GROWTH SUBSTANCES IN RELATION TO THE MECHANISM OF THE ACTION OF RADIATION ON PLANTS

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INTRODUCTION

That radiation exerts a strong formative influence on plants has long been recognized. The blue-violet end of the spectrum is particularly effective in this respect, as has been shown by the work of Schanz (7), Popp (4), Shirley (8), and others (5). With most plants, this part of the spectrum causes decreased stature as compared with the red end. In all the work with ultraviolet radiation carried out by the senior author since 1921 and in many of the researches of other investigators, no effect of such radiation, except perhaps the destructive action of the extremely short wave lengths, has been more consistently found than the decreased stature of plants (6). An exposure of as little as 2 minutes per day, to the unscreened radiation from a quartz mercury-vapor lamp at a distance of 50 cm exerts a marked stunting effect on seedlings otherwise kept in diffused daylight or in darkness.

No satisfactory explanation of the mechanism of this action of radiation has as yet been advanced. Previous attempts in the writers' laboratories to explain it on the basis of changes in such chemical substances as occur in sufficient quantities to be analyzed chemically have so far proved unsuccessful, although investigations in this field have hardly attained a good start as yet. Hare and Kersten (3), as a result of their discovery that ultraviolet radiation destroys indole-3-n-propionic acid, have recently suggested that "a possible action of ultraviolet light upon the tryptophan within the plant may in some part explain the effect of ultraviolet light upon plants." This inference warrants further inquiry, since it has been shown that ultraviolet radiation destroys tryptophan in vitro, and that disagreeable odors such as might result from the breaking down of tryptophan and other indole derivatives have sometimes been observed when seedlings were exposed to distinctly injurious doses of ultraviolet. The recent interest in plant-growth substances or hormones, the rapidly accumulating experimental data on their characteristics and effects, and particularly the technique developed (1, 9, 10) for their quantitative determination, have furnished another means of approach to this problem.

The present investigation was undertaken for the purpose of determining whether the effects on the plant of short-wave radiation could be explained on the basis of the effect of such radiation on plant-growth substances. The work was begun in January 1936, as a part of a more extended investigation of the effects of radiation on plants which has been under way for several years. After about 3,000 tests

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2 Reference is made by number (italic) to Literature Cited, p. 936.
had been completed, a paper appeared by Burkholder and Johnson (2) reporting a somewhat similar investigation.

In general, these men found that intact seedlings or excised tips of seedlings of *Avena* and *Zea* exposed to the radiation from a quartz mercury-vapor lamp transmitted through 5 cm of distilled water in a glass cell or through a Corning No. 986 red-purple Corex A filter of 7 mm thickness, contained less growth substance, as determined by a modified Went method, than did controls kept in darkness, the reduction in growth substance being greater with increased exposure. That the effect of the radiation was not brought about by a downward diffusion of the growth substance away from the direction of the radiation was indicated by the fact that lower sections of treated seedlings showed no commensurate increase in growth substance. Further evidence that the effect of the radiation was a direct one was the fact that irradiated blocks of agar into which growth substance had previously been allowed to diffuse from excised coleoptile tips caused less curvature in decapitated *Avena* test plants than did control blocks not irradiated.

Ultraviolet radiation alone, as transmitted through Corning No. 986 red-purple Corex A glass, caused a marked reduction in growth substance as compared with the amount in controls. The test plants were exposed to radiation through this glass for 60 and 120 minutes at an intensity of 1,888 ergs/cm²/sec., an exposure probably great enough to cause injury to the seedlings, since this glass transmits ultraviolet radiation down to 250 mμ. Burkholder and Johnson do not indicate whether injury occurred, but the writers have never been able to irradiate plants through this glass under the conditions stated, without injuring the exposed tissues. On the other hand, tests with the blue mercury line, 4,358Å, at an intensity of 278 ergs/cm²/sec., indicated to Burkholder and Johnson "only small and perhaps insignificant differences in growth substance as compared with the darkened controls." It is likely that in these tests the intensity of the radiation was too low and the exposure time not great enough, for, if the stunting effect of such radiation as compared with darkness is to be attributed to reduction in growth substances, we should expect a significant difference between irradiated plants and controls. In these tests, 8 seedlings were illuminated for 55 minutes, 10 for 180 minutes, and 10 for 300 minutes. The average curvatures produced by these seedlings were 11.0°, 9.5°, and 8.7°, respectively, as against 10.9° for the controls in darkness. While there was probably too small a number of seedlings used under any condition to warrant reliable inferences, these results do at least indicate a consistent decrease in growth substance with length of exposure.

When excised tips of coleoptiles were subjected to unilateral illumination, the illuminated side contained less growth substance than the shaded side, but, strangely enough, when intact coleoptiles were thus treated, although they bent toward the light, the illuminated side contained more growth substance than the darkened side. No explanation is offered for these contradictory results.

While the Burkholder and Johnson paper was probably only intended as a preliminary one, since few tests were made under any one set of conditions, the results do indicate that the effect of radiation of short wavelengths is to inactivate growth substance. If this is to be used in explanation of the mechanism of the action of radiation
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upon plant growth, it is necessary that the conditions of radiation that produce definite formative effects be the ones that are used in the studies of growth substances. With ultraviolet radiation, for example, the intensity, spectral range, and duration of exposure should be such as to cause the ordinary stunting of growth without seriously injuring the seedlings. In the present investigation an attempt was made to satisfy these conditions.

EXPERIMENTAL PROCEDURE

The test plants used in this investigation were turnips (Brassica rapa L.), variety Purple Top White Globe. Individually selected seeds were germinated on cotton and filter paper in sterilized Petri dishes, 50 seeds to a culture, and kept in darkness at 25° C. except during the radiation treatments. The seeds were evenly spaced in the germinators.

The source of radiation was a mercury-vapor lamp in quartz operated on a 110-volt alternating current circuit with rectifier. The intensity of radiation from this lamp at a distance of 50 cm, without any screen, was 21.65 watts (10^-5/cm²). Three types of Corning glass filters were used: Noviol 0, transmitting down to 389 μm; G586A, transmitting the region 300-436 μm; and red-purple Corex A, transmitting the region 250-415 μm. The total energy transmission of these filters was measured by means of an Eppley pyrheliometer of the Kimball and Hobbs type and the distances from the plants to the mercury-vapor lamp were so adjusted as to equalize the intensities. In some cases, however, the plants were exposed at shorter distances. The lengths of exposures through screens transmitting ultraviolet radiation or to the unscreened arc were such as to minimize injury but to produce stunting effects on the seedlings. These periods of time were determined by the results of several years' work with such radiation. Seedlings irradiated through Noviol 0, which eliminates all ultraviolet, were given longer exposures.

The plants were usually exposed for the first time about 24 hours after being placed in the germinators, and daily or twice a day thereafter, usually for 3½ or 7 days. During irradiation a strong current of air was kept moving over the plants to minimize heating effects, although these were negligible because of the short periods of exposure. Immediately after irradiation the plants were returned to the dark chamber. Controls consisted of plants kept under all the conditions to which the test plants were exposed except the irradiation.

Tests for growth substances were made by a slight modification of the Went Avena method (9). The test plants used for this purpose were a pure-line selection of oats (Avena sativa L.) made by Dr. C. F. Noll of this station and chosen because of their purity and uniformity. In the first series (table 1) the tips (1 mm) of the turnip seedlings to be tested for growth substance were transferred to blocks of 3 percent agar, 2 by 2 by 1 mm, and the growth substance allowed to diffuse into them while they were kept for 2 hours in a moist chamber. These blocks were then cut in half, one half of each block used for the tests and the other half discarded. The halves used were transferred unilaterally to Avena coleoptiles that had been decapitated.

9 Loaned by the Eppley Laboratory, Inc.
2 hours previously and allowed to remain in a moist chamber. About 0.5 mm of the coleoptile tip was again removed before this transfer. *Avena* coleoptiles about 30 mm in length were chosen from seedlings grown in darkness for about 5 days. During all manipulations the *Avena* seedlings were fastened with special holders and glass tubes. After the transfer of the agar blocks to the decapitated coleoptiles, the latter were returned to the dark chamber for 2 hours, at the end of which time the angle of curvature of the coleoptiles was measured directly in a shadow box. All manipulations were carried out in photographically inactive light. In the later tests (table 2) the tips of the turnip seedlings were transferred directly to the decapitated coleoptiles and the angle of curvature measured. For comparison some of the *Avena* coleoptile tips were also transferred to agar and tested for growth substance. Likewise, the agar itself was tested. All results were treated statistically and probable errors determined.

**Table 1.**—Mean curvatures obtained with agar blocks into which growth substance had diffused from tips of turnip seedlings

<table>
<thead>
<tr>
<th>Screen</th>
<th>Daily exposure</th>
<th>Tests</th>
<th>Mean curvature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unscreened arc</td>
<td>5</td>
<td>2,232</td>
<td>0.52±0.008</td>
</tr>
<tr>
<td>Noviol 0</td>
<td>20</td>
<td>240</td>
<td>2.80±0.023</td>
</tr>
<tr>
<td>Control (total darkness)</td>
<td>0</td>
<td>1,808</td>
<td>3.10±0.012</td>
</tr>
</tbody>
</table>

**Table 2.**—Mean curvatures obtained by direct transfer of tips of turnip seedlings to decapitated *Avena* coleoptiles

<table>
<thead>
<tr>
<th>Screen</th>
<th>Semi-daily exposure</th>
<th>Tests</th>
<th>Mean curvature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unscreened arc</td>
<td>5</td>
<td>672</td>
<td>0.03±.005</td>
</tr>
<tr>
<td>Red-purple Corex A 1</td>
<td>5</td>
<td>240</td>
<td>.10±.009</td>
</tr>
<tr>
<td>Red-purple Corex A 2</td>
<td>5</td>
<td>144</td>
<td>.99±.013</td>
</tr>
<tr>
<td>G986A</td>
<td>40</td>
<td>684</td>
<td>.70±.012</td>
</tr>
<tr>
<td>G986A</td>
<td>20</td>
<td>504</td>
<td>1.70±.016</td>
</tr>
<tr>
<td>Noviol 0</td>
<td>20</td>
<td>576</td>
<td>4.90±.017</td>
</tr>
<tr>
<td>Control (total darkness)</td>
<td>0</td>
<td>1,983</td>
<td>5.40±.013</td>
</tr>
</tbody>
</table>

\[1 16 \text{ cm from lamp.}\]
\[2 30 \text{ cm from lamp.}\]

**RESULTS AND DISCUSSION**

In table 1 are given the results obtained by transferring agar blocks to the decapitated coleoptiles. The results shown in table 2 were obtained by direct transfers of the tips of the treated turnip seedlings to the decapitated coleoptiles. The latter method proved to be the better since the authors were more concerned with relative than with absolute amounts of growth substance. The growth substance present in the *Avena* coleoptile tips, as determined by the agar method, and in the agar itself is shown in table 3.
An examination of tables 1 and 2 reveals that in every case irradiated plants produced lower curvatures and hence less growth substance than did the controls kept in total darkness. Furthermore, the shorter the wave lengths to which the plants were exposed, the lower were the curvatures. Thus the lowest curvatures were obtained with plants exposed to the unscreened arc, which transmitted down to about 235 m\(\mu\); the next lowest by the plants screened with red-purple Corex A, which transmitted down to 250 m\(\mu\), and the next by the plants screened with G586A, which transmitted down to about 300m\(\mu\). Increasing the length of exposure with the G586A screen or decreasing the distance with the red-purple Corex A screen still further reduced the curvatures. The plants screened with Noviol 0, which eliminates practically all ultraviolet but transmits the entire visible spectrum, gave less curvature than the controls, indicating that, although the presence of ultraviolet radiation was much more effective in reducing growth substance, this effect of radiation is not restricted to the ultraviolet region. This is in accord with what would be expected from the fact that the seedlings screened with Noviol 0 were distinctly shorter than the controls, though much longer than the plants exposed to ultraviolet radiation. The shortest plants were uniformly those exposed to the unscreened arc.

There was, therefore, a distinct correlation between the stunting effect of the radiation used and the degree of curvature produced in *Avena* coleoptiles. Since the number of tests conducted under each condition was very large, and the differences between test plants and controls were uniformly in the same direction, these results are significant. This is further shown by the statistical analysis. If the Went method of determining growth substance is accepted as accurate, these results indicate that radiation, and particularly the short wave lengths of the spectrum, causes a reduction in the amount of growth substance present in growing tips of exposed plants. If, furthermore, we accept the postulation of Went and others, that the elongation of stems is directly controlled by growth substance, these results indicate that radiation checks elongation of stems through its action on growth substances. In other words, this formative effect of radiation may be at least partly explained on the basis of inactivation of growth substance. The discovery by Burkholder and Johnson, previously referred to, that radiation inactivated growth substance which was allowed to diffuse into agar, lends further support to this conclusion.
SUMMARY

A study has been made of the amount of growth substance present in turnip seedlings kept in total darkness as compared with the amounts present in such seedlings exposed to the radiation from an unscreened mercury-vapor lamp or to this radiation as screened through Noviol 0, G586A, or red-purple Corex A glasses. Irradiated plants uniformly contained less growth substance, as determined by the Went method, than did controls in total darkness. The shorter the wave lengths to which the plants were exposed, the greater was the reduction in growth substance. Although ultraviolet radiation was more effective in reducing the amount of growth substance present in seedlings, plants exposed to only visible radiation likewise showed a lower amount than did the controls in darkness. Since the degree of stunting of the seedlings was definitely correlated with reduction in growth substance, and since growth substance has been shown to exert a controlling influence on stem elongation, these results are believed to support the thesis that the stunting effect of radiation upon plants may be at least partly attributed to the inactivation of growth substances.

LITERATURE CITED

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