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

I. EXTRACELLULAR RESPONSE TO OSCILLATIONS IN OSMOTIC PRESSURE AND AUXIN

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Summary

Bean roots subjected to oscillations in osmotic pressure or in the auxin concentration of their weakly conducting bathing medium exhibit corresponding oscillations in their bioelectric fields with a resonance at the natural period of oscillation (approx. 5 min). The amplitude and phase responses, expressed as a function of the periods of the applied oscillations, are in agreement with those predicted from a theoretical model of a feedback loop. A physiological model compatible with the theoretical one is suggested.

I. Introduction

In a previous paper from this Laboratory, Scott (1957) described spontaneous oscillations in bioelectric potentials produced by bean roots maintained in an unchanging environment. It was suggested that these oscillations could be caused by a closed-loop feedback system of control acting between certain physiological variables. The suggested variables were the electric field, the auxin supply, and membrane permeability. In the present paper experiments which were devised to test this hypothesis are described.

One of the standard methods of investigating a feedback system is to apply an external oscillation of varying frequency to one of the variables of the feedback loop. From the resulting amplitude and phase responses of all the variables, the properties of the loop may be determined. Alternatively, if only one of the variables in the loop can be measured the properties of the loop may be found from its response to oscillations applied separately to each of the other variables in the loop. In the case of the bean root system under investigation, oscillations of other elements in the proposed loop besides the electric field have not been observed. Attempts to measure resistance changes corresponding to the postulated permeability oscillations were successful. Consequently only the second of the methods of investigation mentioned above was feasible.

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If the bioelectric field forms part of a feedback loop it would be expected to show characteristic responses when an oscillatory electric field is applied to the plant root. Preliminary experiments were performed in order to look for this response but it was found that there were considerable practical difficulties in separating the applied oscillatory potentials from any resulting bioelectric oscillations, and the experiments were discontinued.

Two experimental treatments have been found which result in oscillation of the bioelectric potential. In the first of these the osmotic pressure of the root's bathing solution is changed rhythmically. The oscillation in the bioelectric potential probably results from an oscillation in the water content and hence salt concentration of the outer cells of the root. In the second treatment an oscillatory concentration of β-indolylacetic acid (IAA) is applied to the root's bathing solution.

In this paper the results of experiments using these treatments are described and discussed in terms of a simple feedback loop oscillator.

II. Experimental Material and Methods

Broad beans (*Vicia faba* L. cv. Long Pod) were grown at 25°C in tap water which was continuously circulated and aerated. Two-day-old plants with roots about 3 cm long were used.

In most experiments the plants were removed from the culture bath and set up in the bathing solution of the measuring tank only about an hour before the commencement of the experiment. This time would probably not allow for complete equilibration of the plant roots with the bathing solution. However, in some series of experiments, the plants were allowed to equilibrate in the bathing solution for 15 hr or more before measurements were begun. In most experiments 10^{-4}M KCl was used as the bathing solution but in some cases 10^{-4}M $CaCl_2$ was used. The bath temperature was 25°C.

Bioelectric potentials were recorded automatically at five points in the bathing solution close to the plant root, using a six-channel recorder described by Scott (1957). The growth meter also described by Scott (1957) was used in some experiments to record the rate of elongation of the root. A recording chart speed of 3 in./hr was used throughout.

In order to produce an oscillation in osmotic pressure of the plant's bathing solution without changing the environment in any other way, a soluble unionized substance to which the plant membranes are practically impermeable is required. The substance must be unionized otherwise the conductivity of the plant's bathing solution and hence the bioelectric potentials would be affected. Sucrose has been used in most of the osmotic pressure experiments described in this paper. Although plant membranes have only a very low permeability to sucrose, it is, however, a physiologically active substance even in small concentrations once it enters a tissue (Brown and Sutcliffe 1950). Consequently the osmotic pressure experiments were repeated with mannitol which has the advantage of being largely physiologically inactive as well as being almost as non-permeating as sucrose. The results obtained with mannitol and with sucrose were in agreement.

The IAA used in these experiments was weighed out 10 mg at a time and then dissolved in 10 ml of absolute alcohol. This was stored away from light and was made up with 10^{-10}M KCl to the required concentration on the day of use. While in use the stock bottle containing the IAA in aqueous solution was shielded from light to help prevent deterioration. The concentrations of IAA in aqueous solution used in these experiments were 10^{-9}, 10^{-7}, and 10^{-5}M, respectively.

Figure 1 shows a diagram of the apparatus used to provide an oscillatory concentration of sucrose (or IAA) in a solution of constant KCl concentration (10^{-4}M). Two constant-head supply bottles, one containing 10^{-4}M KCl and M/30 sucrose, and the other containing only 10^{-4}M KCl, feed the two containers shown on the left of the diagram. The supply is adjusted so that the levels in each container are at the same constant height throughout. One end of each syphon tube dips into each container so that the rates of liquid supply to the feed tubes are determined by the heights of the syphon-tube outlets. These in turn are determined by the positions of the two eccentric circular cams on which the rigid arms supporting the syphon tubes rest.

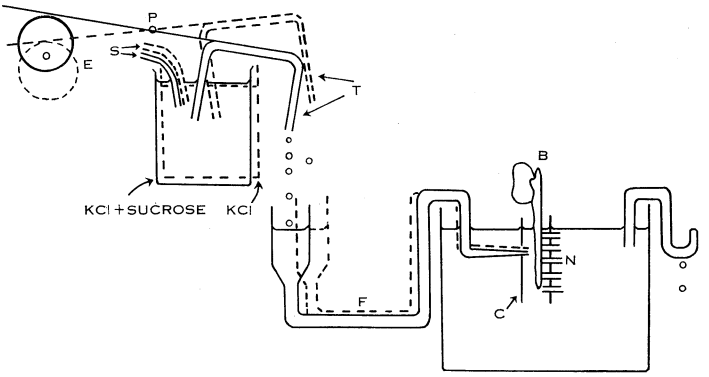

Fig. 1.—Schematic diagram of apparatus used to produce oscillations in osmotic pressure (or IAA concentration) in the bathing solution of plant root. The rear supply path is shown by the dotted lines. *E*, eccentric cams; *P*, fixed pivot; *S*, supply from reservoir bottles; *T*, syphon tubes; *F*, feed tubes; *B*, bean plant; *C*, polystyrene cylinder; *N*, "Nylex" tubes.

The cams are held on a single shaft so that they cannot rotate relative to one another. Further, the cams are opposed so that when one syphon tube is at its greatest height the other is at its lowest. In this way a maximum supply rate of sucrose plus KCl solution is delivered to one feed tube while a minimum supply of pure KCl solution is delivered to the other. When the camshaft is rotated by a half revolution the pure KCl solution supply rate is a maximum while the sucrose plus KCl solution supply rate is a minimum. The solutions are delivered via the feed tubes syphoning into the small polystyrene cylinder containing the bean root, the total rate of supply of solution being approximately constant throughout the cycle. The potential measuring probes placed at various distances along the plant root are held in holes drilled through the cylindrical container.

The camshaft is driven so that it advances by one-hundredth of a revolution every time an electric impulse is applied to the drive relay. The electric impulses are produced by an electronic pulse generator, the pulse repetition frequency of which can be adjusted continuously from 2 to 60/min. In this way the period of rotation of the shaft and consequently the period of the sucrose (IAA) concentration oscillation may be varied from about 1·7 to 50 min. However, the periods used were normally in the range 2–12 min.

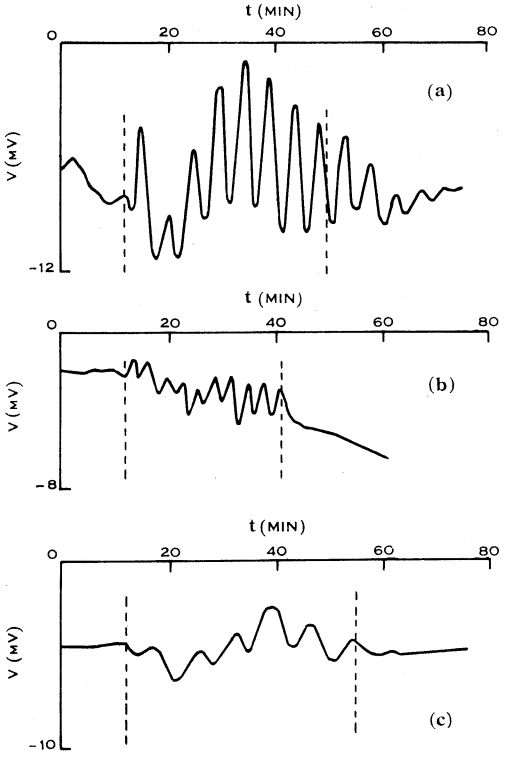

Fig. 2.—Potential response to osmotic pressure oscillations (0–M/30 sucrose) of three periods: (*a*) 4·7 min (the resonant period, T_r); (*b*) 3·0 min; (*c*) 7·5 min. The oscillation is applied between the dotted lines.

The phase of the applied oscillation in concentration (sucrose or IAA) is recorded on the potential record chart by automatically applying a voltage sufficient to deflect the pen to an off-scale position when the supply rate of sucrose plus KCl solution becomes a maximum. Since the total solution supply rate remains approximately constant (20 ml/min) throughout the cycle and the volume of the cylinder surrounding the plant is only about 1 ml, there is only a negligible phase difference (a few degrees) between the sucrose concentration round the plant and the supply rate of sucrose plus KCl solution even for periods as short as 2 min. Further, it

may be shown that the concentration maxima and minima deviate from the nominal values (M/30 and 0 for sucrose; 10^{-9}M and 0, etc. for IAA) by less than 1% even for the 2-min period. The delay involved in the syphon tubes is estimated to be negligible (about 1 sec).

III. Results

(*a*) *Osmotic Pressure Response*

When the osmotic pressure of the plant root's bathing solution is oscillated, the bioelectric potential is forced to oscillate with the same period as the applied osmotic pressure oscillation. Figure 2(*a*) shows the potential response to the application of an osmotic pressure oscillation, the period of which is close to the natural period of oscillation for the plant. The natural period is the period of the transient oscillations which are generally observed when the plant is set up for measurement or its environment is disturbed (Scott 1957; Jenkinson 1958.) After removing the osmotic pressure oscillation, the potential oscillation is seen to continue for a number of cycles though it is damped. Moreover, the amplitude response is much greater than that in Figures 2(*b*) and 2(*c*) where the period of the applied osmotic pressure is considerably different from that of the plant's natural potential oscillation. In such cases (Figs. 2(*b*), 2(*c*)), the potential oscillation disappears as soon as the applied oscillation is removed.

For a particular period the amplitude of the potential response depends on the amplitude of the applied osmotic pressure oscillation. It has been found that for oscillations in sucrose concentration with peak values lower than 10^{-3}M the extracellular potential response is negligible. Between 10^{-3}M and 3×10^{-2}M the potential response increases with molarity and above 3×10^{-2}M it increases only slightly up to the plasmolysis threshold. Since the potential response does not increase appreciably beyond M/30 and since this molarity is well below the plasmolysis threshold, it has been chosen to give the maximum osmotic pressure in most experiments. This maximum pressure is about 0·8 atm.

In Figure 3 the potential responses to five different applied periods of osmotic pressure oscillation are shown. These potentials were recorded simultaneously at three different regions along the root. At all three positions a potential oscillation is produced, its period being equal to that of the applied osmotic pressure oscillation. The oscillatory response is again greater for periods near the natural period of the root (about 5 min), this being particularly noticeable in the potential recorded at the elongating region. Further evidence for a close correlation between the period at which the plant's response to an applied oscillation is greatest (i.e. the resonant period) and the natural period of the plant is given in Figure 4(*a*) where the values obtained for a number of plants are compared.

The response to applied oscillations with resonance near the natural period is generally found to be more marked in the elongating region than in other regions such as the root tip, primary meristem, or root base. Further, the oscillatory potentials at these regions are substantially in antiphase with those produced at the actively resonant, elongating region. It appears that these other regions are

not so actively resonant and that their potential oscillations are of a passive type caused largely by return currents produced in the actively resonant (elongating) region.

By means of the following experiments it has been possible to test the hypothesis that the actively resonant region is located in the elongating zone of the root (between 2 and 12 mm in the bean roots used), and not in the primary meristem nor in the regions where cell elongation has ceased. First the osmotic pressure oscillation

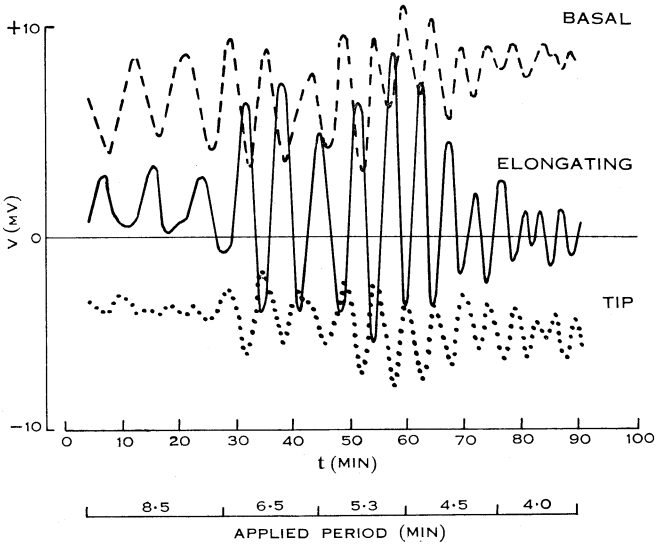

Fig. 3.—Potential response to osmotic pressure oscillations (0–M/30 sucrose) at three regions of the root. The period of the oscillation is reduced in steps from 8·5 to 4·0 min. Note the phase difference between the oscillation at the elongating region and at other parts of the root.

was set at the resonant (or natural) period. This caused the plant to produce enhanced oscillations at the same period. Three millimetres of tissue from the tip end of the root were then cut away thus removing the primary meristem. This treatment did not affect the response of the plant to the resonant period in any way, within 1 or 2 hr of the excision. However, the removal of a further 10 mm greatly inhibited the response to the resonant period, and in some cases completely suppressed the plant's potential response to the osmotic pressure oscillation.

In some experiments the resonant conditions was first evoked with most of the root (*c.* 3 cm) immersed in the bathing solution. Then the plant was raised so that only the last 10 cm remained in the bathing solution. The resonant oscillations continued although the background potential pattern (i.e. the steady potential pattern on which the oscillations are superimposed) was diminished. Excision of the first 3 mm from the tip end again did not diminish the oscillatory potential response.

This type of experiment was repeated using plants with roots only above 15 mm in length. In such roots there is hardly any tissue which has ceased elongating. These plants again exhibited strong resonances which were not inhibited by excising the primary meristem. These experiments show convincingly that the elongating zone of the root is the actively resonant region.

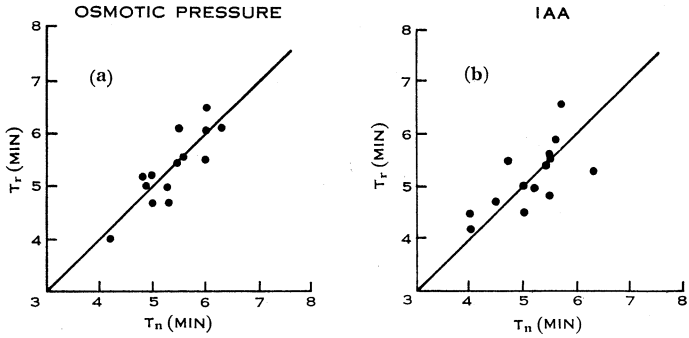

Fig. 4.—Relation between the resonant period (T_r) and the natural period (T_n) for a number of plants used in the osmotic pressure and auxin experiments.

(*b*) *IAA Response*

Resonant responses, very similar to those evoked by oscillations in osmotic pressure, have been observed when the concentration of IAA in the bathing solution is oscillated between 0 and 10^{-7}M. The period of resonance again shows good agreement with the plant's natural period (Fig. 4(*b*)). If 10^{-9}M IAA is used as the peak concentration in the cycle, resonance still occurs but the response to all periods of oscillation is usually diminished considerably. The response to oscillations in IAA with 10^{-5}M peak concentration is small and no resonance is observed. This is probably because at such high concentrations the elongation of the root is completely inhibited (Scott, McAulay, and Jeyes 1955). Since oscillations between 0 and 10^{-7}M IAA evoke the most marked effect, most of the results for IAA in this paper refer to this peak concentration.

For the bioelectric oscillations evoked by IAA oscillations, the actively resonant region is again the zone of elongating cells in the root. This was shown in the same manner as for osmotic oscillations by excising different parts of the root.

Routine observations of growth rate using the growth meter described by Scott (1957) have shown that in certain isolated instances there are irregular oscillations in the rate of elongation of the same period as the resonant potential oscillations evoked by osmotic pressure or IAA. However, it does not appear that potential oscillations at resonance show any general correlation with oscillations in the overall rate of elongation of the root.

(c) *Dependence of Amplitude and Phase of Bioelectric Oscillation on Period*

Figure 5 shows typical examples of the dependence on period of the amplitude and phase (defined below) of the electric response in the active region for roots subjected to oscillation in osmotic pressure and IAA concentration. The natural periods of oscillation, obtained from transient potential data for each of the plants involved, are shown on the period (T) axis. It is seen that the resonant period is in agreement with the natural oscillatory period for each plant, both for osmotic pressure and IAA.

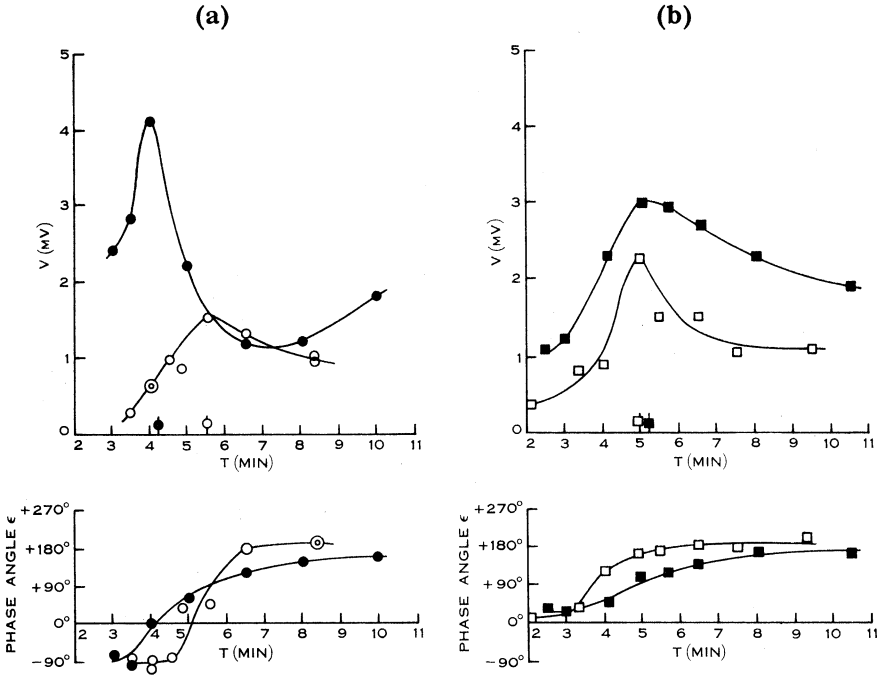

Fig. 5.—Amplitude and phase responses in the elongating region for (*a*) two plants subjected to osmotic oscillation (0–M/30 sucrose) and (*b*) two plants subjected to auxin oscillation (0–10^{-7}M IAA). V is the double amplitude and ϵ the phase (defined in text) of the electric oscillation for applied oscillations of period T. The natural periods are indicated on the horizontal axes.

The phase angles shown in Figures 5(*a*) and 5(*b*) for osmotic pressure and IAA are defined as follows: If the algebraic maximum of potential precedes the maximum concentration of sucrose (or IAA), the potential leads, and the phase lead is shown as a positive phase angle. If the potential lags, the phase angle is negative. It is seen that the phase angle changes as the period is increased, the greatest rate of phase change being near the resonant period. At long periods, the observed potential oscillation is 180° out of phase with the applied oscillation in osmotic pressure or IAA concentration. However, the change in phase angle from short to long periods is greater for osmotic pressure than for IAA. For osmotic pressure the change is about 270° while for IAA it is only about 180°.

In Figure 3 it was seen that the phase of the forced potential oscillations is not the same for all points along the root, the oscillations produced at the elongating region being substantially, though not exactly, in antiphase with those observed at the more passive regions. Hence, if phase response curves such as those in Figure 5(*b*) were drawn for the potential responses at the passive regions, these curves would be displaced by about 180° with respect to those in Figure 5(*b*) for the elongating region. The phase curves for the passive regions, however, still show the same phase change (about 270° for osmotic pressure) from short to long period. In some cases even the phase curves for the elongating regions of different roots

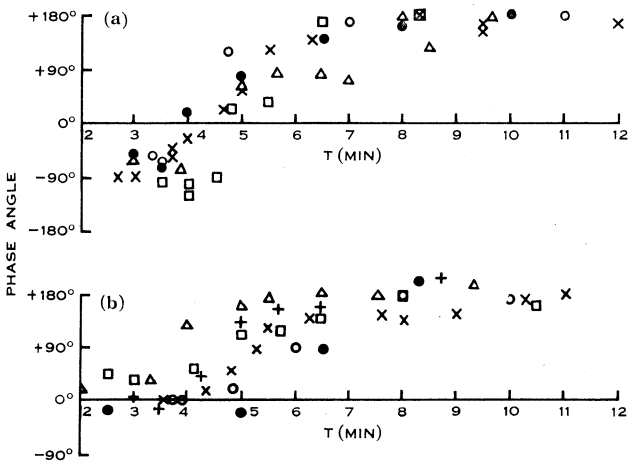

Fig. 6.—Normalized (see text) phase response of the potential oscillations in the elongating regions of several plants to changes in period of (*a*) osmotic pressure oscillations and (*b*) IAA oscillations.

are displaced somewhat with respect to one another although each still shows the same phase change from short to long period. The curves in Figure 5(*b*), however, are the most common type for the elongating or actively resonant region. Consequently to clarify the comparison between phase curves for a number of different plants, they have been normalized so that the phase angle at long periods is +180°. This comparison of such normalized data is shown in Figure 6, different symbols being used to denote different plants. Figures 6(*a*) and 6(*b*) again show the contrast between the phase responses to oscillations in osmotic pressure and IAA concentration.

In all the experiments described so far a solution of 10^{-4}M KCl was used as the roots' bathing medium, the oscillations in sucrose or mannitol concentration (for osmotic pressure) and IAA concentration being superimposed on the constant background of 10^{-4}M KCl solution. In some cases the plant was allowed to equilibrate in this KCl bathing solution for 15 hr or more before the commencement of experiments. The phase and amplitude responses of these plants to oscillations in osmotic pressure and IAA concentration were the same as those for plants which were allowed

to equilibrate for only about 1 hr after removal from the tap water culture medium. This suggests that the bioelectric responses described are independent of the presence or absence of various ion species left in the root tissue, originally obtained from the tap water culture medium. However, it is possible that in both cases (short and long equilibration time in 10^{-4}M KCl) a variety of ion species from the cotyledon material was available to the root throughout the experiments.

In some series of experiments plants were first equilibrated in 10^{-4}M $CaCl_2$ for 15 hr or more before the oscillations in osmotic pressure or IAA concentration were applied, the background solution still being 10^{-4}M $CaCl_2$. The amplitude and phase responses to osmotic pressure and IAA oscillations were the same under these conditions as those already described in which KCl was used throughout.

These results suggest that the oscillatory bioelectric currents and the physiological system responsible for the potential oscillations are not dependent on whether the cation in the bathing solution or the root tissue is monovalent or divalent. The effect of different anions has not been studied.

IV. Discussion

The simplest system which exhibits properties similar to those of bean roots which have been described in this paper and by Scott (1957) is a feedback loop containing three linear, exponential-delay elements. As the authors are not aware of any simple treatment in the literature of this feedback system, and because it may have wider biological application, it has been thought desirable to consider the theory of it in some detail. This is done in Appendix I.

It will be seen that this feedback model has many of the properties which are exhibited by the plant root. It may be stable (i.e. any oscillations produced as a result of stimulation are transient and die out after a few cycles) or may oscillate spontaneously depending on the value of the feedback loop gain K (and also on the relative values of the three time delays). When a forcing oscillation is applied at any point in a stable loop, oscillations of the same period (although differing from it in phase) are caused at other points in the loop. The amplitude of the response to an applied oscillation depends on its period and resonates close to the natural period of the system. It is also found that changes in period of the applied oscillation cause the phase difference between it and the resulting oscillation at different points in the loop to change. The total change in phase in going from very low to very high periods is 180° if there are two delay elements between the point of application of the forcing oscillation and the point of observation; and 270° for three interposed delay elements.

Since all these properties are observed in the behaviour of the plant root, it is considered to be strong evidence that a feedback system of this kind is in operation in the plant.

It is not claimed that the actual processes in the plant can be described in detail in terms of the simple model considered above. There may be more elements and more complex modes of interaction. There is, for example, some evidence for a second oscillation with a natural period of about 90 min which would require an additional

feedback path. In addition the elements are unlikely to be truly linear or to have delay characteristics that are truly exponential. More complex delay elements would raise the order of the differential equation and would, in general, result in oscillations which were not sinusoidal. Since the oscillations produced by the plant are very nearly sinusoidal it is assumed that the elements have characteristics similar to those considered in the model.

A physiological feedback loop for the plant root is now proposed and is shown in Figure 7. This is essentially the same as that proposed by Scott (1957) but has been modified in some respects to fit the results described in this paper.

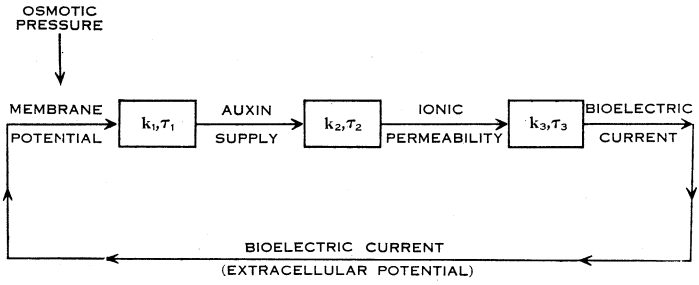

Fig. 7.—A proposed feedback loop with three exponential delays.

It is proposed that osmotic pressure is linked closely with the membrane potentials of the root's outer cells (i.e. epidermal and cortical cells) so that a change in osmotic pressure causes immediate changes in membrane potential. Experimental evidence for this assumption will be discussed in a further paper in this series when intracellular potential responses will be described.

It is proposed then that the electric fields across cell membranes are responsible for the distribution of auxin (IAA) within individual cells or groups of cells with a time-delay constant τ_1. Transport of auxin by bioelectric fields has been proposed by a number of investigators (cf. Schrank 1957), but has been criticized on the grounds that these fields are not sufficiently large. Clarke (1937) has shown that there is no electrophoretic transport of IAA through agar blocks unless the applied fields are greater than 50 V/cm. This does not appear to be a valid criticism for, although the average bioelectric fields in bulk tissue are less than this value by several orders of magnitude, the fields in biological membranes are much larger. For instance, a potential of 1 mV across a membrane 100 Å thick results in an electric field of 10^5 V/cm in the membrane. Such fields are consequently quite sufficient to cause local electrophoretic movement of auxin (e.g. from one side of a membrane to the other), although other processes may well be involved in its movement through bulk tissue.

As the next stage is the feedback loop it is proposed that auxin modifies the properties of cellular membranes in their permeability to ions (time delay τ_2). In a considerable variety of tissues the effect of auxin on membranes permeability to water, ions, and other solutes has been studied. The sensitivity of membrane

permeability to auxin in such plant tissues as bean endocarp, the abscission zone of *Coleus*, and the leaves of *Mesembryanthemum* sp. and *Rhoeo discolor* has been reported by Sacher (1957, 1959) and by Sacher and Glasziou (1959). Ling and Gerard (1949) showed that IAA at $2 \cdot 5 \times 10^{-4}$M and 5×10^{-3}M increases the permeability to potassium in the *Rana pipiens* sartorius fibre membrane. The effect is reversible on removal of the IAA. Bennet-Clark (1955) has suggested that the effect of auxin on the ionic permeability of plant cell membranes is exerted through an acetylcholine-mediated system.

The change in ionic permeability of plant cell membranes, affected by a change in auxin concentration or supply, may be caused primarily by auxin-induced cell wall plasticity. In decapitated *Avena* coleoptiles this cell wall plasticization occurs within a few mimutes after the auxin addition (Adamson, personal communication). This in turn permits the uptake of water by the cell, stretching the plastic cell wall and the cell membrane enclosing the protoplasm (Adamson and Adamson 1958; Van Overbeek 1959). This mechanical stretching of the cell membrane could change its permeability to ions and other solutes.

This type of interaction between auxin and membrane permeability is in accordance with the observation that the actively resonant region of the root is situated in the elongating zone where the cell walls are most sensitive to auxin.

It is possible that variation in permeability could arise in the auxin-sensitive Donnan system of the cell wall (Van Overbeek 1959). This seems unlikely since the constitution of this system depends on whether it contains divalent or monovalent cations, whereas the bioelectric effects were found to be independent of whether the root's bathing solution contained monovalent (K^+) or divalent (Ca^{++}) cations. However, the concentration of these ions in the external solution may have been too low to alter appreciably the ionic composition of the cell wall.

The bioelectric currents which flow in developing biological tissue and through any surrounding conducting media are due to the non-uniform properties of this tissue. As auxin-induced permeability changes are likely to differ in different parts of the root it would be expected that these would modify ionic fluxes in the tissue and hence the magnitude and paths of bioelectric current. This is the final element in the proposed feedback loop and introduces a third delay, τ_3.

To complete the feedback loop it is suggested that the bioelectric current would modify potential differences across membranes within the plant with negligible time delay. Evidence for this is available in experiments on the large algae (cf. Findlay 1959) and is to be expected as an ohmic property of any resistive membrane or barrier. Thus the loop is completed.

From Figure 7, it is apparent that between the auxin supply and the bioelectric current (or the extracellular bioelectric field) there are two delay elements, and three delay elements between the osmotic pressure and the bioelectric current. Thus the phase and amplitude relations for this physiological model are in agreement with those for the plant which have been described in this paper. It is, however, probable that other physiological feedback loops could be envisaged which would be equally in accord with the results described, but the one discussed above appears to be the simplest.

Applying this model to the results obtained and assuming that the three time constants are approximately equal gives values of the feedback loop gain K which range from about -2 to about -5 for the plants investigated, corresponding values of the time constants being in the range $0 \cdot 8$–$1 \cdot 4$ min.

V. Acknowledgment

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Appendix I

ANALYSIS OF FEEDBACK LOOP CONTAINING THREE LINEAR, EXPONENTIAL-DELAY ELEMENTS

This section presents a simplified analysis of the properties of a feedback loop containing three linear, exponential-delay elements. It will be shown that this system has properties similar to those exhibited by the plant root which have been described in this paper. The arrangement of elements is shown in Figure 8(*a*).

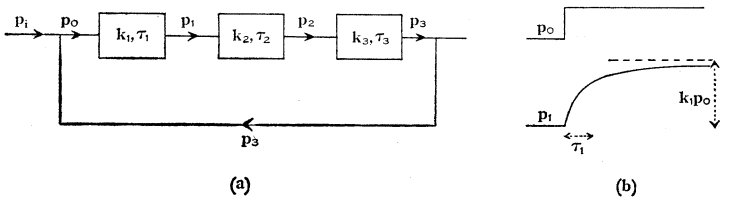

Fig. 8.—(*a*) The three-element feedback loop discussed in the text. (*b*) Illustrating the response p_1 to a step change in the input p_0 to the first delay element. p_1 changes to within 1/e of its final value (k_1p_0) in a time equal to the time constant τ_1.

A disturbance p_0 of the input quantity fed to the first delay element results in a disturbance p_1 in the output quantity. This is fed to the second delay element and then in turn to the third element. The output p_3 from this element is fed back so as to contribute to the input to the first element. The loop may have in addition an external input p_i.

The relation between the input and output of any element is characterized by constants k and τ. If the input to the first element suddenly changes by p_0, the output p_1 changes exponentially to a final value k_1p_0 with a time constant τ_1 (see Fig. 8(*b*)). That is,

$$p_1 = k_1p_0[1-\exp(-t/\tau_1)],$$

or in differential form,

$$p_1+\tau_1(\mathrm{d}p_1/\mathrm{d}t) = k_1p_0.$$

Such a relationship characterizes a linear, exponential-delay element. The constant k_1 may be either positive or negative depending on whether the output tends to increase or decrease when the input increases.

The equations describing the three-element loop (Fig. 8(*a*)) are as follows:

$$\left.\begin{aligned} p_1+\tau_1(\mathrm{d}p_1/\mathrm{d}t) &= k_1p_0, \\ p_2+\tau_2(\mathrm{d}p_2/\mathrm{d}t) &= k_2p_1, \\ p_3+\tau_3(\mathrm{d}p_3/\mathrm{d}t) &= k_3p_2, \\ p_0 &= p_i+p_3. \end{aligned}\right\} \tag{1}$$

From these equations is obtained a differential equation of the third order for any of the quantities of the loop (such as p_3) in terms of p_i. Thus

$$\tau_1\tau_2\tau_3(\mathrm{d}^3p_3/\mathrm{d}t^3)+(\tau_1\tau_2+\tau_2\tau_3+\tau_3\tau_1)(\mathrm{d}^2p_3/\mathrm{d}t^2)+(\tau_1+\tau_2+\tau_3)(\mathrm{d}p_3/\mathrm{d}t)+p_3(1-K) = Kp_i, \tag{2}$$

where $K = k_1k_2k_3$ and is called the *feedback loop gain*. The feedback is positive or negative depending on whether K is positive or negative.

The solution of this equation is the sum of two terms: (i) a *complementary function* characteristic of the feedback loop alone (i.e. the solution of the equation when $p_i = 0$), and (ii) a *particular integral* which depends on the form of the input quantity p_i. The complementary function determines transient behaviour, while the particular integral gives the steady-state behaviour.

For simplicity the mathematical analysis is now restricted to the special case for which $\tau_1 = \tau_2 = \tau_3 (= \tau)$. No serious loss of generality results from this. The differential equation then becomes

$$\tau^3(\mathrm{d}^3p_3/\mathrm{d}t^3)+3\tau^2(\mathrm{d}^2p_3/\mathrm{d}t^2)+3\tau(\mathrm{d}p_3/\mathrm{d}t)+p_3(1-K) = Kp_i. \tag{3}$$

(i) *The Complementary Function* (i.e. the solution when $p_i = 0$)

The complementary function may be written in the form

$$p = P_a \exp(\alpha t/\tau)+P_b \exp(\beta t/\tau) \cos[(2\pi t/T)+\phi], \tag{4}$$

where p refers to any of the quantities p_1, p_2, and p_3. P_a, P_b, and ϕ are arbitrary constants determined by the initial conditions, and α, β, and γ satisfy the relationships

$$\left.\begin{aligned} \alpha+2\beta &= -3, \\ \gamma^2+2\alpha\beta &= 3, \\ \alpha\gamma^2 &= K-1, \end{aligned}\right\} \tag{5}$$

and the natural period of the oscillation, T, is given by

$$T = 2\pi(\gamma^2-\beta^2)^{-\frac{1}{2}}. \tag{6}$$

For a stable feedback loop (i.e. one in which any transient disturbance eventually dies out) it is evident from equation (4) that both α and β must be negative. This is found from equations (5) to be the case provided the loop gain K lies between $+1$ and -8. For more positive values of K the first term increases exponentially, and for values of K less than -8 the oscillatory term increases in amplitude exponentially. In a practical control system the quantity fed back must be amplified (i.e. $K > \pm 1$); hence for stability the feedback must be negative and within the range of K values -1 to -8. If the negative feedback gain is too large the system "hunts" (i.e. produces uncontrolled oscillations).

The degree of stability of the oscillatory component is measured by the decrement, Δ, which is the ratio of successive maximum disturbances. When $K = -8$, $\Delta = 1$ (i.e. oscillations are sustained), and Δ is zero (i.e. the system is critically damped) if $K = 1$. The values of Δ for $K = -2$ and -7 are $0{\cdot}08$ and $0{\cdot}86$ respectively.

If the time constants are not all equal the solution of the differential equation is of the same form, but the range of values of the loop gain K for stability is different. Typical values are set out below. The stable range is smallest when the time constants are all equal.

Relative Values of Time Constants	Range of Values of K for Stability
1 : 1 : 1	+1 to − 8·0
1 : 2 : 2	+1 to − 9·0
1 : 5 : 5	+1 to − 14·4
1 : 10 : 10	+1 to − 24·2
1 : 2 : 4	+1 to − 11·2
1 : 4 : 16	+1 to − 26·6
1 : 10 : 100	+1 to −122·2

Thus it is seen that a stable feedback system may become unstable either if the negative feedback gain increases, or if the relative values of the time constants change so that they are more nearly equal.

(ii) *The Particular Integral* (i.e. the steady-state behaviour)

The particular integral depends on the form of the input function p_i. For a sinusoidal input

$$p_i = P_i \exp(j\omega t),$$

and steady-state solutions of the form

$$p_3 = P_3 \exp[j(\omega t + \epsilon_3)],$$

are obtained for each of the quantities p_3, p_2, p_1, and p_0.

Substituting for p_3 and p_i in equation (3),

$$[\tau^3(j\omega)^3 + 3\tau^2(j\omega)^2 + 3\tau(j\omega) + (1-K)]P_3 \exp[j(\omega t + \epsilon_3)] = KP_i \exp(j\omega t),$$

from which is found by collecting real and imaginary parts that

$$P_3 = [KP_i/(A^2+B^2)] \text{ and } \epsilon_3 = \text{artan}(-A/B),$$

where

$$A = 3\tau\omega - \tau^3\omega^3,$$

and

$$B = 1 - K - 3\tau^2\omega^2.$$

The amplitudes and phase relationships for p_2, p_1, and p_0 are now obtained easily from p_3 using equations (1). For sinusoidal oscillations of angular frequency ω,

$$P_0/P_1 = P_1/P_2 = P_2/P_3 = (1+\tau^2\omega^2)/k,$$

and there is a phase lag of artan $(\tau\omega)$ in each element. If the gain k for an element is negative, this can be expressed as an additional phase shift of π.

Typical graphs of the amplitude and phase relationships for the steady-state values of p_0, p_1, p_2, and p_3 as functions of the period of the sinusoidal input p_i are shown in Figure 9. The cases considered are for loop gains of -2 and -7. It is here assumed that the phase reversal necessary to make K negative occurs in the third element, but it is evident that it could occur in any one of the elements in the loop or in all three of them.

It is noted that resonance behaviour occurs in the vicinity of the natural period throughout the loop and this is more marked when K is -7 (i.e. when the system is approaching the unstable condition for which $K = -8$. The phase changes most rapidly with period in the vicinity of resonance, the effect again being more marked for $K = -7$ than for $K = -2$.

An important quantity for the present paper is the total change in phase (relative to the input) at various points in the loop when the input oscillation changes from very short period to very long period. The graphs show that this quantity (to be called the *total phase shift*) has values 0, $\pi/2$, π, and $3\pi/2$ for p_0, p_1, p_2, and p_3 respectively. The total phase shift therefore indicates the number of delay elements between the input oscillation and the point of observation of the forced oscillation, each element contributing $\pi/2$ to the total phase shift.

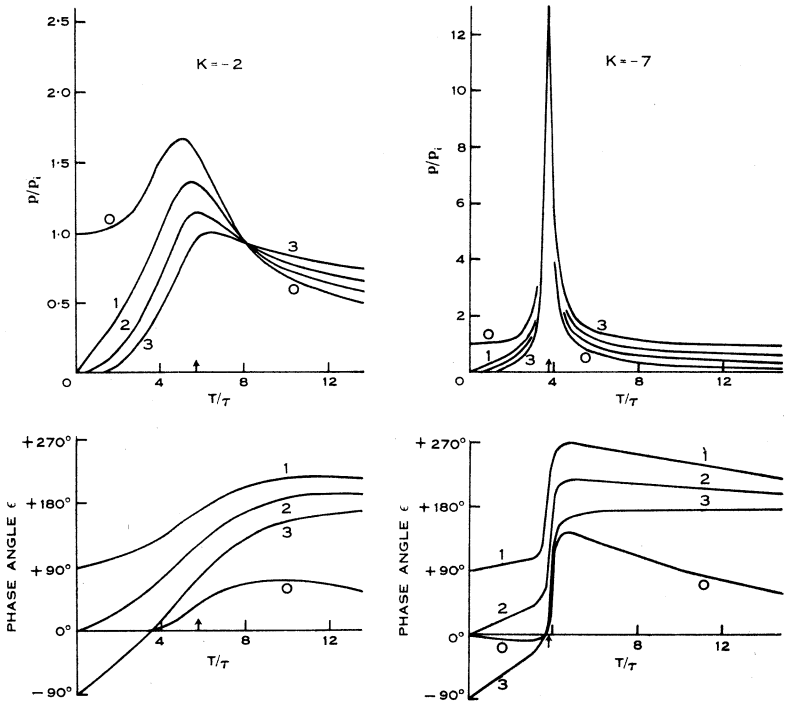

Fig. 9.—Amplitude and phase relationships for a sinusoidal input p_i at different points in the loop for two values of the loop gain, -2 and -7. The numbers on the graphs refer to the various values of the amplitude $p(p_0, p_1, p_2$, and $p_3)$, and phase angle $\epsilon(\epsilon_0, \epsilon_1, \epsilon_2$, and $\epsilon_3)$.

All the features described above for the steady state apply equally well to loops in which all time constants are not equal. In applying the rule concerning the total phase shift, it must be remembered that the periods of the forcing oscillations must extend well beyond, both above and below, the range of time constants in the loop.