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Auxin Does Not Alter the Permeability of Pea Segments to Tritium-labeled Water[1]

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ABSTRACT

The possibility of an auxin effect on the permeability of pea (*Pisum sativum* L. cv. Alaska) segments to tritium-labeled water has been investigated by three separate laboratories, and the combined results are presented. We were unable to obtain any indication of a rapid effect of indoleacetic acid on the efflux of ^{3}HHO when pea segments previously "loaded" for 90 minutes with ^{3}HHO were transferred to unlabeled aqueous medium with indoleacetic acid. We were able to confirm that segments pretreated with ^{3}HHO plus indoleacetic acid for 60 to 90 minutes can show an enhanced ^{3}HHO release as compared with minus indoleacetic acid controls. However, this phenomenon appears to be due to an increased uptake of ^{3}HHO during the prolonged indoleacetic acid pretreatment, and therefore we conclude that auxin does not alter the permeability of pea segments to ^{3}HHO in either short term or long term tests. We confirm previous reports that the uptake of ^{3}HHO in pea segments proceeds largely through the cut surfaces, and that the cuticle is a potent barrier to ^{3}HHO flux.

The process of cell enlargement in response to auxin requires water uptake (14), and in some of the earlier work water uptake was regarded as the primary process influenced by auxin (3, 19). Reports have appeared indicating that auxin has a positive (2, 18, 20), a negative (4, 9), or no effect (11) on permeability to, or exchange of, isotopically labeled water. Kang and Burg (8) recently reported detecting a promotion by IAA of ^{3}HHO exchange by pea stem segments within a few min after exposure to the hormone. In view of its rapidity, the reported effect has attracted our interest in relation to the currently favored concept that IAA may exert its primary action at the plasma membrane (1, 5–8, 10, 15–17, 21). One should note that there is no reason to think that an effect on permeability to water could cause cell enlargement, but the effect would nevertheless be of great interest if it indicated an interaction of IAA with the plasma membrane.

In this paper we report the inability of three separate laboratories to obtain the rapid effect of IAA on exchange of ^{3}HHO reported by Kang and Burg.

MATERIALS AND METHODS

Plant Material. Seeds of *Pisum sativum* L. cv. Alaska were grown in vermiculite in the dark. Source of seeds, and conditions of growth and handling of pea seedlings in the different laboratories represented in this report were as follows: San Diego, seeds from W. A. Burpee Seed Co., grown at 22 C without humidification, with occasional exposure to dim green light, stem segments cut and handled under normal laboratory light (fluorescent lamps); Stanford, seeds from Ferry Morse Seed Co., grown at 25 C without humidification (typical relative humidity about 50%), with exposure to dim red (600 to 700 nm) light every other day and during cutting and handling of the stem segments (except in insert to Fig. 2); Bochum, seeds from Vaughan Seed Co., S. Katrine, Illinois, grown at 22 C without exposure to light, segments prepared and handled in room light. From seedlings 7 to 8 days old, segments 10 mm (San Diego, Bochum) or 8 mm (Stanford) long were cut from the third internode beginning directly below the plumular hook.

For experiments involving abrasion of the cuticle (Fig. 6) the third internode was gently stroked lengthwise, using the thumb and forefinger, with a paste consisting of 1 part of No. 305 emery (Edmund Scientific Co., Barrington, N. J.) suspended in 1 part of water. Each internode was stroked once on opposite sides, and a second time on opposite sides after rotating the internode 90°, than washed thoroughly with water to remove emery prior to excision of a stem segment. The tissue was not injured by this treatment as indicated by a normal elongation response to IAA and normal cytoplasmic streaming in its epidermal cells.

^{3}HHO Uptake Measurements. For experiments at Stanford and San Diego stem segments were placed in dishes containing 5 ml of solution containing 5 mM K-phosphate buffer (pH 7.0), 2% sucrose, 5 μM $CoCl_2$, 0.5 μc/ml ^{3}HHO (Schwarz/Mann), with or without IAA. Initial experiments at Bochum made use of this medium, but later experiments were performed using only 5 mM K-phosphate buffer pH 6.5, with or without IAA. No differences were observed in Bochum with either method.

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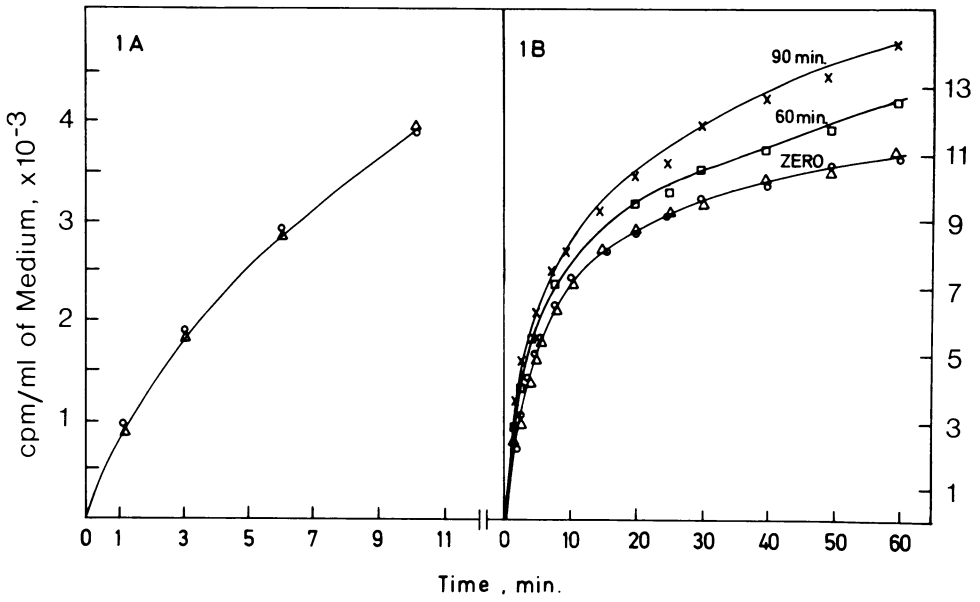

FIG. 1. Effect of IAA on release of ^{3}HHO from Alaska pea stem segments as measured in San Diego, Calif. (A) and Bochum, Germany (B). Segments were "loaded" with ^{3}HHO for 90 min prior to transfer to unlabeled medium for measurement of efflux. A: 10 μM IAA (△) or H_2O (○) added at the time efflux measurements were begun; B: 10 μM IAA added at the time efflux measurements were begun (△) or as a pretreatment (□, ×) added for the times indicated on the graph; minus IAA control (○). A and B are the averages of two and seven separate experiments, respectively.

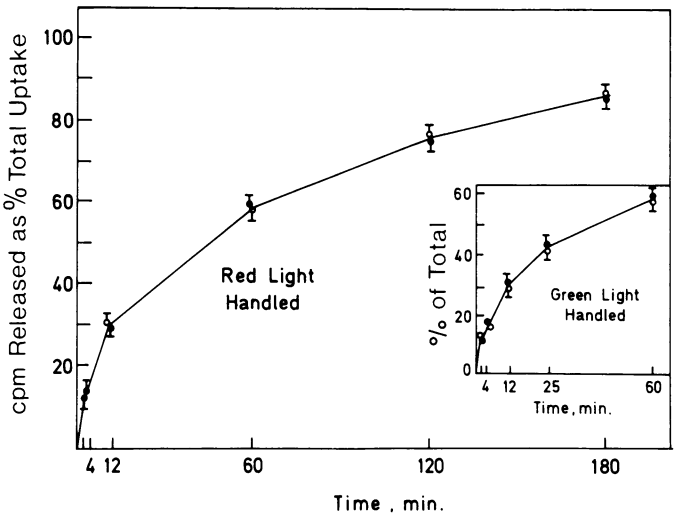

FIG. 2. Effect of 10 μM IAA on efflux of ^{3}HHO from Alaska pea stem segments as measured in Stanford, Calif. IAA (●) added 90 min prior to efflux measurement, *i.e.* at the time of exposure to the ^{3}HHO media; minus IAA control (○). Segments were exposed occasionally to red light. Inset, plants and segments exposed only to dim green (546 nm) light during growth and handling. Bars show the range for triplicate tissue samples. Efflux from each sample expressed as per cent of the total ^{3}HHO taken up by that sample during the pretreatment with ^{3}HHO.

The dishes were agitated gently at 22 C (San Diego, Bochum) or 25 C (Stanford) on a reciprocating shaker. At various times, groups of at least six segments were removed, quickly blotted on tissue paper, and transferred to scintillation vials containing 5 ml of scintillation fluid. The ^{3}HHO in the tissue was allowed to equilibrate with the scintillant overnight before determining ^{3}H using a liquid scintillation spectrometer.

^{3}HHO Efflux Measurements. Stem segments were "loaded" with ^{3}HHO for 90 min under the same conditions described above for uptake measurements. If an auxin pretreatment was desired auxin was included for the appropriate time in the uptake medium. After ^{3}HHO uptake, 10 segments per treatment were removed, quickly blotted, then transferred to 10 ml of a similar buffer solution lacking ^{3}HHO, and placed on the shaker. At the indicated times, 0.2 ml of the medium was removed and transferred to scintillant for counting. At least duplicate aliquots were taken in all cases for each experimental point.

RESULTS AND DISCUSSION

We were unable to obtain any indication of a rapid effect of IAA on efflux of ^{3}HHO when pea segments that had previously been allowed to take up ^{3}HHO for 90 min in the absence of IAA were blotted and transferred to unlabeled aqueous medium with IAA (Fig. 1, A and B). Kang and Burg (8) reported that IAA increased efflux by about 10 to 15%. The half-time for ^{3}HHO efflux, which for tissue segments of a given geometry should be inversely proportional to ^{3}HHO permeability (13), did not differ, in our experiments, between IAA-treated and control tissue, even if tissue was pretreated with IAA for 90 min during the loading period (Fig. 2). According to Kang and Burg a 90-min pretreatment gave a maximum effect of IAA (about 30% reduction of half-time).

Our inability to confirm the IAA effect on ^{3}HHO exchange was not due to the red light treatment used at Stanford rather than to dim green light conditions employed by Kang and Burg (8), because peas raised in the dark with exposure only to very dim 546 nm light during watering and during preparation and handling of segments gave essentially identical results (inset, Fig. 2). Similarly, segments handled under a dim green safelight at San Diego gave results no different from segments exposed to room light during the experiments.

The finding of Kang and Burg that segments pretreated with ^{3}HHO in the presence of IAA for 60 to 90 min can show an enhanced ^{3}HHO efflux as compared with minus-IAA controls was confirmed (Figs. 1B and 3). However, as shown in Figure 3 this may be entirely due to an increased previous uptake of ^{3}HHO by the tissue during IAA pretreatment. The increase

in efflux of ^{3}HHO from IAA-pretreated tissue at any time in the efflux experiment was in close proportion to the increase in total ^{3}HHO uptake caused by IAA during pretreatment, and despite the difference in uptake the half-time for ^{3}HHO exchange did not differ between IAA-treated and control tissue (Fig. 3), thus affording no indication of a real difference in permeability induced by IAA. One should note that an IAA effect on efflux is not seen in Figure 2 because the data are normalized for differences in uptake.

Tissue treated with IAA during preincubation with ^{3}HHO for 90 min took up about 15 to 20% more ^{3}HHO than minus-IAA controls, whereas IAA-treated pea segments increased only 5 to 7% in fresh weight over controls during the preincubation period. If the segments come to equilibrium with the external ^{3}HHO in 90 min as previously implied (8), one would expect only a 5 to 7% difference in ^{3}HHO content. However, the data in Figure 4 indicate that even by 150 min the entire tissue of a pea internode segment does not come to equilibrium with a ^{3}HHO solution. The plus/minus IAA difference in ^{3}HHO uptake at 90 min is greater than the difference in fresh weight. Presumably this is so because promoted uptake of water during IAA-induced elongation leads to an increase in the amount of ^{3}HHO uptake during the time prior to attainment of ^{3}HHO equilibrium between tissue and medium. In agreement with this explanation, sections that are prevented by 0.25 M mannitol from expanding do not show any increase in ^{3}HHO uptake when treated with IAA (Fig. 5).

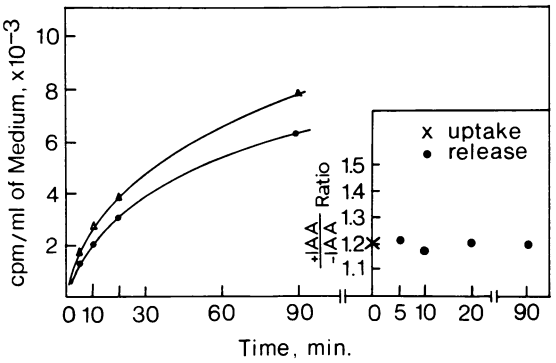

FIG. 3. Efflux of ^{3}HHO from IAA-pretreated pea segments as measured in San Diego, Calif. Segments were "loaded" with ^{3}HHO for 90 min without (○) or with (△) 10 μM IAA. At the end of this pretreatment, 10 segments were removed from each lot for determination of ^{3}HHO uptake, the ratio between plus and minus IAA samples being shown as × in the inset. Closed circles in the insert show the plus-minus IAA ratio for ^{3}HHO release at each time point during the efflux measurement.

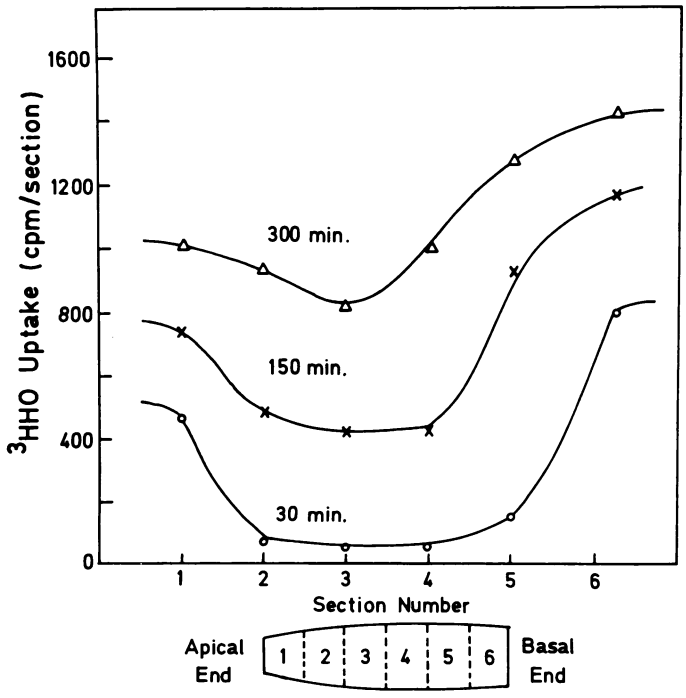

FIG. 4. Distribution of ^{3}HHO within pea segments during uptake from minus IAA media containing ^{3}HHO. At the indicated times two 15-mm segments were removed, blotted, and cut transversely into six pieces as indicated, and counted.

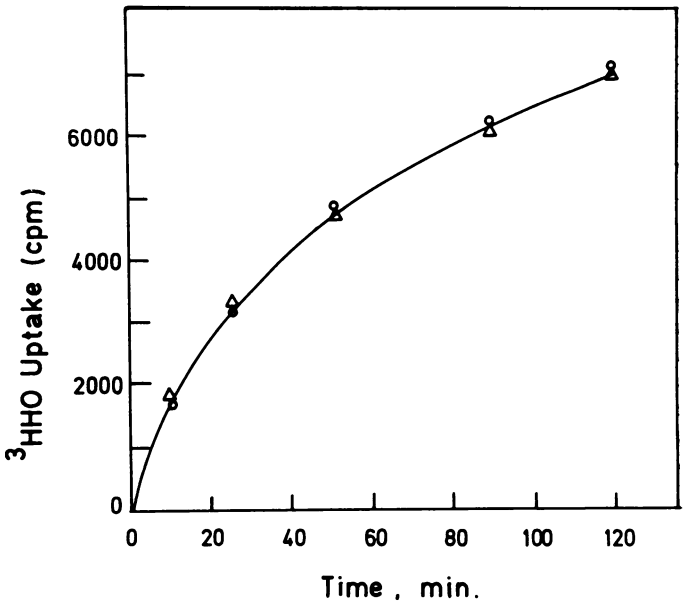

FIG. 5. Uptake of ^{3}HHO in the presence of 0.25 M mannitol with (△) or without (○) 10 μM IAA.

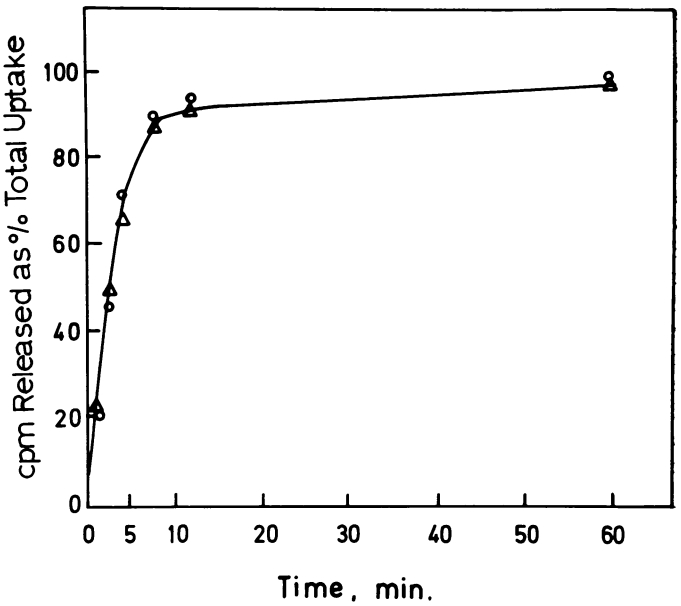

FIG. 6. Release of ^{3}HHO from pea segments with abraded cuticle. Segments were equilibrated for 90 min with ^{3}HHO medium without (○) or with (△) 10 μM IAA then transferred to unlabeled medium for efflux measurement. Peas grown at Stanford, Calif.; compare with Fig. 2.

The data in Figure 4 indicate, as suggested previously (8), that pea internode segments exchange ^{3}HHO mainly via the cut ends and that lateral penetration is largely prevented by the cuticle. This can be clearly shown by abrading the cuticle with emery powder. This "scrubbing" technique was introduced by P. B. Green and R. Cummins (personal communication) to improve osmotic exchange between coleoptile segments and ambient media.

Abrasion of the cuticle has a dramatic effect on the time course of ^{3}HHO efflux from pea stem segments, reducing the half-time for exchange from about 40 min to 2.5 min, for peas grown under the conditions used at Stanford (Fig. 6 compared with Fig. 2); such tissue exchanges ^{3}HHO almost as rapidly as do root segments (8, 12). However, abraded pea segments in which cuticular resistance presumably has been minimized still showed no promotive effect of IAA on ^{3}HHO efflux (Fig. 6) that would indicate a positive effect on permeability to water. Such segments respond to IAA by elongating just as well as do unabraded pea segments.

The kinetics of ^{3}HHO exchange shown in Figures 2, 3, and 5 differ drastically from those found by Kang and Burg (8), who reported half-times of 8 to 12 min, as compared with greater than 30 min for unabraded pea segments in the present data. The long half-times were obtained with peas grown at Stanford and San Diego, whereas a half-time as short as 5 min was obtained in similar ^{3}HHO exchange experiments using peas grown at Bochum (Fig. 1B). All of us were using peas of nominally the same variety. Apparently the differences in growth conditions between the different laboratories result in pea seedlings with considerably different cuticular permeability characteristics (Fig. 6).

An obvious possibility is that pea seedlings grown in less humid conditions, perhaps like those of the California laboratories, develop a thicker, less permeable cuticle. To check this we grew at Stanford a crop of peas under saturated humidity. Stem segments from these plants showed, however, ^{3}HHO exchange characteristics not appreciably different from those of Figure 2. There must be another explanation for the differences between ^{3}HHO exchange characteristics of stem segments from peas grown in different laboratories. Nevertheless, even the peas grown at Bochum, for which the half-time for ^{3}HHO exchange was even shorter than that found by Kang and Burg (8), did not show any indication of an effect of IAA on permeability to ^{3}HHO.

One possible reason for Kang and Burg's IAA effect might be that the cuticle of their peas had properties that caused it to "crack" and increase in permeability to ^{3}HHO when elongation was induced by treatment with IAA. It is also possible that artifactual effects of ^{3}HHO exchange could result from abrasion of the cuticle during handling of pea segments. We checked this possibility by comparing the ^{3}HHO exchange of unscrubbed segments (from peas grown at Stanford) which were transferred from one Petri dish to another using either (*a*) ordinary thumb-depressing forceps with coarse, corrugated, gripping faces, or (*b*) butterfly forceps with smooth, gripping faces and very gentle action. The latter gave a half-time of 37 min for ^{3}HHO efflux while the former showed a half-time of 34 min.

We do not know the true explanation of Kang and Burg's observation of an apparent IAA effect on ^{3}HHO permeability of pea segments, but it seems clear from our inability to obtain the effect, in any of the three laboratories represented in this report, that Kang and Burg's effect must have depended upon special circumstances of their experiments and is probably not of substantial significance in relation to the mechanism of auxin action. We conclude further that their effect was more likely due to changes in permeability of the cuticle than of cellular membranes.

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