The Bursting Response of Isolated *Avena* Coleoptile Protoplasts to Indol-3-ylacetic Acid

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Protoplasts were isolated from dark-grown *Avena* coleoptiles (var. Victory) by using a modification of the method of Power & Cocking (1970). Subapical peeled pieces of coleoptile (1 cm long) were incubated initially in 20% (w/v) sucrose for 20 min to induce plasmolysis. Subsequently to release protoplasts the peeled pieces were incubated at 24°C for 2 h, in the dark, in 20% (w/v) sucrose containing 3% (w/v) of cellulase (Onozuka P3000) and 1% (w/v) of Macerozyme. Each subapical piece was then washed successively in 15% (w/v) sucrose (two changes, each 5 min), 12% (w/v) sucrose (5 min) and 11% (w/v) sucrose, and then it was split into two halves lengthwise; one half was placed in 11% (w/v) sucrose (the control) and the other half was placed in 11% (w/v) sucrose containing a suitable molarity of indol-3-ylacetic acid. Protoplasts were readily released by gentle teasing of the tissue pieces and any bursting response of the isolated protoplasts could be readily observed microscopically.

At 0.01 mM-indol-3-ylacetic acid the isolated coleoptile protoplasts rapidly burst; after 5 min

there was 100% bursting of these auxin-treated protoplasts and in the control there was only 2% bursting of protoplasts. At 0.1 mM-indol-3-ylacetic acid bursting was slightly less rapid, but after 20 min there was again 100% bursting of auxin-treated protoplasts and in the control 3% bursting of protoplasts. At the less physiological concentration of 1.0 mM-indol-3-ylacetic acid there was 94% bursting of protoplasts after 30 min and in the control 3% bursting of protoplasts.

These results clearly indicate that isolated *Avena* coleoptile protoplasts do respond rapidly to added indol-3-ylacetic acid in a manner largely comparable with the response of root and leaf protoplasts isolated from other species (Gregory & Cocking, 1966). The results also suggest that, contrary to the results of earlier work (Ruesink & Thimann, 1965), a direct action of indol-3-ylacetic acid on the protoplast of cells rather than a restricted action on the cell wall may contribute significantly to the overall growth response of *Avena* coleoptiles to auxins.

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DEMONSTRATION**A Continuous Sampler for Use in Studying Digestive Processes in Large Animals**

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