Some Characteristics of Movement of Indoleacetic Acid in Coleoptiles of Avena. I. Uptake, Destruction, Immobilization, and Distribution of IAA During Basipetal Translocation [1, 2, 3]

Mary Helen M. Goldsmith [4, 5] & Kenneth V. Thimann

Biological Laboratories, Harvard University, Cambridge 38, Massachusetts &
Department of Botany, University of London, King's College, London

Indoleacetic acid (IAA) appears to be the major naturally occurring auxin controlling normal vegetative growth of a wide variety of higher plants. In many plants the auxin of the shoot is produced at or near the apex (8, 12, 32) and must be translocated from the site of production to the many sites where it influences growth and differentiation in the shoot. The translocation of auxin, like that of other organic molecules, can not be attributed to diffusion or purely physical processes; it requires the presence of living cells (28, 29, 23). The movement of auxin differs from the translocation of other organic substances in that it occurs predominantly from the apex toward the base of the shoot. This is not just a reflection of auxin production at the apex. In Avena coleoptiles, Went showed that even when auxin is supplied at the base of a section it can not be collected at the apex (31).

Since the initial work of Went (31) and van der Weij (28, 29), information has been obtained on the ability of plant tissues to translocate natural auxins (13) and on the inhibition of translocation by various substances (e.g. 5, 16, 17, 18). These studies, however, have been limited by the sensitivity and adaptability of some bioassay for detecting the auxin. Conclusions had to be drawn from the amount of auxin activity appearing at the opposite end of a section from a source of IAA. This experimental system is a complex one, and one would like to know how the uptake from the source, the destruction, and the retention of auxin within the tissue itself influence the amount of auxin recovered at the base of a section. Only with the advent of radioactively labeled IAA has this type of quantitative study become feasible.

The information presented here on the normal basipetal transport of IAA by excised sections provides a basis for further studies. For example, the effect of altered oxygen tensions and the characteristics of an acropetal, apolar movement have already been examined and will be described in subsequent papers.

Materials & Methods

► I. Preparation of Plants. *Avena sativa* L. (var. Victory) was used for this study. The oats were soaked without hulling for 4 to 5 hours and then planted in sterile damp sawdust. After 48 hours, just as the tips of the coleoptiles protruded above the surface, the seedlings were exposed to 12 hours of red light (18 inches from 25 w tungsten lamp, Ilford bright red filter). At the end of 90 hours, when used for experimental purposes, the coleoptiles were 25 to 30 mm long and their mesocotyls about 10 mm.

All experiments were carried out at 25 C. The plants were handled under red light (as above) and returned to darkness during the period of transport. Coleoptiles were cut 15 to 25 mm below the apex, and the primary leaf was gently removed from the basal end of the section. Sections of the required length were cut. The uppermost 2 mm of the tip were discarded.

Two different experimental arrangements were used to study translocation. The first method was simply a modification of the design used by Went (31) and van der Weij (28). A group of twenty sections (usually 7-mm long) were supported upright by a small lucite frame so that they did not touch each other. The sections could be oriented with their basal ends either up or down with respect to gravity. At each end, the cut surfaces were in contact with an agar block. The block supplying auxin to the sections, referred to as the donor or source, was usually placed on the apical cut surfaces; the block collecting the auxin which traversed the

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[4] Elizabeth Avery Colton Fellow, American Association of University Women (1958–59); Public Health Service Postdoctoral Fellow, National Cancer Institute (1959–61).

[5] Present address: Department of Botany, Josiah Willard Gibbs Research Laboratories, Yale University, New Haven, Conn.

sections, the receiver, was usually placed at the basal cut surfaces of the sections. Each frame supporting 20 sections rested on a glass microscope slide and was placed within a covered petri dish. A piece of damp filter paper was included in the dish. This design was suitable to obtain the transported material that was subsequently assayed for auxin activity (see Results, I). The same design was also used in qualitative experiments (see Results V & reference 7), but was of limited usefulness because the concentration of the source decreased significantly during the experiment and this complicated the analysis. To achieve a more nearly constant donor concentration, the agar sources were changed at frequent intervals. This produced a greater total uptake, but less activity disappeared from each individual donor. To keep the donor concentration within 10 % of its initial value required that blocks be changed at 10 minute intervals. Since changing of blocks was an unwieldy procedure, aqueous solutions of sufficient volume (1 ml of solution per each hour of uptake) to serve as essentially infinite sources were used. Twenty sections, 20-mm long, were held individually in the holes of a flexible plastic mesh. This mesh was supported by the rim of a beaker which contained the solution of IAA. One cut surface of a section just touched the surface of the solution; the section protruded vertically from the solution, and the other cut surface was covered with a piece of 1.5 % agar to prevent evaporation from the end of the section. In this arrangement, when the donor solution was applied to the apical end of the section, the section was inverted with respect to gravity. The small beaker containing the sections in contact with the solution of IAA was placed inside a container lined with moist towelling, and the whole was placed on a shaker.

► II. Preparation of Agar Blocks. The blocks were prepared by mixing equal volumes of the IAA solution and 3 % agar solution. The agar had been soaked in distilled water and rinsed twice daily for 1 week to remove salts. The resulting blocks of 1.5 % agar were 1.5 × 9 × 12 mm (162 mm^3).

► III. Indoleacetic Acid. The sole source of the carboxyl group of IAA was C^{14} cyanide which had a specific activity of 0.96 mC/mmole. The synthetic method of Thesing and Schülde (27) was modified by Dr. Bruce B. Stowe so as to increase the yield of IAA per mole of cyanide supplied.

To determine the degree of purity of the $C^{14}OOH$ IAA, an aliquot containing about 10 μg of the radioactive material was chromatographed on paper with an ascending solvent front [isopropanol : ammonia : water, 8 : 1 : 1; (25)]. Of the total radioactivity on the strip, 80 % was found in a single spot with the Rf and color reaction (ferric chloride-perchloric acid reagent) identical with that of purified C^{12} IAA. The remainder of the radioactivity was in spots which gave a barely detectable violet-pink color. The contaminants proved to be neutral compounds, and they were easily removed from an aqueous solution of the crude IAA by ether extraction at neutral pH. The IAA was then extracted at acidic pH. After the entire extraction procedure had been repeated twice, 97.5 % of the radioactivity (10 μg sample) was found in the IAA spot on the chromatogram. The remaining activity was uniformly distributed. These purification methods were successfully used on amounts as small as 0.5 mg crude IAA. Radioactive IAA stored in acetonitrile (method of Stowe, personal communication) for as long as 2½ years maintained a constant composition.

In experiments which required unlabeled IAA, purified, crystalline IAA (Hoffmann-LaRoche) was used without recrystallization. This material yielded only one spot, that of IAA, on chromatographing and spraying.

► IV. Determination of Biological & Radioactivity. To determine auxin activity the donor or receiver block was cut into 12 equal pieces and assayed in the standard Avena curvature test (32). To determine radioactivity the agar block was melted by gentle heating and the agar sol was spread evenly over the entire area of a planchet. After drying, a uniform, nearly invisible film of agar adhered to the surface of the planchet. Similarly, aliquots of solutions used were spread to cover the planchet, dried and counted immediately.

Immediately after the transport period, the dimensions of the sections and central hollow were determined on duplicate sections with a calibrated ocular micrometer. From these measurements, the volume of the section and hence the average concentration of activity could be calculated. Sections which had been in aqueous donors were rinsed quickly in distilled water; the central hollow of the coleoptile was rinsed using a needle and syringe. The sections were dried for 20 minutes at 80 C. They were then ground in a few drops of freshly redistilled chloroform. Particles of tissue remained suspended in the chloroform and were easy to transfer quantitatively to a planchet. After the chloroform evaporated, the particles were spread uniformly over the surface of the planchet in a drop of water; then the samples were dried.

A windowless gas flow counting tube connected to an appropriate scaling unit served to detect the radioactivity. The efficiency of counting so-called weightless samples was approximately 40 %. In most cases samples were counted long enough to reduce the relative standard deviation of the counting rate (corrected for background & absorption losses) to 5 % or less, but samples with a counting rate of less than 100 cpm were simply counted for a 10 minute period.

In order to compare the activities present in samples of different thickness, the observed activity must be corrected for self absorption. Correction factors were obtained by determining the decrease in the count of a sample of C^{14} IAA mixed and dried with known amounts of either pulverized tissue or agar blocks. Identical factors were obtained if C^{14}

was added in the form of mannitol; this shows that the decline in activity as increasing weights of tissue or agar are added is not peculiar to IAA and so can be correctly attributed to the absorption and scattering of the sample. Determination of the appropriate correction factors accompanied every experiment.

A test of the accuracy of the factors applied for different amounts of tissue is shown in table I. In this experiment groups of 20 intact sections, 7-mm long, transported IAA for 3 hours. At the end of this period each section was cut into two pieces of varying length which were either pooled in a single planchet or divided between two planchets. Each sample was counted, its radioactivity corrected by the factor appropriate for the amount of tissue involved, and the total activity in the original 7-mm section obtained by adding the component samples. Regardless of the size of the component samples, the total activity calculated to be present in the original samples was the same; this result substantiates the validity of the different correction factors.

Each experiment reported in this paper was repeated at least three times with similar results.

Results

► I. Demonstration That Movement of Radioactivity Reflects Movement of IAA. A wealth of literature reports that when IAA is applied to the apex of a section, material with biological activity can be collected at the base of the section. In the case of Avena coleoptiles, this material has been shown to be IAA (22). In order to follow the movement of IAA simply by observing the migration of radioactivity, it is necessary to show that no radioactive products arising from the metabolism of C^{14} IAA are translocated.

In these experiments, the donor concentration was about that necessary to cause maximum curvature in the Avena test (80 μg/l). In 3 hours under these conditions, about 10^{-2} μgs of IAA was found to enter each group of 20 sections; consequently it was not feasible to look for substances other than IAA by chromatographic procedures. If, however, the movement of radioactivity reflects the movement of IAA, then the ratio of radio- to bioactivity, at a given concentration, should be the same in both donor and receiver blocks. The Avena curvature bioassay was used to determine the biological activity in the receivers. This assay is 10 to 1,000 times more sensitive to IAA than to other related molecules (10), and although all the possible products of IAA metabolism have not been tested, it seems likely that the activity of these products is much less than that of IAA itself.

Typical transport experiments were conducted with 7-mm sections, and receivers collected after 3 hours. Usually six replicates of each transport were carried out; the radioactivity of half of the receivers was determined, and the biological activity of the other half assayed by the Avena test. A series of five concentrations of C^{14} IAA (10–80 μg/l) server as standards for each day's experiment, and a curve such as figure 1, relating radioactivity of the C^{14} IAA to its biological activity was constructed. This curve was used to predict a value for the biological activity of the receivers from their radioactivity. If all the radioactivity in the receivers is due to C^{14} IAA, this value should agree with the actual biological activity observed for the receiver. For example, in experiment 142 (table II) receivers averaged 18.4 cpm.

Table II

Translocation of Radioactivity Parallels Translocation of IAA

Experiment No.	142*	144**	162**
Radioactivity in receiver (cpm)	18.4	16.7	16.3
Biological activity expected in receiver (°curvature)	10	15	7
Biological activity observed in receiver (°curvature)	10.4	14.5	8.3

* Calibration curve in figure 1.
** Calibration curve not shown. Because of daily variations in the sensitivity of the bioassay, it differs somewhat from figure 1.

Table I

Test of Correction Factors

Part	Planchet I					Planchet II					Planchet I + II
	No. of samples averaged	Pieces of section	cpm	Factor	Corrected cpm	No. of samples averaged	Pieces of sections	cpm	Factor	Corrected cpm	Total Corrected cpm in 20 sections
A	3	Apical 1mm and basal 6mm	164.6	1.94	320			None			320
B	3	Apical 3.5mm	116.3	1.67	194	3	Basal 3.5mm	72.7	1.73	126	320
C	3	Apical 1mm	61.6	1.25	77.0	3	Basal 6mm	138.4	1.83	254	331

If this amount of radioactivity in the receivers is due to C^{14} IAA, it should produce a curvature of 10 degrees (fig 1). The average curvature actually caused by these receivers was 10.4 degrees (table II). Table II shows two other such experiments, and indicates that under the conditions of these experiments, IAA is the only radioactive substance translocated to the receiver.

In a number of experiments, the crude IAA containing 20 % of a radioactive impurity (see Methods III) was used in the donor. In these cases, the biological activity observed in the receiver was significantly greater (1.5 times) than expected from the crude IAA (7). In other words, the receivers contained a larger fraction of the donor's biological activity than its radioactivity. In this case, the contaminant must be translocated less readily than IAA (or not at all). The detection of a contaminant accounting for 20 % of the radioactivity of the donor demonstrates the validity of this method in detecting differences in the composition of a sample before and after translocation.

► II. Polarity of Movement of C^{14} Indoleacetic Acid. These experiments demonstrate that the movement of IAA is polarized in all the experimental situations presently employed. Sources spanning the range of IAA concentrations used in other experiments were applied to 7-mm sections. Table III shows that activity significantly above background could be detected in receivers when the source was at the apex but not when the source was at the base of the section. The movement is equally polar with both agar and aqueous sources. The existence of this polarity shows that movement in surface films from source to receiver does not occur in these experiments. These experiments are comparable to those of Went (31) and van der Weij (28) which first demonstrated the polarity of auxin transport. Thus, in the classical sense, the movement of IAA studied here is polar.

► III. Recovery of Original Radioactivity at Conclusion of an Experiment. At the close of all experiments the activity in the source, in the tissue, and in the receivers was determined. No activity disappears from the source unless it is in contact with sections, so that in the presence of sections, *the decline in the donor was equated to uptake by the tissue.* Depending on the experimental conditions, the total activity recovered in sections plus receiver was either equal to or less than the uptake; loss of radioactivity during an experiment was expressed as a percentage of the uptake.

When the agar source is changed hourly, activity leaves the source at a nearly constant rate during a twelve hour period. With a donor of 3.5 mg/l, about half the uptake can be recovered at any time in the sections and receivers. At a 20-fold lower concentration, however, the loss is not a constant fraction of the uptake. Initially, nearly all the uptake is accounted for in the sections and receiver, but the fraction lost increases steadily with time, becomes half of the uptake by 4 hours, and remains constant thereafter. Apparently, the higher the donor concentration, the more quickly a constant rate of IAA degradation and consequent loss of radioactivity is achieved.

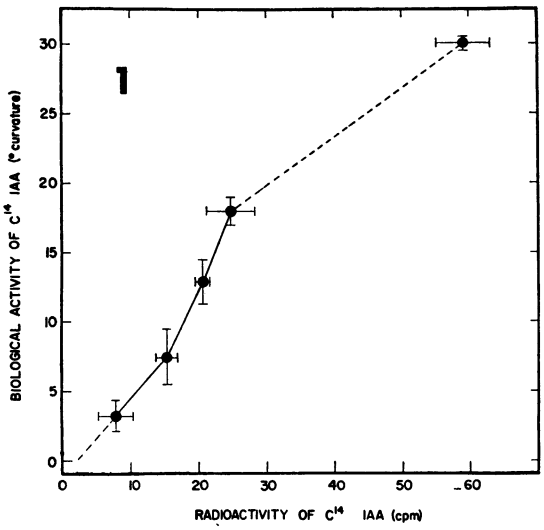
In light of these losses, the possibility that decarboxylation occurs during the heating and drying of samples was examined. The activity of samples treated with NaOH immediately after transport and then dried in the usual manner, and the activity of samples dried in vacuo at −70 C is no different from those dried by the standard procedures. Since alternative methods gave identical recoveries, it is unlike-

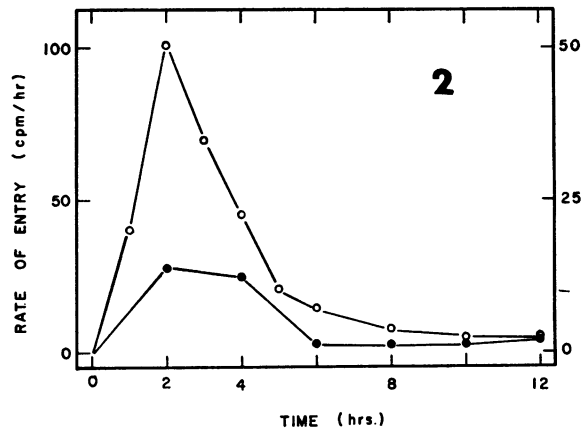
Table III

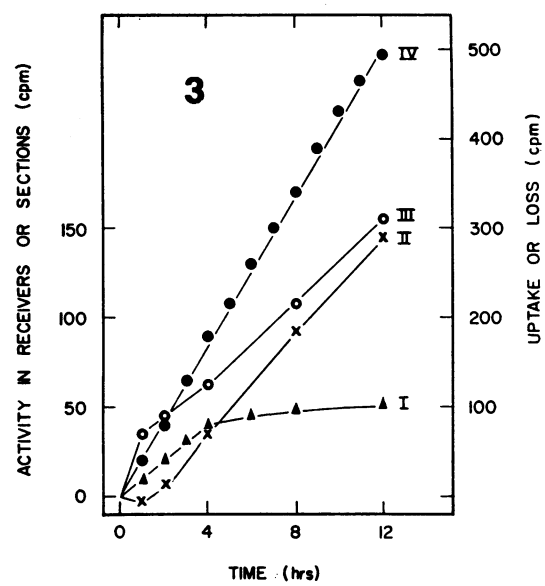
Polarity of Movement of C^{14} IAA

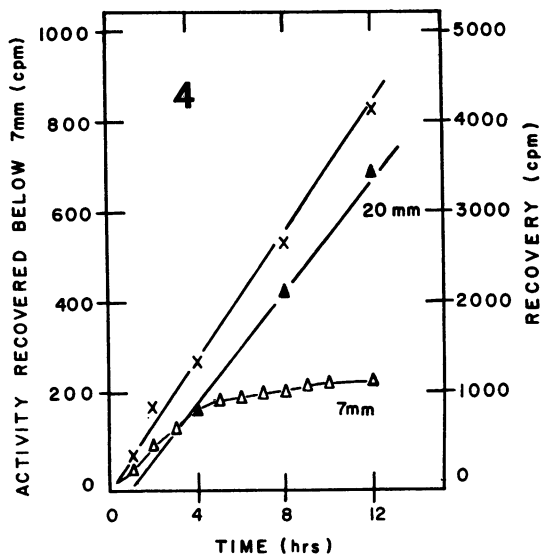
Expt.	Approx. source conc. (M)	Source at:	Transport period (hr)	Original source (cpm/unit volume ± SD*)	Agar block as source	Solution as source
					Receivers (cpm/unit volume ± SD*)	Receivers (cpm/unit volume ± SD*)
I	5×10^{-7}	Apex	3	110 ± 1.7	36.2 ± 1.6	
		Base	3	110 ± 1.7	−1.0 ± 1.1	
II	1×10^{-5}	Apex	2	1290 ± 9	48.0 ± 1.7	
		Base	2	1290 ± 9	+2.2 ± 1.2	
III		Apex	2	1510 ± 10	32.0 ± 1.6	
		Base	2	1510 ± 10	−1.5 ± 1.1	
IV		Apex	3	1125 ± 10	93.5 ± 8.2	119.3 ± 8.9
		Base	3	1125 ± 10	+0.9 ± 1.9	+0.5 ± 2.3
V		Base	3	1040 ± 10		+2.2 ± 1.3
VI	2×10^{-5}	Apex	3	2250 ± 17		127.3 ± 4.6
		Base	3	2250 ± 17		+4.1 ± 2.4

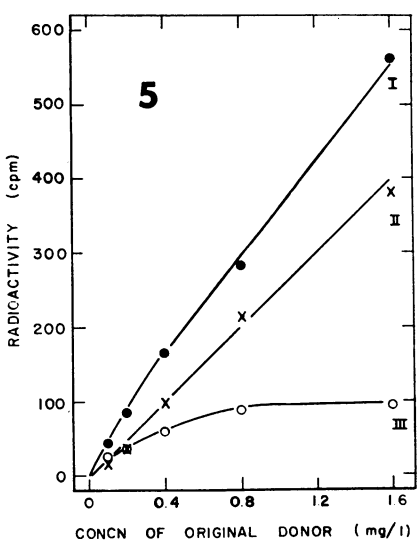
* Standard deviation of counting rate

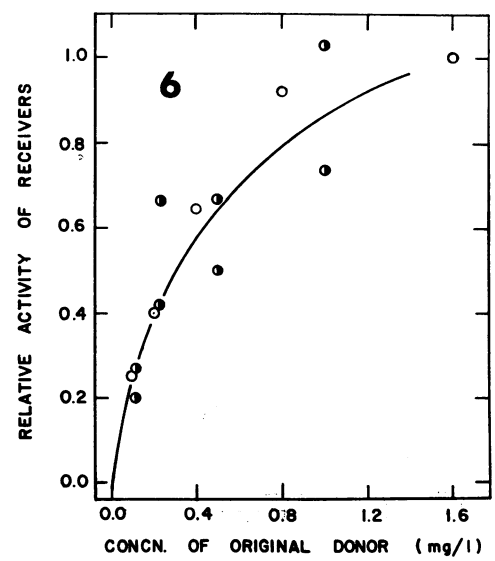

1
BIOLOGICAL ACTIVITY OF C14 IAA (°curvature)
RADIOACTIVITY OF C14 IAA (cpm)


2
RATE OF ENTRY (cpm/hr)
TIME (hrs.)


3
ACTIVITY IN RECEIVERS OR SECTIONS (cpm)
UPTAKE OR LOSS (cpm)
TIME (hrs)


4
ACTIVITY RECOVERED BELOW 7mm (cpm)
RECOVERY (cpm)
20 mm
7mm
TIME (hrs)


5
RADIOACTIVITY (cpm)
CONCN OF ORIGINAL DONOR (mg/l)


6
RELATIVE ACTIVITY OF RECEIVERS
CONCN. OF ORIGINAL DONOR (mg/l)

ly that any loss of activity occurred during preparation of samples.

It seems probable that radioactivity is lost via the enzymatic breakdown of IAA. Using gas phase counting, part of the missing radioactivity has been detected as carbon dioxide released by the coleoptiles during the translocation period. The failure to account for all the missing activity is probably due to technical difficulties which have not been resolved with the present equipment. It has not been determined whether the loss occurs at the cut surface or within the tissue or both, but such information probably would not greatly alter the conclusions below.

► IV. Time Course of Uptake & Transport by 7-mm Sections. In five experiments, the apical ends of sections 7-mm long were placed in contact with a source of radioactive IAA for 12 hours. The agar sources and receivers were replaced hourly. The donor blocks were either of low (0.08 or 0.16 mg/l) or high (1.6 or 3.2 mg/l) concentration.

The rate at which activity appears in the receiver (fig 2) rises to a maximum within 2 hours and then declines. After 8 hours this rate is very low and nearly constant. IAA appears to arrive at the base of the sections at a maximum rate for a somewhat longer time at the lower donor concentration, but a change in the rate of entry with time clearly occurs with either high or low source concentration. Since the rate of entry into the receiver declines to a low level after 4 hours, the total activity in the receiver (Curve I, fig 3) does not increase appreciably after this time. In contrast, the uptake from the source, continues without decline over the 12 hour period (Curve IV, fig 3).

As pointed out in the previous section, at low donor concentrations, loss of activity from the system begins slowly but after 4 hours, increases linearly with time (Curve II, fig 3). With high concentrations, (data not shown in fig 3), no initial lag in loss of activity occurs; loss increases linearly throughout the experiment. Thus the decline in the rate of translocation to the receivers is not correlated with any striking change in either the uptake or rate of loss of activity from the system.

Activity continues to accumulate in the sections even after it no longer appears in the receiver. It is interesting to observe that in all these experiments, the activity in the sections increases more rapidly during the times when little activity enters the receiver; i.e., during the 1st hour of the experiment and again after 4 hours (Curve III, fig 3).

Long sections are more effective than short ones in maintaining the translocation of activity for extended periods of time. The amount of activity translocated 7 mm from the source was compared for 7- and 20-mm sections (fig 4). The source was an agar block replaced at hourly intervals for 12 hours. During the first 4 hours, equal activity was recovered below 7 mm for both short and long sections. As already described, the activity at the base of the 7-mm sections increases only slowly after 4 hours. On the other hand, the activity below 7 mm in the 20-mm sections continues to increase throughout 12 hours without any decline. Since the activity below 7 mm

Data in all figures are for a group of 20 sections.

Fig. 1. The curvature elicited in the Avena test by the purified C^{14} IAA as a function of the amount of radioactivity present. The shape of this curve varied in different experiments reflecting daily variation in the sensitivity of the test plants. In this calibration curve (for experiment 142, table II), each point represents the average cpm of three samples and the average curvature given by three other samples. The standard errors of the mean are given by the extent of the lines through the points. The exact shape of the curve (*solid lines*) was determined only in the region corresponding to the activity of the receivers. Since the radioactivity is a linear function of the amount of IAA present, the curve reflects the response of the test plants to an increasing concentration of IAA.

Fig. 2. The rate at which activity enters a receiver as a function of time. The agar sources were renewed hourly and contained 80 μg/l (*right ordinate, closed circles*) or 1.6 mg/l (*left ordinate, open circles*). Sections 7-mm long.

Fig. 3. A typical experiment showing the activity in the receivers (I) and sections (III) on the left ordinate; and loss of activity from the system (II) and activity leaving the donor or uptake (IV) on the right ordinate. The source was 0.16 mg/l supplied in an agar block and renewed hourly. Sections 7-mm long.

Fig. 4. The recovery of activity further than 7 mm from the source. Solid symbols are for sections 20-mm long; open for 7-mm. X's (*right ordinate*) show total activity recovered in sections plus basal agar receiver. Total recovery was identical for both 7-mm and 20-mm sections. The source was an agar block containing 3.2 mg/l IAA and was changed hourly.

Fig. 5. The radioactivity leaving the donor (I), recovered in the sections (II), and receivers (III), each as a function of the original source concentration. The data were obtained after 3 hours, but similar results were obtained for shorter times. Each symbol represents the mean of 5 samples.

Fig. 6. The radioactivity (*open circles*) and biological activity (*half-filled circles*) of receivers relative to their maximum activity as the source concentration is raised. The data for biological activity are from an experiment using purified, unlabeled IAA in the source. Receivers were appropriately diluted so that their auxin concentration was in the range where the Avena test is maximally sensitive to change in concentration.

Table IV

Distribution of Activity—After 2 Hours' Uptake From Agar Sources of 1.6 mg/l

Expt.	Initial length of section (mm)	Uptake (cpm)	% of Uptake recovered in			% Uptake lost
			Sections		Receiving system*	
			0–3.5 mm	3.5–7 mm		
179	7	1715	49	23	13	15
170	7	1715	50	21	15	14
Average			49.5	22	14	14.5
179	20	1980	52	25	12	11
170	20	1976	55	22	12	11
Average			53.5	23.5	12	11

* i.e., below 7 mm from source; agar block for 7 mm sections; 13 mm of tissue plus agar block for 20 mm sections.

had to be translocated from the source, the longer sections obviously are capable of translocating activity for much longer times than short sections. Although the uptake is often somewhat greater for the long sections, this alone can not be the explanation for the greater amount of translocation. In some experiments the uptake by 20-mm and 7-mm sections was identical, and yet in 12 hours 20-mm sections always translocated at least three times as much activity through the first 7 mm of the section.

► V. Effect of Concentration of Source on Transport of Auxin. In this group of experiments, sections 7 mm in length were supplied with a single agar source for 3 hours or less. With IAA up to 0.4 mg/l, the uptake is not quite proportional to concentration, but rises with decreasing slope as the concentration is increased; above 0.4 mg/l, the uptake is linearly related to source concentration (Curve I, fig 5). The activity in the receiver after 3 hours also rises nonlinearly with increasing concentration of the source, but reaches a maximum when the donor contains 0.8 mg/l (Curve III, fig 5). Other experiments show that the same is true after shorter periods (0.5, 1 hr) of translocation. Experiments with non-radioactive sources of IAA yield exactly similar results for the relation between bioactivity found in the receivers and source concentration (fig 6), and thus confirmed other evidence (Results I) that the appearance of radioactivity in receivers reflects the translocation of IAA.

Although the amount of IAA in the receiver attains a maximum with increasing donor concentration, activity continues to rise almost linearly in the sections (Curve II, fig 5). This observation suggested that the following points should be investigated. A, The distribution of activity along the section between donor and receiver, and the effect of donor concentration and period of uptake on this distribution. B, The influence of the length of the section on the relationship between donor concentration and translocation. C, The translocation of activity out of sections after the external source of IAA is removed.

► VI. Distribution of Activity in Sections & Effect of Length of Section on Amount of Activity Translocated. To determine if the type of source influences the results, some sections were supplied with agar (changed at 10–15 min intervals) and some with aqueous sources. Both sources were applied at the apical ends of sections inverted with respect to their normal orientation to gravity. For both sources, the distribution of activity in the section was the same, and equal, very low levels of activity (less than 1 % of the uptake) were recovered in the agar receiver at the base of the 20-mm section. These results indicate that the two types of source are comparable.[6]

In the following experiments, 20-mm sections supplied with aqueous donors were used. At the close of an experiment each section was cut into 4 to 6 successive lengths and the corresponding pieces of 20 sections were pooled and counted. To study the influence of section length on activity translocated to the receiver, all the activity recovered below any desired length of the section was taken as the activity translocated through that length. Thus with long sections, the receiver was not just a block of agar, but part of the section plus the basal agar block. It will be demonstrated below that whether the section

[6] One small difference was noted; the total activity of sections plus receiver with an aqueous source was only 80 to 85 % of those with an agar source. The explanation of this result is not known. Transport is less sensitive to decrease in oxygen tension than growth (7). Since the growth of sections with aqueous donors was not affected, it is unlikely that oxygen supply is limiting the uptake of the sections in aqueous solutions.

Spreading of the donor solution over the surfaces of the coleoptile is not a problem and does not influence the outcome of experiments with aqueous sources. This is clear from the fact that A, uptake from the solution is not greater than from agar, B, activity recovered in the agar receiver at the base of the 20-mm section was negligible even after 12 hours and C, the movement is polar (table III). No doubt, the hydrophobic nature of the cuticle reduces the tendency of the solution to creep along external surfaces of the sections.

itself or an agar block is the receiver, identical results can be obtained.

The results of experiments with 20-mm sections are summarized in table IV and figures 7 through 12. They lead to the following conclusions.

A. As long as experiments with short sections do not continue longer than 4 hours, the distribution of activity in the section and loss of activity from the system is comparable for 7- and 20-mm sections (table IV). Translocation does, however, persist for a longer time in 20-mm than 7-mm sections (fig 4). Several factors might be involved here; for example, 20-mm sections have a smaller ratio of injured to intact cells and possess larger energy reserves than 7-mm sections. Whatever the causes, the difference between translocation in short and long sections is only manifest after 4 hours. Prior to this time, results obtained with 20-mm sections are completely consistent with those obtained for 7-mm ones.

B. Figure 7 shows that the concentration of activity in the section declines exponentially with distance from the source. This logarithmic distribution is established within an hour throughout the portion of the tissue in which activity is detectable. It is then maintained for at least 12 hours. Some departure from the logarithmic distribution is often noted at the base of the section, where a low level of activity somewhat greater than predicted by extrapolation of the logarithmic curve may appear within the hour. Often, the activity in the basal parts of the sections increases more slowly than in the apical portions.

C. The average concentration of radioactivity in the portion of the section in contact with the donor is given by the y intercept of the curves of figure 7 (i.e., 0 mm from source). Within 4 hours, this concentration *exceeds* that of the source. After 12 hours, it is actually five times the value of the source. The interpretation of this finding is not simple (see Discussion).

D. The slope of the logarithmic decrease with distance is nearly independent of time (fig 7). In some experiments a gradual increase in the slope does occur with time.

E. A striking increase in the slope occurs as the concentration of the source increases (fig 8). For example, in four such experiments the slope was nearly doubled when the source concentration was raised from 0.08 mg/l to 1.6 mg/l.

F. The way in which the amount of activity at any point in the section increased with time varied from one experiment to another. Usually, the activity in a zone of the section increased at a constant rate between about one and four to six hours (fig 9); thereafter, more slowly (fig 10). From the apex to the base of the section, the rate of accumulation progressively decreased (fig 9 & 10). In one experiment, however, the amount of activity in the section increased more rapidly after 6 hours than before. Usually, the initial period of nearly linear increase in activity was more pronounced and persisted for a longer time in the apical part of the section (fig 10).

G. The total activity below any given distance from the source (i.e., in the receiving portion of the section) also declines logarithmically as the length of the section between the source and receiving portion increases (fig 7).

H. The amount of activity translocated through any length of section is the summation of all activity

Table V

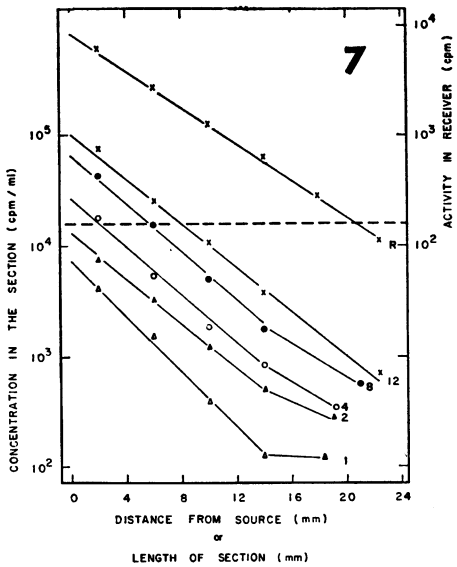
Immobilization & Export of Activity After 2 Hours' Uptake

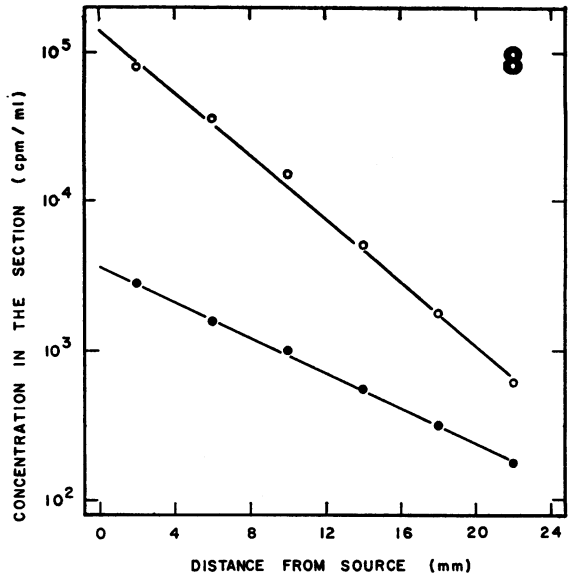
Expt.	Time	cpm Uptake (D_o-D_2)*	% Uptake in section $(100 \cdot S_t/D_o-D_2)$*	% Uptake in receivers $(100 \cdot R_t/D_o-D_2)$*
1	2	3	4	5
	High Donor Concentration (1.6 mg/l)			
71	2 hr uptake	339	82	20
	2 hr uptake + 1 hr export		56	26
182	2 hr uptake	325	84	18
	2 hr uptake + 2 hr export**		72	34
	2 hr uptake + 2 hr export with cold IAA		69	28
186	2 hr uptake	639***	97	20
	2 hr uptake + 2 hr export		74	35
Avg.	2 hr uptake		88	19
	2 hr uptake + 2 hr export		68	31
	Low donor conc. (0.08 mg/l)			
186	2 hr uptake	93***	44	32
	2 hr uptake + 2 hr export		23	47

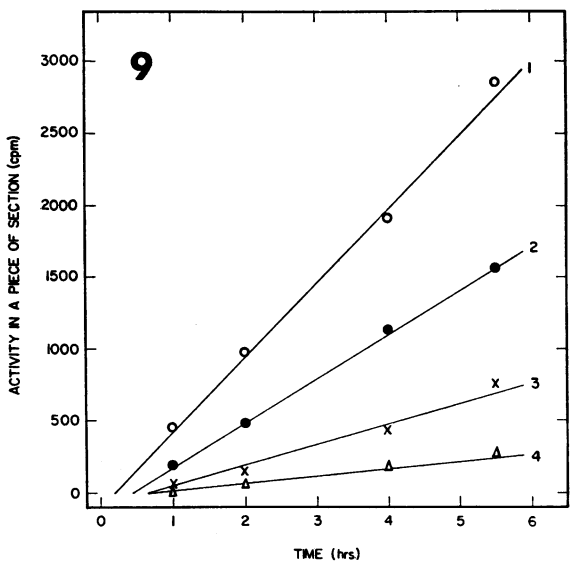
* D_o, cpm in original donor; D_1, cpm in donor after one hours' uptake; D_2, cpm in donor after 2 hours' uptake; S_t, cpm in section at time indicated; R_t, total cpm recovered in receivers at time indicated. (Receivers present at base only during uptake, at both base & apex during export.)

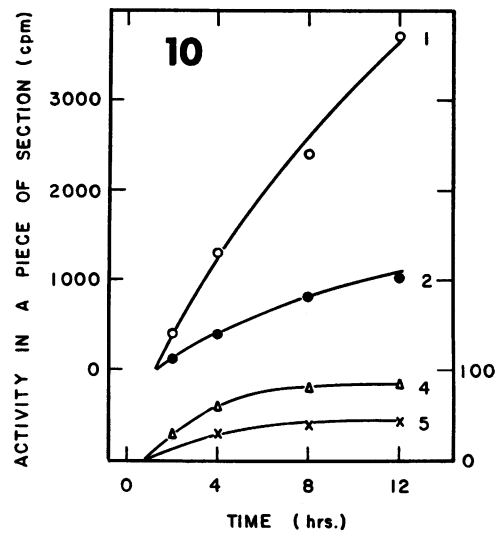
** Export was complete within 2 hours. (fig 13)

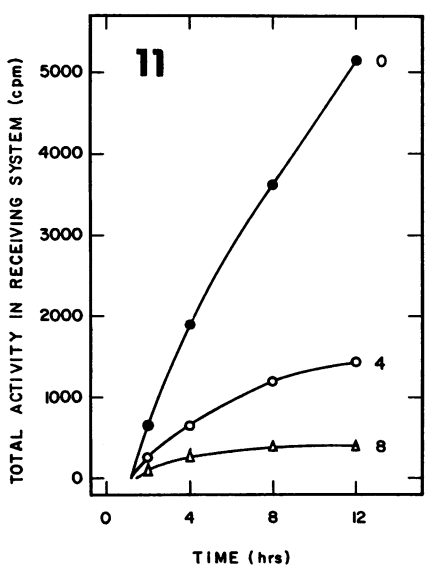
*** IAA of higher specific activity used in this experiment.

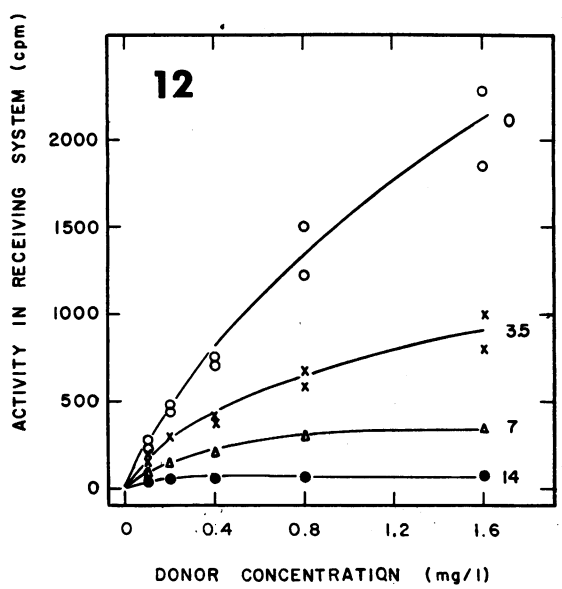

7
CONCENTRATION IN THE SECTION (cpm / ml)
ACTIVITY IN RECEIVER (cpm)
DISTANCE FROM SOURCE (mm)
or
LENGTH OF SECTION (mm)


8
CONCENTRATION IN THE SECTION (cpm / ml)
DISTANCE FROM SOURCE (mm)


9
ACTIVITY IN A PIECE OF SECTION (cpm)
TIME (hrs)


10
ACTIVITY IN A PIECE OF SECTION (cpm)
TIME (hrs.)


11
TOTAL ACTIVITY IN RECEIVING SYSTEM (cpm)
TIME (hrs)


12
ACTIVITY IN RECEIVING SYSTEM (cpm)
DONOR CONCENTRATION (mg/l)

in the zones of the section below it plus that in the basal agar block. Hence, as with the activity in one zone of the section, the activity in the receiving portion of the system increased initially at a near constant rate and then more slowly (fig 11).

I. When the total activity in the receiving system after a fixed time is plotted as a function of the concentration of the source, a family of curves for sections of different lengths does not result. The longer the section, the lower is the source concentration at which the receiver reaches maximum activity (fig 12). In other words, the shape of the concentration-dependence curve depends on the length of section employed in the experiment.

J. In an experiment of 3 hours' duration, the transport through the first 7 mm of a 20-mm section is the same function of the source concentration as is the transport through 7-mm sections into an agar block (Compare fig 5 & fig 12, 7 mm). This extends the evidence in A above, that the receiving system, be it agar block or living tissue, does not influence the translocation.

VII. Evidence for Immobilization of Activity. The purpose of these experiments was to measure how much activity was exported from sections after the donor was removed. After sections 7-mm long had transported IAA for either 1 or 2 hours, the donor and receivers were replaced by empty agar blocks. These blocks were then changed and counted at intervals. Sections were collected for counting at the time the donor was removed and again after export into the receiving blocks ceased.

The results of experiments with a two hour period of uptake are summarized in table V. The fraction of the uptake recovered in the section prior to export (Column 4) doubles (from 44–88 %) as the donor concentration is raised 20-fold. Conversely, the fraction recovered in the receiver during the uptake decreases with the same change in donor concentration (Column 5).

When the donor is removed, activity continues to leave the sections (fig 13). Nearly all of this export occurs in the first hour and approximately half of it within the first 15 minutes. Nearly a third of the exported activity is recovered in the block at the apical end of the section (A, fig 13). This acropetal movement can only be detected when apical blocks are replaced frequently during the export period, presumably because activity which enters this block is thus prevented from re-entering the coleoptile and being carried basally by translocation. Activity appearing in the apical block may have diffused from the external surfaces and intercellular spaces of the section.

The activity collected at the base of the section during export is a rough approximation of the amount available for translocation when the source is removed, and is about equal to the amount that would have arrived there had the source remained in place another hour.

At the end of 2 hours, export has completely ceased, but considerable activity still remains in the sections. A particularly important point is that the fraction of the uptake immobilized in the section

Fig. 7. The average concentration of the activity (*left ordinate, logarithmic scale*) in successive zones from the apex to the base of 20-mm sections. The numbers to the right of each curve give the time in hours. Starting at the apical end, all of the sections at each time were divided into four successive 4-mm pieces. The fifth zone of the sections was that remaining below 16 mm. As a result of growth which occurs during the experiment, the length of this basal piece increases with time. The average concentration in a piece of section was calculated assuming the activity to be uniformly distributed through its total final volume. The average concentration is plotted as the concentration at the midpoint of the piece of section. The concentration of the source is shown by the horizontal dashed line. The curve marked R gives the activity in the receiving system (*right ordinate*) as a function of the length of the section between source and receiving system. Time of translocation was 12 hours.

Fig. 8. The average concentration of activity (*logarithmic scale*) in successive 4-mm zones from the apex toward the base of 20-mm sections. The sections had translocated IAA from an aqueous source of 0.08 mg/l (*closed circles*) or 1.6 mg/l (*open circles*) for 5½ hours.

Fig. 9. The increase in activity during 6 hours in 20-mm sections translocating IAA from an aqueous source of 1.6 mg/l. Curves 1, 2, 3, 4 are for successive 4-mm pieces from the apical end of the section.

Fig. 10. The activity in the sections at different distances from the source during 12 hours. The original sections were 20 mm long. The aqueous source contained 3.2 mg/l. The numbers to the right of the curve refer to the 4-mm pieces into which the section was divided at the end of transport. Curves 1 and 2 (*left ordinate*) are for the apical and subadjacent pieces; 4 and 5 (*right ordinate*) are for pieces 12 to 16 and 16 to 20 mm from the apical surface.

Fig. 11. The activity in the receiving portion of the system during 12 hours transport. This data is from the same experiment as that given in figure 10. The numbers to the right of the curves give the length of the section between source and receiving system.

Fig. 12. The activity in the receiving system as a function of source concentration. Curves are given for the activity recovered below 0, 3.5, 7, and 14 mm from the source in sections which were 20 mm long. Time of translocation was 3 hours.

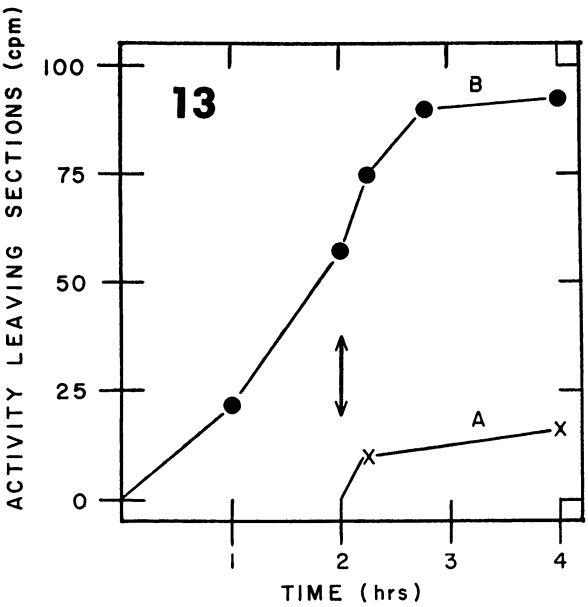


Fig. 13. The total activity (B) recovered in agar receivers at the base of 7-mm sections during and following uptake from an agar source which was renewed hourly and contained 1.6 mg/l IAA. The arrow marks the time that the source and receiver were replaced by empty agar blocks and the period of export began. The activity collected in the block at the basal end of the section during the export period is represented as being added to that collected in the receiver during the 2-hour period of uptake. The activity collected in the empty agar block replacing the donor is also shown (A). The blocks collecting the exported activity were replaced at intervals of 15, 45, and 120 minutes after the source was removed. Data from experiment 182; see also table V.

varies with the concentration; it increases from 23 % to 68 %, i.e., about triples, for a 20-fold increase in the donor concentration. Since as many as six samples were involved, total recovery could not be determined very precisely. In spite of the variability in recovery, the difference between immobilization at high and low concentration is great enough to be significant. Further, the greater the immobilization of the uptake, the smaller the fraction that appears in a receiver (Column 5). This increase in the fraction immobilized could account for the fact that the activity in the receiver reaches a maximum as the source concentration increases (fig 5).

The same amount of activity is recovered in the sections when the source is replaced by an equal concentration of unlabeled IAA, (Expt. 182, table V). Clearly, IAA from the source does not push the translocation or exchange with immobile activity already in the section. This indicates that the immobile IAA has been very tightly bound or has been irreversibly converted to another substance.

The results obtained in experiments with a one hour period of uptake (table VI) confirm those just described.

Discussion

The present findings must be compared with the extensive investigations of van der Weij (28, 29). Although he does not point out that the amount of auxin in the receivers rises non-linearly with concentration, his data for sections 2-mm long fall on a curve similar to that for 3.5-mm sections (fig 12) in this paper. Van der Weij reports that once IAA began to enter the receiver, the rate of entry is the same for sections of different lengths (1–5 mm). This is not so in the present experiments with much

Table VI

Immobilization & Export of Activity After 1 Hour's Uptake

Expt.	Time	cpm Uptake (D_o-D_1)*	% of Uptake in section $(100 \cdot S_t/D_o-D_1)$*	% of Uptake in receiver $(100 \cdot R_t/D_o-D_1)$*
1	2	3	4	5
	High donor conc. (1.6 mg/l)			
180	1 hr uptake	202	79	13
	1 hr uptake + 2 hr export		52	36
	1 hr uptake + 2 hr export with cold IAA donor present		48	26
182	1 hr uptake	155	85	15
	1 hr uptake + 2 hr export		51	31
Avg.	1 hr uptake		83	14
	1 hr uptake + 2 hr export		50	31
	Low donor conc. (0.08 mg/l)			
129	1 hr uptake	27	71	36
	1 hr uptake + 2 hr export		31	84

* See symbols at bottom of table V.

longer sections (7–20 mm) (fig 11). Van der Weij's results suggest that little or no immobilization occurred under the conditions of his experiments (short periods of transport of 100 min or less). As van der Weij did not measure uptake, it is not possible to check this interpretation.

Van der Weij proposed that the x intercept of the line relating the amount in the receiver to the time of transport gave the time for one molecule of auxin to traverse the coleoptile. In this way, he arrived at velocities of auxin transport varying from about 8 to 15 mm/hour. In the present experiments similar values can be obtained by this method. Since some molecules of auxin are immobilized during translocation, this method yields only a minimum value for the velocity of translocation. Canny (3) has carefully considered some assumptions underlying calculations of the velocity of translocation, and concludes that it is preferable to express translocation rates in terms of the mass transfer (g $cm^{-2}hr^{-1}$) until a method can be devised to measure directly the velocity of the translocation stream. The mass transfer of IAA during uptake at 3.2 mg/l was only 0.21 μg $cm^{-2}hr^{-1}$ [7]. As might be expected from its hormonal nature this is some 20 million times less than the value of 5 g $cm^{-2}hr^{-1}$ (3) for the average mass transfer of dry weight in most phloem cells.

Since all the activity in the tissue is clearly not free IAA (Results, VII), the fact that the tissue achieves a higher concentration of radioactivity than the source (fig 7) does not necessarily indicate that IAA enters the tissue against a concentration gradient.

The appearance of relatively large amounts of immobile activity especially at external concentrations above 1 mg/l, are of interest from another point of view. Recently workers in several laboratories (2, 4, 9, 19, 20) have agreed that no significant redistribution of radioactivity could be detected in the tissue of coleoptiles which had previously been given radioactive IAA and were then stimulated geotropically or phototropically. In interpreting these experiments, it should be kept in mind that after a source of IAA is removed most of the activity in a section is no longer free IAA. Furthermore, any redistribution of radioactivity by lateral movement of the small amount of free IAA present could be completely masked by the large amount of immobile radioactivity. It has, in fact, recently been shown that the radioactive IAA which is still being translocated is definitely redistributed under the influence of gravity (6). The extent of the redistribution is comparable whether radioactivity of applied C^{14} IAA or biological activity of endogenous auxin is measured.

Immobile activity also appears during translocation of endogenous auxin. In the growing zone both of the *Osmunda rachis* (24) and of green Alaska pea seedlings (21), transportable auxin decreases at the expense of an immobile substance with auxin activity.

An approximately logarithmic decrease in the radioactivity within a plant with distance from the source is not unique to C^{14} IAA; it has already been observed during translocation of inorganic ions (26), as well as sugars (1, 14, 30) and amino acids (15). To account for such a distribution, Biddulph and Cory (1) suggested a model which has been more rigorously developed by Horwitz (11). The model assumes that an irreversible removal of activity from translocation establishes the logarithmic distribution. Activity is in fact immobilized during translocation of IAA, but other features of the movement do not seem to fit this model.

From Horwitz's derivation, the slope of the logarithmic decrease is determined by the rate constant for the removal reaction (k), the cross-sectional area of the translocation stream (A), and the velocity of this stream (v), and is equal to kA/v. Thus this slope should be independent of the source concentration. In the translocation of IAA, however, this slope increases with increasing donor concentration (fig 8). The model also predicts that at a distance x from the source, the concentration in the translocation stream and thus in the receiver will be proportional to the source concentration. It is a striking characteristic of the translocation of IAA, however, that the total activity in the receiver is not directly proportional to the source concentration (fig 5 & 12); relatively more IAA is retained and immobilized per unit distance at higher donor concentrations.

Figures 5 and 12 show that the activity in a receiver reaches a maximum with increasing source concentration, and that the longer the sections, the lower the source concentration at which this maximum is achieved. These observations are not easy to explain. The cells of coleoptiles can do at least three different things with the IAA they take up: A, they may pass it on to the next cell, B, they may break down the molecules so that radioactivity is lost from the system, or C, they may retain the IAA, perhaps converting it to a substance which can not be translocated or perhaps sequestering it in some part of the cell from which it is not readily removed. Each of these processes, transport, degradation, and immobilization, is probably carried out by a separate enzyme or even a system of enzymes. Probably each process occurs repeatedly in every cell the IAA enters during translocation through the section. The response of the tissue to an increase in auxin probably involves interaction of all the systems using it. The fact that the amount of activity entering the receivers reaches a maximum as the source concentration increases has suggested to some workers that the transport of IAA becomes saturated (13). The experiments reported here indicate quite another explanation, namely the removal of IAA from translocation by destruction and immobilization.

The polarity of translocation of IAA is one of its most puzzling features. A polarity could be im-

[7] Since preliminary experiments suggest that all cells are involved in translocation of IAA, the total cross section of the tissue was used to calculate transfer.

posed by the action of a carrier so localized in the cell membrane that it could move auxin preferentially in one direction—through the basal membrane of one cell into the apical membrane of another. If this system were coupled to a system in the receiving cell which prevented diffusion back by rapidly removing the auxin from the vicinity of the cell membrane, polar transport into a cell and through a section would be achieved.

Summary

By biological assay (Avena curvature test), it was established that when C^{14} carboxyl-labeled indoleacetic acid is applied to a section of Avena coleoptile, the radioactive substance which is translocated through the section is IAA. Uptake and basipetal movement of radioactivity from a source of carboxyl-labeled indoleacetic acid was then studied with sections 7- to 20-mm long supplied either agar or aqueous sources. Under these conditions, the movement of indoleacetic acid is polar.

As the concentration of auxin applied is increased, the activity recovered in a receiver does not increase proportionally and so reaches a maximum. The source concentration at which this maximum is achieved is progressively lower the longer the section of tissue between source and receiver.

The activity within the section itself decreases logarithmically with distance from the source. A simple model of translocation which has been proposed previously to account for this type of distribution is inadequate to account for the effect of source concentration and length of section on the distribution of activity during translocation of auxin.

From the present experiments, it is clear that two other factors besides translocation influence the amount of indoleacetic acid recovered at the base of the coleoptile and the distribution of indoleacetic acid within the coleoptile. In the course of translocation, auxin is also subjected to A, enzymatic destruction and B, immobilization. Destruction is indicated by the failure to recover all the activity leaving the source within the sections and receivers. Immobilization is evidenced by the retention of considerable radioactivity by a section after the radioactive source has been removed or even replaced by unlabeled IAA. The interplay of these three systems for handling auxin—translocation, destruction, and immobilization —is complex.

Acknowledgments

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Potentiometrically Measured Reduction of Low Concentrations of Dye by Illuminated Chloroplasts [1, 2, 3]

Fergus D. H. Macdowall

Plant Research Institute, Research Branch, Canada Department of Agriculture, Central Experimental Farm, Ottawa

According to a wide convergence of evidence indophenol dyes are reduced more simply and rapidly than is ferricyanide, by illuminated chloroplasts (5, 6, 14, 16, 17, 18). In fact it seems probable that the two oxidants are reduced by different sites of electron transport in the chloroplasts. Many kinetic data are now available on the chloroplastic reduction of intermediate concentrations (10^{-4} M) of ferricyanide (7, 11). The following data on the reduction of 2,6-dichlorophenolindophenol (DPIP) by fragmented chloroplasts are submitted to stimulate interest in the use of low, rate-limiting concentrations of oxidants in kinetic studies. Kinetic data obtained when the Hill reaction's terminal electron transfer is rate-determining may distinguish reactions at separate reducing sites.

Materials & Methods

Suspensions of once-washed, partially fragmented chloroplasts were prepared from leaves of spinach (*Spinacia oleracea* L.) or New Zealand spinach (*Tetragona expansa* Murr.) and their chlorophyll contents were determined, as already described (8). The spinach chloroplasts were resuspended in water and kept at 4 C for immediate use. The chloroplasts from New Zealand spinach were resuspended in 0.5 M sucrose and aliquots, each to be separately thawed and partially used for one rate measurement, were stabilized by storage on dry ice after snap freezing (4). An aliquot of suspension was pipetted into a buffered solution of DPIP in a thermostated Warburg vessel fitted with one or two plantinum electrodes. The mixture was depleted of oxygen by shaking in a stream of purified nitrogen, during which the redox potential fell to a steady state as measured vs. a saturated calomel electrode by means of a Beckman Model G pH meter. Upon illumination the redox potential fell further as a result of chloroplastic reduction of the oxidant.

To calculate the rate, the temperature-corrected

[1] Received revised manuscript Dec. 22, 1961.
[2] Data taken from Ph.D. dissertation, Biology Department, Stanford University, 1954.
[3] Contribution No. 149 from the Plant Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Ontario.