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This Week's Citation Classic

Stocking C R. Chloroplast isolation in nonaqueous media. *Plant Physiol.* **34**:56-61, 1959. [Dept. Botany, Univ. California, Davis, CA]

Water soluble solutes are not leached from chloroplasts isolated in organic solvents from frozen-dried leaves. Although difficulties are associated with the use of lipid solvents, the method is a useful supplement to aqueous methods for studying the intracellular distributions of solutes. [The *SCI*® indicates that this paper has been cited over 130 times since 1961.]

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"By the mid-1950s, the essential features of the carbon-reduction pathway of photosynthesis in C_3 plants had been elucidated, and research on the quantum conversion process and photosynthetic electron transport was accelerating. The important role of the chloroplast in cellular metabolism especially with reference to the exchange of solutes between the chloroplast and its cellular environment was becoming appreciated by most investigators in the field. However, in spite of a sustained interest for over 100 years in the movement of water and solutes in plants, little knowledge concerning the intracellular movement of metabolites was available. One of the major difficulties in such investigations was the absence of a reliable method of isolating chloroplasts that still retained their water soluble constituents.

"At the University of California at Davis, T.E. Weier and I had been studying the interrelationship between chloroplast structure and function. In 1955, I received a fellowship in biochemistry to work in the laboratory of R.H. Burris at the University of Wisconsin. While there, I modified Martin Behrens's[1] method of isolating nuclei from frozen-dried animal tissue with organic solvents for use in isolating chloroplasts from frozen-dried leaves. One especially difficult problem was the separation of dried cytoplasm from dried chloroplasts. However, it was found, and subsequently verified by electron microscopy, that the cold nonaqueous solvents weakened the chloroplast envelope and allowed the separation of cytoplasm from chloroplasts during vigorous grinding.

"It is well known that frequently almost identical research occurs simultaneously and independently in different laboratories. Soon after the publication of this article on nonaqueous isolation of chloroplasts, I received a reprint from Ulrich Heber[2] in the Federal Republic of Germany which described his research using an almost identical method. Also in the same year, Behrens[3] published a short paper describing the isolation of chloroplasts by his method. Heber studied the intracellular distribution of sugars rather than of enzymes as I had done. It is interesting that had I been aware of Heber's studies I might not have continued with my research along these lines and some years later Heber told me that if he had not seen my paper dealing with enzymes, he probably would not have continued his studies using this method.

"The frequency of citation of this article, which has more recently been reviewed,[4] undoubtedly is a consequence of its appearance at a time when there was a critical need for a method of studying the intracellular distribution of water soluble metabolites. Effective and reliable aqueous methods of isolating intact chloroplasts that retained their water soluble constituents had not yet been developed. Since the nonaqueous method itself involves technical problems concerning the purity of the isolated chloroplasts, it is understandable that some of the citations have been in papers in which the method has been critically evaluated."

1. **Behrens M.** Untersuchungen an isolierten Zell- und Gewebsbestandteilen. I. Mitteilung: Isolierung von Zelkernen des Kalbsherzmuskels. *Z. Physiol. Chem.* **209**:59-74, 1932.
2. **Heber U.** Zur Frage der Lokalisation von loslichen Zuckern in der Pflanzenzelle. *Ber. Deut. Bot. Ges.* **70**:371-82, 1957.
3. **Behrens M & Thalacker R.** Gewinnung von Chloroplasten in nichtwassrigem Milieu. *Naturwissenschaften* **44**:621, 1957.
4. **Stocking C R.** Chloroplasts: nonaqueous. *Method. Enzymol.* **23**:221-8, 1971.