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# Transport of Indoleacetic Acid in Intact Corn Coleoptiles[1, 2]

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## ABSTRACT

**We have characterized the transport of [$^3$H]indoleacetic acid (IAA) in intact corn (*Zea mays* L.) coleoptiles. We have used a wide range of concentrations of added IAA (28 femtomoles to 100 picomoles taken up over 60 minutes). The shape of the transport curve varies with the concentration of added IAA, although the rate of movement of the observed front of tracer is invariant with concentration. At the lowest concentration of tracer used, the labeled IAA in the transport stream is not detectably metabolized or immobilized, curvature does not develop as a result of tracer application, and normal phototropic and gravitropic responsiveness are not affected. Therefore we believe we are observing the transport of true tracer quantities of labeled auxin at this lowest concentration.**

It has long been thought that the way a plant regulates the flow of IAA to its different parts helps to determine its growth rate and pattern and that plants change the distribution of IAA in response to environmental signals. Experiments dating from the 1930s have shown that the distribution of auxin may change within a seedling when it begins to grow towards a unilateral light source, or away from the source of gravity; but questions remain concerning whether the observed changes in IAA transport or concentration are sufficient to cause the growth changes, or whether they are simply correlates of the responses (4, 5, 9, 11).

A major impediment to understanding the role of IAA in gravitropism and phototropism has been the inability to study the way a plant transports auxin normally, and whether it changes that pattern, or fails to change it, under stimulus conditions. Most investigators have had to study auxin transport in decapitated stem tissues, or in cut segments of stem tissue, in order to be able to get enough tracer, either tritiated or $^{14}$C-labeled auxin, into the plant to measure transport. The amounts of tracer added to these wounded tissues were large, almost certainly perturbing to the plant's growth physiology, because the tracers available were of low specific activity and methods used to detect the tracer were not of the efficiency available now. In addition, the time resolution of previous experiments has been too coarse to be able to apply the methods to the investigation of real physiological responses hypothesized to involve changes in auxin distribution.

Experimenters have used a variety of methods to apply labeled auxins to coleoptile tissues, and the growth responses to the auxin applications have varied accordingly. Sections of corn coleoptiles floated in auxin solutions, for example, show a linear increase in growth rate as a response to logarithmically increasing concentrations of auxin (3), whereas decapitated oat coleoptiles show a linear increase in growth rate with linearly increasing concentrations of auxin applied in agar blocks placed on the cut surface (11). Intact corn coleoptiles show a linear increase in growth rate to spot applications of linearly increasing concentrations of auxin in lanolin (1), but only at low concentrations of applied auxin. One may conclude that the growth responses one sees resulting from auxin application depend on any or all of the following: the concentration of auxin applied; the method of application; and whether the tissues are intact.

The rate of auxin transport also seems to be different in cut tissues and intact tissues. Corn coleoptile sections immersed in auxin solutions or placed between an IAA-containing agar donor block and an agar receiver block, transport IAA basipetally at a rate reported to be between 8 and 15 mm/h (6). By contrast, the transport of auxin in intact corn coleoptile tissue, inferred from the basipetal migration of the growth response to spot applications of auxin in lanolin to intact coleoptiles, has been reported to be as fast as 23 mm/h at a similar temperature (2).

Decapitated coleoptiles and cut sections of coleoptiles do not show phototropism (9), so it has not yet been possible to determine if changes in auxin transport (for example, changes in the concentration of auxin in the transport stream or changes in the rate of movement of auxin) are involved in the generation of this response. Changes in auxin transport in coleoptiles during gravitropism have been studied by a number of investigators (12). Generally results in coleoptiles are consistent with a change in IAA transport occurring during gravitropism.

We have attempted to establish conditions under which labeled IAA can be added to intact coleoptiles in true tracer quantities, and followed through the plant at a fine enough time and spatial resolution to investigate IAA transport before, during, and after the growth changes in phototropism and gravitropism occur. To this end, we have addressed four major questions about the basipetal transport of IAA in intact coleoptiles. What is the rate of IAA transport? Is this rate of transport dependent on the concentration of auxin in the transport stream? Is IAA destroyed, or diverted into storage pools or conjugated forms as it moves down the coleoptile? Finally, are there any effects of the smaller concentrations of added IAA on a coleoptile's gravitropic and phototropic responses?

## MATERIAL AND METHODS

### Plant Material

Corn seeds (*Zea mays* L., cvPX9540, Northrup King) were imbibed in deionized water on a shaker at about 60 cycles per

[1] We dedicate this paper to the memory of Dr. Ruth L. Satter—a wonderful scientist and human being.

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min for 3 to 8 h and planted in 30 ml glass beakers filled with damp vermiculite, 2 or 3 seeds per beaker. They imbibed and grew under constant red light. The light source was a red fluorescent bulb (Sylvania Red F20T12/R, 20W), and its output was filtered through one 3 mm thick layer of red acrylic plastic (Shinkolite A102, Argo Plastics), and yielded a fluence rate at the level of the plants of 0.7 $\mu$mol/m$^2$ s. The temperature was maintained at 22 to 24°C. Plants with coleoptiles 1.3 to 1.9 cm long were used for experiments on the morning or early afternoon of the 4th d, when the average coleoptile was 1.5 cm long and the average primary leaf was 1.2 cm long (the mesocotyls varied in length).

### Transport Studies

[$^3$H]IAA was obtained at a specific activity of 21.7 Ci/mmol, and a concentration of 46 $\mu$M in ethanol from Amersham. Unlabeled IAA was obtained from Sigma Chemical Company. The auxin transport inhibitor NPA[3] was a gift of Dr. Rainer Hertel.

The lowest concentration of label used, 0.92 $\mu$M [$^3$H]IAA, was a 1:50 dilution of primary label into 95% ethanol (v/v), 1% Triton X-100 (v/v). Higher concentrations of auxin, up to 2.3 mM, were made by diluting the [$^3$H]IAA stock 1:20 into varying concentrations of unlabeled IAA in 95% ethanol, 1% Triton X-100. A volume of 0.5 $\mu$L of label was applied to the tip of each coleoptile as a drop with a Hamilton syringe. The applied drop clung to the tip of the coleoptile on the broad side, approximately centered between the two vascular bundles, and covered an area of about 2 mm in diameter. We did not control which side of the coleoptile received the drop. The solution evaporated rapidly—after about 5 min the drop was no longer visible. In some experiments a solution of 2.3 $\mu$M IAA and 1 mM NPA in 95% ethanol, 1% Triton X-100 was used to test the specificity of the observed movement of radioactivity.

At the time of application of the drop, and at 15 min intervals thereafter, plants were harvested. Their tips were wiped upwards several times, gently, with a Kimwipe soaked in 95% ethanol, 1% Triton X-100, and they were then sectioned into 1.1 mm pieces. Preliminary experiments indicated that the amounts of radioactivity extractable from the cut sections with methanol and with ACS (Beckman Instruments) were not significantly different (data not shown). Individual coleoptile and mesocotyl pieces were therefore placed in 4 mL of ACS, allowed to extract overnight in the dark at room temperature, and counted the next day for 5 min each. Profiles of radioactivity in coleoptiles were averaged, and SE computed. Each transport experiment was performed on at least three separate days, with five to seven plants each day harvested for each time point.

We were interested in relating the amount of radioactivity in each counted section to the amount of exogenous IAA present there. We observed that the total uptake of radioactivity into coleoptiles declined as the dilution of [$^3$H]IAA aged, so we made up a new dilution of label for each series of transport experiments, and performed those experiments on d 1, 3, and 5 after the dilution of label, assaying equal numbers of plants each day.

[3] Abbreviations: NPA, naphthylphthalamic acid; ACS, aqueous counting scintillant.

### HPLC

Coleoptiles were cut 10 mm below the tip. Each one was placed upright in 300 $\mu$L of glass distilled water with the basal end down. After 30 min, coleoptiles were each given 0.5 $\mu$L of the 0.92 $\mu$M label solution and allowed to transport it for 3 h. Radioactivity in the water was concentrated by aqueous phase partitioning as follows. The water was collected, pooled, diluted with 0.2 M phosphate buffer (pH 8.5) to 4:1 sample:buffer, and washed twice with diethyl ether. These first ether washes were saved. The aqueous phase was made acid (about pH 3.0) by dilution with 2 N HCl to 5:1 sample:acid, and then shaken against ether two times again. The aqueous fraction was removed and saved. These second ether fractions were pooled and lyophilized nearly to dryness, and the residue was resuspended in a solution of 1 mM unlabeled IAA, 40% methanol, and 0.5% acetic acid for HPLC analysis. Because IAA is significantly soluble in ether only in the protonated form, it should have partitioned into the second ether fractions.

Ether partitioning is often used as a first step in the purification of IAA from other related compounds; however, it also effectively concentrates the IAA in the sample. The first ether washes and the aqueous phase after the acid partitioning were counted to determine whether we were purifying or merely concentrating the radioactivity passed by the coleoptile. The amounts of radioactivity in these fractions were always very small relative to the amount in the final sample, so we believe that the phase partitioning simply concentrated the labeled material.

Samples were run over a reverse phase C-18 column (150 mm Econosphere, 3 $\mu$m bead size, Alltech, Los Altos, CA) in 40% methanol, 0.5% acetic acid, at a flow rate of 0.5 mL/min. Unlabeled IAA was detected by its $A$ at 280 nm, and [$^3$H]IAA was detected by counting fractions taken every 0.4 min from the column efflux. The results were corrected for the difference in time between detection by absorbance and detection by counting the efflux: the analysis was performed on mixtures of authentic IAA and [$^3$H]IAA and the major peaks of absorbance and radioactivity were aligned.

### Efflux Determinations

Coleoptiles were cut 10 mm below the tip. Each coleoptile was placed with just the basal 2 or 3 mm into 300 $\mu$L of glass distilled water. About 30 min later, each coleoptile was given 0.5 $\mu$L of 0.92 $\mu$M [$^3$H]IAA solution. Immediately, and at 10 min intervals thereafter, batches of plants were removed from the water they sat in. The water for each individual plant was put into 4 mL ACS, left in the dark overnight at room temperature, and counted for 5 min the next day. Time courses were averaged, and SE were determined.

### Phototropic Fluence Response

Coleoptiles were chosen to be straight and upright at the base for these experiments. Preliminary experiments determined that phototropic curvature reaches its maximum at about 120 min after irradiation in these plants, and begins to fall off after about 140 min (data not shown). We chose 110 min after irradiation as the time to harvest.

Coleoptiles were exposed to 30 s of blue light. The light source was a 300 W incandescent bulb in a slide projector.

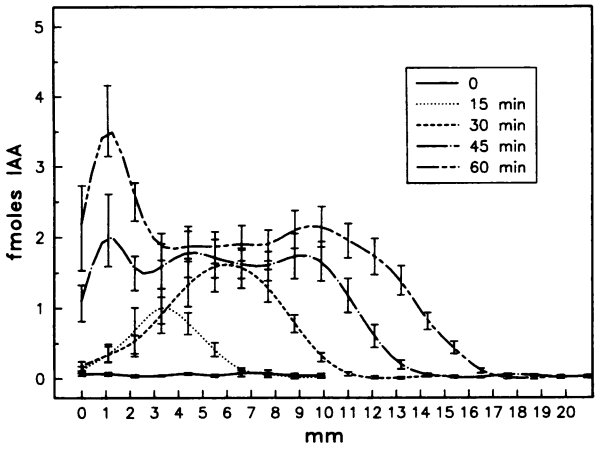


**Figure 1.** Transport of 0.92 μM [$^3$H]IAA over 60 min. Each line represents an average of 15 plants, 5 taken on each of 3 separate days. The *error bars* represent the SEM for each section. The *y*-axis refers to fmol of exogenously added IAA present in each section.

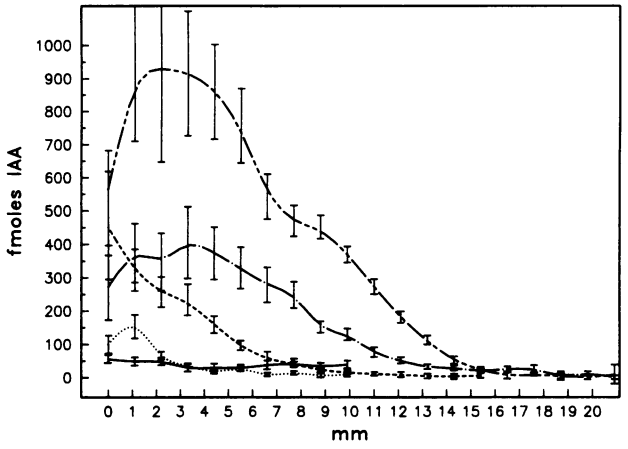


**Figure 3.** Transport of 230 μM IAA over 60 min. Each line represents the average of 14 or 15 plants (see legend for Fig. 1). *Error bars* represent the SEM for each point. Line styles represent time points as in Figure 1.

The light was filtered through a broad band-pass blue filter (Corning B5-60, 5 mm thick), and a heat absorbing filter (kg1, Technical Instrument Co., San Francisco, CA). The narrow side of the coleoptile was illuminated along its whole length, so that the direction of irradiation was parallel with the plane through the vascular bundles, and normal to any gradients of label that may have been caused by the asymmetric placement of the drop of label. Fluence rates were adjusted with reflective neutral density filters (Balzers, Marborough, MA) and were checked with a quantum radiometer (Li-185A, LiCor Corporation).

When IAA was given to plants to determine its effects on phototropic responsiveness, it was applied as before to the tip of the coleoptile 15 min before the beginning of the phototropic stimulus.

At the time of harvest, seedlings were cut off at the base, taped to a thin piece of plexiglass with the direction of irradiation parallel to the plane of the plexiglass, and photocopied at 141% enlargement. Lines were drawn parallel to the apical and basal approximately 0.5 cm, and the angles between the lines were measured on a digitizer pad (Hewlett-Packard 9111A Graphics Tablet, Hewlett-Packard, Sunnyvale, CA).

### Time Course of Gravitropism

As in the phototropism experiments, plants were chosen to be straight at the base. The beakers in which they grew were turned horizontal and taped in place, so that the plane through the vascular bundles was parallel with gravity. Immediately, and at 10 min intervals thereafter, plants were harvested and their curvatures determined as in the phototropism experiments. When IAA was given to determine its effect on gravi-

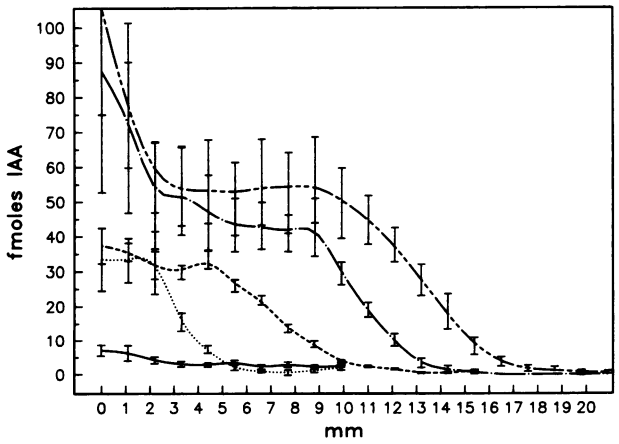


**Figure 2.** Transport of 23 μM IAA over 60 min. Each line represents the average of 15 plants (see legend for Fig. 1). As in Figure 1, the first curve from the left shows the average at 0 min, the next at 15 min, and so on to 60 min for the curve at the far right. Line styles represent time points as in Figure 1. *Error bars* show the SEM for each point.

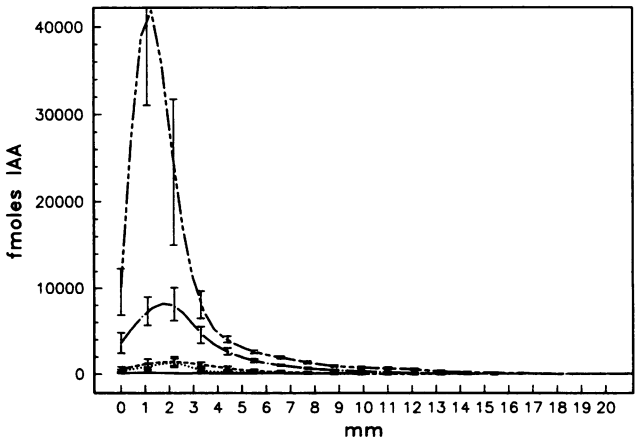


**Figure 4.** Transport of 2.3 mM IAA over 60 min. Each line represents the average of 20 plants, 6 or 7 taken on each of three separate days. *Error bars* represent the SEM for each point. Line styles represent time points as in Figure 1.

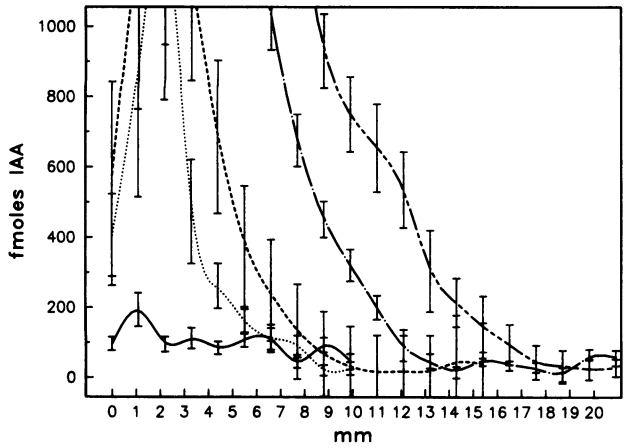


**Figure 5.** A 50-fold *y*-axis expansion of Figure 4.

tropic responsiveness, it was applied as before to the tip of the coleoptile 15 min before the plants were turned horizontal.

## RESULTS

### Transport of IAA

Figures 1 through 4 show the transport of IAA over a period of an hour, applied as 0.5 μL drops of 0.92 μM, 23 μM, 230 μM, and 2.3 mM auxin solutions, respectively. Figure 5 is identical to Figure 4, but the *y*-axis has been expanded 50-fold. At all concentrations the advancing edge of the transport pulse maintains its shape. The fronts of the profiles taken at different times are approximately parallel, so we calculate the velocity of IAA transport as the rate of movement of the *x*-intercept of the line drawn down the front of the transport pulse. By this definition of transport velocity, coleoptiles transport auxin about 20 mm/h at the apex, and the velocity declines down the coleoptile to about 12 mm/h as the front enters the mesocotyl node (this last rate calculated for the 45 to 60 min period after the application of auxin to the tip). These velocities do not vary with the concentration of added IAA.

The fraction of applied radioactivity that the plants absorb over 60 min, 6 to 8%, also does not vary with the concentration of applied IAA, over a range of more than four orders of magnitude. This result indicates that the IAA probably enters the cuticle and initial cell layers of the coleoptile by diffusion.

An interesting feature of the results shown in Figure 1, representing the transport of the lowest concentration of IAA used, is the shape of the transport profile at 15 and 30 min after the application of auxin. While IAA is moving into the coleoptile at a fairly constant rate, the radioactivity in the coleoptile appears to move as a pulse. This apparent pulse movement may result from the superposition of three rates of transport: the slow diffusion of IAA into the coleoptile, the swifter velocity of transport of auxin through the apical tissues, and the declining velocity of IAA transport down the length of the coleoptile.

Coleoptiles given small amounts of IAA (Figs. 1 and 2) transport it quickly out of the tip into a transport stream that reaches a roughly steady-state concentration between 30 and 45 min. The flux of IAA in the transport stream after this (approximately) steady state is reached is directly proportional to the concentration added—at 60 min, at 10 mm from the tip of the coleoptile, the flux of exogenous IAA is about 0.45 and 11 fmol/min for the 0.92 and 23 μM IAA solutions, respectively. These fluxes were calculated from the measured concentrations of labeled IAA per section and the velocity of transport of labeled IAA through the 10 mm region (from Fig. 1). The leveling of the transport stream probably does not represent a saturation of the transport capacity of the coleoptile, but rather a balance between the rates of movement of IAA into the tip and then down the coleoptile.

It should be noted here that these lower concentrations of added IAA only reach an approximately steady state flux. Figure 1 shows an apparent build-up of IAA just behind the wave front by 60 min. While the tip is transporting auxin at a constant flux, the velocity of transport declines down the stem. This means that the concentration of IAA in the transport stream must increase somewhat over time. Had we not observed an increase in labeled IAA concentration behind the front of transport, we might have inferred that IAA destruction or immobilization was occuring.

When larger amounts of IAA (Figs. 3 and 4) are given to coleoptiles the pattern of transport looks quite different. Figure 3 shows the pattern of transport of the 230 μM IAA over 60 min. The flux of IAA through the coleoptile 10 mm below the tip does not appear to reach a steady state, but increases throughout 60 min to about 96 fmoles/min, which is not quite the flux-to-concentration proportionality seen at 10 mm below the tip with the lower concentrations. The coleoptile is still taking up radioactivity at the same proportional rate as at lower concentrations, but is no longer transporting it immediately out of the tip.

The pattern of transport seen with the 2.3 mM IAA is even more extreme (Figs. 4 and 5). At 60 min the IAA flux at 10 mm below the tip is only about 180 fmoles/min, just about twice the flux seen with 10 times less added IAA. Most of the added label remains in the tip of the coleoptile. These results indicate that the IAA transport capacity of the tissue is at or nearing its limit, though the rate of transport does not differ

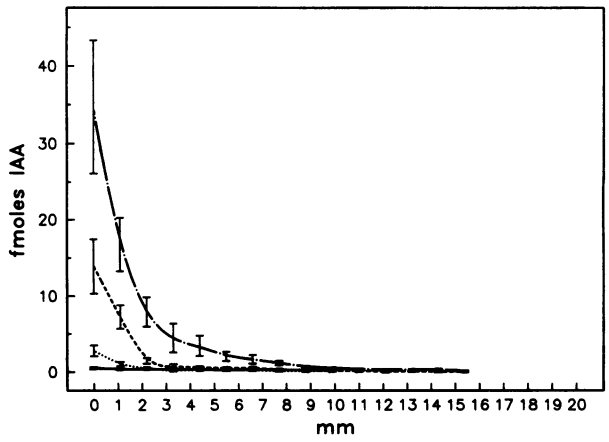


**Figure 6.** Transport of 2.3 μM [$^3$H]IAA over 45 min with 1 mM NPA included in the tracer solution. The sequence of lines represents 15 min time points between 0 min and 45 min. Each line represents the average of 15 plants (see legend for Fig. 1). *Error bars* show the SEM for each section.

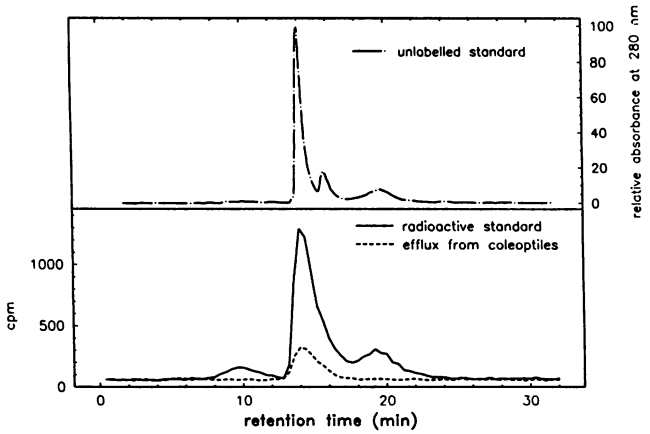


**Figure 7.** HPLC analysis of unlabeled authentic IAA standard, authentic [$^3$H]IAA standard, and radioactive efflux from cut coleoptiles given 0.5 $\mu$L of 0.92 $\mu$M [$^3$H]IAA. The profiles for the radioactive and unlabeled standards shown here were obtained simultaneously from a mixture of authentic labeled and unlabeled IAA. The profile for the collected efflux was obtained the same day, and the absorbance profile for the unlabeled IAA internal standard for that run was similar to the unlabeled IAA profile shown here.

from that of lower concentrations. These two higher concentrations of IAA had very obvious effects on the plants that received them. Seedlings clearly bent away from the side to which the drop was applied.

In the first 2 or 3 mm from the apex of the coleoptile, underneath where the drop of radioactivity was applied, the recoverable radioactivity is probably not all within the transport stream. The amount of radioactivity recoverable from these sections after wiping varied greatly from plant to plant, much more so than the radioactivity present farther down the coleoptile (see the error bars on these sections in Figs. 1–4). We suspect that some of this radioactivity represents material adhering to the cuticle or possibly a seeping of IAA into the coleoptile pore, for we did not control the side of the coleoptile that received the tracer.

Figure 6 shows the pattern of transport obtained when 1mM NPA is included in a solution of 2.3 $\mu$M IAA. The radioactivity does not move normally out of the tip of the coleoptile. Some appears to escape the transport block, however; by 30 min detectable amounts of the tracer are found in the lower parts of the coleoptile. IAA is a smaller and more freely diffusible molecule than NPA, so a small amount of IAA may be expected to escape NPA transport inhibition purely by diffusion. The movement of radioactivity illustrated in Figures 1 to 5 is therefore authentic NPA-sensitive IAA transport, and not seepage of the radioactivity down the outside of the coleoptile, or some internal nonspecific transport.

### HPLC Analysis

Historically, when radioactive IAA has been applied to coleoptile tissue and allowed to move through the tissue into a collection reservoir (either plain water or an agar block) the radioactivity has been found to be associated with authentic IAA, and not breakdown products or metabolites of IAA (6). Our results confirm this finding. Figure 7 shows the results of the HPLC analysis of radioactivity exported by the cut ends of coleoptiles into water over a 3 h period. The retention time of collected label is the same as those of the major peaks in the authentic tritiated and unlabeled IAA standards.

### Storage or Conjugation of the Transported IAA

From the time course of efflux of radioactivity from 10 mm cut coleoptiles into water, we can calculate the proportion of the extractable radioactivity that is available to be transported, and so, by subtraction, the proportion that is either in storage pools or conjugated into nontransportable but extractable forms. Figure 8 shows such a time course for efflux of radioactivity from coleoptiles cut to a length of 10 mm long, and given 0.5 $\mu$L of the 0.92 $\mu$M label. There is a lag period, of about 40 to 50 min, between the application of labeled IAA to the tip of the coleoptile and the significant appearance of radioactivity in the collecting water. From Figure 1 one can predict that after about 45 min the efflux of radioactivity into collecting water from 10 mm long cut coleoptiles should be fairly constant, perhaps rising slowly over time. Linear extrapolation of Figure 8 between 60 and 150 min gives an efflux rate of 20 ± 2 cpm/min. This error is the standard error of the slope, determined from the regression analysis.

From the raw cpm data for Figure 1, 80 ± 10 cpm above background was extractable from the 1.1 mm section at 10 mm below the coleoptile tip between 45 and 60 min after the start of transport. The velocity of transport was about 3.75 mm/15 min at 10 mm below the tip of the coleoptile. One can therefore calculate a flux of 18 ± 2 cpm/min, if all the radioactivity seen in Figure 1 is accessible to the transport stream. This flux is, within error, what we find in the efflux experiments. If auxin sequestering, conjugation into untransportable forms, or destruction is occurring in these coleoptiles, it is within the error of the measurements. We know from the

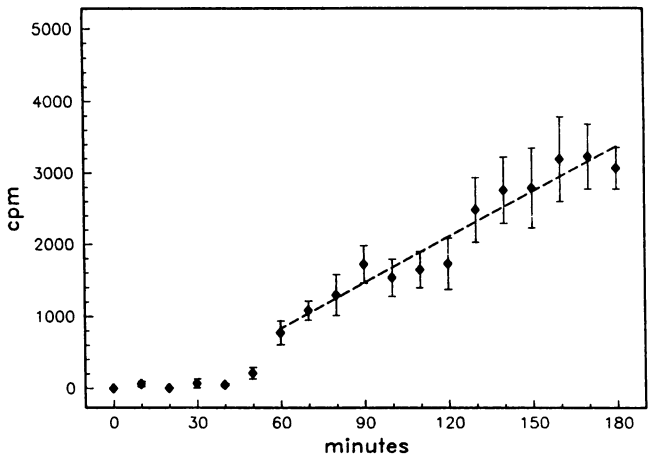


**Figure 8.** Efflux of radioactivity from cut coleoptiles. Coleoptiles were given 0.5 $\mu$L of the 0.92 $\mu$M [$^3$H]IAA at 0 min. Each point represents the average of 9 plants, 3 taken on each of three separate days. *Error bars* represent the SEM, and where they are not visible, they are smaller that the size of the symbols. The line represents the results of a linear regression performed on the data for 60 to 180 min after addition of label, $r^2 = 0.92$. Linear regression performed on a log transform of the data gave nearly identical results.

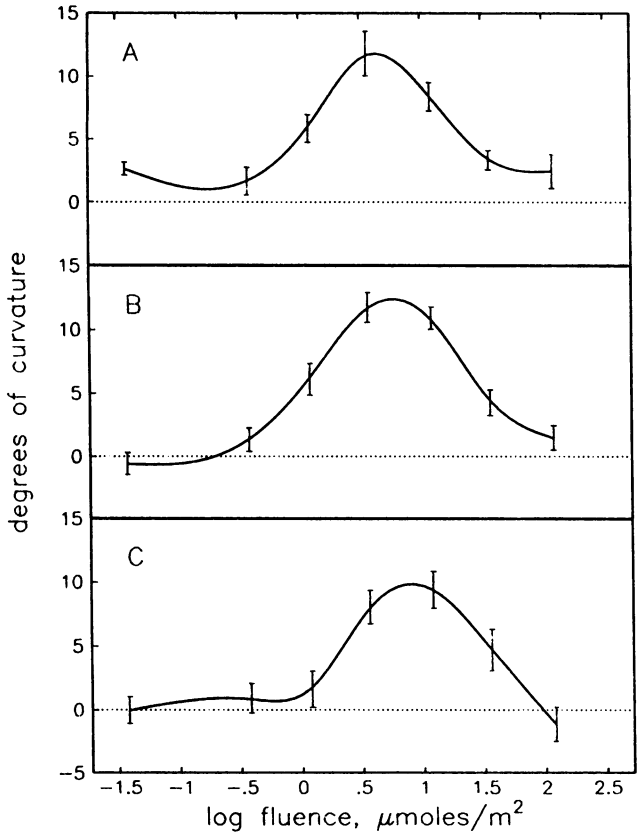


**Figure 9.** Phototropic fluence response of plants with and without prior IAA treatment. (A) Plants not pretreated; (B) plants given 0.5 μL of 0.92 μM [$^3$H]IAA 15 min before the phototropic stimulus; (C) plants given 0.5 μL of 23 μM IAA 15 min before the phototropic stimulus. Each curve represents the average of 30 to 40 plants, taken on 4 or 5 separate days. The *error bars* represent the SEM for each point.

HPLC analysis that the radioactivity that flows from cut coleoptiles is probably IAA. Therefore, the radioactivity in coleoptiles during transport experiments with the 0.92 μM labeled IAA is authentic IAA and is all, within the error of the calculations, in the transport stream.

### Effects of Added IAA on Phototropism and Gravitropism

We were interested in knowing whether, at the lowest concentrations of added IAA, we were observing transport in a physiologically normal plant, or whether the added auxin could be affecting the physiology of the plant; that is, whether, at least at the lowest IAA concentrations, we were observing the transport of a true tracer. Figure 9A shows the fluence response curve for first positive phototropism of untreated plants, harvested 110 min after irradiation.

Adding the solvent/detergent solution alone 15 min before irradiation has no effect on the fluence response characteristics of these plants (data not shown). Adding 0.5 μL of the 0.92 μM label likewise has no effect (Fig. 9B). Giving plants 0.5 μL of the 23 μM label appears to shift the fluence at which maximum curvature is obtainable by about half an order of magnitude to the less sensitive (Fig. 9C). We did not perform time courses for development of curvature for each addition of label, however, so it is possible that the added IAA altered the kinetics of development of curvature somewhat, instead of the sensitivity.

Figure 10A shows the time course of development of gravitropic curvature in untreated plants. As has been seen numerous times before (4, 12), an approximately 40 min lag period is followed by a steady rate of curvature through at least 3 h after the plants are turned horizontal. Adding 0.5 μL of the plain solvent/detergent mix once again has no discernable effect on this response (data not shown). In this case, the addition of 0.5 μL of the 23 μM IAA 15 min before the plants are turned horizontal also has no discernable effect on the kinetics of curvature, or the degree of curvature obtainable over 3 h (Fig. 10B).

## DISCUSSION

The transport of a tritiated IAA tracer down intact coleoptiles proceeds at a velocity that declines down the coleoptile from about 20 mm/h at the apex to about 12 mm/h near the mesocotyl node, and is sensitive to NPA. At the lowest concentration used, 0.92 μM IAA, the added IAA has no discernable effect on gravitropic or phototropic sensitivity or

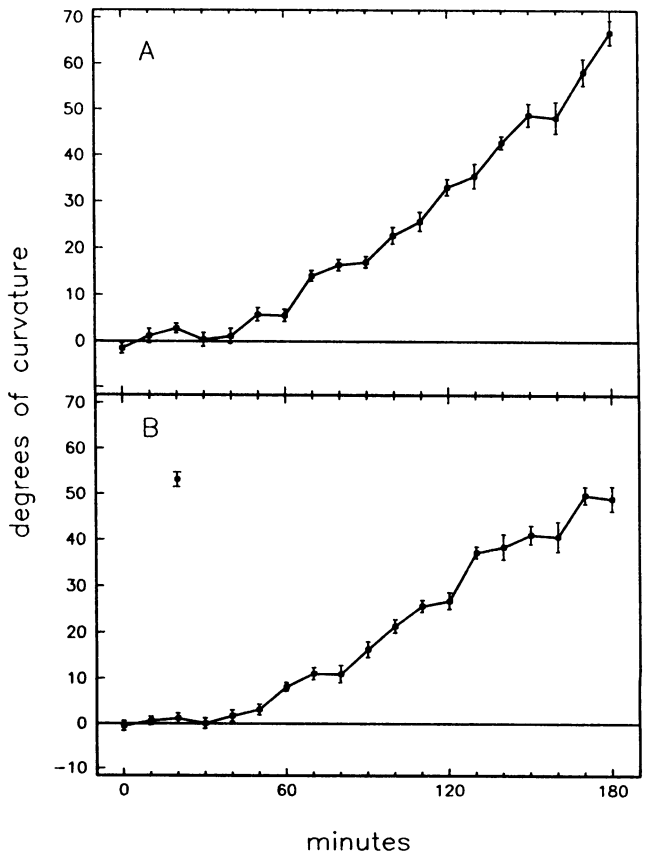


**Figure 10.** Gravitropic response of plants with and without prior IAA treatment. (A) Plants not pretreated; (B) plants given 0.5 μL of 23 μM IAA 15 min before they were turned horizontal at 0 min. Graph A represents the averages of 14 to 16 plants per point taken on three separate days. Graph B represents the averages of 19 to 21 plants per point taken on three separate days. The detached point represents the gravitropic curvature of 20 untreated control plants after 180 min. *Error bars* represent the SEM for each point.

magnitude of response over a standard time interval. As much as 25 times that concentration has no effect on the degree of curvature obtainable during gravitropism, or the kinetic of development of gravitropic curvature. So when we follow the movement of 0.92 $\mu$M IAA applied in a 0.5 $\mu$L drop to the tip of a coleoptile, we are probably seeing a pattern of transport that is representative of the transport of endogenous IAA within the normal coleoptile. We could not detect tracer immobilization or destruction.

The patterns of transport we see with the higher concentrations of IAA are consistent with those reported by other investigators. Newman (8) reported that when [$^{14}$C]IAA is supplied in an agar block to the tip of a decapitated oat coleoptile, the shape of the transport pattern within the tissue varies with the concentration of IAA added. He saw radioactivity decreasing approximately linearily from the tip to the base with the lowest concentrations of added IAA (0.3 $\mu$M), a pattern similar to that shown in Figure 3, and decreasing approximately exponentially with higher concentrations (up to 7 $\mu$M), a pattern similar to that shown in Figure 4.

Like Newman, we have seen changes in the pattern of auxin transport with increasing concentrations of added IAA. Why does the shape of the transport curves change over a concentration range within which the transport capacity of the tissue is not yet at its maximum? Rayle *et al.* (10) reported that the uptake of labeled IAA into tissues that have been pretreated with cold IAA is increased with respect to tissues that have not been pretreated. They also saw that the velocity of movement of the front of a pulse of radioactive IAA through pretreated tissue is the same as through untreated tissue, so pretreatment of coleoptiles with cold IAA increases their capacity to take up and transport labeled IAA subsequently added, but not the velocity at which that labeled IAA flows. It may be that large amounts of added IAA increase the flux capacity of the transport system within the time period of the experiment. These observations underscore the necessity of using very small, physiologically inactive, quantities of tracer to study normal auxin transport.

In Figure 4 it appears that the coleoptile is nearing the limit of its uptake capacity—most of the added IAA is diffusing into the tip of the coleoptile and not being transported out. We do not know whether the radioactivity in the tip of the coleoptiles represented in Figures 3 and 4 is all mobile IAA, or whether some of it is diverted off the overloaded transport stream into storage pools, or into conjugated forms.

Johnson and Morris (7) have recently published a report on the *in vivo* transport of IAA in pea seedlings. Among other experiments, they applied tritiated IAA mixed with varying concentrations of unlabeled IAA in lanolin to the buds. They waited for 6 h, then determined the distribution of radioactivity down the length of the hypocotyl. They found that the distance the front of radioactivity moved over 6 h was invariant with the concentration of added IAA. The shape of the transport profile, however, changed with IAA concentration. The data reported in Figure 3 of that paper also seem to show that, as in our experiments, the ratio of the flux of transported IAA to the concentration of added IAA drops as the concentration of added IAA is greatly increased.

In conclusion, we have characterized the *in vivo* transport of IAA in corn coleoptiles with a view to investigating the role of IAA transport in coleoptile gravitropism and phototropism. At the lowest concentration of IAA added, we observed a pattern of transport different from that reported before, probably as a result of using a concentration of tracer that does not alter the physiology of the tissue to which it has been added. At the higher concentrations of added IAA, our results are similar to those reported previously both in oat and pea. It should now be possible to study *in vivo* auxin transport in corn coleoptiles during phototropism and gravitropism with reasonable temporal and spatial resolution. With more information on the concentration and metabolism of endogenous IAA over time, we will be able to determine the normal movement of IAA through a coleoptile, and so be able to evaluate the significance of any changes we may see in the transport of tracer IAA during light- or gravity-regulated changes in growth pattern.

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