AUXIN RELATIONS IN A DWARF NANA_1 ALLELE OF ZEA MAYS L.

by

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I INTRODUCTION

Genetic dwarfism in corn in relation to auxin production and auxin inactivation was first investigated by van Overbeek (1935, 1938). Using only the diffusion technique, van Overbeek concluded that dwarfism in corn is caused by a lower production of auxin in the dwarfs 1, 2, 3, 7, pigmy, and nana as compared to the normals. Studying inactivation or inhibition of auxin in dwarf nana, he concluded that dwarfism in dwarf nana is enhanced by greater auxin inactivation or inhibition in addition to the lower production of auxin. Later studies have shown that a cause and effect relationship in terms of auxin production is not the reason for the shorter stature of dwarf A (Harris, 1953).

Numerous maize and pea mutants characterized by dwarfism have shown a response induced by gibberellic acid with the resultant phenotype of the normal plant.

The effect of gibberellic acid on dwarf mutants was first studied by Yabuta and Hayashi (1939), Brian and Hemming (1955). Phinney (1956) interpreted the response of four maize dwarf mutants as under the control of single genes. He interpreted the lack of response to gibberellic acid of the other two maize dwarf mutants, that is, dominant-dwarf and dwarf (4963) as an indication that the reason for dwarfism can vary, depending on the particular gene.
controlling the expression of the dwarfing character.

Much care is needed in interpreting the effects of mixtures of auxins, antiauxins or gibberellic acid applied externally; there is evidence that biological antagonism in such mixtures may be exerted on the absorption of the growth promoting substance rather than on the growth process per se.

Because all maize dwarfs including dwarf nana₁ are controlled by single gene loci, according to Emerson, Beadle, and Fraser (1935), the specific gene-specific enzyme theory of Beadle (1945) was considered to be the milestone for the interpretation of dwarfism in maize in relation to the growth promoting-growth inhibiting substances ratio.

For auxin estimation and auxin antagonism in maize segregating for dwarf nana₁, the ether extraction method and the method of ether extraction followed by separation by paper chromatography were conducted in addition to the diffusion method used by van Overbeek.

Studies involving the response of dwarf nana₁ to added gibberellic acid were conducted on the coleoptile, mesocotyl, primary root, primary leaf sheath, and primary and secondary leaf blade.

An experiment to study the interrelationship between light specificity and the length of the coleoptile and the mesocotyl of maize segregating for dwarf nana₁ was conducted to determine whether dwarf nana₁ is light controlled or not. This phenomenon has not been investigated previously.
II MATERIALS AND METHODS

Origin of Mutant

Zea mays seeds segregating for dwarf nana₁ were obtained from Dr. E. G. Anderson, of the California Institute of Technology. The seeds obtained segregate 3:1 for normal and dwarf nana₁, because they were a result of either selfing or crossing between normals, from a stock which segregates for dwarf nana₁.

Germination of Seeds

The seeds were soaked in distilled water for at least 16 hours. The seeds were then laid with the embryo up, between two layers of wet paper toweling in a deep aluminum plate, and covered with a glass cover. Forty-eight to sixty hours after, the germinating seeds were taken out and put on paper toweling, draped on germination racks consisting of hardware cloth 1 1/2" to 2" wide, laid across an aluminum tray 2" deep. The paper toweling dipped down into water in the tray, keeping the paper toweling moist. The chamber in which the seeds were germinated was dark interrupted by periods of about one hour a day of red light. The temperature of the chamber was 24° ± 1.5° C. and the relative humidity was 85 ± 4 per cent.

Selection of Material

The fourth day after soaking the seeds in water the
dwarf nana1 can be distinguished from the normal type. The main distinguishing point at this age is the mesocotyl, that is, the nana1 dwarf does not have a noticeable mesocotyl, whereas the average length of the normal mesocotyl is 25.8 ± 0.5 mm. This characteristic can be used for distinguishing the 5, 6, and 7 day-old seedlings of dwarf nana1 from the normal. For any one experiment, coleoptiles were of the same age and from the same ear. The coleoptile tips used for auxin assays and inactivation studies were 3-5 mm. in length.

Preparation of Material

Avena Test

The seeds of the genetically pure line oats known as Victory were freed from their husks and soaked in water for 2-4 hours. After this period they were laid out groove downward, on wet filter paper in petri dishes in the dark room with a one-hour period of red light each day. They were allowed to germinate in this way for about 40 hours. Following this the seedlings were racked in rows of 12 in glass holders and planted in water. The seedlings were left this way until the coleoptile length had become 25-30 mm. in length. This requires from 70 to 100 hours from the time of soaking the seeds in water.

The uniform coleoptiles were chosen and decapitated. The decapitation was made 3-5 mm. below the coleoptile tip.

Two and a half hours later the coleoptiles were redecapitated below the point of the first decapitation.
The primary leaf was pulled loose and agar blocks were applied unilaterally on the decapitated part and the primary leaf was used here as a support.

The curvature of the *Avena* coleoptiles was directly proportional to the concentration of the indole acetic acid (IAA) applied. This conclusion is the same as reached by Went and Thimann (1937). The data involving Avena curvature in relation to a series of IAA concentrations are shown in Figure 1. This figure shows that within the range of zero to 100 μg/l of IAA, the curvature is proportional to the concentration.

For all experiments the agar used was Fisher Agar-Agar. The agar was prepared as a 1.5% or 3% suspension according to the experiment, by dissolving the agar in hot distilled water and pouring it into vials which were tightly sealed and stored in the refrigerator. The IAA or the extract solution of plant material to be tested for activity was incorporated with this stock agar. The molten agar was then poured into a brass mold to give a block of the dimensions 8.1 mm. x 11.2 mm. x 0.9 mm. All blocks were cast from the same mold. Each block was cut into 12 equal blocks by using a clean safety blade and a cutting tool. Each small block was placed unilaterally on a decapitated *Avena* coleoptile. The blocks remained on the coleoptiles for two hours. Immediately they were shadow photographed and the shadow graphs developed. The protractor was used then
The relationship between IAA concentration and the *Avena* coleoptile curvature. Each value is an average of at least 9 plants.
to measure the coleoptile curvature on the shadow photograph.

**Ether for Extraction**

The ether used in all experiments was diethyl ether. It was made peroxide free by adding to it ferrous sulphate acidified with hydrochloric acid, and it was shaken and stored at 0°C. temperature. Before using it for extraction, the ether was assayed using the standard *Avena* test with a specific amount of IAA.

**Paper for Paper Chromatography**

**Preparation of Eluates Containing Auxin or Inhibitor**

The extracts of plant material were prepared as described by van Overbeek (1941) and Harris (1953). The chromatographic procedure was as described for small chromatograms by Kefferd (1954) with a slight modification. For each experiment IAA was spotted on the starting line as a standard for the location of the growth promoting substance. Following development the strip carrying IAA was sprayed with perchloric acid : ferric chloride : 50 : 1. The location of the IAA was identified and located as a crimson spot. The growth promoting substance in the strips spotted with dwarf *nana* and normal coleoptile tip extracts was considered to have the same size and location as IAA. The strips spotted with extracts of dwarf *nana* and normal coleoptile tips were dried and part of the papers equal to the size and with the same location as IAA were cut from each paper. The portions of the strips containing IAA were cut in-
to pieces, extracted with peroxide free ether, and bioassayed using the standard *Avena* test. The rest of the strips were cut into pieces, extracted with peroxide free ether and bioassayed with a known amount of IAA using the standard *Avena* test, for the estimation of IAA antagonism.

The number of coleoptile tips used for this purpose was 50 tips each for the normal and dwarf nana.

The paper used for chromatography of all experiments was Whatman Filter Paper No. 4.

**Gibberellic Acid**

Gibberellic acid 7.8% potassium salt from the Nutritional Biochemical Company was prepared as an aqueous stock solution of 1,000 μg/ml and used for all experiments with known concentrations.

Gibberellic acid was applied either externally by dropping it on the unfolding leaves of the plant, or internally by planting the plants in solutions of known concentrations of gibberellic acid. Soaking the seeds in known concentrations of gibberellic acid and planting them in water, was also used.

No nutrient solution was given to the plants treated with gibberellic acid, whether the application was external or internal.
**III EXPERIMENTAL RESULTS**

**Morphology of the Coleoptile**

The morphology of the coleoptile of maize has been well described by Avery (1930), Hayward (1951), and Harris (1953).

The corn coleoptile was defined by Avery (1930) as a closed cylindrical structure sheathing the growing point and embryonic leaves. The coleoptile is the first aerial embryonic structure which appears from the corn seed after germination. There is a pore near the end of the coleoptile through which the primary leaf protrudes when coleoptile elongation ceases. There is considerable evidence that the coleoptile is a reduced cotyledon or it is a single leaf rather than the result of fusion of two stipules or other structures. If one considers the coleoptile as the first green leaf of the plant, it is not surprising that it differs from the later leaves (Avery, 1928). Such is also the case in a great many dicots. In fact it is very often true that the first two or three leaves above the cotyledons differ markedly from the later leaves of the plant.

The epidermal cells of the coleoptile in longitudinal section are rectangular in shape and are cutinized on the outer surface.

The coleoptile grows from the time of germination.
until the seventh or eighth day. It has been shown that the growth of the coleoptile is induced by cell elongation. The elongation of the corn coleoptile is evenly distributed over the length of this organ, being different from the _Avena_ coleoptile which does not elongate evenly (Went and Thimann, 1937).

The coleoptile of dwarf nana₁ is shorter than that of the normal sib. The anatomy of the coleoptile of both dwarf nana₁ and the normal sib shows that a noticeable difference between dwarf nana₁ coleoptile and the normal sib is in the length of the epidermal cells, that is, the epidermal cells of the normal being longer than those of dwarf nana₁.

An anatomical study was made to determine the relationship between the length of the coleoptile and cell number of both normal and dwarf nana₁, in the embryonic period and the mature period.

The coleoptile of the embryo was excised from seeds which had been soaked in tap water for 24 hours. The coleoptile was removed leaving the rest of the embryo intact. The seed minus the coleoptile was then planted in vermiculite and given the same number as their fixed and killed coleoptiles. After germination they were classified as to normal and dwarf nana₁. The excised coleoptiles were killed and fixed in formalin-aceto-alcohol (FAA) and in a series of ethyl alcohol and TBA mixture starting from 50 per cent and ending with pure tertiary butyl alcohol (TBA) and TBA with oil, before embedding in paraffin. A median longitudinal section was
taken from each coleoptile. The sections were mounted on slides and stained in triple stain (safranin, fast green and orange G.) according to Johansen (1940) with slight modifications. The stained sections were mounted in Canada Balsam. In addition to that, fifteen complete embryos of unidentified seeds segregating for dwarf nanal were fixed, stained, and sectioned by the same method and for the same purpose.

A median longitudinal section midway between the vascular bundles was used for cell counts. The epidermal cells were counted from the base of the coleoptile to the tip on both sides and the number divided by two. Counts were made on ten separate embryo coleoptiles of each normal and dwarf nanal using an oil emersion objective. Counts also were made on the fifteen intact unidentified embryo coleoptiles. The average cell count of the normal was 83.5 ± 0.82 and that for dwarf nanal was 81.2 ± 0.76. The average cell count of the unidentified coleoptiles was 84.12 ± 0.62.

The above technique was used for sectioning the mature dwarf and normal coleoptiles. They were cut from the seedlings, fixed in FAA and dehydrated with TBA series, embedded in paraffin and a median longitudinal section midway between the vascular bundles was used for cell counts. The average count for twelve mature normal coleoptiles was 85.21 ± 1.3 and that for dwarf nanal was 83.3 ± 0.92.

Results

The average length of 110 normal and 45 dwarf nanal
coleoptiles prior to germination was $1.45 \pm 0.34$ mm. and $1.44 \pm 0.35$ mm.

The results of measurements of the length of the embryonic coleoptiles for normal and dwarf nana suggest that they are indistinguishable at this stage. This would suggest also that the epidermal cell numbers of the two coleoptiles would be the same. To verify this idea a cell count was made for the epidermis of both normal and dwarf nana coleoptiles and of both seed coleoptiles and mature coleoptiles.

A comparison of the values given in Table I shows roughly a difference of 3 per cent or less in the total number. Since the coleoptile increases in length about 20-fold for the normal and about 12-fold for the dwarf, the difference of less than 3 cells becomes negligible. Furthermore, the two mean values are statistically indistinguishable. Thus it may be concluded that elongation of the epidermis of the coleoptile during the growth of this organ is a function of cell elongation.
TABLE I

A comparison between the length and cell number of normal and dwarf nana\textsubscript{1} coleoptiles.

Each value is an average of at least 12 coleoptiles.

<table>
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<tr>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1.45 ± 0.34\textsuperscript{*}</td>
<td>83.5 ± 0.82</td>
<td>30.17 ± 0.77</td>
<td>85.21 ± 1.30</td>
</tr>
<tr>
<td>Dwarf Nana\textsubscript{1}</td>
<td>1.44 ± 0.35</td>
<td>81.2 ± 0.76</td>
<td>18.50 ± 0.65</td>
<td>83.30 ± 0.92</td>
</tr>
</tbody>
</table>

\textsuperscript{*}The Standard Error of the Mean

Auxin Content of Coleoptiles

Van Overbeek determined by diffusion the auxin production of the normal and dwarf coleoptile tips, for the dwarfs 1, 2, 3, 7, pigmy, and nana. The auxin that diffused from the tips into the agar block was assayed by the Avena test. The result of his study showed that the production of auxin in the dwarf coleoptile tips was lower than that of the normal sibs. To confirm van Overbeek's findings and to ascertain whether data from the diffusion technique would be comparable to those obtained by the more recent technique of ether extraction, the diffusion technique, the extraction technique and the technique of separation by paper chromatography were used in studies on nana\textsubscript{1}.

By Diffusion Method

Coleoptile tips 3-5 mm. in length of both dwarf nana\textsubscript{1}
and the normal sibs were removed from the coleoptiles by a clean safety razor blade and a cutting tool. The coleoptile tips were placed cut end down on wet filter paper in a closed petri dish for one hour to rid the cut surface of auxin-destroying substances produced by incision (van Overbeek, 1935). The tips were then removed from the petri dish and placed on an agar block for three hours. The agar block was then bioassayed using the standard Avena test. Six corn coleoptile tips were used for all diffusion experiments, because this number of tips was found to produce a satisfactory amount of auxin which causes a curvature of the Avena coleoptile of at least 50°.

The production of auxin of the dwarf nana₁ compared to the normal has been determined in seedlings from 4 to 7 days old. The lower limit was set because it was practically impossible to distinguish dwarfs from normal plants at a stage earlier than 4 days after the seeds had been soaked in water. The higher limit of 7 days was chosen because it was shown that auxin production after this day decreases rapidly (van Overbeek, 1938).

Results

Avena curvatures obtained from normal and dwarf nana₁ coleoptile tips are given in Figure 2. Each curvature represents an average value of at least 9 Avena test plants in degrees plus or minus the standard error of these test plants. Figure 2 shows that the production of auxin by dwarf nana₁ is
Figure II

The relationship between amount of auxin produced by the coleoptile tips (3-5 mm.) and the age of the seedlings in series segregating nana₁ dwarfs. Each value is an average of at least 9 Avena test plants.
considerably lower than that of the normal sibs, when the plants are 5 days old, but when the plants are seven days old, the contrary is observed, that is, the dwarf nanai plants produce considerably more auxin than the normal sibs. Plants 4 days and 6 days old, however, produce practically the same amount of auxin for nanai and the normal sibs.

The ratio of auxin content of the normal and dwarf nanai coleoptile tips represents the relative rate of production in the respective coleoptiles for the 3 hours left on the agar. This phenomenon was first interpreted by van Overbeek (1941).

The average ratios of diffusible auxin obtained from dwarf nanai compared to that obtained from normal coleoptile tips for 4 days old was 0.935, for 5 days old 0.843, for 6 days old 0.919, and for 7 days old 1.223.

**By Ether Extraction at 0° C.**

**Method**

At least 25 ml. of peroxide free ether was used for extraction at 0° C. The 0° C. temperature was chosen because it was found by Wildman and Muir (1949), that this temperature decreases or prevents enzymatic activities.

The ether extraction was conducted on 45 coleoptile tips, 3-5 mm. in length, for both of the normal and dwarf nanai, separately. The coleoptile tips were immersed immediately in ether, after removing them from their coleoptiles.

A twenty-four to forty-eight hour extraction period
was carried out in the dark at $0^\circ$ C. Following extraction the coleoptile tips were removed from the ether and the extracts evaporated to dryness in a water bath at $75^\circ$ C. The residue was redissolved in about one ml. peroxide free ether.

The extract was then introduced drop by drop onto 0.4 ml. molten agar (1.5%) contained in a 1 ml. volumetric test tube at a temperature of $70^\circ$ C. The ether was evaporated at the surface of the agar and the agar shaken thoroughly. Two portions of 1 ml. each of ether were used to rinse the walls of the evaporation flask and evaporated on the molten agar in the tubes and the tubes again shaken.

In order to determine the amount of auxin present in the ether extracted coleoptile tips, the extract was incorporated in the molten agar and the agar was then bioassayed by means of the standard Avena test.

Five day-old seedlings of both normal and dwarf nana\_1 were chosen for this method, because as shown by the diffusion method the auxin production of nana\_1 at this age was lower than that of the normal sib.

**Results**

The results of auxin determinations by ether extraction are shown in Table II.

The value given for each experiment is expressed in average degree curvature for 12 Avena plants.

The average of the ratios of dwarf nana\_1 to normal for these three experiments is 0.922.
TABLE II

The amount of ether extractable auxin obtained from normal and dwarf nana\(_1\) coleoptile tips expressed as degrees average curvature in *Avena* test. Forty-five tips used in each extraction.

Each value is an average of at least 12 *Avena* test plants.

<table>
<thead>
<tr>
<th>Exp't No.</th>
<th>Curvature of 50 gammas IAA per liter</th>
<th>Curvature of the Amount of Auxin</th>
<th>Ratio of Auxin Extracted</th>
<th>Auxin Extracted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Dwarf Nana(_1)</td>
<td>Dwarf to Normal</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>21.6 ± 0.73*</td>
<td>20.5 ± 0.40</td>
<td>19.2 ± 0.52</td>
<td>0.936</td>
</tr>
<tr>
<td>2</td>
<td>22.7 ± 1.20</td>
<td>16.1 ± 1.42</td>
<td>14.8 ± 0.60</td>
<td>0.919</td>
</tr>
<tr>
<td>3</td>
<td>22.9 ± 0.90</td>
<td>18.0 ± 0.91</td>
<td>16.4 ± 0.30</td>
<td>0.911</td>
</tr>
</tbody>
</table>

* The Standard Error of the Mean

By Separation from Ether Extracts by Paper Chromatography Method

Fifty coleoptile tips of normal and dwarf nana\(_1\) were extracted separately by the method mentioned previously.

As the forms in which auxins occur in plants are largely unknown chemically, methods of extraction remain empirical (Bentley, 1958). Bioassay of crude extracts has little meaning because of the complicated mixtures of growth promoting and growth inhibiting substances which are usually present; consequently paper partition chromatography, paper electrophoresis, and column chromatography, are now
widely employed as methods of purification before bioassay. Gordon (1954) and Larsen (1955) list over 20 papers using these techniques.

Strips 30 mm. in length of Whatman No. 4 chromatography paper were used and the ether extracts were applied to the starting lines of chromatograms from a calibrated micropipette of 2 μl. A strip with a marker spot of IAA applied at one end of the starting line was used as a standard.

The chromatograms were allowed to equilibrate with an atmosphere saturated with chromatographic solvent plus ammonia, for 16 hours and were developed with isopropanol : water : : 10 : 1 descending for time ranging from 4 to 16 hours at a constant temperature of 27° ± 1° C. After development, chromatograms were dried in a draught out of direct sunlight. The paper with the IAA spot was sprayed with perchloric acid : ferric chloride : : 50 : 1, and left to dryness. The crimson spot of the IAA was identified and located. Similar size and location for the extract chromatograms were cut and extracted separately with peroxide free ether, and bioassayed using the standard Avena test to determine the amount of auxin present in the chromatograms for the normal and the dwarf nana1.

Results

The results of auxin determination by ether extraction followed by separation by paper chromatography are shown in Table III.
TABLE III

Amount of ether extractable auxin separated by paper chromatography and obtained from normal and dwarf nana<sub>1</sub> coleoptile tips, expressed as degrees average curvature in the Avena test. Fifty tips used in each extraction.

Each value is an average of at least 10 Avena test plants.

<table>
<thead>
<tr>
<th>Exp't No.</th>
<th>Curvature of 50 gammas IAA per liter</th>
<th>Curvature of the Amount of Auxin</th>
<th>Ratio of Auxin Extracted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Normal</td>
<td>Dwarf Nana&lt;sub&gt;1&lt;/sub&gt;</td>
</tr>
<tr>
<td>1</td>
<td>22.6 ± 0.51*</td>
<td>11.75 ± 0.25</td>
<td>10.5 ± 0.61</td>
</tr>
<tr>
<td>2</td>
<td>23.1 ± 0.35</td>
<td>21.70 ± 0.53</td>
<td>19.4 ± 0.42</td>
</tr>
</tbody>
</table>

* The Standard Error of the Mean

The value given for each experiment is expressed in average degrees curvature for at least 10 Avena plants.

The average of the ratios of curvature of dwarf nana<sub>1</sub> to normal for two experiments is 0.89.

Interpretation of Auxin Content

Diffusion techniques indicate that the dwarf nana<sub>1</sub> coleoptile tip produces from 0.84 to 1.22 as much auxin as the normal coleoptile tip; according to the age of the seedling and over a diffusion time period of 3 hours. Ether extraction technique indicates that the five day-old dwarf nana<sub>1</sub> coleoptile tips contain 0.92 as much ether extractable auxin as normal coleoptile tips. The five day-old dwarf nana<sub>1</sub>
coleoptile tips contain 0.89 as much separated auxin by paper chromatography as normal coleoptile tips.

Diffusible auxin is a measure of the auxin content of the tips at the time of their removal from the coleoptile and the auxin produced in the tips during the period of time that the tips remain on the agar block (van Overbeek, 1941). Ether extraction at 0° C. involves only the auxin present in the tip at the time of removal from the coleoptile (Wildman and Muir, 1949).

The values obtained from crude extracts and those obtained through separation by paper chromatography are statistically indistinguishable. Because the growth promoting substance was separated as IAA, this can lead to the conclusion that the growth promoting substance in the corn coleoptile tips is a substance which is IAA itself or at least acts like IAA (Housely, Booth, and Phillips, 1956).

The difference between the ratios of the above two mentioned methods and that obtained by the diffusion method, leads to the conclusion that the rate of auxin production by dwarf nana is lower than that of the normal. The same conclusion was reached by van Overbeek (1941) for dwarf nana and by Harris (1953) for dwarf A.

**Antagonists and Antiauxins**

**Inactivation of Indoleacetic acid by Corn Coleoptile Tips**

An antiauxin is defined as a substance which inhibits competitively the action of auxins (Tukey, Went, Muir, and
van Overbeek, 1954), though such a competitive inhibition is very difficult to prove, and if it does occur, is probably found at many stages in the uptake, transport, or action of the substances in the tissues (Bentley, 1958).

Auxin antagonists are defined as substances which are themselves inactive, but which counteract externally-added auxin.

A phenomenon similar to auxin antagonism was found by van Overbeek (1935) in dwarf forms of Zea mays. In dwarf nana van Overbeek concluded that the nana inactivated about twice as much auxin as the normal. Went and Thimann (1937) were the first to notice this phenomenon of auxin inactivation of Avena coleoptiles previously treated with auxin. They noticed that if low concentrations were applied, the amount of auxin disappearing was proportional to the resulting growth, but when high concentrations were used, there was a rapid disappearance of the excess auxin without any accompanying growth. Studying inactivation or inhibition in dwarf A₁, Harris (1953) did not find a significant difference in inactivation or inhibition between the normal and dwarf A₁.

Van Overbeek has not mentioned whether the mesocotyl of dwarf nana₁ is light controlled or not.

In order to determine if the light has an effect on the endogenous growth promoting-growth inhibiting substances ratio, the length of the mature coleoptile and mesocotyl of both normal and dwarf nana₁, was measured in white light,
red light, dark with one-hour daily periods red light, and in complete darkness. The measurements of the average length coleoptile and mesocotyl are given in Figure 3.

The existence of an auxin-destroying system in roots was early recognized by Gorter (1927), van Raalte (1936), and by later investigators. Bonner (1949), Galston and Dalberg (1954) showed that the roots may often contain the highest amounts of the IAA-oxidase system, considerable amounts being present in etiolated shoots also, while it seems to be absent from or inactivated by inhibitor in green leaves. Aberg (1956) has tested many compounds which have antiauxin properties and he considers that the hypothesis of a competitive antagonism still best fits the facts as known (Bentley, 1958). On the other hand, the effect of a range of synthetic auxin antagonists and antiauxins on metabolism has been examined by Marre and Dugnani (1954) who suggest that they act by inactivating some fundamental dehydrogenase system, possibly by blocking SH groups even in the absence of auxin. Thimann (1956) points out that the competitive nature of the inhibition caused by so-called antiauxins may be only apparent. The observed effects may be better explained, as may also a number of synergistic effects, as a "natural consequence of the interaction of a group of limiting factors in controlling a multifactor process."

Naturally-occurring inhibitors, although not necessarily having antiauxin functions, are obviously very
The effect of light on the length of the coleoptile and mesocotyl. Each value is an average of at least 25 plants.
widespread. Early reports are summarized by Evenari (1949). Later reports include an inhibitor of shoot elongation and lateral root initiation isolated from pea roots (Howell, 1954) which may be identical with an inhibitor of lateral root tips (Torrey, 1956). Also reported are a stable, water-soluble inhibitor from dormant peach flower buds (Hendershott and Baily, 1955), and a neutral inhibitor in grape internodes and buds (Spiegel, 1954). All these inhibitors are ether soluble. A neutral water-soluble inhibitor in cotyledons of Sinapsis alba seedlings, which controls development of the axillary buds, is also reported (Reinert, 1954).

Some workers have demonstrated the presence of inhibitors by paper partition chromatography. Recent reports include studies on potato (Blommaert, 1954), cabbage (Housely and Bentley, 1956), Avena coleoptiles (Soding and Raadts, 1956), citrus flowers (Stowe, Thimann, and Kefford, 1956), seedling sunflower shoots and pea roots (Audus and Thresh, 1956), two germination inhibitors isolated from lettuce and xanthium seeds (Wareing and Foda, 1956), apple leaves (Luckwill, 1956), black currant berries (Wright, 1956), cauliflower leaves, gooseberries, root nodules and dwarf bean seedlings (Wright, 1956).

Studies were conducted in this thesis to estimate auxin antagonism in the coleoptile tips of Zea mays segregating for dwarf nana. The extraction technique and the technique of separation by paper chromatography, were con-
ducted in addition to the diffusion technique used by van Overbeek.

In all experiments for the estimation of auxin antagonists in the coleoptile tips, 5 day-old seedlings were chosen for both normal and dwarf nana.

**Methods**

1. a. Auxin Antagonism by Diffusion

The diffusion technique described previously was used.

Eight coleoptile tips 3-5 mm in length were placed with their basal cut surface on a 3% agar block without IAA for a period of 3 hours. A similar number with similar length of tips was placed on a 3% agar block containing an equivalent of 100 μg/l of IAA and also left for three hours. The tips were removed from the agar blocks at the end of the three hours and the agar blocks were applied on Avena coleoptiles using the standard Avena test.

**Results**

The data in Table IV show that the auxin antagonism of dwarf nana is about three times as much as that of the normal sibs.

1. b. Auxin Antagonism by Diffusion

The method described in 1. a. was used with a slight modification. The tips were removed from the block without IAA after three hours and this block was then placed in contact with an agar block containing the equivalent of 100 μg/l of IAA for a period of three hours to permit diffusion of
TABLE IV

Antagonism by diffusates from normal and nana\textsubscript{1} coleoptile tips.

Values expressed in average degrees curvature from Avena test.

Sample of eight tips per block.

Each value is an average of at least 10 Avena curvatures.

<table>
<thead>
<tr>
<th>Exp’t Variety</th>
<th>Curvature of Avena</th>
<th>Curvature of Diffusate Only</th>
<th>Observed Curvature of Diffusate Plus IAA per liter</th>
<th>Calculated Curvature of Diffusate Plus 100 gammas</th>
<th>Gain or Antagonism % of Difference of Curvature</th>
<th>A+B</th>
<th>C-(A+B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Used</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>31.8 ± 1.5*</td>
<td>11.10 ± 0.92</td>
<td>37.70 ± 0.98</td>
<td>42.90</td>
<td>-5.2</td>
<td>12.10</td>
<td>19.94</td>
</tr>
<tr>
<td>Nana\textsubscript{1}</td>
<td>9.40 ± 0.48</td>
<td>28.00 ± 1.10</td>
<td>41.20</td>
<td>-13.2</td>
<td>32.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>34.2 ± 1.3</td>
<td>14.20 ± 0.83</td>
<td>44.00 ± 0.41</td>
<td>48.40</td>
<td>-4.4</td>
<td>9.90</td>
<td>18.30</td>
</tr>
<tr>
<td>Nana\textsubscript{1}</td>
<td>12.25 ± 0.94</td>
<td>33.35 ± 0.45</td>
<td>46.45</td>
<td>-13.1</td>
<td>28.20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The Standard Error of the Mean
materials between the two blocks. These blocks were then tested by the standard Avena test. The sum of the average curvatures produced by the two blocks was then taken as the "observed curvature" of the diffusate plus the added IAA.

This method was used to confirm that the growth substances have been destroyed by the method described above under l. a. and not merely transported against the direction of the polarity.

Results

The data given in Table V show the curvature of the 100μg/ℓ of IAA only, the curvature of the diffusate put in contact with the agar block containing 100μg/ℓ of IAA, the "observed curvature" which represents the IAA curvature alone plus the curvature produced by the diffusate put in contact with agar block containing 100μg/ℓ of IAA, and the difference between the "calculated curvature" and the "observed curvature."

2. IAA Antagonism by Extraction

Method

The extraction method described previously for auxin estimation of normal and dwarf nana₁ coleoptile tips, was used in studying antagonism of ether extractable substances. Sixty coleoptile tips 3-5 mm. in length for both the normal and dwarf nana₁ were used for each experiment; then the extract solution was divided into two equal portions; one portion was used for auxin estimation, the other portion,
TABLE V

Antagonism by diffusate from normal and dwarf nana₁ coleoptiles.

Values expressed in average degrees curvatures from *Avena* test.

Sample of eight tips per block.

Values average of duplicate blocks.

<table>
<thead>
<tr>
<th>Exp't Variety</th>
<th>Curvature of Avena Coleoptile</th>
<th>Curvature of Diffusate 100 gammas Only</th>
<th>IAA per liter</th>
<th>Curvature of Diffusate Plus 100 gammas</th>
<th>Calculated Gain or % Loss in Curvature of Diffusate Plus 100 gammas</th>
<th>Inact. Difference or Inhib. nism</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Used</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>32.3 ± 0.4*</td>
<td>12.3 ± 0.35</td>
<td>38.55 ± 0.81</td>
<td>44.6</td>
<td>- 6.05</td>
<td>14.90</td>
</tr>
<tr>
<td>Nana₁</td>
<td>8.5 ± 0.44</td>
<td>31.00 ± 0.76</td>
<td>40.8</td>
<td>- 9.80</td>
<td>24.02</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>29.5 ± 0.61</td>
<td>11.2 ± 0.30</td>
<td>26.60 ± 0.40</td>
<td>40.7</td>
<td>-14.10</td>
<td>34.64</td>
</tr>
<tr>
<td>Nana₁</td>
<td>7.6 ± 0.21</td>
<td>18.40 ± 0.45</td>
<td>37.1</td>
<td>-18.70</td>
<td>50.70</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The Standard Error of the Mean
for auxin antagonism. An equivalent of 50 μg/l of IAA was added to 1.5% agar containing the latter portion.

Results

The curvature of 100 μg/l of IAA only, the curvature of the extract only, the "observed curvature," the "calculated curvature," and the difference between the "observed" and the "calculated" curvatures, are given in Table VI. Table VI shows the difference of antagonism between dwarf nana1 and the normal sib, using the extraction technique.

3. IAA Antagonism

By Separation by Paper Chromatography

The method of separation of the growth promoting substances from antagonists, described previously, was used.

An equivalent of 100 μg/l of IAA was added to 1.5% agar containing the extracts of the strips other than the portions containing the growth promoting substances. The standard Avena test was used for assaying the growth promoting substances and the antagonists.

Fifty coleoptile tips 3-5 mm. in length for both the normal and the dwarf nana1 were used.

Results

The data for the curvature of the 100 μg/l of IAA, and the curvature of the neutral substances plus 100 μg/l of IAA are given in Table VII. Table VII shows the difference of antagonism between dwarf nana1 and the normal sib by separation by paper chromatography technique.
TABLE VI

Antagonism by ether extracts from normal and dwarf nanai coleoptile tips.

Values represent extract of 30 tips in average degrees curvature from the *Avena* test.

Tips extracted for at least 24 hours in the dark at 0° C.

Each value is an average of 12 *Avena* coleoptile curvatures.

<table>
<thead>
<tr>
<th>Exp't Variety</th>
<th>Curvature of Avena Coleoptile Extract</th>
<th>Curvature of Extract Only</th>
<th>Observed Curvature Only Plus 50 gammas IAA per liter</th>
<th>Calculated Curvature Plus 50 gammas IAA per liter</th>
<th>Gain or Loss in Curvature</th>
<th>% Antagonism</th>
<th>% Difference of Antagonism</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Used</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>24.8 ± 0.1*</td>
<td>12.10 ± 0.3</td>
<td>29.0 ± 0.30</td>
<td>33.00</td>
<td>- 4.00</td>
<td>12.10</td>
<td>20.85</td>
</tr>
<tr>
<td>Nana1</td>
<td>10.30 ± 0.1</td>
<td>24.1 ± 1.20</td>
<td>35.10</td>
<td>-11.00</td>
<td>32.95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>23.2 ± 0.3</td>
<td>13.10 ± 0.6</td>
<td>31.2 ± 0.30</td>
<td>36.30</td>
<td>- 5.10</td>
<td>14.05</td>
<td>21.65</td>
</tr>
<tr>
<td>Nana1</td>
<td>11.95 ± 0.4</td>
<td>23.1 ± 0.65</td>
<td>35.15</td>
<td>-12.05</td>
<td>35.70</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The Standard Error of the Mean
TABLE VII

Antagonism by separating the neutral substances by paper chromatography from normal and dwarf nana₁ coleoptile tips.

Values represent the neutral material of 50 coleoptile tips, in an average degrees curvature from the Avena test.

Each value is an average of 12 Avena coleoptile curvatures.

<table>
<thead>
<tr>
<th>Exp't Variety</th>
<th>Curvature of Avena Coleoptile substances caused by 100 gammas IAA per liter only</th>
<th>Curvature of the neutral substances Plus 100 gammas IAA per liter</th>
<th>Gain or Loss in Antagonism Curvature</th>
<th>Per cent Difference of Antagonism</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Used</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>34.3 ± 0.55*</td>
<td>29.2 ± 0.71</td>
<td>- 5.1</td>
<td>114.90</td>
</tr>
<tr>
<td>Nana₁</td>
<td>21.5 ± 0.40</td>
<td>27.8 ± 0.35</td>
<td>- 3.6</td>
<td>11.46</td>
</tr>
<tr>
<td>Normal</td>
<td>31.4 ± 0.30</td>
<td>19.4 ± 0.62</td>
<td>-12.0</td>
<td>38.20</td>
</tr>
<tr>
<td>Nana₁</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The Standard Error of the Mean
Interpretation of Antagonism by Corn Coleoptile Tips

The idea that over-all growth of plants can be influenced by auxin inactivation goes back to some of the early work on auxins, in which the existence of particularly active peroxidase or auxin destruction substances was noticed with dwarf varieties of plants. Van Overbeek (1935) correlated dwarfism in dwarf nana to the greater inactivation of auxins compared to the normal. Von Abrams (1953) compared auxin inactivation in one dwarf and a closely related normal variety of peas, and found there to be no substantial difference, even though treatment with auxin would accelerate the growth of the dwarf more than that of the normal. Galston (1957) has reported markedly greater auxin destroying activity in a dwarf variety of peas.

Galston and Dalberg (1954) have developed the thesis that destruction of auxin limits the zone of elongation in stems, roots, and promotes "ageing" or maturation of plant cells. This was based primarily on the finding that a gradient of increasing IAA-destroying activity appeared with sections of pea stem or root increasingly distant from the apex. It was suggested that towards the lower end of the elongation zone, the rapidly increasing rate of auxin destruction causes the auxin concentration to fall rapidly to the point where growth ceases, and cell maturation occurs. van Overbeek (1935) has reached the same conclusion on the coleoptiles and mesocotyls of Zea mays segregating for dwarf nana.
Jensen (1955) found that peroxidase activity in the zone of elongation of *Vicia faba* roots was strikingly increased by previous treatment with IAA.

Van Fleet (1954) reported that the differentiation of some specialized tissues may also involve the accumulation of phenolic substances which are likely to act either as cofactors for or inhibitors of IAA oxidation. It is an evident possibility, therefore, that the entire gradient in IAA-destroying activity may be simply a consequence of the anatomical changes which take place in the course of development of the stem or root, and might have nothing directly to do with auxin inactivation in the plant (Ray, 1958).

These points illustrate the fact that the observed inversed correlations between activity and auxin content or growth are, as is usually recognized, only correlations and are not necessarily causally connected.

These comments may indicate the many uncertainties which beset the effort to relate IAA-destroying activity in homogenates to processes going on in the intact plants. This makes plain the need for further direct evidence on rates of destruction of native auxin in the intact plants.

For the interpretation of the results of antagonism in corn segregating for dwarf nana, it was tried to avoid many of the previously mentioned difficulties and uncertainties. The coleoptile tips 3-5 mm. in length were chosen for all the experiments, the only variable factor was the
methods and techniques. The lowest antagonism difference between dwarf nana₁ and the normal sib₂ was by the diffusion or l. b. method. It was an average of 12.59 per cent. The highest antagonism difference found between dwarf nana₁ and the normal sib₂ was the method separation by paper chromatography. It was an average of 24.57 per cent. These two results are distinguishable. No better interpretation than saying that the results are dependable to a great extent, on the technique and method used. Still the conclusion of van Overbeek (1935) that dwarfism in nana₁ is related to a lower auxin production and greater inactivation, is valid, if it could be found that auxin production in dwarf nana₁ is consistently less than the normal.

Growth Response to Gibberellic Acid

Not much is known about the effect of gibberellic acid on plant growth. The response of genetic dwarf peas was studied by Yabuta and Hayashi (1939), Brian and Hemming (1955). The response of maize dwarf mutants to gibberellic acid was studied by Phinney (1956). Phinney correlated the maize mutant response to gibberellic acid with single gene action.

The most typical and striking plant response to treatment with gibberellic acid is stem elongation (Stowe and Yamaki, 1957). It is not known if stem elongation is a function of cell elongation or cell division or of both of them.
A differential response to gibberellic acid of varieties of plants within one species was noticed. The most striking instance of varietal response is the effect on certain dwarf plants, first reported by Brian and Hemming (1955). They found that "a few micrograms of gibberellic acid on a leaflet would increase the growth rate of dwarf peas to that of a tall variety." Some peas were more sensitive than others. A similar effect was reported for dwarf varieties of *Vicia* and *Phaseolus*. Phinney (1956) found that in four dwarf mutants of maize, gibberellin brought growth rates up to that of treated normals. Of the other mutants dwarf *nana₁* showed a slight response. When treatment was stopped, the mutants slowly returned to a dwarf growth rate.

In tomato leaf petioles, it was found that there is no response for 1% gibberellic acid in lanolin whereas they are very sensitive to auxin (Kato, 1953).

Pure gibberellic acid was found to increase both length and dry weight of rice leaves (Hayashi and Murakami, 1953). In addition to that an extensive study has been made to show that leaf elongation is a characteristic of the action of gibberellic acid. Yabuta and Hayashi, 1939) on cucumber leaves; Yabuta, Sumiki, and Takahashi (1943) on tobacco leaves, Lockhart (1956) on pea leaves, Brian et al. (1954) on wheat and pea leaves, Radley (1956) on wheat leaves.

It was concluded from all the studies mentioned above that the effects of gibberellic acid vary with leaf and
plant age, and possibly with season as well. There is no
doubt, however, that the capacity to influence leaf growth
is one of the major characteristics of gibberellic acid.

Studies of the effect of gibberellic acid on root
elongation have not reached certainty. Most of the workers
have shown either no influence or inhibition.

The effect of gibberellic acid on Zea mays segre­
gating for dwarf nana, was studied in this thesis. The
studies were restricted to the coleoptile, mesocotyl,
primary leaf blade, secondary leaf blade, primary leaf
sheath, and the primary root.

Methods

Zea mays seeds segregating for dwarf nana, were used
for all experiments.

For the studies of the effect of gibberellic acid on
the length of the mesocotyl and the coleoptile, the seeds
were soaked in 0, 5, 10, 20, 50, 100, 500, 1000, and 2000
$\mu g/l$ of gibberellic acid for 24 hours and planted in aqueous
solutions of the same concentrations of gibberellic acid.
The seeds were planted and grown in darkness with a daily
one hour period of red light. Seven days after the day of
soaking, the coleoptiles and mesocotyls were measured.

For the studies of the effect of gibberellic acid
on the primary leaf sheath and the primary root, the concen­
trations used were 0, 5, 10, and $20\mu g/l$. These concen­
trations were either dropped on the unfolding leaves once every
24 hours when the seeds were planted in water in the white light, or the seeds were planted in the same concentrations of gibberellic acid in the dark with daily periods of one hour of red light. The measurements of the primary root and the primary leaf sheath were taken after at least 8 days of treatment.

For the studies of gibberellic acid on the length of the primary leaf blade and the secondary leaf blade, the seeds were soaked in water and planted in vermiculite. Treatment was started when the plants were 9 days old. Gibberellic acid was dropped on the unfolding leaves once every 24 hours. The concentrations of gibberellic acid used were 0, 10, and 100 \( \mu g/\ell \). Measurements were taken every 24 hours for a period of 8 days.

Results

The results of the effect of gibberellic acid on the mesocotyl and the coleoptile are given in Figure 4. Figure 4 shows no statistically distinguishable difference between the treated coleoptiles and mesocotyls and those untreated.

Figure 5 shows that the externally applied gibberellic acid has no effect on the primary leaf sheath. It shows also that the gibberellic acid has inhibited elongation for both the normal and dwarf nana1. The primary root of dwarf nana1 shows growth promotion for concentrations above 30 \( \mu g/\ell \). The higher concentration such as, 50 \( \mu g/\ell \) has greater influence on the primary root of dwarf nana1 as
The effect of gibberellic acid on the length of the mesocotyl and the coleoptile of the normal and dwarf nana. Each value is an average of at least 16 plants.
The effect of gibberellic acid on the length of the primary root and the primary leaf sheath. Each value is an average of at least 3 plants.
compared to the normal. The treated primary root of dwarf nana₁ is greater in length and significantly different, compared to the standard. The elongation of the primary root of the normal is greatly inhibited by all the gibberellic acid concentrations.

Figure 6 shows that soaking the seeds in gibberellic acid and planting them in an aqueous solution of gibberellic acid in the dark with daily periods of one hour of red light, has no effect on the primary leaf sheath of both the normal and dwarf nana₁. The elongation of the primary root of dwarf nana₁, especially, shows growth promotion, when it reaches almost the same length of the normal.

Figures 7, 8, and 9 show clearly that gibberellic acid has no effect on the elongation of the primary leaf blade. They show at the same time that the gibberellic acid has promoted elongation of the secondary leaf blade especially that of dwarf nana₁.
The effect of gibberellic acid on the length of the primary leaf sheath and the primary root. Each value is an average of at least 5 plants.
The effect of gibberellic acid on the elongation of the first and second leaf blade of maize segregating for dwarf nana. Each value is an average of at least 6 plants.
The effect of gibberellic acid on the length of the first and second leaf blade of maize segregating for dwarf nana₁. Each value is an average of at least 6 plants.
The effect of gibberellic acid on the length of the first and second leaf blade of maize segregating for dwarf nanal. Each value is an average of at least 6 plants.

Figure IX
The morphological studies of *Zea mays* segregating for dwarf *nana_1* have shown that the coleoptile of the normal is indistinguishable in the embryonic period from that of dwarf *nana_1*. The length of the coleoptile of the normal, however, can be distinguished from that of the dwarf *nana_1* from the fourth day after soaking in water. The average length of dwarf *nana_1* mature coleoptiles compared to that of the normal is 0.613. The anatomy of embryonic and mature coleoptiles of both normal and dwarf *nana_1* show no significant difference in the epidermis cell number. Because dwarf *nana_1* coleoptile is much shorter than that of the normal, and because the number of cells in both of them is the same, this leads to the conclusion that the length of the *Zea mays* coleoptiles segregating for dwarf *nana_1*, is a function of cell elongation.

Light has shown a considerable influence on the length of the coleoptile and mesocotyl of maize segregating for dwarf *nana_1*. Almost a straight line can be drawn between the average lengths of the coleoptile, starting from the average value in the white light, the average value in the red light, the average value in the dark and red, and the average value in complete darkness. This is not the case for the mesocotyl of dwarf *nana_1*, because it shows a response
of elongation when planted in complete darkness. The difference in the length of the coleoptile and mesocotyl in the different kinds of light shows that this can be correlated with the effect of light on the ratio of growth promoting-growth inhibiting substances.

Auxin production of the coleoptile tips by the diffusion technique indicates that the ratios of auxin production are dependent on the age of the seedling since from four day-old and six day-old coleoptile tips, no significant difference was observed in auxin amount, between the normal and dwarf nanai. However, dwarf nanai produced significantly less amount of auxin compared to the normal at the age of 5 days. The dwarf nanai produced a significantly greater amount of auxin than the normal sib at the age of 7 days.

The five day old seedlings in which dwarf nanai produces significantly less auxin than the normal, were chosen for all experiments dealing with auxin estimation by methods other than the diffusion method and for all experiments dealing with antagonism. The five day-old seedlings were chosen for all experiments to produce consistent results.

Auxin antagonism was greater at all times in dwarf nanai coleoptile tips than in the normal sib. By diffusion and ether extraction methods auxin antagonism was more than double in dwarf nanai compared to the normal. By the ether extraction method followed by separation by paper chromatography, auxin antagonism was about three times as great in
The lower auxin production and greater auxin antagonism of dwarf nana leads to the same conclusion which van Overbeek (1935) reached, "Dwarfism in corn segregating for dwarf nana is a result of less auxin production and more auxin inactivation by dwarf nana compared to the normal sib." This conclusion is indicated in spite of the fact that the techniques in this thesis have shown a much smaller difference in auxin production and much greater difference in auxin antagonism, in comparison to the technique of van Overbeek.

In any case the results of auxin estimation and auxin inactivation, as demonstrated in this thesis, can be correlated to the auxin promoting-auxin inhibiting, substances ratio; affected either by the existence of substances which inhibit competitively the action of auxins or to the existence of substances which are themselves inactive, but which counteract externally added auxin.

Our evidence for the antiauxin effects was derived from the application of synthetic auxin; because of the difficulties of investigating the natural growth regulators, and the ease of applying synthetic substances to tissues. The difficulties of this approach lie in the interpretations, and until we know more about the chemistry of the cell, it is clear that this type of approach must remain largely empirical.

Gibberellic acid in dwarf nana brought growth rates in the primary root and the secondary leaf blade, up to that
of the treated normal and in some instances to more of the treated normal. It has a very slight effect on both dwarf nana₁ and the normal, for the mesocotyl, the coleoptile, and the primary leaf sheath.

The report of Brian and Hemming (1955) has shown that the growth response of dwarf varieties of beans and peas to gibberellic acid suggests that this response is probably under genetic control according to Phinney (1956). The response of dwarf nana₁ to gibberellic acid and the four maize mutants studied by Phinney (1956) are clear examples of cases in which the ability to respond to gibberellic acid is under the control of single genes. The lack of response of two maize mutants studied by Phinney (1956) indicates that the reason for dwarfism can vary, depending on the particular gene controlling the expression of the dwarfing character.

It has been postulated by Beadle (1945) that the self-reproducing hereditary units, the genes, bring about their effects through determining the specificity or activity of enzymes, one gene being primarily concerned with one enzyme. As was discussed by Horowitz and Leupold (1951), this does not imply that more than one gene cannot be concerned in the production of an enzyme nor that the phenotypic expression of a gene is restricted to a single effect or function. Lockingen and De Busk (1955) have advanced an attractive scheme to account for the transfer of gene specificity to enzymes. According to their model gene-controlled enzyme
specificity is determined by a template synthesis of specific ribonucleic acid.

If we assume that the genes somehow produce their effects through determining enzyme specificity, a genetic difference of the *Neurospora* "blocked reaction" type can be represented as:

\[ \text{gene controlled enzyme} \]

\[ A \rightarrow B \]

where a precursor or substrate molecule A is converted to product B through the activity of a gene-determined enzyme. The presence of the normal versus the mutant allele determines whether the enzyme is active. Such a picture, while it serves a useful function in elementary visualization of gene differences, may be overly simple in two important respects. First the evidence indicates that mutations more frequently involve "somewhat changed" rather than "completely blocked" reactions, and therefore changes in the formation of B can be considered as changes in the rate of conversion of A to B. Second, a gene that influences the conversion of A to B need not operate by a direct effect on the enzyme, although the cases of gene-enzyme relationships that have been studied to date indicate that direct effects are common (Horowitz, 1956).

A large amount of work has been carried out on the mechanism of growth promoting substances, but no firm conclusion, even as to the site of action in the cell, can be reached. There is considerable evidence from recent work on
plant hormones, that the action of plant hormones has returned to the original concept of an effect on the cell wall, though modern work is couched in more elegant terms, invoking an effect via aspects of cell metabolism.
V SUMMARY

1. The length of the mature dwarf nana1 coleoptile compared to the normal sib is 0.61.

2. The length of the seed dwarf nana1 coleoptile is indistinguishable from that of the normal sib.

3. Epidermal cell counts for the seed coleoptile and mature coleoptile of both dwarf nana1 and the normal sib show that there is no significant difference between the epidermal cell number of the mature and seed coleoptile on one hand and dwarf nana1 and the normal sib on the other hand. This leads to the conclusion that the length of the coleoptile is a function of cell elongation.

4. The average ratios of diffusible auxin obtained from dwarf nana1 compared to those obtained from normal coleoptile tips are 0.93 for 4 day-old seedlings, 0.84 for 5 day-old, 0.91 for 6 day-old, and 1.22 for 7 day-old seedlings.

5. The average ratio of ether extractable auxin obtained from dwarf nana1 compared to that obtained from the normal coleoptile tips of 5 day-old seedlings is 0.92.

6. Dwarf nana1 and normal coleoptile tips ether extracted and paper chromatographed gave a ratio of 0.89 as much auxin in the dwarf as compared to the normal.

7. Because the growth promoting substance separated as IAA by paper chromatography was not significantly different
from that extracted by ether extraction only, it was concluded that the growth promoting substance in corn coleoptile tips is a substance which is IAA itself or at least acts like IAA.

8. The elongation of dwarf nana\textsubscript{1} mesocotyl is not light controlled.

9. IAA antagonism by 5 day-old dwarf nana\textsubscript{1} coleoptile tips, all the time and with different techniques, is greater than antagonism by the normal coleoptile tips.

10. IAA antagonism may indicate the many uncertainties which beset the efforts to relate IAA-destroying activity in homogenates to processes going on in the intact plant. This makes plain the need for further direct evidence on rates of destruction of native auxin in the intact plants.

11. Gibberellic acid promotes elongation of the secondary leaf blade to that of the treated normal.

12. Gibberellic acid in lower concentration has an inhibitory effect on the elongation of the primary root on both dwarf nana\textsubscript{1} and the normal sib. Higher concentration promotes primary root elongation especially for dwarf nana\textsubscript{1}.

13. Gibberellic acid has a very slight effect on the elongation of the coleoptile, mesocotyl, primary leaf sheath, and the primary leaf blade of both dwarf nana\textsubscript{1} and the normal sib.
VI BIBLIOGRAPHY


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