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This Week's Citation Classic®

Krogmann D W, Jagendorf A T & Avron M. Uncouplers of spinach chloroplast photosynthetic phosphorylation. *Plant Physiol.* **34**:272-7, 1959.
[McCollum-Pratt Institute and Biology Department, Johns Hopkins University, Baltimore, MD]

We measured the ability of illuminated chloroplasts to reduce ferricyanide and simultaneously form adenosine triphosphate (ATP). These two processes could be uncoupled by ammonium ions. The ratio of ATP formed to ferricyanide reduced suggested multiple sites of phosphorylation. [The *SCI*® indicates that this paper has been cited in over 215 publications since 1959.]

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This work was very much a product of the general thrust of biochemical research of that time. M. Calvin and his colleagues had largely finished elucidating the carbon fixation pathway of photosynthesis.[1] The physicists who sought to rationalize photosynthetic energy conversion had become bogged down in a fruitless controversy over quantum efficiency. Hill's demonstration, much earlier, that illuminated chloroplasts could produce oxygen and reduce ferricyanide[2] had languished—perhaps for want of sensitive assay procedures. Happily, A. Frenkel and D. Arnon made electrifying discoveries of ATP synthesis by illuminated photosynthetic membrane preparations.[3,4]

All of us at Johns Hopkins University were neophytes in photosynthesis research. We had developed spectrophotometric methods to measure chloroplast reduction of ferricyanide and were puzzled by variations in the rate of this reaction between different preparations. Arnon *et al.*[5] observed ATP formation during ferricyanide reduction. Our proximity to the Lehninger school of mitochondrial phosphorylation prompted us to look for similarities in chloroplast and mitochondrial phosphorylation. A similar coupling mechanism was sought. We realized that some of our treatments that stimulated ferricyanide reduction diminished ATP synthesis and so mimicked uncoupling of mitochondrial phosphorylation. While both chloroplast and mitochondrial energy conversion were poorly understood, the uncoupling of mitochondria by dinitrophenol was seen as a keystone to our understanding at that time. We were chagrined that dinitrophenol did not uncouple chloroplasts (later the conditions for its uncoupling action were found), but ammonium ions behaved as classical uncouplers. Later, the mechanism for this uncoupling could be deduced from A.T. Jagendorf's recognition that a Mitchellian proton gradient was the driving force of ATP synthesis.[6] Our measurements of the ratio of ATP synthesized to ferricyanide reduced led to an inference of multiple phosphorylation sites on the chloroplast electron transfer chain, and this has stood the test of time well.

While the execution of the experiments went smoothly as a result of a happy convergence of good methods, good observations, and good ideas, publication encountered obstacles. The reviewers of the first submission wrote pages of objections and questioned the propriety of the first word of the title. Should this really be called coupling? We struggled with revisions and produced a better manuscript without giving too much ground on the new concepts.

The many citations to this work in subsequent years have two causes. First was the discovery of a relation between electron transfer and ATP synthesis that would demand explanation through many years of subsequent work. Second was the rapid growth of research that came shortly after the publication of this paper. Photosynthesis was at the beginning of a golden age.

Subsequent work on energy coupling has been reviewed by A. Trebst,[7] and a more recent account of the chloroplast phosphorylation mechanism by Jagendorf and G. Anthon will soon be published.[8]

1. **Bassham J A, Benson A A, Kay L D, Harris A Z, Wilson A T & Calvin M.** The path of carbon in photosynthesis. XXI. The cyclic regeneration of carbon dioxide acceptor. *J. Amer. Chem. Soc.* **76**:1760-70, 1954. (Cited 200 times since 1955.)
2. **Hill R.** Oxygen produced by isolated chloroplasts. *Proc. Roy. Soc. London Ser. B* **127**:192-210, 1939. (Cited 90 times since 1955.)
3. **Arnon D I, Allen M B & Whatley F R.** Photosynthesis by isolated chloroplasts. *Nature* **174**:394-6, 1954. (Cited 245 times since 1955.)
4. **Frenkel A.** Light induced phosphorylation by cell-free preparations of photosynthetic bacteria. *J. Amer. Chem. Soc.* **76**:5568-9, 1954. (Cited 140 times since 1955.)
5. **Arnon D I, Whatley F R & Allen M B.** Assimilatory power in photosynthesis. *Science* **127**:1026-34, 1958. (Cited 220 times.)
6. **Jagendorf A T.** Acid-base transitions and phosphorylation by chloroplasts. *Fed. Proc.* **26**:1361-9, 1967.
7. **Trebst A.** Energy conservation in photosynthetic electron transport of chloroplasts. *Annu. Rev. Plant Physiol.* **25**:423-58, 1974. [See also: **Trebst A.** Citation Classic. *Current Contents/Agriculture, Biology & Environmental Sciences* **12**(2):16, 12 January 1981.]
8. **Jagendorf A T & Anthon G.** Unresolved problems in photophosphorylation. *Cold Spring Harbor Symp.* In press, 1985.

