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STUDIES ON THE GROWTH OF COLEOPTILE AND FIRST INTERNODE SECTIONS. A NEW, SENSITIVE, STRAIGHT-GROWTH TEST FOR AUXINS[1,2]

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The demand for an improved bioassay of the substances which promote and inhibit growth in plants is apparent from the large number of tests which have been suggested in recent years and has prompted the efforts which will be reported in this paper. The present investigation resulted in (a) the improvement of the well-known coleoptile section test and (b) the working out of a new test using as a test organ the first internode of the oat seedling.

The qualities which one looks for in such tests are: sensitivity, large proportionality range between the concentration of the active substances and the growth response, specificity, reliability, and ease of performance. Let us examine critically the main auxin tests which are based on the measurement of cell elongation to see how well they meet these criteria. From the short and incomplete review summarized in table I it is apparent that the Avena curvature test is a good and sensitive test. It is not well suited, however, to the assay of paper chromatograms for three reasons: 1) the procedure is too delicate, requiring time and skill; 2) the proportionality range is too small; 3) the detection of inhibitors is difficult, although this insensitivity to inhibitors may be an advantage in certain cases. The pea curvature test, on the other hand, would appear to be suitable, but its lower limit of sensitivity is too high for usual paper chromatograms and it does not detect inhibitors readily. Many workers have resorted to the coleoptile straight growth test to detect auxins and inhibitors on paper chromatograms. This seems to be a simple test, requiring little apparatus. Unfortunately, the sensitivity of this test is much less than that of the Avena curvature test. Rietsema (28) reported the development of a sensitive coleoptile cylinder test, but, after six years, no one seems to have used this test, perhaps because of a lack of reproducibility. Roots, which are much more sensitive to auxins than shoots, have been used by several workers, for example Moewus (23), Audus and Thresh (1), etc. Unfortunately, roots generally respond to auxins by a growth inhibition, which makes them unsatisfactory to study growth promoting substances. Very low auxin concentrations, however, promote root elongation. The dome-shaped curve resulting from the succession of the promoting and the inhibiting effect, renders ambiguous the determination of the auxin concentration. Finally, it has been shown that the cress root test of Moewus is not very reliable for quantitative work, the limits of the error made at IAA concentrations below 1 μgm/l being from 50 to 260 % of the actual values (9). Also, the pea root test of Leopold and Guernsey (18) is very variable, to the point that it could not be repeated with a different batch of the same variety of peas (Leopold, private communication).

Screening of Various Test Objects: These observations led us to conclude that a straight growth test was the most desirable type for paper chromatography work and that one should look for a shoot material which would be more sensitive than the stem segments usually employed. Since roots appear to be more sensitive than shoots, we thought of using tissues which would be intermediary between shoots and roots, namely hypocotyls. However, a preliminary

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TABLE I

A COMPARISON OF SOME BIOASSAYS FOR AUXINS

TEST	SENSITIVITY, MINIMUM DETECTABLE IAA			USABLE RANGE * (μGM/L)	RELATIVE ACCURACY **	SPECIFICITY	RELIABILITY	EASE OF PERFORMANCE, SPECIAL EQUIPMENT †
	CONC (μGM/L)	VOL (ML)	TOTAL AMT (μGM)					
A. Tests based on differential growth responses								
Avena curvature test (43)	5	0.3	1.5×10^{-3}	10 to 100	High	Very high	Good	Requires skill, controlled humidity, special small equipment.
Deseeded Avena test (31)	1	0.3	3×10^{-4}	2 to 100	High	High	Good	id
Split pea test (44, 38)	50	25	1.25	100 to 10,000	Medium	Low	Good	Requires little skill, little special equipment.
Quartered Avena test (40)	0.01	20	2×10^{-4}	0.3 to 10,000	Medium	?	Medium ?	id
B. Tests based on straight growth								
Avena section test (4, 7, 29)	10	10	0.1	10 to 3,000	Medium	Low	Good	Requires little skill, little special equipment.
Micro straight growth test (28)	0.01	2	2×10^{-5}	0.01 to 100	Medium	?	?	id
Pea section test (11, 39)	3	5	1.5×10^{-2}	3 to 300	Medium	Low ?	Good	Takes 7 days to grow the test plants. Requires little skill, little special equipment.
Cress root test (23)	0.001	10	1×10^{-5}	1 to 10,000	Low	Very low	Poor	Requires little skill, little special equipment.
Pea root section test (1)	0.001	0.75	7.5×10^{-7}	0.1 to 1,000	Medium	Low ?	Medium ?	id
Pest root test (18)	0.01	10	1×10^{-4}	0.01 to 10	Low	?	Poor	id
Sensitized oat and wheat coleoptile tests (this study)	5	0.5	2.5×10^{-3}	5 to 1,000	Medium	Low	Good	Requires little skill, little special equipment.
First internode test (this study)	1	0.5	5×10^{-4}	1 to 1,000	Medium to high	Low	Good	Requires care, rotating machine.

* For quantitative work.

** The accuracy with which an auxin concentration can be deduced from the measured growth response depends in part on: (a) the type of proportionality between auxin concentration and growth response (linear or logarithmic), (b) the slope of the curve representing this proportionality (a steep slope with large differences in growth corresponding to small changes in concentration tends to give more accurate results), (c) the limits of variability.

† In addition to a temperature-controlled darkroom which is required for best results in all the tests listed.

screening of possible test objects gave a rather discouraging answer as far as dicots are concerned (table II). On the other hand, monocots proved to be much more promising, for the first internodes of oat, sorghum and corn responded rather well to indole-3-acetic acid (IAA). The reason why sorghum and pop corn were tried is that they can develop first internodes up to 15 cm (sorghum) and 20 cm (corn) in length, whereas oat first internodes grow only to 6 cm (14). It was concluded that, among the test objects investigated, the coleoptiles and first internodes of grasses were the most promising ones, and work with other tissues was discontinued. Wheat and oat were selected as candidates for a coleoptile test, sorghum and oat for a first internode test.

On wheat seedlings, no first internode ever develops, even in total darkness. Such a species seemed interesting at first because 1) most of the varieties are hulless, and 2) no red light treatment is necessary to prevent the growth of the first internode. The batches of wheat seeds which were tried first (var. Ceres and Acadia) did not prove to be too sensitive and, also, gave a rather irregular growth with a relatively high standard error. Later, the wheat var. Genesee used by Powell (27) was tried and found to elongate very much at IAA concentrations superior or equal to 10 μgm/l, especially when the sections were soaked in water prior to the actual assay (see below, section 6). As far as oats are concerned, the problem of dehusking thousands of seeds had been solved previously by using hulless varieties, such as Brighton (25). The first internodes of this variety

TABLE II

SURVEY OF POSSIBLE TEST OBJECTS

PLANT	AGE IN DAYS	LOCATION OF SECTIONS	IAA CONC (μGM/L)	$G_{IAA} - G_0$**
Lepidium sativum †	3	Hypocotyl, 2 mm below curved part	10	0.08
		4 mm below curved part	10	0.23
		6 mm below curved part	10	0.27
Helianthus annuus	5	Hypocotyl (9.5-mm sections)	1	0.44
			10	0.99
			100	0.69
Phaseolus vulgaris var. Kentucky wonder	5	Hypocotyl (9.5-mm sections)	1	0.26
			30	1.32
			100	0.88
Zea Mays var. Minhybrid white hullless, pop corn	4	1st internode	10	0.26
			50	0.72
			100	1.47
Triticum vulgare var. Ceres ††	3	Coleoptile, 3 mm below tip	1	0.27
			10	0.30
			100	1.02
Sorghum vulgare var. Western Blackhull kafir, F. C. 9098 ††	4	1st internode, 2 mm below node	1	0.05
			3	0.15
			10	0.53
			30	1.14
			100	1.66
			300	2.34
			1,000	1.95
Avena sativa	3	Coleoptile, 3 mm below tip †	10	−0.15
			100	0.55
			1,000	1.55
		1st internode, 3 mm below node	1	0.57
			3	0.74
			10	1.12
			30	2.24
			100	3.22
			300	3.51
			1,000	3.52

Ten 4-mm sections were grown in the dark for about 24 hours in 0.5 ml liquid (buffer * + 2 % sucrose) in each case, except for bean and sunflower seedlings in which instances ten 9.5-mm sections were grown in 1.0 ml.

* K_2HPO_4 (1.794 gm/l) + citric acid monohydrate (1.019 gm/l).

** This column gives the difference: (Growth in IAA) − (Growth without IAA).

† Exposed to 4 hours of red light during the first day of germination.

†† Obtained through the courtesy of the Agricultural Research Service, U.S.D.A.

proved to be more sensitive than those of sorghum and were finally selected as the best material.

MATERIALS AND METHODS

Oat seeds, var. Brighton, were soaked in tap water for two hours in the laboratory, then laid down on several layers of wet facial tissue (through which roots can grow freely) disposed at the bottom of glass or enamel trays. When coleoptiles were wanted, the trays with the seeds were then placed for 3 to 4 hours at about 35 cm below a concentric pair of G. E. white fluorescent "Circline" lights filtered through a Corning filter No. 2404. This illumination, devised by Thimann (unpublished) to produce an even light field of adequate intensity, suppresses the development of the first internode. The seeds were then grown in a darkroom maintained at 25° C with about 85 % relative humidity. When first internodes were wanted, the seeds were soaked in water for two hours in a closed container to protect them from light and grown in total darkness for 72 hours in moist clean maple sawdust. The subsequent manipulations were performed under the green light of a Westinghouse "green" fluorescent tube wrapped with three layers each of "amber" and "green" cellulose acetate window shading 0.0075 inch thick (obtained from the New England Plastic Shade Co., 80 Boylston Street, Boston, Mass.). This light source, devised by Dr. C. Yocum (unpublished), gives a light having mostly the wavelength of the 546 mμ mercury band which combines the following advantages: (a) it does not appreciably inhibit the growth of the first internode (12, 42); (b) it is phototropically inactive (17); (c) it is in the zone of the maximum sensitivity of the human eye.

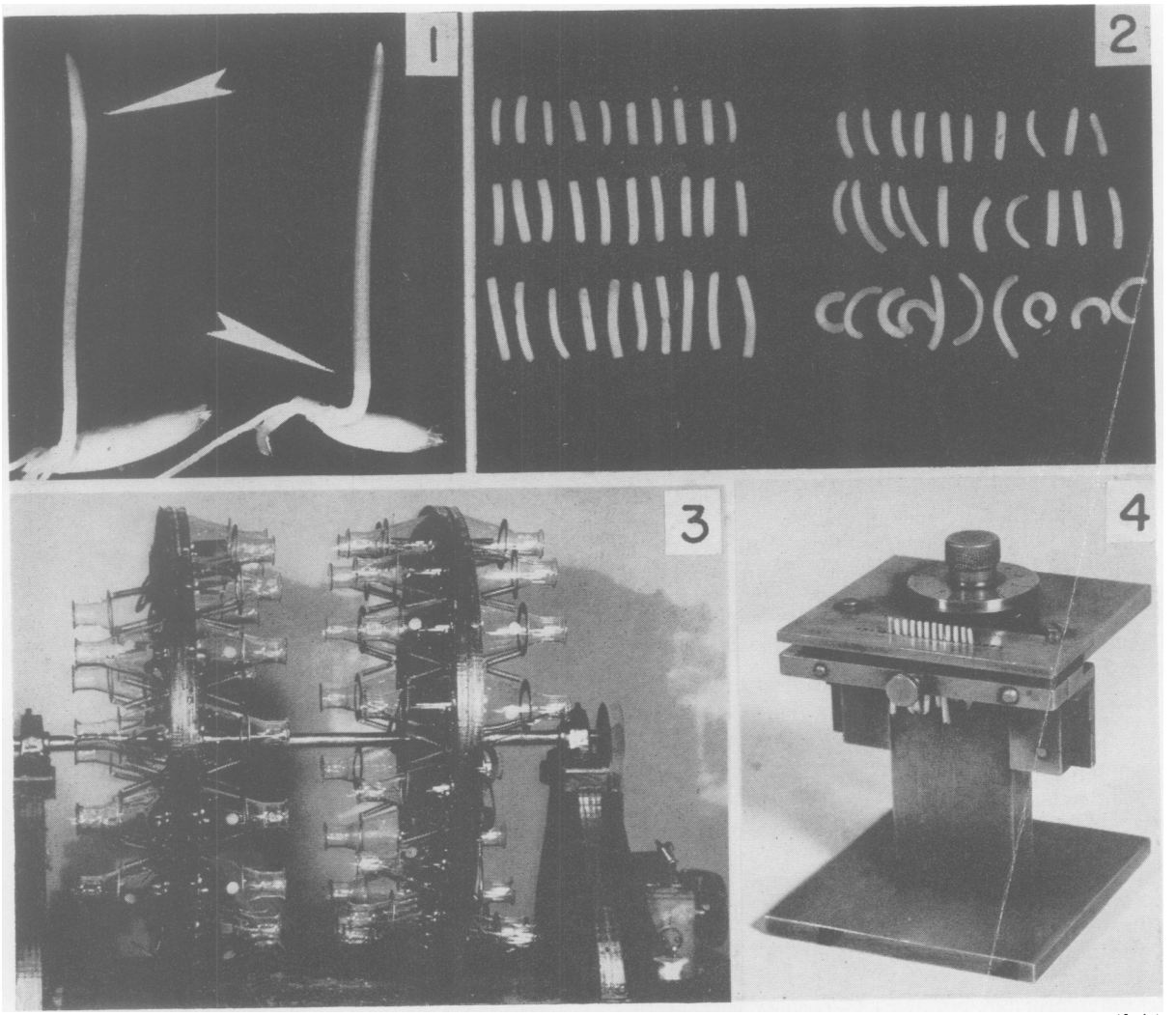
When the seedlings had reached the proper length (about 30 mm total length, fig 1), they were cut with Thimann's improvement of the coleoptile microtome (fig 4) of van der Weij (41). Thus, the error on the initial length of the sections was kept in the order of 0.04 mm.

In the case of coleoptiles, the enclosed first leaf was not removed to simplify the procedure and minimize the injury in handling the sections. Both Han-

cock and Barlow (15) and Bentley and Housley (6) consider that it is better to leave the primary leaf in the coleoptile section. Of course, this point does not apply to the first internodes which are made of solid stem tissue.

Ten replicates were used for each measurement. Generally, the coleoptile sections were floated 3 hours on large volumes (about 150 ml) of glass distilled water containing 1 mg/l of $MnSO_4 \cdot H_2O$ which increases the response to IAA (see below section 6) before being placed in the solutions to be tested. First internodes were presoaked in water for 1 hour only. During this time, they laid on the top of nets of cheesecloth stretched on plastic rings and kept breaking the surface of the liquid, a procedure similar to that devised by Hackett and Thimann (13).

A difficulty arose in the work with the first internodes because they curve very easily in auxin solutions. It was found that rotating the sections at about 1 rpm prevents curving and yields straight regular sections (fig 2). The rotating machine consists of vertical discs on which 125-ml Erlenmeyer flasks are fastened by means of springs (fig 3). The solutions and the sections are put in 13×100 mm Pyrex tubes which are placed in the flasks in which they remain at a slight angle above the horizontal plane. Coleoptile sections, on the contrary, did not need to be rotated, although we confirmed the report



FIGS. 1 to 4. FIG. 1. Oat seedlings, var. Brighton, at the right stages for use in the first internode test (*left*) and the coleoptile test (*right*). The arrows mark the position of the coleoptilar nodes. FIG. 2. Sections of oat first internodes of 4-mm initial length grown in sucrose 2 % + buffer without added IAA (*first rows*), with IAA (10 μgm/l) (*second rows*), and IAA (100 μgm/l) (*third rows*). The sections on the left were rotated, those on the right were not rotated: the latter ones curved. FIG. 3. The rotating machine used to rotate the oat first internodes. The Erlenmeyer flasks are ready to receive the tubes with the sections in 0.5 ml of solution. If the atmosphere is dry, the tubes are closed with vaccine stoppers. FIG. 4. Thimann's improvement of the van der Weij's coleoptile microtome, which allows exact work in the coleoptile and internode section tests.

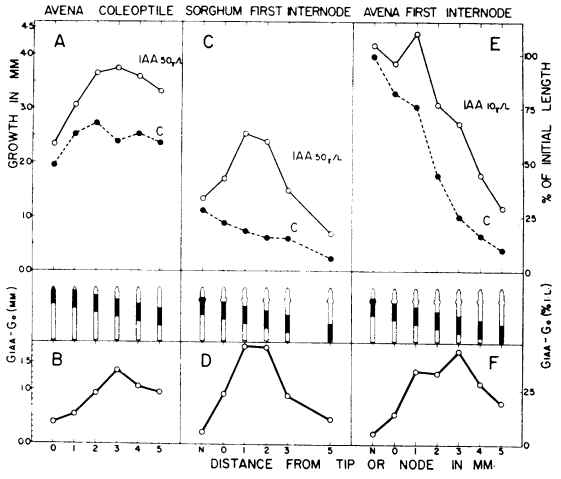


FIG. 5. The effect of location of the original 4-mm section on the growth in sucrose + buffer without added IAA (black circles, dotted lines) and with IAA (white circles, solid lines), and on the difference of these two elongations (curves B, D, and F). *Left.* Scales in mm. *Right.* Scales in % of the initial 4-mm length. On the abscissa of the curves C, D, E, and F, N means that the section includes the coleoptilar node. Each point is the mean of 10 replicates.

of Hancock and Barlow (15) that they grow more if rotated. In our experiments, the coleoptile sections floating on the solutions to be tested were gently shaken on a horizontal "Yankee rotator" shaker rotating at 23 rpm. In general, the sections, whether of coleoptiles or first internodes, were incubated in the test solutions for about 20 hours in the dark. After this time, they were measured to the nearest 0.1 mm by means of a binocular with an ocular micrometer.

Using these general conditions, a search was made to find out what factors would promote an optimal growth response to IAA.

Study of Some Factors Affecting the Response of the Bioassay to Indoleacetic Acid

Throughout the experiments which will now be reported, one must bear in mind that what we are looking for is the largest possible differential between the response to auxin and the response of the controls. In other words, a treatment which increases both the growth of the controls and that of the auxin-grown sections in the same amount does not increase the sensitivity of the test. Thus, in this investigation, the difference: *Growth in IAA – Growth without IAA* is of special importance. Also, we are aiming at a very sensitive bioassay. We will use, therefore, low concentrations of indole-3-acetic acid (10 μgm/l for first internodes, 50 μgm/l for coleoptiles).

1) Effect of the Location of the Section: One of the first points to determine with precision is where in the seedling one should cut the section to get the maximum response to IAA. To clarify this question, the following experiment was made. Four millimeter sections were cut at varying distances from either the tip of the coleoptile or the coleoptilar node and grown in sucrose and buffer (see the composition of the buffer used throughout these experiments at the end of section 9) with and without IAA. The results, represented in figure 5, show that the optimum response to IAA occurs when the 4 mm section is cut 3 mm below the tip of the oat coleoptile, or 1 to 2 mm below the coleoptilar node of sorghum seedlings, and 3 mm below the node of oat seedlings. Therefore, in all the subsequent experiments, the coleoptile sections were always cut 3 mm below the tip. In the case of first internodes, it was decided to cut the sections 2 mm below the node instead of 3 mm below because, in the latter case, the controls without IAA grow so little that they give no indication of inhibitors on paper chromatograms. One observes that first internode sections tend to grow considerably without added IAA if they are cut close to the node. In fact, they double in length if the node is included.

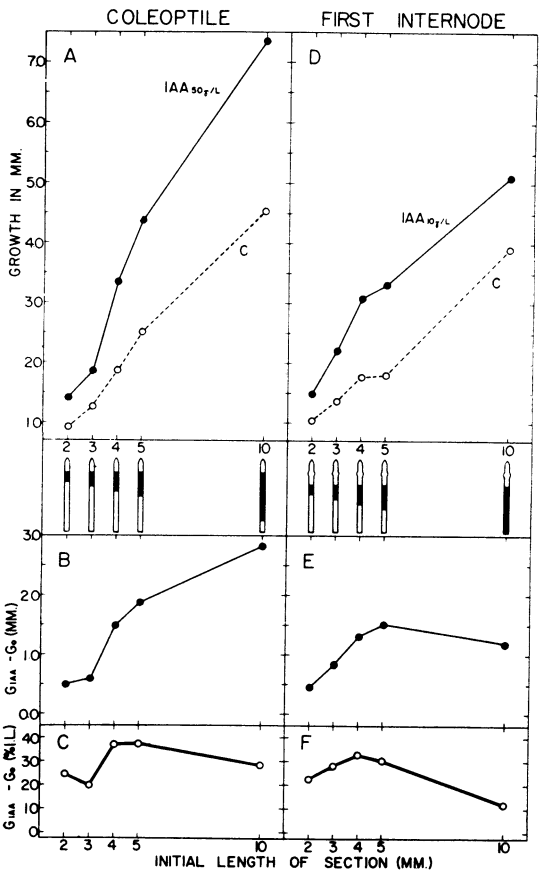


FIG. 6. Effect of the initial length of the oat sections on their growth in buffer + sucrose without added IAA (white circles, dotted lines) and with IAA (black circles, solid lines): graphs A and D. The other curves represent this effect on the difference $G_{IAA} - G_0$, expressed in mm (curves B and E) or in % of the initial lengths (curves C and F).

This effect is probably due to the fact that the meristematic zone at or just above the node functions as an auxin-producing center, a conclusion also arrived at by Mer (21). The fact that growth without added IAA decreases much more steeply in the case of internodes than in the case of coleoptiles as the section is cut further away from the node or tip, indicates that the actively growing zone is narrower in first internodes than in coleoptiles.

2) Effect of the Original Length of the Section: Starting at 3 mm below the coleoptile tip or at 2 mm below the coleoptilar node, we can now cut sections of 2, 3, 4, etc. mm of initial length and measure their growth in sucrose and buffer with and without IAA. The results, shown in figure 6, indicate that, generally speaking, the longer the initial section is, the larger is the growth. One can see also, however, that the longer the initial length of section, the larger is the growth of the controls without added IAA. The differential indicates an optimum of 5 mm of initial length in the case of oat internodes, beyond which no further gain can be obtained. This fact, again, indicates that the growing region of the internode is restricted to a narrow zone. In the case of coleoptiles, this is not the case because the difference $G_{IAA} - G_0$ continues to increase as the length of the initial section is extended. This justifies the use of 10-mm coleoptile sections recommended by many workers. However, the longer the sections, the less straight they are. In addition, if one considers the difference $G_{IAA} - G_0$ expressed in *% of the initial length*, then an initial length of 4 to 5 mm is better than a longer one, at least at the low auxin concentrations used here. For these reasons, an initial length of 4 mm was used throughout this work for both coleoptiles and internodes.

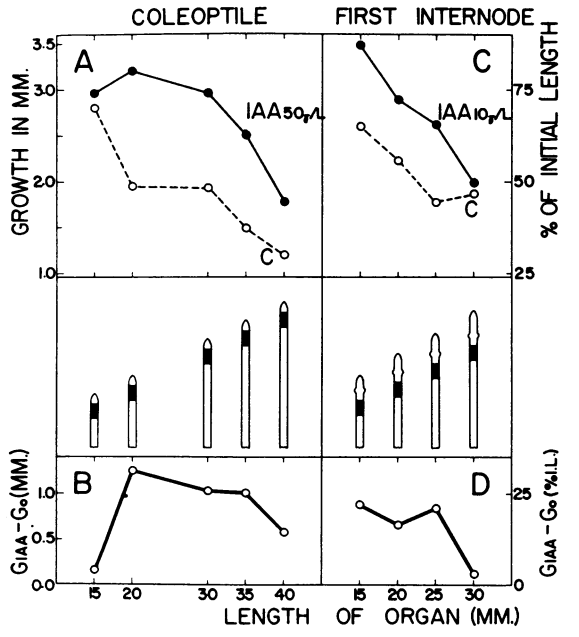
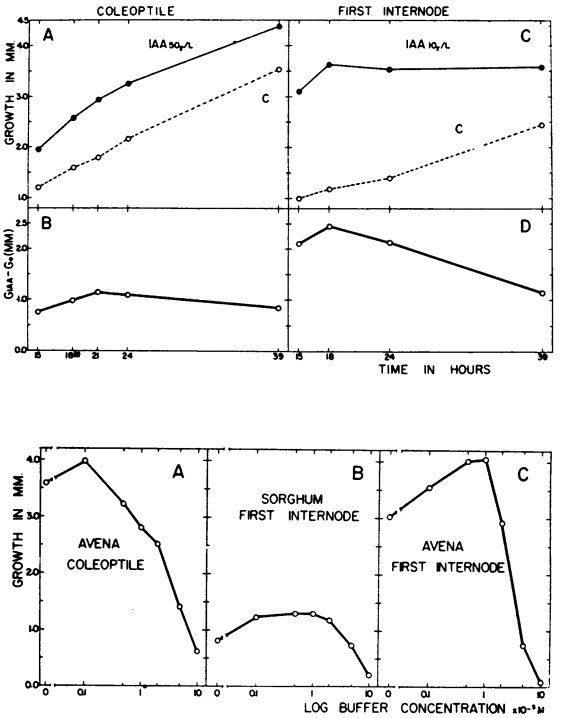


Fig. 7. Effect of the physiological age of the oat seedling (represented by the length of the coleoptile or of the first internode at the time of sectioning) on the growth of the sections in buffer + sucrose without added IAA (white circles, dotted lines) and with IAA (black circles, solid lines), and on the difference between these growths (curves B and D). Scales at the left are in mm, at the right in % of the initial 4-mm length.



Figs. 8 to 9. Fig. 8 (*above*). Effect of time in solutions on the growth of the sections in buffer + sucrose without added IAA (white circles, dotted lines) and with IAA (black circles, solid lines), and on the difference between these growths (curves B and D). Before time 0, the coleoptile sections had been presoaked for 3.5 hours in $MnSO_4 \cdot H_2O$ (1 mg/l) and the first internode sections for 1 hr in water. To exclude the effect of light during the measuring period, the experiment was done as follows: 5 replicate sets of 10 sections were started at the same time and one dish was measured and discarded at the various time intervals. Fig. 9 (*below*). Effect of the concentration of citrate-K phosphate buffer at pH 5.0 in the presence of 2% sucrose on the growth of 4-mm sections. The IAA concentration was 50 μgm/l in the case of Avena coleoptiles and 10 μgm/l in the cases of the first internodes of Sorghum and Avena.

3) Effect of the Age of the Seedling: At what stage of development does the oat seedling give the maximum response to IAA? Many workers in the field have casually studied this point. It has been investigated here by taking into account not so much the actual length of time between soaking of seeds and sectioning, as the physiological stage of development apparent from the length of the coleoptile or internode. In general, the younger and shorter the

TABLE III

EFFECT OF LIGHT ON THE IAA RESPONSE OF OAT COLEOPTILES AND INTERNODES

PLANT PART	TREATMENT	GROWTH OF CONTROLS	IAA CONC	GROWTH IN IAA	$G_{IAA} - G_0$
		mm	*μgm/l*	*mm*	*mm*
Oat coleoptiles	a) Cut under green * light, put for 5 hours in H_2O in the dark, then in the solutions	1.88	50	2.72	0.84
id	b) As a) but with a 30-min exposure to red light ** at the beginning of the 5 hrs in H_2O	1.76	50	2.41	0.65
id	c) As a) but with a 30-min exposure to red light ** while in the IAA solution	1.65	50	2.23	0.58
Oat coleoptiles	d) As a) but put for 5 hrs in buffer + sucrose instead of H_2O	1.78	50	3.43	1.65
id	e) As d) but with a 30-min exposure to red light ** at the beginning of the 5 hrs in buffer	1.42	50	3.06	1.64
id	f) As d) but with a 30-min exposure to red light ** while in the IAA solution	2.06	50	3.31	1.25
Oat coleoptiles	Cut under green * light, soaked for 3 hrs in $MnSO_4 \cdot H_2O$ in the dark and grown in the dark	2.00	10	2.21	0.21
			100	2.73	0.73
			1,000	4.16	2.16
id	Cut under red † light, the rest as above	1.69	10	1.80	0.11
			100	3.04	1.35
			1,000	4.57	2.88
Oat internodes	Cut under green * light, soaked for 1 hr in H_2O and grown in the dark	1.01	10	2.03	1.02
			100	3.26	2.25
			1,000	3.89	2.88
id	Cut under red † light, the rest as above	0.83	10	2.05	1.22
			100	2.85	2.02
			1,000	3.38	2.55

Ten 4-mm sections were used in all cases. Standard deviation from the mean: 0.15 to 0.25 mm for coleoptiles, 0.10 to 0.15 mm for first internodes.

* The green light was the same as that described under Material and Methods.

** This red light was that described under Material and Methods.

† This red light was different from the previous red light. It was obtained by filtering the light given by an incandescent bulb through a red filter transmitting radiations with wavelengths above 596 mμ.

organ is, the larger the growth (fig 7), but the growth of the controls is also high in the young stages. Thus the coleoptile sections taken from a 15-mm long coleoptile grow 75 % of their initial length without added IAA, almost as much as with 50 μgm/l of IAA. On the contrary, in the case of a 40-mm long coleoptile, the controls grow little, but so also do the sections treated with IAA. In short, the optimal differential response is obtained when either coleoptiles or internodes reach 20 to 25 mm in length. In the case of coleoptiles, seedlings up to 35 mm in length may still be used, whereas internodes of that length are too insensitive.

4) EFFECT OF TIME IN THE SOLUTIONS: After what time should one measure the elongation of the sections? This depends on the substance tested. In the case of low concentrations of IAA (fig 8) and in the presence of sucrose and buffer, the elongation of the coleoptile sections may continue for a long time. However, the elongation of the sections without IAA also increases in a parallel manner. The difference $G_{IAA} - G_0$ seems to be at its maximum about 20 hours after the sections have been put in the auxin solution. It is of interest to note that this difference does not increase with time as it does with larger volumes of solution (40 ml), higher IAA concentrations (1 mg/l), no buffer and no presoaking as used by Schneider (29). In the case of first internodes, growth seems to be completed after 18 hours (with low concentrations of IAA); if the sections are kept longer in the solutions, the controls grow more and more as time goes on, thus reducing the difference $G_{IAA} - G_0$. In consequence, if one wants to measure the IAA effect, at concentrations similar to those used here, one does not have to keep the sections in the solutions more than 20 hours. If one wants to measure inhibitors in relation to the controls without auxin, then the longer one waits, the larger will be the difference, because the controls will go on elongating for about two days. These long term experiments, however, may be interfered with by bacterial contamination. In the present investigation, a growth period of about 20 hours was used for both the coleoptiles and the first internodes.

5) EFFECT OF LIGHT: The experiments reported here were performed under the green light described above, with occasional exposure to very feeble intensities of red light due to other workers in the same darkroom. Since it has been reported (19, 30) that red light stimulates the growth of oat coleoptiles in the presence of IAA, an experiment was set up in which 30 min of red fluorescent light (the same light described in Materials and Methods) were given before and after the addition of IAA. As table III

shows, in no case was there a stimulation but rather a slight inhibition. On the other hand, a set of experiments in which the sectioning of the coleoptiles or internodes was done in duplicate under green fluorescent or red incandescent light gave different results (table III). Red incandescent light increased the growth of coleoptiles at concentrations of IAA of 100 μgm/l or above, but reduced it at lower concentrations. On the contrary, the same red light reduced the growth of first internodes in all cases. The discrepancy between the results obtained with the two different sources of red light indicates that the growth of coleoptiles may be affected in opposite ways according to the kind of red light they receive. To avoid this interference, only green light of the proper wavelength should be used.

6) Effect of Presoaking: We have tried to wash coleoptile and internode sections before placing them in the auxin solutions. It became immediately apparent that one hour washing in glass distilled water increases the response of the sections of both coleoptiles and internodes (table IV). It was reasoned that the system responsible for the inactivation of auxins at cut surfaces was in some way washed away. A series of possible inhibitors of this system and of reducing agents was tried (table IV). It was quickly found that sucrose, 2,3,5-triiodobenzoic acid or manganese would markedly increase the response of coleoptile tissues to IAA. The optimum concentration of Mn^{++} (as the sulfate) was about 5.9×10^{-6} M. The effects of Mn^{++} and sucrose given in pretreatment were not additive. The stimulatory effect of Mn^{++} ions on the growth of coleoptiles is well known (8, 10, 20, 32), but this seems to be the first instance demonstrating that a mere pretreatment with manganese increases the auxin response. The duration of the presoaking period is important. As figure 10 shows, the response of coleoptile sections to IAA decreases steadily as the presoaking time is extended over 3 hours. This is also true in buffer (graphs A and B, squares, fig 10). Sucrose, on the contrary, keeps the reactivity of the sections up and seems even to in-

TABLE IV

Effect of Various Pretreatments on the Response to IAA

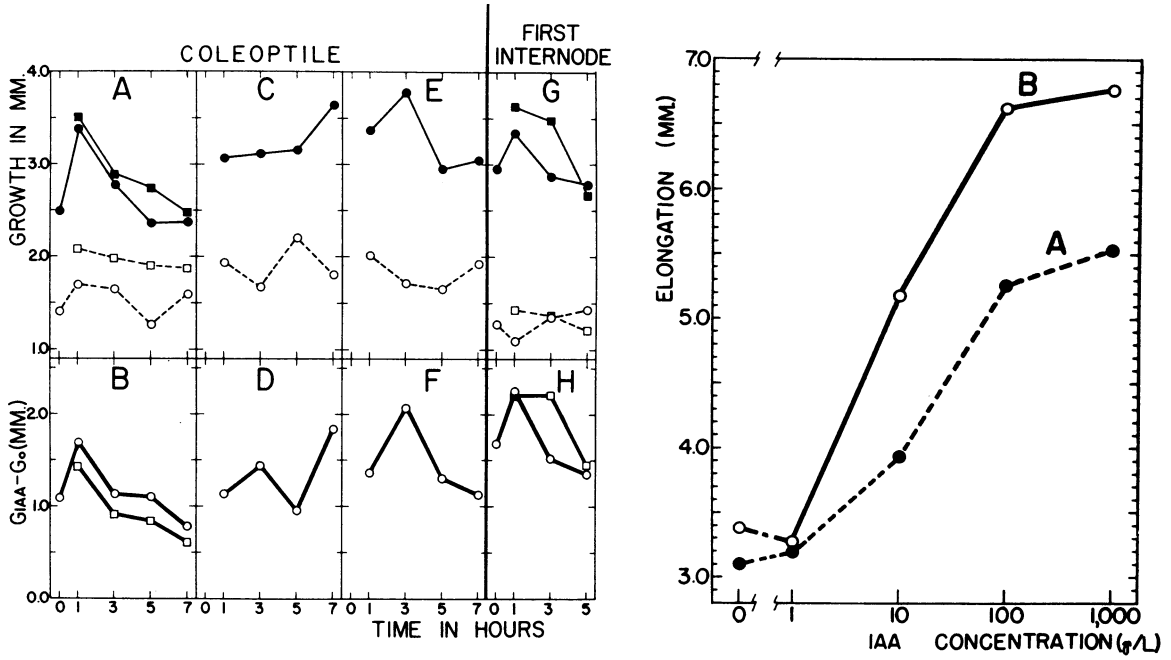
Plant part	Pretreatment	Time	Growth of controls	IAA conc	Growth in IAA	$G_{IAA} - G_o$
		hrs	*mm*	*μgm/l*	*mm*	*mm*
Oat first internodes	None	0	1.27	10	2.95	1.68
	H_2O	1	1.09	"	3.34	2.25
id	H_2O, floating	3	1.30	"	3.25	1.95
	" immersed	"	1.77 *	"	2.28	0.51
	2 % sucrose, floating	"	1.40	"	3.24	1.84
	" immersed	"	1.58	"	3.43	1.85
Oat coleoptiles	None	0	1.40	50	2.49	1.09
	H_2O	1	1.69	"	3.38	1.69
id	H_2O	3	2.00	"	2.47	0.47
	Ascorbic acid 10^{-5} M	"	1.74	"	2.78	1.04
	" 10^{-4} M	"	2.30	"	2.70	0.40
	Glutathione 10^{-4} M	"	2.22	"	2.65	0.43
	$Na_2S_2O_4$ 0.05 mg/l	"	1.99	"	2.39	0.40
	" 0.5 mg/l	"	2.26	"	2.47	0.21
id	H_2O	5	1.88	"	2.72	0.84
	$MnSO_4 \cdot H_2O$ 0.1 mg/l	"	3.00	"	3.98	0.98
	" 1 mg/l	"	2.54	"	4.64	2.10
	" 10 mg/l	"	1.93	"	3.46	1.53
id	$CoCl_2$ 0.01 mg/l	3	1.74	"	2.17	0.43
	" 0.1 mg/l	"	1.80	"	2.19	0.39
	" 1 mg/l	"	1.39	"	1.98	0.59
id	H_2O	3	1.22	"	1.90	0.68
	Sucrose 2 %	"	1.85	"	2.19 **	0.34 **
	$MnSO_4 \cdot H_2O$ 1 mg/l	"	1.59	"	2.57	0.98
	$MnSO_4 \cdot H_2O$ (1 mg/l) + sucrose 2 %	"	1.79	"	2.59	0.80
	2,3,5-Triiodobenzoic acid 0.05 mg/l	"	1.64	"	3.12	1.48
	" 0.5 mg/l	"	1.81	"	2.10	0.29
	Phenylacetic acid 1 mg/l	"	1.52	"	1.83	0.31
	" 10 mg/l	"	1.75	"	2.33	0.58
	" 100 mg/l	"	0.58 †	"	0.52 †	- 0.06

Ten 4-mm sections were used in each case. The sections were always breaking the surface, unless otherwise indicated. Standard deviation from the mean: 0.10 to 0.15 mm for first internodes, 0.15 to 0.25 mm for coleoptiles.

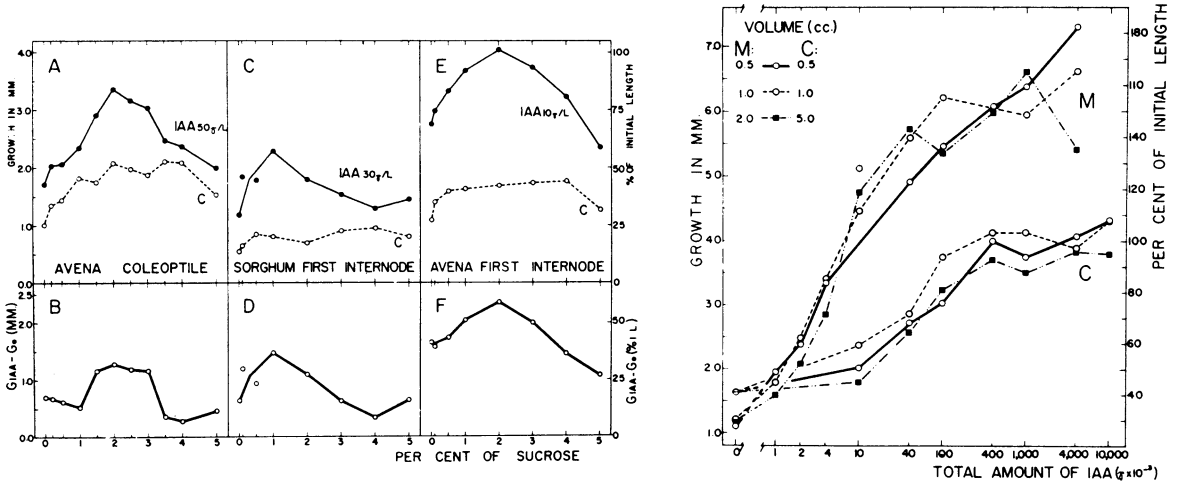
* Immersion in water increases the length of the controls somewhat, but the sections become more slender than when they are floated.

** In this particular series, the response to sucrose was abnormally low. It is generally of the order of the response to $MnSO_4$.

† The sections were dead at the end of the experiment.



FIGS. 10 to 11. FIG. 10 (*left*). Effect of various presoaking treatments on the growth of sections in buffer + sucrose without added IAA (dotted lines) and with IAA (solid lines). The IAA concentration was 50 μgm/l in the case of coleoptiles and 10 μgm/l for first internodes. The abscissa represent the time of presoaking before putting the sections in the final solutions with and without IAA. Curves A and B. Presoaking in water (circles) and in buffer (squares). Curves C and D. Presoaking in 2% sucrose. Curves E and F. Presoaking in $MnSO_4 \cdot H_2O$ (1 mg/l). Curves G and H. Presoaking in water (circles) and in buffer (squares). FIG. 11 (*right*). Effect of presoaking in glass-distilled water on the response to various IAA concentrations of 4-mm sections of wheat coleoptiles (var. Genesee). Black circles, dotted line—no presoaking. White circles, solid line—presoaking in water for 3 hours.

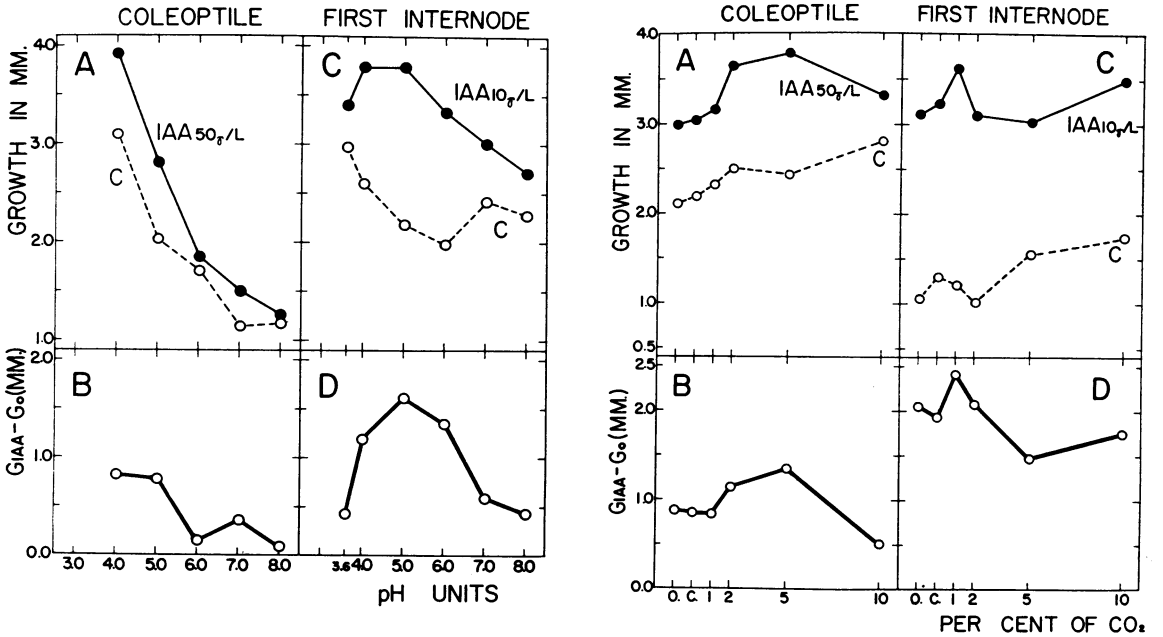


FIGS. 12 to 13. FIG. 12 (*left*). Effect of sucrose concentration on the growth of sections in buffer without added IAA (white circles, dotted lines) and with IAA (black circles, solid lines), and on the difference between these growths (curves B, D, and F). Scales in mm (*left*) and in % of the initial 4-mm length (*right*). FIG. 13 (*right*). Effect of the volume of liquid on the growth in various amounts of IAA of oat first internode sections (curves M) and coleoptile sections (curves C). The coleoptile sections were presoaked for 3 hrs in $MnSO_4 \cdot H_2O$ (1 mg/l) and the internode sections for 1 hr in H_2O. The internode sections were assayed in volumes of 0.5, 1.0 and 2.0 ml, the coleoptile sections in volumes of 0.5, 1.0 and 5.0 ml (buffer + sucrose + Tween 80, 0.1%). The growth of the controls of all three series is the same for first internodes and almost the same for coleoptiles.

crease it after 7 hours (graphs C and D), whereas the optimum presoaking time with manganese seems to be 3 hours (graphs E and F). In the case of first internodes, it is important that the sections be maintained so as to break the surface of the liquid; otherwise, their reactivity is decreased, except when sucrose is present (table IV). In the light of these findings, it was decided that the coleoptile sections would always be presoaked for 3 hours in 1 mg/l of $MnSO_4 \cdot H_2O$ and the internode sections for 1 hour in glass-distilled water. The response of wheat coleoptile sections to IAA is also markedly increased by a preliminary soaking period (see fig 11). In this case, floating the sections for 3 hours in water gave as good a result as floating them on solutions containing various manganese salts.

7) Effect of the Volume of Solution: If one desires a very sensitive test, one has to work not only with low concentrations but also with small volumes. The limiting factor, in the work with plant extracts, is the total amount of auxin available. Starting with given amounts of IAA, we have dissolved them in 0.5, 1.0, 2.0 ml (internodes) and 0.5, 1.0 and 5.0 ml (coleoptiles) of buffer + sucrose + "Tween 80" (0.1 %). The latter substance facilitates the dissolution of IAA in water and does not interfere with the growth of the sections; in fact, it favors it slightly. The coleoptiles were first presoaked 3 hours in manganese sulfate and the internodes 1 hour in water. The results, given in figure 13, demonstrate that there is hardly any benefit gained by using volumes larger than 0.5 ml. Large volumes have been reported to decrease the growth of the controls, perhaps because of the loss of some food factor (29). As figure 13 shows, this effect is completely eliminated when: 1) the sections are washed in large volumes of solutions before the actual test, and 2) sucrose is added to the test solutions. Accordingly, 0.5-ml volumes were used throughout this work and care was taken that no evaporation would take place during the assays. It should be mentioned also that, in the case of coleoptiles, we used flat bottom dishes instead of small beakers whose dome-shaped bottoms do not give a uniform depth of liquid with 0.5 ml.

8) Effect of Sucrose: Sugars, especially sucrose, have been shown by many authors to increase the response of oat coleoptile sections to IAA. On the contrary, Schneider (30) has claimed that sugar increases only slightly the growth of oat first internodes. We have re-examined the effect of sucrose on the elongation of both coleoptile and internode sections using always a series with and a series without IAA for each sucrose concentration. The results (fig 12) show that sucrose concentrations as low as 0.1 % stimulate growth of both the IAA-grown and the control sections. Moreover, it is clear that sucrose has a marked effect on the elongation of first internodes of sorghum and oat. This is at variance with Schneider's results, but it must be remembered that his testing conditions were different from ours (in particular, he used a dif-



Figs. 14 to 15. Fig. 14 (*left*). Effect of pH on the growth of sections in buffer + sucrose without IAA (white circles, dotted lines) and with IAA (black circles, solid lines), and on the difference between the two (curves B and D). Fig. 15 (*right*). Effect of the CO_2 concentration on the growth of 4-mm sections in buffer + sucrose without added IAA (white circles, dotted lines) and with IAA (black circles, solid lines), and on the difference between the two (curves B and D). On the abscissa, O. means open tubes, C. means closed tubes. Of course, the increasing concentrations of added CO_2 were done in closed tubes. Percent CO_2 actually means % of added CO_2. The CO_2 was added a few minutes after the sections had been introduced into the tubes.

ferent oat variety). The sucrose concentration which gives the largest differential response to IAA at low concentrations is 2 % for both oat coleoptiles and first internodes. In the case of sorghum internodes, it seems to be 1 % (fig 12). Wheat coleoptiles also respond well to sucrose; 0.5 % already causes a clear cut increase in length, but the optimum concentration is 2 %.

9) Effect of a Buffer: It is well known that the response of plant tissues depends on the number of undissociated auxin molecules present in the medium. Since an auxin such as IAA is a weak acid, it is important, in a quantitative test, to keep constant the concentration of undissociated molecules of such a substance. This can be done by regulating the pH of the solution. The effect of pH on the response of oat coleoptiles and first internodes to the same overall concentration of IAA is given in figure 14. Elongation decreases markedly as pH increases. Since the controls without added IAA also grow more at low pH's, the difference $G_{IAA} - G_0$ may not necessarily increase with decreasing pH. Figure 14 indicates that this difference is maximum at pH 5.0 for first internodes and close to this value for coleoptiles. A pH of 5.0 was, accordingly, chosen for all our experiments. Of course, the type of buffer used influences also the growth response. We employed here potassium phosphate-citrate buffers. They were chosen for the following reasons: 1) potassium stimulates auxin-induced growth (10, 37); 2) organic acids such as citric acid stimulate also the auxin-induced growth (36); 3) phosphate-citrate buffers have a good buffering capacity around pH 5.0. The effect of buffer concentration on the auxin-induced growth was also investigated (fig 9). In the case of oat coleoptiles, a concentration of IAA of 50μgm/l was used, whereas it was only 10 μgm/l in the case of sorghum and oat first internodes. A concentration of 10^{-3} M of the buffer slightly stimulates growth in coleoptiles, but higher concentrations rapidly inhibit it; in the case of internodes, the optimum lies around 10^{-2} M. Fearing that a 10^{-3} M concentration would not buffer adequately plant extracts separated on paper chromatograms, a concentration of about 10^{-2} M was used, the composition of this buffer being that previously reported (25), namely: K_2HPO_4 1.794 gm/l (about 10^{-2} M) + citric acid monohydrate 1.019 gm/l (about 5×10^{-3} M).

10) Effect of Inorganic Ions: Among the inorganic compounds which have been reported to increase auxin-induced growth are manganese (8, 10, 20, 32, 35), cobalt (22, 33, 35) and perhaps boron (16, 20). Table V shows that manganese sulfate definitely increases the growth of oat coleoptiles, but

Table V

Action of Inorganic Compounds

Plant part	Pretreatment	Solutions	Growth of controls	IAA conc	Growth in IAA	$G_{IAA} - G_0$
			mm	*μgm/l*	*mm*	*mm*
Oat coleoptile	H_2O, 5 hrs	Buffer + sucrose	1.94	100	2.91	0.97
	"	id + $MnSO_4 \cdot H_2O$ (1 mg/l)	2.43	100	4.30	1.87
	$MnSO_4 \cdot H_2O$, 5 hrs	Buffer + sucrose	2.27	100	4.02	1.75
Oat internode	H_2O, 1 hr	Buffer + sucrose	1.00	3	1.14	0.14
	"	id + $MnSO_4 \cdot H_2O$ (1 mg/l)	0.95	3	1.30	0.35
	"	Buffer + sucrose	1.06	10	3.11	2.05
	"	id + $MnSO_4 \cdot H_2O$ (1 mg/l)	1.10	10	2.74	1.64
	"	Buffer + sucrose	1.01	10	2.03	1.02
	"	id + $MnSO_4 \cdot H_2O$ 10 μgm/l	...	10	2.70	...
	"	100 μgm/l	...	10	2.55	...
	"	1,000 μgm/l	...	10	2.47	...
Oat internode	H_2O, 1 hr	Buffer + sucrose	2.23	10	2.89	0.66
	"	id + $CoCl_2$ 0.001 mg/l	1.14	10	2.27	1.13
	"	0.1 mg/l	1.23	10	2.65	1.42
	"	Buffer + sucrose	1.01	10	2.03	1.02
	"	id + $CoCl_2$ 0.01 mg/l	...	10	2.21	...
	"	0.1 mg/l	...	10	2.05	...
	"	1.0 mg/l	...	10	2.65	...
Oat coleoptile	$MnSO_4 \cdot H_2O$ (1 mg/l) 3 hrs	Buffer + scurose	2.10	50	3.88	1.78
	"	id + boric acid 0.1 mg/l	2.43	50	2.38	- 0.05
	"	1 mg/l	2.36	50	2.08	- 0.28
	"	10 mg/l	1.91	50	2.18	0.27
Oat internode	H_2O, 1 hr	Buffer + sucrose	1.40	10	3.53	2.13
	"	id + boric acid 0.1 mg/l	2.02	10	3.20	1.18
	"	1 mg/l	1.60	10	3.05	1.45
	"	10 mg/l	2.08	10	3.02	0.94

Ten 4-mm sections were used in all cases. Standard deviation from the mean: 0.15 to 0.25 mm for coleoptiles, 0.10 to 0.15 mm for first internodes.

TABLE VI

EFFECT OF SOME ORGANIC ACIDS IN RESPONSE TO IAA

COMPOUND	CONC	COLEOPTILES				FIRST INTERNODE			
		GROWTH OF CONTROLS	IAA CONC	GROWTH IN IAA	$G_{IAA} - G_0$	GROWTH OF CONTROLS	IAA CONC	GROWTH IN IAA	$G_{IAA} - G_0$
		mm	*μgm/l*	*mm*	*mm*	*mm*	*μgm/l*	*mm*	*mm*
Buffer + sucrose		2.23	50	3.24	1.01	1.10	10	1.87	0.77
id + Na fumarate	5×10^{-5} M	2.32	"	3.05	0.73	0.82	"	1.99	1.17
id + "	5×10^{-4} M	2.44	"	3.15	0.71	1.52	"	1.99	0.47
id + Na malate	5×10^{-5} M	2.46	"	3.11	0.65	0.85	"	1.92	1.07
id + "	5×10^{-4} M	2.08	"	3.18	1.10	1.33	"	1.73	0.40
id + Na acetate	5×10^{-5} M	2.12	"	3.01	0.89	1.17	"	1.99	0.82
id + "	5×10^{-4} M	1.86	"	2.97	1.11	0.82	"	1.79	0.97

Ten 4-mm oat sections were used in all cases. The coleoptiles were presoaked for 3 hours in $MnSO_4 \cdot H_2O$ (1 mg/l), the first internodes for 1 hr in H_2O. They were then put in the test solutions which always contained the phosphate-citrate buffer described in the text (5×10^{-3} M of citric acid) and 2 % sucrose, with and without IAA. Standard deviation from the mean: 0.15 to 0.25 mm for coleoptiles, 0.10 to 0.15 mm for first internodes.

not always that of first internodes. Furthermore, it shows that a mere pretreatment with Mn^{++} ions before the addition of IAA also increases the growth of coleoptiles. The optimum concentration for such a pretreatment seems to be 1 mg/l of $MnSO_4 \cdot H_2O$ (see table IV), a concentration a hundred times less than that proposed by Bonner (8) in conjunction with arginine and high concentrations of IAA. Cobalt ions and boric acid gave variable results. Without added IAA, boric acid increased growth slightly, but decreased it somewhat in presence of this auxin.

11) EFFECT OF CO_2: Molliard (24) reported that 2 % CO_2 increases the elongation of lupin hypocotyls and pea stems in the light and in the dark. More recently, Yamaki (45) has found that the addition of IAA to oat coleoptile sections causes an immediate uptake of CO_2. We have also studied the action of various concentrations of CO_2 on the auxin-induced growth of the oat coleoptile and first internode. All the experiments were done in the presence of a buffer at pH 5.0, so that an acidification of the medium cannot be responsible for the results obtained. One series of experiments seemed to indicate that CO_2 increases somewhat the growth response to auxin, the optimum concentration being in the neighborhood of 5 % for coleoptiles and 1 % for first internodes (fig 15). However, the results which we have obtained, admittedly with the CO_2 added a few minutes after the IAA, were not always consistent.

12) EFFECT OF VARIOUS ORGANIC COMPOUNDS: We have examined a certain number of organic substances which may increase the auxin-induced growth. We can group some of them under the headings of organic acids, amino acids, vitamins.

An effect of organic acids was not expected since the buffer in which the sections are grown already

TABLE VII

EFFECT OF SOME AMINO ACIDS AND OF GLUTATHIONE ON THE RESPONSE TO IAA

COMPOUND	CONC	COLEOPTILES				FIRST INTERNODES			
		GROWTH OF CONTROLS	IAA CONC	GROWTH IN IAA	$G_{IAA} - G_0$	GROWTH OF CONTROLS	IAA CONC	GROWTH IN IAA	$G_{IAA} - G_0$
		mm	*μgm/l*	*mm*	*mm*	*mm*	*μgm/l*	*mm*	*mm*
Buffer + sucrose		2.03	50	2.81	0.78	0.92	10	1.89	0.97
id + L-arginine HCl	10^{-4} M	2.20	"	4.19	1.99	0.95	"	2.37	1.42
id + "	10^{-3} M	2.26	"	2.58	0.32	0.81	"	2.41	1.60
Buffer + sucrose		3.74	"	4.46	0.72	0.86	"	2.10	1.24
id + L-methionine	10^{-5} M	3.41	"	4.60	1.19	0.90	"	2.18	1.28
id + "	10^{-4} M	3.37	"	4.67	1.30	0.84	"	2.21	1.37
id + "	10^{-3} M	4.92	"	4.88	−0.04	0.76	"	2.37	1.61
Buffer + sucrose		2.23	"	3.24	1.01	1.10	"	1.87	0.77
id + glutamic acid	10^{-4} M	2.00	"	2.86	0.86	0.82	"	2.07	1.25
id + "	10^{-3} M	2.85	"	4.37	1.52	1.17	"	2.23	1.06
Buffer + sucrose		1.99	"	2.99	1.00	0.67	"	1.90	1.23
id + glutathione	10^{-4} M	2.28	"	2.65	0.37	0.75	"	1.73	0.98
id + "	10^{-3} M	2.56	"	3.94	1.38	0.74	"	2.32	1.58
id + "	3×10^{-3} M	2.89	"	5.03	2.14	0.89	"	2.24	1.35

Conditions as for table VI.

TABLE VIII

EFFECT OF VARIOUS VITAMINS ON THE RESPONSE TO IAA

COMPOUND	CONC	COLEOPTILES				FIRST INTERNODES			
		GROWTH OF CONTROLS	IAA CONC	GROWTH IN IAA	$G_{IAA} - G_0$	GROWTH OF CONTROLS	IAA CONC	GROWTH IN IAA	$G_{IAA} - G_0$
		mm	*μgm/l*	*mm*	*mm*	*mm*	*μgm/l*	*mm*	*mm*
Buffer + sucrose		2.23	50	3.24	1.01	1.10	10	1.87	0.77
id + thiamin HCl	10^{-5} M	2.15	"	3.12	0.97	0.97	"	1.54	0.57
id + "	10^{-4} M	2.28	"	3.32	1.04	0.96	"	1.99	1.03
id + riboflavin	10^{-6} M	2.18	"	3.51	1.33	1.48	"	1.72	0.24
id + "	10^{-5} M	1.99	"	3.13	1.14	1.01	"	1.80	0.79
id + Vitamin K_1	10^{-7} M	2.16	"	3.11	0.95	0.89	"	1.76	0.87
id + "	10^{-6} M	2.35	"	3.52	1.17	1.15	"	2.09	0.94
id + "	10^{-5} M	1.88	"	3.93	2.05	1.33	"	2.23	0.90
id + biotin	10^{-7} M	2.54	"	2.82	0.28	0.93	"	1.85	0.92
id + "	10^{-6} M	2.17	"	2.85	0.68	1.04	"	1.83	0.79
id + "	10^{-5} M	2.37	"	2.90	0.53	1.15	"	1.96	0.81
Buffer + sucrose		1.99	"	2.99	1.00	0.67	"	1.90	1.23
id + Ca pantothenate	10^{-6} M	2.31	"	2.82	0.51	0.78	"	1.76	0.98
id + "	10^{-5} M	1.99	"	2.87	0.88	0.65	"	1.91	1.26
id + folic acid	10^{-6} M	2.14	"	2.69	0.55	0.68	"	1.83	1.15
id + "	10^{-5} M	2.17	"	2.71	0.54	0.78	"	1.47	0.69
id + ascorbic acid	10^{-6} M	2.15	"	2.60	0.45	0.71	"	2.07	1.36
id + "	10^{-5} M	2.27	"	2.49	0.22	0.71	"	1.68	0.97
id + "	10^{-4} M	1.97	"	2.45	0.50	0.73	"	1.72	0.99
Buffer + sucrose		3.74	"	4.46	0.72	0.86	"	2.10	1.24
id + choline chloride	10^{-5} M	3.40	"	4.24	0.84	0.84	"	1.90	1.06
id + "	10^{-4} M	3.22	"	4.25	1.03	0.99	"	2.65	1.66
id + "	10^{-3} M	3.19	"	4.36	1.17	0.99	"	1.99	1.00

Conditions as for table VI.

contains about 5×10^{-3} M of citric acid. The results actually obtained did not show any real additional effect of fumarate, malate or acetate (table VI) which are the most stimulatory compounds of this group, as reported by Thimann and Bonner (36). Addition of calcium pantothenate in conjunction with acetate was without effect.

Among amino acids, arginine, methionine and glutamic acid, reported by Bonner (8) to increase the growth of the oat coleoptile, were very active on cole-

TABLE IX

EFFECT OF MISCELLANEOUS ORGANIC COMPOUNDS ON THE RESPONSE TO IAA

COMPOUND	CONC	COLEOPTILES				FIRST INTERNODES			
		GROWTH OF CONTROLS	IAA CONC	GROWTH IN IAA	$G_{IAA} - G_0$	GROWTH OF CONTROLS	IAA CONC	GROWTH IN IAA	$G_{IAA} - G_0$
		mm	*μgm/l*	*mm*	*mm*	*mm*	*μgm/l*	*mm*	*mm*
Buffer + sucrose		3.74	50	4.46	0.72	0.86	10	2.10	1.24
id + adenine sulfate	10^{-5} M	3.27	"	4.85	1.58	0.78	"	2.48	1.70
id + "	10^{-4} M	4.14	"	4.23	0.09	0.78	"	2.42	1.64
id + "	10^{-3} M	4.14	"	5.39	1.25	1.00	"	2.23	1.23
id + hypoxanthine	10^{-5} M	3.57	"	4.54	0.97	1.04	"	2.28	1.24
id + "	10^{-4} M	3.27	"	4.40	1.13	0.76	"	2.03	1.27
Buffer + sucrose		2.10	"	3.88	1.78	1.40	"	3.53	2.13
id + pectic acid	10 mg/l	2.23	"	2.90	0.77	1.60	"	3.15	1.54
id + "	100 mg/l	2.88	"	3.11	0.23	1.40	"	3.28	1.88
Buffer + sucrose		1.88	100	2.95	1.07	1.22	"	2.29	1.07
id + gibberellin X *	1 μgm/l	2.92	"	3.42	0.50	2.34	"	3.15	0.81
id + "	10 μgm/l	3.58	"	3.69	0.11	2.17	"	3.57	1.40
id + "	100 μgm/l	3.72	"	3.89	0.17	2.33	"	3.90	1.57
id + "	1,000 μgm/l	3.57	"	4.52	0.95	2.77	"	3.80	1.03

Conditions as for table VI.

* Kindly supplied by Dr. F. H. Stodola, U. S. Department of Agriculture, Peoria, Illinois.

optiles, somewhat less on first internodes (table VII). In fact, it seems that these amino acids by themselves, at high concentrations (10^{-3} M), stimulate the growth of coleoptiles without added IAA. This situation may create artifacts in the case of chromatograms of aqueous plant extracts, these amino acids acting like auxins. Fortunately, these substances alone are practically without effect on first internodes. Arginine, given only as a 3-hour pretreatment, did not have a marked effect on the growth of oat coleoptiles.

Glutathione at high concentrations (10^{-3} to 3×10^{-3} M) caused a large increase in the response to IAA of coleoptiles, somewhat less in first internodes. Part of this effect may be ascribed to its glutamic acid content since this latter substance is also active.

A series of vitamins were tried also. Some of the results obtained are listed in table VIII. Slight stimulations or inhibitions of growth were observed. For example, Vitamin K_1, stimulated growth somewhat and so did choline chloride in some experiments not reported here. On the whole, however, the vitamins tried here did not show any striking interference with the response to IAA.

Finally, among other miscellaneous organic compounds tested, adenine sulfate had some stimulatory effect, although not consistently. Hypoxanthine and pectic acid were without action, but crystalline gibberellin X had some auxin activity (table IX). A much longer list of compounds active on the growth of oat coleoptiles can be found in Avery and Sargent (3).

13) The Action of a Few Natural Auxins and Related Compounds: Having investigated a large number of variables affecting the response of the coleoptile and of the internode tests to IAA, we could then try to establish standard curves correlating growth response with auxin concentrations. In all cases, the coleoptile sections were presoaked in manganese and the first internode sections in H_2O. Then, the sections were incubated in various concentrations of the auxin to be tested with the phosphate citrate buffer described in section 9, 2 % sucrose and 0.1 % "Tween 80" to facilitate the dissolution of water-insoluble compounds such as the ethyl ester of IAA (IAE). If the results obtained with IAA are plotted on a semi-logarithmic graph (fig 16), it can be seen immediately that the response of the oat internodes to IAA is much more dramatic than that of coleoptiles. It can be seen further that the threshold of response is around $1 \times 5 \times 10^{-9}$ moles of IAA in 0.5 ml which is 0.875 μgm/l in that volume or about 4.3×10^{-4} μgm of actual IAA. This is about the threshold of sensitivity of the standard Avena curvature test. Finally, we can also see on figure 16 that the range of proportionality between elongation of internodes and IAA concentration is large (at least a hundred fold). The disadvantage is that the growth is proportional to the logarithm of the concentration, which diminishes the accuracy, as compared with the Avena curvature test in which the curvature is a linear function of the concentration.

How does the response to other auxins compare with that to IAA? Indoleacetonitrile (IAN), the ethyl ester (IAE) and the amide (IAam) of IAA were tried, as well as cis-cinnamic acid. Figures 17 to 20 give the results of these experiments. They show that, on a molar concentration basis, IAA, IAN, and IAE have similar activities, although, at certain concentrations, IAN and, especially IAE, are slightly more active than IAA. The results concerning IAN are surprising, considering the reports by Bentley and Housley (5) and Thimann (34) who found that IAN was at least 10 times more active than IAA on oat

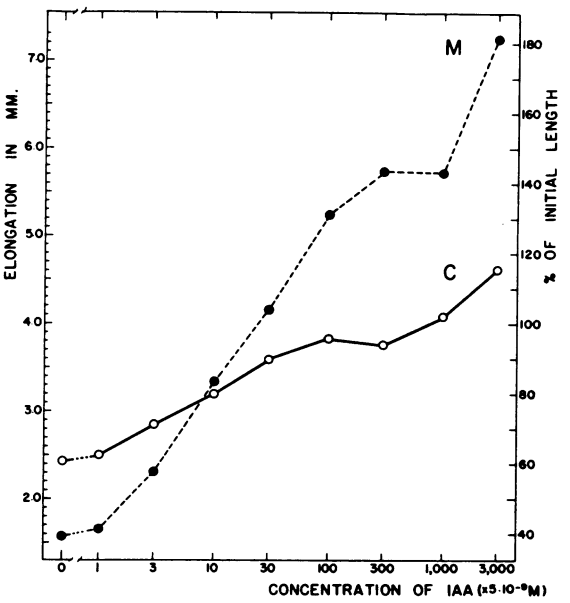
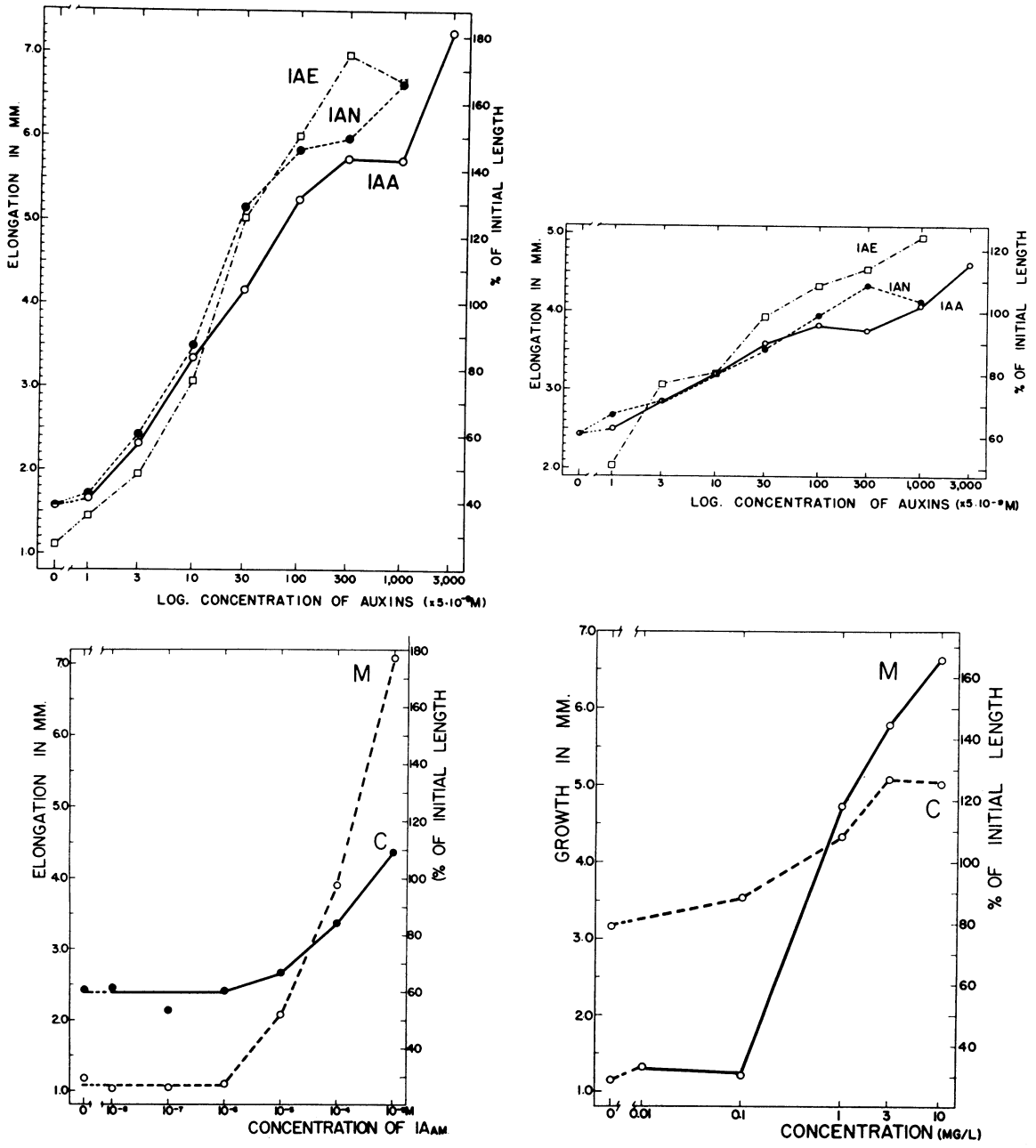


Fig. 16. Growth curves of oat coleoptile (C) and first internode (M) sections in buffer + sucrose + Tween 80 (0.1 %) with various concentrations of IAA. The scale at left gives the growth in mm over the initial 4 mm; the scale at right gives it in percent of the original length. The scale giving the IAA concentrations is logarithmic. The coleoptile sections had been floated 5 hrs on $MnSO_4 \cdot H_2O$ (1 mg/l) and the internode sections 1 hr on H_2O prior to being put into the auxin solutions in which they were left for about 20 hrs.

coleoptiles. This discrepancy may be due to a number of factors; difference in the variety of oat used, difference in the presoaking technique, the buffer, sucrose and Tween, etc. As far as IAam is concerned, it seems to show appreciable activity only at concentrations higher than 5×10^{-6} M in both coleoptiles and first internodes and seems to be from 50 to 100 times less active than IAA (fig 19). The lowest concentration of cis-cinnamic acid which gives an appreciable response seems to be 0.1 mg/l for coleoptiles (fig 20) which is about 6.7×10^{-7} M. Trans-cinnamic acid as well as its esters and hydroxy-derivatives such as ferulic and caffeic acids, were inactive on oat first internodes.



FIGS. 17 to 20. FIG. 17 (*top, left*). Growth curves of oat first internode sections in buffer + sucrose + Tween 80 (0.1 %) and various molar concentrations of indoleacetic acid (IAA), indoleacetonitrile (IAN) and the ethyl ester of IAA (IAE). Scales as in fig 16. The sections had been floated for 1 hr on H_2O before being put in the auxin solutions. FIG. 18 (*top, right*). Growth curves of oat coleoptiles (var. Brighton) in buffer + sucrose + Tween 80 (0.1 %) and varying molar concentrations of IAA, IAN, and IAE. The sections had been floated for 5 hrs on $MnSO_4 \cdot H_2O$ (1 mg/l) before being put in the auxin solutions. Scales as in fig 16. FIG. 19 (*bottom, left*). Growth curves of oat coleoptile (C) and first internode (M) sections in buffer + sucrose + Tween 80 (0.1 %) with various concentrations of indole-3-acetamide (IAam). The coleoptile sections had been pretreated for 3 hrs in $MnSO_4 \cdot H_2O$ (1 mg/l) and the internode sections for 1 hr in H_2O. FIG. 20 (*bottom, right*). Growth curves of oat coleoptile (C) and first internode (M) sections in buffer + sucrose + Tween 80 (0.1 %) with various concentrations of cis-cinnamic acid (1 mg/l = 6.7×10^{-6} M). Pretreatments as in fig 19.

Conclusions

Procedure for the Sensitized Coleoptile Cylinder Test: The steps can be summarized as follows:

1) Soak in tap water oat seeds, var. Brighton, or wheat seeds, var. Genesee, for 2 hours at room temperature.

2) Lay these seeds in parallel rows, the embryos up and pointing in the same direction, on several layers of wet facial tissue in glass or enamel trays.

3) Expose these trays for 3 to 4 hours to red light at 25° C. This step is omitted in the case of wheat seeds.

4) Grow the seeds at 25° C in a moist chamber in the dark for 3 days, the trays being tilted at about a 45° angle, the embryos pointing down. This procedure gives coleoptiles which are straight (G. Curry, private communication).

5) When the coleoptiles reach about 2.5 cm in length, select coleoptiles of equal lengths, cut 4 mm sections, 3 mm below the tip. Leave the primary leaf inside the sections.

6) Float these sections for 3 hours on glass-distilled water containing 1 mg/l of $MnSO_4 \cdot H_2O$. In the case of wheat, glass-distilled water alone is sufficient.

7) Put ten sections in 0.5 or 1 ml of the solutions to be tested, which should contain 2 % sucrose plus a buffer at pH 5.0 (K_2HPO_4 1.794 gm/l + citric acid monohydrate 1.019 gm/l).

8) Incubate about 20 hours in the dark at 25° C. A gentle horizontal shaking may yield a better and more uniform growth. Rotating the tubes around a horizontal axis at about 1 rpm improves growth even more.

9) Measure the length of the sections under a binocular with ocular micrometer, or by projecting their shadows through a photographic enlarger, or by any other suitable means.

Procedure for the First Internode Test: 1) Soak in tap water oat seeds, var. Brighton, for 2 hours at room temperature in a closed, light-tight container.

2) Lay the seeds on about 1 inch of moist, clean sawdust (maple sawdust is good), and cover with about ½ inch of moist sawdust. These operations should be performed under dim light, preferably green light (546 mμ).

3) Grow the seeds at 25° C for 3 days (in sawdust) in complete darkness. From then on, all manipulations should be performed under green light.

4) When the first internodes reach about 2.5 cm in length, they are ready to be used. The coleoptiles, at that time, should be only 0.5 cm long. Longer coleoptiles indicate that the growing seedlings have received light of active wavelengths. Cut 4-mm sections, 2 mm below the coleoptilar node.

5) Wash the sections in glass-distilled water for 1 hour during which the sections should break the surface of the liquid, for example by laying on cheesecloth stretched on plastic rings.

6) Put ten sections per one 13 × 100 or 16 × 125 mm Pyrex tube with 0.5 or 1 ml of the solution to be tested containing the same buffer (1.794 gm/l of K_2HPO_4 + 1.019 gm/l of citric acid monohydrate) and the same sucrose concentration (2 %) as for coleoptiles.

7) Place each tube in an Erlenmeyer flask and rotate around a horizontal axis at about 1 rpm in the dark for about 20 hours, at 25° C.

8) Measure the length of the sections to 0.1 mm.

Value of the Tests Proposed: At the beginning of this article, we have enumerated the qualities that a good bioassay for auxins should have. Let us see if the two tests proposed meet these criteria. A major drawback of the ordinary straight growth coleoptile test was its relatively low sensitivity. By carefully studying the variables that affect this test, we are now able to detect 5 μgm/l of IAA in 0.5 ml instead of 10 μgm/l in 10 ml, which represents a forty fold increase in sensitivity. However, by using the first internode, we can reach a 200 fold increase in sensitivity over the cylinder test as described by Bentley (4). The proportionality range of these tests is good, although the semi-logarithmic relationship between growth and auxin concentration reduces the accuracy. The specificity for auxins is reasonable, but certain substances such as arginine, glutamic acid, methionine, glutathione, etc. also stimulate to some extent the growth of coleoptile sections. First internodes, on the whole, appear to be more specific in this respect. The reliability is good. It should be mentioned that the first internode test gives results that are more uniform statistically than the coleoptile test which has a higher standard error. The standard deviation from the mean is usually 0.10 to 0.15 for first internodes, 0.15 to 0.25 for coleoptiles. Finally, the tests are not too difficult to perform, although the results reflect the care taken in selecting uniform seedlings, cutting the sections exactly where they should be cut and of exactly the same length. The first internode test requires the rotating of the sections, however, but not a humidity-controlled room as does the Avena curvature test. The use of internodes does away with the problem of the primary leaf in coleoptiles. The first internodes have been reported to grow both by cell division and cell enlargement (2) but Schneider (30) concluded that the elongation of sections under the influence of IAA was due to cell enlargement almost exclusively. The first internode test has been used with success in the assay of numerous auxin chromatograms. In particular, it has enabled us to detect clearly the natural auxins present in an extract of as low as 300 mg of fresh plant material (26). Finally, the first internode test is much less sensitive to inhibitors than the coleoptile test, which enables it to clearly detect auxins in an impure extract in which substances inhibitory to coleoptiles would seriously interfere with the auxin picture.

Summary

After a preliminary screening for a good test object for the assay of auxins, the coleoptile of wheat and oat and the first internode of oat have been re-

tained. Among the factors affecting the magnitude of the response of these test objects to indole-3-acetic acid, the following have been studied: the location of the section on the seedling, the length of the initial section, the age of the seedling, the length of time in the auxin solution, the quality of light, the pretreatment in various solutions, the volume of the solution, the concentration of sucrose, the pH and concentration of a citrate-phosphate buffer, inorganic ions, CO_2 concentration, certain organic acids, amino acids, vitamins, auxins, etc. These investigations resulted in the working out of two tests: (a) a more sensitive oat or wheat coleoptile cylinder test, and (b) a new straight growth test using the oat first internode, which is as sensitive as the Avena curvature test of Went.

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PATHWAYS OF OXIDATION IN CELL-FREE POTATO FRACTIONS[1,2]

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Knowledge of the fate and mode of transport of the electrons involved in energy-yielding oxidations is essential for an understanding of physiological activity. Recently, we have studied this problem with special reference to terminal oxidases and plant growth (7, 8). Particular attention was paid to respiratory mechanisms in the potato tuber (31). In the present study, oxidative pathways in the potato were examined by determining the activities of enzymes in isolated cell fractions.

It is now generally assumed that respiration, both in higher plants and animals, is mediated by a series of electron carrier systems, including the pyridine nucleotides, flavoproteins, and cytochromes. Examination of the oxidation-reduction potentials of these systems indicates that the major portion of the energy that is available in organic substrates is released between the level of reduced pyridine nucleotide and molecular oxygen. In view of the importance of these reactions, an attempt was made to reconstruct the pathway of electron transfer from pyridine nucleotide to oxygen; succinate oxidation has also been examined. Studies of this type on isolated animal mitochondria (25) have revealed a characteristic electron pathway from pyridine nucleotide to flavin, thence via an unknown factor to the cytochrome system, and thence to oxygen. Many of these components have been demonstrated in plants, but evidence for the whole sequence of reactions in a single preparation has not been reported. Since plant mitochondria are centers of cellular respiration (16), this study deals primarily with activities associated with isolated particles; some experiments with the soluble fraction are also included. Enzyme activities were determined by following changes in absorption with a spectrophotometer.

MATERIALS AND METHODS

Potato tubers (*Solanum tuberosum*, var. Katahdin) were received monthly from Maine and stored at 10° C. The preparation of cell fractions was carried out in a 4° C cold room. Ten grams of peeled tissue were ground in a glass mortar with sand and an equal volume of 0.05 M phosphate buffer (pH 7.0) which was also 0.2 M with respect to sucrose. After filtering through cheesecloth, the homogenate was centrifuged at 2,000 × g for 10 minutes, the sediment discarded, and the supernatant centrifuged at 10,000 × g for 30 minutes. The resulting supernatant fraction (SF) was poured off; the sediment was washed by resuspending in 15 ml sucrose-phosphate buffer and recentrifuging at 10,000 × g for 30 minutes. The washed sediment was resuspended in 1 to 3 ml of sucrose-phosphate solution. This suspension of particulate material is referred to as the mitochondrial preparation (M_w), on the basis of the centrifugal

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