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Regulation of Root Growth by Auxin-Ethylene Interaction[1]

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ABSTRACT

A large portion of indoleacetic acid (IAA)-induced inhibition of excised root tips and virtually all such inhibition of intact roots are the result of IAA-dependent ethylene production. Under certain conditions an additional effect of IAA accounts for a small portion of the inhibition of excised root tips. Ethylene production in response to applied IAA is governed by the level of applied auxin found inside the root. Evidence is presented to confirm the participation of ethylene in the geotropic response of roots.

Application of IAA to roots invariably causes an inhibition of elongation (3). We have presented evidence (18) that auxin-mediated ethylene production accounts for the inhibition in pea roots, just as it does in pea buds (16) and etiolated pea stems (11). This paper presents additional data supporting the idea that ethylene is the normal intermediate in auxin-mediated root growth inhibition, both in intact plants and in excised root sections, and also that the gas plays a role in root geotropism.

MATERIALS AND METHODS

Seeds of *Pisum sativum* (var. Alaska) were germinated as previously described (18), and all manipulations subsequent to planting were carried out in dim green light to avoid phototrophic and photomorphogenic involvement.

Studies on Tissue Sections. When the roots were 2 to 3 cm long, usually 48 hr after planting, apical 5-mm segments were removed with a two-bladed cutter. Ten of these apices and 2, 5, or 10 ml of medium containing 2% sucrose (w/v), 5 μM $CoCl_2$, 5 mM potassium phosphate buffer (pH 6.8), and an appropriate concentration of IAA were sealed in a 125-ml Erlenmeyer flask by means of a vaccine cap and gently shaken (68 cpm through a 4-cm stroke) in the dark for a predetermined number of hours. In a few cases when very small amounts of evolved ethylene had to be measured, 25- or 50-ml micro-Fernbach flasks were used. At the end of an incubation the ethylene production was determined by gas chromatography (11), and the roots were weighed to the nearest 0.1 mg and measured to the nearest 0.05 mm with an optical stage micrometer and a 10× stereomicroscope. In some experiments ethylene or CO_2 was added initially by injecting a measured amount of gas into each flask with a syringe; in the case of a large volume of CO_2 this operation was preceded by removal of an equal volume of air.

Studies on Intact Roots. Presoaked seeds were planted in 1-quart clay pots filled with moist vermiculite. When the roots had attained a length of 1 cm (usually about 30 hr), the pots were immersed in a solution containing an appropriate concentration of IAA for 3 to 5 min, drained briefly, and placed in a 6-liter desiccator in the presence or absence of applied ethylene. The initial length and weight of the radicals were determined from a sample taken before immersion treatment, and final values after 9 or 18 hr. Ethylene production was measured under similar conditions using tissue grown in 300-ml glass bell jars filled with moist vermiculite and treated with IAA by the technique described above.

Geotropic Studies. Presoaked seeds were planted in moist vermiculite in plastic bins (25 x 6 x 6 cm) and allowed to germinate. When the roots had attained a length of about 2.5 cm, the bins were covered with perforated plastic tops and sealed in 6-liter desiccators, the roots remaining vertical throughout. The pressure in each desiccator was reduced to about 380 mm Hg with a water aspirator, an appropriate amount of CO_2 or C_2H_4 was injected, and then air was admitted until atmospheric pressure was again attained. After a 10-min equilibration period the desiccators were carefully reoriented so that the roots were rotated 90° into a horizontal plane and thus exposed to a maximal geotropic stimulus for the duration of the experiment. At appropriate times the bins were removed from the desiccators, the roots were excised from the seed and placed on glass slides, and the slides were shadowgraphed. The angles of curvature were determined from the resultant prints with a protractor.

Studies with Carboxyl-labeled ^{14}C-IAA. Tracer studies were carried out with carboxyl-labeled ^{14}C-IAA (8 or 33 mc/mmole) obtained from New England Nuclear Corp. and Calbiochem Corp., respectively. The isotopes were chromatographed on paper by the technique described below and found to be in excess of 99% pure. Isolated root tips were incubated with standard media supplemented with ^{14}C-IAA, and at appropriate intervals flasks were removed from the shaker and 10 ml of the gas phase were withdrawn for determination of $^{14}CO_2$ in a Cary-Tolbert ionization chamber by the rate of charge method (23). The solution in the flask was filtered through glass wool, and the radioactivity remaining in solution was determined by gas flow counting (20% efficiency) of 0.1 ml on a planchet. Under these conditions self-absorption was neglibible. The tissue was rinsed with ice-cold distilled water for 1 min, dropped into 95% ethanol, and left overnight at 23°. Two subsequent 24-hr extractions at 23° removed at least 98% of the counts from the tissue; those remaining were determined by crushing the extracted sections, spreading them on planchets (three per planchet), drying the tissue and counting it. The alcohol fractions were combined, evaporated under vacuum to approximately 0.2 ml, and spotted on Whatman No. 20 paper. Subsequently, 0.4 mg of unlabeled IAA or indoleacetyl aspartate was applied to the same spot. Descending chromatography was carried out with a 50:40:10 mixture of chloroform:ethyl acetate:formic acid (25), after which the strips

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were scanned with a Vanguard strip scanner (efficiency 8.0%) and sprayed with a 0.04% solution of bromcresol green (in ethanol) to locate the authentic IAA spot. This spot along with appropriate controls was excised, eluted, and counted on a planchet by the gas flow method. In some cases the spots corresponding to indoleacetyl aspartate and suspected indoleacetyl glucose were also eluted and counted, but in general their activity was sufficient to be determined with the paper scanner.

RESULTS AND DISCUSSION

Evidence That IAA-induced Ethylene Is Responsible for Growth Inhibition Caused by IAA Applied to Isolated Root Tips. Early experiments indicated that a change in the volume of solution in which a given concentration of IAA was applied could alter the duration and magnitude of the growth response. Studies below were carried out using three different solution volumes: 2, 5, and 10 ml. The results obtained with a solution volume of 5 ml have been published (18) and are essentially similar to those obtained with 2 ml.

Applied ethylene gas at an optimally effective concentration (18) prevents pea roots from responding to external IAA at all auxin levels below 10 μM (Fig. 1). At higher auxin levels a second type of inhibition independent of ethylene action occurs, but it can be duplicated by applying equivalent amounts of benzoic acid (Fig. 2). This inhibition may be due to a nonspecific acid toxicity since high concentrations of both IAA (in the presence of ethylene) and benzoic acid cause equal reductions in weight and

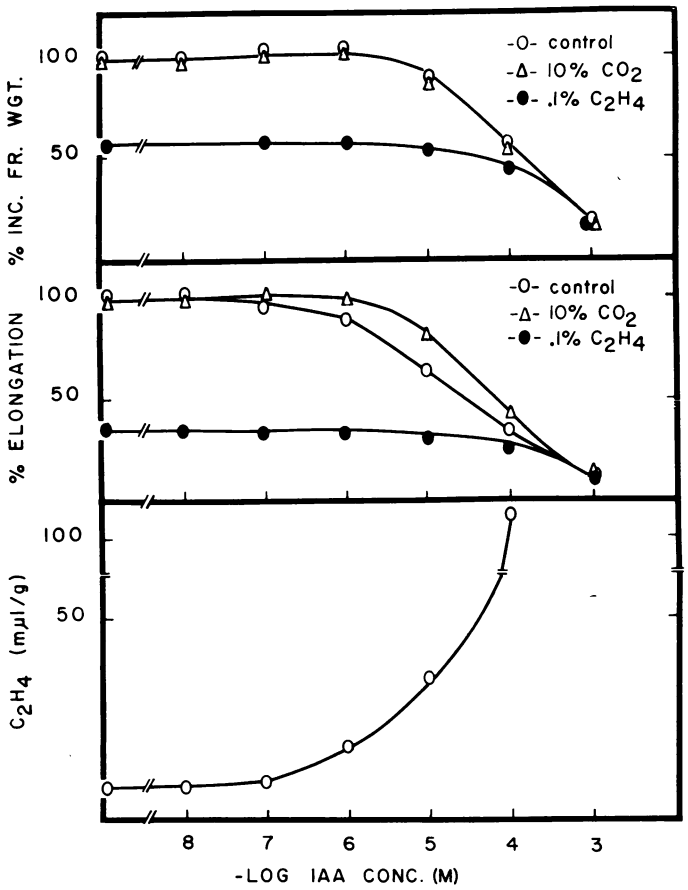

FIG. 1. Effects of IAA, CO_2, and C_2H_4 on excised pea roots during a 24-hr growth period in a 2-ml solution volume. Growth expressed as percentage of net growth in the absence of added IAA, CO_2, or C_2H_4. Similar inhibitions, reversals by CO_2, and ethylene productions were obtained using a 5-ml solution volume (18).

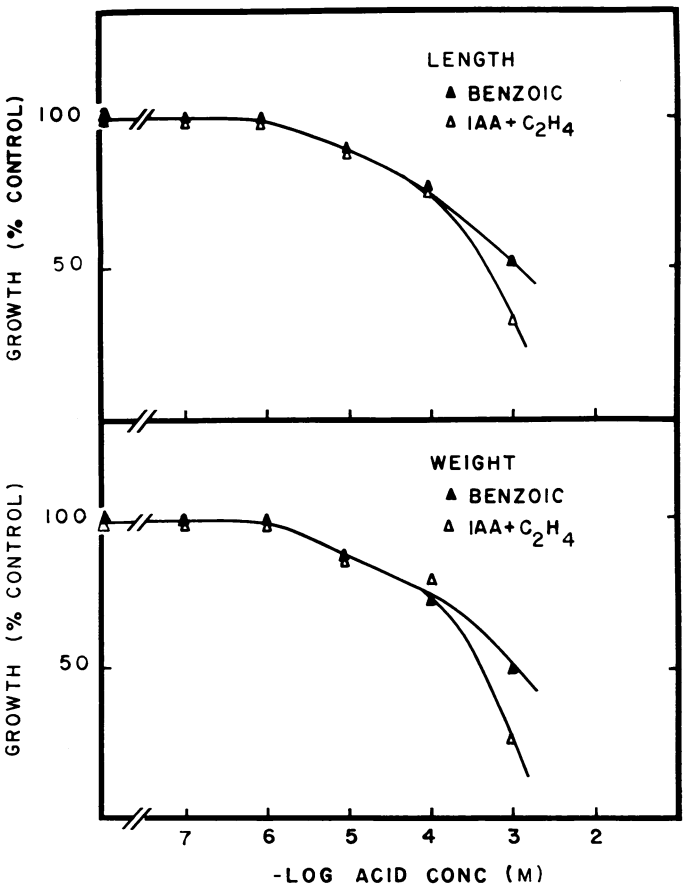

FIG. 2. A comparison of inhibition caused by benzoic acid with that caused by IAA in the presence of 0.1% ethylene. In the latter case control growth was taken to be growth in the presence of 0.1% ethylene.

Table I. *Effect of IAA Concentration on Weight to Length (Swelling) Ratio of Excised Pea Roots in Presence and Absence of 0.1% Ethylene*

The values recorded in this table are calculated from data presented in Figure 1.

−log IAA Concentration	Ratio of Increase in Weight to Increased Length	
	$-C_2H_4$	$+C_2H_4$
0	1.00	1.65
7	1.07	1.65
6	1.13	1.62
5	1.35	1.64
4	1.65	1.66

length (Fig. 2) in contrast to the swelling induced by ethylene alone (Reference 18 and Table I).

IAA also can cause swelling when it induces ethylene formation, but it is evident that IAA *per se* is not responsible for swelling because it produces no such response in tissue treated with ethylene (Table I). Thus when pea roots are treated with IAA in the absence of applied ethylene, the ratio between increase in weight and increase in length approaches but does not exceed the value obtained in the presence of added ethylene. Since IAA does not cause swelling and ethylene does, the gas must be responsible for this effect and therefore for the inhibition of elongation which accompanies it.

Kinetics of IAA and Ethylene-induced Inhibition and Recovery,

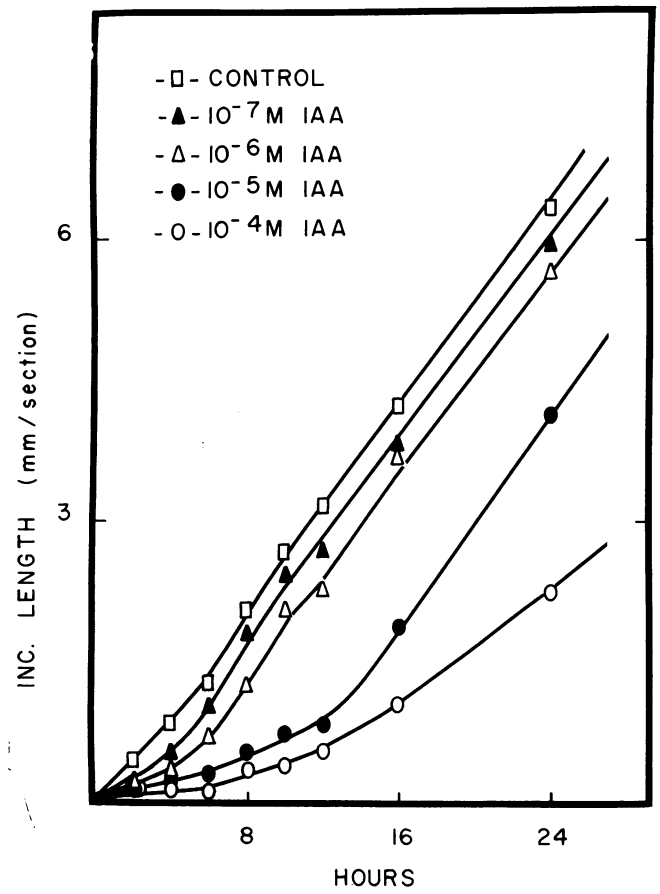

FIG. 3. Time course for the elongation of excised pea roots at various auxin concentrations. Solution volume was 2 ml. Each point represents average value from at least six experiments. Fresh weight increases were similar.

and Ethylene Production. If ethylene evolved after auxin application accounts for auxin-induced growth inhibition in roots, the degree of growth inhibition should be closely correlated at all times with the rate of ethylene evolution. Immediately after 2 ml of IAA solution are applied, growth slows (Fig. 3), and the rate of ethylene production is elevated (Fig. 4) for a period of time which is a function of the IAA concentration. Moreover, during the period in which ethylene is being produced, the absolute rate of growth is correlated with the rate of ethylene production. A precise comparison between rates of growth and ethylene production can be made if the internal concentration of ethylene resulting from an observed production rate is determined. The internal concentration can be converted to a rate of growth since the inhibition resulting from application of the gas externally is known (Reference 18 and Fig. 5B) and occurs quickly (Fig. 6). Direct determination of the internal concentration of ethylene at any given production rate is not feasible, but data obtained from studies on the pea stem (16) indicate that a production rate of 6 $m\mu l \cdot g^{-1} \cdot hr^{-1}$ is equivalent to applying 1 μl/liter ethylene, and a similar relationship has been calculated from data obtained with several other vegetative tissues (9, 10, 12). That this value is applicable to pea roots is indicated by the fact that the tissue acts as though it has been treated with 2 μl/liter ethylene (*i.e.*, it becomes insensitive to applied ethylene) when it is stimulated to produce the gas at an average rate of 5 to 10 $m\mu l \cdot^{-1} \cdot hr^{-1}$. This occurs when slightly in excess of 10^{-5} M IAA is applied (Fig. 1). The conversion factor, 6 mμl/g $\times$ hr, can now be used to calculate (from Fig. 4) internal ethylene concentrations at various times and applied IAA concentrations. Then by reference to Figure 5B, the growth which would result from the application of a like concentration of the gas externally can be determined for each IAA concentration and time. A plot of this theoretical growth based upon ethylene production rate gives rise to a family of curves which represent the growth at each IAA concentration, assuming ethylene to be the sole inhibitory agent. These curves correlate closely with the actual growth curves (Fig. 3) except at very high auxin levels (10 and 100 μM) where nonspecific acid inhibition might be expected in view of the results obtained with comparable concentrations of benzoic acid. If a correction is made for this "acid" inhibition using the percentage inhibition obtained with benzoic acid, the correlation between the curves is improved. Another way of expressing the relationship between growth and ethylene evolution is to plot growth rate (from Fig. 3) *vs.* ethylene production rate (from Fig. 4), using data at all IAA concentrations and elapsed times. The results (Fig. 5A) are in excellent agreement with the assumption that auxin-induced growth inhibition is due to the ethylene produced, for the resultant points can be superimposed upon a curve obtained by plotting growth rate *vs.* concentration of applied ethylene (Fig. 5B).

When auxin is applied, a high rate of ethylene evolution and a substantial inhibition of growth occur within the 1st hr (Figs. 3 and 4). Applied ethylene also causes a marked inhibition of growth within the 1st hr (Fig. 6), which increases progressively and is capable of accounting for a large portion of the inhibition caused by all IAA concentrations (compare Figs. 3 and 6), and

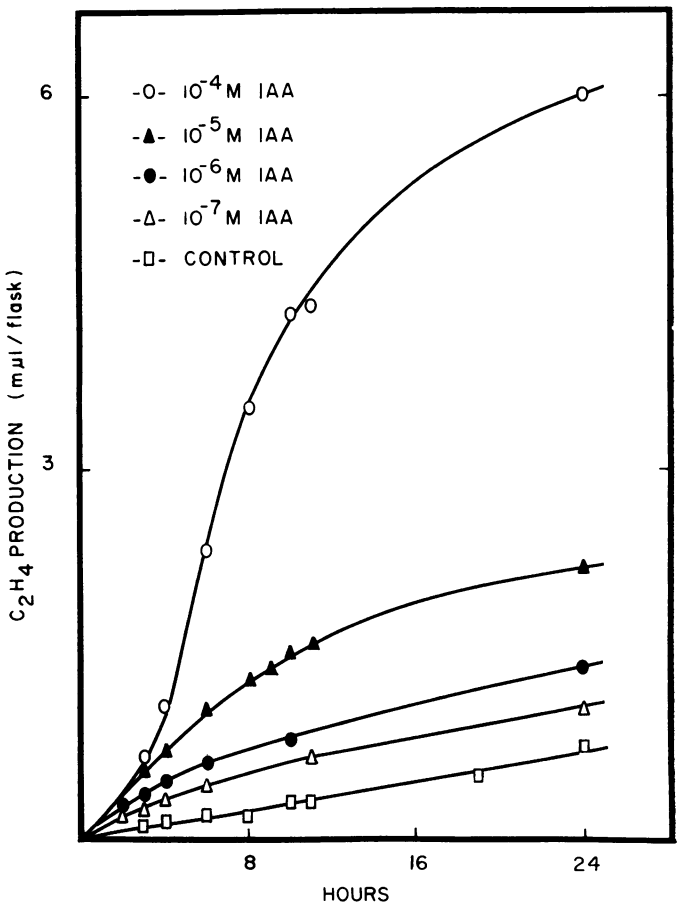

FIG. 4. Time course showing effect of various IAA concentrations on ethylene production. Solution volume was 2 ml. Data for early points at 1-hr intervals were omitted for clarity but are adequately represented by the course of the appropriate curve.

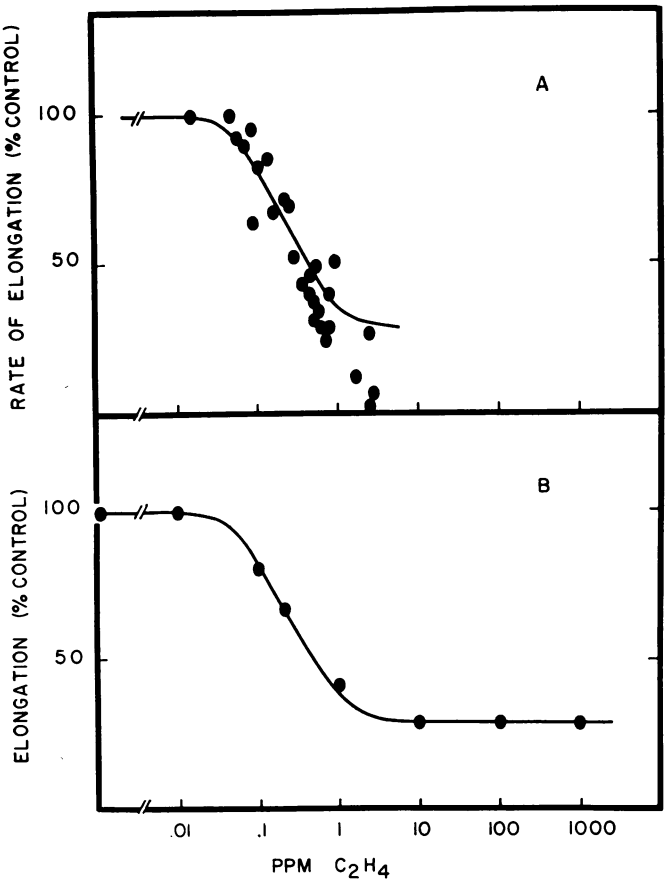

FIG. 5. A: Growth rate at all times and IAA concentrations as a function of internal ethylene concentration (determined from rate of ethylene production by the conversion factor 6 $m\mu l \cdot g^{-1}$ = 1 ppm). The continuous line is identical to that in Figure 10B and is drawn for comparison. B: Growth as a function of applied ethylene concentration in a 24-hr pea root straight growth test.

the entire inhibition at concentrations of IAA less than 10 μM. Similar results were obtained by Andreae *et al.* (5) except that they failed to detect a substantial inhibition due to applied ethylene during the 1st hr and worked mainly with larger solution volumes and higher IAA concentrations. It is clear, however, that a small portion of the inhibition which results after IAA application, especially at high concentrations, is not due to endogenous ethylene. Instead, it may result from one of several other changes caused by IAA: (*a*) induction or activation of the enzyme forming ethylene and of other enzymes (4, 19), (*b*) depletion of substrates such as aspartate by IAA aspartase (4) or methionine by ethylene production (17), or (*c*) production of inhibitory products such as oxindoles (24) by IAA oxidase or IAA conjugates by other enzymes.

Contrary to a report that "the inhibition of pea root growth by ethylene is largely or completely irreversible" (5), it was found that this inhibition, like most other ethylene effects (15), is largely reversible over a long period of time (Fig. 6). Only after 16 hr does the tissue fail to recover completely from ethylene treatment, and this may be due to depletion of endogenous auxin (4) or a slowly developing, irreversible effect upon polar auxin transport such as occurs in stem tissue (13). Since ethylene synthesis does not persist at elevated rates for 16 hr even after application of large volumes of IAA (Fig. 7), except possibly at concentrations in excess of 100 μM, the failure of gassed roots to recover completely after 16 hr might explain why only roots treated with very high IAA concentrations are unable to recover at that time (8), whereas roots treated with lower concentrations of IAA recover completely when the auxin is removed.

The inhibitory phase of the growth-response curve of pea roots is basically similar to that previously reported for pea stem sections (11). In pea stem sections, however, application of ethylene leads to growth inhibition only after a lag period of 3 hr, and consequently exogenous auxin, even though it immediately stimulates ethylene formation, does not inhibit growth for about 3 hr. In pea roots, on the contrary, both auxin and ethylene cause a substantial inhibition within an hour. Another difference in the behavior of root and stem sections arises because of the fact that stem sections inevitably cease to grow after 10 ro 12 hr of incubation, whereas root sections grow at a linear rate for at least 24 hr. Consequently, although auxin-induced ethylene production ceases in both tissues after comparable periods of time (Reference 11 and Figs. 3 and 4), no recovery phase is observed in stem tissue when this happens because growth has already ceased. As a result, in 18- to 24-hr experiments with stem sections, the effect of IAA-induced ethylene is exerted throughout the growth period, whereas under similar conditions in roots the tissue ceases producing ethylene and recovers from its effects during the latter part of an experiment. This explains why stem sections treated with a high enough level of IAA display no obvious response to applied ethylene even though it is present after they cease producing the gas. On the other hand, under similar conditions roots should regain their ability to respond to applied ethylene when they cease producing the gas, and this in part explains why the curves (with

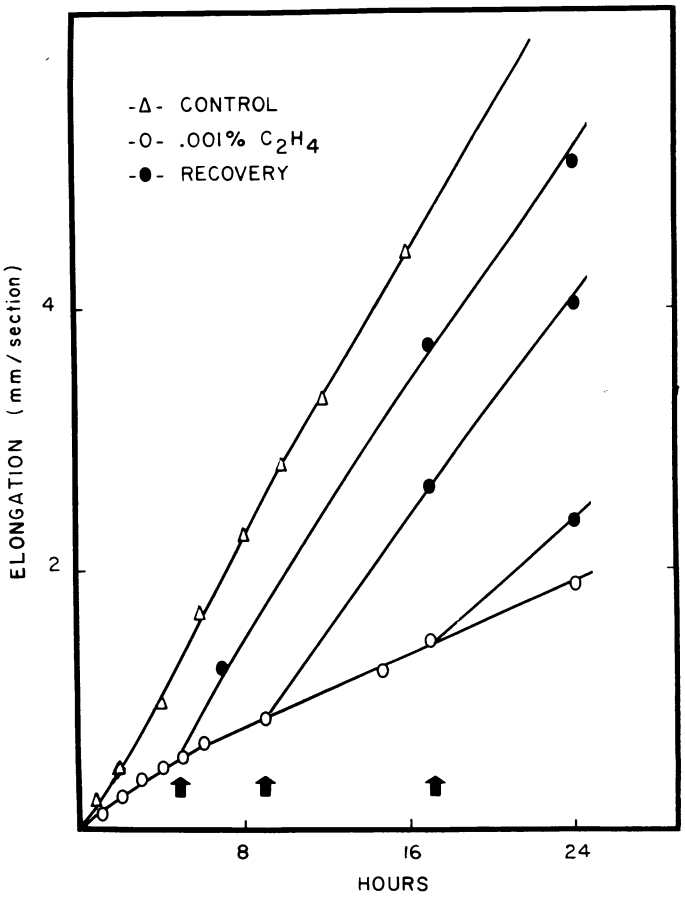

FIG. 6. Time course showing inhibition and recovery of elongation growth in isolated pea roots following treatment with maximally inhibitory concentrations of ethylene. Arrows denote time of transfer to control flasks. Subsequent growth of these roots is indicated by the filled circles. Fresh weight increases were similar.

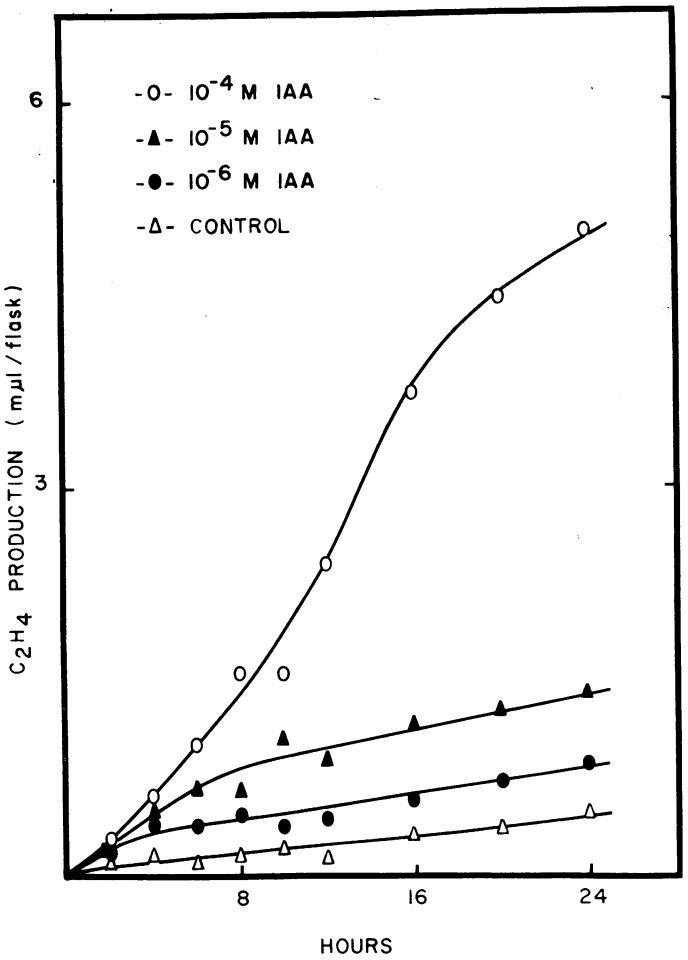

FIG. 7. Time course showing effect of various IAA concentrations on ethylene production by isolated pea roots. Solution volume was 10 ml.

and without ethylene) in Figure 1 fail to intersect in overnight experiments with roots, whereas they do intersect with stems (11, 12).

Effect of CO_2 on Growth of Isolated Root Tips. Carbon dioxide competitively antagonizes ethylene action in pea stems (15) and has the same action in pea roots (18). In the absence of applied IAA, concentrations of CO_2 in excess of 15% progressively inhibit root growth whereas 5 to 10% CO_2 very slightly accelerates it. The growth inhibition resulting from moderate concentrations of applied IAA is largely reversed by CO_2 (Fig. 1) whereas that at high IAA concentrations is not, in accord with the idea that a compound (ethylene) capable of being competitively antagonized by CO_2 mediates the IAA-induced growth inhibition. At substantial IAA levels ethylene production proceeds at so high a rate that too much gas is present to be displaced by a weakly binding competitive inhibitor such as CO_2, and no reversal is observed.

Relationship between ethylene production and internal auxin concentration. Using either 2 or 10 ml of solution, the greatest production of ethylene occurs within the first 4 to 6 hr, and thereafter, within 16 hr, even at the highest IAA levels the production diminishes (Figs. 4 and 7). Because the evolution of ethylene does not continue for longer periods even when the volume of IAA solution applied externally is increased 500%, the diminution cannot be attributed to depletion of the external auxin via destruction, uptake, or conjugation. Instead, it would appear that ethylene production is at all times controlled by the internal IAA concentration (Fig. 8), for regardless of the external IAA concentration (Fig. 9) the production of ethylene approaches a control value when the internal auxin content reaches 1 or 2×10^{-8} meq/g (compare Figs. 4 and 8). The formation of IAA aspartate is believed to be responsible for reducing the internal auxin level in pea roots and other tissue (4, 25) and the observed rates of conjugation of IAA with aspartate and other compounds (possibly glucose or inositol [22]) are adequate to account for the rapid initial and subsequent slower decrease in internal auxin content at each applied IAA concentration (Fig. 10). The loss of auxin from the external solution is mainly dependent upon another enzyme, IAA oxidase (19), and as a result, more than 50% of the radioactivity which disappears from a solution of carboxyl-labeled ^{14}C-IAA is recovered as $^{14}CO_2$ (Fig. 11). Both the loss of IAA from the medium and production of $^{14}CO_2$ cease by 10 to 14 hr, at which time 6 to 8% of the counts still remain in the external solution at each applied concentration of IAA. The bulk of this residual labeled material is not in the acidic indole fraction but instead is chromatographically identified with a conjugate with an R_F close to that reported for IAA-glucose (25) in this solvent system.

Abnormal Effects of Auxin in Large Solution Volumes. If a large volume of solution (10 ml or more) containing IAA is applied, auxin persists long after recovery from the ethylene production phase has been completed and a different type of inhibition manifests itself. This inhibition, which has been studied in detail by others (1, 2, 4, 5, 7) becomes more intense with time (Fig. 12)

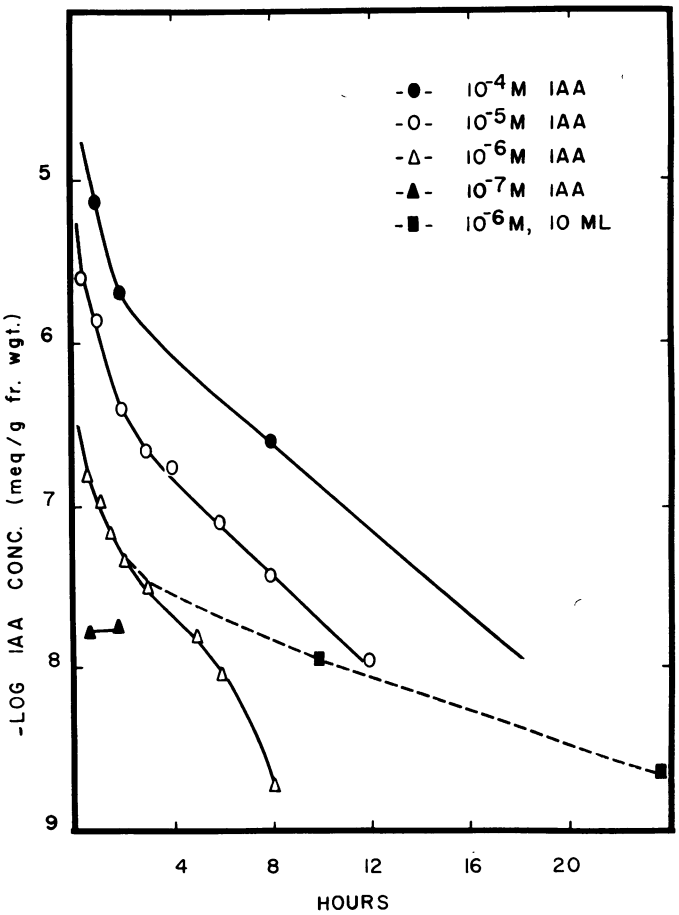

FIG. 8. Time course for decrease in internal auxin concentration following treatment with various exogenous levels of IAA. Internal concentration calculated for entire (fresh wt-dry wt) volume. If this auxin were entirely cytoplasmic, the concentrations could well be 10-fold greater in the cytoplasm. Legend on graph refers to IAA levels applied.

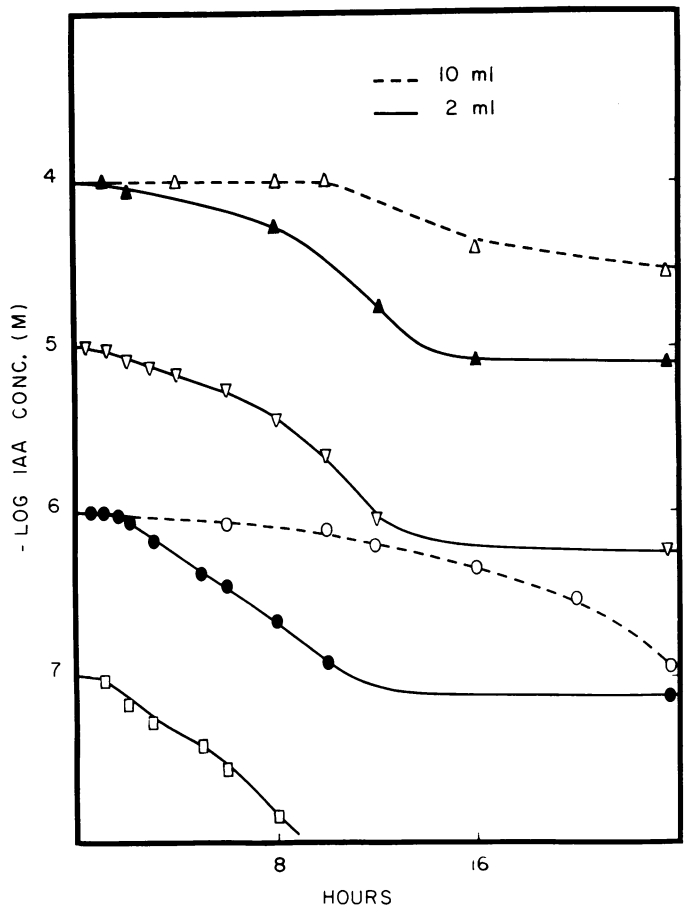

FIG. 9. Time course for decrease in counts in external solution. DPM converted to external concentrations *assuming all counts are free IAA* (see text).

even though the external and internal auxin levels are decreasing. Thus while both 2 ml of 40 μM IAA and 10 ml of 1 μM IAA inhibit growth by about 55% during an 18-hr incubation (Figs. 1, 12, and 13), the kinetics of the effects are quite different in the two cases. At the low volume an initially intense inhibition due almost entirely to ethylene is followed by a recovery phase, whereas at the high volume an initially less pronounced inhibition due largely to ethylene intensifies with time even after ethylene production ceases. The high volume effect is characterized by approximately equal reductions in weight and length (compare additional inhibition of weight and length produced in 10 ml, with total inhibition of weight and length produced in 2 ml), a loss of reversibility by CO_2, and inhibition in the presence of added ethylene even at 0.1 μM IAA (Fig. 13). Surprisingly, the inhibition of length caused by applied ethylene is slightly less at 10 ml than at lower volumes (compare Fig. 1 with Fig. 13), even though the control growth rates are comparable in both cases. However, ethylene production in response to IAA is of similar magnitude and kinetics regardless of solution volume (Figs. 4 and 7), and the tissue responds to this ethylene by swelling.

The internal IAA level must decrease initially at the same rate regardless of the volume of solution applied since most of the decrease in internal auxin occurs before the external concentrations of IAA have become very different at 2- and 10-ml volumes (compare Figs. 8 and 9). A preliminary study at 1 μM IAA confirmed this expectation (Fig. 8). Consequently, it is not surprising that growth inhibition at 2 and 10 ml is closely similar initially; only after 8 hr when the external auxin levels begin to diverge markedly does the high volume effect become apparent (Fig. 14). The cause of the inhibition associated with high volumes of IAA solution is not known, but it should be noted that this is probably not an important mechanism of auxin action under normal conditions *in vivo* (see below).

Evidence That Auxin-induced Ethylene Is Responsible for the Entire Growth Inhibition Caused by IAA in Intact Pea Roots. The application of various concentrations of ethylene to pea seedlings results in an inhibition of root growth (Fig. 15) similar to that obtained with excised pea root tips (Fig. 5B). The roots become obviously swollen, so that in the presence of an optimal concentration of ethylene the ratio, percentage increase in weight *vs.* percentage increase in length, is about 1.58, while in control roots the ratio is 1.0. That no inhibition other than that caused by ethylene occurs when IAA is applied is indicated by the fact that in the presence of applied ethylene growth is not further hindered by any concentration of IAA up to 100 μM (Fig. 16). As is the case with applied ethylene, when auxin alone is added, length is inhibited to a greater extent than weight; the swelling ratios are 1.54, 1.51, and 1.16 at 100, 10, and 1 μM IAA, respectively (compared to 1.58 in ethylene alone). Therefore, all of the swelling and growth inhibition resulting from the application of IAA to intact pea roots can be accounted for by the ethylene produced.

Effect of CO_2 and Ethylene on Geotropism in Intact Roots. Moderately high concentrations of CO_2 (5–10%), which have no effect on the rate of pea root growth or very slightly stimulate it,

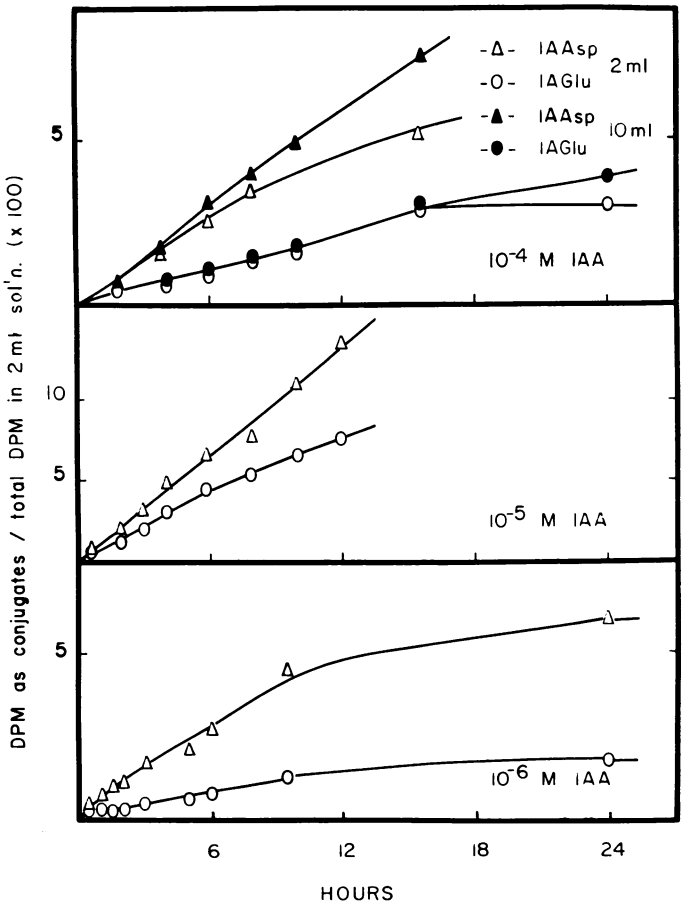

FIG. 10. Effect of various concentrations of IAA on the time course for conjugation of IAA with aspartate and glucose. Data expressed as percentage of total counts in 2 ml of solution regardless of applied volume. IAAsp: Indoleacetic aspartate; IAGlu: indoleacetic glucose.

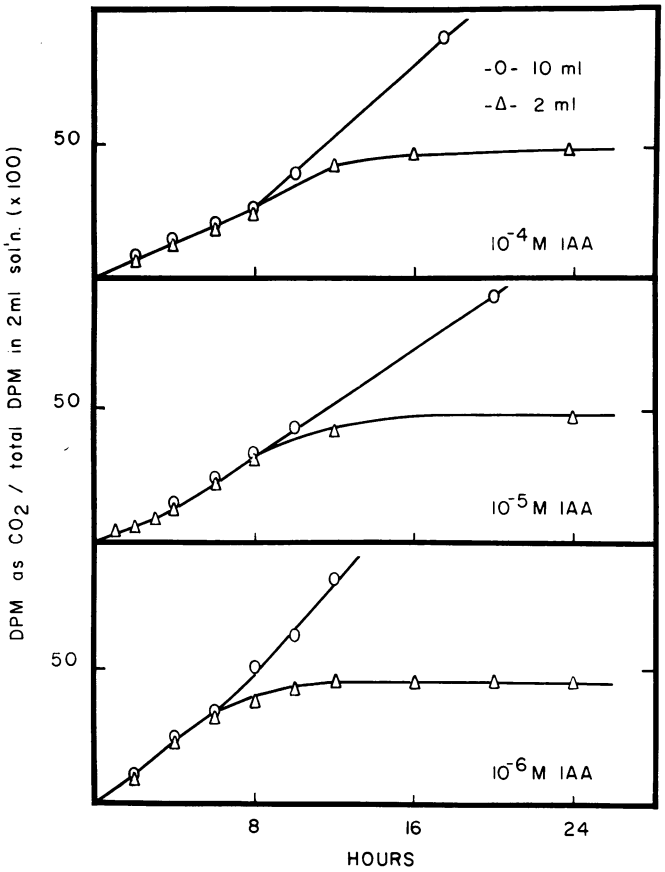

FIG. 11. Effect of various concentrations of auxin on the time course for decarboxylation of IAA. Data expressed as percentage of total counts in a 2-ml solution regardless of applied volume. Decarboxylation in 10 ml of solution continued at a linear rate for at least 24 hr.

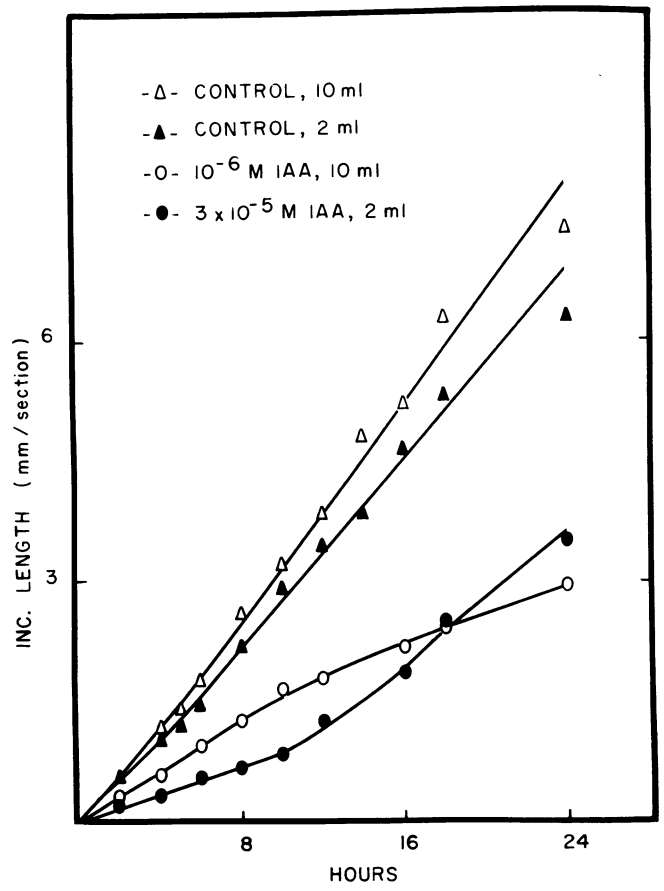

FIG. 12. Effect of solution volume on the pattern of growth inhibition in excised pea roots. The auxin concentrations were chosen to produce approximately equal inhibition at the end of 24 hr.

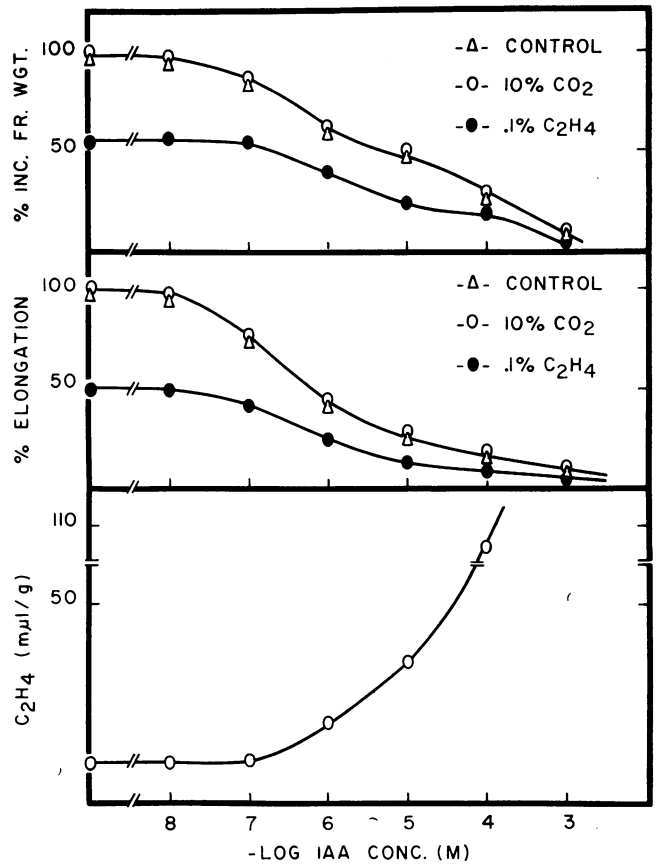

FIG. 13. Effects of IAA, CO_2, and C_2H_4 on excised pea roots during a 24-hr growth period in a 10-ml volume. Growth expressed as percentage of net growth in absence of added IAA, CO_2, or C_2H_4.

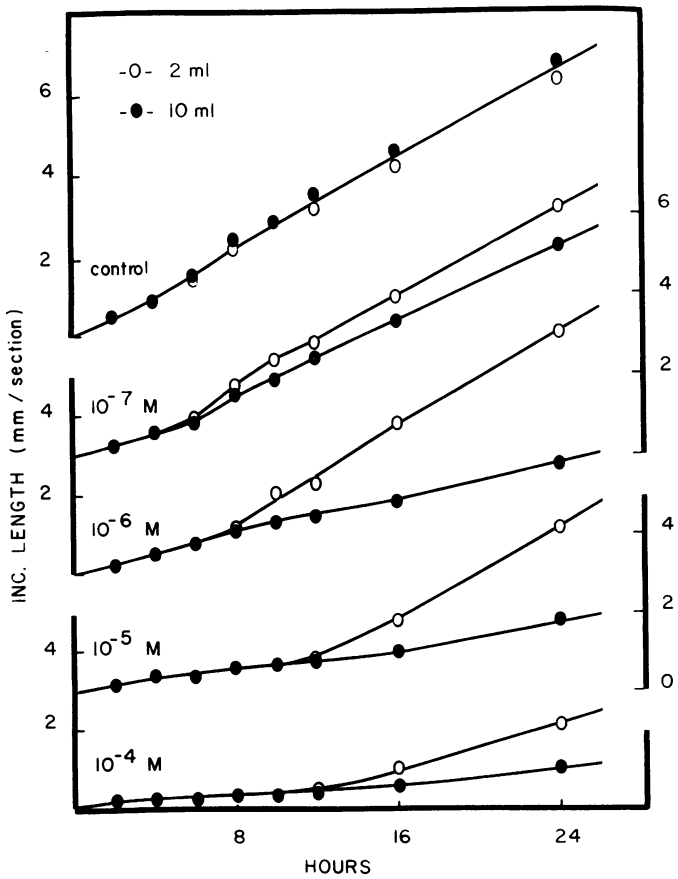

FIG. 14. Effects of various concentrations of IAA on the time course for elongation of excised pea roots in 2 and 10 ml of solution. Each point represents the average of at least six experiments. Similar results obtained for fresh weight increases.

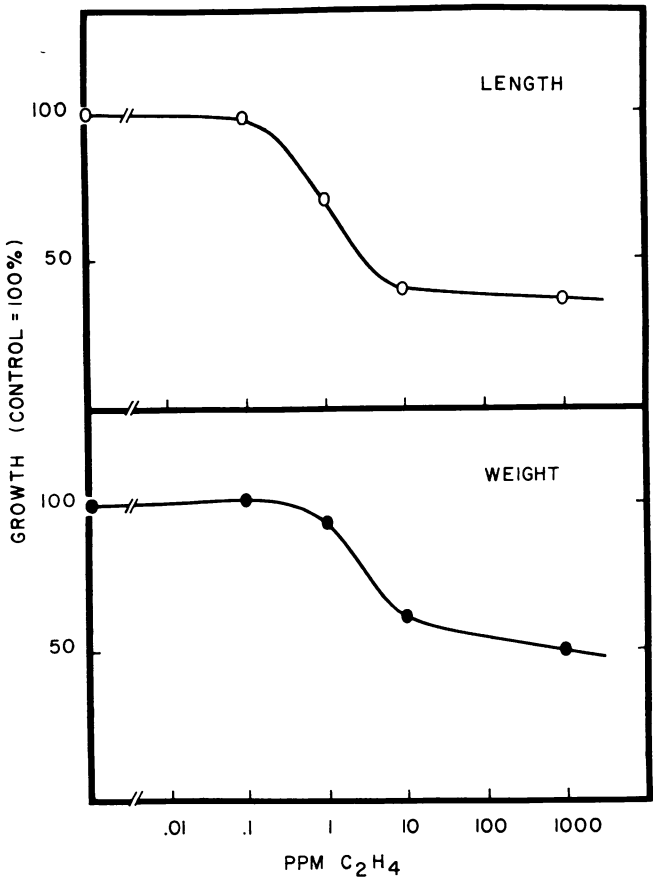

FIG. 15. Growth as a function of ethylene concentration during an 18-hr experiment with intact pea roots.

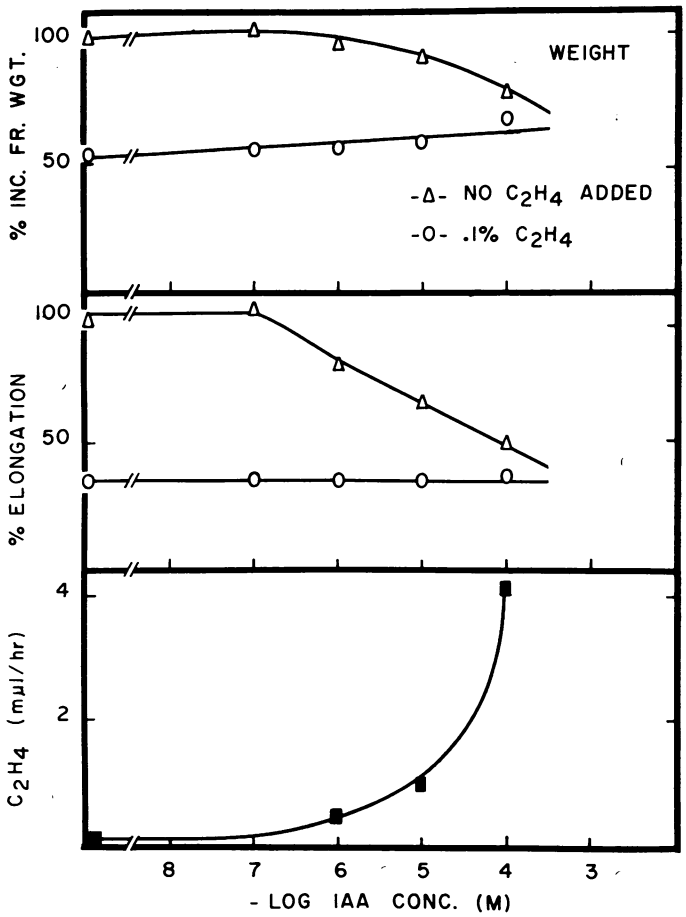

FIG. 16. Effects of IAA and ethylene on intact pea roots after 18 hr. Curves exactly paralleling these were obtained after 9 hr of growth.

markedly retard the geotropic curving of pea roots (Reference 18 and Fig. 17). Since CO_2 is a competitive inhibitor of ethylene action in roots (18) and is commonly used as a diagnostic test for the involvement of ethylene in physiological processes, these results suggest that ethylene mediates root geotropism. Applied ethylene also immediately and completely prevents development of the geotropic response in pea roots (Fig. 17), apparently by a reversible mechanism since the roots regain their geotropic sensitivity when the gas is removed. The geotropic response of stems also is reversibly inhibited by ethylene (11), and here it is known that the mechanism of the effect is a complete, reversible inhibition of lateral auxin transport. However, in roots ethylene participates in the geotropic response and immediately inhibits growth in contrast to stem sections, which do not utilize the gas for geotropic curving and only respond to it after several hours (11). Therefore, the possibility cannot be excluded that the geotropic response of roots is immediately eliminated by ethylene because both the upper and lower side respond equally and maximally to the applied gas and hence cannot discern endogenously produced ethylene gradients.

CONCLUSIONS

Auxin-induced inhibition of root growth is entirely mediated by IAA-stimulated ethylene formation both *in vivo* and in isolated root tips exposed to moderated concentrations and low volumes of auxin solution (*i.e.*, to conditions most closely approximating the *in vivo* situation). If the Cholodny-Went theory of geotropism is correct, it follows that ethylene must mediate root geotropism, whereas it should play no part in stem geotropism since only growth promotion is involved in the latter case. Experiments with CO_2 support this conclusion, for this competitive inhibitor of ethylene action interferes with root geotropism but has no effect on stem geotropism (18). The presence of distinctly swollen cells in the lower portion of the geotroping root also supports this interpretation, for radial enlargement is a common response to ethylene. Involvement of ethylene in root geotropism helps to explain several objections which have been raised against application of the Cholodny-Went theory to roots. Audus and Brownbridge (6), noting that the growth of the upper side is first enhanced and then inhibited during geotropic curving, proposed that IAA is not itself the active inhibitor; instead they suggested that it is responsible for the production of a special inhibitor which can leak from the lower side to the upper side of a geotroping root. Ethylene produced on the lower side as a result of eccentric distribution of IAA would be expected eventually to diffuse to the upper side to a certain extent, thus accounting for the slight inhibition of growth which subsequently occurs there. A second objection, that the observed 70:30 distribution of auxin in horizontally positioned stems (8, 21) and roots (8, 20) is insufficient to account for the extensive growth inhibition on the lower side of a root, can now be countered by data showing that low concentrations of applied IAA cause very extensive initial inhibitions (followed by recovery, Fig. 3) and that the distribution between the upper and lower surfaces may approach 90:10 rather than the lower values previously reported for upper and lower halves of stems (14). The kinetics of inhibition and recovery are identical in excised root tips after application of 0.1 μM IAA (Fig. 3) and in the lower half of a root following a 40-min geotropic stimulation (6). Thus a transient increase in IAA from

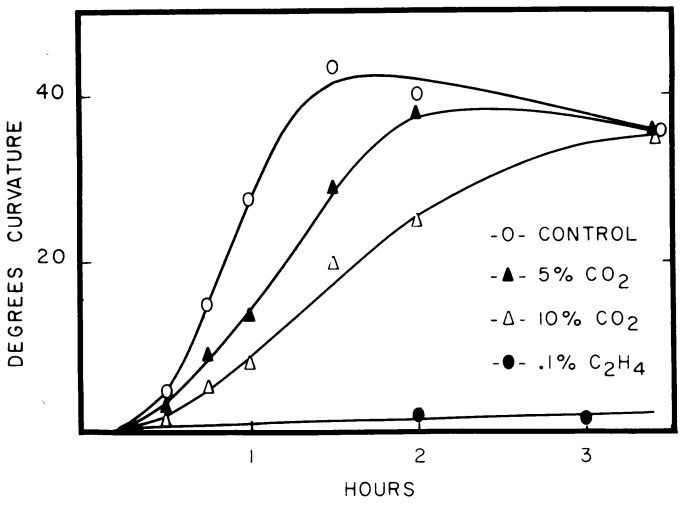

FIG. 17. Time course for curvature of intact pea roots in the presence and absence of ethylene and of various concentrations of CO_2.

a control value of 10 mμM (which does not inhibit growth) to 0.1 μM could account for the geotropic response of the pea root through auxin-induced ethylene formation.

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