INHIBITION OF TRANSPORT OF INDOLE-3-ACETIC ACID IN THE ETIOLATED HYPOCOTYL OF *PHASEOLUS VULGARIS* L.

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[*Manuscript received June* 11, 1956]

Summary

The effect of a number of auxins and auxin analogues on the basipetal transport of indole-3-acetic acid (IAA) in 5-mm segments of hypocotyls of 5-day-old etiolated *Phaseolus vulgaris* L. seedlings has been investigated.

Donor and receptor agar plates were applied respectively to the morphologically apical and basal cut ends of the segments and the receptor plates assayed for their content of auxin by means of the *Avena* curvature test.

Pre-treatment of the hypocotyl segments with either 2,4-dichlorophenoxyacetic acid, 2,4-dichlorophenoxypropionic acid, 2,4-dichlorophenoxybutryic acid, 2,4,6-trichlorophenoxyacetic acid, indole-3-propionic acid, or 2,3,5-tri-iodobenzoic acid decreased the curvature given by blocks cut from the receptor plates. Pre-treatment with either *p*-chlorophenoxy*iso*butyric acid, 2,4-dichloroanisole, coumarin, *o-iso*propyl-*N*-phenylcarbamate, indole, indole-3-acetonitrile, or phenylacetic acid was without effect.

Substances inhibiting transport of IAA also decreased the IAA-induced curvature when incorporated with IAA in agar blocks and tested directly in the *Avena* test. These substances, however, were not transported in the *Phaseolus* segments under the conditions used.

It is concluded that these inhibitors affect the mechanism of the basipetal transport of IAA, and as a tentative explanation it is suggested that this is achieved by competition for sites on hypothetical carriers of IAA.

I. Introduction

Since the discovery of auxins it has been recognized that their transport is polar and requires an energy source. Thus rate of transport can be reduced by inhibitors of respiratory metabolism (du Buy and Olson 1940). As a working hypothesis for this study it has been postulated that, in absorption and transport, auxin becomes attached to a carrier in a specific way. If such carrier sites were occupied by structurally-related compounds another type of inhibition would be obtained.

The literature contains a few reports of inhibition of transport of indole-3-acetic acid (IAA) by auxin analogues. Skoog (1954, 1955*a*, 1955*b*) states that tri-iodobenzoic acid (TIBA) has an inhibitory effect on the transport of IAA. In a short report by Niedergang-Kamien (1955) this property has also been attributed to phenylacetic acid.

This paper deals with experiments planned on the working hypothesis mentioned above. During testing of some auxins and auxin analogues, several of them have been found to inhibit transport of IAA. Whilst this finding alone does not provide sufficient evidence for verification of the hypothesis concerning the transport mechanism, it is thought to add to the understanding of the action of a number of synthetic growth substances.

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During the preparation of this manuscript a fuller account of the work on TIBA by Niedergang-Kamien and Skoog (1956) has come to hand. As will become evident (see Section IV) the results mentioned in the present paper provide an independent confirmation of some of their findings.

II. Materials and Methods

(*a*) *Pre-treatment Experiments*

The main conclusions of this work were drawn from the experiments in which hypocotyl segments were pre-treated with auxins and auxin analogues. For these, seeds of *Phaseolus vulgaris* L. var. Brown Beauty from the 1955 harvest were soaked for 2 hr and then germinated and grown for 5 days at 25°C and 85 per cent. R.H. under weak red light. The hypocotyls were then 10–20 cm long. A uniform batch was selected and, from each, two 5-mm segments were cut from the region just beneath the cotyledonary hook with a double-bladed cutting tool. With the segment still held in the cutter, "Vaseline" was smeared around its circumference midway between the ends, using a hypodermic syringe with a No. 18 needle. This minimized the risk of formation of a water film over the segment's surface. After moistening its morphologically basal end, the segment was placed with this end downwards on an 11 by 11 by 1·5 mm 1·5 per cent. agar plate resting on a microscope slide in a Petri dish lined with moist filter paper. Three other identically prepared segments were placed on the same "receptor" plate. Two segments from the higher region of a hypocotyl were placed on each receptor plate, and were distributed so that all such segments had an equal chance of being allocated to any given plate. The same conditions applied with the lower segments.

Each auxin or auxin analogue was incorporated into an agar plate otherwise identical with a receptor plate. Such "pre-treatment" plates (adhering each to a cover-slip) were placed over the moistened apical ends of the segments. The control pre-treatment plate did not contain growth substance. After 1 hr, these plates were removed without disturbing the segments and were replaced with others identical with them, except that they contained IAA instead of the auxin or auxin analogue. This "donor" plate remained in position for 3 hr; it was then removed, together with the hypocotyl segments, and the IAA collected in the receptor was assayed using the *Avena* curvature test.

(*b*) *The* Avena *Curvature Test*

Victory oats from Svalöf (1949 harvest) were grown at 25°C and 85 per cent. R.H. for 72 hr, using the modification developed by Dr. L. A. T. Ballard of this Division. They were then transferred to modified holders and treated as in the standard test. The 1·5 per cent. agar plates to be tested were cut into blocks 10 mm^3 and applied to the plants held in racks, each of which could hold 12 plants. The number of plants placed in each rack was the same as the number of treatments or a multiple of this number so that each treatment was represented by at least one plant in each rack. Within each rack the treatments were arranged in random order. The advantage of this procedure is that here each plant may be used as a replicate; in the conventional arrangement the mean of 12 plants is a replicate. Unless otherwise

TABLE 1

EFFECT ON BASIPETAL AUXIN TRANSPORT OF 1-HR PRE-TREATMENT OF PHASEOLUS SEGMENTS WITH AUXINS AND AUXIN ANALOGUES

Means of measured *Avena* curvatures (degrees), means of pooled curvatures, and means of pooled transformed curvatures together with difference for significance given by receptor plates after pre-treatment of *Phaseolus* segments with control (plain agar) and 11 auxins and auxin analogues. F for treatments (11 and 264 d.f.) = 14·7, $P < 0{\cdot}001$. Difference for significance between transformed values at the 1 per cent. level = 0·055

	Control	PARA	DCA	Coumarin	Indole	IAN	2,4-D	2,4-DP	2,4-DB	2,4,6-T	TIBA	IPA
Mean curvatures (degrees) (expt. 1)	6·1	6·6	6·2	5·9	7·0	5·1	2·1	3·2	1·6	2·3	2·2	2·3
Mean curvatures (degrees) (expt. 2)	5·7	8·1	8·2	8·2	6·6	7·1	1·6	2·3	2·5	3·1	2·7	2·1
Pooled means	5·9	7·4	7·2	7·1	6·8	6·1	1·9	2·7	2·0	2·7	2·4	2·2
Pooled transformed means	1·208	1·233	1·220	1·232	1·213	1·196	1·066	1·103	1·073	1·102	1·087	1·087
Significant difference from control	—	—	—	—	—	—	**	**	**	**	**	**

** $P = 0{\cdot}01$.

indicated in the text, 12 racks were used in each experiment: thus there were 12 replicate determinations of the auxin concentration of each plate.

The interval between decapitations was 3 hr and the blocks remained in contact with the coleoptiles for 1½ hr before photographing.

The curvatures were measured and the results treated statistically. On testing it was found that the means and variances of the treatments were not independent. A logarithmic transformation of the measured curvatures was made so that this condition of independence was fulfilled. The analysis of variance was carried out on these transformed figures; thus differences between treatments are indicated by comparison of the means of transformed curvatures.

The synthetic growth substances used were *p*-chlorophenoxy*iso*butyric acid (PARA), 2,4-dichloroanisole (DCA), coumarin, indole, *o-iso*propyl-*N*-phenylcarbamate (ISO), 2,4,6-trichlorophenoxyacetic acid (2,4,6-T), 2,4-dichlorophenoxyacetic acid (2,4-D), α-(2,4-dichlorophenoxy)-*n*-butyric acid (2,4-DB), α-(2,4-dichlorophenoxy)-propionic acid (2,4-DP), 2,3,5-tri-iodobenzoic acid (TIBA), indole propionic acid (IPA), indole-3-acetonitrile (IAN), and phenylacetic acid (PAA).

(*c*) *Special Experiments with IAA*

In some experiments IAA was added directly to the receptor plate and after removal of the pre-treatment plate no donor plate was applied. However, the segments remained in position for a further 3 hr; they were then removed and the receptor plate was assayed as in the pre-treatment experiments.

III. Results

The results reported are derived from experiments of three types. The main conclusions come from experiments in which the apical ends of hypocotyl segments were pre-treated with auxins and auxin analogues. These conclusions are extended and supported by experiments in which auxins and auxin analogues were incorporated with IAA into the *Avena* test block and by those in which movement of pre-treatment substance into the receptor plate was investigated.

(*a*) *The Effect of Pre-treatment of Apical Ends of Hypocotyl Segments with Auxins and Auxin Analogues*

The pooled results of two identical experiments in which 11 substances were compared with control are shown in Table 1. The concentration of IAA used throughout was $6{\cdot}0\times10^{-6}$M, TIBA was at $1{\cdot}2\times10^{-4}$M, and the remaining substances at $2{\cdot}5\times10^{-4}$M. On each occasion all 12 *Avena* test blocks were cut from the same receptor plate, but, as the experiment was carried out twice, an estimate of the variance due to differences between receptor plates receiving the same treatment could be made. The analysis of variance showed that this variance was not significant. The results show that pre-treatment with 2,4-D, 2,4-DP, 2,4-DB, 2,4,6-T, TIBA, and IPA reduce curvature markedly compared with the control (plain agar pre-treatment) but that pre-treatment with PARA, DCA, coumarin, indole, or IAN has no significant effect.*

* In another experiment it was found that PAA pre-treatment had no significant effect.

TABLE 2

EFFECT OF AUXINS AND AUXIN ANALOGUES ON IAA-INDUCED CURVATURE IN THE AVENA TEST

Means of measured *Avena* curvatures in degrees and means of transformed curvatures together with significant differences from five experiments. In the first two experiments the curvatures given by control (plain agar) are compared with those from nine substances alone, and the curvature given by $4{\cdot}5\times10^{-7}$M IAA is compared with those given by each of the nine substances in combination with this concentration of IAA. In the remaining three experiments the indicated comparisons are made using three substances both alone and in combination with $3{\cdot}0\times10^{-7}$M IAA

Expt. No.	Auxin or Auxin Analogue	Concn. (M)	Alone			With IAA $4{\cdot}5\times10^{-7}$M			D.F.	*F*	Differences for Significance between Transformed Values
			Mean Curvatures (degrees)	Transformed Means	Significant Difference from Control	Mean Curvatures (degrees)	Transformed Means	Significant Difference from IAA Alone			
1	Control		1·1	1·044	—						0·088*
	IAA	$4{\cdot}5\times10^{-7}$	13·9	1·393	**						0·117**
	2,4-DP	$2{\cdot}5\times10^{-4}$	4·6	1·155	*	3·6	1·137	**			
	2,4-DB	$2{\cdot}5\times10^{-4}$	4·1	1·151	*	2·9	1·114	**	11 and 110	14·7***	
	2,4,6-T	$2{\cdot}5\times10^{-4}$	3·3	1·126	—	3·3	1·102	**			
	IPA	$2{\cdot}5\times10^{-4}$	4·0	1·149	*	5·1	1·188	**			
	PAA	$2{\cdot}5\times10^{-4}$	3·6	1·139	*	6·4	1·218	**			
2	Control		1·6	1·059							0·072*
	IAA	$4{\cdot}5\times10^{-7}$	16·7	1·447	**						0·094**
	DCA	$2{\cdot}5\times10^{-4}$	1·3	1·051	—	15·8	1·428	—			
	Coumarin	$2{\cdot}5\times10^{-4}$	1·7	1·069	—	17·7	1·457	—	11 and 110	50·6***	
	Indole	$2{\cdot}5\times10^{-4}$	1·7	1·062	—	18·0	1·467	—			
	PARA	$2{\cdot}5\times10^{-4}$	1·3	1·046	—	10·1	1·316	**			
	TIBA	$6{\cdot}0\times10^{-5}$	3·1	1·122	—	4·7	1·175	**			

* $P = 0{\cdot}05$. ** $P = 0{\cdot}01$. *** $P < 0{\cdot}001$.

TABLE 2 (*Continued*)

Expt. No.	Auxin or Auxin Analogue	Concn. (M)	Alone			With IAA $3{\cdot}0\times10^{-7}$M			D.F.	*F*	Differences for Significance between Transformed Values
			Mean Curvatures (degrees)	Transformed Means	Significant Difference from Control	Mean Curvatures (degrees)	Transformed Means	Significant Difference from IAA Alone			
3	IAA	$3{\cdot}0\times10^{-7}$	11·4	1·319					—	—	0·089*
	2,4-D	$2{\cdot}5\times10^{-6}$	1·8	1·057		4·8	1·157	**			0·118**
	2,4-D	$2{\cdot}5\times10^{-7}$				6·6	1·210	*			
4	Control		0·9	0·994	—				3 and 33	62·3***	0·069*
	IAA	$3{\cdot}0\times10^{-7}$	12·3	1·335	**						0·093**
	ISO	$2{\cdot}5\times10^{-4}$	1·6	1·059	—	14·4	1·337	—			
5	IAA	$3{\cdot}0\times10^{-7}$	12·9	1·345					4 and 223	17·7***	0·025*
	IAN	$3{\cdot}0\times10^{-7}$	11·6	1·307		16·5	1·418	**			0·039**
	IAN	$6{\cdot}0\times10^{-7}$	12·6	1·336		14·8	1·389	**			

* $P = 0{\cdot}05$. ** $P = 0{\cdot}01$. *** $P < 0{\cdot}001$.

An obvious explanation of this reduction in curvature after certain of the pre-treatments would be that in these cases the movement of IAA through the *Phaseolus* segments has been inhibited. However, if any of these auxins or auxin analogues were to give *Avena* curvatures themselves or to interact with IAA in the *Avena* test, the results might be explained by their transport to the receptors during the pre-treatment period. Accordingly we have carried out one series of experiments to determine whether any of these substances have curvature activity or influence IAA-induced curvature, and another to test for their transport under the pre-treatment conditions.

(*b*) *The Effect of Adding Certain Auxins and Auxin Analogues to IAA in the* Avena *Test*

The results of five experiments in which these substances were tested alone and in combination with IAA in the *Avena* test are shown in Table 2. In two experiments (1 and 2) the IAA concentration was $4{\cdot}5\times10^{-7}$M; in the remaining three it was $3{\cdot}0\times10^{-7}$M. All the substances except TIBA, 2,4-D, and IAN were at a concentration of $2{\cdot}5\times10^{-4}$M, TIBA was at $6{\cdot}0\times10^{-5}$M, IAN at $3{\cdot}0\times10^{-7}$M, and 2,4-D at $2{\cdot}5\times10^{-6}$M and $2{\cdot}5\times10^{-7}$M. In the first two experiments the means are obtained from 11 replicate determinations all taken from the same plate; in the third and fourth from 12 replicates from the same plate; and in the fifth from 12 replicates from each of four plates, i.e. 48 altogether.

It is clear from Table 2 that some of the substances give slight curvature in the *Avena* test. However, it is also evident that IAA-induced curvature is markedly diminished when certain of them are incorporated with IAA in the test block. Those having this property are IPA, 2,4-D, 2,4-DP, 2,4-DB, 2,4,6-T, TIBA, PAA, and PARA. The entries show that 2,4-D is still effective in reducing curvature at concentrations as low as $2{\cdot}5\times10^{-7}$M. The result for PARA indicates that although it does inhibit IAA-induced curvature it is much less effective than TIBA, the other inhibitor used in this experiment. On the other hand, indole, coumarin, DCA, and ISO do not reduce IAA-induced curvature.

In the case of IAN lower concentrations were used, viz. $3{\cdot}0\times10^{-7}$M and $6{\cdot}0\times10^{-7}$M, both alone and in combination with $3{\cdot}0\times10^{-7}$M IAA. It is seen that IAN alone gives considerable curvature, though less than equimolar IAA. When combined with IAA the curvature is greater than that given by either substance alone, but the combination with IAN concentration of $3{\cdot}0\times10^{-7}$M gives a greater curvature than that with IAN concentration of $6{\cdot}0\times10^{-7}$M. These results are in general agreement with the observations of Bentley and Bickle (1952).

Hence it has been shown that some of the auxins and auxin analogues reduce IAA-induced curvature in the *Avena* test. Thus, unless it is demonstrated that these substances do not penetrate to the receptor plates during pre-treatment it cannot be concluded that transport in the *Phaseolus* segments has been inhibited. The following experiments were undertaken to discover whether or not such penetration took place.

(*c*) *Transport of Auxins and Auxin Analogues other than IAA*

In these experiments the results are affected by the fact that contact with the cut surfaces of hypocotyl segments reduces the IAA content of receptor plates. This

is shown in Table 3 which illustrates the increase of this effect with time of contact. In one experiment subsequently described in which there was a 3-hr contact between segments and receptor plates the initial concentration of IAA was increased to compensate for this effect.

TABLE 3

EFFECT OF PHASEOLUS SEGMENTS ON AUXIN CONTENT OF RECEPTOR PLATES

Means of measured *Avena* curvatures in degrees and means of transformed curvatures together with differences for significance given by IAA $3{\cdot}0\times10^{-7}$M and by the same concentration after contact with *Phaseolus* segments

Time of Contact (hr)	Mean Curvatures (degrees)	Transformed Means	Significant Difference from No Contact	D.F.	F	Differences for Significance between Treatment Means
0	11·4	1·325	—	2 and 22	11·7***	0·077*
1½	7·7	1·240	*			0·104**
3	4·4	1·143	**			

* $P = 0{\cdot}05$. ** $P = 0{\cdot}01$. *** $P < 0{\cdot}001$.

In two of these experiments (Table 4) an agar plate containing $3{\cdot}0\times10^{-7}$M IAA was the receptor whilst the pre-treatment plate contained auxin or auxin analogue at $2{\cdot}5\times10^{-4}$M, in all cases, except that of TIBA which was at $1{\cdot}2\times10^{-4}$M. The pre-treatment plate remained in position for 1 hr and was then removed; the segments were left for a further 3 hr and after their removal the receptor was assayed by the *Avena* test. In the third experiment (Table 5) IAA was at $6{\cdot}0\times10^{-7}$M and the pre-treatment plates remained in position for 3 hr before removal together with the segments.

Table 4 shows the results of two experiments in which each treatment mean is obtained from 12 replicate blocks from the same plate. In the first, 10 auxins and auxin analogues have been compared with the control (plain agar pre-treatment plate). If any of the inhibitors of IAA-induced curvature were reaching the receptors we should expect the curvature in these cases to be less than for control or for the non-inhibitor pre-treatment. The variance ratio test indicates that the observed deviations of the treatment means from the general mean would occur by chance more than once in five. Similarly with the three substances of the second experiment the deviations of the treatment means from the general mean could occur by chance more than once in five. Hence in both cases there is no reason to believe that the inhibitors are affecting the curvature.

Each treatment mean of Table 5 is derived from 12 replicate blocks from each of four plates, i.e. 48 altogether. Under the conditions of this experiment the inhibitors would be even more likely to appear in the receptor plates. The results show no evidence of inhibition of curvature and the variance ratio test indicates that

they would be obtained by chance more than once in five. Hence again there is no reason to believe that the inhibitors are affecting the curvature.

TABLE 4

EFFECT OF 1-HR PRE-TREATMENT OF PHASEOLUS SEGMENTS WITH AUXINS AND AUXIN ANALOGUES ON CURVATURE GIVEN BY IAA INCORPORATED IN THE RECEPTOR PLATES

Means of measured *Avena* curvatures in degrees and means of transformed curvatures given by control (plain agar pre-treatment) and pre-treatment with 13 auxins and auxin analogues in two experiments designed to test for transport of auxins and auxin analogues

Expt. No.	Auxin or Auxin Analogue	Mean Curvatures (degrees)	Transformed Means	D.F.	*F*
1	Control	5·8	1·190	11 and 110	<1*
	PARA	5·2	1·178		
	DCA	4·8	1·166		
	Coumarin	5·7	1·178		
	Indole	6·7	1·216		
	IAN	6·3	1·187		
	2,4-D	5·1	1·175		
	2,4-DP	6·4	1·189		
	2,4-DB	6·1	1·197		
	2,4,6-T	4·1	1·143		
	IPA	3·9	1·137		
2	Control	4·4	1·143	3 and 33	1·2*
	ISO	5·7	1·193		
	TIBA	3·9	1·137		
	PAA	4·1	1·137		

* $P > 0{\cdot}2$.

TABLE 5

EFFECT OF 3-HR PRE-TREATMENT OF PHASEOLUS SEGMENTS WITH AUXINS AND AUXIN ANALOGUES ON CURVATURE GIVEN BY IAA INCORPORATED IN THE RECEPTOR PLATES

Means of measured *Avena* curvatures in degrees and means of transformed curvatures given by control (plain agar) and pre-treatment with two substances in an experiment designed to test for transport of auxins and auxin analogues

Auxin or Auxin Analogue	Mean Curvatures (degrees)	Transformed Means	D.F.	*F*
Control	12·2	1·334	2 and 108	2·2*
2,4-D	13·4	1·362		
TIBA	12·0	1·332		

* $P > 0{\cdot}2$.

IV. DISCUSSION

Several of the auxins and auxin analogues used in the experiments involving pre-treatment of apical ends of hypocotyl segments cause a reduction in receptor-

induced curvature compared with the control pre-treatment. Those which exhibit this property are 2,4-D, 2,4,DP, 2,4-DB, 2,4,6-T, TIBA, and IPA, whilst PARA, DCA, coumarin; ISO, indole, IAN, and PAA have no activity.

Although several explanations could account for this effect, our experiments strongly suggest that it is due to a direct inhibition of transport in the *Phaseolus* segments. It has been shown that the reduced curvature after pre-treatment of *Phaseolus* segments with some of the auxins and auxin analogues cannot be accounted for by the demonstrated inhibition of IAA-induced curvature in the *Avena* test by the same substances. Hence it seems likely that the action of these substances is on the *Phaseolus* segments. These are able to destroy IAA, probably by IAA oxidase activity. Goldacre, Galston, and Weintraub (1953) have shown that 2,4-dichlorophenol enhances the activity of IAA oxidase. If all of the inhibitors contained 2,4-dichlorophenol as an impurity our pre-treatment and *Avena* test results might be explained by enhanced IAA destruction. However, such an explanation is not probable, for whilst 2,4-dichlorophenol might be expected as a contaminant of 2,4-D, 2,4-DP, and 2,4-DB it would not be expected in IPA. On the other hand, DCA and PARA which are inactive might be expected to contain amounts comparable to 2,4-D, 2,4-DP, and 2,4-DB.

Thus it is concluded that the inhibitors are affecting the IAA transport mechanism. As indicated in the Introduction, two types of inhibition may be visualized. Substances such as potassium cyanide and 2,4-dinitrophenol inhibit auxin transport by their effect on energy-transfer reactions. On the other hand, all our inhibitors are structurally related to growth substances.

It is noticeable that with the exception of PARA and PAA all the substances which inhibit IAA-induced curvature in the *Avena* test also inhibit transport in the pre-treatment experiments. This appears less remarkable when it is remembered that auxin transport in the coleoptile is involved in the *Avena* curvature test. Indeed we believe that the two effects arise from the same cause, namely the inhibition of auxin transport. A probable explanation of the exceptions is that the *Avena* test interaction is a more sensitive test for transport inhibition than the *Phaseolus* test, and that PAA and PARA are weak inhibitors of transport. Niedergang-Kamien (1955) lists PAA as an inhibitor of auxin transport.

All substances found active in transport inhibition contain a carboxyl group. The importance of this group is illustrated by the contrasting effects of 2,4-D (active) and 2,4-dichloranisole (inactive). However, the mere possession of a carboxyl group does not imply activity. Further experiments, not reported here, show that neither valeric nor benzoic acid reduces IAA-induced curvature of *Avena* coleoptiles and thus exhibit no evidence of inhibition of auxin transport.

It is therefore suggested that the action of the inhibitors is to compete with IAA for specific "carrier sites". This hypothesis may seem difficult to reconcile with the fact that the inhibitors are not themselves transported. However, even after 3 hr no evidence of their transport was obtained.

Niedergang-Kamien and Skoog (1956) have recently published an account of strikingly similar experiments with sunflower stem cylinders. They demonstrate a decrease in basipetal IAA transport in such sections after a preceding immersion in

solutions of TIBA. They relate this result to the morphogenetic effects of TIBA, especially to its effect on the distribution pattern of callus-like outgrowths from tobacco stem segments cultured on a nutrient medium. In TIBA-treated segments these outgrowths occur evenly over the segment surface, in controls they are found only at the basal end. Also the distribution of ether-extractable auxin remains approximately even over the length of the segment after TIBA treatment; in controls the basal concentration becomes higher than the apical.

One criticism which might be made of the work of these authors is the failure to demonstrate that the lowered *Avena* curvatures of the transport experiments were not due to a direct effect of TIBA on IAA-induced curvature in the *Avena* test. Indeed the risk of such an effect would appear to be greater from their immersion pre-treatment than from our agar plate method.

Niedergang-Kamien and Skoog (1956) have suggested that the morphogenetic effects of TIBA can be explained by its effect on basipetal auxin transport alone. However, in the same paper, they also state that other substances have a similar effect on auxin transport. This statement coupled with the results of this paper raises the questions whether other substances give the same morphogenetic effects as TIBA, and if not whether Niedergang-Kamien and Skoog's suggested explanation is sufficient.

The physiological activity of herbicides such as 2,4-D is not well understood. Although many effects of 2,4-D on plants have been demonstrated, this is, in our belief, the first account of its inhibition of basipetal auxin transport. This may be an important part of its herbicidal action.

V. Acknowledgments

The authors wish to express their thanks to Dr. L. A. T. Ballard for continuous interest and for introducing them to the *Avena* test and making available the equipment.

They also wish to thank Mr. G. A. McIntyre for statistical advice, Dr. N. P. Kefford for helpful discussion, Dr. W. Bottomley for the preparation of PARA, Mr. R. O. Kefford, Victorian Department of Agriculture, for the supply of seeds of *Phaseolus vulgaris*, and Mrs. S. Sapiets for valuable technical assistance.

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