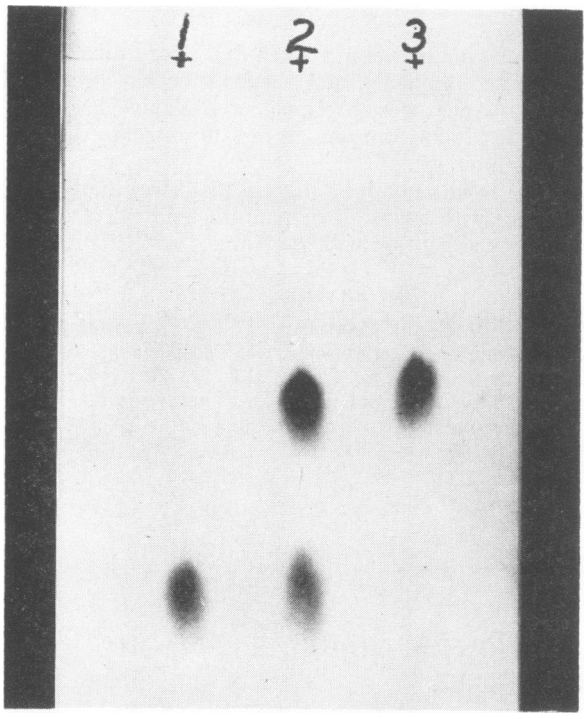

FIG. 1. Photograph of a chromatogram: 1, Gibberellin A (Stodola); 2, Mixture of gibberellin A (Stodola) and gibberellic acid; 3, Gibberellic acid. The chromatogram was developed on Munktells, Cremer-Tiselius electrophoretic paper for 46 hrs at 22° C. 100 μg of each of the gibberellins were used.

rescence produced by concentrated sulfuric acid. We have found the 70 % sulfuric acid treatment preferable since it allows easier handling because the paper disintegrates more slowly. The fluorescent spots may be photographed for a permanent record.

This paper chromatographic system is useful for the qualitative analysis of purified and partially purified preparations of the gibberellins. It satisfies the requirement for a relatively quick, easy method for detection of gibberellin A in gibberellic acid preparations. This has not been possible with the solvents previously reported. The procedure described may be useful for more complete identification of gibberellin-like compounds found in plant extracts.

We wish to thank Dr. F. H. Stodola, N.U.R.B., USDA, Peoria, Illinois, for the sample of purified gibberellin A used for this work.

LITERATURE CITED

1. STODOLA, F. H., NELSON, G. E. N. and SPENCE, D. J. The separation of gibberellin A and gibberellic acid on buffered partition columns. Arch. Biochem. Biophys. 66: 438–443. 1957.
2. TAKAHASHI, N., KITAMURA, H., KAWARADA, A., SETA, Y., TAKAI, M., TAMURA, S. and SUMIKI, Y. Biochemical studies on "Bakanae" fungus, Part XXXIV. Isolation of gibberellins and their properties. Bull. Agr. Chem. Soc. (Japan) 19: 267–277. 1955.
3. PHINNEY, B. O., WEST, C. A., RITZEL, MARY and NEELY, P. M. Evidence for gibberellin-like substances from flowering plants. Proc. Nat. Acad. Sci. U.S. 43: 398–404. 1957.

GROWTH OF ETIOLATED SECTIONS OF PEA INTERNODE FOLLOWING EXPOSURES TO INDOLE-3-ACETIC ACID, 2,4-DICHLOROPHENOXYACETIC ACID AND 2,5-DICHLOROBENZOIC ACID[1]

DAPHNE J. OSBORNE

AGRICULTURAL RESEARCH COUNCIL UNIT OF EXPERIMENTAL AGRONOMY,
DEPARTMENT OF AGRICULTURE, UNIVERSITY OF OXFORD

A striking feature in the assessment of plant growth regulating substances is the discrepancy between results obtained with isolated tissues and whole plants. For instance, indole-3-acetic acid is considered to be more physiologically active than 2,4-dichlorophenoxyacetic acid in most of the standard laboratory tests on excised tissues, but when applied to whole plants it shows low activity, and although a rapid initial epinastic response may occur, it does not normally cause the long-term formative distortions so typical of 2,4-dichlorophenoxyacetic acid and allied herbicidal substances. There is considerable evidence to suggest that the stability of a compound within the plant and the ease with which it can penetrate and be transported contribute to this difference in response. But a further factor might be considered, namely the variation in the length of time the tissues remain in contact with the growth substance.

In laboratory investigations, rapidly elongating sections of stem or coleoptile are most frequently used, and the sections are normally exposed to a known concentration of the growth substance for the whole experimental period. However, when these chemicals are applied to whole plants, only one dose of the growth regulator is usually given and it is the growth response subsequent to this application which is studied. Comparable studies in short-term laboratory tests have received scant attention, although some investigations have been carried out with roots (8, 12). Apart from experiments with supraoptimal concentra-

[1] Received September 6, 1957.

tions on Avena coleoptile sections (9) little is known about the growth responses of excised sections of stem tissue following a single brief period of exposure to growth substances. Experiments were therefore planned to determine the effect upon straight growth of short exposures to suboptimal doses of a number of well known growth regulating substances, each of different chemical structure. Sections of etiolated pea epicotyl were supplied with the compounds for a few hours only. The extension growth was studied when the sections were transferred either to water or to further low concentrations of active growth substances.

By these means clear differences in growth response have been demonstrated between sections which have been treated with indole-3-acetic acid (IAA) and those treated with 2,4-dichlorophenoxyacetic (2,4-D) or 2,5-dichlorobenzoic acids (2,5-DBA).

Material and Methods

Choice of Method: Considerable attention was initially devoted to finding a convenient, reliable and rapid method of assaying growth in sections of plant material. Determinations of the increase in length of sections cut from the elongating portion of stem or coleoptile have been favored by most workers, but since the length of section obtainable from the region is small (usually 1 cm), the growth increments for periods of less than several hours are difficult to measure with great accuracy since they too are small and the measurement is subject to considerable visual error.

Experiments showed that etiolated plants of *Pisum sativum* of considerable uniformity can readily be grown and that a satisfactory elongation response can be obtained from 1-cm sections of the epicotyl when they are grown in aqueous solutions of the sodium salts of IAA, 2,4-D or 2,5-DBA without the addition of sugars or buffers. If the volume of ambient solution is large (40 ml/10 sections) no pH changes are detectable in the external solution during the experiment (24 hours). A simple determination of weight made on a torsion balance is subject to a considerably smaller error than that involved in the measurement of the length of a section, since no correction need be applied for curvature, or other distortion of the section during growth, or for cut ends which are not exactly transverse. Further, the rapidity with which such weight determinations can be made, renders it possible to repeat the operation at frequent intervals with little damage to the tissue.

The increase in length of segments growing in suboptimal solutions of IAA (0.01 to 5.0 ppm), 2,4-D (0.005 to 2.5 ppm) and 2,5-DBA (0.05 to 15.0 ppm) was found to be directly proportional to the increase in fresh weight of the tissues for any test period up to 24 hours from the start of an experiment. Therefore, the conclusions drawn here from measurements of fresh weight are equally applicable to extension growth.

Method: Peas, var. Alaska, were soaked for several hours in distilled water at 24.5° C, then sown in enamel troughs of sterile washed sand. The troughs were kept in a controlled conditioned dark room at 24.5° C and watered with standard volumes of distilled water. Periods of 10 to 15 minutes of red light (Wratten O with 40-watt bulb) were given daily, once the shoots had emerged above the surface. Under these conditions, the 3rd internode was 2 to 3 cm long on the 7th day from planting, and the plants were ready for use. One section, 1 cm long, was cut from the 3rd internode, just below the crook. Ten sections were bulked, weighed on a torsion balance, and then

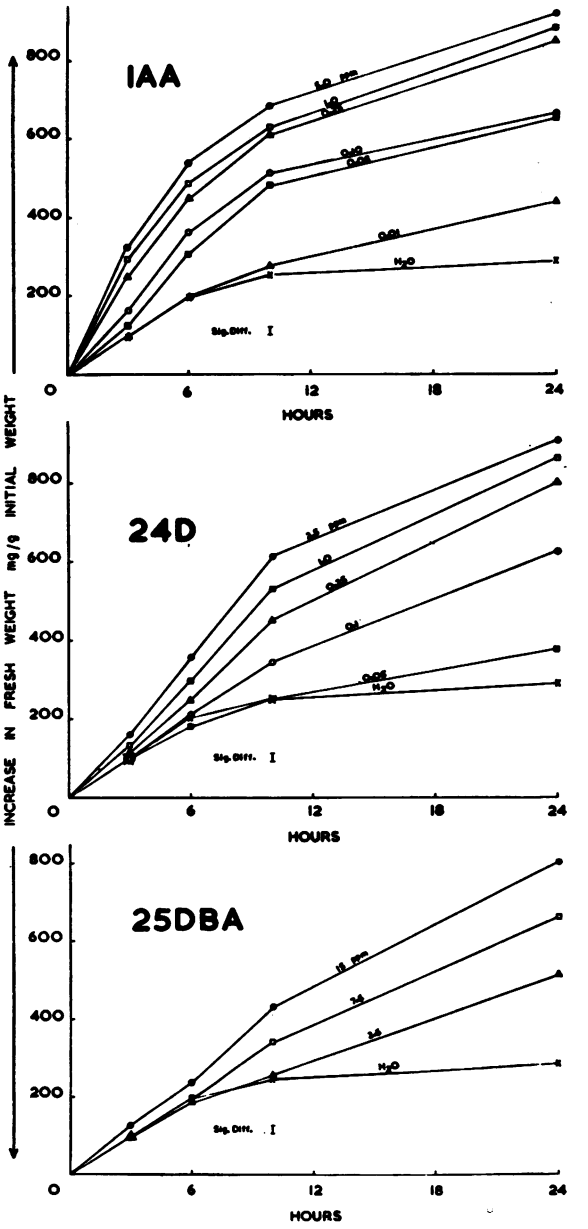

Fig. 1. Extension growth of sections of etiolated pea internode grown for 24 hours in solutions of IAA, 2,4-D or 2,5-DBA, expressed as the increase in fresh weight mg/g initial fresh weight.

floated upon the surface of the operative solution, the volume of which was not less than 40 ml. When the sections were next weighed, they were transferred from the solution to clean blotting paper and the surface liquid gently removed. Tests in which sections were immersed, blotted and weighed several times in succession have shown that the error due to differences in the removal of surface water are in the order of 0.5 to 1 %. Each treatment was duplicated. The technique can be recommended as a useful method for determining small increments of growth.

Results

Growth of Sections of Pea Internode Kept in Solutions of IAA, 2,4-D or 2,5-DBA for 24 Hours: The sections were grown in a range of concentrations of IAA, 2,4-D and 2,5-DBA. Measurements of the fresh weight were made at intervals of 3, 6, 10 and 24 hours from the start of the experiment and the sections were transferred to fresh solution after each weighing. The curves obtained for each compound are shown in figure 1. At these suboptimal concentrations the growth increments of the treated sections are either greater than or approximate to but are never significantly less than that of the controls.

Growth of Sections of Pea Internode Treated with IAA, 2,4-D or 2,5-DBA for Varying Periods and then Transferred to Water: Sections were floated on solutions containing a range of concentrations of growth regulators for periods varying from one to three hours, after which they were removed from the solution, blotted and weighed again. They were rinsed and transferred to dishes of water, and the water changed after each subsequent weighing up to 24 hours from the start of the experiment. A selection of the results is presented in figures 2, 3 and 4. It was ascertained that very little further increase in fresh weight ever occurred after 24 hours in any of the experiments described.

The results for IAA treatments of 1 to 25 ppm for one hour are shown in figure 2 A and 2 B. The rate of growth of some of the treatments eventually falls below that of the control. This is first apparent in sections receiving the smallest dose of IAA (1.0 ppm) and becomes evident somewhat later in those receiving larger doses (5.0, 12.5 and 25 ppm). The rate

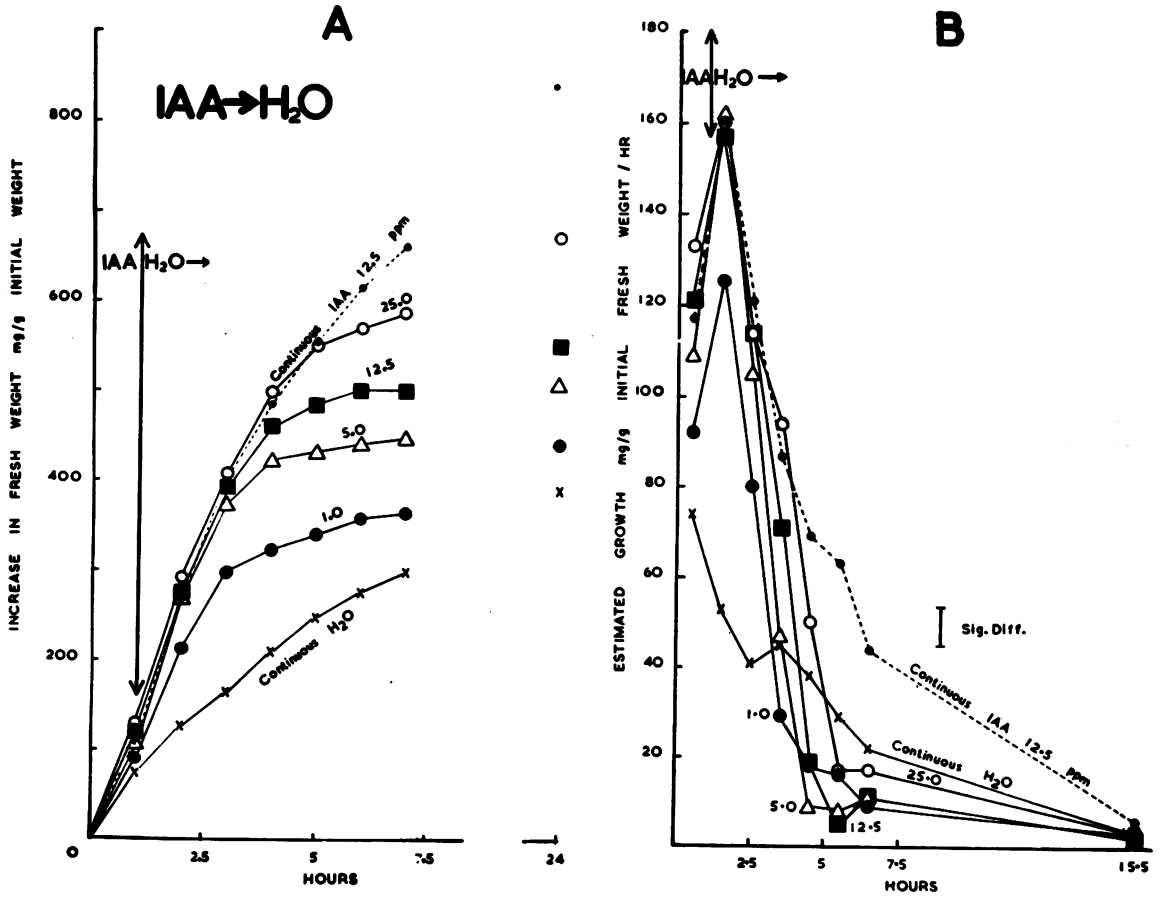

Fig. 2. A. Extension growth of sections of etiolated pea internode treated for 1 hour with different concentrations of IAA followed by 23 hours in H_2O, expressed as increase in fresh weight mg/g initial fresh weight.

B. The data from 2 A plotted as the estimated growth in mg/g initial fresh weight/hr.

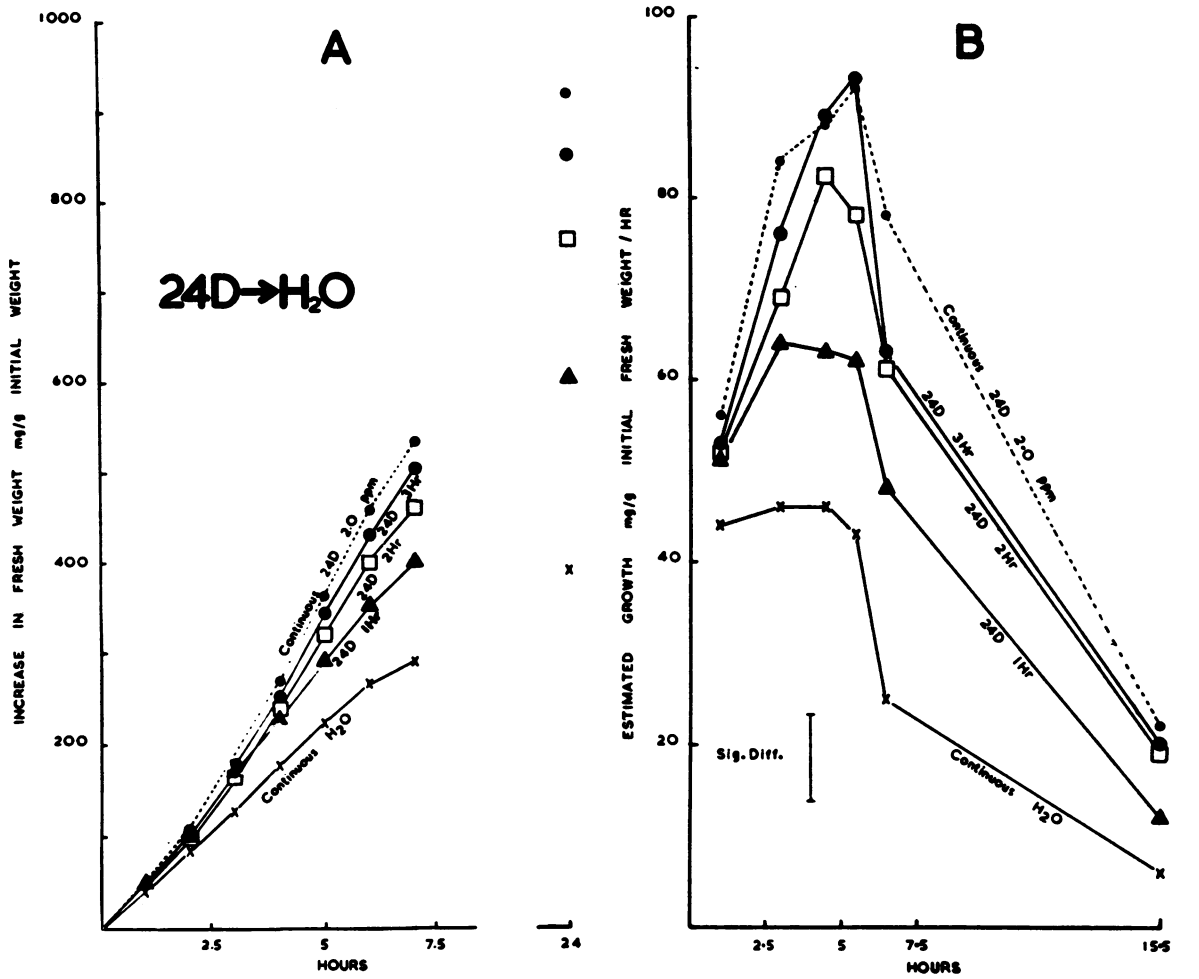

FIG. 3. A. Extension growth of sections of etiolated pea internode treated with 2,4-D 2.0 ppm for 1, 2 or 3 hours then transferred to H_2O, expressed as increase in fresh weight mg/g initial fresh weight.

B. The data from 3 A plotted as the estimated growth in mg/g initial fresh weight/hr.

then remains lower for the duration of the experiment. Sections kept continuously in IAA (12.5 ppm) maintained a higher growth rate than the control. Thus IAA, while first stimulating growth, may subsequently cause a depression in the growth rate. As the initial dose is increased, the fall in growth rate to a value below that of the control is delayed; when IAA is supplied continuously this response cannot be demonstrated (see also fig 1).

Many experiments were carried out over a wide concentration range with both 2,4-D (0.1 to 7.5 ppm) and 2,5-DBA (2.5 to 100 ppm) and in no instance was a depression of growth apparent after transference to water. Two experiments of this kind are shown in figures 3 and 4. These sections were treated with 2,4-D 2.0 ppm for 1, 2, 3 and 24 hours or with 2,5-DBA from 10 to 100 ppm for two hours and then transferred to water. Their rate of growth in water is seen to be either higher than or approaching that of control sections.

It is apparent, therefore, that the growth response of sections following a short treatment with IAA is different from that following a short treatment with either 2,4-D or 2,5-DBA.

It might be concluded that if a tissue is initially induced to grow more rapidly than the water control, it must, once the growth stimulus is removed, show a growth rate below that of the control since available substrates would have become depleted by virtue of the greater growth achieved. The fall in growth rate of IAA treated sections could be explained if there were a rapid depletion of IAA within the cells once the tissue is transferred to water. The response of 2,4-D and 2,5-DBA treated sections in which the growth rate remains either above, or falls to that of the control, could be the result of a continual growth stimulation by residual 2,4-D or 2,5-DBA. However, in a large number of experiments carried out over a wide range of suboptimal concentrations and times of exposure, these latter compounds have been found not

to induce growth rates below those of the controls, and where the rates do fall to that of controls, they may remain at that level for many hours. This suggests that the supply of substrates for further extension growth is not seriously impaired by small initial growth stimulations. This supposition is investigated further in the following experiments.

Response of Sections Initially Treated with IAA, 2,4-D or 2,5-DBA to Further Doses of IAA or 2,4-D: Weighed sections were treated with several concentrations of IAA, 2,4-D, 2,5-DBA and water for 1.75 hours in order to stimulate different amounts of growth. They were then transferred to distilled water for 3.5 hours, during which period the water was changed twice. At the end of this time each batch of sections was weighed and transferred to IAA 0.05 ppm. Twenty-four hours from the start of the experiment the sections were weighed again to obtain the total increase in fresh weight. The results for an experiment of this type, in which the increase in fresh weight of the tissue is expressed in mg/g of the initial weight of the sections is shown in table I.

For clarity, the initial treatment in water, IAA, 2,4-D or 2,5-DBA is henceforth referred to as a pretreatment.

Reference to table I shows that after 5.25 hours, the growth induced by the different concentrations of IAA, 2,4-D and 2,5-DBA covers a closely similar range of stimulations. When these sections are all transferred to the same concentration of IAA it is seen that the subsequent growth made by the sections pretreated with IAA is less than that made by those pretreated with water. The higher the initial dose of IAA (and hence the greater the amount of growth achieved by 5.25 hours) the smaller is the amount of growth in the subsequent IAA treatment. Sections pretreated with 2,4-D or 2,5-DBA however increased in fresh weight in the IAA posttreatments by an amount which approximates closely to that of the water pretreated sections, despite the fact that the pretreatments with these two compounds had caused increases in growth of the same order as those induced by the IAA pretreatment. This suggests that the greater amount of growth achieved in the first 5.25 hours by

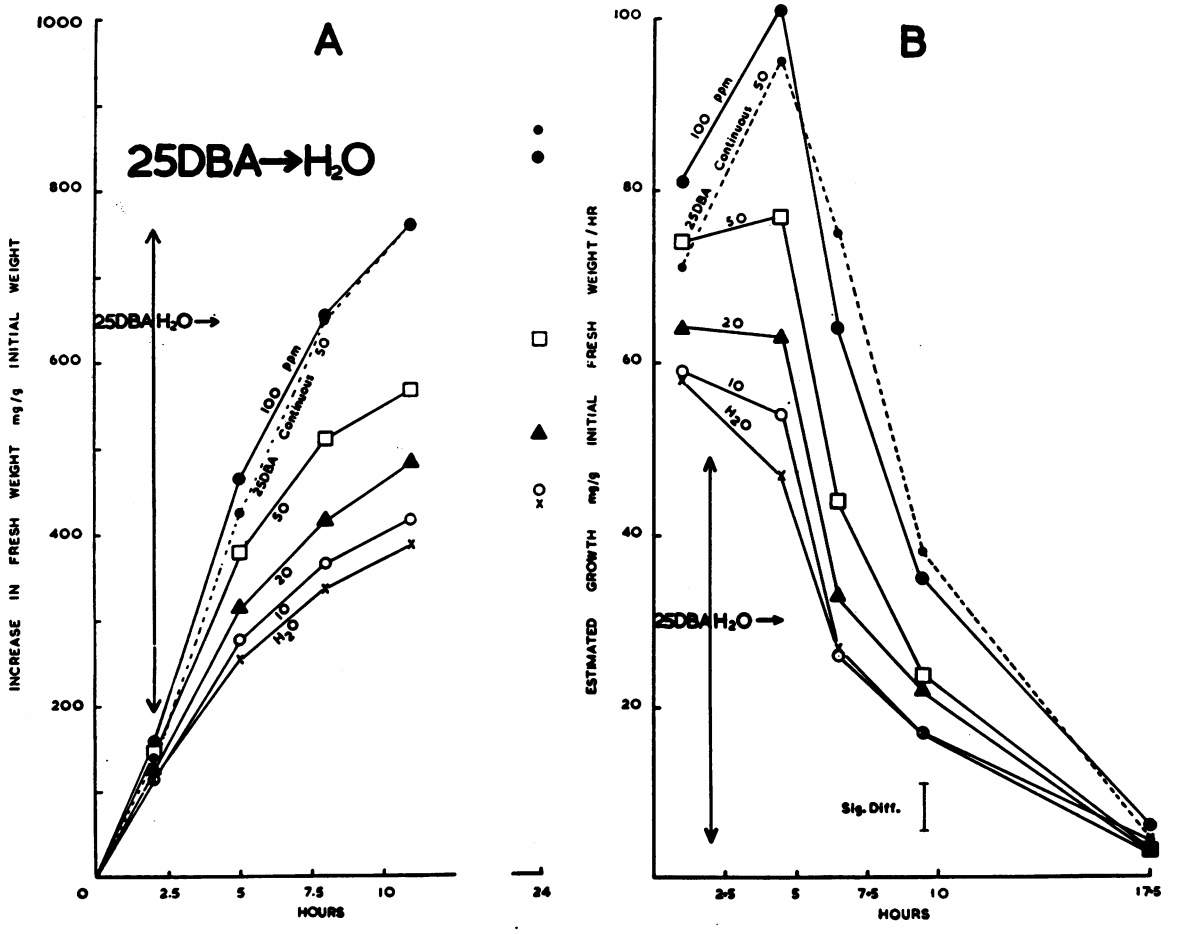

Fig. 4. A. Extension growth of sections of etiolated pea internode treated for 2 hours with different concentrations of 2,5-DBA followed by 22 hours in H_2O expressed as increase in fresh weight mg/g initial fresh weight.

B. The data from 4 A plotted as the estimated growth in mg/g initial fresh weight/hr.

TABLE I

INCREASES IN FRESH WEIGHT OF SECTIONS OF ETIOLATED PEA INTERNODE PRETREATED WITH VARIOUS CONCENTRATIONS OF IAA, 2,4-D, 2,5-DBA OR H_2O AND SUBSEQUENTLY TRANSFERRED TO IAA 0.05 PPM

PRETREATMENT		AVERAGE GAIN IN FRESH WEIGHT AS MG/G INITIAL FRESH WEIGHT			
		BY END OF PRETREATMENT	BY END OF 3.5 HR WATER WASH	BY END OF 18.75 HR IN IAA 0.05 PPM	DURING THE IAA POSTTREATMENT ONLY
		0–1.75 hr	*0–5.25 hr*	*0–24 hr*	*5.25–24 hr*
IAA	2.0 ppm	166	361	684	323
	1.0	152	335	723	388
	0.5	150	327	729	402
	0.25	134	298	735	437
	Mean	*151*	*330*	*718*	*388*
2,4-D	7.5 ppm	109	386	879	493
	3.0	104	343	836	493
	1.0	93	278	789	511
	Mean	*102*	*336*	*835*	*499*
2,5-DBA	100 ppm	114	363	904	541
	50	109	309	809	500
	20	96	250	739	489
	Mean	*106*	*307*	*817*	*510*
H_2O		85	254	732	489
Sig. diff. (P = 0.05) Between two treatments		17.1	33.0	49.2	55.2
H_2O vs 2,5-DBA mean or 2,4-D mean		14.0	26.9	40.2	45.1
H_2O vs IAA mean		13.5	26.1	38.9	43.6

those sections pretreated with 2,4-D or 2,5-DBA compared with those pretreated with water leaves the capacity for further growth unimpaired, whereas a pretreatment with IAA, which causes growth stimulations of the same orders of magnitude, in some way reduces the capacity of the tissue for further growth. It might be advanced that in the case of sections pretreated with 2,4-D and 2,5-DBA the growth made during the IAA posttreatment could be attributed to an additive stimulation given by IAA and by residual 2,4-D or 2,5-DBA within the tissue, and that, in the case of the IAA pretreatments, by the end of the water wash much, or all, of the IAA initially taken up by the sections would have been either lost again to the outside washing water or have been destroyed within the tissue. There would then be little or no additive stimulation during the posttreatment and a reduction in the growth capacity of the IAA pretreated sections compared with those pretreated with 2,4-D or 2,5-DBA should then be anticipated. The data might support this explanation for the growth response following IAA pretreatments, but one might also expect that if the growth response of the 2,4-D or 2,5-DBA pretreated sections are in part dependent upon residual quantities of these substances within the tissues, then the various concentrations of 2,4-D or 2,5-DBA used in the pretreatment should differentially effect the amount of growth during the posttreatment. This, in fact, is not the case. The amount of growth during the posttreatments would appear to be independent of the pretreatment concentration.

There is certain evidence that the pretreatment of plant tissue with growth regulators may effect the subsequent ability of the tissues to take up further growth substance from solution. Unpublished work by W. R. Birch in this Department has shown that a pretreatment of whole *Lemna minor* plants with 2,4-D caused changes in the subsequent uptake of labelled 2,4-D, while Reinhold (11) presents evidence that pretreatment with a high concentration (108.5×10^{-4} M) of 2,4-D depresses the metabolic, though probably not the physical, uptake of IAA by carrot discs. Such effects of pretreatments with growth regulators might be offered as an explanation for some of the results described in the present paper.

Thus, although there appears to be no effect of a 2,4-D pretreatment upon the subsequent total growth of sections transferred to IAA compared with that of sections pretreated with water (table I) the suggestion might be put forward that the smaller growth that occurs during the posttreatment in sections pretreated with IAA could be accounted for by a reduction in the uptake of growth substance during the posttreatment period. If this were so, then the growth during the posttreatment period of sections pretreated with a single concentration of IAA and then subjected to a range of posttreatment concentrations of IAA or 2,4-D should be proportionally lower than that of those pretreated with water and

TABLE II

AVERAGE FRESH WEIGHT INCREMENTS OF SECTIONS OF ETIOLATED PEA INTERNODE GROWING IN A RANGE OF CONCENTRATIONS OF IAA OR 2,4-D FOLLOWING A PRETREATMENT IN IAA OR H_2O

PRETREATMENT 2 HR	AV WT MG/10 SECTIONS AFTER H_2O WASH FOR 3.5 HR	POSTTREATMENT PPM	AV WT INCREMENTS MG/10 SECTIONS DURING POSTTREATMENTS	
			0–2.5 hr	*2.5–16 hr*
		IAA 0.25	46.5	71.5
		0.05	(A) 38.0	(A) 65.0
IAA 1.0 ppm	347.0	0.01	24.0	43.5
		H_2O	16.0	20.5
H_2O	305.5	IAA 0.25	65.0	87.0
		0.05	(B) 61.0	(B) 79.5
		0.01	45.5	55.0
		H_2O	34.5	34.0
			18.5	*15.5*
		Weight increment differences (B – A)	*23.0*	*14.5*
			21.5	*11.5*
			18.5	*13.5*
			Not sig. diff.	Not sig. diff.
			0–18.5 hr	
		2,4-D 7.5	112.0	
		2.5	(A) 106.5	
IAA 1.0 ppm	316.0	0.5	96.5	
		H_2O	17.0	
H_2O	276.0	2,4-D 7.5	135.0	
		2.5	(B) 132.5	
		0.5	120.5	
		H_2O	36.5	
			23.0	
		Weight increment differences (B – A)	*26.0*	
			24.0	
			19.5	
			Sig. diff. 4.86 (P = 0.05)	

given the same posttreatments. The results of an experiment given in table II show, however, that the difference in growth between the water and the IAA pretreated sections during their comparable posttreatments approximates to the same value and appears to be independent of whether the posttreatment is in water (in which case there can be no uptake of growth substance) or in any of the concentrations of IAA or 2,4-D.

The results of direct measurements of uptake during the posttreatments are presented in table III. Sections were pretreated for two hours with IAA, 2,4-D or water, placed in water for 3.5 hours and finally transferred to two concentrations of C^{14}-labelled 2,4-D for the posttreatment. After two and four hours in the labelled 2,4-D the sections were removed, weighed, and the total uptake of 2,4-D determined as radioactive C^{14}. The sections were combusted, the CO_2 collected in barium hydroxide and the barium carbonate counted under standard conditions. The author is indebted to Mr. E. Abeyaratne for carrying out all the analyses. The results demonstrate that there are no major effects of pretreatment with either IAA or 2,4-D on the 2,4-D uptake during the 1st four hours of posttreatment. Such data do not therefore support a view that the depression in growth which follows a short period of treatment with IAA can be explained by differences in the subsequent uptake of a growth regulator.

Further data for growth changes that take place during the course of an experiment are given in table IV. This lists the increments in fresh weight in mg/g initial fresh weight for a complete 24-hour period. The sections were pretreated with various concentrations of IAA, 2,4-D or with water and later transferred to a single concentration of IAA. By the end of the water wash growth of the IAA pretreated sections had slowed down and was less than that of the water pretreated sections. The increases in fresh weight induced by the various concentrations of IAA and 2,4-D were then of the same order of magnitude. The sections were then transferred to IAA 0.05 ppm. The growth increments for consecutive time periods following this transfer show that initially the IAA pretreated sections grow less than the sections pretreated with 2,4-D or water; sections pretreated with the highest concentration of IAA show the smallest growth increments. Another experiment is shown graphically in figure 5. Sections were pretreated with a single concentration of IAA, or 2,4-D or water for two hours. After the water wash batches of sections from each pretreatment were transferred to either

TABLE III

POSTTREATMENT UPTAKE OF LABELLED 2,4-D BY SECTIONS OF ETIOLATED PEA INTERNODE FOLLOWING PRETREATMENTS WITH IAA, 2,4-D OR H_2O

PRETREATMENT	AV FRESH WT MG/10 SECTIONS AFTER 2 HR PRETREATMENT FOLLOWED BY 3.5 HR H_2O WASH (AV INITIAL WT = 237.0)	POSTTREATMENT UPTAKE OF C^{14}-LABELLED 2,4-D EXPRESSED AS 10^{-7} MG 2,4-D/MG FRESH WT AT START OF POSTTREATMENT				
		2,4-D 1.0 PPM		2,4-D 3.0 PPM		
		0–2 hr	*0–4 hr*	*0–2 hr*	*0–4 hr*	*Uptake means*
IAA 1.0 ppm	336.5	6.53	10.98	19.00	31.63	*17.04*
2,4-D 1.0 ppm	315.0	7.26	11.07	17.26	32.68	*17.07*
H_2O	298.5	5.73	11.91	16.84	29.84	*16.08*
Uptake means		*6.51*	*11.32*	*17.70*	*31.38*	

Effect of pretreatment during posttreatment—not significant at P = 0.01.
Effects of concentration and duration of posttreatment—significant at P = 0.01.

IAA 0.05 ppm or 2,4-D 2.0 ppm or to fresh distilled water. The average fresh weight increase in mg/batch of 10 sections is plotted for each consecutive time interval. Again it is seen that towards the end of the water wash the growth increments of the IAA pretreated sections are lower than those of sections pretreated in water. During the different posttreatments the IAA pretreated sections initially grow less than those pretreated with 2,4-D or water although the difference becomes less, the longer the sections remain in the posttreatment solutions.

DISCUSSION

A number of explanations may be proposed to account for the difference in the growth response of sections of pea internode following a short treatment with suboptimal concentrations of IAA, 2,4-D or 2,5-DBA and to account for the reduction in growth that occurs following the short treatments with IAA.

1. An effect on the IAA-oxidase system:

Galston and Dalberg (6) showed that the activity of the IAA-oxidase complex in brei preparations from etiolated pea stems is markedly increased when segments of the tissue are exposed to small doses of IAA or 2,4-D (10^{-7} M), but is decreased when higher concentrations are employed.

The doses of IAA and 2,4-D which have been used for the pretreatments in the present work are those which Galston and Dalberg found would partially inhibit the activity of IAA–oxidase; if this system were operative in controlling growth in the present experiments, one would expect the growth rate of IAA and 2,4-D pretreated sections to fall to a value

TABLE IV

SUCCESSIVE FRESH WEIGHT INCREMENTS OVER 24 HOURS OF SECTIONS OF ETIOLATED PEA INTERNODE PRETREATED WITH IAA, 2,4-D OR H_2O AND SUBSEQUENTLY GROWN IN IAA 0.05 PPM

PRETREATMENT	CONSECUTIVE FRESH WEIGHT INCREMENTS MG/G INITIAL FRESH WEIGHT SUCCESSIVE TIME INTERVALS IN HOURS										GAIN IN WEIGHT MG/G INITIAL FRESH WEIGHT		
	DURING PRETREATMENT	DURING H_2O WASH			DURING POSTTREATMENT IN IAA 0.05 PPM						0–5.5 HR	0–24 HR	DURING IAA POSTTREATMENT ONLY
	2	2	¾	¾	¾	¾	¾	¾	½	15			
IAA 0.5 ppm	176	142	18	18	54	54	44	56	36	258	358	860	502
1.0 "	184	162	17	21	41	35	36	47	27	237	384	807	423
2.0 "	207	167	14	14	36	36	32	44	32	227	402	809	407
Mean	*189*	*158*	*16*	*18*	*44*	*42*	*37*	*49*	*32*	*241*	*381*	*825*	*444*
2,4-D 2.0 ppm	124	158	41	59	77	69	59	55	33	263	382	938	556
3.5 "	126	181	48	53	72	64	52	54	29	257	408	936	528
5.0 "	134	190	55	59	71	59	59	49	29	278	438	983	545
Mean	*128*	*176*	*48*	*57*	*73*	*64*	*57*	*53*	*30*	*266*	*407*	*952*	*543*
H_2O	96	110	36	38	74	66	65	61	34	249	280	829	549
Sig. diff. (P = 0.05) Between two treatments	18.9	15.8	11.5	8.9	10.7	11.6	8.6	7.1	5.4	29.2	40.6	59.7	38.0
Between H_2O treatment and means	15.4	12.9	9.3	7.2	8.7	9.4	7.0	5.7	4.4	23.8	33.1	48.7	31.0

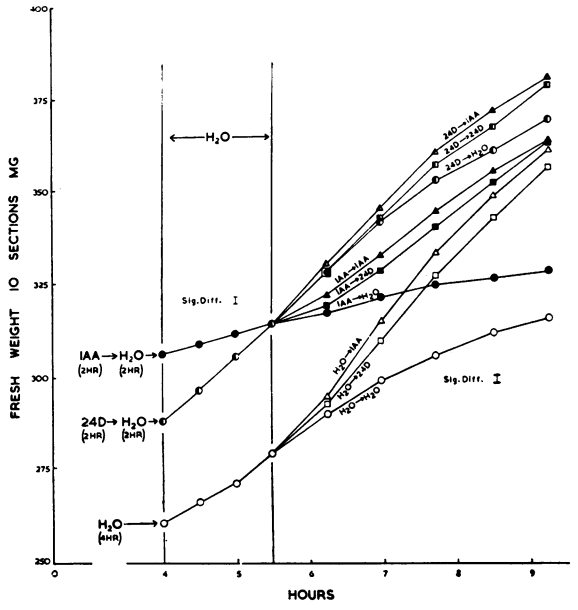

FIG. 5. Extension growth in mg/batch of 10 sections of etiolated pea internode treated for 2 hours with either IAA 1.0 ppm, 2,4-D 2.5 ppm or H_2O, followed by 3.5 hours in H_2O, then by a continuous posttreatment in either IAA 0.075 ppm, 2,4-D 2.0 ppm or H_2O.

which is higher than that of untreated controls when they are all transferred to water, since less IAA should be destroyed within the tissues. This is not so for IAA, since doses of 1.0 to 25 ppm for one hour cause a subsequent and increasing reduction in the growth rate to a value ultimately below that of the control. The continued higher rate which follows the more concentrated 2,4-D or 2,5-DBA pretreatments could be explained by residual growth substances in the tissues. However, the growth rates resulting from one-hour treatments with 2,4-D from 0.1 to 0.5 ppm (which should increase the oxidase activity) or 2,5-DBA from 2.5 to 10 ppm have been found, after a small stimulation for 4 to 5 hours, to fall to, and be maintained for many hours at, values which are not significantly different from the growth rate of the water controls. This suggests that if changes have occurred in the IAA–oxidase activity of these pretreated sections, the resulting effect on their further rate of growth does not fulfill the expectations of an adaptive enzyme system, even though the concentrations of IAA and 2,4-D employed are those with which Galston and Dalberg obtained marked effects on their brei estimations of IAA–oxidase. It would seem, therefore, that any changes that may occur in the oxidase activity of brei preparations of etiolated pea stems as a result of IAA or 2,4-D treatment, are not necessarily reflected in the subsequent extension growth or water uptake of the tissues in vivo.

2. Competition for essential growth metabolites:

The possibility that the growth capacity is reduced in sections which have been made to grow rapidly for the first few hours of an experiment has been discussed already and it is clear that the actual amount of growth achieved in these early stages does not necessarily reduce the growth capacity, since sections pretreated with 2,4-D or 2,5-DBA are still able to respond to further growth substance as readily as if they had grown only as much as water pretreated controls (table I). The reduction in growth that occurs following an IAA pretreatment must therefore be attributed to some special function of IAA. If some metabolic processes were stimulated more by IAA than by 2,4-D or 2,5-DBA, the competition for substrates between the various reactions could result in less of some essential factor being available to the elongation growth processes. This difference in the metabolic balance of the tissues might be a possible explanation for the reductions in growth that occur following an IAA pretreatment compared with pretreatments in 2,4-D, 2,5-DBA or water.

The results could, however, also be attributed to a production of growth inhibitory substances during the IAA treatment and this possibility is now explored more fully.

3. The formation of a growth inhibitor:

Further experiments have shown that when sections treated with IAA, 2,4-D or 2,5-DBA are washed and transferred at once to small volumes of water, biologically determinable amounts of growth stimulating substances pass out of the sections into the water. Since the growth rate may remain higher than that of water treated sections for many hours even when the sections are transferred to large volumes of water (fig 2 B, 3 B and 4 B), it is suggested that some of the originally applied growth regulator remains within the tissues and acts as a supply pool. Blackman (5) has found that *Lemna minor* plants treated with isotopically labelled 2,4-D lose 90 % of the 2,4-D initially taken up when they are transferred to a culture solution for three hours. Johnson and Bonner (7) also using labelled 2,4-D have shown that a certain fraction of the 2,4-D taken up by Avena sections is lost again from the tissue within minutes of the sections being transferred to water and that a further fraction of the 2,4-D remaining could be released only when the sections were transferred to unlabelled 2,4-D. However, they do not quote results for the growth of sections following the treatments. The present experiments with 2,4-D and 2,5-DBA suggest that as long as the growth regulator is present in the supply pool, extension growth remains above that of sections grown continuously in water, but once the pool is exhausted, growth falls to that sustained by endogenous auxin only, and is not significantly different from that of the water controls. When sections are treated with IAA, however, it is proposed that a stimulation of certain metabolic reactions may take place which results in the production of a substance which can dislocate the processes involved in growth by elongation. The observed

growth which follows an IAA treatment would then be the result of a balance between the growth promoting action of IAA, which may be similar to that of 2,4-D and 2,5-DBA, and the growth depressing action resulting from the development of the inhibitor. When sufficient IAA (or other growth regulating substances) are continuously present in the external solution (10), the supply pool is being continually replenished so that the proposed growth inhibitor may never attain a concentration high enough to cause a measurable reduction in growth. When IAA treated sections are transferred to water, the pool should steadily become exhausted until the only source of growth regulator is the auxin formed endogenously. The growth of these sections should then be less than that of the water treated sections because of their higher content of the suggested inhibitor. At a certain critical initial dose of IAA the amount of inhibitor formed should completely counteract the growth stimulating effect of the endogenous auxin and any residual IAA and the extension growth of the tissue should then cease (12.5 ppm IAA, fig 2, approaches this condition). A diagrammatic representation of such a system is shown in figure 6.

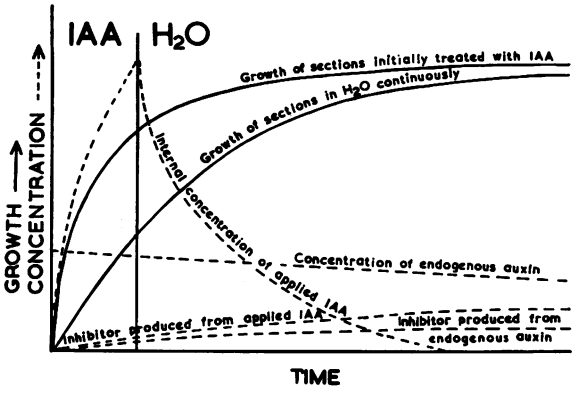

FIG. 6. Diagrammatic representation of the suggested growth stimulatory and growth inhibitory action of both endogenous auxin and applied IAA in sections of etiolated pea internode.

The difference in growth between IAA pretreated sections when they are given further IAA or 2,4-D and those pretreated with water has been shown to approximate to a constant (table II) and to be independent of the posttreatment concentration. This constant could reflect the amount of the growth inhibitor formed in the tissue as the result of the IAA pretreatment.

The development of a growth inhibitor in the pea sections treated with IAA offers a reasonable explanation for the results described. The evidence suggests that either the proposed growth inhibitor is not formed as the result of similar treatments with 2,4-D or 2,5-DBA or that if it is, the amount produced under the influence of these compounds is too small to be detected by the present methods. A survey of some of the data from other workers lends support to the proposed inhibitor hypothesis.

IAA in solution is readily decomposed by bacteria and it seems probable that in experiments involving very small volumes at low concentrations, the IAA may remain at the original concentration for a short while only. This, coupled with uptake by the test tissue, may cause the concentration to decline almost to zero, and might therefore offer conditions essentially similar to some of those described in the present paper. This might explain why sections of Avena coleoptile grown in very low concentrations of IAA (10^{-3} to 10^{-6} ppm) may show a growth stimulation when measured after five hours but a reduction in growth compared with a water control when measured after 20 hours (3).

It is also tempting to speculate that some of the effects ascribed to continuous low concentrations of IAA on the extension growth of roots may possibly be due to a loss of IAA from the external solution and the actual IAA treatment should, perhaps, be considered to be of short duration only.

While investigating endogenous rhythms in plants Ball and Dyke (2) measured the growth rate of whole seedlings of Avena which had previously been subjected to a short period of immersion in IAA and noted that the growth rate was initially stimulated but eventually fell below the level of control plants. Similar immersion treatments with 2,4-D resulted in growth stimulations but no subsequent growth inhibition, the rate falling only to that of the control values. They suggested that a growth inhibitor might be the cause of the reduced growth rate of the IAA treated plants, but did not extend their investigations further. Ball has, however, developed the view that IAA may induce the formation of a growth inhibitor in Aegopodium rhizomes, and has suggested that this might explain some of the effects of IAA on the geotropic responses of these organs (1). Bennet-Clark and Kefford (4) also suggested a conversion of IAA into an inhibitor to explain the rapid decrease in growth rate which succeeds the large initial growth stimulations in Avena sections which are exposed continuously to very high concentrations of IAA (70 and 100 ppm). They were, however, unable to demonstrate the diffusion of inhibiting substances into the external solution. It has been shown in the present work (fig 1 and 2 A) that growth inhibitions cannot be demonstrated while there is considerable IAA in the external solution, or when there is still enough IAA within the tissue to give appreciable growth stimulations. The presence of a growth inhibitor was therefore unlikely to be expressed in Bennet-Clark and Kefford's experiments as long as the sections were kept in high concentrations of IAA even if such a substance were to diffuse into the external solution. Thus it would seem that any attempt to demonstrate the diffusible nature of an inhibitor should be carried out when the IAA concentration within the tissue is minimal. Since the present work

has shown that growth inhibitions can be demonstrated after five hours, in pea sections treated for two hours with 1.0 ppm IAA, a number of experiments were carried out to determine if a diffusible inhibitor was present within such sections. Two principal methods were employed. The 1st method was carried out with the co-operation of Dr. C. C. McCready of this Unit, who developed the apparatus which was finally adopted.

One-cm pea internode sections were treated for two hours with 1.0 ppm IAA, washed and then grown for a further three hours either in water or on glass slides in a saturated atmosphere. A 2nd set of sections which received a water treatment were used as controls. At the end of the five hours the sections were weighed in batches of 10 and placed vertically through small holes in sheets of Perspex mounted over Petri dishes of water so that the greater length of the section was immersed in the water. Ten IAA and 10 water treated sections were mounted in this way in each dish. A disc of 2 % agar, 1 mm thick was then placed on the top (apical end) of each section and a 2nd Perspex rack with holes aligned above those in the lower rack was screwed into position. Freshly cut sections of pea internode were weighed and then dropped through these holes so that their basal ends were sealed to the upper surface of the agar disc below. The dishes were kept at 100 % humidity at 24° C and at the end of 24 hours the upper and lower sections were weighed again. The upper sections increased in weight by the same amount independently of whether the water they took up passed first through the IAA treated or through the water treated sections to which they were sealed. The lower water treated sections continued to take up water during the 24 hours, but the weight of the IAA treated sections remained practically unchanged. The inhibition of these latter sections was therefore effective, although no inhibition of growth occurred in the sections sealed above them indicating that no measurable diffusion of an inhibitor took place from the IAA treated sections.

A 2nd method was an attempt to collect the possible inhibitor by diffusion into a very small volume of water. Sections which had been treated for two hours with water or IAA at 1.0 ppm were washed, and then grown in water for a further three hours. Batches of 10 sections were arranged in the bottom of small beakers in the minimum amount of water (2.0 ml) and kept at 24° C and 5° C. At intervals from two to 24 hours, the sections were removed from a number of the beakers containing the water or IAA treated tissues. To the residual solution a freshly cut, weighed batch of 10 sections was added, and the increase in weight of these sections determined after 8 to 18 hours. No difference could be detected between the growth stimulating effects of the diffusates from the IAA and the water treated sections. It must be concluded, therefore, that if an inhibitor is formed within the tissue as a result of a treatment with IAA, it cannot be demonstrated by diffusion and it is possible that it may influence only the growth of the cells in which it is produced.

THE ROLE OF AN INHIBITOR IN THE INTACT PLANT: Evidence has been presented which supports the proposal that IAA may have a dual action in controlling growth when it is applied to sections of etiolated epicotyl. It remains to consider how this concept might help to explain the growth regulating mechanism of an intact plant.

It is generally accepted that there is a gradient of auxin from the shoot apex. As cells differentiate, they become displaced from the apex and may therefore receive a continually falling supply of auxin. During this period it is suggested that the growth inhibitor may slowly accumulate and finally reach a concentration which counteracts the auxin present. At this stage, extension growth of the cell should stop. It may be demonstrated, however, that such tissues still possess a capacity to grow, for if they are excised and placed in relatively high concentrations of IAA, further extension growth will occur (7).

The formation of a growth inhibitor following the treatment of a plant with IAA might offer an additional explanation for its poor herbicidal activity compared with other synthetic growth regulating substances, although ease of penetration, photochemical decomposition and bacterial breakdown should also be considered. The apparent failure of 2,4-D to induce the formation of an inhibitor together with its relative stability within the plant may explain why completely unrestrained growth results from an application at herbicidal concentrations.

It is therefore proposed that the formation of a growth inhibitor might be one of the ways in which the plant has developed an elegant safety mechanism to protect itself from its own production of auxin and that this safety mechanism is not developed against completely foreign synthetic growth substances such as 2,4-dichlorophenoxyacetic or 2,5-dichlorobenzoic acids.

SUMMARY

1. A simple, rapid and reliable method is described for measuring small increments of growth in sections of etiolated pea internode. The results can be interpreted in terms either of extension growth or increase in fresh weight.

2. The growth of sections kept continuously in solutions of suboptimal concentrations of IAA, 2,4-D or 2,5-DBA for 24 hours, was, over measured periods of time, always either greater than, or the same as that of sections kept in water.

3. The growth of sections treated for a few hours only with suboptimal concentrations of IAA and then transferred to water for the remainder of the 24 hours, ultimately fell below that of the water controls. The reduction in growth that occurred and the time at which it became apparent were dependent upon the concentration of the original IAA treatment.

4. No reduction in growth was observed in sec-

tions similarly treated with 2,4-D or 2,5-DBA. Over measured intervals of time growth remained either higher than or equal to but was never less than that of the water controls.

5. Sections which received an initial treatment with IAA did not respond as much to further applications of a growth regulator as sections which had received an initial treatment with either 2,4-D, 2,5-DBA or water.

6. The nature of the growth depression which follows an IAA treatment is discussed. It is suggested that IAA may have a dual role in controlling growth. It may function as a growth promotor in a way similar to that of 2,4-D or 2,5-DBA. In addition, it may stimulate the formation of a growth inhibitor. It is proposed that one of the effects of IAA upon cell metabolism is the formation of a substance which accumulates within the tissue and which can in some way dislocate the growth processes and render the tissue less sensitive to further applications of growth regulators. The formation of such an inhibitor could be a safety mechanism to protect the plant from its own production of auxin, and might offer a partial explanation for the weak activity of IAA when applied to intact plants.

7. The results suggest that pea sections treated with 2,4-D or 2,5-DBA either do not develop this safety mechanism or develop it only to a small extent and it is proposed that this difference in response to IAA and the synthetic growth regulators might be a further factor to account for the much greater effectiveness of the latter compounds in whole plants.

The author wishes to thank Miss Pamela Clements and Mrs. Ruth Kasasian for technical assistance, and the Unit of Biometry, Oxford, for their advice on statistical analyses. She also thanks Professor G. E. Blackman for his suggestions and encouragement and Dr. E. K. Woodford and other members of the Agricultural Research Council Unit of Experimental Agronomy for many helpful discussions.

Literature Cited

1. Ball, N. G. The effects of certain growth-regulating substances on the rhizomes of *Aegopodium podagraria*. Jour. Exptl. Bot. 4: 349–362. 1953.
2. Ball, N. G. and Dyke, I. J. The effects of indole-3-acetic acid and 2,4-dichlorophenoxyacetic acid on the growth rate and endogenous rhythm of intact Avena coleoptiles. Jour. Exptl. Bot. 7: 25–42. 1956.
3. Barlow, H. W. B., Hancock, C. R. and Lacey, H. J. Studies on extension growth in coleoptile sections. I. The influence of age of coleoptile upon the response of sections to IAA. Ann. Bot. 21: 257–271. 1957.
4. Bennet-Clark, T. A. and Kefford, N. P. The extension growth-time relationship for Avena coleoptile sections. Jour. Exptl. Bot. 5: 293–304. 1954.
5. Blackman, G. E. Interrelationships between the uptake of 2,4-dichlorophenoxyacetic acid, growth and ion absorption. In: The Chemistry and Mode of Action of Plant Growth Substances, R. L. Wain and F. Wightman, eds. Butterworth, London 1956.
6. Galston, A. W. and Dalberg, L. Y. The adaptive formation and physiological significance of indoleacetic acid oxidase. Amer. Jour. Bot. 41: 373–380. 1954.
7. Johnson, H. P. and Bonner, J. The uptake of auxin by plant tissue. Physiol. Plantarum 9: 102–119. 1956.
8. Macht, D. I. and Grumbein, M. L. Influence of indoleacetic, indolebutyric and naphthaleneacetic acids on roots of *Lupinus albus* seedlings. Amer. Jour. Bot. 24: 457–460. 1937.
9. Marinos, N. G. Responses of Avena coleoptile sections to high concentrations of auxin. Aust. Jour. Biol. Sci. 10: 147–163. 1957.
10. McRae, D. H., Foster, R. J. and Bonner, J. Kinetics of auxin interaction. Plant Physiol. 28: 343–355. 1953.
11. Reinhold, L. The uptake of indole-3-acetic acid by pea epicotyl segments and carrot discs. New Phytol. 53: 217–239. 1954.
12. Thimann, K. V. and Lane, R. H. After-effects of the treatment of seed with auxin. Amer. Jour. Bot. 25: 535–543. 1938.

THE RELATION OF THE COTYLEDONS TO ROOT DEVELOPMENT OF PINE EMBRYOS GROWN IN VITRO[1]

CLAUD L. BROWN[2] AND ERNEST M. GIFFORD, JR.[3]
Harvard University, Cambridge, Massachusetts

Attempts to obtain successful seed germination of certain conifers have resulted in the adoption of modified cultural techniques. Aside from certain commonly practiced procedures, e.g., stratification, some workers have attempted, and have been successful in some instances, in growing excised embryos to fairly large trees. Embryos are initially cultured on artificial media with or without the surrounding nutritive female gametophytes, and then transplanted to soil as done by Stone and Duffield (16) and Haddock (5). If successful, this technique also has its obvious advantages for forest geneticists who may wish to obtain seedlings of hybrid origin from a small crop of seeds. The realization that embryos can be grown outside their normal environment has likewise presented

[1] Received September 6, 1957.

[2] Research Assistant, Maria Moors Cabot Foundation for Botanical Research, Harvard University. Present address: Forest Genetics Laboratory, Texas A. & M. College, College Station, Texas.

[3] Senior Merck Post-doctoral Fellow, National Research Council, and Research Fellow in Biology, Harvard University. Present address: Department of Botany, University of California, Davis, California.