BUD REGENERATION AND ELECTRICAL POLARITIES
IN PHASEOLUS MULTIFLORUS

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(WITH FIVE FIGURES)

Introduction

Various investigators have reported experiments on the relationship between the growth substance and the inhibition of the lateral buds in seedlings of Leguminosae. Thimann and Skoog (24) and Skoog and Thimann (20) have demonstrated that the growth hormone when applied to the cut surface of decapitated plants of Vicia faba reversibly inhibits the lateral buds. Hitchcock (5) has shown that lateral bud inhibition could be produced by the application of various chemicals, including indole-acetic acid, indole-propionic acid, or ethylene or propylene gases to decapitated tobacco plants. Thimann and Skoog (24) have advanced the hypothesis that the growth substance is transported directly to the buds where it prevents the buds from producing their own hormone, which, according to these investigators, prevents the growth of the buds. Laibach (9), on the other hand, supposes that the hormone does not act directly on the buds but produces a secondary reaction which in turn causes the inhibition. In this connection it is of interest to note that LeFanu (10) was unable to detect any appreciable amounts of auxin in inhibited lateral shoots of Pisum sativum. Boyesen Jensen (1) obtained no release of inhibition when he applied growth hormone in various ways to the inhibited buds of several plants. Snow (22) has demonstrated that there is an inhibiting influence that may travel both apically and basipetally. However, in a recent paper Snow (23) found that the growth substance could travel upwards and cause both growth and inhibitory effects. Most of the workers in this field have found that the growth substance in physiological concentration travels only downward. Obviously the mechanism by which the growth hormone may bring about the inhibition of the lateral buds is one that has not been completely explained.

The present paper is concerned with the relationship between bioelectric currents and bud inhibition. A number of investigators have presented evidence to show that the hormone may be transported electrophoretically by the inherent potential differences. Went (25) has proposed, on the basis of his experiments on the uptake of dyes (which experiments have been confirmed by Clark, 4), that the apices of these plants are negative to their bases and that this electrical polarity transports the hormone. A discussion of this theory will be postponed until later. It must be kept in mind, however,
that the longitudinal transport of the growth substance is not necessarily the only rôle that bioelectric potentials might play in these phenomena. On the basis of the present status of the work in this field, it is possible that bioelectric potentials may influence the distribution of the growth hormone in the nodes of plants with inhibited lateral buds, or they may be involved in this phenomena in some as yet undetermined manner.

In a previous paper (17) the writer reported that he found in intact vegetative plants of Phaseolus multiflorus electrical polarities of relatively constant orientation in the regions of the axillary buds. In that paper a method was described for measuring potential differences between various loci of the plant. The loci between which the potential differences were measured are labelled in figure 3A. The potential differences between A1B1, A1P1, and A1P1′ were designated as the first node potentials, similarly, A2B2, A2P2, and A2P2′ as the second node potentials. In “unstimulated” plants, A was found to be negative in the external circuit to the other loci at a given node and this orientation was referred to as the normal one.

A portion of the work reported here was done at the University of Texas. For the experiments done there, the same method was used as that described in the previous article. The plants were raised in a greenhouse and placed in a light chamber 24 hours before an experiment; the potentials were measured either with a potentiometer or with a Compton Quadrant Electrometer. During this last year the writer has been continuing this work as a guest of the Department of Botany of the University of Chicago. He wishes to take this opportunity to thank Professor CHARLES A. SHULL for the many courtesies extended to him. At Chicago the plants were grown and measured in a greenhouse, the potentials being measured with a Compton Quadrant Electrometer. Because of the variation of the temperature during the day, experiments involving short periods of time were performed at night in the greenhouse. Under these conditions the temperature did not usually vary more than 2° C. during any given experiment. It must be emphasized that the plants used in the experiment reported here were all definitely in the vegetative state. Essentially the same results were obtained under the conditions of the light chamber and under the conditions in the greenhouse.

Experimentation

INTERNAL POLARITIES

The first problem that the present paper is concerned with is an attempt to investigate the internal electrical polarities of the node. LUND (11) found, in his experiments on the Douglas fir, an internal polarity that was oriented oppositely to the external one. The possibility that the hormone
may be transported by the electrical currents makes it particularly pertinent to investigate the nature of the internal polarities.

For the purpose of measuring internal electrical polarities, small glass "pipes" of about one centimeter length were inserted into the plant so that the end of the pipe was at the center of the stem or petiole. Figure 1 shows the method of leading off the potentials. The pipe was filled with Shive's solution and inserted into the plant. A contact cup of the same kind as shown in the previous paper (fig. 1) was filled with Shive's solution and brought close to the bowl of the pipe so that a drop of solution bridged the gap between the pipe and the contact without the contact actually touching the pipe. This arrangement permitted the plant to move without the stimulation that a rigid contact would produce. A Zn:ZnSO₄ electrode was placed in the contact cup and the potentials were measured in the usual way. The first measurement after placing one of these contacts in the stem showed a decided negativity of the internal contact with respect to an external contact. That is, for example, A₁ was always markedly positive in the external circuit to X₁ immediately after the pipe contact X₁ was placed in the stem. The potential difference between two such contacts decreased with time and in ¾ hour to 2 hours reached a fairly constant value. After some preliminary experiments the following definitive experiment was performed. Four intact plants were taken and contacts were placed as shown in figure 1. Since the potential difference between an outside contact and an inside contact reached

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**Fig. 1.** Diagram on the left shows the position of the pipe contacts. The middle diagram shows the manner in which contact was made between the pipe and the contact cup. The diagram on the right represents a simplified portrayal of the possible path of current through the plant.
a constant level within 2 hours, it was assumed that 4 hours would allow time for the immediate effects of injury to disappear. Consequently, 4 hours after placing the contacts on and in the plant, readings were taken between the various loci at fifteen-minute intervals over a period of 4 hours. These experiments were performed in the greenhouse at night. The temperature varied from 23° C. to 24° C. The results are shown in table I. It will be noted that the node potentials $A_1B_1$ and $A_1P_1$ possessed the normal orientation during this period. The potential differences $X_1Y_1$ and $X_1Z_1$ are all negative, that is, $X_1$ is negative in the external circuit to $Y_1$ and $Z_1$. This demonstrates that the internal polarities measured by this method possess the same orientation, i.e., apical negativity, as do the polarities measured on the outside of the plant. The potential differences between an outside contact and the corresponding internal one are given in columns 5 and 8. The radial polarities, as can be seen from an examination of these measurements, are not as constant as the longitudinal polarities. On the basis of these results, a simplified diagram that would be consistent with both the internal and external longitudinal polarities having the same orientation is presented in figure 1. This diagram represents the possible paths of current flow. It may be pointed out according to this diagram that the measured potential differences may be only a small fraction of the total RI drop in the plant. It would also follow that the total amount of current that can be drawn off from the plant may represent only a small fraction of the actual current density in certain regions within the plant. On the basis of the present experiments it is obviously impossible to say just where the cells producing the electrical potentials are located or whether or not there may be oppositely oriented gradients between the pith and the cortex. In this simple diagram no attempt has been made to represent the radial polarities.

1 For evidence that the individual cells give rise to the measured potential differences in plants the reader is referred to the work of LUND (12), MARSH (14), and ROSENE (18).

<table>
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<tr>
<th>PLANT</th>
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<th>$A_1P_1$</th>
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<th>$X_1Z_1$</th>
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</table>

ORIENTATION AND MAGNITUDE OF THE INTERNAL AND EXTERNAL ELECTRICAL POLARITIES IN THE FIRST NODE. LOCI OF THE VARIOUS CONTACTS INDICATED IN FIGURE 1. POTENTIAL DIFFERENCES IN MILLIVOLTS. FOR FURTHER EXPLANATION SEE TEXT.
Effect of decapitation on the node potentials

In determining the effect of decapitation on the node potentials, it is necessary to eliminate the effect of mechanical stimulation at the contacts. A sharp movement of the stem or petiole against one contact caused the stimulated region to become more negative with respect to an unstimulated region. That is, if the plant was stimulated in the region of A₁, this contact became more negative with respect to B₁; and if stimulated at B₁, B₁ became more negative to A₁. After stimulation, the potentials returned to normal in 5 to 15 minutes. To prevent this type of stimulation the plant was clamped between the node where the potentials were measured and the point of decapitation. A clamp with a large diameter was placed around the plant and the space between was filled with modelling clay; this procedure did not injure the plant in any observable way. The transmission of electrical variations produced by a mechanical stimulus has been reported for various plants by several investigators (Bose, 2, Houwink, 6). However, in these experiments it was found that bending the plant above the clamp or giving it a sharp blow with a glass rod produced no observable effect on the node potentials. It must be pointed out that the period of the instruments employed in the experiment reported here was 15 seconds at the minimum and that electrical variations of shorter duration than this may have escaped detection. However, these experiments in which no effect was observed in the node potentials serve as an adequate control for the following experiments on the effect of decapitation.

Decapitation of the plant produced an immediate response in the potentials of the node below the point of decapitation. In the course of various experiments, more than 30 plants were decapitated below the second node and the potentials of the first node measured. At least 5 plants were used in each of the other experiments of this group. The plants were always clamped to prevent movement against the contacts. The plants were decapitated directly above the clamp with a sharp razor. The temperature at which the various experiments were carried out varied from 20° to 28° C., though the temperature did not usually change more than one degree during the course of any one experiment.

Figure 2 shows the typical effect of decapitation below the second node on the potentials of the first node. At the time indicated by the first arrow the plant was decapitated and the node potentials greatly increased in magnitude, A₁ becoming more negative with respect to B₁, P₁, and P₁'. The magnitude of the potentials then diminished, and in about an hour they had returned to their former magnitude. The next experiments were designed to determine if this response was caused by the loss of the apex or simply by the effects of cutting. A typical response to cutting of the stem of a decapitated plant is shown in figure 2A. At the time indicated by the second arrow
a section was cut from the top of the decapitated plant. This response was similar to the first, although of smaller magnitude and duration. Even though much longer periods of time (up to 24 hours) elapsed before the second cut, this response was always of smaller magnitude than that following the original decapitation.

It was mentioned above that certain mechanical stimuli (bending and hitting) failed to elicit a response when applied above the node. However, cutting or crushing the stem of an intact plant produced a response in the node potentials. B, figure 3, shows a typical response to pressure on the stem. In this experiment the stem was squeezed with a pair of forceps with glass tubes placed over their tips. Gentle and fairly large pressures failed to produce a response in the node potentials. At the time indicated by the arrow, however, the stem was crushed by applying great pressure to the forceps. As can be seen from the figure, this caused an increase in the magnitude of the node potentials with a rather slow recovery. It was observed in plants decapitated between the second and third nodes that the buds of the first node often grew out as rapidly as those of the second node. Therefore the next step was to investigate the effect of decapitation on the first and second node potentials.

A, figure 3, shows the effect of decapitation below the third node on these potentials. The response of the second node potentials is seen to be similar
in magnitude and duration to the response of the first node potentials as represented in figure 2. In the first node potentials of the plant in A, figure 3, there was a small response in which the node potentials became slightly greater in magnitude. For convenience of presentation the response of the node potentials $A_1P_1$ and $A_1P_1'$ are omitted in this figure; the response of these potentials was similar to that of $A_1B_1$. A similar change was found in the second node when the plant was decapitated below the fourth node. These responses of the second node below the point of decapitation were always small and sometimes were within the limits of normal variation of the

![Graph](https://www.plantphysiol.org/draft.png)

**Fig. 3.** A, the effect of decapitation below the third node on the node potentials of the first and second nodes. At the time indicated by the arrow, the plant was decapitated. $A_1P_1$ and $A_1P_1'$ are omitted from this figure for the sake of clarity. The response of $A_1P_1$ and $A_1P_1'$ was similar to that of $A_1B_1$.

B, shows the effect of crushing a portion of the stem on the first node potentials. $A_1P_1$ is omitted; its response was similar to that of $A_1B_1$ and $A_1P_1'$. The position of the clamp is shown in this figure.

node potentials. When the plant was decapitated below the fourth node, the potentials of the first node (i.e., of the third node below the point of decapitation) did not change. At this juncture it may be pointed out that in 4 of these plants decapitated between the third and fourth nodes the buds of the first node grew out as rapidly as the buds of the higher nodes. In all experiments where the potentials of the node immediately below the point of decapitation were measured, a typical response, as shown in figure 2, and in A, figure 3, was observed. However, the particular shape of the recovery curves varied somewhat. The difference between the shapes of recovery curves in figure 2, and in A, figure 3, is not typical for the first and second nodes; the recovery curves of both nodes usually showed several plateaus or
dips. The experiment represented by A, figure 3, was selected in order to demonstrate that the recovery curves sometimes were suggestive of a diphasic variation. A discussion of the rôle of these responses in bud regeneration is postponed until later.

One important point to be brought out here is that, after the immediate response to decapitation, the node potentials return to approximately their former magnitude. The question then arises, is there any later response of the node potentials to decapitation, and if so, is there a relationship between it and regeneration?

**Regeneration of buds and the node potentials**

**Procedure.**—In eleven plants the node potentials and the increase in length of the buds were measured for periods of from 80 to 120 hours after decapitation. Six of these plants were measured in the light chamber described in the preceding paper, and the other 5 were measured in the greenhouse. In 3 of the plants measured in the light chamber, the potentials were measured approximately every hour over a period of 100 hours. For the rest of the 11 plants the measurements were less frequent; usually readings were made at 1- or 2-hour intervals for two 6-hour periods each day. Ten of the plants, including the 3 plants measured at 1-hour intervals, were decapitated below the second node, and the first node potentials and the increase in length of the buds of the first node were measured. One plant was decapitated below the third node, and the buds and potentials of the first and second nodes were measured.

**Change in node potentials.**—For convenience of presentation the results are summarized as follows. The immediate effect of decapitation was typical in all plants. The node potentials showed, after the immediate effect of decapitation was over, spontaneous fluctuations similar to those described for intact plants in the previous paper. However, it was observed after averaging the readings of each successive 6- or 10-hour interval that the average magnitude of the node potentials in each case gradually diminished after decapitation. This diminution extended over a period of from 20 to 40 hours, and in the course of this time at least one and sometimes all of the node potentials became positive. The potentials continued to fluctuate at or above zero, though in some plants they definitely returned to normal before the end of the experiment.

Figures 4 and 5 show the typical course of the node potentials after decapitation. These curves represent 2 of the 3 plants referred to above in which readings were taken at approximately 1-hour intervals throughout the experiment. B, figure 4, represents the course of these potentials in one of the plants from the time of decapitation until about 40 hours afterward. Each point corresponds to a single reading. As can be seen in this graph,
Fig. 4. Relation between the node potentials and the regeneration of the buds. A. Each point represents the average potential difference for ten hours. B. Each point represents a single reading.

These potentials fluctuate, and may return to a normal orientation for several hours, even after they have been inverted. For A, figure 4, and figure 5 the readings were averaged, each point representing a 10-hour interval. The change in the potentials shown in figure 5 is of average magnitude and illustrates the points brought out above. A, figure 4, representing the same
Fig. 5. Relation between node potentials and regeneration of the buds. Each point represents the average potential difference for ten hours.

plant as B, figure 4, also illustrates these points; but in this instance the change in the magnitude of the potentials was greater than usual.

**TABLE II**

Comparison of the average of the node potentials in decapitated and intact plants. Each number represents the average of three node potentials, A1B1, A2P1, and A3P1', for an individual plant. Potential differences in millivolts. For further explanation see text

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<tr>
<th>PLANT</th>
<th>Decapitated plants</th>
<th>Intact plants</th>
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<tr>
<td></td>
<td>20 HR.</td>
<td>1ST 30 HR.</td>
</tr>
<tr>
<td></td>
<td>BEFORE DECAP.</td>
<td>AFTER DECAP.</td>
</tr>
<tr>
<td></td>
<td>mv.</td>
<td>mv.</td>
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<td>10</td>
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<tr>
<td>Avg.</td>
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<td>-9.3</td>
</tr>
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</table>

In table II the 3 node potentials of all 10 decapitated plants were averaged for 3 periods: (a) before decapitation, (b) the first 30 hours after decapitation, (c) and the second 30 hours after decapitation. For controls, the node potentials of 10 intact plants (from previous paper, 17) were averaged for three periods: (a) the first 20 hours, (b) the next 30 hours, (c) and the succeeding 30 hours. In each of the decapitated plants the average...
of the node potentials was more positive for the first 30 hours after decapitation than for the period before decapitation, and more positive for the second 30 hours than for the first 30 hours. On the other hand, in the intact controls the average of the node potentials was approximately the same for all three periods. As a final check on the effect of decapitation on the node potentials, the potentials of the first and second nodes of two plants raised and measured in the greenhouse were followed for 100 hours. One of these plants was decapitated below the third node, while the other one was left intact. In the intact plant, all of the node potentials remained normal throughout the entire experiment, while in the decapitated plant the potentials of both nodes exhibited the typical change after decapitation.

Node potentials and bud growth.—The length of the buds was measured with a pair of calipers which were adjustable by a fine screw. A mark was made with India ink on the node at the base of the bud, and the distance between the top of the mark and the tip of the bud was recorded as the length of the bud. The limit of accuracy of this method was about 0.25 millimeter.

In 4 of the 11 plants in which both bud growth and node potentials were measured, the buds did not start to grow out until 60 or more hours after decapitation. However, the node potentials in these plants showed the typical change after decapitation and were therefore diminished or inverted for 40 or more hours before any observable increase in the length of the buds. In 7 of these 11 plants the buds started to grow comparatively soon after decapitation. In 4 of these 7 plants both buds grew out; and in the remaining 3 plants only one of the buds definitely grew out. In every one of the 7 plants referred to above the node potentials definitely diminished before the buds started to grow. However, a given bud may start to grow before the corresponding node potential has become inverted. This is illustrated in B, figure 4; bud P1' started to grow before the node potential A1P1' had become inverted, although this potential had diminished and was of comparatively small magnitude.

In 4 plants in which both buds grew out, the node potentials A1P1 and A1P1' followed one another fairly closely from the time of decapitation to the end of the experiment. This is illustrated in A, figure 4. On the other hand, in the 3 plants in which only one bud definitely grew out, there was a marked difference between the node potentials A1P1 and A1P1'. In each of these plants the node potential corresponding to the inhibited bud was definitely less positive than the node potential corresponding to the growing bud. In two of these plants the inhibited bud did not increase in length during the entire experiment. However, in the third plant, illustrated in figure 5, the "inhibited" bud started to grow, but became inhibited before the end of the experiment. In this plant the node potential A1P1, cor-
responding to bud P<sub>1</sub>, returned to the normal orientation simultaneously with the inhibition of bud P<sub>1</sub>. Later the node potential A<sub>1</sub>P<sub>1'</sub> also returned to the normal orientation. In fact, as can be seen from an examination of the results presented in table III, the node potentials frequently return to normal after the release of the buds.

**TABLE III**

**Comparison of the node potentials and growth of the buds in decapitated plants.**

Columns 5 and 6 represent the length of the buds in centimeters. Columns 7 and 8 represent the increase in length of the buds during the four-hour interval. Column 9 gives the potential difference from P<sub>1</sub> to P<sub>1'</sub>.

<table>
<thead>
<tr>
<th>Plant</th>
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<th>A&lt;sub&gt;P&lt;/sub&gt;</th>
<th>A&lt;sub&gt;P'&lt;/sub&gt;</th>
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</table>

For further confirmation of the relation between the inhibited bud and the corresponding node potential, 10 decapitated plants were selected, 5 of which had one bud growing and the other bud inhibited, while the remaining 5 had both buds growing. The node potentials and the increase in length of the buds of each plant were measured for 4 hours. This work was done in the greenhouse, and because of the variation of temperature during the day, the experiments were performed at night. The temperature did not usually vary more than one or two degrees during any experiment. The temperature limits for this group of experiments were from 20° C. to 24° C. In this series of experiments the length of the buds was measured with a horizontal microscope, giving an accuracy of about 0.1 millimeter. The results of these experiments are shown in table III. The average values of each of the node potentials are given in the second, third, and fourth columns. Columns 5 and 6 give the initial length of the buds in centimeters, and columns 7 and 8 give the increase in length of the buds during the four-hour period, also in centimeters. It can be seen from columns 7 and 8 of the table that, in the first 5 plants, the bud P<sub>1</sub> remained practically or completely inhibited throughout the experiment, while bud P<sub>1'</sub> definitely increased in length. On the other hand, in the last 5 plants, both buds increased in length. In the first 5 plants, A<sub>1</sub>P<sub>1</sub>, corresponding to the inhibited bud, was
definitely more negative than $A_1P_1'$, corresponding to the growing bud. In contrast with this, in the last 5 plants the average node potentials were of approximately the same magnitude. To clarify these results the potential difference between $P_1$ and $P_1'$ was calculated from $A_1P_1$ and $A_1P_1'$ and is given in the last column of the table. That this procedure is permissible has been amply demonstrated by LUND and BUSH (13), RAMSHORN (16), and CLARK (4). It may be pointed out here that the writer has tested this principle in Phaseolus and has found that it always holds. It can be seen that $P_1P_1'$ is definitely positive in all of the first 5 plants, while in the last 5 plants it is comparatively small. That is, in plants with both buds growing out, the node potentials $A_1P_1$ and $A_1P_1'$ were of approximately the same magnitude, and therefore $P_1P_1'$ was of small magnitude. In plants with one bud inhibited and the other one growing, the node potential corresponding to the growing bud was definitely more positive than the node potential corresponding to the inhibited bud. For example, if bud $P_1$ was inhibited and bud $P_1'$ was growing, $A_1P_1'$ was more positive than $A_1P_1$ and therefore $P_1P_1'$ was definitely positive. Since these last experiments showed a definite correspondence between the electrical polarity from petiole to petiole and the conditions of the buds, the question arose as to whether or not there was also a correspondence between the electrical polarity of the buds themselves and the state of the buds.

In order to answer this question, 8 plants were chosen, 4 of which possessed buds of approximately equal size, while in the other 4 one bud was definitely larger than the opposite bud. In these experiments contacts were placed on the buds 1.5 centimeters from the center of the corresponding node. Measurements were taken between $A_1$ and these contacts. In table IV, $A_1C_1$ refers to the potential difference between $A_1$ and the contact on bud $P_1$; similarly $A_1C_1'$ refers to the potential difference between $A_1$ and the contact on bud $P_1'$. Measurements were taken at night in the greenhouse every 15 minutes over a 4-hour period and each value in the table represents the average for this period. The temperature limits for this series of experiments were from 18.5° C. to 21° C.

It can be seen in this table that $A_1$ is definitely positive in every case to the contact on the bud. In each one of the first 4 plants in which a large difference in the size of the buds occurs, the potential difference represented by $A_1C_1$ ($C_1$ representing the smaller bud) was less positive than the other potential difference represented by $A_1C_1'$. This relationship is similar to the one found between the size of the buds and the node potentials $A_1P_1$ and $A_1P_1'$. The average potential difference between $C_1$ and $C_1'$ was calculated from $A_1C_1$ and $A_1C_1'$ and is given in the last column. This potential difference is definitely positive in the first 4 plants, i.e., the base of the smaller and more inhibited bud is positive to the base of the larger bud. In the last 4
plants, the actual magnitudes of the potential differences of $A_1C_1$ and $A_1C_1'$ are greater than in the first 4 plants. On the other hand, the difference in magnitude between $A_1C_1$ and $A_1C_1'$ is definitely less; that is, $C_1C_1'$ is of smaller magnitude in the last 4 plants. This relationship again parallels that found between the node potentials $A_1P_1$ and $A_1P_1'$ and the size of the buds.

The question then arose, is there a definite electrical polarity between the apical region of a large growing bud and a homologous region of the opposite smaller bud? Four plants were chosen that possessed buds of unequal size; in each case the large bud was over twice the size of the smaller bud. Contacts were placed on the stem directly below the apex of each and the potential differences were measured between these two loci at 15-minute intervals for a 4-hour period. These experiments were also performed in the greenhouse at night. The temperature limits were from 19.5° C. to 21° C. The apical region of the larger bud was found to be strongly negative to that of the smaller bud. The average potential differences for the four plants were $-32$, $-37$, $-42$, and $-51$ millivolts respectively. It is well known that the inhibitory influence travels from the growing bud to the inhibited bud. Since the inhibitory influence travels downward in intact plants, the question then arose, is there a similar polarity between the apex and the base of intact plants?

Eight intact plants were selected with 2 nodes developed and with an average height of about 1 1/2 feet. In each plant one contact was placed on the stem directly below the apex and another contact on $B_1$, directly below the first node, and readings were taken every half-hour over a 4-hour period. The experiments were performed at night in the greenhouse; the temperature limits were from 21° C. to 25° C. The averages of these readings are pre-

<table>
<thead>
<tr>
<th>PLANT</th>
<th>$A_1C_1$</th>
<th>$A_1C_1'$</th>
<th>$P_1$</th>
<th>$P_1'$</th>
<th>$C_1C_1'$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+10</td>
<td>+34</td>
<td>3.1</td>
<td>12.2</td>
<td>+24</td>
</tr>
<tr>
<td>2</td>
<td>+13</td>
<td>+24</td>
<td>3.3</td>
<td>16.8</td>
<td>+11</td>
</tr>
<tr>
<td>3</td>
<td>+28</td>
<td>+37</td>
<td>8.6</td>
<td>15.0</td>
<td>+9</td>
</tr>
<tr>
<td>4</td>
<td>+19</td>
<td>+32</td>
<td>5.4</td>
<td>11.0</td>
<td>+13</td>
</tr>
<tr>
<td>5</td>
<td>+45</td>
<td>+46</td>
<td>5.6</td>
<td>8.5</td>
<td>+1</td>
</tr>
<tr>
<td>6</td>
<td>+42</td>
<td>+48</td>
<td>8.6</td>
<td>9.7</td>
<td>+6</td>
</tr>
<tr>
<td>7</td>
<td>+38</td>
<td>+36</td>
<td>4.3</td>
<td>4.5</td>
<td>-2</td>
</tr>
<tr>
<td>8</td>
<td>+40</td>
<td>+32</td>
<td>4.7</td>
<td>5.2</td>
<td>-8</td>
</tr>
</tbody>
</table>
sent in table V. In all of the plants except the second, in this table, the apical contact was definitely negative to the basal one.

**TABLE V**

POTENTIAL DIFFERENCE IN MILLIVOLTS BETWEEN THE Apex AND BASE OF EIGHT INTACT PLANTS

<table>
<thead>
<tr>
<th>Plant</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>P.D.</td>
<td>mV.</td>
<td>mV.</td>
<td>mV.</td>
<td>mV.</td>
<td>mV.</td>
<td>mV.</td>
<td>mV.</td>
<td>mV.</td>
</tr>
<tr>
<td></td>
<td>-51</td>
<td>+4</td>
<td>-70</td>
<td>-41</td>
<td>-48</td>
<td>-14</td>
<td>-32</td>
<td>-28</td>
</tr>
</tbody>
</table>

The question then arose: Is there a continuous potential gradient along the stem or are there polarities that are oppositely oriented to the main one? For an answer to this question the polarity of the second internode, i.e., B₂A₁, was calculated from the data presented in table IV of the previous paper (17) and is presented in table VI of the present paper. It will be noted that the apical region of the second internode is positive to its base in all but one of the plants, although it was of small magnitude in plant number six.

**TABLE VI**

AVERAGE POLARITY OF THE SECOND INTERNODE, CALCULATED FROM DATA PRESENTED IN TABLE IV OF PREVIOUS PAPER (17)

<table>
<thead>
<tr>
<th>Plant</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>B₂A₁</td>
<td>mV.</td>
<td>mV.</td>
<td>mV.</td>
<td>mV.</td>
<td>mV.</td>
<td>mV.</td>
<td>mV.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+22.1</td>
<td>+19.5</td>
<td>-0.7</td>
<td>+21.9</td>
<td>+18.3</td>
<td>+1.7</td>
<td>+20.4</td>
<td></td>
</tr>
</tbody>
</table>

**Effect of Applied Current on the Inhibition of the Buds**

It is well known that the inhibitory influence can pass through a dead region in the plant (Snow, 21). This was also found to be true in the plants used in this investigation. Therefore, since the potential differences are dependent on the living cells (17), the electrical current is not necessary for the transport of the inhibition, at least over short lengths of the plant. Nevertheless, the possibility remains that the bioelectric potentials may influence the transport of this inhibition in the living tissue. This is in a sense similar to the passage of the hormone in the oat coleoptile. The hormone can pass from the decapitated tip through agar to the body of the coleoptile. The hormone apparently passes through the agar by diffusion but the transport of the hormone in the coleoptile itself is too rapid to be accounted for in terms of ordinary diffusion (Van der Weij, 26).

Current from an external source was applied to intact plants in various ways, in order to find out whether or not an applied current could release the inhibition of the buds. The results were negative as far as the release of the buds is concerned, and are briefly summarized in the following paragraphs.
The strength of the currents varied from 1 to 50 microamperes and were sent through the plant with contacts similar to the ones used for measuring the potential differences. They were washed at regular intervals to prevent the products of electrolysis from coming in contact with the plant. The currents were sent in the following directions: A₁ to B₁, B₁ to A₁, A₁ to P₁, P₁ to A₁, P₁ to P₁', B₂ to A₁, and A₁ to B₂. The buds never grew out unless the stem between the apex and the node was killed and had dried out, and then only after the buds had grown out in control plants decapitated at the same time the current was first applied to the intact plant.² If only a short portion of the stem was killed, the buds remained inhibited. Sometimes the injury extended to the buds themselves and killed them (failure to regenerate after decapitation). Currents up to 5 microamperes produced little injury up to the time that the buds of the decapitated control plants started to grow out.

Although the applied current did not release the inhibition of the buds, several other effects of interest were observed. Injury always occurred first at the locus at which the current entered, even when these loci through which the current was sent were continually washed. This observation is similar to that of Schecter (19) on the polarity of the lethal action of electric currents on Conocephalus. When current was sent from P₁ to P₁', epinasty occurred in the petiole toward the positive pole, and hyponasty in the one toward the negative pole. This effect was most pronounced in young intact plants, definite effects being produced within 6 hours by a current of 50 microamperes and within 20 hours by a current of 5 microamperes. It was also observed that when current was sent from petiole to petiole of the first node of young plants with a growing second internode, bending occurred in the second internode (a region through which presumably no current was passing). The internode would first bend with the convex side towards the positive contact, i.e., the apex itself would move toward the petiole on which the negative contact was placed. The bend would later become reversed, the convex side moving toward the negative contact, and eventually (while the current was still flowing) the stem would become straight again. With a current of 50 microamperes, the stem would show a definite bend within 10 minutes after application of the current, and the whole process would last for about 2 hours. Currents of 5 microamperes would produce a definite bend within 30 minutes.

² Injury appears sooner at the loci where the current enters and leaves the plant than in the stem or petiole in between the contacts. Therefore, except in preliminary experiments, current was not actually sent at these loci but at a region 4 centimeters from the node, i.e., 3 centimeters from the original contacts; and when the first signs of injury appeared the contacts were shifted slightly.
Discussion

The experiments presented in this paper indicate that there is a positive correlation between the electrical polarities and the inhibition and regeneration of the buds. However, many more experiments need to be performed before anything approaching a complete picture of the patterns of bioelectrical potentials in this plant can be presented. Nevertheless several definite relationships have been brought to light by these experiments. The fact that decapitating the plant produced an almost immediate effect on the node potentials which lasted for about an hour demonstrates that events happening at one locus of the plant can definitely modify the electrical processes at a distant locus. The hypothesis presented itself that this particular variation in the node potentials may in some way be a factor in the release of the inhibited buds. Aside from other considerations, it is evident from these experiments that this increase in the magnitude of the node potentials is not a necessary prerequisite for the regeneration of the buds of that particular node, since in many plants decapitated above the third node the buds of the first node regenerated as rapidly as those of the higher nodes, whereas the effect of decapitation of the node potentials was small at the second node below decapitation, and entirely absent in the third node below. Also, crushing the stem produced a response somewhat similar to, though of not as great magnitude, the response to decapitation; and the buds remained inhibited for long periods afterward. From these considerations one can at least tentatively conclude that this immediate response of the node potentials to decapitation is not directly related to bud regeneration.

The experiments on the later effects of decapitation indicate that there is a definite relationship between regeneration and the orientation and magnitude of the potential differences. One of the interesting observations on the later effects of decapitation on the node potentials is that they diminish almost to zero, or become inverted before visible elongation of the buds occurs.

The results on the orientation of potential differences in this plant tend to confirm Went's hypothesis that the apices of this group of plants are negative to their bases. He has also postulated that the negative radical of the hormone is transported electrophoretically towards the positive pole. The fact that the orientation of the potential difference in the second internode is opposite to the main polarity of this plant indicates on first inspection that the hormone could not be transported in the manner postulated by Went in this internode. However, if one assumes that these potential differences existing in the plant give rise to currents and that a current flows downward, say in the region of the nodes, then there must obviously be a return current.

Ramshorn (16) has reported what amounts to an apical positivity for several plants. In this connection it may be pointed out that both Wilks (27) and Clark (4), working on the oat coleoptile, have reported an apical negativity in this plant.
flowing in the opposite direction. The experiments on the internal distribution of polarities suggest that the return circuit for current flowing upward in the region of the node is through the cells themselves. Obviously it becomes difficult to decide, from potential difference measurements alone, in what direction the hormone could be transported by these currents unless the exact path through which the hormone is transported and through which the current flows is known.

The observation that applied currents did not release the inhibition of the buds indicates that the bioelectric currents may not play a rôle in the longitudinal transport of this inhibition. In this connection it may be pointed out that Clark (4) has presented evidence to show that the longitudinal transport of the hormone in the \textit{Avena} coleoptile is probably independent of the measured bioelectric potentials. However, Clark points out that there may be some subtle relationship between the bioelectric potentials and the transport of the hormone that escaped detection by his experimental methods.

The writer feels that certain considerations must be kept in mind when interpreting the results of applied current on living organisms. If we assume that the potential differences give rise to currents, then, as has been pointed out above, there must be a return flow of current. It is obvious that it would be impossible to duplicate exactly a particular pattern of currents in a complex tissue by the application of a current from an external source. An E.M.F. from an external source when applied to the plant will tend to send current in the same direction throughout that portion of the plant to which it is applied. Therefore it is possible to duplicate at best only one direction of current flow while at the same time the applied current is of necessity opposing the return flow of the inherent current. Thus while the duplication of one portion of the electrical pattern may accomplish some result that that portion of the inherent current may itself accomplish in the normal plant, this applied current is also producing an abnormal physiological state in adjoining regions. It is therefore interesting that even currents of small magnitudes do produce visible injury when applied to the plant.

The fact that certain morphological changes similar to those produced by the hormone are produced by the application of small currents, \textit{i.e.}, epinasty, hyponasty, and bending, indicates that the bioelectric currents may in some cases alter the distribution or effect of the hormone in this plant, or in some other way affect the growth processes. The work of Brunner and Amlong (3), Koch (7), Ramshorn (16), Wilks (27), and others indicates that there is some causative relationship between the bioelectric potentials and the growth process in the phenomena of phototropism and geotropism. However, not all of the results of these workers are consistent with the hypothesis that bioelectric potentials transport the hormone to the
measured positive pole. Clark (4) has demonstrated that the hormone is transported in agar blocks only under comparatively large potential gradients.

**Summary**

1. The internal electrical polarities in the region of the nodes were found to possess the same orientation as the external ones.

2. Decapitation of a plant caused a large increase in the magnitude of the node potentials in the node directly below the point of decapitation, a small effect on the 2nd node below, and no effect on the third node below. Cutting a portion from the stem of a decapitated plant produced a similar response, but of smaller magnitude. Crushing the stem of an intact plant also produced a somewhat similar response to decapitation, although of smaller magnitude.

3. After decapitation, the node potentials returned to their former magnitude in about one hour and then steadily decreased until they were either inverted or of small magnitude. This decrease in the node potentials always preceded the first signs of regeneration of the buds.

4. In decapitated plants with both buds growing, the potential difference between the base of the petiole on the side of the growing bud was found to be negative to the base of the petiole corresponding to the inhibited bud; while in plants with both buds growing, the potential difference between the loci were found to be of small magnitude. Similarly, in decapitated plants the base of the growing bud was found to be negative to the base of the inhibited bud; and in plants with both buds growing, this potential difference was of relatively small magnitude. It was also found in decapitated plants that the top of the growing bud was negative to the top of the inhibited bud.

5. In intact plants, the apex was found to be negative to the base.

6. In intact plants, the apex of the second internode was found to be positive to the base of this internode.

7. Currents applied in various ways across the nodes of intact plants failed to release the inhibition of the buds except after a portion of the stem was killed and had dried out. Certain effects of the applied current not directly related to bud inhibition were observed.

8. In the discussion it is concluded that the response of the node potentials to the immediate effect of decapitation is probably not a causative factor in the regeneration of the buds. It is pointed out that there is a definite correlation between the inhibited and regenerating buds and the bioelectric potentials. The interpretation of the experiments on the effect of applied current is discussed.

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