BIOELECTRIC OSCILLATIONS OF BEAN ROOTS: FURTHER EVIDENCE FOR A FEEDBACK OSCILLATOR

III. EXCITATION AND INHIBITION OF OSCILLATIONS BY OSMOTIC PRESSURE, AUXINS, AND ANTIAUXINS

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Summary

After bean roots have been subjected to prolonged application of oscillations in osmotic pressure or β-indolylacetic acid (IAA) at the natural period, they exhibit an increased tendency for oscillatory behaviour. The amplitude of transient oscillations, resulting from sudden changes in osmotic pressure or in IAA concentration, is increased and the damping is decreased. In addition, the application of oscillations in osmotic pressure or in IAA of period considerably different from the plant's natural period, tends to evoke oscillations of the plant's natural period as well as those of the applied period. These effects are greatest at the root's elongating zone and have been interpreted in terms of an increase in the gain of a physiological feedback system responsible for oscillations in potential.

A second phenomenon is described which is again interpreted in terms of the same feedback system. In this, the application of auxin (IAA) or auxin antagonist (2,6-dichlorophenoxyacetic acid) in weak concentrations (less than 10^{-5}M) either in oscillatory or stepwise form, forces the plant potential to oscillate. At high concentrations (10^{-5}M or greater), the auxin variable in the feedback system becomes an invariant and no oscillatory responses appear. The auxin transport inhibitor 2,3,5-triiodobenzoic acid at 10^{-5}M also inhibits oscillations.

I. INTRODUCTION

In this paper it is proposed to describe two oscillatory bioelectric effects which are consistent with the postulated feedback oscillator discussed by Jenkinson and Scott (1961). It is thought that the function of such a feedback loop is to control the physiological variables involved, so maintaining them at certain optimum values. If the physiological condition of the biological system is altered then the dependence of one variable on the next may be changed. This would change the loop parameters such as the gain, so that the amplitude of either spontaneous or resonance oscillations would alter also.

The first effect (see Section III(a)) involves an increase in the total loop gain, resulting in an increased tendency for the evocation of potential oscillations at the natural period. This occurs after the plant root has been subjected to prolonged excitation at the resonance by the application of an oscillation in osmotic pressure or β-indolylacetic acid (IAA) concentration of the plant's natural period of oscillation. This effect is similar to some plasticity phenomena exhibited by nerve cells (Eccles 1953) in which the threshold of response is lowered and in some cases the response is increased after prolonged stimulation.

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The second effect (Section III(*b*)), although superficially quite different from the first, is fundamentally related to the operation of the feedback loop. In this it is considered that oscillations are suppressed by changing the auxin variable into an invariant, thereby effectively breaking the feedback loop at this point.

In this respect, the effects of substances, known generally as "antiauxins", on the bioelectric potentials will be described and discussed in terms of their known physiological action. The physiological interpretation is shown to have considerable bearing on the relation of auxin to the feedback loop responsible for bioelectric oscillations.

II. Materials and Methods

The experimental material and methods were identical with those described by Jenkinson and Scott (1961).

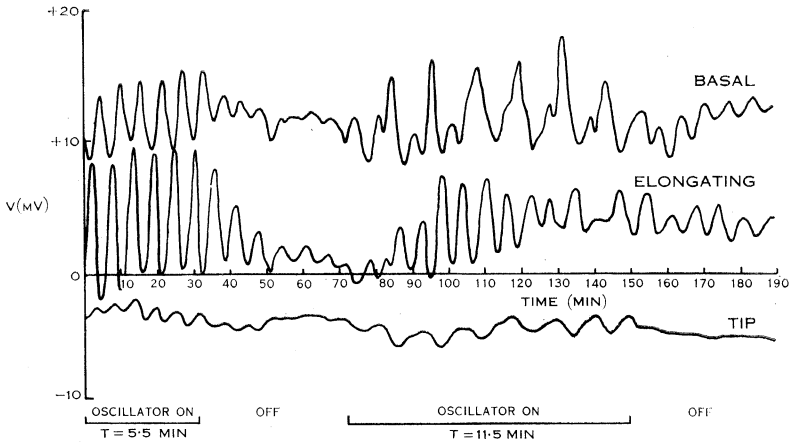

Fig. 1.—Potentials recorded simultaneously at the tip, the elongating zone, and the basal region of a bean root in response to prolonged oscillation in osmotic pressure at the natural period and subsequently to a considerably different period.

III. Results

(*a*) *The Bioelectric Plasticity Effect*

In a previous paper, Jenkinson and Scott (1961) described the extracellular potential response to applied oscillations in osmotic pressure or in auxin concentration. This response was found to consist in a forced bioelectric oscillation of the same period as the applied oscillation. It was shown further that the maximum amplitude of the response or resonance occurs at the plant's natural period of oscillation in potential. In addition to this, another type of response occurs in some cases. It is quite frequently found that oscillations of the natural period are evoked by an applied oscillation (either in osmotic pressure or IAA) of considerably different period. This type of response often occurs in plants showing a very marked resonance and the effect is most pronounced at the elongating region of the root. In many plants the effect only occurs after being subjected to excitation at the natural period for a considerable number of cycles.

Figure 1 shows the effect quite clearly. Initially, resonance was evoked by applying an osmotic pressure oscillation of the natural period (= 5·5 min) for a considerable number of cycles. After this oscillation was removed and the natural oscillation had been damped out, a period of 11·5 min was applied. At the tip, only the applied period appears in the response whereas at the basal region both the applied and the natural period appear in the resulting variations in potential. At the elongating region the applied period of 11·5 min is almost completely absent from the response, only oscillations of the natural period being excited. On removal of the 11·5-min oscillation, the potentials at the elongating and basal regions continue to oscillate at the natural period only, while at the tip no oscillation ensues.

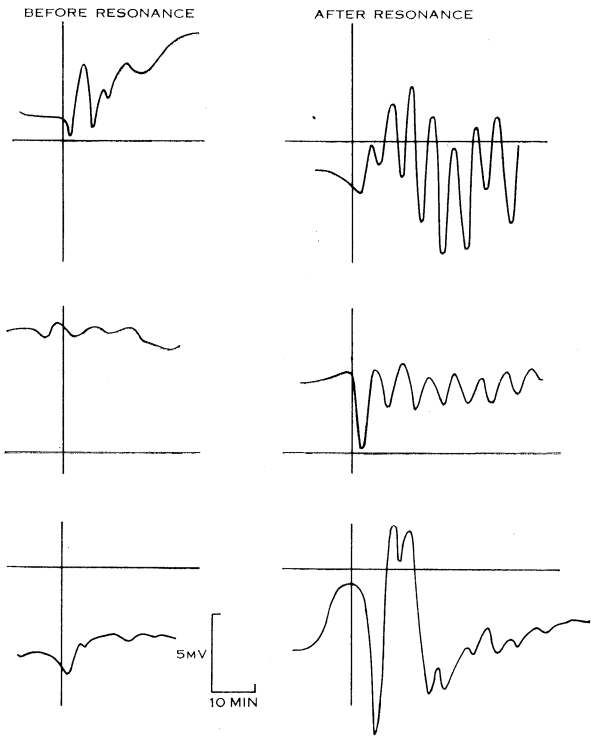

Fig. 2.—Responses in potential to sudden changes in osmotic pressure (shown by vertical lines) before (traces on left) and after (right) osmotic excitation at resonance. Both tracings in each pair were recorded at the same distance along the bean root from the tip. In the top and middle pairs, the osmotic pressure change was from M/60 to M/30 while for the bottom pair, from 0 to M/30.

It has been found that damped oscillations of the natural period are frequently evoked by a sudden change in osmotic pressure or auxin concentration. Hence it is not surprising that any variation, such as an oscillation, in these two environmental variables, should cause oscillations in potential of the natural period. However, the increased tendency for oscillations of the natural period to appear

after the plant has been subjected to prolonged excitation at resonance is a separate phenomenon. To study this effect in more detail the plant was stimulated to evoke transient oscillations before and after prolonged excitation at the resonance.

It was found that after resonance excitation, the natural oscillations in the transient were of greater amplitude and were less damped than those evoked by the same stimulus before resonance excitation. The traces on the left and right of Figure 2 show the transient potential oscillations evoked by suddenly increasing the osmotic pressure by a constant amount before and after osmotic excitation at the resonance, for three different bean roots. Both tracings (left and right) were recorded at the same

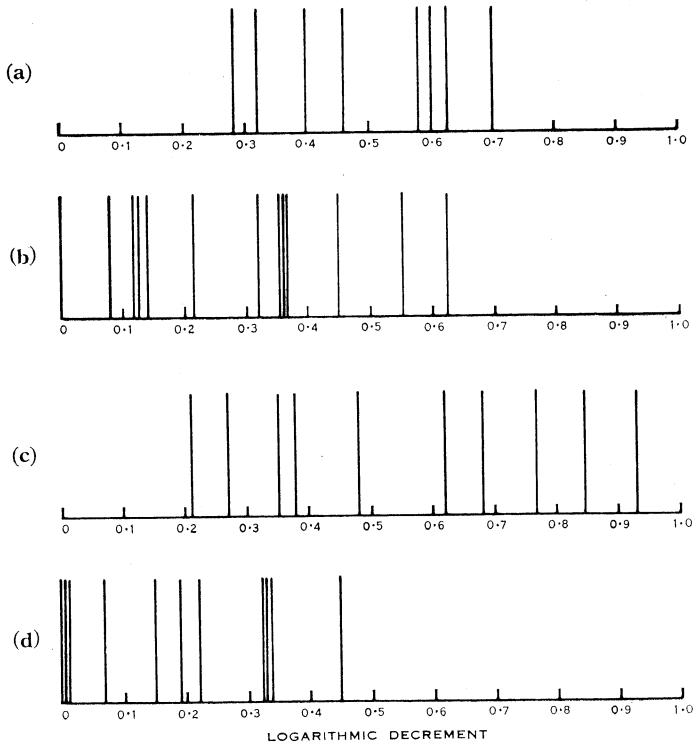

Fig. 3.—Occurrence of logarithmic decrements (shown by vertical lines) of free oscillations, as in Figure 2, for a number of bean roots: (*a*) transient potential oscillations evoked before osmotic excitation at the resonance; (*b*) oscillations evoked after resonance; (*c*) and (*d*) show the corresponding results for the logarithmic decrements before and after resonant excitation by oscillation in the concentration of the IAA solution (from 0 to 10^{-7}M).

position along the plant (i.e. the same distance from the root tip). The main feature is the increased amplitude and the decreased damping of the transient oscillations on the right (after resonance) compared with those on the left (before resonance). Results similar to those in Figure 2 were obtained when IAA was used to evoke the transients and the resonant oscillations, by suddenly increasing its concentration in the former and oscillating its concentration at the natural period in the latter.

The damping coefficients (logarithmic decrements) of the transient oscillations evoked by various stimuli have been measured both before and after resonance excitation. It has been found that the damping of these transient oscillations does not depend on the stimulus with which they were evoked, whether this be mechanical, electrical, brief exposure of the root to air, or changes in osmotic pressure or IAA concentration. In Figure 3, these damping coefficients, expressed as logarithmic decrements, have been grouped according as the transient oscillations were evoked before or after resonance excitation by osmotic pressure or IAA concentration. Before resonance, the logarithmic decrements, as a group, are larger than they are after prolonged excitation at the resonance, whether by means of osmotic pressure or IAA concentration oscillation.

(*b*) *The Excitatory and Inhibitory Effects of Auxin and Antiauxins on Bioelectric Potentials*

The oscillatory potential responses evoked by oscillating the concentration of auxin (IAA) in the bathing solution of a plant root were described by Jenkinson and Scott (1961). The oscillations shown in Figure 4(*a*) were produced in response

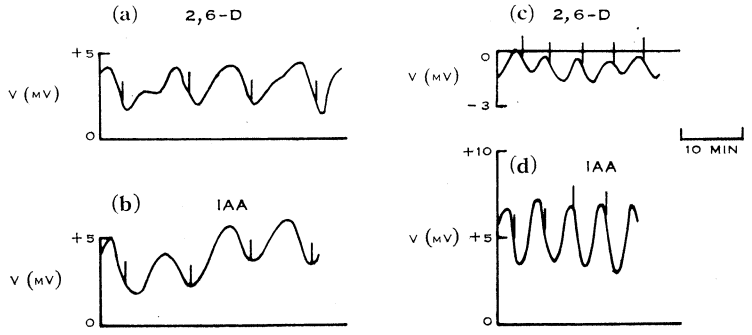

Fig. 4.—Responses in potential to oscillations in the concentration of (*a*) the auxin antagonist 2,6-dichlorophenoxyacetic acid (2,6-D) and (*b*) the auxin IAA, recorded on the same bean root and at the same period of oscillation. Similar results are shown in (*c*) and (*d*) for a different period of oscillation. In all cases the concentration of the auxin or antagonist was oscillated between 0 and 10^{-7}M (shown by the vertical strokes on the traces).

to an applied oscillation in the concentration of an auxin antagonist 2,6-dichlorophenoxyacetic acid (2,6-D) in the plant root's bathing solution. The times of maximum 2,6-D concentration (viz. 10^{-7}M) are indicated by the vertical marks on the record. Immediately after this record was obtained the 2,6-D was replaced by IAA and some minutes later, the record of the potential response from the same plant (Fig. 4(*b*)) was obtained. In both cases the maximum concentrations of 2,6-D and of IAA in the cycle were 10^{-7}M. The responses in the two cases are very similar as regards amplitude and phase with respect to the applied oscillation.

Figures 4(*c*) and 4(*d*) show oscillations evoked by oscillations in 2,6-D and IAA concentration at another period. Again the responses were recorded from the same plant but in this case the IAA response was obtained about 4 hr after the 2,6-D

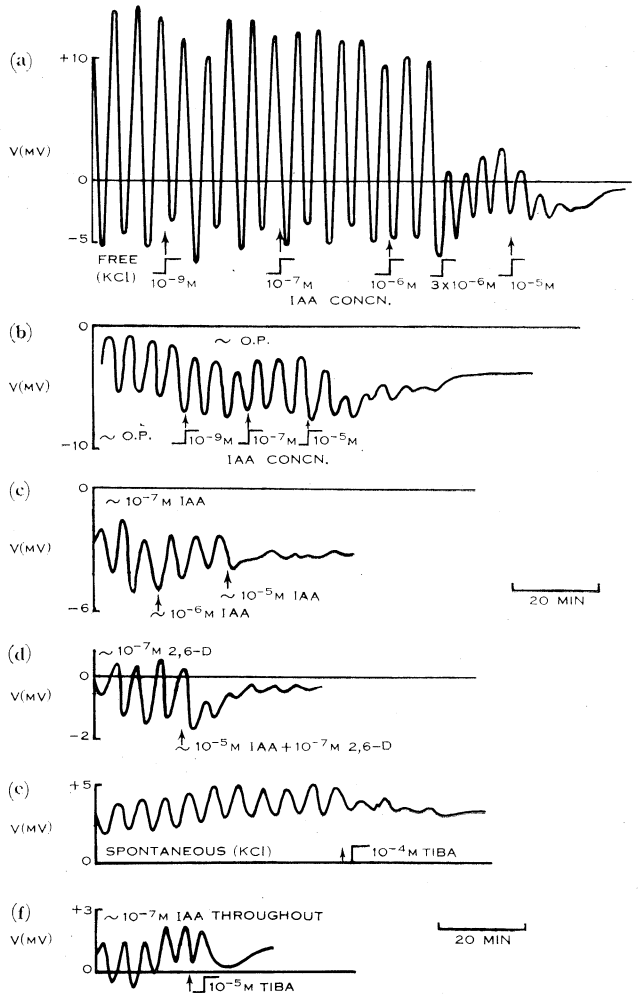

Fig. 5.—(*a*) Effect of increasing the bathing solution concentration of IAA in successive steps. Initially the bean root was producing spontaneous oscillations in potential in 10^{-4}M KCl following resonance. The IAA concentration was increased suddenly to the values shown at the times indicated. (*b*) Effect of increasing the concentration of IAA in the bathing solution in successive steps to a root producing resonant oscillations in potential evoked by an oscillation in osmotic pressure applied throughout, represented by ~O.P. (*c*) Effect of successively increasing the peak value of the oscillatory concentration of IAA applied to the bathing solution of the root. The applied period of oscillation was the natural period for the plant root. (*d*) Effect of applying an oscillation in IAA concentration (0–10^{-5}M) in addition to and in phase with the oscillation in 2,6-D (0–10^{-7}M) applied throughout. Both oscillations in concentration were applied at the plant's natural period. (*e*) Effect of adding 10^{-4}M 2,3,5-triiodobenzoic acid (TIBA) to the bathing solution (10^{-4}M KCl) of a plant producing spontaneous oscillations. (*f*) Effect of adding 10^{-5}M TIBA to the bathing solution of a plant producing resonant oscillations in response to an oscillation in IAA (0–10^{-7}M) applied throughout. In all cases (*a*) to (*f*) the constant background bathing solution was 10^{-4}M KCl and the potentials were measured at the elongating zone of the root.

response. Although the average value of the potential changed sign during this time and the oscillations in potential differ in amplitude, it is evident that the same phase relations exist with respect to the applied oscillations in concentration in both cases.

The relation of the amplitude and phase responses to the applied period of oscillation in 2,6-D concentration (0–10^{-7}M) is very similar to that for IAA. The main features again are: resonance at the natural period of oscillation and a phase change for short to long periods of 180°.

It is found that the addition of relatively weak concentrations (below 10^{-6}M) of the auxin IAA to the root's bathing solution does not affect spontaneous oscillations in potential. It was seen, however, to stimulate oscillations in cases where the oscillations were small or non-existent. At 10^{-6}M, however, the amplitude of the oscillations is decreased, whilst the addition of 10^{-5}M IAA inhibits the oscillations completely. This is shown in Figure 5(*a*) in which successively increasing steps of IAA concentration were added to the bathing solution of a root producing spontaneous oscillations following resonance. These results are paralleled by the fact that routine observations of growth rate have revealed no changes in the normal extension of the plant root in concentration of IAA less than 10^{-6}M. The elongation of the root ceases, however, on addition of 10^{-5}M IAA.

The effect of adding IAA to the bathing solution of a plant root producing oscillations in potential (at resonance) in response to an oscillation in osmotic pressure is the same as that for spontaneous oscillations in potential. The potential record shown in Figure 5(*b*) was obtained by applying an oscillation in osmotic pressure (by varying the concentration of mannitol in the bathing solution between M/30 and 0) to the plant root and successively adding increased concentrations of IAA to the bathing solution. The osmotic pressure oscillation at the resonant period was continued throughout. The addition of 10^{-9}M and 10^{-7}M IAA caused no appreciable change in the amplitude of the oscillations in potential but at 10^{-5}M IAA the oscillations were damped and disappeared after a few more cycles.

In Figure 5(*c*) the results of applying oscillations of the resonant period in the concentration of IAA to the root's bathing solution are shown. Initially the oscillation in IAA concentration was between 0 and 10^{-7}M. This was then changed to an oscillation between 0 and 10^{-6}M IAA. This produced no appreciable change in the amplitude of the oscillations in potential. The IAA concentration was then oscillated between 0 and 10^{-5}M. The oscillations in potential ceased.

In Figure 5(*d*) the initial potential oscillation was evoked by applying an oscillation in 2,6-D concentration between 0 and 10^{-7}M in the bathing solution. IAA at 10^{-5}M was then added to the 10^{-7}M 2,6-D so that the concentrations of auxin plus antagonist of the bathing solution oscillated between 0 and 10^{-5}M IAA plus 10^{-7}M 2,6-D. This inhibited the oscillations in potential.

From these results it is apparent that the addition of IAA at concentration greater than about 10^{-6}M, either in constant or oscillatory concentrations, inhibits oscillations irrespective of the manner in which they were evoked.

The action of the auxin antagonist 2,6-D is very similar to that of IAA. For a plant capable of producing oscillations, the addition of fairly weak concentrations

of 2,6-D applied either as sudden changes or oscillation will initiate sustained oscillations in the same manner as for changes in osmotic pressure or IAA concentration.

Again in similarity to IAA, 2,6-D at strong concentrations inhibits oscillations regardless of whether they are spontaneous or evoked by changes, either oscillatory or stepwise, in osmotic pressure, IAA, or 2,6-D concentration. The concentration, either constant or oscillatory, at which 2,6-D becomes inhibitory to oscillations is about 10^{-4}M, i.e. 1 or 2 orders of magnitude higher than for IAA. Thus the excitatory and inhibitory actions of the auxin antagonist 2,6-D are very similar to those of the auxin IAA.

It was found that the addition of 2,3,5-triiodobenzoic acid (TIBA) inhibited both spontaneous oscillations (Fig. 5(*e*)) and those evoked by oscillating the IAA concentration between 0 and 10^{-7}M at the natural period (Fig. 5(*f*)). In Figures 5(*e*) and 5(*f*) the constant concentrations of TIBA were 10^{-4}M and 10^{-5}M, respectively. These results show that the auxin transport inhibitor (TIBA) (Hay 1931) also inhibits oscillations, whether spontaneous or forced.

IV. Discussion

(*a*) *The Bioelectric Plasticity Effect*

Jenkinson and Scott (1961) showed that the damping of natural oscillations in the variables of the feedback system is related to the total loop amplification factor, K. For a negative feedback system, with all time delays being equal, it was shown that as the magnitude of K increases, the damping decreases until at $K = -8$ there is no damping and the system may oscillate spontaneously. Hence the increased amplitude and decreased damping of transient oscillations following resonant excitation may be formally ascribed to an increase in the magnitude of K, the total amplification of the feedback loop. This in turn could be caused by an increase in the magnitude of one or more of the individual amplification factors k_1, k_2, or k_3. A change in the time delays τ_1, τ_2, or τ_3 could also alter the damping.

Such alterations in the amplification factors or the time delays would cause some change in the natural period of oscillation but this would probably be small. No significant changes in period have been observed in oscillations before and after resonance. Jenkinson and Scott (1961) showed that the change in the natural period for a change in K from -2 to -7, though not negligible, is not large. Further, this change in K, from -2 to -7, means a change from very heavy damping (logarithmic decrement of 0·9) to zero damping. Such a change in damping is considerably more than the damping decreases normally observed after prolonged resonance excitation.

The biological significance of these changes in the formal parameters of the model may be sought in a facilitation of the influence of one physiological variable on another, e.g. the effect of auxin on membrane permeability to ions. By subjecting the membranes to large oscillatory variations in auxin concentrations, as envisaged at resonance, the membrane structure may well be modified so as to render its permeability more sensitive to changes in auxin concentration. The facilitation of other such interactions between variables suggested in the model is also conceivable.

(*b*) *The Excitatory and Inhibitory Effects of Auxin and Auxin Antagonists on Bioelectric Oscillations*

Before discussing the bioelectric effects evoked by auxins and auxin antagonists in the root's bathing solution either in constant or oscillatory concentrations, it is necessary to consider briefly some of the known biochemical and physiological facts concerning these substances. These have been summarized by McRae and Bonner (1953).

The biochemical action of auxin in producing cell wall plasticity is thought to consist initially in the attachment of an enzyme to the auxin molecule, the attachment taking place at two points on each molecule. Cell enlargement then results from the osmotic uptake of water, so stretching the plasticized cell wall.

It is possible, however, for an auxin molecule to become attached to its enzyme at only one point, in which case cell wall plasticization does not ensue. The molecules of auxin antagonists are similar to auxin molecules but one of the sites of attachment to the enzyme is either absent or inactivated. Consequently, the auxin–antagonist molecule can only form a one-point attachment to an auxin enzyme. Thus if auxin molecules are present in the tissue, either naturally or artificially supplied, the added antagonist competes for the auxin enzymes, so inhibiting the true auxin action.

Auxin in sufficiently strong concentrations is self-antagonistic in that the auxin molecules compete for the enzymes, one site of one auxin molecule becoming attached to one point on the enzyme molecule and one site of another auxin molecule becoming attached to the other point on the same enzyme molecule.

The exact physiological and biochemical action of auxin in relation to bioelectric potentials is of course not known. However, the relation of auxin to bioelectric potential oscillations and that of auxin to cell wall plasticity and consequent cell enlargement occur in the same morphological region of the root. Further, in the experiments described, there is a close parallel between the actions of artificially supplied auxins and antagonists on both the bioelectric behaviour and on root extension. Hence it seems probable that a similar biochemical action of auxin is involved in both cases. It may well be that the same initial chemical action between auxin and its enzyme leads eventually to both cell enlargement and the effects of auxin on the oscillatory bioelectric potentials.

Measurements of the concentration of auxin inside intact roots suggested that this is higher than the concentrations of auxin which produce increased elongation in root segments where the auxin concentration can be controlled (Aberg 1957). It seems probable therefore that the addition of IAA in concentrations even as low as 10^{-9}M is partially inhibitory to intact root extension. That is, the total concentration of auxin molecules is sufficiently high to cause partial inhibition of the two-point attachment by the auxin to its enzyme. In other words, the addition of auxin acts in the same way as the addition of auxin antagonists. Hence an increase in concentration of either auxin or auxin antagonist decreases the number of two-point attachments taking place between auxin molecules and their enzymes in the root tissue. Conversely, a decrease in concentration of either increases the number of two-point attachments

taking place. Consequently it is quite reasonable that the action of both auxin and auxin antagonists should be very similar so far as their effects on the bioelectric potentials of roots are concerned.

This would explain the fact that the application of oscillatory concentrations of IAA and 2,6-D (provided the peak concentrations are sufficiently weak so as to cause no appreciable inhibition of root elongation) evokes oscillations in the plant root potentials, the phases of the oscillations being the same for IAA and 2,6-D for a given period of the applied oscillation.

In Section III(*a*) it was seen that if the concentrations of IAA or 2,6-D are weak (i.e. sufficiently low that no appreciable inhibition of root elongation occurs), changes (either increasing or decreasing) in the concentration of either auxin or antagonist will initiate damped, and occasionally sustained, oscillations. If sustained oscillations, either spontaneous or resonant, are already present, changes in the concentration of IAA or 2,6-D have no effect. These results, again the same for auxin and antagonist, may be explained in terms of the change in the number of two-point attachments taking place between auxin molecules and their enzymes caused by a change in the amount of either auxin or antagonist applied. That is, a change in either causes a change in the effective auxin concentration in the tissue. If the auxin supply (and distribution) be considered as one of the variables in a negative feedback loop, a change in effective auxin concentration would lead to changes in the other variables of the loop such that the auxin supply would be adjusted to oppose the initial change. If the loop is at all unstable, this automatic adjustment would be of a damped oscillatory form. Hence oscillations, either damped or possibly sustained, would appear in all the loop variables such as those observed in the bioelectric potential.

If the variables of the loop, including the bioelectric potential are already in oscillation, either spontaneous (because of loop instability) or in forced resonance, the amplitude of the oscillations is controlled by the feedback loop parameters such as the total gain and possibly by non-linear characteristics. Consequently, a change in auxin concentration (or an effective change by addition of an auxin antagonist) would not be likely to change the amplitude of the oscillations, unless the loop parameters were also changed. This again is unlikely if the auxin and auxin antagonist concentrations are weak enough not to affect the elongation of the root appreciably.

If the concentration of either auxin or antagonist is increased so much that two-point attachments between auxin molecules and their enzymes are totally inhibited, then the feedback loop is effectively broken at the point where auxin action is involved. For the auxin supply then becomes an invariant so that the feedback action of the loop is prevented. The prevention of auxin transport by means of TIBA so prohibiting variations in the auxin supply would have the same effect of inactivating the feedback oscillator. Consequently, no oscillations in the bioelectric field would be produced.

It may be concluded that the bioelectric oscillatory phenomena described in this paper are all consistent with the proposed physiological feedback oscillator.

V. Acknowledgments

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VI. References

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