This Week's Citation Classic®

Hager A & Meyer-Bertenrath T. Die Isolierung und quantitative Bestimmung der Carotinoide und Chlorophylle von Blättern, Algen und isolierten Chloroplasten mit Hilfe dünnschichtchromatographischer Methoden (Extraction and quantitative determination of carotenoids and chlorophylls of leaves, algae and isolated chloroplasts with the aid of thin-layer chromatography). *Planta* 69:198-217, 1966.
[Institute of Botany, University of Munich, Federal Republic of Germany]

This paper describes new thin-layer chromatography techniques that allow for fast separation and quantitative determination of all principle carotenoids and chlorophylls localized in the thylakoid membranes of chloroplasts. Furthermore, with these methods, isomeric carotenoids of chloroplasts, which differ only in the position of a double bond (as for instance α- and β-carotene, or lutein and zeaxanthin), could be separated in one step for the first time. [The *SCI*® indicates that this paper has been cited in over 245 publications.]

Chloroplast Pigment Chromatography and Xanthophyll Cycle

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In the 1960s research on photosynthesis received a new impulse from P. Mitchell's chemiosmotic hypothesis.[1] Attention was focused on the conversion of light energy into chemical energy in the thylakoid membrane. Consequently, it seemed necessary to get more information about the structure of the membrane and especially the pigments that are involved in light perception. Therefore, a method for fast isolation and identification of the photosynthetic pigments became extremely necessary.

In addition, another necessity arose during my studies on alpine plants at the Institute of Botany of the University of Munich. A mechanism, which some Russian researchers[2] were already working on, had attracted my attention: in chloroplasts the amount of a few carotenoids appeared to change very drastically upon illumination. Later on we learned that this change is connected to the conversion of the xanthophyll violaxanthin into zeaxanthin, a reaction that is triggered by H^+ accumulation within the thylakoid lumen and that is reversible in the dark ("xanthophyll cycle").[3] Research on this process was long delayed since there was no convenient method for separating zeaxanthin from its isomer, lutein, which is abundant in chloroplasts.

For those reasons I tried to develop a new method for separation and identification of leaf pigments. At the beginning I used columns of potato starch for chromatography; however, their handling was very complicated. Therefore, I changed to glass-fiber papers and finally to thin layers of silica gels. Using this method we (Thea Bertenrath, a PhD student, and I) came up with a very precise separation. Unfortunately, we recognized that the chlorophylls and xanthophylls changed due to the acidity of the silica gels and also by oxidation. These difficulties were overcome by buffering the pH of the layers and by the addition of an antioxidant (ascorbic acid).

However, the separation of isomeric carotenoids was still not possible with this system. We hoped to solve this problem by using highly absorbent materials. From alkaline-earth metal oxides we produced hundreds of different thin-layer mixtures and also tried a lot of solvent mixtures for the chromatographic procedure. Our colleagues in the lab were not pleased at all by our efforts since very fine dust settled all over. Finally, we found a mixture of three components that allowed excellent separations of all carotenoids.

But meanwhile the question of auxin-induced growth captured my interest, and I could only return to the problem of the xanthophyll transformations after publishing results on the primary action of this hormone, which too became a *Citation Classic*.[4]

Then, the progress in chromatography in the 1960s gave way to a full description of the mechanism of the light-dependent, reversible xanthophyll cycle. At the Annual Meeting of the German Botanical Society at Würzburg, September 23-29, 1974, I presented these results in a plenary lecture[5] (also published in an English version[6]).

I believe that the reason for the frequent citation of our paper is that now, 20 years later and even after the introduction of HPLC, there is still no method for isolating pigments (especially isomers of carotenoids) that is faster, cheaper, and more effective.

[Editor's note: Among recent papers citing Hager's work and applying the technique described above are those by G.W. Francis and M. Isaksen[7] and by N. Suzuki and colleagues.[8]]

1. **Mitchell P.** Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. *Biol. Rev. Cambridge Phil. Soc.* 41:445-502, 1966. (Cited 1,675 times.) [See also: Mitchell P. Citation Classic. (Barrett J T, ed.) *Contemporary classics in the life sciences. Volume 2: the molecules of life.* Philadelphia: ISI Press, 1986. p. 193.]
2. **Sapozhnikov D I, Krasovskaya-Antropova T A & Mayevskaya A N.** Changes observed in the relation between the main carotenoids in the plastids of green leaves exposed to light. *Dokl. Akad. Nauk. SSSR* 113:465-7, 1957. (Cited 65 times.)
3. **Hager A.** Lichtbedingte pH-Erniedrigung in einem Chloroplasten-Kompartiment als Ursache der enzymatischen Violaxanthin—Zeaxanthin-Umwandlung; Beziehungen zur Photophosphorylierung (Light-dependent decrease of the pH-value in a chloroplast compartment causing the enzymatic interconversion of violaxanthin to zeaxanthin; relations to photophosphorylation). *Planta* 89:224-43, 1969. (Cited 75 times.)
4. **Hager A, Menzel H & Krauss A.** Versuche und Hypothese zur Primärwirkung des Auxins beim Streckungswachstum (Experiments and hypothesis concerning the primary action of auxin in elongation growth). *Planta* 100:47-75, 1971. (Cited 285 times.) [See also: Hager A. Citation Classic. (Barrett J T, ed.) *Contemporary classics in the life sciences. Volume 1: cell biology.* Philadelphia: ISI Press, 1986. p. 285.]
5. **Hager A.** Die reversiblen, lichtabhängigen Xanthophyllumwandlungen im Chloroplasten (The reversible, light-induced conversions of xanthophylls in the chloroplast). *Ber. Deut. Bot. Ges.* 88:27-44, 1975. (Cited 40 times.)
6. ———. The reversible, light-induced conversions of xanthophylls in the chloroplast. (Czygan F C, ed.) *Pigments in plants.* Stuttgart, FRG: Fischer Verlag, 1980. p. 57-79. (Cited 15 times.)
7. **Francis G W & Isaksen M.** Thin-layer chromatography of carotenoids with tertiary alcohol-petroleum ether solutions as developing solvents. *J. Food Sci.* 53:979-80, 1988.
8. **Suzuki N, Saitoh K & Adachi K.** Reversed-phase high-performance thin-laye. chromatography and column liquid chromatography of chlorophylls and their derivatives. *J. Chromatography* 408:181-90, 1987.

14 - 15