abscission at low concentrations (e.g., 10^{-5} M) and inhibit at high concentrations (e.g., 10^{-3} M) within a wide variety of conditions and ages. The promotion effect was not obtained with young explants held in darkness, or with older explants which had a low carbohydrate reserve unless substrate was added. 4) It is concluded that auxin generally has a two-phase action on abscission, but that its action is mediated by leaf age, light, and the supply of substrates such as sugar.

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GROWTH RESPONSES OF CRUCIFERS TO INDOLEACETIC ACID AND INDOLEACETONITRILE[1]

BURLYN E. MICHEL

Department of Botany, State University of Iowa, Iowa City, Iowa

Housley and Bentley (10) have summarized the problem of indoleacetonitrile (IAN) activity in plant growth. Although there is widespread belief that growth responses to IAN are in reality responses to the indoleacetic acid (IAA) derived from it, before final judgment concerning the physiological role, if any, of IAN can be rendered, it would seem that careful study should be made of some species in which IAN occurs naturally. This paper reports some of the initial efforts in such an attempt.

The only reports of extensive comparisons of IAA and IAN are those of Bentley's group in England using Avena coleoptile sections. It is the purpose of this paper not only to compare the effects of IAA and IAN on crucifers but also to contrast these results primarily with those of the English group.

[1] Received June 4, 1957.

Materials and Methods

Hypocotyls from etiolated seedlings of the following crucifers were used: cabbage (*Brassica oleracea* var. *capitata* L. horticultural strain Golden Acre), radish (*Raphanus sativus* L. horticultural strain Crimson Giant), and turnip (*Brassica rapa* L. horticul-

tural strain Purple Top White Globe). For comparative purposes, etiolated coleoptiles of *Avena sativa* L. strain Victory were used.

Seeds were planted in rows in polystyrene trays of sand. Seedlings were grown and experiments were performed in a room that was maintained at a temperature of 24° C and a relative humidity near 85 %. The room was kept dark except for occasional red illumination from incandescent safelights (Rubylites of 4 C.P.). Germination of the crucifers was somewhat uneven; however experiments were started when most of the seedlings averaged about 30 to 35 mm in height —cabbage requiring about 5 days, radish and turnip, 4 days. Only those within 5 mm of the average were used. With a cutter of the author's design, one 10-mm section was cut from each hypocotyl beginning, as closely as could be determined in the dim red light, 5 mm below the cotyledonary node. For Avena, 10-mm sections were cut beginning 5 mm below the undecapitated tips of 25- to 35-mm coleoptiles. As standard procedure 10 sections were floated on 20 ml of test solution in each 20 × 100-mm Petri dish. When testing extracts and on a few other occasions 5 ml of solution in 15 × 60-mm dishes were used, the smaller volume not giving significantly different results.

The author is well aware that both final length after some arbitrary period of time and growth rates during that time are influenced by many factors including the composition of the basal medium. The test solutions used, except to test effects of pH and buffers, contained no additives other than regulator (or/and extract) and the NaOH or HCl necessary to give a pH of 6 for the following reasons: Radish hypocotyl sections responded essentially identically to IAA and to IAN in 0.005 M sodium maleate solutions of initial pH's of 5 and 6 (final pH's of 5.3-5.4 and 6.2, respectively); however optimal 24-hour growth for both regulators was 25 % less in this buffer than with none, initial pH adjusted to 6 (final pH 6.6 to 6.9). Bentley's group did not adjust initial pH; however, as will be indicated later, this proved important only with the highest IAA concentrations used. Sucrose was not added primarily because the English group, with whose work this was to be compared, did not do so.

Lengths of sections were determined either by direct measurement or photographically as previously described (14). The growth in water alone was subtracted from total growth before plotting the graphs; however the values for water growth appear in the legends. It may be noted that growth in water of crucifer hypocotyls is much less than that of Avena coleoptiles. Greater variability often was found among crucifer sections of a test dish than usually reported or found by the author for Avena; nevertheless average values were quite consistent and reproducible. Representative results have been chosen for presentation in this paper.

Cabbage extract preparations tested were prepared using the following procedure: Freshly harvested etiolated hypocotyls were macerated with mortar and pestle, 1 *N* HCl being added to give pH 2. Following high-speed centrifugation the supernatant, which contained the significant bulk of growth stimulating activity, was decanted and brought to pH 9 to 12 by adding 1 *N* NaOH. This alkaline aqueous supernatant was extracted 4 to 5 times with redistilled, peroxide-free ethyl ether to produce the neutral fraction. The aqueous portion was reacidified with HCl to pH 2 to 3 and extracted 4 to 5 times with ether to produce the acid fraction. The water was frozen out of both neutral and acid ether fractions in a deep freeze. The ether extracts were decanted into distilled water in beakers in a water bath of about 55° C and heated for one hour after visual disappearance of the ether. The now aqueous fractions were adjusted to pH 6 as necessary and diluted and tested as desired.

All water used in preparing test solutions was redistilled from Pyrex after distillation from a tin-lined still and passage through an Amberlite MB-2 column. The IAN was synthesized using a slightly modified procedure of Henbest, Jones, and Smith (9). The final steps in purification of the IAN were completed in the Department of Chemistry at the State University of Iowa under the direction of Prof. S. Wawzonek.

Results and Discussion

To establish common ground, present another base for comparison of results obtained with crucifers, and point out a pH effect mentioned earlier, it is desirable to compare the results of Bentley and Housley (2, 3)

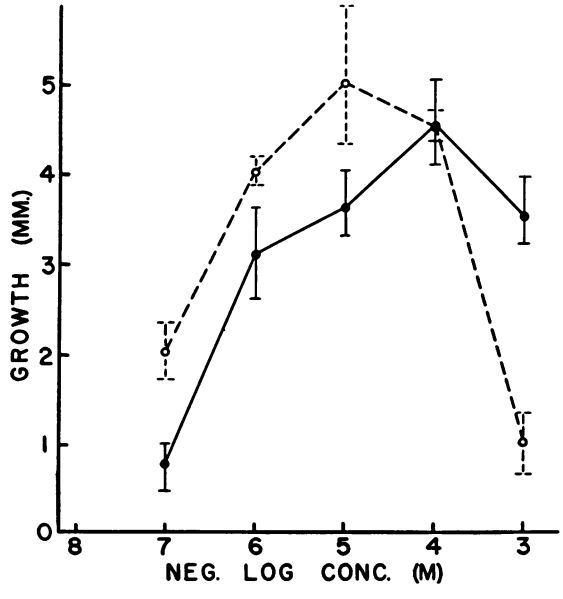


Fig. 1. Twenty-four-hr growth of Avena coleoptile sections in IAA (solid lines) and IAN (broken lines). Each point is the mean of 4 dishes from 2 trials. Vertical lines show extremes of single dish means. Water growth (subtracted before plotting) was 3.6 mm.

with those obtained for Avena by the author. Figure 1 shows the latter, which, with one major exception, closely resemble the published results of Bentley and Housley. The exception is their finding that after 24 hours the average length of sections in 5.7×10^{-4} M

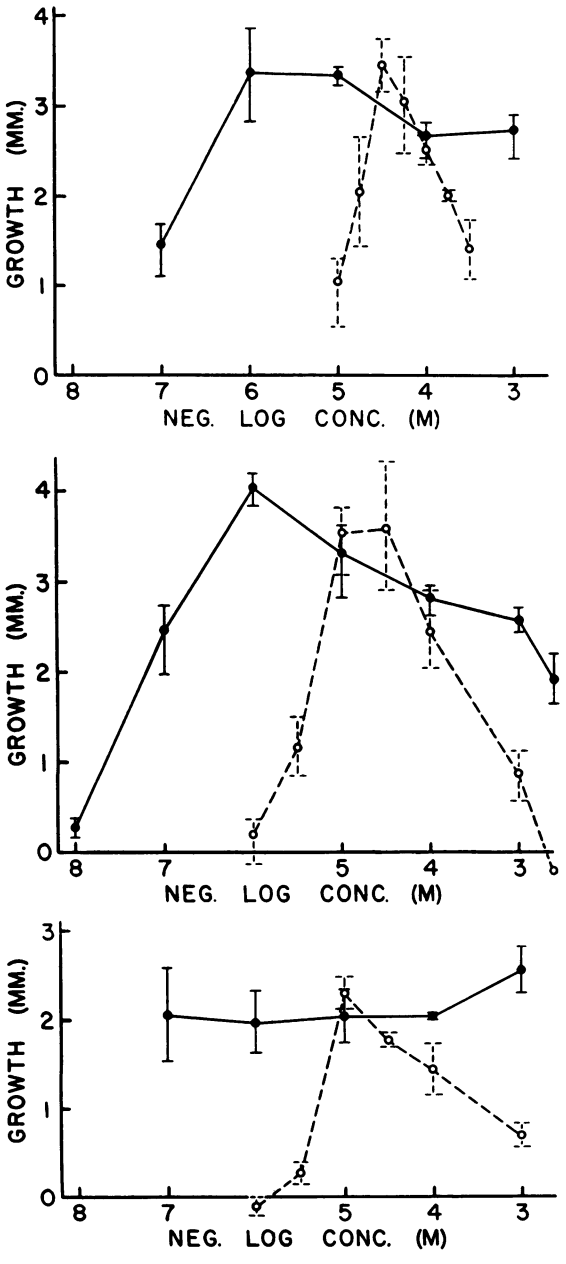


FIG. 2. Twenty-four-hr growth of various crucifer hypocotyl sections in IAA (solid lines) and IAN (broken lines). Each point is the mean of 1 to 5 dishes from 1 to 3 different trials. Vertical lines show extremes of single dish means. *Upper,* cabbage with water growth of 0.6 mm. *Middle,* radish with water growth of 0.9 mm. *Lower,* turnip with water growth of 0.4 mm. Water growth subtracted before plotting.

IAA was about the same as that for water controls. Initially growth was greater in IAA but this was followed by shrinkage. These workers concede that this might be related to the low pH of 4.1 measured for this concentration however they point out that the connection is not clear since no shrinkage of sections occurred in succinic acid solution at pH 3.5. That this is related to low pH is indicated in figure 1 by the lack of or at least low toxicity at high IAA concentrations when the initial pH is adjusted to 6. Low pH itself may not be toxic, for Nitsch and Nitsch (15) reported greater growth of Avena coleoptile sections in sucrose and buffer with and without IAA (2.9×10^{-7} M) at pH 4 than at higher pH values. The data of Bonner and Foster (4) show little toxicity to Avena coleoptiles even of high concentrations of IAA at the relatively low pH of 4.5 in the presence of sucrose and buffer. It is possible that sucrose and/or buffer have some protective value against low pH. Still, it seems certain that the toxicity of high IAA concentrations reported by Bentley and Housley (2, 3) are either related to low pH or anomalous.

A low pH not only increases the proportion of undissociated IAA, in which form IAA is presumably more readily absorbed, but also seems to have other effects. Reinhold (17) has found that pH effects do not parallel the dissociation of IAA and that pH and regulator absorption are not clearly related. Van Overbeek (21) has recently and briefly discussed this problem. In this connection it might be mentioned that radish hypocotyl sections grew about half as much in 0.006 M citrate-phosphate (1 : 2) buffer at pH 4.5 as at 7.0, although, as mentioned earlier, they grew about the same in maleate buffer at pH's 5 and 6. About all that can be said at this point is that the effects of pH and buffers are not limited to regulator penetration and that the dependence of regulator absorption on pH is not clear.

With the growth curve for IAA, as shown in figure 1, essentially like that for IAN over the entire concentration range except for displacement to the right, any necessity for invoking different inhibitory mechanisms at high concentrations as was done by Bentley and Housley (2, 3) is obviated. The possibility that high concentrations of both IAA and IAN may possess general or non-specific cellular toxicity unrelated in a direct way to growth mechanisms in addition to probable self-competitive inhibition should not be disregarded.

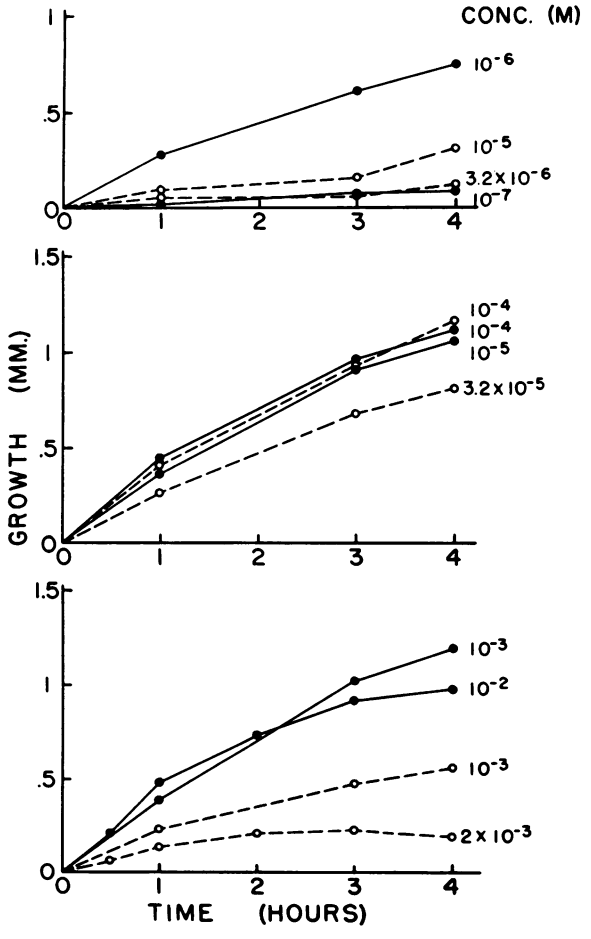
The patterns of growth responses among the crucifers tested, as shown in figure 2, while quite different from those of Avena, are similar to each other except for the response of turnip to IAA. The nearly equal growth of turnip hypocotyl sections over a 10,000-fold IAA concentration range is difficult to understand, though a partial explanation may be indicated by results to be presented later. Germination of the turnip seeds was not uniform, the diameter of the hypocotyls was small, and the sections tended to curve markedly in the test solutions; therefore work with turnip was not continued.

There are several differences between the growth curves for the crucifers and for Avena. Crucifers respond to lower concentrations of IAA. The optimal concentration of IAA for crucifers is 10^{-6} M; for Avena, 10^{-4} M. The growth curves for crucifers do not decline as rapidly at supraoptimal concentrations of IAA as does that for Avena. Crucifers are less sensitive to low concentrations of IAN than Avena; however, the concentrations producing maximum growth are the same. The growth curves for crucifers span less of a concentration range of IAN than does that for Avena.

With the above noted differences in comparative 24-hour growth responses of Avena and crucifers to IAA and IAN, it seemed of interest to compare the growth rates of one of the crucifers with those of Avena reported by Bentley and coworkers (3, 12). These investigators found no appreciable differences between growth rates induced by IAA or IAN at any but the highest concentrations used, if comparisons are made between concentrations giving nearly equal final lengths.

If IAN possesses activity only after hydrolysis, the growth rates observed throughout the entire period of growth would be expected to vary from those of IAA only as a result of side effects, such as general toxicity, or sufficiently slow conversion to cause a lag period. However, if IAN possessed activity without hydrolysis, it would seem logical also to expect nearly the same growth curves as for IAA because of the structural similarity of the two molecules. Bonner and Foster (4) found both IAA and 2,4-D to give linear growth rates that are apparently strictly comparable even though no assumption of conversion of the 2,4-D is made. Without the operation of substantially different growth mechanisms or of side effects or lag periods, one would expect active compounds to produce quite comparable growth rates over long periods of time.

The growth rates of radish hypocotyl sections for a range of concentrations of IAA and IAN during 24 hours are shown in figures 3, 4, and 5. As was mentioned earlier, the values plotted represent the differences between growth in water and in regulators.



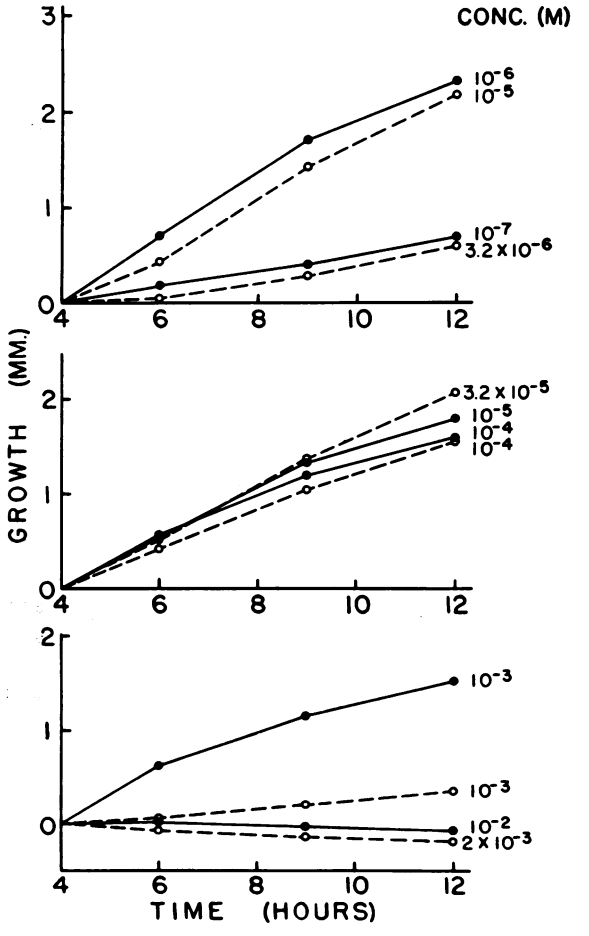


FIG. 3 (*left*). Growth rates of radish hypocotyl sections in IAA (solid lines) and IAN (broken lines). Water growth (subtracted before plotting) given in figure 6. 0 to 4 hrs.

FIG. 4 (*right*). As figure 3 except 4 to 12 hrs.

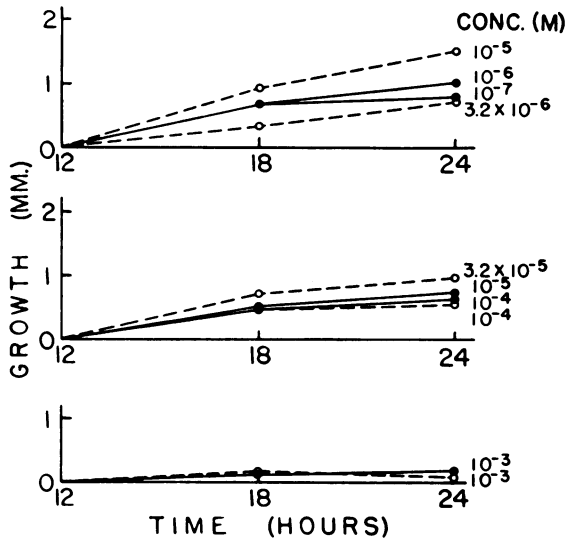


FIG. 5. As figure 3 except 12 to 24 hrs.

In this connection it should be noted that the negative slopes in these graphs do not reflect shrinkage of sections—shrinkage never having been observed—but only growth rates less than those of control sections in water. To enable more accurate representation of early growth and better comparison of actual rates and changes in rates, the curves were broken into three figures covering the periods of 0-4, 4-12, and 12-24 hours; and the figures were broken into three parts, grouping concentrations that produced comparable 24-hour growth. Although it was necessary that the scales for the abscissas in the figures be different, the scales of the ordinates were ajusted so that equal growth rates have equal slopes throughout, e.g., a growth rate of 0.5 mm per hour would have a slope of 1 in each figure.

The results are about the same as those found for Avena (3, 12) and what would be expected according to the preceding discussion. The apparent lag period for the first three hours with 10^{-5} M IAN is an anomalous result as indicated by other similar trials. Using fewer dishes and larger photographs taken 15, 30, and 60 minutes following transferral of sections, the initial growth rates in 10^{-6} M IAA and 10^{-5} M IAN were found to be nearly identical. Both showed slight lag periods of 30 minutes or less. With the bulk of the data showing that in both radish and Avena the initial growth rates in response to IAN do not lag measurably behind those to IAA, no conclusion regarding the necessity of conversion of IAN can be reached. It can be concluded that if such conversion is required, it occurs with sufficient rapidity to escape detection by measuring growth rates.

The possibility of high concentrations of IAA and IAN possessing general cellular toxicity in addition to probable self-competitive inhibition has been suggested. Evidence for such toxicity is illustrated in figures 3 and 4 in the rapid reduction from a high to an extremely low growth rate at the highest concentrations of both IAA and IAN. Toxicity symptoms appear at a lower concentration of IAN than of IAA. Initial growth rates in IAA continue to rise with increasing concentration, but this is not true for IAN. One more evidence of general cellular toxicity of IAN was the flaccid and water-soaked appearance of the ends of radish sections in 2×10^{-3} M IAN, the only concentration in which this was noted. Housley et al (12) found high concentrations of IAA and IAN also to exhibit toxicity symptoms in the growth rates of Avena coleoptile sections, although, as has been pointed out, their reported IAA toxicity seems related to low pH.

The data of figures 3, 4, and 5 are combined and plotted as a function of regulator concentration in figure 6. This emphasizes the limitations of results of the kind presented in figures 1 and 2 since the concentrations producing maximum growth and the shapes of the curves change with time. The points of maximum growth for IAA at the different times plotted on figure 6 sweep from lower right (1 hr, 10^{-2} M) to upper left (24 hr, 10^{-6} M) and for IAN from

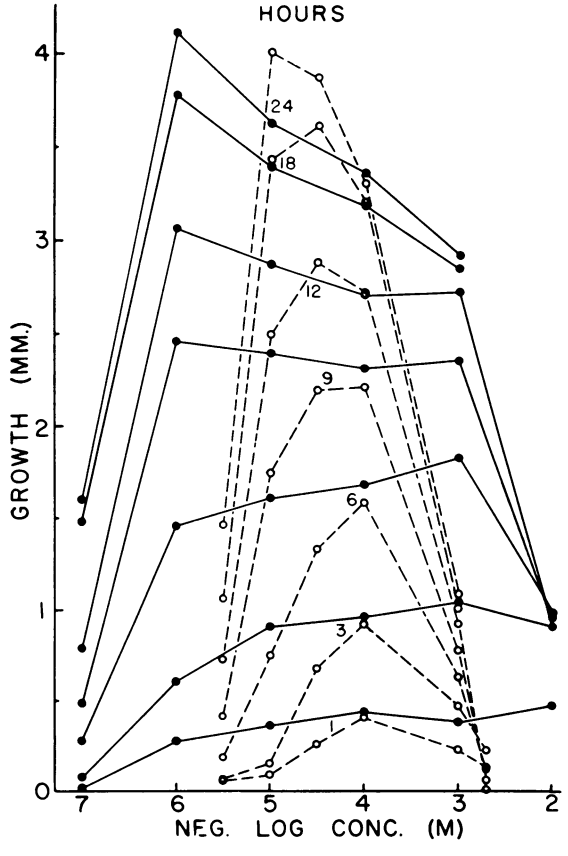


FIG. 6. Growth of radish hypocotyl sections in IAA (solid lines) and IAN (broken lines) after various periods of incubation. Combination of data from figures 3, 4, 5. Water growth (subtracted before plotting) in mm: 1 hr-0.17, 3 hr-0.22, 6 hr-0.35, 9 hr-0.43, 12 hr-0.46, 24 hr-0.57.

lower center (1 hr, 10^{-4} M) to upper center (24 hr, 10^{-5} M). Both shift to lower regulator concentrations with increasing time; however there is a 10,000-fold concentration change for IAA to only 10-fold for IAN. No appreciable change with time in concentration of IAA or IAN stimulating maximum growth in Avena has been reported (3, 4).

Perhaps most surprising is the plateau of uniform response to such a wide range of IAA concentrations —1000-fold even after nearly 12 hours. These results may help explain the apparently anomalous equal growth of turnip sections for such a wide IAA concentration range referred to earlier and recorded in figure 2. That there are no comparable plateaus in the IAN growth curves reflects a combination of two phenomena: the lack of response of radish sections to low concentrations of IAN and the greater general toxicity of IAN than of IAA at high concentrations. The latter has been discussed, but the former needs to be examined in more detail and in relation to the results obtained for Avena.

An attempt to establish a framework that might serve as a guide for interpretation of the data of this paper and also be in agreement with other available data led to the scheme presented in figure 7.

The potentially active IAA was suggested by and is meant to correspond with the "physical uptake" of Reinhold (17), the "diffusible" and "exchangeable" fractions of Johnson and Bonner (13), and the "outer space" of anionic diffusion of Epstein (6). The concentration of potentially active IAA within tissue is presumably the same as or directly proportional to the external concentration. In contrast to the above, Andreae and Van Ysselstein (1) found the uptake of IAA from external solution to be wholly dependent on aerobic respiration in pea stems.

The metabolic removal is based mostly on the findings of the above authors (1, 6, 7, 13, 17) and others. It is also presumed that the actual utilization of IAA in growth is metabolic, with the IAA so used never returned as again potentially active. The metabolic uptake might by-pass the diffusible internal pool of potentially active IAA in the epidermal cells. However, as pointed out by Epstein (6) in regard to uptake of ions, this could hardly be expected in the

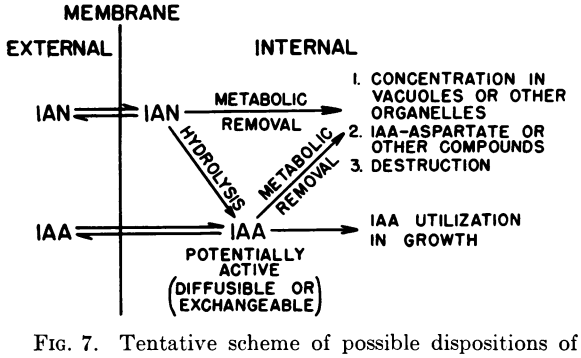


FIG. 7. Tentative scheme of possible dispositions of IAA and IAN in plant sections placed in solutions of these compounds.

internal cells. With an internal pool filled to at least a steady state by diffusion within 20 to 30 minutes (6, 13), inclusion in the diagram of metabolic uptake directly from the external medium seemed superfluous or even misleading.

Direct utilization of IAN in growth is not included since there is no clear evidence that it occurs; however, reference will be made from time to time to this possibility.

The rates of reactions and even some of the reactions presented in figure 7 certainly vary from species to species. It is this variability that must be used in any attempt to interpret or possibly explain the differences in responses noted. As one example may be cited the lack of significant responses of corn and peas to IAN, which indicates not only absence of direct IAN utilization but also absence of an IAN hydrolyzing enzyme or enzyme system in these species (19). Another example is the variability, both qualitative and quantitative, of metabolic products of IAA found in 12 plant species (7).

The belief, which seems to have become so widespread, that IAN activity in Avena depends on hydrolysis rests not only on proof that hydrolysis does occur but also on the assumption that the internal potentially active concentration of IAA is 3 to 10 times greater in any IAN solution than in an IAA solution of the same concentration. An apparently reasonable explanation has been offered to account for the latter (18)—a greater rate of uptake of IAN than of IAA coupled with considerable IAN hydrolysis.

Throughout the lower concentration range Avena requires about 5 times more IAA than radish for comparable growth. This greater sensitivity of radish could reflect differences in one or more of the actions which add IAA to or remove it from the potentially active pool. Whether movement across the membrane is sufficiently rapid to allow establishment of near equilibrium and not be limiting or is no more than a limiting steady state flow is undetermined. Greater penetrability of IAA through radish than through Avena membranes could be a reason for occurrence of the above phenomenon. Lower non-growth metabolic removal of IAA in radish than in Avena also could account for the greater sensitivity of radish since the effect would be to increase the relative amount of potentially active IAA. The most probable explanation, however, might lie in the relative reactivities in growth utilization of IAA or any complexes formed.

In view of the greater sensitivity of radish than of Avena to low IAA concentrations, it was somewhat surprising to find that radish requires about 100 times as much IAN as Avena for response. From the discussion above it certainly would appear that radish differs considerably from Avena in its ability to produce or maintain from external IAN a pool of potentially active IAA, if in fact it does so at all. If radish membranes are no less permeable than those of Avena to IAA, could they be expected to be less so to IAN? More reasonable would be a greater metabolic re-

moval of the internal IAN or a less concentrated hydrolyzing enzyme or one with a lower turn-over number. Steady state conditions for any hydrolysis that does occur must be rapidly established because, as indicated earlier, no greater lag period in growth response is found than for IAA alone. Still another possibility exists if one is ready to assume that radish membranes are more permeable to IAA than those of Avena. Perhaps a greater proportion of any IAA produced from IAN leaks to the external solution (where the concentration of IAA remains essentially zero) from radish than from Avena. Much of the IAA produced from IAN by Avena has been found in the external solution (18).

The difficulty of evaluating extractable auxins is well known (8). One possible lead in consideration of the hydrolysis to IAA of exogenously supplied IAN would be knowledge of the endogenous concentrations of IAA and IAN. Whether or not IAN is a natural precursor of IAA has not been shown; however the data of this paper show that if IAN is not active per se, it can be a precursor of IAA in hypocotyl cells. The early work of van Overbeek (20) with radish seedlings and the extremely limited growth of sections in water reported in this paper make it appear that any IAN found in hypocotyl cells originated in the upper portions of the seedlings as IAN or one of its precursors. Thus, IAN supplied exogenously in solution might be expected to find the same fate in hypocotyl cells as that originating within the seedlings. This should be true at low external concentrations at least, though Andreae and Van Ysselstein (1) found the patterns of internal metabolism and disposition of IAA in pea stems to be different at low and moderately high external concentrations.

Activity in the Avena coleoptile section test, described earlier, was found in the acid fraction from an aqueous extract of cabbage hypocotyl equivalent to approximately 2×10^{-7} M IAA in the original hypocotyls. The use of extract dilution and admixture with known concentrations of IAA indicated the presence of one or more inhibitors in the acid fraction that strongly inhibited growth of cabbage hypocotyls. They, however, had little effect on Avena coleoptile growth. No attempt has been made to determine the nature of these inhibitors.

The color reaction of this acid fraction in a Salkowski test produced an off shade of reddish pink that had considerable yellow in it as indicated by the absorption spectrum, which was somewhat reminiscent of the curve observed by Platt and Thimann (16) with a mixture of catechol and IAA. The intensity of the color reaction was several times greater than would have been obtained from a pure IAA solution of the concentration indicated by biological activity. Holley et al (10), while reporting IAA in ether extracts of cabbage leaves, also found much color developing that was not attributable to IAA. Although it was not proved that IAA was present in the extract from cabbage hypocotyls, the biological assay and color reaction point to the presence of some active, indole compound; and the fractionation procedure used makes it highly unlikely that it was other than an acid. Housley and Bentley (11) did not find IAA in cabbage leaves; however they stated that any present might have been destroyed during extraction.

The activity in the Avena coleoptile section test of the neutral fraction from the aqueous extract was equivalent to approximately 10^{-5} M IAN in the original hypocotyls, which is about the same as found in cabbage leaves by Henbest et al (9). The results of Housley and Bentley (11) indicate that almost all the activity found in the neutral fraction of an ether soluble extract is attributable to IAN.

The neutral fraction was negative in the Salkowski test and exhibited little evidence for the presence of inhibitors of Avena coleoptile growth, though it inhibited growth of cabbage hypocotyls nearly 100 %.

Since the growth responses of cabbage hypocotyls to 2×10^{-7} M IAA and to 10^{-5} M IAN are more than double the growth in water (fig 2), it seems certain that not all of the extractable auxin is immediately or potentially available for growth. Johnson and Bonner (13) report similar findings with 2,4-D and Avena coleoptiles. This could be interpreted as demonstrating that a continuing supply of regulator from a source external to the growing cell is essential for appreciable continued growth by elongation and that much of the regulator passing into a cell becomes unavailable for growth by elongation, though it conceivably could produce other of the recognized growth regulator effects. This might be expected if the regulator effect in growth by elongation is limited to the loosening of the cell wall, as most recently supported by Cleland and Bonner (5). It is also consistent with the scheme presented in figure 7.

Whether or not the 50 to 1 ratio of IAN to what is probably IAA reflects a limited hydrolysis of IAN is, therefore, uncertain. Such a ratio does, however, correlate quite well with the lesser sensitivity of crucifers to IAN than to IAA and the assumption that IAN must be hydrolyzed to be active in growth.

Analytical studies of the fates of externally supplied IAA and IAN are underway at the present time.

Summary

The 24-hour growth of cabbage, radish, and turnip hypocotyl sections was compared with that of Avena coleoptile sections. The crucifers were more sensitive to IAA but less sensitive to IAN. Whereas the growth response ranges for IAA and IAN were nearly identical for Avena, for crucifers the range of response to IAA was much greater than to IAN.

As had been reported for Avena, the growth rates of radish in response to IAA and IAN were essentially alike. Growth rates changed with time, increasing then decreasing at low concentrations and decreasing at medium to high concentrations. The concentrations of IAA and IAN producing maximum growth shifted to lower values with increasing time up to 24 hours, the shift for IAA being much greater than for IAN.

The concentrations of endogenous IAN and an active, acid, indole compound—presumably IAA—were found to be of the magnitude of 10^{-5} M and 2×10^{-7} M equivalents, respectively.

A scheme indicating the probable or possible fates of IAA and IAN following external presentation of these to plant sections was presented and the data discussed in relation to this scheme. Since several possibilities for explanation of the results exist, whether or not IAN is active in promoting growth before or only after hydrolysis in crucifers cannot now be determined.

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THE RELATION OF OPTICAL FORM TO THE UTILIZATION OF AMINO ACIDS. II. UTILIZATION OF STEREOISOMERIC VARIETIES OF ASPARTIC ACID AND ASPARAGINE BY CARROT ROOT DISKS[1]

E. D. H. EL-SHISHINY and M. A. NOSSEIR

Botany Department, Faculty of Science, Alexandria University, Moharrem Bey, Alexandria, Egypt

The numerous studies, that have been made, of the use of the amino acids as nitrogen sources for growing plants, embryos, plant tissues, and the limited number of investigations of the utilization of stereoisomeric varieties of amino acids by higher plants were referred to in the first paper of this series by El-Shishiny and Nosseir (3). The recent work of Webster and Varner (11) showed that lupine seedlings absorb and utilize aspartic acid.

The study of the metabolism of the stereoisomers of amino acids in living organisms has increased our knowledge of the range and nature of oxidative and synthetic reactions available to the body. More-

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