Plant Physiol. (1967) 42, 415–420

An Explanation of the Inhibition of Root Growth Caused by Indole-3-Acetic Acid[1]

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Received November 29, 1966.

Summary. Low concentrations of indole-3-acetic acid inhibit the growth of pea root sections by inducing the formation of the growth regulator, ethylene gas. Ethylene is produced within 15 to 30 minutes after indole-3-acetic acid is applied and roots begin to swell immediately after they are exposed to the gas. Carbon dioxide competitively inhibits ethylene action in roots, impedes their geotropic response, and partially reinstates auxin inhibited growth. It is concluded that ethylene participates in the geotropic response of roots, but not that of stems.

Relatively low concentrations of auxin inhibit the growth of roots, and somewhat higher concentrations that of buds and most, but not all (9, 15) stems. If this inhibition of growth is comparable in nature to that of certain enzymes by high concentrations of substrate, it would be a natural consequence of 2-point attachment between auxin and its receptor (14). However, the kinetic data which gave rise to this theory have been questioned (6), and it has proven unacceptable as an explanation of auxin induced growth inhibition in etiolated pea and sunflower stem sections (9). In these tissues excess auxin induces the formation of a growth regulator, ethylene gas, which causes cellular swelling. We now present evidence that a similar mechanism accounts for auxin inhibited growth in roots. This feedback system, in which the action of auxin is modified by a hormone-induced regulator, must mediate the geotropic response of roots if, as proposed by Cholodny (11) and Went (22), geostimulated roots curve downwards when a growth inhibitory concentration of auxin accumulates in their lower side.

Materials and Methods

Seeds of *Pisum sativum* (var. Alaska) were soaked in water for 6 hours, planted in moist vermiculite, and grown in darkness at 23°. After 48 hours, when the roots were 2 to 3 cm long, the apical 5 mm was removed in dim red light. Ten sections and 5 ml of medium containing 2 % sucrose (w/v), 5 μM $CoCl_2$, 50 mM 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 in the dark for 18 hours at 23° before ethylene production was determined by gas chromatography (9). The tissue was then removed, blotted dry, weighed, and measured. In a few experiments ethylene or CO_2 was added initially by injecting a measured amount of gas into each flask with a syringe. Lima bean (var. Prizetaker), sunflower (var. Mammoth Russian), *Avena sativa* (var. Victory II), *Ricinus* (var. Red Spire), and mung bean (var. Oriental Jumbo) roots were grown and handled in the same manner as pea roots, and tomato seeds (var. Homestead 24) were germinated on wet filter paper and used after 3 days when the roots were about 1 cm long.

Curvature of intact roots was studied in 10 liter desiccators saturated with water vapor. Seeds were placed in petri dishes containing damp vermiculite and the roots aligned horizontally, their tips extending over the edge of the dishes. Various concentrations of CO_2 were introduced into the desiccators and at appropriate time intervals the roots were removed, shadowgraphed, and their angle of curvature determined with a protractor.

Results and Discussion

Control roots produce small amounts of ethylene (fig 1) but within 15 to 30 minutes following application of 0.1 mM IAA the rate is at least doubled, and thereafter it increases rapidly. The amount of

[1] This investigation was supported by research grant EF-00782 from the United States Public Health Service, Division of Environmental Engineering and Food Protection, and was carried out while S. P. Burg was the recipient of Career Research Development Award 1-K3-GM-6871. A. V. Chadwick was supported in part by Predoctorial Fellowship 1-F1-GM-33614 from the USPHS, and also by NIH Training Grant TO1 GM 649-06.

ethylene produced is controlled by the IAA concentration (fig 2, lower) and is closely related to the growth inhibition which auxin causes (fig 2, upper). Since ethylene retards root growth (fig 3), might not the gas account for the inhibitory action of auxin? To test this hypothesis root

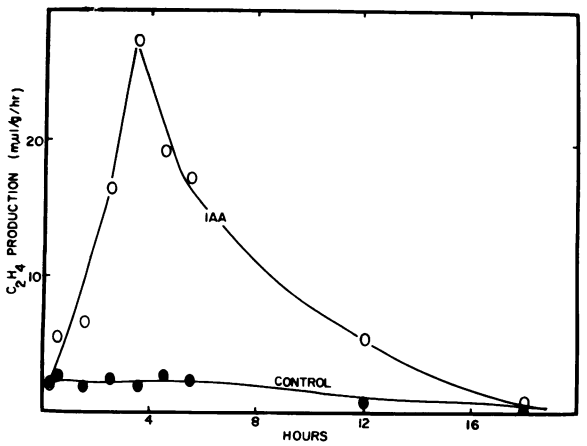

FIG. 1. Time course showing the induction of ethylene synthesis after 0.1 mM IAA was applied at the start of the experiment.

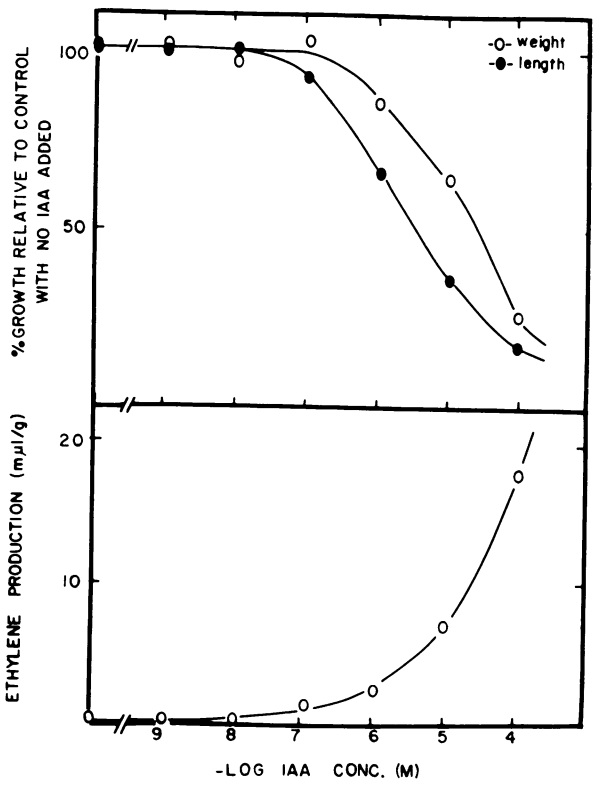

FIG. 2. Dependence of the rate of growth and ethylene formation on IAA concentration during an 18 hour pea root straight growth test. Control 5 mm roots without auxin added increased 130 % in length and 170 % in fresh weight.

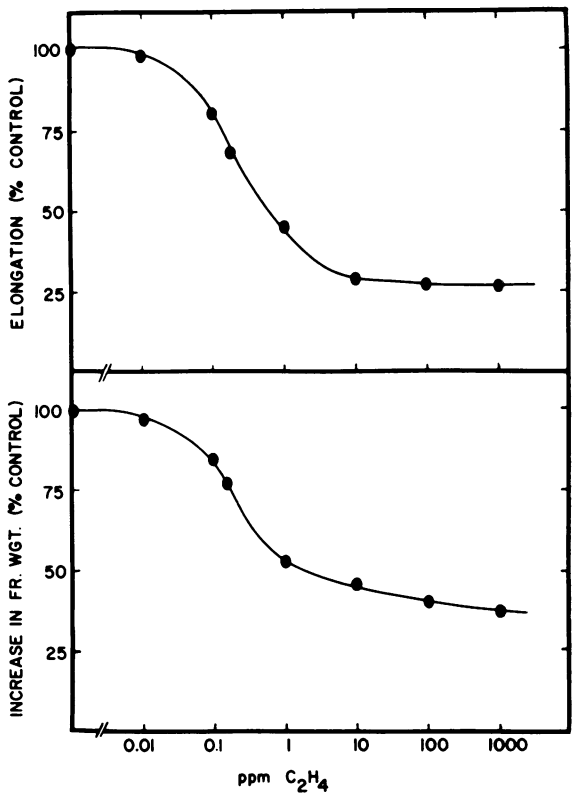

FIG. 3. Growth as a function of ethylene concentration during an 18 hour pea root straight growth test.

sections were grown in various concentrations of IAA in the presence and absence of 1000 ppm ethylene, an amount of gas causing a maximum retardation of root growth (fig 3). If ethylene is involved in the auxin induced inhibition of growth, the presence of a supraoptimal concentration of the gas should prevent the roots from responding to applied auxin. This was the case at concentrations of IAA ranging from 10 mμM to 1 μM, but higher levels still inhibited root growth in spite of the presence of exogenous ethylene (fig 4, lower curve). However, at these high concentrations the growth inhibition is of a different type than that induced by a slightly supraoptimal level of growth hormone. This is indicated by the fact that the ratio between the absolute percentage increment in weight versus the absolute percentage increment in length increases from a control value of 1.3 at auxin concentrations lower than 10 mμM to 2.3 in sections exposed to 5 μM and all higher concentrations. Thus the inhibition which occurs at concentrations between 10 mμM and 5 μM IAA involves swelling whereas that at higher levels of IAA consists of an equivalent reduction in length and weight. Andreae (3) also has shown the growth inhibition curve in pea roots to be biphasic. He reports that 1 phase starts at IAA concentrations lower than 0.1 μM and is nearly saturated by 10 μM as indicated by an inflection in the growth

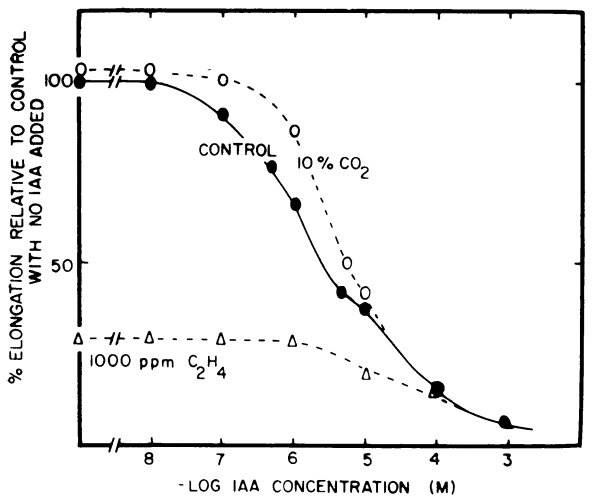

FIG. 4. Effect of 10 % CO_2 or 1000 ppm ethylene on the elongation of pea root sections grown in various IAA concentrations for 18 hours.

curve at that point (also see inflection in fig 4, control). A second phase occurs at IAA concentrations in excess of 10 μM, rendering the tissue bleached or discolored and preventing all growth by 1 mM. The first phase Andreae was able to reverse by rinsing treated roots in an auxin free medium and by lowering the pH of the solution to 4.5; the second is irreversible. Since the high concentration inhibition can be duplicated with equivalent amounts of acetic and benzoic acids (3) it is not related to any physiological action of IAA, and therefore we have only concerned ourselves with the inhibition which occurs between 10 mμM and 5 μM IAA. It is this inhibition which is lacking in tissue treated with 1000 ppm ethylene, suggesting that these concentrations of auxin retard growth solely by inducing ethylene formation. Several other arguments prove this to be the case. For example when pea roots are treated with ethylene their length is reduced more than their weight (fig 3) so they become swollen. The ratio between the absolute percentage increase in weight versus the absolute percentage increase in length changes from 1.3 in control tissue to 2.3 at all concentrations of ethylene in excess of a few ppm. Therefore, the 2.3 ratio obtained when auxin causes swelling is quantitatively consistent with the measured response to ethylene. Ethylene also accounts for essentially the entire auxin induced inhibition of elongation. Tissue containing a few ppm ethylene cannot respond to applied gas (fig 3) and consequently the average internal concentration of ethylene must be a few ppm within root sections treated with just enough auxin (0.1 mM) to render them unresponsive to added ethylene. This amount of ethylene reduces elongation by 75 % and thus would explain more than 90 % of the inhibition caused by 0.1 mM IAA [the remainder of the effect presumably is due to nonspecific toxity at the excessively high auxin concentration (2, 3)]. Roots would have to contain 0.3 ppm ethylene when they produce ethylene at a rate of 1 mμl/g hour in order to have an internal content of 2 ppm when they are treated with 0.1 mM IAA. This is closely similar to the ppm per mμl/g hour ratio calculated for both pea and sunflower stem sections (9). Finally we find that auxin inhibited growth in roots is reinstated by CO_2, a competitive inhibitor of ethylene action. Carbon dioxide antagonizes ethylene action in pea stems (8, 10), and the results illustrated in figure 5 demonstrate that it has the same action in pea roots. The data for root growth as a function of ethylene concentration (fig 3, upper) yield a straight line (fig 5, lower curve) when expressed as a double inverse Lineweaver-Burk plot. As the CO_2 concentration is raised a family of intersecting curves arise which exactly describe a classical case of competitive inhibition (fig 5). Reversal of ethylene action by CO_2 has also been demonstrated for fruit ripening (16, 25), flower fading (20, 21), leaf abscission (1), epinasty (13), and auxin inhibited growth in stems (10), and is predicated on the fact that CO_2 is a close analogue of allene, itself an analogue of ethylene capable of mimicking the action of the olefin in inducing fruit ripening as well as growth changes in pea tissue (9, 10). In the absence of applied auxin, concentrations of CO_2 in excess of 20 % progressively inhibit root growth, whereas 5 to 10 % CO_2 very slightly accelerates it (fig 3, see data at less than 0.1 μM IAA). The growth inhibition caused by low concentrations of IAA is largely reversed by 10 % CO_2 as would be expected if ethylene mediates the response. For example

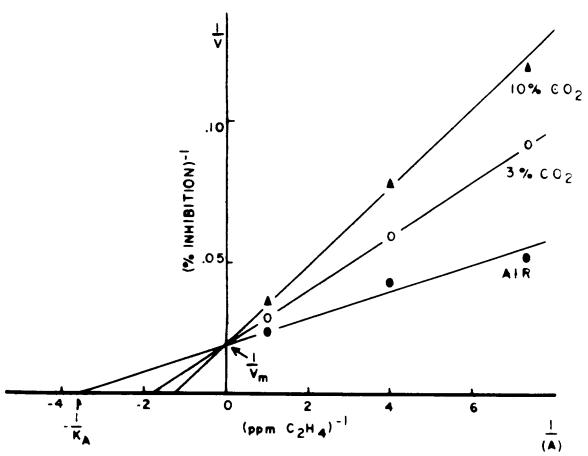

FIG. 5. Lineweaver-Burk plot (1/V vs 1/A) at various concentrations of CO_2 showing competitive inhibition of ethylene action by CO_2. The effect measured (V) is the % change in the rate of elongation caused by ethylene (A = activator), and the maximum effect (V_m) is a 70 % inhibition. The Michaelis-Menten constant for ethylene (K_A) is 1.5 mμM, and that for CO_2 (K_I) is 0.8 mM, corrected for solubility of each gas in water.

1 μM IAA and 0.1 ppm ethylene both reduce elongation by 35 %, and 10 % CO_2 reverses the inhibition by 60 % in each case. Similarly, the growth inhibition caused by 10 μM IAA can be duplicated by applying 1 ppm ethylene, and 10 % CO_2 causes only a slight reversal in both cases. Thus the results with CO_2 substantiate the conclusion that auxin inhibits the growth of pea roots by inducing ethylene formation.

Preliminary studies with germinating tomato seeds and root sections of mung bean, sunflower, *Avena* and *Ricinus* indicate that IAA inhibits root elongation in each case by inducing ethylene formation. Stimulation of ethylene production occurs coincident with the first detectable inhibition of root elongation at between 0.1 and 1 μM IAA in these roots, and in the 1 tissue investigated, sunflower, the sensitivity to ethylene is comparable to that of pea roots.

The proposed role of ethylene as mediator of IAA inhibitions in roots is consistent with numerous other effects which have been attributed to auxin. For example when IAA retards longitudinal growth in roots it leads to radial enlargement of the cortical cells of the expanding zone (12). These swellings, which become covered with root hairs (12), largely compensate for the volume decrease, and as a result the fresh weight of many roots is not altered by IAA except at a very high concentration (2). Auxin also does not decrease the dry weight, frequency of mitoses, or plastic extensibility of roots except at a "toxic" level, so it has been concluded that "the auxin inhibition of longitudinal growth is certainly not the result of a decreased intensity of the growth processes in general, but rather the outcome of a change in their course (2)". The same may be said of ethylene action, for the gas does not prevent auxin from stimulating growth and respiration (8, 10); instead it removes the restraint on radial expansion, causing swelling of the cortical cells of the expanding zone (8, 17), and little if any change in fresh and dry weight (9) or the number of mitoses (17). Moreover the swollen cortical cells of gassed roots also become covered with root hairs (24 and fig 7), thus accounting for the apparent ability of auxin to produce the same effect.

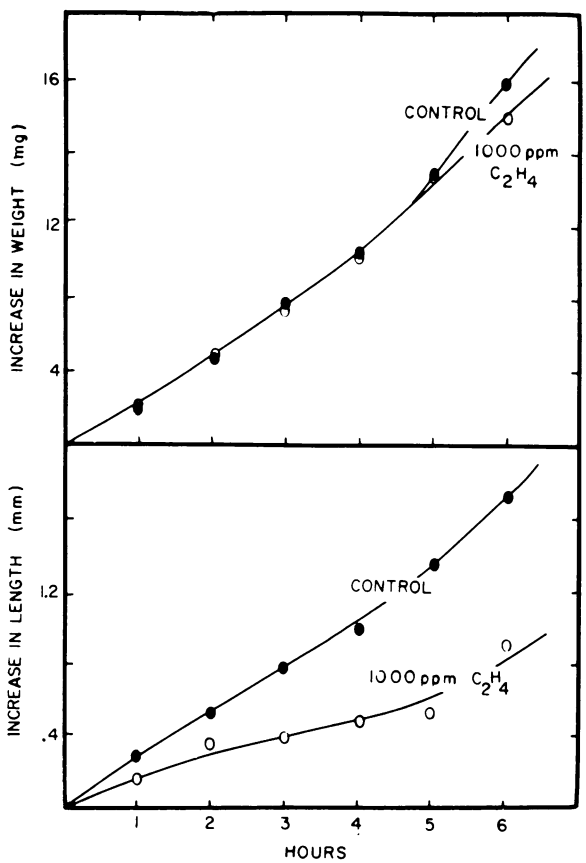

FIG. 6. Time course showing the effect of 1000 ppm ethylene on the elongation and rate of increase in fresh weight of pea root sections. Ethylene was added at the start of the experiment.

Role of Ethylene in Root Geotropism. If ethylene participates in the geotropic response of pea roots, formation of the gas would have to be induced and the tissue affected by it within about 15 minutes, the time which elapses between geostimulation and the first detectable curvature in these roots (4). The data in figure 1 suggest that ethylene formation may be induced within the requisite time and that in figure 6 depicts a response to ethylene which is essentially instantaneous.

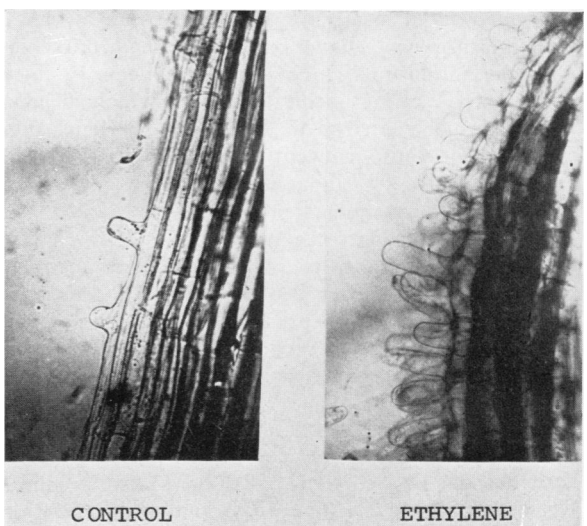

FIG. 7. Photomicrographs (100 X) of freehand longitudinal sections through the zone of root hair formation in 2 day old pea roots. Control root hairs, shown emerging from elongated epidermal cells, are rarely found at this time but become numerous on the third day. Treated roots were exposed to 1000 ppm ethylene between the twenty-fourth and forty-eighth hours. Note the swollen cortical and epidermal cells, and extensive formation of root hairs.

Table I. *Inhibition of Geotropism by CO_2*

Time (hrs)	Degrees curvature* 1	2	3	4
Control	6.4 ± 2.2	16.9 ± 1.1	20.3 ± 4.0	35.0 ± 3.5
5 % CO_2	5.2 ± 2.8	13.6 ± 1.7	15.8 ± 6.2	27.8 ± 4.7
10 % CO_2	1.1 ± 1.4	7.0 ± 6.6	12.2 ± 1.8	23.0 ± 5.8

* ± Standard deviation.

During a 5 hour period after ethylene is applied, elongation is inhibited without any concomitant reduction in tissue fresh weight (fig 6). This interval encompasses essentially the entire time needed for geotropic curvature development in this tissue (4) and therefore the lower half of the curving zone should possess swollen cells due to ethylene action. Microscopic examination of pea roots which have undergone geocurvatures reveals this to be the case. As a further test of the hypothesis that ethylene production is required for root geotropism, a competitive inhibitor of ethylene action, CO_2, was applied to geostimulated roots. The growth of vertically oriented, intact roots was slightly accelerated by 5 to 10 % CO_2, but the geotropic curving of horizontally positioned roots was markedly retarded (table I), and similar results were obtained with intact lima bean roots. Carbon dioxide at a concentration of 7 % had no effect on the geotropic bending of pea stem sections suspended horizontally between a donor agar block (1 μm IAA) and basal receiver (9), but this is in accord with the Cholodny-Went theory which proposes that stem geotropism does not involve growth inhibition by excess auxin but rather growth promotion. Several other puzzling observations can be explained if ethylene mediates root geotropism. For example when pea roots curve, their upper side is slightly accelerated in growth rate at first but then becomes progressively inhibited (4). An overall decrease in growth during geotropic curvature of various other roots has also been reported (2, 7, 18, 19) and cannot be due solely to auxin redistribution, so it has been concluded that the release or synthesis of a growth-inhibiting substance must be involved (4, 5, 19). We believe ethylene to be this substance, and that the eventual inhibition of growth on the upper side of the root is a natural consequence of the back diffusion of the gas into this region. If ethylene formation ceased once the roots regained a vertical orientation [and it soon slows down, even when auxin is continuously present (9, and fig 1)] a normal growth rate immediately would be restored since the gaseous inhibitor has no lasting effect on growth unless continuously present (9), and is rapidly dissipated from tissue.

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