CC/NUMBER 6
FEBRUARY 6, 1984

This Week's Citation Classic ™

Rottenberg H, Grunwald T & Avron M. Determination of ΔpH in chloroplasts. 1.Distribution of [^{14}C]methylamine. *Eur. J. Biochem.* **25**:54-63, 1972.
[Department of Biochemistry, Weizmann Institute of Science, Rehovot, Israel]

A new method for the determination of ΔpH across chloroplast thylakoid vesicles which is based on the distribution of [^{14}C]methylamine was developed. It was demonstrated that under optimal conditions for light driven ATP synthesis a large ΔpH (acidic inside) is established and appears to be a competent intermediate of photophosphorylation [The *SCI*® indicates that this paper has been cited in over 235 publications since 1972.]

Hagai Rottenberg
Department of Pathology
and Laboratory Medicine
School of Medicine
Hahnemann University
Philadelphia, PA 19102

January 11, 1984

"In the fall of 1969, after completing my PhD thesis at Harvard University, I arrived at the Weizmann Institute of Science for postdoctoral work with Mordhay Avron. My thesis work at Harvard, under the guidance of A.K. Solomon, was concerned with the mechanism of active potassium transport in mitochondria. During the course of this work, I became a 'convert' to Mitchell's chemiosmotic mechanism, and considered it important, therefore, to develop methods for the quantitative determination of the proton electrochemical gradient in mitochondria in order to test the predictions of the chemiosmotic hypothesis.[1]

"When I arrived at the Weizmann Institute, Avron, together with his then student Steve Karlish, was investigating light-induced proton transport in chloroplasts. It was apparent from their work that there was no quantitative relationship between the extent of proton uptake and photophosphorylation, but there was no way to measure the proton electrochemical gradient in chloroplasts. Since chloroplasts pump protons *into* the interthylakoid space, in contrast to mitochondria which pump protons out, the ion distribution methods which proved useful in mitochondria could not be employed. However, it seemed to us that the principle, namely, that the equilibrium distribution of permeable ions can be used to indicate the magnitude of the membrane potential and that of permeable acids (or bases) to indicate ΔpH, must also be valid in this case.

"Considering the polarity of the chloroplast, we reasoned, we should use anions (rather than cations) for the measurement of membrane potential, and amines (rather than carboxylic acids) for the measurement of ΔpH. Since it was already known that chloroplasts can pump ammonium in the light, we felt confident that our approach must work. It was our great fortune, at that point, to be joined by a very young and eager Dutch student, Tilly Grunwald (now Bakker-Grunwald) who came to spend a year with us. Grunwald used all her considerable experimental skill to perform the long series of experiments which proved our basic assumptions. We know today that we were also aided by lucky accidents and guesses. First was the lucky choice of ^{14}C-methylamine, which was the only commercially radiolabeled aliphatic amine available then. As it turns out, it is also the best. Second was the use,of ^{14}C-sorbitol as a marker for the extra-thylakoid space, which turns out to be the best marker in this system. Third, it also happened that a few weeks before we started these experiments, the new Beckman microfuge arrived. With transparent cover and transparent tubes we had no trouble in illuminating the chloroplasts during centrifugation, which we know today to be essential for detection of a large "pH. After publishing a short report[2] on our initial experiments, we proceeded together with Simon Schuldiner, who had just arrived as a fresh graduate student, to develop faster methods for the measurement of ΔpH based on the same principles. Schuldiner is now at Hebrew University in Jerusalem.

"These studies resulted in a series of papers of which this work was the first.[3,4] In the decade that followed these studies, proton pumps have been found in many cells and organelles and have proved to play a central role in ATP synthesis and active transport. Moreover, in many of these systems, as well as in vesicle preparations and reconstituted pumps, protons are pumped into the internal space, simulating the case of the chloroplast. Hence, the methods which we developed for the measurement of ΔpH in chloroplasts have found wide use in all of these systems."[5,6]

1. **Rottenberg H.** ATP synthesis and electrical membrane potential in mitochondria. *Eur. J. Biochem.* **15**:22-8, 1970.
2. **Rottenberg H, Grunwald T & Avron M.** Direct determination of ΔpH in chloroplasts and its relation to the mechanism of photoinduced reactions. *FFBS Lett.* **13**:41-4, 1971.
3. **Schuldiner S, Rottenberg H & Avron M.** Determination of ΔpH in chloroplasts. 2. Fluorescent amines as a probe for the determination of ΔpH in chloroplasts. *Eur J. Biochem.* **25**:64-70, 1972.
4. **Rottenberg H & Grunwald T.** Determination of ΔpH in chloroplasts. 3. Ammonium uptake as a measure of ΔpH in chloroplasts and sub-chloroplast particles. *Eur. J. Biochem.* **25**:71-84, 1972.
5. **Pick U & McCarty R.** Measurement of membrane ΔpH. *Meth. Enzymology* **69**:538-47, 1980.
6. **Rottenberg H.** The measurement of membrane potential and ΔpH in cells, organelles, and vesicles. *Meth. Enzymology* **55**:547-69, 1979.