

Oaks A & Bidwell R G S. Compartmentation of intermediary metabolites.

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Critical experiments using tracer methodology showed that  $^{14}\text{C}$ -labelled metabolites added from outside were not treated in a random fashion. For example, one compound synthesized by different routes was typically metabolized at different rates. Patterns of metabolism were also shown to be quite different at different developmental stages. This led in a general way to ideas of compartmentation. The vacuole was considered to be the storage reservoir of plant cells. [The  $\text{SC}^{\circ}$  indicates that this paper has been cited in over 120 publications since 1970.]

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"In the 1960s, a number of publications from the laboratories of Harry Beevers,<sup>1</sup> R.G.S. Bidwell, and F.C. Steward<sup>2</sup> had established beyond a doubt that radioactive tracers were not handled in a random fashion. My own contribution to the overall field related to the role that an amino acid supplied by the endosperm of a cereal seed might have in its own synthesis in the embryo. This required an experimental definition of a 'metabolic' pool versus a 'storage' pool and the role of transported amino acids in maintaining these pools. This was a relatively new area of research for me. The influence of working at Purdue University, with its strong emphasis in molecular biology, and in Beevers's laboratory cannot be underestimated.

"The technology permitting the isolation of intact organelles was in its infancy at that time. For

example, the 'Good Buffers' were just coming on the market. These buffers permitted the isolation of intact chloroplasts, peroxisomes, and mitochondria, and hence studies that would clarify the location of the carbon path in photosynthesis. Compartmentation was shown to permit the activation of ribulose-1,5-bis phosphate carboxylase-oxygenase by increasing pH and  $\text{Mg}^{2+}$  concentration within the chloroplast stroma in the light<sup>3</sup> and the partitioning of metabolites in photorespiration.<sup>4</sup> In broad terms, the review anticipated some of these discoveries, and this, I think, helped to keep it contemporary for a relatively long period of time.

"In 1975, Wagner and Siegelman<sup>5</sup> published a method for large-scale isolation of intact vacuoles from protoplasts. About the same time, a number of other workers established alternate methods for sequentially extracting the cytosol and vacuolar contents. In an interesting study using both of these techniques, Delmer<sup>6</sup> was able to show that malic acid, methyl red, and betaine were, in fact, localized in the vacuole and that the metabolic pool of tryptophan was in the cytosol. Properties of the membranes that permit differential uptake and release of metabolites from particular organelles are currently under intense investigation. Solutions to problems of membrane selectivity will help to define the actual role of compartmentation in the regulation of metabolism.

"In the review, we suggested that there might be compartments less permanent than mitochondria or chloroplasts that could isolate specific metabolic events in the cytosol. For example, the inhibition of leucine biosynthesis in maize roots by the leucine metabolic pool could represent the localization of leucine within the cytosol.<sup>7</sup> I think the era for defining these temporary compartments may just have started.<sup>8,9</sup> It is my prediction that this represents the next stage in investigations designed to clarify the regulation of metabolic events in cells."

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