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This Week's Citation Classic

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. [Radiation Laboratory, Department of Chemistry and Division of Plant Nutrition, University of California, Berkeley, CA]

Early products of photosynthetic $^{14}CO_2$ fixation by green algae and higher plants separate readily on two-dimensional paper chromatograms. Exposure of X-ray film produced radioautographs revealing chemical properties and relative amounts of all the radioactive components of the extract. The radiochromatographic method facilitated identification of intermediates and delineation of their metabolic sequences in photosynthesis. Chemical identification of phosphoglyceric and malic acids as first products of CO2 fixation and of sugar phosphates, sugars, and amino acids are described. [The *SCI*® indicates that this paper has been cited over 265 times since 1961.]

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" 'The Path of Carbon in Photosynthesis' included some 26 publications delineating the sequence and identity of the intermediates between atmospheric carbon dioxide and the sugars, proteins, and lipids of all plants. 'Path V described the radiochromatographic method which greatly accelerated progress toward that goal.

"With Martin Kamen and Sam Ruben in I 1942,had recognized the importance of liquid-liquid partition for revealing properties of 'trace' or 'carrier-free' amounts of radioactive products of photosynthesis. [1] Melvin Calvin, too, had had similar experiences in his development of novel chelating agents for transuranic elements.[2] We recognized at once the power of the then new partition procedure, paper chromatography, for separating the products of $^{14}CO_2$ fixation. Exposure of X-ray film to the pattern of compounds on the paper gave 'radiograms' recording the amounts and location of the radioactive products, a visible wealth of information. We could correlate position on the paper with partition and chemical properties. We could elute the compounds and re-chromatograph with authentic compounds. Radiochromatography greatly enhanced the resolving power for separation of known compounds from unknown labeled ones. Exact correspondence of the film image with the spot produced by a spray reagent was convincing indeed. Procedures for sugars and their phosphates reported in 'Path V led to identification of ribulose and sedoheptulose phosphates and of phosphoglycolate, all intermediates in the carbon reduction cycle of photosynthesis which became clear by 'Path XXI.'

"The resolving power of large two-dimensional paper chromatograms is remarkable. They separated sugars, sugar phosphates, oligosaccharides, amino acids, peptides, carboxylic acids and their esters as well as phospholipids, glycolipids, sulfo-and arsenolipids from their 'lyso' derivatives. The first of these were described in 'Path V.

"Melvin Calvin's recognition of the dibasic nature of the 'first product' from its strong binding on ion exchange resins clearly indicated it must be phosphoglycerate which plants produce first. This met with resistance in contemporary scientific circles where a more direct photochemical CO_2 reduction had been anticipated. We chose 'Path V' as a site for final burial of both the opposition and the documented identification and direct isolation of 'PGA.'

"Elegant radiochromatographic studies by Bassham and Calvin went on to establish the factors regulating photosynthetic metabolism. The method spread in many directions using tritium and other radioisotopes. Even without radioactivity we could 'activate' the chromatogram with neutrons and prepare radiograms for sensitive and selective analyses. It yielded the identity of the plant lipids and established their function. Now we are returning to the same methods to delineate the path of arsenate in oceanic food chains. By similar 'radiograms' we recognize the water-soluble and lipid components in the reduction, methylation, and ultimate degradation of the arsenolipids of marine algae by higher animals.

"Citations to this paper stem from its value as a guide to the Radiochromatographic Method. Though I spent considerable effort on developing solvents and understanding their partition properties the real weight of the work is buried in the untold hours and nights of effort by its many authors to find the first product of photosynthesis and to document its identity as phosphoglycerate. That step, once taken, passed attention to the next. Like so much history of discovery, it was frightening to write and painful to read. The Radiochromatographic Method, though, continues to spread on film before us new and exciting vistas of metabolic scenery."

1. **Benson A A.** Philosophy of the tracer method. R*adioisotopes* **26**:66-74,1977.
2. **Martell A & Calvin M.** *Chemistry of the metal chelate compounds.* New York, NY: Prenlice-Hall. 1952. 580 p.