 11:18-9, 1986. (Cited 10 times.)
5. **Hoff H F, Chisolm G M, Morel D W, Jürgens G & Esterbauer H.** Chemical and functional changes in LDL following modification by 4-hydroxynonenal. (Cerutti P A, Fridovich I & McCord J M, eds.) *Oxyradicals in molecular biology and pathology.* New York: Liss, 1988. p. 459-72.

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