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This Week's Citation Classic

Jensen R G & Bassham J A. Photosynthesis by isolated chloroplasts.
Proc. Nat. Acad. Sci. US 56:1095-101, 1966.
[Lawrence Radiation Laboratory, University of California, Berkeley, CA]

A method is described for rapid isolation of intact chloroplasts from spinach leaves capable of high rates of complete photosynthesis with CO_2. Not previously possible, the rates with isolated chloroplasts now resembled those of the intact leaf. [The *SCI*® indicates that this paper has been cited in over 485 publications since 1966.]

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"After finishing my graduate research in 1965 at the C.F. Kettering Research Laboratory, Ohio, I went to the biodynamics laboratory on the University of California, Berkeley campus, under Melvin Calvin and his group to continue my studies on photosynthesis. Calvin had received the Nobel prize in 1961 for his elucidation of the path of carbon in photosynthesis. I elected to work with J.A. Bassham who was directing the research on photosynthetic carbon assimilation. Starting with the isolation procedures of D.A. Walker[1] with pea chloroplasts, we optimized the conditions for isolation of chloroplasts from spinach which retained their ability to do complete photosynthesis with CO_2. It was apparent that Tris buffer was inhibitory so we used one of the then new N.E. Good zwitterion buffers. Rapid isolation and the use of fresh spinach also increased the rates of light dependent CO_2 uptake. Although not sustained for more than ten to 15 minutes, the rates were greater than half of the rate of CO_2 uptake by the intact leaves and did not require added metabolites. The products labeled with ^{14}C were those expected for the Calvin cycle.

"At that time, the rate of CO_2 fixation with isolated chloroplasts was less than five percent compared to the intact leaf. Our vigorous rates of CO_2 fixation in the absence of any added metabolites gave us confidence that our observations were more like those expected of chloroplasts in the intact leaf. We later published observations on the effects of added cofactors and intermediates, the diffusion of labeled photosynthetic intermediates out of the chloroplast, and the apparent effect of light on the carboxylation reaction.[2-4] An overview of the biochemistry of the chloroplast has recently appeared.[5]

"At that exciting time our improvement was considered a breakthrough because it was the first demonstration that the entire process of photosynthesis from O_2 evolution to CO_2 fixation could operate at significant rates outside of the intact plant cell. Since then, each laboratory has added their particular improvements to the isolation with the elucidation of many important mechanisms pertaining to the regulation of photosynthesis. The excitement continues today as many in this field visualize the eminent potential for optimization of this key life process for increasing plant production."

1. **Walker D A.** Improved rates of carbon dioxide fixation by illuminated chloroplasts. *Biochemical J.* 92:22C-3C, 1964.
2. **Bassham J A, Kirk M & Jensen R G.** Photosynthesis by isolated chloroplasts. I. Diffusion of labeled photosynthetic intermediates between isolated chloroplasts and suspending medium. *Biochim. Biophys. Acta* 153:211-18, 1968.
3. **Jensen R G & Bassham J A.** Photosynthesis by isolated chloroplasts. II. Effects of addition of cofactors and intermediate compounds. *Biochim. Biophys. Acta* 153:219-26, 1968.
4. ----------. Photosynthesis by isolated chloroplasts. III. Light activation of the carboxylation reaction. *Biochim. Biophys. Acta* 153:227-34, 1968.
5. **Jensen R G.** Biochemistry of the chloroplast. (Tolbert N E, ed.) *The biochemistry of plants. Volume 1. The plant cell.* New York: Academic Press, 1980. p. 273-313.