More work is needed to explore additional physiological and biochemical aspects of low temperature injury.

Summary

Chilled and non-chilled sweetpotato roots were compared with respect to leakage from tissues, and mitochondrial activity during 10 weeks of storage at 15 and 7.5° C (the chilling temperature).

There was approximately five times as much leakage from the chilled tissue slices as from the non-chilled tissue slices. Almost all the leakage consisted of potassium ions.

During the 1st four weeks, the mitochondria from chilled and non-chilled tissues, when assayed in complete systems at 25° C, showed approximately equivalent rates of oxidation and phosphorylation. However, after the 5th week there was a rapid decline in oxidative and phosphorylative activity in the chilled tissues and at the 10th week the mitochondria from the chilled tissues were practically inactive.

In the late stages of degeneration there was a build up of chlorogenic acid (and similar polyphenols) and a decline of ascorbic acid in the chilled tissue.

We gratefully acknowledge the assistance of A. Specht of the Plant Industry Station, who determined the potassium concentrations in the sucrose and water solutions surrounding the tissue slices.

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AUXIN ACTIVITY OF SOME INDOLE DERIVATIVES[1,2]

KENNETH V. THIMANN

The Biological Laboratories, Harvard University, Cambridge, Massachusetts

During the past 20 years, in numerous laboratories, very large numbers of compounds have been synthesized and tested for their activity as auxins or as modifiers of auxin action. The majority of these have been acids of the aryloxy series. Curiously enough, although indoleacetic acid (IAA) is the most widely occurring natural auxin, very few of the compounds tested have been indole derivatives. Kostermans in 1934 tested a number of indole derivatives, but only in the Avena curvature test with agar blocks, where the ability of the substance to be transported largely controls its activity. More recently,

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using Avena coleoptile sections in solution, Muir and co-workers (14, 15) tested 4-chloroindoleacetic acid and found it to have 80 % (15) or 140 % (14) of the activity of IAA; 2-methyl-IAA was found to have 1.5 % (14) or 45 % (15) of the activity of IAA, and introduction of two chlorine atoms into the ring did not raise this. Hansen (7) found 6-chloro-IAA more active in inhibiting root growth and root cell elongation than IAA. Hansen et al (8) and Fawcett et al (5) have tested indoleisobutyric acid (see below), and van de Westeringh (28) has shown that 3-indolemethylsulfonic and phosphonic acids and 3-indolemethyltetrazole have some activity, though unfortunately his data were not converted to relative activity or any other uniform quantitative measure. With the exception of these few cases, no systematic testing of indole derivatives has been carried out.

The present paper is a beginning at making up this deficiency; it reports tests which have been carried out at various times over a period of 8 years on 23 synthetic indole derivatives or closely related compounds. In a number of cases the results are of special interest for their bearing on current hypotheses or controversies. They illustrate again some of the striking specificities in the relation between structure and auxin activity.

Materials and Methods

Three bioassays have been employed: straight growth of 10-mm subapical sections of 72-hour Segrehaver Avena coleoptiles, straight growth of 20-mm sections from the immediately subapical part of the 3rd internode of etiolated 7-day-old Alaska pea stems grown in the presence of occasional weak red light, and curvature of longitudinally slit sections of the same pea stems.

Details of the methods have previously been given (21, 24, 25). Since the slit pea stem curvature is, on the whole, the least selective (25) it was generally used for the 1st screening. The Avena tests were carried out with 10 to 12 10-mm sections floating on 4 ml of solution in a 4-cm Petri dish. The solution contained 2 % sucrose, M/200 or M/1000 KCl or, in later work, potassium phosphate M/400 brought to pH 4.5 with HCl. $MnCl_2$ 10^{-4} M was added in a few of the earliest tests, and after 1954 (21) 3×10^{-5} M $CoCl_2$ was used.

The pea tests were usually carried out in 20 ml of solution in a Petri dish, without additions other than the test substance, but in a few instances M/100 KCl or buffer was added. Although the addition of $CoCl_2$ plus sucrose to pea sections increases the growth (sucrose alone does not do this under our conditions) the increment caused by addition of an auxin is not proportionately increased.

The elongation or curvature was routinely measured after 22 hours of gentle rotary shaking in darkness unless otherwise indicated. Some variation in the growth of controls between different experiments is due largely to the various small changes in technique which have been made during the 8-year period. Changes of a few tenths of a degree in the temperature of the dark room are also responsible for appreciable variations in growth rate.

Indoleacetic acid (IAA) in several concentrations was included in all assays, and wherever the activity of a compound was appreciable, it is expressed as percent of the activity of IAA, by interpolation on the IAA curve, as exemplified in tables III and IV. To simplify the formulae, the indole radical is presented as *Ind.* wherever possible.

The compounds tested and their sources were as follows: Indole, Eimer and Amend Co.; 5-methylindole, Sigma Chem. Co.; 6-methoxyindole, synthesized by Fritz E. Bader of Harvard; skatole, Eastman Kodak Co.; indole-3-glycolic acid, sample of Na salt supplied by Professor Marvin D. Armstrong and synthesized by K. N. F. Shaw of the University of Utah; indole-3-glyoxylic ethyl ester, melting point 188 to 189° C (higher than in the literature), synthesized by Bruce B. Stowe of Harvard; N-acetylindoxyl, samples from Bios Ltd. and Delta Chemical Co.; five oxindole derivatives, synthesized by J. B. Hendrickson of Harvard; 3-methyldioxindole, melting point 167 to 168° C, synthesized by Bruce B. Stowe of Harvard; α-indole-3-isobutyric acid, supplied by Professor H. Burström (3) and synthesized by G. N. A. Jönsson of the Royal Institute of Technology, Stockholm; 5- and 7-hydroxyindoleacetic acids, synthesized by A. Ek and B. Witkop (4); indole-3-lactic acid, melting point (sharp) 100° C, isolated from a culture of *Oidium lactis* on a tryptophane medium by Bruce B. Stowe of Harvard; indolemethylenemalonic acid and three imidazole derivatives, synthesized by Bernhard Witkop of the National Institutes of Health; indene-3-propionic acid, synthesized by Professor G.

Table I

Action of Indole Alone and in Combination with 2-Carbon Compounds on Growth of Avena Coleoptile Sections *

Control **	Plus indole 1×10^{-4} M	Plus addition	3×10^{-3} M	10^{-3} M	3×10^{-4} M	10^{-4} M
38.3	45.2	Glycolate	37.9	41.5	45.4	... ***
38.7	40.3	Chloracetate	6.4	17.2	32.0	34.6
38.7	40.3	Glycine	35.2	40.6	38.8	38.2

* All figures are elongation after 22 hours as percent of initial length.
** Sucrose 2 %, KH_2PO_4 pH 5.5 M/200, $CoCl_2$ 3×10^{-5} M.
*** No promotion in another experiment.

TABLE II

ACTION OF INDOLE ALONE AND IN COMBINATION WITH D-RIBOSE ON GROWTH OF AVENA COLEOPTILE SECTIONS *

CONCN OF INDOLE	CONCN OF RIBOSE		
	0	10^{-4} M	10^{-3} M
0	34.3 **	35.0	38.2
3×10^{-5} M	40.0	37.0	40.0
1×10^{-4} M	41.6	...	...
3×10^{-4} M	50.9	50.1	47.4

* All figures are elongation after 22 hrs as percent of initial length. Mean of 2 complete experiments.
** Control: sucrose 2 %, KH_2PO_4 M/200, pH 5.0, no $CoCl_2$.

R. Clemo of King's College, Newcastle-on-Tyne; indole-4-acetic acid, melting point 205° C, synthesized by H. Plieninger and K. Suhr (15a) and supplied by W. A. Andreae of Science Service Laboratory, London, Ontario; 7-azaindole-3-acetic acid, melting point 213 to 214° C, synthesized by M. M. and B. L. Robison (17) of Amherst College, Massachusetts.

The reference sample of IAA was from Hoffmann La Roche, Basel, Switzerland.

1. INDOLE: On a number of occasions a small but real growth-promoting effect of pure indole has been observed, particularly on Avena sections. For instance, in one test, controls in sucrose-KCl grew 45 % while sections in 3×10^{-4} M indole grew 66 %, an increase of more than one third. As a mean of four other experiments, controls grew 36.5 ± 1.7 % and sections in 3×10^{-4} M indole grew 48.7 ± 3.2 %, again an increase of one third. Other data appear in tables I and II. The optimum concentration of indole was found to be 3×10^{-4} M in all experiments.

Since indole is unlikely to be an auxin itself, it seemed reasonable to suppose that it might be acting as substrate for the in vivo synthesis of indoleacetic acid. As likely participants in such an in vivo reaction, three substituted acetic acids, namely glycolic acid, chloracetic acid and glycine were tested in combination with indole. However, table I offers no evidence to support the occurrence of a reaction of this type in Avena. The participation of D-ribose in such a synthesis (cf. the synthesis of indole in bacteria from diphospho-ribose and anthranilate (31)) is made improbable by table II.

It must be concluded that if indole is in fact converted by the plant to an auxin, then the material furnishing the side-chain is not any one of the substances tested. Greenberg et al (6) have demonstrated a non-enzymatic condensation of indole with glyoxylic acid, but their product was considered to be indole-3-glycolic acid, and this has only very low activity on Avena, as shown below.

The possibility remains that indole reacts in some way with the endogenous IAA, either protecting it from destruction or synergizing with it. Marked synergism between indole and IAA in root formation was observed by van Raalte (29), but in his experiments indole by itself was without effect.

2. 5-METHYLINDOLE AND 6-METHOXYINDOLE: Both of these compounds are inactive. At 10^{-4} M and above they were found to cause marked browning of Avena sections, especially in presence of $CoCl_2$.

3. SKATOLE: This compound bears the same relation to IAA as 2,4-dichloroanisole does to 2,4-D, namely it is the carboxyl-free analogue. In 1949 Bonner reported (2) that 2,4-dichloroanisole acted as an auxin antagonist. The deduction was made that the interaction between dichloroanisole and IAA, at IAA concentrations below 1 mg per liter, was competitive. However, the published data (table 11 of ref. (2)) show that there was an increase in the percentage of inhibition with increasing concentration of IAA and therefore do not support this claim. Later, Audus and Shipton (1) could find no evidence of competition, but McRae and Bonner (13) supplied more extensive data which did show competition both with IAA and with 2,4-D. However, since it is IAA, not 2,4-D, which is the natural auxin, it seemed much more worth-while to see whether the carboxyl-free analogue of IAA, namely skatole, had inhibiting action, and if so, whether it was competitive with IAA.

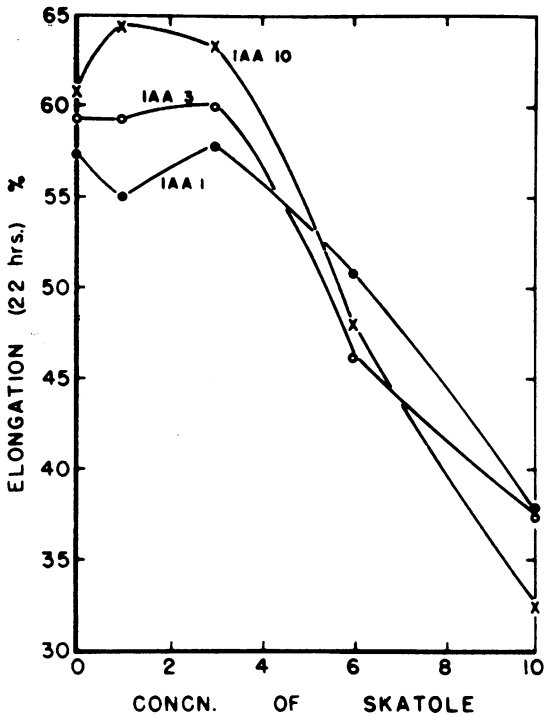
In preliminary experiments (which were reported in 1953 at the Auxin Conference held at Lund, Sweden) skatole was found to inhibit growth in all the test methods, the effective concentration range lying between 10^{-3} and 10^{-4} M. More extensive results with the straight growth of pea stem sections are summarized in figure 1 and data with pea curvature are shown in table III. The straight growth tests show that when the IAA concentration is raised from 1 to 10 mg per liter, neither the percentage of inhibition nor the absolute change in the elongation of the sections is decreased. In fact, the reverse is the case; if, for instance, we take skatole at 6 mg per liter, and calculate the decrease in growth as percent of the control, we find that when IAA is at 1 mg per liter the decreases are 11.5 and 10.3 % in two tests,

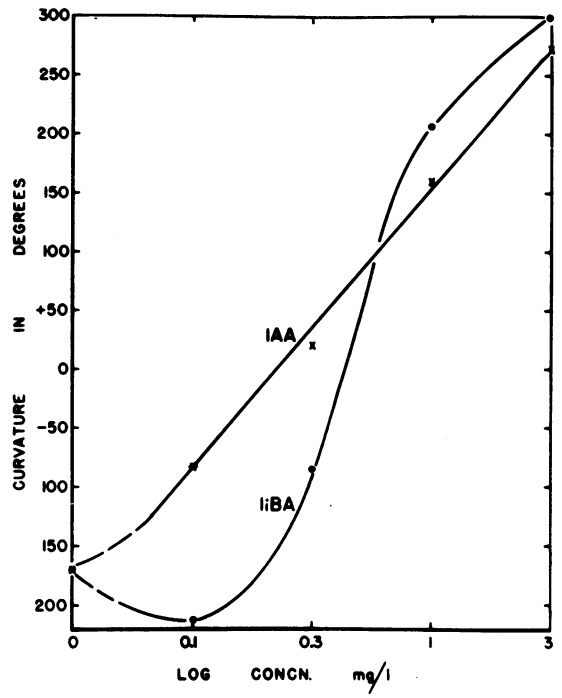
TABLE III

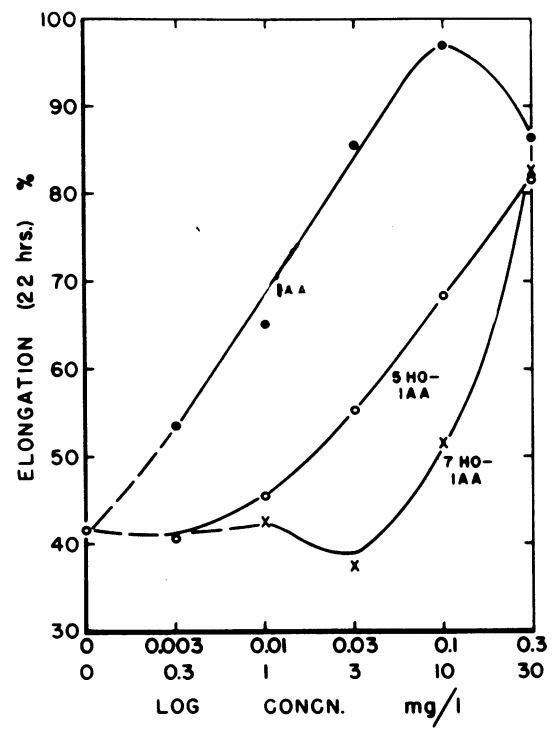
CURVATURE OF SLIT PEA STEMS IN A SERIES OF CONCENTRATIONS OF IAA AND SKATOLE. TEST OF 10/18/49

CONCN OF IAA $\times 10^{-6}$ M	CONCN OF SKATOLE $\times 10^{-4}$ M					
	0	1	3	Δ *	10	Δ *
0	−156	−181	−168		−196	
				− 12		− 40
6	+ 90	+157	+221		−111	
				+131		−201
18	+194	+166	+289		−158	
				+ 95		−352
60	+401	...	+218		− 62	
				−183		−463
180	+447	...	+368		−124	
				− 79		−570

* Change due to skatole.







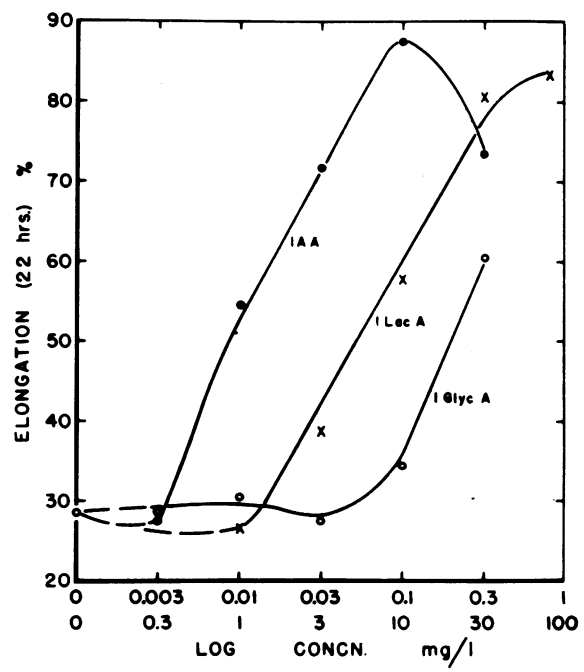


FIG. 1 (*top left*). Inhibiting action of skatole on straight growth of Pisum stem sections at three levels of IAA. (Expt. of 26/10/49). A 2nd experiment gave closely similar results.

FIG. 2 (*top right*). Curvature in the pea test of indole-3-isobutyric acid (IiBA) and IAA. All points from a single experiment (of 13/2/57).

while when IAA is at 10 mg per liter the decreases are 20.8 and 16.4 %. Corresponding figures can be calculated from the data at a skatole concentration of 10 mg per liter. (There is evidence of slight but real growth promotion when the concentration of IAA is high and that of skatole is low.) These data give no indication of any competition between skatole and IAA. Even when the lengths were measured at 6 hours, which is within the period of linear elongation rate for pea stem sections, the inhibition was not decreased in the least by the presence of IAA at 1 or 10 mg per liter. On the whole it tended to be greater rather than less. A Burk-Lineweaver plot of the data of figure 1 and of other similar experiments shows no convergence whatever of the lines for different skatole concentrations.

The curvature tests (table III) lead to the same conclusion. Again there is some promotion or synergism at low skatole concentrations (1 and 3×10^{-4} M). Indeed, it is clearer here than with the straight growth tests. At 3×10^{-3} M, skatole was toxic at all levels of IAA. Readings with the curvature test were not made during the period when the curvature is developing linearly, but nevertheless the results are very clear; high levels of IAA show no tendency to overcome the inhibition caused by skatole. Indeed, the decrease in curvature caused by a given level of skatole is actually greater at high than at low IAA concentration; in other words the behavior is the opposite of that to be expected for competition.

It is concluded that skatole is a growth inhibitor, but that the inhibition is not competitive with auxin, at least in pea stems. The inhibition tends rather to resemble that caused by arsenite, which is greatest at high auxin concentration (21).

4. Indole-3-glycolic Acid: As mentioned above, this substance, *Ind*.CHOHCOOH, has only slight, though real, activity on Avena (see table IV and fig 4). The two tests summarized in table IV yielded relative activities (IAA = 100) of 0.5 and 0.3 % respectively. Minimal or threshold activity is observable at 10 mg per liter. The greater growth in experiment II is largely due to the presence of cobalt, at its optimal concentration, in this experiment.

On Pisum straight growth the relative activity of indole-3-glycolic acid was somewhat lower than on Avena, being about 0.2 %. These data are consistent with the figures given by Greenberg et al (6), from which a relative activity of less than 0.2 % can be calculated. In Pisum curvature, however, a figure of about 2 % was found. It must be concluded that the activity of this compound in all assays, though real, is very low.

5. Indole-3-glyoxylic Ester: Tests with this substance, *Ind*.COCOO · C_2H_5, were inconclusive.

Table IV

Relative Activity of Indoleglycolic Acid on Avena Coleoptile Sections

Substance	Concn mg/l	Elongation in percent I	Elongation in percent II
Control solution *	...	51.4	59.8
Indoleacetic acid	0.1	...	89.9
	0.3	63.8	118.6
	1.0	75.3	101.5
Indoleglycolic acid	0.3	48.6	59.7
	1	47.7	61.4
	3	49.3	58.3
	10	49.5	65.2 **
	30	57.8	91.6
	90	69.6	84.0
Equivalence ∴ Relative activity		IAA 0.3 = IGA 60 0.5 %	IAA 0.1 = IGA 30 0.3 %

* Sucrose 2 %, KH_2PO_4 M/200; expt. I without cobalt; expt. II with $CoCl_2$ 3×10^{-5} M.
** Mean of three.

Whether used directly as the ester, or after a few minutes of hydrolysis with warm dilute NaOH, the activity was seldom proportional to concentration. In one Pisum curvature test a relative activity of 0.7 % was indicated, but in another it was zero. High concentrations slightly inhibited Avena growth. A relative activity of a few tenths of 1 %, with slight toxicity at 30 mg per liter, would be consistent with the results. The sample was not large enough for extensive purification, and the danger that it might be decomposing in the solution made a further detailed study unwarranted.

6. N-Acetylindoxyl: This substance has been reported active on Avena (18) and indeed the sample obtained from Bios Ltd. showed slight but irregular traces of activity on Avena. At 10 and 30 mg per liter, however, growth was inhibited about 10 % (as with indole-glyoxylic ester). On the other hand, the sample from the Delta Chemical Co. showed no activity whatever, nor did either sample show any activity on Pisum, either for straight growth or for curvature. It is concluded that N-acetylindoxyl has no auxin activity.

7. Oxindole Derivatives: Since it appears that the product of enzymic oxidation of IAA is an oxindole derivative (16, 19), a number of oxindole compounds were examined. None of the following showed activity on pea curvature in concentrations up to 30 mg per liter, or about 2×10^{-4} M; oxindole-3-acetonitrile, oxindole-3-pyruvic oxime, oxindolene-3-ace-

Fig. 3 (*lower left*). Straight growth of Avena coleoptile sections in 5-hydroxy and 7-hydroxy-indoleacetic acids, compared with IAA. The scale for the two hydroxy acids is the lower one, i.e., their concentrations are 100 times those of IAA.

Fig. 4 (*lower right*). Straight growth of Avena coleoptile sections in indole-3-lactic (ILacA) and indole-3-glycolic (IGlycA) acids, compared with IAA. The scale for ILacA and IGlycA is the lower one.

tonitrile, oxindolene-3-pyruvic acid and oxindolone (3-dehydrodioxindole).

Special attention was paid to 3-methyldioxindole, since this compound has many properties in common with the IAA oxidation product (19). Tested on Avena up to 30 mg per liter in the presence of IAA concentrations from 0 to 5 mg per liter, it showed neither auxin activity, synergism nor true inhibitory effect. Pea curvature tests showed the same lack of all three activities.

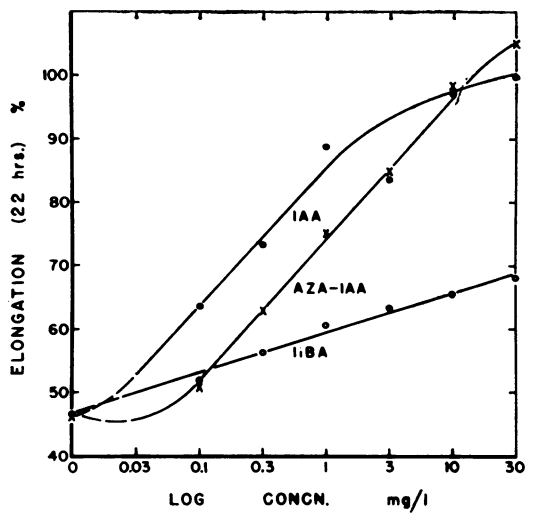
8. Indole-3-isobutyric Acid: Burström (3) and Hansen (7) have shown indole-3-isobutyric acid (IiBA), or *Ind.*$C(CH_3)_2COOH$, to be a powerful "root auxin," promoting the elongation of wheat roots by about 50 %, and opposing the growth-inhibiting action of IAA. It would be expected, therefore, to act as an auxin antagonist in the present tests. The reverse is the case. On Avena its effect is weak, but the slight activity is growth-promoting—about 0.3 % of IAA; however, the very slow increase in growth with increase in IiBA concentration makes precise comparison impossible. The linear relation and gradual slope are shown in figure 5, which confirms the curve given by Hansen et al (8). Two pea straight growth tests likewise showed real growth promotion, with relative activities of 1.5 % and 2 % respectively.

It is the activity in the pea curvature test that is remarkable. Here IiBA actually produces larger curvatures, at high concentrations, than does IAA (fig 2). This behavior, coupled with the low activity and unusual concentration-dependence in the Avena section test, makes it impossible to ascribe the activity of IiBA to traces of indole-*n*-butyric acid as an impurity.

In the pea curvature test, the difference in slope of the curves for IAA and IiBA (see fig 2) makes it difficult to assess the relative activity of IiBA. Using as criterion the concentration needed to reduce the outward curvature by 100° (25), the relative activity would be 33 %; however, using the concentration needed to produce 100° inward curvature, it would be over 100 %, i.e., IiBA would be more active than IAA. This high activity in differential growth compared with the much lower values for straight growth indicates that IiBA is probably transported from cell to cell only with difficulty. It is of interest, also, that in the similar curvature test with slit Avena coleoptiles (24), IiBA is significantly more active than on Avena straight growth, the relative activity approaching 25 %. Thus it is largely the curvature method per se which is responsible for the high relative activity.

At no concentration did IiBA inhibit growth, in the absence of added IAA. However, the increased growth of Avena due to IAA was clearly inhibited by IiBA. A concentration 10 times that of the IAA reduced the growth increment by about 10 %. At lower ratios, and especially at low absolute concentrations of IAA, there is some evidence of synergism, especially in pea straight growth.

In contrast to its action on roots, IiBA is thus clearly an auxin for shoots. A similar conclusion has been drawn by Fawcett et al (5).



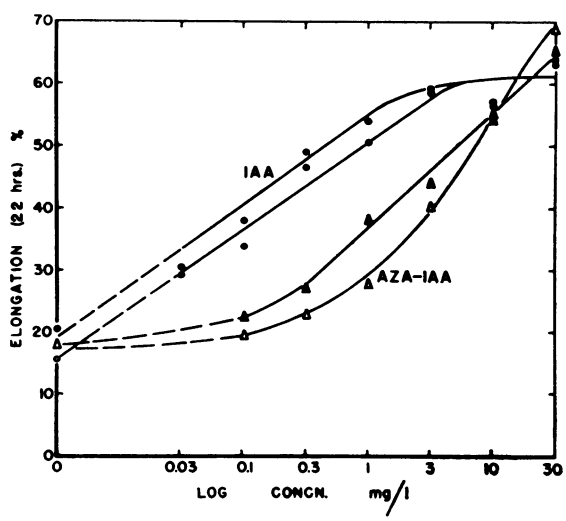


Fig. 5 (*above*). Straight growth of Avena coleoptile sections in 7-azaindoleacetic acid (Aza-IAA) and indole-3-isobutyric acid (IiBA). Tests of 16/7/56 (Aza) and mean of 25/11/57 and 2/12/57 respectively (IiBA). All on same scale.

Fig. 6 (*below*). Two pea stem straight growth bioassays of 7-aza-IAA, each with its reference curve for IAA (31/10/56 and 1/11/56).

9. 5-Hydroxy- and 7-Hydroxyindoleacetic Acids: The former of these two acids has recently come into prominence as the product of the physiological oxidation of 5-hydroxytryptamine or serotonin. Quantities up to 400 mg per day may be excreted in human urine in certain pathological conditions (26), and the feeding of tryptophane also leads to its excretion (27). Furthermore, 5-hydroxy-IAA occurs in plants, having been found in seeds of *Piptedenia peregrina* (cited in (27)). It is also of importance

TABLE V

CURVATURE OF SLIT PEA STEMS CAUSED BY 5- AND 7-HYDROXYINDOLEACETIC ACIDS

CONCN, MG PER LITER	CURVATURE IN IAA (MEAN OF 3)	CURVATURE IN 5-HO-IAA (MEAN OF 2)	CURVATURE IN 7-HO-IAA
100	...	+201	+194
30	...	+172	- 34
10	+395	+ 3	- 42
3	+228	-118	-342
1	+104	-221	-234
0.3	+ 2	-175	-221
0.1	- 83	...	...
H_2O	-190	-190	-198
Relative activity		4 %	1.6 %

that both these acids can be attacked by the very specific indoleacetic acid oxidizing enzyme from *Omphalia flava*, albeit the action is slow and the products are not the same as those formed from IAA (16).

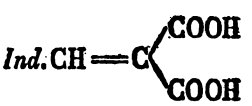
On Avena coleoptile sections both compounds show weak activity. In figure 3 the scale for the hydroxy acids is 100 times that for IAA; the relative activity of 5-hydroxy-IAA averages 0.12 % of that of IAA, while 7-hydroxy-IAA appears less active, perhaps 0.06 %. A feature of these tests is the marked deposit of purple breakdown products at the cut surfaces of the sections. This is particularly marked with 7-hydroxy-IAA. This suggests that if inactivation at the cut surface could be reduced, without inhibiting growth, the relative activity might well be higher.

Pea curvature assays are shown in table V. The small sample available of the 7-hydroxy acid did not permit much repetition of tests, but it is clear that both substances possess real, though low, activity, and that the 5-hydroxy acid is about twice as active as the 7-hydroxy acid. The relative activities of 4 % and 1.6 % represent a slight downward revision of the figures cited in (4). On pea straight growth, both substances were virtually inactive up to 30 mg per liter (1.6×10^{-4} M).

10. INDOLELACTIC ACID: The very great lowering of activity occasioned by introduction of a hydroxyl group in the two acids just mentioned lent special significance to a study of indole-3-lactic acid (ILacA), or *Ind.*$CH_2CHOHCOOH$. This compound has recently been identified (12) as among the products formed from tryptophane by *Agrobacterium tumefaciens*. On Avena sections the growth increment caused by ILacA is satisfactorily proportional to concentration, and the slope of the curve is parallel to that of IAA (fig 4). The relative activity is only 0.16 %. On the straight growth of pea stems the activity was higher, averaging 0.4 % in two tests. As usual, a higher value for pea stem curvature, about 1.8 %, was found.

Although this sample of ILacA had the correct melting point (100° C; given in the literature as 100 to 101° C) it was not a synthetic product but was obtained from *Oidium lactis* (*Geotrichum candidum*); it is difficult, therefore, to rule out the possibility of its containing a minute trace of IAA. Careful chromatography of quantities of 100 μg each, in several different solvents, did not reveal any sign of IAA, so that the amount of IAA present, if any, is probably less than 0.2 %. In any case, since the relative activity of ILacA is significantly different in different tests, and especially since in the pea curvature test it approaches 2 %, the whole of its activity cannot be ascribed to contaminating IAA. However, the activities quoted may perhaps be regarded as maximal.

11. INDOLEMETHYLENEMALONIC ACID: In the chlorophenoxyacetic series it has been shown (10, 11; see discussion in (20)) that the introduction of a 2nd carboxyl group almost completely destroys activity in the pea test. The availability of a small sample of indole-3-methylenemalonic acid,



(used as a synthetic intermediate), made possible another test of this rule. Both on pea curvature and pea straight growth it was found virtually inactive. The relative activity on pea curvature, if real, was clearly less than 0.2 %.

12. IMIDAZOLE DERIVATIVES: The point was made some years ago (20) that thus far no synthetic compounds having only a 5-membered ring have been found active as auxins. Pyrrole-3-acetic, thiopheneacetic, furylacetic and both isomers (Δ_1 and Δ_2) of

TABLE VI

RELATIVE ACTIVITY OF INDOLE-4-ACETIC ACID (I-4-AA) ON AVENA COLEOPTILE SECTIONS, AND TEST FOR INTERACTION WITH INDOLE-3-ACETIC ACID

CONCN, MG PER LITER	ELONGATION IN PERCENT AFTER 22 HOURS				
	I-3-AA	I-4-AA	I-3-AA	I-4-AA	BOTH
Control solution *	32.8		44.3		
0.01	44.2	...	...	...	
0.03	69.0	...	78.3	...	93.3
0.10	70.1	...	...	...	
0.30	71.5	29.9	...	55.5	
1.0	76.9	45.7	...	...	88.1
3.0	72.4	64.8	93.4	...	
10.0	...	69.8	...	...	
30.0	...	71.4	...	...	

Equivalence:

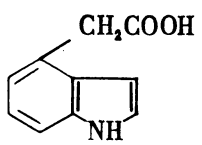
I-3-AA 0.01	= I-4-AA 0.9	or relative activity =	1.1 %
0.03	9		0.3 %
0.1	14		0.7 %
0.3	30		1.0 %

* Sucrose 2 %, KH_2PO_4 M/200 pH 5.0, $CoCl_2$ 3×10^{-5} M, Penicillin G 2×10^{-6} M.

cyclopenteneacetic acid are all inactive. Yet the same structures when fused with 6-membered rings (i.e., the acetic derivatives of indole, thionaphthene, benzofurane and indene) are highly active, at least on pea curvature. The behavior of the three imidazole derivatives so far tested supports this generalization, and although they are not indole compounds it is appropriate to record them here. Imidazoleacetic acid, imidazoleacrylic acid (or urocanic acid) and imidazoleacetonitrile were all completely inactive in the pea curvature test up to 100 mg per liter. They also had no effect on straight growth.

13. Indene-3-propionic Acid: This compound is also not an indole, and is only of interest here because it is so much less active than its acetic homologue. Indene-3-acetic acid has been shown to have 20 % relative activity in the pea curvature test, though less in other tests (see discussion in ref. (20)). Two pea curvature tests with indene-3-propionic acid gave relative activities of only 3 and 4 %. On pea straight growth it was nearly inactive.

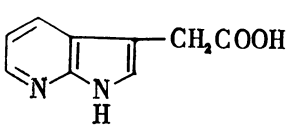
14. Indole-4-acetic Acid: This acid:



was found to have unexpectedly low activity. Representative bioassays, shown in table VI, indicate that on Avena its activity is exactly 1 % of that of IAA. On Pisum curvature (in accord with the usual lesser selectivity of this test) it is about 2 %. In the straight growth of Pisum the curve is steeper than for IAA so that a relative activity can be only roughly estimated. In Avena, however, the slope of the line relating concentration to growth increment closely parallels that for IAA.

In the presence of low concentrations of IAA, indole-4-acetic acid gives no indication of synergism, and in the presence of high concentrations there is little evidence of antagonism (table VI) although some slight interaction is not excluded.

15. 7-Azaindole-3-acetic Acid: Although this compound:



seemed of special interest as a possible auxin antagonist, figure 5 shows that it acts as a powerful auxin on Avena, and indeed, it is easily the most active of the substances described in this paper. Its relative activity, derived from several points on the curve, averages 50 %. In combination with IAA, 7-azaindole-3-acetic acid (7-Aza-IAA), showed no synergism or antagonism.

The two tests on Pisum straight growth (fig 6) show that, at 30 mg per liter, 7-Aza-IAA is at least as active as IAA, while at low concentrations the relative activity falls to 10 %. In pea curvature the activity is essentially equal to that of IAA throughout.

Discussion

The best values for the relative activities are summarized in table VII. The first conclusion to be drawn from the data is that the structural requirements for auxin activity in the indole series are rather demanding. This extends also to the requirements for activity as an antagonist or synergist. It makes a refreshing contrast to the situation with purely aromatic compounds, in which good activity is found in structures ranging all the way from 2,3,6-trichlorobenzoic acid to 1-naphthalenephosphinic acid and 2,4-dichlorophenoxymethyltetrazole (28, 29).

The second conclusion is that the concept of "anti-auxin" or auxin antagonist needs considerable modification. The non-competitive inhibition of pea stem growth and curvature by skatole shows that the suggestion that carboxyl-free analogues should act as anti-auxins is not borne out in this case. The failure of 7-Aza-IAA to act as an antagonist is unexpected in view of the antagonistic activity of other 7-Aza-indoles in other systems (17). The clear growth-promoting activity of indoleisobutyric acid shows that an anti-auxin in one test may be an auxin in another. Similarly, triiodobenzoic acid can be an antagonist in one test, an auxin in another and a synergist in a third (see discussion in (23)). While there certainly can be antagonism in specific tests, there is probably no one substance that can safely be regarded as a

Table VII

Summary of Relative Activities (IAA = 100) in the Three Bioassays

	Avena straight growth	Pisum straight growth	Pisum (slit) curvature
Indole	0.02	...	...
Skatole	Inhib	Inhib	Inhib
Indole-3-glycolic	0.4	0.2	2
Indole-3-glyoxylic	? 0.1	ca 0	? 0.3
Indole-3-isobutyric	0.6 to 5.0 *	1.7	33 to 130 *
5-hydroxy-IAA	0.12	ca 0	4
7-hydroxy-IAA	0.06	ca 0	1.6
Indole-3-lactic	0.16	0.4	1.8
Indole-3-methylene malonic	0	0	0.3
Indole-4-acetic	1.0	1 to 6 *	2
7-Aza-IAA	50	6 to 100 *	ca 100
Indene-3-propionic	...	ca 0	3.5

5-Methyl and 6-methoxyindole; N-acetylindoxyl; oxindole-3-acetonitrile and -3-pyruvic oxime; oxindolene-3-acetonitrile and -3-pyruvic acid; 3-dehydrodioxindole; 3-methyldioxindole; imidazoleacetic acid, imidazoleacetonitrile and imidazoleacrylic acid were all inactive.

* Depending on which part of the curve is used for comparison.

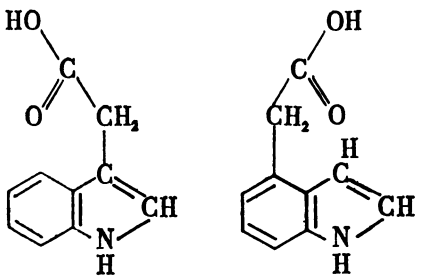
general "anti-auxin." Auxin antagonists should be defined by the specific bioassay in which they function.

It is satisfactory to observe that a number of the rules relating structure to activity, formulated originally from the aryl and aryloxy acids, hold good for indole derivatives also. Most striking is the effect of a hydroxyl group in lowering the activity. Whether in the side-chain, as in IGlycA and ILacA, or in the ring, as in 5-HO-IAA and 7-HO-IAA, the hydroxyl reduces the auxin activity for straight growth by at least 100 times. The activity for pea stem curvature is reduced 25 to 50 times. The similar example of mandelic, atrolactic and tropic acids has previously been discussed (20). One is tempted to deduce, modifying an earlier proposal of Veldstra, that the structure, and especially the ring, must not be made hydrophilic at any point other than the carboxyl, but can only be substituted by such lipophilic groups as Cl and CH_3. The inactivity of the oxindoles and the dicarboxylic acids would support this view, but, like so many other attractive proposals, it disagrees with an important part of the facts, namely the high activity of the phenoxy acids. For instance, 2,4-dichloro*phenoxy*acetic acid is about 50 times as active on pea curvature as 2,4-dichloro*phenyl*acetic acid. The phenolic oxygen, although it is in ether linkage, must certainly exert some degree of hydrophilic action. Thus the strong effect of a hydroxyl group, clear-cut as it is, remains unexplained.

Another characteristic cause of lowered activity, namely the introduction of a 2nd carboxyl, is exemplified by indolemethylenemalonic acid, compound no. 11 in this series. A third point, namely the inactivity of 5-membered ring compounds, is extended to three more instances by the imidazole derivatives here tested. Except for their ring structure these compounds would have been predicted to have good activity. Their complete inactivity makes a sharp contrast with the activity of the 6-membered pyridoxyacetic acids recently reported by Veldstra (30). Fourthly, the interchangeability of carbon and nitrogen in the ring, exemplified previously by the high activity of indene-3-acetic acid, receives additional strong support from the equally high activity of 7-Aza-IAA.

One of the early theories, especially emphasized by Wain, was that the side-chain must contain at least one hydrogen atom. The remarkably high activity of indoleisobutyric acid in pea curvature, and its real auxin activity on Avena, clearly disprove this view.

Finally, the very low activity of indole-4-acetic acid (I-4-AA), bears unfavorably on two current theories. In the first place, the position *ortho* to the side-chain is just as free in I-4-AA as in I-3-AA, so that the theory of Muir and Hansch (14) that activity depends on a free *ortho* position is satisfied in a qualitative way; however, quantitatively it offers no basis for a difference in activity of 100 times between the two compounds. In the second place, both I-4-AA and I-3-AA equally fulfill the proposed requirement of Jönsson (9) that the side-chain should be able to fold in such a way as to bring the carboxyl near the center of the compound ring system and in the same plane:



Hence this theory also provides no basis for a difference between the activities of the two acids of 100 times.

The only fair conclusion is that all the rules so far proposed are too simple, and none of them takes sufficient account of the varied features of an organic molecule. It is evident that there are rules, and strict ones, but their formulation is elusive. The interaction between the auxin molecule and its substrate evidently does not occur simply "at two points," or "in a plane," etc., but involves delicate gradations of electron density and spatial configuration, for the adequate description of which our present theories are too crude.

Summary

The activities of 20 indole derivatives and four related compounds have been determined in three bioassays; straight growth of Avena coleoptile sections, straight growth of Pisum stem sections and differential growth (curvature) of slit Pisum stem sections.

Indole itself promotes growth significantly but no evidence could be found for its conversion to IAA in vivo. Skatole inhibits growth in all three systems, and in pea stems the inhibition is clearly not competitive with IAA.

The four hydroxy derivatives, indolelactic, indoleglyoxylic and 5- and 7-hydroxy-IAA all have very low activity, which corresponds well with the low activity of hydroxy-aromatic acids. Six derivatives of oxindole and three of imidazole are inactive, the latter confirming the rule that compounds with 5-membered rings are inactive. Indolemethylenemalonic acid is virtually inactive, confirming the rule that dicarboxylic acids are generally inactive. Indenepropionic acid is much less active than indeneacetic acid.

Indole-3-isobutyric acid has low but real auxin activity on straight growth, and has activity close to that of IAA on curvature; it is therefore a true "shoot auxin."

Indole-4-acetic acid has only 1 to 2 % of the ac-

tivity of I-3-AA, which accords poorly with two theories of the structural requirements for auxin activity.

7-Aza-indole-3-acetic acid has high activity, approaching that of IAA, in all three systems.

It is concluded: 1) that auxin antagonists should be defined by the specific bioassay in which they function, 2) that the empirical rules relating structure of aromatic compounds to activity also hold good in the indole series, but 3) that the requirements for activity, though evidently stringent, are not well described by any of the recently proposed theories.

Excellent and faithful technical assistance was rendered successively by Mrs. Irma Slankis, Mrs. Berit Ørning, Mrs. Lisa Wilkinson, Mrs. Eileen Bladon, and Miss Irmgard Winkler. Thanks are due to Mr. W. R. Porter for carrying out the chromatographic analysis of indoleacetic acid. I wish also to thank Dr. Bruce B. Stowe for valuable discussions, for supplying three of the tested substances and for a critical reading of the manuscript.

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DEPTH CONTROLLED DEUTERON IRRADIATION OF LACTUCA SATIVA SEEDS. I. EFFECTS ON GERMINATION AND GROWTH[1]

S. KLEIN[2] AND JOHN W. PREISS[3]

DEPARTMENT OF BOTANY AND DEPARTMENT OF BIOPHYSICS, JOSIAH WILLARD GIBBS RESEARCH LABORATORY, YALE UNIVERSITY, NEW HAVEN, CONNECTICUT

Although plant response to photon and particle irradiation is well studied, we are unaware of experiments involving an accurate control of the penetration depth of charged particles in multicellular organisms. Lateral restriction of such particles has been accomplished by micro-beam techniques (15). Longitudinal restriction can be accomplished only by fixing the kinetic energy of the particles before they reach the tissue to be irradiated. Preiss (11) has used the latter technique in obtaining the spatial distribution of intracellular yeast invertase. The charged particles were electrons with kinetic energies such that their trajectories ended well within the dimensions of the individual yeast cells.

The experiments described in the present paper are concerned with the germination and subsequent root growth effects produced by deuterons of various penetrating abilities in lettuce seed tissue. Analogous work by Busse and Daniels (2) involved placing corn seeds in a cathode ray beam with the embryos or endosperms facing the source.

The physiology of lettuce seed germination has been described in detail (for reviews see 4, 8, 14). Under the experimental conditions, the germination percentage of seeds kept in complete darkness after imbibition is usually less than 30. However, complete germination can be achieved when a short illumination is given briefly after imbibition. This photoreaction has a peak response at about 6700 Å, and can be reversed by less energetic photons (7000 to 7500 Å).

The embryo of the seed is surrounded by a number of layers. As described by Borthwick and Robbins (1) and by Evenari and Neuman (6) these include a pigmented fruit coat which contains large air spaces and is traversed by ridges of parenchymatous cells, a degenerated seed coat, and a dense endosperm two to three cell layers thick. The induction of germination is thought to be associated with layers surrounding the embryo (8), while subsequent growth is governed by factors in the embryo itself. Therefore, one would expect radiation induced changes to depend on the penetration depth of ionizing particles. Deuterons with energies up to about 4 Mev are excellent projectiles for such studies because their range in protein or cellulose is of the order of 100 μ or less (9). Alpha particles with about twice this energy meet these requirements; however the cyclotron beam currents are much smaller than those obtained by accelerating deuterons.

The aim of this work is twofold: to establish the usefulness of depth controlled irradiation as a tool in radiation biology and physiology on the macro scale, and, in the special case of the lettuce seed, to contribute to the understanding of germination. This report will deal mainly with the biological aspects; dosimetry, range measurements and calculations related to physical problems are discussed elsewhere (12).

MATERIALS AND METHODS

Lettuce seeds, var. Grand Rapids, obtained from Pieters-Wheeler were used exclusively in these experiments. The seeds were stored, prior to use, in a desiccator at 3° C. All particle bombardments were carried out on dry seeds in vacuo at room temperature. Immediately after irradiation batches of 50 to 100 seeds were deposited on wet filter paper in Petri dishes and kept in complete darkness at 25° C. The filter paper was thoroughly soaked with 2.5 cc of water. Light treatment, whenever desired, was given two hours after imbibition. Red light was obtained by the use of cellophane-wrapped fluorescent tubes giving an intensity maximum near 6500 Å. With a light intensity in the illumination plane of about 19 kiloerg/cm^2 × min, one minute of illumination was sufficient to promote complete germination of unirradiated controls. Germination percentage was noted, and root length was measured 48 hours after imbi-

[1] Received January 20, 1958.

[2] Present address: Hebrew University, Jerusalem, Israel. Participation of S. Klein in this joint interdepartmental work was supported by grants from the Anna Fuller Fund and from the Yale University Atypical Growth Committee (American Cancer Society) to A. W. Galston.

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