

# This Week's Citation Classic

CC/NUMBER 32  
AUGUST 8, 1983

**Spanswick R M & Williams E J.** Electrical potentials and Na, K, and Cl concentrations in the vacuole and cytoplasm of *Nitella translucens*. *J. Exp. Bot.* 15:193-200, 1964.  
[Biophysics Department, University of Edinburgh, Scotland]

The membrane potentials across the tonoplast and plasmalemma of *Nitella translucens* were measured separately. The concentrations of sodium, potassium, and chloride in the cytoplasm and vacuole were also measured. Assuming flux equilibrium, it was possible to identify the membranes at which active transport of each of the ions occurs. [The *SCI*<sup>®</sup> indicates that this paper has been cited in over 110 publications since 1964, making it the 11th most-cited paper published in this journal.]

Roger M. Spanswick  
Section of Plant Biology  
Division of Biological Sciences  
Cornell University  
Ithaca, NY 14853

May 19, 1983

"This project was undertaken with the encouragement of Elwyn Williams and Jack Dainty soon after I began my work for my PhD degree. Dainty had begun to introduce a biophysical approach to ion transport in plants a few years earlier<sup>1</sup> and my task was to investigate the dependence of the membrane potential on external ion concentrations.

"It quickly became evident that an understanding of the membrane potential required information about the potential across the plasmalemma, as opposed to the plasmalemma and tonoplast in series, and the ion concentrations in the cytoplasm. The giant internodal cells of the Characeae are the most convenient system on which to make these measurements. It is possible to centrifuge the larger cells so that the flowing cytoplasm collects at one end. Up to 1  $\mu$ l of cytoplasm can be collected from the end of the cell and analyzed for Cl<sup>-</sup> by electrometric titration and for Na<sup>+</sup> and K<sup>+</sup> by atomic absorption spectroscopy. It was during this time that I learned the importance of not

leaving the atomic absorption spectrophotometer unattended. A power failure turned off the air compressor and the acetylene flame deposited a layer of sticky black soot over everything in the instrument room.

"The potential measurements were complicated by the 'sealing' phenomenon in which a new cell wall is formed around the tip of the cytoplasmic electrode and eventually excludes it from the cytoplasm. However, there was plenty of time to make the measurements and the results were consistent.

"Perhaps more important than their significance for electrophysiology, the results, and MacRobbie's work on ion fluxes,<sup>2</sup> made it possible to draw conclusions as to the sites of active transport for the three ions in *Nitella*. The site of active transport for Cl<sup>-</sup> was at the plasmalemma rather than the tonoplast as had previously been believed. This helped to establish that the plasmalemma was the main permeability barrier in the cell, contrary to Briggs's hypothesis<sup>3</sup> that the cytoplasm was part of the free space. It was also evident that Na<sup>+</sup> was transported out of the cytoplasm across the tonoplast into the vacuole as well as out of the cell across the plasmalemma. It is for these reasons that the paper has been quoted in several plant physiology textbooks.

"However, the information was also useful in establishing the magnitude of the diffusion potential across the plasmalemma. This, in turn, was important for setting a limit on the value of the diffusion potential when it became clear that the potential is controlled not by passive diffusion but by an electrogenic ion pump.<sup>4</sup>

"The idea of an electrogenic H<sup>+</sup> pump is now widely accepted<sup>5</sup> and recently we have isolated and reconstituted ATPases from both the tonoplast<sup>6</sup> and plasmalemma (O'Neill and me, unpublished) and demonstrated that they transport H<sup>+</sup>."

1. Dainty J. Ion transport and electrical potentials in plant cells. *Annu. Rev. Plant Physiol.* 13:379-402, 1962.
2. MacRobbie E A C. Ionic relations of *Nitella translucens*. *J. Gen. Physiol.* 45:861-78, 1962.
3. Briggs G E & Robertson R N. Apparent free space. *Annu. Rev. Plant Physiol.* 8:11-30, 1957.
4. Spanswick R M. Evidence for an electrogenic ion pump in *Nitella translucens*. I. The effects of pH, K<sup>+</sup>, Na<sup>+</sup>, light and temperature on the membrane potential and resistance. *Biochim. Biophys. Acta* 288:73-89, 1972.
5. ...., Electrogenic ion pumps. *Annu. Rev. Plant Physiol.* 32:267-89, 1981.
6. Bennett A B & Spanswick R M. Solubilization and reconstitution of an anion-sensitive H<sup>+</sup>-ATPase from corn roots. *J. Membrane Biol.* In press, 1983.