This Week's Citation Classic

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Bendall D S & Bonner W D, Jr. Cyanide-insensitive respiration in plant mitochondria. *Plant Physiol.* 47:236-45, 1971.
[Johnson Research Foundation, University of Pennsylvania, Philadelphia, PA]

Five different hypotheses concerning the nature of the alternative cyanide-resistant oxidase system of plant mitochondria were critically tested by experiment. It was demonstrated that the pathway does not involve cytochromes and that the respiratory chain bifurcates on the substrate side of the antimycin-sensitive site. [The *SCI*® indicates that this paper has been cited over 130 times since 1971.]

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"Nobody interested in plant respiration can fail to wonder at the behaviour of the aroid spadix. Here is a tissue that may squander in a single afternoon all its accumulated reserves and literally burn itself to death. The rates of respiration are one to two orders of magnitude greater than those characteristic of most other plant tissues. The ease with which mitochondria could be prepared in large yield from spadix tissue seemed to make it the ideal source for studies on electron-transport systems involving not only the classical cytochrome system but also cyanide-resistant respiration, which is widespread in plants but about which those working with mammalian mitochondria obviously could say nothing. A major hindrance was the shortness of the flowering season of both *Arum maculatum* in Europe and *Symplocarpus foetidus* in the US. The crucial experiment always seemed to be the next one to be done when the season ended. A variety of competing theories had consequently grown up; was the cyanide-resistant oxidase a flavoprotein, a cytochrome *b*, a type of cytochrome *a*, or was it rather a mirage caused by incomplete inhibition of cytochrome oxidase?

"By the mid-1960s, answers to these questions seemed within reach, and the opportunity was provided by an invitation from Walter Bonner to visit his laboratory in the Johnson Research Foundation. Here I exchanged the hard-won pleasures of direct vision spectroscopy for the equally satisfying experience of cytochrome difference spectra mechanically drawn with Britton Chance's split-beam spectrophotometer. The latter instrument had the immense advantage of providing quantitative results that could be readily communicated to others. An early morning ritual of the laboratory was the harvesting of the mung-bean seedlings for the day's supply of mitochondria. Everyone joined in, including casual visitors,[1] and I believe this simple communal activity made an important contribution to the success of the laboratory.

"During the skunk cabbage season of 1966 we were able to show conclusively that the alternative oxidase pathway diverged from the normal respiratory chain on the substrate side of cytochrome c, but none of the suggestions for the chemical nature of the oxidase could be substantiated. This was a disappointing situation to be in, but during the last week of my stay in Philadelphia, James Bahr, a student in the laboratory, provided the one new element of the paper when he demonstrated specific inhibition of the cyanide-resistant pathway by a high concentration of KCNS, a result which supported the involvement of a non-haem iron compound. This idea led to the discovery that hydroxamic acids are relatively potent and specific inhibitors,[2] but in itself seems too tentative to explain the popularity of the paper. I suspect this is largely due to the fact that we were able to make a clear-cut choice between five conflicting hypotheses.

"The real breakthrough came considerably later when Peter Rich[3] and John Palmer[4] independently demonstrated that the cyanide-resistant enzyme can use artificial quinols as substrates, so opening up the possibility of purification."

1. **Bonner W D.** A general method for the preparation of plant mitochondria. *Methods Enzymol.* 10:126-33, 1967.
2. **Schonbaum G R, Bonner W D, Storey B T & Bahr J T.** Specific inhibition of the cyanide-sensitive respiratory pathway in plant mitochondria by hydroxamic acids. *Plant Physiol.* 47:124-8, 1971.
3. **Rich P R.** Quinol oxidation in *Arum maculatum* mitochondria and its application to the assay, solubilisation and partial purification of the alternative oxidase. *FEBS Lett.* 96:252-6, 1978.
4. **Huq S & Palmer J M.** Isolation of a cyanide-resistant duroquinol oxidase from *Arum maculatum* mitochondria. *FEBS Lett.* 95:217-20, 1978.