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CC/NUMBER 11
MARCH 12, 1984

Halperin W & Wetherell D F. Adventive embryony in tissue cultures of the wild carrot, *Daucus carota*. *Amer. J. Bot.* 51:274-83, 1964.
[Department of Botany, University of Connecticut, Storrs, CT]

Carrot embryos develop from cultured cells on a medium consisting of minerals, sucrose, vitamins, and hormones. Photographs illustrate the development of embryos through filamentous stages to the cotyledonary stage. The absence of a requirement for coconut milk or other complex additives in the medium is significant. [The *SCI*® indicates that this paper has been cited in over 120 publications since 1964.]

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January 9, 1984

"As a graduate student I was interested in both plant physiology and chemotaxonomy. I was naive enough to assume that tissue cultures might provide material for chemotaxonomic study free of the problems associated with intact plants. My interest in umbellifers (carrot family) led me to attempt the culture of many species found growing wild in the countryside around the University of Connecticut campus.

"Cultures of wild carrot often produced tiny, green plantlets. Don Wetherell, my faculty adviser, borrowed a culture for some other purpose which I no longer recall. He transferred a callus culture to rotating liquid medium, something which I had not done, and noticed that the liquid suspension was full of discrete white bodies. They turned out to be embryos—the obvious source of the plantlets which I had been observing. We went on to describe the form of the plantlets and the range of media on which they appeared.

"This paper has no doubt been cited so often because it contained the first extensive photographic documentation of tissue culture embryos at a number of stages and because it took issue with a number of prevailing ideas about the ontogeny of plantlets in such cultures, as well as the chemical environment which was required for their development.

"The frequency of citation of this paper is unfortunate since it does contain a number of errors. A series of later papers contain an accurate description of how such embryos originate from proembryogenic clumps, the histological nature and self-perpetuation of such clumps, how embryos become polarized, and some quantitative and autoradiographic data on the distribution of cell divisions in clumps and the embryos which emerge from them when the auxin level is lowered.[1-3]

"A major contribution of the paper was to call into question the notion that coconut milk was required for, or specifically stimulatory for, embryogenesis in cell cultures. This idea was advanced by F.C. Steward and his co-workers at Cornell University. Steward's work in plant tissue culture was highly original and beautifully described in a series of stimulating and insightful papers beginning in the 1950s.[4] The routine use of coconut milk by the Cornell group, however, led to the hypothesis that this liquid endosperm (which normally provided for the nutrition of coconut embryos) contained substances specifically responsible for embryogenic behavior by cultured plant cells. We showed this not to be the case.

"Our understanding of embryogenesis in cultures remains at a very elementary level. The synthetic hormone, 2,4-D, is the most efficient in causing the appearance of proembryogenic clumps in a proliferating explant. Clumps perpetuate themselves by continued growth and fragmentation. Lowering the 2,4-D level (or removing it) allows embryos to organize at the periphery of the clumps. *This is not the initiation of embryogenesis*, but the release of embryogenic tissue from auxin inhibition. Why 2,4-D is so efficient and why carrots, and a few other species, form embryos so readily is not known."

1. **Halperin W.** Alternative morphogenetic events in cell suspensions. *Amer. J. Bot.* 53:443-53, 1966. (Cited 100 times.)
2. **Halperin W & Jensen W A.** Ultrastructural changes during growth and embryogenesis in carrot cell cultures. *J. Ultrastruct. Res.* 18:428-33, 1967. (Cited 90 times.)
3. **Halperin W.** Embryos from somatic plant cells. (Padykula H A, ed.) *Control mechanisms in the expression of cellular phenotypes*. New York: Academic Press, 1970. p. 169-91.
4. **Steward F C, Mapes M O & Mears K.** Growth and organized development of cultured cells. II. Organization in cultures grown from freely suspended cells. *Amer. J. Bot.* 45:705-8, 1958. (Cited 210 times.)

