INHIBITION OF CURVATURE RESPONSES BY SHUNTING THE INHERENT ELECTRICAL FIELD

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(WITH FOUR FIGURES)

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Introduction

In studies of the bioelectric fields of the onion root, Rosene (2) found that the magnitude of the longitudinal polarity was decreased or shunted when a jacket of tap water was placed around a segment of the root between the contacts. When the shunt was removed, the polarity quickly returned to practically its former level. Similar results were obtained by Wilks and Lund (7) from their experiments with the Avena coleoptile. They observed that filling isolated sheaths (taken from 34 to 36 millimeter seedlings) with Shive's solution caused the magnitude of the external longitudinal polarity to be decreased from 40 to 60 to less than 10 millivolts. As in the onion root, when the solution was removed from the inside of the sheath, the electrical polarity was promptly restored. These facts were presented as proof of internal shunting of some of the polar cells which contribute to the longitudinal electrical polarity of the sheath.

Wilks and Lund (7) also reported preliminary experiments in which the central cavities of isolated coleoptiles were filled with various solutions and then exposed unilaterally to 400 meter candles of white light. Coleoptiles that were filled with Shive's solution did not show any bending response, and those filled with distilled water curved only slightly when compared to the curvature of the air-filled sheaths. Since it is known from previous data that electrolytic solutions, such as Shive's, short-circuit the electrical polarity, these experiments indicate that the inherent electrical field is a fundamental prerequisite for the growth curvature processes.

The purpose of the present paper is to present supplementary data which show the quantitative effects of short-circuiting the inherent electrical field, using different concentrations of Shive's solution, on the various tropic responses of the Avena coleoptile.

Experimental procedure

Isolated coleoptiles of Avena sativa, Victory Strain (U. S. Department of Agriculture C. I. 2020) were used in all experiments. Etiolated seedlings were grown and prepared in the manner described in previous publications (3, 4). The laboratory conditions and the apparatus used were also the same as in preceding investigations (3, 4).

The coleoptile sheaths which were used in these experiments were isolated from intact seedlings that were 30 (± 1) millimeters long, perfectly

1 Supported by the University of Texas Research Institute.
straight, and had a well-developed root system. A cut was made all the way around the coleoptile about five millimeters apical to the seed so that the primary leaves could be pulled out of the basal end. The hollow coleoptile cylinder was next filled with one of the solutions. Either a glass pipette with a capillary tip or a hypodermic syringe with a blunt needle was used for the filling operation. The sheath was then placed in a glass holder with its cut end submerged in Shive's solution (6)\(^2\) and permitted to remain undisturbed for one hour to recover from the stimulation of handling. At the end of this time one of the several types of stimuli was applied. After stimulation, growth curvature was measured by following the tip position with an ocular micrometer. At the end of each experiment the total angular curvature was determined by the Judkins method (1). The techniques for making the electrical measurements have been published in preceding papers (3, 4).

**Results**

**Stimulation by gravity**

In this first series of experiments the coleoptiles were stimulated by placing them in the horizontal position for one hour. The upward curvatures that were observed are shown in figure 1, in which each curve represents an average of six or more experiments. The initial downward curvature, common in all of the curves, is due to the weight of the coleoptiles.

Curve I shows the average curvature of the sheaths which were not filled with any solution so that only air occupied the inside space. This curve serves as a sort of control indicating the maximum geotropic curvature that was observed under these conditions. Curves II, III and IV were obtained from sheaths filled with Shive's solution X \(\frac{1}{2}\), X 1 and X 2, respectively. Sheaths filled with Shive's solution X 2 show only slightly more than half as much curvature as air-filled coleoptiles. The curves in figure 1 clearly show that both the rate and the final angular geotropic curvature of isolated sheaths are inhibited by Shive's solution, and that the extent of the inhibition is dependent on the concentration of the electrolyte with which the cylinder was filled.

Since the higher concentrations of Shive's solution inhibit geotropic bending most effectively, there is the possibility that the inhibition could be mediated by osmotic mechanisms. Results of an additional series of experiments, designed to test this possibility, are presented in figure 1 by curve VI. In this series the isolated cylinders were filled with a solution of glycerol in distilled water, with the concentration selected to give an osmotic pressure of 3.5 atmospheres. This should be at least equal to the pressure of Shive's solution X 2, which was the most concentrated electrolyte used in any of the present experiments. It is evident from the comparison of curves VI and I that the glycerol solution on the inside of the cylinder in no way limits the upward bending of the isolated coleoptile.

\(^2\) Shive's solution R582 was used in all experiments.
Table I gives the final angular curvatures of the individual experiments in order to illustrate the uniformity of the results. The numerals were arranged in descending order to facilitate making the comparisons.

![Graph](image)

**Fig. 1.** Upward curvatures of isolated Avena coleoptiles which were placed in the horizontal position for 60 minutes. Each curve represents an average of six or more experiments. Internal cylinders were filled with the following:

- Curve I: Air
- Curve II: Shive's solution X ½
- Curve III: Shive's solution X 1
- Curve IV: Shive's solution X 2
- Curve VI: Glycerol solution
TABLE I

DEGREES OF UPWARD CURVATURE OF ISOLATED AVENA COLEOPTILES WHICH WERE FILLED AS INDICATED AND KEPT IN THE HORIZONTAL POSITION FOR 60 MINUTES

<table>
<thead>
<tr>
<th>SHEATH FILLED WITH</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>VI</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>AIR</td>
<td>SHIVE'S</td>
<td>SHIVE'S</td>
<td>SHIVE'S</td>
<td>GLYCEROL</td>
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<tr>
<td></td>
<td></td>
<td>X ½</td>
<td>X</td>
<td>X 2</td>
<td>SOLUTION</td>
</tr>
<tr>
<td>42</td>
<td>39</td>
<td>32</td>
<td>30</td>
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<tr>
<td></td>
<td>15</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>30</td>
<td>27</td>
<td>21</td>
<td>35</td>
</tr>
</tbody>
</table>

STIMULATION BY UNILATERAL ILLUMINATION

The curvature responses of isolated coleoptile sheaths to unilateral illumination of 200-meter-candle-seconds of incandescent light (10 watt G. E. Mazda lamp) are shown in figure 2. Each curve is an average of six or more experiments. As in the previous series, the internal cylinders were filled with various dilutions of Shive's solution. Curve I shows the average bending of sheaths which were filled with air. The average maximum angular curvature at the end of the experiments was 16.3 degrees. Curves II, III, IV and V represent the bending when the sheaths were filled with Shive's solution X ½, Shive's solution X 1, Shive's solution X 2 and distilled water, respectively. All curves show a significant degree of inhibition of bending toward the light. Curves II, III and V are practically identical, and there is no expressive difference in the final angular curvatures. In order to check the validity of the similarity of these curves, duplicate series were performed for curves II and III. Table II presents the angular curvature data of the individual experiments. Comparison of column II A with II B and III A with III B gives an indication of the degree of reproducibility of these experiments. When the sheath was filled with Shive's solution X 2, bending toward the light was inhibited more effectively than by any of the other solutions. Curve IV reveals this fact.

Curve VI shows the phototropic curvature of sheaths filled with a glycerol solution of the same concentration as used in the previous experiments. This curve reveals two surprising facts. During the first 80 minutes the average bending toward the light is slightly greater than it is for the air filled coleoptiles, and appreciably more than for the sheaths filled with distilled water. The inhibitory effect of the distilled water on bending toward the light seems to be completely masked by the presence of the glycerol. Data that are available do not warrant an explanation of this observation. These results substantiate a previous suggestion that the inhibition of curvature caused by various concentrations of Shive's solution...
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apparently is not directly dependent on an osmotic factor. The second feature of curve VI is that the curvature practically stops after 80 minutes. This type of response is not apparent in any of the other curves. The presence of the glycerol limits the maximum curvature attainable by stopping the bending toward the light earlier than the other solutions.

Results, which are presented in figure 2 and table II, indicate that the rate and magnitude of curvature toward the light are both influenced by the concentration of the electrolyte solution in the internal cylinder. The

![Graph](image)

**FIG. 2.** Curvatures of isolated Avena coleoptiles toward 200-meter-candle-seconds of unilateral illumination. Each curve represents an average of six or more experiments. Internal cylinders were filled with the following:

- Curve I, Air
- Curve II, Shive's solution X \(\frac{1}{4}\)
- Curve III, Shive's solution X 1
- Curve IV, Shive's solution X 2
- Curve V, Distilled water
- Curve VI, Glycerol solution
TABLE II

DEGREES OF CURVATURE OF ISOLATED AVENA COLEOPTILES WHICH WERE FILLED AS INDICATED AND STIMULATED BY 200-METER-CANDLE-SECONDS OF UNILATERAL ILLUMINATION

<table>
<thead>
<tr>
<th>SHEATH FILLED WITH</th>
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<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIR</td>
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<td>B</td>
<td>A</td>
<td>B</td>
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<td></td>
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<td>9</td>
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<td>8</td>
<td>-2</td>
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<td>6</td>
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<tr>
<td>11</td>
<td>5</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Average</td>
<td>16.3</td>
<td>10</td>
<td>11</td>
<td>9.9</td>
<td>10.5</td>
<td>4</td>
</tr>
</tbody>
</table>

relationship of degree of inhibition to concentration is somewhat irregular, because distilled water, Shive's solution $X \frac{1}{2}$ and Shive's solution $X$ 1 are equally effective curvature inhibitors.

STIMULATION BY ELECTRICAL CURRENT

Previous work has shown that direct current of the order of 10 microamperes applied transversely for two minutes to the apical region causes the coleoptile to establish a transverse electrical polarity and to bend toward the electropositive pole of the current applying circuit (5). Figure 3 shows the effect of filling the inside of the sheath with different dilutions of Shive's solution on electrically induced curvature. Each curve is an average of five or more experiments and is plotted above the zero line to indicate bending toward the electropositive pole of the circuit. Curve I represents the temporal sequence of the bending process resulting from the application of 10 microamperes for two minutes at a level five millimeters below the apex. The coleoptiles in these experiments were filled with air. Under the conditions of these experiments (Curve I) 70 minutes are required to attain the maximum curvature in the initial direction. After this, the seedlings start to bend in the opposite direction (5). At the end of 110 minutes the remaining average angular curvature is 10.1 degrees. When the coleoptile is filled with distilled water and stimulated electrically (Curve V), bending in the first direction is initiated more slowly. The maximum curvature that is reached is about the same as shown by curve I, but an additional 25 minutes are required to reach this maximum. Because of this fact the average angular curvature in the initial direction at the end of 110 minutes is larger than the curvature corresponding to curve I. Shive's solution $X \frac{1}{2}$ inhibits the rate and magnitude of bending toward the
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electropositive pole of the current-applying circuit. This fact is apparent from curve II. Filling the inside cylinder of the coleoptile with Shive's solution X 1 and Shive's solution X 2 inhibits electrically induced bending more effectively than Shive's solutions X $\frac{1}{2}$ or distilled water. This is made evident by comparing curves III and IV to curves II and V. It is

![Diagram showing curvatures of isolated Avena coleoptiles induced by 10 microamperes of direct current applied transversely for two minutes at a level five millimeters below the apex. Each curve represents an average of five or more experiments. Internal cylinders were filled with the following:

- Curve I, Air
- Curve II, Shive's solution X $\frac{1}{2}$
- Curve III, Shive's solution X 1
- Curve IV, Shive's solution X 2
- Curve V, Distilled water
- Curve VI, Glycerol solution]
noted that curves I, II, III and IV reach the maximum in about the same time. From this it appears that the time required to attain the upper limit

![Graph showing transverse electrical polarities established in isolated Avena coleoptiles by 10 microamperes of direct current applied transversely for two minutes at a level five millimeters below the apex. Each curve represents an average of five or more experiments. Internal cylinders were filled with the following:
- Curve I, Air
- Curve II, Shive's solution X ½
- Curve III, Shive's solution X 1
- Curve IV, Shive's solution X 2
- Curve V, Distilled water
- Curve VI, Glycerol solution](image-url)
of curvature in the initial direction is not dependent on the concentration of the solution with which the cylinders were filled. Curvature responses of coleoptiles filled with the glycerol solution (Curve VI) are practically identical to the responses of sheaths filled with distilled water. This indicates that glycerol is entirely without effect on electrotropic bending.

In this series of experiments the transverse electrical responses resulting from the applied current were also measured. The curves in figure 4, which are numbered to correspond to the curves in figure 3, show the results. Application of 10 microamperes for two minutes at a level five millimeters basal to the apex causes air-filled coleoptiles to establish an average transverse electrical polarity of 31 millivolts. This polarity gradually decreases to about 10 millivolts at the end of the experiments (Curve I). The curves in figure 4 fall in the same sequence as the corresponding bending curves in figure 3. Coleoptiles that had been filled with Shive’s solution X 2 manifest the smallest transverse electrical polarity throughout most of the experiment.

Discussion

Results of the preliminary experiments published by Wilks and Lund (7) indicate that the growth curvature responses of isolated Avena coleoptiles to unilateral light were inhibited by filling the internal cylinder with electrolytes such as Shive’s solution. Non-electrolytes did not inhibit phototropic bending. It was also demonstrated by the same investigators that the longitudinal electrical polarity of the sheath disappears when it is filled with a conducting solution. Apparently the electromotive forces of the internal surface are short-circuited by the electrolyte. These results were interpreted to indicate that the inherent electrical potentials are a necessary prerequisite for phototropic responses.

Supplementary data, which are presented in this paper, show that curvature responses of isolated Avena sheaths to light, as well as to gravity and to electrical stimulation, are definitely inhibited when the internal cylinder is filled with Shive’s solution. It turns out that the degree of inhibition is, to a large extent, dependent on the concentration of the electrolyte used. There are some differences in the degree of effectiveness of a given concentration on the various growth responses, but Shive’s solution X 2 is generally a more effective inhibitor of curvature than the more dilute components. Furthermore, the present experiments show that the magnitude of the transverse electrical polarity, which is induced by the applied direct current, is also lowered when the sheath is filled with Shive’s solution. The extent of the inhibition of this polarity is again a function of the solution concentration.

One of the first questions to be answered would be whether or not the various concentrations of salt solution exert their effect on growth by altering the osmotic relations within the cells. Control experiments were included in which the cylinders were filled with a water solution of glycerol.
Glycerol was selected because it is essentially a non-electrolyte, and the concentration was adjusted to exceed the maximum osmotic pressure of Shive's solution X 2. The results show that the glycerol solution did not inhibit the curvature in any one of the series of these experiments. One tentative explanation of this would be that a decrease in turgidity of the tissue is not responsible for the inhibition of the bending by Shive's solution. Such interpretation, however, involves the question of permeability of the cell membranes to glycerol as compared to the inorganic salts in Shive’s solution, and therefore must be made with caution. More data are obviously necessary for the clarification of this point.

It is also clear from figure 4 that glycerol has no effect on the transverse electrical polarity which is induced by applied current, while Shive’s solution does. Thus the present results are consistent with the thesis that the inherent electrical field is an essential requirement for the various curvature responses, and that a decrease in these polarities by shunting them always results in less bending.

Summary

1. Geotropic curvature of isolated Avena coleoptiles is inhibited by filling the internal cylinder of the sheath with an electrolyte, Shive’s solution. The degree of inhibition is directly dependent on the concentration of the solution used.

2. Bending toward 200-meter-candle-seconds of unilateral illumination is also inhibited by filling the sheath with Shive’s solution. Shive’s solution X 2 causes most inhibition, while distilled water, Shive’s solution X \( \frac{1}{2} \) and X 1 are about equally effective.

3. Similarly, filling the isolated coleoptiles with Shive’s solution inhibits the curvature induced by 10 microamperes of direct current applied transversely for two minutes at a level five millimeters basal to the apex. The degree of inhibition is, to some extent, dependent on the concentration of the solution. The transverse electrical polarity induced by current, applied as indicated, is also decreased by Shive’s solution; the degree of inhibition is dependent on the concentration used. Glycerol (14.4 grams per liter of distilled water) has no effect on the transverse electrical polarity induced by direct current.

4. Growth curvature responses of the coleoptile sheath to stimulation by gravity, unilateral illumination, and transversely applied current are not inhibited when the cylinders are filled with a solution of non-conducting glycerol with the concentration adjusted to exceed the osmotic pressure of Shive’s solution X 2.

5. Previous investigations have shown that the longitudinal electrical polarity of the Avena coleoptile can be shunted by filling the sheath with an electrolyte. Since the present experiments show that the use of elec-

\[ ^3 \text{The specific conductances (measured at 25° C.) of the various solutions used are as follows: Distilled water, 0.0000098; Shive's solution } X \frac{1}{2}, 0.0023; \text{ Shive's solution } X 1, 0.0042; \text{ Shive's solution } X 2, 0.0075; 0.145 \text{ M glycerol solution, 0.000024.} \]
trolytes always results in less curvature, they support the hypothesis that the existence of an inherent electrical field is an essential requirement for growth responses.

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