SOME EFFECTS OF DECAPITATION ON ELECTRICAL AND ELONGATION PHENOMENA IN THE AVENA COLEOPTILE

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(With four figures)

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Introduction

Growth of the Avena coleoptile, including elongation and curvature responses, is dependent on the auxin which is produced only by the cells in the extreme apex (4, 12, 13). A polar force is required to direct and control the distribution of this auxin. It frequently has been suggested that the inherent electrical field could function as this force and there is evidence that is consistent with this thesis (9). However, if the inherent electrical field is dependent on the auxin-regulated elongation process, then this hypothesis would be untenable. For this reason, it is important to test this possibility by experimentation.

Preliminary evidence, from experiments which were not specifically designed to evaluate this point, indicates that the longitudinal electrical polarity of the Avena coleoptile is not directly dependent on the presence of auxin that is required for elongation (4, 14). It also has been demonstrated that a transverse electrical polarity, in response to mechanical and electrical stimulation, can be established in the etiolated oat seedling in which the auxin has been decreased by removal of the apex before stimulation (7, 10). However, no previous attempt has been made to measure simultaneously the elongation rate, as a manifestation of the functional auxin content, and the longitudinal electrical polarity of the Avena coleoptile.

It is generally accepted that decapitation of the coleoptile removes the source of auxin and results in its gradual decrease in the remaining tissue, because auxin disappears in the process of growth (2). Since growth of the coleoptile is proportional to the amount of auxin added (2, 11, 13), it is permissible to use the rate of elongation as an index of the quantity of auxin used in this process. The present experiments were performed to determine the effects of decreased auxin supply resulting from decapitation, as measured by the elongation rate, on the longitudinal component of the electrical pattern of the coleoptile.

Materials and methods

The experimental material was the coleoptile of the Victory strain of Avena sativa (U. S. Department of Agriculture, C.I. 2020). All seeds were

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2 Supplied by the U. S. Department of Agriculture.
husked, soaked in tap water with the pH adjusted to 7.1 by a phosphate buffer and sprouted in Petri dishes on filter paper moistened with buffered tap water. The Petri dishes were kept 38 cm. from a 60-watt lamp (dark Ruby Mazda which emitted only wave lengths longer than 5,700 Å). They were then placed in a dark box in an aquarium containing the same buffer solution. Only plants that had a good general appearance, straight cole-

Fig. 1. Photograph of experimental equipment (see text for description).

optiles and fully developed root systems were selected for experimentation. In the apparatus, each seedling was placed in a holder similar to that in the aquarium and supported in the same buffer solution. After handling, before an experiment, the plant was always allowed to rest at least 30 minutes to recover from the mechanical stimulation.

The electrical measuring device was a duBridge (5) amplifier in a light-
tight box with a Rubicon galvanometer (Model 3403 HH) as the indicating instrument. In this circuit the galvanometer had a sensitivity of 1.08 millivolts per scale division. Variation of the zinc–zinc sulphate electrodes was never more than 2.2 millivolts during an experiment. The electrodes were placed in glass cups which were filled with Shive's solution made with distilled water. Figure 1 shows the spatial relationship of the coleoptile to the contacts and attached glass cups. In all instances the contacts were placed so as to obtain the maximum longitudinal electrical polarity but to maintain approximately uniform lengths of included segments. The apical contact was always connected to the control grid and the basal contact was grounded. Glass ring contacts and the combination contact-holder were used for the isolated coleoptiles (sheaths separated from primary leaves and seed). Actual contact with the tissue was made by a water meniscus.

**Fig. 2.** Mechanical decapitator (see text for description).

Elongation was measured by observing a spot of India ink, placed near the apex, with a horizontal microscope equipped with a Filar micrometer ocular.

All experiments were performed in a dark room equipped with a temperature- and humidity-controlling device, which maintained the temperature within ± 3 of 24°C. The temperature never varied more than 1°C during an experiment. A ruby glass neon tube, which emitted only wavelengths longer than 6,200 Å, was the light source.

Various attempts at decapitation in preliminary experiments made it evident that some device was needed to permit precise control of decapitation of the coleoptile with little effect on the electrical polarity. (Curve II in figure 3 B shows the type of electrical response that is inevitable when the plants are decapitated by hand.) Figure 2 shows the construction details of a mechanical decapitator designed to meet the essential requirements.
On one end of the brass bar was a small block of lucite (A), which had two grooves. The vertical groove (L) was slightly larger than the coleoptile and was designed to encircle it on three sides during decapitation to prevent movement to either side or away from the blade. Groove (M) was horizontal to allow the passage of the decapitator blade through the plant.

A second unit suspended from the brass bar consisted of two \( \frac{3}{8} \) by \( \frac{1}{4} \) inch brass blocks (B), three inches in length, and three \( \frac{3}{4} \) inch square iron rods. Two of the iron rods (C) were screwed between the brass blocks (B) so that a square hole remained down the center of this combination. The square rod (D), six inches long, was lapped to fit snugly into this hole, thus eliminating sway in the movable decapitator rod. This sliding rod (D) was rounded and threaded for one-half inch on each end. A compression spring (E) was placed on the blade end of the shaft. Nuts (F), placed in front of the spring, permitted adjustment of tension. The knife (G), part of a thin razor blade, was mounted at an angle with DeKhotinsky cement. Two nuts (H) were placed on the back thread of this movable bar (D) to allow adjustment of the cutting depth. A soft iron cap (I) was drilled and tapped to fit the end of the threaded bar (D) to serve as a bumper and to add surface for the electromagnet to hold. Each adjustment was held securely by a locking nut.

The third element of this decapitator was an electromagnet (J) with its wires connected to terminals (K).

In operation, the decapitator circuit was completed, the cutter mechanically cocked, the blade and plastic coleoptile rest moistened with distilled water, the cutting depth adjusted, and the decapitator moved onto the coleoptile at the desired level. Movement of the decapitator was accomplished by a micromanipulator. Opening the circuit allowed the spring to drive the blade through the plant. The circuit was then recompleted, the cutter recocked and the decapitator removed.

Experimental

Effect of decapitation on rate of elongation and longitudinal electrical polarity

In this series intact coleoptiles 34 (± 2) mm. in length were used. The ink spot was placed about 5 mm. below the apex. Figure 3A shows the characteristic elongation rates (measurements at 5-minute intervals). Curve I is a typical example of six experiments in which the plants were not decapitated. The gradual decrease in the rate of elongation is normal for coleoptiles of this age and length.

Curve II of figure 3A was chosen as the typical representative of eight experiments. In this set the coleoptiles were decapitated by hand, using a quick stroke with a thin razor blade and supporting the opposite side of the apex with a finger. Three (± 2) millimeters of the apex were removed. The initial rate of elongation, prior to decapitation, is less than that shown by the control curve I. This is due to inherent variation of the individual
coleoptiles. After decapitation (indicated by an arrow in the figure) and lasting for 80 minutes, there is a period of slight decrease in the rate of elongation. From 80 to 155 minutes after decapitation the rate is greatly decreased. This is followed by increased elongation beginning 155 minutes after decapitation.

Curve III of figure 3A is the characteristic example of five experiments. Coleoptiles were mechanically decapitated as described in the previous section (decapitated segment length, 3 to 4 mm.). The initial rate of elongation is practically equal to that of the control curve I. After decapitation the sequence of rate changes is the same as in curve II, but the time inter-

vals are slightly different. These changes in rates of elongation (curves II and III) are in general agreement with earlier work (4, 12).

Figure 3B shows the concurrent electrical polarities of the same plants whose elongation rates were presented in figure 3A. The differences in magnitudes of electrical polarities shown in figure 3B are due to individual variation in the plants. Two other phenomena manifested by these curves warrant consideration.

First, in curve II there is a large rapid increase in the electrical polarity immediately after decapitation, but the voltage always recedes to the original level within a few minutes. In other experiments of this series the polarity frequently decreases beyond the original average before returning to this level. These electrical changes are apparently the result of mechani-
cal stimulation caused by bumping the coleoptile against the apical contact in the process of manual decapitation. In curve III (coleoptile mechanically decapitated) the initial electrical response is greatly reduced, while in other experiments of this kind it was completely eliminated.

A second feature, appearing in the curves in figure 3B, is the gradual decrease of the electrical polarity shown by curves I and III toward the end of the experiment. This is also apparent in some of the duplicate curves, which are not included. However, in every case the decline in polarity started only after the decrease in the elongation rate was noted.

Several observations can be made from the data in figure 3. A large difference in the initial rates of elongation is shown by curves II and III, while the initial electrical polarity indicated by curve II is only slightly less than is shown by curve III. There is always a decrease in the rate of elongation of the decapitated plants but never a concurrent decrease in the longitudinal polarity. Toward the end of the experiment the decapitated plants show an increased elongation rate due to a functional regeneration of the apical cells (3). A parallel increase in the electrical polarity was never observed. These results clearly indicate that the longitudinal electrical polarity is independent of the auxin-controlled elongation rate. Results of a corresponding series of experiments on isolated coleoptile sheaths are in agreement with these observations.

Effect of Mechanical Stimulation of Cutting on Rate of Elongation and Longitudinal Electrical Polarity

Previous investigations have demonstrated that the electrical pattern of Avena is sensitive to mechanical stimulation (7). The data in figure 3 show that the initial electrical response caused by manual decapitation can be substantially reduced by use of a mechanical decapitator. One other remote but real possibility remains. Theoretically, a prolonged response to the mechanical stimulation of cutting could be equal but opposite in orientation to the effect of the absence of the apical cells with the net result that the average magnitude of the electrical polarity would remain unchanged. The present experiments treat this possibility.

Intact plants 33 (±1) mm. long were used. The decapitator was adjusted so the blade would cut only 3/4 of the way through the coleoptile. The ink spot was placed about 5 mm. from the apex while the level of the cut was 3 (±1) mm. below the apex. Preliminary tests had shown that the best way to keep the cut wet and to keep its edges from spreading apart was to place a piece of wet filter paper (4 mm. long and 2 mm. wide) over the cut so that the paper strip would extend above the apex. Distilled water was added to the filter paper strip from time to time and thus to the incision. This arrangement functioned as a diffusion bridge for the transport of auxin from the apex to the basal cells.

Curve Ia in figure 4A is another one of the control series. It shows a uniform rate of elongation throughout the 240-minute period. Curve IV,
representative of six experiments, is from a "partially decapitated" intact plant in which the incision was kept wet as previously indicated. This curve shows only a slight decrease in the elongation rate during the period of 210 minutes. Some of the control curves (curve I, fig. 3 A) from coleoptiles that were not decapitated show decreases of similar magnitude in the rate of elongation.

The corresponding curves in figure 4 B represent the longitudinal electrical polarities of the same plants. Curve Ia, the control, maintains approximately the same average magnitude for the 240-minute period. Curve IV shows an increase in the potential difference after decapitation which lasts for a period of about 55 minutes. Then there is a gradual decrease in the E.M.F. toward the end of the experiment. However, there is no consistent difference between these electrical curves and those in figure 3 B.

The results of these experiments (uniformly supported by a parallel series using isolated coleoptiles), in comparison to the data in figure 3, demonstrate that mechanical stimulation of cutting does not have exactly the opposite effect of removing the tip in so far as the potential difference is concerned. Therefore, the maintenance of the longitudinal electrical polarity is not a prolonged result of mechanical stimulation, nor is it in any way dependent on the presence of the apical cells of the coleoptile.
Effect of decapitation on the spontaneous variation of the longitudinal electrical polarity

Continuous bioelectrical fields manifest spontaneous variations in magnitude even though all known experimental conditions are constant. Curve III in figure 3 B and curve IV in figure 4 B indicate that neither the absence of the apex nor the mechanical stimulation of decapitation influences the inherent variability of the electrical polarity. Since shorter (younger) plants exhibit greater electrical variation (14), they could conveniently be used to check this observation.

Intact plants, 20 (± 1) mm. in length, were selected. The ink spot was placed about 3 mm. from the apex, the apical ring contact 5 (± 0.5) mm. below the apex, and the basal contact was placed so that a segment of tissue 12 (± 2) mm. remained between the contacts. A segment 2 (± 1) mm. long was removed. Each experiment was continued until there was first a decrease and then an increase in the elongation rate.

Curve V in figure 4 A, from a decapitated coleoptile, is typical of a group of four experiments. It is noted that the changes in the elongation rate are in the same sequence as in the corresponding previous curves. The total cycle is somewhat shorter for the 20-mm. coleoptile primarily because the duration of the first decreased rate, soon after decapitation, is shorter than for the longer plants.

Curve V in figure 4 B represents the electrical changes of a short decapitated plant. This curve shows a gradual increase in the magnitude of the polarity and indicates a decrease in electrical variability during the latter half of the experiment. However, the decrease in variability starts several minutes after the decreased elongation rate is evident, and the electrical variation does not reappear at the end of the experiment when an increase in the elongation rate is manifested. These results (supported by a parallel series using isolated coleoptiles) further indicate that the inherent variation of the electrical polarity is not directly dependent on the rate of elongation.

Discussion

Experimental results, which have recently been reported, demonstrate the magnitude, orientation and variability of the various components of the inherent electrical field of the Avena coleoptile (14). As an incidental part of this investigation the coleoptile was isolated from the seed, decapitated and then removed from the primary leaves. These data (14) indicate that such treatment had no effect on the longitudinal electrical polarity. Since it is not known how long after decapitation the electrical measurements were made, these observations cannot be evaluated in terms of effective auxin content of the tissue. The results in the present paper confirm some of the electrical pattern characteristics previously reported (14) and present simultaneous and continuous records of the functional auxin content (indexed by elongation rate) and the characteristics of the longitudinal electrical polarity before and after decapitation.
When the coleoptile was decapitated by hand, it was sometimes impossible to keep the plant from touching the apical contact. This mechanically stimulated region immediately became more negative with respect to the basal and grounded contact region. Such increase in negativity of the stimulated region is in agreement with results from the onion root (6), from Chara (1), and from Avena (7). Because of the orientation of the inherent electrical polarity, this stimulation resulted in a rapid increase in the potential difference. When the mechanical decapitator was used this initial electrical response was reduced from 75 to 100%. In a few experiments the initial increase in voltage was followed by a more gradual decrease. Evidence, currently available, is inadequate to reveal the nature of this second prolonged electrical change. It could be a "conduction phenomenon" such as reported for Chara (1). It might also be due to stimulation of the basal contact region or extended recovery from stimulation of the apical region.

These data prove that the absence of the apical cells causes no characteristic change in the magnitude or orientation of the longitudinal potential of intact coleoptiles or isolated sheaths. The possibility that a prolonged secondary electrical response to the mechanical stimulation of cutting is equal but opposite in orientation to the effect of the absence of the cells of the apex has been ruled out by experiment (fig. 4). The present findings also show that spontaneous variations in electrical polarity are not directly dependent on the presence of the apical cells. Therefore, it now appears that the origin and characteristics of the continuously maintained bioelectrical field are independent of the metabolic mechanism that is specifically responsible for elongation of the Avena coleoptile.

Summary

1. In order to investigate the effect of auxin on the longitudinal electrical polarity of the Avena coleoptile simultaneous measurements of elongation rates and voltages were made before and after decapitation.

2. A description of the decapitating instrument, devised to diminish mechanical stimulation of the coleoptile, is presented.

3. Decapitation of intact plants results in two decreased, but different, elongation rates which are followed by an increased rate. Elongation rates are used as an index of the functional auxin. No significant effect of decapitation on the orientation or magnitude of the longitudinal electrical polarity was observed. Isolated sheaths gave similar results.

4. The data show that the longitudinal electrical polarity of the Avena coleoptile is independent of the auxin-regulated elongation process.

5. Spontaneous variations of the longitudinal electrical polarity are also not directly dependent on the elongation mechanism.

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