

This Week's Citation Classic®

Pedersen T A, Kirk M & Bassham J A. Light-dark transients in levels of intermediate compounds during photosynthesis in air-adapted *Chlorella*. *Physiol. Plant.* 19:219-31, 1966.

[Chemical Biodynamics Division, Lawrence Radiation Lab., Univ. California, Berkeley, CA]

The unicellular algae, *Chlorella*, photosynthesized and respired with $^{14}\text{CO}_2$ and H^{32}PO_4 during alternate periods of light and dark. Changes in the labeling of metabolites during transitions between dark and light indicate that at least three key enzymes of photosynthetic carbon reduction, active in the light, become inactive in the dark. [The SCI® indicates that this paper has been cited in over 160 publications.]

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The biochemical pathway of carbon dioxide fixation and reduction during photosynthesis in green plants was mapped in Berkeley by Melvin Calvin and his associates by the early 1950s.¹ A major contribution to that achievement came from analyzing the radioactively labeled products of photosynthesis with $^{14}\text{CO}_2$ by two-dimensional paper chromatography and radioautography.² Even after the Calvin Cycle (reductive pentose phosphate [RPP] cycle) gained general acceptance, there remained questions, particularly about its quantitative importance and the stoichiometry of the carboxylation reaction, which some scientists thought might be reductive.

In order to evaluate these possibilities, we perfected methods of maintaining steady-state photosynthesis with algae in the presence of labeled CO_2 and sometimes ^{32}P -labeled phosphate, followed by perturbation of the physiological state. Pool sizes of labeled metabolites, quantified by their labeling during steady-state photosynthesis, changed rapidly following perturbation, such as the light to dark switch.

By taking many samples before and rapidly after perturbation, and by quenching and subsequently analyzing these samples, we could follow such transient changes. This method already had been used in mapping the RPP cycle,¹ but was considerably refined with the assistance of my highly skilled long-time associate, Martha Kirk.³

By 1965 the RPP cycle was well established (although a special pathway for incorporating CO_2 into C_4 acids and transporting carbon therein to the site of the RPP cycle was discovered in Hawaii and Australia in plant species we had not examined in Berkeley). It was becoming evident that chloroplasts carry out glycolysis in the dark and that futile cycles would exist if all the RPP cycle enzymes plus the glycolytic enzymes were simultaneously active. A Norwegian scientist, Tor A. Pedersen, joined our laboratory for a year and with our assistance carried out more precise studies of light-dark and dark-light transients in labeled metabolites following steady-state photosynthesis.

Until that time, the only known effect of light on the RPP cycle was via the production of ATP and NADPH, needed for the activation and reduction of the carboxylation product, 3-phosphoglycerate to triose phosphate and ATP for the conversion of ribulose 5-phosphate to the carboxylation substrate. The transient changes we found from dark to light clearly showed that the RPP enzymes fructose 1,6-bisphosphatase and sedoheptulose 1,7-bisphosphatase were inactive in the dark, becoming active during the first 30 seconds in the light. When the light first came on, substrates for the reactions mediated by these enzymes piled up; products disappeared. Other data showed that the carboxylation enzyme also becomes inactive in the dark.

Elegant biochemical studies in many countries have since established the mechanisms of light activation of these enzymes,⁴ although questions still remain. Since our paper was the first to indicate such regulation *in vivo*, it may have been cited for that reason. Also, in this paper we updated our description of the steady-state and transient kinetic tracer experiments and of analyses by paper chromatography and radioautography. We have often since referred to that paper for such details. Perhaps others have done the same.

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. Benson A A, Bassham J A, Calvin M, Goodale T C, Haas V A & Stepka W. The path of carbon in photosynthesis. V. Paper chromatography and radioautography of the products. *J. Amer. Chem. Soc.* 72:1710-18, 1950. (Cited 425 times since 1955.)
3. Bassham J A & Kirk M R. Dynamics of the photosynthesis of carbon compounds. I. Carboxylation reactions. *Biochim. Biophys. Acta* 43:447-64, 1960. (Cited 90 times.)
4. Buchanan B B. Role of light in the regulation of chloroplast enzymes. *Annu. Rev. Plant Physiol.* 31:341-74, 1980.