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IAA-Induced Growth Responses of Decapitated Corn Seedlings

INDICATIONS OF TWO APPARENT ADAPTATIONS WITH A POSSIBLE ROLE IN GRAVITROPISM[1]

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ABSTRACT

The vertical growth responses of corn seedlings (*Zea mays* L. Mo17 × B73) were determined over an 8-hour period. When seedlings were decapitated 3 millimeters from the coleoptile's tip and supplied with indole-3-acetic acid (IAA) in 1.5% agar blocks, the response was dependent both on time and IAA concentration. The dose-response curves changed in shape and magnitude depending on the total time of IAA application. High concentrations (>3.2 × 10^{-6} molar) initially produced high relative growth rates that decreased back to the intact rate (0.03 millimeter per hour per millimeter) after 3 hours. Low concentrations (<1.0 × 10^{-6} molar), or agar blocks without IAA, resulted in a rapid decrease from the intact rate to a level that stabilized at 0.01 millimeter per hour per millimeter until the growth rate began to recover after 3 to 4 hours. Intermediate concentrations produced responses similar to that of the intact organ, though some features of these responses were unique.

The coleoptile curvature in response to gravity depended upon whether the coleoptiles were intact, decapitated, or decapitated and supplied with IAA. Coleoptiles decapitated and not supplied wth IAA showed little or no curvature for 3 hours after decapitation. By this time an adaptation, evoked by the low IAA level, had developed and the coleoptiles began to curve steadily. When 1.0 or 3.2 × 10^{-6} molar IAA was supplied, curvature was initiated within the first 30 minutes and reached a maximum rate before decreasing and stopping after 3 to 4 hours. The sequence of events in response to these concentrations was similar to the intact sequence but the curvature rate was reduced to one-third to one-half. A model for the autotropic response involving an auxin concentration-dependent, growth-modulating mechanism capable of two modes of adaptation is described.

The Cholodny-Went theory of gravitropic responses, as applied to plant shoots, suggests that the plant growth regulator IAA is transported laterally from the upper to the lower side of a gravitropically stimulated plant. This lateral movement is believed to cause a decrease in concentration on the upper side, resulting in a decreased growth rate. On the lower side, the concentration increases, thus stimulating growth. Recent criticism of this theory focuses on the controlling role of IAA (3, 6, 13). Although it has been demonstrated that lateral transport of IAA does occur in corn coleoptiles (8, 11), the magnitude of this transport has been suggested to be inadequate to stimulate growth to the extent that is observed on the lower side (3, 6).

Much of the criticism is based on dose-response curves obtained with *Avena* coleoptiles. Recent re-evaluation of such dose-response curves has shown that the curves are sigmoidal with maximum growth rate over the range of 3.0 × 10^{-7} to 1.0 × 10^{-3} M IAA (2). Similar results were obtained for corn coleoptile segments; however, the range of IAA concentrations that stimulated growth was much narrower, 1.0 to 3.0 × 10^{-7} M.

In most dose-response determinations, little attention has been given to relating observed growth rates to the growth rate of an intact part under the same conditions. Recent investigations of the SGR[2] by Evans *et al.* (4, 5) and Vesper and Evans (12) have shown that coleoptile tissue can adapt to low levels of IAA. Prior to their work, it was generally believed that the SGR was a manifestation of the regeneration of the physiological tip, which resumed production of IAA and thus restored the growth rate. They concluded, on the contrary, that the SGR was not due entirely to a restored synthesis of IAA, but to an increase in tissue sensitivity to the IAA present. This conclusion is supported by the work of Iino and Carr (9), who have shown that the production of IAA does not recover in conjunction with the recovered growth rate.

Though much work has been done with regard to growth responses of corn-coleoptile segments in IAA solutions (2, 4, 5, 10, 12), intact coleoptiles have received little attention. In this investigation, a comparison was made between IAA-induced vertical growth responses of decapitated corn coleoptiles and vertical growth responses of intact coleoptiles. By selection of a concentration of IAA that closely mimicked intact growth for use in gravitropic experiments, it was hoped to further elucidate the role of IAA in all phases of the gravitropic response.

MATERIALS AND METHODS

Corn seeds (*Zea mays* L. Mo17 × B73) were soaked for 12 h in running tap water (30 ± 1°C) and placed embryo up on moist paper towels. After 24 h, uniform seedlings were selected and planted in zinc trays containing moist vermiculite. Seedlings were grown in closed containers under red light (0.1 μw m^{-2}, integrated over the range of 600 to 725 nm). Experiments were performed 60 h after completion of the 12-h soak. For some experiments involving gravistimulation, seedlings were grown under the same conditions except that they were placed in individual glass tubes. This allowed for easier manipulation of seedlings to ensure uniform gravity stimulation.

IAA solutions were prepared in citrate-KOH buffer (20 or 100 mM, pH 5.65) as was the stock agar solution for each experiment. IAA solutions of various concentrations were applied to corn seedlings in the form of agar blocks (3 × 3 × 1.2 mm). The final agar concentration for each IAA solution was 1.5%.

For all experiments, seedlings were placed in a large (8 m^3) humid chamber (95–100% RH) and kept in the dark for the duration of the experiment except during photography. Relative growth rates were determined by photographing sets every 30

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[2] Abbreviations: SGR, spontaneous growth response; L_{T1}, coleoptile length at T1; L_{T2}, coleoptile length at T2; T1, time at the beginning of a growth measurement period; T2, time at the end of a growth measurement period.

min for 8 h using a green safelight for a 12-s exposure. All rates were calculated relative to the length of the tissue at the beginning of a length measurement period utilizing the equation $(L_{T2} - L_{T1})/((T2 - T1)(L_{T1}))$. A small resin bead was attached to the node of the seedling with a 3% gelatin solution to facilitate measurement of the coleoptile. Coleoptile measurements were made by projecting the photographic negative (enlarging it 2.5 ×) and measuring the image in mm. For decapitated seedlings, the 3-mm tip was removed and replaced immediately with an agar block. In gravitropic experiments, the seedlings were rotated 90° after treatment. Curvature of an individual coleoptile was determined by the intersection of two tangents, one at the node and one at the tip. This effectively allowed for determination of the curvature of the coleoptile alone and not the whole seedling.

RESULTS AND DISCUSSION

Dose-Response for Vertically Oriented Seedlings. To determine the relationship of IAA-induced growth to intact growth, the growth response of vertically oriented decapitated corn coleoptiles supplied with various concentrations of IAA was compared to that of intact coleoptiles. High concentrations of IAA (Fig. 1A) caused an increase in growth rate within the first 0.5 h, which peaked between 1 and 2 h. The gradual decrease in rate that followed stabilized between 3 and 4 h after decapitation at a growth rate similar to that of intact coleoptiles. This decreased growth rate was not due to depletion of the effective level of IAA in the agar blocks, since there was no renewed increase in the growth rate when fresh blocks were applied after 4 h.

Figure 1C illustrates what might be considered a typical SGR (5). There was a rapid decrease in growth rate that stabilized at about 0.01 mm h^{-1} mm^{-1} for 1.5 h, after which there was a gradual recovery that exceeded the intact rate (compare with Fig. 1A). This response was similar when 1.0×10^{-7} and 1.0×10^{-8} M IAA were applied. The growth response to 1.0×10^{-8} M IAA differed from 1.0×10^{-7} M by more closely resembling the 0.0 concentration in the last 2 h in that the growth rates remained above the intact control level. The most significant difference between 0.0 and a low concentration of IAA would seem to be the slightly longer recovery time at the low concentration. The rate of recovery was approximately the same but the initiation of the recovery was delayed by about 1 h when 1.0×10^{-7} and 1.0×10^{-8} M concentrations were provided. This delay in recovery was similar to the delay of the SGR caused by IAA application to corn coleoptile segments observed by Evans *et al.* (5). They found that application of IAA delayed the SGR until the IAA source was removed.

The intermediate concentrations of IAA caused a slight, early stimulation of growth (Fig. 1B) that gradually declined to the same level as seen for the low concentrations. Growth at intermediate concentrations showed a near-linear decrease over a 2- to 3-h span, but growth at the 1.0×10^{-6} M concentration fell at a much faster rate and reached a minimum roughly 1 h before that at the 3.2×10^{-6} M concentration. At approximately 4 h, there was a sharp increase in the growth rate so that it exceeded the intact rate. It then declined to a level equal to or slightly below the intact rate. The sharp increase in growth rate cannot be attributed to replacement of the agar block, as experiments in which blocks were replaced every 2 h produced the same response pattern (data not shown). Though no concentration of IAA exactly mimicked the coleoptile tip, 1.0 and 3.2×10^{-6} M most closely approached the intact growth rate when measured over an 8-h growth period.

The responses shown in Figure 1C would support the earlier conclusions of Vesper and Evans (12), namely, the coleoptile tissue has become sensitized to the low level of IAA, thus producing increased growth rates. However, the responses to high concentrations of IAA suggest an opposite mechanism, one

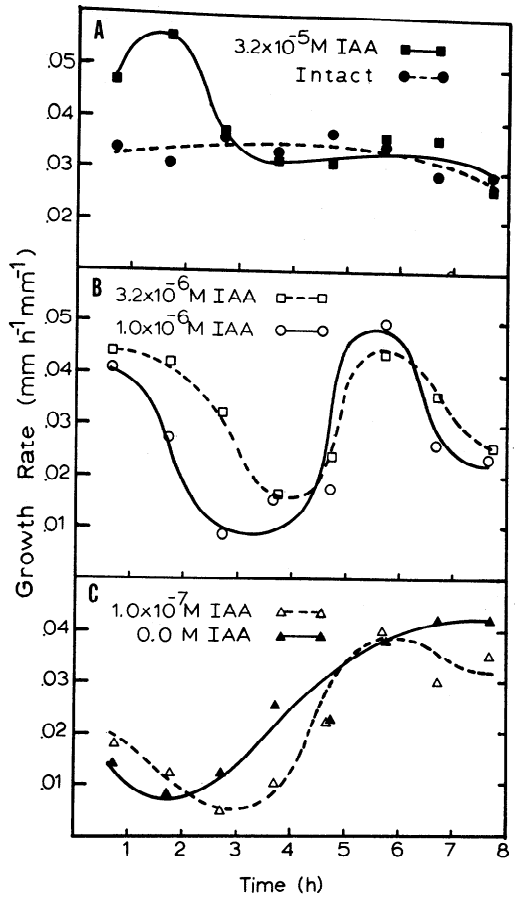

FIG. 1. Growth responses of vertically oriented decapitated corn coleoptiles to IAA. Decapitated coleoptiles were supplied with agar blocks each containing a specific concentration of IAA (3.2×10^{-5}, 3.2×10^{-6}, 1.0×10^{-6}, 1.0×10^{-7} M, and 0 shown). Agar blocks were made up with 100 mM citrate-KOH buffer (5.65) but other repetitions with 20 mM buffer gave similar results. Fresh agar blocks were supplied at 4 h. Only selected responses are shown that are representative of general IAA concentration groups. Part A is representative of the high concentrations, 1.0×10^{-5} to 1.0×10^{-4} M, part B the intermediate concentrations, and part C the low concentrations including 0, 1.0×10^{-8} and 1.0×10^{-7} M IAA. All values are the mean of three separate experiments with four to five coleoptiles per treatment. Individual points represent the average of two consecutive 0.5-h measurements. The average standard error of the mean of each IAA concentration at each time period is equal to ±0.006 where $n = 3$. Analysis of variance was performed to test significance of the differences in growth curves using the time by IAA interaction. This interaction was found to be significant at the 99% level based on the F test.

in which the tissue becomes 'desensitized' to IAA. This observation coupled with the oscillations in growth rate after the adaptation period (3 h) seem to indicate a complex interaction between IAA and the tissue, regulated by a feedback mechanism. All concentrations, except two, gave oscillatory patterns that were similar in timing though different in amplitude. These were most pronounced at 1.0 and 3.2×10^{-6} M IAA. The two exceptions were 0.0 and 1.0×10^{-8} M IAA, which seemed to be more damped than the others or had periods too prolonged to be detected in 8 h.

The differences between the initial responses obtained with different levels of IAA occurs as a result of an interaction of added IAA with the normal SGR. Addition of low (Fig. 1C) to intermediate (Fig. 1B) concentrations of IAA delays the SGR but does not prevent the response, as do supraoptimal (Fig. 1A)

concentrations. The 0.0 IAA treatment failed to show the sharp oscillations most clearly demonstrated at the intermediate concentrations. This would seem to be a further indication of the close interaction between IAA concentration and changes in tissue sensitivity to applied IAA.

To determine if increases in tissue sensitivity to IAA could be occurring at low IAA concentrations, experiments were conducted in which either 0.0 or 1.0×10^{-8} M IAA was supplied for 4 h. At the end of this period, blocks containing 3.2×10^{-5} M IAA were added and the growth responses were determined. Figure 2 illustrates the response of tissues preconditioned with 0.0 and 1.0×10^{-8} M concentrations. The increased growth rate after addition of the higher IAA concentration reached a maximum above that observed when the same concentration was added imediately after decapitation (Figs. 1A and 2A). The per cent increases in maximum growth rate over the initial response to 3.2×10^{-5} M IAA in the three individual experiments were 34, 28, and 20%. The timing of these maximum responses varied from experiment to experiment by up to 1 h. Figure 2 therefore de-emphasizes the individual maximum responses. These results would seem to confirm the increased tissue sensitivity previously described by Vesper and Evans (12). However, this increased growth rate was not as great as shown by them and seems to be very transient, dropping rapidly after reaching a maximum.

Analysis of dose-response curves indicates how this adaptation occurs. The temporal change in dose response can be seen clearly in Figure 3 in which these responses are plotted on an individual

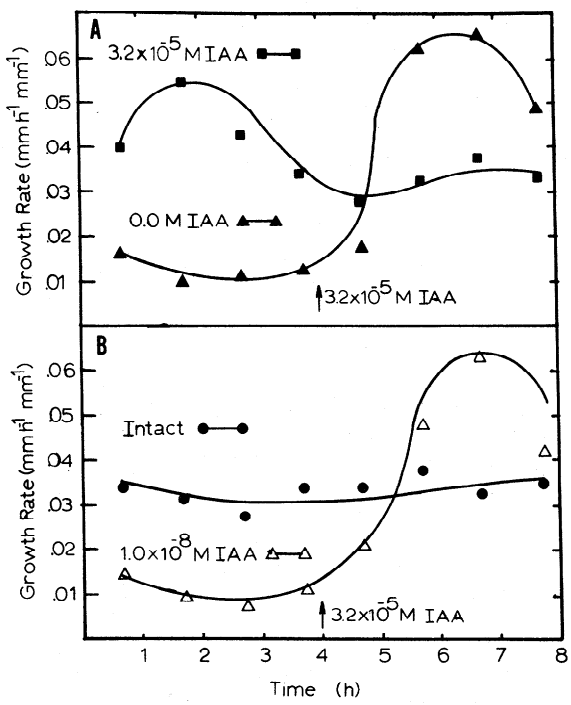

FIG. 2. Growth response of decapitated coleoptiles supplied with a high IAA concentration after preconditioning with a low one. Coleoptiles were decapitated and supplied with agar blocks containing 0 or 1.0×10^{-8} M IAA. After 4 h, blocks were replaced with ones containing 3.2×10^{-5} M IAA. Controls consisted of a set of intact coleoptiles as well as a set of decapitated coleoptiles supplied with 3.2×10^{-5} M IAA. Agar blocks contained 20 mM citrate-KOH buffer (5.65 pH). Appropriate agar blocks were exchanged every 2 h. All values represent the mean of three separate experiments with four to five coleoptiles per treatment. The standard error of the mean by IAA concentration is equal to ±0.0022 where $n = 24$. An F test of the time by IAA interaction indicated this interaction was significant at the 85% level during the first 4 h and at the 99% level during the last 4 h of the experiment.

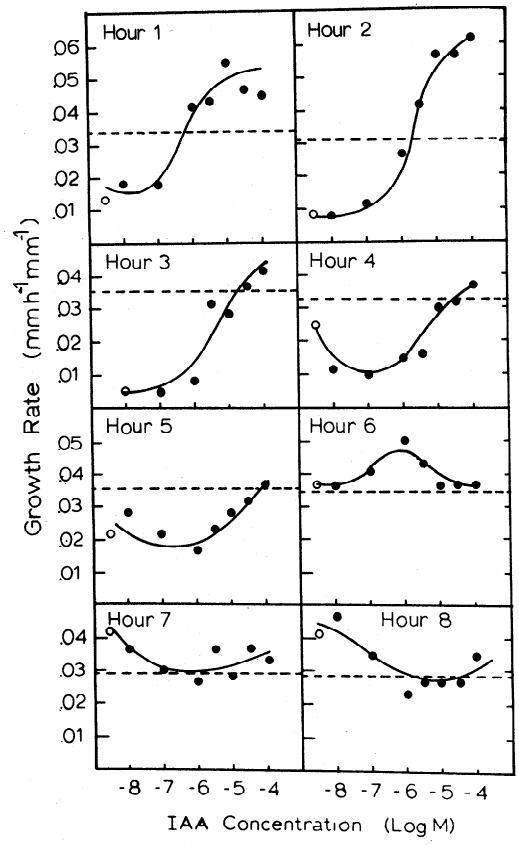

FIG. 3. IAA dose-response curves for vertically oriented decapitated corn seedlings. Application of IAA as in Figure 1. The growth rate (mm h^{-1} mm^{-1}) for each IAA concentration is calculated on an individual hourly basis. The initial point (O) on each curve represents the growth response to 0.0 IAA and the horizontal dashed line represents the average intact coleoptile growth rate for each time period. All values are the mean of three separate experiments. The standard error of the mean by IAA concentration is equal to ±0.002, $n = 51$. In an analysis of variance of this data, it was determined that the dose-response curves after each of the first 4 h were significantly different from each other at the 99% level based on the F test, whereas after each of the last 4 h the curves differed only at the 75% level.

hourly basis. From these plots, it is seen that rates at higher concentrations drop rapidly. By 4 h, these rates were approximately equivalent to the intact coleoptile rate, and remained at this level for the remaining 4 h of the experiment, though some oscillation around the intact level occurred. During the second 4-h period, the growth rates observed at the lower concentrations increased so as to equal or exceed the intact growth rate. The large changes in the dose-response pattern clearly indicate that the tissue was undergoing some major metabolic changes. An adaptation process that serves to alter the sensitivity to IAA would account for these changes within the tissue.

The dose response of decapitated corn seedlings to IAA exhibited a strong dependence on time. As the duration of time after IAA application increased, the observed range of growth rates decreased. The data presented here suggest that adaptation processes can occur in corn coleoptile tissue resulting in a sensitization or desensitization to IAA within the tissue. That these two processes have distinct and separable roles was illustrated by a different dependence on concentration, timing, and the direction of the ultimate effect on growth. The findings reported here indicate that a narrow range of IAA concentrations may exist within the tissue and that fluctuations outside of this range initiate the appropriate adaptation response. This would suggest

that the sequence of events in the growth-regulating process could be subject to a feedback mechanism to regulate growth of the tissue.

Gravitational Curvature of Decapitated Coleoptiles. As already discussed, the dose response of decapitated seedlings varied with time. However, two of the concentrations tested in this study (Fig. 1B) most closely approached and bracket the intact growth rate. For this reason, concentrations of 1.0 and 3.2×10^{-6} M were selected for use in the gravitational experiments. These experiments were conducted for a maximum of 5 h with gravitational stimulation beginning immediately after decapitation.

Figure 4A shows curvature as a function of time for intact coleoptiles and coleoptiles decapitated and supplied with agar blocks containing no IAA. The intact coleoptile response was a relatively linear increase for the first 3 h of gravitational stimulation, after which the curvature decreased for 2 h. This increase and decrease in curvature reflect the autotropic response documented most recently by Firn and Digby (6). As growth rate on the upper side begins to increase, the curvature decreases in rate and eventually stops resulting in a straightening of the coleoptile. The timing of the autotropic response (Fig. 4A) agrees reasonably well with that reported by Firn and Digby.

Of most importance is a comparison of the responses of intact coleoptiles with those of decapitated coleoptiles supplied with 0.0, 1.0, and 3.2×10^{-6} M IAA concentrations (Fig. 4). When no IAA was supplied in the agar blocks placed on decapitated coleoptiles, virtually no curvature was observed for the first 3 h (Fig. 4A). After this initial period, curvature began but remained at a rate approximately one-half that of the intact coleoptiles. In contrast, coleoptiles supplied with IAA (Fig. 4B) showed an initiation of curvature very similar to that of the intact coleoptiles, but the rates of curvature were approximately one-half the intact rate. The rates of curvature of decapitated coleoptiles with and without IAA were the same, but without IAA, curvature was delayed 3 h. Both concentrations of applied IAA showed the greatest rate of curvature during the first 3 h, then decreased or stopped altogether. When IAA was supplied, the timing of the gravitropic response was very similar to that of the intact coleoptiles; only the magnitude of the response was affected.

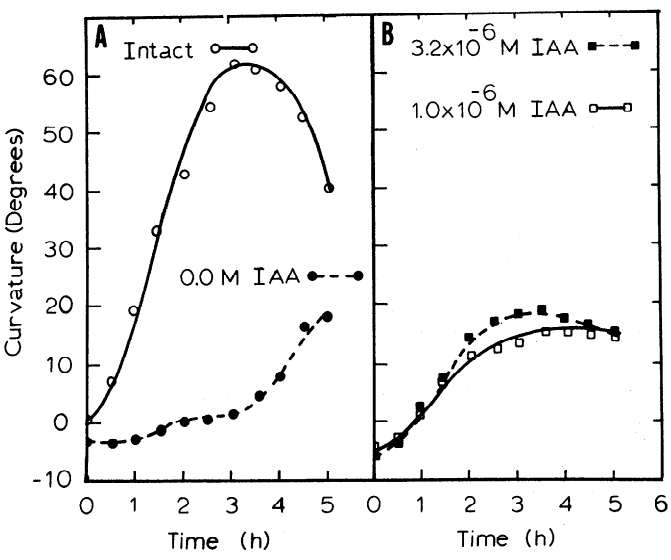

FIG. 4. Gravitropic response of intact and decapitated corn coleoptiles. Agar blocks were made up containing 20 mM citrate-KOH buffer (5.65 pH). All blocks were exchanged every 2 h for the duration of the experiment. For each experiment, a set of four intact seedlings was used as a control group. Each point represents the mean of three separate experiments. The standard error of the mean by IAA concentration is equal to ± 1.7, $n = 33$. An F test of the IAA effect indicated significance at the 99% level.

In light of the Cholodny-Went theory, one possible explanation of these observations could be that *Zea* coleoptile tissue requires a specific, tightly controlled level of IAA for an optimal rate of curvature. Anker's work with *Avena* coleoptile segments (1) suggests that a narrow range of applied auxin concentrations gave optimum curvature. It is possible that the 3.2×10^{-6} M concentration provides too much IAA, preventing an adequate asymmetric distribution of IAA to be established and preventing complete development of the asymmetric growth pattern. This would result in less total curvature than observed in coleoptiles of intact seedlings. The 1.0×10^{-6} M concentration would be inadequate to provide sufficient stimulation of growth resulting in limited curvature. From the data, it is not possible to determine if these explanations are correct since both concentrations gave nearly the same response. It is equally likely that both concentrations provide a sufficient amount of IAA for the initiation of curvature but other factors become limiting in the expression of the total gravitropic curvature.

The observation that curvature occurred only after 3 h when decapitated coleoptiles were supplied with no IAA in the agar blocks suggests that an adaptation to the low level of IAA present in the tissue has occurred. This contention is supported by the work of Iino and Carr (9), who have shown that the level of diffusible IAA from corn coleoptiles falls very rapidly after decapitation of the 3-mm tip. The level of diffusible IAA is maintained at about one-half that of the intact coleoptiles until a slow recovery begins after 4 h, but the recovery does not seem to reach the original level. This observation supports an adaptation phenomenon since the recovery of diffusible IAA observed by Iino and Carr was of a different magnitude than the recovered growth rate reported here (Fig. 1C), and by Evans *et al.* (5). In terms of the observed gravitropic response of decapitated coleoptiles, the lack of complete recovery after the adaptation period (3 h) suggests a complex interaction of the tissue, IAA, and the transverse differential in growth resulting from gravity stimulation.

Though no concentration exactly mimicked the intact growth response, concentrations of 1.0 and 3.2×10^{-6} M were found to be reasonably close. When decapitated coleoptiles were supplied with these concentrations and given a gravitational stimulation, the curvature response was restored to at least one-third that of intact coleoptiles. However, timing of the initiation of curvature was the same as that shown by intact coleoptiles. The curvature of decapitated coleoptiles not supplied with IAA was delayed for 3 h. These observations would seem to indicate that IAA has an essential role in the gravitropic response, though the increased growth on the lower side may be more complex than can be accounted for by a simple increase in IAA concentration alone.

Conclusions. Firn and Digby (6) established the importance of the autotropic response in determining the final overall orientation of gravitropically responding tissue. The data presented here suggest an explanation for the autotropic response in corn coleoptiles. Growth responses of coleoptiles supplied with high or low concentrations of IAA indicate that adaptations to the level of IAA can occur within this tissue, whether sub- or supraoptimal, to maintain or regain an endogenous growth rate. It seems feasible, therefore, that the autotropic response, which is initiated by an increase in growth rate on the upper, slow growing side and a decrease on the lower, fast growing side, is due to tissue adaptation.

If one assumes that the change in growth rate observed on the upper and lower sides of a gravitropically stimulated coleoptile is due to the lateral movement of IAA, then the autotropic response may be due to the adaptations described. The rapid movement of IAA away from the upper epidermal cells may account for the rapid decrease in growth rate there. This decrease

in auxin level may initiate a time-dependent adaptation resulting in an increased sensitivity to auxin. When the adaptation process is fully realized after 3 to 4 h, the growth rate on the upper side will begin to increase, serving to initiate the autotropic response. These events on the upper side are paralleled by a desensitization of the tissue on the lower side, accounting for the observed reduction in growth rate that also contributes to the autotropic response. The timing of the adaptations corresponds well with the timing of events of the autotropic response (6) and suggests a role in this response.

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