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# The Decrease in Auxin Polar Transport Down the Lupin Hypocotyl Could Produce the Indole-3-Acetic Acid Distribution Responsible for the Elongation Growth Pattern

**José Sánchez-Bravo*, Ana M Ortuño, Juana M Botía, Manuel Acosta, and Francisco Sabater**

*Department of Biología Vegetal, Faculty of Biología, University of Murcia, E-30001, Murcia, Spain*

## ABSTRACT

**The variation of indole-3-acetic acid (IAA) transport along *Lupinus albus* L. hypocotyls was studied using decapitated seedlings and excised sections. To confirm that the mobile species was IAA and not IAA metabolites, dual isotope-labeled IAAs, [5-$^3$H]IAA + [1-$^{14}$C]IAA, were used. After apical application to decapitated seedlings, the longitudinal distribution of both isotopes at different transport periods showed that the velocity of IAA transport was higher in the apical, elongating region than in the basal, nongrowing region. This variation in velocity was not a traumatic consequence of decapitation because after application of IAA to the basal region of decapitated seedlings, both the velocity and intensity of IAA transport were lower than in the apical treatment. The variation in IAA transport down the hypocotyl was confirmed when it was measured in excised sections located at different positions along the hypocotyl. The velocity and, to a greater extent, the intensity of IAA transport decreased from the apical to the basal sections. Consequently, if the amount of IAA reaching the apical zones of lupin hypocotyl were higher than the IAA transport capacity in the basal zones, accumulation of mobile IAA might be expected in zones located above the basal region. In fact, an IAA accumulation occurred in the elongating region during the first 4-h period of transport after apical treatment with IAA. It is proposed that the fall in IAA transport along the hypocotyl might be responsible for the IAA distribution and, consequently, for the growth distribution reported in this organ. An indirect proof of this was obtained from experiments that showed that the excision of the slowly transporting basal zones strongly reduced the growth in the remaining part of the organ, whereas excision of the root caused no significant modification in growth during a 20-h period.**

The frequently described correlations between the longitudinal distribution of IAA and growth in stem axes suggest that in some tissues, cell elongation is regulated mainly by the endogenous levels of auxin (15, 18, 22, 26, 28, 29). In plant systems, such as coleoptiles or hypocotyls, the apex is the main source of auxin. Therefore, it can be suspected that basipetal polar auxin transport might be involved in supplying auxin for growth in these systems. Auxin transport has been intensively investigated for decades, and several hypotheses have been formulated to account for the characteristics of the process (see reviews by Goldsmith [2], Kaldewey [7], Rubery [17]). However, the actual mechanism responsible for IAA distribution, which presumably causes the growth distribution, remains uncertain.

Contrary to those reported for sunflower hypocotyls (1), some data suggest that mobile auxin is involved in the regulation of elongation growth in etiolated lupin hypocotyls. Thus, [5-$^3$H]IAA-fed decapitated seedlings showed a wave of unaltered IAA in the elongation zone that correlated with the distribution of growth (12), as well as with the distribution of endogenous IAA content (18). Moreover, in a recent article (20), we showed that radiolabeled IAA polarly transported in the stele moved sideways to the outer tissues, where the IAA-sensitive cell growth seems to be located (8, 14, 16), and maintained normal growth in decapitated lupin hypocotyl.

The concept of a continuous stream of auxin molecules moving at a uniform and constant v[1] and I is based on the pioneering studies of van der Weij (27). This picture of polar transport has been challenged by much evidence that points to a considerable flexibility of the auxin transport system. Most of this evidence is derived from studies that showed variations in the transport characteristics along the axes of corn and oat coleoptiles (3, 11, 23, 24), pea seedlings (22), sunflower hypocotyls (25), and prefloral flower stalks of *Fritillaria* (5, 6). Recently, Parker and Briggs (13) showed that the transport of tritiated IAA down intact corn coleoptiles proceeds at a velocity that declines from 20 mm $h^{-1}$ at the apex to 12 mm $h^{-1}$ near the mesocotyl node. These velocities do not vary with the concentration of added IAA (between 0.92 $\mu$M and 2.3 mM IAA). Since, at the lowest concentration used, the added IAA has no effect on graviotropic or phototropic response, the authors conclude that the observed pattern of transport is representative of the transport of endogenous IAA.

Whether the variation of IAA polar transport can be the cause of the growth-correlated IAA distribution reported in lupin hypocotyls (12, 18) is the subject of the present article. To check this hypothesis, we have studied the transport of IAA in two systems: decapitated seedlings and excised sections located at different positions along the hypocotyl. Because previous reports showed that lupin hypocotyls exhibited a high capacity to metabolize exogenously added IAA (19–21), experiments applying a double isotope treatment ([5-$^3$H]IAA and [1-$^{14}$C]IAA) were carried out to confirm that the mobile species was IAA and not IAA metabolites. Using this procedure, it was also possible to confirm that the described changes in the capacity to metabolize IAA along the

[1] Abbreviations: v and I, velocity and intensity of IAA polar transport.

hypocotyl (19, 20) do not interfere with the possible variation of IAA transport down the organ.

## MATERIALS AND METHODS

### Plant Material and Measurement of Growth

Seeds of *Lupinus albus* L. cv Multolupa were allowed to imbibe for 24 h in water and were grown in damp vermiculite at 25 ± 0.5°C in darkness. All experiments were carried out on uniform 6-d-old seedlings. At this age, the hypocotyls (65 ± 5 mm in length) were marked with ink every 5 mm. The growth of each zone was expressed as the mean relative elongation (in percent). Decapitated plants were obtained by cutting the hypocotyl starting 5 (for apical treatment) or 35 mm (for basal treatment) from the cotyledons.

To study the effect produced by excision of the root and the basal zones on hypocotyl growth, seedlings were marked with ink every 10 mm and placed vertically in glass tubes containing 0.01 M potassium phosphate buffer, pH 6.4. To avoid disruption of the xylem stream, the cut zone remained immersed in the buffer during the cutting. Sectioned plants were hung by their cotyledons from the edge of tubes, and the basal 5 mm remaining were immersed in the buffer. In control plants, the root was immersed in the buffer. During the growth period, plants were kept at 25 ± 0.5°C in darkness. At different times, the length of the zones was measured without removing the plants from the buffer.

### Application of Labeled IAA to Decapitated Seedlings and Extraction of Radioactivity

A drop (10 μL) of aqueous solution containing 1.54 kBq of [5-$^3$H]IAA (specific activity 925 GBq mmol$^{-1}$, 0.17 μM) and 0.78 kBq of [1-$^{14}$C]IAA (specific activity 2.18 GBq mmol$^{-1}$, 36 μM) was applied either to the apical or to the basal cut surface of decapitated seedlings. To prevent the loss of basipetal polar transport of IAA produced by decapitation (10), IAA treatments were carried out immediately after decapitation. The plants were kept in the culture trays during treatment under the conditions indicated above. At different time periods, the hypocotyls were divided into sections of 5 mm. Samples of four sections with the same localization in four different plants were extracted with acetonitrile as described by Sánchez-Bravo et al. (21). A HPLC analysis of the acetonitrile extracts (21) showed that $^{14}$C activity corresponds mainly to unaltered IAA, whereas $^3$H activity includes decarboxylation products and a small percentage of IAA (3%) conjugates in addition to IAA. According to this, a decrease of the ratio of $^{14}$C to $^3$H in the acetonitrile extracts compared to the ratio in the applied IAA solution can be considered as an index of IAA decarboxylation (19), as was already demonstrated (21). From the distribution of radioactivity along the hypocotyls, the velocity of IAA transport was calculated either from the displacement of the radioactive pulse or from the position of the radioactive front. In the latter case, the velocity of IAA transport was calculated according to Parker and Briggs (13) as the rate of movement of the $x$ intercept of the line drawn down the front of the radioactive profiles.

### IAA Transport in Excised Sections of the Hypocotyls

The transport of IAA in hypocotyl sections was studied by the intercept method of van der Weij (27) with some modifications. Sections (15 mm long) from different locations along the hypocotyl (see diagram in Table I) were treated with 10 μL of aqueous solution containing 1.47 KBq of [5-$^3$H]IAA (specific activity 803 GBq mmol$^{-1}$, 0.18 μM) and 0.8 KBq of [1-$^{14}$C]IAA (specific activity 2.04 GBq mmol$^{-1}$, 39 μM). IAA was applied to the apical cut surface of the sections 10 to 15 min after cutting to prevent the loss of IAA polar transport capacity of the sections (10). The application of IAA in a drop placed on the cut surface of sections prevents the intensive IAA metabolism observed when IAA is applied through a donor agar block (19). The basal end of the sections was put on a cylindrical (8 mm diameter, 5 mm thickness) receiver block of agar (1%). Sections were maintained vertically with the aid of a conical plastic tube, whose maximal and minimal diameters were the same as those of the agar block and the hypocotyl, respectively. During the transport period (up to 8 h), the sections were kept in darkness at 25 ± 0.5°C. At different times, the agar block was replaced. The same transport sections were used for different time periods. Radioactivity in the receiver agar blocks was extracted with 0.5 mL of 96% ethanol for 2 h at 4°C in darkness. The radioactivities of replaced agar blocks were totaled and plotted against time, thus obtaining the transport curves (Fig. 3). The linear parts of these curves were adjusted by the least squares method. From the linear regression equations, the intercepts with the time axis ($t_o$) served to determine v (in mm h$^{-1}$): length of sections (15 mm)/$t_o$. The slope of the equations represents I (in Bq h$^{-1}$).

### Radioactivity Measurement

The activity of $^{14}$C and $^3$H in the acetonitrile extracts was measured in a Rack Beta model 1211 liquid scintillation counter (LKB, Turku, Finland). The scintillation solution and the channels used for the simultaneous counting of both isotopes were as described previously (19).

### Chemicals and Radiochemicals

Radioactive IAA, [1-$^{14}$C]IAA and [5-$^3$H]IAA, were obtained from Amersham International (Amersham, Buckinghamshire, UK). Aqueous solutions were obtained as described by Sánchez-Bravo et al. (19). The radiochemical purity was checked periodically by TLC and only unaltered solutions were used. Unlabeled IAA was from Merck (Darmstadt, Germany). Acetonitrile was HPLC grade. Other reagents and solvents were of analytical grade.

## RESULTS

### Variation in IAA Transport along the Hypocotyl of Decapitated Seedlings

When IAA was applied to the apical cut surface of decapitated seedlings, the distribution of $^{14}$C (mainly unaltered IAA) was similar to that of $^3$H (Fig. 1). Figure 1 suggests that the velocity of basipetal movement of both isotopes varied

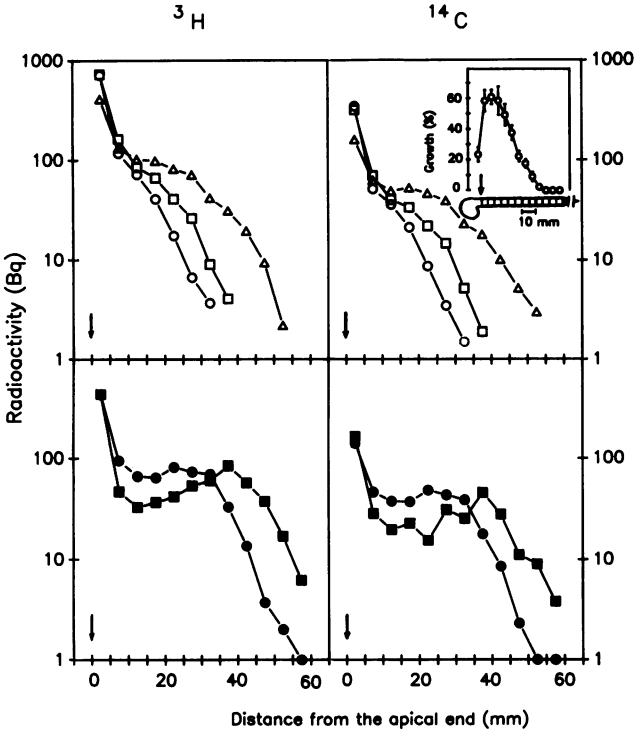



**Figure 1.** Time course of $^3$H and $^{14}$C movement in lupin hypocotyls. Longitudinal distribution of $^3$H (left) and $^{14}$C (right) measured in the acetonitrile extracts after the application of [5-$^3$H]IAA + [1-$^{14}$C]IAA to the apical cut surface of decapitated seedlings obtained at different times: 1 h (○); 2 h (□); 4 h (△); 6 h (●); and 8 h (■). Data correspond to pooled samples from four plants and are expressed as Bq per 5-mm section at each time. Longitudinal distribution of growth during 24 h (from age 6 to 7 d) in intact plants is indicated (inset). Data represent the mean relative elongation of four plants ± SD. The arrows denote the point of decapitation.

along the hypocotyl. Thus, in the basal 15-mm region, v of the radioactive fronts between 6 and 8 h was about 5 mm $h^{-1}$, whereas in the immediately superior zone, this v was 7.5 mm $h^{-1}$ (calculated for the 2- to 4-h period after IAA application), very close to the v of the radioactive pulse, which moved a distance of 15 mm between 6 and 8 h. No radioactive pulse could be detected along the hypocotyl before 6 h due to the accumulation of $^{14}$C and $^3$H in the elongating region (see inset in Fig. 1) during this period. Therefore, it was not possible to calculate the v of the radioactive pulse in the apical fast-growing region. According to the position of the radioactive fronts, average vs of 30, 20, 14, 9, and 7.5 mm $h^{-1}$ could be deduced for 1, 2, 4, 6, and 8 h of transport, respectively, for both isotopes. That is to say, v decreased as the IAA transport progressed down the hypocotyl.

After application of IAA to the cut surface of the basal region of decapitated seedlings, the distribution of $^{14}$C and $^3$H showed minor changes during the first 2 h of transport (Fig. 2). During this time, the radioactive fronts moved about 20 mm, that is, half the distance run by the fronts during the same period when IAA was applied to the apical zone (Fig. 1). From the above, it can be concluded that v was higher in the apical growing region than in the basal, nongrowing region of the hypocotyl.

I also varied along the hypocotyl. Thus, radioactivity extracted at 2 h from the application zone (first 5-mm section from the cut end) was higher in the basal treatment (949 Bq of $^3$H and 414 Bq of $^{14}$C, Fig. 2) than in the apical treatment (735 Bq of $^3$H and 324 Bq of $^{14}$C, Fig. 1). The double isotope treatment permitted us to conclude that the results obtained in these assays cannot be imputed to a different capacity to metabolize IAA between the apical and the basal zones. In fact, the ratio of $^{14}$C to $^3$H extracted from the application zone was very close in both assays, and, consequently, the lower activity of $^{14}$C in the apical treatment was not due to a greater decarboxylation of IAA in the apical zone compared to the basal zone. Obviously, the lower activity of $^3$H in the apical treatment corroborates this conclusion. Taking into account that in both assays, the radioactivity recovered at 2 h in the acetonitrile extract was similar (about 70% of $^3$H applied) and that the radioactivity reaching the roots was undetectable, the radioactivity exported from the application zone as a percentage of radioactivity recovered in the whole hypocotyl was 23.0% $^3$H and 24.6% $^{14}$C for the basal treatment, and 34.3% $^3$H and 37.3% $^{14}$C for the apical treatment. The results of these experiments clearly indicate that I was higher in the apical than in the basal region of the hypocotyl.

## Transport of IAA in Excised Sections Located at Different Positions along the Hypocotyl

The intercept method of van der Weij (27) was used to evaluate IAA transport in zones excised from the hypocotyl and apically treated with [1-$^{14}$C]IAA + [5-$^3$H]IAA (see graph in Table I). No significant differences were detected in the v of both isotopes, $^3$H and $^{14}$C, in the same zone (Table I). On the other hand, some discrepancies were observed between

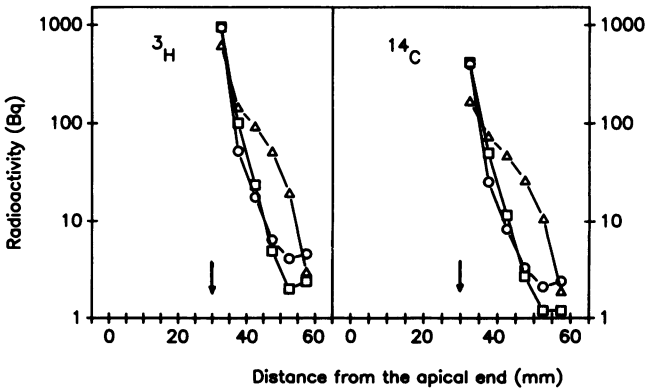
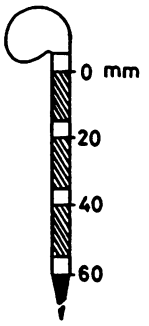



**Figure 2.** Time course of $^3$H and $^{14}$C movement in lupin hypocotyls. Longitudinal distribution of $^3$H (left) and $^{14}$C (right) measured in the acetonitrile extracts after application of [5-$^3$H]IAA + [1-$^{14}$C]IAA to the cut surface of the basal region of decapitated seedlings obtained at different times: 1 h (○); 2 h (□); and 4 h (△). Data correspond to pooled samples of four plants and are expressed as Bq per 5-mm section. The arrows indicate the point of decapitation where IAA was applied.

**Table I.** *I and v in Sections Excised from Different Regions of the Lupin Hypocotyls*

The diagram shows the location in the hypocotyl of the sections (shaded zones). According to the intercept method of van der Weij (25), data of v and I for $^{3}H$ and $^{14}C$ were obtained from the equations given in the legend to Figure 3, as described in "Materials and Methods." From the values of $^{14}C$ I, and taking into account the initial value of the $^{14}C$:$^{3}H$ ratio added to the sections (0.54), the value of the $^{3}H$ I′ that might be expected if decarboxylation products (tritiated) remained immobilized in the section was calculated.




| Location | $^{14}C$ | | $^{3}H$ | | |
|---|---|---|---|---|---|
| | v | I | v | I | I′ |
| | *mm h$^{-1}$* | *Bq h$^{-1}$* | *mm h$^{-1}$* | *Bq h$^{-1}$* | *Bq h$^{-1}$* |
| Apical | 6.1 | 24.8 | 6.1 | 53.4 | 45.9 |
| Middle | 5.3 | 16.0 | 5.1 | 35.2 | 29.6 |
| Basal | 4.3 | 8.9 | 4.2 | 21.3 | 16.5 |

the values of $^{14}C$ and $^{3}H$ I in the same zone. The ratio of $^{14}C$ to $^{3}H$ in the radioactivity accumulated in the receiver agar blocks up to 5 h of transport (Fig. 3) was in general very close to that in the IAA solution applied (0.54), suggesting that the species mobilized was unaltered IAA. After longer periods of transport, the ratio decreased, perhaps due to the diffusion of decarboxylated products (tritiated) from the basal end of the sections to the receiver block. This might be the reason for I being slightly higher than I′ according to the data for $^{14}C$. Table I shows that v and, to a greater extent, I of both $^{3}H$ and $^{14}C$ transport decreased from the apical to the basal zones, the difference in I being double the difference in v. In the apical zone, the v was 1.4 times and the I 2.8 times higher than in the basal zone.

### Effect on Hypocotyl Growth Produced by the Excision of the Root and the Basal Hypocotyl Zones

Table II shows the elongation at different times of the remaining part of the hypocotyl after excision of the root and basal hypocotyl zones of variable length. Although the excision of the root did not modify hypocotyl growth compared with the control during the first 20 h, the excision of basal zones produced a significant decrease in hypocotyl elongation so that the longer the zone excised, the greater the reduction in growth. As regards the distribution of growth in control plants after a 20-h period (Fig. 4), the basal 10- or 25-mm zones exhibited almost no growth. Therefore, the reduction in growth produced by excision of these zones was not due to the elimination of elongating cells. Although the basal 35- and 45-mm zones contained growing cells (Fig. 4), the growth in the remaining part of the hypocotyl after excision of both zones was lower than the growth exhibited by the equivalent part of the hypocotyl in control plants (compare data under Δ growth and in Table II).

After excision of the basal zones, growth was maintained for a period of time that depended on the length of the

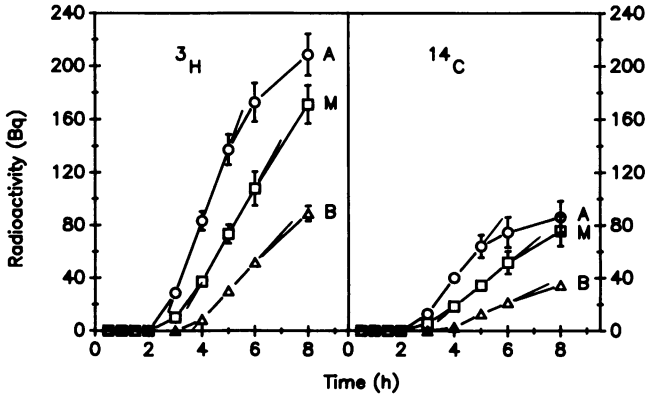



**Figure 3.** Time course of basipetal IAA transport through sections excised from different regions of lupin hypocotyls. The accumulated activity of $^{3}H$ (left) and $^{14}C$ (right) in the agar receivers at different times of transport after application of [5-$^{3}H$]IAA + [1-$^{14}C$]IAA to the apical cut end of the sections is shown. Sections (15 mm long) located at different positions along the hypocotyl were used: apical (A, O), middle (M, □), and basal (B, Δ) (see diagram in Table I). Data represent the mean values of three sections. Vertical bars denote SD when larger than symbols. The linear parts of the transport curves were adjusted by the least-squares method.

The following equations were obtained for $^{3}H$ transport:
Zone A $y = (53.4 \pm 0.6)\ x - (131.0 \pm 0.8)$ ($r = 0.998$)
Zone M $y = (35.3 \pm 5.0)\ x - (103.7 \pm 7.1)$ ($r = 0.980$)
Zone B $y = (21.4 \pm 0.1)\ x - (75.6 \pm 0.1)$ ($r = 0.985$).

The following equations were obtained for $^{14}C$ transport:
Zone A $y = (24.8 \pm 0.2)\ x - (61.0 \pm 0.2)$ ($r = 0.998$)
Zone M $y = (16.1 \pm 3.0)\ x - (45.3 \pm 4.3)$ ($r = 0.987$)
Zone B $y = (8.9 \pm 0.1)\ x - (31.1 \pm 0.1)$ ($r = 0.997$).

**Table II.** *Effect of the Excision of the Root and Basal Hypocotyl Zones on the Growth of the Remaining Part of Lupin Hypocotyls*

The root and basal hypocotyl zones of variable length (10, 25, 35, or 45 mm) were excised in 6-d-old seedlings as described in "Materials and Methods." The basal 5 mm of treated plants and the root in control plants were immersed in 10 mM (pH 6.4) phosphate buffer. The initial length of the hypocotyls after treatment, measured starting 5 mm from the cotyledons, is indicated ($L_0$). At different times, the growth (elongation) of the remaining part of the hypocotyls (the apical 5 mm excluded) was measured. Data correspond to the mean value ± SD of five plants. Variation of growth (Δ) at 20 h was calculated as percentage of increase (+) or decrease (−) in relation to the growth exhibited by control plants.

| Treatment | $L_0$ | Elongation | | | | Δ Growth | |
|---|---|---|---|---|---|---|---|
| | | 4 h | 6 h | 8 h | 20 h | Percent[a] | Percent[b] |
| | *mm* | *mm* | | | | | |
| Control | 58.5 ± 6.7 | 1.8 ± 0.3 | 2.8 ± 0.3 | 3.8 ± 0.2 | 7.2 ± 0.6 | | |
| −Root | 58.8 ± 9.4 | 2.4 ± 0.6 | 3.2 ± 0.9 | 4.4 ± 0.6 | 7.5 ± 1.5 | +4.2 | +4.2 |
| −10 mm | 48.1 ± 7.2 | 1.3 ± 0.2 | 2.3 ± 0.8 | 3.0 ± 1.0 | 4.1 ± 1.2 | −43.0 | −43.0 |
| −25 mm | 32.7 ± 4.4 | 1.4 ± 0.9 | 2.1 ± 1.0 | 2.5 ± 1.0 | 2.8 ± 0.8 | −61.1 | −58.3 |
| −35 mm | 22.6 ± 3.3 | 0.6 ± 0.9 | 1.2 ± 0.7 | 1.4 ± 0.7 | 1.5 ± 0.6 | −79.2 | −73.5 |
| −45 mm | 12.0 ± 2.4 | 0.3 ± 0.4 | 0.5 ± 0.6 | 0.6 ± 0.7 | 0.7 ± 0.7 | −90.3 | −74.0 |

[a] Percentage calculated on the basis of the whole hypocotyl growth; [b] Percentage calculated on the basis of the growth in a hypocotyl region with identical location and size ($L_0$).

excised zone. According to Table II, the longer the zone excised, the shorter the growth period. This fact confirms that the suppression of basal zones was the real cause of growth reduction in the remaining part of the hypocotyl. The distribution of growth was also modified after excision of the basal zones (Fig. 4). The reduction in growth was first detected in the first subapical cm. When the length of the excised zone increased, growth in the second cm was also reduced.

## DISCUSSION

The experiments described here show that the v of basipetal IAA movement in decapitated seedlings and in excised sections of etiolated lupin hypocotyls is in the range of 3 to 30 mm $h^{-1}$. This agrees with the v of polar IAA transport measured in other plant materials (see reviews by Goldsmith [2] and Kaldewey [7]). The v's of IAA polar transport in excised sections (Table I) were lower than those that could be deduced for decapitated seedlings (Fig. 1). The same phenomenon has been observed in other plant materials. For example, corn coleoptiles placed between an IAA donor and a receiver agar block have been reported to have a v of 8 to 15 mm $h^{-1}$ (2), whereas in decapitated corn coleoptiles supplied with a pulse of IAA, the v has been reported to be as fast as 41 mm $h^{-1}$ (23).

From the distributions of $^3H$ and $^{14}C$ at different sampling times after application of double-labeled IAA to the apical cut surface of decapitated lupin hypocotyls (Fig. 1), it is deduced that IAA transport varied along the hypocotyl, as the apical, elongating region exhibited faster IAA movement than the basal, nongrowing region. The higher v in the apical region was not a traumatic consequence of decapitation because after application of IAA to the basal region, both the v and the amount of IAA exported from the application zone were lower than in the apical treatment (compare Figs. 1 and 2). Measurement of IAA transport in excised sections located at different positions along the hypocotyls confirmed that the v and, to a greater extent, the I decreased continuously from the apical, elongating region to the basal, nongrowing region (Table I). With the use of the intercept method of Van der Weij, it has been reported recently that IAA transport was greater in the younger, upper segments than in older, basal segments of etiolated *Helianthus annuus* hypocotyls (25). These results agree with the accepted view that polar IAA transport is faster in young than in aged tissues (7, 13, 17).

As regards the cause of the basipetal decrease in IAA transport along the lupin hypocotyl, at least two possible explanations can be deduced from the available literature: (a) there is a basipetal increase of endogenous inhibitors of IAA transport (according to Jacobs and Rubery [4], flavonoid compounds including quercetin, kaempferol, and apigenin, which are widely distributed in plants, can specifically compete for the naphthylphthalamic acid binding site of the exit carrier, thus inhibiting the polar IAA transport); (b) alternatively, a basipetal decrease in the concentration of exit carriers could occur in a similar way to that reported in maize root (9). In a very recent article, Suttle (25) demonstrated that the progressive loss of basipetal IAA transport capacity in etiolated *H. annuus* hypocotyls with advancing physiological age was directly correlated with the loss of function of the IAA efflux carrier. Suttle suggests that biochemical changes in the auxin transport system with advancing age are at least partially responsible for the observed reduction in polar IAA transport.

The existence of a basipetal decrease of IAA transport along the lupin hypocotyl might produce the so-called "barrier effect" first suggested by Kaldewey (7). The consequence of this phenomenon would be the accumulation of IAA in zones located above those exhibiting lower IAA transport. If the amounts of IAA applied to the apical zone of decapitated seedlings were higher than the capacity of IAA transport in lower zones, the expected IAA accumulation would be located in the elongation zone of the hypocotyl. In fact, Figure 1 shows that after apical application of a 0.36-nmol IAA pulse, a temporary accumulation of radioactivity occurred during the following 4 h of transport, localized in the elongation zone of the hypocotyl (see inset in Fig. 1). In a previous

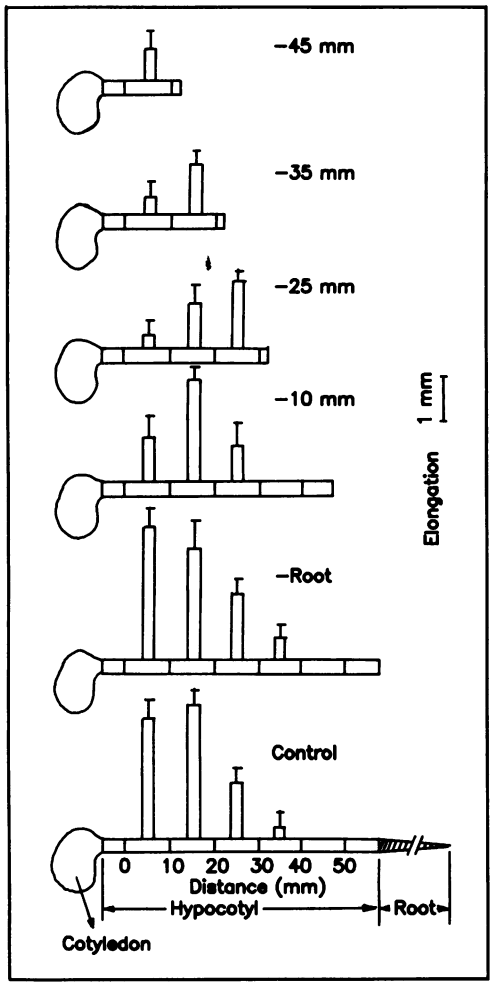



**Figure 4.** Effect of the excision of the root and basal hypocotyl zones on the distribution of growth. Prior to the treatment, hypocotyls were marked with ink every 10 mm starting 5 mm from the cotyledons. Histogram bars represent the growth (elongation) of each zone during the 20-h period after treatment. Data represent the mean values ± SD of five plants and correspond to the experiment in Table II.

article (20), we stated that decapitation of lupin seedlings drastically reduced hypocotyl elongation unless decapitated seedlings were apically treated with exogenous IAA. The application of a 0.34-nmol IAA pulse maintained normal growth in the hypocotyl compared to intact seedlings during the 6-h period following decapitation; the growth at 24 h was half that of the control. On the other hand, the application of two 0.34-nmol IAA pulses with a 4-h interval completely restored hypocotyl growth in the 24-h period following decapitation (20). These data indicate that the amount of IAA added to decapitated seedlings in the present experiments could temporarily substitute for the endogenous source of auxin (cotyledons and/or apical meristem) to maintain cell elongation. The requirement of a second IAA treatment 4 h after the first IAA application to saturate the growth response of the organ is justified by data in Figure 1, which show that radioactivity in the elongating region following the application of labeled IAA decreased progressively after 4 h. From the above, it is concluded that IAA must be accumulated in the elongating region of the hypocotyl for cell elongation to be maintained. Previous studies (12, 18) showed that during lupin hypocotyl growth, the IAA content of intact plants was greater in the apical, growing zones than in the basal, nongrowing zones. Though a lower IAA responsiveness in basal tissues cannot be discarded, the available data suggest that variation in IAA content along the elongation region of lupin hypocotyl could play an important role in the growth pattern found.

An indirect proof that the barrier effect might play a decisive role in the distribution of growth is shown in Table II and Figure 4. The data clearly indicate that in the experimental conditions, growth remained essentially unmodified for 20 h in the absence of root. This means that there was no limitation of water for growth and that compounds or stimulants (i.e., cytokinins) coming from the root were unnecessary for hypocotyl growth during this period. On the other hand, the excision of basal hypocotyl zones produced a significant reduction of growth in the remaining part of the hypocotyl (Table II). Because the basal zones have low IAA transport capacity (Table I), their elimination can be expected to reduce the barrier effect and, consequently, IAA accumulation in the elongation region. Therefore, the progressive reduction of the barrier effect seems to be the cause of the reduction in growth noted in the experiment in Table II. This interpretation is supported by the fact that after excision of basal hypocotyl regions, the reduction in growth was located mainly in the fast-growing zones (first and second subapical cm, Fig. 4), which contained the highest IAA concentrations in intact seedlings (18). The shortening of the growth period when the length of the excised zones is increased (Table II) supports the view that the reduction of growth might be due to a progressive decrease of IAA level in these zones.

In previous articles, it was reported that longitudinal distribution of unaltered IAA in [$^3$H]IAA-fed decapitated lupin hypocotyls (12) exhibited a wave-like pattern similar to that obtained with endogenous IAA (18), the main wave of IAA being located in the elongation zone of lupin hypocotyls at any age. From these correlations between elongation and IAA, a working hypothesis was formulated in which a role in growth is ascribed to IAA polar transport (12). Recently (20), we demonstrated three basic points in which the hypothesis was sustained: (a) there is no noticeable basipetal IAA movement in the cortex + epidermis tissues where the growth-sensitive cells are located; (b) the IAA added to and transported into the stele diffused sideways to the cortex + epidermis; (c) the high capacity of cortex + epidermis for IAA metabolization might allow a gradient of IAA that maintains the lateral auxin flow. The results of the present work suggest that the barrier effect caused by decreased IAA transport down the lupin hypocotyls might produce the growth-correlated IAA (endogenous and exogenous) waves found in lupin hypocotyls. Because lateral IAA migration from the stele to the outer tissues (cortex+epidermis) was dependent on the IAA concentration in the stele (20), a spillover of IAA from the transport system could be expected to occur in the IAA wave zone for elongation to be maintained in the growth-sensitive outermost cells. Further experiments study-

ing the radial distribution of endogenous IAA along lupin hypocotyls are needed to confirm the proposed model.

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## LITERATURE CITED

1. **Firn RD, Tamimi S** (1985) Auxin transport and shoot tropisms: The need for precise models. *In* M Bopp, ed, Plant Growth Substances 1985. Springer-Verlag, Berlin, Heidelberg, New York, Tokyo, pp 236–240
2. **Goldsmith MHM** (1977) The polar transport of auxin. Annu Rev Plant Physiol **28:** 439–478
3. **Goldsmith MHM** (1982) A saturable site responsible for polar transport of IAA in sections of maize (*Zea mays*) coleoptiles. Planta **155:** 68–75
4. **Jacobs M, Rubery PH** (1988) Naturally occurring auxin transport regulators. Science **241:** 346–349
5. **Kaldewey H** (1968) Transport und Verteilung von Indol-3-(essigsäure-2-$^{14}$C) in nickenden SproBachsen. Vortr Gesamtgeb Bot **2:** 90–106. Cited in Kaldewey H (1984)
6. **Kaldewey H** (1971) Geoepinasty, an example of gravimorphism *In* SA Gordon, MJ Cohen, eds, Gravity and the Organism. University of Chicago Press, Chicago, London, pp 333–339. Cited in Kaldewey H (1984)
7. **Kaldewey H** (1984) Transport and other modes of movement of hormones (mainly auxins). *In* TK Scott, ed, Hormonal Regulation of Development II. Springer-Verlag, Berlin, pp 80–148
8. **Kutschera U, Briggs WR** (1987) Differential effect of auxin on *in vivo* extensibility of cortical cylinder and epidermis in pea internodes. Plant Physiol **84:** 1361–1366
9. **Martin HV** (1988) Carriers for IAA in maize roots: localization and possible roles. *In* M Kutácek, RS Bandurski, J Krekule, eds, Physiology and Biochemistry of Auxins in Plants. Academia, Praha, pp 247–252
10. **Morris DA, Johnson CF** (1990) The role of auxin efflux carrier in the reversible loss of polar auxin transport in the pea (*Pisum sativum* L.) stem. Planta **187:** 117–124
11. **Newman IA** (1970) Auxin transport in *Avena*. I. Indoleacetic acid-$^{14}$C distributions and speeds. Plant Physiol **46:** 263–272
12. **Ortuño A, Sánchez-Bravo J, Moral JR, Acosta M, Sabater F** (1990) Changes in the concentration of indole-3-acetic acid during the growth of etiolated lupin hypocotyls. Physiol Plant **78:** 211–217
13. **Parker KE, Briggs WR** (1990) Transport of indoleacetic acid in intact corn coleoptiles. Plant Physiol **94:** 417–423
14. **Pearce D, Penny D** (1983) Tissue interactions in indoleacetic acid-induced rapid elongation of lupin hypocotyls. Plant Sci Lett **30:** 347–353
15. **Pengelly WL, Hall PJ, Schulze A, Bandurski RS** (1982) Distribution of free and ester indole-3-acetic acid in the cortex and stele of *Zea mays* mesocotyl. Plant Physiol **69:** 1304–1307
16. **Penny D, Miller KF, Penny P** (1972) Studies on the mechanism of cell elongation of lupin hypocotyl segments. N Z J Bot **10:** 97–111
17. **Rubery PH** (1987) Auxin transport. *In* PJ Davies, ed, Plant Hormones and Their Role in Plant Growth and Development. Kluwer Academic Publishers, Dordrecht, pp 341–362
18. **Sánchez-Bravo J, Ortuño A, Acosta M, Sabater F** (1986) Distribution of indole-3-acetic acid in relation to the growth of etiolated *Lupinus albus* hypocotyls. Physiol Plant **66:** 509–514
19. **Sánchez-Bravo J, Ortuño A, Acosta M, Sabater F** (1988) *In vivo* metabolism of labelled indole-3-acetic acid during polar transport in etiolated hypocotyls of *Lupinus albus*: relationship with growth. Plant Growth Regul **7:** 271–288
20. **Sánchez-Bravo J, Ortuño A, Botia JM, Acosta M, Sabater, F** (1991) Lateral diffusion of polarly transported indoleacetic acid and its role in the growth of *Lupinus albus* L. hypocotyls. Planta **185:** 391–396
21. **Sánchez-Bravo J, Ortuño A, Botía JM, del Rio JA, Caballero M, Acosta M, Sabater F** (1990) Identification of the metabolites of indole-3-acetic acid in growing hypocotyls of *Lupinus albus*. Plant Growth Regul **9:** 315–327
22. **Scott TK, Briggs WR** (1960) Auxin relationships in the Alaska pea. Am J Bot **47:** 492–499
23. **Shen-Miller J** (1973) Rhythmicity in the basipetal transport of indoleacetic acid through coleoptiles. Plant Physiol **51:** 615–619
24. **Shen-Miller J** (1973) Rhythmic differences in the basipetal movements of indoleacetic acid between separated upper and lower halves of geotropically stimulated corn coleoptiles. Plant Physiol **52:** 166–170
25. **Suttle JC** (1991) Biochemical bases for the loss of basipetal IAA transport with advancing physiological age in etiolated *Helianthus* hypocotyls. Plant Physiol **96:** 875–880
26. **Thimann KW** (1934) Studies on the growth hormone of plants. VI. The distribution of the growth substances in plant tissues. J Gen Physiol **18:** 23–34
27. **Van der Weij HG** (1932) Der Mechanismus des Wuchsstofftransportes. Recl Trav Bot Néerl **29:** 379–496
28. **Went FW** (1942) Growth, auxin and tropisms in decapitated *Avena* coleoptiles. Plant Physiol **17:** 236–249
29. **Wildman SG, Bonner J** (1948) The chemical nature and formation of auxin in the *Avena* coleoptile. Am J Bot **35:** 740–746