MEDIATION OF GEOTROPIC RESPONSE BY LATERAL TRANSPORT OF AUXIN [1, 2]

BARBARA GILLESPIE [3] & WINSLOW R. BRIGGS

DEPARTMENT OF BIOLOGICAL SCIENCES, STANFORD UNIVERSITY, STANFORD, CALIFORNIA

A suggestion concerning the mechanism of geotropic response was offered independently by Cholodny in 1924 (12) and by Went in 1926 (27). It was known by this time that a growth promoting hormone (an auxin) is produced in tips of coleoptiles and seedlings and moves in a continual stream toward their bases. Cholodny and Went postulated that auxin moves also from the upper to the lower side when a shoot is placed horizontally. As a result, the growth rates of the upper and lower halves would decrease and increase, respectively, and the shoot would curve upward. In 1927 Cholodny (13) generalized his hypothesis to cover the phototropic response, and in 1928 Went (28), again independently, arrived at the same explanation. The concept of hormonal coordination of plant tropisms has come to be called the Cholodny-Went Theory, in honor of both men. The development of the theory has been reviewed by Went and Thimann (29).

Several workers performed experiments which demonstrate that geotropic curvature of a shoot is the result of a decrease in growth of the upper half accompanied by an increase in growth of the lower half. Among these, the careful measurements of Navez and Robinson (20) on the Avena coleoptile seem particularly reliable.

Direct studies of auxin distribution following phototropic induction were first performed by Went in 1928 (28). He placed agar blocks basipetal to each half of a coleoptile tip which had been longitudinally split at the base. He illuminated the coleoptile unilaterally, perpendicular to the split, and, after a suitable length of time, measured the relative amounts of auxin in the two blocks by means of the Avena test. He found that the illuminated side released much less auxin than did the shaded side.

Shortly afterward, Dolk (15) performed similar experiments on both *Avena sativa* and *Zea mays* coleoptiles which had been induced by gravity. More auxin was obtained from halves which had been lowermost than from those which had been uppermost, while the total auxin yield of each organ was unaffected by stimulation. A number of workers repeated Dolk's experiment with other plant organs, and found similar distributions of auxin between the top and bottom halves (4, 14, 19, 20, 25). Other workers obtained similar distributions when auxin was obtained by extraction (5, 26).

Bünning et al (9), Gordon and Eib (18), Reisener (21), Reisener and Simon (22), and Ching and Fang (10), applying carbon 14-labeled indoleacetic acid to oat coleoptiles and to other plant parts, have recently been unable to demonstrate lateral movement of the labeled hormone during or following either geotropic or phototropic stimulation. Numerous other workers have expressed lack of confidence that lateral auxin transport occurs (1, 2, 3, 5, 11, 23), and some have proposed other mechanisms to explain the differential growth of the two sides (2, 7, 9). In view of the recent experiments of Briggs et al (8) suggesting that lateral transport of auxin may indeed mediate the phototropic response of coleoptiles of *Zea mays* to certain dosages of unilateral light, it seemed worth while to repeat these experiments, using gravity instead of light as the stimulus.

MATERIALS & METHODS

The methods for growing corn (*Zea mays* L. Burpee Snowcross) were almost identical with those described by Briggs et al (8). Auxin was collected from excised 7-mm corn coleoptile tips by setting 1.5 % agar blocks against the cut surfaces of the tips for 2.5 hours. Three similar tips were placed either vertically or horizontally against an agar block in order to determine whether or not the orientation of the tips with respect to gravity had any effect on the total amount of auxin diffusing from them. Next, six tips were partially bisected, leaving the apical 2 mm intact. The bases of each tip were separated by a glass cover slip, and agar blocks were set against the bases in such a way that auxin diffusing from bases on opposite sides of the glass could be collected separately. The assembly was placed so that the plane of bisection was horizontal. Halves from three completely bisected tips were laid horizontally with the convex side up, so that each would be comparable with the top half of a complete horizontal tip. Halves from three more bisected tips were laid horizontally with the convex side down, so that they would be comparable with the bottom halves of complete horizontal tips. In every experiment three intact tips were placed vertically on an agar block, and the

[1] Received revised manuscript January 30, 1961.

[2] This work was supported by National Science Foundation Grants nos. G-2832 and G-8688 to W.R.B. and by a National Science Foundation predoctoral grant to B.G.

[3] Present address: Biological Laboratories, Harvard University, Cambridge 38, Mass.

amount of auxin obtained was thus used as a measure against which the percent recovery of auxin from the various experimental situations could be determined.

Following the auxin diffusion procedures, all agar blocks were assayed by means of the standard Avena curvature test (29). Control blocks containing 25 and 250 γ/liter indoleacetic acid were assayed with each test to determine whether or not the experimental values were within the proportionality range of the bioassay. The block containing 250 γ/liter was calculated to produce the maximum possible curvature obtainable in the time allotted for curvature development (110 min). Experience in this laboratory has indicated that as long as this curvature is in excess of 40 degrees, curvatures between about 5 and 30 degrees, obtained from other blocks assayed simultaneously, were directly proportional to auxin concentration.

Although manipulations were made rapidly in a room in which the humidity was 92 %, and the assemblies were placed immediately in petri dishes in which a saturated atmosphere was maintained, it was found desirable to keep the surface area of agar exposed to air the same in all cases. This was achieved by backing the upright blocks with glass. Water was added to the agar blocks to prevent drying. It tended to run off the vertical blocks, however, and to seep by capillary attraction between the coleoptile tips and the glass on which the tips rested. This problem was not serious for partially split tips, since they tended not to lie flat against the glass which separated their bases. In the case of the totally split coleoptiles, the drops of water placed on the blocks were small, and the tips were supported by a glass slide which did not touch the agar. The air gap between the agar and glass prevented water from being drawn out under the tips and carrying auxin away from the agar. If these precautions were not followed, recovery of auxin was reduced and the ratio of auxin between upper and lower halves was thus untrustworthy.

It might appear from this description that, since different amounts of water were applied to the receptor blocks of the various assemblies, the auxin yields from different assemblies should not be compared. However, the total amount of water added was regulated so that the same moisture content was maintained in each block, as far as could be determined visually. Furthermore, members of each pair of assemblies received identical treatment, so that the critical comparisons between upper and lower halves of partially split tips and between upper and lower halves of totally split tips are valid.

Results

The results are presented in tables I, II, and III. Each value represents the average degrees curvature of 12 Avena coleoptiles to which the blocks from a single auxin diffusion from three intact tips (or 6 half tips) were applied. The different numbered experiments were done on different days and the average of the standard errors for the values shown in these tables was ±2.0°; the standard error never exceeded ±3.3°.

Table I

Diffusible Auxin Obtained From Vertical & Horizontal Coleoptile Tips*

Expt.	Vertical tips	Horizontal tips
1	18.2	17.9
	18.3	18.9
	21.8	25.0
2	24.3	28.5
3	25.3	27.9
	29.8	28.2
	31.9	32.6
	35.1	35.8
4	39.7	36.1
Average:	27.2	27.9

* Degrees Avena curvature

Table I shows that equal amounts of auxin are obtained by diffusion from vertical and horizontal coleoptile tips. Although the range in the amount of auxin obtained from each set of three tips was considerable, and there were on some occasions statistically significant differences between amounts obtained from vertical and horizontal tips in a single experiment, these differences can be ascribed either to variability in the experimental material or to variability in technique from day to day. On different occasions either horizontal or vertical tips might yield the greater amount of diffusible auxin, and if the results of the several experiments are averaged, little difference between the yields is apparent. Thus Dolk's observations (15) are confirmed.

The distribution of diffusible auxin obtained from the upper and lower halves of partially split horizontal coleoptile tips is shown in table II. There

Table II

Distribution of Diffusible Auxin Obtained From Horizontal Partially Split Coleoptile Tips*

Expt.	Intact tips	Upper halves	Lower halves
5	29.2	20.6	33.2
6	28.0	17.4	39.8
	...	21.3	29.6
2	24.3	18.5	27.7
	28.5	...	...
4	39.7	16.8	36.9
	36.1	19.3	33.8
7	28.5	11.0	37.3
Average:	30.6	17.7	34.0

Per cent recovery from split tips: 85
Per cent of recovered auxin in upper halves: 34
* Degrees Avena curvature

is clearly far more auxin obtained from the lower than from the upper halves. Furthermore, in every case, the difference was clearly significant. It should be noted that only 85 % as much auxin was obtained, on the average, from horizontal split tips as from vertical intact ones. It might well be argued that the 15 % loss was entirely from the upper sides. Even if this had been the case, however, inspection of the table reveals that the difference between yields from upper and lower halves is still significant. Indeed, it seems far more likely that the loss is an artifact reducing the apparent amount of auxin obtained from the lower halves, since many of the values shown for the lower halves are somewhat beyond the proportionality range of the bioassay.

Finally, the distribution of diffusible auxin obtained from totally split horizontal coleoptiles is shown in table III. The averages shown indicate that approximately equal auxin quantities are obtained from upper and lower halves. Although the differences between the halves are sometimes significant, they can again be ascribed to technical causes since they do not consistently appear, and may be in either direction. In no case are they of the magnitude of those found between upper and lower halves of partially split tips.

TABLE III

DISTRIBUTION OF DIFFUSIBLE AUXIN OBTAINED FROM HORIZONTAL TOTALLY SPLIT COLEOPTILE TIPS*

EXPT.	INTACT TIPS	UPPER HALVES	LOWER HALVES
2	24.3	15.6	22.6
4	39.7	29.0	33 9
	36.1	18.8	26.8
	...	28.5	23.8
8	28.5	18.8	22.3
	...	23 8	22 8
Average:	32.2	22.4	25.4

Per cent recovery from split tips: 74
Per cent of recovered auxin in upper halves: 47
* Degrees Avena curvature

The lateral gradient of diffusible auxin in geotropically stimulated coleoptiles (table II) and the equality of total diffusible auxin production in stimulated and nonstimulated coleoptiles (table III) suggest that auxin is not being produced or destroyed as a consequence of a geotropic stimulation, but rather that auxin or an auxin precursor is moving from the upper to the lower sides. This suggestion is supported by the demonstration that complete separation of lower and upper sides virtually eliminated the appearance of an auxin gradient across the coleoptile. These results are closely in accord with those of Briggs et al (8) for phototropically stimulated coleoptiles.

DISCUSSION

The evidence above might be used to support the alternative hypothesis that an inhibitor moves from the lower to the upper side of a stimulated coleoptile. Unless a special undiscovered polar transport system is postulated for such an inhibitor, it must be assumed that this agent operates by releasing auxin into the auxin transport system in the lower side and by removing an equal amount of auxin from the transport system on the upper side. Such a hypothesis seems unlikely, since it demands the existence in the corn coleoptile of an inhibitor with curious properties unlike any which have been reported in the literature, and since it is no easier to understand why an inhibitor should be laterally translocated than why auxin should be laterally translocated. Similar reasoning argues against the participation of an auxin synergist.

The further possibility of lateral translocation of a substance participating in auxin synthesis in some capacity other than that of an immediate precursor is made unlikely by Dolk's observation: when auxin, collected in agar from corn coleoptile tips, is permitted to diffuse from the agar into horizontal decapitated oat coleoptile sections, more auxin may be obtained from agar receptor blocks which have been applied to the lower basal cut surface of the oat coleoptile sections than from blocks applied to the upper basal surface (15).

If the results of these experiments are valid, the failure of several workers to obtain evidence for lateral transport of exogenous radioactive indoleacetic acid must be explained. In some cases (10, 21, 22), organs with the tips intact were submerged in a solution of radioactive auxin. Following geotropic stimulation, the distribution of the auxin removed from solution by the organ was studied by measuring the radioactivity of the tissue. It was never proven in these experiments that all or even a substantial fraction of the labeled auxin entered the transport system. Yet it was shown in Dolk's experiments and in all experiments modeled after these that, in coleoptiles, it is auxin moving in the transport system which is correlated with tropic behavior. If the auxin in the transport system is partially endogenous and partially exogenous, and if a relatively large amount of exogenous auxin is present in the tissues without participating in transport, any lateral redistribution of moving auxin would be extremely difficult to detect by measuring tissue radioactivity, considering that the absolute quantities are exceedingly small.

On the other hand, some workers (9) decapitated coleoptiles and supplied labeled auxin at the cut surface. Following phototropic stimulation auxin was again assayed by measuring tissue radioactivity. It has been demonstrated that labeled auxin supplied in this manner enters the transport system (17). However, it has also been demonstrated that, in corn,

phototropic lateral redistribution probably occurs entirely within less than 0.4 mm of the coleoptile tip and possibly within less than 0.2 mm (W. R. Briggs, unpublished). An oat coleoptile is capable of perceiving and reacting to gravity along its entire length. However, the greater part of the lateral transport occurs in the top 3 mm; more basal regions can effect only a slight redistribution (15). In view of all these considerations, studies of redistribution of labeled auxin in decapitated coleoptiles are of doubtful value, unless they measure only auxin in the transport system. The best way to do this is to measure labeled indoleacetic acid diffusing from coleoptile tips into agar blocks. Indeed, experiments by Gillespie and Thimann (16) clearly show that radioactive indoleacetic acid applied in agar blocks to the apical ends of Avena coleoptile sections is laterally redistributed by gravity in horizontal sections, but that such redistribution is restricted to that portion of the applied auxin which has actually entered the transport system (in their experiments, less than 20 % of the total entering the tissue). It can be demonstrated clearly only by using auxin diffusion techniques.

Brauner and Appel (6) have recently published experiments which are particularly pertinent to the present discussion. They found that if the apices of detached Avena coleoptiles were split to a depth of 2 mm, and a small piece of mica inserted into the split, subsequent geotropic response was far lower if the coleoptiles were induced with the split horizontal than with the split vertical, suggesting blockage of some transport pathway by the mica in the horizontal case. Although they did indeed obtain some curvature with the mica horizontal, this is not surprising, since Dolk (15) has shown that a certain amount of geotropic induction is possible below the apex itself.

These authors also demonstrated that if half the apex of detached coleoptiles is excised by a vertical and a horizontal cut, and agar blocks appressed against the remaining vertical cut surfaces, significant amounts of auxin could be collected in such blocks (as measured by the Avena curvature test) if the coleoptiles were geotropically stimulated with the blocks on the lower side, but not if they remained vertical. Their experiments thus suggest that gravity induces the transverse migration of auxin itself, rather than a precursor, since the activity of the transversely migrating substance is detected immediately as auxin in the Avena curvature test. A lag in such a test would have suggested the presence of a precursor.

The fact that geotropic response is mediated by lateral transport in the corn coleoptile does not mean that lateral transport is the only mechanism by which plants respond to gravity. For example, the amount of auxin which can be obtained from grass nodes (24) and from the meristematic growth rings of sugar cane nodes (26) increases when these plants are placed horizontally. There is no evidence, however, that auxin production or release is unilateral; lateral transport may concentrate the newly appeared auxin in the lower half.

Summary

The distribution of auxin during geotropic stimulation of corn coleoptiles has been determined by means of the Avena test. It has been verified that equal amounts of auxin diffuse out from vertical and from horizontal coleoptile tips, and that the lower halves of partially split coleoptile tips release a larger amount of auxin than do the upper halves. When coleoptile tips are completely bisected prior to stimulation, however, the same quantity of auxin is obtained from each half. The simplest interpretation of these results is that auxin, or possibly an auxin precursor, is translocated from the upper to the lower side of a stimulated coleoptile.

Literature Cited

1. Anker, L. 1956. The auxin concentration rule for the geotropism of Avena coleoptiles. Acta. Botan. Néerl. 5: 335–341.
2. Audus, L. J. & M. E. Brownbridge. 1957. Studies on the geotropism of roots. I. Growth-rate distribution during response & the effects of applied auxins. J. Exp. Botan. 8: 105–124.
3. Bennet-Clark, T. A., A. F. Younis, & R. Esnault. 1958. Geotropic behavior of roots. J. Exp. Botan. 10: 69–86.
4. Boysen-Jensen, P. 1933. Die Bedeutung des Wuchsstoffes für das Wachstum und die geotropische Krümmung der Wurzeln von *Vicia faba*. Planta 20: 688–698.
5. Boysen-Jensen, P. 1936. Über die Verteilung des Wuchsstoffes in Keimstengeln und Wurzeln während der phototropischen und geotropischen Krümmung. Kgl. Danske Videnskab. Selskab, Biol. Med. 13: 1–31.
6. Brauner, L. & E. Appel. 1960. Zum Problem der Wuchsstoff-Querverschiebung bei der geotropischen Induktion. Planta 55: 226–234.
7. Brauner, L. & A. Hager. 1958. Versuche zur Analyse der geotropischen Perzeption. I. Planta 51: 115–147.
8. Briggs, W. R., R. D. Tocher, & J. F. Wilson. 1957. Phototropic auxin redistribution in corn coleoptiles. Science 126: 210–212.
9. Bünning, E., H. J. Reisener, F. Weygand, H. Simon, & J. F. Klebe. 1956. Versuche mit radioaktiver Indolylessigsäure zur Prüfund der sogenannten Ablenkung des Wuchshormonstromes durch Licht. Z. Naturfors. 11-B: 363–364.
10. Ching, T. M. & S. C. Fang. 1958. The redistribution of radioactivity in geotropically stimulated plants pretreated with radioactive indoleacetic acid. Physiol. Plantarum 11: 722–727.
11. Ching, T. M., R. H. Hamilton, & R. S. Bandurski. 1956. Selective inhibition of the geotropic response by *n*-1-naphthylphthalamic acid. Physiol. Plantarum 9: 546–558.
12. Cholodny, N. 1924. Über die hormonale Wirkung der Organspitze bei der geotropischen Krümmung. Ber. deut. botan. Ges. 42: 356–362.
13. Cholodny, N. 1927. Wuchshormone und Tropismen bei den Pflanzen. Biol. Zentral. 47: 605–626

14. DIJKMAN, M. J. 1933. A quantitative analysis of the geotropical curvature in dicotyledons. Proc. Kon. Akad. Wetensch. Amsterdam 36: 748–758.
15. DOLK, H. E. 1936. Geotropism & the growth substance. Translated by F. Dolk-Hoek & K. V. Thimann. Rec. trav. botan. néerl. 33: 509–585.
16. GILLESPIE, B. & K. V. THIMANN. 1961. The lateral transport of indoleacetic acid-C^{-14} in geotropism. Experientia 17: in press.
17. GOLDSMITH, M. H. M. 1958. Some characteristics of the transport of indoleacetic acid in Avena coleoptiles (abstract). Plant Physiol. 33 suppl.: xxi.
18. GORDON, S. A. & M. EIB. 1956. Auxin transport in the phototropic response (abstract). Plant Physiol. 31 suppl.: xiv.
19. HAWKER, L. E. 1932. Experiments on the perception of gravity by roots. New Phytol. 31: 321–328.
20. NAVEZ, A. E. & T. W. ROBINSON. 1932. Geotropic curvature of Avena coleoptiles. J. Gen. Physiol. 16: 133–145.
21. REISENER, H. J. 1957. Versuche zum Geotropismus mit radioaktiver β-Indolylessigsäure. Naturwiss. 44: 120.
22. REISENER, H. J. & H. SIMON. 1960. Weitere Geotropismus-Versuche mit radioactive β-Indolylessigsäure. Z. Botan. 48: 66–70.
23. RUFELT, H. 1957. Influence of the composition of the nutrient solution on the geotropic reactions of wheat roots. Physiol. Plantarum 10: 373–396.
24. SCHMITZ, H. 1933. Über Wuchstoff und Geotropismus bei gräsern. Planta 19: 614–635.
25. VAN DER LAAN, P. A. 1934. Der Einfluss von Aethylen auf die Wuchstoffbuildung bei Avena und Vicia. Rec. trav. botan. néerl. 31: 691–742.
26. VAN OVERBEEK, J., G. DAVILA OLIVO, & E. M. SANTIAGO DE VAZQUEZ. 1945. A rapid extraction method for free auxin & its application in geotropic reactions of bean seedlings & sugar-cane nodes. Botan. Gaz. 106: 440–451.
27. WENT, F. W. 1926. On growth-accelerating substances in the coleoptile of *Avena sativa*. Proc. Kon. Akad. Wetensch. Amsterdam 30: 10–19.
28. WENT, F. W. 1928. Wuchstoff und Wachstum. Rec. trav. botan. néerl. 25: 1–116.
29. WENT, F. W. & K. V. THIMANN. 1937. Phytohormones. Macmillan, New York.

INVOLVEMENT OF ADENYLATE KINASE IN PHOSPHORYLATION OF RIBOSE-5-PHOSPHATE BY ADENOSINEDIPHOSPHATE IN MITOCHONDRIA-FREE PREPARATIONS FROM ROUGH LEMON LEAVES[1]

D. VAN NOORT & A. WALLACE

DEPARTMENT OF HORTICULTURAL SCIENCE, UNIVERSITY OF CALIFORNIA, LOS ANGELES

In some of our previous studies it was noted that ADP[2] was effective as the ATP source in crude homogenates for the carboxylation enzyme system (4) when R5P was used as substrate. The overall reaction is as follows:

$$R5P + ATP + HCO_3^- \xrightarrow{Mg^{++}} 2\ PGA + ADP$$

This reaction is itself a 3-enzyme sequence (6, 8). Studies were undertaken to elucidate the nature of the ADP replacement of ATP. Specifically, phosphorylation of ADP by P_i, by labile phosphates such as creatinophosphate and acetylphosphate, and by an adenylate kinase were investigated as possible means of formation of ATP from ADP in the preparations.

MATERIALS & METHODS

The K salts of R5P and G6P were prepared from the Ba salts by precipitating the Ba with an equivalent amount of K_2SO_4, and centrifuging at 1,000 × *g* at 0° C to remove the precipitate. Stock solutions of 0.03 M were prepared in water which had been demineralized in a Barnstead Bantam demineralizer. The K salt of acetylphosphate was prepared similarly from the di-Ag salt using an equivalent amount of KCl to precipitate the Ag.

In all the experiments rough lemon leaves (*Citrus jambhiri* Lush.), washed in distilled water, dried on paper towels and with the midrib removed, were used in the ratio of 2 g fresh weight of leaves per 5 ml tris-sucrose buffer (0.2 M tris plus 0.5 M sucrose, pH 8.0) for the homogenates. After grinding the leaves with a Virtis homogenizer at 75 % maximum speed for 1 minute, the homogenate was strained through a double layer of cheesecloth, centrifuged at 1,000 × *g* for 10 minutes at 0° C to remove unbroken cells, cell debris, and large particles. A mitochondria-free supernatant was obtained by centrifuging the crude supernatant at 30,000 × *g* at 0° C for 20 minutes.

[1] Received revised manuscript February 18, 1961.

[2] Abbreviations used in this paper: AMP for adenosinemonophosphate; ADP for adenosinediphosphate; ATP for adenosinetriphosphate; PGA for 3-phosphoglycerate; R5P for ribose-5-phosphate; G6P for glucose-6-phosphate; tris for tris(hydroxymethyl)aminomethane; P_i for inorganic phosphate; EDTA for ethylenediaminetetraacetic acid.