Physiological Evidence for Auxin-induced Hydrogen-ion Secretion and the Epidermal Paradox

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Summary. Peeled Avena coleoptile sections will respond to auxin only if the molarity of the incubation buffer at pH 6.2 is less than 5 mM. This inhibition of auxin-induced growth is not due to toxicity or to a reduction of turgor below the critical value needed for extension but rather appears to be related simply to buffering capacity. These data therefore serve as physiological evidence that H⁺-secretion is an integral part of auxin-induced cell wall loosening. Other data obtained utilizing peeled plant sections and epidermal strips suggest that the epidermis does not directly control cell extension growth. A model is proposed to explain the curvature response in split-segments tests in terms of a H⁺ gradient across the section. As far as tested this model appears to be an alternative to an older concept which implied that the curvature phenomenon in split sections was mediated by special properties of the epidermal layer. Our results suggest that the curvature response may be more directly attributable to the presence of the cuticle.

Introduction

It has recently been shown that indole-3-acetic acid (IAA) can rapidly induce hydrogen ion secretion in Avena coleoptile segments (Cleland, 1973; Rayle, 1973). Since the magnitude of auxin-induced H⁺ secretion is sufficient to initiate considerable cell extension growth it appears likely that auxin-induced cell wall loosening is mediated, at least in part, via H⁺ secretion (Hager et al., 1971; Rayle and Cleland, 1972; Rayle, 1973). In this paper certain physiological predictions based on the secretion theory of auxin action were tested.

Material and Methods

Plant Material. Seedlings of Avena sativa L., cv. Victory, or Secale cereale L., cv. Petkuser Kurzstroh-Roggen, were grown in plastic trays containing vermiculite in a dark cabinet at 22°C. After 5–6 days the coleoptiles had reached a length of 25–35 mm and 10-mm sections were harvested for the growth assays. Seedlings of

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**Pisum sativum** L. cv. Alaska were grown under the same conditions. When the seedlings had reached a stage where the third internode was 40–50 mm in length, sections were cut for straight growth tests or split section assays. In those experiments utilizing peeled sections the epidermis and cuticle were physically removed using fine forceps (Rayle, 1973).

**Straight-growth Assays and Split-Section Tests.** Two methods were used to monitor cell extension growth. For long-term experiments a standard straight-growth assay was used (Method A). With *Avena*, groups of 10 sections (initially 10 mm in length) were placed in 25-ml flasks containing 10 ml of the test solution. After incubation (usually 4–6 h) in a shaker water bath (26°C), the sections were measured with a fine millimeter ruler or were shadowgraphed and the image subsequently measured. With *Pisum*, 10-mm sections were harvested immediately below the apical hook from seedlings at the 3rd-internode stage. For each treatment 10 sections were incubated in 25 ml of the appropriate solution for 4 h in a shaking water bath at 30°C. Extension was determined by measuring shadowgraphic images obtained before and after incubation in the test solution. Harvesting of all segments as well as the straight-growth assays were performed under room light. In some cases continuous growth records were obtained (Method B) by using a recording device similar to that described by De la Fuente and Leopold (1970) and Durand (1973).

The extension of epidermal strips was monitored by method B, but modifications were made so that single strips could be fixed within clamps and an external load (1–10 g) applied (see Rayle and Cleland, 1972; Durand, 1973). Epidermal strips were obtained by physically peeling with fine forceps the epidermis from segments of the species to be tested. The size of these strips varied but we tried to obtain strips which were not torn and which were 1.5–3 mm in width and 15–20 mm in length. After clamping into the extension-measuring apparatus, the length of strip capable of extension was approximately 10 mm. Various methods were used to ensure that the epidermal strips did not dry out during isolation or clamping. For example, in many trials physical removal of the epidermis and clamping was accomplished completely under water and during the time involved in transferring the strips to the extension apparatus (1–2 min) the strips were sprayed with the appropriate solution. Isolation of the epidermal strips and all manipulations prior to the growth assay itself were conducted under room light. The assay was performed under green light.

Split-section tests were performed using segments from the third internode of *Pisum*. Sections 40–50 mm in length were harvested, the hooks removed, and the sections peeled (if desired). Beginning at the apical end the segments were then slit down the middle for a distance of approximately 35 mm, using a razor blade. The split sections (usually 5–10) were placed in large dishes containing 50 ml of the appropriate solution. During the incubation period the dishes were put on a shaker under room light. The temperature of the room was 22–23°C. After the desired incubation period representative segments were removed and photographs taken.

**Results**

The most obvious physiological prediction based on the H⁺-secretion theory of auxin action is that auxin-induced growth should be prevented when sections are floated on buffer solutions of sufficient strength to prevent acidification of the cell wall region. As can be seen in Fig. 1, *Avena* sections from which the cuticle and epidermis have been removed