It was found that the process of cellular absorption of Rb in corn leaf tissue resembles that in excised roots of barley in the following respects: absorption at the concentration used is a linear function of time for at least 1 hour; the accumulation ratio reached in 1 hour, with an external concentration of 0.02 mM Rb, is over 250: 1; the rate of absorption at 4.5° is 10 % or less of the rate at 30°.

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Transport and Distribution of Auxin during Tropistic Response
II. The Lateral Migration of Auxin in Phototropism of Coleoptiles [1, 2]

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Since the pioneering experiments of N. G. Cholodny and F. W. Went (11, 12, 27; also see 28) it has been known that phototropic curvature in the asymmetrically illuminated oat coleoptile is associated with a difference between the amounts of auxin passing through the lighted and shaded sides. This was at first ascribed to lateral migration of auxin across the plant. No obvious decrease in the basipetal transport of applied auxin could be found. Another explanation was that some auxin is photolytically destroyed on the lighted side. This was supported by the frequent observation that total yields of endogenous auxin from phototropically stimulated plants were less than from dark controls (26, 27, 28) and also by much work on model systems (14, 23). Nevertheless, 3 lines of evidence point away from this interpretation.

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Firstly, at least in corn coleoptile tips, light causes no decrease in the total yield of auxin obtained in agar receiving blocks (7, 8). This was shown both for the "first positive" and "second positive" curvature (see 26), and discrepant results of other workers could be explained by the observation (6) that brief exposure of corn or oat coleoptiles to phototropically ineffective red light decreases the subsequent auxin production by the tips. Since most experimenters had not excluded red from their safe-lights, the decreased auxin yields which they reported do not necessarily constitute evidence for a role of auxin destruction in phototropism.

Secondly, calculations made by Bünning (9) and by Thimann and Curry (26) show that an incident photon causing the "first positive" curvature of Avena would have to be capable of destroying at least several hundred IAA molecules in order to account for the observed asymmetry. Such a high quantum yield certainly rules out a direct photolytic effect.

Thirdly, von Guttenberg (19) showed that although decapitated oat coleoptiles give only a very slight response to 3 hours' stimulation by unilateral

white light, the same plants subsequently develop a marked curvature when the physiological tip becomes regenerated or when IAA is supplied to them. In fact, the plants remember a previous exposure to light for at least 8 hours in the dark in the absence of auxin, giving up to 15° curvature when auxin is subsequently provided. These observations also rule out any direct photolytic action.

Evidence of another kind supports the idea that in phototropism auxin is actually moved across the plant. Two years before Went's experiments, it had been shown by Boysen-Jensen and Nielsen (4) that the insertion of a fragment of mica in the tip of a coleoptile, in the plane perpendicular to the incident light, nearly prevented the development of phototropic curvature. But when the cut surfaces were permitted to remain in contact with each other, or when the cut was made parallel to the plane of the incident light, curvature comparable to that in unslit controls ensued. Briggs et al. (8), after showing that under their conditions the corn coleoptile tip gave a 71: 29 distribution of auxin between shaded and lighted sides, repeated and extended the findings of Boysen-Jensen by completely bisecting a coleoptile tip perpendicular to the incident light, and separating the halves with a thin glass cover-slip. The glass eliminated the auxin differential entirely. Not only does this experiment strongly support the lateral transport hypothesis, but the data also show that the total amount of auxin entering a receiver block at the base of the tip was the same in phototropically active light as in inactive light; evidently, therefore, the decrease in auxin on the lighted side of the tip is approximately balanced by an increase on the dark side.

Recent studies with IAA-C^{14} have, however, given results in sharp disagreement with those found for endogenous auxin. A number of workers have attempted to find asymmetric distribution of radioactivity when the labeled auxin was applied to phototropically stimulated oat coleoptiles (10, 18, 24). Their results have been uniformly negative, whether the IAA was applied to intact or to decapitated plants, and whether the light dosage was low or high. Two possible explanations suggest themselves. The first is that an auxin precursor or other factor involved in auxin synthesis is moved across the plant, rather than the IAA itself. This possibility has been discussed in detail by Thimann and Curry (26). The second is that only a special endogenous auxin migrates, resulting in an asymmetry measurable by bioassay of this endogenous growth hormone, while the radioactivity of the IAA-C^{14} remains uniformly distributed. Since Gordon and his coworkers were able to find asymmetry of endogenous auxin under conditions apparently the same as those leading to symmetrical distribution of IAA-C^{14}, search is continuing for additional endogenous auxins in coleoptile diffusate (18, 22).

However, the meaning of these experiments with radioactive IAA is subject to 2 major reservations. First, several workers have been unable to find evidence for lateral transport of applied radioactive IAA during the geotropic response, yet we have shown (15, 16) that when purified IAA-C^{14} is properly applied in low concentrations to horizontally-placed coleoptile sections the distribution of radioactivity becomes markedly asymmetrical, agreeing well with the original bioassay data of Dolk (13). Hertel and Leopold (19a) also showed lateral transport of C^{14}-IAA through the cut surface of halved horizontal coleoptiles. The data of Goldsmith and Wilkins (17) in which C^{14}-IAA applied asymmetrically to the apical cut surface of horizontal coleoptile sections became symmetrically distributed at the base, gives further demonstration that lateral transport occurs in geotropism. Second, as has been stressed by Thimann and Curry (26) and by Briggs (7), the light-sensitive region for the "first positive" response of the corn coleoptile is strictly localized within 0.5 mm of the tip, so that the significance of any experiments in which the tip was removed or mutilated, or in which the IAA-C^{14} was not applied strictly to the apex, is doubtful. Even for the "second positive" response, the tip is more sensitive than the lower regions of the coleoptile.

The nature of the factor which migrates across the phototropically stimulated coleoptile tip therefore remains in question. The present paper is a re-examination of this problem with the aid of radioactive IAA. The results show clearly that the factor moving laterally is IAA itself.

Materials and Methods

The techniques for growing corn (*Zea mays*, var. Burpee's Barbecue Hybrid), for purifying the C^{14}-carboxyl-IAA, and for studying its distribution in subapical coleoptile sections have all been described in the preceding paper (16). Since enzymic IAA destruction results in loss of the carboxyl group, carboxyl labeling assures that the radioactivity represents undecomposed IAA. In order to study the distribution of IAA-C^{14} in apical sections, it was necessary to modify the procedure slightly as follows: the tips of corn coleoptiles which had just received at least 2 hours of red light were cut to a length of 6.5 mm and lined up on a razor blade which bisected their bases to a depth of 1 mm. Agar receiver blocks were set against the halved bases on either side of the blade. The side of the assembly to receive illumination was determined randomly, and, if the exposure was to be only 1000 meter-candle-seconds, the tips were then illuminated. Immediately afterwards, agar blocks containing the IAA-C^{14} were fixed to a plastic support which was lowered over the row of coleoptile tips until it just made contact with the intact apices. If the dosage was to be greater than 1000 meter-candle-seconds the donor blocks were lowered into place before the assembly was illuminated. The rest of the procedure was unchanged.

Corn seedlings to be used for dose-response studies were germinated as described previously (16) but

were transferred to vials of 0.7 % agar about 48 hours after soaking, when the roots were 1 to 2 cm long. They were then exposed as usual to red light for about 4 hours to inhibit mesocotyl development. Two hours before use, they were placed under a red light again in order to lower the rate of synthesis of endogenous auxin. The program of illumination was thus identical with that for the plants used for auxin distribution studies. Both groups of plants were ready for experimentation about 90 hours after soaking.

To raise oat seedlings for curvature response measurements, the seeds were hulled, soaked for 2 hours in distilled water, set out on moist filter paper, immediately irradiated with red light for 5 hours to inhibit mesocotyl development, and about 32 hours after soaking were set into vials of 0.7 % agar. About 40 hours later they were illuminated with red light for 2 hours and then used for curvature experiments.

The light source for phototropic exposures was a 150-w incandescent bulb. Light of 500 meter-candle intensity was measured either with a General Electric or Weston exposure meter, or with an electronic galvanometer and thermopile calibrated against a C-620 lamp from the Bureau of Standards. At the lower intensities measurements were made with the thermopile because of its greater sensitivity. Readings with the thermopile were correlated with those of the photocells at intensities high enough for both to be reliable; it was calculated that 1 meter-candle on the exposure meters corresponded to 39 ergs/cm^2 second. The temperature in the room containing the light source was maintained between 25° and 27°. The humidity in this room was not regulated, but coleoptile sections to be illuminated for more than a minute were placed in moist transparent chambers which prevented drying out. Slight variations in the humidity of the room did not noticeably affect the reproducibility of the curvature response.

The counts per minute have been corrected for self-absorption only in those cases where total recovery is quoted, that is, in tables IV and V. The correction factors differ from experiment to experiment, due to different planchet diameters and different volumes both of agar and of sections (which may or may not have been halved as well). There are of course random errors in the sizes of the donor blocks and in the actual counting, the latter being especially serious when the counts are small. It was in order to minimize the effects of all these unavoidable errors that so large a number of experiments has been felt necessary.

Results and Discussion

The Response to Low Light Dosages. The classical work on phototropism was mainly concerned with the dose range in which curvature is a function of the product of intensity and time (see 26). Both in oats and in corn this "first positive curvature" occurs at white light dosages below 5000 meter-candle-seconds.

To select an appropriate light dosage within this

Table I

Asymmetric Distribution of IAA-C^{14} Entering Agar Blocks from Corn Coleoptile Tips Unilaterally Exposed to 1000 Meter-Candle-Seconds White Light ("First positive" range of light dosage)

Light dose	IAA in donor	Experiment No.	Cpm in agar*		Avg amount of exogenous IAA transported** (μg/tip-hour)	% of radioactivity on lighted side
			Lighted receiver	Shaded receiver		
100 m-c, 10 sec	2.2 × 10^{-6} M; specific activity 16.9 c/mole	1	10.9	28.4	1.4 × 10^{-5}	28
		2	14.3	50.4	2.5 × 10^{-5}	22
		3	15.2	50.6	2.5 × 10^{-5}	23
= 1000 m-c-s		4	22.1	61.9	3.2 × 10^{-5}	26
						Average 25
		5	39.0	66.2	3.7 × 10^{-5}	37
		6	35.2	82.0	4.2 × 10^{-5}	30
		7	48.3	158.	7.0 × 10^{-5}	23
		8	91.	170.	8.6 × 10^{-5}	35
		9	118.	157.	9.8 × 10^{-5}	43
						Average 34
50 m-c, 20 sec	7.5 × 10^{-5} M; specific activity 0.96 c/mole	10	2.3	7.9	0.6 × 10^{-4}	23
		11	4.3	15.4	1.1 × 10^{-4}	22
= 1000 m-c-s		12	6.1	19.5	1.4 × 10^{-4}	23
		13	8.9	22.8	1.7 × 10^{-4}	28
						Average 24

* The cpm have not been corrected for absorption by the agar; the correction factor for 0.16 ml agar is 1.1. Counting efficiency was 30 %. There were 22 tips per experiment. In the 100 meter-candle series the transport period was 165 minutes, in the 50 meter-candle series 180 minutes.

** The average amount of endogenous IAA transported per tip per hour under the conditions of these experiments is 8 × 10^{-4} μg [see table I of the preceding paper (16)].

Table II

Asymmetric Distribution of IAA-C^{14} in Corn Coleoptile Tips Unilaterally Illuminated with 1000 Meter-Candle-Seconds White Light

Experiment No.	Cpm in tissue*		Tissue weight, mg		% of radioactivity in lighted half
	Lighted side	Shaded side	Lighted side	Shaded side	
1	16.4	27.2	9.8	9.6	38
2	28.3	44.0	10.4	9.4	39
3	19.1	45.6	9.6	8.9	30
4	37.7	67.8	9.4	9.4	36
5	62.9	94.4	9.7	9.1	40
6	25.5	61.7	8.9	9.6	29
7	68.1	141.0	...	...	33
8	84.3	159.0	10.4	10.0	35
				Average	35

* The cpm have not been corrected for absorption by the tissue; the correction factor is 1.3. Counting efficiency was 35 %. Experiment numbers correspond to those of table 1.

range, rows of intact corn coleoptiles (preilluminated for 2 hours with red light) were subjected for different periods to white light of 50 meter-candle intensity. They were returned to darkness for 100 minutes, then shadowgraphed for measurement with a goniometer. The resulting dose-response curve had a shape similar to that obtained with Burpee's Snowcross corn (5). In each of 4 duplicate experiments the dose of maximum "first positive" effectiveness was about 1500 meter-candle-seconds, which produced curvatures of approximately 13°. Exposure to 1000 meter-candle-seconds, which was selected for the auxin distribution studies, produced 9 or 10°, and was within the range where response is roughly proportional to the logarithm of light dosage.

With this light dosage the distribution of IAA-C^{14} was determined as described above. Table I shows that the amounts of radioactivity transported into the receivers on the lighted and unlighted sides are indeed unequal. The data of the first series of experiments fall into 2 groups. In the first group, on the average, only 25 % of the label appears in the agar block from the illuminated side, with 75 % on the darker side. The second group of experiments, though performed at the same time as the first, was segregated from it because relatively more auxin was carried into the receivers. The variability of the data in this second group is high, and the average ratio is only 34: 66. The reduction of asymmetry cannot be due to an overloading of the lateral transport system because the second series (experiments 10 to 13) in which still more auxin is transported, produced an average ratio of 24: 76. The decreased asymmetry is probably due to the fact that if some of the tips protruded too far into the donor block, IAA-C^{14} would have entered below the tip region. In a preliminary series of experiments, in which coleoptile tips were permitted to push into the agar donor as they grew so that the total amount of auxin transported was high, the average ratio between the lighted and unlighted sides was only 47: 53. For this reason, in all subsequent experiments the support to which the donors were fixed was raised once or twice during the 165 or 180 minute transport period to prevent the growing tips from penetrating the donors. In any event, table I establishes the regular occurrence of a strongly asymmetric distribution of IAA-C^{14}.

The extent of this asymmetry of exogenous auxin compares closely with that of the endogenous auxin diffusing from lighted and unlighted sides of coleoptile tips. Using another variety of sweet corn, Briggs (7) has reported a 35: 65 asymmetry, whereas ratios of 26: 74 and 17: 83 were obtained earlier by Went (27) and Wilden (29), respectively, with oats. Both the average values of table I, namely 25: 75 and 34: 66, fall within the range of these data. It is difficult to make exact comparisons with the earlier work, however, since the plants are not the same and the light dosage in the present experiments was somewhat submaximal. As a matter of fact, the isotope method might be expected to indicate even more pronounced asymmetry than the bioassay method, for 2 reasons. First, the bioassay measurements are interfered with by the uniformly distributed endogenous auxin which continues to diffuse symmetrically into the receivers for the first half hour or more after illumination, before the wave of redistributed auxin reaches the base of a 6.5 mm tip. Radioactivity determinations are free from this complication. Second, Briggs found with Burpee Snowcross corn that the region of auxin production extends more than 1.5 mm farther down the tip than does the region of lateral translocation (7). If this applies to the present variety of corn, then IAA-C^{14} which is carefully applied to the extreme tip may become even more completely redistributed than the endogenous auxin.

For the sake of completeness, the distribution of radioactivity within the coleoptile tissue of experiments 1 through 8 was assayed; table II shows that, as expected, more auxin is found in the shaded than in the lighted halves of the plants. The asymmetry here only amounts to 35: 65, but since it is difficult to bisect the rather unsymmetrical, tapering intact tips rapidly and accurately, these data may be less

quantitatively reliable than those of table I. Below the region of IAA-C^{14} entry and redistribution, the asymmetry of radioactivity in the tissue should not vary appreciably with distance from the tip, and should be the same as the asymmetry of radioactivity emerging into the receivers. Nevertheless, table II is important because it shows that failures of previous workers to find asymmetric distribution of applied IAA-C^{14} were not due to their assaying of the radioactivity in the tissue rather than in receiver blocks. It appears, instead, that these failures must be attributed to improper application of the radioactive auxin.

The Curvature Response to Continuous Light. The responses to small and to large amounts of light are different in several ways. Firstly, the curvature resulting from large light dosages, or "second positive response," does not seem to be dependent on the total amount of light received, but rather on the length of time the light remains on. Secondly, the region of sensitivity to high light dosage extends several millimeters below the apex. Thirdly, in oats at least, the "second positive" curvature response begins almost immediately, whereas there is a 20 minute lag before a response can be observed following a "first positive" exposure. It thus seems clear that the coleoptile has 2 distinct systems responsible for the "first positive" and "second positive" curvatures. The 2 types of response have been compared and contrasted by Thimann and Curry (26) and by Zimmerman and Briggs (30).

In order to relate measurements of auxin distribution to the curvature which would occur under the same experimental conditions, a few dose-response studies were carried out. The first dealt with Avena. Rows of oat seedlings growing in agar, with mesocotyls shaded, were exposed to 500 meter-candles of light for times varying from 15 to 100 minutes and then allowed 40 more minutes for curvature to develop. (This curvature time was kept short in order to minimize geotropic reversal.) The response was confirmed to be linearly dependent on the duration of illumination, as predicted (26, 30). The longest exposure produced an average curvature as high as 68° in spite of the fact that 40 minutes is well below the 90 minute time previously reported optimal for the development of curvature under these conditions (26).

In another experiment, rows of plants were exposed for 2 hours to light of intensities from 10 to 600 meter-candles. It was confirmed that the response is at least approximately independent of light intensity over this 60-fold range. Thus the continuous, high intensity light which brings about asymmetric IAA-C^{14} distribution in the subapical coleoptile sections causes in the intact seedling a phototropic curvature which is clearly characterized as the "second positive" response.

The responses of corn coleoptiles are slower than those of oats. Time lapse photographs taken with red light showed that maximal curvature occurred in corn 2 hours after the end of a 45 minute illumination with white light. By exposing for 20, 40, and 60 minutes to unilateral 500 meter-candle light and allowing 2 hours for curvature development, it was ascertained that induction begins immediately after the light is turned on and proceeds until, at the end of an hour's exposure, a 40° curvature has been induced. However, if following exposures greater than an hour, 2 hours were allowed for curvatures to develop, then geotropic straightening interfered. Therefore, in order to study response to exposures of more than an hour, it was necessary to place the plants on a clinostat after illumination. By this means it was possible to show that induction is an increasing function of exposure time for at least 2 hours. The behavior of plants illuminated longer than 2 hours was impossible to assess in this way because such large phototropic curvatures had developed during the induction period, before the plants were placed on the clinostat, that geotropic counter-induction confused the results. For the auxin experiments, such a constraint is placed on curvature by the short length of the sections employed that only insignificant geotropic effects might be expected. The plants were, therefore, generally exposed to 500 meter-candles for a full 3 hours.

If the upper 2 mm of the coleoptile tips were covered by opaque black caps, a curvature of only 11 to 12° was induced by an hour's illumination. Controls bent about 40°. Thus the tip does play an important part in receiving the stimulus for the second positive curvature.

Auxin Distribution under Continuous Light. For the initial studies of auxin distribution, subapical sections were used. One advantage of this is that auxin enters decapitated sections readily, whereas in order to get a small amount of exogenous auxin through the cuticle of an intact tip, such a high concentration must be applied that any photolysis and consequent loss of isotope might be overlooked. Another advantage is that decapitation absolutely excludes interference by the "first positive" response.

Preliminary auxin distribution experiments were performed on oats. Coleoptile sections were cut, mounted on a razor blade, and provided with agar donors and receivers exactly as described in the first paper (16). Instead of being placed horizontal, however, the assemblies were subjected to unilateral white light of 500 meter-candle intensity for the whole duration of the 3-hour transport period. Table III sum-

Table III

Percentage of Transported Auxin Entering Agar Blocks from Lighted Side of Subapical Oat Coleoptile Sections Exposed to 500 Meter-Candles White Light for 3 Hours

The donor blocks contained 2.3×10^{-6} M IAA-C^{14}, of specific activity 0.96 c/mole. The data are the summary of 3 series of experiments.

Series	Experiments per series (20 sections each)		% of C^{14} on lighted side
1	11		45.7 ± 1.1
2	20		46.2 ± 0.8
3	12		47.2 ± 1.1
Total	43	Weighted average	46.4

Table IV

Asymmetric Distribution of IAA-C^{14} Entering Agar Blocks from Subapical Corn Coleoptile Sections Unilaterally Exposed to 500 Meter-Candle White Light for 3 Hours ("Second positive" range of light dosage)

Donor blocks contained 9.3 × 10^{-6} M IAA-C^{14} of specific activity 0.95 c/mole, corresponding to 985 cpm per pair of donors. This amount gave rise in the receiver to 11 × 10^{-4} μg/section hour, which is 1.4 times as high a value as would be expected from intact tips. Two assemblies of 16 sections were pooled for each experiment. To correct for self-absorption, cpm in agar samples have been multiplied by a factor of 1.05 and cpm in tissue have been multiplied by 1.60 (tissue of each experiment was counted in 2 lots of 16 sections each). Counting efficiency was 31 %.

Counts per minute				% of radioactivity in lighted receiver	% recovery of radioactivity
Left in donor block	Found in Tissue	Entering lighted receiver	Entering shaded receiver		
318	...	104	127	45.0	...
320	307	167	190	46.7	100
264	324	180	206	46.6	99
317	302	163	205	44.4	100
354	305	162	175	48.0	101
avg 316	310	155	181	46.1	100

Table V

Demonstration That No Auxin Is Destroyed in Corn Coleoptile Sections Unilaterally Exposed to 500 Meter-Candle White Light for 3 Hours

Series 1 and 3: There were 24 sections in each experiment (2 assemblies of 12 each), and the transport period was 3 hours. The specific activity of the IAA was 0.95 c/mole. In the 1st series, the amount transported was 0.8 times normal; the correction factor for absorption by the agar (0.32 ml) was 1.45 and that for tissue absorption 2.16. In the 3rd series, the amount transported was 1.2 times normal; the correction factor for agar was 1.00 and that for tissue 2.40.

Series 2: There were 10 sections in each experiment (single assemblies), the transport period was 3 hours, the specific activity 5.6 c/mole, the amount transported 0.9 times normal, and the counting efficiency 44 %. The correction factor for absorption by agar was 1.00 and that for tissue was 1.90.

	Counts per minute			% radioactivity recovery of
	Left in donor	Found in tissue	Entering receiver	
Series 1				
3.7 μM donors				
(413 cpm per assay)				
Illuminated				
(9 experiments)	189	87	148	104
Not illuminated				
(6 experiments)	174	85	147	98
Series 2				
3.7 μM donors				
(1665 cpm per assay)				
Illuminated				
(10 experiments)	839	342	590	105
Not illuminated				
(10 experiments)	863	332	570	106
Series 3				
9.3 μM donors				
(1007 cpm per assay)				
Illuminated				
(5 experiments)	586	214	219	101
Not illuminated				
(5 experiments)	574	205	225	100

marizes the asymmetric distribution of auxin resulting: on the average, 46 % of the auxin was found on the lighted side, 54 % on the shaded side.

More detailed experiments were carried out on the corn coleoptile, because of its conveniently larger size. Table IV shows that when sections cut 2 mm below the apex were unilaterally exposed to 500 meter-candle light for 3 hours only 46.1 % of the radioactivity emerging at the base was on the side which faced the light. This figure agrees with that found for oats. Since in these experiments the average amount of auxin transported was 1.4 times normal, it may be judged from the data of figure 1 of the preceding paper that the capacity of the redistributing system was not being exceeded.

Furthermore, the recovery of radioactivity in the experiments of table IV was virtually complete. In order to confirm this crucially important point, a

Table VI

Asymmetric Distribution of IAA-C^{14} Entering Agar Blocks from Subapical Sections When Different Amounts of Auxin Are Being Transported Basipetally. Unilateral 500 Meter-Candle White Light for 3 Hours

Full details for the 1st series are presented in table IV. For series 2, 4, and 5, the correction factor for absorption by agar would be 1.1, the specific activity of the auxin was 16.9 c/mole, the counting efficiency 34 %, and the transport period 3 hours. Per cent recovery was calculated to be 105 % for series 5. Series 2 and 4 were carried out simultaneously. Series 3 and 6 were also carried out simultaneously but about a year later than the others. The counting efficiency then was 44 %. The absorption factor would be 1.0 for series 3 and 1.1 for series 6; the specific activity for series 3 was 3.94 c/mole and 16.9 c/mole for series 6.

Series	Amount of auxin transported	Number of experiments averaged	Number of sections per experiment	Counts per minute		% of Radioactivity found on lighted side
				Lighted receiver	Shaded receiver	
1	1.4 × normal	5	32	147	172	46.1
2	0.5 × normal	7	12	367	416	46.9
3	0.4 × normal	5	10	135	135	50.0
4	0.1 × normal	6	20	114	134	46.0
5	0.02 × normal	12	22	21.5	29.8	41.8
6	0.003 × normal	5	40	7.1	9.6	42.5

Table VII

Asymmetric Distribution of IAA-C^{14} Entering Agar Blocks from Intact Tips Exposed to "Second Positive" Light Dosages

The specific activity of the IAA was 0.95 c/mole. The transport period was 3 hours, including the exposure to white light. Experiments of the 1st series involved 44 (6.5 mm long) tips. After multiplying the cpm by a factor of 1.2 to correct for absorption of radioactivity by the agar and a factor of 3.2 to convert cpm to dpm, it can be calculated that on the average the amount of exogenous IAA transported per tip per hour was 0.3 × the amount of endogenous auxin transported per tip per hour. Experiments of the 2nd and 3rd series involved 48 tips. The amount of exogenous IAA transported per tip per hour was calculated to average 0.1 × the normal amount in the 2nd series and 0.2 × the normal amount in the 3rd.

Light dosage	Donor concentration	Counts per minute		% radioactivity in lighted receiver
		Lighted receiver	Shaded receiver	
3 hr at 500 m-c	30 μM	34.0	67.1	34
		34.8	75.7	32
		52.6	85.3	38
		18.4	48.8	27
		42.6	76.2	36
		53.4	111.0	32
		21.6	47.1	32
		46.1	102.0	31
		37.9 avg	76.7 avg	33 avg
30 min at 50 m-c	20 μM	3.4	7.3	36
		16.0	25.8	38
		2.9	6.1	37
		14.3	22.4	39
		9.5	21.5	31
		10.6	16.2	37
		6.8	18.5	27
		1.9	3.5	35
				35 avg
30 min at 50 m-c	60 μM	19.3	41.6	32
		36.7	68.9	33
		16.0	25 6	38
		23.4	51.1	31
		23.9 avg	46.8 avg	34 avg

comparison was made between assemblies of sections illuminated with phototropically active white light and controls placed either under dim red light (series 1, 3) or in darkness (series 2). Table V summarizes the results of these 3 series of experiments in which 80 %, 90 %, and 120 % of the normal amount of auxin was transported into the receivers. In the first series, the average value for recovery of radioactivity in the illuminated assemblies was 104 % while the average for the controls was 98 %. In the second series the values were 105 % and 106 %, and in the third series 101 % and 100 %, respectively. These

slight deviations from 100 % recovery are within the expected range of error. It must be concluded that little or no IAA is destroyed by light during 3 hours' strong illumination. More important yet, inspection of table V also shows that the partition of the radioactivity between the donors, tissue, and receivers is essentially the same in all the series. Thus, it appears that light-induced changes in the *rate* of transport cannot be invoked to account for the lateral asymmetry.

It is of interest to ask whether, as the donor concentration is lowered, the lateral transport system redistributes a fixed amount of the auxin or a fixed proportion of it. Therefore, the experiments of table IV were repeated with donors containing lower concentrations of IAA-C^{14}. Table VI shows the striking result that when 0.5 and 0.1 times the normal amount of auxin is transported out of each section, the ratio of radioactivity entering receivers from the lighted side to that from the shaded side is the same as in table IV, when the sections were transporting 1.4 times the normal amount. (An anomalous failure to detect asymmetry occurred in series 3: this single case, involving fewer sections than the other series, probably falls within the expected range of variation.) Series 5 and 6 suggest that the asymmetry may rise slightly at very low auxin levels, but the experiments are too few and the radioactivity too low to be certain of their meaning. The main conclusion is that over a wide range of concentrations, the asymmetry which develops under continuous illumination is independent of the amount of auxin undergoing basipetal transport.

Although one of the most interesting features of the "second positive" response is its occurrence in the subapical region, the tip of the coleoptile also shows conspicuous bending. Therefore, the experiment was duplicated with intact tips protruding about 0.5 mm into donors containing 3×10^{-5} M IAA-C^{14}, again illuminating with 500 meter-candles for 3 hours. The average ratio between the amounts of auxin which emerged into the receivers on the lighted and shaded sides, as can be seen in table VII, was 33: 67. Using a light intensity of only 50 meter-candles and a 30-minute duration of exposure, donors containing 2 and 6×10^{-5} M IAA gave similar ratios, averaging 35: 65 and 34: 66. This similarity persists in spite of the fact that the experimental conditions were varied, and that the depth of insertion of the tips into the donor blocks was not precisely controlled.

The difference between the results with the tip on and those with subapical sections agrees with the observation that even for the second positive stimulus the sensitivity of the tip of the corn coleoptile is greater than that of the regions below; both the auxin asymmetry and the curvature were about 4 times as great when the tip participated in the response as when the subapical region alone took part.

Although tables IV and V make it clear that no auxin is destroyed in continuously illuminated subapical sections, the radioassay methods of table VII are inadequate to prove that destruction is unimportant in the apices. This is in part because the amount of radioactivity in the donor is large enough to obscure any small losses within the tissue, and in part because the amount entering and passing through the tissue depends on the highly variable area of contact between donor block and coleoptile tips, making comparisons of transport through stimulated and unstimulated tips very uncertain. It was felt necessary, therefore, to make measurements of the endogenous auxin. The bioassays of Briggs (7) indicate that destruction of endogenous auxin by phototropically stimulated coleoptiles of Burpee's Snowcross corn is negligible. Similar bioassays have now been carried out on our Burpee's Barbecue Hybrid corn. Sets of four 6.5-mm coleoptile tips cut from plants which had received the usual 2-hour illumination with red light were set on plain agar receivers. Three sets remained for 3 hours under dim red light as controls, and 3 sets were subjected to 3 hours of unilateral irradiation with white light of 500 meter-candle intensity. The agar receivers were assayed with the Avena curvature test. The average curvature produced by the control blocks was 14.4°, while the blocks from the illuminated tips gave 16.4°. The difference has a $p = 0.3$. This test thus extends Briggs' measurements and makes it clear that destruction of endogenous auxin plays no role in the mediation of the "second positive" phototropic response of these coleoptiles.

Conclusions

The results of these experiments all point unequivocally to the lateral movement of auxin from the lighted to the shaded side of the corn coleoptile as the prime cause of its phototropic curvature. The lateral movement appears to occur most readily in the tip, whether the illumination is such as to cause the first positive or the second positive type of response. However, a smaller but perfectly clear-cut lateral movement giving 46: 54 distribution could be established in basal sections given long exposures. For these conditions, oat and corn coleoptiles gave the same values. While it is difficult to rule out a small amount of auxin inactivation, especially in the experiments with intact tips, the recoveries in many experiments are close enough to 100 % to rule out photodestruction of auxin as a necessary participant in phototropism.

With the exclusion of photodestruction the principal argument for the participation of riboflavin in phototropism falls to the ground, since this was based primarily on the ability of riboflavin to catalyze photolysis of IAA in vitro. Other ingenious schemes involving growth inhibition by IAA photolysis products (e.g. that of Meyer and Pohl, 20, 21) can also now be discarded. The existence of an auxin asymmetry both in the tissue and in the agar receivers appears furthermore to rule out a difference in the *rate of transport* as cause either of phototropism or of geotropism (cf. 2). Rather, the occurrence of true *lateral transport* is well supported.

The dominant role of lateral auxin transport in

tropisms accords well with the central position which the transport of auxin, and its susceptibility to various types of modifying factors, is beginning to assume in the morphogenesis and growth control of seedlings.

Summary

Apical sections of coleoptiles of corn (*Zea mays* L., var. Burpee's Barbecue Hybrid) with the tips intact were supplied at the extreme tips with indoleacetic acid-C^{14} and unilaterally illuminated with 1000 meter-candle-seconds of white light—a dosage which caused "first positive" phototropic curvature in controls. The ratio of the radioactivity which was transported into agar receivers set under the lighted and shaded sides of the sections was found to be 25: 75.

A ratio only slightly lower was measured in the 2 halves of the coleoptile tissue.

Similar apical sections of corn coleoptiles supplied with indoleacetic acid-C^{14} were unilaterally illuminated with 50 meter-candle white light for 30 minutes or 500 meter-candles for 3 hours, dosages which led to "second positive" type curvatures in control plants. The auxin emerged asymmetrically into agar receivers at the bases of the sections, with about 34 % on the lighted side and 66 % on the shaded side.

Bioassay confirmed that there is no appreciable difference between the amounts of endogenous auxin transported out of coleoptile tips in continuous 500 meter-candle white light and control tips kept in dim (phototropically inactive) red light.

Subapical corn coleoptile sections similarly supplied with indoleacetic acid-C^{14} and unilaterally illuminated with 500 meter-candle white light for 3 hours transported the auxin asymmetrically into basal agar receivers, the ratio measured being 46.1: 53.9. Similar experiments with Avena coleoptile subapical sections also gave a ratio of 46: 54.

All of the radioactivity supplied to such phototropically stimulated subapical sections was recoverable at the end of the experiments; hence, the asymmetry was not produced by auxin destruction. The partition of radioactivity between agar donors, tissue, and agar receivers was not measurably different from that in controls.

No differences in the magnitude of the asymmetry developed by these subapical sections could be distinguished whether they transported 1.4, 0.5, or 0.1 times the normal amount of auxin, although there was some evidence that with extremely low auxin concentration the asymmetry may be increased.

It is concluded that photodestruction of auxin is not the cause of phototropic curvature in these coleoptiles, but that indoleacetic acid is translocated laterally across the corn coleoptile during both the "first positive" and "second positive" phototropic responses.

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Comparison of Lignin-Like Products Found Naturally or Induced in Tissues of Phleum, Elodea, and Coleus, and in a Paper Peroxidase System [1, 2]

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While the biosynthetic pathway in a grass, wheat, appears to be similiar to that observed in woody materials (3, 12), certain peculiarities of grass lignins or lignin-like products need to be elucidated. One peculiarity is the relative insolubility in alkali of the lignin-like product of peroxidation of an exogenous source of coniferyl alcohol in contrast to that formed by ferulic acid or found naturally within a grass (19, 20). The other is the presence of ferulic and *p*-hydroxycinnamic acids, but not sinapic acid, after alkaline extraction and hydrolysis of cell wall residues containing lignin (21).

In order to explore these problems, comparisons were made of natural and induced lignin-like products in a system containing filter paper infiltrated with peroxidase (17) and in tissue sections of selected herbaceous plants. Tissues studied in detail were Phleum and Coleus, a highly lignified monocot and dicot respectively, and Elodea, an aquatic monocot without detectable lignin.

Materials and Methods

Plants used were generally greenhouse grown from seed or plants obtained commercially. Most of the work here and in preceding papers (19, 20, 21) was done with the hexaploid *Phleum pratense* L. var. climax. In addition, seeds of a tetraploid line of *Phleum pratense* were obtained from E. L. Nielsen. Other plants used were: *Triticum aestivum* L. (wheat), *Phyllostachys aurea* Riv. (bamboo), *Zea mays* L. (corn), *Avena sativa* L. (oats), *Saccharum officinarum* L. (bagasse or sugarcane), *Coleus blumei* Benth., *Pelargonium cultivar* (geranium), *Nicotiana tabacum* L. (tobacco), *Impatiens holstei* Engler and Warb. (balsam), *Helianthus annuus* L. (sunflower), *Tradescantia virginiana* L., *Elodea densa* Planch., *Lemna minor* L., *Apium graveolens* L. (celery). The native lignin preparation from bagasse was the same as used previously (19), but the preparation was hydrolyzed in 0.5 N NaOH as in the regular treatment.

Phenolic compounds were purchased commercially. Ferulic and *p*-hydroxycinnamic acids were recrystallized from ethanol by the addition of water. The eugenol was an aged preparation with a strong phloroglucinol test.

Histochemical tests on sections and biochemical analyses on water and ether extracted tissue (called cell wall residue) were described previously (19, 20, 21). One change was that the fluorometric analyses were done in 0.05 M tris buffer or in 0.05 M NaOH. The change in OD at 350 mμ is a measure of the peak in the ionization difference spectrum in the region near 350 mμ. For this determination, the OD at wave lengths between 340 and 360 mμ of a solution

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