Experiments on the Transport of Auxin
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Source: *Botanical Gazette*, Vol. 100, No. 3 (Mar., 1939), pp. 465-484
Published by: The University of Chicago Press
Stable URL: https://www.jstor.org/stable/2471726
Accessed: 10-04-2020 17:52 UTC

EXPERIMENTS ON THE TRANSPORT OF AUXIN[1]

F. W. WENT AND RALPH WHITE

(WITH TEN FIGURES)

Introduction

The purpose of the work described in this paper was to measure quantitatively the transport of auxin, using a new experimental technique. The original method of measuring the rate of auxin transport required the determination of the amount transported through the tissue to an agar block during a series of time intervals, thus determining the time at which the first auxin arrived (6). Since with the photokymograph (4) the beginning of the curvature can be accurately determined, and since this initial curvature occurs a known time interval after application of auxin to the recording plant, it is possible to determine for each coleoptile when auxin reaches its cut surface. Thus by placing a piece of tissue one-sidedly on a recording plant, and applying auxin to the other end of this tissue, the rate of auxin movement through it can be determined.

Method

Preliminary experiments were all unsuccessful for one of two reasons. When coleoptile sections were interposed between the recording coleoptile and the agar block containing auxin, either the curvatures started simultaneously with the controls as if no section were present, or no curvatures appeared at all. Finally, however, the technical difficulties were overcome, and transport velocities of the same order of magnitude as VAN DER WEIJ (6) described were obtained. To this end the following improvements in technique had to be made, for the rest adhering to the procedure previously outlined (4). Figure 1 gives a sketch of the new set-up.

The grass peduncle inserted in place of the primary leaf did not give a satisfactory support of the applied tissue plus agar block, and

[1] This work was carried out with the aid of the Works Progress Administration, Official Project no. 165-03-6999, Work Project no. 6330-6989.

the latter often stuck to it. The method by which the leaf is partly pulled out and a fine metal wire (*D*) is inserted gave good results, provided the agar block (*A*) and the top of the transport tissue (*B*) did not touch the wire. It was also found that copper wire gave irregular results when not properly inserted and touching the coleoptile walls; nichrome wire and especially silver wire nr. 36 (diameter 0.12 mm.) were very satisfactory.

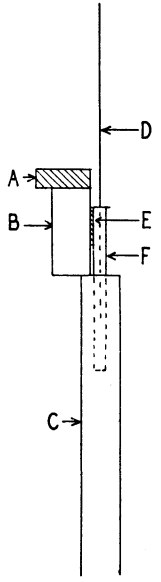

FIG. 1.—Schematic picture of test plant *C*, with silver wire *D* inserted in the partly pulled out primary leaf *F*. On one side of its cut surface the transport section *B* is stuck by means of petroleum jelly *E* against the primary leaf *F;* on top is an agar block *A*, containing the substance to be transported.

The primary leaf (*F*), after pulling loose, was cut off so that the transport tissue would protrude about 2 mm. above it. A thin film of petroleum jelly (*E*) was applied to the upper part of the protruding part of the primary leaf, care being taken not to come in contact with the cut surface of the coleoptile as it would interfere with the transport of auxin. The transport tissue (*B*) was then placed on the cut surface of the recording coleoptile (*C*) and held in place by the petroleum jelly (*E*) against the primary leaf (*F*). The contact between the tissues is very essential and was therefore inspected with a magnifying glass. The agar blocks (*A*) containing the growth promoting substances were then placed on top of the transport tissue, so that they would not touch the wire and the contact between transport tissue and recording plant would not be broken. In all experiments, blocks of 2 × 2 × 1 mm. 2 per cent agar were used, which were washed in the solutions for 1–2 hours.

To remove the auxin still contained in the tissues through which transport had to be measured, and for which mostly *Avena* coleoptile sections were used, these were placed with their basal cut surface on wet filter paper for 3 hours. After that they did not induce curvatures by themselves when no auxin was applied.

The most important departure from the standard conditions as described by SCHNEIDER and WENT (4), however, was by decreasing the relative humidity of the air to 75–77 per cent during the test. This unfortunately causes premature drying of the agar blocks, but for the first 3 hours the decrease in volume is not considerable. This lowering in humidity is essential to prevent leakage of auxin along the surface of the transport tissue. That leakage actually occurred at high humidities was found by placing inverted coleoptile sections on the recording plants and applying high concentrations of indoleacetic acid in agar on them. When this was done at 85–90 per cent humidity strong curvatures resulted; at 76 per cent humidity there was no curvature at all, and therefore no transport through the inverted sections. In normal sections, of course, considerable transport took place. At 88 per cent humidity lanolin paste containing 0.1 per cent indoleacetic acid gave no inverse auxin transport through 6 mm. coleoptile sections, since no auxin would leach from the paste. Further details of this polar transport are given later in this paper.

For the transport test, the recording plants (as well as the sections) were even more carefully selected than in the standard *Avena* test. Among other factors, this selection was for thick coleoptile walls, which gave a better contact surface. Plants 30 mm. long were found best.

In general the reaction of the recording plants was delayed, owing to the extra time required for the auxin to reach the plant through the transport tissue. Since in the *Avena* test plants, sensitized through a double decapitation, the "regeneration" opposes the auxin curvature after 80 minutes, the standard double decapitation method (10) was not suitable for this transport test. Therefore the coleoptiles were decapitated only once, and the transport tissue with auxin was applied immediately, so that the curvature could occur before the regeneration (4, p. 477). To make the control plants comparable, the agar blocks without transport tissue were applied 40 minutes (mean transport time) after decapitation.

Evaluation of results

All these transport experiments have been repeated several times; the reaction of a total of more than 2000 plants has been recorded in

all. Only about one-sixth of this material is here presented, since all results were closely similar. Some of this additional material, however, is incorporated in tables 2 and 3.

Before the experiments are discussed in detail, certain difficulties in the interpretation of the data should be pointed out.

On account of the variability of the material, some method of averaging is essential. As previously described (4), the position of

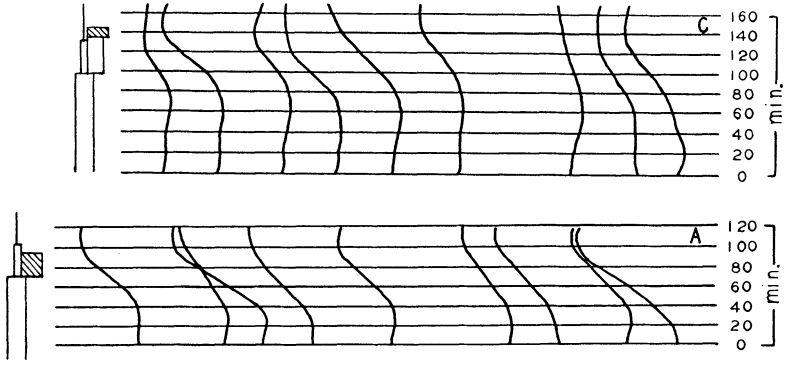

FIG. 2.—Retracing of part of record of experiment represented in fig. 3. Parallel lines drawn at 20 min. intervals, extending between them. Where they are perpendicular to the time lines the plants remain straight; slope toward left means negative (growth) curvature. Curve *A:* controls, curvatures start 20–40 min. after first recording. Time elapsed between putting on of agar block (containing 0.1 mg./l. indoleacetic acid) and first recording, 4 minutes. Curve *C:* 6.3 mm. coleoptile section inserted between agar block (containing 4 mg./l. indoleacetic acid) and test plant. Curvatures start 60–80 min. after first recording, which occurred 8 minutes after putting on the agar blocks.

the tip of the test plant is recorded once every 4 minutes. As long as the plant does not curve, this position remains the same, and a straight line is recorded (fig. 2). Although in general the onset of the curvature is very sudden, so that it should not be difficult to determine in which 4 minute period the curvature starts, yet unfortunately the nutations which are always present to some extent make it difficult to measure this point with the desired accuracy. When the rate of curvature is small, it is also difficult to tell when the curvature started. By taking the mean of the reactions of eight to ten plants, these nutations are ruled out and the onset of the curva-

ture can be determined with greater accuracy from the mean curve, as shown in figures 3–10.

But a new difficulty arises: in these mean curves the beginning of the curvature will not be the mean of the onset of the individual plants, but will depend upon the moment that the first one starts to bend. Therefore there must be a systematic difference between the two types of calculation (table 1). Figure 2 is a retracing of the record of the experiment represented in figure 3, curves *A* and *C*.

TABLE 1

TIME BETWEEN APPLICATION OF AUXIN AND (1) ONSET OF CURVATURE OR (2) INTERSECTION POINT, MEASURED FOR INDIVIDUAL TEST PLANTS AND CALCULATED FROM AVERAGE CURVES OF FIGURE 3. FOR A DESCRIPTION OF INDIVIDUAL TREATMENTS (*A*, *B*, *C*, *D*), SEE FIGURE 3

CURVE	TYPE OF READING	INDIVIDUAL TIMES IN MINUTES (MEASURED FROM FIGURE 2)	MEAN	CALCULATED FROM FIG. 3	MEAN OF THREE SHORTEST TIMES
A.....	Onset	20, 28, 36, 32, 32, 32, 28, 28, 44 ..	31	26	28
	Intersection	34, 42, 44, 38, 46, 38, 42, 40, 52 ..	42	41	37
B.....	Onset	38, 42, 30, 46, 34, 34, 34, 42, 46 ..	38	32	33
	Intersection	36, .., 44, 46, 48, 32, 38, 58, 46 ..	43	40	35
C.....	Onset	76, 68, 72, 60, 72, 80, 76, 80	73	67	67
	Intersection	80, 86, 96, 84, 84, 94, 96, 94	89	87	83
D.....	Onset	60, 64, 56, 72, 64, 60, 56, 80, 64, 64	64	55	57
	Intersection	62, 76, 72, 74, 76, 78, 74, 80, 86 ..	75	73	69

The individual reactions are all similar, and in no single case can there be doubt that the transport section greatly delayed the onset of the curvature. For the rest the type of reaction is the same. After a short start phase, the rate of curvature becomes constant (eumotoric phase), remains so for 30 to 60 minutes, and then decreases. The eumotoric phase can be used for an unambiguous determination of the beginning of the curvature. If a line is drawn extending this eumotoric phase it will cross the line which extends the original position of the plant, and the intersection between the two lines is easy to determine and is a much more definite point than the onset of the curvature (points *A* and *D*, fig. 3). This point also depends

on the moment of arrival of the auxin at the cut surface. But the mean of the individual intersections does not significantly differ from the intersection derived from the average curve (table 1). Besides, this point of intersection is more or less independent of the auxin concentration. This is not true of the time of onset of curvature, which can be detected the sooner the greater the rate of curva-

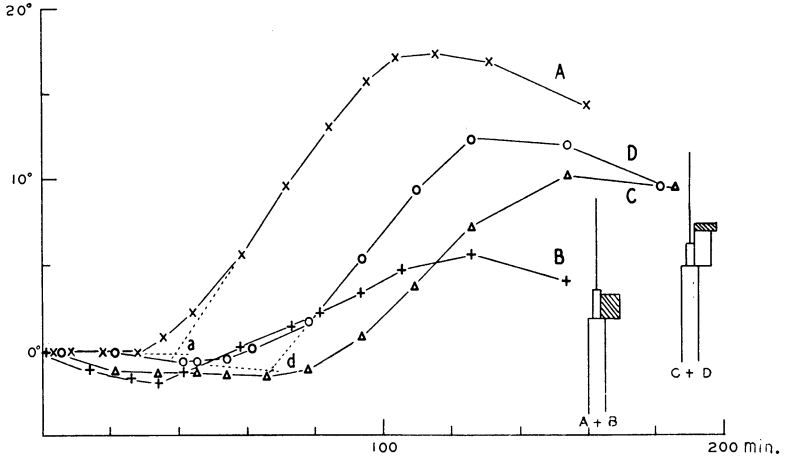

FIG. 3.—Abscissa: time in minutes after application of auxin. Ordinate: movement of tip of test plants in degrees. Curves *A:* 0.1 mg./l. indoleacetic, applied directly; *B:* 0.05 mg./l. indoleacetic acid, applied directly; *C:* 4 mg./l. indoleacetic acid, applied on intervening 6.3 mm. upright coleoptile section; *D:* as *C*, but concentration 2 mg./l. indoleacetic acid.

ture (= rate of auxin arrival; table 1). These considerations sufficiently justify the use of this intersection point throughout this paper.

BASIC EXPERIMENTS

Figure 3 shows the course of the curvature of the test plants when agar blocks containing auxin were placed on directly, or with an intervening coleoptile section of 6 mm. Each curve is calculated as the mean of eight to ten test plants. Figure 2 and table 1 give data on the individual reactions in this experiment. A few conclusions, substantiated by other experiments which will not be described here, can be drawn from figure 3. In the first place it is seen that the slope of the curve (that is, the rate of curvature) depends upon the con-

centration of the auxin in the agar block (curves *A* and *B*; see also curves *A* and *B*, fig. 6). This is the same as described before (**4**, p. 488). There is no difference in the rate of curvature of the test plants carrying the transport sections (curves *C* and *D*), indicating that they represent "maximum angles"; that is, the auxin concentration reaching the test plants was so high that they curved at a

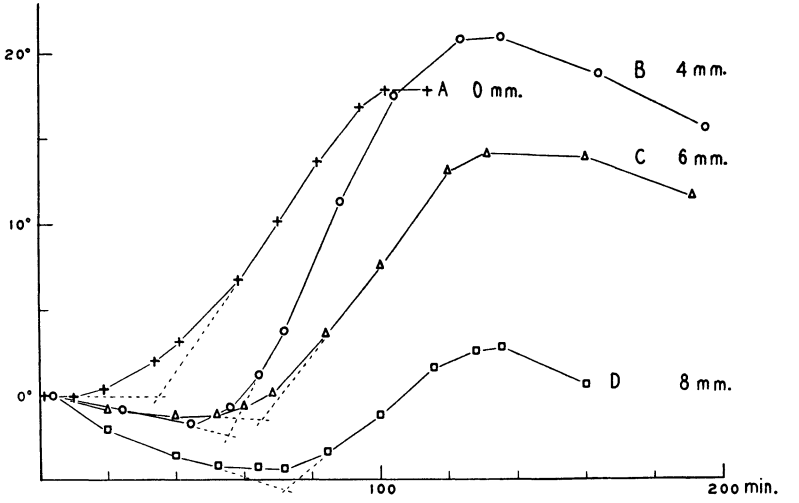

FIG. 4.—Abscissa: time in minutes after application of indoleacetic acid 0.1 mg./l. (curve *A*) and 2 mg./l. (curves *B*, *C*, *D*). Ordinate: movement of tip of test plants, in degrees. Curves *A:* agar directly applied to test plant; *B:* applied on 4.2 mm. transport section; *C:* on 6.3 mm.; *D:* on 8.4 mm.

maximal rate. The type of the curves *A*, *C*, and *D* is similar, with the exception of the moment when the plants start to curve. The transport time of auxin through 6.3 mm. sections was 39.5 minutes in this experiment, corresponding to 9.6 mm./hour.

The experiment of figure 4 compares well with that of figure 3. Each curve was calculated as the mean of eight to ten test plants. The transport time through 4.2 mm. sections (curve *B*) was 55–35 = 20 minutes, for 6.3 mm. sections (*C*) 65–35 = 30 minutes, and for 8.4 mm. sections (*D*) 77–35 = 42 minutes. Thus the conclusion of VAN DER WEIJ (**6**) is confirmed, that the velocity of transport is independent of the length of the coleoptile section, in this experi-

ment being 12.4 mm./hour. The amount transported, however, decreases with the longer sections.

Figure 5 shows the auxin transport through sections cut from different levels of the coleoptile. In agreement with the experiments of VAN DER WEIJ (6) with transport of an auxin concentrate from urine, it was found that the sections cut nearest the tip (*B*) transported somewhat more auxin than middle sections (*C*), but basal

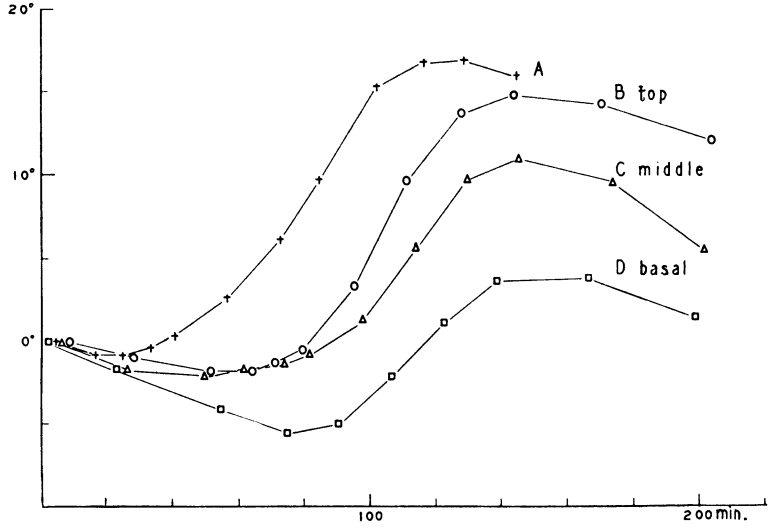

FIG. 5.—Abscissa: time in minutes after application of indoleacetic acid 0.1 mg./l. (curve *A*) and 2 mg./l. (curves *B*, *C*, *D*). Ordinate: movement of test plants in degrees. Curves *A:* control, agar directly applied to test plant; *B*, *C*, *D:* with interposition of 6.3 mm. coleoptile sections (*B*, cut 3–9 mm. from tip, *C* 9–15 mm., and *D* 16–22 mm.).

sections gave distinctly less transport (*D*). The velocity differed very little in the three regions of the coleoptile. Since VAN DER WEIJ did not find differences in transport capacity at various heights in the coleoptile, when *Zea* tip auxin was used, it would indicate that his auxin concentrate resembled the indoleacetic acid which we used in our experiments rather than the auxin *a* or *b* from *Zea* coleoptile tips. These experiments indicate that never more than the upper 9 mm. of the decapitated coleoptile should be used for transport sections.

Since only a small fraction of the cut surface of the transport sec-

tion is in contact with the test plant, it was of importance to re-investigate the auxin transport through the tissues containing the vascular bundles, and through the parenchyma. On the cross section the coleoptile is elliptical, and the two vascular bundles extend along the narrow side. In two experiments, one of which is summarized in figure 6, the transport sections were attached to the test

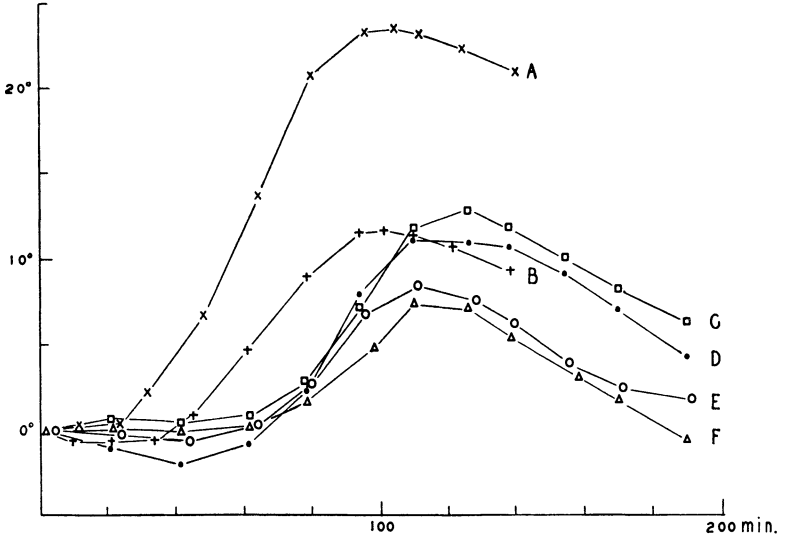

FIG. 6.—Abscissa: time in minutes after application of indoleacetic acid 0.25 mg./l. (curves *A*, *C*, *D*) and 0.1 mg./l. (curves *B*, *E*, *F*). Ordinate: curvature of test plants in degrees. Curves *A* and *B:* controls, agar directly applied to plant. Curves *C–F:* 4.2 mm. coleoptile section between agar and test plant, in *C* and *E:* vascular bundle of transport section in contact with test plant, *D* and *F*, parenchyma only.

plants either with their broad (*D* and *F*) or with their narrow (*C* and *E*) sides. Both experiments gave identical results, showing that the auxin transport is practically independent of the position of the transport section; in other words, that the presence of the vascular bundle does not materially increase the auxin transport. This is in agreement with the results of VAN DER WEIJ (6) and WENT and THIMANN (10), but differs from the conclusions of LAIBACH and KORNMANN (3). Although no difference was found in regard to the position of the transport sections, still in all experiments care was taken to place them with their narrow side against the test plant.

Polarity of transport

There were many reasons for investigating the polarity of auxin transport with the new technique. In the most accurate transport experiments (**6, 7**) the mean transport of twelve coleoptile sections was always determined, so that occasional leakage through one or more sections could not be detected. Besides, as mentioned under Method, the previous experiments on auxin transport in the *Avena* coleoptile were all carried out at a high humidity, which al-

TABLE 2

Concentrations of indoleacetic acid applied dissolved in agar or in lanolin, tested with respect to their ability to move from base to apex through Avena coleoptile sections of differing length

Length of coleoptile section (mm.)	No inverse transport with concentrations		Inverse transport with concentrations	
	mg./l. solution in agar	mg./gm. lanolin	mg./l. solution	mg./gm. lanolin
2.1	.....	.....	.....	10
3.1	50, 200	2, 1	1000	5?, 10
4.2	100	10	1000	.....
6.3	2, 4, 10, 50, 200, 1000	1	.....	.....
10.5	4	.....	.....	.....

lows a certain amount of auxin leakage along the surface of the coleoptile sections. This leakage must have been considerable, especially in Jost and Reiss' (**2**) experiments, since their coleoptiles were made to adhere to the wall of the glass vessel by capillary action of the solutions containing auxin. Thus it is not surprising that they failed to obtain clear evidence for polar auxin transport. Also it would now seem that part of the inverse transport reported by Went (**9**) might have been due to leakage along the surface of the coleoptiles, since the transport experiments were carried out in closed petri dishes in a high humidity. However, the falling off of the transport beyond concentrations of 1 mg. indoleacetic acid/cc. is proof that not all of the inverse transport was due to leakage.

At humidities above 80 per cent, applying auxin in agar, no transport tests showed either polarity of the auxin movement

through the tissues or an appreciable time lag of the auxin curvature when a coleoptile section even in normal position was interposed between agar block and test plant. However, when not auxin agar but auxin paste (1 mg. indoleacetic acid per gram lanolin) was used, even

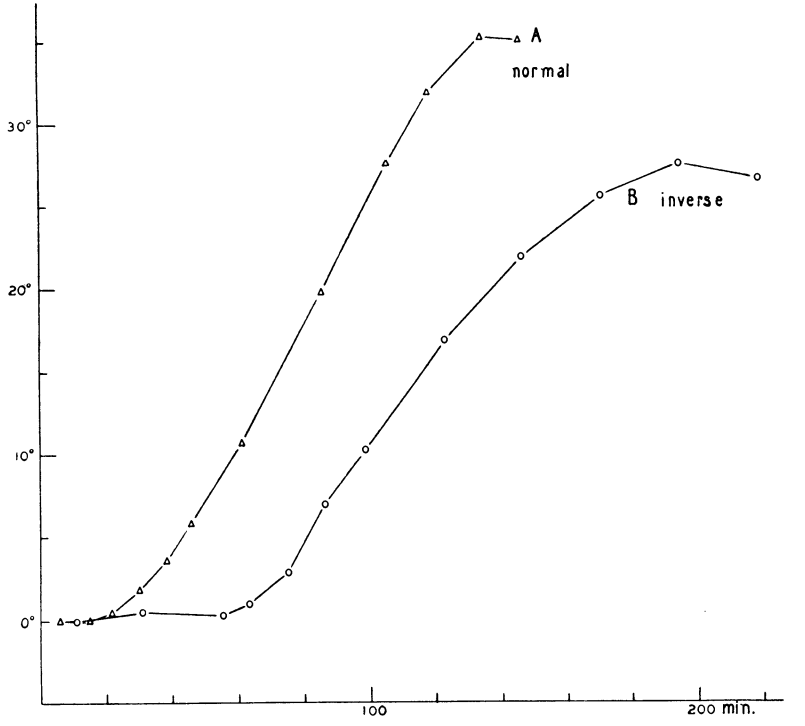

FIG. 7.—Abscissa: time in minutes after application of indoleacetic acid 0.5 mg./l. (curve *A*) and 1000 mg./l. (curve *B*). Ordinate: curvature of test plants in degrees. In curve *A*, a 3.1 mm. coleoptile section was placed apex up between test plant and agar; in curve *B* this section was placed apex downward (inverse transport).

at a humidity of 85–90 per cent no trace of leakage or non-polar transport was observed.

Since auxin concentrations are physiologically better defined in agar than in lanolin, most transport experiments were carried out with auxin-agar at humidities of 70–80 per cent, with occasional checks with auxin-lanolin pastes. Table 2 summarizes the experiments on inverse auxin transport (each determination is the mean of

ten to twenty test plants). In all cases control determinations were made with coleoptile sections in the normal position, and in every case good transport of a 0.5, 1, or 2 mg./l. solution of indoleacetic acid was obtained, indicating that the lack of transport through the inverted sections was not due to unfavorable experimental conditions. The data in table 2 leave no doubt that the longer the sec-

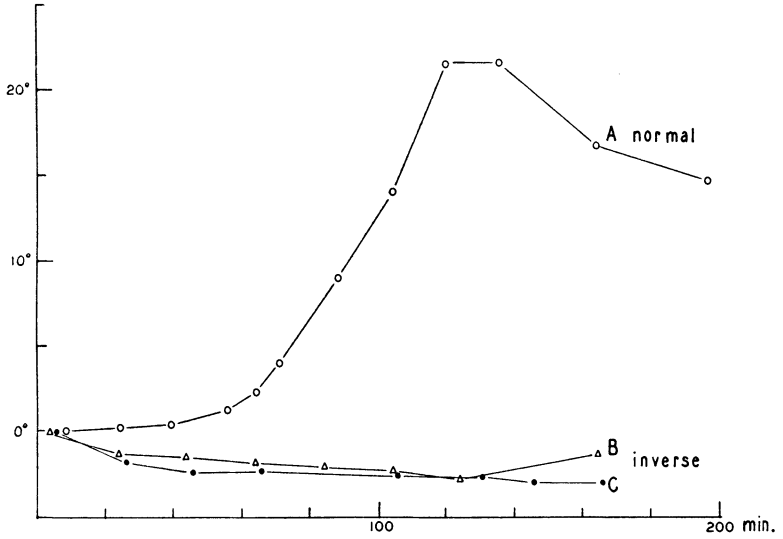

FIG. 8.—As fig. 7, but length of coleoptile section 6.3 mm. Curves *A:* indoleacetic acid 1 mg./l., section apex upward; *B:* indoleacetic acid 200 mg./l., section apex downward; *C:* indoleacetic acid 1000 mg./l., section apex downward.

tions the more strictly polar is the transport. Whereas the 3.1 and 4.2 mm. sections still permit the passage of 1000 mg./l. indoleacetic acid solutions, the 6.3 mm. sections do not do so any more. Figures 7 and 8 give the results of two actual experiments. In each case it was observed that the velocity of inverse transport was considerably less than of normal transport (4–6 mm./hour against about 10 mm./hour), whereas the capacity was greatly less, especially when the enormously increased concentration gradient is considered. Thus the inverse transport differs from the normal transport in more than one way.

Transport of different compounds

With the described test it is possible to measure the velocity and capacity of transport through coleoptile tissues of all substances active in the *Avena* test. This has been done with indole(3)acetic acid (figs. 3–8), indole(3)butyric acid, anthraceneacetic acid (fig. 9), naphthaleneacetic acid (fig. 10), indole(3)propionic acid, and

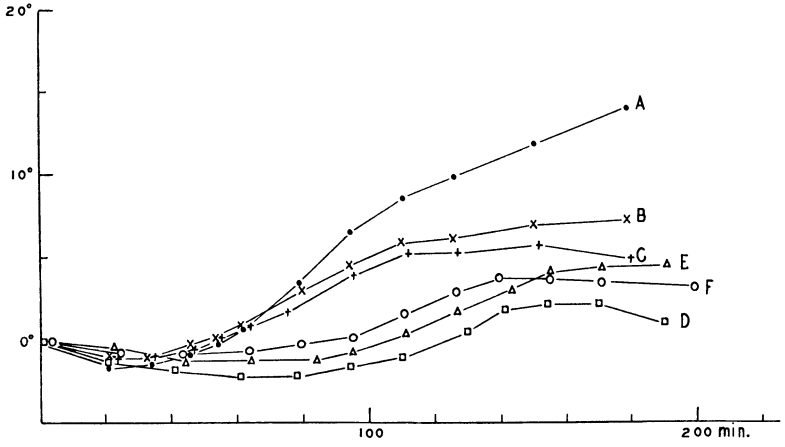

Fig. 9.—Abscissa: time in minutes after application of anthraceneacetic acid. Curves *A–C*, no sections; *D–F*, 4.2 mm. sections between agar and test plant. *A:* concentration 4 mg./l.; *B:* 2 mg./l.; *C:* 1 mg./l.; *D:* 100 mg./l.; *E:* 50 mg./l.; *F:* 20 mg./l. Ordinate: curvature of test plants in degrees.

cis-cinnamic acid. The last two did not show any transport at all under the conditions described, and will not be considered further. This was not due to a lack of response of the test plants, since these substances gave curvatures in the standard *Avena* test. In the first four compounds mentioned, however, good values for transport velocity were obtained, and these are given in table 3. The figures in this table are means of three to ten separate determinations with ten plants each.

The figures of line *A* are calculated from the controls in each experiment, and indicate the time required for the beginning of the curvatures after application of the agar containing the active substance. These figures give an indication of the velocity of the growth reaction in which these compounds must take part.

The second line (*B*) is the reciprocal value of the transport velocity of these compounds. It will be seen that, although there is great variation in the individual determinations, still there is no doubt that the transport of naphthaleneacetic acid is considerably slower than that of indoleacetic acid. This is not due to differences in the applied concentrations, since figures 3, 6, 9, and 10 show that there is no systematic difference between the transport velocities, or the moments of incipient curvature in the controls, when different concentrations are tested.

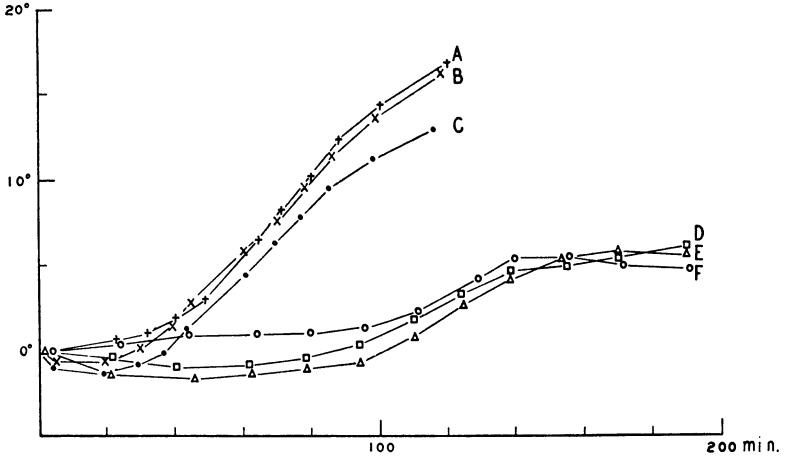

FIG. 10.—Abscissa: time in minutes after application of naphthaleneacetic acid. Curves *A–C:* agar applied directly; *D–F:* 4.2 mm. coleoptile sections between agar and test plants. *A:* concentration 4 mg./l.; *B:* 2 mg./l.; *C:* 1 mg./l.; *D:* 50 mg./l.; *E:* 20 mg./l.; *F:* 10 mg./l. Ordinate: curvature of test plants in degrees.

Line *D* is a summary of results obtained with the standard *Avena* test, and indicates the length of the curved zone 90 minutes after one-sided application of the various compounds. Also in these experiments no differences in length of curvature were observed if different concentrations were applied, which also indicates that transport velocity is independent of concentration. VAN DER WEIJ (6) has come to the same conclusion.

It is interesting to calculate with the aid of lines *A* and *B* how long the curved zone in an *Avena* test would be. Since the transport experiments were carried out with *Avena* plants which were decapitated only once, which retards the onset of the curvature 10 to 20

minutes (4, p. 477), and since the apparent transport is slowed down owing to two extra cut surfaces, the calculations were made for a reaction time of 118 minutes. The calculated values obtained in this way (line *C*) are in good agreement with the actually measured length of the curved line in the standard *Avena* test (line *D*).

There is another point which is of interest. Applied indoleacetic acid may give a maximal curvature, even when it reaches the test plant through a transport section, indicating that the amount

TABLE 3

RATE OF GROWTH REACTION (A); TRANSPORT RATE (B); LENGTH OF CURVED ZONE AS CALCULATED FROM A AND B (C); AS DETERMINED IN STANDARD AVENA TEST (D); AND ACTIVITY OF FOUR GROWTH PROMOTING SUBSTANCES IN AVENA COLEOPTILES

	Indole(3)acetic acid	Indole(3)butyric acid	Anthracene-acetic acid	Naphthalene-acetic acid
A. Time required for beginning of curvature in controls..	37 min.	53 min.	47 min.	34 min.
B. Time required for transport through 4.2 mm. sections..	28± 1.9 min.	38±5.3 min.	47±1.8 min.	66±3.8 min.
C. Calculated length of curved zone after 118 minutes...	12.1 mm.	7.2 mm.	6.3 mm.	5.4 mm.
D. Length of curved zone in standard Avena test.....	11.8 mm.	7.8 mm.	5.5 mm.	6.0 mm.
Concentration giving 5° curvature in standard Avena test	0.05 mg./l.	1 mg./l.	1 mg./l.	2.5 mg./l.

transported is considerable. From figure 6 it would appear that almost as much indoleacetic acid reaches the test plant through the interposed tissue as from the agar block directly. This is not true for indolebutyric acid, naphthaleneacetic acid, and anthraceneacetic acid. At concentrations of 10–20 mg./l. practically as much active material reaches the test plant through the transport section as at a five times higher concentration. But the controls indicate that the plants are able to give a much larger reaction. Thus it is clear that, for those substances, the amount transported (or the transport capacity) is limited.

TRANSPORT THROUGH DIFFERENT TISSUES

The method described in this paper can be used to measure the auxin transport through other plant tissues also. Only they should be small enough so that they can be fitted on an *Avena* coleoptile. In a few cases very rapid transport was obtained through *Nitella* internodal cells.

Small parallelepipeds were cut from potato tubers in longitudinal and radial direction, but in no case was any transport found, neither of 10 nor 2 mg. indoleacetic acid per gram lanolin.

In corn coleoptiles good transport was found, but only when high concentrations were applied (10 mg. indoleacetic acid per gram lanolin). In one experiment the transport of low auxin concentrations through 4.2 mm. *Avena* coleoptile sections gave maximum angles, with 59 and 62 minute intersection points in two sets of ten plants each, and no inverse transport occurred. In the 4.2 mm. corn coleoptiles only small curvatures resulted with high auxin concentrations, intersection point 87 minutes (eight plants). Also inverse transport of this same concentration took place, intersection point 102 minutes (ten plants).

Through 4.2 mm. papaya leaf stalks and midribs only very little was transported (120, 128, and 132 minute intersection points), but good results were obtained with 3.1 mm. sections of the same material.

Through young hypocotyls of tobacco plants no transport was observed with 1 mg. indoleacetic acid per gram lanolin, but the same concentration very clearly moved only from apex to base through *Tropaeolum* leaf stalks and flower stalks.

Thus this technique allows the determination of auxin transport through many different types of tissues. It is interesting to note that in general the velocity and capacity of transport were much less than in *Avena* coleoptiles. This work will be continued.

Discussion

It is not necessary to repeat the discussion on polarity of the auxin transport, as given by WENT and THIMANN (10). The present paper gives additional and rigid proof of the almost absolute polarity of the *Avena* coleoptile tissue for the transport of indoleacetic acid. It

also indicates why some investigators, especially JOST and REISS (**2**), did not observe this polarity, since it is apparent only when leakage along surface films is small or excluded. Of course it is still possible that the inverse transport of the highest auxin concentrations through 3 and 4 mm. sections was due to some leakage. This does not seem likely, however, considering the long transport time and the sudden onset of the curvature.

With regard to the remarkable differences in the amount of auxin leakage between the older transport experiments with the original technique (**8, 6, 9**) and these new experiments, something more has to be said. Whenever the auxin moves to a recipient in the form of an agar block, diffusion through tissues or the agar greatly exceeds leakage along the surface. The latter must occur of necessity, since the auxins are surface active; that is to say, they will accumulate in a water-air interphase. How relatively unimportant a movement along this interphase is in competition with diffusion is demonstrated by the value obtained for the diffusion constant of auxin. This was determined by letting the substance diffuse from a thin agar layer into a stack of three other agar layers. The value obtained in this way did not differ greatly from the value expected, on account of the molecular volume, so that the spreading along the agar surface could not have been more than a fraction of the total amount diffusing.

To account for the relative importance of surface leakage in the type of experiments described in this paper, the following fact has to be remembered. Although movement of auxin in an interphase will take place in a monomolecular film, it may be exceedingly fast, provided all auxin arriving at one end is immediately removed (**1**). This will not be the case if at this end the auxin has to diffuse into agar. But if the arriving auxin is completely removed by the apical cells near the cut surface of the coleoptile (which is quite possible since the auxin transport inside the coleoptile is equally large with, as against, a concentration gradient, **7**), a large amount of auxin might be moved in the moist surface film along the epidermis of the coleoptile. Thus the discrepancy in the results might be in the type of receiving material of the auxin, whether agar or living plant cells.

The transport rate experiments with different substances have

been the first to allow a critical test of THIMANN's hypothesis (5) that the activity of a substance in the *Avena* test depends not only on its growth activity per se, but also on its transport rate. Thus substances with a slow rate of transport would seem only slightly active in the *Avena* test. As far as this work goes it has borne out THIMANN's assumption. If the substances tested are placed in order of their molar activity in the *Avena* test, indoleacetic > indolebutyric, anthraceneacetic > naphthaleneacetic > indolepropionic > cis-cinnamic acids, then this series represents the order in which the transport rates of these substances decrease. For the last two it could not be measured at all. This is not surprising, since the curvatures they induce in the *Avena* test are shorter than 4 mm.

At the same time it is apparent that the difference in transport velocity is not the only factor which determines the difference in activity of these substances in the *Avena* test. First there is a difference in the time of onset of the curvature, which is independent of transport velocity. Therefore, this moment of beginning of the curvature must be determined by the rate of penetration of the compound into the cell and/or the rate of the growth reaction proper. Especially such growth promoting substances as have a benzene nucleus only (as phenylacetic acid) have a very slow rate of reaction. In addition, the fact must be considered that the capacity of the transport of growth promoting substances is very limited. This means that if less is transported than is necessary for a growth response no curvature at all will result. This is the case with most growth promoting compounds with a benzene nucleus, which all give S-shaped curvatures in the *Avena* test. Take phenylacetic acid as an example. That it is transported downward is indicated by the positive curvatures which it induces down to about 1 cm. below the cut surface, when an agar block containing a 10^{-4} molar solution is put one-sidedly. When sections are submerged in this concentration they will grow as much as they do in the considerably lower optimal indoleacetic acid concentrations. In the *Avena* test, however, the negative curvature due to the growth promoting properties of phenylacetic acid is limited to a zone of not more than 2 mm. length. These facts can be correlated in the following way: To get equal increases in growth, about a 100× higher concentration of phenyl-

acetic than of indoleacetic acid is required. Since the amount which can be transported inside the *Avena* coleoptile is limited, apparently not enough phenylacetic acid reaches the growing cells farther down to produce a visible growth increase, although the positive curvature indicates that some phenylacetic acid was present. In another paper this curious positive curvature will be considered in greater detail.

Summarizing this discussion, it may be said that the extent of the *Avena* test curvature depends on several different properties of a growth promoting substance: (1) the rate of transport of the substance inside the coleoptile tissue; (2) the rate of growth reaction, indicated by the time of beginning of the growth curvature; (3) the amount transported in connection with the activity of the substance in *Avena* growth. Point 3 must be investigated with more direct methods, but until we have a method which will allow the analysis of extremely small amounts of such phenyl compounds, no direct determinations seem possible.

Summary

1. Adapting the photokymograph recording of the *Avena* test, a new technique for measuring the velocity and capacity of the transport of growth promoting substances through different tissues has been worked out. For this the rate of bending and the moment of beginning of curvatures are compared for a normal *Avena* test and one in which the agar block is separated from the test plant through the tissue of which the transport properties are to be determined. Thus for individual pieces of tissue these properties can be calculated.

2. In general the results of VAN DER WEIJ (**6**) were confirmed, as far as the transport properties of the *Avena* coleoptile are concerned. Transport velocity is independent of length of tissue, but the amount transported decreases with increased length of coleoptile section. Transport through the narrow or wide side of a coleoptile is practically the same. The lower part of the coleoptile has slightly different transport properties from the more apical zones.

3. The polarity of indoleacetic acid transport is far more pronounced than most of the earlier investigators found. In our experiments leakage along moist surfaces was more nearly excluded. Only

the highest auxin concentrations (1000 mg./l.) were transported from base toward top through 3.1 and 4.2 mm. sections, but not through 6.3 mm. coleoptile sections.

4. There are great differences in transport velocity among various growth promoting substances. Owing to this and to differences in the rate of the growth reaction and limited transport capacity of the coleoptile cells, a wide variation results in the apparent growth activity of these substances in the *Avena* test.

5. Transport of indoleacetic acid through other plant tissues was determined, with positive results in corn coleoptile, papaya leaf stalks and midribs, *Tropaeolum* leaf stalks and flower stalks, and *Nitella* internodal cells.

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