Quantitative determination of the increase in potassium concentration in the guard cells of tobacco leaf tissue during stomatal opening using an electron microprobe revealed that sufficient potassium accumulated to account for the increased osmotic pressure in the guard cells that causes stomatal opening in the light. [The SCI indicator that this paper has been cited over 120 times since 1969.]

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“The stomatal pores of leaves act as diffusion resistors and affect the rate of COT assimilation during photosynthesis and water loss by transpiration, a relationship previously studied using chemicals to control stomatal widths by Zelitch and Waggoner.1 By the late-1960s, it was well established that stomatal opening in the light results because of the osmotic swelling of the guard cells that surround the stomatal pore, and the predominant view was that increased soluble carbohydrate was the main solute in the guard cells responsible for stomatal opening. One of us (Zelitch, a plant biochemist who had done research on stomatal physiology) had seen a 1967 paper by Fujino2 indicating there was more potassium in the guard cells of leaf epidermal strips containing open versus closed stomata, but cytochemical staining methods were used and quantitative data were lacking.

“Meanwhile, Sawhney (the senior author and a soil scientist) had developed a convenient technique for investigating the diffusion of cations from small mica particles during chemical and biological weathering by using an electron probe X-ray microanalyzer located in the geology department at nearby Yale University.3 Following a weekly lunch club seminar at the Station by Zelitch during which Fujino’s observations were undoubtedly mentioned, Sawhney told Zelitch about the sensitivity of the electron microprobe and indicated it might be useful for accurately determining the potassium concentration of guard cells. He asked Zelitch how large guard cells were, and Zelitch asked Sawhney how small an area the instrument could assay. Once it was determined that the dimensions were completely compatible, it was agreed that an experiment should be conducted immediately to determine whether sufficient potassium moved in and out of guard cells to explain stomatal movement.

“Zelitch carried out the experiments on tobacco leaf discs and isolated samples of epidermal tissue taken at different stages of stomatal opening and closing. Sawhney prepared the samples for analysis by the electron microprobe, standardized the system using membrane filters impregnated with known concentrations of KCl solution, and carried out the analyses. Within several weeks the experiments and analyses were completed and were promptly written up for publication.

“The guard cells accumulated about 0.3 M potassium during opening, and during closing a similar efflux of potassium was observed. These quantitative results demonstrated for the first time by direct measurement that sufficient potassium movement took place to account for stomatal opening and closing. The results were confirmed with tissue of Vicia faba by Humble and Raschke using similar methods, and the importance of potassium transport in stomatal movement was thus clearly established. The article may also have been highly cited because it was one of the first publications demonstrating the use of the electron microprobe technique for quantitative elemental analysis in plant tissues. The paper resulted because of a fortuitous scientific collaboration at the right time between scientists of different disciplines.”