Influence of Previous Temperature on Stomatal Response to Osmotica

It has been reported by ZELITCH[1] and by DRAKE and SALISBURY[2] that the stomata of leaves that have been growing at higher temperature open wider in the light than do those of leaves grown at lower temperature when compared at a standard assay temperature. The latter authors also demonstrated that the phenomenon has survival value to the plant, in that leaves with wide-open stomata remain cooler on a hot day and those with more nearly closed stomata remain warmer on a cool day. The purpose of this note is to extend these data by demonstrating that whether pre-treatment with high temperature causes greater or lesser opening depends on the water potential of the solution outside the cells.

Methods. Assays for stomatal opening in the light were carried out essentially as developed by ZELITCH[3]. At mid-morning, leaves of 30 cm length or greater were cut from greenhouse-grown *Nicotiana tabacum* cv. Havana seed and placed in the dark at room temperature for 30 min. 12-mm discs were then cut from the lamina between the major veins and pairs were floated in the light in 100 ml beakers containing 40 ml aliquots of KCl, NaCl, or fucose solutions of various strengths. The light was provided by quartz-iodide lamps at an intensity of about 4×10^5 erg cm^{-2} sec^{-1} at the upper surface of the leaf and was reflected by mirrors below the beakers so that the intensity coming to the under (wet) surface of the leaf from below was about 2×10^5 erg cm^{-2} sec^{-1}. A rapidly circulating water bath maintained the temperature of the solutions in the beakers at 30 °C; preliminary experiments yielded similar though slightly less dramatic results at 22 and 26 °C. At the end of 90 min, the under surface of each disc was replicated and measured according to the method of SAMPSON[4] and of ZELITCH[3].

Results. In the course of a year's work, it became apparent that the opening response varied with the season. Representative experiments are shown in Figure 1. During the autumn and winter, opening increased with increased KCl concentration, never falling off except after treatment with concentrations of 0.1 *M* KCl or greater. In the spring and summer, on the other hand, opening of stomata on discs floated in low concentrations of KCl decreased in comparison with the opening of stomata floated on deionized water. In the plot from 27 April 1967, the effect was readily measurable at 10^{-5} *M* KCl, and reached a minimum in the range between 10^{-4} and 10^{-3} *M* KCl. However, the slopes and the position of the minimum varied considerably from experiment to experiment; similarly, there was variability in the rising plots of the autumn and winter experiments. The apertures of stomata in the water controls was yet another inconstant feature of the experiments.

Because the plants had been grown under supplementary light to provide a standard 16 h daylength, it seemed likely that the seasonal shift in temperature was the factor responsible for the observed differences. Therefore, tobacco seedlings with leaves about 3 cm long were divided into 2 lots and placed in Sherer Model CEL 257 HL growth chambers with identical illumination (mixed incandescent and fluorescent sources, almost 10^5 erg cm^{-2} sec^{-1} at plant level) and daylength (16 h) but with differing temperatures of 18 and 28 °C. It was checked with a thermocouple that leaf temperatures did not deviate as much as a degree from air temperature; such deviations were caused by leaves shading one another from the circulating stream of air and from the light. The plants in the 2 chambers of course grew at different rates, and had dramatically different internode length and leaf shape; leaves from the warm chamber had rounded tips and those from the cool chamber had pointed tips. When 20 cm leaves were available from both chambers, stomatal behavior was assayed.

Figure 2 confirms that discs cut from plants grown at 18 °C respond much like those from plants grown in the greenhouse during the autumn and winter, and

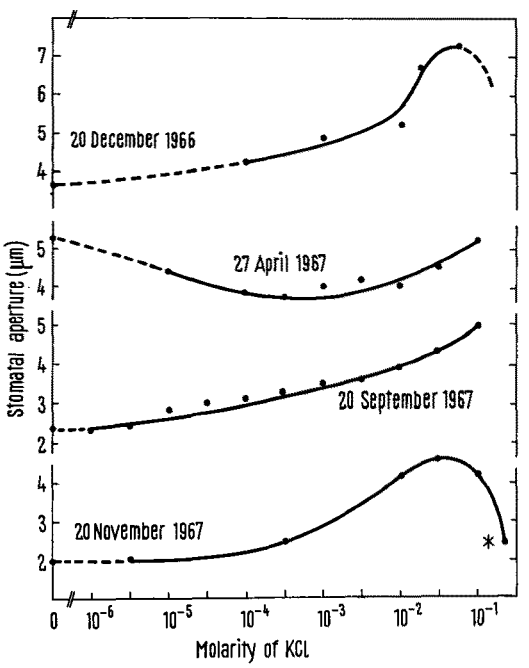

Fig. 1. Plots representing seasonal variation of stomatal opening in the light as a function of the concentration of the KCl solution on which the leaf tissue was floated. Asterisk indicates that tissue was wilted.

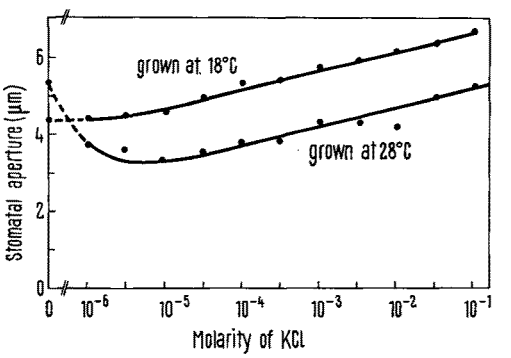

Fig. 2. Plots of stomatal aperture in the light at 30 °C as a function of the concentration of the KCl solution on which the leaf tissue was floated; the upper curve represents stomata from plants grown at 18 °C, and the lower represents stomata from plants grown at 28 °C. Each point averaged from 2 identical experiments performed on consecutive days.

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plants grown at 28 °C respond much like those grown during the spring and summer. It was consistently found that stomatal aperture was wider in the water control for discs taken from the warm chamber, in accord with the observations of ZELITCH[1] and of DRAKE and SALISBURY[2]. However, the presence of even a micromolar concentration of salt in the bathing solution caused the stomata from the warm chamber to open less than those from the cool chamber, an effect that was observed for all higher salt concentrations as well.

In order to check whether the effect was specific for the salt or due to lowering of the water potential, osmotically comparable concentrations of KCl, NaCl, and fucose were compared. The results (Figure 3) show that the effect is osmotic for all concentrations below 2×10^{-2} eq. l^{-1}. Above this concentration (at least in the experiment of Figure 3) the effect of K^+ is partially specific,

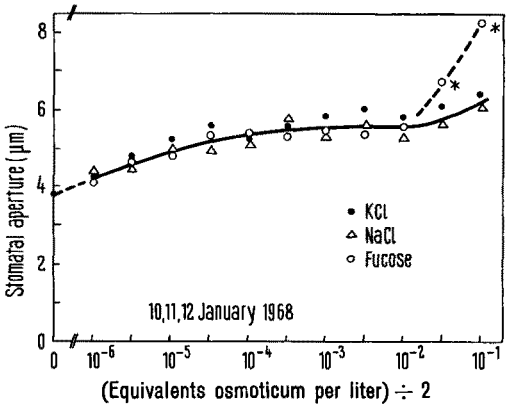

Fig. 3. Comparison of influence of KCl, NaCl, and fucose in the standard assay for stomatal opening. Each point averaged from 3 identical experiments with greenhouse-grown plants. Asterisks indicate wilting of leaf tissue, evidenced only by wrinkles in the replica of epidermal cell surfaces for the first point but for the second point by flaccidity of the leaf disc when it was removed from solution.

as is not surprising in light of the studies of IMAMURA[5], YAMASHITA[6], FISCHER[7,8] and others[9–13].

These experiments suggest that the dependence of stomatal opening on previous temperature described by DRAKE and SALISBURY[2] may be strongly modified by any other variables which influence the water potential of the extracellular solution. If this be true, the adaptive consequences of the temperature effect may be more complex than originally envisioned.

Zusammenfassung. Die Spaltöffnungsweiten in der Epidermis von Blattscheiben aus *Nicotiana tabacum* werden durch osmotisch wirksame Substanzen unterschiedlich beeinflusst, wenn die Pflanzen vorgängig bei verschiedenen Temperaturen aufwachsen. Bei 28 °C sind die Spaltöffnungen der Blattstücke in osmotisch wirksamen Lösungen weniger geöffnet als in reinem Wasser, während sich bei einer Temperatur von 18 °C die Spaltöffnungsweiten entgegengesetzt verhalten.

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A New Actinomycin-Like Antibiotic Produced by a Mutant Strain of *Streptomyces indicus*

Since the discovery of *Streptomyces antibioticus* in 1941[1], at least a dozen[2] different *Streptomyces* spp. have been reported for actinomycin-producing cultures. Here we report a pigmented compound, antibiotic MT-10, closely related to actinomycin group of antibiotics. It was isolated from a morphological mutant, strain No. MT-10[3], which was obtained through UV-irradiation of *Streptomyces indicus* CHAKRABARTY[4] (ATCC 25397), a newly described species. It differs from the pink coloured wild type, having yellowish mycellium and spores, and also is capable of diffusing a yellow pigment into the culture media. It also shows antimicrobial activity against bacteria as well as plant and human pathogenic fungi.

The active material was produced in Pridham and Gottlieb's medium (modified) containing $(NH_4)_2SO_4$, 2.64 g/l; KH_2PO_4, 2.38 g/l; K_2HPO_4, 5.65 g/l; $MgSO_4$, 7 H_2O, 1.0 g/l; maltose 20.0 g/l, and pH was adjusted to 7.0 before sterilization. The substance was produced in stationary flask cultures at 28 °C after 10 days of incubation. The antibiotic was extracted by ether and purified over alumina column chromatography using ether as the eluting solvent. The active material was obtained as orange yellow crystals from methanol by drying in vacuo.

The homogeneity of the active material was established by paper chromatography and TLC. The compound is readily soluble in ether, acetone, methanol, ethanol, butanol, chloroform, benzene, ethyl acetate, methyl ethyl ketone and acetic acid, but only slightly soluble in carbon tetrachloride and ethylene glycol. It is insoluble in water and petroleum ether. It is decomposed at 204°. Specific rotation is −205–215° (C = 0.25% in ethanol). UV-absorption spectrum shows maxima at 420 and 442 nm. The IR-absorption spectrum shows maxima

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