ON THE BIOELECTRIC FIELDS OF PLANT ROOTS AND THEIR POSSIBLE ROLE IN ORGANISATION.

THESIS SUBMITTED FOR EXAMINATION FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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by

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PREFACE

The investigation described in this thesis was conducted as a part time research between 1949 and 1955. Except for a year spent at the University of British Columbia, all the work was done at the University of Tasmania under the supervision of Professor A.L.McAulay.

In the early years much exploratory work was necessary to overcome difficulties in experimental technique which only became apparent as the work progressed and which had been the cause of much inconsistency in the results of previous investigations. The early work is described only briefly in this thesis. The major part of the thesis is devoted to the results obtained in the past three years, using methods developed as a consequence of the earlier work.

An outline of the experimental work to be described in this thesis is not given until section I.5 (page 7) of the Introduction. It was thought desirable that the work should be considered in relation to the general problem of organisation and in the light of the achievements and difficulties of earlier investigations, and these matters are discussed first.

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Scott, B.I.H., McAulay, A.L. and Jeyes, Pauline (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.

It is proposed shortly to prepare for publication papers embodying the work described in sections IV and V of this thesis.

The author finds some difficulty in acknowleding adequately his indebtedness to Professor A.L.McAulay in this investigation. Professor McAulay first suggested the problem, collaborated closely in the early stages, and maintained a strong and critical interest throughout. His assistance is gratefully acknowledged.

The vibrating probe electrometer (Section II.3) suggested by the author was constructed and tested in the University of British Columbia in collaboration with Dr. O.Blüh, whose interest and help is here acknowledged.

Much of the success of the experimental work is due to the efforts of Mr. D. Le Souef and Mr. D.Millwood, technicians in the Department of Physics, who gave valuable assistance to the author in the design and construction of apparatus.

Thanks are also due to: Mrs. P. Jeyes, Miss G.Newell and other assistants in the Biophysics Laboratory for their aid in the compilation of numerical data;

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I. INTRODUCTION

I.1 The Organisation of Living Systems

One of the fundamental problems of biology is the method whereby undifferentiated cells organise to form specific organs and tissues. The influences which govern the way a cell develops are to some extent not predetermined but are dependent on the environment in which the cell finds itself.

Experiments with animals which have led to this point of view are discussed by Huxley and De Beer (1934). (See also Agar 1943). For example, if undifferentiated tissue is transplanted from one part of a living system to another it may develop in a manner characteristic of the region into which it is transplanted and not as was predestined according to its original location. In other cases a system has been said to establish a 'polarity' which controls its subsequent development.

Few comparable studies have been made of plants, but here too there is some evidence that similar control is exerted by the system as a whole over further development within that system.

This differentiating influence has been called the 'biological field' but little is known of its nature or of the mechanisms involved in the controlling processes. This is especially true in plant systems.

Research work in the Biophysics Laboratory, at the University

of Tasmania, a section of which is described in this thesis is directed at finding out more about the biological field. In particular, experiments will be described in this thesis designed with the ultimate aim of testing whether the bioelectric field of plant roots has the necessary properties of a biological field.

I.2 Bioelectricity - Historical Discussion.

For a great many years it has been known that an intimate relationship exists between electricity and living systems. Galvani (1791) observed that muscular contractions of a frog's leg occurred when it was touched with joined leads of iron and copper. Even earlier it had been established that the shock received from certain fish such as the torpedo and electric eel was in fact due to an electric discharge. Volta (1800) described his first Voltaic pile as an 'Articifical electric organ' because of its similarity to the electric organ of these fish.

In the nineteenth century a large number of papers appeared which indicated that living tissue of every type was affected by, and itself apparently generated, electric fields. Much of this early work was very crude and unreliable, being hampered by the lack of suitable experimental techniques and measuring apparatus. In the early years the only method sufficiently sensitive to detect a small potential difference was by the contraction

it caused in a frog's muscle.

In medical therapy, electric treatments were widely used in the nineteenth century, and as Curtis (1950) has remarked "at one time or another it is probable that electric currents have been advocated as a remedy for practically every disease that afflicts mankind". Parallel developments took place in the field of agriculture and a great volume of unreliable and contradictory literature appeared dealing with the effects of applied electric fields on the growth and yield of agricultural crops (reviewed by Briggs,1926).

As the reliability and sensitivity of the measuring apparatus increased it became possible to make measurements of the small bioelectric potential differences generated by plant and animal tissues, including in some cases those associated with single cells. The response time of the measuring instruments was decreased, permitting the study of the rapid changes in potental (called 'action potentials') which take place in nerve and muscle. These studies have led to great advances in our knowledge of the behavior of the nervous system (cf.Eccles 1953). The use of valve circuits since about 1920 has produced considerable improvements in the techniques of measuring bioelectric potentials.

I.3. Causes of Electric Fields associated with Living Organs.

The reasons why electric potential differences occur in and around living tissues are reasonably well understood, although there are

still many differences of opinion over the detailed mechanisms especially in plant systems. It is not proposed to discuss these processes in any detail in this thesis, but a brief comment on the cause of bioelectric fields appears to be desirable.

In the case of a single cell, it is known that the ionic concentrations inside the cell are very different from those in the surrounding medium. Respiratory energy is used to maintain these concentration differences. The ions tend to diffuse so as to equalise concentrations inside and outside the cell, but because the ionic mobilities in the membranes and phases through which the ions have to pass generally differ markedly from each other and from their values in free solution, diffusion potential differences are usually set up. In addition the presence of fixed ions of one charge in one phase or membrane causes unequal concentrations of diffusible anions and cations in that phase. This leads to the establishment of a potential difference (known as a Donnan potential difference) across the boundary of that phase with another containing no fixed ions.

The potential differences and ionic concentrations associated with the cell adjust themselves so that in equilibrium the total flux across any boundary of each ion due to respiratory processes ("ionic or molecular pumps"), concentration gradients, plus the electric field, is zero.

These phenomena are discussed in detail by Höber et al (1947).

In the case of most cells which have been measured, the steady potential inside the cell is of the order of 50-100 mv more negative than that of the external medium (Bunning 1937). This difference of potential difference depends on the ionic concentration of the external medium as well as other factors.

A single cell placed in a uniform environment probably would exhibit the same potential difference across its boundaries on all sides and no external field would be expected. However, if a gradient of some factor affecting the potential of the cell (e.g.ionic concentration) exists in the external medium, symmetry is no longer present and a small residual field may be observed around the cell. With an aggregate of cells in such a gradient, these residual potential differences would integrate, setting up an observable electric field in the surrounding medium.

It is obvious that such gradients of ionic concentration must occur throughout developing systems, in which salts are continually being absorbed through one region and transferred to another region where they are required by enlarging cells. Thus it is to be expected that characteristic potential differences would be found in association with any specific organ or tissue in the process of development.

I.4 Possible role of the Bioelectric Field as an Organiser.

In view of the universal presence of electric fields in the

around living systems, due to the processes outlined above, it has occurred to some workers (cf. Lund,1947; Burr, 1947; McAulay et al 1951) that these fields might be responsible for the organisation of differentiating tissue. The bioelectric field established by an organ in a characteristic pattern might itself control the further development of that organ. In other words the bioelectric field was suggested as a possible biological field.

It is well known that electric forces are of great importance in the organisation of the inanimate world. An example, superficially resembling the postulated role of electric forces in living systems, occurs in the growth of a crystal. New atoms attach themselves to the crystal in a pattern which is controlled by the pattern of the electric field of atoms already forming the crystal.

In order to test whether the bioelectric field is indeed responsible for the organisation and development of biological systems the following questions clearly must be answered:-

(1) Does the electric field in and around a biological system in a particular stage of its development form a recognisable and characteristic pattern?

(2) If this is so, and if the electric pattern is suitably modified (say by short-circuiting, or by using an external source of P.D.) do corresponding changes occur in the biological development of the system?

If the answers to these questions are in the affirmative there can be no doubt that the bioelectric field is a biological field,

possibly the only biological field.

A number of investigators claim to have shown that consistent and characteristic electric fields are associated with particular living organs. Unfortunately the results obtained by different research groups seldom agree and are often completely contradictory. A typical example of this is given in Section II.2 for the case of plant roots.

Again, the effect of applied electric fields has been the subject of controversy between observers for many years (see Lund et al 1947 Ch.IV). Although Schrank and co-workers (e.g. Wiegand and Schrank 1955) have recently performed experiments which appear to indicate a positive correlation between applied electric currents and plant bending, it is still open to some doubt whether their techniques are sufficiently satisfactory to permit the definite conclusion that the electric field is the primary cause. For example the bending which was observed by Schrank's group when a current was passed transversely across a plant shoot might have been caused by the accumulation of a particular ion from the bathing solution at one point of electrical contact, due to the method of passing current. Other unsatisfactory features of Schrank's technique are discussed later (Section IV.1).

I.5 Reasons for Present Investigation and Outline of Work Described in Thesis

In the early stages of the present research it was realised that a critical analysis of techniques used for making measurements of bioelectric fields was a necessary preliminary to a detailed study

of these fields if a significant contribution to knowledge of them was to be hoped for.

A number of possible sources of artefacts in measurement, were considered, many of which had been overlooked by previous investigators. These considerations led to the development of four new methods of measurement of bioelectric fields in which these possible sources of error were largely eliminated. These will be described in section II of this thesis.

In what was considered to be the most satisfactory of the new methods a plant root was immersed in a weakly conducting solution and measurements made of potential differences in the water near the root due to currents generated by the root and flowing through the water.

This method was applied to the study of the bioelectric fields of bean roots, and in section III it is shown that an actively elongating root develops a characteristic and usually steady potential pattern in the external medium. Furthermore it is shown that by inhibiting elongation of the root by two methods, the magnitudes of the potential differences along the root were significantly reduced, and with one method the process was reversable.

In section IV, the study of this steady external potential pattern is continued. Techniques are described which permit the mapping of the field lines in the surrounding medium. Estimates are made of the electric currents flowing from the root, and the electric power dissipated by the root in the external medium. The

amount of electric power produced is considered in relation to the total power available in the root through respiration.

Experiments to test the effects of different ions in the external medium are also discussed in section IV, and consideration is given to which ions are responsible for carrying the electric current, in particular across the plant surface.

In 1953 it was decided that some aspects of bioelectric fields could better be studied from automatic recordings of bioelectric potential differences. Accordingly apparatus was constructed which recorded automatically the potential differences between several points near a root growing in water at frequent intervals over long periods of time. This is described in section V.

In the same section, a very sensitive recorder of the rate of elongation of a root is described. It is believed that this apparatus detects smaller increases in length than any other growth meter that has been described elsewhere. The rate of elongation and potential pattern are measured simultaneously and recorded on the same chart.

With the automatically recording apparatus it has been found that under some conditions bean roots generate sinusoidal electric oscillations with periods of about 5 minutes which are superimposed on the steady or background potential pattern. They may continue for several hours. These regular oscillations which have not been observed by other investigators are described in section V and the conditions under which they arise are discussed.

The mechanisms for these and similar oscillatory processes in living systems are also discussed in this section, and a particular feedback mechanism is postulated to account for the observed electric oscillations.

In the concluding section (VI) the results of the whole investigation are discussed in relation to the general problem of organisation and control, and consideration is given to proposals for further investigation.

I.6 Electric Fields within Living Tissue

It will be noted that throughout this investigation, bioelectric measurements have been confined to the conducting medium external to the root. No experiments to determine the electric pattern and current paths within the plant have been described. Although preliminary observations of internal potential differences have been made by the author and by others in the laboratory (McAulay, Ford and Hope 1951) and reasonably consistent results obtained, the interpretation of these results is difficult and they have not been included in this thesis.

Interpretation is difficult because even the finest practicable microelectrode damages many cells during its insertion into the root, and it is difficult to be certain of the material in the immediate vicinity of the microelectrode tip. At first it is probably largely the sap of broken cells, but gradually salt would be absorbed by surrounding cells and healing tissue would form. The electric changes resulting from these processes are likely to be

complex and much larger than the relatively small potential differences causing internal currents to flow. Further analysis has been postponed until the external pattern has been studied more completely.

II. DISCUSSION OF POSSIBLE SOURCES OF ERROR IN MAKING BIOELECTRIC MEASUREMENTS, AND NEW EXPERIMENTAL METHODS.

II.1 Potential Differences measured in Plant Systems.

Experiments made at the University of Tasmania and elsewhere show clearly that electric potentials measured on the surface of plant organs depend on a number of causes. In addition to those due to the plant itself when in its normal steady state of metabolism, there are a number of other sources of potential difference, some of them generated by the plant itself due to treatment it receives during measurement, and others introduced from outside.

By using suitable experimental techniques which will be discussed later, it is possible to eliminate effects associated with the process of measurement and other external factors, and measure the electric fields generated by the plant alone.

The electric fields generated by plant organs may be divided into two types:-

(a) Fields due to static or quasi-static charges within the plant system. These fields are conservative (i.e. electric energy is not being dissipated). They may, however, separate ions within cells and hold them in this polarised condition.

(b) Fields due to active electromotive forces generated by the plant which are supplying power. These dissipative fields cause currents to flow continuously through closed circuits within and

around the plant.

In seeking a correlation between electric field and morphogenesis, a study of the second type of field appears to offer the best immediate prospect.

It must be recognised however, that the conservative type of field, possibly by polarising cells or creating double layers, may well affect morphogenesis. Tropism due to gravitation provides an example of an undoubted effect on plant growth produced by a conservative field.

The object of the present section is, in the first place, to show how potential differences due to the plant itself can be distinguished from those introduced from outside and during the process of measurement. In the second place, its object is to describe methods of measurement in which the fields due to the plants themselves have been isolated. In the first of these methods, only the dissipative field due to the active electromotive force supplying power is measured, while in the others, the conservative type of field is measured also.

In section III of this thesis, results of experiments using the first of these techniques are described. It is there shown that a correlation exists between the observed electric potential pattern along a bean root and its rate of elongation for bean roots whose growth is controlled by certain treatments.

II.2 Conditions Required to Distinguish Normal Plant Electromotive Forces from Artefacts.

A number of investigations of the electric potential differences associated with plants have been made in the past. An examination of the literature shows very little agreement between the results obtained.

Some of the results that have been obtained using roots as material, are compared in Figure 1 , in which the potential of the root relative to the tip is plotted against the distance from the tip. It is evident that there is a wide diversity between the results of different observers. It is true that these results are not directly comparable since they have been obtained under a variety of conditions, and some of the variation between them is certainly due to such factors as the type of plant used, its age, and the state of its environment. Nevertheless a critical examination of the experimental techniques employed in the investigations shows that in most cases the measured potential differences were due not only to the plant e.m.f's but also to factors introduced by the measuring techniques.

In view of the lack of complete recognition of these difficulties in the past, it is now proposed to examine in some detail the sources of error which may occur in making electric measurements on plant material.

(a) Local potential differences may be set up at the points of contact of the measuring tubes and the plant surface due to

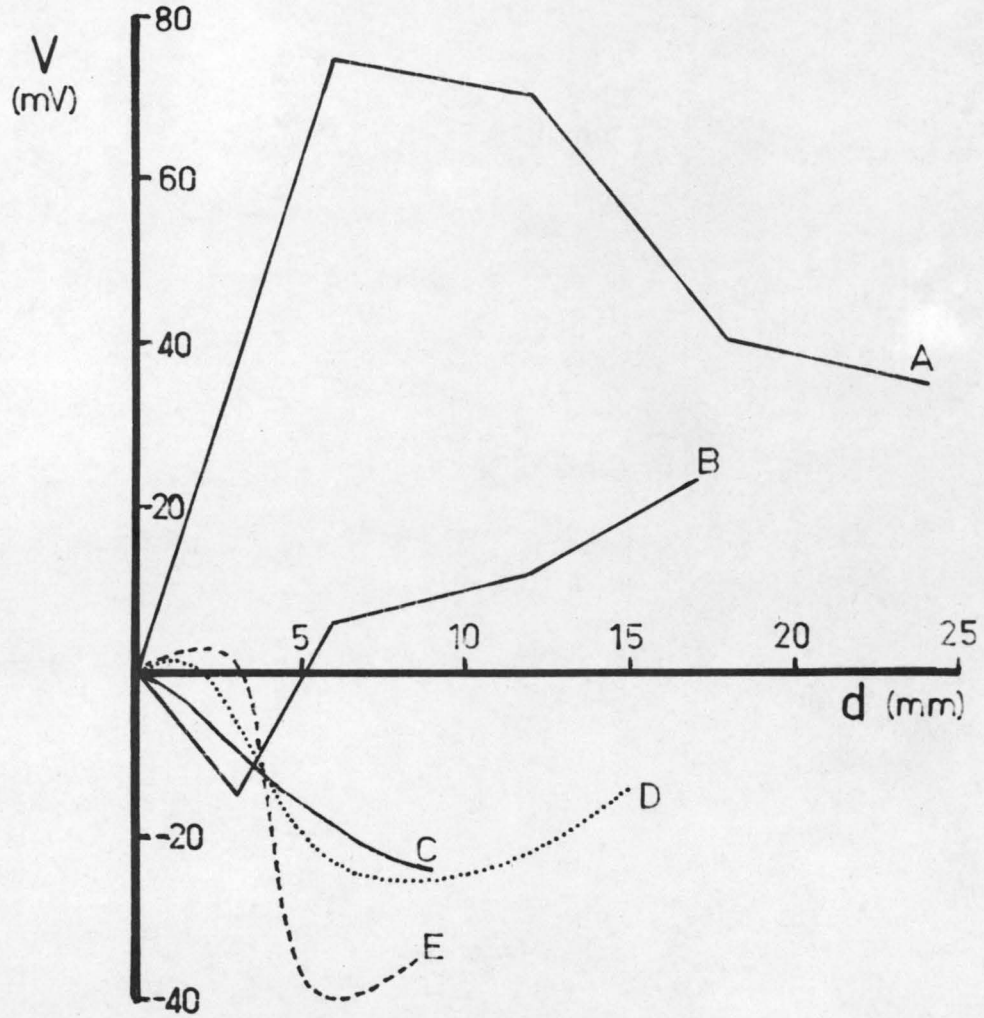


FIG. 1. Comparison of the results obtained in several investigations of the relation between the external potential of the root, V, relative to the root tip and the distance d from the tip along the root. Data obtained from A, Thomas (1939) (bean); B, Lundegarh (1940) (wheat); C, McAulay, Ford and Hope (1951 (maize); D, Lund and Kenyon (1927) (onion); and E, Ramshorn (1934) (bean).

(Section II.2)

differences in concentration of ions in the contacting medium.

It is well known that plant tissue absorbs inorganic ions differentially from the bathing solution. A phase boundary exists between the solution and the cytoplasm of the tissue and for reasons given in I.3 boundary potentials are set up which depend on the nature of the tissue, and the nature and concentration of the ions.

In measurement of plant potentials, salt concentration changes frequently occur due to the following two causes.

(i) Evaporation of the liquid at the point of contact may take place causing an increase in salt concentration.

(ii) Salts may be removed from the contacting liquid due to salt uptake by the plant causing large decreases in its salt concentration.

To illustrate the magnitude of the potentials set up in this way, it is found that if the concentration of KCl at one point of contact on the bean root is changed by a factor of ten (the concentration at the other contact remaining unchanged) the potential difference between the contacts changes by more than 30 millivolts. (Hope 1951).

Unless special precautions are taken, such sources of error are nearly always found to be present. In spite of the magnitude and importance of these effects, little consideration has been given to their elimination in the past and many of the published results have been obtained using stagment water or agar contacts. This

must be taken into account in evaluating the results obtained in previous investigations.

(b) Local conditioning of the plant surface may occur at the point of contact. The point of contact may differ from neighbouring points on the plant surface in a number of ways. It may be wetter and less well aerated than surrounding points, and there are likely to be differences in temperature and lighting conditions. The condition of cytoplasm is changed when salt concentration is changed of a solution which is in contact with it. As a consequence of this, a potential difference is shown between two regions otherwise identical which have been subjected to different histories such as exposure to accidental differences in salt concentration of the contact points.

(c) Variation of the overall state of the plant's environment preceding and during measurement may give rise to transient potential patterns that obscure the desired steady state pattern. Important factors which may have to be controlled are temperature, humidity, salt concentration, degree of aeration, and possibly light and gravitational influences.

It is clearly necessary to disturb as little as possible the uniformity, either locally or generally, of the plant's environment in the process of measurement.

(d) Mechanical or electrical stimulation of the plant when being set up for measurement, and local injury caused by the contacting probes influence plant potentials.

It is usual for an area on a plant surface to become more negative if it is stimulated. The effect of stimulation usually disappears in a few minutes, but in some cases may remain much longer. Extreme care must be taken when making contact with a plant surface that no local injury occurs as quite large potential variations may result, and electromotive forces associated with healing are likely to continue as long as tissue repair continues.

(e) The electrometer may be influenced if alternating current pick-up occurs in the measuring circuit. A signal due to this cause is usually partially rectified (probably at the electrometer valve grid) and a d.c. potential is registered by the instrument, superimposed on the potential being measured. Pick-up can be prevented by adequate shielding of the input circuit.

(f) Errors are introduced if too much current is drawn from the plant. Unbalanced and possibly variable potential boundaries in the electrical connections to the measuring instrument may also cause errors. As is well known, these effects can be eliminated by the use of suitable electrometers with high input impedence, and reversible non-polarisable electrode systems. High insulation of the input circuit is of course necessary to avoid d.c. leakage.

II.3 Some New Methods of Measuring Plant Potentials.

Four new experimental methods have been devised and tested with a view to measure true plant potentials when the plant is

growing normally.

Most of the sources of error listed above are liable to occur if the measuring probes are in mechanical contact with the plant. To avoid this measurements are made without mechanical contact with the plant in three of the new methods. In the fourth a liquid contact is established, but this is kept flowing to avoid the bad features discussed above in (a) with stagnant contacts.

The first method (Figure 3A) was to immerse a bean root in water and measure potential differences between points in the water adjacent to the root, the potentials being measured through liquid filled (or in some cases agar filled) tubes, the salt concentration in which was the same as that in the bulk water. In this way, ohmic potentials were measured along currents produced by the plant organ in a homogeneous medium. This medium, which has, up to the present produced the most promising results is described in detail in section III (also Scott, McAulay and Jeyes 1955). With suitable precautions, this method enables potential differences to be measured which are due to the plant organ alone, and if appropriate care is taken, the plant can be in a normal steady state of metabolism and unaffected by the process of measurement. The measurements give information about active electromotive forces generated by the plant and capable of supplying power.

In methods two and three electric fields were measured in the air in the immediate vicinity of the plant and for this reason the

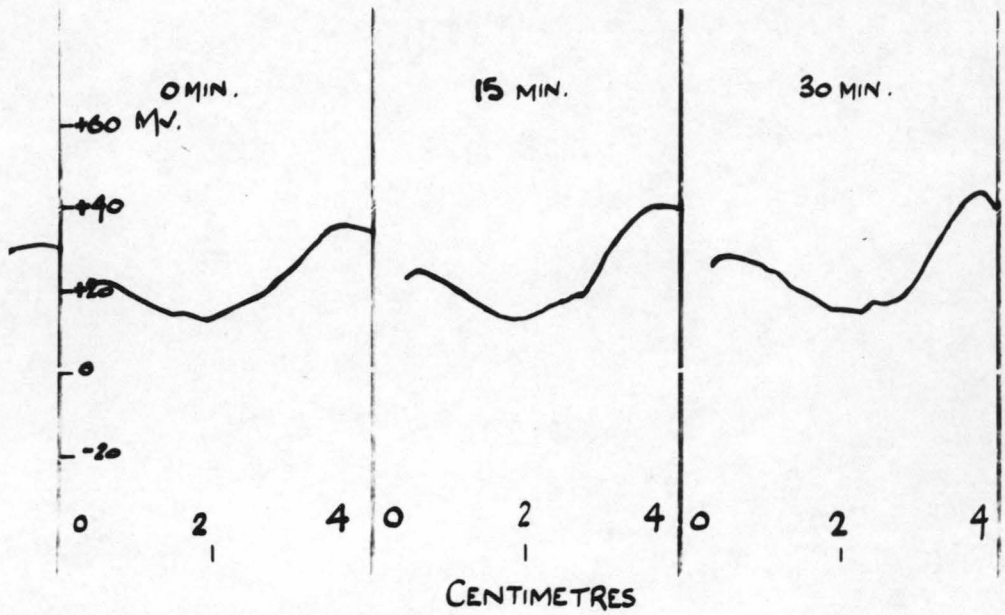
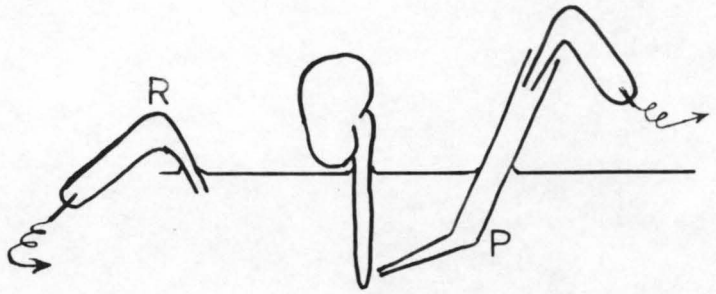
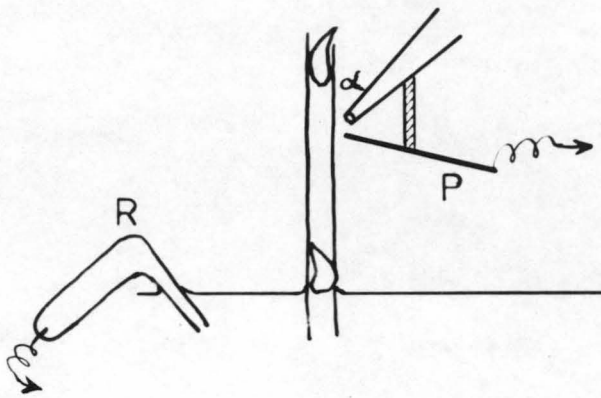


FIG.2. Potential pattern along a 5 cm. length of grass stem between two nodes obtained by the ionized air method. Each section of the figure records a single sweep along the plant, the potential being recorded automatically at 15 minute intervals.

(Section II,3)



A



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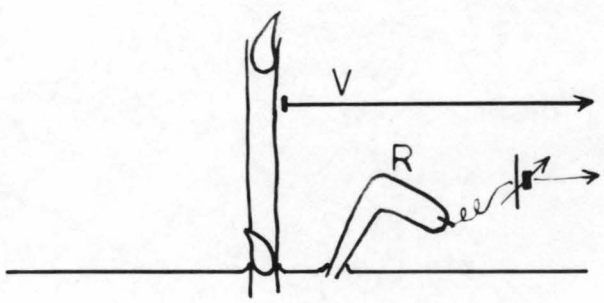

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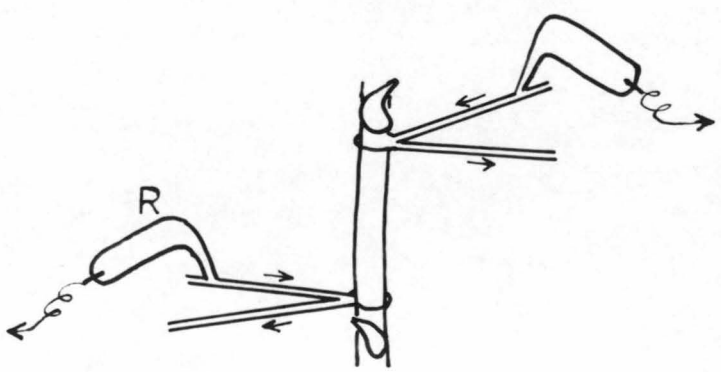


D

potentials, though due to the plant in a condition unaffected by the measurements, did not select only the electromotive force generated which was capable of supplying power.

Method two (figure 3B) was suggested by Professor A.L.McAulay and a simple apparatus for making automatic measurements of the bioelectric field along a grass stem by means of it was constructed by the author. In this method an electrometer probe was placed a few millimetres from the plant surface and was arranged so that it could scan the surface. A source of α-particles was held near the electrode and ionised the air between plant and electrode thus creating a conducting path between them. The electrode reached equilibrium in a few seconds and during this time currents drawn from the plant source were less than 10^{-13} amps.

The source of α rays was a few drops of very dilute radium bromide solution evaporated on the end of a glass rod and covered by a glass film whose stopping power was equivalent to about 2 cm. of air. The probe was of platinum. A contact potential difference undoubtedly existed between probe and air, but this appeared to be steady and the experiment was concerned with differences only. On moving the electrode relative to the plant reproducible potentials were recorded.

Figure 2 shows a typical record of the potential pattern along a portion of grass stem between two nodes made with the automatic scanning apparatus.

The full possibilities of this technique have not been explored as far as it has been set aside temporarily in favour of the first

method.

Method 3 (Fig.3C) was suggested by the author and was tested by him in collaboration with Dr. O. Blüh at the University of British Columbia. With this method no currents were drawn from the source and purely static charges could be observed. A vibrating probe connected to an electrometer was brought close to the plant surface and an alternating current was induced in the input circuit if a P.D. existed between plant and electrode. This was amplified and displayed on a cathode ray oscillograph screen. A compensating potential was applied to the input circuit and this was varied until no A.C. was observed on the screen. The probe was then assumed to be at the same potential as the adjacent plant surface. By moving the probe relative to the plant and observing the change in the compensating potential necessary for a null reading, it was possible to measure the potential pattern along the plant surface without drawing any current from it. This method has been described in more detail elsewhere. (Blüh and Scott 1950)

In the fourth method which was suggested by the author (Figure 3D) contact was made through a stream of running water which was applied to a point on the surface of the stem. The stream of water flowed down one tube about 2 mm. in diameter and was sucked up another similar tube. The two tubes which comprised one contact were adjacent to one another and attached to the surface on the plant. In this way the plant was wet by a flowing stream of constant

composition, and no water escaped down the plant surface. Each stream was part of a separate highly insulated circuit. By this method changes of concentration due to uptake by the plant or due to drying are avoided. Conditioning of the point of contact on the plant is not prevented but is as nearly as possible the same at both contacts. This method has some advantages in the measurement of potential differences on plant stems.

The mechanisms which produce electromotive forces in plant tissues may be such that some can supply more power than others. Whether the field produced by one of these will be observed outside the plant in a particular case depends on the magnitude of the current generated in the conditions of measurement. If this current is too large, the field may not be measured, either because the e.m.f. itself is polarised or because the potential drop in the tissue is so great that the external field is insignificant.

Method 1 selects for measurement only those bioelectric processes which are capable of supplying a relatively large amount of power to the external medium. By using one of the other methods it is possible to investigate less powerful bioelectric processes taking place in the organ and so gain a more complete picture of the bioelectric behavior of plants.

III. THE STEADY ELECTRIC POTENTIAL PATTERN GENERATED BY A BEAN ROOT GROWING IN WATER AND THE CORRELATION WITH RATE OF ELONGATION OF THE ROOT.

III.1 Outline of Observations.

When a bean root grows actively in aerated water it generates an electromotive force which passes current through the water. This section of the thesis describes measurements which have been made of the ohmic potential differences due to these currents in the water adjacent to the root. The pattern of these potentials is characteristic and reproducible when the root is growing strongly.

The section further describes experiments in which the growth of the root has been controlled and a correlation which has been found between the change in potential pattern and the rate of elongation in certain cases.

By addition of auxin of suitable concentration to the bath in which the root is grown, it is possible to arrest growth and observe the change in potential pattern. This process proves to be reversible, as removal of the auxin allows the plant to grow again and the potential pattern to recover.

A similar correlation is observed when the temperature at which the roots are grown is raised, but the root cannot be restored to its original condition by lowering the temperature.

III.2 Experimental Method.

The method used was the first of those suggested in section II of this thesis. It was designed to eliminate from the observed potentials any effects introduced by the method of measurement,

and also to maintain the plant organ in its normal steady state of metabolism. A fortunate property of the root is its ability to grow strongly and healthily in aerated water. If the conductivity of the water is sufficiently low, potential differences between points in the water adjacent to the root can be observed due to the currents generated by the root e.m.f's. For this reason it was decided to grow bean roots vertically in a 10^{-4}N KCl solution under controlled conditions and observe the potential pattern in the water adjacent to the root and its relation to the rate of elongation of the root.

In section II a number of possible artefacts were discussed. It is believed that the method outlined above eliminates all of these.

Since the measuring tubes are not in contact with the plant, no effects due to injury by them can occur. As the salt concentration in the measuring tubes and in the bath is the same, the point of measurement on the plant is not in a different condition from neighbouring points due to local diffusion or other local variations.

Errors introduced in the measuring circuit have been prevented by the use of a suitable vale electrometer and mercury-calomel electrodes. The whole input circuit is insulated with polystyrene and shielded to prevent A.C. pick-up. The plants are left in the measuring bath for at least an hour before measurements are commenced to avoid stimulation effects which may occur in setting

up the plant for measurement. The bath is aerated, stirred, and its temperature controlled to avoid changes in environment. No effect was observed on either rate of growth or the potential pattern of bean roots due to light.

As the potential measured is an ohmic drop along a current, its source is evidently an active e.m.f. involving energy change in the plant organ.

III.3 Material.

For the experiments described in this thesis, the broad bean, Vicia faba L., Johnson's Long Pod variety was used. After soaking the seeds overnight in water, the seed coats were removed and a number of seeds impaled on stainless steel rods 1/16" in diameter. Mounted in this way, the seedlings could be handled easily for growing in water baths and could be transferred to the measuring bath with a minimum of stimulation. Impaling the seed did not appear to affect the development of the plant in any way.

For most experiments the beans were prepared by growing in baths of aerated distilled water at 25°C. The water in the tank was slowly changed from a reservoir tank. The rods supported the plants so that the shoot was above water level and the roots were submerged and grew vertically. Plants 2-3 days old with roots 20-30 mm. long were used in experiments.

In one series of experiments discussed later, roots were prepared by growing in air saturated with water vapour and minute water

droplets. The fine spray was produced by a simple atomiser using compressed air. In this bath the roots grew quite strongly but the root surface appeared rather different from that of a root grown in water and more like what is observed for a root grown in dry soil or sphagnum moss.

III.4 Apparatus.

The "Perspex" measuring bath is shown in Figure 4. It was 7" long, 6" wide and 3" deep and was insulated with polystyrene. The bath was aerated and the water in the tank circulated by passing compressed air through a sintered glass plug 'P' mounted in the tank. The small bubbles passing through provided much more efficient aeration than with a larger air jet.

Heating of the bath presented some problems. Electrical interference had to be avoided and an arrangement giving rapid response to the thermostat was essential. It was decided to heat the bath by means of a coil of nichrome wire stretched across the top of the bath just above the water level. A 12 volt A.C. supply was connected to the coil and heat radiated downwards warmed the water. Surprisingly little A.C. pick-up was experienced, and this arrangement with some shielding of the heater from the measuring circuit, proved very satisfactory. The thermostat was a mercury-alcohol switch 'C' which controlled the heater through a relay. Temperature stability was $\pm$ 0.5°C.

The 10^{-4}N KCl solution used in the measuring bath was changed

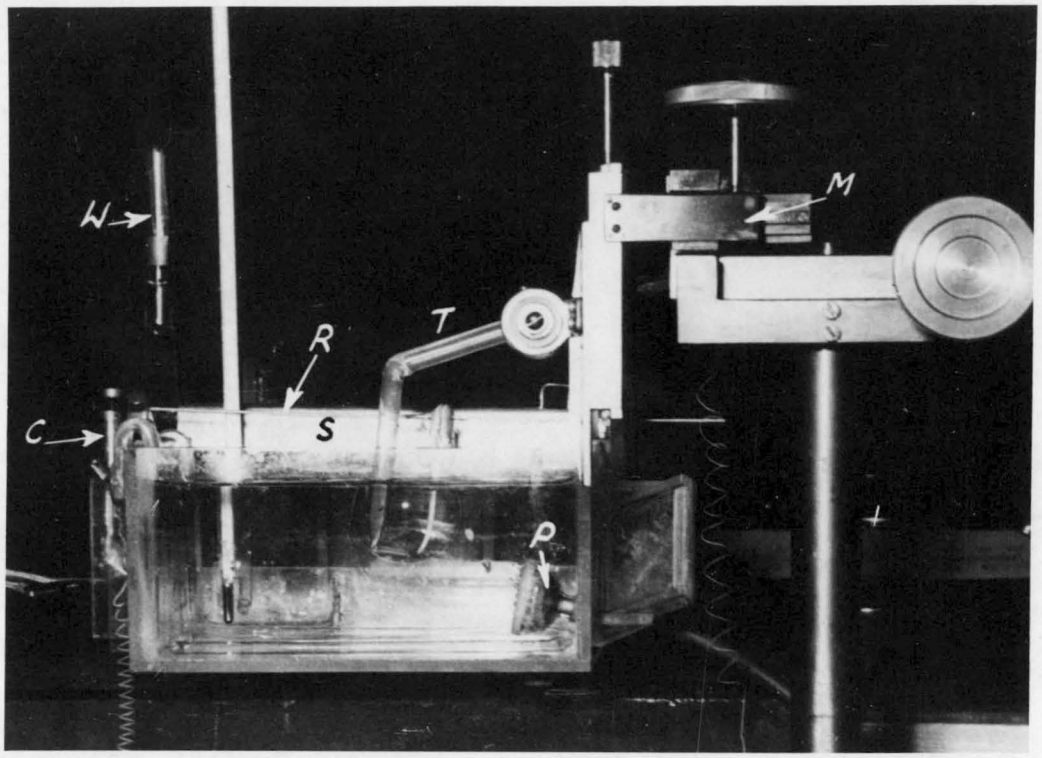


FIG.4 The measuring bath used in the experiments described in Section III. The following parts are referred to in text: P, sintered glass plug; S, shield for A.C. heater; C, mercury-alcohol switch control; R, stainless steel rod supporting plant; M, micromanipulator; T, measuring tube; W, water inlet; G, metal slide for moving box.

(Section III.4)

from a reservoir . The water was made to drip into the bath, and to drip out at the overflow to facilitate electrical insulation of the bath.

The beans were set up in the measuring tank on a stainless steel rod passing through the cotyledon with only the roots immersed and held vertically.

The electrometer used in these experiments employed a pair of matched M.E. 1400 Mullard electrometer valves in a balanced circuit. The instrument was designed to be insensitive to fluctuations in the high tension voltage and in the valve heater current. A Cambridge spot galvanometer (full scale deflection about 2μa) was used and the maximum sensitivity of the electrometer was such that an input of 1 mv. produced a deflection of 3 cm. on the scale. Currents flowing in the input circuit of the electrometer under the conditions of operation were not greater than 10^{-12} amperes. The circuit used is similar to that shown in Figure 17.

The connections to the measuring bath were made using mercury-calomel electrodes. The reference electrode was connected directly to the bath. The other electrode made contact with the bath through a measuring tube, 'T', containing either 10^{-4}N KCl agar or 10^{-4}N KCl solution. This tube was mounted on a micromanipulator 'M', allowing it to be moved easily in the vicinity of the plant root. Since 10^{-4}N KCl has a low conductivity (about 1.5×10^{-5} mho. cm.$^{-1}$ at 25°C) the tip of the measuring tube had to be coarse or hand capacity effects became troublesome due

to the high resistance of the input circuit. In most of these experiments the diameter of the tip was about 0.5 mm. corresponding to a tip resistance of about 15 MΩ.

Elongation of the root was measured by projecting a greatly enlarged image on a wall. A 5" Waterworth projection lens was used, mounted in front of the box with its axis horizontal. For most experiments, back lighting was employed, giving an enlarged shadow of the root, but in some experiments the root was marked, and strongly illuminated from the front so that the regions of elongation of the root could be found. In this way, changes in length of the order of 0.02 mm. could be observed. The light had no apparent effect on either the growth or the potential pattern of the root.

The whole box could be moved laterally on a metal slide, 'G', so that each bean in the box could be placed in turn in front of the lens for measurement.

III.5 Results.

The present section comprises a study of the potential pattern close to a bean root actively growing in water contrasted with that which appears when growth is inhibited.

The aspect of growth that has been selected is elongation and attempts have been made to control this by a variety of means and study the resulting potential changes.

Several treatments have been applied to the growing root, such as subjecting it to mechanical vibration, controlling its oxygen supply, growing it at varying temperatures and inhibiting growth by

the application of auxin.

The last of these methods, auxin treatment, was considerably the most successful. By this means elongation could be inhibited and the inhibition subsequently removed and growth restored. The method of temperature control also provided results of interest which are described below, but preliminary results by the other methods were not so promising and study of these has not yet been followed up.

In both cases studied, the pattern of the active e.m.f. was correlated with rate of elongation in spite of the fact that subsequent growth was very different in the two cases. This suggests a direct link between elongation and potential pattern. Of course it is possible that both elongation and potential pattern are more dependent upon some third change common to both methods of control.

(a) Control of Growth with Auxin.

(i) Bean seedlings with roots approximately 25 mm. long were transferred to the 10^{-4}N KCl measuring bath at 25°C and allowed to reach equilibrium with their environment.

After about 2 hours, the potential pattern and rate of elongation of the roots were measured, but those whose growth rate was less than 0.4 mm/hr. discarded. 2 mg. of indole acetic acid (IAA) per litre of solution was then added to the bath containing the rapidly growing beans. After about an hour the potential pattern had reached a new steady state and the average potential and rate of elongation over the next two hours were

recorded.

The results of this experiment are shown in Figure 5. In this and subsequent diagrams throughout this thesis, the vertical lines through the points mark the 95 per cent confidence limits; that is, the probability is 0.95 that the mean of the population represented by the sample lies within the limits given by the ends of the line. The limits for the mean growth rate also are the 95 per cent confidence limits.

(ii) Bean seedlings were transferred to the measuring bath at 25°C containing 10^{-4}N KCl and 2 mg/litre IAA. After about two hours elongation had practically ceased. The potential pattern was then observed, and the water in the bath replaced with KCl solution without indoleacetic acid. One to three hours after the removal of IAA when the root was again elongating, the mean potential pattern and growth rate were measured.

The results of this experiment are given in Figure 6.

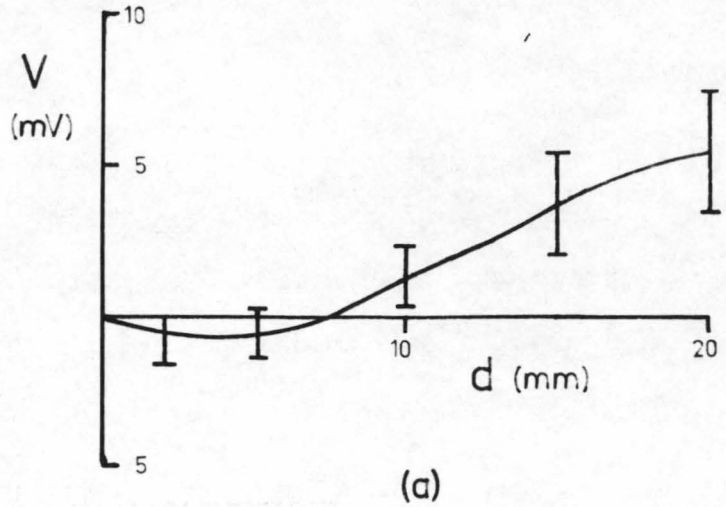
An examination of these graphs shows that the inhibited roots have a markedly different potential pattern from those growing strongly. The normal potential pattern of a growing root shows a region 2-5 mm. from a tip most negative with the base of the root approximately 6 mv. more positive. Suitable treatment with indoleacetic acid inhibits the elongation of the root and reduces the potential gradients along the root by a significant amount. Removal of the auxin allows the root to

grow again (although the recovery is not complete in two hours, the growth rate being well below that of untreated roots) and the potential gradient along the root returns almost to its value prior to auxin treatment.

(b) Control of Growth by Temperature.

Graph 7 (a) shows the normal potential pattern for roots grown in 10^{-4}N KCl at 25°C. In this case, and in the following one at 37°C, the growth and potentials were averaged for a 24 hour period following the initial settling down.

Graph 7 (b) shows the potential pattern for roots measured in 10^{-4}N KCl at 37°C. Inhibition of growth at this temperature was most marked in the case of roots pre-treated by growth in a saturated atmosphere for 24 hours before exposure to 37°C. The graph refers to roots treated in this way. It is seen that the roots have almost entirely stopped elongating and the potential differences along the root are not significantly different from zero. Roots measured at this temperature for 24 hours and then returned to a 25°C bath do not recover normal growth. The activity of the primary meristem is suppressed and the main root stops growing, but pronounced initiation of secondary roots quite close to the primary root tip is observed. (See Figure 8). Electromotive forces are once more produced by the root when it is returned to the 25°C bath, but they are not the same as those for a root which has not been treated at 37°C.



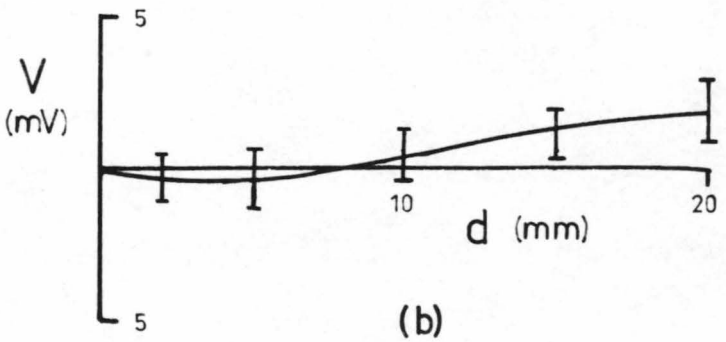
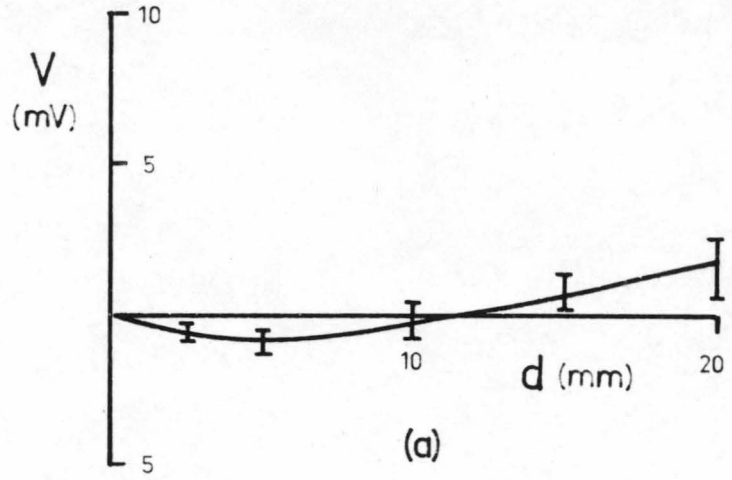


FIG.5. Control of growth and potential pattern of bean roots by the application of indoleacetic acid (IAA). (a) Roots at 25°C untreated with IAA. Mean growth rate 0.82 ± 0.060 mm/hr. (b) Same roots at 25°C 2 hr after treating with IAA (2 mg/1). Mean growth rate 0.027 ± 0.077 mm/hr. V is the potential relative to that of the root tip and d is the distance along the root from the tip.

(Section III.5)



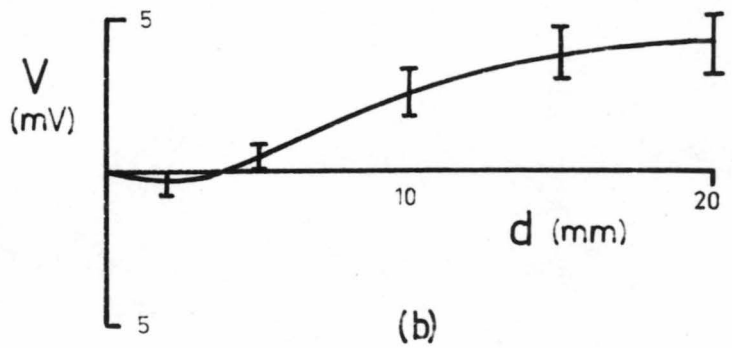
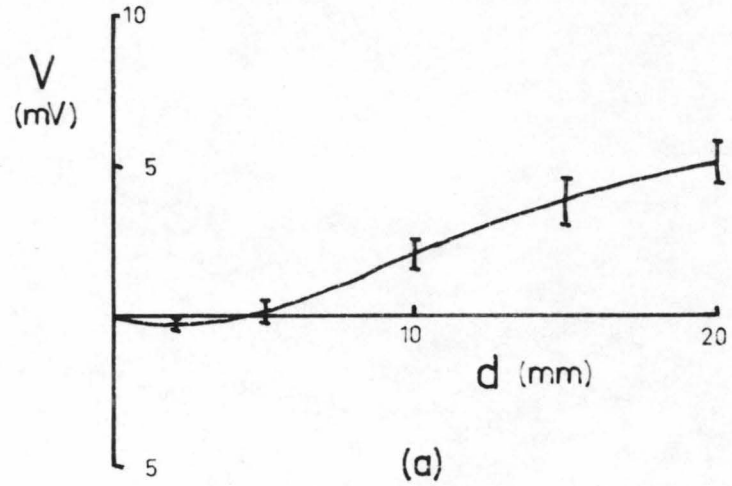


FIG.6. Recovery of growth and potential pattern on removal of IAA. (a) Roots at 25°C 2 hr after treating with IAA (2 mg/1). Mean growth rate 0.037 ± 0.043 mm/hr. (b) same roots at 25°C 2 hr after removal of IAA from bath. Mean growth rate 0.36 ± 0.072 mm/hr.

(Section III,5)



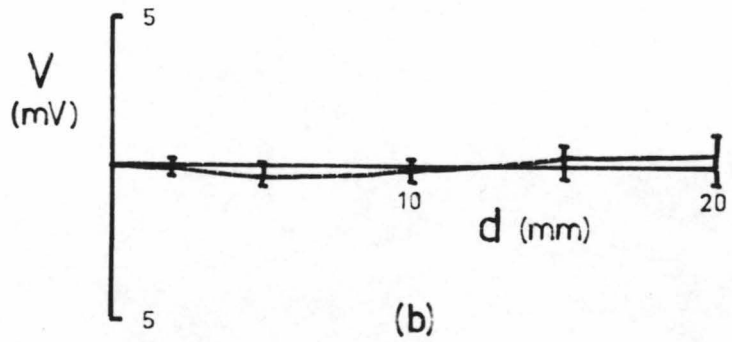


FIG.7. Control of growth and potential pattern by temperature. (a) Roots grown and measured in water at 25°C. Mean growth rate 0.532 ± 0.076 mm/hr. (b) Roots grown in saturated air and measured in water at 37°C. Mean growth rate 0.007 ± 0.041 mm/hr.

(Section III.5)

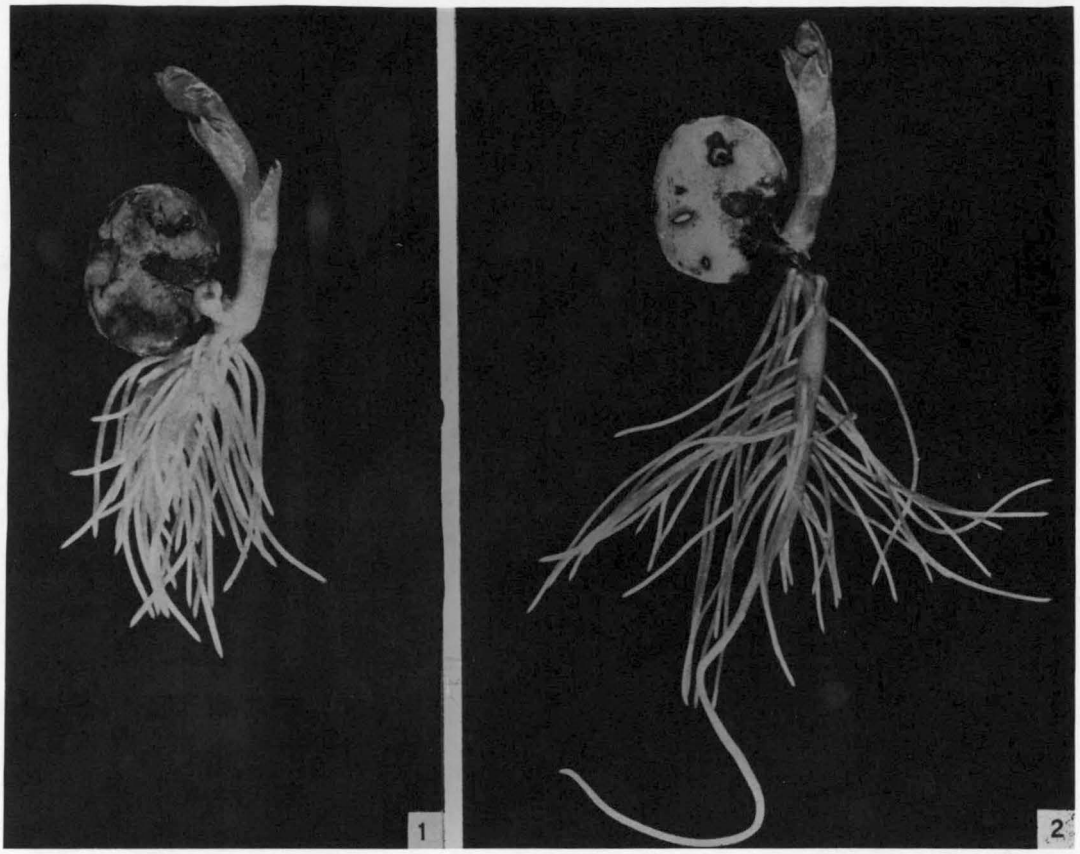


FIG.8. Abnormal subsequent growth of roots treated for 24 hrs at 37°C.

1. Root one week after treatment at 37°C. Notice lack of development of primary root with secondary roots appearing quite close to primary tip.

2. Untreated root of same age.

(Section III.5)

IV. FURTHER STUDIES OF THE STEADY BIOELECTRIC PATTERN AROUND A BEAN ROOT GROWING IN WATER INCLUDING MEASUREMENT OF THE ELECTRIC CURRENT AND POWER GENERATED

IV.1 Introductory Discussion

Measurements of potential differences in the water close to a root give only a partial description of the bioelectric pattern external to the root. In order to describe this more completely further information is required, including the magnitudes and directions of the currents flowing out from the plant surface, and the paths they take through the water before re-entering the root (i.e. the paths of the field lines in the water), and the amount of electric power dissipated in the water. In addition it is of interest to know which of the ions present are actually carrying the current, especially across the plant surface.

The experiments described in this section were designed to provide some of this information.

The long range plan of the present research is to test the hypothesis that the bioelectric field has a formative influence on the bioelectric pattern (see section I.4). It is therefore important to know the amount of electric power being developed by the plant and the relation of this to the amounts involved in other power producing and power consuming processes within the tissue.

The knowledge of current paths is needed to give a sound basis to a subsequent investigation now being planned of the effect on plant development of currents from an external source. For these experiments

it will be of considerable importance to know the manner in which the external field modifies the bioelectric field of the plant, and in particular the amount of current from the external source which passes through the plant and the regions on the plant surface through which the current enters and leaves.

In some previous investigations of the effect of externally applied currents, this information does not appear to be known with any certainty. For example in Schrank's (1944) experiments, it does not appear possible to state how much of the current from the external source actually passed through the plant and how much by-passed the plant through the external medium. (see tracing in Lund et al 1947 p 221).

The mapping procedure developed in this paper would allow this information to be obtained.

Little consideration appears to have been given in the past to the measurement of the electric power generated by bioelectric sources in plants or its importance. (see discussion by Crane 1950). Blinks (1933) made some measurements of the power output of a single Halicystis cell. McAulay, Ford and Hope (1951) estimated the power dissipated in an external circuit when two contacts were made on the surface of turnip hypocotyls and couch grass shoots. They estimated that the current flowing in the external circuit through these points of contact was of the order 10^{-8} amperes (depending, of course, on the resistance of the external circuit) and the external power dissipation was about 10^{-9} watts. However, it must be pointed out

that the objections to the method of measurement through isolated contacts of salt solution discussed in section II. 2 still apply in this case, and part of the relatively large amount of power observed may be due to processes resulting from lack of equilibrium on the contacting system. For example, the energy changes associated with an evaporating liquid contact are likely to be considerably higher than the energy associated with the bioelectric source.

IV.2 Estimation of Current and Power.

Consider an area dA of a plant surface immersed in a medium of conductivity σ (Figure 9a). If the electric field strength in the medium adjacent to the area is $\bar{E}$, in a direction inclined at an angle θ to the surface normal, the density $\bar{J}$ of the electric current near the surface is given by

$$\bar{J} = \sigma \bar{E}$$

Thus the total current di passing through dA is given by

$$di = JdA \cos \theta$$

$$= - \sigma \frac{\partial V}{\partial r} dA \qquad \ldots\ldots (1)$$

where $\frac{\partial V}{\partial r}$ is the radial potential gradient (i.e. at right angles to, and away from the surface of the root).

If the maximum potential gradient in the plane of dA is also known, the direction of current flow may be obtained in addition. To obtain the total current leaving the root the quantity $- \sigma \frac{\partial V}{\partial r} dA$ is integrated for all areas where $\frac{\partial V}{\partial r}$ is negative; and the total current entering the root is obtained by integrating the same quantity for areas

where $\frac{\partial V}{\partial r}$ is positive. If the bean and bath are carefully insulated so that other current paths are eliminated, these two integrals must, of course, be equal.

Consider now a current tube along which a current di is flowing (Figure 9b). The current leaves the plant surface through dA_1 and re-enters it through dA_2. The potentials (relative to an arbitary origin) close to these areas are V_1 and V_2 respectively.

The electric power dP dissipated in the current tube is therefore given by

$$dP = di\ (V_1 - V_2)$$

$$= -\sigma\ (V_1 \frac{\partial V_1}{\partial r}\ dA_1 + V_2 \frac{\partial V_2}{\partial r}\ dA_2)$$

$$\text{since } di = -\sigma \frac{\partial V_1}{\partial r}\ dA_1 = +\sigma \frac{\partial V_2}{\partial r}\ dA_2$$

Thus the total power dissipated in the surrounding medium,

$$P = -\sigma \oint V \frac{\partial V}{\partial r}\ dA \qquad \ldots\ldots \qquad (2)$$

i.e. the total power dissipated in the external medium by the bioelectric source can be estimated if the values of potential and radial potential gradients are known at all points close to the root surface.

IV.3 Method of Measurement and Computation.

It was decided to make measurements based on the relationships derived in IV.2 to determine the total current and power generated by the root in the surrounding medium.

Two measuring probes were mounted on the same manipulator so that they could be moved together in the vicinity of the root. The

FIG.9. (a) The electric field strength $\underline{E}$ in a medium of conductivity $\underline{c}$ adjacent to an area $\underline{dA}$ of the plant surface. The plant is shaded.

(Section IV.2)

(b) A tube of current flow in the external medium.

(Section IV.2)

(c) A simplified model of the bioelectric current circuit inside and outside the root. E is the effective bioelectric emf., and $\underline{r}$ & $\underline{R}$ are the effective internal and external resistances respectively. The current i and the power dissipated in the external medium are given by

$$i = \frac{E}{r+R} \quad \text{and} \quad P = R\left(\frac{E}{r+R}\right)^2$$

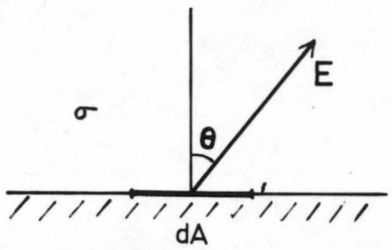
(Section IV.4b)

(d) Diagram showing the "sheath" (dotted line) surrounding the root. The two circles a distance $\underline{d}$ apart represent the ends of the scanning probes, and the sheath lies between these probably a little closer to the inner probe.

(Section IV.3)

(e) Illustrating the postulated ionic movements forming the electric current. In the external KCl solution the current is mainly carried by K^+ and Cl^-, but in the root and across the boundary into the external solution it is carried by H^+ and HCO_3^- (or CO_3^{--})

(Section IV.5)



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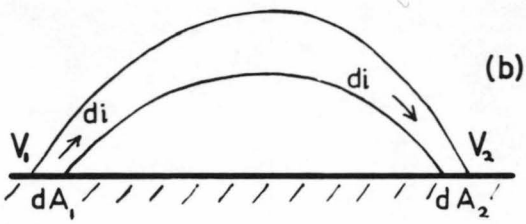

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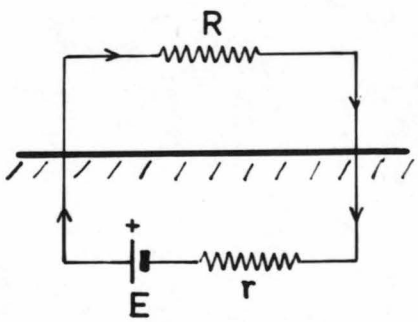

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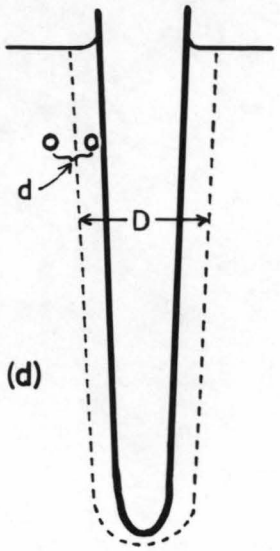

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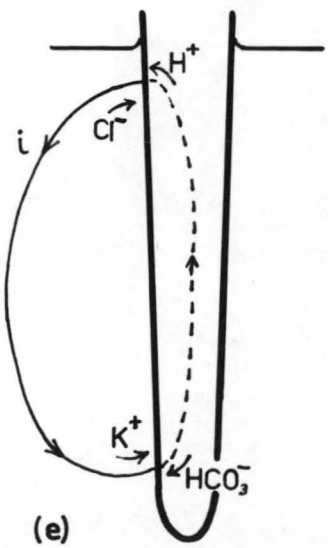

(e)

probe tips were a small constant distance apart, the line joining them being always normal to the adjacent plant surface. (Figure 9d) The plant was mounted on an insulated holder with only its root immersed and could be rotated about the vertical axis of the root so that all sides of it could be measured. In other respects the apparatus was the same as in section III.4. The temperature of measurement throughout was 25°C.

The probes were first moved along the root as close as possible to its surface and the potential V of the nearer one measured relative to a distant point in the bath. The electrometer was then switched to read the potential δV between the tips, and the scanning was repeated. Measurements of V and δV were obtained on four sides of the root by rotating it, and finally the first side was rechecked in order to find whether the potential of the plant had changed significantly during the period of measurement. Typical curves for V and δV along one side of the root are shown in figure 10a. Note that V and δV are zero in this case at about the same position on the plant indicating that the zero equipotential surface is normal to the plant surface at this point. This, however, is not always found to be the case.

For the reasons given in section III.2, the measuring probes were filled with the same solution as was in the bath. Because this was frequently only weakly conducting the tips were made rather large in diameter (0.5-0.7 mm) in order to keep their electric resistance reasonably low and so avoid undue electric pick-up in the measuring circuit. The separation of the tip centres, d, was 1.5 to 2.0 mm.

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It therefore must be made clear that the quantity $\frac{\delta V}{d}$ does not measure the radial potential gradient at the plant surface, but at a point somewhere between the tips, estimated in most cases to be 1.2-1.5 mm from the plant. These measurements therefore do not permit the determination of the total currents flowing from the root or the total power it dissipates, but only those currents which pass through a sheath surrounding the root nearly 1.5 mm from its surface, and the power dissipated in the medium beyond this sheath (see figure 9d). The average root diameter is about 3mm. Calculations of total current and power beyond the sheath therefore underestimate the total output of the root by an amount which would be considerable in a strongly conducting solution, in which most of the current presumably flows close to the plant surface.

In the case of the salt concentrations used in the present experiments, practically all the current flows well out from the root (the radial component of E being in most regions greater than the component along the root) (see figure 11), and it is estimated 90 percent or more of the total current flows through the sheath and is therefore measured.

Some further slight inaccuracy is to be expected due to the distortion of the field caused by the presence of the measuring probes in it.

In spite of these limitations, the use of the method is considered to be justified, and further improvements of technique should increase the accuracy of the results.

In the computation of current, the total current passing through

FIG.10 Computation of current and power

(a) Graphs of the potential V near the plant surface, and the potential difference, δV, between two probes with their tips arranged radially, plotted against the distance, l, from the root tip along the plant.

(Section IV.3)

(b) The current per unit length per quadrant flowing through the plant surface $\left(\frac{\partial i}{\partial l}\right)_A$, plotted against distance from the root tip.

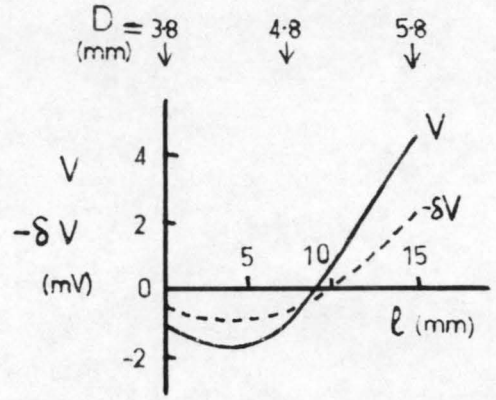
(Section IV.3)

(c) The power per unit length per quadrant $\left(\frac{\partial P}{\partial l}\right)_A$, plotted against distance from the root tip.

(Section IV.3)

0·0001 N KCl

Quadrant A



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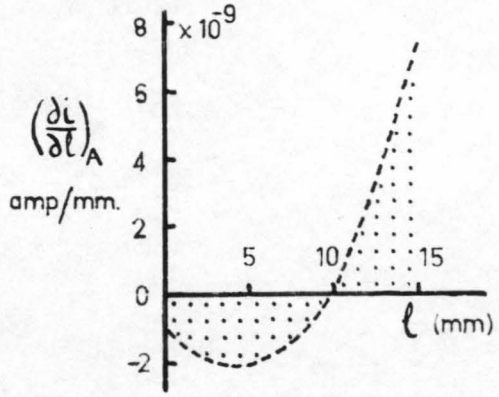

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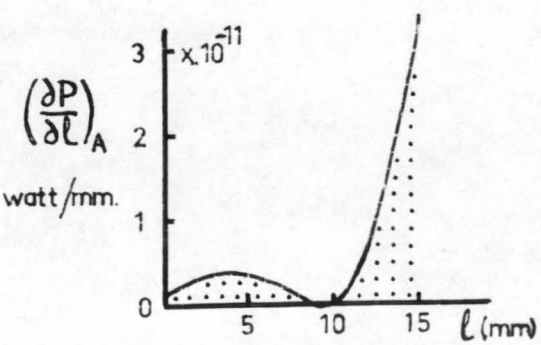

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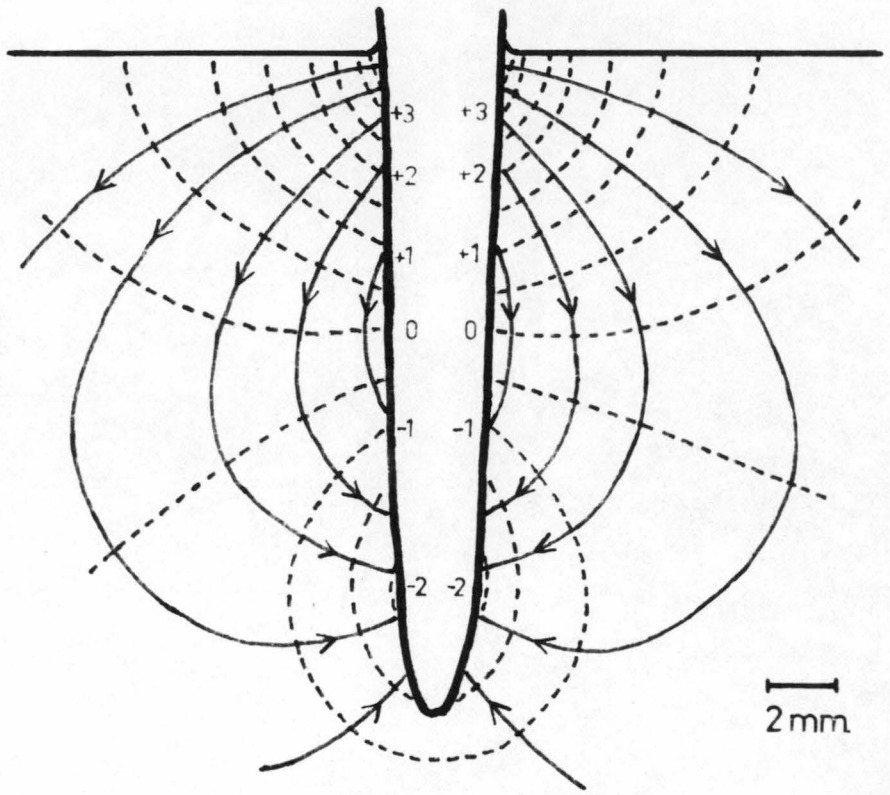


FIG.11. Typical simplified pattern of the current paths through the 10^{-4}N KCl solution in which bean root is growing. The full lines are typical current paths (lines of force) and the dotted lines are equipotentials. The numbers refer to the potential in millivolts of the adjacent equipotential lines in the solution.

(Section IV.4a)

Although in other investigations external current paths have been largely eliminated by having the root surface only slightly moistened it is not considered that this would cause the polarity of the root to change. In fact preliminary experiments by the author with roots along which only a thin film of water was running have shown rises in the external potential differences and no suggestion of a reversal of polarity. Roots of different kinds of plants might show a reversed polarity. The only other material measured by the author is the Onion root and this has a pattern very similar to that of the bean root. It seems unlikely that the plants used by other investigators (maize and wheat) would differ from this significantly. In the opinion of the author the reversed readings of other investigators have been due to unsatisfactory measuring procedures discussed previously (section II).

The field lines for actual roots are seldom confined to a plane as shown in figure 11. The potential pattern is usually slightly different on each side of the root, indicating that there is a component of the field strength at right angles to a plane containing the root axis. This, of course, means that the current paths will not be planar but will spiral around the root.

These complex features have been displayed in three dimensional models, a photograph of a typical example being given in figure 12. Equipotential lines have been mapped on the surface of the model, the contours being 1 mv. apart. The heavy black line is at zero potential

relative to a distant point in the bath. The magnitude and direction of the currents are represented by the length and direction of wires projecting from the model at typical points, the current leaving through a circle, and entering through a cross.

An examination of these models shows that there are often pronounced solenoidal components of the field, and frequently small areas of the plant surface are observed through which the current densities may be as much as 20 times the average for the whole plant surface. The current density in this most active region may be as high as 1.10^{-9} amp/mm^2 for a bathing solution of 0.0001 N KCl, and 3.10^{-9} amp/mm^2 for 0.001 N KCl.

It is to be expected that these asymmetric bioelectric patterns would show a correlation with asymmetric patterns of growth or development, such as bending. Although a preliminary examination has so far shown no such correlation, further study is being planned.

(b) Effect of concentration of KCl in the external solution

A series of measurements were made to determine the effect of concentration of KCl in the external solution on the potential pattern and power and current supplied by the root.

Only two concentrations of KCl were used, these being 0.001N and 0.0001N. The conductivity of more dilute solutions was inconveniently low; while in more concentrated solutions, external potential differences were very small, and in addition poor estimates of current and power were expected owing to the relatively large amount of current which would flow entirely within the measuring sheath.

A separate set of roots was used for each concentration. The bath temperature was 25°C and the roots were allowed to settle in the bath for at least two hours before measurement. Roots which were not growing rapidly were discarded.

Data obtained in these measurements are summarised in figure 13 and in the first two rows of table 1.

Salt	Conc.	Conductivity mho cm^{-1}	Number of roots	Mean Growth Rate mm/hr.	Total Current per Root $x10^{-7}$ Amps.	Total Power per Root $x10^{-10}$ Watts.
KCl	0.0001N	$1.50\ 10^{-5}$	14	0.65	0.66 ± 0.21	2.39 ± 1.53
KCl	0.001N	$1.47\ 10^{-4}$	26	0.89	2.16 ± 0.23	3.50 ± 0.60
NaCl	0.001N	$1.24\ 10^{-4}$	5	1.17	1.39 ± 0.19	1.65 ± 0.63
KI	0.001N	$1.47\ 10^{-4}$	5	0.86	2.25 ± 0.24	2.57 ± 0.61
$\frac{1}{2}K_2SO_4$	0.001N	$1.48\ 10^{-4}$	7	1.05	2.80 ± 0.98	3.15 ± 1.80
$\frac{1}{2}MgSO_4$	0.001N	$1.10\ 10^{-4}$	6	0.99	2.79 ± 0.79	4.51 ± 1.86

Table 1: Table giving the mean values of the total current and total power supplied by bean roots to external solutions differing in ionic composition and concentration. The limits given are the 95 percent confidence limits in the mean values

It is seen that the magnitude of the potential difference along the root is approximately halved when a tenfold increase takes place in the external concentration (and hence, approximately, in conductivity). The total external current correspondingly increases by a factor of about 3.2 and the total external power increases by a factor of 1.5.

These changes are not unreasonable, as it must be remembered that

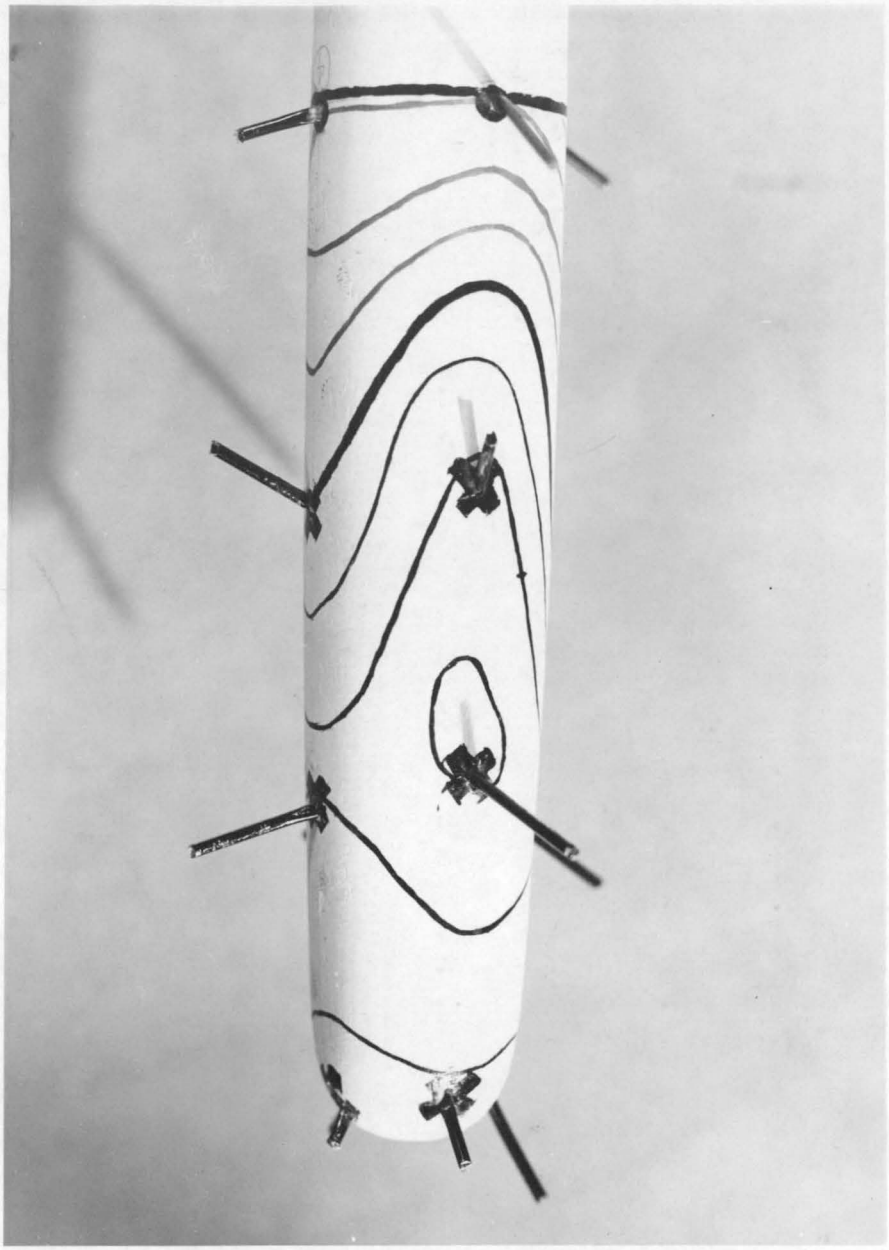


FIG. 12. Photograph of three dimensional model of the bioelectric field in the external medium (10^{-4}N KCl) at the plant surface. The lines are equipotentials 1 mv. apart (the thick line being the zero equipotential) and the currents passing through the plant surface are represented by the wires projecting from the model. The length of the wires is a measure of the current density. The current enters the plant at a cross and leaves at a circle.

(Section IV.4a)

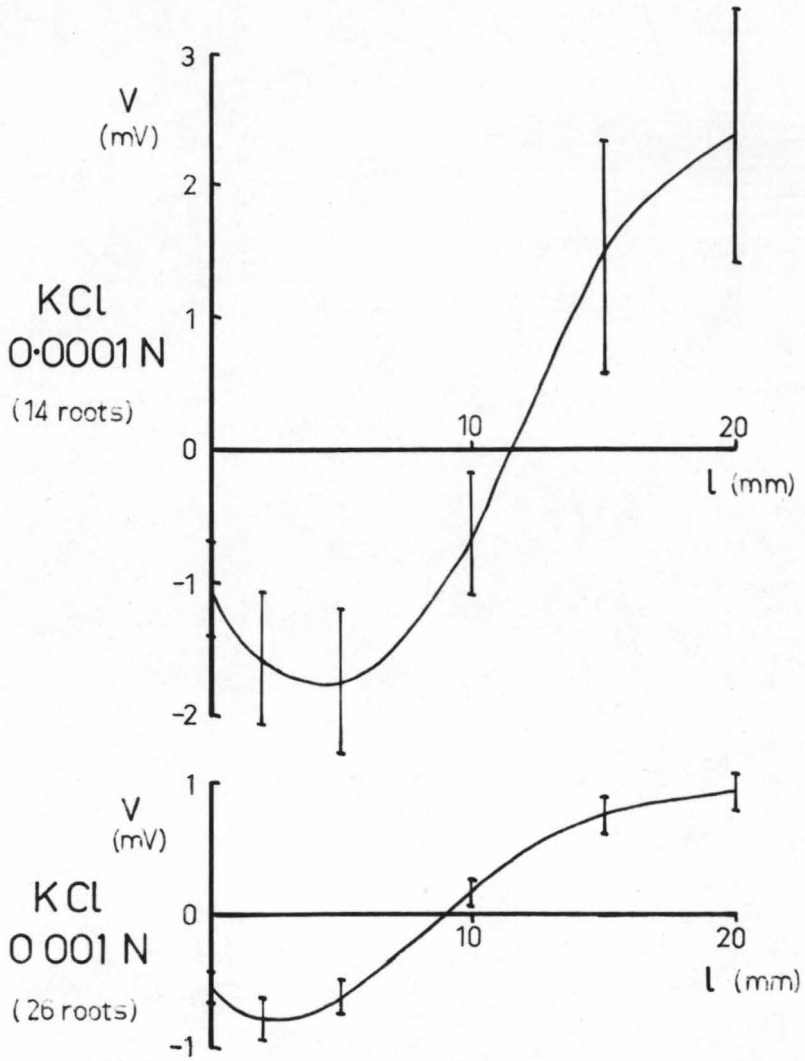


FIG.13. Comparison of the potential pattern in 0.0001 N KCl and 0.001 N KCl. V is the potential near the root relative to a distance point in the bath, and it is plotted against the distance l from the root tip.

(Section IV.4b)

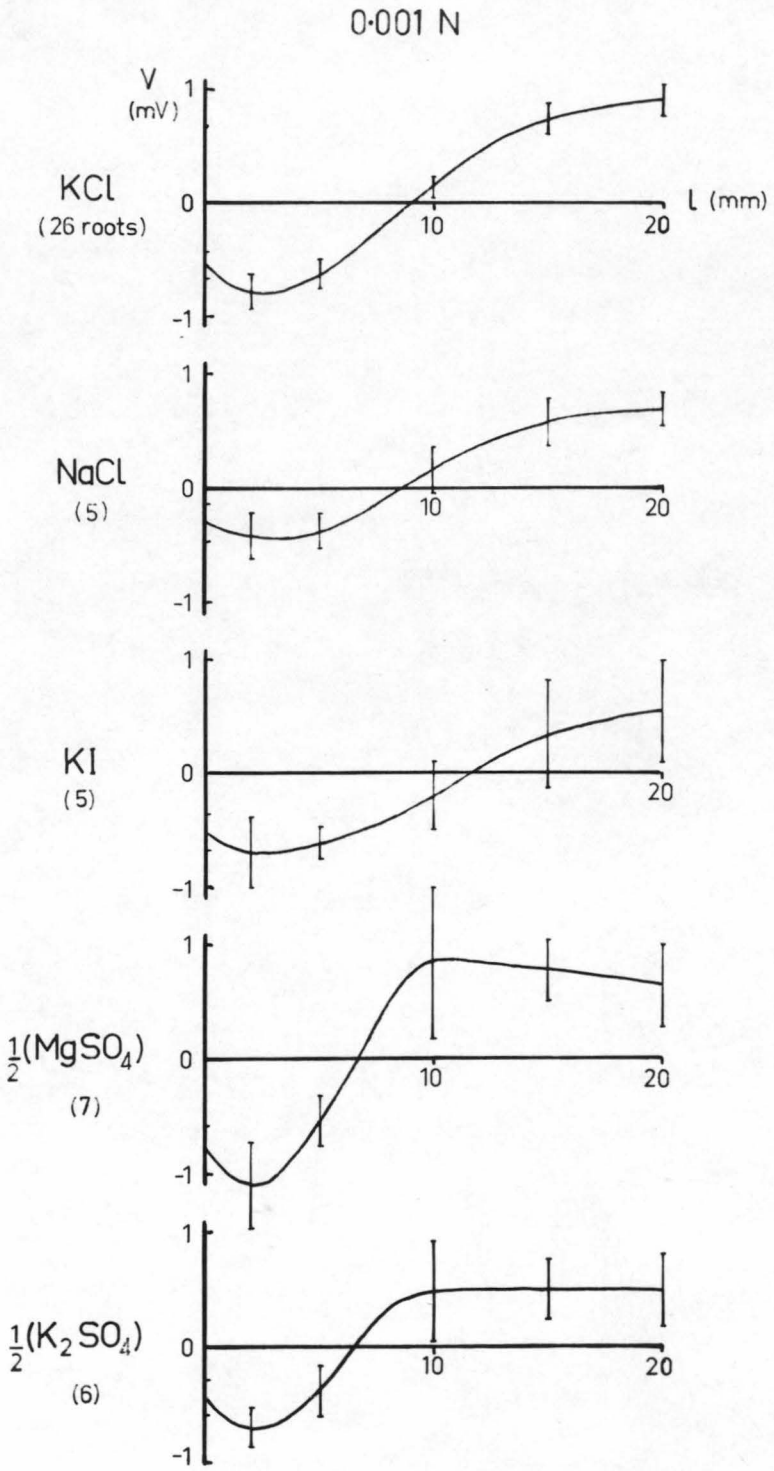


FIG.14. Comparison of the potential pattern for roots measured in solutions of different salts.

the currents are flowing in closed loops through the external medium and returning along paths inside the plant. In these experiments only the properties of the external path are being changed appreciably.

If it is permitted to represent the current path by a simple loop (figure 9c) and to assume that a tenfold concentration change in the external solution causes a tenfold resistance change in the external circuit, but no change either in the internal resistance r or the effective e.m.f. E, it is possible to calculate values of E and r using the measured values of external currents and powers. If this is done, E is found to be about 5.6 mv, and r is 19,000 ohms which is intermediate between the values of the effective external resistance for the two concentrations.

The power dissipated within the root is about $5. 10^{-11}$ in the case of 0.0001 N KCl, and $9. 10^{-10}$ in the case of 0.001 N KCl.

However, it must be pointed out that the above mentioned assumptions considerably over-simplify the problem and it is doubtful whether calculations based on them have any real significance.

(c) Effect of various ions in the external medium.

A series of experiments has been performed to test the effect of different ions in the external solution on the potential pattern and the power and current supplied to the external medium. The results are summarised in figure 14 and table 1.

The external concentration was in each case 0.001N.

In the case of divalent ions the molarity was 0.0005 M so that the conductivities of the external solution were all approximately the

same. HCl was also tried, but roots were found not to grow in either 10^{-3}N or 10^{-4}N solutions.

A different set of roots was used for each salt and these were allowed to settle for at least two hours before measurement. The bath temperature was 25°C. As in previous experiments only rapidly elongating roots were used. The measuring probes, of course, in each case contained the solution being used in the bath.

It will be seen that there is considerable similarity between the potential patterns for roots grown in each of the salt solutions. In each case the most negative region is about 3 mm. from the root tip with the root base 1.1 to 1.7 mv more positive. Only a few roots have been used in some salt solutions giving rise to rather large confidence limits; nevertheless there is no suggestion that major changes in potential pattern result from the use of solutions of different ionic constitution.

The values for total current and total power also appear to be of the same order for each salt. The value for NaCl appears to be significantly low, and although this may be a real difference, the sample is hardly large enough to warrant the inference that less current and power are generated in NaCl than in other ionic solutions.

IV.5 Ions responsible for carrying the bioelectric current.

At this stage it is relevent to consider which of the ions present in the system are responsible for carrying the electric currents generated by the root, and flowing in closed paths partly in the external medium and partly within the root.

In the external medium, the mobilities of all ions used in the present series of experiments are of the same order; hence approximately half the external current is earned by the anions and half by the cations. Cations migrate in the external medium from the vicinity of mature parts of the root to the rapidly elongating region where the potential is most negative, and anions in the reverse direction. Had H^+ or OH^- been present in the external solution in appreciable concentrations, these ions would have carried a proportionally higher fraction of the current because of their much high mobilities.

The situation is different across the root surface. Here the relative concentrations of the ions are not the same as those in the external solution, and their mobilities in the external membranes through which the current must flow are likely to be very different from their values in free solution, and to differ greatly from ion to ion.

Unfortunately there does not appear to be much reliable information about the movement of ions within plant tissue under the action of electric forces. Osterhout (1936) and Blinks (1949) working with large algal cells, and Hope (1951) working with roots have made estimates of the relative mobilities of various ions in membranes by observing the changes in potential between the plant and external solution which occur when the salt concentration in the external solution is changed suddenly.

These calculations are made on the assumption that the simple

Nernst equation for diffusion potentials is applicable. For example Blinks (1949) gives the following values for the ionic mobilities in the outer membrane of Halicystis relative to Cl^- taken as unity:- K^+, 17; Mg^{++}, 1.9; Cl^-, 1.0; Na^+ 0.2; SO_4^{--}, 0.1.

Hope (1951) estimated that K+ was about three times more mobile than Cl^- in the outer surface of bean root.

However, it is not now considered by most investigators that the observed potential changes are due solely to diffusion processes. The presence of immobile anions in the outer membranes almost certainly gives rise to Donnan potentials across the external boundary and these are also functions of the ionic concentration of the external solution. (Teorell (1935), Briggs and Robertson (1948), Hope (1953))

Thus the relative mobilities quoted above may be far from correct. However the Donnan equilibrium does not discriminate between various anions, or between various cations, so that the large difference between the values quoted by Blinks (1949) for K+ and Na+ and for Cl^- and $(SO_4)^{--}$ in the case of Halicystis are likely to be real.

The present investigation has shown that the external electric field, and the total current generated by the root apparently do not depend on the nature of the ions present in the external solution, provided that the normality is not changed. In view of the high probability that the ions investigated would differ greatly in their mobilities within the root membranes, it is therefore suggested that none of them play a major part in carrying the electric current in or out of the root.

The most likely ions within the root which could carry the bioelectric

current are those derived directly from respiration. Starch and sugars are oxidised continously within the tissue with the liberation of carbon dioxide, largely in the form of H^+ and HCO_3^- (or CO_3^{--}). Kosychev (1927) states that the amount of CO_2 liberated per day by roots is equal to about 10 percent of their weight.

These ions are likely to pass through plant membranes more readily than other ions present, and are therefore likely to carry the bulk of the current to the external solution.

The following description of the current-carrying process is therefore postulated (see figure 9e)

When an electric field is set up within and around a root (i) in the external solution cations (say K+) move to the more negative region outside the plant, while anions (say Cl^-) move to the more positive region.

(ii) H+ ions move in the direction of the return current path within the root and emerge from the root in the more positive part of the external solution. HCO_3^- (or CO_3^{--},) ions move in the reverse direction within the root and emerge into the more negative external region.

If this is the case, and the external solution were prevented from mixing it would be expected that the external region with a positive potential would become more acid, and the region with a negative potential would become more alkaline. A simple test with litmus failed to show this, which is to be expected as any effect would be swamped by a general outflow of H_2CO_3 from the root.

The movements of ions carrying the electric current should of course, not be confused with ionic movements due to salt accumulation which may be taking place at the same time.

IV.6 The Amount of Electric Power generated compared with the Total Amount available from Respiratory Processes.

It is of interest to compare the amount of electric power generated by the root with the total amount being released by respiratory processes within the root.

For the case of a root growing in 0.001 N KCl it was estimated earlier (section IV.4b) that 3.10^{-10} watts of electric power is dissipated in the external medium and perhaps another 9.10^{-10} watts within the root.

Doyer (1915) made an estimate of the energy released in respiration based on the amount of CO_2 liberated by young wheat rootlets. He assumed that the CO_2 had arisen from the complete decomposition of starch, according to the equation

$$C_6H_{12}O_6 + 6\,O_2 = 6\,CO_2 + 6\,H_2O + 674000 \text{ cals.}$$

On this basis he calculated the total energy released as about 6.10^3 cal/hr/kgm. of roots which corresponds to a total power of 7.10^{-3} watt/gm. This is likely to be the upper limit of power production and would be less if the starch were not completely broken down.

He also measured directly the rate of liberation of heat energy by the roots. This was about 3.10^{-3} watts/gm.

If these results also apply for bean roots, a typical root (20 mm long, 3 mm diam) would liberate a maximum of 1.10^{-3} watts through respiration, about 4.10^{-4} watts of this being the form of heat. Most of the difference would be used in metabolism.

Thus it is seen that only about one millionth of the total energy produced in the root by respiratory processes is used to cause a current to flow through a circuit partly in the external medium. In this estimate, current circuits wholly within the root have not been taken into account.

It is, of course, true that electric forces are doing work across membranes of individual cells associated with salt accumulation, and the energies associated with these may be considerable. However, they are not the primary concern of this research. It is concerned with the electric fields associated with aggregates of cells and the possible role of these fields in establishing a pattern for the future development of the system.

It might be argued that the electric energy would have little bearing on the development of the root, being so small a part of the total energy of the root. Although this may be so it should be borne in mind that this form of energy has special properties which make it convenient for communication and pattern formation. The oscillatory and wave-like behavior of the electric field described in section V suggest special features of this kind.

If the electric field controls development, considerable power

amplification is necessary. This could be available if the electric field controls the movement of the plane hormone (auxin), minute quantities of which are capable of causing considerable changes in a plant system.

V. RHYTHMIC OSCILLATION OF THE ELECTRIC PATTERN GENERATED BY A BEAN ROOT GROWING IN WATER AND A POSSIBLE FEEDBACK MECHANISM RESPONSIBLE FOR IT.

V.1 Introductory Discussion

In sections II and III a new method of measuring the bioelectric fields generated by plant roots was described (see also Scott, McAulay and Jeyes, 1955). A root was grown vertically in a bath of water made weakly conducting with KCl. Potential differences were then observed between points in the water adjacent to the root due to electric currents generated by the root and flowing through the water.

This method was claimed to be an improvement on those employed in previous investigations. Interference with the normal growth of the root by the measuring process was avoided, leading to results which appeared to be more consistent than those reported previously.

In earlier sections the steady electric pattern generated by a normal, growing root was described. A more detailed study of this pattern has shown that under some circumstances large periodic fluctuations in the electric potentials are generated (McAulay and Scott, 1954). These oscillations are superimposed on the steady or background pattern. They are sinusoidal in form and have periods of the order of five minutes, amplitudes of a few millivolts and may persist for several hours.

The observed oscillatory pattern and the conditions under which it is generated will be described in detail in section V.3.

Study of these variations in electric pattern has been made practicable by the construction in this laboratory of apparatus which automatically

measures and records at frequent intervals electric potential differences between a number of points in the bath adjacent to the root. This apparatus will be described in section V.2.

A new apparatus for measuring and recording the rate of elongation of a root simultaneously with the measurement of its electric potential pattern is also described in section V.2. This apparatus was constructed in order to discover whether rhythmic oscillations of rate of growth accompanied the electric oscillations. Observations made with this equipment are described in section V.3d.

The observation of rhythmic changes in the electric field of roots has raised the general question of the cause of these and similar oscillatory phenomena associated with biological material which do not appear to be related to any corresponding oscillatory changes in the environment. Possible mechanisms for the generation of such oscillations will be discussed in section V.4 and in particular a possible source of the electric oscillations described in this paper will be suggested.

V.2 Apparatus and Materials.

(a) Automatic Apparatus for Measuring and Recording Potential Differences between a Number of Points near Plant Roots grown in Water.

The apparatus used for automatically recording bioelectric potential differences near bean roots growing in weakly conducting water is shown in figure 15. A schematic diagram is given in figure 16.

The basic recording instrument used was a Cambridge six

channel, quick acting recorder with a maximum full scale deflection of 1 mV. This recorder produced dots of different colours to distinguish the different channels. The time between successive dots was five seconds so that each channel was recorded at half minute intervals. For most of the work described in this paper the chart was driven at 1 • 5" per hour.

The recorder had to be modified so that it could be used with an electrometer stage input. This was necessary because the input resistance of the recorder was only 1000 ohms, whereas the input resistance should be of the order of 10^{11} ohms for bioelectric measurements in order to prevent undue current drain and resulting polarisation of the source. The electrometer used a pair of matched ME 1400 valves in a balanced circuit and was designed to be as insensitive as possible to changes in HT or LT voltage. Currents flowing in the input circuit of this stage were never more than 10^{-12} amperes under the condition of measurement. The circuit diagram of the electrometer stage is shown in figure 17.

A photograph of the measuring bath is shown in figure 18. It was constructed of "Perspex" and had the dimensions 11"x5"x3". It was filled with 0.0001 N KCl solution which was aerated, stirred, and temperature controlled. For most of the measurements described in this paper the temperature was controlled at 25°C, although one or two experiments were conducted at temperatures as low as 17°C.

The plant under investigation, P, was mounted in the tank with the root immersed vertically (figure 19). The ends of lengths of

transparent "Nylex" tubing, N, (3 mm bore) were brought close to the surface of the root at various points along its length. The other end of each tube dipped into an insulated plastic cup C, the cups and tubes being also filled with 0.0001 N KCl.

Tubes came from as many as five points near the plant. A sixth tube came from a point in the bath some distance from the plant; this was used as a reference (see later).

Each of these cups was connected in turn to the measuring circuit by means of a liquid switching arrangement. Associated with each cup was a glass bridging tube B (also filled with the same KCl solution) which could dip into it and make a connection across to a small 0.0001 N KCl bath, R, at the rear of the main bath and insulated from it. The bridging tube made this connection when the solenoid, S, on which it was mounted was energised. The six solenoids were energised in succession, synchronised with the recorder output channels (colour of trace on chart).

One of the electrodes E (calomel half cells) dipped into the rear bath, and the other into the main bath at a point some distance from the plant. These electrodes were connected to the electrometer.

Thus it is seen that each point near the plant was connected in turn to the rear electrode via a path which was entirely of 0.0001 N KCl.

Water was nowhere allowed to become stagnant. A fresh supply dripped into the rear bath and passed through the bridging and "Nylex" tubes to the main bath where the excess dripped out at an overflow.

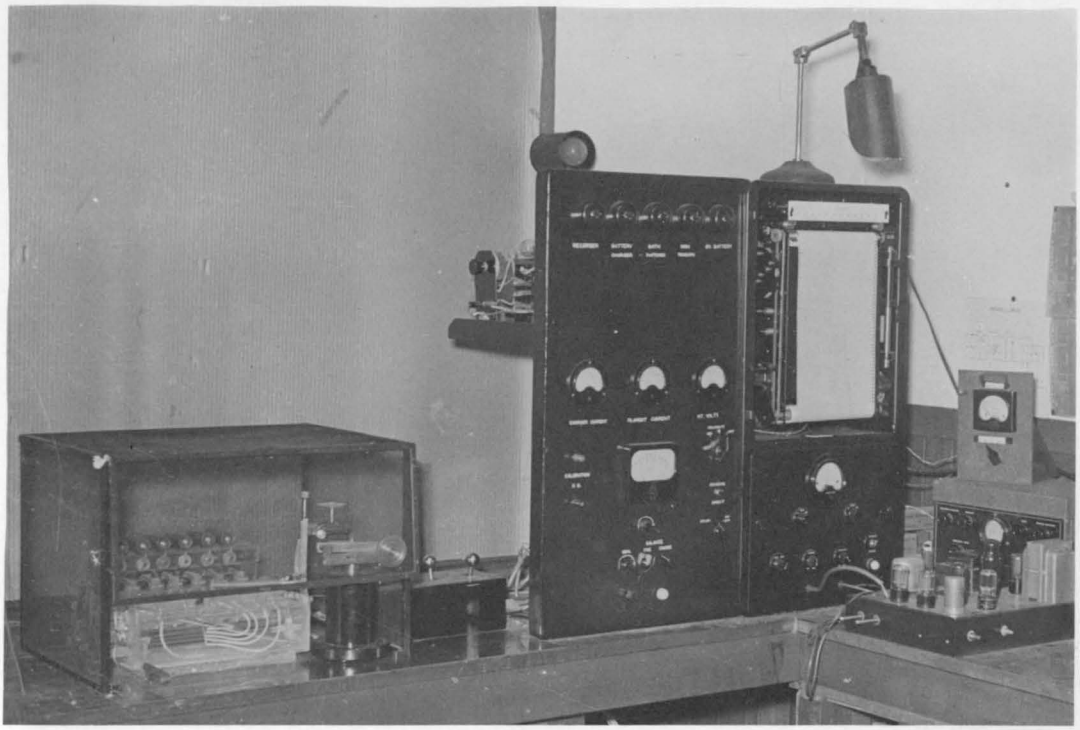


FIG.15. Apparatus for making automatic records of plant potential differences.

(Section V.2a)

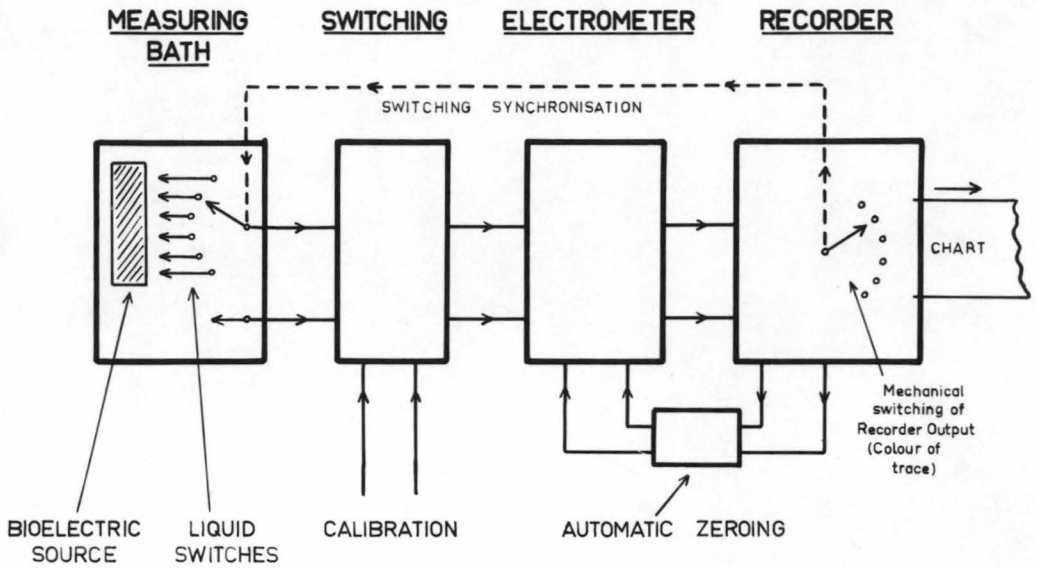


FIG.16. Schematic diagram of the automatic apparatus for measuring plant potential differences.

(Section V.2a)

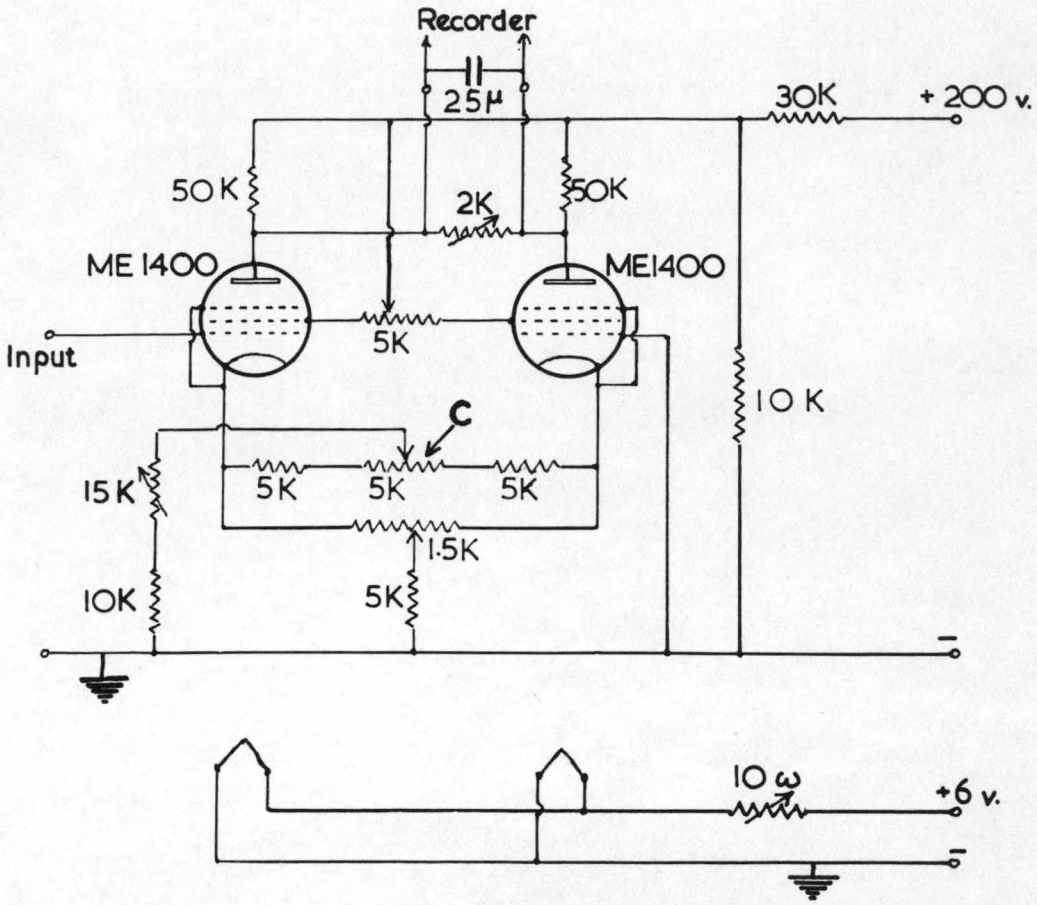


FIG.17. Circuit diagram of the electrometer stage used in the automatic recording apparatus.

(Section V.2a)

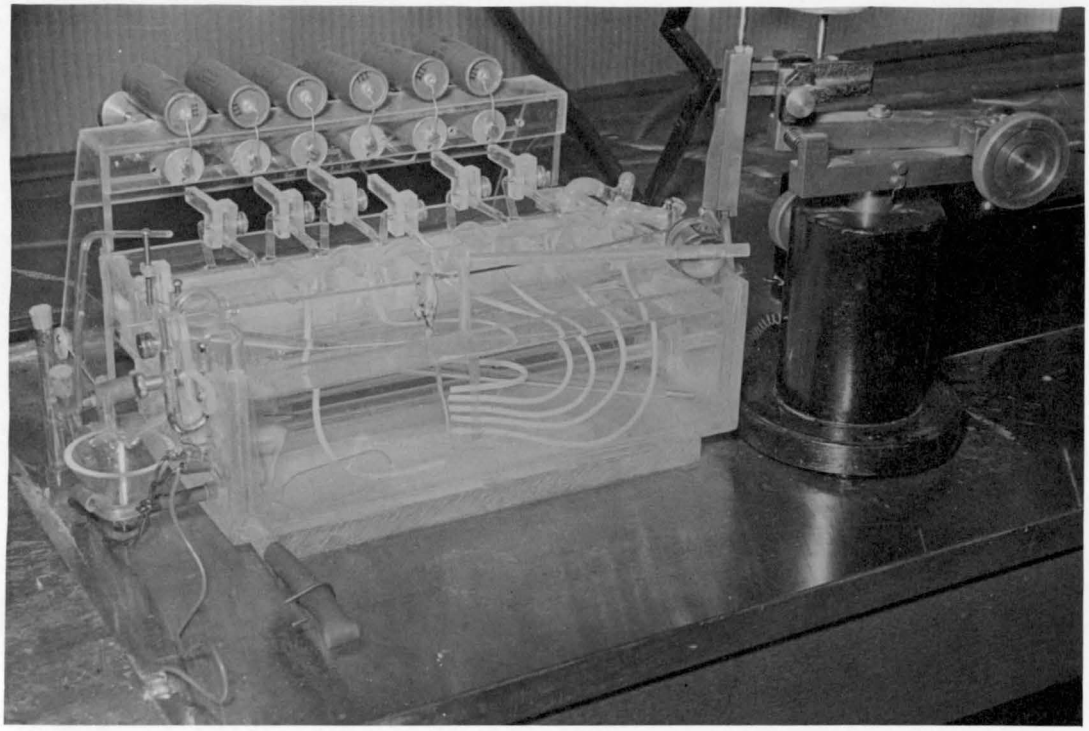


FIG.18. Photograph of measuring bath showing a bean set up for measurement.

(Section V.2a)

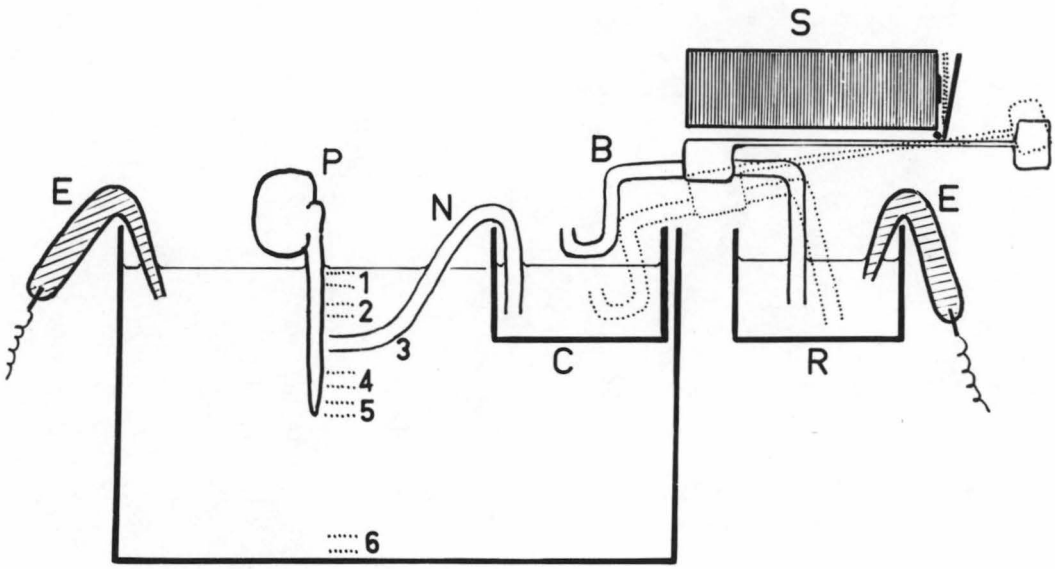


FIG.19. Section through the measuring bath to illustrate the liquid switching arrangement. For simplicity only one of the liquid conducting paths from the plant to the rear bath is shown.

(Section V.2a)

It might appear that the rather complicated bath switching arrangement described above which required high insulation could have been avoided if, instead of using a single pair of electrodes and a single electrometer, separate electrometer stages and electrodes were used for each channel. Selection of the various channels for measurement could then be done by a relatively simple metallic selector switch in the recorder itself.

The former system was used, partly to avoid duplication, but more especially because electrometer circuits and half cells are never absolutely stable, but show slow potential drifts over long periods of time. In order to determine whether an observed change in potential is a genuine plant change or due merely to a drift in the measuring apparatus, it is necessary to "switch the plant out", i.e. to measure the potential at a point in the bath remote from the plant and where the potential is presumably unaffected by it. To obtain a reference reading with an automatic recorder it is therefore necessary either to move the measuring probe away from the plant to a distant point at frequent intervals, or to use some sort of bath switch such as that described above, and for one channel to switch to a distant point.

In the present case the reference potential was recorded at 30 second intervals. For convenience in reading the charts any drifts in the measuring apparatus were automatically compensated for, so that the reference trace always showed no deflection from zero on the chart. A phase sensitive motor which drove the zeroing control, C,

(Fig 17) in the electrometer was energised through contacts in the recorder whenever the reference potential differed from zero.

(b) Growth meter which Automatically Records the Rate of Elongation of Plant Roots growing in Water.

It was desired to find a sensitive method of measuring the rate of elongation of a root in order to test whether rhythmic variations occurred in this rate and if so whether they correlated with the electric rhythms detected with the recording apparatus (section IIa) and described in detail in section III.

The rate of growth of a moderately active root is about 10μ/min. Consequently in order to observe small oscillatory variations in this rate, the periods of which might be only a few minutes, it was desirable that increases in length of about 1μ should be detected. In addition to this requirement the method had to be suitable for use with roots growing in water, it should not interfere with the potential measuring apparatus and it should lend itself readily to automatic recording of the growth rate.

None of the conventional methods appeared to be suitable. Optical methods could not easily be applied. Auxanometers of the type described by Koningsberger (1922) and recently modified by Ranson and Harrison (1955) could not be used because of electric interaction and the difficulty of keeping the switch contacts dry.

The method which was finally chosen was suggested by Dr. D.B. Idle of the University of Brimingham, (personal communication). Fig. 20 is a photograph of the apparatus, the principle of which is shown diagrammatically in figure 21. 0.0001 N KCl solution from

a constant head (about 120 cm of water) flowed along a tube in which there were two constrictions X and Y which restricted the flow of water by about the same amount. The construction at Y was variable being covered by a flexible flap against which the root tip pressed lightly.

The method made use of the fact that a small increase in the resistance to the flow of water at Y caused a considerable increase in the water pressure between X and Y. This increase caused electric contacts in the mercury manometer to close, and the lift mechanism was activated. The plant was then raised through a small increment (1μ) thereby reducing the pressure on the flap and breaking the electric contact in the manometer. Thus the growing plant was raised up in 1μ steps each time the manometer contacts were closed.

The lift mechanism employed the screw and ratchet of a Cambridge rocking arm microtome and this was operated by means of a solenoid. Power to energise the solenoid came from a relay circuit (Fig.22) as an electric pulse each time the manometer contacts closed. In the event that a single upward step by the plant was insufficient to break the contacts (as would be the case, for example, if the root grew several microns suddenly) it was arranged that pulses from the relay should continue to energise the solenoid at one or two second intervals until the contact broke. The number of increments was counted on a mechanical register.

In order to facilitate comparison of the electric pattern with

the rate of growth, it was decided to record the number of growth increments each minute on the same chart as was used for the potential difference measurements. Each pulse from the relay turned the moveable contact of a variable resistance through a small angle. Because a constant current was passed through this resistance the potential difference across it was proportional to the number of increments. This potential difference was registered by the recorder each minute, one of the channels being used for the purpose. The integrator was then automatically reset to zero to count the number of increments during the next minute.

It is believed that this is the most sensitive apparatus that has been constructed to observe rates of elongation of plants. A rapidly growing root is raised by 1μ increments at intervals of 2 or 3 seconds.

(c) Material

The material used in the experiments described in this thesis was Vicia faba Johnson's Long Pod variety which was grown in water baths at 25°C. In most cases tap water was used, but in some the growth medium was 0.0001 N KCl. The water was circulated and aerated. Plants 2 - 3 days old with roots about 30 cm long were used in most experiments.

V.3 Results

(a) Root as source of electric Oscillations.

Whenever a new phenomenon is being studied it is especially important to check whether all possible artifacts have been accounted for and removed. In the present case careful tests were made to

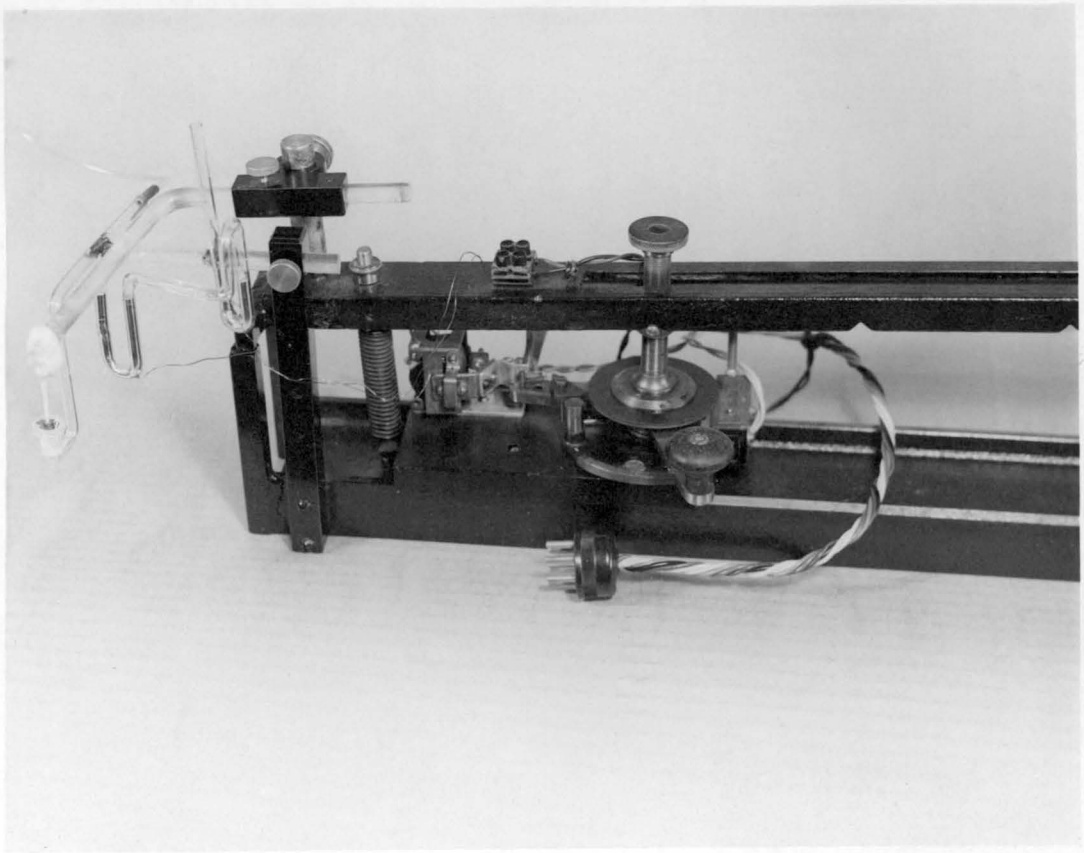


FIG. 20. Apparatus used for measuring the rate of growth of plant roots.

(Section V. 2b)

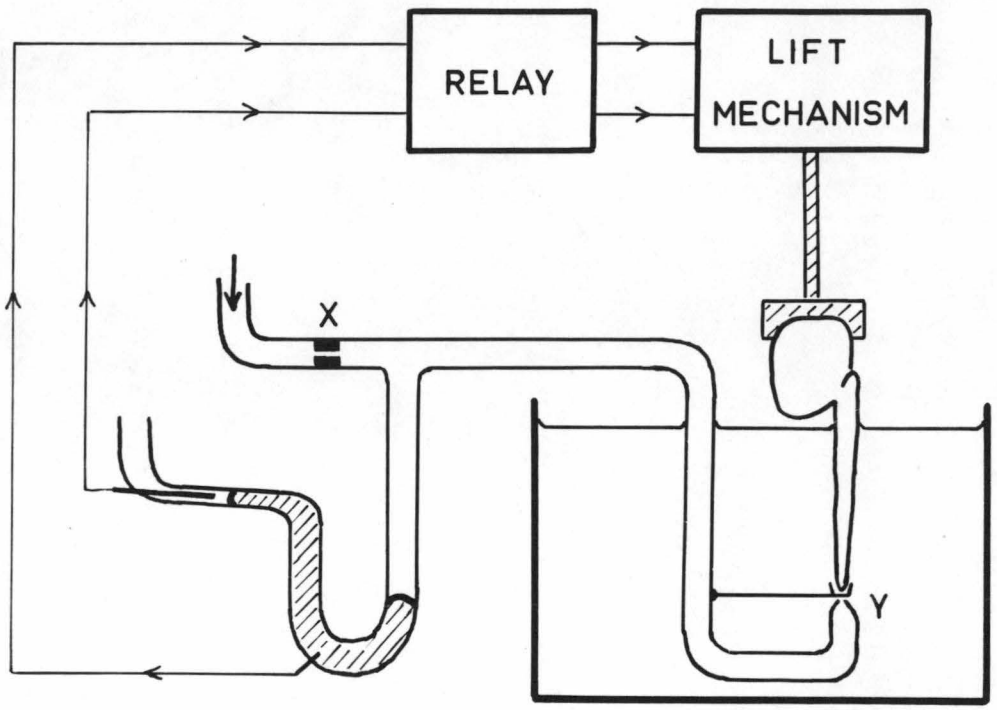


FIG. 21. Simplified diagram illustrating the principle of the rate of growth meter.

(Section V.2b)

RELAY FOR GROWTH METER

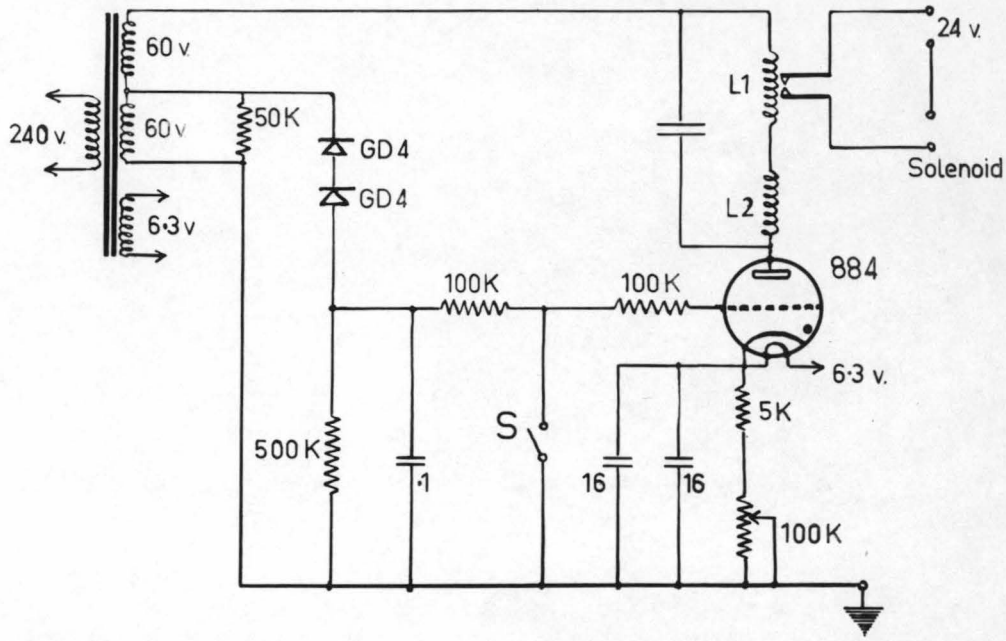


FIG.22. Diagram of the relay circuit used with the rate of growth meter. Pulses energise the solenoid raising the root when switch S is closed. The interval between pulses is determined by the setting of the 100 K rheostat.

(Section V.2b)

eliminate processes other than those due to the plant which could have given rise to the observed electric oscillations.

Such oscillations clearly could not have arisen in the electrodes, electrometer or recorder, as the reference trace giving the potential at a point remote from the root in the bath showed no oscillations.

Since the temperature of the measuring bath was thermostatically controlled it was necessary to check whether the electric oscillations correlated with the switching off an on of the heat. No such correlation was found; in fact the electric oscillations still took place when the thermostat was disconnected. In a similar way it was shown that small fluctuations of height of the water in the bath were not responsible for electric fluctuations. Interaction with neighbouring electrical circuits was also ruled out.

That the root was itself the source of the electric oscillations could be seen most conclusively by moving the measuring probes in the vinicity of the root. The amplitude of the oscillations became smaller as the probes were moved away from the root and varied with the position of the probes along and around the root.

It is therefore concluded that the electric oscillations were generated by the plant itself.

(b) Descriotion of the Oscillatory Pattern.

The results obtained in 26 cases of bean roots which were observed to generate regular and persistent electric oscillations have been analysed. The general features of this oscillatory

pattern will now be described.

When a plant was taken from the growth bath to the measuring bath and allowed to settle for some time (about 1 hour), the electric pattern in the water surrounding the root usually became steady, although slow changes occurred over long periods of time. This steady pattern agreed with that described in sections III and IV, the region in the vicinity of the root tip being more negative than the root base. This indicated that an electric current flowed out from the plant at the root base, entering it again in the vicinity of the tip.

In the case of the group of plants now under consideration, oscillatory variations were observed to be superimposed on this normal steady potential pattern. These oscillations commenced from one to ten hours (with an average of 4.7 hours) after setting the root up for measurement, and continued for an average of 5.8 hours. The average number of oscillations generated by the plant during this time was 55.8.

It was noticeable that the oscillations were very nearly sinusoidal in form. Certainly they never resembled relaxation oscillations. For a particular plant the period of oscillation at all points in its vicinity was the same and remained remarkably constant while the plant was in the oscillatory state. For a typical root, the average period for 42 cycles was 5.68 minutes with a standard deviation of 0.60 minutes.

For the majority of plants studied the period was of this order,

although in four cases it was longer, ranging from 12 to 32 minutes. If these slowly oscillating cases are excluded the mean period for the main group was 5.75 minutes with a standard deviation of 1.02 minutes.

The amplitude of oscillation varied with position along the root. The most active region was nearly always eithin eight mm of the tip. The average over a number of plants for the maximum peak-to-peak amplitude of the oscillation in the most active region was 1.7 millivolts although in two cases amplitudes as large as 5 mv were recorded.

Although the amplitude of oscillation at a particular point usually remained approximately constant over many cycles, it was observed in five cases that the amplitude slowly built up to a maximum value, then fell away to zero, the process being repeated several times. The period of the 'beats' was about 2 hours.

A study of the phase relationships of the oscillations taking place at several points along the root has revealed some interesting data.

In most cases the oscillatory pattern was in the form of a standing wave, the oscillations taking place above and below a particular point (node) along the root being reversed in phase (Fig.23). In some cases two nodes were observed, the oscillations near the tip and the root base being in phase, but opposite in phase to those of a region in between (Fig. 24).

Such phase reversals are to be expected, since a rise in potential of the water near one part of the root must be accompanied by a fall somewhere else along the root, if the electric current

leaving the root is always to balance exactly the current entering it. As large oscillations were often observed near only a relatively small part of the root it appeared that the active source of the electric oscillations in these cases was confined to this region. Other parts of the root were inactive, small oscillations being observed there merely because of the return path of the oscillatory current through them.

In a few cases, (Fig. 25) the phase relationship was considerably more complicated suggesting that a wave of activity was moving along the plant. It is seen that the oscillations were in phase near the root base but closer than 15 mm to the tip, the phase changed with position and was not related in a simple manner to that at the root base.

A pattern of this type might be produced by a root having more than one active source. If this were so, some mechanism would be required to synchronise the sources to oscillate at very nearly the same period, as the phase differences were found to remain almost constant for more than 30 cycles. As suggested above a more plausable explanation is that a disturbance generated by a source moved in the form of a wave along the root thus causing electric oscillations which lag more and more in phase as the distance from the source is increased. In the present example, the observed phase relationship would be consistent with that caused by a wave travelling up the root from the tip with velocity 3.0 mm/min or alternatively down the root with velocity 1.4 mm/min. The phase relationships warrant further study, particularly to test whether the phase changes continuously along the

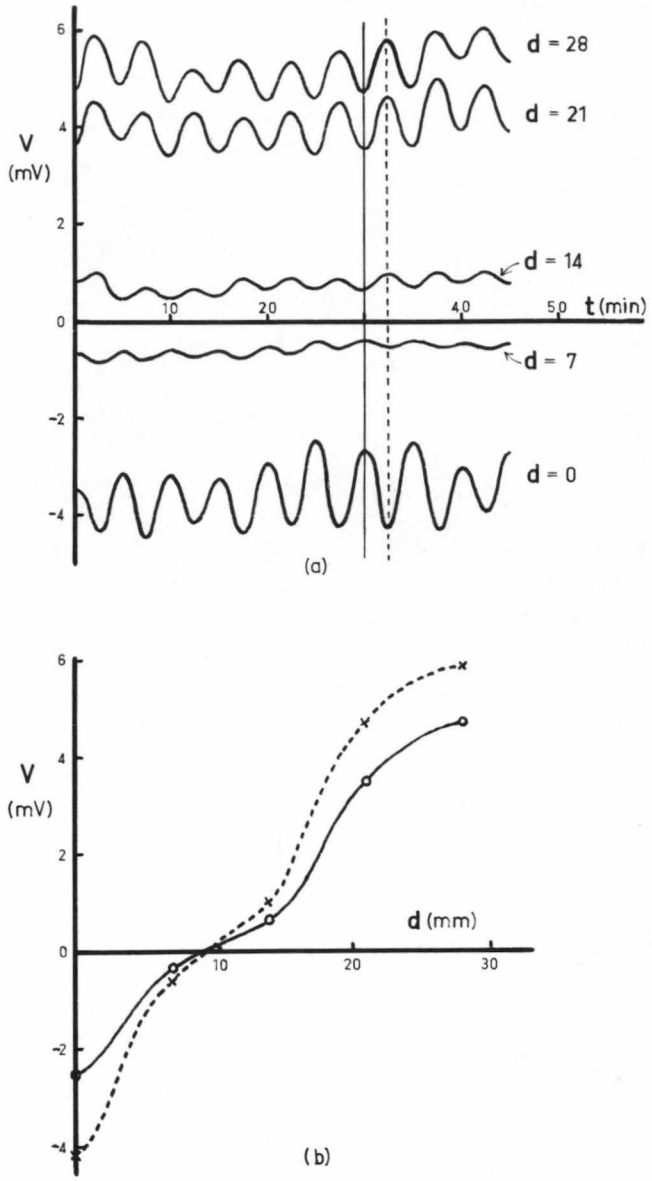


FIG.23. (a) Electric oscillations generated by a bean root. Each graph shows the time variation of the potental V (measured relative to a distant point in the bath) at a point close to the root a distance d (mm) from the tip.

(b) Corresponding graph of V against d. The two curves are the extreme forms of the standing wave pattern a half cycle apart. Note the phase reversal about the node 9 mm from the tip.

(Section V.3b)

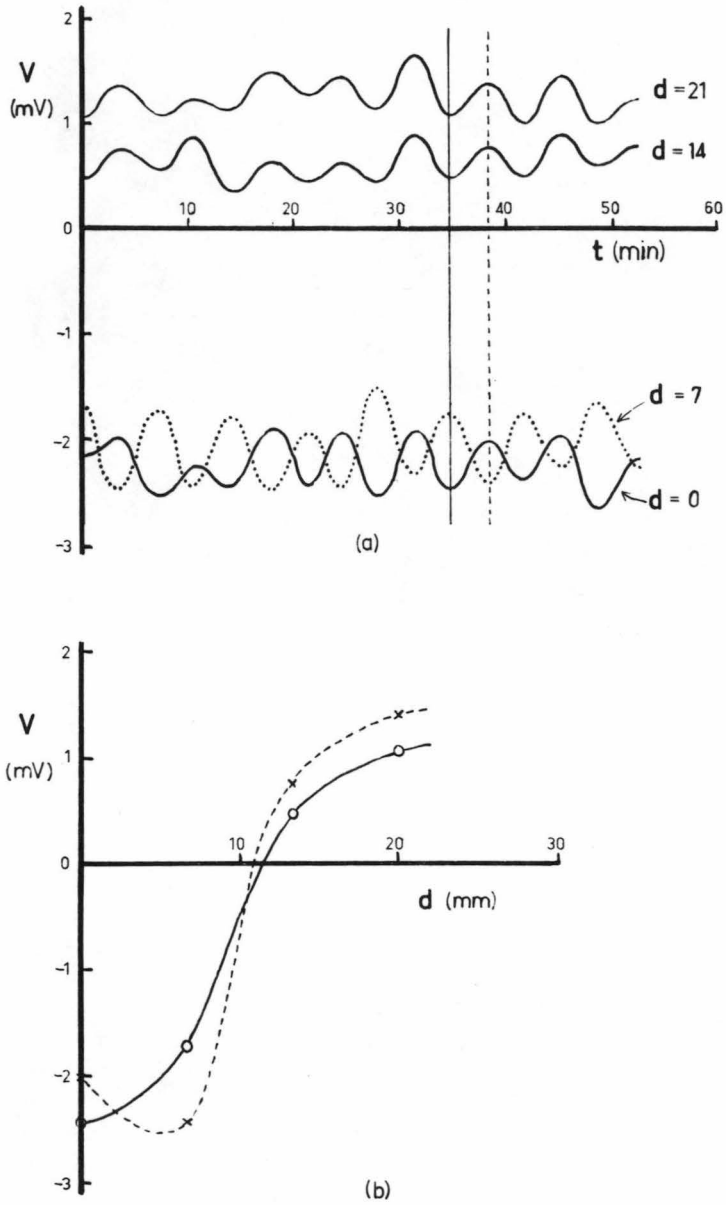


FIG.24. As for figure 23, except in this case the standing wave pattern has two nodes.

(Section V.3b)

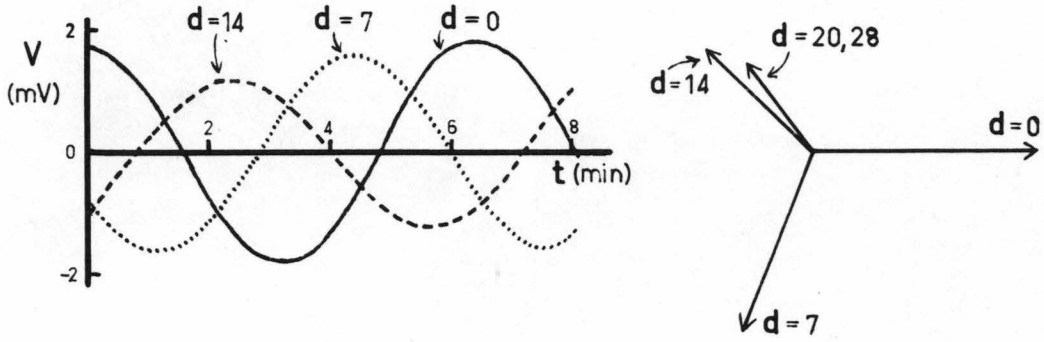


FIG.25. Illustration of the complex phase relationship sometimes observed.

(a) The time variation of the potential V at points along the root d mm. from the tip. For simplicity only the oscillatory component of V is shown.

(b) A vector diagram illustrating the amplitude and phase relationship of the oscillations taking place for various values of d.

(Section V.3b)

root in the active region.

(c) Transient Oscillations resulting from Stimulation.

The electric pattern of a bean root was very sensitive to stimulation and changes in environment, large changes in the electric potential along the root being produced immediately. When the cause of the disturbance was removed, the pattern again becomes practically steady after a time which might range from a few minutes to an hour, depending on the degree of stimulation.

During this transitory time before the steady pattern is restored, the potential changes were frequently in the form of damped oscillations (Fig. 26). Such oscillations almost always occurred when a plant was taken from the tank in which it was grown and set up for measurement. Overshoot processes followed by damped oscillations were also observed to result from such treatments as mechanical stimulation of part of the root, sudden changes in the salt content of the water in the bath, addition of indole-acetic acid to the bath and exposure of the root to air for a short time.

In a preliminary study of these patterns it was found that the period of the transient oscillations was of the same order as the maintained oscillations described in section V.3b, although the variability was greater, both during the oscillation of one plant, and from plant to plant.

Further study of these transient electric changes is planned.

(d) Observations of the Rate of Elongation of the Root.

Since section III of this thesis had shown some correlation between

the steady electric pattern and the mean rate of elongation of the root, it was considered possible that the observed oscillatory electric changes might be accompanied by corresponding oscillations in the rate of elongation.

The rate of elongation of the root was measured and recorded simultaneously with the electric potential measurements, using the apparatus described in section V.2. No special tests were made of the accuracy and reliability of the growth meter, but this was considered to be good judging by the small amount of scatter (less than 5 percent) which was observed in the rate of growth record of some roots. Other roots showed more random scatter than this, and this was considered to be due to a genuine variability in the rate of growth, although no exhaustive tests have yet been made of other possible sources of scatter.

On a few occasions, variations which were approximately periodic were observed, (fig. 27) but these did not occur while electric oscillations were being generated and there appeared to be no correlation between the two. It is of course possible that growth oscillations too small to be detected were taking place during the time of electric oscillation. If this were so the amplitude of the growth oscillations must have been less than about 15 percent of the mean growth rate in order to escape detection.

The lateral movements of bean roots have been studied using time lapse photography. It has been found that the growing root usually spirals around a vertical axis with a period of about 2 hours. So

far there is no indication of a five minute rhythm superimposed on this.

(e) Correlation with pressure applied to the root tip.

A large number of treatments have been applied to a root producing its normal steady electric pattern with a view to causing it to start generating electric oscillations. None of these treatments has been entirely satisfactory. Nevertheless there is some evidence that pressure applied to the root tip tending to prevent elongation is one important factor required for the oscillatory state.

On only two occasions were roots which were not experiencing tip pressure observed to be generating electric oscillations. On 18 other occasions, oscillations were in each case found to be associated with the presence of an upward force on the root tip. In some cases oscillations were produced when the root grew against a fixed obstacle, while in others they were produced when the root tip experienced a constant upward force of about 100 dyne.

These oscillations usually commenced soon after pressure was applied to the tip, and lasted for some time after it was removed, although in one or two cases oscillations ceased while the root was still experiencing pressure. Permanent bending of the root frequently resulted from these tip pressures.

Although the above results suggest that tip pressure is an important contributing factor for the oscillatory state it is not true to say that oscillations always arise if tip pressure is applied. On a large number of occasions roots were subjected to tip pressure and no

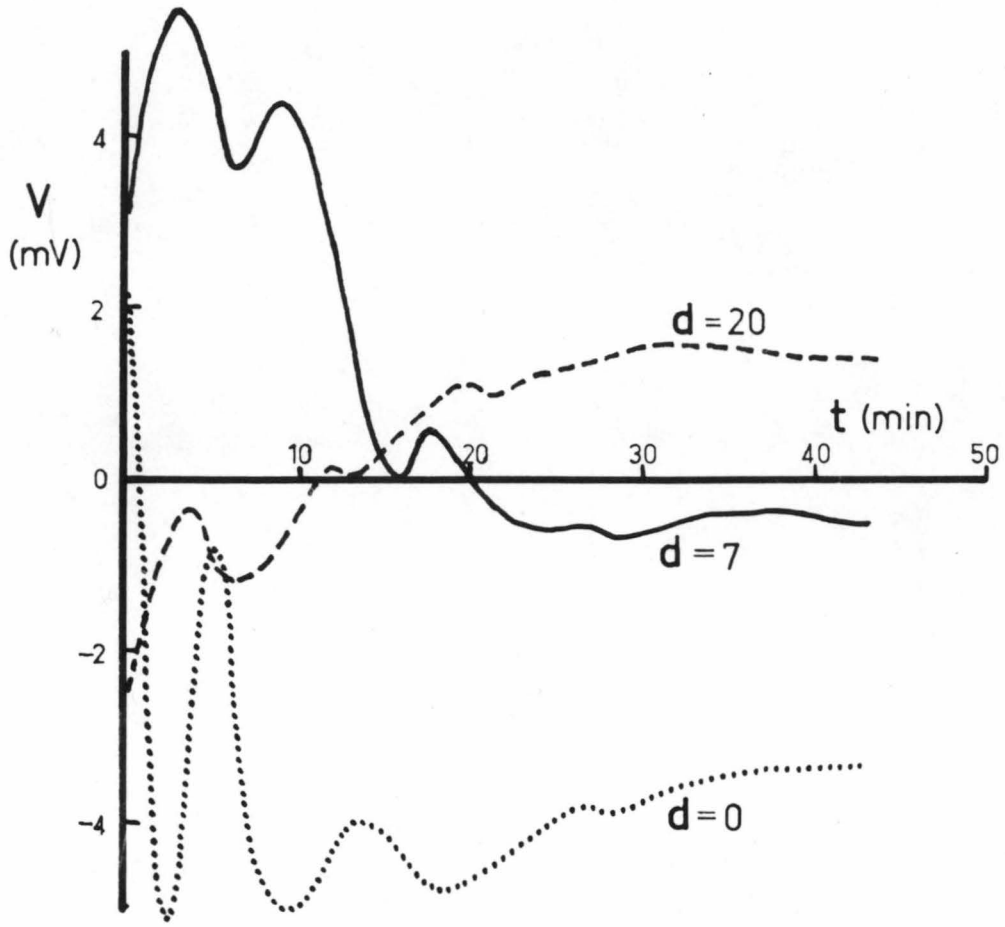


FIG.26. Damped oscillations in the potential pattern following stimulation. The symbols have the same meanings as in previous graphs.

(Section V.3c)

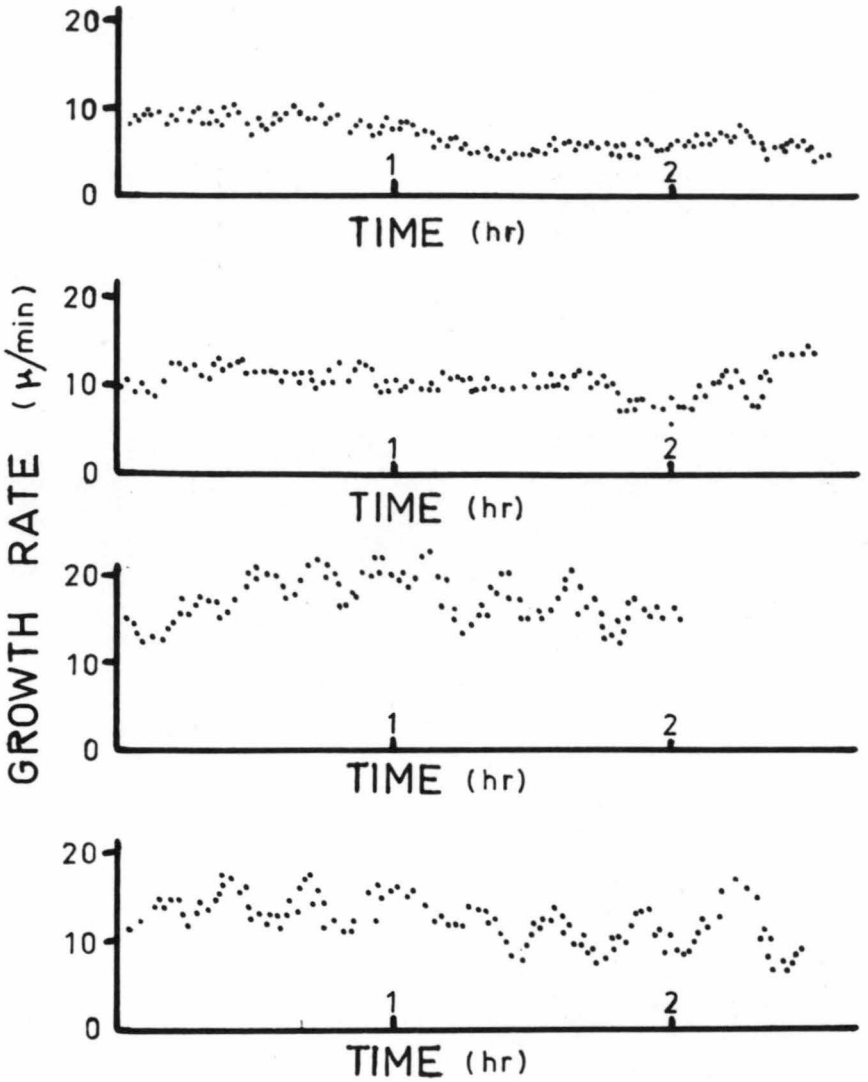


FIG.27 Tracings from four charts showing the rate of growth of bean roots as measured and recorded by the apparatus described in section V.2b. Note the presence of oscillations in the rate of growth in the two lower graphs.

These were not found while electric oscillations were being generated by the root. The period of the growth oscillations is rather longer than most of the electric oscillations.

(Section V.3d)

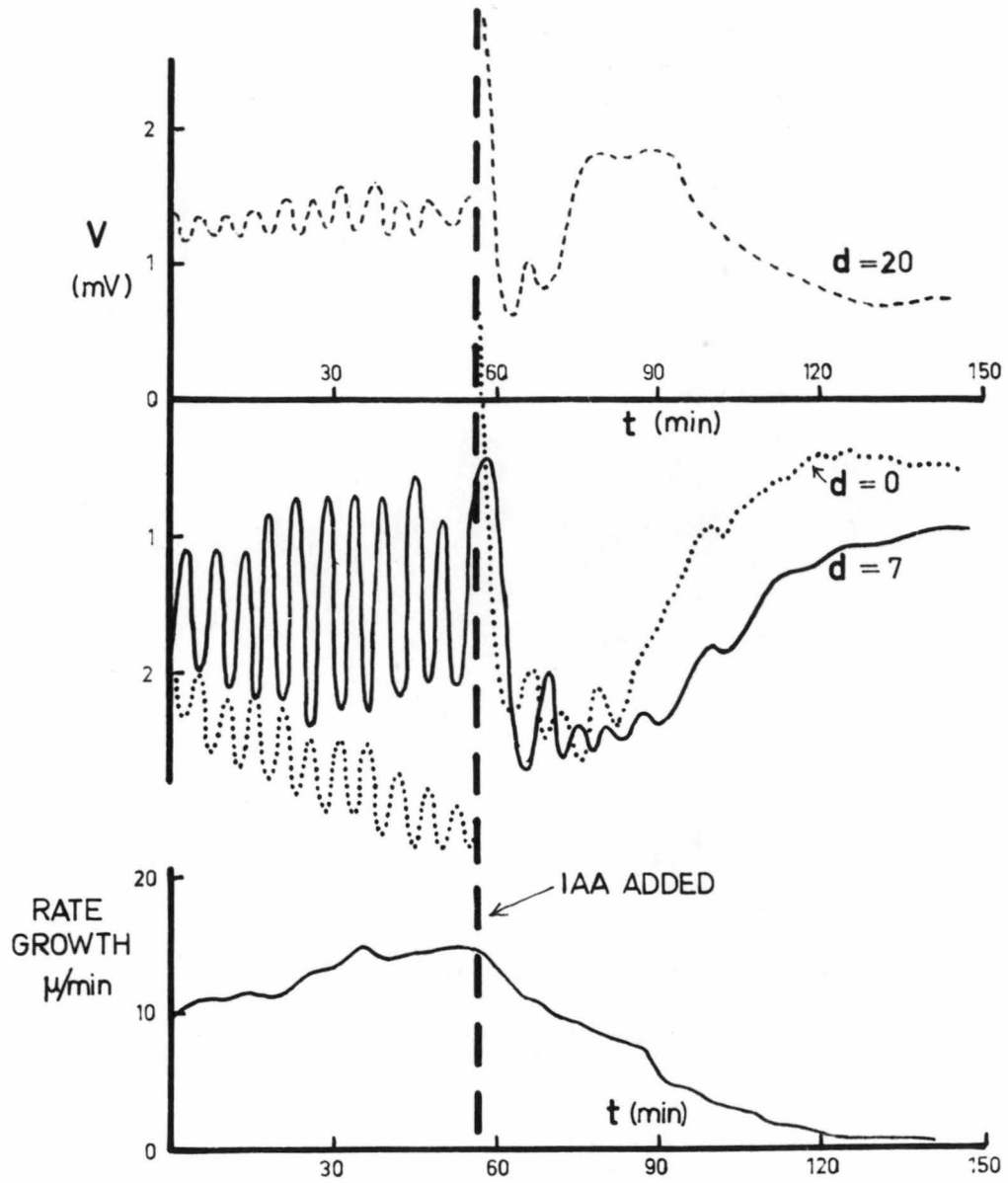


FIG.28. Effect of indole acetic acid on the potential pattern and rate of growth of the root. I.A.A.(2 mg./itre) was added to the bath containing a root generating electric oscillations at a time indicated by thick broken line. The symbols have the same meaning as in previous graphs.

(Section V.3f)

electric oscillations were observed.

These preliminary investigations suggest that the plant must comply with certain other conditions, as yet not ascertained, before it has the capacity to generate electric oscillations. Only then will it oscillate when pressure is applied to the tip.

(f) Effect of Auxin.

In one series of experiments indole acetic acid (IAA) was added to the measuring bath in which a root was generating electric oscillations. The concentration of IAA was usually made 2mg/litre. At this strength the auxin inhibits elongation of the root (see section III.5)

In each case it was observed that IAA had a marked effect on the electric oscillations. A typical example is shown in figure 28. It will be seen that the electric potentials were immediately altered (stimulation effect) after which the oscillation was rapidly damped.

At the same time the rate of growth fell until the root had practically stopped elongating about an hour after the addition of auxin.

After several hours the plant was sometimes observed to generate an oscillation again and at about the same time the root in some cases resumed elongating slowly. As the experiment was performed in daylight it was thought possible that the auxin effect was reduced due to destruction of the auxin by light.

V.4. Discussion.

Rhythmic oscillatory processes are frequently observed in living

systems. Although most of these rhythms are clearly related to rhythmic changes in the environment (diurnal, annual tidal, periodic variations in light or temperature) (Kleitman,1949), several cases are known of rhythms in systems living in an apparently unchanging environment. For example the autonomic to-and-fro or circular movements of elongating shoots, roots, tendrils and rhizomes (Darwin 1880; De Groot 1938; Bennett-Clark and Ball 1951) and the periodic changes in colour and oxygen consumption of the fiddler crab to a controlled environment (Brown, Bennett and Webb 1954). In the above examples the periods of the rhythms are long compared with those of the electric oscillations described in this paper which are also apparently due in no way to rhythmic processes external to the plant. More rapid spontaneous rhythms are also found in biological systems. The heart beat and involuntary breathing of animals and the various electric variations associated with brain and nervous tissue, for example, have periods ranging from a few milliseconds to a few seconds.

Cases of overshoot and sometimes damped oscillation in biological systems following stimulation or a change in environment are also recorded in the literature: Burton (1939); Idle (1955).

Little attempt appears to have been made to explain the mechanism of the internal processes which give rise to the observed spontaneous rhythms, or in any case those with periods of the order of minutes or hours. Although oscillatory behaviour is frequently observed in simple physical systems due to some inertial property (mass, moment of

inertia, inductance etc) the periods of these oscillations are seldom more than a few seconds. Sinusoidal oscillations with periods of the order of five minutes have so little energy associated with them that this would normally be dissipated in a very short time unless it is replenished at just the correct rate.

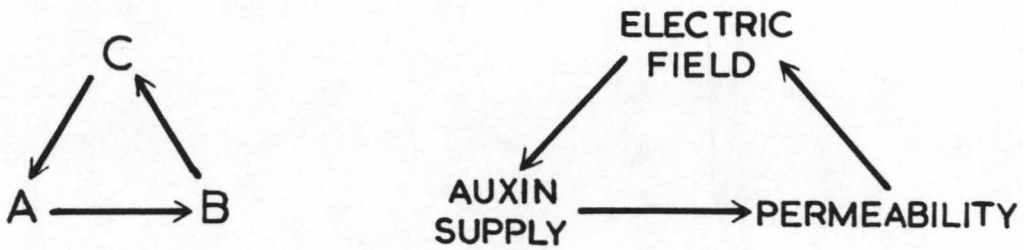
Burton (1939) has shown that overshoot processes may be exhibited by steady state systems, but it does not seem possible to extend his treatment to cover the case of continuous oscillations. Indeed Landahl (1955) has shown that linear steady state systems do not exhibit an observable periodicity.

In considering the cause of the slow electric oscillations described in this paper the possibility that these are purely electric in origin is first of all examined. It is well known that the electric impedance of cell membranes has resistive and capacitative components and it might be suggested that these could form a RC oscillator, say of the Wien Bridge type. For a period of 5 minutes typical values of R and C are 5 megohms and 10 microfards respectively. These values appear to be rather higher than those observed in plant membranes (0.25 megohm cm^2 and 1 microfarad cm^{-2} respectively for Nitella - Blinks 1930 and 1936). Even if it is conceded that the discrepancy in these values is due to the different material, it is difficult to see how the resistances and capacities could combine in the correct manner to form an oscillator. An amplifier with exactly the correct gain would have to be present, and the outputs of the individual cellular oscillators would have to be synchronised so as to give an observable oscillation in the external medium.

The most likely source of sinusoidal oscillations as slow as those described in this paper is a feedback system in which processes that are not purely electric are included in the feedback loop. It is not proposed to consider here in any detail the behaviour of these systems as they have been studied fully in connection with servomechanisms and automatic control processes (Porter 1950). The following simple example (Fig. 29a) serves to illustrate the basic principles.

A, B, and C are three variable quantities which are interrelated in such a manner that a change in A causes a change in B which in turn causes a change in C. If now the change in C causes A to alter in such a manner as to oppose its original change, the system is said to be a closed loop control system or a negative feedback system. In order to gain adequate control the signal fed back is usually amplified (by a factor known as the feedback loop gain) and some external energy source is necessary. It will be seen that negative feedback tends to stabilise the system again disturbing influences and because of this is widely used in automatic control systems. A familiar example is the therostat which is controlled by the temperature of the room, and which in turn governs the rate of flow of fuel to the furnace which heats the room.

Control by negative feedback can never be instantaneous as some time delay in the various processes in the feedback loop cannot be avoided. If either the time delay or the feedback loop gain is too large the system may become unstable and start to oscillate spontaneously.



(a) (b)

FIG.29. (a) General form of a simple feedback loop.

(b) Possible feedback loop responsible for the electric oscillations described in this paper.

(Section V.4)

These oscillations are sinusoidal provided the quantities involved in the feedback loop are related linearly to one another. They may be of any period depending on the time delays occurring in the loop.

Thus it is seen that a system which under normal circumstances is kept stable and resistant to external disturbing factors by negative feedback may lose control and start to oscillate if one or more of the relationships in the feedback loop is altered. If the equilibrium of a system on the verge of instability is disturbed, it executes a damped oscillation before finally returning to the equilibrium state. The rather delicate balance which exists between stability and instability is familiar to those who handle feedback controlled systems.

It will be noted that the root which generates electric oscillations has many properties in common with the systems discussed above. The electric field of the root may be almost steady for several hours when suddenly quite large sinusoidal variations commence. If a plant producing a steady electric field is stimulated, damped electric oscillations result.

It is therefore attractive to suggest that some automatically controlled process involving negative feedback is associated with the growing root. Under normal circumstances the system is stable but only just so, since transient changes are in the form of damped oscillations. Occasionally some change in the properties of the system causes it to become unstable, thereby upsetting the control process.

Not enough is known to say what aspects of the growth of the root

are being controlled and what processes are involved in the feedback loop assuming that one is present. So far no oscillatory variations in the root's behaviour have been found associated with the electric variations. It is possible that the electric field itself is not an active element in the loop but is merely coupled to some other process which is directly involved. If this is the case, the electric field is acting as a very convenient and sensitive indicator of the stability or otherwise of the control system.

Further discussion of feedback processes in living systems will be given in section VI.2.

To conclude this discussion some tentative suggestions as to the sequence of processes involved in the proposed feedback loop will be made. Suppose the auxin concentration in the elongating region of the root controls the permeability of the cell walls to salts (Thimann 1949). This would undoubtedly affect the electric resistance of the plant tissue and hence the magnitude of the electric currents flowing in the plant root and returning via a path in the external medium. If it is now supposed that the electric field modifies the rate of supply of auxin within individual cells or groups of cells to sites which it affects permeability, a feedback loop is thereby completed (Fig. 29b). Under some conditions the feedback system might become unstable and give rise to the observed oscillations.

The possibility that the bioelectric field plays a part in the movement of auxin in plants was suggested many years ago, Went (1932); Kögl (1933).

Although the relationship is certainly not as simple as it first appeared, recent experiments by Schrank and Backus (1951) have suggested that the diffusible fraction of auxin may well be moved electrophoretically.

The fact that swamping the water around the root with indoleacetic acid quenches the electric oscillation suggests that auxin plays some part in the feedback process.

Further search for other changes in the root while electric oscillations are being generated is necessary before the feedback system can be specified with any certainty. Some estimate of the time delays in the processes would then have to be made in order to see whether they are compatible with 5 minute electric oscillations.

VI. CONCLUSIONS AND FURTHER DISCUSSION.

This thesis should be considered as a report of progress in a continuing investigation of the broad problem of the electric fields of plants and their possible roles as organisers. It is not claimed that any large section of the investigation has yet been completed. In the course of the research many matters have arisen which require further study.

It remains now to ask what main conclusions may be drawn from the work so far completed, how do these relate to the general problem of organisations and control, and what further lines of investigation could most fruitfully be followed up at this stage.

VI.1 Main Conclusions from the Present Investigation.

The following main conclusions may be drawn from the work described in this thesis:-

(a) The present method of measuring bioelectric fields can be accepted with confidence, the observed electric pattern and the associated amounts of current and power being in fact those generated by a bean root growing in water when it is in a condition undisturbed by the process of measurement.

(b) The electric pattern of an actively growing bean root is normally steady and persistent and retains its polarity under a variety of conditions (for example of ionic composition and concentration in the external solution). The polarity described in this thesis is a complete reversal of that frequently quoted in the literature (most recently by

Rosene and Lund 1953). In spite of this, it is concluded that the present results, which have been found consistently over a period of four years during which the measuring techniques have developed steadily, are more reliable. Possible causes of erroneous measurements by other investigators have been discussed in section II.

(c) It is concluded that a correlation exists between the potential pattern of the root and its rate of elongation, or some related process.

(d) Only a small fraction (about one millionth) of the total respiratory energy of the root is in the form of electric energy associated with a bioelectric source capable of passing a current through the external medium. This must be borne in mind when considering the ability of the bioelectric field to act as an organiser or controller of plant development.

(e) It is concluded that the ions responsible for carrying the bioelectric current across the plant surface are not those in the external solution, but are probably ions released within the root during respiration.

(f) It has been shown that under some circumstances the root generates regular sinusoidal oscillations with periods of about 5 minutes. Although no great progress has been made in the study of these oscillations, there is an indication that they may have considerable significance. The existence of these oscillations together with the indication in one or two cases of a 'wave of activity' moving along the root (section V.3b) suggest possible feedback control and

communication processes hitherto unsuspected in plant systems.

VI.2 Further Discussion of Feedback Processes in Living Systems.

Many self regulating processes employing negative feedback are now recognised in the higher animals (Wiener 1948; Walter 1953). Examples of these are the control of body temperature, balance, blood pressure, and the CO_2 concentration in the blood. These are all controlled automatically without conscious effort, the controlled state being described as homeostasis. Another example of unconscious control occurs in the coordination of muscular movement, effected also by negative feedback (Eccles 1953).

Walter (1953) rightly stresses the importance of homeostasis to the higher animals. He considers that the development of internal processes which automatically compensate for environmental and other fluctuations has been a necessary factor in the survival of the mammals. Because in man particularly this control is achieved in the lower brain without conscious effort, the main part of the brain is kept free for other functions including the development of mind.

Of course there are innumerable examples of feedback processes requiring conscious effort. For instance, when a pencil is to be picked up, an error signal (the separation of hand and pencil) is fed back to the brain via the eye and this results in the appropriate muscular movement.

Although these feedback processes in man are usually adjusted to

achieve positive control, occasionally overcorrection occurs, resulting in loss of control and oscillatory behavior.

For example, the balance controls acting in an unfamiliar situation may introduce too high a feedback loop gain (a child learning to ride a bicycle 'wobbles'). For a person with the disease known as 'purpose tremor' the feedback process employed in reaching to pick up an object is overcorrected and the hand moves up and down in an uncontrolled oscillation. (Wiener 1948). Recently Cherry, Sayers and Marland (1955) reported that stammering could be almost entirely suppressed, even in bad cases, if the subject were prevented from hearing what he was saying. This can be interpreted in terms of a feedback process. In normal individuals, speech is subject to feedback control through the ear (we tend to shout when wearing earphones). In stammerers the feedback appears to be too great (the stammering being a form of uncontrolled oscillation) and normal speech requires the suppression or reduction of aural feedback.

In view of the widespread occurrence of feedback controlled processes in the higher animals, from which great benefit is derived, it is to be expected that plants and the lower animals lacking nervous systems would have developed rudimentary feedback mechanisms in their struggle for survival which would permit some regulation of their own development and some compensation for environmental changes. Yet hardly any evidence for this has been put forward. Walter (personal communication) has pointed out that in mechanisms of control surprisingly few valid

analogies have been found between plants and animals.

One case in which feedback control probably is involved is the tropic response of plants. For example, in phototropism, unilateral illumination of a shoot tip causes bending as a result of unequal growth on the two sides of the shoot in the elongating region several millimetres below the tip. Schrank's experiments (see for example Backus and Schrank 1952) suggest that a transverse electric potential difference is established in the shoot by unilateral light and this modifies the diffusion of auxin from the tip to the elongating region. Similar mechanisms are possible in geotropism and other tropic responses.

It is of interest to consider what happens when a root, which is growing vertically because of geotropism, encounters an obstacle. It is not known how the information that the root can no longer grow vertically is transmitted from the tip to the elongating region. Although the mechanism has had little study, the root's behavior does not suggest that the bending stimulus is due merely to the transmission of mechanical stresses within the root.

The suggestion from V.3e that electric oscillation often accompanies tip pressure might provide an explanation of this behavior. If the geotropic response is indeed controlled by negative feedback processes involving the bioelectric field, then tip pressure frequently would appear to upset the feedback loop in some way causing the system to become unstable and oscillate. As a result of this the geotropic

response would be temporally suspended and the root tip free to make exploratory movements and so "feel its way around the obstacle".

Although there is little evidence to support these speculations it is of interest to note that almost exactly the same procedure was incorporated in Walter's (1953) mechanical 'imitation' of a living system to suppress temporally its phototropic response when it encountered an obstacle.

VI.3 Proposals for Further Investigation.

It is now proposed to discuss some problems which have arisen in the present investigation on which further work is planned.

It is proposed to study further the electric oscillations of plant roots in the hope of finding definitely whether they are due to an overcorrected feedback system, and if so what sequence of processes comprises the feedback loop. If periodic changes in the electric resistances of the plant's outer membranes are involved it should be possible to detect these by passing a current from an external source through the root. No rhythmic oscillation of rate of elongation has been found which correlates with the five minute electric rhythms. It is nevertheless possible that small lateral movements of the root tip occur with this period. If so they should be detectable with a modified version of the growth meter described in V.2b. At this stage it is difficult to see what other processes possible involved in the feedback loop (such as movement of auxin) would show changes which could be detected if the periods of variation were as short as five minutes.

It is also proposed to study the bioelectric pattern of roots and shoots not immersed in a bulk liquid medium. In preliminary experiments, measurements have been made on roots coated with a thin uniform sheath of water. The water was flowing continuously to avoid the effects of stagnation described in II.2a. This has been achieved by depositing on the root a thin layer of short fibres (made by completely disintegrating filter paper) and the water film being maintained around the root by surface tension. With this technique the fine structure of the potential pattern close to the root surface can be studied more easily. It is hoped later to apply it to smaller structures such as adventitious buds and roots in an early stage of development. The use of one of the other new methods of measurement described in II.3 might also be considered here.

In the near future it is also proposed to apply the present techniques to the study of the effects of externally applied electric fields on plant development. Schrank's results indicating that electric fields induce bending should be confirmed with a careful analysis which indicates how much of the external current passes through the root, what ions are involved and where the current enters and leaves the root.

External fields in a variety of patterns should be applied to the plant to test whether development is affected. In particular the the effect of alternating fields with periods in the vicinity of the natural oscillatory period of the root (about 5 minutes) might be of great interest. One recalls the effect of flickering lights at specific 'resonant' frequencies on epileptics (Walter 1953).

Finally there is the problem of the potential differences and current paths within the root. It is hoped to develop techniques which are considered to be sufficiently reliable to study the internal bioelectric field but this is likely to be postponed until a later stage.

In conclusion it may be stated that the question "Is the bioelectric field a biological field?", is still far from answered with any certainty. The results of the work described in this thesis provide some support for an affirmative answer.

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Vibrating Probe Electrometer for the Measurement of Bioelectric Potentials

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(Received May 22, 1950)

THE instrument described in this paper is intended for the measurement of bioelectric, membrane, and diffusion potentials. The principle of the method has been suggested previously and applied to the study of contact potentials and electrical properties of adsorptive films.[1] Apparently it has not been used for the purposes mentioned above.

The construction of the instrument is shown diagrammatically in Fig. 1. An insulated metallic probe is set into vibration by means of a telephone T, and brought close to the surfaces of the object to be investigated, in this case a plant. As long as a potential difference obtains between probe and surface, the induced charge on the former undergoes alterations with the change of capacity due to the vibration of the probe. Corresponding potential changes are produced on the grid of the electrometer tube E, the output of which is fed to an amplifier A and displayed on a C.R.O. screen as a sinusoidal curve. The amplitude of this curve depends on the potential difference between S and P. The action of the instrument can also be compared to that of a generating voltmeter.

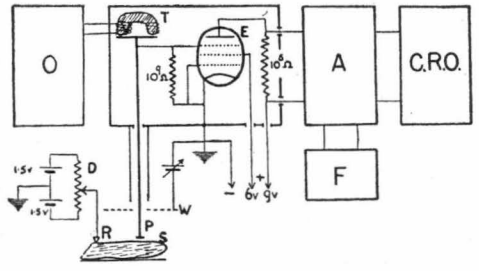

FIG. 1.

With the help of a potential divider D an opposing potential is introduced between surface and ground, which is varied until the trace on the C.R.O. screen is reduced to zero amplitude, when the probe and the opposite region of the surface are at equal potentials. Alternatively the compensation voltage could be introduced into the circuit between cathode and earth.

A photograph of the apparatus is shown in Fig. 2. Telephone and electrometer tube, carefully shielded from each other, are enclosed in a tinplate box which is mounted on a microscope rack allowing vertical movement of the probe with the help of the coarse and fine controls. The object is placed on the mechanical stage of a microscope, and can be moved in the horizontal plane. A reference electrode R is in contact with the object, and connects it to the compensating circuit D. Both platinum and silver-silver chloride electrodes have been used and found sufficiently stable.

The probe vibrates at a frequency of 280 sec.$^{-1}$. A 959-acorn tube operated at reduced potentials with $G3$ as its control grid to reduce grid currents[2] was chosen as

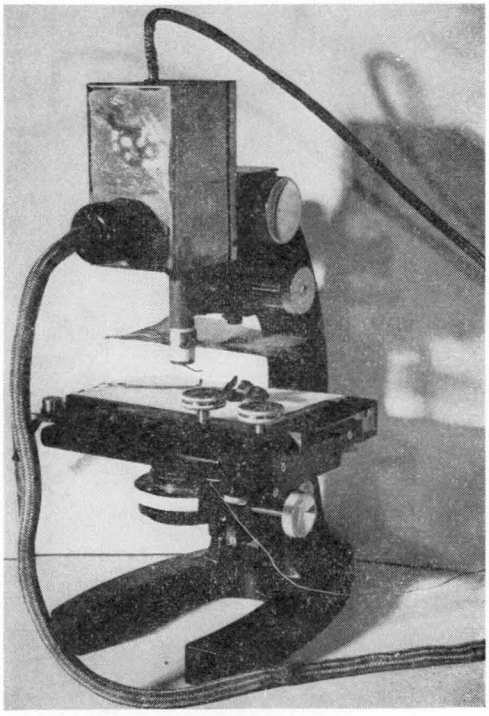

FIG. 2.

* On leave of absence from the University of Tasmania, Hobart, Tasmania, Australia.

[1] Zisman, Rev. Sci. Inst. **3**, 7 (1932); Frost and Hurka, J. Am. Chem. Soc. **62**, 3335 (1940).

[2] C. E. Nielson, Rev. Sci. Inst. **18**, 18 (1947).

electrometer tube. The amplifier A is a three-stage battery operated General Radio Company Model 1231-A.[3] A parallel antiresonant filter F tuned to 280 sec.$^{-1}$ is used to reduce the noise level and 60 sec.$^{-1}$ alternating current interference. The object is shielded with wire gauze W to reduce a.c. pick-up.

One of the main advantages of the instrument is the possibility of using a.c. amplification. Further, since the probe does not make contact with the surface, no galvanic current is drawn from the source of potential and no diffusion potentials are introduced, as by the use of conventional electrodes. The possibility of producing injury potentials through the mechanical contact between electrode and object also does not arise. The use of the instrument is, of course, limited to surface measurements. The vibrating probe electrometer can be used for the investigation of resting potentials and their slow changes in time (growth, external influences), or for the study of the electric potential distribution across an extended surface; it could also be adapted for the measurement of action potentials. With the present arrangement and a probe area 2-mm^2 readings could be obtained only within 2–3 mv. It is hoped that the noise level can be still further reduced with more adequate shielding, and by using an electrometer tube of lower input capacity. This would permit the use of smaller probes, and even microprobes for the mapping of microscopic electrical structures. The instrument has been used in preliminary experiments for the mapping of surface potentials of various plant materials, and the observation of diffusion processes in ionic solutions.

[3] The amplifier has been placed at the disposal of one of us (O.B.) by the National Research Council of Canada in connection with another research problem.

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By B. I. H. Scott,* A. L. McAulay,* and Pauline Jeyes*

[*Manuscript received September* 6, 1954]

Summary

Methods of measurement of the electric fields produced by plants have been developed which eliminate artefacts commonly present in such investigations.

The normal potential pattern in the water surrounding actively growing bean roots is described.

When rates of elongation of roots are controlled by two methods which produce very different types of overall metabolic change, the potential pattern is shown to be correlated with the rate of elongation.

Values are given of the electric power dissipated in water in which a bean root is growing, and of the current generated by the root in the water.

I. Introduction

Experiments made here and elsewhere show clearly that electric potentials measured on the surface of plant organs depend on a number of causes. In addition to those due to the plant itself when in its normal steady state of metabolism, there are a number of other sources of potential difference, some of them generated by the plant itself owing to treatment it receives during measurement, and others introduced from outside.

In this paper a new approach is made to the problem of the bioelectric phenomena associated with plant metabolism. The effect of the process of measurement on the observations made is entirely eliminated by measuring the potential fall down currents produced by the plant in an external medium.

When a bean root grows actively in aerated water it generates an electromotive force which passes current through the water. This paper describes measurements which have been made of the ohmic potential differences due to these currents in the water adjacent to the root. The pattern of these potentials is characteristic and reproducible when the root is growing strongly.

The paper further describes experiments in which the growth of the root has been controlled and in which a correlation has been found between the change in potential and the rate of elongation.

By addition of auxin of suitable concentration to the bath in which the root is grown, it is possible to arrest growth and observe the change in potential pattern. This process proves to be reversible, as removal of the auxin allows the plant to grow again and the potential pattern to recover.

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A similar correlation is observed when the temperature at which the roots are grown is raised, but the root cannot be restored to its original condition of normal growth by lowering the temperature.

II. Some Causes of the Conflicting Results Obtained in Previous Investigations of the Electric Fields of Plants

A number of investigations of the electric potential differences associated with plants have been made in the past. An examination of the literature shows very little agreement between the results obtained.

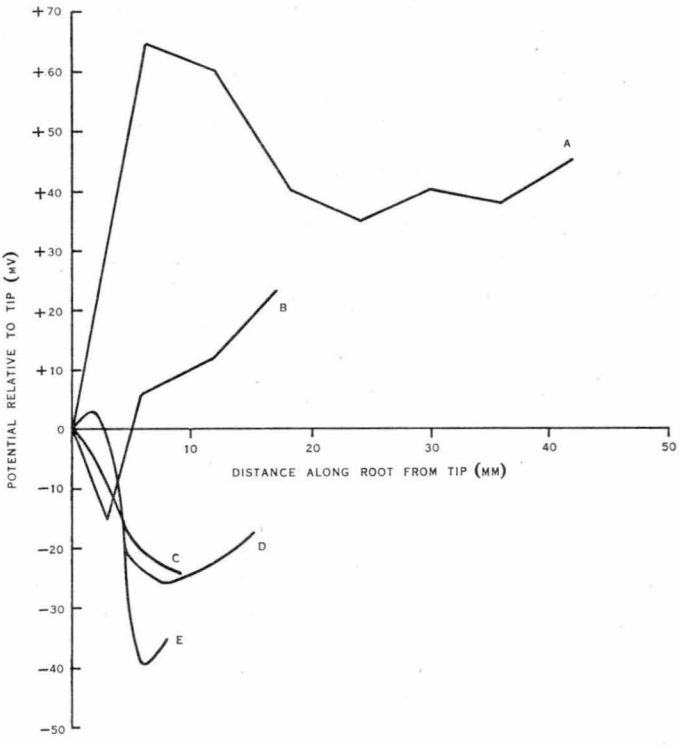

Fig. 1.—Comparison of the results obtained in several investigations of the relation between the external potential of the root relative to the tip and distance from tip along the root. Data obtained from *A*, Thomas (1939) (bean); *B*, Lundegårdh (1940) (wheat); *C*, McAulay, Ford, and Hope (1951) (maize); *D*, Lund and Kenyon (1927) (onion); and *E*, Ramshorn (1934) (bean).

Some of the results obtained using roots as material are compared in Figure 1, in which the potential of the root relative to the tip is plotted against the distance from the tip. It is evident that there is a wide diversity between the results of different observers. It is true that these results are not directly comparable since they have been obtained under a variety of conditions, and some

of the variation between them is certainly due to such factors as the type of plant used, its age, and the state of its environment. Nevertheless, a critical examination of the experimental techniques employed in the investigations shows that in most cases the measured potential differences were due not only to the plant e.m.f.'s but also to factors introduced by the measuring techniques. In particular, the following causes can be recognized as responsible for many of the variations in the results recorded.

The effect of variations in salt concentration at the point of contact has been almost invariably overlooked in previous investigations. Salt concentration changes are produced by drying and are due also to salt uptake by the plant. The resulting potential changes can be quite large, and are often greater than the plant potential it is desired to measure.

To illustrate the magnitude of the potentials set up in this way, it is found that if the concentration of KCl at one point of contact on a bean root is changed by a factor of 10 (the concentration at the other contact remaining unchanged) the potential difference between the contacts changes by more than 30 mV (Hope 1951).

Other factors which may obscure the true steady state electric pattern generated by the plant are variations in environment, either of the plant as a whole, or local variations at the point of contact. These may include temperature, humidity, salt concentration, the amount of aeration, and light and gravitational influences. In addition, stimulation or injury by the measuring probes can produce large potential differences.

III. Experimental Methods

Most of the sources of error listed above are liable to occur if the measuring probes are in mechanical contact with the plant. To avoid this, three methods have been developed and used to measure plant potentials without making mechanical contact with the surface of the plant.

Two of these methods are suitable for measuring electric potentials of plant surfaces in air, while the other is suitable for use with plants grown in water. In the first, which has been described elsewhere (Blüh and Scott 1950), a probe is set into vibration close to the plant, and its potential is adjusted until there is no field between the probe and plant. This condition is reached when no alternating current flowers in the probe circuit. In the second method, air in the neighbourhood of a probe and plant surface is made conducting by α-particle irradiation and a current flows between probe and plant to bring them to electrical equilibrium.

The third method, which forms the subject of the present paper, will now be described in more detail.

A fortunate property of the root is its ability to grow strongly and healthily in aerated water. If the conductivity of the water is sufficiently low, potential differences between points in the water adjacent to the root can be observed, owing to the currents generated by the root e.m.f.'s. For this reason, it was decided to grow bean roots vertically in a 10^{-4}N KCl solution under controlled

conditions and observe the potential pattern in the water adjacent to the root and its relation to the rate of elongation of the root.

Since the measuring tubes are not in contact with the plant, no effects due to injury by them can occur. As the salt concentration in the tubes and in the bath is the same, the point of measurement on the plant is not in a different condition from neighbouring points resulting from local diffusion or other local variations.

Errors introduced in the measuring circuit have been prevented by the use of a suitable valve electrometer and mercury-calomel electrodes. The whole input circuit is insulated with polystyrene and shielded to prevent A.C. pick-up. The plants are left in the measuring bath for at least an hour before measurements are begun to avoid stimulation effects which may occur in setting up the plant for measurement. The bath is aerated, stirred, and its temperature controlled to avoid changes in environment. No effect was observed on either rate of growth or the potential pattern of bean roots due to light.

As the potential measured is an ohmic drop along a current, its source is evidently an active e.m.f. involving energy change in the plant organ.

IV. Material

For experiments described in this paper, the broad bean, *Vicia faba* L., Johnson's Long Pod variety, was used. After soaking the seeds overnight in water, the seed coats were removed and a number of seeds impaled on stainless steel rods 1/16 in. dia. Mounted in this way, the seedlings could be handled easily for growing in water-baths and could be transferred to the measuring bath with a minimum of stimulation. Impaling the seed did not appear to affect the development of the plant in any way.

For most experiments the beans were prepared by growing in baths of aerated distilled water at 25°C. The water in the tank was slowly changed from a reservoir tank. The rods supported the plants so that the shoot was above the water-level and the roots were submerged and grew vertically. Plants 2-3 days old with roots 20-30 mm long were used in the experiments.

In one series of experiments discussed later, the roots were prepared by growing in air saturated with water vapour and minute water droplets. The fine spray was produced by a simple atomizer using compressed air. In this bath the roots grew quite strongly but the root surface appeared rather different from that of a root grown in water and more like what is observed for a root grown in dry soil or sphagnum moss.

V. Apparatus

The "Perspex" measuring bath is shown in Plate 1. It was 7 in. long, 6 in. wide, and 3 in. deep and was insulated with polystyrene. The bath was aerated and the water in the tank circulated by passing compressed air through a sintered glass plug *P* mounted in the tank.

Heating the bath presented some problems. Electrical interference had to be avoided and an arrangement giving rapid response to the thermostat must

be used. It was decided to heat the bath by means of a coil of nichrome wire stretched across the top of the bath just above water-level. A 12-V A.C. supply was connected to the coil and heat radiated downwards warmed the water. Surprisingly little A.C. pick-up was experienced, and this arrangement, with some shielding of the heater from the measuring circuit, proved very satisfactory. The thermostat was a mercury-alcohol switch, *C*, which controlled the heater through a relay. Temperature stability was $\pm 0{\cdot}5°C$.

The 10^{-4}N KCl solution used in the measuring bath was changed from a reservoir. The water was made to drip into the bath, and to drip out at the overflow to facilitate electrical insulation of the bath.

The beans were set up in the measuring tank on a stainless steel rod passing through the cotyledon with only the roots immersed and held vertically.

The electrometer used in these experiments employed a pair of matched M.E. 1400 Mullard electrometer valves in a balanced circuit. The instrument was designed to be insensitive to fluctuations in the high tension voltage and in the valve heater current. A Cambridge spot galvanometer (full scale deflection about 2 μA) was used and the maximum sensitivity of the electrometer was such that an input of 1 mV produced a deflection of 3 cm on the scale. Currents flowing in the input circuit of the electrometer under the conditions of operation were not greater than 10^{-12} A.

The connections to the measuring bath were made using mercury-calomel electrodes. The reference electrode was connected directly to the bath. The other electrode made contact with the bath through a measuring tube, *T*, containing 10^{-4}N KCl agar. This tube was mounted on a micromanipulator, *M*, allowing it to be moved easily in the vicinity of the plant root. Since 10^{-4}N KCl has a low conductivity (about $1{\cdot}5 \times 10^{-5}$ mho cm^{-1} at 25°C) the tip of the measuring tube had to be coarse or hand capacity effects became troublesome owing to the high resistance of the input circuit. In most of these experiments the diameter of the tip was about 0·5 mm, corresponding to a tip resistance of about 15 MΩ.

Elongation of the root was measured by projecting a greatly enlarged image on a wall. A 5-in. Waterworth projection lens was used, mounted in front of the box with its axis horizontal. For most experiments back lighting was employed, giving an enlarged shadow of the root, but in some experiments the root was marked, and strongly illuminated from the front so that the regions of elongation of the root could be found. In this way, changes in length of the order of 0·02 mm could be observed.

The whole box could be moved laterally on a metal slide, *G*, so that each bean in the box could be placed in turn in front of the lens for measurement.

VI. Results

The present paper consists of a study of the potential pattern close to a bean root actively growing in water contrasted with that which appears when growth is inhibited.

The aspect of growth that has been selected is rate of elongation and attempts have been made to control this by a variety of means, and study the resulting potential changes.

Several treatments have been applied to the growing root, such as subjecting it to mechanical vibration, controlling its oxygen supply, growing it at varying temperatures, and inhibiting growth by the application of a suitable concentration of auxin.

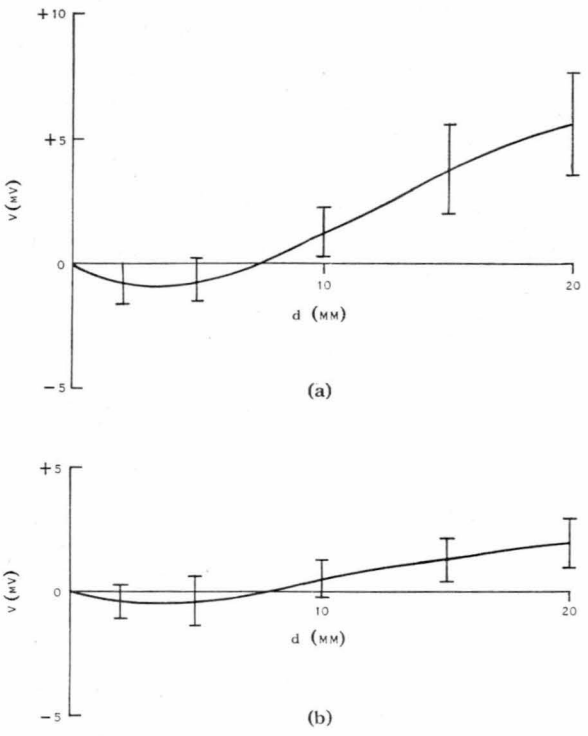

Fig. 2.—Control of growth and potential pattern of bean roots by the application of indoleacetic acid (IAA). (*a*) Roots at 25°C untreated with IAA. Mean growth rate $0{\cdot}82 \pm 0{\cdot}060$ mm/hr. (*b*) Same roots at 25°C 2 hr after treating with IAA (2 mg/l). Mean growth rate $0{\cdot}027 \pm 0{\cdot}077$ mm/hr. V is the potential relative to that of the root tip and d is the distance along the root from the tip.

The last of these methods, auxin treatment, was considerably the most successful. By this means, elongation could be inhibited, and the inhibition subsequently removed and growth restored. The method of temperature control also provided results of interest which are described below, but preliminary results by the other methods were not so promising and study of these has not yet been followed up.

In both cases studied, the pattern of active e.m.f. was correlated with the rate of elongation. The fact that the correlation was the same in both cases suggests a direct link between elongation and potential pattern. Of course, it is possible that both elongation and potential pattern are more dependent upon some third change common to both methods of control.

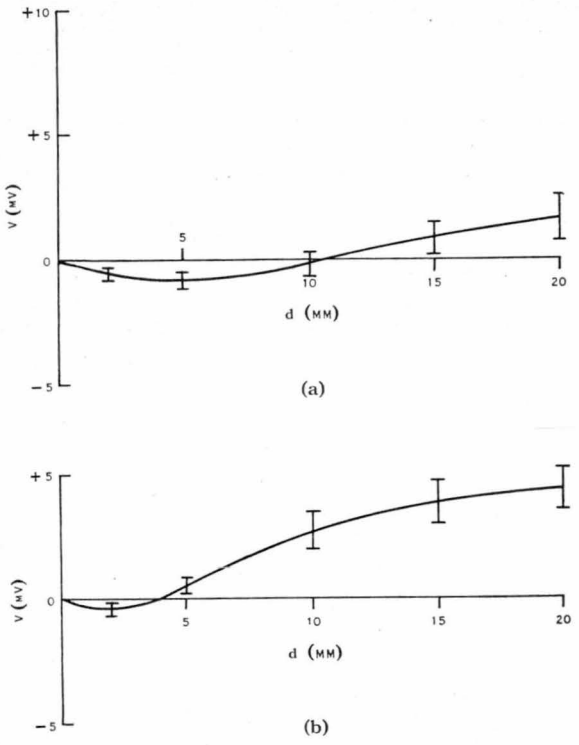

Fig. 3.—Recovery of growth and potential pattern on removal of IAA. (*a*) Roots at 25°C 2 hr after treating with IAA (2 mg/l) . Mean growth rate 0·037 ± 0·043 mm/hr. (*b*) Same roots at 25°C 2 hr after removal of IAA from bath. Mean growth rate 0·36 ± 0·072 mm/hr.

(*a*) *Control of Growth with Auxin*

(i) Bean seedlings with roots approx. 25 mm long were transferred to the 10^{-4}N KCl measuring bath at 25°C and allowed to reach an equilibrium with their environment.

After about 2 hr, the potential pattern and rate of elongation of the roots were measured, and those whose growth rate was less than 0·4 mm/hr discarded. Indoleacetic acid (IAA) (2 mg/l of solution) was then added to the bath containing the rapidly-growing beans. After about an hour the potential pattern had reached a new steady state and the average potential and rate of elongation over the next 2 hr were recorded.

The results of this experiment are shown in Figure 2. In this and subsequent diagrams the vertical lines through the points mark the 95 per cent. confidence limits; that is, the probability is 0·95 that the mean of the population represented by the sample lies within the limits given by the ends of the line. The limits for the mean growth rate also are the 95 per cent. confidence limits.

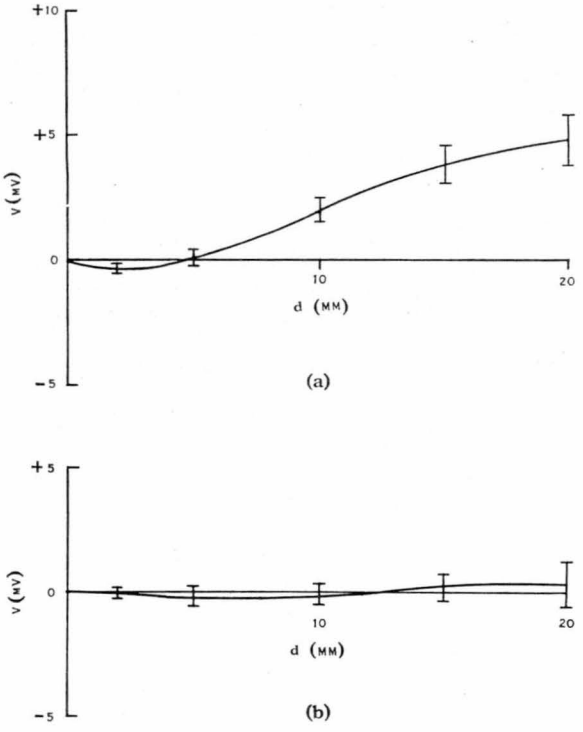

Fig. 4.—Control of growth and potential pattern by temperature. (*a*) Roots grown and measured in water at 25°C. Mean growth rate 0·532 ± 0·076 mm/hr. (*b*) Roots grown in saturated air and measured in water at 37°C. Mean growth rate 0·007 ± 0·041 mm/hr.

(ii) Bean seedlings were transferred to the measuring bath at 25°C containing 10^{-4}N KCl and 2 mg/l IAA. After about 2 hr elongation had practically ceased. The potential pattern was then observed, and the water in the bath replaced with KCl solution without IAA. At 1-3 hr after the removal of the IAA, when the root was again elongating, the mean potential pattern and growth rate were measured.

The results of this experiment are given in Figure 3.

An examination of Figures 2 and 3 shows that the inhibited roots have a markedly different potential pattern from those growing strongly. The normal potential pattern of a growing root shows a region 2-5 mm from the tip most negative, with the base of the root approx. 6 mV more positive. Suitable

treatment with IAA inhibits the elongation of the root and reduces the potential gradients along the root by a significant amount. Removal of the auxin allows the root to grow again (although the recovery is not complete in 2 hr, the growth rate being well below that of untreated roots) and the potential gradient along the root returns almost to its value prior to auxin treatment.

(*b*) *Control of Growth by Temperature*

Figure 4 (*a*) shows the normal potential pattern for roots grown in 10^{-4}N KCl at 25°C. In this case, and in the following one at 37°C, the growth and potentials were averaged for a 24-hr period following the initial settling down.

Figure 4 (*b*) shows the potential pattern for roots measured in 10^{-4}N KCl at 37°C. Inhibition of growth at this temperature was most marked in roots pre-treated by growth in a saturated atmosphere for 24 hr before exposure to 37°C. The graph refers to roots treated in this way. It is seen that the roots have almost entirely stopped elongating and the potential differences along the root are not significantly different from zero. Roots measured at this temperature for 24 hr and then returned to a 25°C bath do not recover normal growth. The activity of the primary meristem is suppressed and the main root stops growing, but pronounced initiation of secondary roots quite close to the primary root tip is observed (see Plate 2). Electromotive forces are once more produced by the root when it is returned to the 25°C bath, but they are not the same as those for a root which has not been treated at 37°C.

(*c*) *Power and Current*

More detailed experiments have been made from which the current density and current direction in the neighbourhood of the root can be deduced. In addition, the total power dissipated in the solution due to current produced by the root can be calculated.

These data have been obtained by measuring the radial and longitudinal components of the potential gradient in different orientations around the root. With this information, it is possible to map the equipotential surfaces and hence the current paths throughout the external medium. A typical simplified pattern is shown in Figure 5, in which the full lines are current paths and the dotted lines are equipotentials.

For a root growing actively in 10^{-4}N KCl at 25°C, the total current leaving the root (which, of course, must be equal to the total current entering it, since leakage paths have been eliminated) is of the order of 5×10^{-8} A. If the root is taken to have a surface area of 2·5 cm^2 immersed, the mean current through unit area is 4×10^{-8} A/cm^2, although, of course, it varies considerably over the whole root surface. In certain regions of an active root it may be as high as 2×10^{-7} A/cm^2. The total power dissipated in the surrounding medium is of the order of 2×10^{-10} W.

It is found that small variations with time of the potential pattern occur even when all environmental factors known to affect the plant are controlled.

Under certain conditions, rapid rhythmic oscillations of potential take place. These oscillations can be induced experimentally.

Investigations of these phenomena are continuing and it is proposed to make them the subjects of later papers.

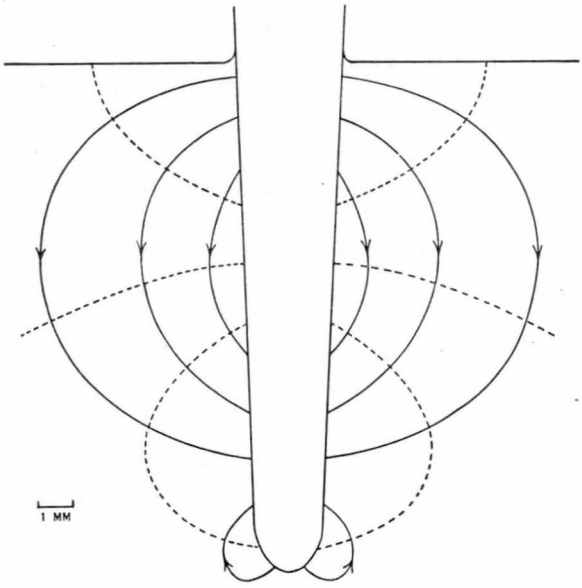

Fig. 5.—Typical simplified pattern of the current paths through the 10^{-4}N KCl solution in which bean root is growing. The full lines are typical current paths (lines of force) and the dotted lines are equipotentials.

VII. Discussion

The experiments described in this paper were made with the object of providing definite reproducible information about the electric currents produced by a plant organ acting as a unit. The long-range object is to obtain information about the morphology and behaviour of the electric patterns that characterize integrated plant structures and their relationship to the morphology and development of the material structure.

It is found that the most negative part of the potential pattern for a rapidly growing root corresponds with the region which is elongating most rapidly (2-7 mm from the tip). A possible mechanistic explanation is now given.

The greatest uptake of salt might be expected to take place in the region of greatest rate of elongation, where newly vacuolated cells must fill up to a salt concentration which is many times greater than that of the external solution.. It is known that the mobility of K^+ is greater than that of Cl^- in tissue (Hope 1951). The K^+ will penetrate the root more rapidly, forming an electric double layer, and a slight excess of Cl^- will build up in the region outside the elongating part of the plant. This will make the region electrically negative. With this

hypothesis it would also be expected that a root which had not elongated for some time would be absorbing salt at a slower rate and more uniformly over the whole root surface. Under these conditions the observed reduction in the external field is to be expected.

The mechanisms which produce electromotive forces in plant tissues may be such that some can supply more power than others. Whether the field produced by one of these will be observed outside the plant in a particular case depends on the magnitude of the current generated in the conditions of measurement. If this current is too large, the field may not be measured, either because the e.m.f. itself is polarized, or because the potential drop in the tissue is so great that the external field is insignficant.

The present method selects for measurement only those bioelectric processes which are capable of supplying a relatively large amount of power to the external medium. By employing other methods which were mentioned earlier in this paper (Section III) it is possible to investigate less powerful bioelectric processes taking place in the organ and so gain a more complete picture of the bioelectric behaviour of plants.

VIII. Acknowledgment

Our thanks are due to the Commonwealth Scientific and Industrial Research Organization, a grant from which aided part of this work.

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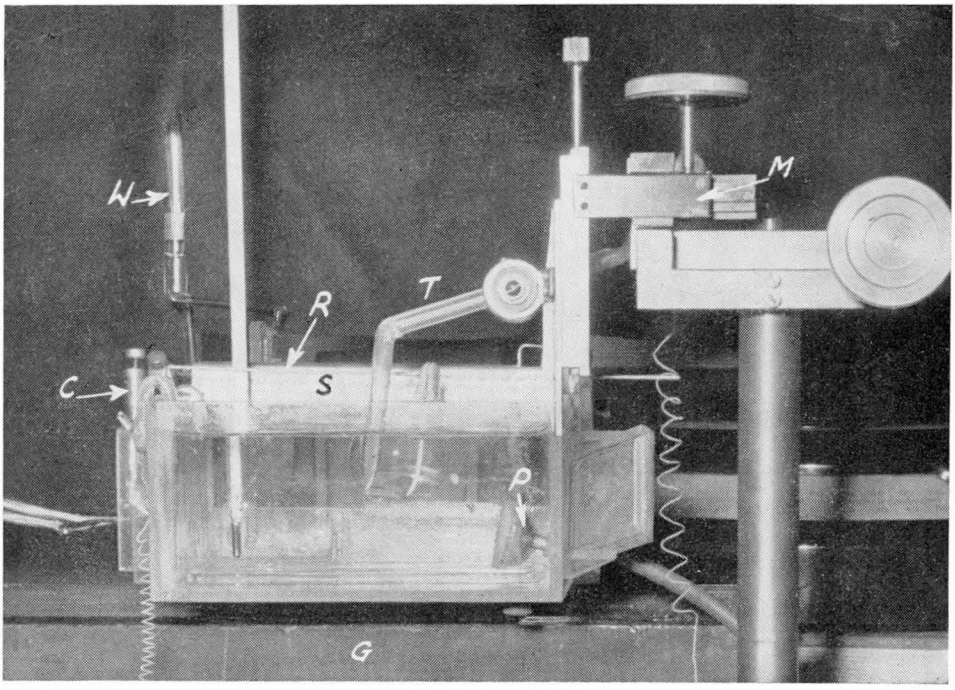
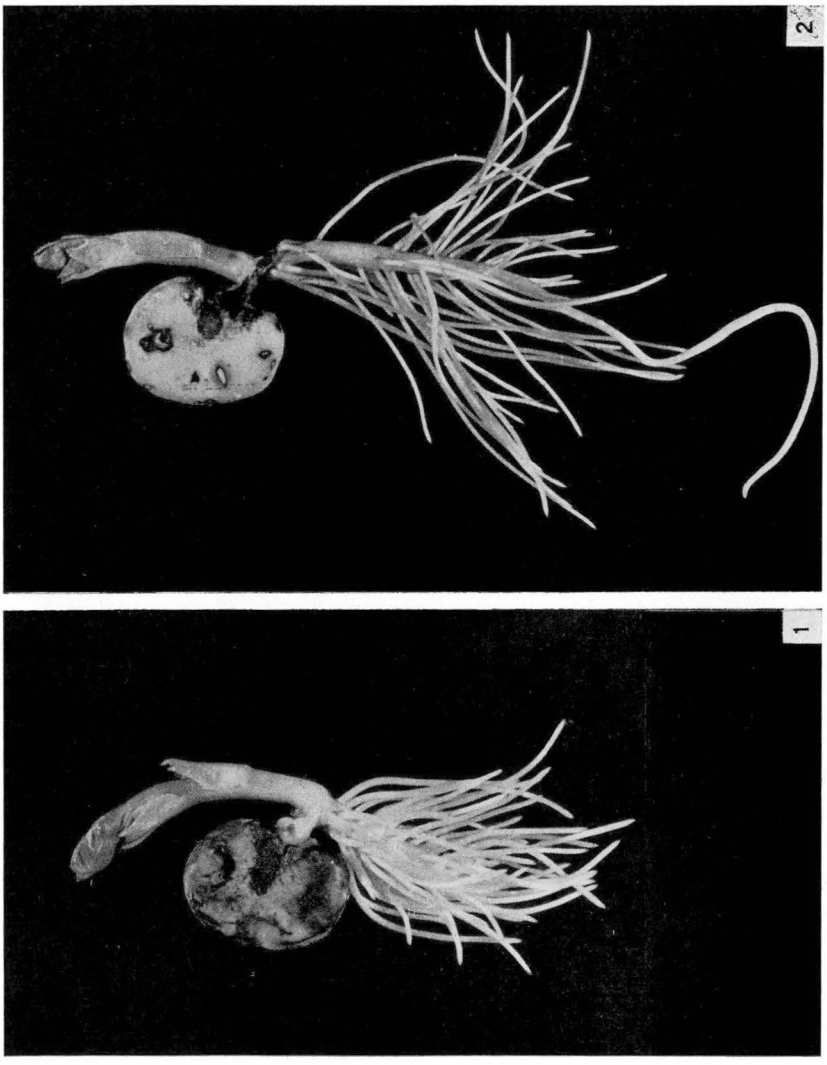
ELECTRIC CURRENT GENERATED BY ROOT GROWTH



The measuring bath used in the experiments desribed in the paper. The following parts are referred to in the text: *P*, sintered glass plug; *S*, shield for A.C. heater; *C*, mercury-alcohol switch control; *R*, stainless steel rod supporting plant; *M*, micromanipulator; *T*, measuring tube; *W*, water inlet; *G*, metal slide for moving box.

ELECTRIC CURRENT GENERATED BY ROOT GROWTH



Abnormal subsequent growth of roots treated for 24 hr at 37°C.

Fig. 1.—Root one week after treatment at 37°C. Notice lack of development of primary root with secondary roots appearing quite close to primary tip.

Fig. 2.—Untreated root of same age.

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A New Approach to the Study of Electric Fields produced by Growing Roots

THE electric fields primarily developed across the walls of plant cells give rise to an integrated field associated with a growing plant organ. Information from the literature with regard to such electric fields associated with plant organs is contradictory and has led to conflicting theories ; and the conflict in experimental evidence has never been satisfactorily resolved. An investigation of the literature seems to us to show that artefacts developed at points where measuring probes make contact with the plant would, in most cases, swamp the normal field which it is desired to investigate.

We have made an essentially new approach by studying the currents which are generated by a plant organ in a weakly conducting medium. Potential differences are measured between points in the medium near the plant without disturbing its normal

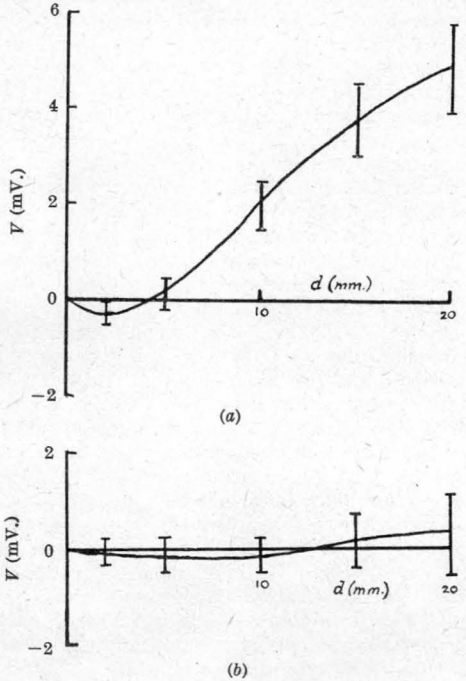


Fig. 1

growth in any way. If these measurements are made in a suitable pattern, information about current density and direction, and the electric power developed by the plant, can be obtained. Consistent results have been obtained with bean and onion roots grown in water. As a first step in the investigation of the electrical processes associated with an organ as a whole, the changes have been observed in the pattern of electric potentials in the water which occur when growth is rapid and when it is inhibited.

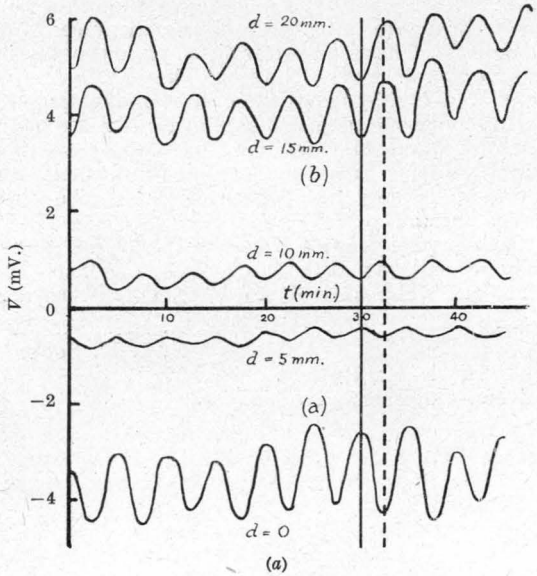
Fig. 1(*a*) shows the pattern observed with bean roots growing rapidly and uniformly in aerated water at 25° C. V is the potential relative to that of the root-tip of points close to the root in the external medium (0·0001 N potassium chloride solution) and this is plotted against the distance d from the tip. The growth-rate is about 0·8 mm. per hour.

Fig. 1(*b*) shows the corresponding pattern produced when growth is inhibited. Inhibition has been produced by methods as different as the addition of indole acetic acid to the water in which the root was growing, and raising the temperature of the root. In the case of the addition of indole acetic acid, the effect was reversible, and when growth was resumed the electrical pattern returned to that associated with normal growth.

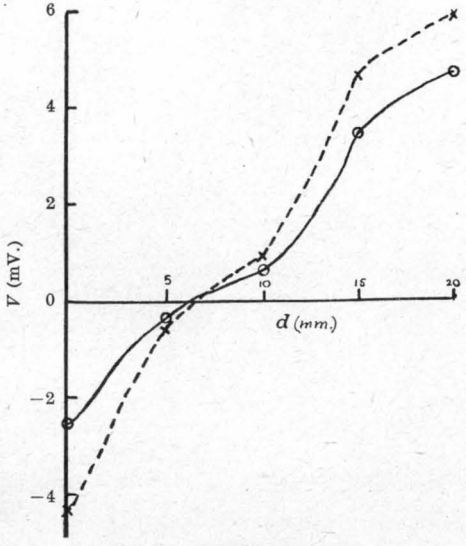
Figs. 1(*a*) and 1(*b*) show averages in experiments each of which comprised about twenty-five plants, the vertical lines through the points giving the 95 per cent confidence limits.

The electrical pattern is seldom static and shows small slow variations with time even when all environmental factors known to affect the electric field are controlled. In conditions other than that of straight rapid growth, the potential pattern may differ considerably from that discussed. In some conditions the root is observed to produce persistent and relatively rapid oscillations in its electrical pattern. These oscillations are often practically sinusoidal, with periods ranging from 4 to 15 min. or more. The more rapid oscillations are more common.

Observation of these rapid changes of electrical pattern has been made possible by the development in this laboratory of an automatic apparatus which records at frequent intervals the electric potential differences between a number of points adjacent to the root grown in water. The oscillations can be induced by experimental treatment, such as by introducing an obstacle which impedes elongation of the root, or by inducing bending by wounding. They also appear spontaneously, accompanying variations in growth such as spontaneous bending. They do not occur when the root grows rapidly and evenly.


d = 20 mm.
d = 15 mm.
(b)
d = 10 mm.
t (min.)
d = 5 mm.
(a)
d = 0
V (mV.)
6
4
2
0
−2
−4
10
20
30
40

(a)


V (mV.)
6
4
2
0
−2
−4
5
10
15
20
d (mm.)

(b)

Fig. 2

Fig. 2(*a*) shows a graph of potential against time in a typical case where electrical oscillation accompanied bending. Each trace shows the potential V relative to a distant point in the bath plotted against time t, for a point near the root at a distance d from the root-tip. The graph shows simultaneous potentials for several values of d. Note the phase differences at a and b. Fig. 2(*b*) shows the corresponding graph of potential plotted against distance from the root-tip. Two traces are shown giving potentials at times corresponding to a crest and a trough of the oscillatory potential. Where the two traces cross, the phase changes by π.

These investigations, which are part of a general study of patterns of morphogenesis, are being continued and will be described in more detail elsewhere.

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B. I. H. Scott

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University of Tasmania. July 8.

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