

TRANSIENT BIOELECTRIC POTENTIALS PRODUCED BY ELECTRICALLY STIMULATED BEAN ROOTS

By I. S. JENKINSON*

[Manuscript received April 17, 1958]

Summary

It is shown that following electric stimulation, bean roots immersed in a weakly conducting salt solution produce transient bioelectric potentials characteristic of the type of electric stimulus applied.

The transient potentials caused by constant-amplitude alternating voltages are shown to depend uniquely on the number of cycles applied, irrespective of the frequency, provided that a sufficient amount of electric charge (10^{-4} C approximately) is passed through the plant during stimulation. The plant adapts itself to the stimulus applied so that the root's potential pattern is disturbed less after the application of a large number of alternating voltage cycles than after relatively few cycles.

The transients resulting from the application of direct voltage are found to depend to some extent on the voltage polarity. However, the transient potentials caused by direct voltages of opposite polarity yield a common component in close agreement with that found for a small number of cycles of alternating voltage. The same minimum charge as for alternating voltage is required for maximum stimulation.

I. INTRODUCTION

In an earlier paper, Scott (1957) has described spontaneous oscillatory potentials and also transient potentials following stimulation, observed at points adjacent to a bean root immersed in a weakly conducting salt solution. It was suggested that such oscillatory potentials could be explained in terms of a feedback system of control acting between certain functional variables of the biological system, including the electric field.

Since the spontaneous oscillations occur infrequently, attention has been directed to a more detailed study of transient potentials which frequently exhibit overshoots and damped oscillations before the potentials return to the steady state again. These transients are observed to result from such treatments as mechanical stimulation, sudden changes in salt concentration of the bathing solution, addition of indoleacetic acid to the bath, and exposure of the root to air for a short time.

A more readily controlled stimulus consists in the application of electric voltage between the cotyledons and the plant's bathing solution. In this paper correlations between different electric stimuli and the transient bioelectric fields occasioned on removal of such stimuli, are described. It is thought that from a study of such correlations, knowledge of the mechanism of initiation of oscillatory potentials produced by plant roots in an unchanging environment may be revealed.

* Biophysics Laboratory, Physics Department, University of Tasmania.

In this paper it is shown that the transient potential pattern of the root observed immediately after alternating voltage stimulation depends only on the number of cycles applied, irrespective of the alternating voltage frequency, provided that a sufficient quantity of electricity is passed through the plant during stimulation. The form of the relation is such that the bioelectric field of the plant root is disturbed less after a large number of alternating voltage cycles than after relatively few cycles. This indicates that the plant adapts itself to the alternating voltage, the extent to which it does so being determined solely by the number of voltage cycles to which it has been subjected.

II. EXPERIMENTAL MATERIAL AND METHODS

The material used in the experiments described in this paper was a Long Pod variety of the broad bean, *Vicia faba* L., which was grown in continuously circulated and aerated tap-water at 25°C. Plants 2–3 days old with roots about 4 cm long were used in most experiments.

The plant under investigation was mounted vertically in the measuring tank with the root, but not the cotyledons, immersed in a bathing solution of 10^{-4} N KCl. The cotyledons in contact with moist cotton wool were held securely by an insulated stainless-steel clamp which acted also as one of the electrodes for the application of electric voltage stimulus, the other electrode being immersed a few centimetres below the root tip in the bathing solution.

Bioelectric potentials were measured by means of probes consisting of lengths of transparent "Nylex" tubing (2-mm bore), the ends of which were placed close to the surface of the root at various points along its immersed length. The other end of each tube dipped into an insulated plastic cup, the cups and tubes being filled with the same solution as that of the bath. Tubes came from five points near the plant while a sixth came from a distant point in the bath thus acting as a reference zero of potential. Each of these cups was connected in turn via a calomel half-cell to a six-channel automatic recording apparatus described by Scott (1957). A recording-chart speed of 3 in./hr was used throughout.

While the external voltage was applied to the plant, the electrometer grid was earthed. On removal of the applied voltage the electrometer was switched in immediately to record the transient potentials of the plant root. Voltages, either direct or alternating, were applied between the two stainless-steel electrodes, a microammeter being included in the circuit to record the total current passed through the plant.

Direct voltages were supplied by dry cells, the applied voltage stimulus being referred to as plus or minus according as the cotyledons were made positive or negative with respect to the electrode in the bathing solution. Voltages of 9 and 18 V, producing total currents through the plant of 100–250 μ A, were employed. The duration of direct voltage application ranged from 0.01 to 300 sec. A relay circuit was employed to control the applications of short duration.

Alternating voltages of 10 and 20 V peak amplitude in the frequency range 0.1–10 c/s were supplied by a stable, low-frequency resistance-capacitance oscillator

while a step-down transformer acting from the mains supply provided alternating voltages of frequency 50 c/s. Alternating voltages were applied for durations appropriate to provide numbers of cycles from 1 to 3000.

In order to assess the importance of the proximity of the bath electrode to the plant root and to determine which part of the root was most affected by the passage of current during electric stimulation, experiments were conducted to determine the distribution of applied current density flowing out from the plant root surface and into the bathing solution. This was done by exploring the region around the root with a measuring probe to determine the equipotential pattern. From this the current paths were determined and the current strengths passing out from various regions along the root were calculated.

It was found that the current density passing through the region of the root just below the bathing solution surface was about 100 times as great as that passing through the tip region, the current density falling off exponentially between the two regions. Further, the distribution of the applied current passing out from the root was found to be independent of the position of the bath electrode provided that it was situated more than 2 cm below the root tip. In all experiments reported in this paper the separation between the root tip and the bath electrode was greater than this distance. The bathing solution could then be regarded as constituting the bath electrode.

General observations of growth rate and also microscopic examination of plant root sections revealed no injurious effects which might have been caused by the accumulation of ions during the passage of current within the range of currents and durations employed.

In some experiments the rate of elongation of the root was recorded using the growth meter described by Scott (1957). No significant changes were observed in the rate of elongation either during or following electric stimulation.

III. RESULTS

(a) General Form of Transient Potentials

Following stimulation, the plant's potential pattern recovers eventually to a state not significantly different from that preceeding stimulation, provided the stimulus is not too great. The form of the transient potential pattern depends on the particular type of stimulus applied and is independent of any previous electric stimuli which the plant has undergone provided sufficient time has been allowed for the potential pattern to recover to the normal unstimulated state.

Figure 1 shows typical time courses of potentials near two representative points along the same plant root for three different successive electric stimuli. Figures 1(a) and 1(b) refer to direct voltage applications of opposite polarity while Figure 1(c) refers to alternating voltage application. Values of potentials prior to stimulation are shown at $t = 0$. From this example it may be seen that the potential transients frequently overshoot the steady value before recovering. In some cases slow damped oscillations of periods up to 40 min have been observed. The general

form of the transients suggests that the potential recovery is of a damped oscillatory nature.

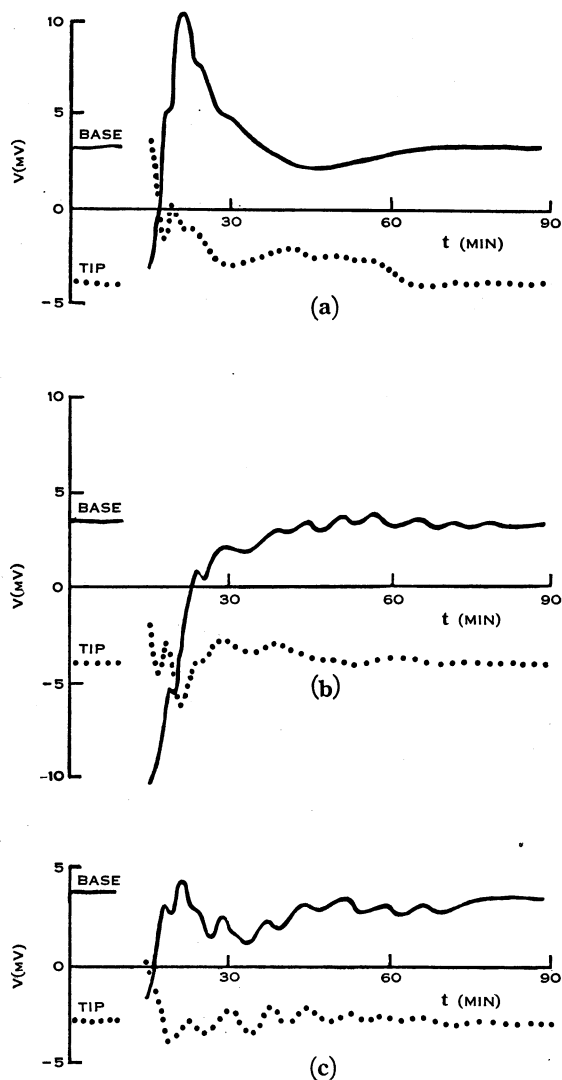


Fig. 1.—Time courses of transient potentials observed at the tip and basal regions of the same root. The stimuli applied are (a) +10 V, (b) -10 V, and (c) 10 V peak alternating voltage, each applied for 300 sec. Steady potentials before stimulation are shown in each case, the gap in the record indicating the time of stimulation. For simplicity only two of the five transients normally recorded are shown. The three intermediate transients show a gradual transition in form between the two extremes shown.

As well as these long period oscillations, less heavily damped oscillations of shorter period (approx. 5 min) are frequently observed superimposed on the slowly

varying recovery. These shorter period oscillations are similar to the spontaneous oscillations occasionally produced by bean roots (Scott 1957).

In Figures 1(a) and 1(b) it is apparent that the transients following the application of +10 V for 300 sec is different from those following -10 V for the same duration. It is shown in Section III(c) that the direct voltage transients may be regarded as containing a component which is not dependent on the polarity of the applied voltage and a component which is.

Since the transients resulting from alternating voltage stimulation are simpler in form than those for direct voltages, the results of the former will be described first.

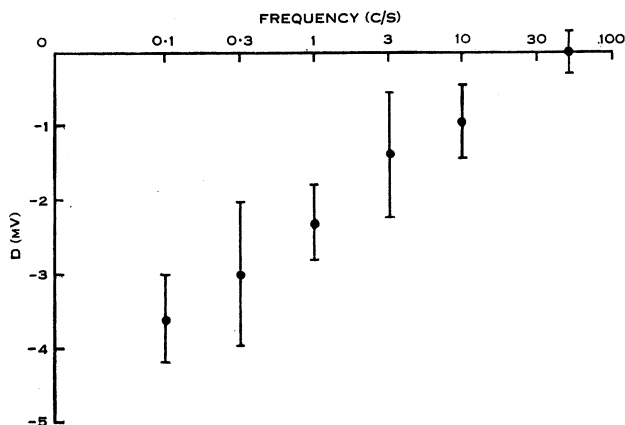


Fig. 2.—Relations between D (at the root base) and the frequency of the 10 V peak voltage applied for 300 sec. Ninety-five per cent. confidence limits are indicated (10–20 plants).

(b) *Alternating Voltage Stimulation*

In these experiments it was necessary to ascertain first that the resulting transient was not dependent on the phase in the cycle at which the alternating voltage stimulus was switched off. It might be reasonable to expect, for instance, that after applying one cycle of relatively long period, such as 10 sec, that the recovery transient would differ if the cycle were applied between two positive peaks (maxima), or between two negative peaks (minima). This was tested by averaging the appropriate transients obtained from 10 plants, each of which received a number of stimuli of the two extreme types cited. It was found that the corresponding pairs of average potential recovery curves did not differ significantly from one another. Hence it was concluded that transients caused by alternating voltage application are not dependent on the phase of the alternating cycle (or cycles) applied.

As shown in Figure 1(c), the characteristic feature of the alternating voltage transients is that the root tip potentials are algebraically increased while those of the root base are decreased immediately after stimulation, i.e. the electric polarity of the root tends to reverse. In general, the potential is decreased only at the root

base, i.e. in the near vicinity of the bathing solution surface where most of the applied current flows through the root surface, the rest of the potentials being increased, though often to a lesser extent.

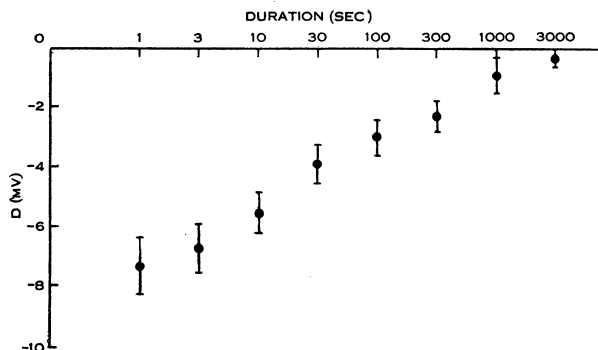


Fig. 3.—Relation between D (at the root base) and the duration of the applied 10-V stimulus at 1 c/s. Ninety-five per cent. confidence limits are indicated (20 plants).

It is evident that an index of the effect caused by stimulation is given by the deviation in plant potential (at a particular point along the root) immediately after removal of the stimulus. This deviation (D) with sign attached, may then be used to compare the effects caused by different electric stimuli.

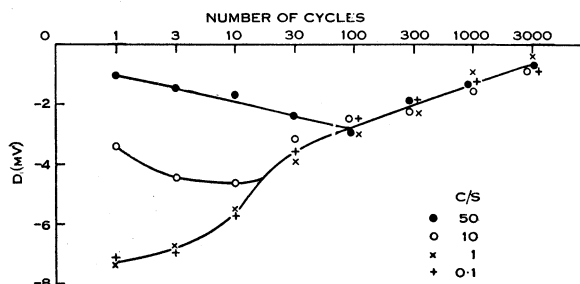


Fig. 4.—Relation between D (at the root base) and the number of cycles of the applied 10 V peak voltage at frequencies of 50, 10, 1, and 0.1 c/s. Ninety-five per cent. confidence limits are similar in magnitude to those in Figure 3 (20 plants).

In Figure 2, D (at the root base) is plotted against the frequency of the alternating voltage applied for a constant duration of 300 sec, while, in Figure 3, D is plotted against duration of application at a constant frequency of 1 c/s. In both cases the peak voltage was 10 V throughout.

It is seen that the magnitude of D decreases with both increasing frequency and duration of application. This suggests that the magnitude of D decreases with the number of cycles of alternating voltage applied. In Figure 4, D is plotted against

the number of cycles, employing frequencies of 50, 10, 1, and 0.1 c/s, applied for appropriate durations.

It appears that D depends uniquely on the number of cycles applied at a constant peak voltage, except for the cases in which the stimulus is applied for durations less than about 1 sec (viz. 50 c/s for 1–30 cycles, and 10 c/s for 1–10 cycles).

The fact that for very brief applications of the higher frequency alternating voltages, the curve departs from the unique relation between D and the number of cycles, implies that insufficient electricity is passed through the plant during such short applications. It is possible to pass this required amount of electricity through the plant by applying a single initial cycle of 1 sec period. If this is done and then

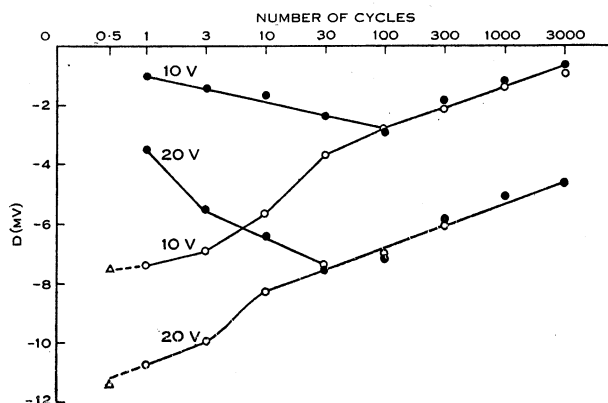


Fig. 5.—Relations between D (at the root base) and the number of cycles at 50 c/s (●) of 10 V (upper curve) and 20 V (lower curve). ○ Values of D obtained when a single cycle of period 1 sec is applied immediately before the 50 c/s voltage application. Ninety-five per cent. confidence limits for all points are similar to those in Figure 3 (15 plants). Points at 0.5 cycles (△) are referred to in Section IV.

followed immediately by a number of cycles of frequency 50 c/s, the relation between D and the number of cycles then becomes identical with that for lower frequencies such as 1 and 0.1 c/s (Fig. 4) even if the number of cycles is small.

This is shown in Figure 5 in which the 10 V 50 c/s curve from Figure 4 is redrawn. The other 10 V curve (open circles) shows 10 V 50 c/s data obtained after first applying an initial cycle of 1 sec period. It is apparent that this curve is identical with that in Figure 4 for the 1 c/s and 0.1 c/s points.

Data obtained similarly for 20 V 50 c/s is shown in Figure 5 also. The relations between D and the number of cycles at 20 V are obviously similar to those at 10 V but the magnitude of D is greater. The point at which the upper and lower constant voltage curves join indicates the number of cycles for which just sufficient charge has been passed through the plant to make the relation between D and the number of cycles independent of frequency. For 50 c/s at 10 V this point is at 100 cycles, while for 20 V, only 30 cycles is sufficient.

This suggests that the passage of a constant quantity of charge is necessary before the dependence of D on the number of cycles becomes independent of the

frequency. As is shown below, it appears that this required quantity of charge is independent of the applied voltage and its frequency.

Values of this quantity of charge Q have been determined from measurements of the average value of alternating current passed through plants during stimulation. Q is then given by the product of the average current and the necessary duration for the dependence of D on the number of cycles to become independent of frequency. Three cases have yielded the following average values of Q (Table 1). Twenty plants were used in each case. Considering the logarithmic type of relation between D and the number of cycles, there is quite good agreement between the values of Q obtained.

TABLE 1
INDEPENDENCE OF QUANTITY OF CHARGE ON APPLIED
VOLTAGE AND FREQUENCY
Each result is the mean of 20 plants

| Voltage (V) | Frequency (c/s) | $Q \times 10^5$ (C) |
|----------------|--------------------|------------------------|
| 10 | 10 | 9 ± 5 |
| 10 | 50 | 14 ± 5 |
| 20 | 50 | 10 ± 5 |

The form of the relations between D and the number of cycles at other points along the root is identical with those described for the basal regions (i.e. at points on the root just below the surface of the bathing solution). However, as mentioned previously in this section, D changes sign and decreases in magnitude a few millimetres below the bathing solution surface.

(c) *Direct Voltage Stimulation*

From sets of transients such as those of Figures 1(a) and 1(b) obtained from a number of plants, it is found that the average recovery curves of potential are significantly different depending on whether the direct voltage is applied in a positive or in a negative sense with respect to the bathing solution. However, the averages of corresponding pairs of transients induced by equal but opposite direct voltage stimuli in a number of plants are found to be very similar to those obtained from alternating voltage stimulation. These average curves will be referred to as the "common" components of the direct voltage transients and may be regarded as their components not dependent on the sign of the applied voltage. Only the common components will be discussed in this paper. As in the alternating voltage results, the characteristic feature of the common components is that the potentials of the very basal end of the root are decreased while those of the regions nearer the tip are algebraically increased immediately after removal of the applied direct voltage. Again, an index of the common effect may be defined as the initial deviation,

D_c , following stimulation of the common potential curve from the unstimulated plant's potential.

Figure 6 shows D_c (at the base) plotted against duration of application of both 9 and 18 V. These graphs show that the magnitude of D_c increases with duration up to about 1 sec for 9 V and about 0.3 sec for 18 V after which D_c is independent of the duration of voltage application but its magnitude increases with voltage.

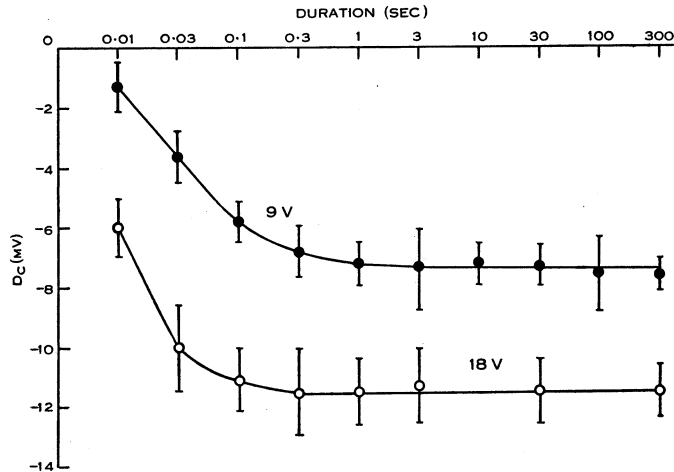


Fig. 6.—Relations between D_c (at the root base) and the duration of application of 9 and 18 V. Ninety-five per cent. confidence limits are indicated (20 plants).

This again indicates a charge requirement before D_c becomes independent of duration, the average value of this charge Q_c for the two cases (9 and 18 V) being 9×10^{-5} C. This may be compared with the average value of $Q = 11 \times 10^{-5}$ C, obtained in the alternating voltage case (Section III(b)).

IV. DISCUSSION

In Section III(b) it was seen that D depends uniquely on the number of cycles of constant peak voltage applied, provided that the amount of charge passed through the plant during stimulation exceeds a definite quantity Q ($= 11 \times 10^{-5}$ C approximately). This quantity of charge would be transported through the plant by 11.4×10^{-10} g-equiv. of ions. It was shown further (Fig. 5) that after this amount of charge has been passed by applying a single cycle of period 1 sec, the relation between D and the number of cycles at 50 c/s applied subsequently is identical with the unique relation between D and the number of cycles applied at lower frequencies such as 1 and 0.1 c/s, at which frequencies the required amount of change is passed during only one cycle.

The relation between D_c and duration of 9- and 18-V pulses was shown to exhibit a similar effect in that the magnitude of D_c increases with duration until

a charge of Q_c ($= 9 \times 10^{-5}$ C) has been passed, after which D_c remains constant (Fig. 6).

A single direct voltage pulse of about 10-V amplitude might be interpreted as half a cycle of 10-V peak alternating voltage. In this case it would be expected that D_c should be independent of the pulse length (provided it is 1 sec or longer) since the number of cycles (viz. a half cycle) remains the same whatever the pulse length is. This may be compared with the alternating voltage case in which single cycles of period 1 sec or longer were seen to cause identical values of D .

Figure 5 shows the 10- and 20-V curves relating D at the base and the number of cycles. The points shown at 0.5 cycle indicate the values of D_c for 9- and 18-V pulses of duration greater than 1 sec. It is apparent that these points are close to the extrapolations (dotted) of the 10- and 20-V alternating voltage curves respectively.

This, together with the fact that the values of Q ($= 11 \times 10^{-5}$ C) and Q_c ($= 9 \times 10^{-5}$ C) are in agreement, suggests that the direct voltage common effect and the alternating voltage effect are identical.

Having combined the two effects in this way, the phenomenon may be summarized as follows. The transient potentials observed immediately after the removal of an electric stimulus depend on two factors: the total quantity of charge passed through the plant, and the number of alternations of the applied voltage. As the quantity of charge passed through the plant is increased the magnitude of the transients (measured by D or D_c) increases until its maximum value is reached with the passage of approximately 10^{-4} C. This quantity of charge and the value of D or D_c is independent of the manner in which the charge is passed although the value of D or D_c increases with the magnitude of the applied voltage.

In addition to this effect there is that of the number of cycles in which the magnitude of D decreases approximately logarithmically with the number of cycles applied.

Marsh (1930) showed that following the passage of direct current along onion (*Allium cepa*) roots, the potential changed and eventually returned to the unstimulated state. The potential change was found to increase with the amount of charge passed along the root while the polarity of the potential change depended solely on the direction in which the current flowed, i.e. no common change was observed even though the quantities of charge passed through the roots ranged from 10^{-5} C to values as large as 10^{-3} C. However, these experiments were conducted in a rather different manner from those described in this paper. The electrodes, used both for measuring root potentials and for the application of electric current, consisted of ring electrodes containing tap water placed at various positions along the root growing in air saturated with water vapour.

It is to be expected that both the stimulated and the unstimulated root's potential pattern should differ for the two methods of measurement, since potentials, either steady or transient, produced in a bathing solution depend on the flow of bioelectric current through the solution about the root (Scott, McAulay, and Jeyes 1955), whereas for a root in air, a more static potential is measured.

Danisch (1921) showed that *Vorticella nebulifera* adapts itself to the application of a succession of mechanical shocks of known energy. For instance, after nine impulses, each of 500 ergs, the organism no longer responds to repeated impulses, whereas for 2000-erg impulses, 420 are required. Danisch concluded that the effect was one of habituation rather than fatigue. These results appear to be similar to those described in this paper.

V. ACKNOWLEDGMENTS

The author wishes to express his gratitude to Professor A. L. McAulay and Dr. B. I. H. Scott to whom he is indebted for much valuable advice and helpful criticism during the preparation of this paper. This work was carried out during the tenure of a C.S.I.R.O. Studentship in Biophysics for which the author is grateful.

VI. REFERENCES

- DANISCH, F. (1921).—Ueber Reizbiologie und Reizempfindlichkeit von *Vorticella nebulifera*. *Z. allg. Physiol.* **19**: 133–88.
- MARSH, G. (1930).—The effect of applied electric currents on inherent cellular E.M.F. and its possible significance in cell correlation. *Protoplasma* **11**: 447–74.
- SCOTT, B. I. H. (1957).—Electric oscillations generated by plant roots and a possible feedback mechanism responsible for them. *Aust. J. Biol. Sci.* **10**: 164.
- SCOTT, B. I. H., MCAULAY, A. L., and JEYES, P. (1955).—Correlation between the electric current generated by a bean root growing in water and the rate of elongation of the root. *Aust. J. Biol. Sci.* **8**: 36.