# Transport & Distribution of Auxin during Tropistic Response. I. The Lateral Migration of Auxin in Geotropism [1, 2]

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A central tenet of the theory of tropisms is that shoots are enabled to bend toward light and away from the earth by a migration of auxin across the stimulated plant. Following Cholodny's proposal of such a lateral migration (12), Went in 1928 (31) showed directly that when the tips of Avena coleoptiles are illuminated from one side, more auxin diffuses out of the darker than out of the lighter side. The evidence was almost simultaneously extended to geotropism by Dolk, whose doctoral thesis (15) provided a careful demonstration that when Avena coleoptile tips are held horizontal more auxin diffuses out of the lower than out of the upper side. Dolk's experiments further showed that when auxin is externally applied to horizontal sections cut from decapitated coleoptiles, it undergoes a similar asymmetric distribution. For both phototropism and geotropism the asymmetric distribution of endogenous auxin was soon confirmed by other workers, and the observations were extended both by diffusion and by extraction to dicotyledonous seedlings as well (see Went & Thimann (33) and Thimann & Curry (27) for reviews). Subsequently a large aggregate of concepts, though actually not many additional data, have crystallized around this simple notion of lateral auxin transport.

In recent years, however, the lateral transport hypothesis has been seriously questioned, primarily because a number of workers failed to find any asymmetric distribution within the tissue when radioactive indoleacetic acid was applied to stimulated plant parts, and radioactivity, instead of growth-promoting activity, was the criterion. Negative results with plants placed horizontally or illuminated unilaterally were reported for more than one species by several groups of workers, notably Bünning et al. (10), Gordon and Eib (19), Reisener (21, 22), Reisener and Simon (23), and Ching and Fang (11). On the other hand the original measurements, based on bioassay of *endogenous* auxin diffusing from the basal surface of stimulated plant sections into agar blocks, have been confirmed and extended: Briggs et al. (9) and Gillespie and Briggs (16) have found clear-cut asymmetric distribution. Briggs (8) presents still more evidence for the reproducibility of these results. Much of this work has recently been reviewed by Anker (2).

Such striking disagreement between two methods of measurement calls for explanation. If a plant indeed translocates its native indoleacetic acid, it must without discrimination move exogenous indoleacetic acid in the same way, providing that the applied auxin is made accessible to the transport system. It is of fundamental importance that when IAA-$C^{14}$ is applied to oat coleoptile sections, the radioactivity transported out into agar blocks is indeed biologically active as auxin (18). On the other hand, several interpretations of the discrepancy may be considered.

In the first place, the endogenous auxin which becomes asymmetrically distributed might not be indoleacetic acid (IAA). This is, however, made improbable by the repeated demonstrations that IAA is the auxin produced by Avena coleoptile tips (34, 20, 25) and by the fact that it does restore geotropic sensitivity to decapitated Avena coleoptile sections placed horizontally in solutions (1). As a second alternative, the material which becomes asymmetrically distributed might not be the auxin itself, but an auxin precursor or some other factor controlling the synthesis of auxin.

Thirdly, it is important to note that the measurements of radioactivity have invariably been made on the tissue itself or on tissue extracts, while most of the measurements of biological activity have been made on agar blocks receiving the transported auxin. Thus gravity or light might conceivably cause the lateral migration of a factor controlling polar transport, so that although the amounts of auxin on the two sides of the plant remained the same, the amounts transported through the tissue into receiver blocks might be different. It would, indeed, be surprising if careful determinations of radioactivity in the tissue failed to reveal some change, but it is not impossible.

Mechanisms of this and other kinds involving changes in auxin production, release, transport, or utilization on one side (without change in the amounts present at any moment) have been postulated

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by several workers, but as will be brought out below, no mechanism which does not involve lateral transport of some substance is compatible with the data based on bioassay.

The authors have therefore set out to resolve the discrepancy. The arguments, which apply equally well to geotropism and phototropism, will be evaluated by similar types of experiments for both cases. This first paper deals with the geotropic response of two types of shoot; tropisms of roots have not been included in the present work. Some preliminary results on geotropism have already been published (17).

## Materials & Methods

Carboxyl-labeled indoleacetic acid (IAA-$C^{14}$) was synthesized by the method worked out by Stowe (26). The specific activity was 0.95 curies per mole. In addition, we are grateful to Dr. Stowe for providing a sample of IAA with a higher specific activity, namely 16.9 curies per mole; this greatly facilitated certain of the more delicate measurements. The methods of purifying the IAA-$C^{14}$ and of measuring low radioactivity and self-absorption both in agar blocks and in tissue were similar to those of Goldsmith and Thimann (18). The purity of the IAA of lower specific activity was checked chromatographically, using as solvent isopropyl alcohol: 28 % ammonia: water :: 8:1:1. On one occasion Bitancourt's method using water in an atmosphere saturated with acetic acid vapor (3) was substituted. At least 97 % of the total radioactivity on the paper chromatograph strip was associated with a single well-defined Salkowski-positive spot; the remaining 3 % was uniformly spread between the spot and the solvent front. No other Salkowski-positive regions appeared, although it was shown that as little as 2 % of the Salkowski-positive material could have been detected under the conditions employed, had it occurred in a localized spot elsewhere on the paper. With the alcohol-ammonia-water method, the $R_f$ of the spot was generally close to 0.35; in those instances where it deviated, a control spot of unlabeled pure IAA deviated to the same extent. The hormone solution used in the experiments presented in table VII was found to be slightly less pure than the other lots; two spots appeared, one with an $R_f$ of about 0.3 which contained 96 % of the total radioactivity and one with $R_f$ of about 0.7 which contained 1.7 % of the radioactivity. The sample of IAA of high specific activity received from Dr. Stowe was reported by him to be 97 % pure, and was further purified as above.

For the experiments with oats, radioactivity was measured with a windowless Geiger counter with an efficiency of 44 % and a background which was typically 17 cpm. Each sample was counted for 10 minutes immediately after it was dried down. For later experiments with corn and sunflower seedlings, a counter with a background of only 3 cpm became available; the efficiency was approximately 33 %, changing slightly whenever the window was replaced. This machine held larger planchets than did the former counter, permitting thinner spreading of samples, with consequent reduction in the absorption factors. It was found also that the samples did not need to be counted immediately after drying, since they lost no measurable activity after a week at 25 C or a month at 3 C, when dried down on a planchet either alone or with agar or pulverized tissue. This stability permitted long counting periods, and the value recorded for each sample is based on not less than 1000 counts. For critical determinations of auxin ratios between the upper and lower sides of the plants, 2000 to 6000 counts were usually accumulated for each sample. For all these reasons, the consistency of the data obtained with corn and sunflowers was somewhat improved over that reported for oats.

Coleoptiles of both *Avena sativa* L., var. Segerhavre, and *Zea mays* L., var. Burpee's Barbecue Hybrid (lot 6241), were grown in darkness interrupted occasionally by dim red light (Corning Filter No. 2408), at 25 C, with relative humidity of 83 % to 87 %. Unhusked oats were soaked in water for about three hours and sown in damp sawdust. Twelve hours of red light, administered during the third night of growth, sufficed to inhibit mesocotyl elongation, and the plants were ready for use 90 hours after planting. The corn seeds were surface-sterilized for about ten minutes in 10 % commercial hypochlorite (Chlorox), rinsed for three hours in running tap water, and set out on moist paper towels. A red light was turned on for three or four hours during the third night of growth in order to inhibit mesocotyl elongation. If molds began to develop, the germinating seeds were sprinkled lightly with Phygon (2,3-dichloro-1,4-naphthoquinone, Naugatuck Chemical Co.). About 90 hours after soaking, when the plants were ready for use, they were illuminated for 2 hours with light from a fluorescent bulb covered with a red filter (i.e. red light free from far-red). This preillumination reduced the endogenous auxin to a low and consistent level as described in detail by Briggs (7) and confirmed in the present experiments (unpublished data). The distribution of IAA-$C^{14}$ induced by gravity was found to be the same in sections cut from plants pre-exposed to red light as in those grown in total darkness after 60 hours and manipulated under a far-red source [incandescent bulb, Corning Filter No. 7-69(2600)]. Polar auxin transport, too, appeared to be unchanged. Since some of the experiments were tedious, twelve hours often elapsed between the cutting of the first and last coleoptiles, but corn coleoptiles between 2.5 and 4 cm long and oat coleoptiles between 2.5 and 3.5 cm long were always selected. Although auxin production, growth rate, and tropistic reactivity have long been known to vary conspicuously with coleoptile age (33), the behavior of both polar and lateral auxin

transport systems seemed remarkably constant in oat and corn coleoptiles within this length range.

Seeds of *Helianthus annuus* L., var. Mammoth Russian (lot 170 of the Joseph Harris Co.) were soaked in water for about three hours, set out in moist vermiculite, and grown for 110 hours at about 25 C under white light measuring 12,000 metercandles at plant level. The daylength was 16 hours. The hypocotyls selected were between 3.5 and 5 cm high, about 2 mm in diameter, and bore dark green cotyledons. Preliminary investigations showed no gross difference in lateral distribution of IAA-$C^{14}$ by seedlings cut at the end of the day and those cut at the end of the night. The plants were transferred to the dark room at least two hours before sectioning and thereafter received only minimal red light during manipulations.

All sections were cut 6.5 mm long. The apical cut removed the auxin-producing zone (31, 8, 6); it was made 1.5 mm below the tip of Avena and at least 2 mm below the tip of Zea. Sections of Helianthus hypocotyl were cut 2 mm below the cotyledons.

In all experiments, blocks of 1.5 % washed agar were cut to a standard size (12 × 9 × 1.5 mm). For the Avena curvature test of table II these were cut into 12 to provide the standard 10 $mm^3$ blocklets for setting on the assay plants. Blocks used for the growth test of figure 2 were also cut into twelve pieces, only eight of which were used for measurements at each auxin concentration. In other experiments the blocks were usually subdivided into halves, thirds, or fourths, but were recombined at the end of each experiment.

## Results & Discussion

Coleoptiles of Oats (*Avena sativa*): The first experiments were modeled after the classical investigation of Dolk (15). A razor blade, coated with stopcock grease, was mounted in a lucite holder. On either side of the blade, agar blocks were set in such a way that the cutting edge of the blade protruded 1 mm above the surface of the blocks. Next, 20 Avena coleoptile sections were pressed onto the blade so that the basal ends were bisected in a plane perpendicular to that of the vascular bundles, and rested on the agar. Finally, agar blocks containing 2.3 × $10^{-6}$ M $C^{14}$-labeled IAA were set on the apical ends of the coleoptile sections; these blocks were held in place by the lid of the plastic apparatus. The assembly was then enclosed in a small humid chamber, and set so that the Avena sections were held either horizontal (experiment) or vertical (control). After 165 minutes, the coleoptile sections, the donor agar block, and the upper and lower (or in controls the right and left) receiver agar blocks were spread uniformly in separate planchets, dried, and counted. Unused duplicates of the donor blocks were similarly treated. The results of these experiments have been published in detail (17) and are given here only in summary form in table I. Each figure is an average from 11 or 12 complete experiments, each comprising 20 coleoptiles. First, the results with vertical sections (series I) show highly symmetric distribution of the transported label and nearly complete recovery of applied radioactivity. Series II and III show that, after application of the same auxin concentration to horizontal sections, there appears an asymmetry in the distribution amounting to 40.1 : 59.9 in one series, and 39.1 : 60.9 in the other. Six other preliminary experiments gave similar results. The combined data leave no doubt but that the transport of IAA-$C^{14}$ is asymmetric in Avena coleoptile sections held horizontal.

The discrepant results reported by other workers have without exception been obtained by halving coleoptiles supplied with IAA-$C^{14}$ and determining the radioactivity in the tissue (either directly or by extraction). This procedure measures not only the

**Table I**

Summary of Distribution of IAA-$C^{14}$ Entering Agar Blocks Applied to Base of 6-mm Sections of Avena Coleoptiles and in the Tissue Itself.

| | cpm in donor block | cpm in tissue | | cpm in receiver blocks | | % recovery | % in upper block or tissue |
|---|---|---|---|---|---|---|---|
| | | Upper half | Lower half | Upper | Lower | | |
| I Sections vertical, receivers divided | 298 | 143 | | 26.7* | 28.0* | 97 | 49.1* ± 1.4 |
| II Sections horizontal, receivers divided | 269 | 124 | | 25.4 | 37.1 | 90 | 40.6 ± 1.1 |
| III Identical with II | 2 × 456** | 142 | | 29.2 | 45.5 | 96 | 39.1 ± 1.3 |
| IV Sections horizontal, tissue divided | 294 | 66.4 | 72.5 | 62.5 | | 98 | 47.8 ± 0.7 |

Each number representing cpm has been corrected for self-absorption of agar or tissue. Initial activity of donor block was 511 cpm in series I, II and IV, 586 in series III. Diffusion time was 165 min.

* For *upper* and *lower* read *left* and *right* sides respectively in series I with sections vertical.

** Two donor blocks were used instead of one in series III.

## Table II

Amount of Auxin Diffusing out of Corn Coleoptile Tips Pre-illuminated with Red Light

| Experiment | Auxin source | Auxin yield, degrees Avena curvature | Calculated amount of IAA transported per tip per hour in $\mu g$ |
|---|---|---|---|
| 1. | 4 (6 mm) tips for 3 hr = 12 tip-hr | 15.7<br>18.0<br>11.9<br>18.5 | |
| | | 16.0 avg | $8.3 \times 10^{-4}$ |
| | 0.025 mg/l IAA in agar | 7.1<br>6.0 | |
| | | 6.5 avg | |
| 2. | 6 (3 mm) tips for 2 ¼ hr = 13.5 tip-hr | 14.5<br>15.4<br>15.9<br>14.3<br>14.6 | |
| | | 14.9 avg | $7.0 \times 10^{-4}$ |
| | 0.025 mg/l IAA in agar | 5.8<br>7.0 | |
| | | 6.4 avg | |
| | | | $7.7 \times 10^{-4}$ avg |

IAA in transit, but also any additional IAA which is not being transported. Since most evidence indicates that only transportable auxin is correlated with geotropism in the Avena coleoptile (14, 32), a fourth series was carried out in order to determine whether the presence of immobilized auxin in the tissue might under some conditions obscure a lateral gradient in the moving auxin. In this series the sections were bisected in the plane which had previously been horizontal. Table I, series IV, shows that while the total radioactivity found in the tissue and in the receivers agrees very well with the results in series I and II, the asymmetry of distribution within the tissue is very small, and though it is in the expected direction, it is too slight to be convincing. As control on the technique of bisection, five other sets of coleoptile sections were bisected and weighed; the average difference between 20 upper and 20 lower halves was found to be 2.2 % with a maximum difference of 5.3 %. Thus, even though the amounts of auxin diffusing out of the upper and lower sides of the bases of the sections show a clear difference, the auxin gradient within the tissue might easily be overlooked. This fact evidently goes a long way toward explaining the failure of other workers to obtain asymmetrical auxin distribution under the influence of gravity.

Asymmetric IAA Distribution in Coleoptiles of Corn (*Zea mays*): Because the Avena coleoptile is the classical object for studies on auxin and tropisms, and especially because the apparent discrepancy between the distribution of endogenous diffusible auxin and applied IAA-$C^{14}$ had been reported mainly for Avena, it was felt essential to carry out the first experiments with that coleoptile. The coleoptile of corn is, however, preferable for this work, both because of its larger size and because of the more complete recovery of radioactivity obtained with corn coleoptile sections. (A certain amount of radioactivity disappears during most 3-hour experiments with oat sections.)

It was first necessary to determine the amount of endogenous auxin normally diffusing from the corn coleoptile tip under the conditions used. This was done with the standard Avena curvature test. The results (table II) show that the corn coleoptile tip, when pretreated with red light, yields $8 \times 10^{-4}$ $\mu g$ of auxin per hour. Next was determined by trial and error, for each series of experiments, the concentration of IAA-$C^{14}$ which, applied in a donor block to a given number of corn coleoptile sections, would result in this "normal" amount of auxin being moved into the receiver by each plant during a diffusion period of 165 minutes.[3] This made it possible to relate the amount of auxin supplied by donor block to the auxin that the tip would have produced under the conditions of the experiment, had it been left on the section. (These figures appear in the notes to the tables).

The amounts of IAA-$C^{14}$ diffusing out of the upper and lower halves of the cut surfaces of horizontal 6.5 mm corn coleoptile sections were then determined as with oats. The basal ends of 22 sections were pressed onto a horizontal razor blade so that they were bisected to a depth of less than 1 mm, donor agar blocks containing $5.7 \times 10^{-6}$ M IAA-$C^{14}$ were placed against the apical surfaces, and plain agar blocks were set at the basal surface on either side of the blade as receivers. The radioactivity in the upper and lower receiver blocks is shown in the first two columns of table III, part A. In the third column the radioactivity found in the upper of the two receivers is expressed as per cent of the total amount transported; the average value is 30.4 %. An important feature of the manipulations—randomization of the sides of the completed assemblies which were to face downward—guarantees the absence of a bias in this average.

Part B of table III shows that there is a clear difference between the radioactivity of the upper and lower halves of the tissue as well; 40.3 % of the radioactivity found in the tissue is on the upper side.

[3] Thirty minutes of this period are required for the first IAA-$C^{14}$ molecules to cover the 6.5 mm distance to the base of the sections. Hence the amounts of such exogenous IAA per hour must be computed with an effective diffusion period of 135 minutes rather than the actual period of 165 minutes. Furthermore, to simplify the calculation it was assumed that IAA-$C^{14}$ enters the tissue at a constant rate.

After the dried samples had been counted they were weighed; the sixth column indicates that the weights of the upper and lower halves were in every case nearly equal. By adding the radioactivity in receivers and tissues to that remaining in the donors and comparing the sum with the 632 cpm supplied in the donors, as has been done in the table, it can be seen that on the average 93 % of the radioactivity present initially was still present at the end of the experiment. The asymmetric distribution of IAA moving through the tissue must be considered conclusively demonstrated.

Degree to which the Asymmetric Distribution of Moving IAA Is Masked by Immobilized IAA: The difference between the auxin content in the upper and lower halves of the tissue, in corn as in oats, is less marked than that in the agar receiver blocks. This is to be expected, since as the auxin is transported longitudinally, more and more of it moves into the lower half of the section as a result of lateral transport. The difference between the auxin content in the upper and lower halves of a section thus represents an integration from the apical end of the section, where lateral transport is just beginning, to the basal end where a larger proportion of the auxin has accumulated on the lower side.

A factor more important for the present discussion, however, is the extent to which the auxin present in the tissue may be in bound form. It was indicated above that in Avena coleoptile sections such bound axin can mask some or all of the asymmetric distribution of the auxin being transported. If the amount bound were directly proportional to the amount transported, there would be no masking except that due to integration effects noted above. As Goldsmith and Thimann have shown (18), radioactivity is bound uniformly along the length of an oat coleoptile section in proportion to the amount of applied auxin transported through it, when that amount is comparable with the endogenous level. However, as the concentration of IAA-$C^{14}$ is raised, the proportion of auxin bound increases; most of the binding occurs within the uppermost third of the tissue. Such an increase in binding would cause serious masking of the true asymmetry. Since this phenomenon could well be of general occurrence, a study was made of the amounts of binding and of transport in corn coleoptile sections as a function of auxin concentration in the donor block.

Coleoptile sections were set between donor and receiver blocks in the manner of Van der Weij (29), using a series of IAA concentrations in the donors.

## Table III

Asymmetric Distribution of Auxin in Corn Coleoptile Sections Transporting Normal Amount of Auxin*

Part B. Tissue (halved lengthwise)

| | Radioactivity, cpm** | | % radioactivity in upper receiver | cpm remaining in donor | % recovery of radioactivity*** |
|---|---|---|---|---|---|
| | Upper receiver | Lower receiver | | | |
| 1 | 62.0 | 139.0 | 30.8 | 195 | 92 |
| 2 | 48.2 | 112.9 | 29.9 | 261 | 93 |
| 3 | 49.3 | 115.0 | 30.0 | 233 | 93 |
| 4 | 55.7 | 131.8 | 29.7 | 220 | 91 |
| 5 | 40.7 | 114.0 | 26.4 | 310 | 98 |
| 6 | 47.5 | 108.2 | 30.5 | 396 | 95 |
| 7 | 50.9 | 91.2 | 35.8 | 294 | 92 |
| avg | 50.6 | 116.0 | **30.4** | 259 | 93 |

Part B: Tissue (halved lengthwise)

| | Radioactivity, cpm** | | % radioactivity in upper half | Dry wt, mg | | % weight in upper half |
|---|---|---|---|---|---|---|
| | Upper half | Lower half | | Upper half | Lower half | |
| 1 | 77.7 | 106.0 | 42.3 | 8.4 | 10.0 | 46 |
| 2 | 63.5 | 99.0 | 39.2 | 10.8 | 9.3 | 54 |
| 3 | 68.1 | 121.1 | 36.0 | 9.2 | 9.1 | 50 |
| 4 | 64.3 | 103.8 | 38.3 | 11.5 | 9.8 | 54 |
| 5 | 66.9 | 89.5 | 42.8 | 9.9 | 9.0 | 52 |
| 6 | 64.6 | 82.0 | 44.0 | 10.5 | 9.9 | 51 |
| 7 | 56.4 | 86.4 | 39.6 | 8.8 | 10.7 | 45 |
| avg | 65.9 | 98.3 | **40.3** | 9.9 | 9.7 | 50 |

* Concentration of IAA-$C^{14}$ in donor was $5.7 \times 10^{-6}$ M, corresponding to 632 cpm. This amount was chosen so that it gave rise in the receivers to $8 \times 10^{-4}$ μg IAA per hour (cf. table II).

** The cpm in receivers and donors have been multiplied by a self-absorption factor of 1.25 for agar; the cpm in the tissue have been multiplied by a self-absorption factor of 1.74. Specific activity of IAA was 0.95 c/mole; counting efficiency was 33 %. Diffusion time was 165 min. There were 22 sections/receiver.

*** Obtained by adding activity in donor and receiver blocks shown in Part A to that in tissue shown in Part B.

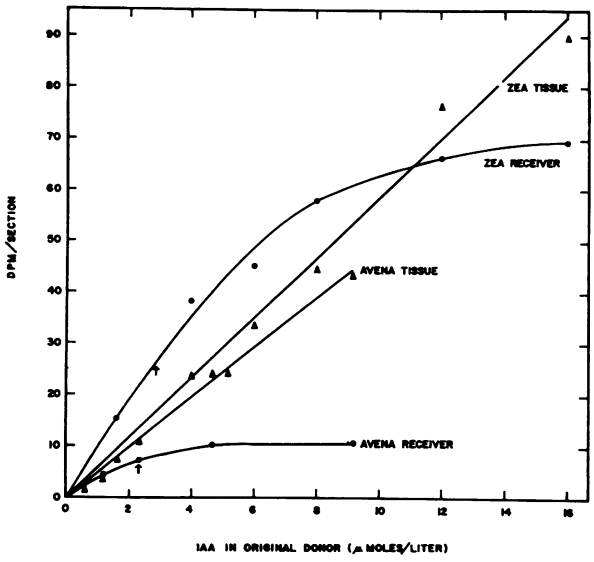



FIG. 1. The dependence of polar transport of IAA through coleoptile sections on donor concentration. Upper curves: data for 6.5 mm Zea coleoptile sections. Lower curves: data for 7 mm Avena coleoptile sections, adapted from Goldsmith and Thimann (18). Arrows indicate amounts of IAA transported in the oat experiments of table I and in the corn experiments of table III. The donor concentration which would produce normal transport by the corn is $2.5 \times 10^{-6}$ M; this value is not known for the oats, but $0.5 \times 10^{-6}$ M agar blocks produce almost maximal response in the standard Avena curvature test. The data have been normalized by calculating disintegrations per minute on a single-plant basis; in both cases the specific activity of the IAA-$C^{14}$ was 0.95 c/mole. All agar donor and receiver blocks were the same size, and diffusion time was 3 hours for both plants, but there were 20 sections per assembly of Avena as compared to 12 for Zea.

In figure 1 the counts found in the tissue and in the receivers are plotted against the counts applied as IAA-$C^{14}$. For comparison, the data of Goldsmith and Thimann on oat coleoptile sections are plotted on the same scale. It is clear that, although the amounts of auxin in both tissues increase linearly with increasing donor concentration, the ability of the sections to transport auxin into receivers soon approaches saturation. The amounts of auxin transported into the receivers in the geotropic experiments of tables I and III are shown by arrows in the figure. Since the arrow for corn lies well below the beginning of saturation, a decrease in the donor concentration of table III would not change the proportion of auxin that remains in the tissue. Little if any masking should therefore have occurred in the experiments of table III. The arrow for oats, on the other hand, lies much closer to the saturation level, and this probably explains the failure to detect a significant asymmetry in the oat tissue. Whether the lateral transport becomes saturated at the same IAA level as the basipetal transport is not known, but even if it does not, overloading of the basipetal system would diminish the asymmetry of the auxin at the base of the section.

The binding of auxin probably accounts in large part for the failures of other workers to measure asymmetric distribution of applied IAA-$C^{14}$, for none of them assessed the proportion of bound and moving auxin. Reisener and Simon (23) did make such an attempt; by drying treated coleoptile sections over $P_2O_5$ and extracting with ether, they concluded that only about half the label applied was fixed in the tissue. However, their method is inapplicable to this problem since it was shown long ago (28) that when IAA is added to tissue which is subsequently dried, the auxin becomes fixed in a non-physiological way. Such auxin fixation can be reversed by adding water, but this was not done. The status of the free and bound auxin in their experiments thus remains unknown.

Asymmetry Independent of IAA Supply in Unsaturated Sections: It remains possible, of course, that the absolute amount of auxin undergoing lateral movement is always the same for a given stimulation period, regardless of the auxin level in the tissue. In such a case, the asymmetry would become increasingly conspicuous as the donor concentration was lowered. If, on the other hand, under normal conditions the activity of the induction system is limited by the auxin available to it, the asymmetry should be independent of donor concentration over a considerable range.

Therefore, the distribution of IAA-$C^{14}$ was studied in two series of experiments similar to table III except that the donor concentration was lowered. The results of the first series, in which the donors were adjusted to produce 70 % of the normal auxin transport, are shown in table IV, while table V shows the results of a second series in which only 40 % of the normal amount of auxin was carried. The distribution of radioactivity in the receivers of table IV is 31.5:68.5 and that in table V is 28.9:71.1, agreeing very closely with the 30.4:69.6 of table III. Indeed $t$-tests gave $p=0.5$ and 0.3, respectively. In regard to the tissue, the auxin asymmetry shown in table IV, i.e. 38.7:61.3, can be compared with the 40.3:59.7 of table III; again, there is no significant difference ($p=0.4$). However, in a third series of experiments, in which the average amount transported by the sections was only one sixth of the normal amount, the distribution found was 23.2:76.8. (For this test the IAA employed was of higher specific activity than in the other experiments.) A $t$-test showed that the difference between this ratio and the ratio of 30.4:69.6 in table III is highly significant, $p$ being well below 0.01. Thus if the donor concentration is low enough, the proportion of auxin found on the lower side does increase somewhat.

It will be noticed that these experiments provide an independent check that the proportion of radioactivity remaining in the tissue does not change appreciably at these auxin levels. In table IV, 678—314=364 cpm entered the tissue, and 147 cpm re-

## Table IV

Asymmetric Distribution of Auxin in Corn Coleoptile Sections Transporting Seven tenths of the Normal Amount of Auxin.* Conditions otherwise as in Table III.

| | Radioactivity in receivers, cpm | | % of radioactivity in upper receiver | Radioactivity in halved tissue, cpm | | % radioactivity in upper tissue half |
|---|---|---|---|---|---|---|
| | Upper | Lower | | Upper | Lower | |
| 1 | 76.0 | 151 | 33.4 | 45.6 | 90.5 | 33.5 |
| 2 | 60.5 | 130 | 32.0 | 51.6 | 79.4 | 39.5 |
| 3 | 61.9 | 150 | 29.2 | 58.6 | 88.5 | 39.8 |
| 4 | ... | ... | ... | 42.0 | 54.8 | 43.4 |
| 5 | 62.2 | 145 | 30.0 | 51.5 | 75.5 | 40.5 |
| 6 | 56.5 | 116 | 32.8 | 41.5 | 75.5 | 35.5 |
| avg | 63.4 | 138.4 | **31.5** | 48.5 | 77.4 | **38.7** |

| | cpm remaining in donor | cpm in apical tissue segment | % recovery of radioactivity | Weight of halved tissue, mg | | % of weight in upper tissue |
|---|---|---|---|---|---|---|
| | | | | Upper | Lower | |
| 1 | 243 | 19 | 94 | 17.5 | 16.6 | 51 |
| 2 | 301 | 18 | 99 | 17.5 | 16.1 | 52 |
| 3 | 282 | 25 | 99 | 17.5 | 16.2 | 52 |
| 4 | 396 | 25 | ... | 15.5 | 15.8 | 50 |
| 5 | 303 | 21 | 97 | 17.6 | 16.9 | 51 |
| 6 | 326 | 20 | 94 | 16.8 | 16.9 | 50 |
| avg | 314 | 21 | 95 | 17.1 | 16.4 | 51 |

* The concentration of IAA-$C^{14}$ in donors was $3.0 \times 10^{-6}$ M, corresponding to 678 cpm. This amount was chosen so that it gave rise in the receivers to $5.6 \times 10^{-4}$ μg IAA per section per hour; i.e., 70 % as much auxin as was transported in table III. The cpm in receivers and donors have been multiplied by a self-absorption factor of 1.33 for agar; the self-absorption factor for tissue halves was 1.56. Diffusion time 165 minutes, counting efficiency 33 %, and specific activity of IAA 0.95 c/mole. There were 20 sections per assembly, and two assemblies were pooled for each experiment.

## Table V

Asymmetric Distribution of Auxin Produced by Corn Coleoptile Sections Transporting Four Tenths of the Normal Amount of Auxin.*

| | Counts per minute | | | | % recovery | % cpm in upper receiver |
|---|---|---|---|---|---|---|
| | Upper receiver | Lower receiver | Tissue | Donor | | |
| 1 | 44.4 | 124.0 | 151 | 236 | 101 | 26.4 |
| 2 | 32.4 | 71.3 | 110 | 318 | 97 | 31.2 |
| 3 | 23.4 | 51.6 | ... | 335 | ... | 31.1 |
| 4 | 35.8 | 91.5 | 84 | 282 | 93 | 28.1 |
| 5 | 50.9 | 125.5 | 109 | 219 | 92 | 28.9 |
| 6 | 47.1 | 109.0 | 99 | 244 | 91 | 30.2 |
| 7 | 32.4 | 90.1 | 89 | 298 | 93 | 26.6 |
| avg | 38.1 | 94.7 | 107 | 276 | 95 | **28.9** |

* The concentration of IAA-$C^{14}$ in donors was $2.7 \times 10^{-6}$ M, corresponding to 547 cpm per pair of donor blocks. This amount gave rise in the receivers to $3.2 \times 10^{-4}$ μg IAA per section per hour, i.e., 40 % as much auxin as was transported in table III. The cpm in receivers and donors have been multiplied by a self-absorption factor of 1.19 for agar, while the cpm in tissue have been multiplied by a self-absorption factor of 2.02. Diffusion time 165 minutes, counting efficiency 33 %, and specific activity of IAA 0.95 c/mole. There were 22 sections per assembly, and two assemblies were pooled for each experiment.

mained there. Thus 40 % of the entering counts were found in the tissue. In table V the figure is 40 %, and in table III it is 44 %. In addition, in table IV the apical 2/3 mm of the sections was removed before halving the tissue and counted separately, to confirm that the picture is not distorted by excessive binding of radioactivity near the surface in contact with the donor. When dry these segments weighed (as an average of the six experiments each comprising forty sections) 3.4±0.4 mg each, and contained only 14 % of the radioactivity in the tissue.

An Alternative Method of Demonstrating Lateral Auxin Transport: A modification of the geotropic type of transport experiment which shows at least qualitatively the increased lateral movement of auxin caused by gravitational stimulation was designed by Brauner and Appel (5). These workers removed half of the apex of an Avena coleoptile and replaced

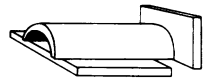
it with a small agar block; the seedling was then arranged horizontally so that the block was on the lower side. It was anticipated that during a 90 minute diffusion period enough auxin would be moved downward across the cut surface into the agar block to be detected by the Avena curvature test and, indeed, such blocks caused test plants to curve 4°. Control blocks, which replaced the halved apices of plants placed upright, produced only 1° curvature. The results of this experiment seem plausible, but since the curvatures were very small, the experiment has been refined using corn coleoptiles and radioactive IAA of high specific activity.

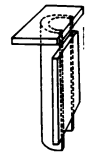
Corn coleoptile sections were slit in half and set with their longitudinal cut surfaces facing downward and touching agar receiver blocks 4.5 mm wide. Donor blocks were provided at the apical cut surfaces, and an air gap 1 mm wide was carefully maintained between donors and receivers (see sketch in table VI). The basal 0.5 mm of the sections extended past the edge of the receiver block. On each receiver block rested the halves of five coleoptile sections, which together received auxin from half a donor block. Horizontal and vertical assemblies were, of course, produced in alternation. Contact between tissue and agar was carefully checked at the beginning and end of the 150 minute diffusion period; all of the sections seemed to maintain excellent contact and, when removed, left slight impressions on the agar.

## Table VI

Movement of IAA-$C^{14}$ from Longitudinally Halved Corn Coleoptile Sections into Laterally Applied Agar Blocks.*

| | Counts per minute | | | % recovery |
|---|---|---|---|---|
| | Receiver | Tissue | Donor | |
| | | | Horizontal Series | |
| 1 | 166<br>199 | 492 | 2190 | 90 |
| 2 | 206<br>228 | 565 | 2100 | 92 |
| 3 | 138<br>177 | 407 | 2410 | 93 |
| 4 | 108<br>133 | 424 | 2720 | 100 |
| 5 | 122<br>118 | 375 | 2410 | 90 |
| avg | 319 | 453 | 2366 | 93 |
| | | | Vertical Series | |
| 1 | 84<br>82 | 743 | 2220 | 93 |
| 2 | 78<br>39 | 628 | 2300 | 91 |
| 3 | 53<br>47 | 530 | 2420 | 90 |
| 4 | 39<br>38 | 455 | 2720 | 96 |
| 5 | 27<br>36 | 490 | 2623 | 94 |
| 6 | 48<br>34 | 332 | 2538 | 87 |
| avg | 101 | 530 | 2471 | 92 |





* Donors were halved, each half supplying a whole assembly. Receivers were counted separately, but the two halves of a donor and the corresponding tissue lots were pooled. The cpm of agar samples have been multiplied by a self-absorption factor of 1.14; the cpm of tissue samples have been multiplied by a self-absorption factor of 1.51. Each assembly contained 20 halves from 10 corn coleoptile sections. Diffusion time 150 min; concentration of auxin in donor $3.3 \times 10^{-6}$ M, corresponding to 3380 cpm; counting efficiency 16 %; specific activity of the auxin 16.9 c/mole.

The data are presented in table VI. Although there is some variation in the amounts of radioactivity found in the receivers, column one shows that no receiver from a vertical assembly contained as much auxin as did the least radioactive of the horizontal ones. On the average three times as much auxin moved into receiver blocks beneath horizontal halves of coleoptile than into those on the sides of vertical halves. In terms of the amounts entering, 32 % of the label leaving the donors entered the horizontal receivers, as contrasted to 11 % entering the vertical receivers. This result gives very strong support to the idea that IAA actually moves from the upper to the lower half of the coleoptile.

Total Amounts of Auxin Transported in the Horizontal and Vertical Positions: In itself the existence of a gradient of auxin across the horizontal coleoptile does not rigorously prove that IAA moved from the upper to the lower side. Facilitation of polar transport on the lower side of a horizontal coleoptile, for example, could be proposed to explain the data. Such an effect could not explain the results of table VI. Furthermore, it would mean that horizontal coleoptile sections should transport more auxin in a given time than vertical ones. For example, table III shows that 70 units of auxin diffuse out of the basal end of the lower half of the horizontal section for every 30 units diffusing out of the upper half. The total amount of auxin recovered in the receivers is thus 100 units. Since the facilitation hypothesis assumes that transport is unchanged in the upper half, each side of a vertical section must transport 30 units; only 60 units should be transported in all. Such large differences should be easily detected.

The amounts of transport in 165 minutes through vertical and horizontal sections were therefore compared directly. All experimental conditions were the same as before, except that the sections and receivers were intact. Table VII shows no significant difference in the amounts of $C^{14}$ collected in these experiments; a $t$-test by the group comparison method gave $p=0.45$. An analogous calculation eliminates the possibility of significant hindrance of polar transport on the upper side. These radioactivity measurements with sections are in complete agreement with the bioassays of Dolk (15) and of Gillespie and Briggs (16) in which no difference could be detected between the amounts of endogenous auxin entering receiver blocks from horizontal and vertical

coleoptile tips. In Gillespie and Briggs' experiments, it was also shown that if the tips were bisected in the horizontal plane the auxin differential was eliminated completely. Thus the geotropic behavior of the coleoptile is coordinated by the passage of some substance from one side to the other. That this substance was not related to the synthesis of auxin was clear, for Dolk had already shown that replacing the tip of a horizontal coleoptile section, where auxin is synthesized, with a donor block containing diffusate from other tips did not interfere with the appearance of a differential when the applied auxin diffused out into agar blocks at the upper and lower basal surfaces of the section. It is apparent, therefore, both from bioassay and radioassay, that auxin must decrease in the upper half of the horizontal coleoptile to the same extent that it increases in the lower half.

Growth Limitation by IAA: One other point remains, it cannot be taken for granted that the asymmetry in auxin distribution leads to asymmetric growth of the coleoptile. The response of corn coleoptile sections to a range of concentrations of applied auxin was therefore investigated. Following the standard preillumination with red light, the plants were decapitated and allowed to remain under dim red light 2 hours in order to reduce the level of their endogenous auxin (7). Sections were then cut and set upright, capped individually with donor blocks of one twelfth standard size, and measured with a traveling microscope. After 3 more hours had passed, the sections were measured again. In figure 2 the per cent elongation is plotted against the concentration of IAA applied. Within the range studied, which includes concentrations three times that known to supply auxin in normal amounts, it is clear that growth does increase substantially with concentration. It may therefore be concluded that the asymmetry of auxin distribution must decrease the growth rate of the upper half of the coleoptile and increase that of the lower half.

## Table VII

Comparison of Net Polar Transport in Horizontal and Vertical Sections of Corn Coleoptiles.

| Donor concentration | Counts per minute | | | | | |
|---|---|---|---|---|---|---|
| | Horizontal | | | Vertical | | |
| | receiver | tissue | donor | receiver | tissue | donor |
| 2.6 μM | 46 | 26 | 73 | 49 | 23 | 70 |
| (144 cpm) | 46 | 26 | 78 | 49 | 33 | 68 |
| Series 1a* | 49 | 30 | 70 | 46 | 23 | 76 |
| | 48 | 23 | 80 | 45 | 23 | 72 |
| | 49 | 31 | 69 | 43 | 19 | 76 |
| | 39 | 19 | 92 | 35 | 23 | 82 |
| | 52 | 31 | 70 | 40 | 19 | 76 |
| | 38 | 22 | 80 | 46 | ... | 82 |
| 2.6 μM | 30 | 16 | 105 | 37 | 17 | 76 |
| (144 cpm) | 43 | 23 | 77 | 41 | 21 | 84 |
| Series 1b* | 35 | 19 | 98 | 36 | 16 | 93 |
| | 30 | 22 | 96 | 32 | 13 | 93 |
| avg | **42** | **24** | **82** | **42** | **23** | **79** |
| avg recovery | | | 103 % | | | 100 % |
| 5.6 μM | 80 | 40 | 180 | 89 | 34 | 165 |
| (366 cpm) | 83 | 41 | 168 | 98 | 36 | 162 |
| Series 2a** | 83 | 43 | 159 | 94 | 35 | 154 |
| | 79 | 52 | 158 | 89 | 37 | 156 |
| | 79 | 55 | 172 | 79 | 34 | 170 |
| | 71 | 41 | 171 | 75 | 34 | 170 |
| | 84 | 36 | 166 | 76 | 35 | 182 |
| 5.6 μM | 76 | 35 | 174 | 68 | 25 | 172 |
| (366 cpm) | 69 | 38 | 168 | 78 | 37 | 164 |
| Series 2b** | 68 | 38 | 180 | 65 | 32 | 172 |
| | | | | 67 | ... | 204 |
| | 73 | 41 | 184 | 83 | 34 | 163 |
| avg | **76** | **43** | **171** | **79** | **34** | **170** |
| avg recovery | | | 95 % | | | 93 % |

Specific activity of IAA was 0.95 c/mole, counting efficiency 16 %. Each number represents the cpm corrected by an appropriate self-absorption factor: 1.14 for agar, 1.50 for tissue. 12 sections/experiment.

* Series 1: Diffusion time 180 minutes; sections transporting about the same amount of auxin as transported out of intact tips under similar conditions.

** Series 2: Diffusion time 165 minutes; sections transporting about twice as much auxin as transported out of intact tips under similar conditions.

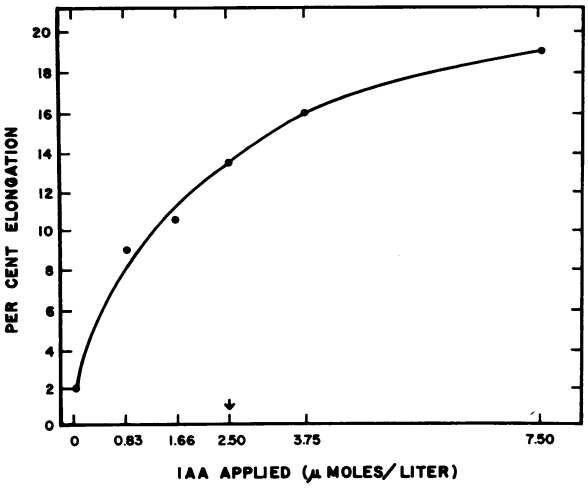



FIG. 2. The dependence of growth rate on auxin concentration. $2.5 \times 10^{-6}$ M is the donor concentration known to result in a normal amount of transport (cf. fig 1). Each point represents measurements of 8 sections; a duplicate experiment produced very similar results.

Asymmetric IAA Distribution in Hypocotyls of Sunflower (*Helianthus annuus*): It was long ago shown by bioassay that in dicotyledonous seedlings placed horizontal an asymmetric distribution of endogenous auxin similar to that in coleoptiles becomes established (4, 13, 24, 30). Therefore, it was desirable to extend the work with IAA-$C^{14}$ to such a seedling, and light-grown hypocotyls of *Helianthus annuus* were selected. The upper part of the seedling was discarded, the apical surface of the 6.5 mm section being cut 2 mm below the point where the cotyledons bulge out of the hypocotyl. The basal ends of the sections were pressed onto a razor blade (with the cotyledonary plane perpendicular to it) and donor blocks, which contained 0.46, 1.3, or $2.3 \times 10^{-6}$ M IAA-$C^{14}$, were applied at the apical end. Receiver blocks were similarly applied to the basal cut surfaces on both sides of the razor, and the assemblies were placed horizontal in small humid chambers. The data are presented in table VIII.

It is evident that lateral auxin transport occurs in sunflower hypocotyls as well as in coleoptiles. It will be noted that at the lowest donor concentration,

## Table VIII

Asymmetric Distribution of IAA-$C^{14}$ Diffusing out of Horizontal Sections of Light-grown Sunflower Hypocotyls

| Donor concentration | Counts per minute | | | | % recovery | % of transported radioactivity in upper receiver |
|---|---|---|---|---|---|---|
| | Upper receiver | Lower receiver | Tissue | Donor | | |
| 0.46 μM | 29.9 | 47.5 | 415 | 376 | 92 | 38.8 |
| (946 cpm)* | 39.8 | 64.7 | 357 | 406 | 92 | 38.0 |
| Series 1 | 56.4 | 76.6 | 440 | 386 | 101 | 42.4 |
| | 29.5 | 37.2 | 431 | 430 | 98 | 44.3 |
| | 35.4 | 47.4 | 453 | 424 | 101 | 42.7 |
| | 48.0 | 68.1 | 489 | 401 | 106 | 41.4 |
| | 18.8 | 28.1 | 362 | 468 | 93 | 40.0 |
| | 36.4 | 51.0 | 479 | 380 | 100 | 41.7 |
| | 40.9 | 50.8 | 443 | 415 | 100 | 44.6 |
| | 26.4 | 41.5 | 417 | 488 | 103 | 39.0 |
| avg | 36.2 | 51.3 | 429 | 417 | 99 | **41.3** |
| 1.3 μM | 134 | 172 | 806 | 714 | 94 | 43.4 |
| (1942 cpm)** | 118 | 128 | 728 | 796 | 91 | 47.9 |
| Series 2 | 104 | 122 | 816 | 911 | 101 | 46.0 |
| | 105 | 122 | 725 | 996 | 100 | 46.4 |
| | 87 | 133 | 697 | 1020 | 100 | 43.3 |
| avg | 110 | 135 | 754 | 887 | 97 | **45.4** |
| 2.3 μM | 124 | 167 | 1460 | 2575 | | 42.8 |
| (3900 cpm)*** | 146 | 183 | 1920 | 2240 | | 44.5 |
| Series 3 | 118 | 118 | 1870 | 2380 | | 50.0 |
| | 129 | 142 | 1830 | 2515 | | 47.6 |
| | 112 | 143 | 2100 | 1930 | | 43.9 |
| | 141 | 150 | 1750 | 1830 | | 48.5 |
| | 126 | 158 | 1895 | 2310 | | 44.5 |
| | 133 | 156 | 2040 | 2235 | | 46.0 |
| avg | 129 | 152 | 1858 | 2252 | | **46.0** |

Specific activity of IAA-$C^{14}$ in each series was 16.9 c/mole. Diffusion time was 3 hours.

* Series 1: The cpm of agar samples have been multiplied by a self-absorption factor of 1.15; the cpm of tissue samples have been multiplied by a factor of 2.36. Each experimental unit contained 12 sections. Counting efficiency was 34 %.

** Series 2: The agar correction factor was 1.07, while the tissue factor was 1.83. Each experimental unit contained 13 sections. Counting efficiency was 25 %.

*** Series 3: The agar correction factor was 1.11, while the tissue factor was 2.20. There were 12 sections per assembly. Per cent recovery could not be computed, because of a counter breakdown. The efficiency was approximately 28 %.

41.3 % of the transported auxin was found on the upper side, whereas at the two higher concentrations the less striking values of 45.4 % and 46.0 % were obtained. This suggests that the lateral transport system is approaching saturation at these IAA levels. If one compares the total amounts of auxin being transported in the three series, it appears that, although the retention of label by the tissue increases linearly, the basipetal transport system is approaching saturation. If the basipetal transport system were nearly saturated, then, as discussed above for the coleoptile, a decrease in the asymmetry would occur regardless of whether or not the lateral transport system were approaching saturation. A bioassay of the endogenous auxin produced by intact tips of the seedlings—that is, 6 mm segments of hypocotyl with apex and cotyledons attached—permitted the calculation that, on the average, sections of series 1 were transporting half as much IAA as would be transported by an intact tip. Sections of series 2 and 3, on the other hand, were transporting two times as much IAA. Thus the behavior of this tissue is in principle similar to that described in figure 1.

## Conclusion

There are three aspects to the study of any physiological response to an external stimulus: the initial detector of the stimulus, the ultimate effector which causes the response, and the mechanism which links them. This paper is concerned with the second of these, namely, the nature of the asymmetry which leads to geotropic curvature. Specifically the question asked has been: does active auxin, at growth controlling levels, become asymmetrically distributed in the tissue, and if so how is this brought about? Such an auxin asymmetry is experimentally shown to occur, and is brought about by lateral movement of the auxin. In this work, complications due to possible changes in endogenous auxin production have been avoided by the use of exogenous IAA-$C^{14}$, radioactivity being the sole criterion of hormone distribution. Such changes doubtless occur in certain instances, but in the tissues chosen for study they are quantitatively negligible. Changes in constituents other than auxin, such as ions or metabolites, are of course not ruled out as causative, independent or resultant events. But the lateral transport of IAA here demonstrated is probably the principal cause of the geotropic curvature of seedling shoots.

## Summary

I. IAA-$C^{14}$ applied at the apical ends of horizontal 6.5 mm sections of the coleoptiles of corn (*Zea mays*) or oat (*Avena sativa*) emerges asymmetrically into receiver blocks at the basal ends.

II. In the case of corn, the ratio of the radioactivity found in the upper and lower receiver blocks was 30:70.

III. A lateral auxin gradient is also demonstrable within the corn tissue itself, the ratio of radioactivity in the upper and lower halves of the sections being 40:60.

IV. It is shown that the system transporting radioactivity readily becomes saturated at auxin concentrations not far above the physiological level.

V. Within a wide range of auxin concentrations, below the level of such saturation, the magnitude of the asymmetry does not depend on the amount of auxin supplied, but at extremely low auxin levels the asymmetry does increase significantly.

VI. When halved corn coleoptile sections are placed horizontally, about three times as much radioactivity emerges into a receiver block in contact with the longitudinal cut surface as when they are placed vertically.

VII. Gravity has no appreciable effect on the total amount of radioactivity transported basipetally.

VIII. The growth of the coleoptile is limited by IAA at the concentrations normally present. Therefore, the asymmetric auxin distribution must lead to the observed upward curvature.

IX. Horizontal hypocotyls of sunflower (*Helianthus annuus*) are also capable of laterally redistributing IAA-$C^{14}$. For a 6.5 mm section the ratio of the radioactivity found in upper and lower receiver blocks was 41:59.

X. Lateral redistribution of IAA is the major means by which seedling shoots carry out their geotropic curvature.

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