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This Week's Citation Classic®

Pitman M G. The determination of the salt relations of the cytoplasmic phase in cells of beetroot tissue. *Aust. J. Biol. Sci.* **16**:647-68, 1963.
[Botany Department, University of Cambridge, England]

Analysis of the rate of isotope exchange was used to determine amounts of K^+, Na^+, and Br^- in the cytoplasmic phase of red beetroot cells. Fluxes into and out of the cell were estimated for K^+ and Br^- showing that salt saturation was due mainly to increase in efflux from the vacuole but partly to decrease in influx at higher vacuole salt concentration. [The *SCI*® indicates that this paper has been cited in over 170 publications since 1963.]

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In these days of rapid expansion—and aging—of scientific information, it's very pleasing to have a paper cited that has celebrated its 21st anniversary. The work described began as part of my PhD thesis in the Botany School, University of Cambridge, with G.E. Briggs and was completed there before I moved to Australia in 1962. It owed much to conceptual developments in ion transport in plant cells generated by application of biophysical concepts to the giant cells of algae in the Characeae by Jack Dainty and Enid MacRobbie[1] at Edinburgh and Alex Hope and Alan Walker[2] in Australia. At that time, too, Cambridge had a strong group in animal physiology concerned with biophysics of transport across membranes. Of particular current interest then was the exchange properties of the "free space" (walls, intercellular spaces) of plant tissues and whether the plasmalemma of the cytoplasm was an effective barrier to transport to the cell. The studies with giant algae cells reinforced the view that active ion transport occurred at the membrane boundaries of the cytoplasm.

This paper was the first attempt to extend to higher plant cells the "characean" approach using kinetic analysis of isotope exchange. Particular problems were the validity of extrapolating from a collection of cells in a tissue to a cell "ideotype" and demonstrating that one of the kinetic components could be identified as "cytoplasmic." This "proof" involved showing that the kinetic phase was in a series between the vacuole and the free space and that ion fluxes had characteristics of active ion transport.

Experimental approaches depended primarily on isotope exchange, making use of short-lived isotopes of K, Na, and Br as well as the long-lived isotope of Na. Access to these isotopes enabled me to concentrate on exchange as well as net uptake, estimated from chemical analysis of the isotope decay. In this way, plant cells could be studied at flux equilibrium or in the transition to equilibrium, which was a great advantage over earlier approaches based on net ion uptake alone. A valuable development in another area at that time was the measurement of cell electrical potential differences in higher plant cells by Bud Etherton and Noe Higinbotham,[3] which brought the higher plant cell model for ion transport closer to the sophistication of studies with *Chara*.

These early measurements with beetroot cells have been extended to carrot cells, plant cells in suspension culture, and more complex tissues such as plant roots, where the flow through the root has to be included, in addition to the fluxes into cortical cells. Refinements have occurred in the mathematical treatment of flux and tracer exchange, while computing power has facilitated analysis. For a review on applications see reference 4.

Looking back on this work, I recall both the strains of working for several days nonstop to get all possible data from a delivery of short-lived isotopes and the disadvantage to an impecunious student of using beetroot disks instead of carrot disks, as there seemed to be more recipes to use the rest of the carrot than the remaining beetroot!

1. **MacRobbie E A C & Dainty J.** Ion transport in *Nitellopsis obtusa. J. Gen. Physiol.* 42:335-53. 1958. (Cited 140 times.)
2. **Hope A B & Walker N A.** Ionic relations of cells of *Chara australis*. III. Vacuolar fluxes of sodium. *Aust. J. Biol. Sci.* 13:277-91. 1960.
3. **Etherton B & Higinbotham N.** Transmembrane potential measurements of cells of higher plants as related to salt uptake. *Science* 131:409-10. 1960. (Cited 100 times.)
4. **Walker N A & Pitman M G.** Measurement of fluxes across membranes. (Lüttge U & Pitman M G. eds.) *Transport in plants II. Part A. Cells.* Berlin: Springer-Verlag, 1976. p. 93-126.

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