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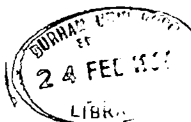
BIOELECTRIC POTENTIALS IN PLANTS.

A THESIS SUBMITTED BY KEITH JAMES PARKINSON, B.Sc., DUNELM.,

FOR THE DEGREE OF

DOCTOR OF PHILOSPHY IN THE UNIVERSITY OF DURHAM.

DEPARTMENT OF BOTANY,
DURHAM COLLEGES IN THE
UNIVERSITY OF DURHAM.
1963.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NOTES ON PRESENTATION OF THIS THESIS.

Each Chapter considers a separate topic and is self contained. Each Chapter has been individually numbered and the page numbers are preceeded by the number of the Chapters to which they belong.

In a similar manner the Figures, Plates and Tables have the Chapter number followed by a number representing their positions in that Chapter.

The Chapter Bibliographies are individually and consecutively numbered in Roman numerals and these also are preceeded by the Chapter number.

When a Plate, Figure or Table is referred to in the text, it will be found immediately following the page on which it is first mentioned.

INTRODUCTION.

This research was concerned with the measurement and interpretation of electrical potentials existing between different parts of intact plants. The topic was originally chosen because although the observations of Schrank on the relationship between tropisms and changes in the bioelectric potential pattern of the oat coleoptile were often mentioned in passing in the literature, they had not in general been independently confirmed, and it seemed that they were worthy of a more detailed study. Experiments on changes in potential in fungi under tropic stimulation, where auxins do not complicate the picture, also offered possibilities.

As no previous work on bioelectric potentials had ever been carried out in this department, it was first necessary to develop apparatus which would give accurate and reproducible measurements of such potentials. Using this apparatus it was hoped:-

(a) To repeat previous published work to assess the suitability of the techniques employed, and also, as further experience was gained, to review the validity of some published results.

(b) To investigate the bioelectric potentials reported to

develop prior to tropic responses in plant tissue and ascertain if these were the cause or effect of the responses.

(c) To investigate bioelectric potentials in fungi.

All these objects have to some extent been achieved, but in the course of this research several other topics have been investigated, for it was felt that, as the development of the apparatus had occupied such a considerable time, a broad survey of the different aspects of bioelectric potentials, which would indicate the potentialities of the apparatus and the range of possible applications, would be or more value at this stage than a detailed study of one aspect.

In writing this thesis, it seemed desirable to devote a separate chapter to each topic. Each chapter is self contained, having its own summary, literature survey, conclusion and reference section. The chapters, with the exception of the first, are as nearly as possible in chronological order. Chapter one gives details of all contact systems and electrodes developed, even though some were not developed until late in the research.

It must be stated right at the beginning of this thesis that the phenomenon of bioelectric potentials appeared **far** simpler at the commencement than on completion of the research,

and whereas in the opening stages the problems of measurement loomed large, and the interpretation seemed straight forward, now the opposite view holds.

CHAPTER.1.

CHAPTER 1.

THE MEASUREMENT OF BIOELECTRIC POTENTIALS

SUMMARY

Problems involved in the measurement of bioelectric potentials are discussed, then the various possible methods of measurement are surveyed; comprehensive constructional details for the electrodes and contacts used in this work, and specifications of the chosen potential measuring equipment are given.

A major problem involved in the measurement of electrical potentials in plant tissues is the high resistance of the tissues. It can be proved that this necessitates the use of a measuring circuit whose input resistance exceeds that of the source by several orders of magnitude if the potentials measured are to bear a close relationship to those actually existing.

<u>To show that the resistance of the measuring instrument must be large compared with that of the source, if the voltage measured is to approximate to the actual E.M.F.</u>

Let the E.M.F. of the source be E and its internal resistance R.

Let the voltage measured by the instrument be V and its internal resistance be R^*.

When the instrument is in the circuit let a current I flow.

Therefore the voltage drop across the instrument $= V = R^*I$. –(1) (By Ohm's Law) and the voltage drop across the source $= RI$.

Therefore $E = R^*I + RI = I(R^* + R)$ –(2) (By Kirchhoff's Law) and substituting (1) in (2) $E = V + RI$.

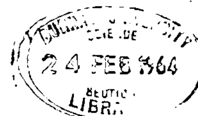
If V is to be approximately equal to E then RI must be small in comparison, i.e. R^* must considerably exceed R.

If R^* is one hundredth of R, then $V = 0.99\%$ of E.

R^* is one tenth of R, then $V = 9.09\%$ of E.

R^* is R, then $V = 50\%$ of E.

R^* is ten times R, then $V = 90.9\%$ of E.



R^* is one hundred times R, then V= 99% of E.

R^* is one thousand times R, then V= 99.9% of E.

Below are listed some crude resistance measurements made on Oat coleoptiles, using a flowing liquid contact and Zinc/Zinc Sulphate electrodes, as usually employed in this work. The measuring instrument used was an Avo Model 8.

Resistance of a pair of electrodes = 1,150 ohms.

Resistance of a pair of electrodes and liquid contacts = 9×10^5 ohms.

Resistance of a pair of electrodes and liquid contacts, with a centimetre length of coleoptile tissue between = 1.4×10^6 ohms.

Therefore the resistance of the coleoptile tissue = 5×10^5 ohms.

The measuring circuit therefore should have a resistance greater than 10^8 ohms, when the discrepancy between the actual and measured potential will be no greater than 1%.

Observing this principle will ensure the least disturbance in the magnitude and pathways of the natural current flow.

Bearing the above in mind, there are three possible methods of measuring the potentials, each of which will be dealt with separately in the following sections.

Section A. Attachment to the plant of contacts leading via non-polarizable reversible electrodes to the measuring instrument.

This is the method that had generally been used and was the one chosen for the major part of this research. There are three aspects to

consider with regard to this method:-

(a) Type of measuring instrument.

(b) Type of electrode system.

(c) Type of contact system making attachment to the plant.

(a) Measuring Instruments.

There are four possible types of measuring instrument.

(i) Electrostatic instruments.

(ii) Galvanometers.

(iii) Potentiometers.

(iv) Electronic instruments.

(i) Electrostatic instruments have been widely used as they have the advantage that there is no current flow through them, other than by way of insulation leaks, and their input resistance is that of their insulation.

Examples of their use are:-

Haake's (1892), Lippman's capillary electrometer.

Clark's (1938), Wulf String electrometer.

Lund & co-workers' (1947), Compton-type quadrant electrometer.

Their main disadvantages are the problem of devising a method of continuous recording and their relatively slow response.

(ii) Galvanometers are current measuring instruments and therefore there must be a flow of current in the measuring circuit. These too have been widely used in the past. Waller (1900), used a Thomson

mirror galvanometer which from his published results may be calculated to have had an input resistance of 5×10^5 ohms. His quoted resistances for the plant tissue are 5×10^5 to 10^6 ohms. The magnitudes of his reported results must have been less than 50% of the true potentials.

Waller (1925), used a D'Arsonval galvanometer of input resistance of only 1,800 ohms. Since his results are not greatly different from those of other workers, it seems that his electrodes, contacts and plant tissue, must have had extremely low resistances! Rehm (1936), also used a galvanometer but no resistance values are given. He quotes a value of 10^5 ohms for the tissue resistance. The recording methods used with sensitive galvanometers are generally photographic. Their main disadvantages are their relatively low input resistance and the difficulties of continuous recording.

(iii) In the use of potentiometers, the current led off from the plant tissue is balanced against an external source, using a calibrated potentiometer circuit and a galvanometer or other current-detecting instrument to determine the null point. This is the point when the external potential applied by the potentiometer is exactly equal to the E.M.F. of the plant tissue; no current is then flowing in the leads from the tissue. Either side of the balance point a current is applied to or drawn from the tissue; (its magnitude and duration depend on the distance from the balance point, the time to find the balance and the resistances of the null point detector and plant tissue.)

Using suitable valve circuitry the instrument resistance can be made very high and the current therefore very small. There is this one advantage with the method, that at the balance point no current is being drawn from the tissue, and the E.M.F. of the plant tissue is measured. However, there are a number of disadvantages; small currents may be applied to the tissue, continuous recording is difficult, and where self-balancing potentiometers are employed, the input resistance does not exceed 10^6 ohms.

The potentiometer method was used by Rosene (1937), and also by Schrank (1947), in some work. Galvanometers were used as null point detectors.

(iv) Electronic instruments have almost entirely been used in recent work. A normal valve voltmeter has an input resistance of approximately 10^7 ohms whilst electrometer circuits using selected tubes have input resistances up to 10^{12} ohms.

Into a special class in this group comes the Vibrating Reed Electrometer which is based on a dynamic condenser and has an input resistance as great as that of the insulation. It shows a greater stability than valve electrometers. All the instruments have outputs suitable for external recorders and cathode ray oscillographs.

A vibrating reed electrometer was the obvious choice and the instrument selected was a Vibron 33B whose input resistance exceeds 10^{14}

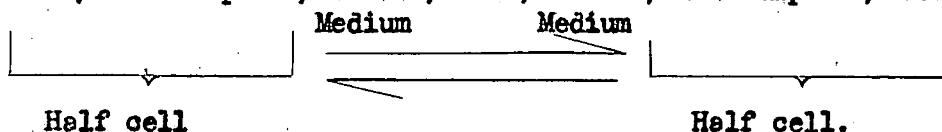
ohms. The output from this was fed into an Elliot pen recorder.

The high and low input terminals of the Vibron have been used in this work, the low generally being connected to earth. The leads from the electrodes were connected to these two terminals; the potential and polarity indicated by the Vibron is that of the high terminal with respect to the low. In the results, when the electrodes and contacts are referred to as high or low, it is indicated that the potentials and polarities being measured were those of the high contact with respect to the low. This has been abbreviated to High/Low in some results; e.g. 2/1 indicates the potential of contact 2 with respect to contact 1.

(b) Electrode Systems. The electrode is necessary for the transfer of the charge from the contact medium to the input wires of the measuring instruments. The electrodes used must be non-polarizable, i.e. the passage of a current through the electrodes must lead to no changes in the potential measured by the electrodes brought about by changes in the electrodes themselves. They must also be reversible. In a reversible electrode, when no current is flowing no reaction is taking place, and when a current is flowing in one direction, the reaction is exactly equal and opposite to that when flowing in the other. It is necessary to use a pair of such electrodes to measure the potential, for when this is being done a current is flowing in one direction through one electrode, and in the other direction through the other. If the two reactions are not equal and opposite, this in

itself could affect the measured potential.

e.g. Zinc/Zinc Sulphate/Contact/Plant/Contact/Zinc Sulphate/Zinc



It is also essential that there should be no potential produced due to the electrodes, yet, since a reversible half-cell shows a potential characteristic of the metal and solution composing it, it is necessary to use two similar half-cells in the circuit so that their potentials will be equal and opposite.

The two types of non-polarizable reversible half-cells of use in biological work are (a) A metal dipping into a solution of its ions e.g. Ag/AgCl. Zn/$ZnSO_4$. These are reversible with respect to cations.

(b) A metal in contact with one of its insoluble salts immersed in a solution of soluble salt of the same anion e.g. Hg/$HgCl_2$/KCl -Calomel half-cell. This is reversible to anions.

The importance of the difference between electrodes reversible to cations and anions will be mentioned in the chapter on the sources of bioelectric potentials.

It was felt that as the measuring instrument was to have an input resistance of 10^{14} ohms and the maximum potentials expected were 100 millivolts (when the current flowing would be 10^{-15} amps., and the number of electrons involved would be only 6,290/second), that

polarization and reversibility could be ignored and some new and simpler electrodes tried.

The first system tried was stainless steel/deionised water. At first stainless steel hypodermic needles with the tips ground flat and mounted normally on hypodermic syringes were used. The hypodermic syringe could be used to expel a drop of liquid and thereby make contact. The leads to the electrometer were attached directly to the stainless steel needles. When two such electrodes were brought into contact, potential differences (P.D's.) from 10 to 100 millivolts were found to exist between them. This, it was suspected, could have been due to the brass rubbed off from the cleaning wires on to the inside of the needle bore, setting up small electrolytic cells. The needles were then washed with concentrated nitric acid, when it was revealed that in fact the needles bases were of plated brass!!

Undeterred, needles were made up using narrow stainless steel tubes pushed into polythene blocks into which the hypodermic syringe could also be pushed. These still gave potentials, though not as large as before; the potentials did not disappear after the most careful cleaning of the needles.

Another idea then tried was the use of stainless steel/graphited oil contacts. If these had worked then there would have been no problems from the evaporation of contact liquid. Unfortunately, a mixture of colloidal graphite and white paraffin gave zero conductivity.

Acheson Collids Limited were approached and in a most helpful letter said that dispersions of graphites in liquid form have low conductivities but kindly provided a sample of "Gredag" graphited conducting grease, Grade Y.B., for us to try; the resistance of a 2mm., portion extruded from a size 20 hypodermic needle was 10^7 ohms but was variable. Using such a graphite contact, a tapping was taken off a length of resistance wire giving a P.D. in the 100 millivolt range. The P.D. measured using the Vibron was highly variable and sensitive to vibration (c.f. Carbon microphone). Even with the input resistance of the Vibron, variations in the resistance of the graphite contact were apparently affecting the measured P.D.

A detailed survey of the literature was now made to see what other electrodes had been tried.

Waller (1900), used DuBois-Reymond's electrodes (Zinc/Zinc-Sulphate). He stated that D'Arsonval's (Silver/Silver cloride) electrodes showed a P.D. of 2 to 3 millivolts on illumination by a 16 candle-power lamp at 10 centimetres, whilst under similar conditions the Zinc/Zinc Sulphate electrodes gave a response of only one tenth of a millivolt.

Lund (1923), found that for the measurements of potentials of less than one millivolt $Zn/ZnSO_4$ electrodes were not sufficiently stable. He also found that Calomel electrodes were susceptible to light, a variation of 0.2 millivolts being experienced, and that $Cd/CdSO_4$

ZINC/ZINC SULPHATE ELECTRODE AND CONTACT SYSTEM.

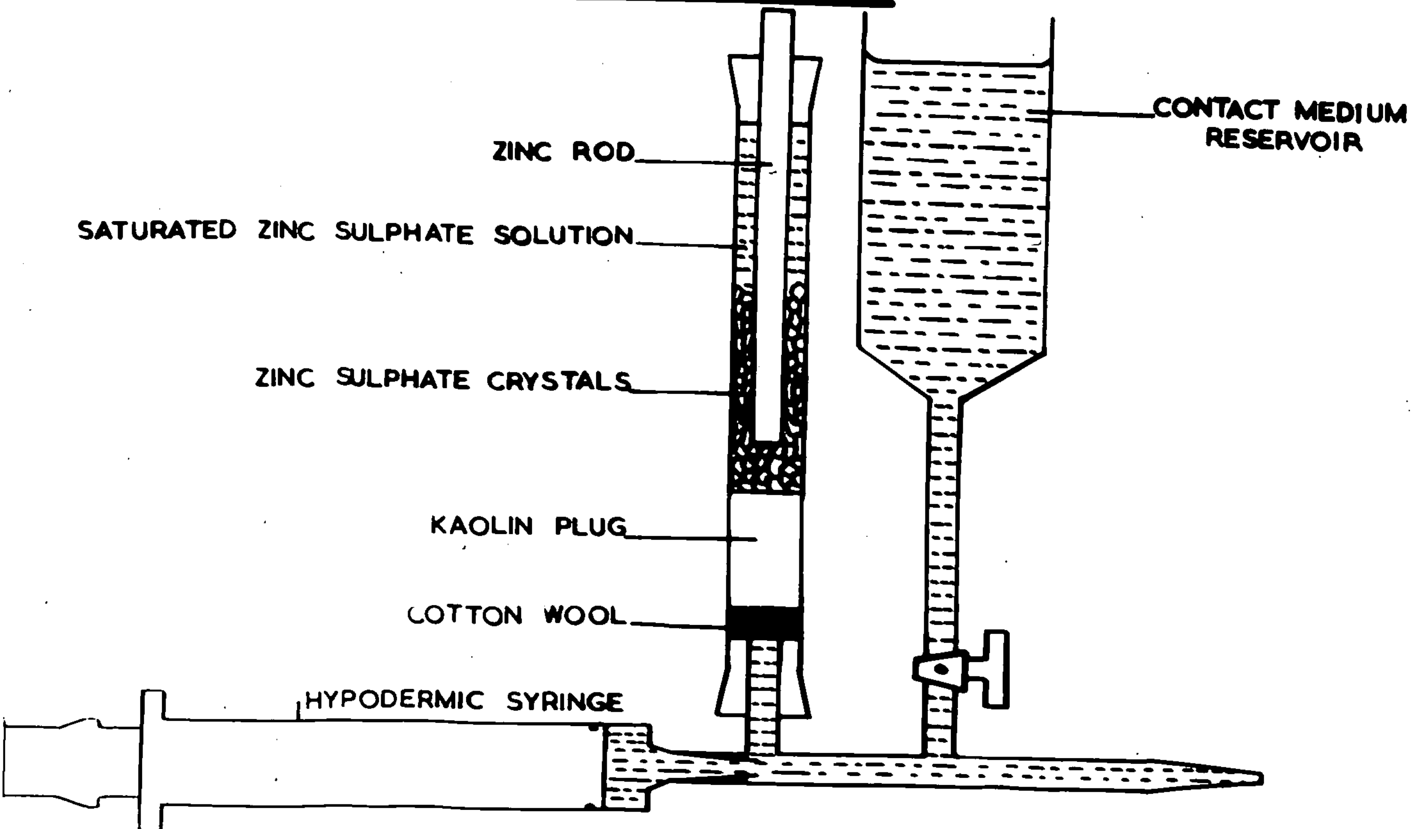


FIG. 1.1.

DIAGRAM OF ZINC ZINC/SULPHATE ELECTRODE.

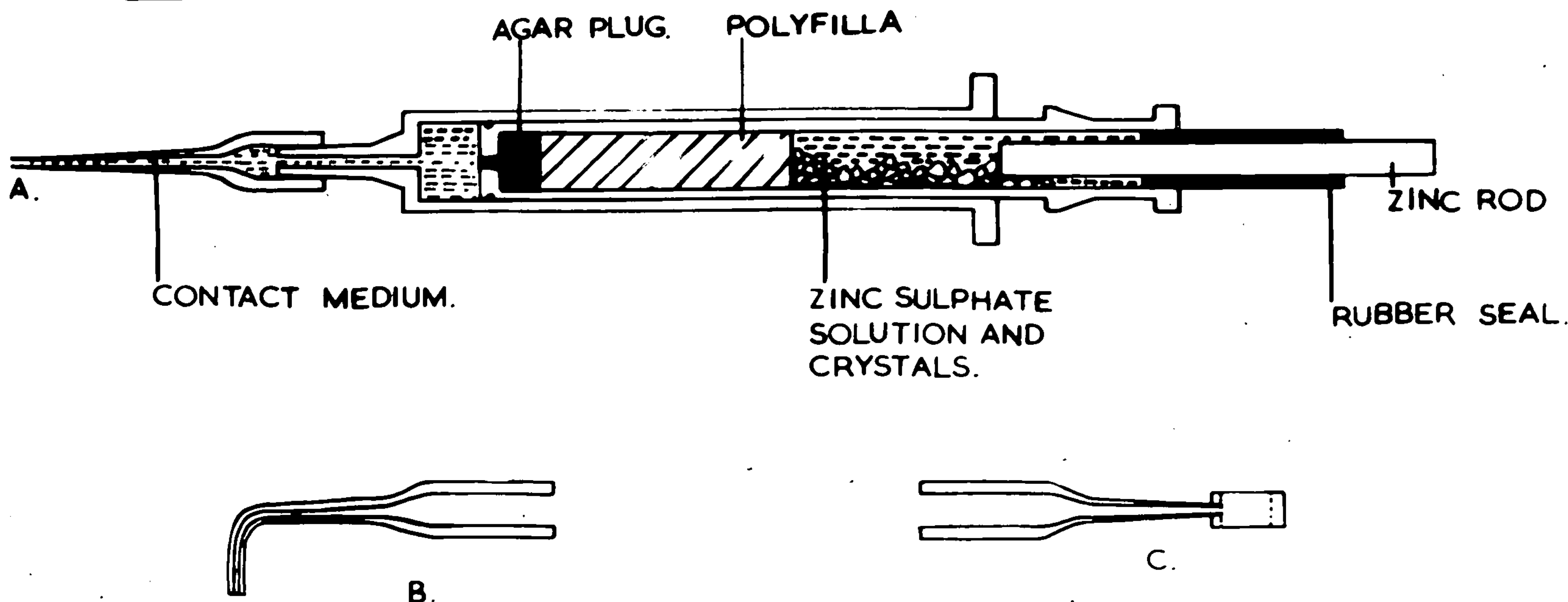


FIG. 1.2.

sulphuric acid. Later, Atlas syringes of 5 millilitres capacity were obtained and were used in the remainder of the research (Figure 1.3.). The Zinc rods in these are of 0.5 cms diameter and are durable after amalgamation. Electrodes of this type rarely need attention more frequently than once a month. Early versions had no agar on each side of the "Polyfilla", cellulose plaster plug, but this was later introduced to prevent erosion of the plug. In the latest types this agar has been replaced with cotton wool plugs. Fortunately with this size of hypodermic syringe, the plunger is hollow, with a screwed cap, facilitating the electrode conversion.

(c) Contact Systems

An extremely wide variety of contact systems have been used by other workers, and some of these are listed below:-

Agar contacts	Clark (1937).
	Hope (1951).
	Siniuchan and Stolarek (1962).
	Grahm and Hertz (1962).
Acid free kaolin	Sheard and Johnson (1930).
Moistened felt brushes	Wilcox, Knight and Bless (1953).
Moistened absorbent cotton	Rosene (1937).
Moistened linen threads	Rehm (1936).
Moistened starch paste	Nishizaki (1960).
Still liquid drop contacts	Schrank (1947).

DIAGRAM OF ZINC/ZINC SULPHATE ELECTRODE.

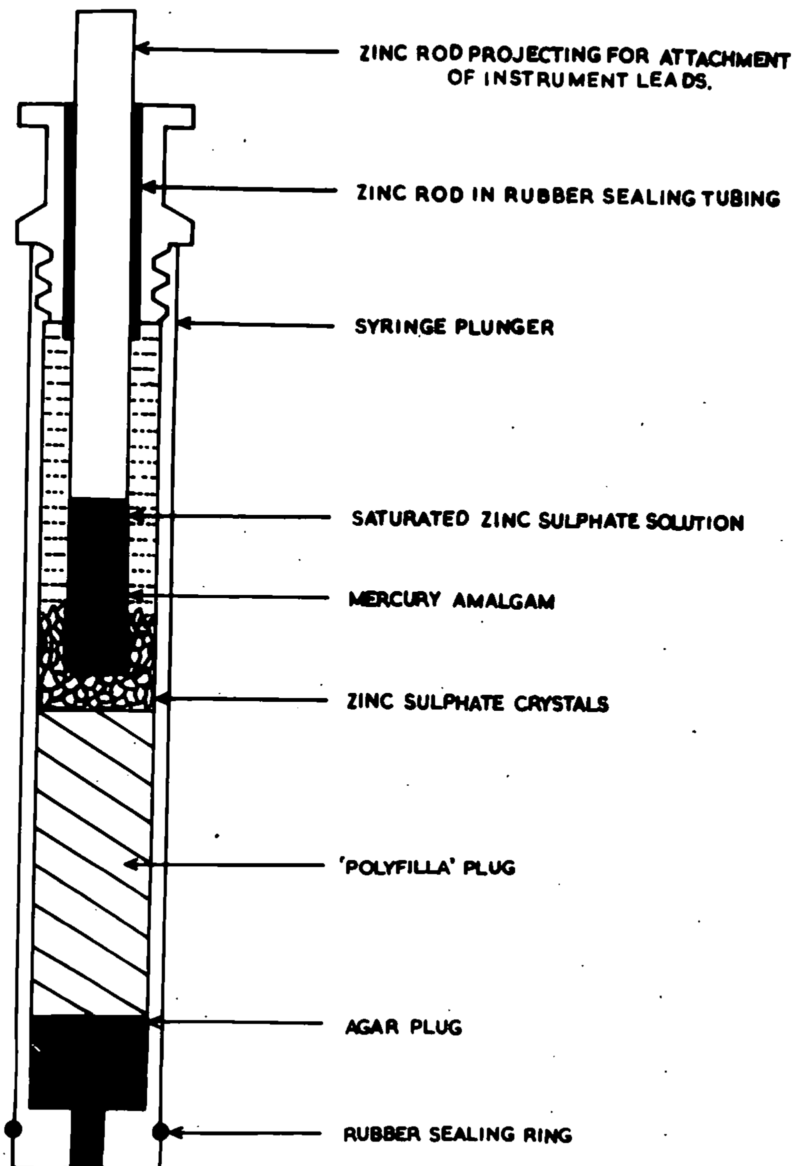


FIG. 1.3.

It has been widely reported in the literature that large potentials due to injury can be generated in plant tissues (Heilbrunn, 1943). It is essential therefore that the contact method used should cause as little damage as possible to the tissues and also be little different from a natural situation. It was thought that a liquid drop contact system similar to that of Schrank, would be appropriate, for there is no difference between his contact drop and a natural rain-drop. The system tried at first is shown in Figure 1.1. A drop of contact medium could be expelled on to the plant surface using the hypodermic syringe, and contact made via this. The reservoir enabled the system to be washed out between experiments. This contact was soon superseded by the system in Figure 1.2. The hypodermic syringe acted as the medium reservoir; contact was still made via a drop of liquid expelled by means of the plunger but the plunger itself contained the electrode. This was used in experiments reported in Chapter 2. In future it will be referred to as a "liquid drop" contact. A number of different contact tips were developed for different purposes, e.g. Figure 1.2.

A. Tip for making a side contact with plant tissue.

B. Tip for making contact at the apices of plants.

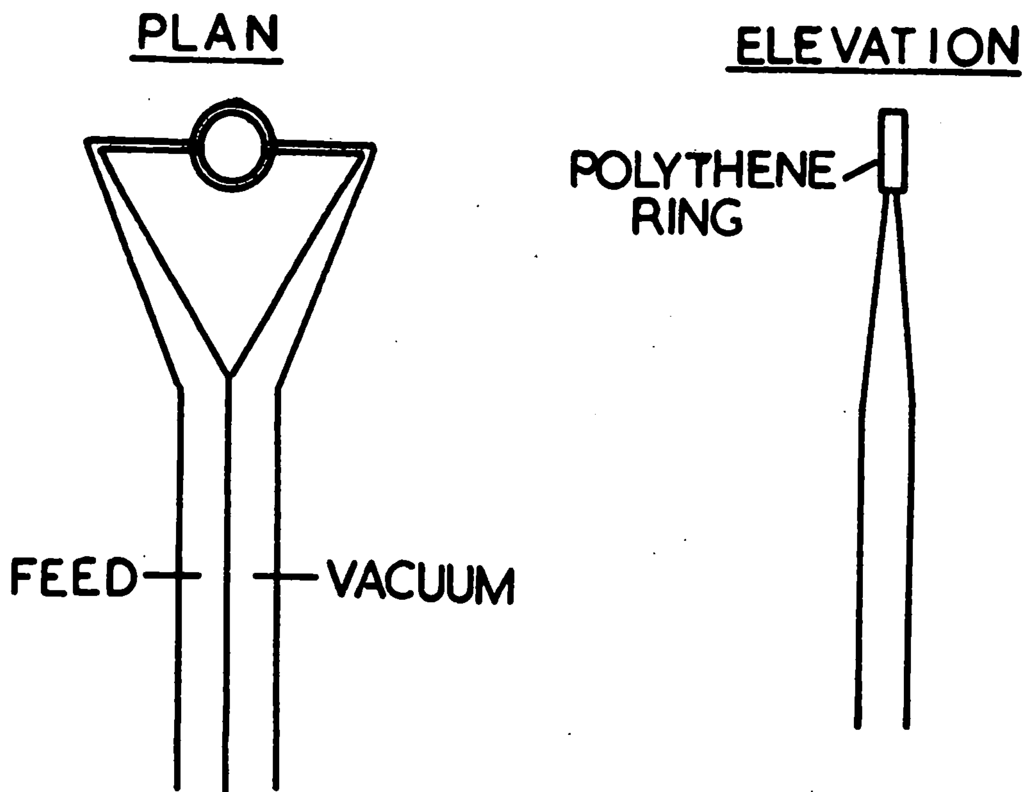
C. Tip for dipping into and making contact via the plant growth medium.

The usual system adopted in this research was to have one contact on the aerial portion of the plant and another in the growth

medium. This latter contact, of course, was little affected by evaporation or ion absorption, as the volume of medium involved was large. A short glass tube led directly from the growth medium to the electrode. However, as will be related in more detail later, experimental evidence indicated that when the aerial contact drop was renewed by expelling more liquid from the reservoir the measured potential was changed. This drop renewal was necessary to compensate for evaporation. Long experiments could not be contemplated using this type of contact as constant attention was necessary.

Scott, McAuley and Jeyes (1955), state that "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". They avoided the difficulty by measuring the potentials existing in a water bath, close to a root surface, due to the electric currents generated by the roots. Osterhout and co-workers (1927), in several papers reported that they used flowing liquid contacts of various types in order to maintain a contact medium of constant composition.

A new type of aerial contact was therefore envisaged using a continuous circulation of the contact medium. The first system tried is shown in Figure 1.4A. This was completely unsuccessful, for the



ELEVATION

B

VACUUM

PLAN

FEED

POLYTHENE RING

RUBBER SUPPORT.

FIG. 1.4.

HYPODERMIC SYRINGE ELECTRODE

liquid ran round the periphery of the ring and did not form the expected drop.

Then a system using two concentric tubes, the inner a feed tube and the outer a vacuum tube (Figure 1.5.), was tried. This was successful and was used in much subsequent work. It was found that the size of the drop formed on the plant surface could be controlled by adjustment of the feed rate, the vacuum, and the position of the end of the vacuum tube.

In the early experiments attempts were made to recirculate the contact medium. This is shown in Figure 1.6. A single contact medium reservoir was used, the medium dripping into funnels feeding the contact systems. There was a single collecting flask for the exhausted contact medium, and from this the contact medium was pumped back into the reservoir. This system, surprisingly enough, could be made to work but adjusting the rates of flow to attain true recirculation was a formidable task, and it was abandoned in favour of the system shown in Figure 1.7.

At this stage it is instructive to consider the rates of renewal of solutions in the contact drops. The inner contact tube had an internal tip diameter of about 0.5 millimetres. The diameter of the contact drop was also of this order. The contact was usually placed at a distance of 1 millimetre from the plant surface so the volume of this contact drop was:-

DIAGRAM OF FLOWING DROP CONTACT.

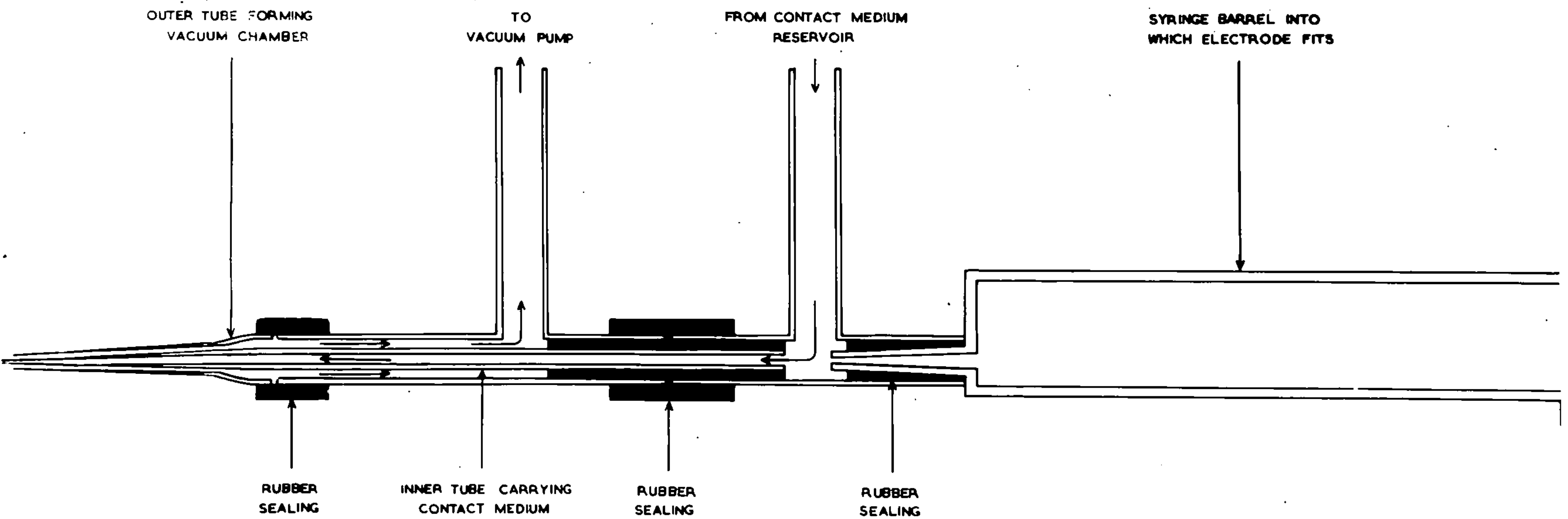


FIG. 1.5.

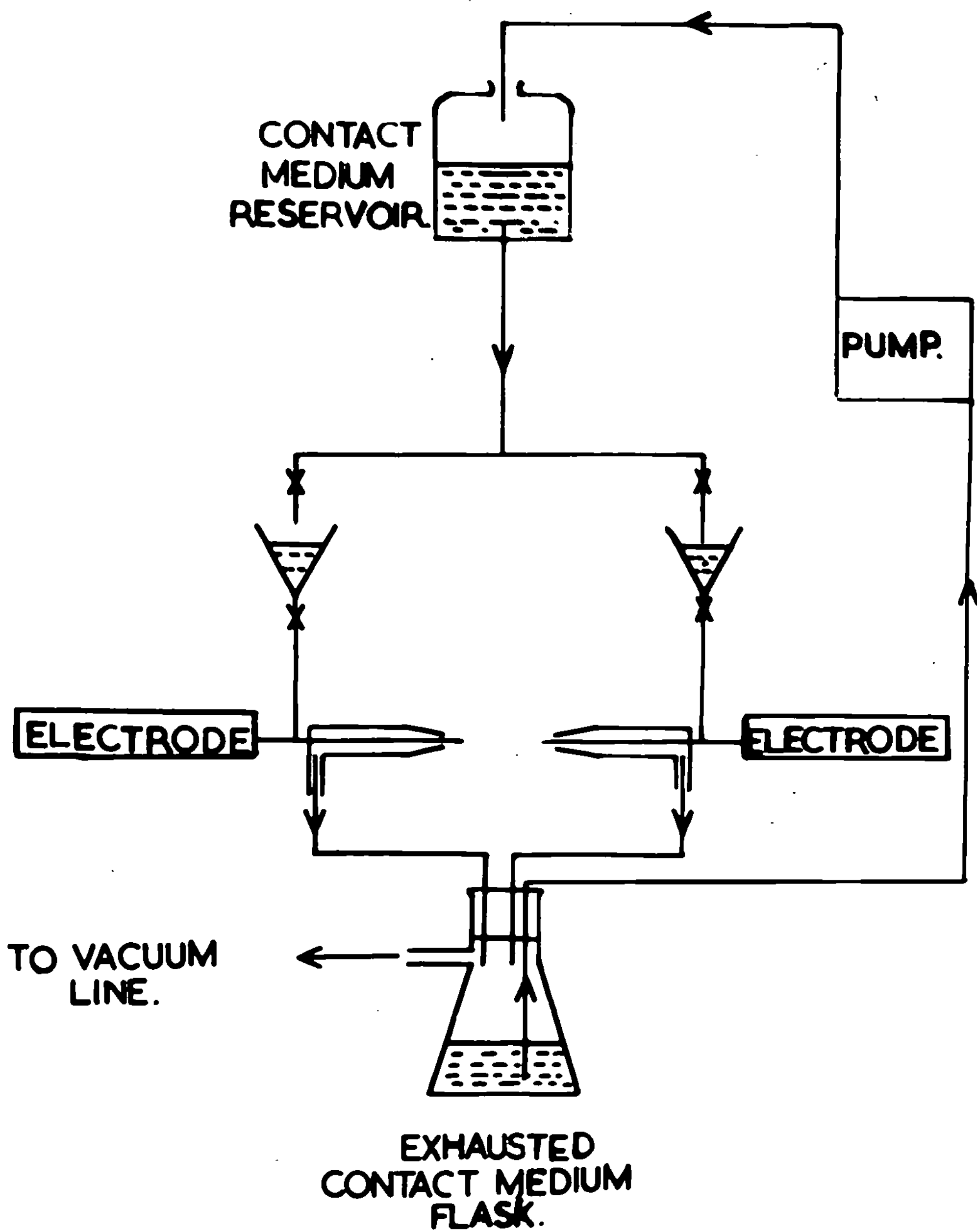


FIG. 1.6.

DIAGRAM OF ELECTRODE AND CONTACT SYSTEMS.

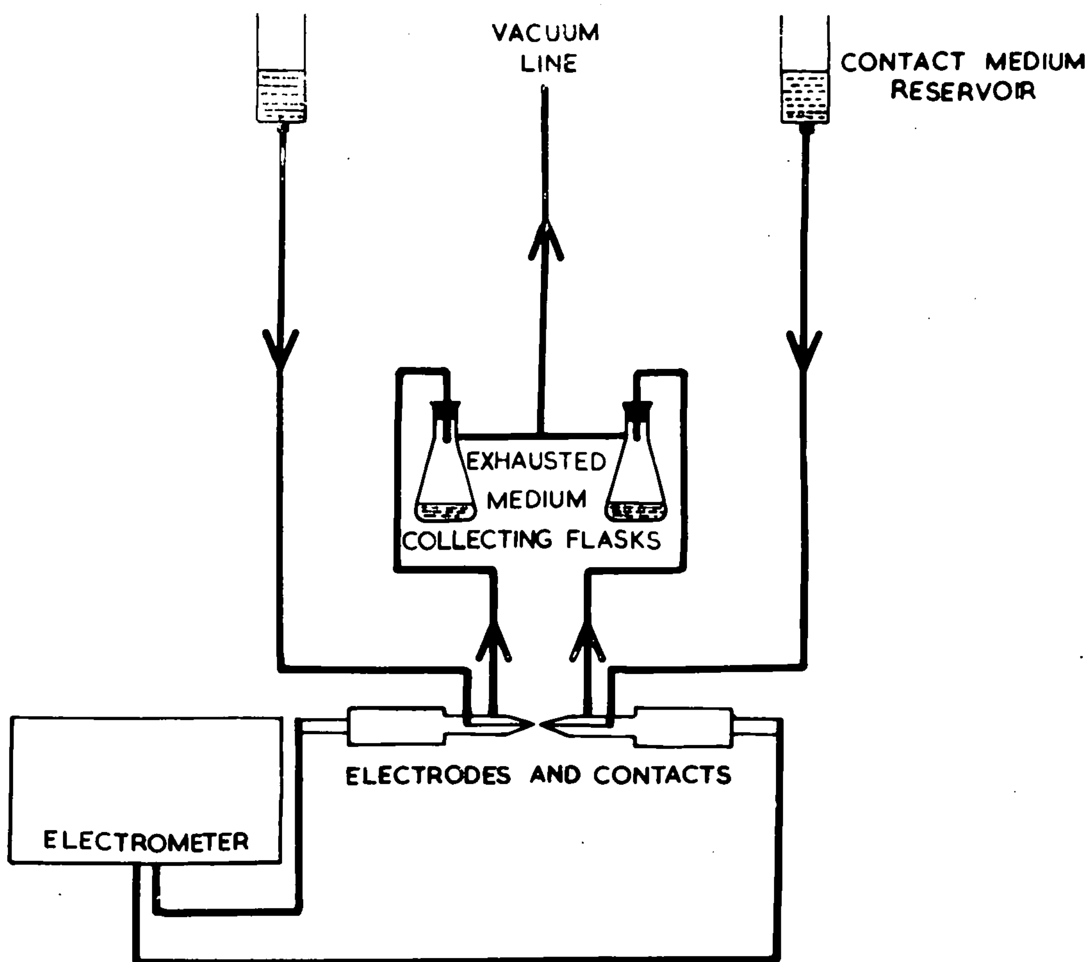


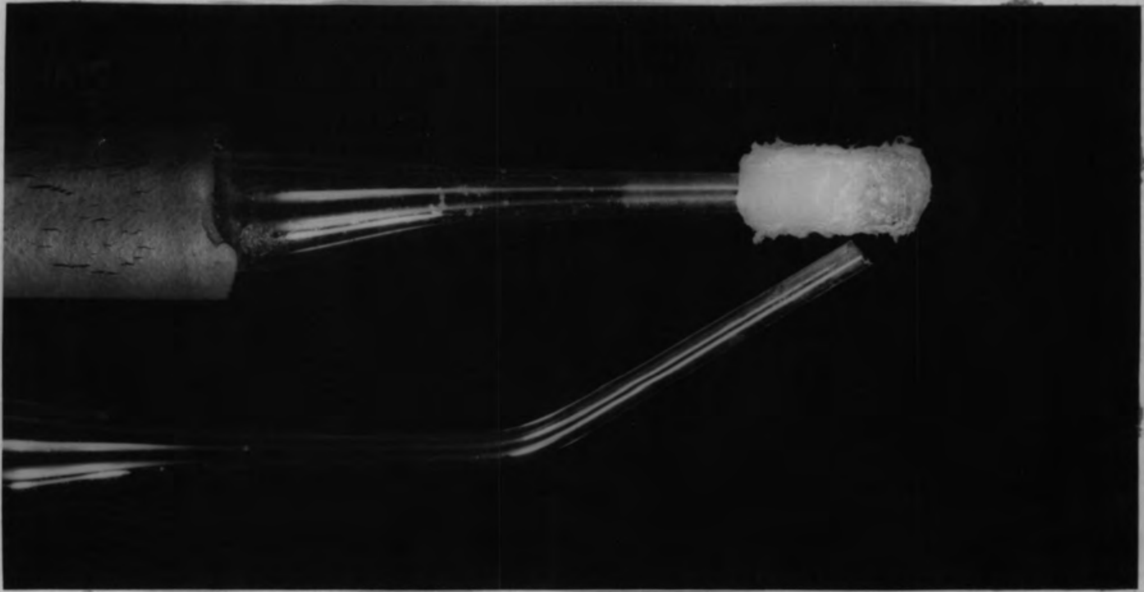
FIG. 1.7.

$\Pi \times 0.025 \times 0.025 \times 0.1$ millilitres.

$= 1.96 \times 10^{-4}$ millilitres.

The contact medium reservoirs were of 300 millilitres capacity, and the feed rate was usually of the order of 1 millilitre per minute but in longer experiments it was dropped to $^1/6$th of this rate. This gives a rate of renewal of the contact drop in the first case of 84 per second, and in the second of 14 per second. It is obvious that this was quite sufficient to prevent the contact medium showing any changes due to outward movement of ions from the plant tissue or inward movement from the contact medium, and concentration changes due to evaporation. This contact system was extremely good for most purposes and will in future be referred to as a "flowing drop contact". It had however rather a small contact area with the plant, and also the plant, due to nutation movements etc., could move away from it. For some experiments a ring contact such as we had attempted to develop before, seemed more suitable. Such a contact was later most successfully developed (Figure 1.4B), and will in future be referred to as a "flowing drop ring contact".

The position of the vacuum tube was highly critical for the successful formation of a drop. The photographs (Plate 1) show the position in a successful contact. As will be reported later this type of flowing drop ring contact has also been used to apply auxin to coleoptiles and it is felt that the use of such a system offers many



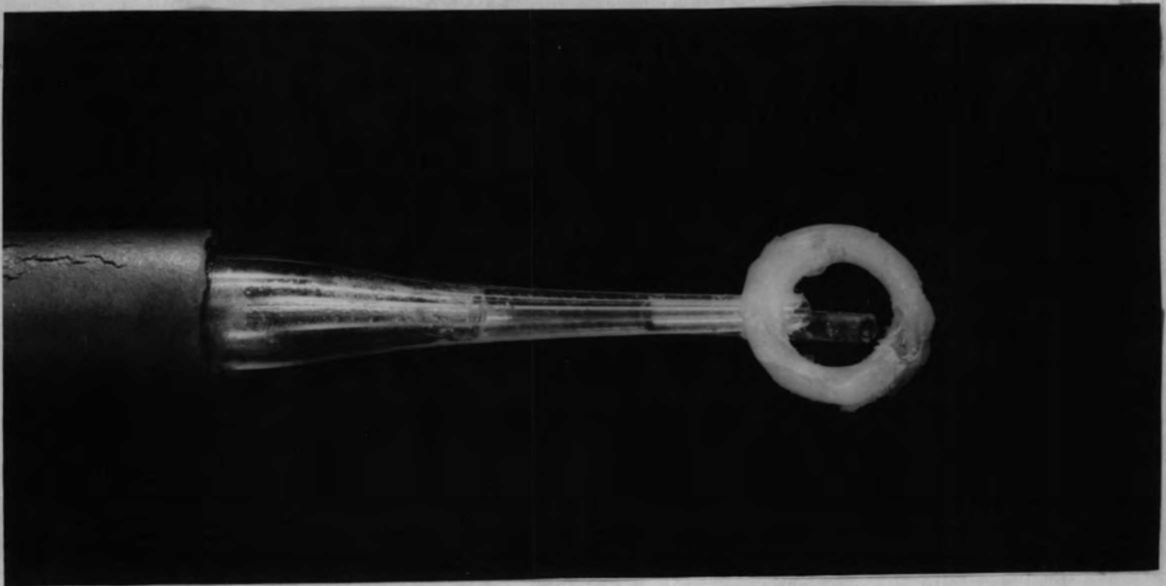


PLATE. I.I.

advantages over the conventional agar block. This will be discussed in more detail in Chapter 6.

The polytheme rings were of about 0.5 centimetres diameter, and assuming a perfectly hemispherical drop, with the previously quoted flow rates, the rates of contact medium change were 30 per minute and 5 per minute respectively. Using this contact, readings over periods of 48 hours have been obtained without disturbing the plant in any way.

Subsequent to these developments, a reference was found to a system similar to the flowing drop contact (Newman, 1959 and 1960). His differed in that the electrode was placed on the exhaust rather than on the feed side of the contact. Newman's flow rate was low compared with that reported above. Osterhout and co-workers used several types of flowing contact but none comparable with that developed by ourselves.

The flowing contact medium contacts have proved extremely reliable in use, giving reproducible results. It was feared at one time that electro-kinetic potentials, set up due to the contact medium flow, might have interfered, but this does not seem to have been the case. For instance, the P.D. that existed between a flowing drop ring contact, and a contact in the equivalent of the growth medium was zero, when the plant was represented by a moistened piece of string.

Also the following calculations on this point were worked out. The equation for electro-kinetic potentials between the ends of a tube, due to the flow of a solution through it, is:-

$$E = \frac{\zeta D P}{4 \pi \eta \chi}$$

where ζ is the zeta potential difference between the solution and the tube.

D is the dielectric constant of the solution.

P is the pressure difference between the two ends of the tube in dynes.

η is the viscosity of the medium

χ is the specific conductance of the solution.

Since D, η, χ, ζ are constants, depending only on the solution and tube in question, then E should be directly proportional to P. Kruyt and Willigen (1918), found the following results using dilute KC1 and a glass capillary tube.

P in centimetres of mercury	E in millivolts	$\frac{E}{P}$
40	486	12.15
50	605	12.10
60	725	12.08
70	834	11.91
80	943	11.79

Examination of a graph plotting $\frac{E}{P}$ against pressure, shows that $\frac{E}{P}$ tends to the constant value of 12.2 at low pressures. (Figure 1.8).

Therefore the equation of this graph of P.D. against pressure in centimetres of mercury, for low pressures is E = 12.2 P millivolts,

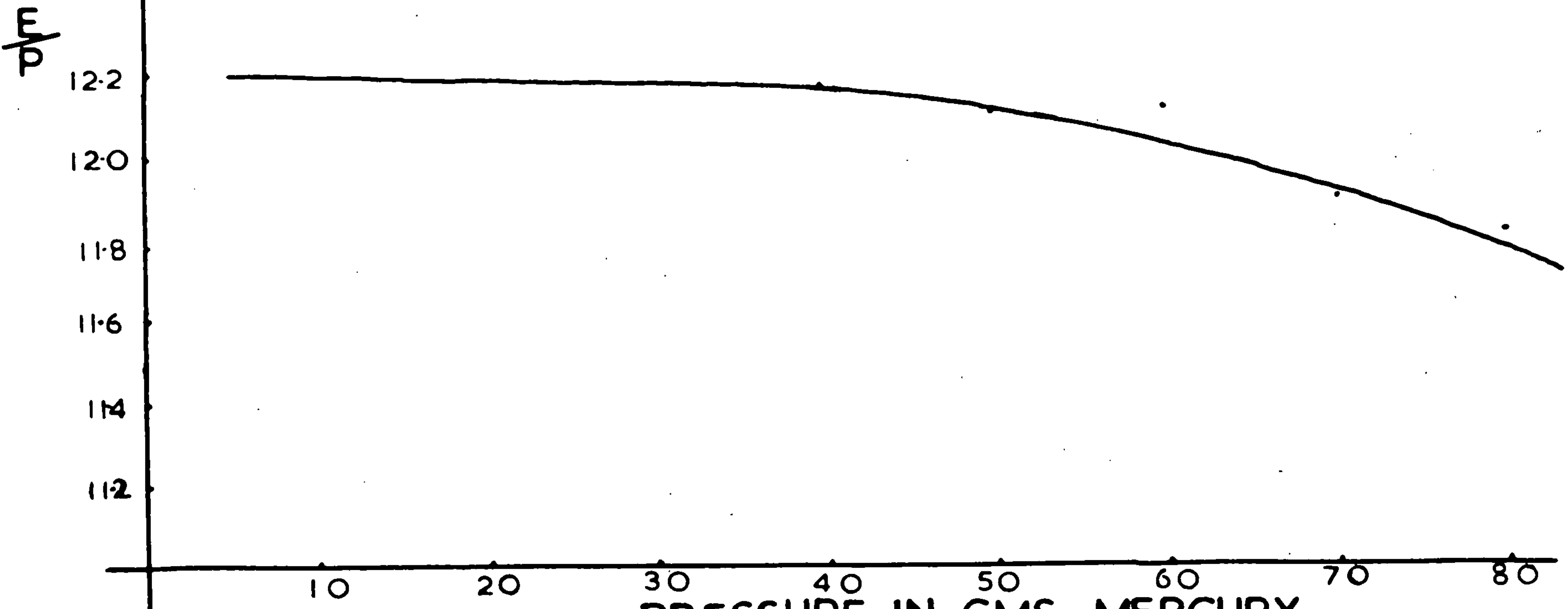


FIG. 1.8.

where P is the pressure in centimetres of mercury or $E = \frac{12.2x}{13341.6}$ mv.
where x is the pressure in dynes per square centimetre.

We have used these results to calculate an approximate value for the kinetic potentials that could be generated. It is only necessary to calculate the pressure drop in the tube between the electrode and the actual contact to determine the potential directly.

The pressure drop is calculated using Poiseuilles' Law.

i.e. $P = \frac{Q 8 \eta l}{\pi r^4}$ where P is the pressure drop in dynes per square centimetre.

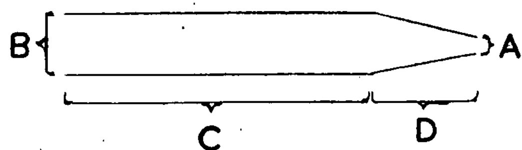
Q is the volume of liquid flowing in millilitres per second.

η is the viscosity of the liquid.

r is the radius of the capillary contact.

For water η = 0.01.

<u>Consider the situation with regard to our contact tube.</u>



A is 0.025 centimetres radius.

B is 0.1 centimetres radius.

C is 5.0 centimetres long.

D is 2.5 centimetres long.

Since the tube is drawn out into a point capillary, and is therefore non uniform, it has been treated as follows:-

(a) The pressure drop along the uniform portion C has been calculated.

(b) For the remaining non-uniform part of the tube, D, the minimum pressure drop, as if it were not drawn out into a capillary, and the maximum drop, as if this part of the tube was of the tip diameter throughout its length, has been calculated.

(c) Addition of the values for C and the maximum and minimum values for the tapered region, D, gives the maximum and minimum kinetic potential.

Calculated pressure drop along C with a flow rate of 1 millilitre per minute = 21 dynes per square centimetre.

Therefore P.D. in millivolts = $\frac{12.2 \times 21}{13341.6}$ generated between the ends of C.

which approximately equals 2.0×10^{-5} millivolts.

Minimum pressure drop along remainder of tube, D,

= 10.5 dynes per square centimetre

therefore the minimum P.D. generated between the ends of D, is approximately equal

to 1.0×10^{-5} millivolts.

<u>Therefore minimum kinetic potential generated along Contact tube is 3.0×10^{-5} millivolts, approximately.</u>

Maximum pressure drop along remainder of tube, D,

= 2710 dynes per square centimetre.

therefore the maximum P.D. generated between the ends of D is approximately equal to 2.5 millivolts.

<u>therefore the maximum kinetic potential generated along the contact tube could be 2.5 millivolts approximately.</u>

The P.D. actually existing would be smaller than the maximum, as this is calculated making pessimistic assumptions. The flow rate on which these calculations are based was the highest normally used.

Slower rates were more usual and the potential therefore smaller. Furthermore, where a pair of flowing contacts were used, the kinetic potentials would tend to cancel out and even where the bioelectric potential was measured between a stationary and a flowing contact the value of the kinetic potential could be no greater than that which is often accepted as reasonable for electrode asymmetry.

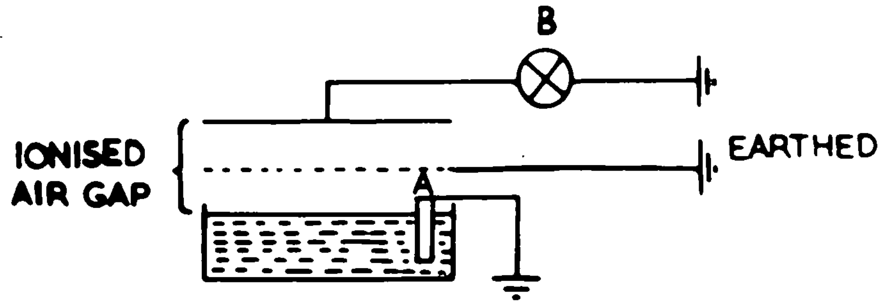
Chapter 1. Section B - Measurement of electrical potential differences employing an ionising radiation.

The potential at a point on the surface of a plant may be measured by positioning a metal plate a short way off the plant surface opposite this point, and making the air between it and the plant conducting, by means of an ionising radiation (alpha particles or gamma rays).

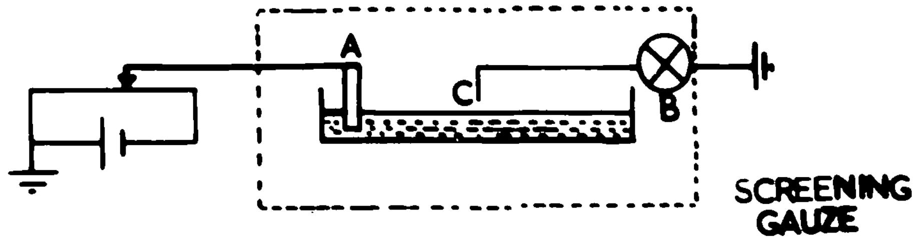
This system was first suggested by Bluh and Scott (1950), and again by Scott McAulay and Jeyes (1955). It has however never been used on plant tissue.

Andauer (1927), appears to have been the first to use the principle when he attempted to measure the absolute potential of the Calomel electrode. His circuit is shown in Figure 1.9 (1). He used the rate of discharge of the charged electrometer (B), as a measure of the

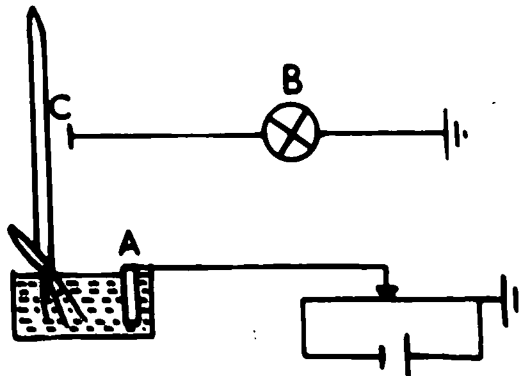
① ANDAUER'S METHOD



② SURFACE POTENTIAL METHOD



③ IONISING PROBE APPLIED TO PLANTS



A — REVERSIBLE ELECTRODE.

B ELECTROMETER

C IONISING PROBE.

FIG. 1.9.

absolute potential but ignored the contributions of the air/liquid and air/metal plate interfaces. His first value of the absolute P.D. of the calomel electrode (A), was +0.51 volts. In a later paper (1928), he introduced corrections for these interfacial potentials which altered the value to -0.2 volts.

The method has also been used to measure the surface potential of a film of an insoluble substance on a liquid. This is measured by the effect of a surface film of the substance on the potential difference at the air/liquid interface. (Figure 1.9(2)). The tip of the air electrode (C), is coated with a radio-active material providing alpha particles. The arrangement constitutes a complex electrolytic cell with two electrolytes, the liquid in the trough and the ionised air, and three surfaces, the reversible electrode in the liquid (A) the air/liquid surface, and the surface of the air electrode (C). Only one of these, the air/liquid surface, has its P.D. altered by the presence of an insoluble and non volatile film on the liquid. In practice therefore, the overall P.D. without the film, is found by adjusting the potentiometer to give a null deflection on the electrometer (B). The film is then applied and the new balance point found. The difference between the two readings gives the surface potential.

Returning to plant tissue, it seems that there would be two possible methods of approach, the first of which is shown in Figure 1.9 (3). This could not be used to give directly the potential of the point with

respect to the base, for there are the following unknown interfacial potentials in the circuit which exist only as a result of the attempt to measure the potential and which are not counter-balanced.

:- Reversible electrode (A) Growth medium surface Air/metal surface (C).

This method is best suited to the study of potential changes at one point. By taking readings at two points, the difference between them gives the absolute potential of one point with respect to the other, for the interfacial potentials now cancel out. By the use of two ionising probes, the ptential of one point with respect to another could be measured directly. No attempt has been made to use this method in this research.

Chapter 1 Section C. - Measurement of potential employing a dynamic condenser.

This method is based on a dynamic condenser, one plate of which is formed by the plant surface and the other by a metal plate vibrating a short distance from it.

When a charge exists on the condenser, due to the potential of the plant surface, an alternating current will flow through a circuit connecting the vibrating plate and the plant, at the same frequency as the vibrations of the plate. This may be amplified and displayed on a cathode ray oscillograph. Using a potentiometer, a potential is applied to the plant until this A.C. is zero, then the applied potential is

equal and opposite to the original potential existing on the plant. This method has been successfully applied to a study of geoelectric responses of plants by Hertz (1960), and Grahm and Hertz (1962). During this research it was deemed desirable to develop a vibrating probe, and a more detailed discussion is left to the chapter devoted to this.

CHAPTER 1.

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CHAPTER.2.

CHAPTER 2.

PRELIMINARY EXPERIMENTS ON SYCAMORE SEEDLINGS, ON WHEAT AND ON OAT COLEOPTILES.

SUMMARY

Preliminary experiments on Sycamore seedlings and on Wheat coleoptiles are described. In more detailed experiments on Oat coleoptiles, changes in the potential magnitudes and patterns are noted on alterations in the growth and contact media. Since graphs of the potential magnitudes against contact and/or growth medium concentrations show straight line relationships, it is inferred that the potentials measured are diffusion potentials in their broadest sense.

This chapter is concerned with what at the time of experimentation were intended to be preliminary experiments to confirm the accuracy of the apparatus, but, as will be shown, they developed into investigations in their own right. In these experiments both Sycamore (Acer pseudoplatanus) and Wheat (Triticum Sp) seedlings were employed. There had been no previous work on these plants. The Sycamore seedlings were collected in the field, and they had cotyledons present but no leaves. The seedling's roots were placed in pots containing Shive's 3 salt solution and the plant held in place by cotton wool.

Shive's solution (Shive, 1915) consists of 18 millilitres of Molar potassium dihydrogen phosphate, 15 millilitres of Molar magnesium sulphate, 5 millilitres of Molar calcium nitrate, 1,000 millilitres of distilled water.

In later experiments where various dilutions of Shive's solution have been used, the complete Shive's, described above, has been taken and diluted in the usual manner to give a percentage dilution.

The rooting medium formed the basal contact. Contact was made at different parts on the surface of the Sycamore seedlings by means of drops of Shive's solution expelled from the syringe electrode, ("liquid drop contact"; Chapter 1, Section A) and the potential of these points measured with respect to the base. For completeness sake the following results are included. In this, as in all other experiments reported in this thesis, the polarities referred to are those existing

in the external circuit.

The apex of the Sycamore seedling was usually positive, but in some, after disturbance by knocking, etc., was negative. The longitudinal P.D. was small, usually in the region of one millivolt but occasionally was as high as five millivolts. Changes in potential were noted when the preparation was approached and it was thought advisable to screen the apparatus. Half-inch wire mesh was used for this purpose and the whole screening cage earthed. The shielding is shown in plate 2.1.

Experiments were then commenced on Wheat coleoptiles. Complete details of the growing technique are given below as this was followed throughout the research when either Wheat or Oat (Avena Sp) were involved.

Oat grains were first dehusked, but this was not necessary in the case of Wheat. The grains were then placed in Büchner funnels and tap water allowed to drip on them for two to three days in normal daylight. The exact time depended on the water temperature. When the grains had germinated they were planted out on rafts made of one inch plastic curtain rings with nylon mesh stretched across (Plate 2.2.). The rafts were supported by glass slides in such a way that the growth medium just covered the net. The grains were placed at an angle on the rafts so that the coleoptiles grew straight upwards and they were left in red light (above 6000 Å) for the remainder of their growth. The

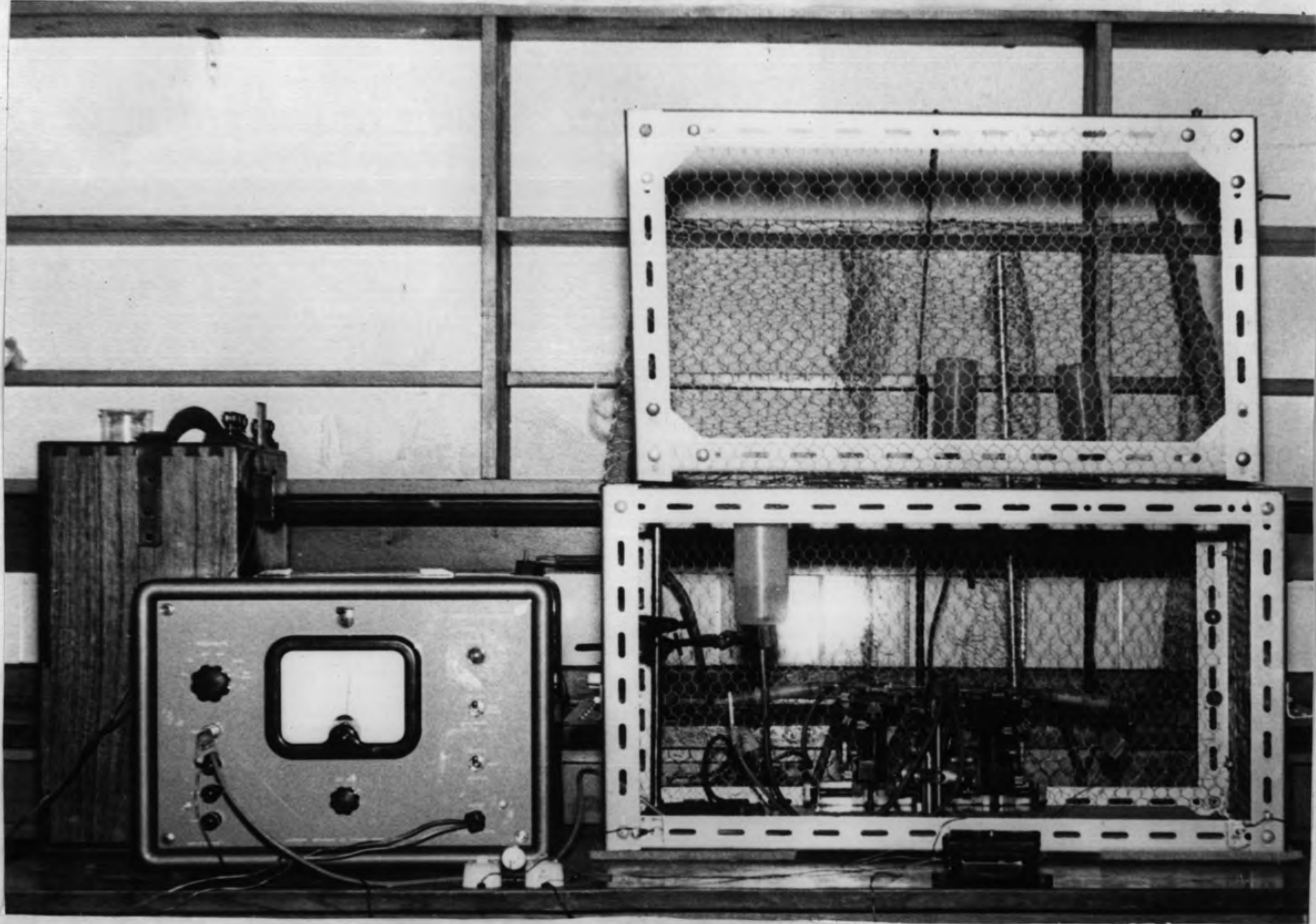


PLATE. 2.I.

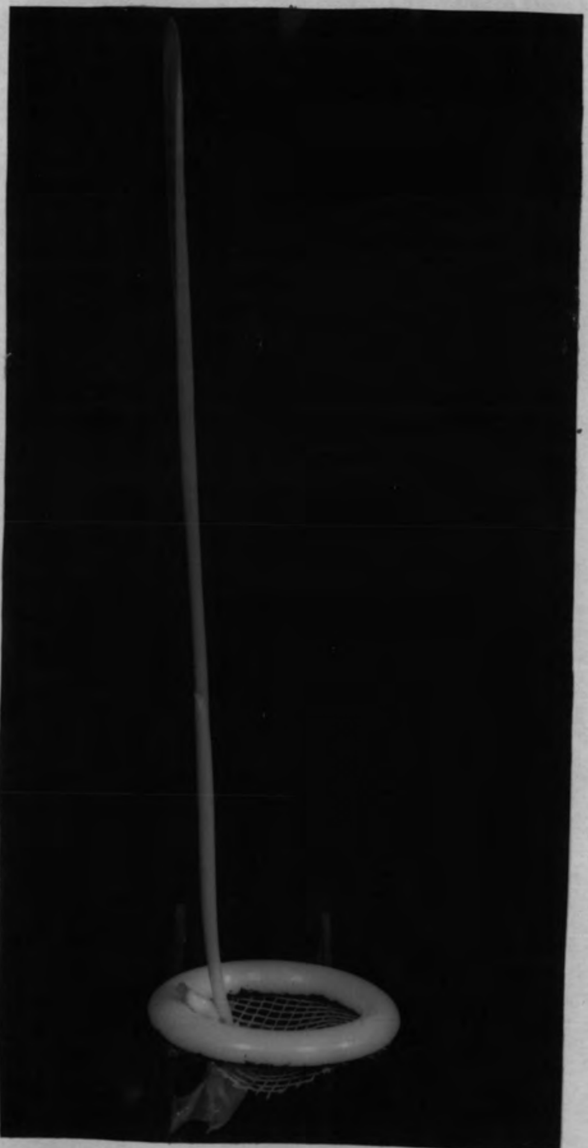


PLATE. 22.

advantage of growing the seedlings on rafts was that they could be moved to the experimental chamber without disturbance, by simply transferring the rafts.

The Morphology of the Grain Seedling.

Upon germination of the grain, the primary root begins to grow out and is followed within a short time by two lateral roots. Meanwhile the shoot also starts to elongate; it consists of a growing point, a very short stem with two partially developed leaves, and a surrounding sheath, the coleoptile.

Between the base of the coleoptile sheath and the actual grain there develops an internode, the mesocotyl, whose length is very dependent upon the growth conditions.

The growth of the coleoptile, which is a hollow cylinder with a solid conical top, takes place almost entirely in the longitudinal dimension. In its early growth, up to a length of one centimetre, cell divisions of the parenchyma accompany the elongation, but the epidermal cells cease dividing at a very early stage and grow only by extension. From a length of one centimetre up to its final length, cell divisions are virtually absent and growth is entirely by cell elongation. On this account the coleoptile is a particularly suitable subject for studies of growth uncomplicated by cell division.

In transverse section, the coleoptile is elliptical, with vascular bundles running up on either side. The cells at the tip of

the coleoptile are morphologically distinguishable from the others for they do not elongate and are almost isodiametric. The epidermal cells of the extreme tip stain more heavily than the rest and are probably richer in protoplasm. The region of isodiametric cells is limited to the apical 0.5 millimetres. The growth of the primary leaf closely follows that of the coleoptile under natural conditions so the coleoptile is almost completely filled.

When the coleoptile has attained its full height, it ceases to grow and the primary leaf breaks through. The whole growth of the coleoptile occupies about 5 days and the maximal rate of elongation, one millimetre per hour, is attained during the stage when the coleoptile is 20 to 35 millimetres high.

Throughout the growth, the apical 2 millimetres hardly elongates. The zone of maximum growth is first located near the base and migrates upwards so remaining about 10 millimetres below the tip. These details of morphology have been taken from "Phytohormones" (1937) by Went F.W. and Thimann K.V.

Under the condition of growth in red light, the first internode, (mesocotyl), elongates only a little, due to the inhibiting effect of this wavelength of light, to which its growth is especially sensitive. The coleoptile sheath, however, grows long for it is the blue end of the spectrum that inhibits its elongation. ((Avery Burkholder and Creighton, 1937). Coleoptiles are not phototropically sensitive to red

light and in it, grow vertically under the influence of gravity. The red light was provided by fluorescent tubes wrapped in 8 layers of red cellophane. Spectroscopically tested they gave light above 6000 Å only. During the period of growth and in the experimental chamber, the temperature was controlled at $20^{\circ}C \pm 1^{\circ}C$.

The apparatus used in the Wheat and original Oat experiments is shown in Plate 2.3.

In the first experiments on Wheat coleoptiles the readings were commenced 10 minutes after making contact. They indicated that with Shive's contact and growth medium, that the apex of the coleoptile was positive with respect to the base. Wilks and Lund (1947) and Schrank (1947) found the apex was negative with respect to the base in Oat coleoptiles.

In another series of experiments readings were commenced immediately after making contact and it was noted that large changes in potential were taking place upon making contact, and a constant value was not attained for about 20 minutes. (Figure 2.1.A.). If contact was broken for a period of only five minutes an exactly similar change again occurred on remaking contact.

Wilks and Lund (1947), had plotted the longitudinal potential of Oat coleoptiles by passing a ring contact rapidly down the outside of the sheath. They state that each time the electrode was moved to a new position, a <u>slight</u> change in potential was noted, and the

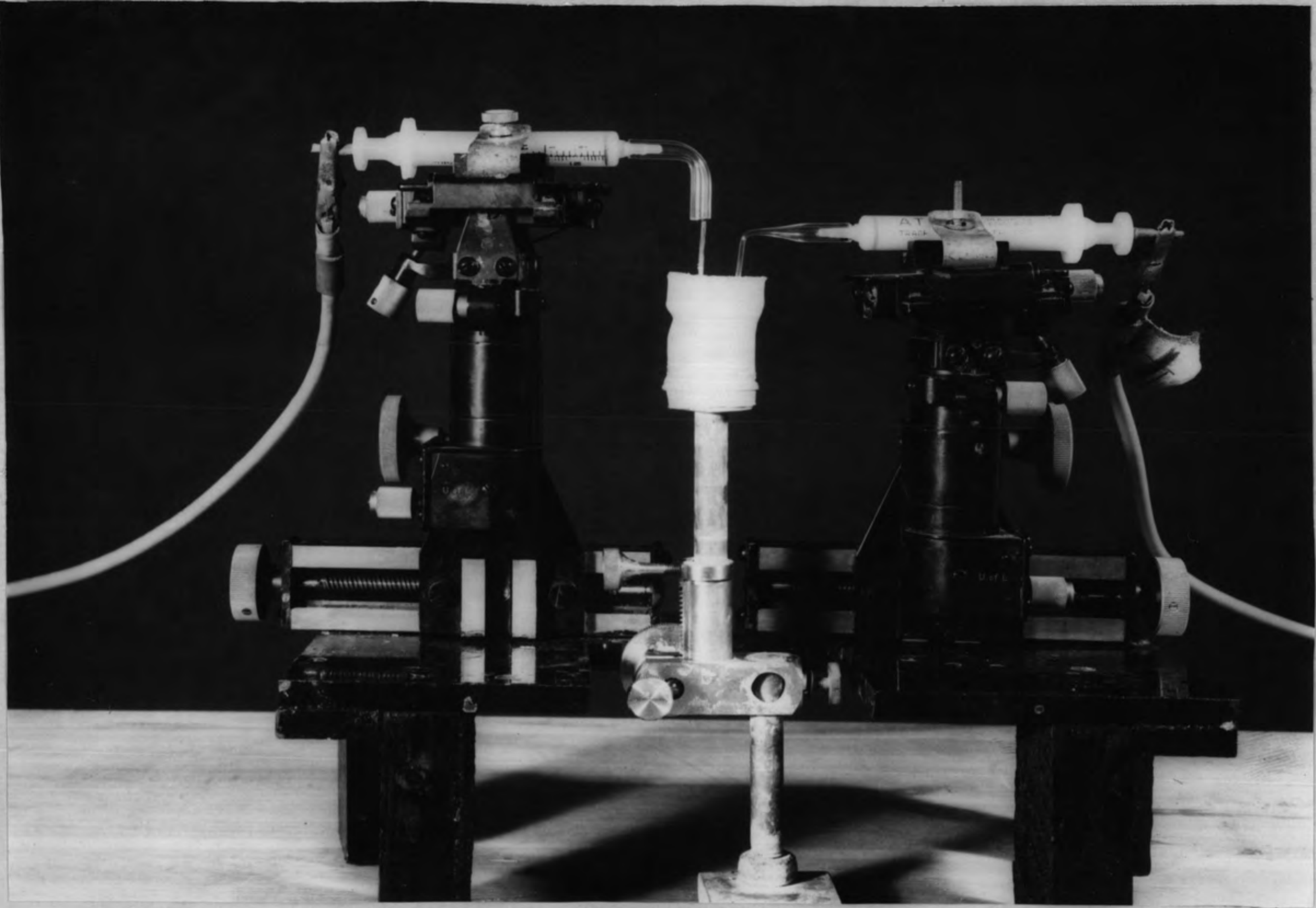


PLATE. 2.3.

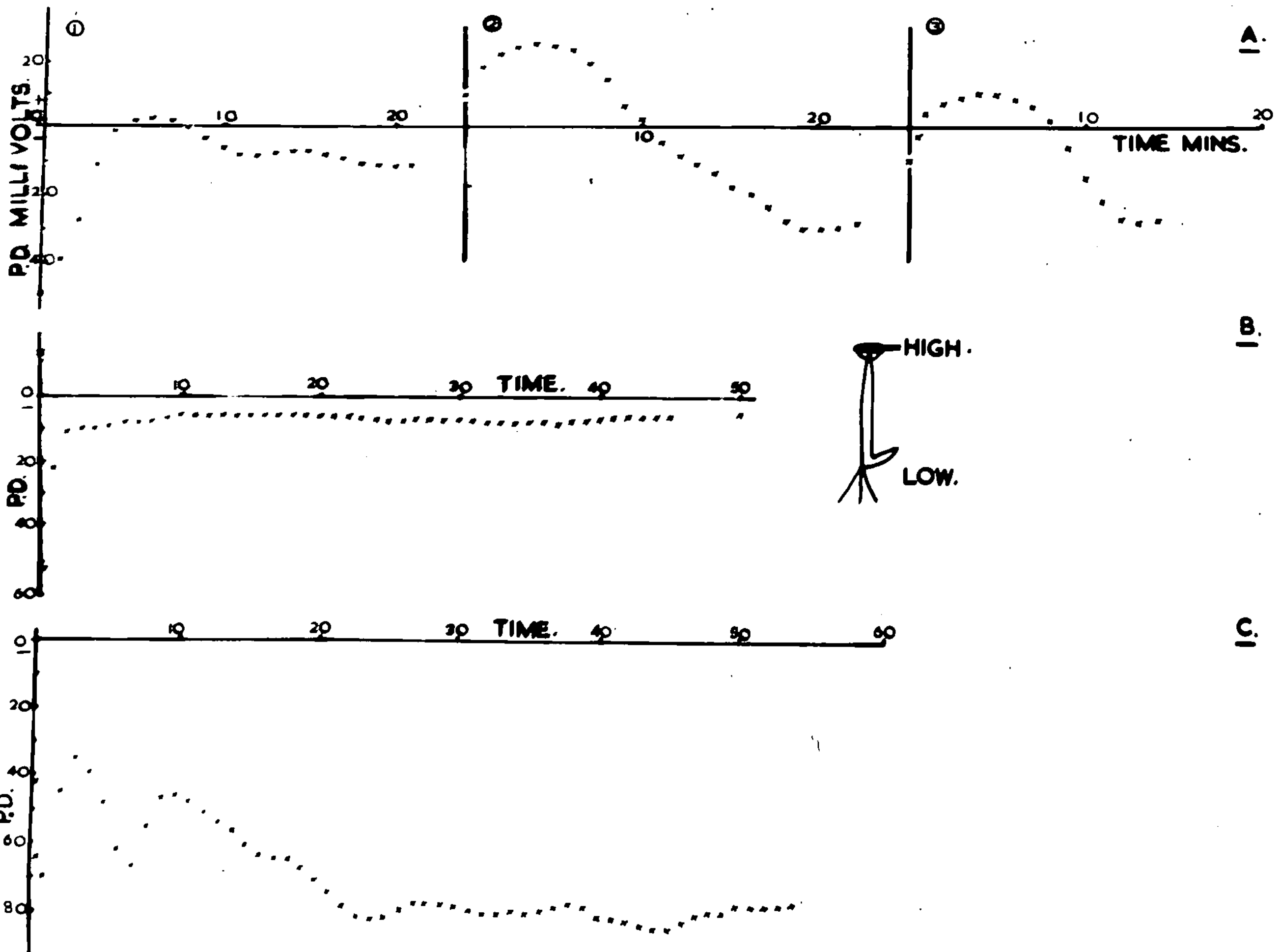


FIG. 2.I.

electrometer reading was not recorded until the potential was "relatively" stable.

A similar experiment to that of Wilks and Lund (1947), was therefore performed, the ring contact (Figure 1.2.C.) being moved down the coleoptile and the potential recorded with respect to the base. It was found that the potential pattern of the sheath was not simple, maxima and minima in the potential occurring several times along its length. Large changes in potential followed the movement of the contact to a new position, and if the contact was returned to a previously measured position the potential had altered. If however the positions where the potential was a maximum or minimum, the points of inflexion, were plotted, it was found that when the contact was returned to such a position, though the value of the potential had altered, it was still a point of inflexion.

A series of readings were taken on coleoptiles, plotting points of inflexion only. The results are seen in Figure 2.2. The values of the P.D. measured at the time of plotting are noted, but due to the changes mentioned above these are of little significance. "A" is a group of coleoptiles showing similar potential patterns. "B" is an anomalous example whilst "C" is the average of group "A". The P.D. values on the left of the coleoptiles are based on the apex as zero whilst those on the right with the base as zero. The coleoptiles varied in size but have been scaled up to the same height.

DIAGRAMS OF RESTING ELECTRICAL POTENTIALS IN WHEAT COLEOPTILES.

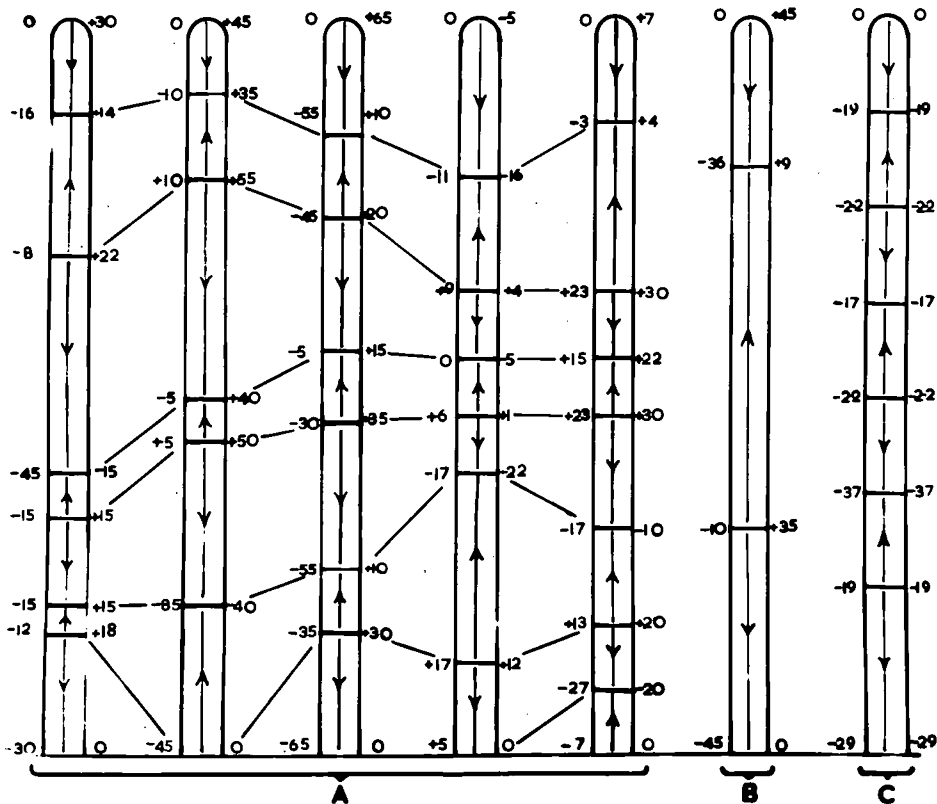


FIG. 2.2.

The most important points to emerge from these results are:-

(a) The similarity of the potential pattern in the examples.

(b) The apex is generally positive with respect to the base.

(c) There are at least six positions along the length of the coleoptile at which the potential is a maximum or minimum.

These results were rather startling, for if we are to believe, as Schrank (1947), has claimed, that transverse polarities induced by gravity, light, etc., cause a migration of auxin to the positive side, how then can the apex be positive, when auxin is known to be leaving it? Also how can the potential show such variations along the length of the coleoptile yet auxin pass steadily down?

Characteristic changes in potential on first making contact consistently occurred in all these observations with Wheat, but they were not found by Wilks and Lund (1947) and Schrank (1947), in Avena. However, Clark (1935), noted that measurements of Avena electrical potentials obtained by contacts moved up and down the coleoptile, were extremely variable, and it was impossible to obtain constancy over any considerable time. Only plants with fixed contacts gave the constancy of potential desired, but even then it took 90 to 120 minutes to stabilise: the changes previous to this consisted of a slow decrease, followed by a slow increase in the negativity of the tip with respect to the basal contact.

Since we had so far used Wheat coleoptiles whilst all published

work was upon Oat, we now decided to use Oat coleoptiles. Oat coleoptiles, in this, as in other fields, have been well investigated and though many papers are concerned with the application of currents, the effects of gravity and of unilateral light, there are also several papers upon the distribution of the electric field in the unstimulated Avena coleoptile. Listed below are the papers and authors, with the more important points from each paper:-

Ramshorn (1934), claimed that the apex was positive with respect to the base.

Clark (1935, 37, 38), stated that the apex of the coleoptile was negative with respect to the base and the internal polarity of the sheath was similar.

Wilks and Lund (1947), stated that they found the apex negative with respect to the base, and an inversion of polarity occurred partway down the coleoptile. The internal polarity was opposite to that of the external. They published the first potential map.

Schrank (1947 and 1951), claimed the results of Wilks and Lund, (1947), were correct and published a more detailed potential map.

Our work was started using Avena sativa variety Barnwell. Growth and experimental procedures were as previously described and contact and growth medium were again Shive's solution.

With Avena also, large changes in P.D. occurred on first making contact. These were more complex than those of Triticum, consisting of

a rapid increase in the negativity of the apex with respect to the base followed by a slower fall and then a gradual slight increase in negativity again (Figure 2.1.B.). The potential stabilised after about 30 minutes at a value of about 10 millivolts; with the apex negative to the base.

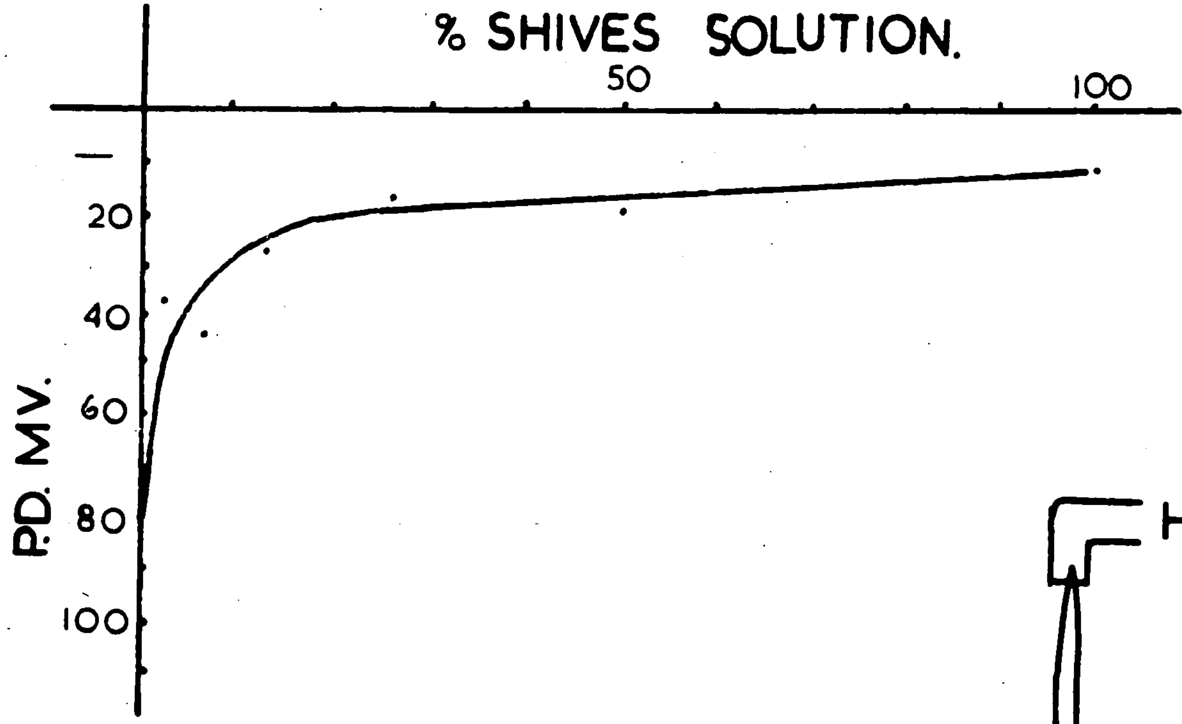
An experiment was carried out to determine if the original changes were due to the current, however small, drawn off by the "Vibron". The potential was allowed to stabilise and then the Vibron leads removed, the electrodes and contacts being left in place on the plant. On remaking the circuit, there was no change in the potential. Another possibility was that these changes were due to diffusion potentials set up by ion movements into and out of the contact drop, a stable potential finally being attained. This was to some extent borne out by the knowledge that renewing the contact drop also led to a small change in the potential. However, when the contact was removed for only 10 minutes and then reapplied without changing the contact drop, an exactly similar change to that originally observed upon first applying the drop, occurred. With Shive's solution, the stabilised potential (about -10 millivolts), was much lower than that recorded by Wilks and Lund (1947), for Avena (-50 millivolts). They used tap water as contact medium and it seemed possible that this could be a cause of the difference. (It is difficult to determine from the publications of Schrank (1947 and 1951), who like ourselves used Shive's solution, whether he repeated the work

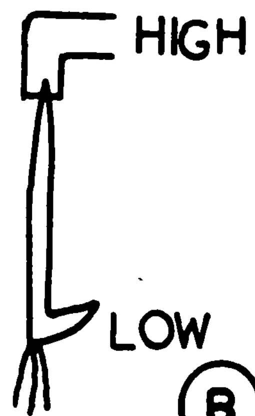
of Wilks and Lund (1947), and also found an apical negativity of 50 millivolts or he simply echoed their results).

A series of experiments was therefore tried using deionised water as growth and contact medium. This was intended to achieve two results. It would give an indication whether the results of Wilks and Lund (1947), were due to the use of a tap water contact and growth medium; secondly, since with deionised water all ion movements on making contact must have been into the contact drop, a comparison with results obtained with Shive's solution would make possible an assessment of the importance of ion movements.

The typical result is shown in Figure 2.1.C. The change on first making contact differed from that with Shive's, in that, there was little or no primary increase in negativity of the apex. It also differed in that the final P.D. was in the region of -80 millivolts, i.e. closer to the results of Wilks and Lund (1947).

A series of experiments measuring the P.D. of the apex/base with a wide range of dilutions of Shive's solution as the growth and contact medium was carried out. Figure 2.3.A. shows that the graph of the P.D./Log of the ion concentration is a straight line over the concentration range, Shive's to 0.1% Shive's. This result led to the conclusion that the potentials being measured were mainly of the nature of diffusion potentials and not as suggested by Lund and co-workers (1947), reduction/oxidation potentials. However we had unwittingly complicated





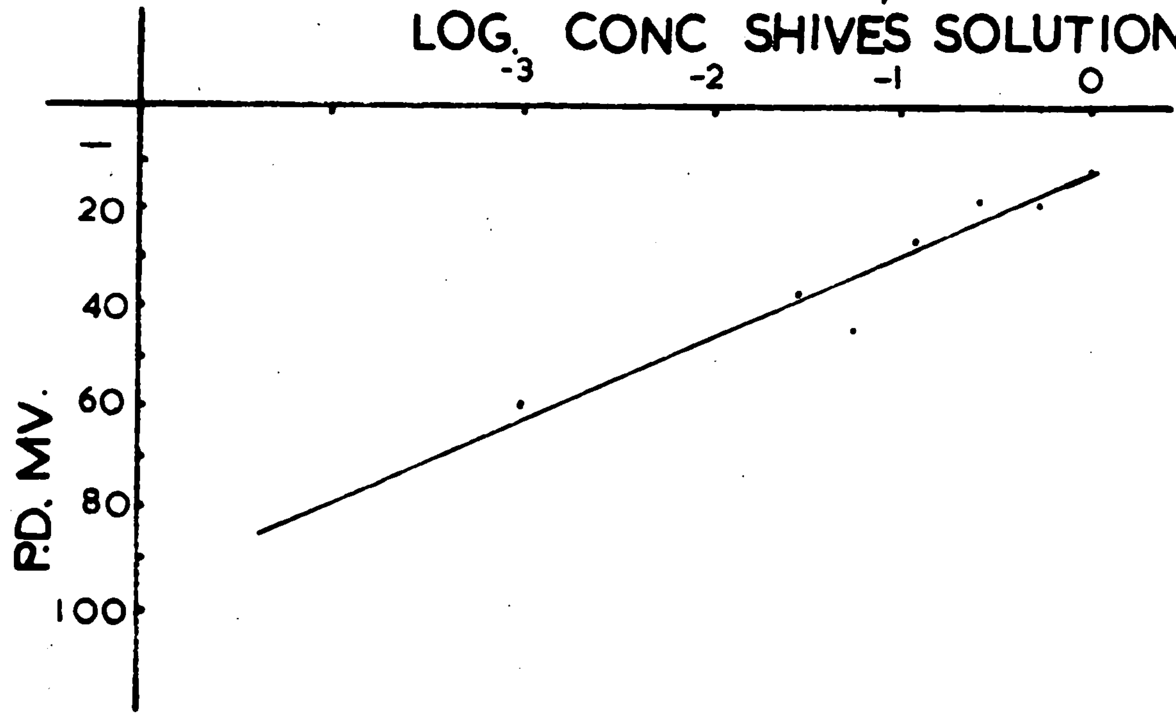
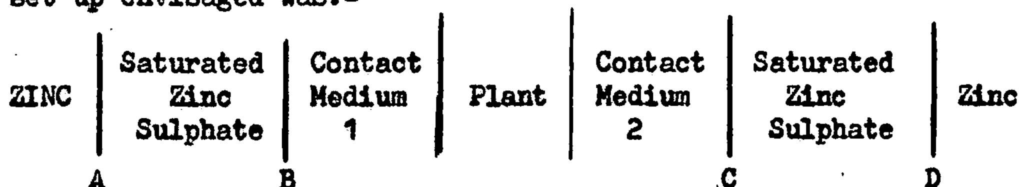


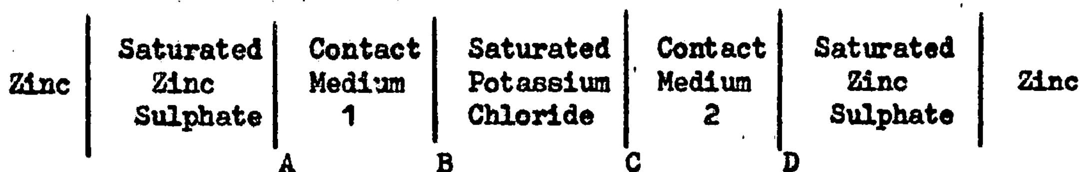
FIG. 2.3.

the interpretation of the above results and also some of those that follow, by not realising at that time, that they were indicative of diffusion potentials. Not only was the contact medium changed, but also at the same time the growth medium; deionised water (log $\frac{1}{\infty}$) was used in some of the experiments, and also mixed mineral salt solutions were used where several ions were involved.

In the next experiment it was hoped to separate the effect of the growth medium from that of the apical contact medium. Since the set up envisaged was:-



it was necessary to determine the contribution to the potentials by the B and C liquid junctions.



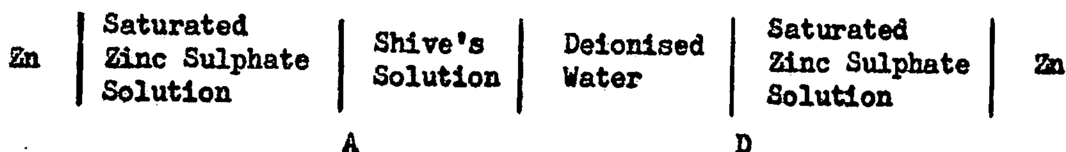
was assembled; using saturated KCl it was hoped to minimise any potentials that were generated at B and C.

With the above the following results were recorded.

TABLE 2.1.

Contact Medium 1.	Contact Medium 2.	P.D.½
Shive's	Deionised water	-1.5 mv
Shive's	1% Shive's	-1.3 mv
Shive's	10% Shive's	- 0.8 mv

With the cell:-



the P.D. Shive's/Deionised electrode is -12 mv and since the A and D junctions are only contributing -1.5 mv, then the Shive's solution/ Deionised water diffusion potential is approximately -10.5 mv.

The low values of the A and D junctions may be due to a saturated zinc sulphate swamping effect. Even though the mobilities of the ions are different ($\frac{1}{2}Zn^{++}$ = 47 reciprocal ohms, $\frac{1}{2}SO_4^{--}$ = 68.5 reciprocal ohms) the effects due to them will be the same at both electrodes. It seems therefore that it will be permissible to ignore the A and D junctions.

The following results were recorded on the coleoptiles grown in Shive's solution and deionised water.

TABLE 2.2.

Growth medium and basal contact medium.	Apical Contact Medium	Mean P.D. Apex/base mv.	Standard error mv.
Shive's	Shive's	-10	$\pm$ 8
Shive's	Deion	+53	$\pm$ 14
Deion	Shive's	-101	$\pm$ 24
Deion	Deion	-80	$\pm$ 11

Each result is the mean of ten experiments.

From these it may be concluded that:-

(a) Increase in the concentration of the contact medium at the apex caused the apical negativity to increase. This is opposite to the effect on the potentials of changing both the growth and contact media. The apical effect must be opposing the basal, and from this, it follows that the changes in the potential difference between the base and growth medium must be similar but larger than the changes between the apical part and the contact medium, on dilution of both these media.

(b) There is a difference in properties between plants grown in deionised water and in Shive's solution. The magnitude of the differences in the potentials measured on the Shive's grown coleoptiles, with deionised water and Shive's solution as the apical contact, are not the same as the magnitude of the differences of the deionised water grown coleoptiles with the same contact media. Therefore the properties of the coleoptiles vary with variation in the growth medium.

The next two experiments employed two electrodes making contact on the coleoptile sheath and a third electrode placed in the growth medium. Flowing drop contacts were used on the sheath. The apparatus is shown in Plate 2.4. This type of apparatus was used in all experiments with flowing drop contacts reported in this thesis, with the exception of the geo-electric experiments (chapter 8) and the experiments on Fungi. (Chapter 12).

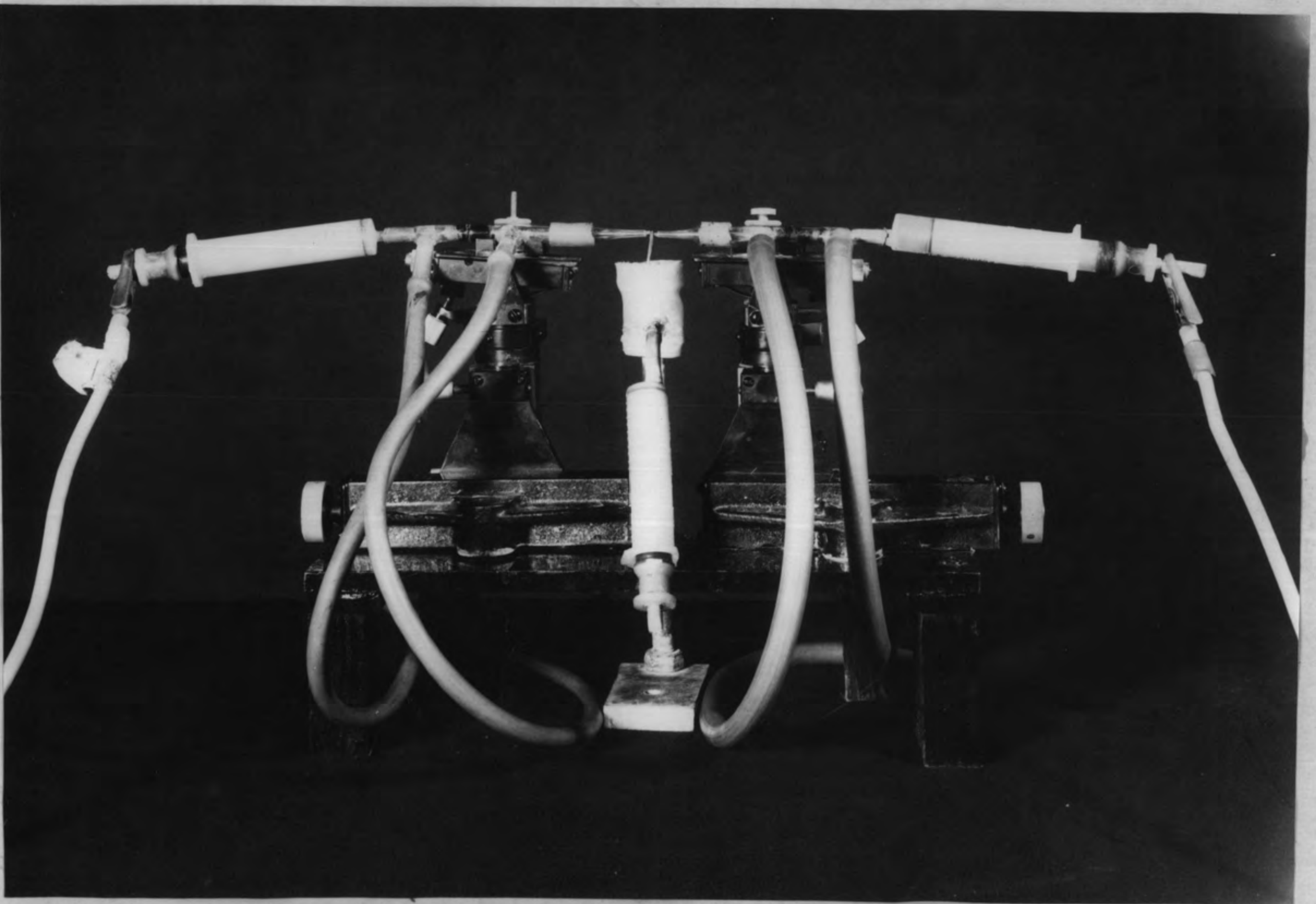


PLATE. 2.4.

Differing concentrations of growth medium were employed and the effect of this on the P.D. measured between points on the sheath was determined. Later, simultaneous changes in the contact medium concentration of the flowing drop contacts were made to see whether these altered the P.D. measured between points on the sheath. The results are shown in the following. Table 2.3. A & B.

In experiment C a single flowing ring drop contact was used on the apex of the coleoptile and the potential measured with respect to another electrode in the growth medium.

The apparatus used for this experiment is shown in Plate 2.5. This type of apparatus was used in all experiments with flowing ring drop contacts reported in this thesis.

TABLE 2.3.

Basal contact & Growth Medium	Contact Media	Potential between Apex/base mv.	Potential between Mesocotyl/ base.	Potential between Apex/Meso-cotyl mv
Shive's	Shive's	+18 +8 +13 +9 +6 -1 +10	+48 +42 +43 +43 +30 +33 +40	-30 -34 -30 -34 -36 -36 -34 -30
		mean +7.3mv	mean +39.8mv	-32.5mv
	10% Shive's	+28 +11 +15 +14 +9	+68 +65 +61 +61 +57	-40 -54 -46 -47 -48
		mean +15.4mv	+62.4 mv	-47 mv
10% Shive's	Shive's	-37 -18 -23 -20	+2 -1 +9 -3	-39 -17 -32 -17
		mean -24.5mv	mean +1.75mv	mean -26.25mv
	10% Shive's	-3 -4 -19 -18	+51 +27 +37 +22	-54 -31 -56 -40
		mean -11mv	mean +34 mv	mean -45
1% Shive's	Shive's	-20 -59 -79 -70	-15 -45 -71 -49	-5 -14 -8 -21
		mean -57 mv	mean -45 mv	mean -12 mv
	10% Shive's	-15 -30 -30 -51	-5 -10 -8 -33	-10 -20 -22 -18
		mean -31.5mv	mean -14mv	mean -17.5mv

A

No results were taken at lower contact medium concentrations as the resistance of the electrodes and contacts was so high as to be very susceptible to interference.

At other times the two following sets of data were collected showing similar trends but different magnitudes.

Growth Medium and basal contact medium	Contact Medium	Potential between Apex and Base	Potential between Mesocotyl and base	Potential between Apex and Mesocotyl	
Shive's	Shive's	-18 -7 -15 +8 +20 +7	+16 +28 +10 +28 +26 +27	-34 -35 -25 -20 -6 -20	B
		mean -1 mv	mean +21 mv	mean -22 mv	
0.1% Shive's	Shive's	-61 -73 -27	-60 -57 -24	-1 -16 -3	
		mean -53.6mv	mean -47 mv	mean -6.6mv	
Shive's	Shive's			-18 -28 -19	C
				mean -22 mv	
10% Shive's	10% Shive's			-45 -52 -34 -56 -35	
				mean -44 mv	
1% Shive's	1% Shive's			-67 -79 -60 -93	
				mean -75 mv	
0.1% Shive's	0.1% Shive's			-90 -120 -85 -65	
				mean -90 mv	

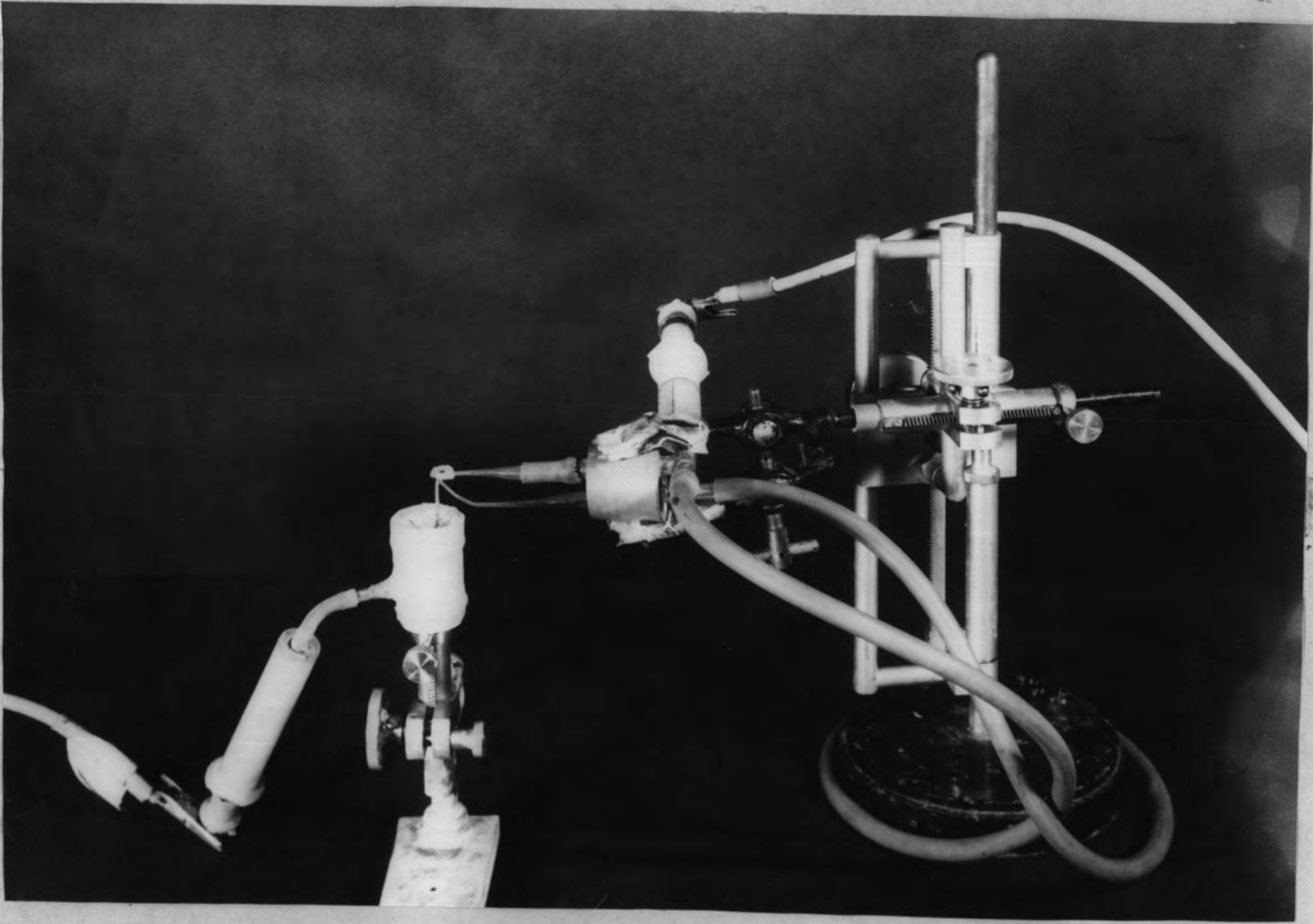


PLATE. 2.5.

The results show:-

(a) <u>With constant growth medium concentration</u>

With decrease in the contact medium concentration at the apex, the P.D. of the apex/base becomes more positive. With decrease in the contact medium concentration at the mesocotyl, the P.D. of the mesocotyl/base also becomes more positive. However for the same decrease in concentration at the apex and mesocotyl, the effect is greatest at the mesocotyl, therefore the P.D. of the apex with respect to the mesocotyl becomes more negative.

(b) <u>With constant contact medium concentration at the apex and the mesocotyl.</u>

With decrease in the growth medium concentration, the P.D. of the apex/base becomes more negative. With decrease also the P.D. of the mesocotyl/base becomes more negative.

However the effect at the mesocotyl is greater than at the apex, and therefore the P.D, of the apex/mesocotyl becomes more positive. This indicates that changing the growth medium has effects throughout the coleoptile and furthermore that these effects are not the same at all points. It also confirms that with decrease in concentration of the growth medium the base becomes more positive with respect to the others regions. Therefore the potential of the apex, mesocotyl and base with respect to an external Shive's solution undergoes a similar change upon dilution of this solution, though the magnitude of the change differs in each case.

The results show that in a concentrated growth medium, it is possible for the apex to be positive to the basal roots as found by Ramshorn, (1934); this effect is increased by dilute contact media. The published potential patterns of Wilks and Lund (1947), and Schrank (1947,51) only hold for dilute growth media (less than 1% Shive's) for they show the mesocotyl negative with respect to the base. With concentrated contact media, this effect is emphasised, but the apex then tends to become positive with respect to the mesocotyl. These workers therefore must have used dilute growth and contact media in obtaining their potential patterns (Tap Water?), although Schrank in the same work reports using Shive's solution.

Hope (1951), found that broad bean root tips behave as hydrogen electrodes in solutions of different hydrogen ion concentration. The influence of pH upon the plant potentials had to be shown to be of small significance in this work, before the changes upon contact medium dilution could be ascribed to changes in the mineral salt concentration, for we may at the same time be altering the pH of the solution. No experiments were carried out dealing specifically with pH from this aspect, but an experiment to see if there was any influence of pH on the changes that occurred when electrode contact with a coleoptile was first established, gave the necessary information. (For details of changes involved see Chapter 4).

	$0.1M\ KH_2PO_4$ + CH_3COOH Till pH2.5		pH 5.2 $0.1M\ KH_2PO_4$		pH 8.5 $0.1M\ K_2HPO_4$	
	Magnitude 1st peak millivolts	Magnitude 2nd trough millivolts	Magnitude 1st peak millivolts	Magnitude 2nd trough millivolts	Magnitude 1st peak millivolts	Magnitude 2nd trough millivolts
	-26 -5 -19	-36 -28 -42	-12 -21 -29	-42 -67 -49	-61 -21 -61 -35 -32	-63 -46 -85
Means	-16.6	-35	-21	-52	-42	-54

Shive's solution was used as growth and basal contact medium in all cases. The potentials recorded are those of the apex with respect to the base.

It seems that in the lower pH the apex is more positive, though as well as the influence of pH there are changes in other ion concentrations.

The pH of Shive's solution is 4.7 and of 1% Shive's it is 5.7, but with Shive's solution as the apical contact the apex is more negative than with more dilute solutions, an opposite change to the above.

With KCl solution the pH is that of the solvent medium which with deionised water is pH 6.1. This will not change upon dilution with deionised water. Furthermore, in the experiments reported in Chapter 4 with KCl solution, a flowing ring drop contact was used at the apex.

The pH of this contact medium could not have been changed significantly by the outward movement of H^+ from the plant tissue. The concentration of the apical contact medium was changed over a range of concentrations 0.1N to 0.0001N KCl and the potential measured with respect to the base. The basal contact medium and growth medium was in all cases Shive's solution. With 0.1N the apex was 10 millivolts negative whilst with 0.0001N KCl the apex was 48 millivolts positive, with respect to the base. These changes can only be ascribed to change in KCl concentration.

The change is of the same polarity as that with Shive's dilution.

Although the potentials measured may have been influenced by changes in hydrogen ion concentration, in this work it seems clear that pH changes can have played no significant part in bringing about the potential changes when the contact medium concentration was altered.

<u>Conclusions.</u>

1. The magnitude of electrical potentials measured in oat seedlings are dependent on the concentration of ions in the contact and growth media.

With dilutions of Shive's solution, a region of a plant in contact with a more concentrated solution will be negative with respect to the same region in contact with a more dilute.

The magnitude of the above potential difference depends on the region of the plant involved, being greater in the basal regions than

in the apical regions. It also depends on the growth medium, being larger in the more dilute.

Where the magnitude of potential differences in plant tissue are quoted, they are only of value where both the growth medium and contact media are specified. This also applies to potential pattern diagrams, for changes in the growth and contact media cause drastic changes in these.

It is possible to explain some of the discrepancies between the the results of the various other workers on the bases of the various contact and growth media they have employed.

2. Graphs plotting the magnitudes of the measured potentials against the log, of the growth and/or contact medium concentration show straight line relationships. This is taken to indicate that diffusion potentials in the broadest sense are involved.

Assuming these potentials are diffusion potentials then we can draw the following conclusions.

(a) Since with decrease in the external contact medium concentration, the potential of the plant tissue relative to the contact medium becomes more negative, with Shive's solution at least, the tissue permeability to cations is greater than to anions.

(b) Since the P.D. change with change in contact medium is greater in the basal than the apical regions, the relative cation permeability is similarly greater in the basal regions.

(c) With alteration in the growth medium concentration there is an alteration in tissue properties throughout the plant.

This no doubt involves changes in the tissue ion concentration but it also involves changes in the ion permeabilities for the magnitude of the potential change, upon change in contact medium concentration at a particular point on the plant surface, varies between the different growth medium concentrations. Also these changes are greater in the basal regions than the apical regions of the coleoptile.

3. It is impossible to believe that auxin is being transported down the plant by the potential gradient we measure, when, under suitable conditions of measurement, the gradient may have either a positive or a negative or a zero value, yet the plants continue to grow.

This does not however preclude the possibility that transverse potential gradients may develop under unilateral stimulation causing changes in auxin distribution.

CHAPTER 2.

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CHAPTER.3.

CHAPTER 3

A DISCUSSION ON THE ORIGINS OF BIO-ELECTRIC POTENTIALS

SUMMARY

The various possible sources of electric potentials in coleoptiles are considered and the magnitudes of their contributions to the measured potentials are calculated. The conclusion is, that in all but the measurement of the transverse apical potential, the major contribution to the measured potential comes from potentials generated across cell membranes. Even in the apical situation, though there is a shorting out by other phases, changes in the potential probably come from changes in the membrane cytoplasmic potentials.

It is pertinent at this point to enter into a discussion concerning the origin of the potentials measured in whole plant tissues. Reference will be made to experiments reported later in the thesis, but for an explanation of the foregoing results, the origin is now considered.

General reviews of the origin of potentials have been written by Heilbrunn (1943) and Crane (1950), whilst Hober (1945), Briggs, Hope and Robertson (1961), and Dainty (1962), have given detailed reviews of Donnan, diffusion and membrane potentials in plant tissue.

The potentials measured arise from three sources:-

(A) They may be generated as functions of the measuring system.

(B) They may result from the physical properties of plant material not being directly dependent on the metabolic activities of the plant (except in so far as these materials were produced by the plant).

(C) They may be potentials which are wholly dependent on metabolic activities for their existance. These potentials may arise from physical structures metabolicly maintained.

These 3 classes are considered individually in the following discussion.

Class A.

Consider the situation used in the measurement of the potential:-

Zinc/Saturated/Contact/Plant/Contact/Saturated/Zinc.
Zinc Medium Medium Zinc
Sulphate (1) (2) Sulphate

a b c d

Class A consists of potentials set up at the phase bounderies "a,b,c,d". A pair of isoelectric Zinc/Zinc Sulphate half-cells are always selected and therefore "a" and "d" cancel out. Where the contact media are of the same constitution and concentration "b" and "c" will also be equal, but different contact media have occasionally been employed and asymetric potentials will then exist. Placing saturated potassium chloride salt bridges between the electrodes and the contact media will reduce these, but at least with Shive's solution, they seem to be small anyway, as previously reported.

Electro-kinetic potentials generated due to contact medium flow also come into this class.

In practice the aim is to reduce all these potential to the minimum, and it can be confidently stated that in no experiment reported in this thesis could they have exceeded 2 millivolts.

It is necessary now to consider the structure of the coleoptile in relation to the contacts before we can enter into a discussion of the Class B and C potentials.

Consider the structure of the coleoptile in relation to the contacts. (Figure 3.1.A.). Each of the different layers of the coleoptile may make a contribution to the potential in ways discussed later, but the question arises as to how great is the contribution of each region.

Consider the regions to be batteries of E.M.F., E^1, E^2 and E^3 and internal resistance R^1, R^2 and R^3 (See Figure 3.1.B.). The resistance of the external circuit is R and a current I flows through it, and the potential measured across the terminals of the circuit is E.

$$I = i.1. + i.2. + i.3. \quad \text{by Kirchhoff's Law.}$$

Where i.1., i.2., and i.3., are the currents through each of the batteries.

Therefore $i.1. = \frac{E^1}{R^1}$

$$i.2. = \frac{E^2}{R^2}$$

$$i.3. = \frac{E^3}{R^3}$$

but $i.1. + i.2 + i.3 = I = \frac{E}{R}$

Therefore $\frac{E}{R} = \frac{E^1}{R^1} + \frac{E^2}{R^2} + \frac{E^3}{R^3}$

but R is the instrument resistance and constant

Therefore E is proportional to $\frac{E^1}{R^1} + \frac{E^2}{R^2} + \frac{E^3}{R^3}$

Therefore the contribution by any region potential, to the total measured potential, is inversly proportional to its resistance.

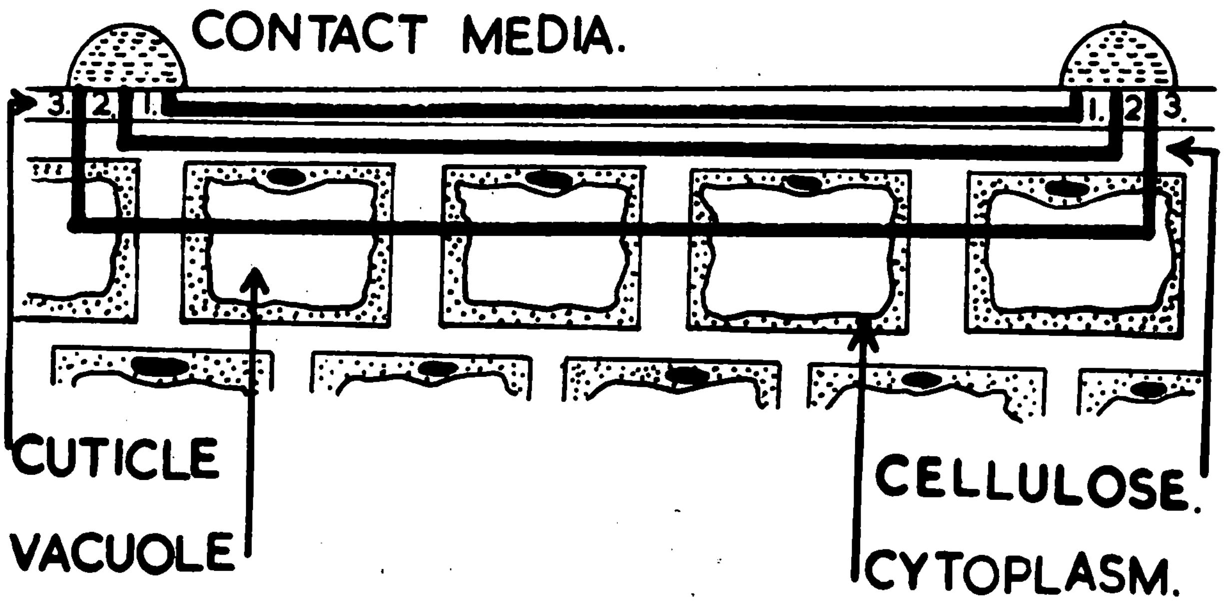


FIG. 3.1.

It is now necessary to calculate the relative resistances of the different regions to get an indication of which phase contributes most to the potential.

Examination of T.S. and L.S. of coleoptile sheaths show

(a) that the cuticle is very thin and occupies only a very small fraction of the total area of the tissue.

(b) the cellulose walls occupy rather more of the area than the above.

(c) the cytoplasm occupies a greater area than the above and in the non-vacuolated tip forms the greater part of the material. In the vacuolated region where it just lines the wall the area is smaller but still exceeds that of the cellulose.

(d) the vacuole in well developed regions occupies the greater part of the tissue. The three pathways in which we are interested for the purposes of resistance calculation are shown in Figure 3.1.A. One important thing to notice is that the pathways 2 and 3 include contributions by the transverse components of the cuticle, and the cuticle and cellulose wall, respectively.

Figure 3.2. gives details of some measurements made from sections of coleoptile tissue. Measurements of the vacuole are not included since it appears that there is little resistance to the passage of ions through the tonoplast membrane from the cytoplasm and the two can therefore be treated as one for the purpose of these calculations.

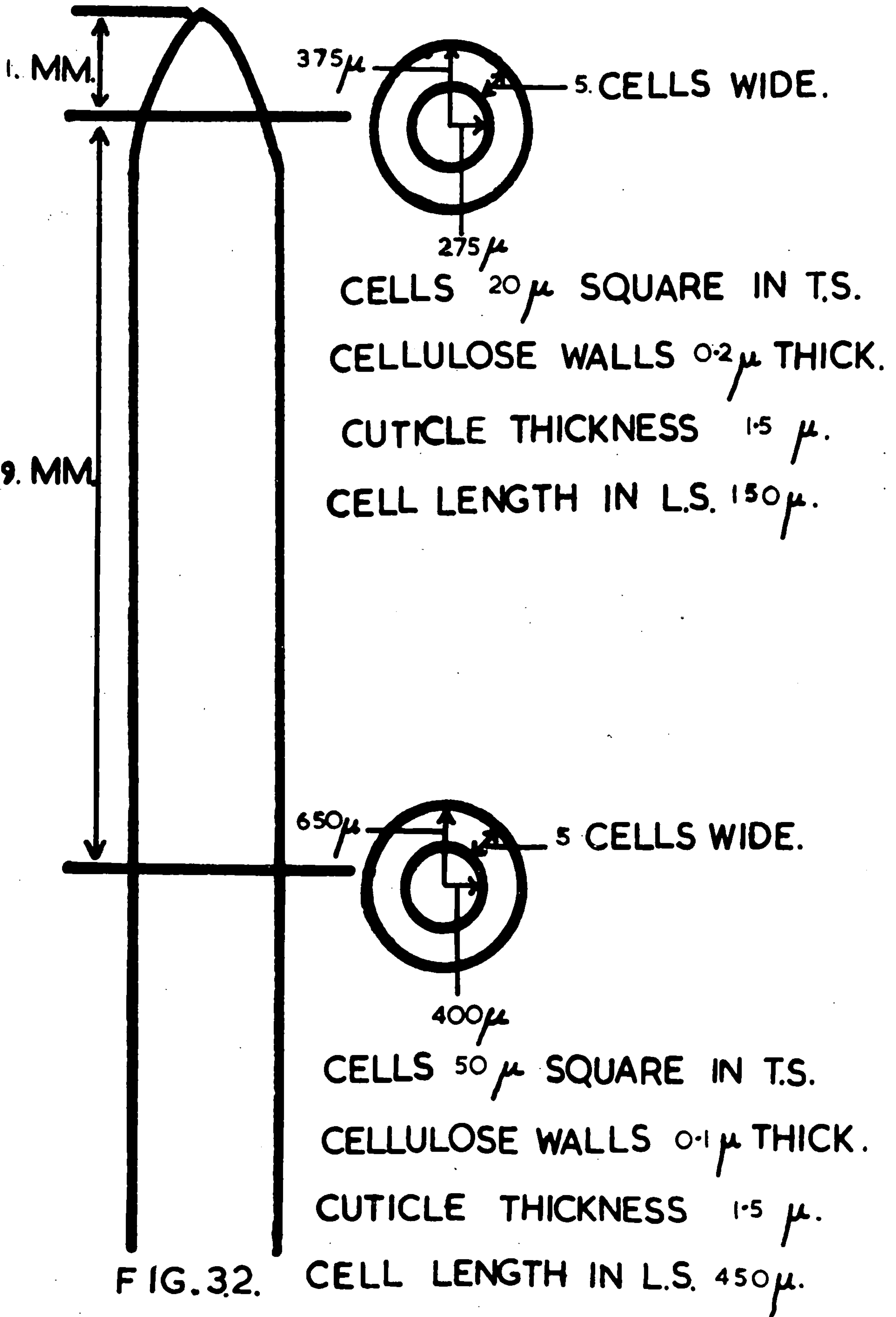


FIG. 32.

For ease of calculation the transverse electrical resistances calculated apply to a piece of tissue the same length as half the circumference of the coleoptile and 5 cells thick. The depth chosen is that of the number of cells to give approximately a 1 millimetre depth of tissue. The cell sizes are as shown in Figure 3.2.

The longitudinal electrical resistance is calculated for a piece of tissue 5 cells wide by 5 cells broad and 1 centimetre deep in the mid-region of the coleoptile with the contacts at the top and bottom.

A. Transverse electrical resistance at the apex of a coleoptile.

1. Along the cuticle.

$$\text{Cuticle length} = 0.375 \times \pi \text{ centimetres.}$$

$$\text{width} = 0.00015 \text{ centimetres.}$$

$$\text{depth} = 0.015 \times 5 \text{ centimetres.}$$

Let resistivity of cuticle material be ρc.

$$\therefore \text{Resistance} = \frac{\rho c\ 0.0375\ .\ \pi}{0.00015 \times 0.015 \times 5} \text{ ohms.}$$

$$= 10^4\ \rho c \text{ ohms (approx.).}$$

2. Through the cell walls and including the transverse cuticle resistance at the contact positions. The contact area is considered 5 cells square.

$$\text{Resistance across cuticle} = \frac{2 \times \rho c \times 0.00015}{0.015 \times 5 \times 5 \times 0.002} \text{ ohms.}$$

$$= 0.4\ \rho c \text{ ohms.}$$

Resistance of cellulose walls of one cell.

$$= \frac{\rho w\ 0.002}{2(0.015 \times 0.00002) \times 2(0.002 \times 0.00002)} \text{ ohms}$$

where ρw = resistivity of the cellulose wall.

But there are 25 rows of cells, each row $0.0375 \times \pi$ cms. long and therefore containing

$$\frac{0.0375 \times \pi}{0.002} \text{ cells.}$$

$\therefore$ Total resistance of cellulose = $\frac{pw\ 2.95 \times 10^3}{25} \times \frac{0.0375 \times \pi}{.002}$ ohms.

$\therefore$ Total resistance of transverse path across cuticle and through cellulose cell walls = $(0.4\ pc + [6.95 \times 10^3\ pw])$ ohms.

3. Through the cytoplasm of the cells including transversing cell membranes, cell walls and the cuticle at each end of the tissue. Contact area is considered to be 5 cells square.

Cuticle resistance = 0.4 pc ohms.

Resistance of cytoplasm, cell walls and membranes of one cell

$$= \frac{2pw \times 0.00002}{0.015 \times 0.002} + \frac{pct \times 0.002}{0.015 \times 0.002} \times \frac{10^4 \times 2}{0.015 \times 0.002}$$

$$= [1.3\ Pw + (6.65 \times 10\ pct). + (6.65 \times 10^8)]\ \text{ohms.}$$

Where Pct = resistivity of the cytoplasm and the resistivity of the cell membranes is taken as 10^4 ohms.

There are 25 rows of cells composed of $\frac{0.0375 \times \pi}{0.002}$ cells in each row.

$\therefore$ Total resistance of the transverse path across the cuticle, across the cellulose cell walls, and through the cytoplasm and cell membranes is:-

$$[0.4pc + 3pw + (1.6 \times 10^2 pct) + 1.6 \times 10^9]\ \text{ohms.}$$

The cuticle is relatively unwettable and is probably composed of a waxy material which one suspects would have an extremely high resistivity. However from plasmolysis experiments and observations on coleoptile wilting, reinforced by some results reported later which indicate water-movement influencing the potentials, it seems that the cuticle is water permeable, but no doubt the

resistivity will be quite high. Let us assume for the purpose of these calculations, the resistivity is 10^6 ohms (distilled water). The resistivity of the cellulose wall water will be that of a dilute mineral salt soltuion (10^4 ohms $\equiv$ 0.001 N KCl) whilst that of the cytoplasm, a lower resistance with a higher salt concentration, say 10^2 ohms which is approximately equivalent to .01N KCl.

Using these values, resistance of the paths is as follows:-

1. Cuticle 2.95×10^9 ohms.
2. Cellulose 7×10^7 ohms.
3. Cytoplasm 1.55×10^9 ohms.

It follows therefore that in the measurement of transverse potentials at the apex the membrane potentials contribute little, the major part of the potential being due to the cellulose wall phase.

B. Transverse electrical resistance mid-way down coleoptile.

1. Along the cuticle.

Cuticle length = $0.065 \times \pi$ cms.

Thickness = 0.00015 cms.

Depth (2 cells deep) = 0.045×2 cms.

$$\text{Resistance} = \frac{pc\ 0.065 \times \pi}{.00015 \times 0.09}\ \text{ohms.}$$

$$= 1.5 \times 10^4\ pc\ \text{ohms.}$$

2. Through cellulose walls and including a transverse path across the cuticle at each end of the tissue. The contact area is considered to be 5 cells broad by 2 deep. Note that the walls are thinner here than at the apex.

$$\text{Resistance of cuticle} = \frac{2 \times 0.00015 \times pc}{0.09 \times 0.025}\ \text{ohms}$$

$= \underline{0.13 \text{ pc ohms}}.$

Resistance of cellulose walls of one cell

$$= \frac{pw \times 0.005}{(2 \times 0.45 \times 0.00001) + (2 \times 0.005 \times 0.00001)}$$

$$= \underline{5 \times 10^{13} \text{ pw ohms}}.$$

But there are 10 rows of cells, each row, $0.065 \times \pi$ centimetres long and therefore composed of $\frac{0.065 \times \pi}{0.005}$ cells

Total resistance of walls

$$= \frac{pw5 \times 10^{3} \times 0.065 \times \pi}{10 \times 0.005}$$

$$= \underline{1 \times 10^{5} \text{ pw ohms}}.$$

Total resistance of the transverse path across the cuticle and along the cellulose walls is $(0.13 \text{ pc} + 10^{5} \text{ pw})$ ohms.

3. Through the cytoplasm of the cells including transversing cell membranes, cell walls and the cuticle at each end of the tissue. Contact area is considered 5 cells broad by 2 cells deep.

Cuticle resistance = 0.13 pc ohms.

Resistance of cytoplasm cell walls and membranes of one cell

$$= \frac{pct \times 0.005}{0.045 \times 0.005} + \frac{2pw \times 0.00001}{0.045 \times 0.005} + \frac{2 \times 10^{4}}{0.045 \times 0.005} \text{ ohms}$$

$$= [0.09 \text{ pw} + (2.2 \times 10 \text{ pct}) + (8.9 \times 10^{7})] \text{ ohms}.$$

But there are 10 rows of cells, each row composed of $\frac{0.065 \times \pi}{0.005}$ cells

Total resistance of the transverse path across the cytoplasm of the cells including transversing cell membranes, cell walls, and the cuticle at

each end of the tissue is:-

$$[0.13\ pc + 0.4\ pw + 80\ pct + (3.6 \times 10^{8})]\ ohms.$$

Substituting the previously suggested values for the various resistivities we find the following approximate resistance values for the three pathways.

1. Cuticle - 1.5×10^{10} ohms.
2. Cellulose walls - 10^{9} ohms.
3. Cytoplasm - 10^{8} ohms.

In the measurement of transverse potentials mid-way down the coleoptile, the major contribution to the measured values will be the cytoplasm and its membranes, along with any transverse components of the cellulose and cuticle potentials.

C. Longitudional electrical resistance of a piece of coleoptile tissue from the mid-region, 5 cells wide by 5 cells broad with a length of 1 centimetre. The contact areas at the two ends of the tissue are considered to be 5 cells wide by 2 cells deep.

1. Longitudinal cuticle resistance.

$$= \frac{pc.\ 1.}{.00015 \times 0.025}\ ohms$$

$$= 2.6 \times 10^{5}\ pc\ ohms.$$

2. Longitudional resistance through the cellulose walls including the cuticle transverse resistance at the contact positions.

Transverse cuticle resistance = 0.13 pc ohms. (This was calculated for a similar contact area in B.1.)

$$\text{Longitudinal cellulose resistance} = \frac{pw.l.}{4 \times 0.00001 \times 0.005 \times 25}\ ohms$$

$$= 2 \times 10^{5}\ pw\ ohms.$$

<u>Total longitudinal resistance through the cellulose walls = $(0.13\ pc + 2 \times 10^{5}\ pw)$ ohms.</u>

3. <u>Longitudinal resistance through the cytoplasm and across the cell membranes, including the transverse resistances of the cellulose walls and cuticle at the contact positions.</u>

Transverse cuticle resistance = 0.13 pc ohms.

Longitudinal resistance of cytoplasm, cellulose cell walls and membranes of one cell,

$$= \left(\frac{2pw\ 0.00001}{.005 \times .005} + \frac{pct \times 0.045}{.005 \times .005} + \frac{2 \times 10^{4}}{.005 \times .005}\right) \text{ ohms.}$$

$$= (0.8\ pw + [1.8 \times 10^{3}\ pct] + [8 \times 10^{8}]) \text{ ohms.}$$

But there are 25 rows of cells composed of $\frac{1}{0.045}$ cells each.

<u>Total resistance approximately equals $(0.13\ pc + 0.7\ pw + 10^{5}\ pct + [7 \times 10^{8}])$ ohms.</u>

Substituting the estimated values of resistivity,

1. Longitudinal cuticle resistance is approximately 2.6×10^{11} ohms.
2. " cellulose " " " 2×10^{9} ohms.
3. " cytoplasmic and membrane resistance is approximately 7×10^{8} ohms.

In the measurement of longitudinal potentials the cytoplasmic/membrane potentials will form the major contribution to the measured values.

It is realised that these values are all approximate and in fact the measured values of the tissue resistance are lower than any calculated, being in the region of 10^{6} to 10^{7} ohms, but they do indicate that only in the apical regions, when measuring transverse potentials, is there any likelihood of changes in the membrane/cytoplasmic potentials being masked by shorting out through the other

pathways. This is where the value of the calculations lies.

From these calculations it appears that potentials arising along the length of the cuticle can contribute little, though transverse potentials may contribute to the potentials of other phases. Potentials arising longitudinally in the cellulose walls are of more significance than the above but it seems that using external contacts on the surface of plant tissues, a considerable proportion of the measured potential is accounted for by potentials existing across cell membranes.

The potentials arising in the different phases are now considered separately. In the case of the cuticle and cellulose wall phases, transverse potentials, which contribute to measured membrane potentials are considered, as well as longitudinal potentials.

Class B potentials.

When contact is made on the outside of the coleoptile, the contact medium is separated from the living cytoplasm by the cuticle and cellulose cell walls. Although the cuticle surface is relatively unwettable, the rate of coleoptile wilting on removal of its water supply indicates that it is relatively permeable to water, as does also the ease of plasmolysis. It therefore probably contains water. The cellulose walls contain water. The water within the cellulose walls may be divided into two components, that forming the so-called water free-space (W.F.S.), where free diffusion with the external medium applies, and that forming the Donnan free-space (D.F.S.) where the position is complicated by the presence of fixed charges in the walls. Dainty and Hope (1959) estimate the W.F.S. for Chara cellulose walls as 46% of the whole.

In this particular instance we are interested only in the W.F.S.

When the contact medium is applied to the surface of the plant, since its ion concentration is unlikely to be the same as that in the cuticle and W.F.S. on the wall, diffusion potentials may be set up at the contact. This will only occur if the mobilities of the anions and cations differ. Osterhout (1927), suggested, and rightly so, that across the thickness of the cuticle and wall these will only be transient. Dainty and Hope (1959), report that Mannitol had a half time of 3 seconds for diffusing from Chara cell walls 16 μ thick, and Macrobbie and Dainty (1958), report a half time for KCl diffusing from the W.F.S. in Nitellopsis of one second. This transverse potential contributes to the

potentials of the other pathways. Even if identical contact media are involved, as long as the composition and concentration of ions within the W.F.S. at the two points of contact differ, or even if these are identical and yet the contacts are applied at different times, then a transient potential will exist until equilibrium is attained.

Where there is a considerable length of cuticle and cellulose wall between the contacts the diffusion potentials could maintain steady values for considerable periods of time (Osterhout, 1927). These potentials due to longitudinal diffusion can only arise if the contact media at the two contacts differ, for otherwise the effects cancel out.

Where the volumes of contact media are sufficiently large to show no change in concentration during the course of the experiment, the equation for the magnitude of this potential will reduce to that for the diffusion potential existing between the contact media being used.

For a univalent electrolyte this is $E = \frac{U - V}{U + V} \frac{RT}{F} \log_n \frac{C_1}{C_2}$

where U = mobility of cation.

V = mobility of anion.

and C_1 and C_2 are the concentrations of the solutions between which the potential is measured

and E is the potential of C_2 with respect to C_1. This is the Nerst equation.

The mobilities within the cuticle, and W.F.S. of the wall should be the

same as those in free solution, and therefore if KCl is used as a contact medium there will be only a small potential generated as the mobilities of K+ and Cl- are nearly the same. Furthermore, upon dilution of one of the contact media there should be very little change in the potential. Experimentally it has been shown that there is a considerable change. The Shive's /Deionised water diffusion potential has been shown experimentally to be only - 12 millivolts and yet when the contact medium at the apex of the coleoptile is changed from deionised water to Shive's or vice-versa the P.D. change is several times greater than this, though in the expected direction.

Therefore, although these longitudinal potentials must make some contribution to the results when contact media differ, the potentials measured are far greater than they can explain. As predicted by calculations, they cannot be the dominant potential measured. The transverse component will show up on changes in the contact media and upon the original application of contact, but it is only transient, contributing nothing to the final potential.

We may now consider the potentials generated by the D.F.S. As mentioned before, a large part of the cellulose wall water is under the influence of fixed negative charges and in this region, the D.F.S., Donnan potentials will be set up with the contact media. The wall is a weak cation exchange resin whose charges probably arise from the ionisation of the carboxyl groups of Pectic substances (Briggs, Hope and Robertson, 1961.)

Donnan potentials can also arise in the cytoplasm due to fixed ions in the

cytoplasm or a membrane impermeable to either anions or cations. Another possible source of Donnan potentials is if the cuticle is impermeable to some ions. Whether this is so is not known; in both these cases the following equations and observations will still apply.

Donnan membrane potentials cannot provide a continuous supply of electrical energy, and could therefore not be responsible for ion transport in plants. Another important characteristic is that an electrode sensitive to any ion to which the membrane is permeable cannot record the corresponding Donnan membrane potential. However this only arises where the electrodes are inserted directly into the bathing media and not when they are connected by salt bridges. (Crane, 1950) and therefore this limitation would not apply in our work. Considering the longitudinal Donnan phase potential with contacts applied to plant tissues as in Figure 3.1.A. With regard to the D.F.S. we have a continuous Donnan phase whose exchange capacity may vary between the two contact positions. This phase is in contact with the two contact media whose concentration may or may not be identical. For a univalent salt e.g. KCl and a cation exchange resin

$$E = \frac{RT}{F} \ln\left(\frac{a_o}{\frac{1}{2}\left(\sqrt{A^2 + 4a_o^2} + A\right)}\right)$$

where a_o = external concentration of the salt.

A = exchange capacity of the Donnan phase.

E = P.D. of A with respect to the external medium.

This equation neglects the terms in the electrochemical potential for the hydrostatic pressure.

Consider a Donnan Free Space of uniform exchange capacity A, in contact with two contact media of concentrations CM_1 & CM_2.

P.D. of CM_1 with respect to A = $-\frac{RT}{F}\log n \frac{CM_1}{\frac{1}{2}\left(\sqrt{A^2+4CM_1^2}+A\right)}$

P.D. of CM_2 with respect to A = $-\frac{RT}{F}\log n \frac{CM_2}{\frac{1}{2}\left(\sqrt{A^2+4CM_2^2}+A\right)}$

P.D. of CM_1 with respect to CM_2 =

$$\frac{RT}{F}\left(-\log n\frac{CM_1}{\frac{1}{2}\left(\sqrt{A^2+4CM_1^2}+A\right)}+\log n\frac{CM_2}{\frac{1}{2}\left(\sqrt{A^2+4CM_2^2}+A\right)}\right)$$

$$= \frac{RT}{F}\left(\log n\frac{CM_2}{CM_1}+\log n\frac{\sqrt{A^2+4CM_1^2}+A}{\sqrt{A^2+4CM_2^2}+A}\right)$$

Where CM_1 and CM_2 are considerably greater than A the P.D. = 0.

Where CM_1 and CM_2 are considerably smaller than A the P.D.

$$= \frac{RT}{F}\log n\frac{CM_2}{CM_1}$$

which compares with the Nerst equation

$$PD = \frac{RT}{F}\frac{(U-V)}{(U+V)}\log\frac{CM_2}{CM_1}$$

∴ The equation is that for a diffusion potential with an immobile anion.

This implies that the anion exchange resin attracts cations and repels anions. When the external concentration is high the resin is saturated and the Donnan potential zero. We will then, however, have a diffusion potential as in free solution, when all the resin is saturated.

The same will still apply even if the exchange capacity is non-uniform and we see that the effect of the Donnan phase is to modify the mobilities of the ions from the values that obtain in free solution.

Where the contact media are identical we would expect no potential. If we consider the change in the potential due to a change in the concentration of

the contact medium at one contact only, the potential being under the control of the Donnan phase, the change will, for a univalent salt, be 58 millivolts for a tenfold change in concentration; with increased dilution, the diluted contact goes more positive.

Consider now the transverse Donnan phase potentials of the cellulose wall which will contribute to the measured potential of the cytoplasmic phase. The magnitude of this P.D. will be determined by the difference between the magnitude of the two Donnan potentials at the points of contacts.

i.e. for a univalent salt.

$$E_{CM_2}^{CM_1} = \frac{RT}{F} \log n \frac{CM_2}{CM_1} + \log n \frac{\left(\sqrt{A_1^2 + 4CM_1^2} + A_1\right)}{\left(\sqrt{A_2^2 + 4CM_2^2} + A_2\right)}$$

Where A_1 is the exchange capacity of the wall in contact with contact medium concentration CM_1 and A_2 is the capacity in contact with contact medium concentration CM_2.

The situation is far more complicated in practice, for there are the cytoplasmic/Donnan phase potentials to consider which will oppose the above.

However, as plasmolysis experiments indicate that water readily moves through the cellulose, and uptake and loss of ions from cells can be readily shown, this suggests there must be many free pathways through the cellulose walls which will short out any transverse Donnan phase potentials and therefore reduce their influence so they have probably been unimportant in this work.

All these equations over-simplify the case, for they consider only a univalent salt, whereas in actual fact, mixtures of di- and univalent ions are

involved. However they indicate what may influence and determine the magnitude of the Donnan potentials.

Macrobbie and Dainty (1958), quote a half-time of 1 hour for the exchange of KCl with Donnan Free Space ions (said to be cytoplasmic) in Nitellopsis.

Dainty and Hope (1959), found in Chara australis that Na^+ and Ca^{++} exchange could be divided into fast and slow components, the fast component having a half time of about two minutes for Na^+ from a 1 milli-equivalent per litre Na^+ solution, the slow, a half time of about 30 minutes. 75% of the total exchange occurred by the faster component. Ca^{++} was about twenty five times as slow. This was a cellulosic exchange as cell walls alone gave similar results.

One suspects that similar changes in the potential could occur on change in contact medium and on initial application of the contacts. This would imply a fast change followed by a slower change but both of the same polarity, and the equilibrium potential would not be attained till all ion exchanges were completed. It was wondered if the changes taking place on the original application of the contacts could be due to these adjustments.

However with Donnan exchanges and diffusion potentials in the W.F.S. and cuticle one could not expect a multi-spiked potential curve. The next chapter (4) shows that these changes are ascribable to changes in membrane permeabilities.

Our calculations have shown that the longitudinal Donnan and diffusion potentials in the cellulose and cuticle could only be important in the measurement of transverse potentials when using differing contact media; whenever

transverse potentials have been measured in this investigation identical contact media have been used, and so it is likely that these potentials have contributed only little to our results. However the presence of the Donnan cellulose wall phase may influence the concentration of the various ions in the region external to the cytoplasm and could therefore influence the membrane potentials, and this is probably the main effect, if any, of the Donnan phase of the cellulose walls on the potentials measured.

Class C

These are potentials existing across cell membranes as a result of metabolic activity.

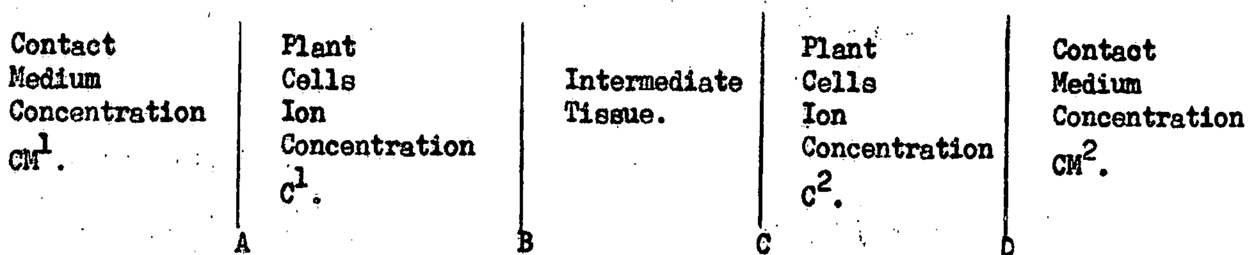
Lund (1928), considered the potentials measured to be due to oxidation/reductions systems in the cell, certain sites in the cell membrane acting like "noble metal" electrodes. However this is no longer the current view and the potentials are now generally ascribed to diffusion potentials in the broadest sense (Dainty, 1961), the membrane integrity being maintained by metabolic activity.

Results of Lund and co-workers (1947), which were put forward as evidence for the oxidation/reduction theory may in fact all be interpreted as changes in metabolic activity leading to changes in the membrane permeability or to effects on ion pumps which will both affect the measured potentials.

Our preliminary results suggested that membrane, diffusion or Donnan potentials were being measured. In the previous section, diffusion and Donnan potentials have been considered and this section considers Membrane Potentials only.

Membrane potentials have usually been theoretically treated using the Goldman equation, which is difficult to apply to this work for neither the concentration of ions within the coleoptile cells nor ions to which the membranes are permeable are known.

The following simple solution has been used. Consider details of the plant and contacts:-



The measured P.D. will be the algebraic sum of the P.D.'s at the membranes "A,B,C,D" and at all the membranes of the intermediate tissues.

All equations for membrane potentials indicate a direct relationship between the P.D. and the log of the ratio of the ion concentration across the membranes, and assume that the potentials are also function of the membrane permeabilities to ions (or ion mobilities with the membrane).

i.e.

$$E = a \log \frac{(C\,1)}{(C\,2)} + B$$

where (a) is dependent on the ion mobilities in the membrane, C 1 and C 2 are the ion concentrations on the two sides of the membrane and B is a constant.

The P.D.'s at B and C and the intermediate membranes reduce to a value based on the ion concentration C 1 and C 2 and a function of ion mobilities through all the intermediate membranes.

Therefore for the system in the diagram:-

$$E = a \log \frac{CM_1}{C_1} + a_1 \log \frac{C1}{C2} + a_2 \log \frac{C2}{CM2} + B.$$

where E is the potential of CM2 with respect to CM1 and a, a_1 and a_2 are the membrane permeabilities.

If $a = a_1 = a_2$.

Then $E = a \log \frac{CM1}{CM2} + B$.

and where $CM1 = CM2$, $E = B$.

Thus if the contact media are of similar concentration and are simultaneously changed to a new and similar concentration, E should remain constant but this is not so in these experiments.

$$\therefore a \neq a_1 \neq a_2$$

If CM1 is constant and CM2 is changed to a new concentration CM^*_2 then P.D. change

$$= a_2 \log \frac{CM^*_2}{CM_2}$$

If CM^*_2 is more concentrated than CM_2, our experiments show that the P.D. becomes more negative. (At the apex, upon change from Deionised water to Shive's solution, P.D. change is from + 40mv. to -10mv.

P.D. change = 40 - - 10 = + 50mv.)

a_2 is positive.

The same may be shown for a^1.

i.e. Cation permeability exceeds anion.

This is in agreement with Hope (1951), working on Bean roots.

If the mobility of the anion or cation through the membrane is zero then a Donnan potential exists. As long as the internal concentration of the indiffusible ion is considerably greater than the external there will be a potential difference of 58 millivolts existing between contact media differing ten-fold in

concentration; the less the ratio between the concentration of indiffusible ion and the external concentration, the lower this potential becomes till, when the external concentration is considerably greater than the internal the Donnan potential is zero. If the contact media are identical this potential will be zero.

The relationships between these 3 classes of potentials are extremely complex. For instance, upon original application of contacts, or change of contact media, until the ions have diffused through the W.F.S. there can be no effect on the other phases, so in the very first stages changes in the W.F.S. potentials are the only ones occurring. This does not mean that the potential of the W.F.S. will predominate but that only in this phase will changes in the potential occur.

Once exchange with the D.F.S. ions commences new diffusion potentials will exist in the W.F.S. and these potentials are bound to continue till the D.F.S. is in equilibrium. The Donnan phase will influence the ion concentration in the region of the cell membrane and therefore influence this potential. Also until the Donnan exchanges, if any, of the cytoplasm are completed, diffusion potentials will exist and the true membrane potential will not be developed. There is the further complication that in the rapidly growing tissue which constitutes the coleoptile there will be a net incorporation of ions which the cells will take up wherever available, and the fluxes of ions into and out of the cells will not be equal. Thus permanent diffusion potentials could exist between the medium in

contact with the cytoplasm and the actual external contact medium so long as growth continues.

The following is an attempt from the point of view of this work to simplify the above consideration. As suggested above, the greater part of the potential is probably contributed by the following system.

Electrode	Contact Medium	W.F.S. Cuticle and cell wall	D.F.S. cell wall and Cytoplasm	Membrane P.D. across Plasma Membranes	D.F.S.	W.F.S.	C.M.	Electrode

Note.

This will not apply in the apical regions in the measurement of transverse potentials, where the dominant phase is the cellulosic. However one would expect similar cellulose Donnan capacities at the two contact positions; moreover similar contact media have been used. Therefore this cellulosic phase should not generate any measurable Donnan potential, and changes in the potentials measured must be due to cytoplasmic potentials, though these will be smaller than theoretical due to shorting out by the cellulosic phase. It is therefore to changes in the above system that we must look to explain changes in the transverse potentials.

The transient changes due to the original penetration of ions into the W.F.S. of the cuticle and cell wall will be the first changes that take place. Also, as pointed out above, later changes due to ion exchanges of the Donnan Free Space will cause diffusion potentials in the W.F.S. which will persist till equilibrium is attained. Once equilibrium is attained the W.F.S. will not be influencing the potential unless there is ion uptake by the plant leading to steady diffusion potentials. The Donnan phases of the cellulose wall will influence the potential

by altering the nature and concentration of ions in the region of the membranes as well as by their own contributions to the P.D. The final potential will be dependent on the Donnan phase and the cytoplasmic membrane potentials, but as suggested before this Donnan contribution may be largely shorted out and its effect on the ion distribution also largely nullified.

It is assumed in this work that any changes in the vacuolar and cytoplasmic free ion concentrations in the course of the experiments are small. (Macrobbie and Dainty, (1958), found a half time for vacuolar ion equilibrium of 10^3 hours). We also have obtained some experimental evidence that the half time must be long in coleoptiles, for where the contact medium has been changed through a series of concentrations and is then returned to the original concentration the P.D. value is close to that originally observed. This consideration may not hold in the root region.

Changes in the Donnan equilibrium will be of an exponential form and one would also expect similar changes in Donnan potentials.

It is important to realise that all ions within the cells will influence the potential and it is only because certain ions predominate that it is possible to apply simple equations to the results.

CHAPTER 3.

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CHAPTER.4.

CHAPTER 4

FURTHER EXPERIMENTS ON THE ELECTRICAL POTENTIALS IN OAT COLEOPTILES

SUMMARY

A more detailed experiment investigating the characteristic changes in the measured potentials subsequent to making contact on Oat Coleoptiles is reported. It is shown that the changes are dependent upon changes in membrane permeabilities, with perhaps some water movement leading to changes in the tissue ion concentrations. Another experiment reports the investigation of the magnitude of the potentials measured with various dilutions of Shive's solution as apical contact medium, but with Shive's solution always as growth and basal contact medium. From the similarity of the graphs of the magnitudes of the measured P.D's plotted against the Log of the concentration of Shive's solution and KCl, it seemed possible that the P.D. was largely determined by the K^+ concentration; however, an experiment is described which shows this is not so.

Some further more detailed experiments to elucidate the nature of the potentials and changes in them, are now to be described.

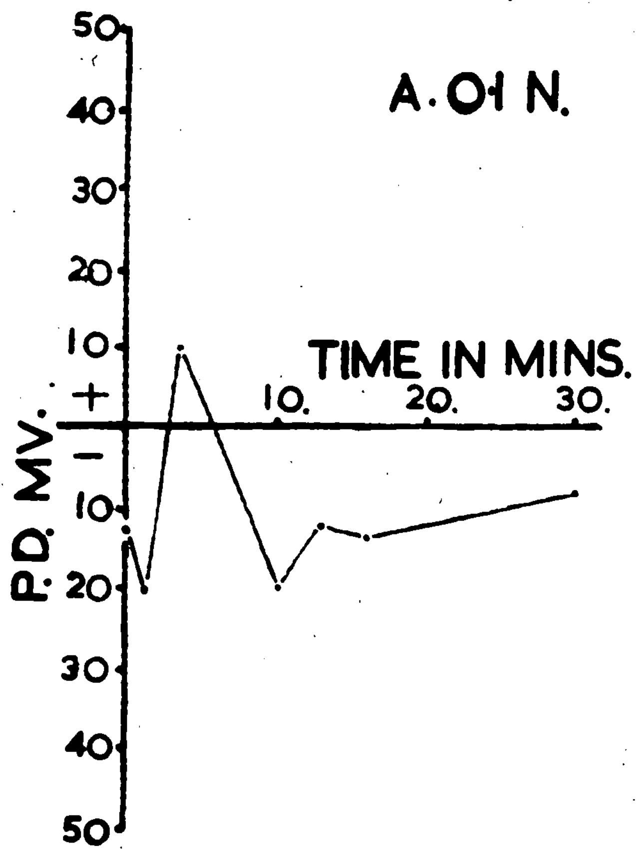
A new experiment to clarify the potential changes upon making contact was carried out. Shive's solution was used as growth and basal contact medium but a flowing drop ring contact was used at the apex with various concentrations of KCl solution as contact medium. In every case the osmotic pressure was adjusted with mannitol to be equivalent to 0.1N KCl. The potential of the apical flowing drop ring contact was measured with respect to the base. Recording continued for 30 minutes subsequent to contact application. The apparatus used is shown in Plate 2.5.

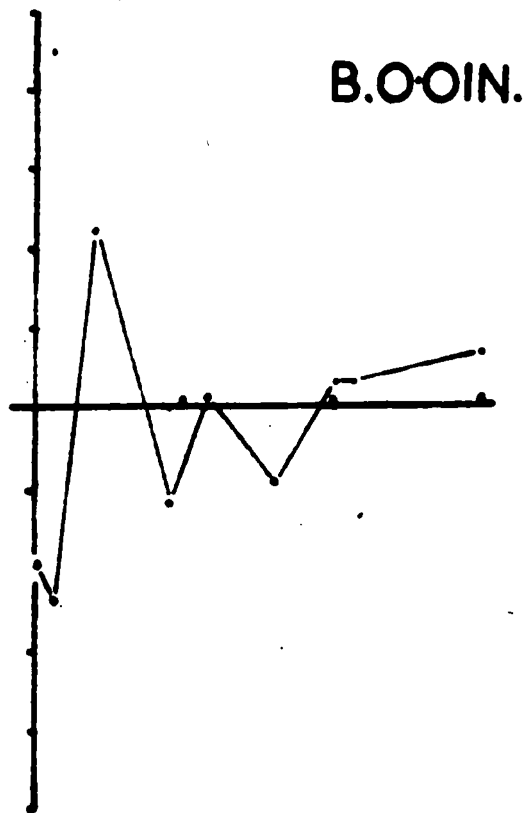
The results for each concentration are plotted in Figure 4.1. and each result represents the mean results of three experiments. The same coleoptiles were used for "A" and "C" and another, similar set, for "B" and "D". Figure 4.2. plots the magnitudes of the various peaks and troughs in the potentials, against the log concentration of KCl and here one can see from "Peak 1", that the "A" and "C" groups are distinct from the "B" and "D" but that a straight line relationship is indicated.

The important points to note are:-

1. With decrease in concentration, the positive potential of the peaks increases, as does also the final positive potential.

2. Similarly the potential of the troughs also shows an





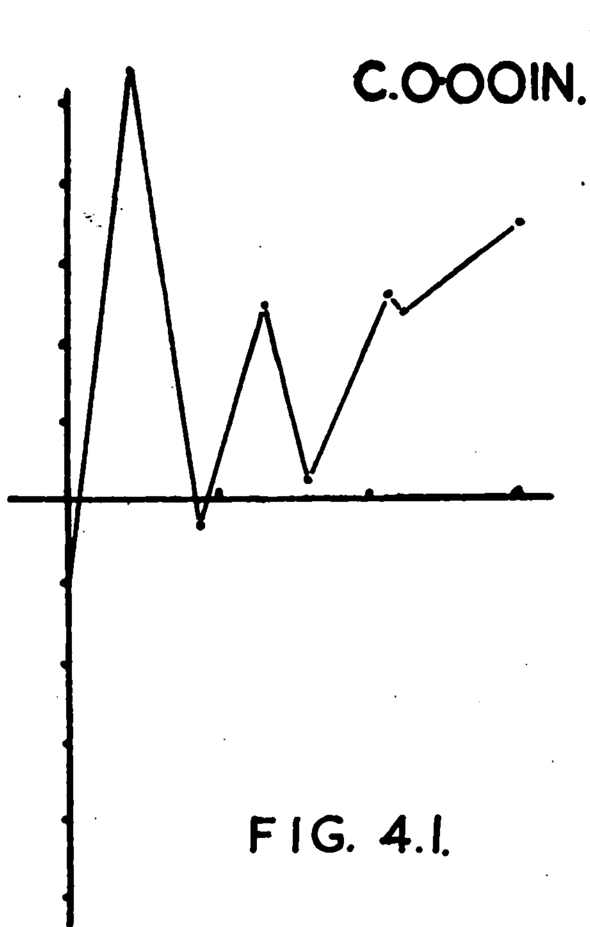
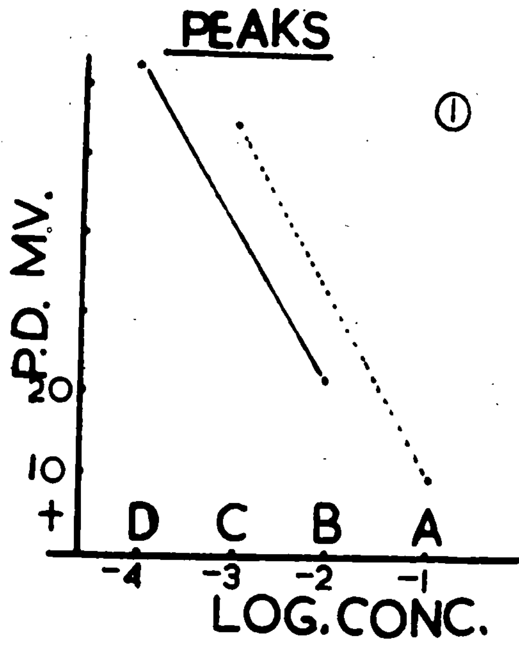
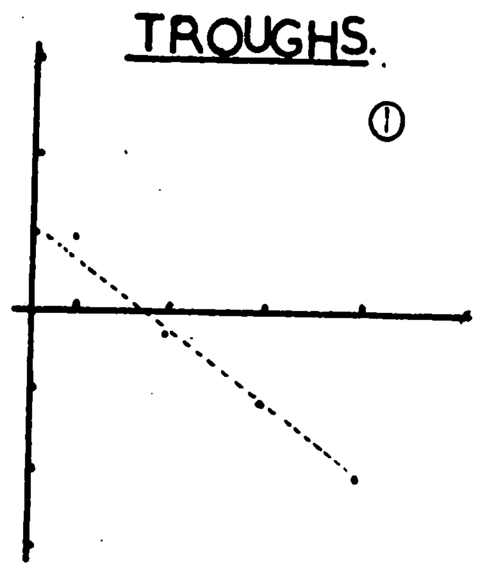


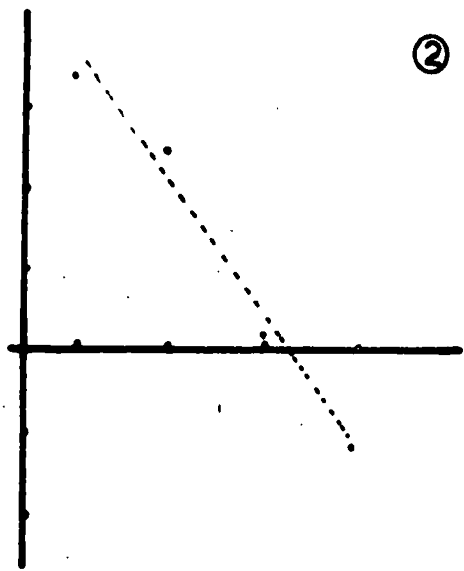
FIG. 4.1.

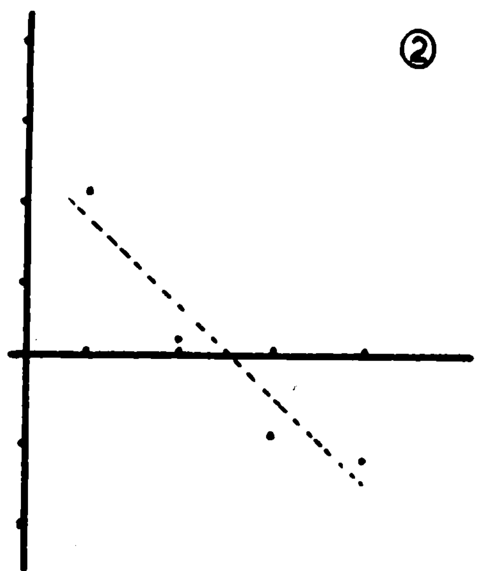
PEAKS

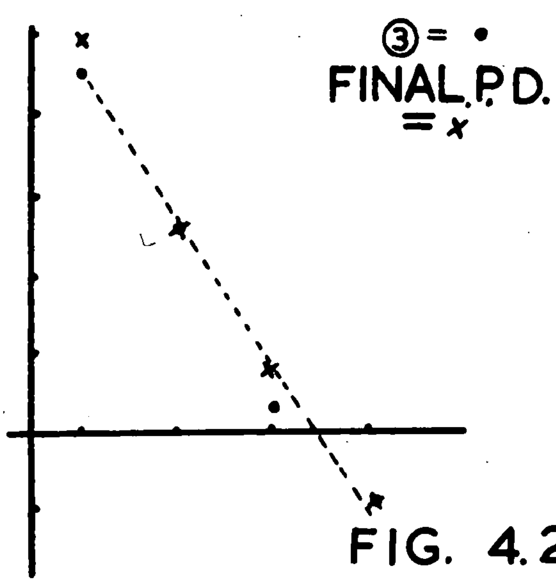
TROUGHS.











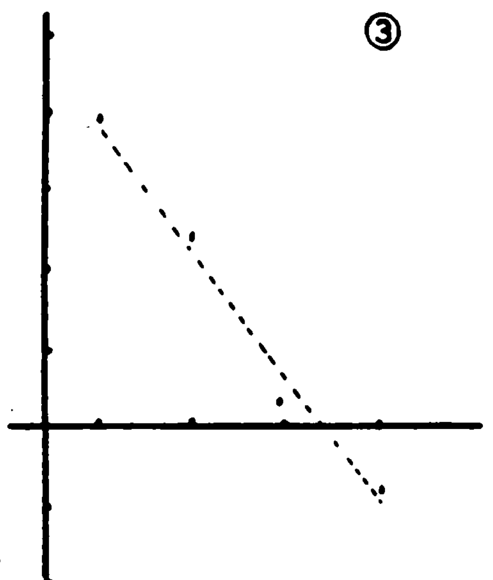


FIG. 4.2.

increase in positivity.

3. When the peak and trough potentials are plotted against log of the concentration straight line relationships show up.

4. There is an increase in negativity in "A" and "B" immediately after making contact, reaching a peak at 30 seconds and this is greater in "A" than "B" and absent in "C" and "D".

5. The oscillations show a periodicity of 4, 5, 4, 3, 4, and 2 minutes and they are damped in form.

Consider the equations of the lines of the troughs and peaks plotted against the log of the KCl concentration.

	A & C	B & D	Mean
Starting P.D.			$E = +5.5 \log_{10}KCl - 7.5$
1st trough A & B only			$E = +3 \log_{10}KCl - 18$
1st peak	$E = -22 \log_{10}KCl - 12$	$E = -19.5 \log_{10}KCl - 17$	$E = -20.75 \log_{10}KCl - 14.5$
2nd peak	$E = -18 \log_{10}KCl - 30$	$E = -16.5 \log_{10}KCl - 32$	$E = -17.25 \log_{10}KCl - 31$
3rd peak	$E = -17.5 \log_{10}KCl - 26.5$	$E = -21 \log_{10}KCl - 39$	$E = -19.25 \log_{10}KCl - 32.75$
1st trough	$E = -9 \log_{10}KCl - 30$	$E = -11 \log_{10}KCl - 34$	$E = -10 \log_{10}KCl - 32$
2nd trough	$E = -7 \log_{10}KCl - 20$	$E = -15 \log_{10}KCl - 40$	$E = -11 \log_{10}KCl - 30$
3rd trough	$E = -15.5 \log_{10}KCl - 24.5$	$E = -17.5 \log_{10}KCl - 32$	$E = -16.5 \log_{10}KCl - 28.25$
Final P.D.	$E = -17.5 \log_{10}KCl - 26.5$	$E = -21.5 \log_{10}KCl - 36$	$E = -19.5 \log_{10}KCl - 31.25$

Consider the following theoretical equations and their agreement with the above experimental results.

Theoretical equation	0.1N KCl		0.01N KCl		0.001N KCl		0.000-1N KCl	
	Exp.	Theor.	Exp.	Theor.	Exp.	Theor.	Exp.	Theor.
	PD in mv		PD in mv		PD in mv		PD in mv	
1st peak $E = -20 \log_{10} KCl - 15$	+10	+5	+22	+25	+54	+45	+61	+65
2nd peak $E = -17.5 \log_{10} KCl - 30$	-12	-12.5	+1	+5	+24	+22.5	+34	+40
3rd peak $E = -19 \log_{10} KCl - 30$	-9	-11	+3	+8	+26	+27	+45	+46
1st trough $E = -10 \log_{10} KCl - 30$	-21	-20	-13	-10	-3	0	+10	+10
2nd trough $E = -12.5 \log_{10} KCl - 30$	-13	-17.5	-10	-5	+1	+7.5	+20	+20
3rd trough $E = -17.5 \log_{10} KCl - 30$	-9	-12.5	+3	+5	+24	+22.5	+38	+40
Final P.D. $E = -20 \log_{10} KCl - 30$	-9	-9	+7	+10	+26	+30	+50	+50
Starting P.D. $E = +7.5 \log_{10} KCl - 5$	-13	-12.5	-20	-20	-10	-27.5	-35	-35
1st trough A & B only $E = +5 \log_{10} KCl - 12$	-20	-17	-24	-22		-27		-32

Let us consider what these equations actually represent. If this is a simple membrane potential then $E = a \log c + b$ where (a) contains terms dependent upon the membrane permeabilities to ions in the contact medium, of which (c) is the concentration. (b) contains terms dependent upon the ion concentration of the tissue and upon potentials due to other regions of the plant as well as upon other ions within the tissues. Also the transient diffusion potentials set up on first making contact are included in the b term, for the mobilities of K^+ and Cl^- are similar and other ions in the tissues will be at the same concentration no matter what the external concentration of KCl and therefore these potentials will be the same

in all cases and not proportional to KCl concentration.

The theoretical equations give results in good agreement with the experimental potentials. This is not really surprising as the theoretical equations have been derived from the experimental, but it is believed they can explain the results more clearly.

When the contact is first applied to the coleoptile tips the cell membrane shows slightly greater permeability to anions and the potential contribution from other phases etc., (b), is small.

Due to the application of the contact the permeability becomes greater for cations (particularly K+) than anions (particularly Cl-) and at the same time the potential contribution of other phases is increasing. This continues till the first positive peak. As shown by the calculations, in the higher concentrations of contact media this will give rise to a fall in the potential after the first contact application, followed by increase in the positivity. At the first positive peak, the process of permeability change reverses and the permeability to cations begins to decrease. (All cases of permeability change represent a relative change in the permeability to ions. For instance, an increase in cation or a decrease in anion gives the same effect. By measurement of membrane resistances it would be possible to obtain a clearer indication of which change had occurred.) The potential contribution of (b) continues to increase till it reaches a maximum at the first trough and then

remains constant. The first trough marks the point when there is again an increase in cation permeability though it does not attain its previous value. This new peak is the seoond positive peak. There is then a further decrease (second trough), not as great as previously, an increase to a value greater than the second peak but not as great as the first (third peak), a slight decrease, (third trough), followed by an increase to a final value similar to that of the first peak. We therefore have damped oscillations in the membrane permeability giving rise to damped oscillations in the potential. These are summarised in Figure 4.3.A. The changes upon original application of 0.1N KCl resemble the changes found with Shive's whilst 0.000 1N KCl resembles deionised water so the same explanation also applies with Shive's or deionised contact media.

It is now necessary to consider the changes in (b).

These will be brought about by:-

1. The transient diffusion potential upon first making contact.

2. Changes in the potential existing within the tissues, brought about by changes in permeability of membranes (It is possible that the permeability changes are not confined to the membranes in contact with the contact medium only but that changes also occur in the other cell membranes. If this were so one could expect (b) to reflect the oscillations of (a) which it does not seem to do, but since (b) is composed of several other potentials as well, it may be the experiment

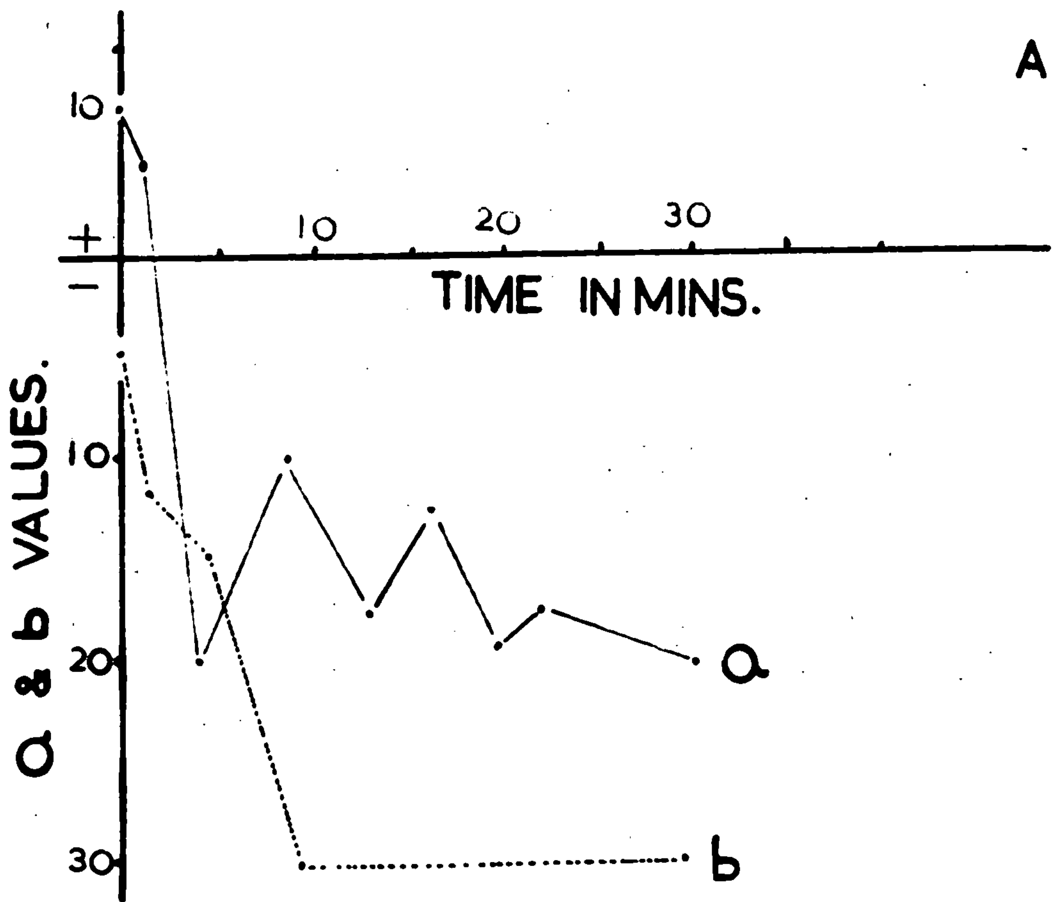


FIG. 4.3.

was not sensitive enough to detect the oscillations.

The evidence in favour of this permeability change is the close agreement in time and magnitude of (a) and (b) Figure 4.3.A.

3. Changes due to water movement leading to changes in internal ion concentrations. It has been shown that the suction force of the coleoptile is osmotically equivalent to a 5 to 10 x Shive's solution, which is equivalent to 0.2 to 0.4 N KCl. In this experiment the osmotic activity of the various contact media was adjusted to be equivalent to 0.1N KCl. Water may enter the coleoptile tissue leading to a dilution of the internal concentration and this entry must be the same in all cases. (This assumes the tissue is not fully turgid, which, since the humidity is not controlled, is possible.) If this were so, then it takes about 5 to 10 minutes for the completion of water uptake, after which it remains constant. The dilution of the internal concentration must lead both to a change in the potential between the tissue and contact medium and the potential within the tissue.

The graph dilution with time one would expect to be of expotential form and it is seen that the points of (b) are in reasonable agreement with this.

There is one factor which rules out this change as one of major importance, and that is, that the change in potential is proportional to the log of the ratio of the internal ion concentration before and after the change. For a 10x change in concentration, i.e. a 10x change in volume, the maximum potential change is 58 millivolts, and

it would probably be less, for this magnitude of change can only occur if there is a Donnan potential between the tissue regions. The volume changes must in practice be relatively small, so it seems that changes in membrane permeability and not ion concentration, are the predominant factor bringing about the changes in (b).

However, there is one way of testing the water movement theory, for using a constant concentration for KCl solution as contact medium and altering the osmotic pressure of the contact medium by addition of mannitol we can alter any water movement pattern.

Such an experiment was tried. The value (b) will depend on factors such as coleoptile height and growth conditions and as the experiments were carried out on a different coleoptile batch from the above, the results have been analysed by calculating (b) using the values of (a) from the above experiments. Each concentration is the mean of three experiments.

	0.01M Mannitol		0.1M Mannitol		1M Mannitol	
	Measured P.D.mv	b	Measured P.D.mv	b	Measured P.D.mv	b
Starting P.D.	+25	+50	+38	+63	+43	+68
trough	+19	+25	NOT PRESENT			
1st peak	+57	-9	+57	-9	+76	-10
1st trough	+9	-24	+15	-18	+19	-14
2nd peak	+24	-34	+27	-31	NOT PRESENT	
2nd trough	+14	-27	+23	-18		
3rd peak	+27	-36	NOT PRESENT			
3rd trough	+24	-34				
Final P.D.	+33	-33	+36	-30	+49	-17

Considering (b)

In all cases, there is a rapid decrease in its value after making contact, and all show the same value at the 1st peak. After this, in the .01M mannitol the decrease is still rapid, reaching a constant value between the first trough and 2nd peak. In 0.1M mannitol the result is not so clear, but the final value is similar to that of 0.01M mannitol, attained at a similar or perhaps a slightly later time. With the 1M mannitol the value decreases only slowly and slightly from the 1st peak. The results are also shown graphically in Figure 4.3.B.

With M mannitol the net water movement must have been outwards for it is equivalent to 13 x Shive's. Etherton and Higinbotham (1960), report coleoptile tissue as osmotically equivalent to 0.2M mannitol.

The results show that the first changes in (b) are similar in all osmotic solutions and therefore cannot be due to water movements, but are ascribable to diffusion potentials, or changes in membrane permeability leading to changes in membrane potentials. The changes subsequent to the above do however differ in the higher and lower osmotic solutions, and are probably ascribable to outward water movement in the former, leading to an increase in the ion concentrations of the tissues.

A factor emerging from the results is that after the potential has stabilised, (b) can be treated as a constant over a range of contact media concentrations, as long as the most concentrated does not exceed the equivalent of 0.1M mannitol.

Presumably with all solutions below this osmotic concentration the cells of the tissue are fully turgid and therefore the internal concentrations similar.

One unexpected feature of the results is that immediately contact is made the mobility of the anion appears to exceed that of the cation, but later the reverse is true. Typically for plant tissues, the cation permeability has been reported to be greater than the anion. There is no immediate explanation of this phenomenon and more experiments involving further contact media, other concentrations, and the accumulation of many more results, would be necessary before one was forthcoming.

Since the hypothesis put forward involves no ion movements into the tissue, but only small water movements, this explains why after changing through various concentrations of contact media, the potential on return to the original concentration is similar to the original potential.

The conclusions from these experiments may be summarised as follows:-

1. The potential changes upon first making contact are ascribed to changes in membrane permeabilities, transient diffusion potentials and perhaps small water movements into the tissues.

2. Regular oscillations in the membrane permeability set up by contact application, induce oscillations in the potential measured, but a discussion of the factors inducing the permeability oscillations is left to the chapter dealing with potential oscillations in general. (Chapter 12).

3. The equilibrium potential is a membrane potential, with cation permeability exceeding anion.

Apart from the results with KCl recorded above and a few with 10% and 100% Shive's solution, no results were available for the change in P.D. upon change in contact medium, with constant growth medium. In nearly all previous experiments we have more than one variable and it has not seemed sensible to carry out calculation on those results, but far more advisable to carry out a few further

experiments with a constant growth medium. A flowing drop ring contact was applied to the apex and various Shive's concentrations were used successively as contact media on the same coleoptile. Shive's solution formed the basal contact and growth medium. A saturated KCl salt bridge was placed between the electrode and the variable apical contact medium and the potential measured with respect to the base.

The potential after a period of 30 minutes from contact application was recorded. The results below are for two coleoptiles.

Apical Contact Medium	P.D. millivolts	
Shive's	-14	-20
10% Shive's	+4	-14
1% Shive's	+34	+4
0.1% Shive's	+57	+28
Shive's	-12	-19

Figure 4.4 plots the mean P.D. against log-concentration.

The equation of the line is $E = -21.5 \log_{10}C + b$ which compares with the $E = -20 \log_{10}C + b$ found for KCl solution. This suggested that the potential across the plant apical region was determined largely by the K^+ concentration in the external solution. If this were so, then the permeability to Ca^{++} and Mg^{++} must be low.

The equation shows that the cation permeability exceed anion but the anion permeability cannot be zero, for the P.D. change for a tenfold concentration change would then be 58 millivolts.

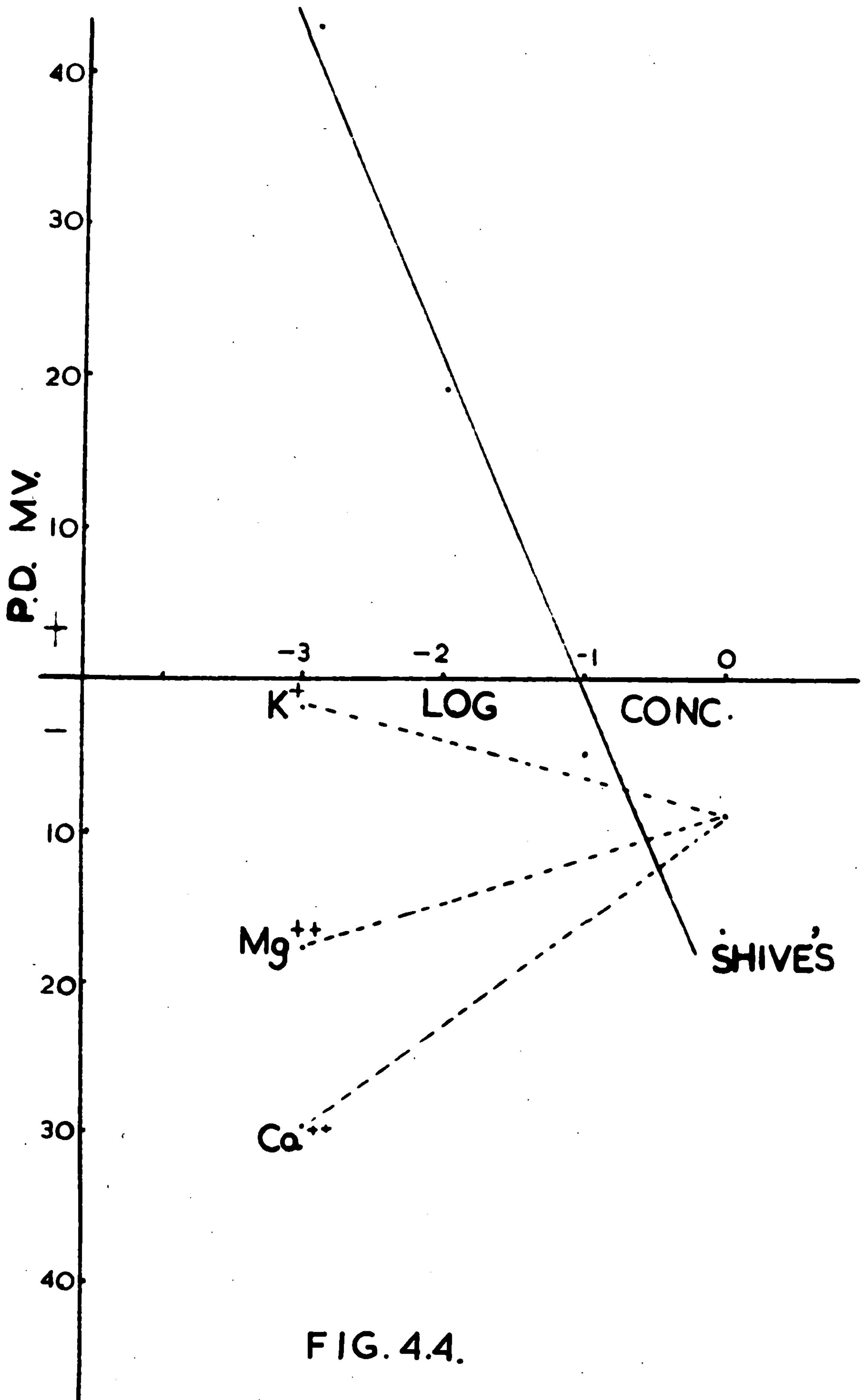


FIG. 4.4.

In an effort to determine if Ca^{++} and Mg^{++} have an influence on the potential, the effect of using Shive's solution with only 1/1000th of the normal concentration of these salts was tried.

Apical Contact Medium	P.D. mv	Mean P.D. millivolts
Shive's	-8,-14,-6	-9.3mv
Ca++(1/1000th)	-28,-36,-26	-30mv
Mg++(1/1000th)	-11,-22,-22.5	-18.5mv
K+ (1/1000th)	0, -14, +10	-2mv
Shive's	-8,-20,-10	-12.6mv

The results are also shown in Figure 4.4.

The equations for the respective salts are:-

$$K^{+}\ E = -2.4 \log_{10} C_{K^{+}} - 11.3$$
$$Mg^{++}\ E = +3 \log_{10} C_{Mg^{++}} - 9.3$$
$$Ca^{++}\ E = +6.9 \log_{10} C_{Ca^{++}} - 9.3.$$

It is now obvious that agreement between the data for KCl and Shive's upon dilution, was fortuitous.

To explain these results, it is suggested that the membrane permeability is a function of the K+, Mg++ or Ca++ ratio. When we dilute whole Shive's the ratio is constant, and so is the permeability. The potential is then proportional to the concentration. The same applies with KCl alone. If we just dilute the K+ in Shive's the K+/Ca++ falls and permeability to cations decreases, which will decrease the potential change from that with K+ alone. However, when we try to explain the situation with Ca++ and Mg++ dilution, we have to assume that when these are decreased the cation permeability still decreases relative to the

anion, yet we know that with KCl alone the cation/anion permeability must be the same as with Shive's. More experiments on this are necessary before anything conclusive can be shown but it seems likely that interactions of ions on the membrane permeabilities are involved. There may of course be effects of the anions to be taken into account.

Etherton and Higinbotham (1960) have shown that with cells of the Oat coleoptile, when the external concentration of K+ is decreased, then the external solution becomes more positive with respect to the vacuole. This is the same as our results.

Etherton (1963), (personal communication), found that the effect with Ca++ was opposite, agreeing with our Ca++ dilution experiment.

Examination of Etherton and Higinbothams' published data (1960), gives the equation for KCl as

$E = -(20 \text{ to } 30) \log_{10}(KCl) + b$ which is in reasonable agreement with our results, confirming that our potentials are composed of trans-membrane ones.

Hope (1950), for bean roots gives the equation as

$$E = -32 \log_{10}(KCl) - 38$$

Ueda (1961),

$$E = -29.7 \log_{10}(KCl) - b \text{ for Opalina Sp.}$$

This suggests that $E = -(20 \text{ to } 30) \log_{10}(KCl)$ may be generally applicable to plant and perhaps animal tissues.

Conclusion

(a) An hypothesis is presented which indicates that the changes in potential noted subsequent to making contact with the plant are due to changes in membrane permeabilities and to water movements which bring about changes in the internal ion concentration.

(b) The equilibrium potential with Shive's solution and KCl is a membrane potential with the cation permeability exceeding the anion.

(c) Comparison between our results and those of Etherton and Higginbotham (1960) confirms that the potentials we measure are membrane potentials.

(d) The equation $E = -(20 \text{ to } 30) \log_{10}(KCl) + b$ may be generally applicable in plant and perhaps animal tissues.

(e) The membrane permeabilities are dependent on the ratios of various ions in the contact media. This agrees with other workers e.g. McCutcheon and Lucke (1928), who found that high external Ca^{++} decreased permeability whilst high K^{+} increased permeability of membranes. This also agreed with the suggestion on ion antagonism, that only in certain ratios of mono and divalent ions is membrane integrity maintained.

CHAPTER 4.

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CHAPTER.2.

CHAPTER 5

CHANGES IN THE BIOELECTRICAL POTENTIALS OF GREEN PLANT TISSUES BROUGHT ABOUT BY CHANGES IN LIGHT INTENSITY

SUMMARY

The accidental discovery of characteristic changes in the bioelectrical potentials of green plant tissue, produced by changes in light intensity, is described. Further work on the influence of the intensity change, and the timefactor is detailed. Subsequent to this work, a considerable body of literature on the topic, was located which is now reviewed. The results of previous workers are compared with our own. It is shown that it is changes in membrane permeabilities that bring about the response, with perhaps the involvement of some changes in ion concentrations. The effects of the application of ammonia and carbon dioxide, on the plant potentials are detailed and these responses are compared with those of light. It is concluded from these experiments that the illumination responses are mediated through pH changes brought about by changes in the photosynthesis rate.

It is shown that coleoptile tissue responds in a similar manner to green plant tissue on application of ammonia and that the ion balance of the contact medium influences the magnitude of the changes in green plant tissue potentials on changes of light intensity.

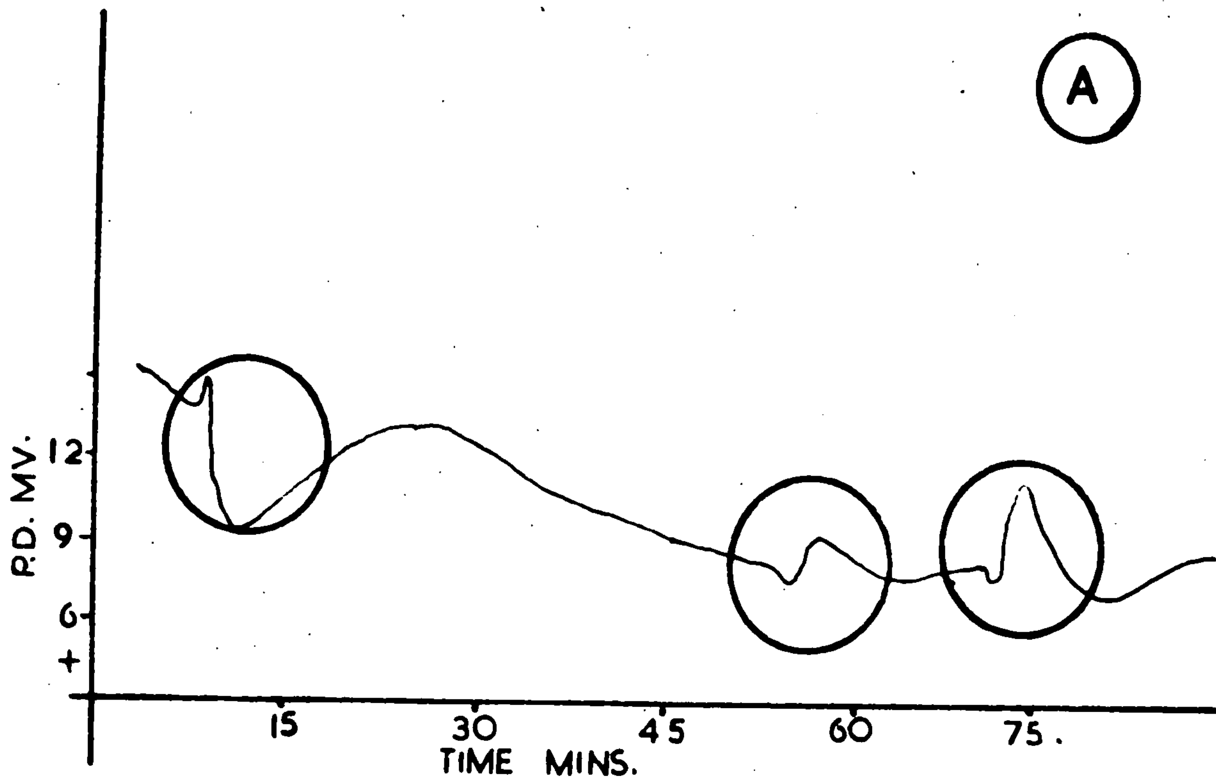
This effect was discovered by a complete accident during work upon the resting potentials of Oat seedlings. It was noted that odd characteristic potential changes had occurred during the course of an experiment measuring the P.D. between the Oat primary leaf and seedling base (Figure 5.1.A). From calculations of the times at which these changes occurred, it was found that they coincided with entry into the laboratory to examine the recording. It was then suspected that light might be involved, for though the entrance was light trapped, this was by no means fully efficient.

We had no knowledge at that time of any previous observations of a bio-electric response by green tissue to brief illumination, and we had not, till then, envisaged a study on these lines. However, the effects were so fascinating that a fairly extensive study was carried out, the first part of which was undertaken before earlier literature on this topic had come to our notice. Subsequently a considerable body of literature has been located which confirmed some of the observations we had by then made. In the description that follows there are however some completely new observations.

Experimental Methods

The Oat grains were soaked, germinated, and then grown in red light at 20°C as previously described. Seedlings with coleoptiles about 25 millimetres long were selected and allowed to continue their growth in white tungsten filament light of 2,500 metre-candles intensity at 25°C. When the seedling height had attained about 11 centimetres the seedlings were transferred to the experimental assembly, the form of which is shown in

PLATE/



B

1 SEC.

2 SECS.

5. SECS.

10 SECS

30 SECS.

2 MINS

5 MINS.

LIGHT OFF.

LIGHT OFF.

LIGHT. OFF.

PD 3. MV. INTERVALS.

TIME 15. MIN. INTERVALS.

FIG. 5.1.

PLATE 2.5. The base contact was made via the growth medium and a "flowing drop ring" contact was applied to the tip of the primary green leaf. The apical contact was connected to the low input lead of the Vibron. Experiments were carried out in continuous weak red light (1 metre-candle), and the stimulating light was given by white light of 2,500 metre - candles intensity, unless otherwise stated. Heat was always filtered out by a glass trough containing 5 centimetres depth of water. The light source was positioned vertically above the seedling. Unless otherwise stated Shive's solution has been used as growth and contact medium.

The light that reached the seedling when the door open must have been of short duration. The first experiment therefore consisted of exposing the plant to varying periods of vertical white light. The results are shown in Figure 5.1.B. It was found that with increase in the exposure time the response increased. Even with only one second of light there was a detectable response. It seemed from the form of the curve of P.D on the resulting chart, that when the light was turned on a change started, which was modified when the light was turned off. This was not an "All or None" response such as the action potential of Nitella Species.

It was now necessary to discover at which of the two contact positions the changes were taking place. A new electrode and contact were designed which enabled a very narrow beam of high intensity light to be incident on the tissue precisely at the position of the contact. This is shown in Figure 5.2. With this arrangement there exists the possibility of the contact medium being heated and this could be suspected of

causing/

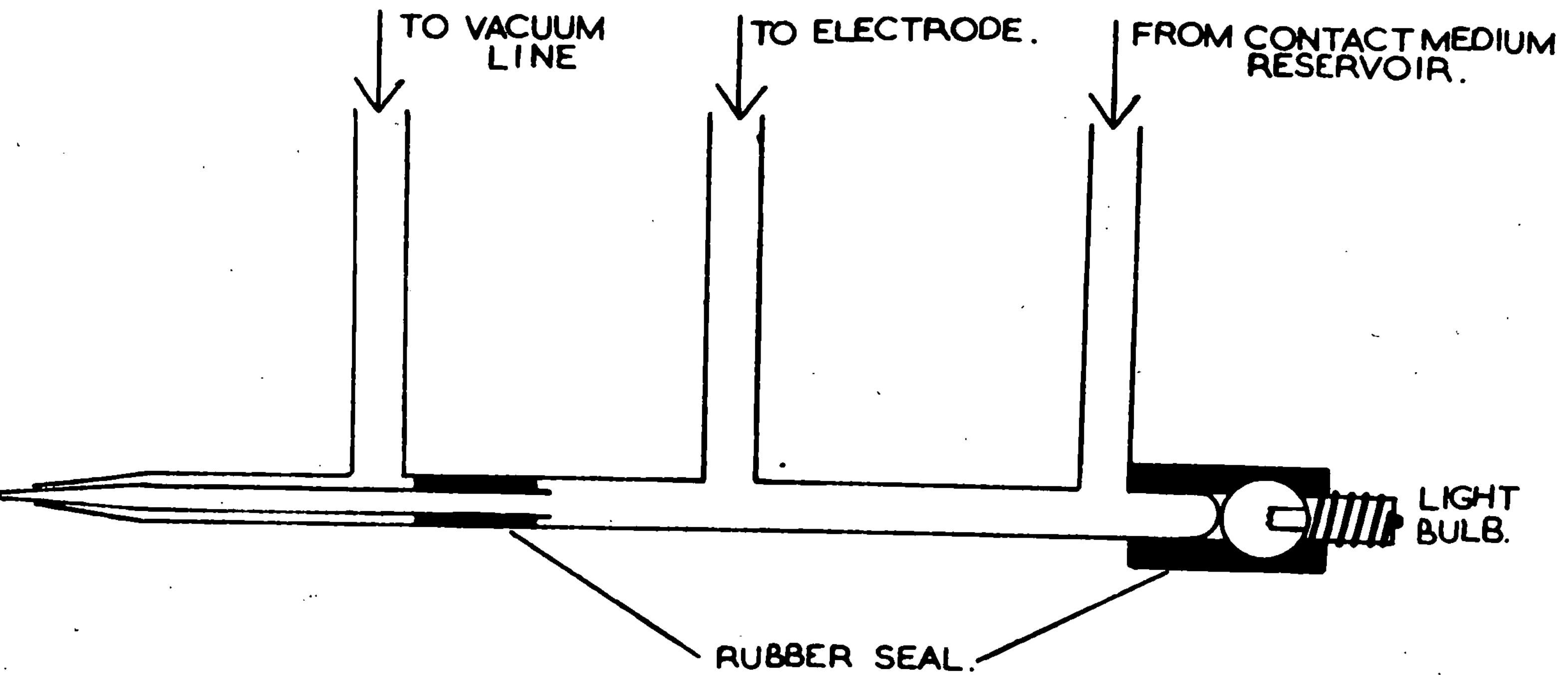


FIG. 5.2.

causing the response.

The light bulb used was rated at 0.6 watts and the rate of contact medium flow was not less than one millilitre per minute. 0.6 watts is a rate of energy flow equivalent to 0.144 calories per second. This could raise the water temperature by 8.64°C, if all the heat was transferred to the flowing contact medium stream. However the area of the contact between the bulb and the actual flowing contact tube was small so radiated heat would make the major contribution to heating the medium. All parts of the contact tube were painted black, making it an efficient radiator. Also the rate of air flow to the vacuum line was made large, which would help to cool the medium. It is doubtful therefore whether the temperature rise would in practice be significant.

Using such a contact on the primary leaf and a basal contact in the growth medium, the light effect was identical with that recorded before, indicating that the basal parts could be contributing little if anything to the effect, for in this experiment, the light can be confined to the apical contact.

An experiment using illumination with white light, interspersed with varying dark periods, was then carried out. (The weak red light was continuous throughout). The results are shown in Figure 5.3. The times noted on the graphs are the lengths of the dark periods.

This showed that there were both light anddark effects which were opposite in form, and that a period of approximately 30 minutes between the two was necessary for the system to be able to give the full response

to/

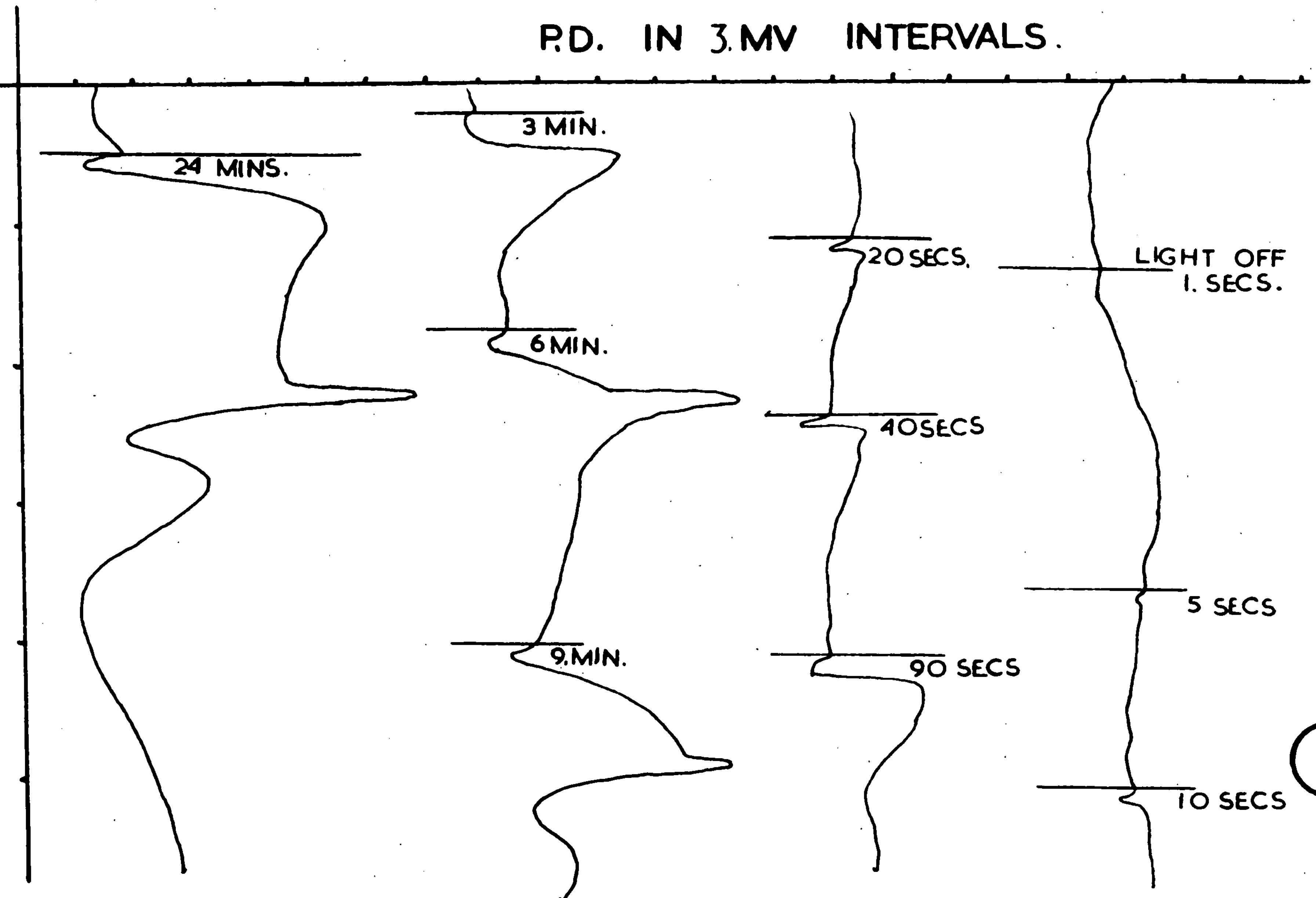


FIG. 5.3.

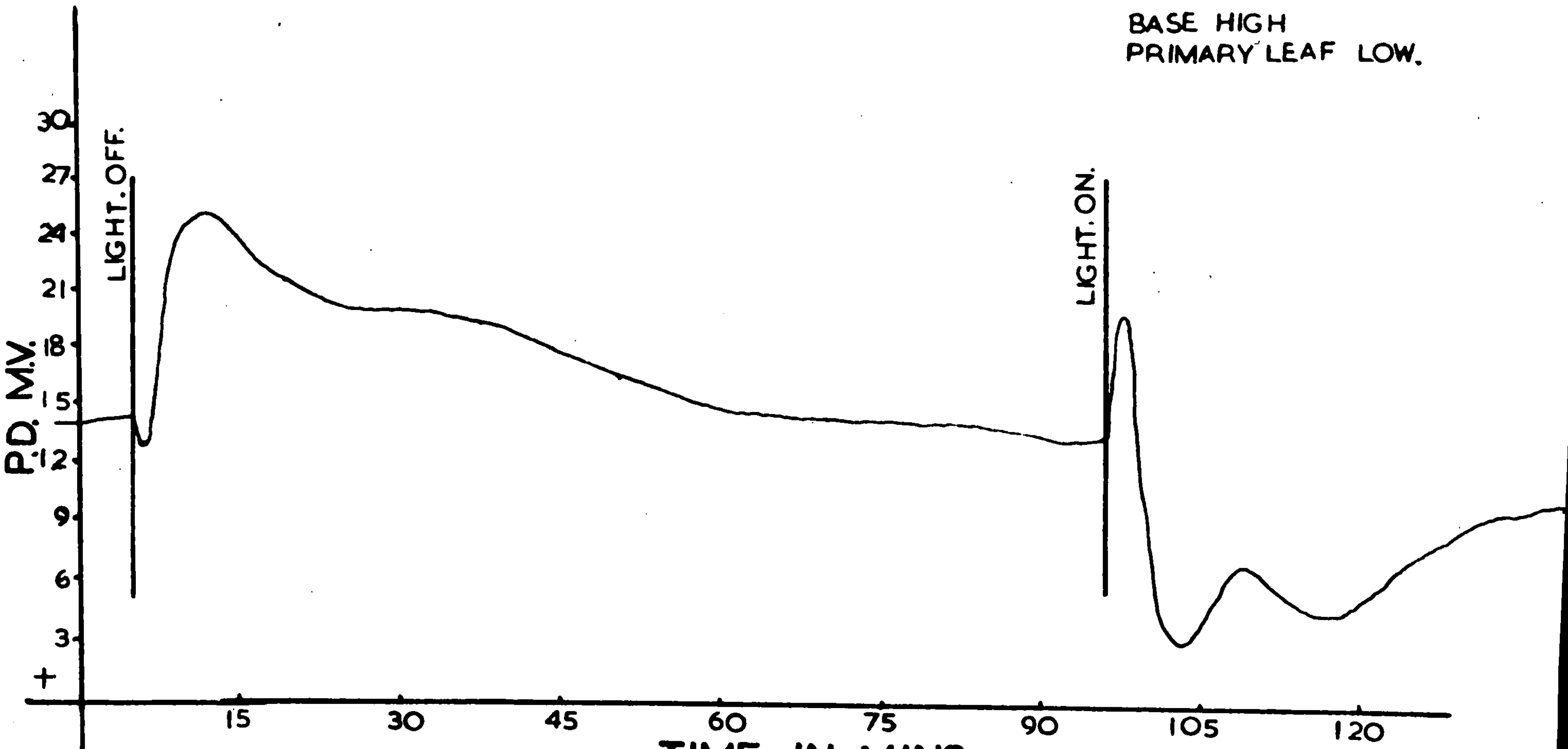


FIG. 5.3.

to a change to light or to darkness. At shorter intervals the effects observed were a combination of the two responses.

As the recorder used was not particularly fast responding, a hand recorded experiment had to be carried out to determine the exact time after the light was turned on or off, that the response commenced. This showed that there was a lag time of approximately 15 seconds.

From the results it also seemed that illuminated tissue was positive in potential with respect to similar unilluminated tissue, at equilibrium.

The effects of the light intensity on the magnitude of the response were now investigated. It should be pointed out that the light intensities quoted are those measured perpendicular to the leaf surface and to the direction of the light at the point of contact. The actual light absorption by the leaf will be much less than that for plant tissue perpendicular to the direction of the light. In this experiment the illuminated primary leaf was the High. The results are recorded in Figure 5.4. Over the rather limited range of intensities used in this experiment (1000 to 3,500 metre-candles), the magnitudes of the effects increased with increase in the intensity change. This confirmed that it was not an "All or None" reaction, and showed that it was not a light/dark effect but an intensity change response.

The other aspect considered in this series of experiments was fatigue. Figure 5.5 shows the resulting graph. With 90 second periods of darkness every 30 minutes and 2000 metre-candles illumination, there was no evidence of fatigue. The magnitude of the effects were also remarkably constant.

Therefore/

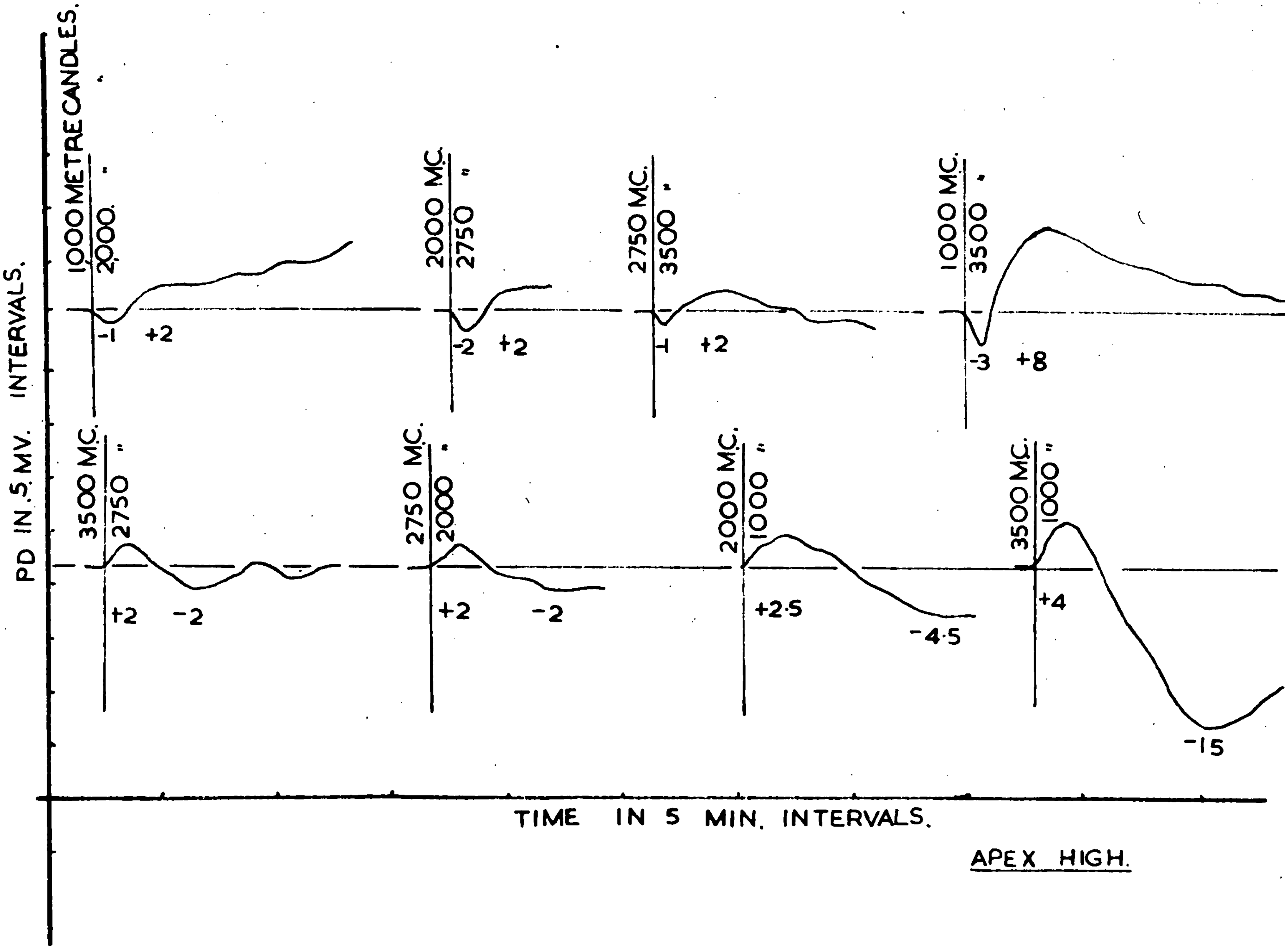


FIG. 5.4.

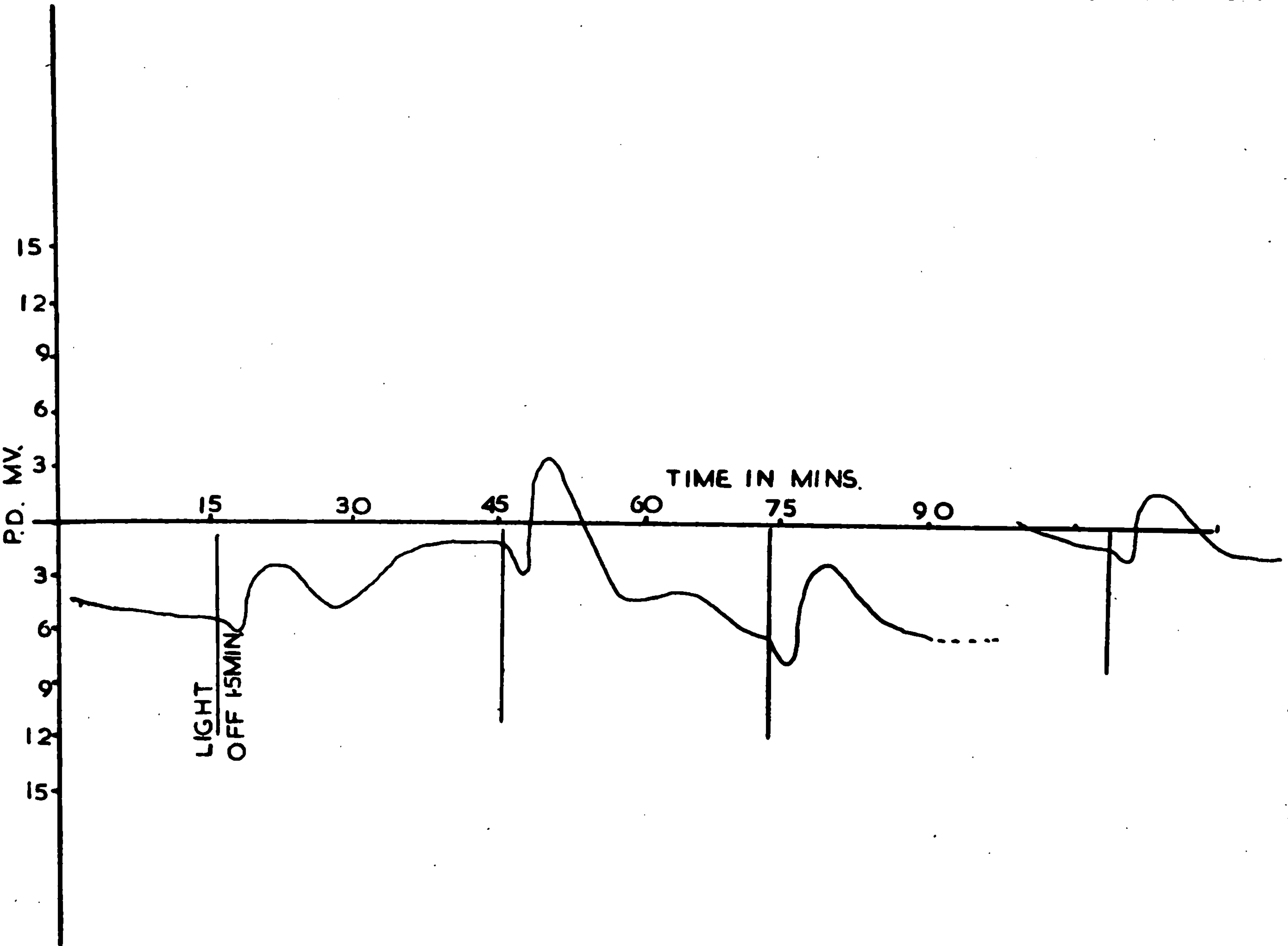


FIG. 5.5.

Therefore we may ascribe changes in the response of the same plant at 30 minute intervals, to changes in the experimental conditions.

Summary of the Experimental Results

Changes in the potential measured between an oat seedling primary leaf and the basal growth medium occur on changes in light intensity. These changes take place in the leaf tissue not the basal regions of the seedling. Below are given details of the typical responses, the upper contact being high. The polarities recorded therefore are those of the primary leaf with respect to the base. (In most of the graphs so far shown the reverse has been the case). The bracketed figures are the abbreviations which will be used in the work when referring to the various parts of the results.

The light Response

This consists of an increase in negativity commencing about 15 seconds after the increase in light intensity and reaching a minimum between 30 seconds and two minutes (1st -). This is followed by an increase in positivity to a maximum at 3 to ten minutes (1st +) and then another minimum (2nd -). Any subsequent oscillations are small and rapidly damped.

The Dark Response

There is again a lag time of approximately fifteen seconds followed by an increase in positivity to a peak between 30 seconds and 2 minutes (1st +). This is followed by a minimum (1st -) at 3 to 10 minutes.

This/

This is followed by a 2nd positive peak (2nd +) and then another minimum (2nd -). These oscillations are more marked than on increase in light intensity but after the 2nd - they are rapidly damped.

The changes upon increase in intensity are seen to be opposite to those on decrease and with a decrease in time between intensity changes, the responses overlap and cancel out, therefore it is believed that the same system is involved in both responses.

They are not "All or None" responses but increase with increase in the intensity change.

The final potential of tissue in the high intensity light is positive with respect to the same tissue in the lower.

There is a lag time of about 15 seconds subsequent to the intensity change, before potential changes are detectable and the potential takes about 30 minutes to stabilise.

No fatigue is detectable under constant experimental conditions, when the intensity changes are separated by 30 minute intervals; this allows scope for experiments on changes in the treatment applied to the same plant. Below are listed the various papers that were located subsequent to the above work.

Haake (1892) appears to have been the first to observe alterations in the electric potentials in plants as a result of illumination. He found effects both on commencement and cessation of illumination but only in chlorophyll containing tissues. He associated these changes with carbon dioxide assimilation but also suggested that water movements might be involved, though he thought

their/

their influence would be slight. He tried altering the external concentration of carbon dioxide but obtained no positive results.

Klein (1898) published results showing fluctuations of potentials caused by illuminating and darkening leaves of various plants. However he was concerned to establish a relationship between the polarity existing between the electrodes on first making contact and that induced by light.

A.D. Waller in several papers around 1900, recorded light and dark effects in several plant species but whereas the two above workers had illuminated the whole plant, he illuminated at the position of one electrode only. He found differences between individual plants of the same species, (but as these were detached leaves from garden or pot plants this is not surprising), and between different species. He found with a slight increase in CO_2 there was an increase in response, whilst with pure CO_2 the response disappeared. There was no response in the absence of CO_2. The effects were found in chlorophyll containing tissues only. There was a latent period of 3 to 10 seconds before the response occurred.

Bose (1907) considered the effects arose from the natural "excitability" of plant tissue due to the stimulus of light. He noted an effect in an etiolated celery petiole containing no chloryphyll.

Waller, J.C. (1925-29) found the effects were restricted to green tissues except for a confirmation of the response in celery petioles. He also confirmed Waller A.D.'s observations of CO_2 effects and roughly established that the action spectrum was similar to that of photosynthesis. In the later paper, he suggested a redox origin of the potential changes. He noted that the magnitude

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of the response altered with previous growth conditions and that the form of the response varied from species to species. He also recorded effects with atmospheres of nitrogen and hydrogen, and suggested that the results were due to the lack of oxygen, but as there was no addition of carbon dioxide to the gases these conclusions are not valid. This point is emphasised by the use of an entirely different plant species in the carbon dioxide experiments. He found with increase in the intensity change an increase in response.

Sheard & Johnson (1930) found that with Sunflower and Poinsettias leaves and stems there were rapid and large changes in potential (300 millivolts) between the tips and bases on illumination with infra-red light. Visible light at the violet end of the spectrum and ultra-violet produced similar changes but of smaller magnitudes (5 to 100 millivolts). They also found that if ozone and oxides of nitrogen produced by passing air through a quartz mercury arc were passed into a container enclosing the leaf there was a similar response. Therefore they suggested that the effects of the I.R. and U.V. light were to ionise the air. With Sunflower, they found upon changes in illumination, a close correlation between the P.D. developed and temperature changes produced by the illumination. They maintained the temperature constant and then illuminated with U.V. free light and found potential effects which they ascribed to Infra-red light. They also found that illumination with U.V. and with I.R. decreased tissue resistance, whereas visible light had little effect.

Glass (1933) published details of the effects of light on the electrical potentials in Elodea leaves. He found that when a light was shone on the leaf, there was a period of about one minute in which there was either no change or sometimes a negative peak. This was followed by an increase in positivity of

the/

the illuminated tissue. Upon turning off the light, there was a sharp immediate fall in potential, followed by a slowing down of the fall, or even a small rise, followed by a further fall to the original dark potential. Onion roots gave no response.

Marsh in a series of papers between 1935 and 1939 investigated the effects of light upon the electrical potentials in Valonia. He found that the action spectrum for the response resembled that of photosynthesis and also that the potential change/light intensity curve was similar to that of rate of photosynthesis/light intensity. He noted that CO_2 concentrations affected the results, in that light could restore the potential when it had been depressed by increasing the CO_2 concentrations. The changes he recorded consisted of a drop in the P.D. across the protoplasm on transfer to a lower light intensity, and a restoration in higher intensitits. The P.D/Time graph for this change shows several characteristic spikes which Marsh does not explain.

Brauner L and M. Brauner (1937), working on Helodea densa, attributed the potential changes produced by light to alterations in the ionic permeabilities of the leaf surface. They based this hypothesis on the results of experiments involving different concentrations of contact media.

Blinks and Skow (1938) used a glass electrode to show that upon illumination the first pH response of the protoplasm of green tissues consisted of an increased acidity, followed later by a decreased. There was an "alkaki gush" upon cessation of illumination. He suggested that these "gushes" were responsible for the original rapid potential changes, opposite in polarity to the final responses, upon changes in illumination intensity.

Brown (1938) found that the potential response action spectrum for Chara

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resembled that of photosynthesis, and he also found effects of CO_2 and O_2 concentrations upon the response. His graphs resembled thoese of Glass. He found, with alternating 10 minute periods of light and dark, no signs of fatigue.

Blinks (1939) also noted effetcs of light upon the potentials of Halicystis and Valonia. He related these to pH changes brought about by CO_2 consumption. He found no effects of Ultr-viloet and Infra-red. and the action spectrum resembled that for photosynthesis. His changes consisted of a rapid negative peak followed by a slower increase in the positive potential of the outside of the cell with respect to the vacuole, upon illumination. He found effects with both CO_2 and O_2 concentration changes and claimed that acetic acid had a similar effect as high CO_2 concentration upon the potential, implying a pH effect. He also stated that ammonia altered the light response. From results with various KCl contact media concentration, he concluded that the ultimate effect of light was an alteration of the surface properties of the cells.

Nishizaki (1958 -) in a series of papers has investigated the light and dark potential responses of Phaseolus vulgaries leaves. He has shown there is a similarity between the action spectra of the potential changes and photosynthesis and he has investigated the intensity/time relationships of the response. He has also investigated the influence of alterations in the KCl concentration of the contact media on the magnitude of the potential response.

He concludes that the first changes may be attributed to changed permeabilities of the cell membranes to ions but later changes may not and are perhaps attributable to ion or water movements.

Labrique, (1960) found an on/off light effect upon potentials in Mimosa pudica.

Walker (1962) reported the accidental discovery of a light/potential effect in Chara cells. Light increased the magnitude of the potential difference measured across the plasma lemma and greatly reduced its resistance. He found that for this effect to be shown, it was essential to have calcium and bicarbonate ions in the bathing medium, and that it was dependent upon photosynthesis. He ascribed the decreased resistance to increased permeability to the bicarbonate ions. He stated that, "This appears to be the first demonstration of a large effect of light on membrane resistance".

Comparing the above abstracted results with our own, we find that both Waller A.D. and J.C. had similar responses to ours with some plants (Geranium), but with others (Iris and Cabbage), the first negative peak on turning on the light was missing.

Brown and Glass both found a rapid original change in potential upon change in light intensity but there were no subsequent oscillations. Marsh's description of a curve with several spikes resulting from changes in illumination intensity suggest a resemblance to our own, and Blinks found a rapid change followed by a slower of the opposite polarity which is also similar. In all these cases the polarity changes reported agree with those recorded by ourselves.

Nishizaki, whilst producing a very similar curve to our own, notes opposite polarity changes throughout; we hope to clarify this discrepancy by correspondence.

There are two possible explanations for the potential changes :-

(A) Changes in the ionic permeabilities of cell membranes.

(B) Changes in internal ion concentrations due to ion or water movements.

As pointed out before (Chapter 4) water movements are unlikely to contribute significantly to the potential, and changes in ion movements will probably in any case involve changes in membrane permeabilities. There is one paper which does suggest that there might be a case for a link between photosynthesis and water movement. Glinka and Reinhild (1962) found that high CO_2 reduces the permeability of the plant tissue to water and high O_2 increases the permeability by 40%. This effect was not due to pH changes and the response was rapid. The relatively high CO_2 condition might correspond with the state of tissue in the dark and the high O_2 with the illuminated state, in experiments in which potential changes were observed.

A simple way to discriminate between systems A and B is to compare the

effects/

effects with different concentrations of contact medium.

The apparatus and method used was as described before but various concentrations of KCl solution were used to make contact upon the primary leaf, though the osmotic concentration was adjusted, using mannitol, to be always the equivalent of 0.1N KCl. The basal contact and growth medium were Shive's solution. The potential recorded is that of the primary leaf with respect to the base.

The experimental procedure was :-

(1) Potentials were allowed to stabilise in light.

(2) The light was turned off for 20 minutes.

(3) The light was turned on and the potential allowed to stabilise again.

(4) The contact medium was changed and the potential allowed to stabilise again, when the above was repeated.

Figure 5.6. shows a graph plotting the magnitudes of the points of inflexion of the response curves against the KCl concentration. The results all give reasonable straight lines. The equation of such a line is :-

$E = a \log C + b$ where E is the measured potential
C is the concentration of the apical contact medium and
b is a constant.

If the phenomenon observed arises predominantly from a membrane potential, then "a" contains terms dependant on the ion permeability of the tissues

and/

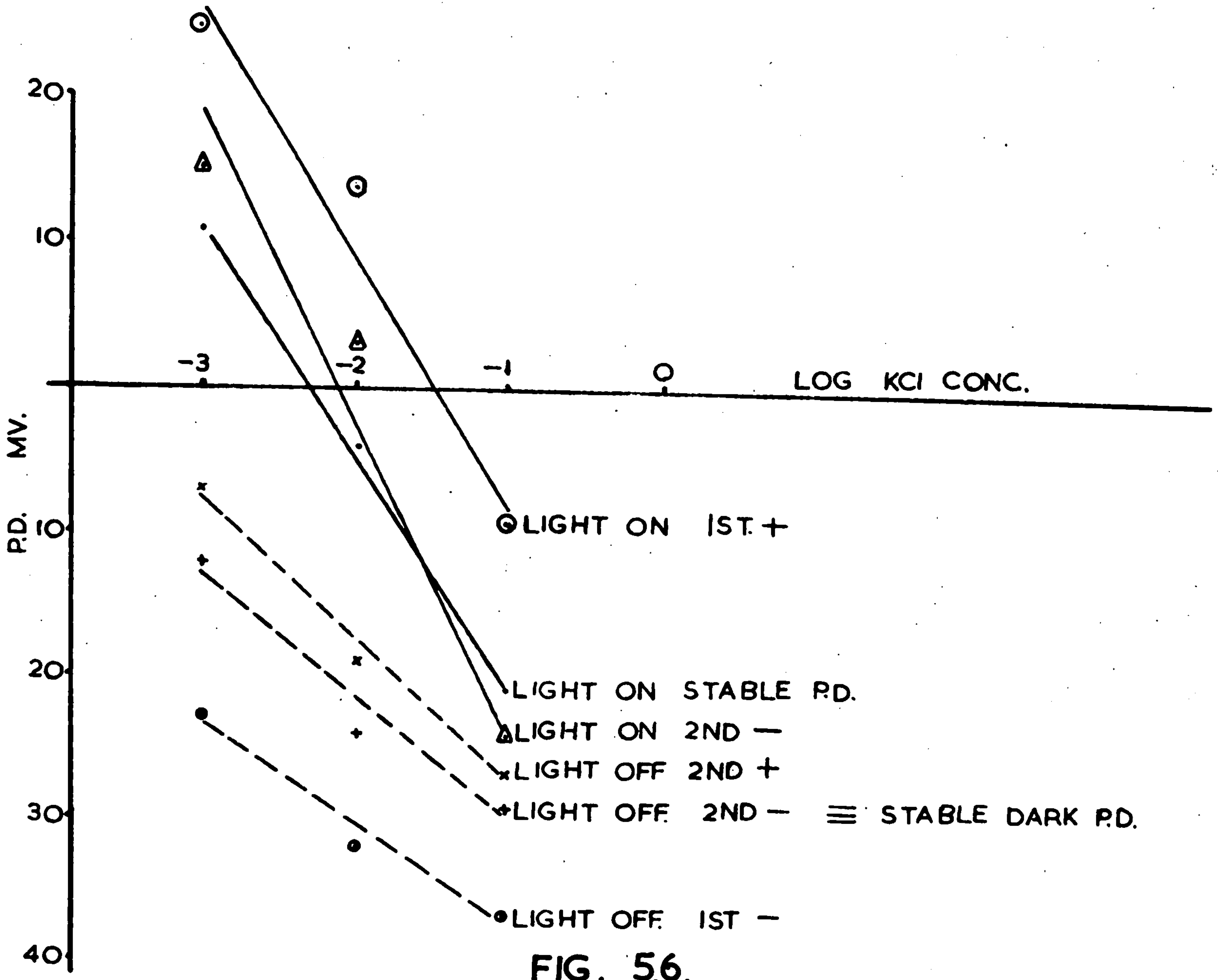

FIG. 5.6.

and "b" contains terms dependent upon the potentials existing within the tissue, as well as upon the ionic concentrations of the tissues.

Assuming the potentials are membrane potentials, then calculations of "a" and "b" for the differing peaks and troughs of the light responses will give an indication of whether changes in permeabilities or ion concentrations are involved.

The equations of the lines have been calculated using the method of least squares.

The equations are :-

Stable P.D. in light	$E = -16 \log_{10} KCl - 36.6$
Light off 1st -	$E = -7 \log_{10} KCl - 44.6$
" " 2nd +	$E = -10 \log_{10} KCl - 37.6$
" " 2nd -	$E = -8.5 \log_{10} KCl - 38.6$

The 2nd - occurs about 20 minutes after the light is turned off and corresponds closely with the time the light is turned on again. Therefore this equation can be considered to approximate to the stable potential in the dark.

Light on 1st + $E = -17 \log KCl - 24$

2nd - $E = -19.5 \log KCl - 41$

When the light was turned off, there was no marked 1st +, though in two there was a small peak of less than 0.5 millivolts and in the other, the negative change did not occur immediately. Similarly when the light was turned on there was no 1st - peak but there was a lag of about one minute before the 1st + began.

Since these results are based on one experiment only we must not

place/

place too much reliance on them. However they clearly demonstrate the greater relative permeability to cations particularly K^+ in the light than in the dark. (As stated before in Chapter 4, where increase in cation permeability is suggested, it could just as easily be decrease in anion, etc.). There is also an alteration in the potential contribution of "b", which since the internal ion permeabilities should also alter is what one would expect.

With the "light off" effect, oscillations in the permeability bring about oscillations in the potential and they are also reflected in corresponding oscillations in "b".

With the "light on" effect the 1st + peak is explicable from the permeability change but the 2nd - peak is due to a change in (b) independant of (a). This was also noted indirectly by Nishizaki who states that this peak is independant of the contact medium concentration. It may be due to a change in internal ion concentration.

We are left with the first changes in potential not present in these graphs to consider. In another experiment these were present at 0.025N KCl concentration but not at 0.5N and in yet another, not in 0.025N KCl but in deionised water. It seems therefore that with increase in contact medium concentration these also decrease in magnitude. (Nishizaki's graphs show a similar result). These peaks can be interpreted as a momentary increase in cation permeability on darkening and a decrease on illumination.

Having assumed that these changes are largely brought about by changes

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in the membrane permeability, we must now consider what factors may influence the membrane permeability. Early in the work on the effects of light, we were struck by the similarities between the potential changes and stomatal opening.

e.g. (1) Both are dependant on photosynthesis.

(2) Neither is an "all or none" response.

(3) Stomata have been reported to open in some cases within one minute.

The accepted hypothesis for stomatal opening is :-

(a) Light $\rightarrow$ photosynthesis $\rightarrow$ CO_2 utilisation

(b) pH shift as $H_2CO_3 \rightarrow H_2O + CO_2$

(c) Shift in starch/sugar balance or imbibitional changes due to the pH shift, leading to greater osmotic pressures, water uptake and stomatal opening.

It has been shown that by exposure to ammonia vapour, stomates can be induced to open, and by exposure to acetic acid, to close. It was therefore decided to try the effects of applying acetic acid and ammonia in the contact medium.

It was first necessary to determine if this would produce an electrode effect and an experiment using a water soaked string to represent the plant, was tried. When the contact medium was changed by means of a two way tap, from 0.1M.K_2HPO_4, solution to a similar solution containing 0.15N ammonia, there was only a small change in the potential (less than 2 MV).

Mixtures of potassium di-hydrogen phosphate and potassium hydrogen phosphate with acetic acid or ammonia, or unsupplemented, were chosen as contact media, as these allowed large pH changes with little change in the other ion concentrations. In all experiments where mixtures of KH_2PO_4 and K_2HPO_4 alone were employed, contact media changes from one pH to another never produced changes resembling light effects, though of course there were changes brought about by the differing ion concentrations.

When ammonia was added to the contact medium, effects very similar to a "light on" response were produced. These consisted of a negative peak followed by a positivation of the potential of the tissue to which it was applied, with respect to the base.

These results are shown in Figure 5.7. along with the "light on" effects upon the same plants. When the ammonia was removed there was no effect. Only in one case, when the ammonia was used in a rather high concentration (0.15N), the effects could not be repeated after the original response. Also in this case a subsequent light effect could not be produced so it looks as though permanent injury had been caused. However at this high concentration the initial result was particularly like that produced by light (Figure 5.8). In the graphs of the results the apical contact was low.

Below is given a table comparing the light and ammonia effects in the different experiments. Since it is difficult to discover exactly when the ammonia reaches the plant surface due to the changing tap being at some distance from the actual contact, the time of the first visible change/

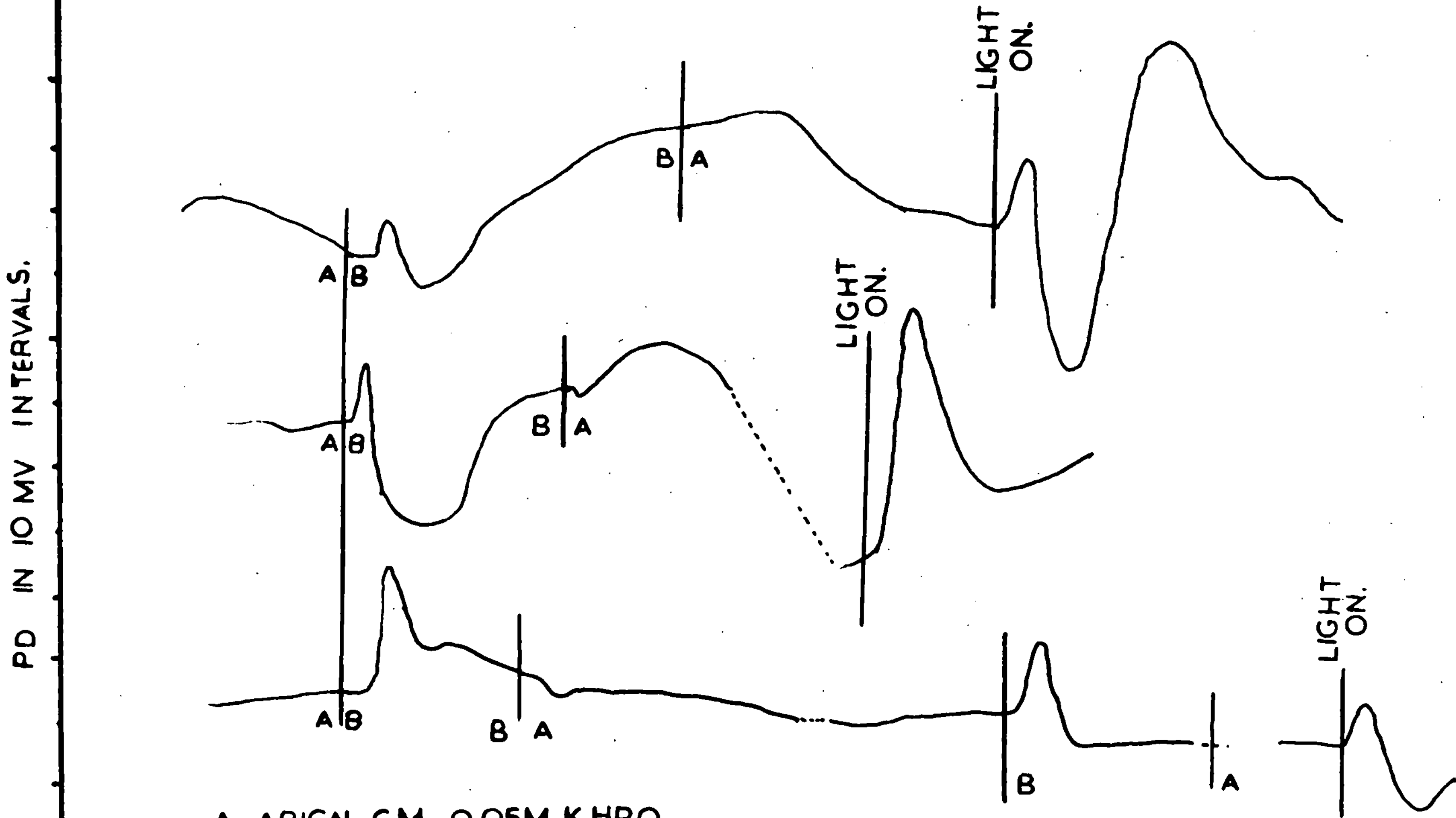


FIG. 5.7.

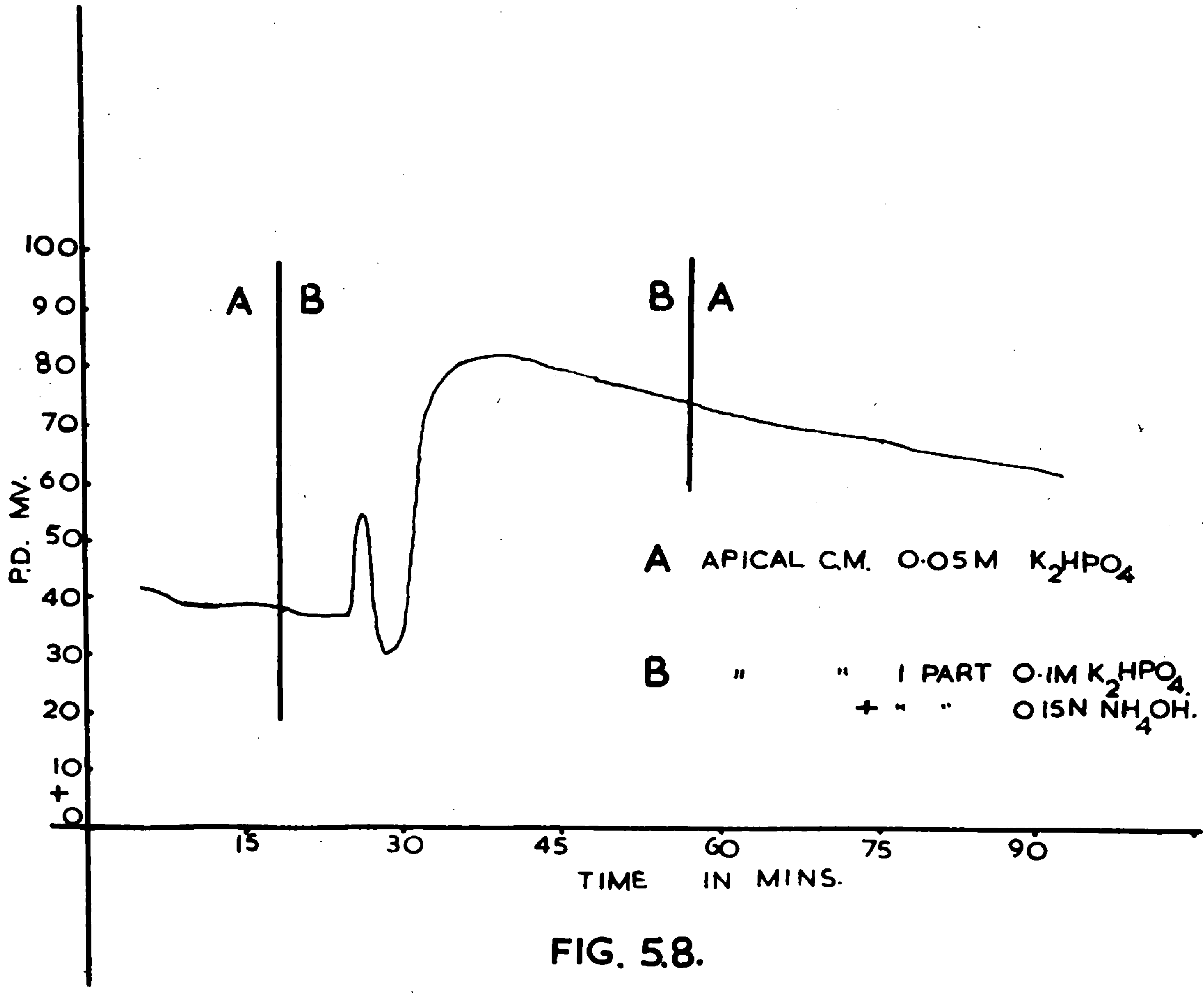


FIG. 5.8.

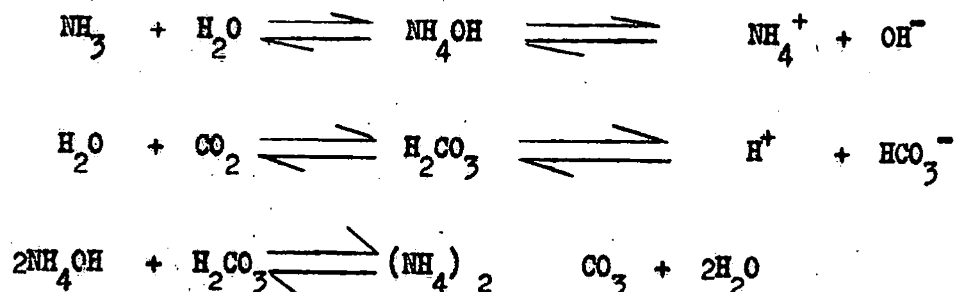
change is taken as zero. With light, the instant when this is turned on is taken as zero. The magnitudes of the potentials recorded represent for the 1st negative , the potential difference between the stable potential and the 1st trough, and for the 1st positive, the potential difference between the first trough and first peak.

TABLE 5.1.

Light ON				Ammonia applied			
Time 1st- mins.	Mag.1st- mv.	Time 1st+ mins.	Mag.1st + mv.	Time 1st- mins.	Mag.1st- mv.	Time 1st+ mins.	Mag.1st+ mv.
2.0	40.0	10.0	30.0	0.5	10.0	6.0	26.0
—	—	5.5	9.0	1.5	19.0	4.0	25.0
2.0				1.5	22.0	4.5	16.0
				1.5	12.0	6.0	17.0
After NH_3 removed							
1.5	7.0	6.0	18.0				
1.0	2.0	4.5	21.0	1.5	10.0	4.0	5.0
				1.5	5.0	6.0	5.0
After NH_3 removed							
1.5	2.0	6.0	17.0				
1.0	0.5	4.0	8.0	0.5	0.25	3.5	11.0
				1.0	1.0	5.0	8.0
After NH_3 removed							
1.0	1.0	6.0	21.0				

There is seen to be a quite good agreement between the two and it therefore looks as though the ammonia can to some extent reproduce the changes induced by light. With ammonia, however, there is rarely a 2nd - which supports the previous suggestion that this is due to changes in internal tissue potentials which the surface application of ammonia may not induce, but light does.

In one experiment with ammonia, the contact medium had been left exposed to the atmosphere for several days and when this was used, there were no responses, even though the same seedling gave typical light responses. When the pH of the contact media was measured it was down to 8.5 compared with the usual 10+ due to the absorption of CO_2 . When this medium was replaced by fresh, typical responses were obtained.



$$NH_3 + H_2O \rightleftharpoons NH_4OH \rightleftharpoons NH_4^+ + OH^-$$

$$H_2O + CO_2 \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

$$2NH_4OH + H_2CO_3 \rightleftharpoons (NH_4)_2 CO_3 + 2H_2O$$

The concentration of ammonium ions would be approximately the same in both the fresh and stale solutions (some will have been lost as ammonia gas at the surface, but at these concentrations, this will only be small). Therefore, the response must be induced by the hydroxyl ions, unless bicarbonate ions can inhibit the response. Such an inhibition seems unlikely for later it will be shown that a contact medium containing a high concentration of carbon dioxide has little effect on the response magnitude.

If a high external hydroxyl ion concentration (possibly in combination with ammonium ions) is required to produce a sufficient shift in the internal pH for a response, this would explain why no other pH changes brought about the response.

Experiments were now commenced with acetic acid in the contact medium, giving a PH of 2.5, in the hope that a dark response would be induced upon a continuously illuminated plant. There were no resemblances to dark responses. One wondered then if the dark response was a result more of carbon dioxide built up than of decrease in pH. It was therefore decided to try the change from a normal contact medium to one made up with an aqueous carbon dioxide solution. If carbon dioxide build up was involved, this might produce a dark response on a continuously white light illuminated seedling.

0.0005N KCl, with and without carbon dioxide, was used as the contact medium. When the carbon dioxide medium was applied, there was a large and rapid fall in the potential of the primary leaf with respect to the base (45mv) reaching a minimum at 15 minutes. This was followed by a slow recovery to a potential of +25 mv compared with the original +50 mv. When the carbon dioxide medium was removed there was a small negative trough followed by a rapid rise in potential to +40 mv in 5 minutes. This resembled a "light on" effect.

The result agreed with Blinks who found that Carbon dioxide at increased concentration, decreased the positive potential. If carbon dioxide had been limiting in photosynthesis, it was possible that any build up was too small to produce a typical response. We would then

have to assume that upon the removal of the carbon dioxide medium the internal concentration drop was sufficient to produce a response; also in this range of intensities, increase in intensity gives an increase in response, which if carbon dioxide is limiting should not be the case.

Experiments were also tried on the "light on & off" effects with and without carbon dioxide enrichment of the contact medium which gave the following results.

Before CO_2 Application							
Light Off				Light On			
1st+	1st-	2nd+	2nd-	1st-	1st+	2nd-	2nd+
1mv	13mv	6mv	9mv	1mv	14mv	19mv	7mv
With CO_2 Present							
1st+	1st-	2nd+	2nd-	1st-	1st+	2nd-	2nd+
0mv	12mv	4mv	8mv	0	20mv	21mv	13mv
After CO_2 Removed							
1st+	1st-	2nd+	2nd-	1st-	1st+	2nd-	2nd+
0	12 mv	7 mv	-	0	18mv	-	

The peak magnitudes were calculated as recorded before.

The carbon dioxide seems to have little influence on the light effects, if anything, intensifying the "light on" response. This does not agree with Blinks who found that high carbon dioxide concentrations reduced the response. However upon the evidence of our one result it is impossible to draw any true comparisons.

A similar experiment was carried out but with ammonia present in the contact medium. Again there was no obvious influence. This again disagrees with Blinks who found that ammonium salts reversed the polarity of his light response.

There are many unexplained features of the results and obviously much more work would be necessary to produce a sensible explanation, but as a tentative hypothesis, it is suggested that the "light on & off" effects are due to internal pH changes brought about by changes in photosynthesis. External pH changes only influence the internal pH when there is high external concentration of hydroxyl ions as the membranes are relatively impermeable to H ions but permeable to OH ions.

Summary of results so far reported

(1) Application of ammonia to oat seedlings can bring about changes in potentials similar to those produced by light.

(2) The higher the ammonia concentration, the greater the response in a similar manner to increased light intensity.

(3) Even though treatment with ammonia involves only a simple increase in the external pH, a first negative and first positive change are detectable; it is reasoned that a similar simple pH change brought about by light could induce similar effects.

(4) With ammonia, a 2nd negative change is not visible, but since previous results have indicated that this change is a function of potential changes within the tissue, one could expect this for the applied ammonia cannot be having much more than a surface effect.

(5) Neither removal of ammonia nor application of low pH media would induce a typical "Dark-response", nor did application of carbon dioxide saturated contact medium.

(6) It is suggested that light brings about photosynthesis with utilisation of carbon dioxide leading to an increase in pH which affects the cell membranes and produces the potential changes. In the dark the reverse process takes place. This also applies with changes in light intensity which will,influence the photosynthetic rate.

There is a large body of literature upon the influence of light on ion absorption and tissue permeabilities to ions. The following details are taken from Electolytes & Plant Cells by Briggs, Hope & Robertson (1962).

Hoagland Hibbard & Davies (1927), showed that in Nitella there was a greater accumulation of Cl^- & B_r^- in light than in the dark. Jacques & Osterhout (1934), found light increased the absopption of K^+ by Valonia macrophysa, and Ingold (1936), showed that the same applied for Elodea canadensis. The latter also found the external pH was greater in the dark than in the light.

Arisz (1947), showed that for Vallisneria spiralis, the uptake of chloride was increased in the light. MacRobbie & Dainty (1958), investigated the effects of light on Na^+ & K^+ flures in Rhodymenia palmata and found that those of Na^+ were little affected, but there was a marked increase in the efflux of K^+ and probably in its influx.

All evidence shows that light increases ion movements across the membranes and therefore for the purpose of our investigation increases the membrane permeability. This applies to both anions and cations but from our results this increase must be greater for cations than anions.

Another interesting observation recorded by Curtis & Clark (1950), is that alkalis increase permeability whilst acids decrease, which would agree with our interpretation.

There is a similarity between the "light on" response and the potential changes resulting from the application of contacts to Oat coleoptiles. It seemed possible that the latter response was due to the pH of the applied contact medium. However, over a pH range of 2-10 the response was similar in form so it can have nothing to do with pH.

Another experiment tried was the effects of ammonia on oat coleoptiles. The result is shown in graph in Figure 5.9. Ammonia induced a small negative change followed by a large positive potential, similar to the light effect and also the ammonia effect on primary leaves. The property of membranes to respond in a characteristic manner to ammonia is not the prerogative of photosynthetic tissue, but is present in others.

An odd feature of the results of the bio-electric responses of oat seedlings to illumination was that in no other type of contact medium, at any concentration, had the 1st- upon commencement of illumination, approached in magnitude that originally found with Shive's. It was felt that this possibly indicated an interaction of ions on the membranes.

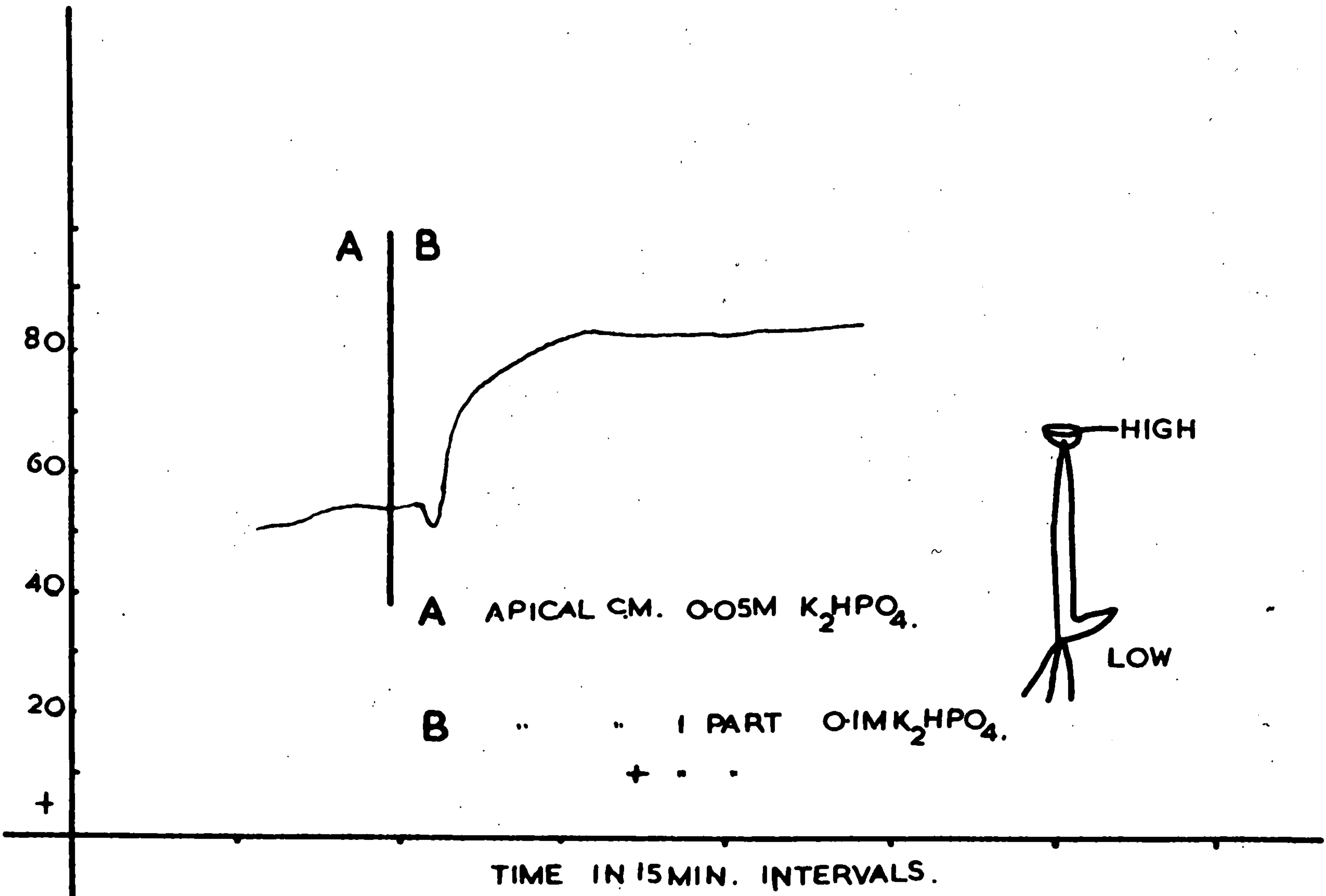


FIG.5.9.

To test this suggestion the following experiment was carried out. Shive's solutions containing low concentrations of calcium magnesium, or potassium salts were, in turn, used as contact media on the same oat seedling. With each contact medium the potential was allowed to stabilise in the dark, then the light was turned on, left for 30 minutes, then turned off. The potential was then allowed to stabilise again, then the contact medium was changed etc. The basal contact and growth medium was normal Shive's solution.throughout. This contact was below. The results are shown in Figure 5.10. These are for the "light on" response only as the "light off" was atypical.

There is too much variation in the normal Shive's results at the beginning and end of the experiments to draw any conclusions other than with regard to the 1st- peak. With KCl alone as contact medium, decrease in concentration increases the peak magnitude, but apparently in the presence of $Ca(NO_3)_2$ and/or $MgSO_4$, there is a decrease. Also the peaks are amazingly large with low $Ca(NO_3)_2$ & $MgSO_4$ solutions. It is believed that these results indicate an interaction of ions influencing the membrane properties. It would seem that the light response would be a useful tool for the investigation of the interaction of ions on the plasma-membranes.

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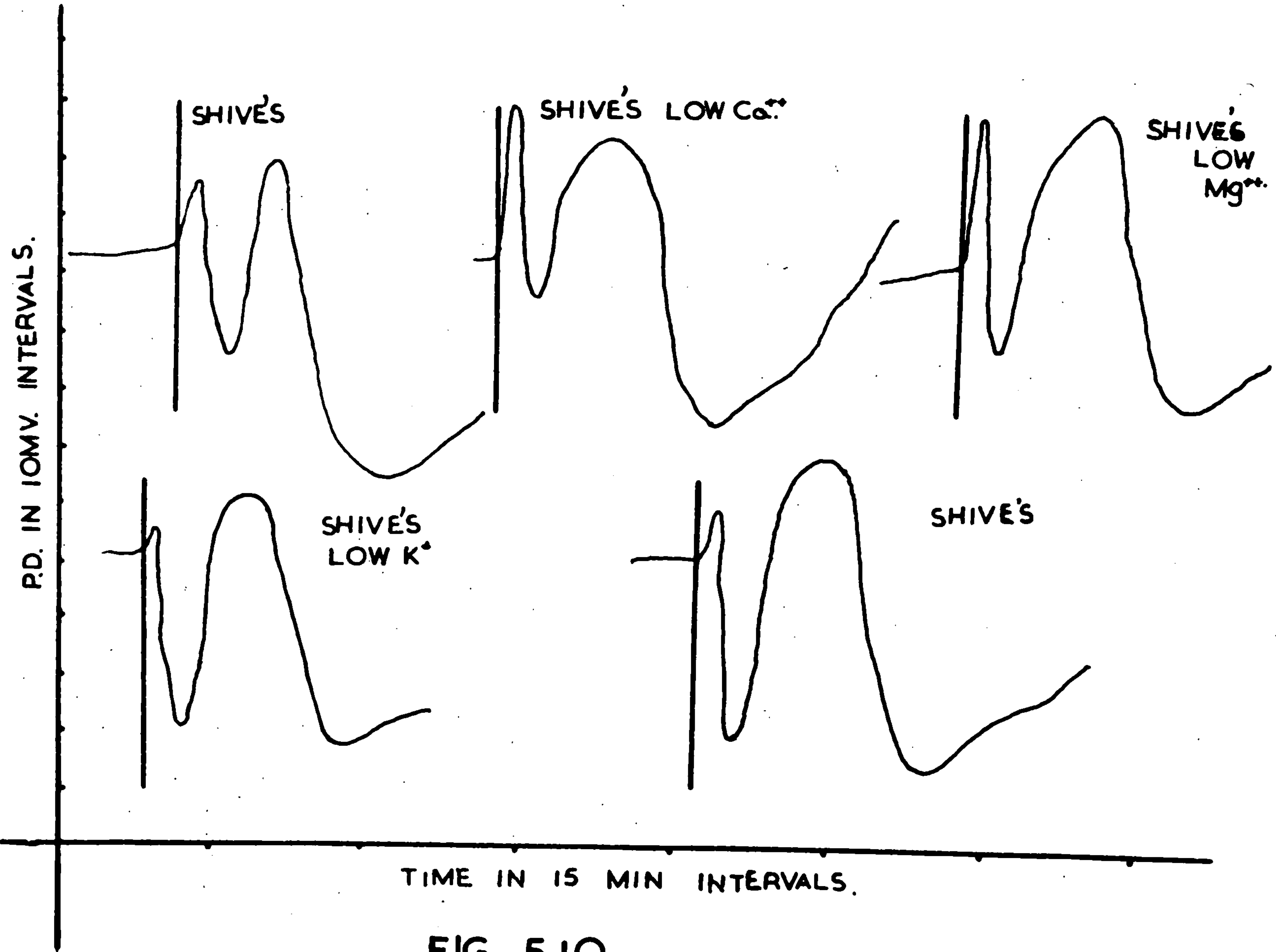

FIG. 5.10.

CONCLUSIONS

(1) Changes in light intensity cause characteristic changes in the electrical potentials of green tissues whose magnitudes over a limited range at least are proportional to the intensity changes. It is not an "all or none" response.

(2) From observations of the influence of changes in KCl contact medium concentrations, the potential changes are interpreted as due to alterations of membrane permeabilities with a greater cation permeability (particularly K^+) in light than in dark.

(3) Since ammonia can to some extent reproduce the effects of light, it is suggested that the light response is mediated through pH changes brought about by photosynthesis.

(4) Evidence is presented that shows the composition of the contact medium can influence the membrane permeability changes.

CHAPTER 5.

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CHAPTER.6.

CHAPTER 6

THE EFFECT OF AUXIN ON THE BIOELECTRIC POTENTIALS OF OAT COLEOPTILES

SUMMARY:

Details of previous work in this field are given. Preliminary experiments on the application of IAA/agar blocks to the apices of double decapitated coleoptiles are described. These are followed by details of more comprehensive experiments in which the IAA was applied in solution, using a flowing drop ring contact. The results indicate that a longitudinal transport system moving at a rate of 12 millimetres per hour exists in coleoptiles. Application of Shive's solution or IAA leads to the transmission of a stimulus at this rate, back along the coleoptile, which causes the potential of tissue it is passing, to become more positive with respect to other tissue. The changes are ascribed to changes in membrane permeability induced by changes in the IAA content or ion balance of the tissues.

An original observation on a surface property of auxin is described.

Growth of higher plants is dependent upon the presence of small concentrations of certain chemicals. These are known as auxins. The most common of these substances is indolyl acetic acid (IAA). In the oat coleoptile this substance is manufactured in the apical cells and then transported back to the growing region which is located a few millimetres below the extreme tip. If the apical cells are removed (First decapitation), the source of IAA is removed and growth ceases. However, a new physiological tip develops, after about 2½ to 3 hours and growth then recommences.

If 3 hours after first decapitation the apical 1 millimetre of coleoptile tissue is removed (second decapitation), there is then no further development of a new tip and the coleoptile is left depleted of its IAA. This process is known as double decapitation. If IAA is now applied to the cut tip of the coleoptile, growth is resumed. Since there is known to be an intimate connection between IAA and many plant processes one could perhaps expect a relationship between IAA and plant electrical potentials.

Newman (1959 & 1960) reported that it was possible to detect transport of indolyl acetic acid (IAA) down an Avena coleoptile by changes in potential. Under constant conditions he found that the potential, between electrodes placed longitudinally 2 millimetres apart on the surface of an intact coleoptile, underwent regular oscillations of 15 minute periodicity and a few millivolts amplitude. If the coleoptile was "double decapitated", these oscillations disappeared and if half an hour after the second decapitation an IAA/agar block was placed on the tip, the primary leaf having been removed, a series of changes in the potential occurred between the electrodes on the plant. These consisted of a positivation of the upper contact, followed by damped oscillations. The wave of potential changes passed down the coleoptile at a rate of 14 millimetres per hour, which approximates to that recorded for auxin transport. He also found that upon illumination of the apical 2 millimetres of the coleoptile with white light, a similar wave followed.

This seemed to us to be a very interesting and important result, since it suggested that a tissue of higher auxin content will be positive

in electrical potential with respect to a tissue of lower auxin content. This is the result found in studies of geo- and photo-induced potentials. It seemsd possible that geo- and photo-electric potentials could thus be due to differences in auxin concentration rather than vice versa. It was decided to attempt to confirm Newman's observations. There was one weakness, it was felt, in the use of IAA/agar blocks, and that was that the concentration of IAA is continually decreasing, due to the diffusion out. It was thought that this might explain the damped oscillations, which, if the concentration could be maintained, might become stabilised. Also there is always the problem of getting close contact between the block and the plant tissues.

The flowing drop ring contact was admirably suited to the application of a drop of liquid at a constant concentration, and was used in this work.

The potential was measured between a pair of flowing drop contacts situated a few millimetres apart along the length of the coleoptile, and the apparatus was similar to that shown in Figure 2.4 The contact and growth medium used throughout was Shive's solution; (Newman used 0.01. N.KCl). The first series of experiments investigated whether oscillations in potential do disappear after decapitation.

Figures 6. 1. A.B.& C. show graphs recording the potential measured between two flowing drop contacts placed on the coleoptile before decapitation, after first decapitation and after second decapitation three hours later. The upper of the two contacts was high and Shive's solution was used as growth and contact media. Where oscillations exist before

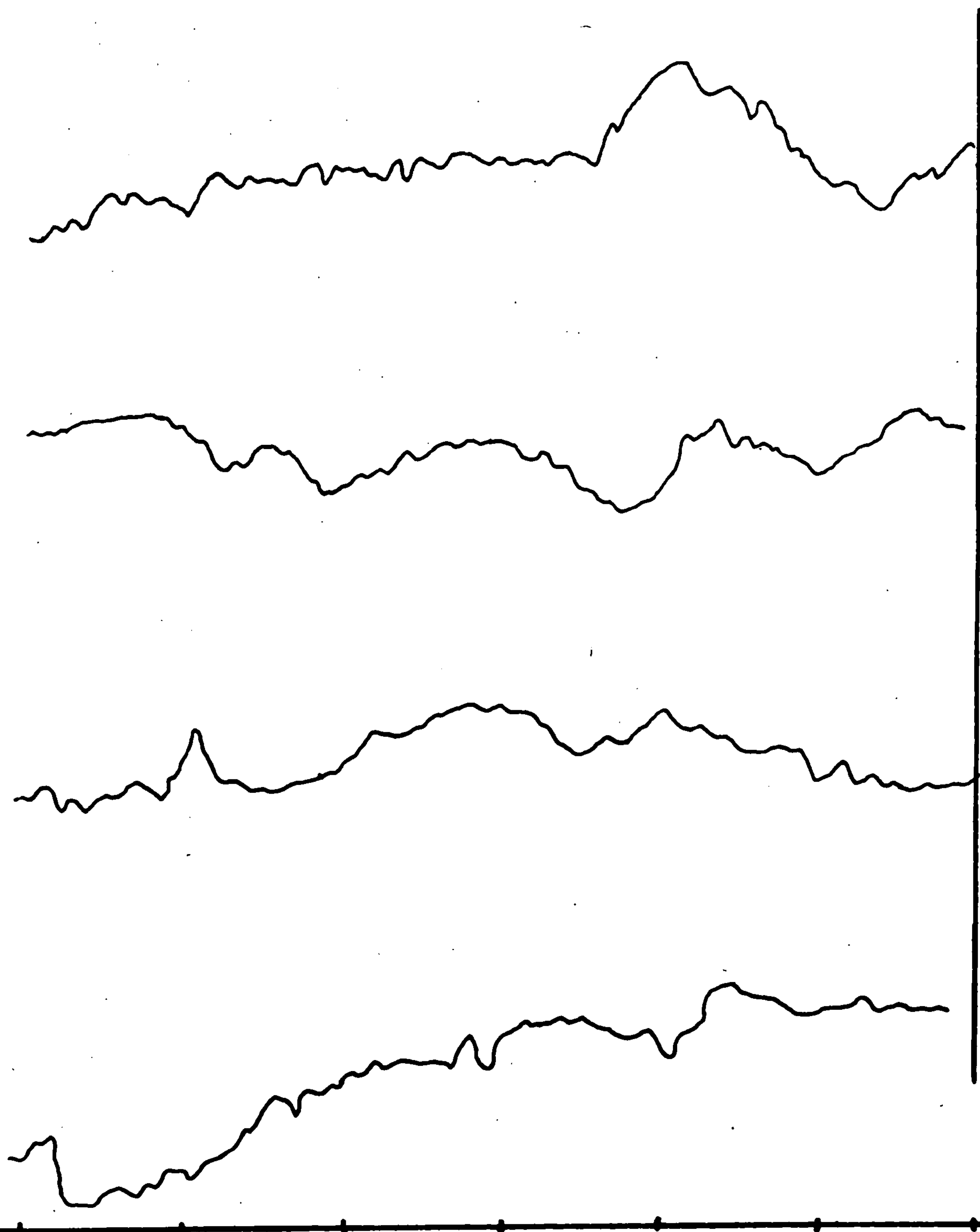


FIG. 61. A.

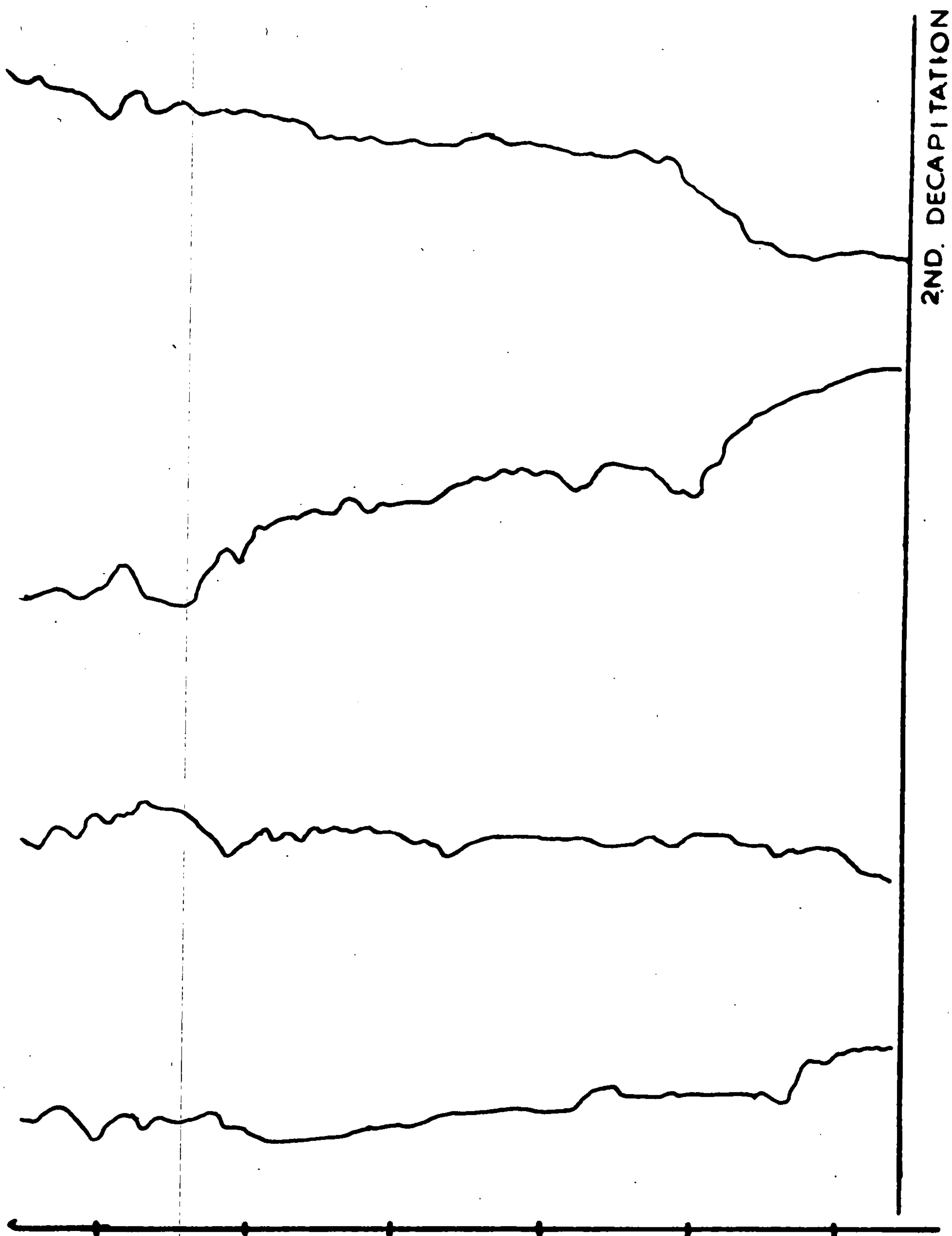


FIG. 6.1.B.

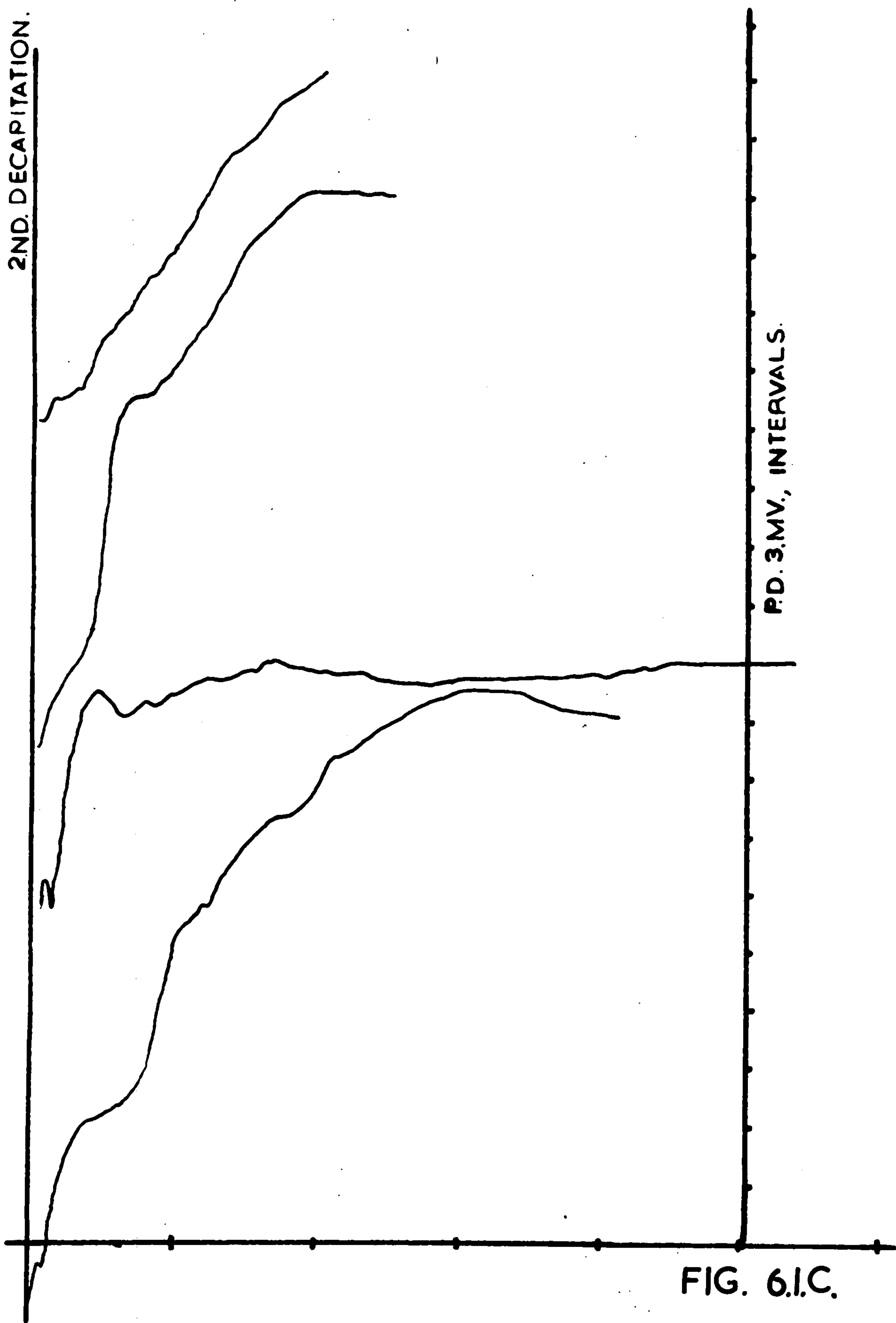


FIG. 6.I.C.

decapitation they may disappear subsequent to the first decapitation and in no case were oscillations present after second decapitation. However decapitation causes large changes in the potential and it takes about 30 minutes for these to subside after second decapitation. There is no correlation between the distance of the contacts from the apex and the disappearance of oscillations after first decapitation, which we thought might have been the case, if the disappearance of oscillations was correlated with removal of auxin by decapitation. However, the differences observed between the coleoptiles may be connected with differences in tissue auxin content.

The oscillations are not of sine-wave form but consist of rapid positivation to a plateau maintained for about 15 minutes, then a rapid decrease, followed almost immediately by a further increase. The periodicity is 15 to 25 minutes and the amplitude 2 to 3 millivolts.

Even when such oscillations were not present, the potential was never really stable. This is a result of using the small contact area, flowing drop contacts, where the potential is never as stable as with ring contacts. Ring contacts form a lower resistance contact and also average the potential over a large area, giving a more stable value. Figure 11.1. shows one result of the measurement of the potential between a flowing drop ring contact on the coleoptile and the growth medium, in a "double decapitated" coleoptile, and reveals the remarkable constancy of potentials. Also, an experiment with ring contacts on an intact coleoptile, showing the sinusoidal wave form typical of later work, is seen in Figure 7.4.B.

One other interesting result was found with a coleoptile in which the primary leaf emerged during the experiment; in this the coleoptile had stopped growing and without any decapitation the potential showed stability. Since the coleoptile was only 19 millimetres tall when the leaf emerged, the most likely reason was a cessation of auxin production.

The above results are consistent with Newman's observations. The implications seem to be as follows:-

Decapitation, with consequent auxin depletion of the tissue, eliminates oscillations.

Where the oscillations are observed, they might arise from any of four circumstances:-

A. Fluctuations in rate of transport of auxin to the tissues.

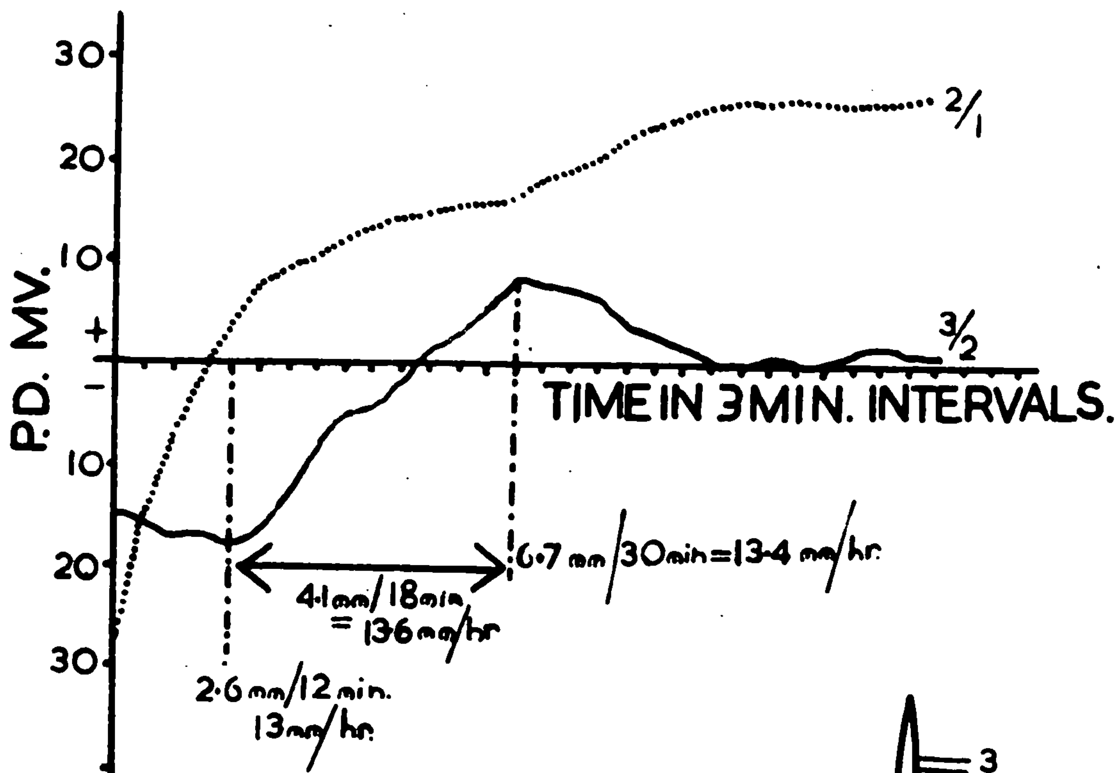
B. Fluctuation in rate of auxin production at the source.

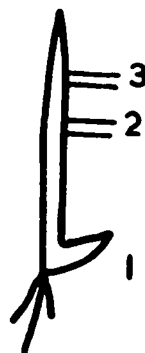
C. Fluctuations in auxin content of the tissue brought about by a fluctuating rate of inactivation, (e.g. IAA-oxidase system).

D. Fluctuations in a bio-electric potential generating system which operates only in the presence of auxin.

The next two experiments involved the application of agar blocks containing two parts per million IAA to the apices of "double decapitated" coleoptiles. These gave the graphs shown in Figure 6.2.

The positive potential of the upper contact increases relative to the lower, commencing at a time corresponding with the arrival of a wave of stimulation, presumably due to IAA travelling from the apex at a rate of 10 to 13 millimetres per hour.





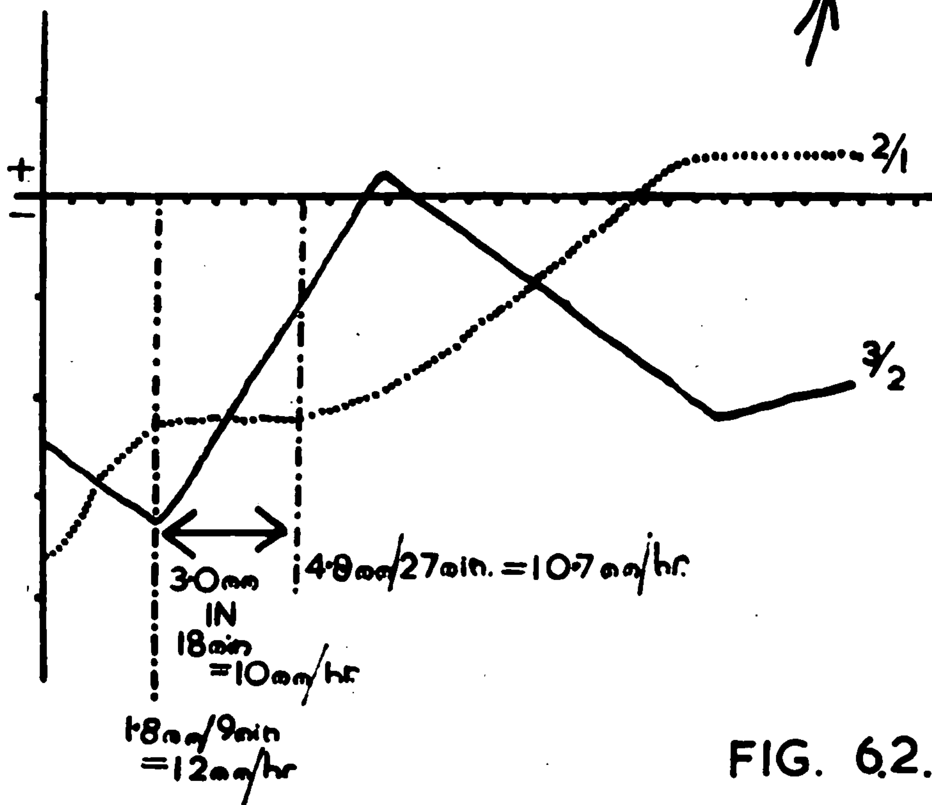


FIG. 6.2.

Corresponding with the increase in the positive potential of the lower contact with respect to the base, there is a decrease of the positivity of the upper contact with respect to the lower. This is what one would expect as the IAA wave passes down. In these experiments however there is no evidence of oscillations, but just an increase in positive potential with the arrival of IAA, followed in the case of the upper contact by a subsequent decrease.

With the agar, however, drying out rapidly occurred, and this was when it was decided to use a flowing ring contact to apply IAA. (In fact the contact was originally developed for this purpose and later adapted for the electrode contact). (Figure 6.3.)

In using a flowing ring contact, it was first necessary to determine the effect of the carrier liquid on the potentials. Deionised water was applied alone to the apex of a "double decapitated" coleoptile, but in the two experiments where this was done, the potential changes measured at the contacts subsequent to the application were large. Shive's solution was also tried and in the preliminary experiment the changes did not seem as large, so a whole series of experiments was carried out using Shive's solution.

In this series of experiments the upper contact was placed 3 to 6 millimetres below the decapitated apex. The primary leaves had been plucked out, yet in only one case did the solution penetrate down the centre of the coleoptile to the contact level, and then it took 45 minutes to penetrate 3 millimetres.

DIAGRAM OF APPARATUS USED TO APPLY FLOWING DROP TO APEX OF COLEOPTILE.

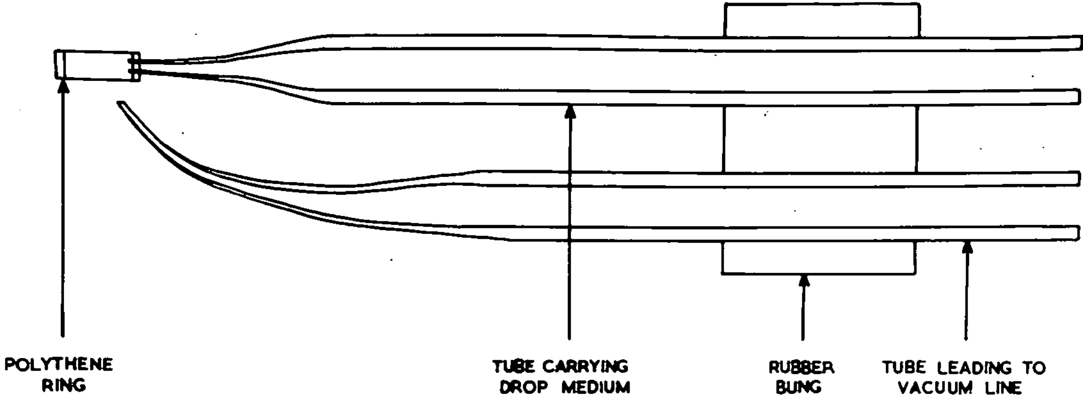


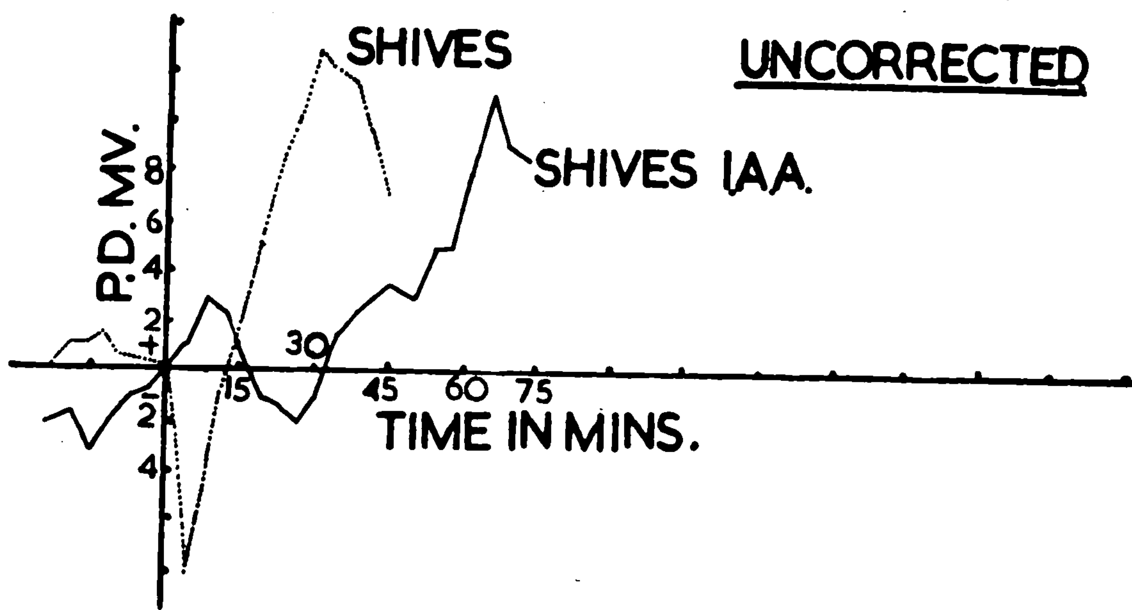
FIG. 6.3.

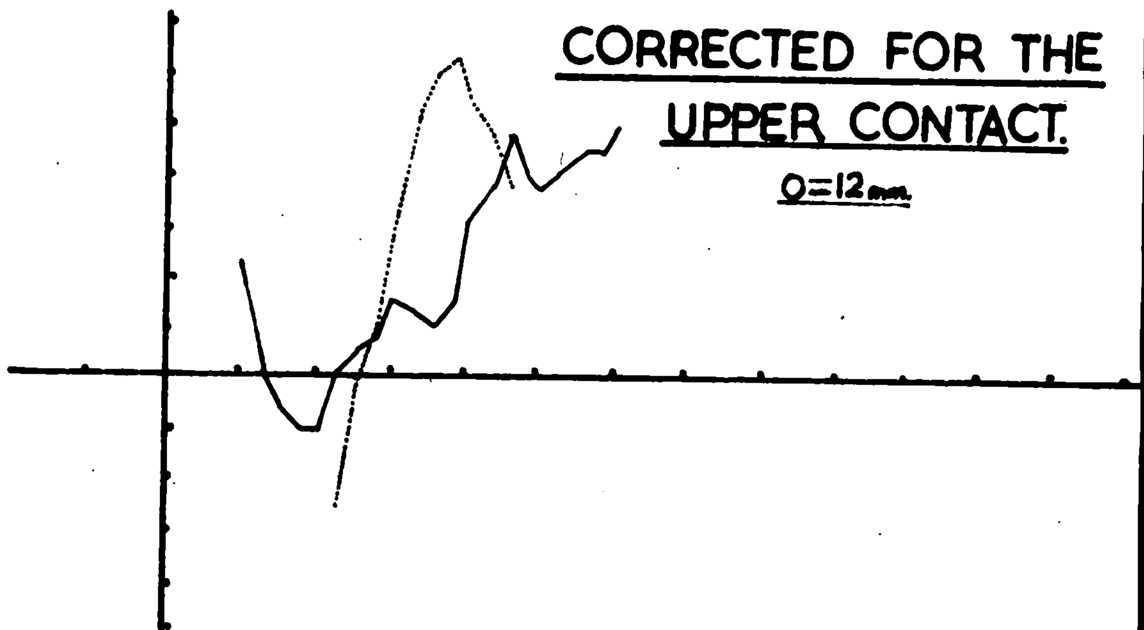
With Shive's solution containing one part per million IAA, the penetration was far more rapid, and in an effort to prevent it reaching the contacts within the experimental period, the contacts were positioned 7 to 10 millimetres below the apex, but penetration was so rapid (10 millimetres per hour), that nearly always the auxin solution reached contact level within the experimental time. When the solution reached contact level, there was generally a small jump in potential, but the direction in which the potential was changing before the arrival continued after the arrival, and even when the liquid had passed the level of both contacts, potential changes occurred and the potential was not zero.

According to the observation of Wilks and Lund (1947) and Schrank (1950&1953) the IAA-Shive's solution should have shorted out potentials, but even if the solution was acting as the pathway of least resistance, at the contact position, coleoptile tissue separated it from the contacts. Asymmetric potential could be generated here.

As recorded later, this penetration difference between Shive's solution and the same solution plus one part per million of IAA was further investigated.

Figure 6.4.A. shows the results for Shive's and IAA/Shive's. They are the mean of 7 and 5 results respectively, and the average was found by taking the time and P.D. when the liquid was applied to the apex as zero, and working out the mean potentials at 3.75 minute intervals.





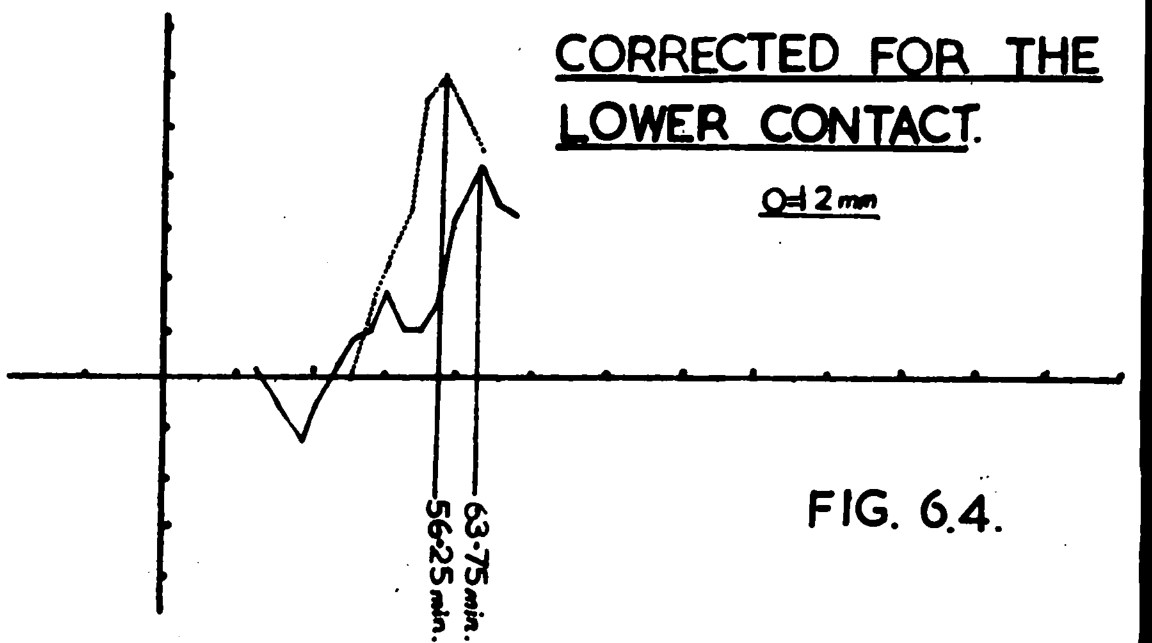


FIG. 6.4.

With Shive's, the potential after application of the liquid is generally slightly more negative than with IAA/Shive's, though in the figure the difference is accentuated by the very high negativity of one Shive's result. This may be a result of the closer proximity of the electrodes to the apex when Shive's solution was applied, but this implies the rapid conduction from the apex of a stimulus whose strength diminishes with distance. Such a stimulus is also indicated by the large changes subsequent to decapitation. It is difficult to generalise on the potential changes subsequent to the initial negativation in Shive's, other than to say that a peak positive potential is reached between 19 and 37 minutes (mean 30 minutes) after applying the solution. With IAA/Shive's this peak is not reached before 60 minutes after application. In both cases it is difficult to say when the change to a positive potential begins, but, as suggested before, when this reaches its peak, it may be supposed, if due to an auxin wave, to correspond with the arrival of the auxin wave at the lower contact. Since there was a considerable variation in the contact positions, a rate of transport of auxin of 12 millimetres per hour was assumed and the coleoptile results then corrected for the positions of their contacts. For a coleoptile with a contact 12 millimetres below the apex the correction is t=0 but where the contact is 10 millimetres the graph commences at t= +10 minutes and at 8 millimetres t= +20 minutes. Figure 6.4. B.C. shows the mean results for both upper and lower contact corrections. With the correction, the peaks of positive potential of the Shive's and Shive's/IAA more closely correspond. If we assume the peaks correspond with the arrival of the solution at the lower contact, we get values for the rate

of transmission from the graph corrected for the lower contact position, of 12.8 millimetres per hour for Shive's, and 11.3 millimetres per hour for Shive's/IAA.

This suggests that:-

(a) A longitudinal transport system operating at a rate of approximately 12 millimetres per hour exist in coleoptiles.

(b) Shive's solution applied to the apex produces a wave of positive potential passing down the coleoptile at the above rate.

It is therefore impossible to conclude from these results that IAA will also bring about this change, though from the observations with agar blocks it seems reasonable to infer that it will.

These changes in potential, it is suggested, are due to changes in membrane permeabilities to ions brought about by IAA or by the ions of Shive's solution.

Since the original change is an increase in positive potential, it is due to a relative increase in cation permeability. What brings about the rapid changes after decapitation or application of a solution to the decapitated apex is not known, but since the cytoplasm throughout the plant is continuous, it is possible that a change similar to the action potential of Nitella or an animal nerve, is conducted away along the cytoplasm.

This is conjecture without evidence, though experiments with various concentrations and compositions of contact media might lead to a tenable hypothesis.

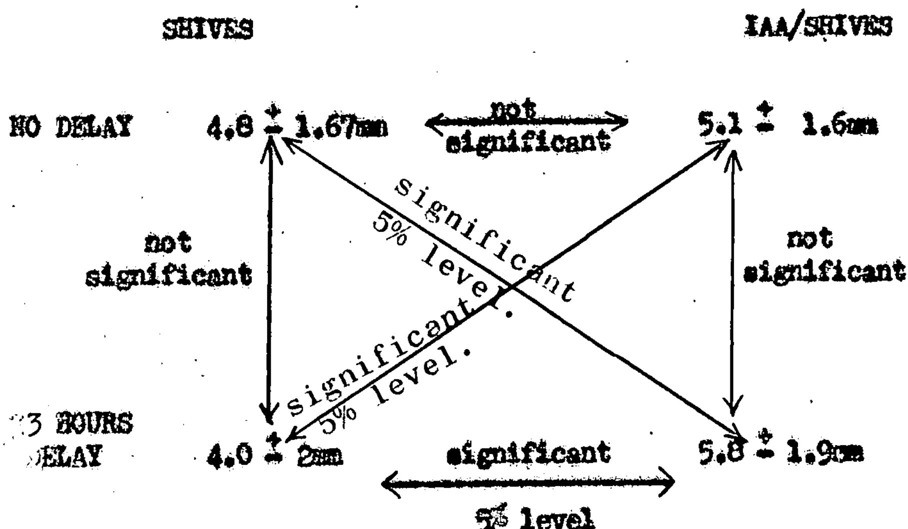
Van der Weij (1932) and Went (1937) both record rates of transport of auxin of 10 to 12 millimetres per hour which agrees with the rate of transport we found for the Shive's solution and IAA stimuli.

Some brief experiments on the penetration of IAA solutions into coleoptile cylinders were tried.

Coleoptiles between 25 to 30 millimetres long were decapitated and had the primary leaf withdrawn and were then divided into two groups. The first group had the "seeds" and roots removed and the coleoptiles were then placed in petri dishes containing either plain Shive's solution or Shive's with one part per million IAA. The second group was treated likewise, three hours later, after a "second decapitation". One hour after immersing in the solution the degree to which it had penetrated was measured.

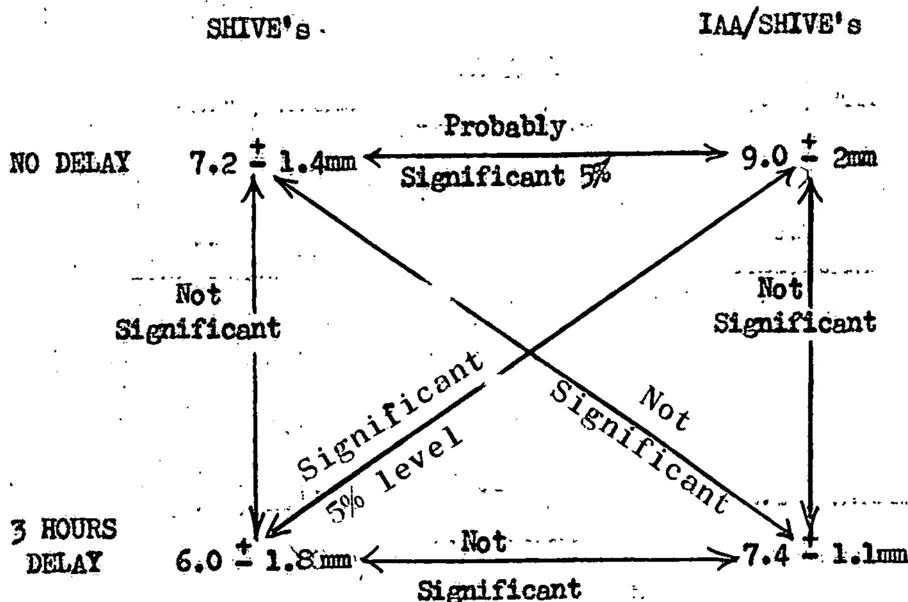
The results are shown in the following table:

TABLE 6.1



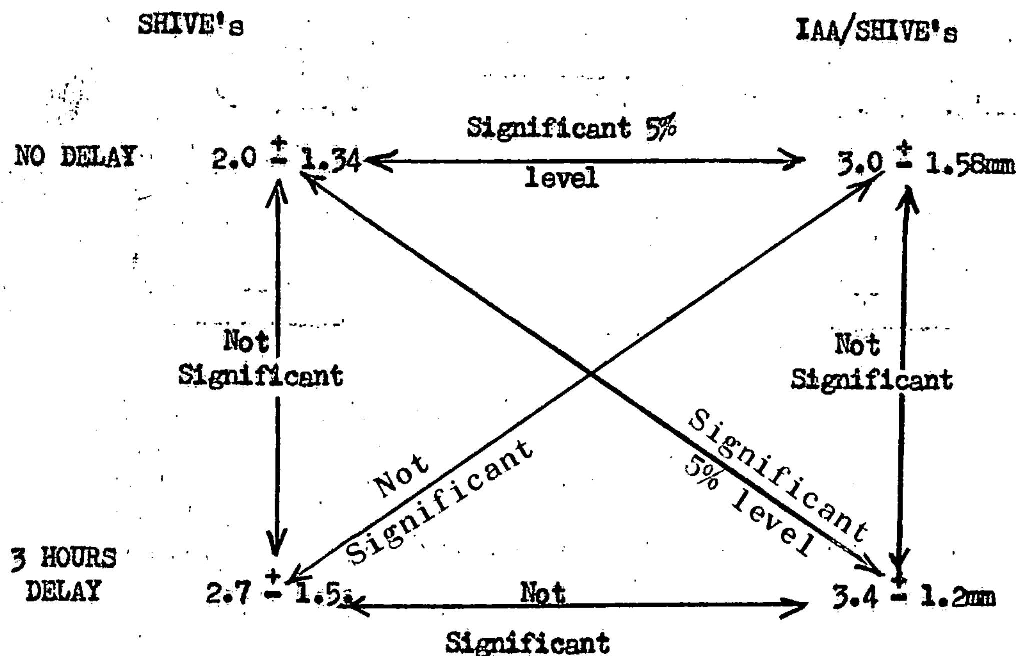
Since the penetration was not as great as with a flowing drop contact, it was thought possible that aeration might be important, so the above experiment was repeated, but this time, the dishes were shaken. Penetration was considerably greater. It is however possible this was due to a more efficient immersion of the coleoptile cylinders by shaking. In the first case they tended to float to the surface.

TABLE 6.2.



In order to ensure a good contact between the coleoptile apices and solution, in the next experiments, the cylinders were pushed inverted through polyurethane foam, floating on the surface of the solution. This ensured that the apices were immersed. This method gave the smallest penetration of all experiments.

TABLE 6.3.



For all experiments Student's t test has been applied but rarely are the results significant by this test. However in all experiments solutions containing IAA show greater penetration than solutions without. If the penetration depends on a surface property of IAA then one would expect the IAA/Shive's solution always to show similar penetrations and the Shive's solution likewise. This is the picture that emerges from the significant differences that do exist. If penetration depends on internal auxin concentrations the expected results would be:-

Penetration with IAA/Shive's - no delay (high internal auxin, high external)
greater than Shive's - no delay (high internal, low external)
greater than IAA/Shive's - delay (low internal, high external)
greater than Shive's - delay (low internal, low external)

where - as the three results are:-

Immersed	Shaken	Inverted
IAA/Shive's - delay	IAA/Shive's - no delay	IAA/Shive's - delay
IAA/Shive's - no delay	IAA/Shive's - delay	IAA/Shive's - no delay
Shive's - no delay	Shive's - no delay	Shive's - delay
Shive's - delay	Shive's - delay	Shive's - no delay

This again suggests that it is a property of the IAA in the solution rather than auxin in the plant, that sets limits to the penetration.

Solution aeration also seems to be important, for:-

(i) The flowing ring contact shows more rapid penetration than any of the above

(ii) When the solution is shaken the penetration is greatest.

No references have been found to this auxin phenomenon, and, apart from these few experiments, no further work has been carried out, as it was felt to be rather too far outside the limits of the work. It has however been thought worth recording, as this appears to be the first observation of IAA acting as a surface active agent.

CONCLUSIONS

(i) Decapitation of coleoptiles leads to a reduction in oscillations in the potential and after second decapitation they disappear completely.

(ii) Application of an agar block containing auxin (IAA. two parts per million) leads to a wave of positive potential passing down the coleoptile at the accepted rates of auxin transports, 12 millimetres per hour.

(iii) Application of Shive's solution leads to a similar wave.

(iv) The response is ascribed to changes in membrane permeability brought about by IAA and changes in ion balance of the cells.

(v) There is evidence also for a very rapid stimulus passing down as a result of decapitation and application of the solutions. The mechanism of this is unknown.

(vi) IAA solutions penetrate further down the interior of the coleoptile than do solutions lacking IAA. It is suggested that this may be due to a surface active property of IAA, rather than to the internal auxin concentration.

CHAPTER 6.

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CHAPTER.7.

CHAPTER 7

PHOTO-ELECTRIC RESPONSES OF COLEOPTILE TISSUE AND THEIR RELATIONSHIP TO PHOTO-TROPIC CURVATURES PRODUCED BY REDISTRIBUTION OF AUXIN.

SUMMARY

A general description of photo-tropic responses of coleoptiles and then a detailed discussion of previous work on the effects of light on the electrical potentials of oat coleoptiles are given.

Experiments we have carried out, which indicate potential responses both on turning on and off white tungsten lamps, and also provide evidence of the involvement of auxin in the changes, are described.

In a discussion of the results, it is shown that the transverse potential gradient to which had previously been ascribed the function of auxin redistribution is in fact itself caused by the auxin redistribution.

Photo-tropisms of plant tissue are in general due to differing growth rates of the tissue on the illuminated and shaded sides.

The Avena coleoptile shows an extremely complicated photo-tropic response, for with increasing energy of illumination, it passes through a "first positive phase", "first negative", "second positive", "second negative", ending finally with a further positive phase of tropic response.

The "second positive", is the tropism present in normal daylight and is the one used in this work, because, for reasons given later, it was felt necessary to use continuous light.

As the coleoptile tissue shows a direct light growth response it has been suggested that the differing responses on the light and shaded sides could account for the resulting bend, but it has been shown that they are insufficient by a factor of 4. This light growth reaction has a fast and slow component; the former, the fast response, has been likened to a shock reaction; it is not proportional to the energy change and has been ascribed to a transient decrease of tissue sensitivity to auxin; the latter component is dependent on tip illumination and is supposed to be due to a reduction in the auxin leaving the tip.

The maximum region of photosensitivity for inducing tropisms is restricted in the coleoptile to the extreme apex and the further one moves away from the tip, the lower is the sensitivity. (2 millimetres down the sensitivity is 1/36,000 of that at the extreme apex, according to Sierp and Seybold (1926). Blue light is the most effective, whilst red light is without direct effect, though it may modify the sensitivity to shorter spectral regions.

The photo-tropic response occurs in the region somewhat below the tip. Galston (1959), quotes a figure of 1 centimetre, but from films we have taken the region commences from 3 to 5 millimetres below the tip. There must be conduction downwards of the stimulus to this region.

The basal regions of the coleoptile are also photo-sensitive, but require higher dosages for response than the apical, and the action spectrum differs, which may imply a different receptor.

It has been shown that under unilateral illumination giving positive curvatures, although there is little reduction in the total auxin (IAA) leaving the apex, there is a redistribution. The greater quantity of auxin is on the shaded side and it is this which gives rise to the positive tropism.

Effects of light on the Electrical potentials of Oat Coleoptiles

There are only a few papers on this topic and yet on these observations partly rests the hypothesis that tropisms are brought about by the lateral transport of auxins along potential gradients set up across tissues by unilateral stimulation. Auxin is an anion and will move to the positive side. Cholodny (1926-27) first suggested this with regard to geo-tropism, and it was then suggested by Went (1932) that the speed and polarity characteristics of hormone transport could be explained on the basis of basipetal migration of auxin by electro-phoresis, the bases of plant that had then been investigated usually being positive with respect to the apex. Clark (1937) claimed that the measured electrical potentials in the plant had no parallel with auxin movement, but Schrank (1944-52) in a series of papers, showed that where tropisms exist so do transverse potentials, and suggested that these caused the auxin redistribution which the Cholodny-Went theory of plant tropism required.

Waller (1925) performed some experiments on the light effects on etiolated seedlings, which are comparable to dark grown oat coleoptiles. He found no characteristic changes in potential. He did however find effects with blanched celery petioles, confirming Bose's observation on this point. Illumination increased the positivity of the illuminated part, whilst turning off the light had the opposite effect. Unfortunately, the periods of light and darkness were of only five minutes' duration, periods too short to give an indication of the full effect. Bose (1907) is completely unhelpful on the form of the changes, just mentioning that he found effects in tissues lacking chlorophyll and citing the celery petioles as an example. He was seeking to prove his hypothesis that all tissues are sensitive to light stimulation and to discredit Waller's (1900) observations that only green tissues were light sensitive.

Clark (1935) found that with etiolated oat coleoptiles, there was absolutely no effect on the potentials on turning on a light (100 watt placed 40 centimetres vertically above the coleoptile), but 5 to 30 minutes later, when the light was turned off there was a period of damped oscillations commencing with a positivation of the apex. The effect was most marked in the apical region of the coleoptile.

This paper does not appear to have been fully understood by Wilks and Lund (1947), for they observe "Clark has reported an increase in the negativity of the apex of the coleoptile of Avena as a result of illuminating the plant from above." (Bioelectric Fields and Growth. P.26.) and yet the original paper clearly showed that the changes occurred only on a cessation of illumination, and that they consisted not of a simple increase in the

negativity of the apex but rather oscillations commencing with a positivation of the apex and a final return to the original potential.

Wilks and Lund (1947), also record that "Unilateral illumination of the apical 2 millimetres of the apex of the coleoptile in the normal position caused the shaded side to become more negative with respect to the illuminated sides at a point 5 to 6 millimetres below the apex." This result therefore had nothing to do with the direct effect of light on the coleoptile tissue but indicated that a stimulus perceived at the apex could in some way be conducted back to the base. Unfortunately they publish no data on the time scales involved.

Openoorth (1941), had reported that with a light dosage of 500 metre-candles, giving a positive tropic response, the lighted side became positive with respect to the shaded, and Schrank (1946 and 51), published data showing that under continuous unilateral illumination giving a positive curvature a P.D. was developed across the apical cells of coleoptiles, such that the lighted side was first positive and then later negative to the shaded side.

Backus and Schrank (1952), published detailed graphs of changes in longitudinal and transverse polarities under different light dosages giving both positive and negative tropic responses. They used only 2 minutes of illumination and it is not possible to say whether the results were due to the commencement or cessation of illumination. They knew of Clark's work on this point, in fact citing the reference in this paper. Backus and Schrank (1952), make one important contribution, for they show

that the changes on the two sides of the coleoptile were similar in form but differed in magnitude, so giving rise to the transverse potential difference.

Newman (1959) noted that when a light was shone on the apex of a coleoptile a wave of change in the electrical potential passed down the coleoptile at a similar rate to that of auxin transport. As the wave passed a contact, this contact became more positive with respect to the reference contact.

Summary of these Papers

(a) Both commencement and cessation of illumination cause changes in the potentials of coleoptiles. These changes take place throughout the coleoptile but are largest at the apex.

(b) Stimuli may be transmitted back from the apex to cause potential changes further down the coleoptile.

(c) Similar changes in potential occur on the front and back faces of the coleoptile but differ in magnitude.

Any experiments attempting to be of value should distinguish between the direct effects of light and the effects conducted from some other part and also between light on and off effects.

The latter problem was solved by the use of light continuously applied for a time period sufficient to complete any electrical response and then turning the light off for a similar period.

Clark (1935), had reported that there was more variation of potential in red light than in complete darkness. It therefore seemed advisable, as it was hoped to use red light as background lighting during these white light experiments, to check this observation. The results obtained

showed no difference in the degree of variation of the potential measured in red light and complete darkness. (It should be noted that Blaauw-Jansen (1958) reported a different photo-tropic response in red-light-grown coleoptiles from that given by dark grown, and claimed that this was due to a chemical factor produced by the primary leaves).

The next experiment tried was that of shining a light vertically on to the tip of the coleoptile where a flowing drop ring contact was placed and measuring the potential difference between this ring contact and the basal growth medium. In this series of experiments the white tungsten light had an intensity of 4000 metre-candles at the level of the contact and the heat was filtered out by a trough containing 5 centimetres depth of water. Shive's solution was used both as growth and contact medium, and the apparatus was that always used with a flowing drop ring contact. Plate 2.5.

The mean graph was obtained by taking the potentials at regular intervals from the continuous recorder graphs for a number of coleoptiles and taking the arithmetical mean. The P.D., and the time, when the light was turned on or off was taken as zero.

There are large variations in the potential after setting up in this, and other reported experiments, but in the 15 minutes before the light was turned on, the potential variation was less than 2 millivolts (Figure 7.1).

Consider the graph of events after turning on the light. The fractions quoted above each peak or trough on the graph indicate in how many experiments this peak or trough distinctly occurs, e.g. "A" occurs

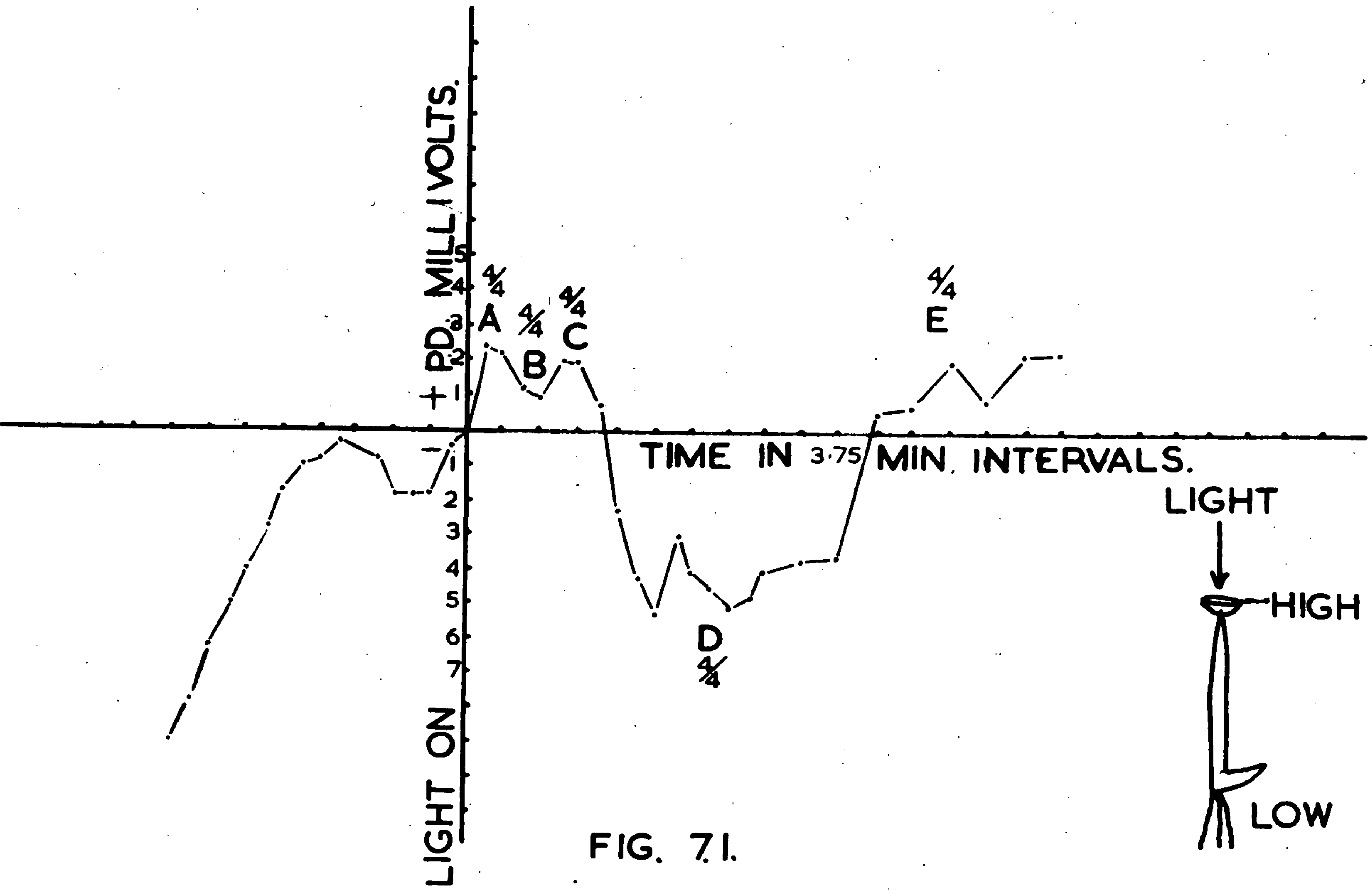


FIG. 7.1.

in 4 out of 4 experiments. In none of the 4 experiments is the graph exactly of the form indicated and in 3 of the 4 experiments "B" is below the value of the potential when the light was originally turned on. In 3 out of 4 experiments "C" is smaller than "A" and in all the experiments "D" is below the zero line. The relatively positive peak in the "D" trough occurs in only one experiment.

All experiments show an increase in the positive potential of the apex after 30 minutes but in only 2 experiments is the value attained greater than the original potential. (After this there is no similarity in the graphs). Figure 7.2.A. shows what really represents a more typical curve.

The important things to note are:-

(a) White light has an effect on the electric potential of the apex of the coleoptile with respect to the base (C.F. Clark ; 1935).

(b) The effect starts immediately the light is turned on.

The question now is why do these results differ from Clark's? There is the possibility that the coleoptile, being grown in red light in contrast to Clark's which were grown in total darkness, may contain a factor, (e.g. a pigment), causing the change in potentials. If we assume this factor is rapidly formed in the light, then although Clark obtained no light response, he might have dark effects after a period of illumination. However, even if this were the case, since it has been shown by Filzer (1930), that completely dark-grown coleoptiles are the most phototropically sensitive, it would infer that the potential changes on turning on light have nothing to do with the subsequent tropisms.

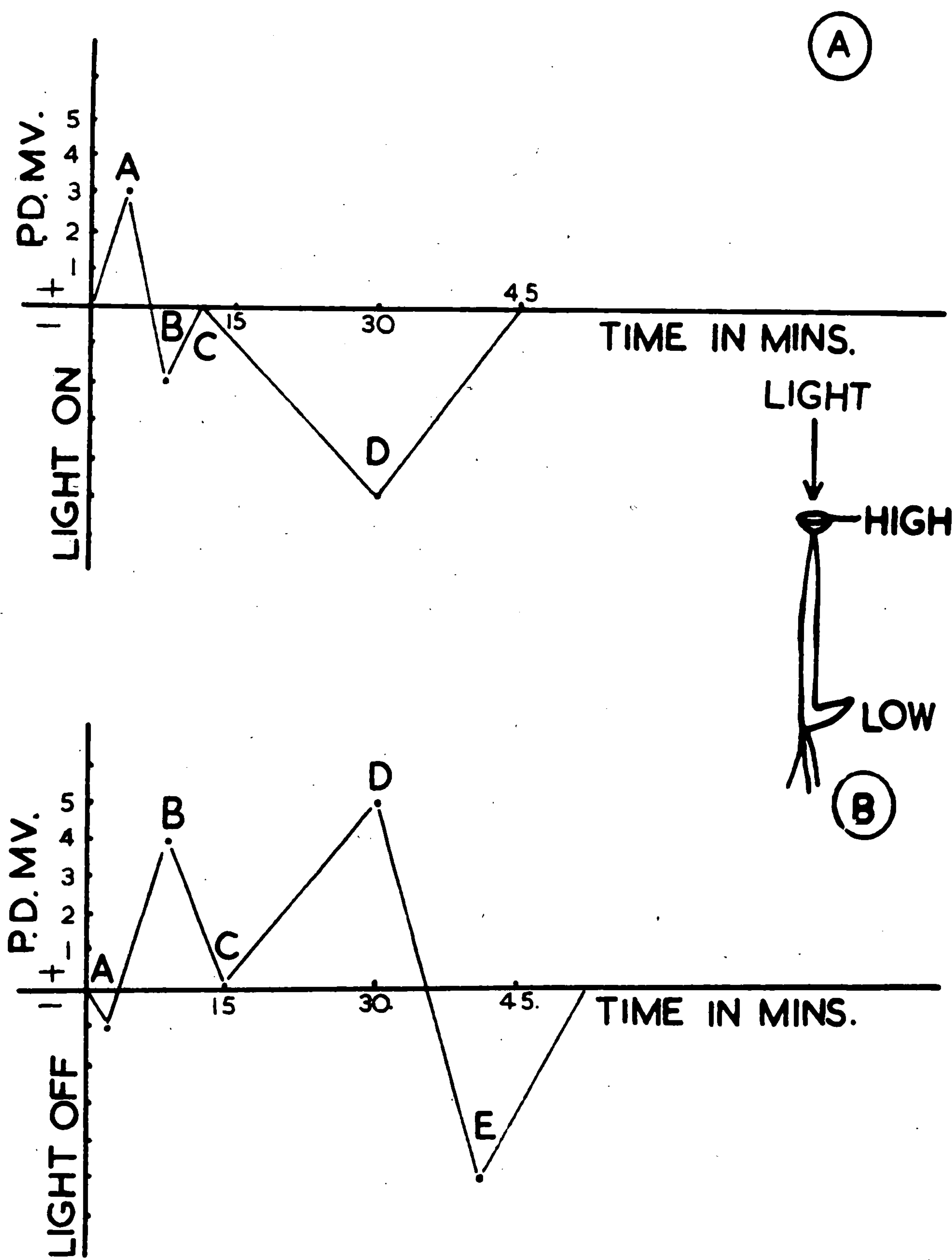


FIG. 7.2.

Now we consider the dark effect and again the P.D. and time when the light is turned off is taken as zero. (Figure 7.3.)

3 out of 5 show a negative slope as soon as the light is turned off, and in another graph there is a change to a less positive slope so "A" should probably be below zero. In only one graph does the peak in trough "C" occur. "B" and "D" are approximately the same magnitude in 3 out of 5 of these experiments and are positive in all but one. "E" is always more negative than "C". In two graphs recording went on for longer periods of time and oscillations continued till recording ceased which in one case was 2 hours later. The oscillations had a periodicity of approximately 30 minutes $\pm$ 7 and an amplitude of about 5 millivolts.

Figure 7.2.B. shows what is thought to represent a more usual response than the arithmetical average of these few results.

Comparing this with Clark's (1935) results, we find that "B, C, D" correspond with his first positive peak, "E" with his first negative, "F" with the next positive; thus there is some agreement between the two. Since he took readings only every 5 minutes he would miss "A", whilst "C" may have been too small for detection by his electrometer, or may not have existed in his variety of oat. In conclusion, then, Clark's (1935) results with reference to the dark response are confirmed, but we also find a light response which may be due to a difference in growth conditions.

When one compares the light "on" and "off" results in Figure 7.2., it is seen that there is a quite close agreement on the times of the various peaks and troughs, but their polarities are reversed. This suggests that both of the effects are mediated through a similar system. This is

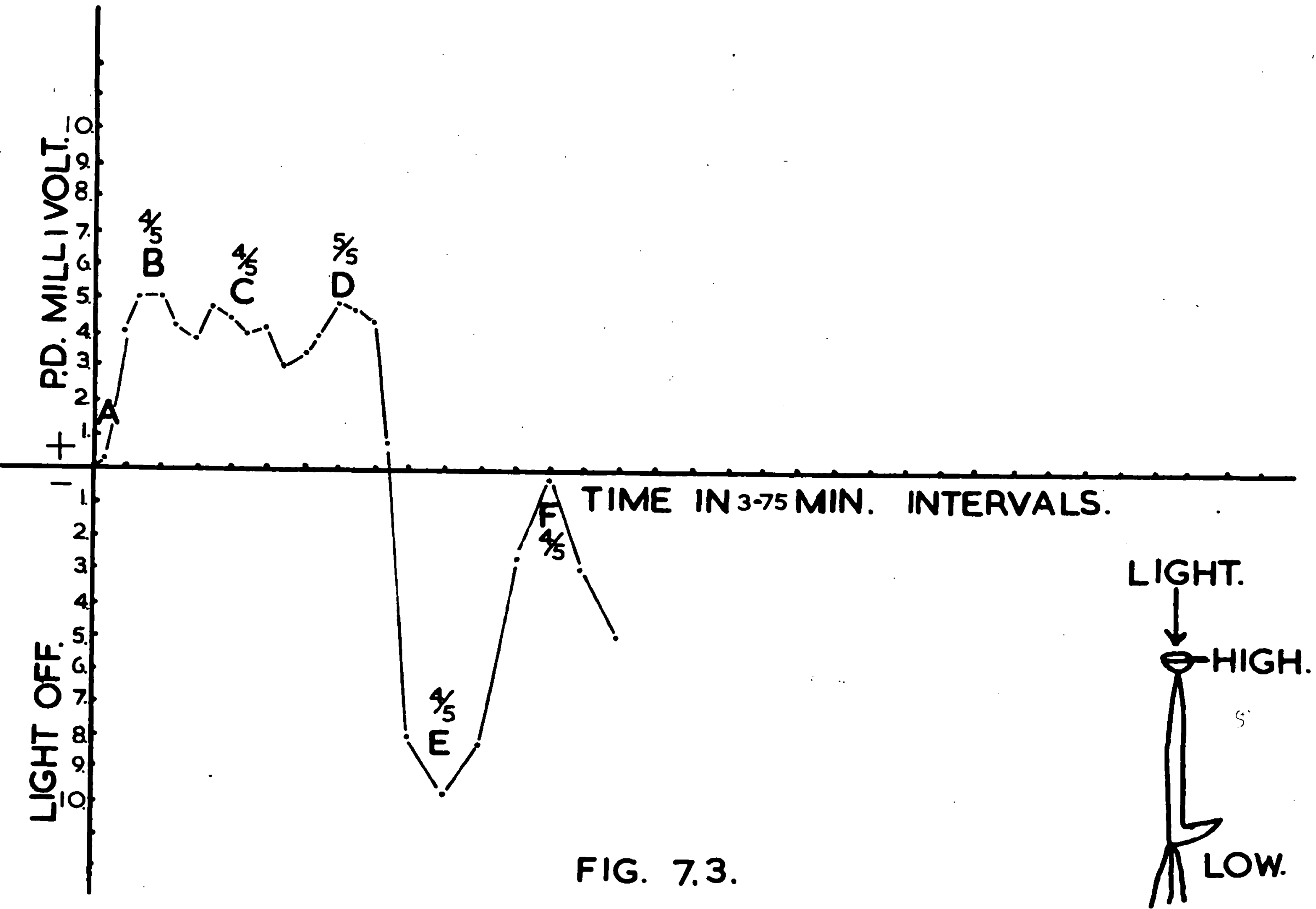
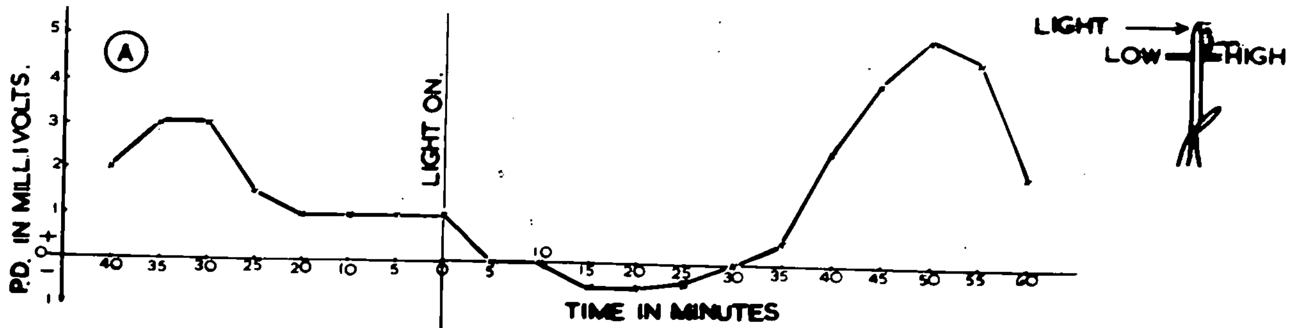


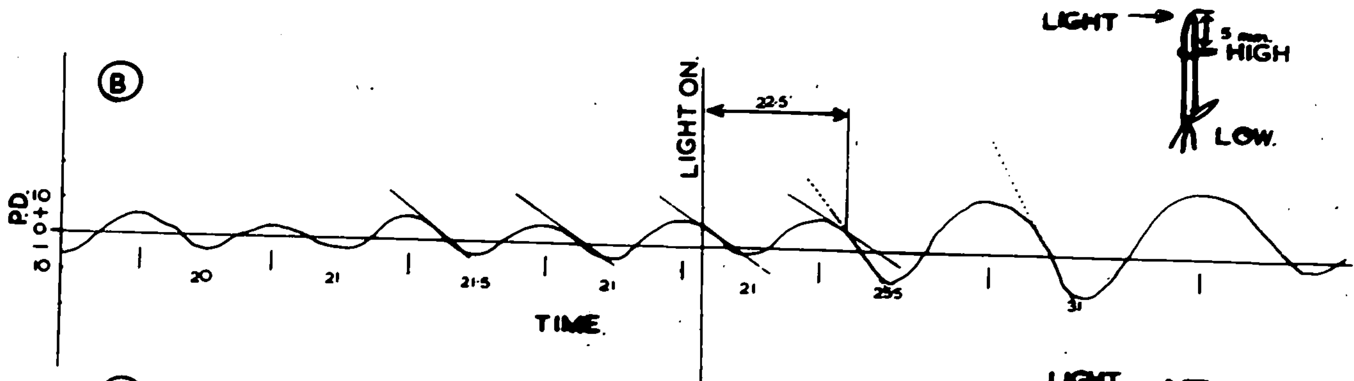
FIG. 7.3.

also borne out by the "D" troughs and peaks after 30 minutes of illumination and lack of, respectively, being the largest in magnitudes of any part of the responses.

The position at the apex is complicated by the fact that it is impossible to distinguish between the direct effect of light on the tissue and changes due to the stimulus reported to pass down the coleoptile; other experiment separating these two factors were devised.

Flowing drop contacts were positioned transversely 3 millimetres below the apex, and then a narrow beam of light was projected from one side on to the apical 1.5 millimetres of the coleoptile tip. This white tungsten light had an intensity of 2,500 metre-candles, and gave positive photo-tropic curvatures. The results, the mean of 4 graphs, are shown in Figure 7.4.A. The slight negativity immediately after turning on the light is believed not to be due to the light, as 2 out of the 4 graphs show this change commencing before the light was turned on, and another shows no change immediately after the light is turned on. The stimulus, in the form of a rapid potential change, reached the contact level in approximately 20 minutes, giving a rate of transport of 9.0 millimetres per hour at 20°C. Newman (1959), found a 3 minute time lag between commencement of illumination and beginning of the downward conduction of the stimulus, at 25°C. This delay, which will no doubt be greater at 20°C, will give a rate of transport greater than 9.0 millimetres per hour. Newman (1959), found a transport rate of 8 millimetres per hour at 15°C. and 14 millimetres per hour at 25°C. for the stimulus. Van der Weij (1932), recorded a rate of transport of auxin in decapitated coleoptiles of 10 to





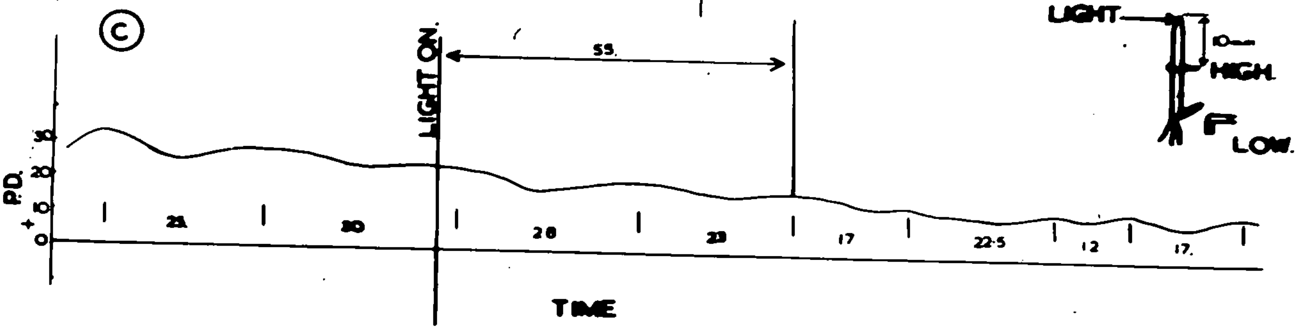


FIG. 7.4.

12 millimetres per hour at 25°C, though Went (1935), claimed that in intact coleoptiles the rate is higher at 15 millimetres per hour. It is suggested therefore, that the stimulus recorded in our results is in fact an auxin wave.

The result confirms Newman's (1959) observation that upon illumination of the apex a stimulus is conducted back along the coleoptile. However, in our experiment the electrodes were transversely placed. This indicated that the stimulus at the two sides of the coleoptile is not the same and a wave of asymmetric change has been translocated down. The dark side is positive to the light side as the stimulus passes. Auxin was found by Newman (1959), and ourselves, to increase the positivity of the tissue to which it is applied. It is suggested therefore, that our result is exactly that to be expected, for it has been shown that there is greater auxin concentration on the dark side of coleoptiles showing positive phototropism, and that this gives rise to the subsequent photo-tropic curvature. The result differs from that of Wilks and Lund (1947), but without any knowledge of the times involved in their experiments, it is impossible to make comparisons.

Also two individual experiments were carried out with a light on the apex and a flowing drop ring contact situated part way down the coleoptile, the potential being measured with respect to the base. In one experiment for a period of 2 hours before the light was turned on, oscillations with a periodicity of 21 ± 1 minute and an amplitude of approximately 12 millivolts had occurred. 22.5 minutes after the light was turned on, there was an obvious change in the oscillations whose

period and amplitude increased. (Figure 7.4.B). Taking the change in the oscillations as the arrival of the stimulus, a rate of transport of 13 millimetres per hour at 20°C is indicated. Similarly, in the second graph (Figure 7.4.C), the oscillations alter about 55 minutes after the light was turned on, though the alteration was not as marked as the above. This gives a rate of transport of 11 millimetres per hour at 20°C. The odd thing in both these results, is that the first change appears to be an increase in negativity of the area the wave is passing. Newman (1959), gives no details of the position of his contacts with respect to the light, except that only the apex was illuminated, and that they were point contacts. Consider the set up where two contacts are placed one above the other on the dark side of a coleoptile. There will be a redistribution by lateral transport of auxin in the apical regions, so that there will be a greater concentration on the dark side than on the illuminated, and a wave of increased auxin concentration will pass down the dark face of the coleoptile. The upper contact will be positive with respect to the lower as the wave passes it.

However, using a ring contact which averages the potential over a large area completely encircling the coleoptile, as there has been shown to be some reduction in the total auxin passing down illuminated coleoptiles when the illumination is of intensities giving the second positive curvatures, one would expect a primary increase in negativity as the curve passes.

In conclusion, these results indicate that oscillations in electric potentials measured between various parts of coleoptiles, may be influenced by the transport of something (probably auxin) from above, and that light brings about alterations in the transported stimulus.

The next step was to investigate the effects of light on coleoptile tissue sufficiently far below the apex, so that a reasonable length of time should elapse before the potential was influenced by the conducted stimulus.

Flowing drop ring contacts were placed 6 to 10 millimetres below the apices of coleoptiles and the potential was measured with respect to the base. Vertical illumination was used, being of two intensities, either 4,000 metre-candles or 250 metre-candles. The results obtained are shown in Figure 7.5. 1 to 4.

They indicate that the stimulus of light leads to an immediate alteration in pre-existing potential oscillations or to an initiation of new oscillations in the potentials measured. The degree of change varies from plant to plant and from the few results available, it is impossible to draw conclusions with regard to the differences between 4,000 and 250 metre-candles of illumination. From a cursory examination of the "Light on" responses, one might infer the arrival of a stimulus about 30 minutes after the light was turned on, as there is then a pronounced negative trough. However, this represents the "D" trough of our original "light on" experiment and must therefore be a direct effect of light on the tissue. Also, with the "light off" response,

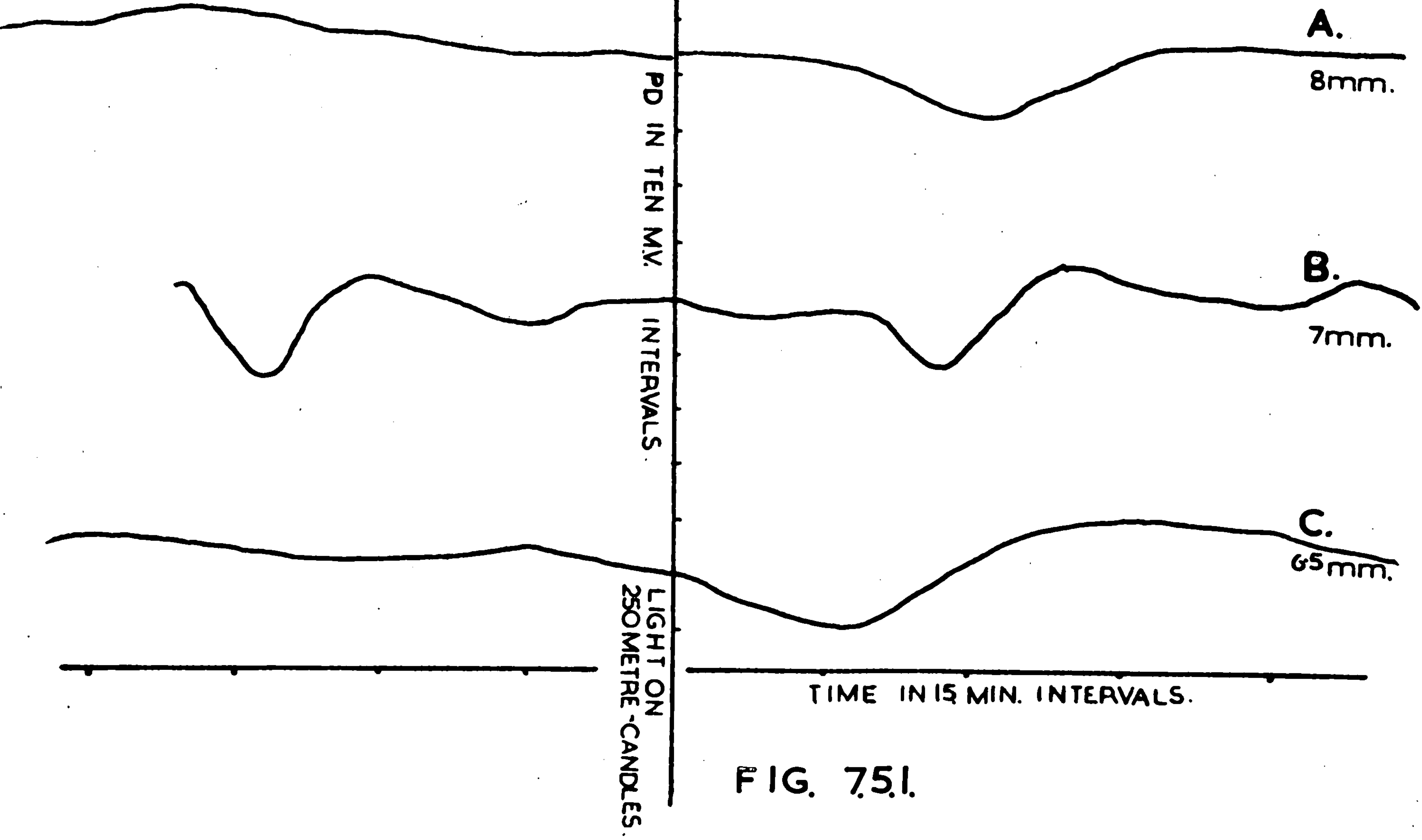
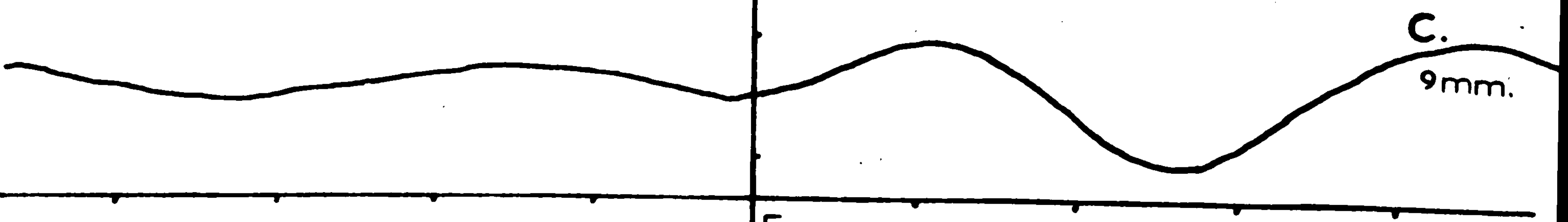


FIG. 7.51.



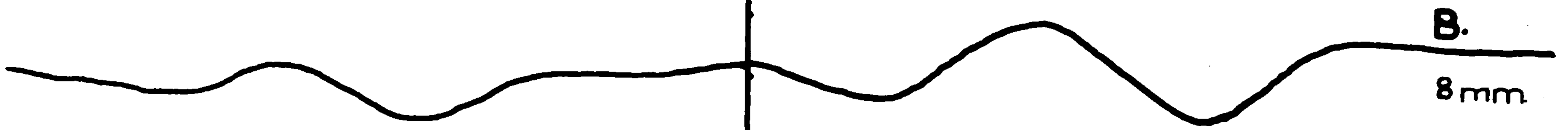
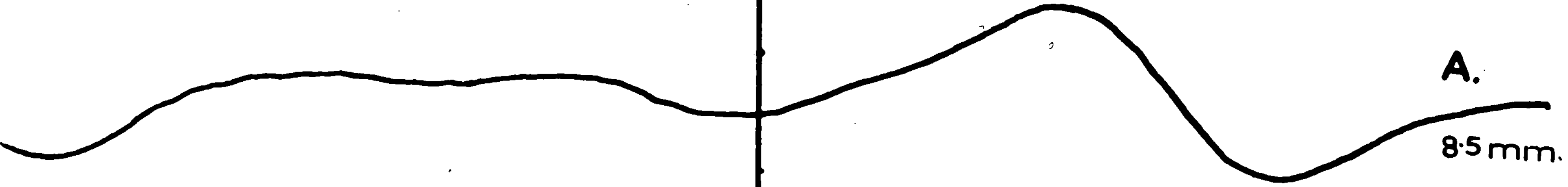



FIG. 7.52.

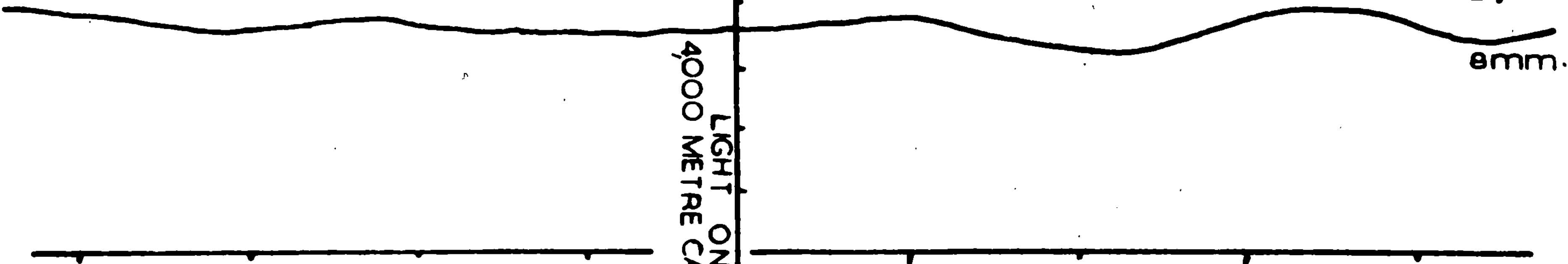


FIG. 7.5.3.

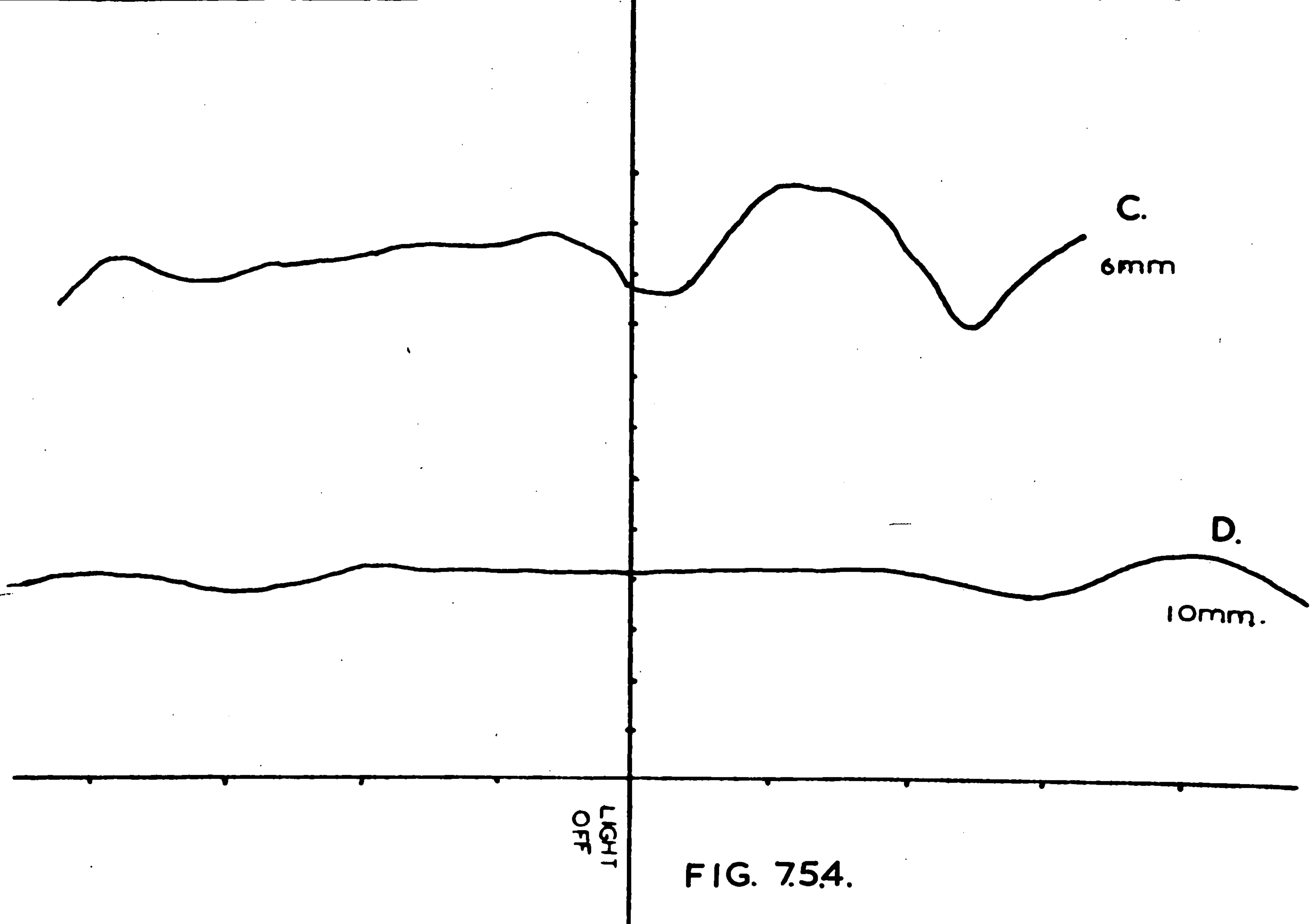


FIG. 7.54.

the peak that occurs in all results after 30 minutes and the trough at 45 minutes, correspond with the "D" peak and "E" trough of the apical response. However, with both the "on" and "off" responses, apart from the correlations mentioned, it is difficult to correlate other details of the responses with those of the apical regions. (The original typical apical responses are shown in Figure 7.2.). Also, from the graphs, it is not possible to pick out any obvious changes brought about by conduction from above.

It has been shown that commencement and cessation of illumination have direct effects on the coleoptile tissue at least to a distance of 10 millimetres below the apex. It has been possible to separate the direct effects from any conducted stimulus and there are similarities and differences between these more basal responses and the responses in the apical regions.

The correlation between the apical response and the response in this region occurred only after a period of 30 minutes after the light was turned on. If the changes in potential at the apex are responsible for a redistribution of auxin, it will be changes within a very short period after the light was turned on that are involved. Therefore a new experiment was devised to further investigate the potential changes in the apical regions.

For the experiment the light flowing drop contact described in Chapter 5 and illustrated in Figure 5.2., was used. This contact was placed about 1 millimetre below the apex of the coleoptile and the

potential measured with respect to the base. The illumination at the contact position was intense but fell off towards the apex. Using this method, it was reasoned that the tissue response should be large and yet even though close to the apex, the conducted response small in comparison. The light at this position still gave strong positive curvatures.

A series of seven results were obtained and were analysed as before, taking the time and the P.D. when the light was turned on as zero.

Figure 7.6. shows the resulting graph. Immediately the light was turned on, there was a positivation "A" of the illuminated area, reaching a maximum in 4 minutes. This was followed by a reversal of polarity.

One would suggest from previous results that the auxin wave should pass a point 1 millimetre below the apex about 10 minutes after the light was turned on, and the change in potential should reach a maximum about 30 minutes later. It should consist of a wave of negativity as the auxin concentration will be reduced on the illuminated face which is the "High" contact in this experiment.

The "B", "C", "D" trough of the mean graph must have been influenced by the auxin wave, but this cannot be the result of the wave for it commenced at 3.75 minutes, far too early for auxin change at the contact level. (This would have required a rate of transport of 16 millimetres per hour for the auxin).

It is perhaps not surprising that it is difficult to detect the auxin wave potential change, for changes in the longitudinal potential are bound to be smaller than the transverse potential, for the latter consists of changes on the two sides of the coleoptile in opposite directions giving a greater response.

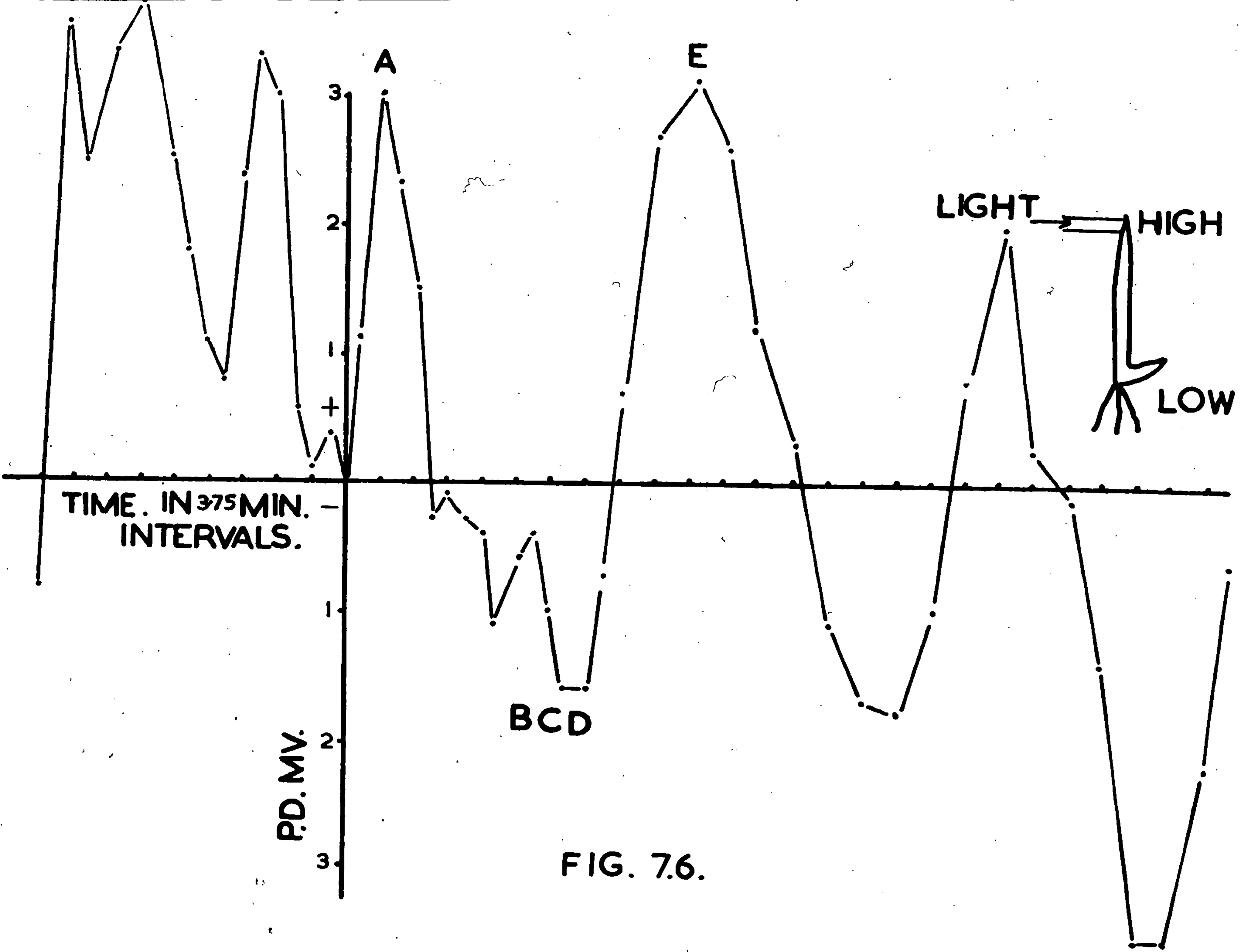


FIG. 7.6.

Comparing the results of this experiment (Figure 7.6.) with the result from a flowing drop ring contact on the apex (Figure 7.2.A), it is seen that they both agree on the "A" peak, but the "B" and "D" troughs and "C" peaks clearly distinguishable in the apical result, are not readily discerned in this one.

In a later experiment, using a very intense light, with a contact positioned one millimetre below the apex, it was shown that in fact the results at the apex and at this position were the same (Figure 7.7.) With the apical result, oscillations subsequent to "C" were difficult to detect, though they are such an obvious feature of the result with the contact 1 millimetre below. The point about which the potential is oscillating appears to become more negative with respect to the base.

This experiment has shown that the responses of the coleoptile tissue to light, are similar in the apical regions and regions just below the apex. It has also confirmed that light may induce oscillations in the measured potential differences; there are also indications that the mean potential of the lighted side becomes more negative with respect to the base. This would agree with the dark side becoming positive with respect to the illuminated side.

Therefore the next experiment involved the measurement of transverse potentials developed under the influence of unilateral light. A pair of contacts were positioned on opposite sides of the coleoptile about 2 millimetres below the apex, one contact being the "light" type. The potential between the two electrodes was measured, the light contact being "High".

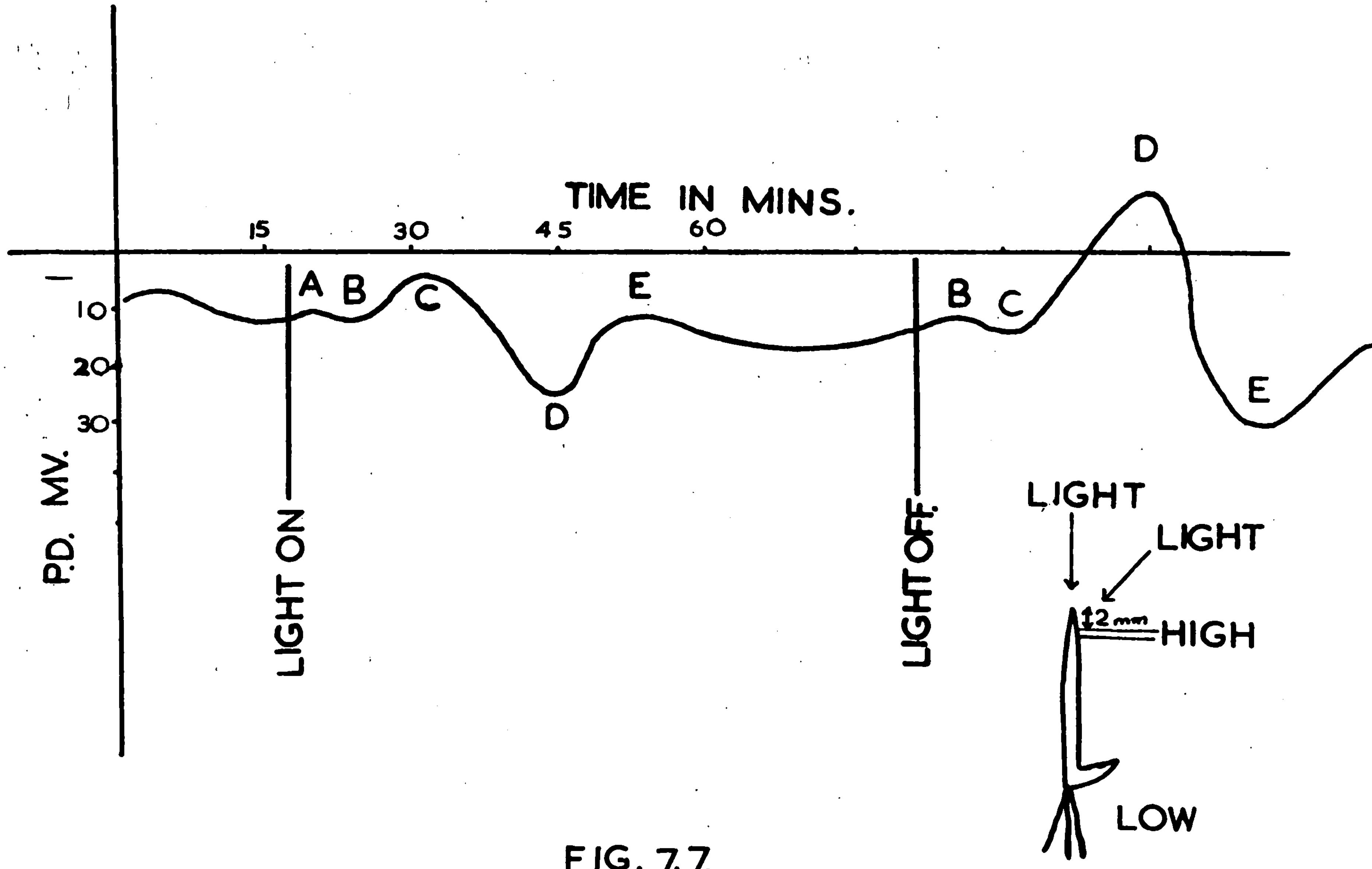


FIG. 7.7.

As discussed more fully in Chapter 3, with such a situation there would have been a considerable shorting out of any membrane potentials. Also, as the changes were reported by Backus and Schrank (1952), to be similar on both front and back faces of the coleoptile, the transverse potentials being due to their differing magnitudes, we could only expect to measure small transverse potentials.

The graph in Figure 7.8 shows the changes, plotted by the same method as before, upon turning on the light. 4 out of 5 of the results show that immediately after the light was turned on, the lighted side became negative with respect to the dark. This negativity reached a maximum at about 20 minutes. Also 4 out of 5 results show a gradual decrease of this negativity (2 show an actual reversal of potential) to a peak at about 30 minutes. This is followed by a further increase in negativity. All results show the final negativity.

If this result is compared with that of the "light" contact when the potential was measured with respect to the base (Figure 7.6.) we find that the original negativity of the former corresponds with the "B.C.D." trough of the latter, and the positive peak with peak "E" of the latter. Since however, the transverse potential of the majority of the coleoptiles remains negative throughout, it must mean that the changes occurring at the two faces with respect to the base are similar, but the changes at the lighted face are greater. The calculated P.D. of the dark side with respect to the base is shown in Figure 7.9.

It has been shown in a previous experiment that the greatest difference in auxin concentration as judged by the changes in potential,

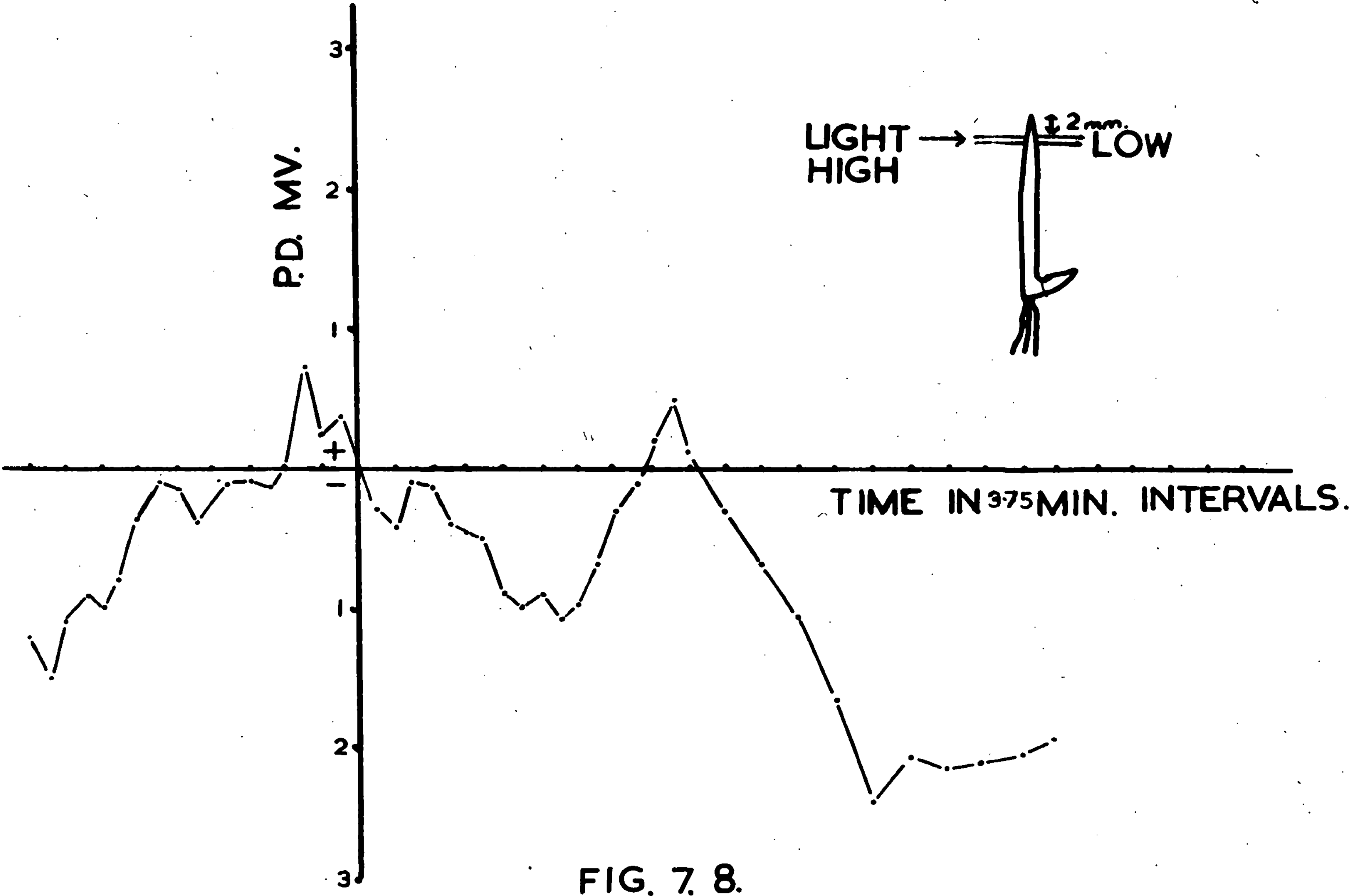


FIG. 7. 8.

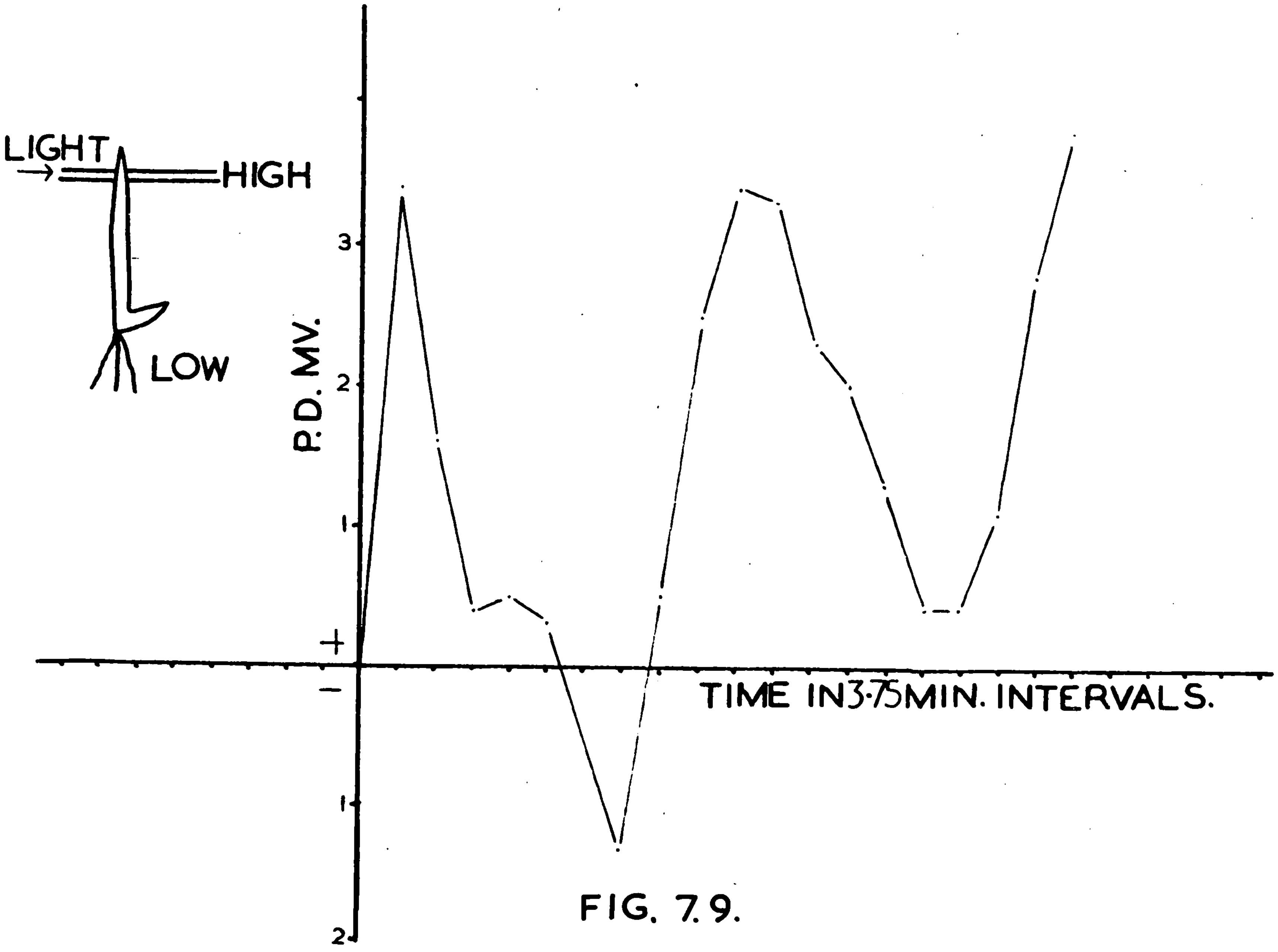


FIG. 7.9.

occurs at a time of 50 minutes after unilateral illumination, 3 millimetres below the apex. Therefore, 2 millimetres below the apex with a rate of transport of 12 millimetres per hour, this would occur at 45 minutes. This is close to the time when the potential difference between the two sides becomes really marked. It is suggested therefore, that the development of this marked transverse potential is due to differences in auxin concentration (it is, as expected, lighted side negative with respect to the shaded side).

Schrank's graph (1951) with contacts transversely placed 0.5 millimetres below the apex, should show the auxin effect reaching the maximum at about 37 minutes. This is about the time when his marked P.D. develops. The same conclusion also applies to similar graphs published by Backus and Schrank (1952).

We must therefore look to the P.D.'s that develop in the first 20 to 30 minutes of illumination if we are to explain auxin redistribution as a function of the potentials. These potentials are only small, and in Schrank's results (1951), are of the wrong polarity. In our experiment the polarity is correct, but the magnitude is small.

Schrank (1951) claims that the potential we ascribe above to the auxin concentration gradient, is that responsible for the auxin redistribution, for it precedes his bending response by approximately 15 minutes. He has failed to realise that in coleoptiles the region of perception and response differ and in fact from time lapse photographs we have taken of coleoptiles bending under unilateral light, it is seen that the first bending responses occur in a region 3 to 5 millimetres below the apex.

If it was the peak of the auxin wave that was detected by Schrank (1951), 0.5 millimetres below the apex, it still has 2.5 millimetres to travel before any bending will be visible. At a rate of 12mm/hour, this will take 12.5 minutes, which agrees well with his 15 minute delay time.

In our own experiments the auxin wave was just detected after 20 minutes of illumination 3 millimetres below the apex. It only reached a maximum after 50 minutes and since bending responses are detected after 40 minutes of illumination, this again agrees with the suggestion that the potential changes are a result of, rather than the cause of the changes in auxin concentration.

The question arises as to why, even before the auxin wave arrived, there were differences in the magnitude of responses on the two sides of the coleoptile. The obvious explanation is that the different intensities of light on the two sides of the coleoptile give responses of different magnitudes.

In the experiment mentioned above, where a 150 watt projection lamp as well as the 4,000 metre-candles of light were shone on the coleoptile at the contact position, the response was considerably greater than anything we had recorded before for a similar contact position, (Figure 7.7.), which bears this out.

There is also the question of how potential changes are brought about by the direct effect of light.

Using an apically placed flowing drop ring contact and 4,000 metre-candles of illumination, a series of observations with different concentrations of KCl as the apical contact medium were made.

Figure 7.10 shows the results. They indicate that with decrease in contact medium concentration there is an increase in the response.

It is suggested that the best interpretation of the results is that light brings about changes in the membrane permeabilities to at least K^+ and Cl^-. Since the results with KCl 0.001N resemble closely in magnitude those with Shive's solution (K^+ 0.018n) other ions must have an influence besides K^+.

Conclusions:

There is a response by potential changes in coleoptiles both on turning ON and OFF lights; with increase in intensity change there is an increase in response.

Similar effects take place on the illuminated and shaded sides of the coleoptile, but those on the former are greater.

There is evidence that upon illumination a stimulus passes down the coleoptile from the apex at a rate of approximately 10 millimetres per hour, and it is suggested that this is due to a change in auxin supply.

It is suggested that the potential changes due to commencement and cessation of illumination have brought about by changes in the permeability of membranes to ions. There may be a pigment intermediary, but unfortunately it has not been possible to determine the action spectrum. There is evidence that the light "on" and "off" effects are mediated through the same system.

We could find no evidence to support the hypothesis that the potential changes are bringing about a redistribution of auxin and

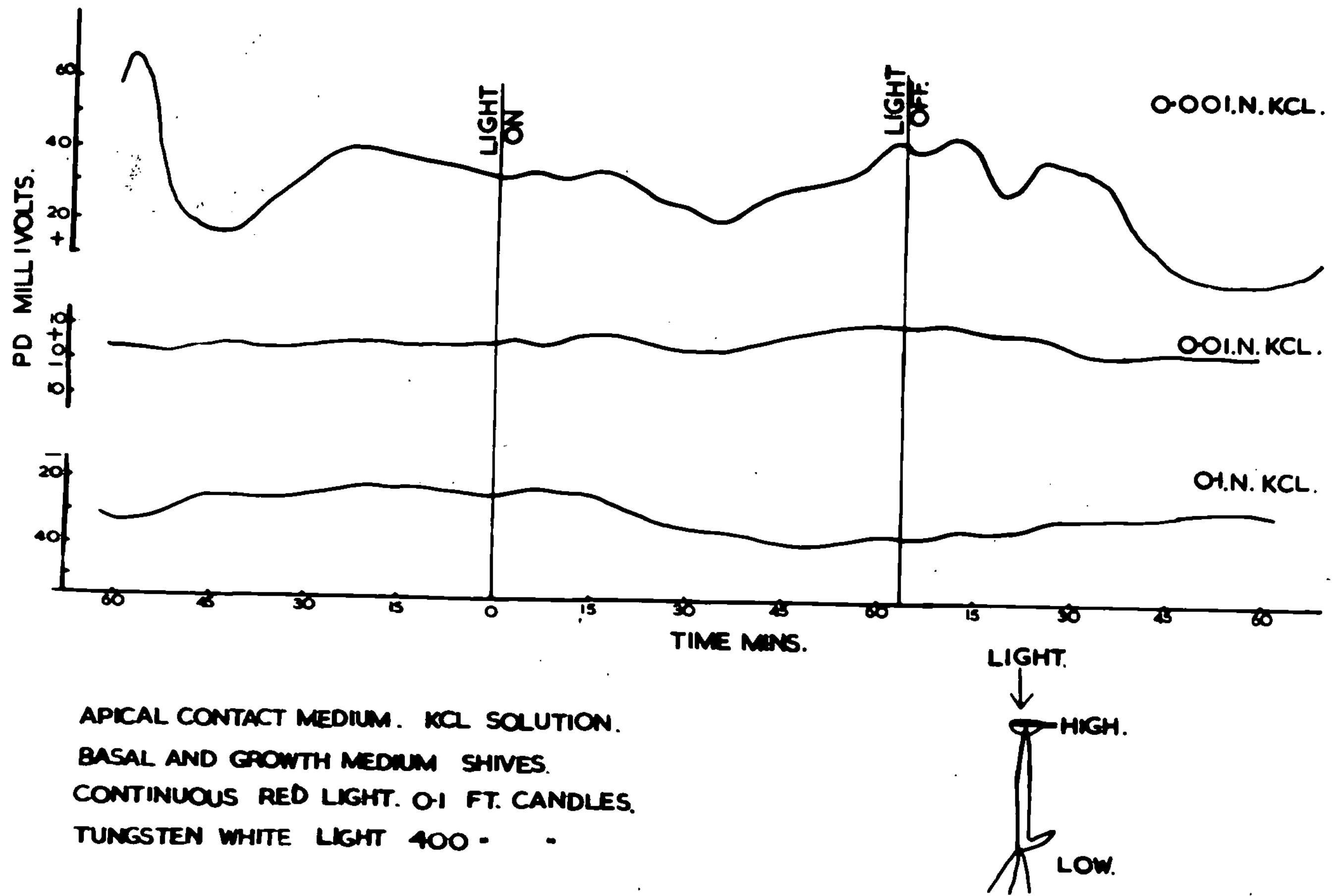


FIG. 7.10.

contend that the potential to which this function has been ascribed is in fact a function of the auxin redistribution.

Oscillations induced by light are discussed in more detail in Chapter 11.

CHAPTER 7.

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CHAPTER.8.

CHAPTER 8

GEO-ELECTRIC RESPONSES OF COLEOPTILE TISSUE AND THEIR RELATIONSHIP TO GEO-TROPIC CURVATURES PRODUCED BY REDISTRIBUTION OF AUXIN.

SUMMARY

A general description of geo-tropism of coleoptiles and then a detailed survey of previous work on the development of transverse electrical potentials across plant tissues, and on changes in the potential pattern, under the influence of gravity, are given.

Experiments we have carried out, which show the development of a transverse polarity and similar changes in the longitudinal potentials of the upper and lower surfaces of the horizontal coleoptile, though differing in magnitude, are described.

In the discussion, these results are compared with those of the other workers and it is concluded that, because of the many discrepancies between the various results, much more work is necessary.

Coleoptiles show a marked negative geo-tropism and within a period of 20 minutes after turning on their sides upward curvatures are visible. Below are listed some of the characteristics of the geo-tropic responses of coleoptiles and some of the ways in which it is generally held to differ from the phototropic response. These details are taken from Went and Thimann's monograph, Phytohormones (1937), and from Anker's (1962), contribution to the Encyclopedia of Plant Physiology.

(1) The geo-response is brought about by unequal growth rates, on the upper and lower sides of the coleoptiles, which are themselves due to a redistribution of auxin. There is no reduction in the total amount of auxin leaving the apex; however there is a greater concentration on the underside of the sheath.

(2) There is a detectable redistribution of auxin within 15 minutes of turning the coleoptile to the horizontal from the vertical.

(3) The auxin redistribution may occur in any region of the coleoptile but there is a decrease in sensitivity with increase in the distance from the apex.

(4) In coleoptiles turned on their sides for less than 30 minutes the curvature starts at the apex and passes down to the base. However with longer periods of horizontal exposure the coleoptile bends simultaneously through all but the most basal zones.

(5) There is no geo-growth reaction comparable with the light-growth reaction.

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The geo-electric effect was first discovered by Bose (1907) when he found that the underside of the peduncle of the lily uriclis became positive to the upper side within one minute after turning to a horizontal position.

Brauner (1926,27,28), reported that the stems of Bean, Sunflower, Tradescantia, Tulip and Narcissus all became electropositive on the underside with respect to the upper on turning to the horizontal. He claimed that stems boiled for 10 minutes gave similar responses and also electolyte solutions separated by parchment membranes reproduced the effect. He concluded that the effects were non vital phenomena and had a physical basis in steaming potentials.

Brauner & Amlong (1933), improved the technique and confirmed the results. Brauner (1942) extended the research on artificial membranes and still considered the effects in plants to be non-vital. In 1959 however, he showed that the effects observed in plants were due to a different mechanism than that previously suggested.

Cholodny (1926,27), was the first to suggest that the transverse movement of auxin in geo-tropism was produced by the electrical potential gradient which was itself established by the action of gravity.

Wilks and Lund (1947), detected a geo-electric potential in Avena coleoptiles with the underside positive to the upper. Also Schrank (1947 & 1951), extended this work on the Oat coleoptile showing that the potential developed within one minute of turning the plant to the horizontal. He also showed that a transverse potential developed along

the full length of the coleoptile but was smaller in the basal than in the apical regions. The maximum value of the transverse potential found was 15 millivolts. He found that similar changes in the longitudinal potential of the upper and lower sides were taking place after displacement to the horizontal position. These were greater in magnitude on the lower side than on the upper which gave rise to the transverse geo-electric potential.

Hertz and Grahm (1958), published an account of experiments demonstrating the development of geo-electric P.D.'s across ion exchange membranes. With a 10^{-3} N. KCl solution above and below the membrane, a P.D. of 20 millivolts with the lower side positive to the upper, was developed, when particles of solid $CaCO_3$, sparingly soluble but capable of exchanging Ca^{++} ions with the membrane, settled onto the upper membrane surface.

In plants it has frequently been demonstrated that freely falling starch grains are characteristically present in some of the cells of the geo-perceptive regions of responding organs. (Audus 1962). Starch grains and not insoluble inorganic salts therefore seem to be the characteristic statoliths of plant cells.

In 1960 Hertz measured the geo-electric potential in Avena coleoptiles using a vibrating condenser system (see Chapter 13) and concluded that the geo-potential only developed 15 minutes after the plants were turned on their sides and had values of up to 60 millivolts.

In 1962 Grahm & Hertz expanded this work and claimed that using gelatine contacts and $Zn/ZnSO_4$ electrodes, they could only detect the effect after 15 minutes as with the vibrating plate.

Wartenberg (1957), doubted the existence of geo-potentials and ascribed the results to the diffusion of ions from the cuticles of the plants. When turned on their sides, he suggested, the heavier salt solution would settle into the electrode of the lower contact but would rest on the plant surface at the upper, giving a concentration difference across the plant surface, leading to the geo-potential. Grahn & Hertz (1962), suggested that with gelatine contacts this would not occur, and that this explained why in their experiments, the potential only developed after 15 minutes. However, with gelatine, even at 15% concentration, the physical properties with regard to the diffusion of ions are little different from those of water, so ions will still diffuse from the cuticle, though there cannot be the mass movement suggested by Wartenberg.

Another point against Wartenberg's suggestion is that even with Shive's solution, Shrank (1947), obtained geo-electric potentials. Shive's solution is relatively concentrated (3 x Shive's plasmolyses coleoptiles), so one would have thought that there would be little net tendency for ions to diffuse into the contact medium from the cuticle.

Also it is difficult to imagine a salt solution concentration gradient across the coleoptile of sufficient magnitude to give the measured values.

Jantsch (1959), is reported as having confirmed geo-electric potentials in Iris japonica and to have found a latent period between exposure and response by change in potential, of 8 hours. The potentials he measured were in the region of 60 millivolts.

If the reported results of Grahm & Hertz (1962), are correct and there is a latent period of 15 minutes, since it has been shown that auxin redistribution has commenced within this period of time, it is obviously impossible for the potential change to be a cause of the redistribution. The experiments reported now using conventional electrodes and liquid contacts were designed to investigate the time course of any development of geo-electric potentials rather than to ascertain their cause.

These experiments were designed to enable the longitudinal potentials of the upper and lower sides to be measured and to eliminate assymetrical salt accumulation in the contact drops. Flowing drop contacts were used which solved any problems due to the latter point; they also enabled measurement of the potentials at room humidities. The importance of this lay in the suggestion of Grahm & Hertz (1962), that the magnitudes of the potentials reported by Schrank (1947), were lower than their own because of the shorting out of the potentials by a surface film of water present at the high humidities used by Schrank. However, having raised this point, they later used a 100% relative humidity to prevent their gelatine contacts drying out, and still they reported values 3 times greater than those of Schrank. As mentioned in Chapter 3, the major contributing transverse potentials in the apical region are probably those of the Water and Donnan free spaces of the cellulose walls. However, with transversly placed contacts and identical contact media these potentials will be zero. Furthermore, one cannot envisage any changes in them as a result of gravity and therefore changes which do occur must be due to the cytoplasmic potentials. As these are contributing little to the measured potential

they may be said to be shorted out, but this applies with gelatine contact also.

The apparatus constructed is shown in Plate 8.1. The construction was of an extremely rigid form so that upon turning from the horizontal to the vertical there was no movement of the contacts relative to each other. As stated above, flowing drop contacts were used.

Schrank (1947) said that when his apparatus was turned it had been necessary to remake contact due to a sagging of the coleoptiles. However in our experiments the base of the coleoptile, the grain and the roots were wrapped in moist cotton wool and firmly inserted into a tube containing Shive's solution and this allowed little sag. Also, with flowing drop contacts, it was possible to position what was to be the lower contact, relatively far off the coleoptile, and still maintain contact. This allowed for any sag when turning the coleoptile on its side, and by this means uninterrupted observations were possible.

All measurements were made in red light at 20°C with Shive's solution, as contact and growth medium.

The first experiments were to determine the electrode effects if any. The electrodes were brought into close proximity so there was a fusion of their individual drops, with no material between. When they were now turned from the horizontal to the vertical, a potential of 0.9 millivolts developed in one case between them, the upper contact positive to the lower, and in another experiment 0.6 millivolts, the lower contact positive. It is difficult to say where the potential was developing. It was unlikely to be at the contact tips where mixing of

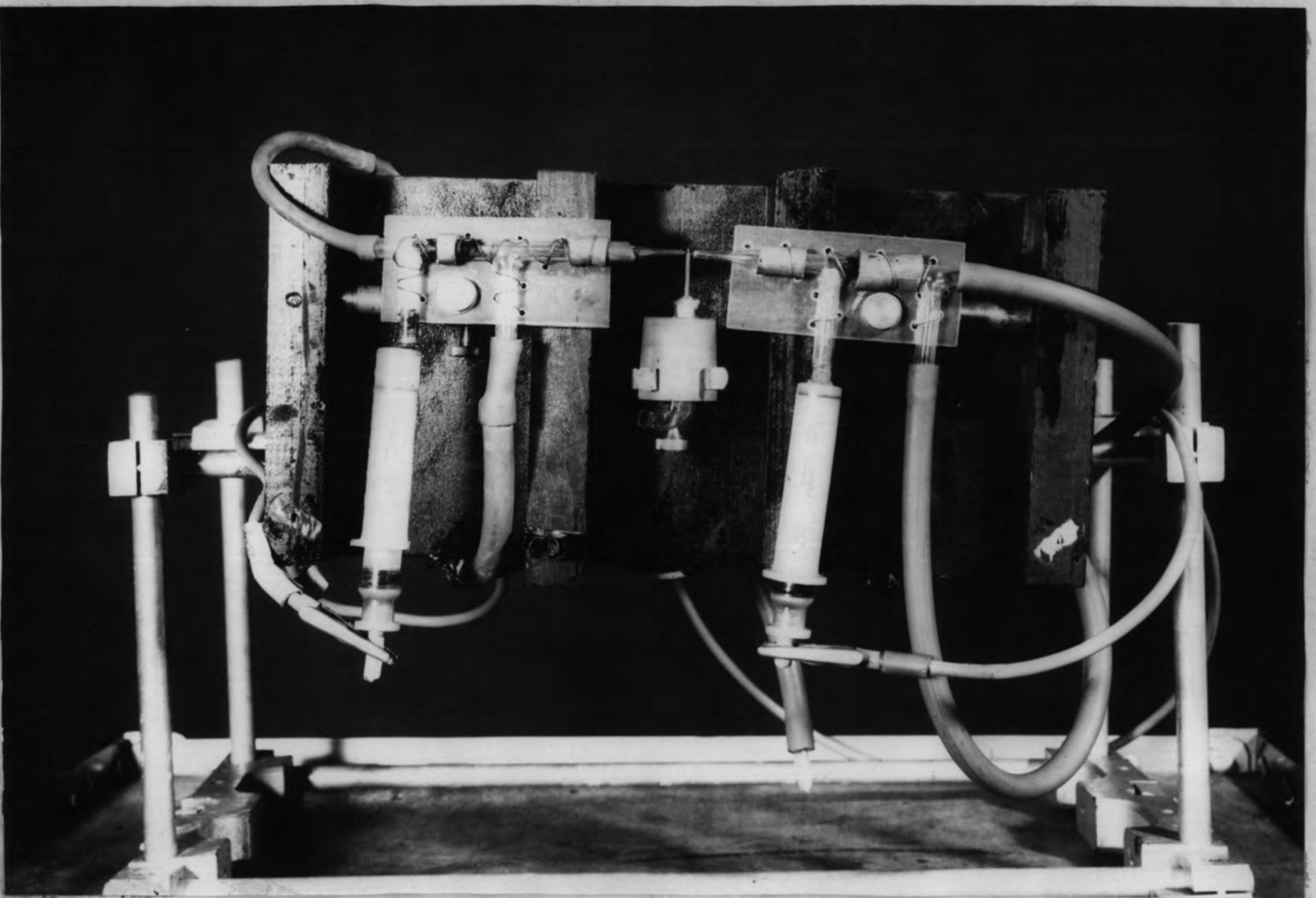

PLATE.8.1.

the two contact media appeared complete and it is probably a junction of the electrodes. The maximum potential developed after 15 minutes but as its value was small and of unpredictable polarity, no further experiments were deemed necessary upon the effect.

For the transverse gravity experiment the contacts were positioned 2 millimetres below the coleoptile apex and the potential left to stabilise. The whole apparatus was then turned through 90 degrees (Plate 8.2.) and readings continued till contact was broken by a positive bend or for other reasons. 12 experiments were performed but 3 showed no tropism (and no transverse potentials) and are not included. Another experiment showed extremely large oscillations in potential before and after turning on its side and would have had an undue influence on the mean and yet another had only been on its side 15 minutes when contact was broken. Both these have been excluded. The remaining 7 have been analysed by taking the time and the potential when the coleoptiles were turned to the horizontal as zero and averaging the potentials read off from the continuous recorder graphs at frequent intervals.

The results are shown in Figure 8.1 in the lower graph.

For the 30 minutes before turning on their sides, the transverse pontential of the coleoptiles is relatively close to zero. Within 3 minutes of turning on their sides the potential of the upper side is negative to the lower. This actually occurs in 5 graphs and in only 1 result was there no development of this negative potential within the 45 minute period.

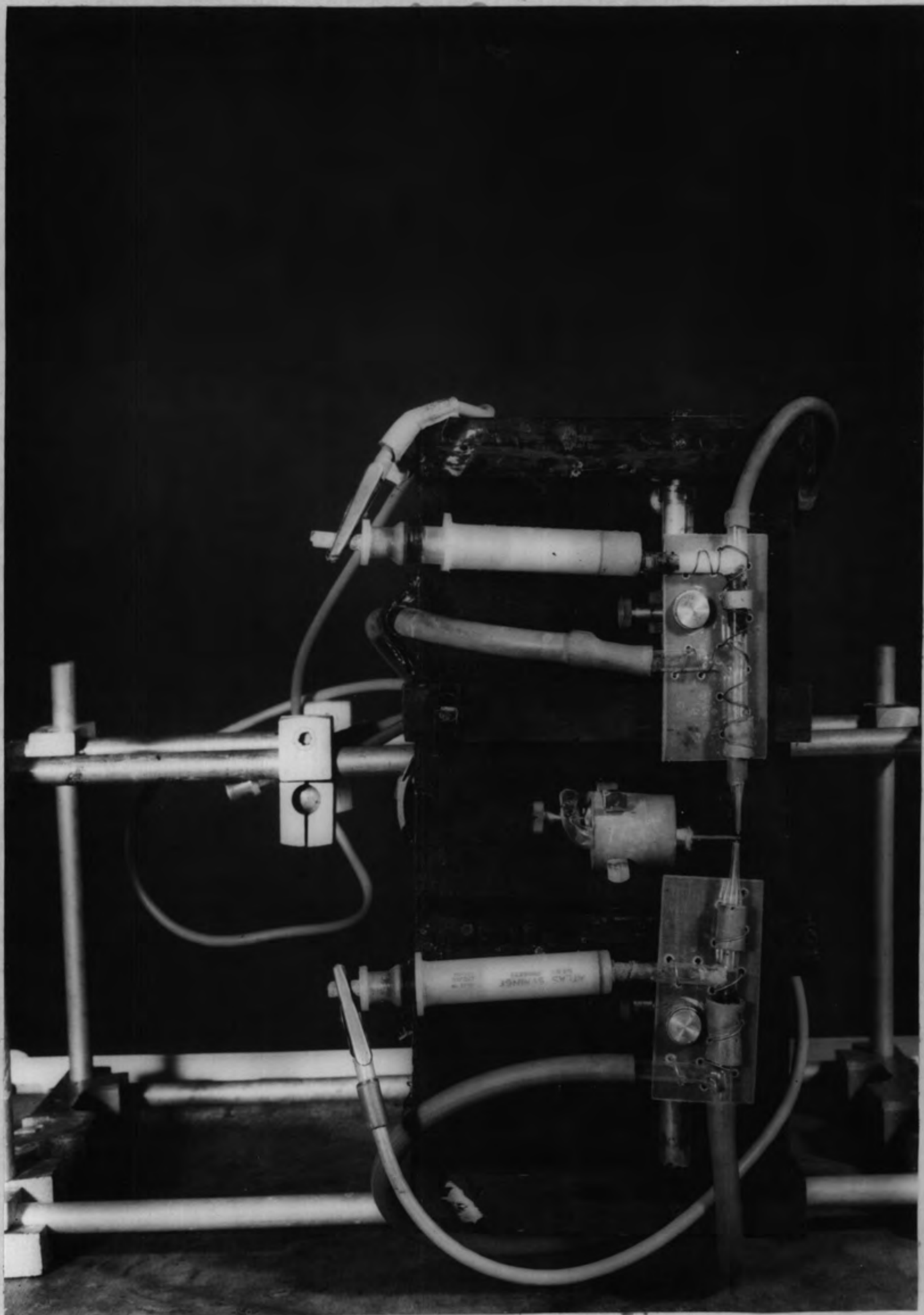

PLATE.8.2.

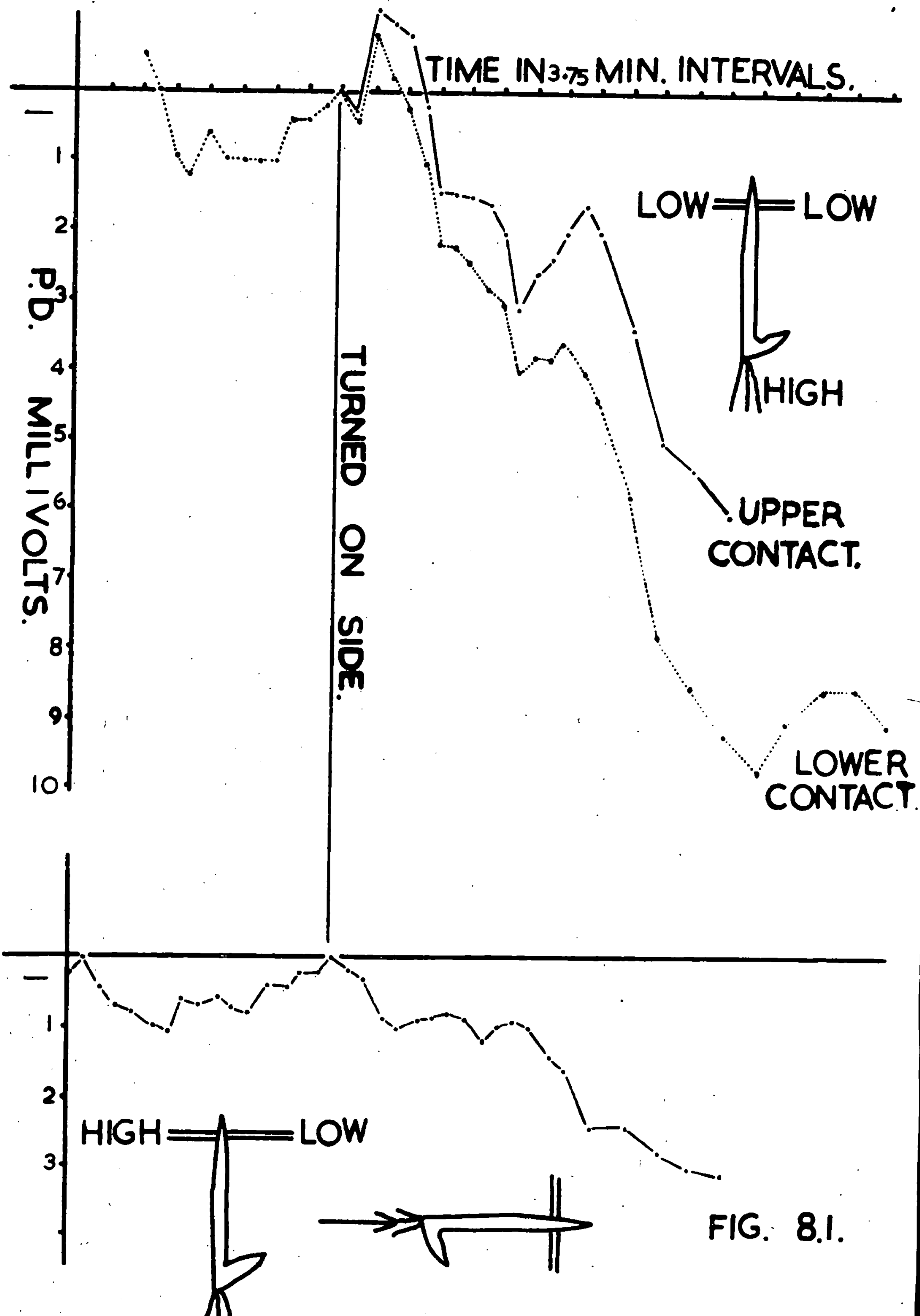

FIG. 8.1.

In all the 5 experiments the negative potential develops within 3.75 minutes but in every case, later on, there is an acceleration of the development of the negative potential which then becomes more marked. This occurs after -

approximately 15 minutes in 2 results,

approximately 22 minutes in 2 results, and

approximately 30 minutes in 1 result.

The mean graph shows the marked acceleration at 20.5 minutes.

Comment on these results is reserved until after consideration of changes in the longitudinal potential.

A contact was placed 2 millimetres below the apex on what would subsequently be the underside and the potential of the base was measured with respect to it. The apparatus is shown in Plate 8.3.

8 graphs were obtained but 3 were discarded for (a) showing large changes in potential before and after turning on the side, indicative of a poor contact; (b) showing no bending response, and (c) broken contact soon after turning to horizontal.

The remaining 5 graphs were analysed as above and the results are shown in Figure 8.1. upper graph. (The potential change of the base with respect to the upper contact, has been calculated from the transverse, and the longitudinal potential of the lower contact).

The true negative potential does not begin to develop in any of the results till after 3.75 minutes and in all of them has developed by 11.25 minutes. The mean graph indicates the negative potential beginning to develop after 3.75 minutes. The slight positive P.D. prior to this is due to one result only.

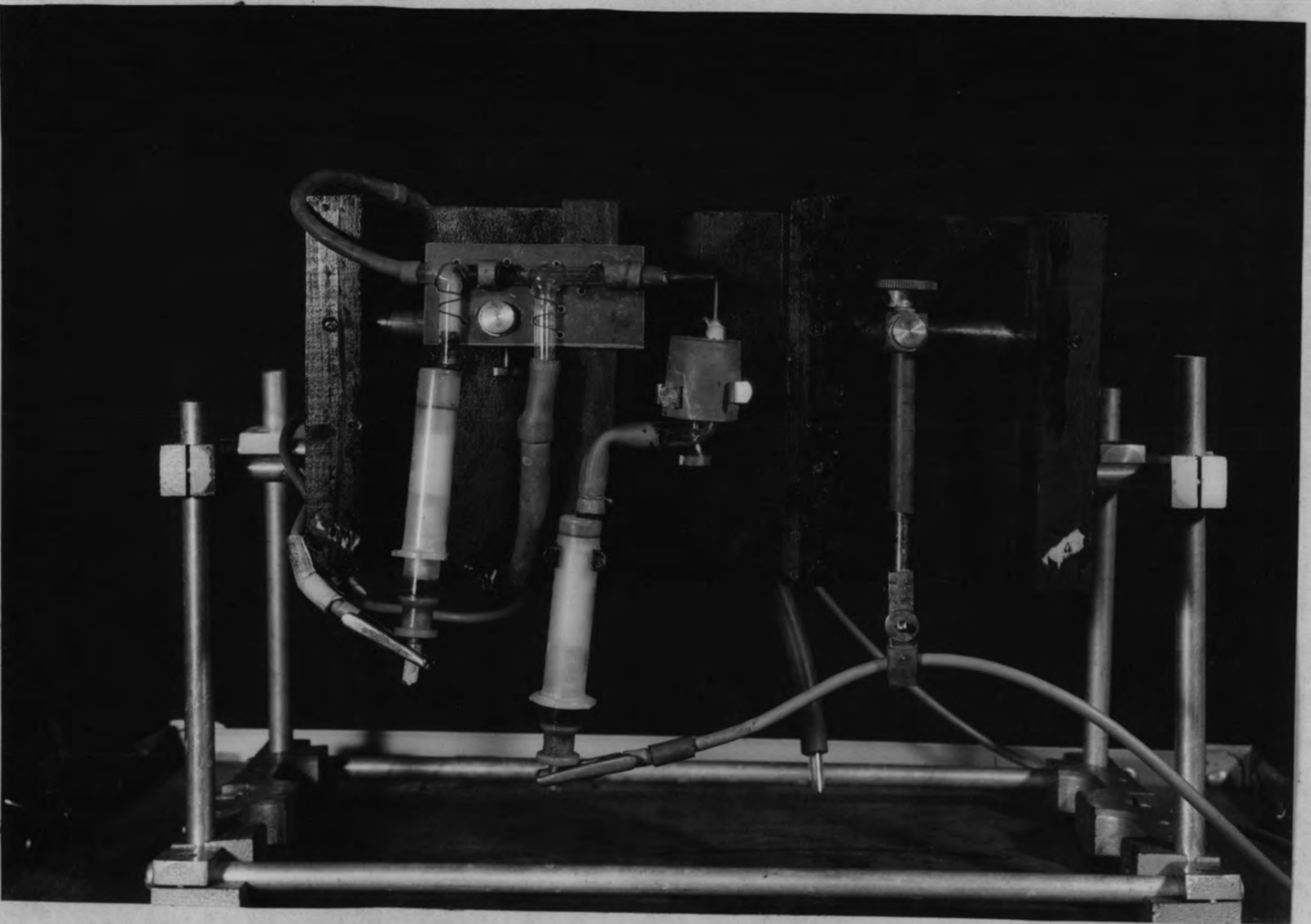

PLATE. 8.3.

In 4 out of 5 results there is an increase in the negative potential from its commencement to a maximum in about 45 minutes and all of these agree on a more rapid increase in the potential between 20-30 minutes after turning on their sides. The other result shows a peak negativity at 20 minutes and then a decrease till recording stopped at 35 minutes.

Returning to the upper longitudinal potential, we find that it has a similar form to that of the lower but differs in magnitude especially after 20 minutes.

When we compare these results with previous workers we find that they are unique, agreeing with some in some details, and others in others, but not showing complete agreement with any other published work.

(1) Our results show a latent period before the development of any potential of approximately 3 minutes but there is a marked development of the transverse potential only 20 to 30 minutes after turning to the horizontal.

Schrank (1947), claimed the potential developed within one minute and his results show a gradual increase up to a maximum at 25 to 30 minutes.

Grahm & Hertz (1962) found no change until 15 minutes after the positional change and a maximum 30-45 minutes later.

(2) The results of the upper and lower longitudinal potentials are similar differing only in magnitude. They indicate that the potential gradually increases from its commencement at 3 minutes to its peak at 45 minutes after the positional change. However the change accelerates at 20 to 30 minutes which is the time when the marked transverse

potential is developing. Both the upper and lower sides show a change to a more positive potential with respect to the basal contact but the lower shows the greater positivity throughout so the lower side becomes positive with respect to the upper.

We therefore agree with Schrank that the changes were similar on the upper and lower sides, but disagree on the form of the change.

The lower side positive with respect to the upper agrees with all workers, but the magnitude (5 millivolts) is smaller than that recorded by Schrank (1947), (10 millivolts) and Grahm & Hertz (1962), (60 millivolts).

It is suggested that the marked potential developing after 20 to 30 minutes and attaining a maximum in about 45 minutes in the horizontal position, corresponds with the potential measured by Grahm & Hertz (1962).

Considering the potentials found in this work during the initial 20 minutes after displacement. From an examination of the transverse potential alone, one might be led to believe it was an electrode effect, for it has a value of less than 1 millivolt and reaches a peak in about 15 minutes. However, examination of the longitudinal results indicates that this cannot be the explanation, for the magnitude is then 3 to 4 millivolts and similar in both the upper and lower contacts. The explanation of Wartenberg (1957), cannot hold, for flowing drop contacts were used and also Shive's solution was the contact medium.

The method of Grahm & Hertz (1962) differs in two respects from that employed by Schrank (1947), and ourselves:-

(a) they use gelatine contacts not liquid drop contacts.

(b) Schrank and ourselves have both used Shive's solution as contact medium. Grahm & Hertz do not record the composition of their gelatine contacts. In earlier experiments, they used KCl solution as contact media, but they do not state if KCl was incorporated in the gelatine contacts.

There are no obvious reasons why these differences should give rise to the different results, i.e. the earlier development of the potential in our own and the far greater magnitude in Grahm & Hertz's.

This research is incomplete but it indicates that a far more detailed study involving several types of contact, contact media and electrodes, as well as further experimentation with vibrating condensers, will be necessary, before any real advances can be made.

It had been hoped to try the geo-effect with other contact media and at various concentrations, but so many experiments failed, more than in any other series of experiments. This was because often contact broke on turning the apparatus through the necessary 90° and this means that not only is that experiment lost, but also the time waiting for the preparation to stabilise. It was felt, with the objects of the research in mind, that it was best to spend the available time on other projects.

In conclusion, it may be said that though geo-electric potentials exist, there are no obvious explanations at present for the discrepancies between the times of development, magnitudes and form of the potential changes, reported by different workers. There is agreement on only one point, the underside becomes positive with respect to the upper. This positivity is not a function of auxin redistribution for the potential

changes are similar top and bottom, whereas, if auxin were involved, since there is an increase in auxin on the lower side and a decrease on the upper, one would have expected the P.D. changes on the two sides to be opposite. What brings about the P.D. changes is not known, though from our work on the influence on their geo and phototropism of filling the coleoptile with mineral salt solution (Chapter 9), it has been suggested that changes in membrane permeabilities are involved in bringing about the geo-response. If this were the case, then, since our measured P.D.'s depend partially on the membrane permeabilities, we would expect these also to change.

CHAPTER 8.

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CHAPTER.9.

CHAPTER 9

THE EFFECTS UPON THE GEO AND PHOTO RESPONSES OF OAT COLEOPTILES OF FILLING THE HOLLOW COLEOPTILE WITH MINERAL SALT SOLUTIONS.

SUMMARY

A detailed survey of the literature upon the effects of filling coleoptile cylinders with various solutions is given and then experiments we have carried out are described. From the experiments it is concluded that the decrease in phototropic curvature in coleoptiles filled with Shive's solution is due to changes in the optical situation but there is also a reduction in geotropic curvature. This is ascribed to an interference of the medium filling the hollow of the coleoptile with the permeabilities of the cell membranes.

In 1934 Du Buy reported that if a hollow coleoptile cylinder was filled with water, the curvature resulting from unilateral illumination was reduced and he claimed the light gradient across the coleoptile was also reduced. He used decapitated coleoptiles, withdrawing the primary leaf, and then filling the cylinder with the liquid.

Sierp and Seybold (1926), reported that the apex of the coleoptile was many thousands of times more sensitive to light than a few millimetres below the apex, yet filling the coleoptile cylinder with a liquid will not alter the optics at the apex, so it is interesting to note that Du Buy reported the effects on decapitated coleoptiles where changes in the light gradient may be effective.

Wilks & Lund (1947), claim that apically intact coleoptile cylinders, cut off above the mesocotyl and filled with Shive's solution, show zero phototropism and with distilled water only a slight positive curvature. With paraffin they gave the same response as when left empty. They also report that when a coleoptile cylinder is filled with tap water or Shive's solution the external longitudinal electrical potential is reduced almost to zero and they claim that it is this shorting out effect that is preventing the development of the tropic responses. Their conclusions were based on only 5 coleoptiles in each group. Schrank (1950), in a more ambitious experiment compared both light and gravity and claimed the reduction in curvature

was proportional to the Shive's shorting solution concentration. He also claimed that the measured shorting out of the longitudinal potentials was similarly proportional to the concentration of salt solutions employed. In a second paper (1953), he found that excised Avena coleoptiles filled with various salt solutions showed a decreased geotropic response. The solutions used were KCl, NaCl and LiCl over a concentration range 0.1 to 0.01 Normal. These results are not straightforward for LiCl had an inhibitory effect late in the response whilst NaCl had its effect early in the response. However, he found that as the concentration of each solution was increased then so was the inhibition, but there was no correlation between the degree of inhibition of the different salt solutions and their relative conductances.

Brauner (1954) criticizes the photo-tropic experiments on the grounds that no attention was paid to the optical effects of the solutions.

Our own criticism rests on the very few coleoptiles involved and on the experimental technique. Schrank had filled the coleoptile cylinders by pushing tapered glass tubes on to their bases and forcing the solution up the middle of the coleoptile, air escaping presumably through the apical pore. Since the liquid was forced in under pressure it may have caused damage to the apical region of the coleoptile and this may have led to a reduction in the response. In particular there

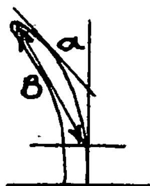
may have been infiltration of the liquid into the apical regions, which Bünning (1955), showed reduced the apical absorption of light.

In the experiments reported now, coleoptiles between twenty and thirty millimetres long were nicked with a razor-blade just above the mesocctyl, and then sharply bent over, when they became neatly severed. The coleoptile cylinder was then carefully pulled away, leaving the primary leaf attached to the mesocotyl. A narrow hypodermic needle with the end ground flat was then inserted into the hollow until its end was seen to lie close to, but not touching, the tip. The plunger was then depressed when the liquid entered the cylinder. The needle's outside diameter was narrower than the inside diameter of the coleoptile cylinder and therefore there was no build up of pressure in the cylinder. Still depressing the plunger, the needle was withdrawn, leaving the cylinder completely filled with solution.

The bases of the coleoptiles were now wrapped in moist cotton wool and individually inserted into small ignition tubes containing Shive's solution, which were placed in a rack within a humidity chamber. It was found necessary to maintain a very high humidity, for in an experiment when this was not done, the control sheaths, containing air, showed no responses, for they had wilted, whilst those containing Shive's solution showed strong responses, not having wilted. The Shive's solution up the centre had provided a capillary water supply to all cells, whilst the severed vascular bundles presumably could not

provide an adequate water supply.

After preliminary experiments, a time of 75 minutes was allowed for the development of the geo-response. For the photo-response, when the coleoptiles were exposed to a continuous unilateral tungstem light of 500 metre-candles intensity, two series of experiments were carried out with 3 hours and 5 hours exposure respectively. After these times, the coleoptiles were laid on a glass sheet with the curvature in the horizontal plane, and placed in a photographic enlarger which gave a three times magnified image on the base board. The outlines of the coleoptiles were then carefully drawn round to give a permanent record and from these the following measurements recorded.



α = the angle of bend.

β = distance the bend has travelled down the coleoptile.

The following table gives details of the results.

TABLE 9.1.

Geo-response

Air filled controls	Statistical Significance	Shive's filled experimental
Angle of Bend = 25.3° Standard error = ± 6.299°	Difference significant at 0.1% level	Angle of bend = 19.14° Standard error = ± 6.89°
Distance bend has travelled down the coleoptile = 11.04 mm Standard error = ± 2.3mm	Difference significant at 5% level	Distance bend has travelled down the coleoptile = 9.3mm Standard error = ± 3.36mm

Photo response. 3 hours exposure.

Air filled controls	Statistical Significance	Shive's filled experimental
Angle of bend = 37.5° Standard error = ± 12°	Difference not significant.	Angle of bend = 35.1° Standard error = ± 8.94°
Distance bend has travelled down the coleoptile = 11.2 mm. Standard error = ± 2.3	Difference significant at 5% level	Distance bend has travelled down the coleoptile = 12.9mm Standard error = ± 3.4

Photo response. 5 hours exposure.

Air filled controls	Statistical Significance	Shive's filled experimental
Angle of bend = 51° Standard error = ± 11.8°	Difference significant at 1% level	Angle of bend = 43° Standard error = ± 8.2
Distance bend has travelled down the coleoptile = 14mm Standard error = ± 2.1mm	Difference significant at 10% level	Distance bend has travelled down the coleoptile =13.1mm Standard error = ± 1.6mm

The statistical analysis is based on the application of Student's t, test for small samples.

The Geo-response

There is a highly significant difference between the degree of bending of the Shive's filled and the empty coleoptile cylinders, being

greater in the latter. There is also a significant difference in the extent to which the bend has passed down the coleoptile, the distance being greater in the empty sheaths.

This in general agrees with Schrank's observations, (1950). He found in the empty controls a curvature of 35.4^{o} (compared with our 25.3^{o}) and with Shive's a curvature of 27° (19.14^{o}). He also noted that coleoptiles filled with solutions formed their curvatures nearer the apex, and that the more concentrated the solution the nearer the apex the curvature developed.

It is suggested that the bend has travelled further down in the controls because there has been a reduction in the geo-sensitivity of those coleoptiles which have been filled with Shive's solution.

They now require longer periods of exposure before showing any responses. This also means that at any time, the degree of curvature of the Shive's filled will probably be smaller than that of the empty sheaths.

Assuming that permeability changes of the cell membranes are involved in the redistribution of auxin, then it is possible that the solutions introduced into the coleoptiles may interfere with these changes. Shive's solution contains both di-and monovalent ions and one would expect ion antagonism to apply, so that the inhibition produced will be smaller than one might expect from its individual ion concentrations. From Schrank's results (1950), with 0.5 x Shive's

solution in which the K^+ concentration alone is 0.009N (total ion concentration 0.029N), there is a bending response of 30° but with 0.01N KCl (1953), he had a bending response of 26°. With 2 x Shive's K^+ concentration 0.038N (total ion concentration 0.116N) the bending response is 21° yet with 0.05N KCl a bending response of approximately 25° is obtained, however with 0.1N KCl the bending response is only 19°. There is therefore some evidence for the theory in these results.

Further evidence is obtained from the result of Schrank's experiment (1953), of placing the air filled coleoptile cylinders in growth media containing the salts, which in other experiments he had used to fill the cylinders.

Comparing their bending responses with those of similar cylinders placed in deionised water, he found that their responses were reduced. It therefore appears to be a direct effect of ions on the tissues rather than any shorting effect on the bio-electric potentials.

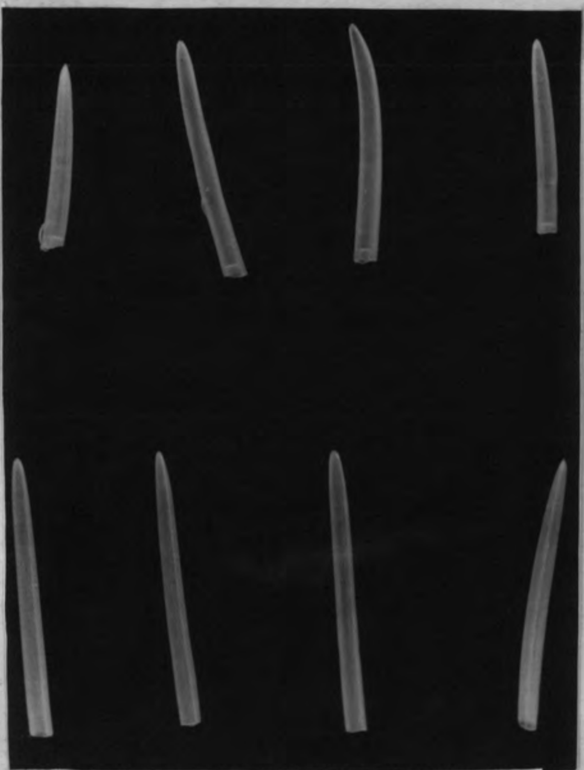
The photo-response.

After three hours exposure to the unilateral illumination, the curvature of the empty sheaths is slightly greater though the difference is not significant. There is, however, a significant difference in the extent to which the bend has passed down the coleoptile, being greater in the Shive's filled sheaths.

After five hours, the empty sheaths now show a significantly greater curvature and the bend has passed further down the coleoptile than in the Shive's filled. This, however, is hardly significant.

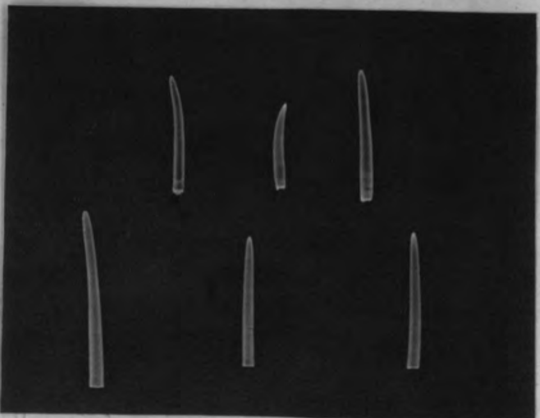
These observations do not confirm those of Schrank who found differences in the curvature after only 2 hours. The curvature of the air filled controls was the greater. It is suggested that Schrank's method of filling the sheath has caused an infiltration of water into the apical tissues, whilst in our experiments this is not possible. This infiltration of water will directly alter the light gradient and influence the response. In our experiments the apical response has been normal, and in up to three hours there is little difference between the two series. (Details of Phototropisms of Avena coleoptiles are given in Chapter 7).

After 3 hours the light dosage has been sufficient to give strong basal curvatures which, as the light gradient is reduced in the sheaths filled with Shive's solution, will be smaller in these sheaths. Plate 9.1. shows the results of laying coleoptile cylinders, air filled or Shive's filled, upon sheets of light sensitive photographic bromide paper, and exposing to parallel light from above. Where the light transmission has been greater the print will be darker. This clearly shows that the light transmission through the filled is greater than the empty, resulting in the darker line down the middle of the filled coleoptiles. This means that filling the cylinders with liquid has reduced the transverse light gradient and therefore one could expect that it would reduce light responses dependent upon recepter mechanisms in this region.



(A).

(B).



(C).

(A). PRINT OF ENLARGED PROJECTED IMAGE.

(B)&(C) CONTACT PRINTS.

IN ALL CASES UPPER ROW OF COLEOPTILES FILLED WITH SOLUTION, LOWER ROW EMPTY.

PLATE. 9.1.

There may of course again be an interference with membrane permeabilities, but such an interference is more likely to be important in the later basal response than in the rapid apical, for the region of apical sensitivity and auxin redistribution is separated from the coleoptile hollow by many cell layers.

There is an important distinction between the geo-and photo-receptive regions, which has a bearing on this theory. It has been shown that with 30 minutes horizontal exposure to gravity, the coleoptile responds by a simultaneous bend throughout the upper regions, so the receptor is not localised at the apex. One would therefore expect with exposure to gravity of longer than 30 minutes any interference with the membrane permeabilities, of any tissues in the coleoptile would be important.

As yet little importance can be attached to the significant difference between the distance the bend has travelled down in the two photo-tropic series after 3 hours for it is only at the 5% level. Further experiments would be necessary to show if this is a real difference.

Conclusions.

1. Coleoptile cylinders filled with Shive's solution show a reduction in the degree of geo-tropic curvature and also in the distance the bend has travelled from the apex, when compared with the air filled controls.

It has been suggested that changes in membrane permeabilities following transverse gravitational stimulation lead to changes in auxin distribution, and therefore any factor that alters membrane permeability is liable to modify auxin redistribution and hence the growth response.

The experimental introduction of salt solutions into the cavity of the coleoptile sheath may not only have some shorting out effects on the electrical potentials, but may also result in a modification of the cell membrane permeabilities and therefore modify the geo-tropic response.

Furthermore any changes in cell membrane permeability may change the bioelectric potential gradients that have been measured and the recorded results of the influence of salt solutions within the coleoptile cavity on the bioelectric potentials may have been determined by this fact rather than by a postulated shorting out effect.

2. There was no significant difference in the phototropic responses of coleoptiles filled with Shive's solution as compared with air filled controls within a period of 3 hours, but after 5 hours the air filled showed a significantly greater response. This differs from Schrank's results and it is suggested that this is due to his experimental method which may have caused the solutions to infiltrate the apex and altered the optical situation. The differences after 5 hours are explained as a basal response when the differences in the optical situation due to the presence of the solution will be of importance.

CHAPTER 9.

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CHAPTER. 10.

CHAPTER 10

EXPERIMENTS UPON THE INFLUENCE ON THE MEASURED ELECTRICAL POTENTIALS, OF JACKETING THE REGION BETWEEN THE CONTACTS WITH MINERAL SALT SOLUTIONS AND INVESTIGATIONS OF THE EFFECTS OF SUCH TREATMENTS ON THE UPTAKE OF P^{32} AND Ca^{45} BY THE PLANTS.

SUMMARY

A detailed survey of previous literature on the influence of mineral salt solutions on bio-electrical potentials in whole plants, is given. Experiments are then described which indicate that the alteration of the potentials by the solutions, is a function, not of the ion conductances, but of the ion species involved. With $CaCl_2$ solution the potential change is ascribed to the setting up of a Donnan potential, whilst with KCl, to a diffusion potential. It is suggested that the Ca^{++} reduces the membrane permeability to anions to zero.

Experiments investigating the influences of the mineral salt jackets on the uptake of P^{32} and Ca^{45} are then described. Changes in uptake that do occur, are ascribed to changes in transpiration rate and exosmosis from the leaves into the jacketing medium, and not to any influence of the salt solutions on the potential.

Rosene (1935), found that if, when measuring the potential between two parts of an onion root, part of the region between the contacts was surrounded by water, the measured potential was reduced, but if surrounded by paraffin then there was no effect. She suggested that the potentials may be responsible for:-

1. Ion transport
2. Absorption of water and solutes
3. Transpiration
4. Growth,

and she concluded "The fact that electric energy is available for the transport of ions, and the additional fact that the E.M.F. of the root is modified only when an electrolyte solution comes in contact with the root, indicates that the available output of electric energy by the root is related to the conductivity of the solution in which it grows."

Wilks and Lund (1947), recorded that when a coleoptile cylinder was filled with tap water or Shive's solution, the external longitudinal polarity was reduced to zero.

Breazeale, McGeorge and Breazeale (1951), suggested that as mineral nutrients are absorbed as ions, and ions carry an electrical charge, then the absorption is an electrical phenomenon, and applying potentials should influence it. They found, using a polargraphic technique and applying the half-wave potential for the cation in which they were interested, that they could increase the absorption of cations when the instrument's negative electrode was connected to the

aerial parts and the positive to the roots. If applied in the reverse direction there was no effect and if voltages other than half-wave potential were applied, there was no influence at all. Anion absorption was not influenced. The potentials applied were several colts. They used both Tomato seedlings and Eucalyptus plants.

Opritov (1958), found that stimulating doses of IAA and NAA applied to Tomato plants increased the uptake of P^{32} and also increased the electrical potential differences. He also found that the same applied to C^{14} uptake from $NaHC^{14}O_3$ containing solutions. Using Wheat seedlings, he altered the P.D.s by applying cadmium chloride solution (5×10^{-6}N), which has the effect of making the region to which it is applied more positive, and found that increased apical negativity increased the uptake of P^{32} from $KH_2P^{32}O_4$ solutions, and vice versa. He also used shunts of deionised tap water, and 0.01N NaCl solutions by laying wicks soaked in the appropriate solution along the side of the plant, and found greatest uptake when the shunting was least, that is, with deionised water.

Gorlanov (1960), showed that shunting Onion roots by immersing in dilute salt solutions reduced the transport of P^{32} labelled phosphate from the leaves, to which it was applied, to the roots. The reduction was greater, the greater the conductivity of the shunting medium. He also measured the electrical potential differences and claimed that these varied in a similar manner to the above. He used

distilled water, tap water and 0.01N sodium chloride as shunting solutions.

Anderson, Wittwer and Buckovac (1961), using Phaseolus vulgaris, found that by applying 240 volts across the primary leaves, the current being applied by copper leads inserted into a moistened charcoal paste on the leaf, they could influence the transport of Ca^{45}, applied as $CaCl_2$ solution between the leaf bases. They found Ca^{45} showed a preferential transport to the anode and suggested that it must be attached to an anionic carrier. In many cases the tissues showed damage.

The preceding reference section is believed to be complete with regard to papers showing the link between ion absorption and transport, and the experimental alteration of Bioelectric potentials in whole plant tissues.

From Chapter 3, discussing the origin of the potentials, it is obvious that if a pathway of lower electrical resistance is provided between the contacts, then the contributions of the other pathways will automatically be reduced. When one puts a liquid shunt round the tissue, one is providing such a pathway. One would expect therefore that the alteration in potential to be inversely proportional to the shunt resistance or directly proportional to the shunting liquid conductance. There are however further complicating factors.

The current pathway before the shunt was inserted would be through the cells of the tissue and the potential measured largely a membrane potential. When the shunt is inserted the current pathway will now be through the cells lying outside the shunted region, and in the shunted region, through the shunt, to an extent depending on the shunt resistance. Now membrane potentials will be set up where the pathway passes out of the cells into the shunt. There will also exist at these points temporary Donnan and diffusion potentials between the solution and the "Donnan and water free space" of the cell walls, and perhaps permanent Donnan potentials with the cytoplasm. All these will depend upon the nature and concentration of the ions in the shunt, and since these will be included in the measured potentials, the effect of the shunt on the potentials will not be dependent solely on its conductance, but also on its ionic composition and concentration.

The first experiments were designed to test the above conclusions. Oat seedlings were used as the experimental material. Dark-grown coleoptiles, which had reached a height of 25 to 30 millimetres were inserted through a hole drilled in the screw cap of a polythene 2oz bottle and were sealed in place with vasaline. They were then left to grow in white tungsten light of approximately 4000 metre-candles intensity at 20°C, till the primary leaf was about eleven centimetres long. They were then ready for use.

The screw cap was screwed onto an inverted polythene bottle so that the primary leaf protruded through a hole in the bottle base. (Figure 10.1). A glass tube welded into the side of the bottle near to the cap allowed the shunting medium to enter and leave. Shive's solution was used as contact and growth medium throughout and one electrode contact was placed in the growth medium, and the other, a flowing drop ring contact, placed on the primary leaf. The apical contact was always the high and the potential measured with respect to the base.

4000 metre-candles of vertical illumination by white tungsten light was used and the polythene shunting bottle was painted black, inside and out, to ensure that changes in illumination intensity brought about by absorption of light by the shunting medium were negligible. The apparatus is shown in Plate 10.1.

After setting up, the potential was allowed to stabilise and then the shorting medium was run into the bottle. The potential was allowed to stabilise again and then the shorting medium was either replaced immediately with another, or the bottle left empty.

The stable potential before any shunt was applied was taken as zero, (on the primary leaf it generally was close to zero) and the stable potential when the shunt was applied was noted as positive or negative with respect to this zero. When one solution was immediately replaced by another, the same zero was still used. There is

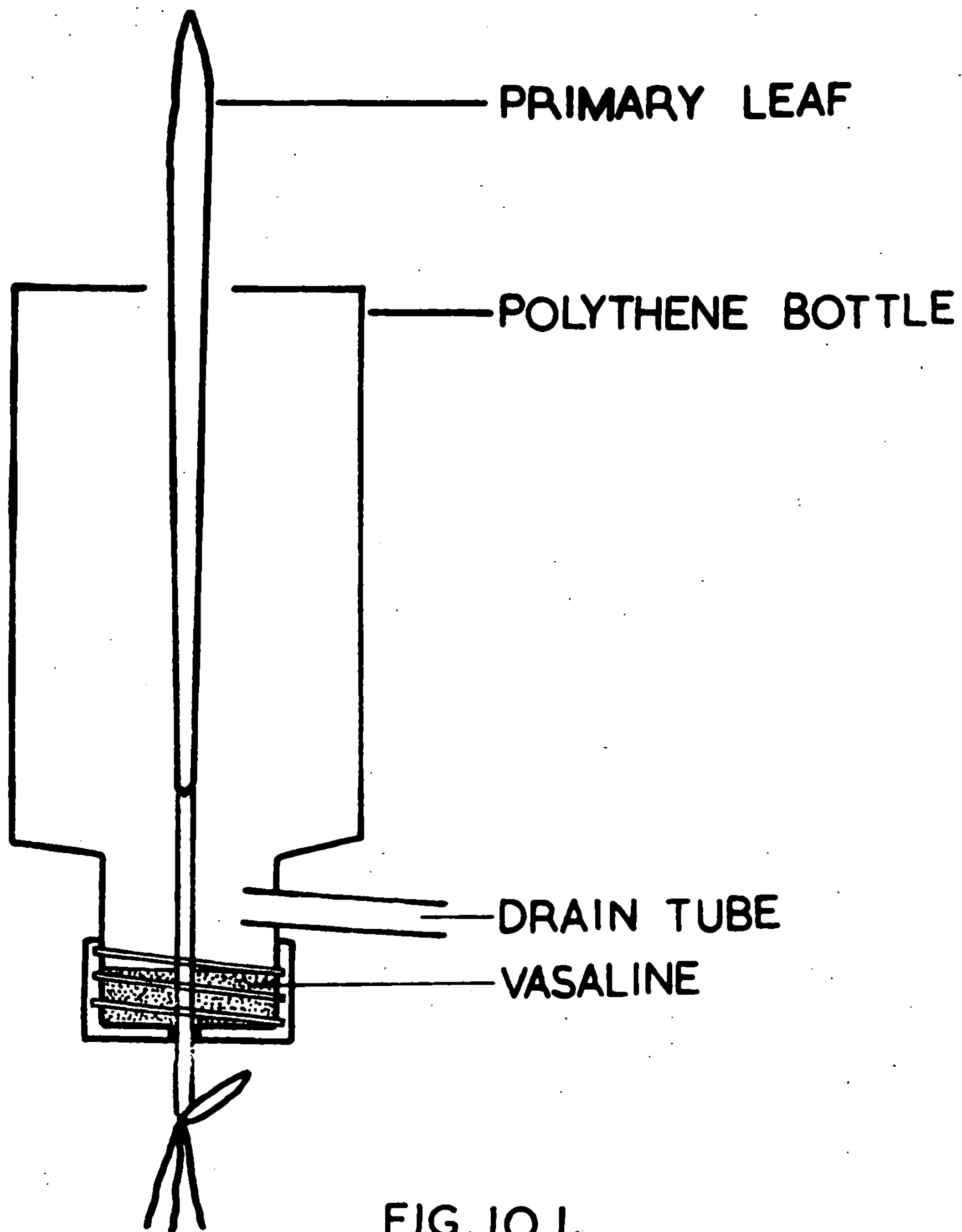

FIG. 10.1.

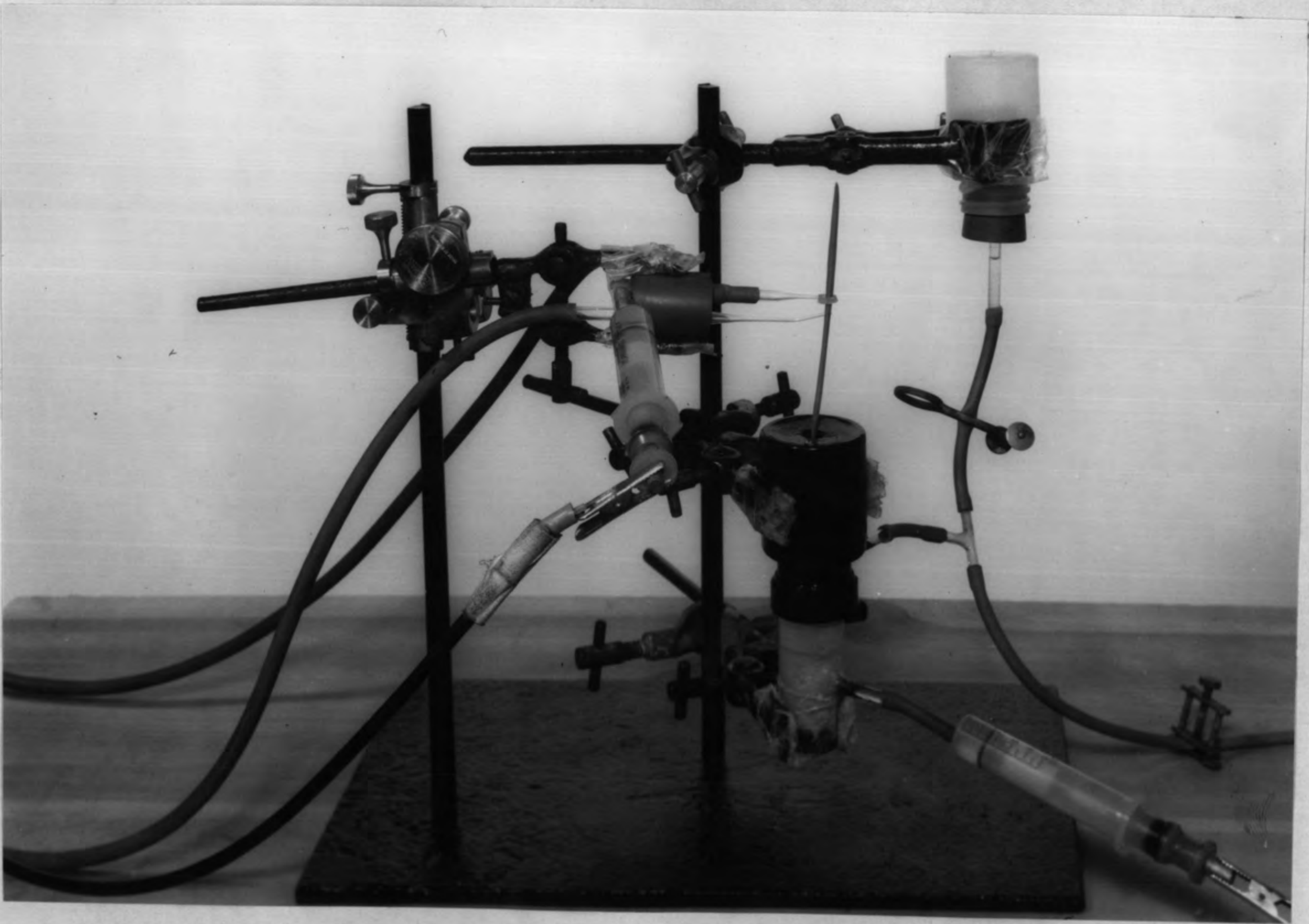

PLATE.10.1.

justification for this procedure. In one experiment when a new zero was attained with no shunt, between each shunt application of different concentrations of potassium chloride solution, in a run of 7 hours, the zero changed at no time more than $\pm$ 1.5 millivolts. In another experiment in which during a period of six hours four different concentrations of potassium chloride solution were successively applied, when finally emptied the zero had shifted by only 2 millivolts.

These, in themselves, are important results for they imply that there is no permanent effect on the measured potentials. This suggests there is little interchange of ions between the plant tissue and the shunting medium.

Table 10.1 shows the results of the first few experiments.

Analysis of Results.

There appears to be little predictable effect with either Shive's solution or deionised water. KH_2PO_4 solution seems to make the potential more negative, but there is no consistent effect of change in concentration.

Dilute solutions of KCl make the potential more negative than the more concentrated.

	Type of Shunt applied	P.D. Change	Type of Shunt applied	P.D. change	Type of Shunt applied	P.D. change	Type of Shunt applied	P.D. change
1	Shive's Solution	-5 mv	Deionised Water	+2 mv				
2	Deionised Water	-3 mv	Shive's Solution	+11 mv				
3	Deionised Water	+5 mv	Shive's Solution	+19 mv				
4	0.0001N KCl	-1 mv	0.001N KCl	+11 mv	0.01N KCl	+3 mv	0.1N KCl	+9 mv
5	0.0001N KCl	-20.5mv	0.001N KCl	-12 mv	0.01N KCl	-4 mv		
6	0.0001N KCl	-1.5mv	0.001N KCl	-10mv	0.01N KCl	-11mv	0.1N KCl	-5 mv
7	0.001N KCl	-14 mv	0.01N KCl	-4 mv	0.1N KCl	+10mv		
	0.001N KH_2PO_4	No change	0.01N KH_2PO_4	No change	0.1N KH_2PO_4	-7 mv		
8	0.001N KH_2PO_4	-7 mv	0.01N KH_2PO_4	-7 mv	0.1N KH_2PO_4	-9 mv	1.0N KH_2PO_4	-9 mv
9	0.001N $CaCl_2$	+10mv	0.01N $CaCl_2$	+27 mv	0.1N $CaCl_2$	+55mv	0.01N KCl	+42 mv
10	0.001N $CaCl_2$	-18mv	0.01N $CaCl_2$	0 mv	0.1N $CaCl_2$	+30 mv		
	0.001N $CaCl_2$	+8 mv	0.01N $CaCl_2$	+12mv	0.1N $CaCl_2$	+25 mv		

TABLE 10.1.

Calcium chloride solution has a far larger effect on the potential than potassium chloride and in all concentrations tried (except for one result) makes the potential more positive. With increase in concentration the positivity increases.

The next experiment compared the effects of different salt solutions but all at the same concentration with respect to the anion.

TABLE 10.2.

Solution	P.D.Change
0.1N KCl	+5 mv
0.1N $MgCl_2$	+18 mv
0.1N $CaCl_2$	+12 mv

It seemed possible, from these results that there was a Valency influence so another experiment was tried.

TABLE 10.3.

Solution	P.D. change	Valency
0.1N KCl	+10 mv	Univalent/Univalent
0.1N NaCl	+6 mv	" "
0.1N $CaCl_2$	+15 mv	Divalent/Univalent
0.1N $BaCl_2$	+13 mv	" "
0.1N $FeCl_3$	-25 mv	Trivalent/Univalent

This clearly confirmed the Valency influence with the uni and divalent salts, but the strong negativation with $FeCl_3$ was unexpected

and further experiments were carried out with this salt.

TABLE 10.4.

Conc of $FeCl_3$	P.D. Change
0.1N	-12 mv
0.1N	-9 mv
0.01N	-1 mv
0.001N	+1 mv
0.1N	-13 mv

These results confirmed the $FeCl_3$ effect and showed that with increase in concentration there was an increase in negativity. This is the opposite of the effect with KCl and $CaCl_2$.

The shunting effects of 0.1N $FeCl_3$ and 0.1N $FeCl_2$ (≡ Cl^- concentration) and 0.1N $FeSO_4$ and 0.1N $Fe_2(SO_4)_3$ (≡ SO_4^{--} concentration) were now compared. Although some oxidation of the Fe^{++} to Fe^{+++} will take place, within the 30 minute experimental period there will have been no great change. Even if oxidation does occur the chloride and sulphate ions will still be at equivalent concentrations and the ferric ions not.

TABLE 10.5

Solution	P.D.Change
0.1N $FeCl_3$	-4 mv
0.1N $FeCl_2$	-3 mv
0.1N $FeSO_4$	+5 mv
0.1N $Fe_2(SO_4)_3$	+5 mv

These results are taken to indicate that the Fe^{++} or Fe^{+++} are not alone responsible for the changes but that the Cl^{-} and SO_4^{--} are also involved. The Cl^{-} ion makes the potential more negative, the SO_4^{--} ion more positive than the unshunted potential. Whether there is an independent effect of Fe^{++} and Fe^{+++} is not shown, but if there is it must be identical for the two ions.

In the next series of experiments, more results on the differing effects of similar concentrations of different ions were obtained.

TABLE 10.6.

Solution	P.D. change		
0.1N KCl	-12	+16)	Univalent/Univalent
0.1N NaCl	-6	+20)	
0.1N $MgCl_2$	+5	+21)	Divalent/Univalent
0.1N $CaCl_2$	+15	+25)	
0.1N $Ca(NO_3)_2$	+11	-)	
0.1N $FeCl_3$	-12	0	Trivalent/Univalent
0.1N $CaCl_2$	+11	-	Divalent/Univalent

The results so far indicate:-

1. It is possible to alter the measured electrical potentials between the primary leaf and bases of Oat seedlings by jacketing part of the region between the contacts with an electrolyte solution.

2. There may be an increase or decrease in the normal potential depending on the nature and concentration of ions in the shunting medium.

3. Taking a standard concentration of 0.1N, it is possible to say that calcium, magnesium, sodium, and potassium chloride solutions all make the potential more positive, but that the effects of calcium and magnesium are similar but greater than those of potassium and sodium which are themselves similar.

We may now consider whether there is any relationship between the specific conductances of the solutions and their shunting effects.

At 0.1N concentration the specific conductances are in the order $KCl > NaCl > CaCl_2 > MgCl_2$ so this shows that there is no relationship between the magnitudes of the potential change and the relative specific conductances of the shunting media.

Figure 10.2. shows a graph plotting the potential change produced by shunting with KCl and $CaCl_2$ against the log., of the shunting medium concentration. The potential changes plotted are the means of all the results available for the respective salts at these concentrations. Also plotted on the same graph are their Equivalent conductances and Activity coefficients at these concentrations.

There is some agreement between the P.D. change and equivalent conductance graphs but the agreement between the P.D. change and Activity coefficient is even more close.

It should in all fairness be pointed out that for dilute solutions the equivalent conductance and activity coefficient graphs usually approximate in form though this is not necessarily so.

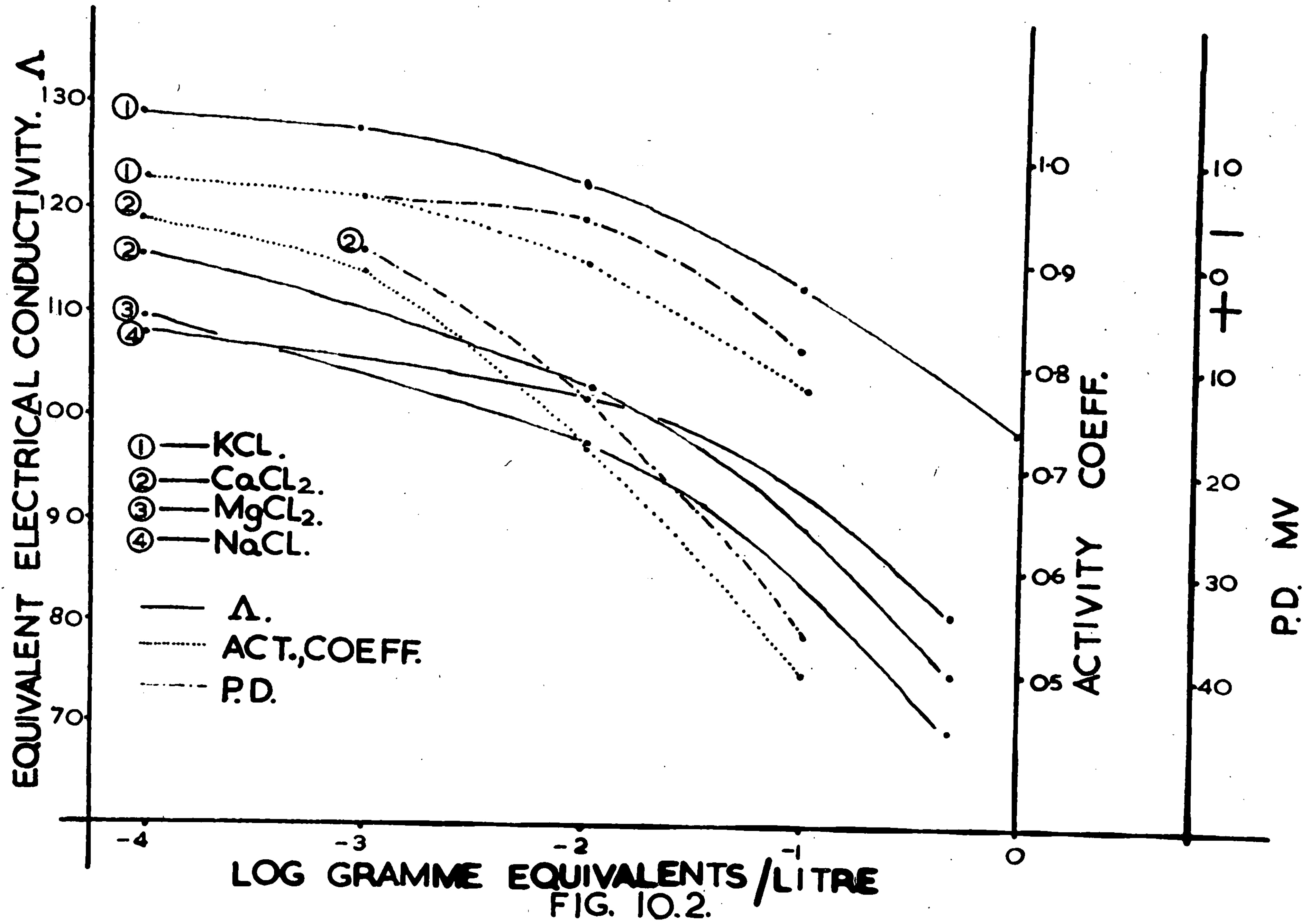

FIG. 10.2.

One important point, however, is that the activity coefficients of the mono-valent salts (K and NaCl) are very alike and considerably different from those of the divalent (Mg, Ba and $CaCl_2$). This is the same as the effect on the potential, so it does appear that our attention should be focussed on the activity coefficients.

The activity coefficient is defined as the ratio of the physically active, thatis apparent concentration, to the actual concentration. In dilute solutions it approaches unity. It may be regarded as a measure of the extent to which an ion or molecule departs from ideal behaviour.

Let us consider a single membrane separating two solutions. The P.D. across this membrane will be proportional to the log of the Activity ratios.

Let the outside be infinitely dilute (Activity $A\infty$), and the inside solution at a concentration C^1, Activity AC^1.

$$\therefore \quad PD\infty = k \log \frac{A\infty}{AC^1}$$

Consider a change to an external concentration C^2. Activity AC^2, the internal concentration remaining the same.

$$\therefore \quad \text{New } PD_{c2} = k \log \frac{AC^2}{AC^1}$$

$$\therefore \quad \text{PD Change} = PDC^2 - P.D\infty$$

$$= k \left(\log \frac{AC^2}{AC^1} - \log \frac{A\infty}{AC^1} \right)$$

$$= k \log \frac{AC^2}{AC\infty}.$$

But $\frac{AC^2}{AC\infty}$ is the activity coefficient at concentration C_2.

$\therefore$ PD change is directly proportional to the log., of the Activity coefficient.

Figure 10.3.A. shows the result of plotting our P.D. change results against the log of the Activity coefficient for KCl(2) and $CaCl_2$(1). It is seen that they both give reasonable straight line graphs.

Diffusion, Donnan and membrane potentials will all give graphs of this form, for all depend on a log concentration ratio. An odd feature of the results is that the effect is the opposite expected for it is at the higher concentrations, when the actual activity is lower than expected from the theoretical concentration, that we get the largest potential changes. This will be discussed later.

There are certain features of the results which give clues to the actual systems involved. Immediately after changing or applying the shunt there were rapid changes in potential, but only in a very few cases was the potential then established maintained at this value.

A case in point is shown in Figure 10.4.A. The more usual effect was a rapid change in potential followed by slower changes of which the most frequent form is shown in Figure 10.4.B and C. Large changes occur on emptying but are of no characteristic form. We would expect the rapid changes on filling and emptying, as we are causing immediate

A

PD CHANGE MV

+

−

50

30

10

10

−3000 −2000 −1000 0

LOG. ACTIVITY COEFF. ×10⁴

①

②

B

PD CHANGE MV

+

−

100

80

60

40

20

20

−1 −2 −3 −4

LOG KCl CONC.

③

①

②

FIG. 10.3.

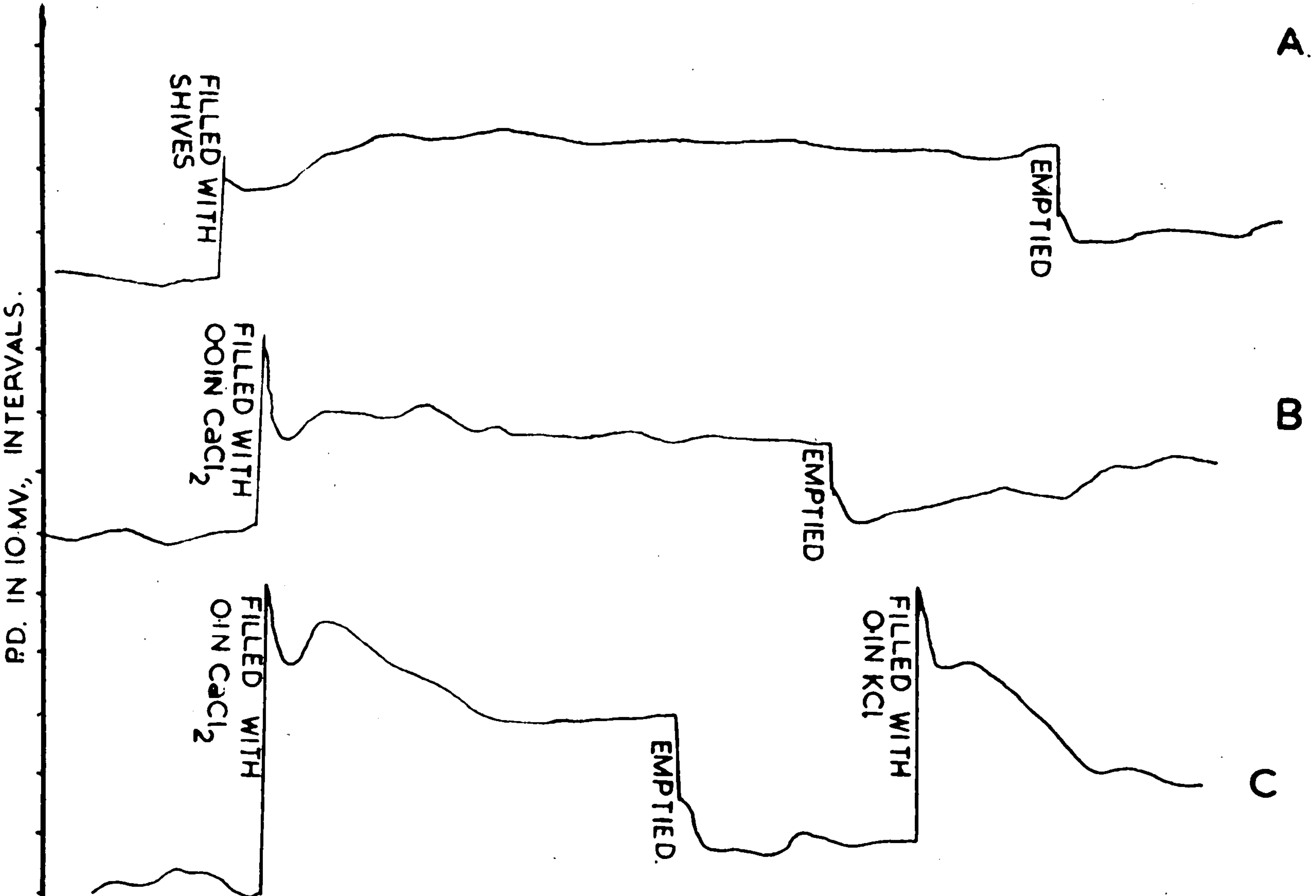

FIG. 10.4.

alterations in the potential contributions of the other pathways and if this were the only effect, the changes should be proportional to the specific conductances of the solutions.

It seemed possible that the P.D. changes immediately on applying the shunt would be proportional to the specific conductances of the solution. It was however impossible to obtain sufficient results from the present data, where the usual experiment was to change from one salt solution to another, rather than emptying between each change.

Our results show that the final P.D. change is not dependent upon the specific conductance, and as the shunt is so large compared with the tissues, it is reasonable to assume that in all concentrations employed, its resistance has been so much lower than that of the tissues and therefore its actual shorting out effect relatively constant. We must therefore consider the other ways in which it can influence the potentials.

From previous observations it seems likely that transverse diffusion potentials between the shunt and the tissue will be transient, lasting only a few seconds, and cannot explain the results. Longitudinal diffusion potentials could last longer but the effect will be similar at the two ends of the shunt and will cancel out. If the changes are due to membrane potentials then the permeabilities to Na^{+} and K^{+} must be the same yet differ from Ba^{++}, Ca^{++} and Mg^{++} which

must also be similar. Furthermore it would be necessary to assume that the internal Na^{+} and K^{+} concentrations are alike and also the Ba^{++}, Ca^{++}, and Mg^{++}. These assumptions are unlikely to be true and therefore membrane potentials, though no doubt contributing something to the results, are unlikely to be the main explanation. We are therefore left with Donnan potentials which may well be responsible, for with Donnan potentials one would expect ions of similar valencies and activity coefficients to have similar effects at the same concentrations.

Since at 0.1N concentration the chloride ions were all in the same concentrations, the P.D. effects can be ascribed to cations, with anions as the fixed charges.

The following Table 10.7 shows the potential existing between Donnan phases of various activities and various concentrations of KCl and $CaCl_2$ solutions corrected for activity.

TABLE 10.7

Donnan Phase Activity	0.1	1.0	10	100	1000	10000	Milli - equivalent/litre
Conc of KCl .0001N	-12.5	-59	-116.7	-175	-233	-290	(A)
.001N	-1.3	-12.5	-59	-117	-175	-233	
.01N	0	-1.3	-13.3	-60.5	-118	-177	P.D. in millivolts
.1N	0	0	-1.55	-15.3	-64	-122	

Donnan Phase Activity	0.1	1.0	10	100	1000	10000	milli - equivalents/litre
Conc of $CaCl_2$ .0001N	-6.25	-29.25	-58.5	-87.5	-116	-145	
.001N	-0.7	-6.8	-30.5	-59.5	-88	-117.5	(B)
.01N	0	-0.78	-7.7	-32.25	-61	-90	
.1N	0	0	-1.12	-10	-36.2	-65	

TABLE 10.8

Activity of Donnan Phases between which P.D. exists	0.1 & 1	1 & 10	10.100	100 & 1000	
Concentration of KCl .0001N	-46.5 (+35) ↓	-57.7 (+11) ↓	-58 0 ↓	-58 0 ↓	(A)
.001N	-11.2 (+10) ↓	-46.5 (+34) ↓	-58 (+11) ↓	-58 (+1) ↓	
.01N	-1.3 (+1) ↓	-12.0 + (+10) ↓	-47.2 (+33) ↓	-57.5 (+8) ↓	
.1N	0 ↓	-1.55 ↓	-14 ↓	-49 ↓	

(B)

Activity of Donnan Phases between which P.D. exists		0.1 & 1	1 & 10	10 & 100	100 & 1000
Concentration of $CaCl_2$	.0001N	-23 (+17)	-29 (+6)	-29 0	-29 0
	.001N	-6 (+5)	-23 (+16)	-29 (+5)	-29 0
	.01N	-1 (+1)	-7 (+6)	-24 (+15)	-29 (+3)
	.1N	0	-1	-9	-26

(C)

Activity of Donnan Phases between which P.D. exists		0.1 & 10	1 & 100	10.1000
Concentration of KcL	.0001N	-104 (+146)	-116 (+11)	-116 0
	.001N	-58 (+45)	-105 (+46)	-116 (+11)
	.01N	-13 (+11)	-59 (+44)	-105 (+43)
	.1N	-2	-15	-62

(D)

Activity of Donnan Phases between which P.D. exists		0.1 & 10	1 & 100	10 & 1000
Concentration of $CaCl_2$	.0001N	-52 (+22)	-57 (+5)	-58 (0)
	.001N	-30 (+23)	-52 (+21)	-58 (+5)
	.01N	-7 (+6)	-31 (+21)	-53 (+18)
	.1N	-1	-10	-35

Consider if we are dealing with only one Donnan phase at either top or bottom of the shunted region. If the external medium was diluted, therewould be changes in potential which would be small at the more concentrated concentrations and larger between the more dilute reaching a maximum of 58mv for a tenfold change in KCl concentration and 28 mv for $CaCl_2$. The actual effect observed was a large change between the more concentrated and small changes between the more dilute.

Table 10.8 shows the potentials existing between pairs of Donnan phases, as though the pathway of least resistance was through Donnan phases of differing exchange capacities at the top and bottom of the shunt, whose potentials would be opposed. Also shown in brackets are the changes in potential on changing from one shunting concentration to another. These show that at certain Donnan phase concentrations the potential changes would be greater between more concentrated solutions than dilute.

If the upper phase had the greater activity, upon application of the shunt, the apex would become more negative. Upon dilution of the shunt this negativity would increase. This is not what experiments have shown. The reverse, when the apex would be positive on application of the shunt and yet more positive on dilution is also not found.

This enigma it is suggested, is due to the shunted area contributing a large negative potential to the apical P.D., before the application of the shunt. Considering just the shorting out effect,

when the shunt is applied the apex will become more positive. If the upper phase has the greater Donnan activity then this will generate an apical negativity opposing the above. Assuming the positive potential to be greater, the overall potential will be positive. Upon dilution of the shunting medium the negative Donnan potential will increase and therefore the measured positive potential decrease.

This also would explain why one gets positive effects with concentrated KCl solutions and the negative effect with the dilute.

Considering the Donnan phase activities to be 1.C and 100 millequivalents per litre and the shorted out potential as -50 millivolts. With $CaCl_2$ solution (Table 10.8D) the P.D. changes would be:-

conc	Theoretical P.D. change mv	Actual experimental value mv.
0.0001N	-7	-5
0.001N	-2	0
0.01N	+19	+15
0.1N	+40	+35

The agreement is very close.

However with KCl solution the respective values are, from Table 10.8.C:-

Conc	Theoretical P.D. change mv	Actual experimental values mv
0.0001N	-66	-8
0.001N	-55	-6
0.01N	-9	-4
0.1N	+35	+10

In this case there is a considerable divergence. In fact, whatever values are chosen for the Donnan phase activities, the effects

with KCl will always be greater than with $CaCl_2$, yet in the experiments the reverse was the case. Also the results with $FeCl_3$ are not explicable as Donnan potentials, for, since Donnan potentials generated with a trivalent cation will be smaller than any of the others, the overall potential should be more positive than the others. experiments have shown that the potential change is always negative.

It is suggested that with $CaCl_2$, the permeability to chloride ions is zero and the potential, a Donnan potential; with KCl, the permeability to chloride ions is not zero, and the potential is a simple membrane potential.

We have evidence that this may be the case for KCl, from the experiment reported in Chapter 4 which investigated the influence of changes in the concentration of the KCl contact medium at the apex of a coleoptile. This showed that there was a 22 millivolt change per tenfold change in the KCl concentration over a range of concentrations. If the permeability to Cl^- was zero this value would have been 58 millivolts, at least in the more dilute.

Since the apical region of the coleoptile forms the basal part of the shunted region, we may use the experimental values obtained before, to calculate values of the potential change in the upper region of the shunt. The results of doing this are seen in Figure 10.3.B. (1) is the line for the coleoptile apex. (2) is the line for the experimental P.D. change on application of KCl shunting media. (3) is

the theoretical line for the P.D. change in the shunt upper region. This line has a slope of 27 millivolts/tenfold KCl change. The positivity increases with dilution confirming the greater relative cation permeability. Also, since the slope of the upper region is slightly greater than that of the basal, so also must be its relative cation permeability.

Since the P.D. of the seedling we measure before the application of shunts is approximately zero, if we allow for the 50 millivolts change due to the shorting out, and the P.D. change at the basal region of the shunt, then the resultant P.D. will be that of the upper region with respect to the shunt. This in fact has been done in producing (3). This indicates that the P.D. of the upper region is considerably greater than that of the lower at all concentrations, and because the permeabilities are not very different, this indicates that the ion concentration of the upper regions of the shunt must be greater than those of the basal. This bears out the result with $CaCl_2$, where the upper region had the greater anion concentration.

With the $FeCl_3$ solution, the effect is ascribed to a small permeability to the ferric and ferrous ions with a larger permeability to the chloride ions. This is borne out from one experiment upon changing the concentration of $FeCl_3$ which gave the result

TABLE 10.9

Conc	P.D. change
0.1N	-9 mv
0.01N	-1 mv
0.001N	+1 mv

i.e. the opposite effect to $CaCl_2$ and KCl but what one might expect if the membranes are more permeable to anions than to cations.

CONCLUSIONS

The changes in potential produced by shunting are ascribed to the production of membrane potentials. In the case of calcium chloride and related salts, there is low permeability to the anion and the potentials approximate to a Donnan potential. The anion concentration in the apical region has a value of approximately 100 mille-equivalents/litre, about one hundred times as large as the basal region of the shunt.

With KCl the permeability to Cl^- is increased but that of K^+ is still greater. The ion concentration in the upper region of the shunt is greater than that in the basal. The suggestions with regard to KCl and $CaCl_2$ are in agreement with the accepted effects of these salts on protoplasm (See Heilbrun 1943).

With $FeCl_3$ there is lower permeability to Fe^{+++} than to Cl^-.

Although hitherto, the potential change has been related to the activity coefficient, and though this relationship will hold for a single membrane potential, the relationship in these experiments seems to have been largely fortuitous, for with both $CaCl_2$ and KCl a similar result would have been obtained without any allowance for activities.

More information upon ion uptake was sought by the use of radio-active tracers.

Oat seedlings were grown exactly as before. In the first series of experiments on P^{32}, they were then placed one through each of sixteen holes in the bases of polythene drinking tumblers. Unfortunately the difficulties of efficiently sealing the bases of sixteen seedlings with vasaline, were so great, that after several experiments had been ruined due to shorting medium leakage into the growth medium, the method was abandoned, and recourse made to plants mounted individually in inverted polythene bottles as described before.(Second and third experimental series).

For the first series of experiments, after the plants had been placed in the growth medium containing P^{32}, with the various "shunting" solutions in place, for 24 hours, they were divided into portions. The separate fractions were weighed and then they were digested in 5 millilitres of boiling 10% nitric acid for four hours; the volume was then made up to 10 millilitres and the radio-activity counted in a Veal liquid counter tube (Mullard 124/01). The count was then expressed as rate/grm, fresh plant material. This was unsatisfactory, in that, on the mid-regions vasaline was present, so later each result was expressed as rate/plant.

In the second series of experiments Ca^{45} was used in the growth medium and in the third series Ca^{45} and P^{32} were used simultaneously.

In all these cases, the plant parts were separated and digested as above. The volume was then made up to 5 millilitres of which one was withdrawn, placed on an aluminium planchet, dried, and then the radio-activity counted.

In all cases the results have been corrected for background and decay. In most cases the total count was sufficient to give a standard deviation of less than 2.5% on the actual count (i.e. count exceeds 1000).

Experiments of the first Series.

The P^{32} growth medium was made up from 100 millilitres of Shive's solution to which was added 0.5 mls of a P^{32} solution. This had been prepared by adding the supplied 1.65 mls of carrier-free P^{32} phosphate solution in dilute hydrochloric acid, activity 1 millicurie to 10 mls of distilled water. The P^{32} activity present in the 100 mls of Shive's solution was therefore $\left\{\frac{1 \times 0.5}{11.65}\right\}$ millicuries.

In this series of experiments, $MgSO_4$ was often used as a shunting medium as it gave a slightly greater positive potential change than $MgCl_2$ and it was thought undesirable to use $CaCl_2$, as later Ca^{45} experiments were to be carried out.

Experiment 1.

To compare the uptake of P^{32} by plants shunted with N $MgSO_4$, with unshunted plants.

The plants were divided into three regions after treatment:-

(a) Portion of leaf above the shunting liquid.

(b) The plant tissue within the shunted region.

(c) The plant tissue below the shunted region including the grain and the roots.

The results are shown in Table 10.10.

Analysis of the results.

The P^{32} content in all regions of the shunted plant is lower than that in the control. As could be expected, the basal regions contain more P^{32} than the apical.

Since N $MgSO_4$ is rather concentrated it may have affected the tissues osmotically, so the next experiment made use of a more dilute solution.

Experiment 2.

To compare the uptake of P^{32} by plants shunted with 0.1N $MgSO_4$, with unshunted plants.

TABLE 10.10

Background = 280/20 min = 14 cpm

N $MgSO_4$ shorting medium = 296/20 min = 14.8 cpm

Corrected for 5 days decay.

Shunts used	Regions of Plants	No. of Plants	Wt. of Plants in Mgs	Actual Count	Count per minute per Plant	Counts per minute per grm fresh wt.
Control	Basal Region	7	425	13,245 /2 mins	1.214	39,870
Plants	Shunted Region	7	310	6,069 /10 mins	108	4,110
Unshunted	Apical Region	7	210	1,504 /10 mins	25	1,661
Plants	Basal Region	8	470	11,419 /2 mins	912	31,070
Shunted with N $MgSO_4$	Apical Region	8	140	819 /15 mins	7.5	861
Shunted with N $MgSO_4$	Shunted Region	8	400	6,184 /10 mins	96.5	3,800

The results of this experiment are shown in Table 10.11.

Analysis of these Results

It is difficult to discern any differences between the control and treated plants with regard to the P^{32} uptake. However the uptake of P^{32} in this experiment is much greater than that which occurred in the previous one, indicating that although growth conditions were controlled as closely as possible it will be difficult to make valid comparisons of the actual magnitudes of the activities taken up in the successive experiments.

Discussion of Experimental Results

TABLE 10.11.

Background. 831/60 mins = 13.7 cpm.

0.IN $MgSO_4$ shunting medium = 138/10 mins = 13.8 cpm.

Corrected for 7 days decay.

Shunting Solutions applied.	Regions of Plant.	No. of Plants.	Wt of Plants. Mgs.	Actual Count.	Counts per minute per plant.	Counts per minute per gramme fresh weight.
Unshunted	Basal	4	300	12,204/ 2 mins.	2080	27,810
	Region	4	270	12,205 / 2 mins.	2080	30,890
Control	Shunted	4	220	6,829/ 5 mins.	465	8,418
Plants	Region	4	220	8,256 /5 mins.	555	10,200
	Upper Region	4	150	5,941 / 5 mins.	395.	10,730
		4	90	3,085 /5 mins.	208	8,971
	Basal	4	280	11,096 /2 mins.	1892	27,070
Plants	Region	4	270	13,809 /2 mins.	2360	34,950
		5	280	31,736 /5 mins.	1735	30,980
Shunted	Shunted	4	160	5,953 /5 mins.	405	10,080
with	Region	4	220	17,222/ 10 mins.	585	10,640
0.IN		5	220	15,843/ 10 mins.	430	9,777
$MgSO_4$.	Upper	4	160	8,300/ 10 mins.	280	6,987
	Region	4	80	3,092/ 5 mins.	206	10,340
		5	120	8,118/ 10 mins.	225	9,109

The experiments have shown so far that placing a liquid shunt around an Oat seedling may lead to a reduction in P 32 uptake when compared with one without such a shunt. This difference is not marked. Since by shunting, the area of leaf surface available for transpiration will be considerably reduced, one would expect the transpiration stream up the xylem vessels also to be reduced and therefore the transport of ions up the plant. Perhaps their uptake by the roots could also be reduced. Therefore the result obtained is perhaps that which we might have expected.

An experiment was now carried out to see if there was any significant reduction in the size of shunted plants when compared with normal.

Two batches of 15 plants were prepared in the usual manner, one batch was shunted with O.INKCL and the other left empty, 48 hours later the plants were harvested and their sizes compared using Student's t method for small samples. The results are shown in Table 10.12.

TABLE 10.12.

	Empty Control.	Shunted with 0.INKCL
Overall plant length. (Base of grain to primary leaf tip).	15.6cms. ± 1.002.	15.5 cms. ± 0.7.
	Not Significant.	
Length of 2nd leaf. (Base of grain to 2ndry leaf tip).	11.15 cms. ± 1.48.	9.06 cms. ± 0.63.
	Significant at the 1% level.	

Analysis of Results.

The 2nd leaf was immersed intthe shunt and this has caused a significant reduction in its size, due presumably either to oxygen lack or to a reduction in translocation of essential materials because of the lack of transpiration.

This suggested that in subsequent experiments more significant results might be obtained if the leaf tips were sub-grouped according to size. The area of primary leaf not immersed in the shunt will vary according to its length and hence the magnitude of the transpiration stream will also vary.

The groups chosen for the next experiment were :-

(A) Bases of all plants.

(B) Shunted regions of all plants.

(C) The primary leaves of plants in which the secondary leaves

had grown up above the shunting liquid surface. It seemed that in these cases the growth of the primary leaf was low or zero.

(D) Secondary leaves which had grown up above the shunting liquid surface.

(E) Primary leaves 0 to 3 centimetres above the shunting liquid surface.

(F) Primary leaves 3 to 5 centimetres above the shunting liquid surface.

(G) Primary leaves over 5 centimetres above the shunting liquid surface.

Since this grouping greatly complicates the results, the next three experiments, the corrected activity per plant only has been recorded.

Experiment 3.

To compare the uptake of P 32 by plants, shunted with 0.IN $MgSO_4$ solution, deionised water and with untreated controls.

TABLE 10.13.

Background. = 2096/160 min = 13.1 cpm.

0.IN $MgSO_4$ shunting Medium. = 162/10 min = 16.2 cpm.

Deionised water shunting Medium. = 148/10 min = 14.8 cpm.

	Basal Region A.	Shunted Region B.	Primary leaves C.	Primary leaves E.	Primary leaves F.	Primary leaves G.
Control.	1802 cpm plant.	568	197	88	237	
Shunted with 0.IN $MgSO_4$.	1370	453	184		1.1	50
Shunted with Deionised water.	1468	530	238	5.75		93 cpm/plant

Activity reported is in counts per minute per plant.

Analysis of Results.

It is difficult to draw any conclusions from this experiment except that with increase in size of the primary leaf the greater has been the uptake of P 32. There is again a tendency for the untreated control to have the greater P 32 content as suggested before, but little can be said of differences between the plants shunted with deionised water and 0.IN $MgSO_4$ solution.

Experiment 4.

This experiment was to confirm that the reduction in P^{32} uptake shown by shunted plants is independent of electrical potential changes induced by the presence of the shunt. To this end, Deionised water, which we have shown has only small and non predictable effects on the potential gradient, was used as shunting medium.

TABLE 10.14.

Background 12.8 cpm

	Basal Region A	Shunted Region B	Primary Leaves C	Secondary Leaves D	Primary Leaves F	Primary Leaves G	
Control	2140	540	293	14		163	
Shunted with Deionised Water	1335	444	269	8	177	149	

Analysis of Results.

Again the untreated control has a higher P^{32} throughout. It was decided in all subsequent experiments to use deionised water "shunted" plants as controls.

Experiment 5.

To compare the uptake of P^{32} by plants shunted with a medium which increases the positivity of the apex (0.1N $MgSO_4$), with a medium which decreases it (0.1N $FeCl_3$) and with a deionised control.

TABLE 10.15.

Background = 133/10 mins = 13.3 cpm

$FeCl_3$ Shorting medium 143/10 min 14.3 cpm

$MgSO_4$ " " 647/50 min 12.9 cpm

Deionised water Shorting Medium 144/10 min 14.4 cpm

	Basal Region (A)	Shunted Region (B)	Primary Leaves (C)	Primary Leaves (E)	Primary Leaves (F)	Primary Leaves (G)
Shunted with Deionised Water	1218 Cpm / Plant	380	214	155	90	76
Shunted with 0.1N $MgSO_4$	2028	337	137	30	58	124
Shunted with 0.1N $FeCl_3$	2270	411	183	70	39	113

Analysis of Results.

No consistent picture emerges from these results.

It was decided in future to use individual plants for the experiments rather than the groups of 16 arranged in one polythene vessel.

EXPERIMENTS OF THE SECOND SERIES.

In the following experiments on Ca^{45}, plants in individual pots were used. Also planchet counting was used, for the β energy of the Ca^{45} is too low for efficient counting with the liquid method.

The Ca^{45} growth medium was made up from 100 millilitres of Shive's solution to which was added 1.0 millilitres of a Ca^{45} solution. This Ca^{45} solution had been prepared from the supplied 1.05 millilitres of Ca^{45} solution, containing a total activity of one millicurie, and 10 millilitres of distilled water.

The Ca^{45} activity present in the 100 millilitres of Shive's solution was therefore $(\frac{1 \times 1}{11.05})$ millicuries.

The plants used in the experiments were selected for uniformity. Seedling heights were carefully measured before and after the experiments and the results, for individual plants within one experiment, are strictly comparable, for the heights and subsequent growth were similar in all cases.

In these experiments the plants were divided into three portions only, for determination of the Ca^{45} uptake, namely,

(a) Grain, roots and region of coleoptile below the shunting medium.

(b) Tissue in the shunted region.

(c) Primary and any secondary leaves above the level of the shunting liquid.

<u>Experiments 6 and 7</u>

To compare the uptake of Ca^{45} by plants shunted with 0.1N $MgCl_2$, 0.1N KCl, 0.1N $FeCl_3$ and with deionised water.
The results are shown in full in Table 10.16.

<u>Analysis of Results.</u>

In experiment 6, the plant shunted with 0.1N KCl shows the greatest uptake of Ca^{45} in all but the mid-region, when it is displaced to second place. In all but the mid region, uptake by the deionised shunted plant is least.

In experiment 7, there is no plant showing a consistently greater uptake, but in all regions, the plant shunted with deionised water shows the least uptake.

TABLE 10.16

Expt. 6. Corrected for 82 days decay.

Background. 505/50 minutes = 10.1/min.

Shorting media. $MgCl_2$ = 11,305/970 mins = 11.6/min

KCl = 794/60 mins = 13.2/Min

$FeCl_3$ = 993/90 mins = 11.03/min

Deionised water = 3242/300 min = 10.81/min.

	BASAL REGION		SHUNTED REGION		UPPER REGION		
Shunting Media	Actual Counts	Counts/ min/ plant	Actual Count	Counts/ min/ plant	Actual Count	Counts/ min/ plant	
$MgCl_2$ 0.1N	24,215/ 45 mins	747	7,485/ 45 mins	231	5,193/ 30 mins	215	
KCl 0.1N	26,325/ 30 mins	1235	9,180/ 45 mins	287	10,405/ 60 mins	215	
$FeCl_3$	16,667/ 30 mins	740	8181/ 40 mins	273	4,816/ 35 mins	168	
Deionised Water	15,627/ 35 mins	619	9045/ 40 mins	301	2,308/ 30 mins	87	

TABLE 10.16 (Cont'd)

Expt. 7. Corrected for 87 days decay

Background 11.6 cpm

Shorting media $MgCl_2$ = 3546/295 mins = 12.0 cpm
$FeCl_3$ = 1429/121 mins = 11.8 cpm
KCl = 17058/1350 mins = 12.6 cpm
Deionised water = 13109/1020 mins = 12.8 cpm.

	BASAL REGION		SHUNTED REGION		UPPER REGION	
Shunting Media	Actual Counts	Counts/ Min/plant	Actual Count	Counts/ Min/plant	Actual Count	Counts/ Min/plant
0.1N $MgCl_2$	22,186/ 15 mins	2120	13,389/ 60 mins	303	4596/ 65 mins	85
0.1N KCl	12,222/ 10 mins	1745	2537/ 10 mins	355	1630/ 30 mins	61
0.1N $FeCl_3$	9549/ 15 mins	905	7961/ 30 mins	366	2717/ 30 mins	114
Deionised Water	7908/ 15 mins	746	2043/ 10 mins	277	1432/ 30 mins	52

Experiments of the third series.

In this series of experiments Ca^{45} and P^{32} were simultaneously supplied in the growth medium.

The respective β energies are

	energy	(M.ev)
P^{32}	max	1.718
	av	0.695
Ca^{45}	max	0.255
	av	0.08

Thus there is a big difference in their β energies. From a graph of Energy (Mev)/Range in Aluminium (Mg/cm^2), it is seen that all β particles of energy below 0.35 M.ev will be absorbed by 100 mg/cm^2 of aluminium (Principles of Radio-Isotope Methodology by G.D. Chase).

An absorber of 116 mg/cm^2 aluminium was used and the following experiments carried out on samples of P^{32} and Ca^{45}.

TABLE 10.17.

	Without Absorber	With Absorber	% transmitted after background correction.
P^{32}	9558/min	4655/min	49%
Ca^{45}	80,331/min	36/min	0.045%

The technique therefore was to count the sample with the absorber in place, from which the P^{32} activity was determined, and

then without it, when, by subtraction the Ca^{45} activity was given.

As in the previous Ca^{45} experiments the seedlings were carefully selected for uniformity and the shunting media used were given 0.1N $MgCl_2$, 0.1N KCl, 0.1N $FeCl_3$ and deionised water.

The growth media was made up from 100 mls of Shive's solution, the same volumes of Ca^{45} and P^{32} solutions being added as for the individual P^{32} and Ca^{45} growth solutions described before. It therefore contains the same Ca^{45} activity as the previous Ca^{45} growth medium and the same P^{32} activity as the previous P^{32} solution.

Experiments 8, 9 and 10.

To compare the uptake of P^{32} and Ca^{45} by plants shunted with 0.1N $MgCl_2$, 0.1N KCl, 0.1N $FeCl_3$ and with deionised water.

The results are shown in full in Table 10.18.

TABLE 10.18.

Expt. 8. Background = 11.6 cpm.
P^{32} corrected for 12-16 days decay.
Ca^{45} corrected for 91-95 days decay.

Shunting Medium	Region of Plant	Actual Count With absorber	Actual Count without absorber	P^{32} activity counts per min/ plant	Ca^{45} activity counts per min/ plant	P^{32}/Ca^{45}
0.1N $MgCl_2$	Base	18,849/ 155 mins	5851/ 11 mins	406	435	0.93
	Shunted	2167/ 15 mins	2349/ 60 mins	106	112	0.95
	Upper	609/ 33 mins	4111/ 120 mins	25.1	12.9	1.95
0.1N KCl	Base	8025/ 75 mins	4,352/ 10 mins	352	338	1.04
	Shunted	2274/ 20 mins	2102/ 60 mins	95	80	1.19
	Upper	661/30min	1600/31 mins	38.5	27.6	1.39
0.1N $FeCl_3$	Base	3481/ 30 mins	7803/ 15 mins	474	440	1.07
	Shunted	3501/ 40 mins	1832/ 60 mins	85	55	1.54
	Upper	1448/ 99 mins	6053/ 230 mins	13.5	12.8	1.05
Deionised Water	Base	12,736/ 75 mins	11,417/ 15 mins	717	636	1.12
	Shunted	2546/ 25 mins	3126/ 105 mins	82	79	1.04
	Upper	2244/ 130 mins	1645/ 40 mins	23.7	44.4	0.53

TABLE 10.18 (Contd)

Expt. 9. Background = 11.6 cpm

P^{32} corrected for 20 to 22 days decay

Ca^{45} corrected for 99 to 101 days decay.

Shunting Region	Region of Plant	Actual Count with Absorber	Actual count without Absorber	P^{32} Activity counts per min/plant	Ca^{45} Activity counts per min/plant	P^{32}/Ca^{45}
0.1N $MgCl_2$	Base	1944/ 20 mins	2407/ 5 mins	500	454	1.1
	Shunted	1245/ 47 mins	6972/ 91 mins	61	52.5	1.16
	Upper	1273/ 91 mins	4119/ 190 mins	14.4	7.8	1.8
0.1N KCl	Base	4310/ 55 mins	19800/ 60 mins	360	274	1.33
	Shunted	1377/ 60 mins	4082/ 45 mins	79.5	87.5	0.91
	Upper	1880/ 100 mins	5281/ 100 mins	39.5	41	0.96
0.1N $FeCl_3$	Base	4026/ 46 mins	45257/ 101 mins	407	425	0.96
	Shunted	922/ 45 mins	1226/ 15 mins	63.5	81	0.79
	Upper	918/ 65 mins	1263/ 31 mins	14.6	37	0.4
Deionised Water	Base	11092/ 120 mins	15373/ 40 mins	435	314	1.38
	Shunted	1702/ 60 mins	9494/ 95 mins	97	84	1.15
	Upper	1863/ 96 mins	2590/ 60 mins	47	24	1.95

TABLE 10.18 (Contd)

Expt. 10. Background = 12.0 cpm
P^{32} corrected for 52 days decay
Ca^{45} corrected for 131 days decay

Shunting Region	Region of Plant	Actual count with Absorber	Actual count without Absorber	P^{32} Activity counts per min/plant	Ca^{45} Activity counts per min/plant	P^{32}/Ca^{45}
0.1N $MgCl_2$	Base	2010/ 25 mins	23569/ 30 mins	1855	1110	1.67
	Shunted	1393/ 60 mins	3570/ 30 mins	299	148	2.02
	Upper	3977/ 240 mins	1815/ 35 mins	118	54	2.19
0.1N KCl	Base	2006/ 25 mins	15598/ 20 mins	1850	1101	1.67
	Shunted	4925/ 190 mins	10823/ 130 mins	372	76	2.11
	Upper	2642/ 140 mins	1838/ 35 mins	181	47	3.85
0.1N $FeCl_3$	Base	4549/ 60 mins	5498/ 8 mins	1731	956	1.81
	Shunted	2050/ 80 mins	2399/ 30 mins	364	70.5	5.17
	Upper	1017/ 60 mins	11339/ 210 mins	128	56	2.28
Deionised Water	Base	1248/ 15 mins	7782/ 10 mins	1931	1081	1.79
	Shunted	(135 mins/ (2925	(70 mins/ (5582	257	85	3.2
	Upper	14416/ 940 mins	1173/ 30 mins	85	36	2.35

1cc growth medium P^{32} = 1056 cpm
Ca^{45} = 880 cpm
$\therefore P^{32}/Ca^{45} = 1.2$

The results are complicated and it is difficult to determine any clear pattern. In an attempt to simplify matters, the relative order of uptake of the Ca^{45} and of the P^{32} ions, with the different shorting media, are listed below, for all the reported Ca^{45} experiments and for the three above P^{32} experiments.

TABLE 10.19.

Ca^{45}

	Base Region					Shunted Region					Upper Region				
Expt.	6	7	8	9	10	6	7	8	9	10	6	7	8	9	10
$MgCl_2$	2	1	3	1	1	4	3	1	4	1	2	2	3	4	2
KCl	1	2	4	4	2	2	2	2	1	3	1	3	2	1	3
$FeCl_3$	3	3	2	2	4	3	1	4	3	4	3	1	4	2	1
Deion	4	4	1	3	3	1	4	3	2	2	4	4	1	3	4
Average:	Mg > K > Fe > Deion.					K > Deion > Mg > Fe					K > Fe > Mg > Deion				

P^{32}

	Base Region			Shunted Region			Upper Region		
Expt.	8	9	10	8	9	10	8	9	10
$MgCl_2$	3	1	2	1	4	3	2	4	3
KCl	4	4	3	2	2	1	1	2	1
$FeCl_3$	2	3	4	3	3	2	4	3	2
Deion	1	2	1	4	1	4	3	1	4
Average	Deion > Mg > Fe > K			K > Mg=Fe > Deion			K > Deion > Mg=Fe		

The major conclusion that can now be drawn from the results is that with both P^{32} and Ca^{45} there is a greater uptake into the upper regions of the plants when the shunting medium is KCl than when any of the other shunting solutions are used.

This uptake cannot be dependent on the measured changes in the primary leaf/base electrical potentials that are brought about by shunting, for KCl only slightly increases the positivity of the tissue, whilst $MgCl_2$ greatly increases it and deionised water has little effect.

The results in the basal region can probably be ignored as small differences in the area of root would, with the method employed, markedly affect the results. The root sample was blotted before digestion but there must have been variations in the "carry over" of the active growth medium.

Having shown that there is no relationship between the measured potential gradient between the tip and base of the seedling and the uptake of P^{32} and Ca^{45}, it is necessary to consider by what means the presence of the shunt may bring about the alterations in the uptake of ions that do occur.

1. The shunt may lead to a decrease in the transpiration stream by reducing the area of leaf exposed to the air. This would lead to a decrease in the transport of water and perhaps ions up the plants and therefore perhaps a decrease in ion uptake. This seems to be a definite effect but then there should be no difference in uptake between the

different shunting media if it was the only effect.

2. The shunt could provide an easier pathway for ions than the tissue, the ions moving either by diffusion (very slow) or under the influence of the admittedly small potential gradient between the ends of the shunt, generated by the bio-electrical activity of the tissue. This should lead to an increase in ion uptake.

3. Ions taken up by the roots could leak out into the shunting medium in preference to passing further up the plant tissue, and be lost to the plant, leading to a reduction in ion transport to the upper regions.

4. Ions composing the shunting medium might be absorbed, so reducing the absorption of ions from the growth medium.

If any of the three above suggestions were correct one would expect to find tracer material in the shunting medium, for in the first, the medium is the actual pathway, in the second, there is a leakage into the shunting medium and in the third, one would expect some tracer to enter the shunting medium if ions can so readily pass into the tissues. However, in all cases, the shunting media hardly show significant activities above background.

TABLE 10.20.

Shunting Media	Count/minute above background/ml of Shunting medium		
$FeCl_3$	0.2 cpm	0.9 cpm	1.3 cpm
$MgCl_2$	0.4 cpm	1.5 cpm	
KCl	1 cpm	3.1 cpm	
Deion water	1.2 cpm	0.7 cpm	
$MgSO_4$	0.1 cpm	3.1 cpm	

Furthermore, substantial movements of ions to or from the tissue into or out of the shunting medium would lead to changes in the tissue ion distribution and therefore to permanent changes in the bioelectric pattern.

Although in the experiments the shunts were applied for only 3 hours, there was never a permanent change in the potential due to the shunt application.

5. The shunting medium might permeate the cellulose walls and cause changes in the membrane permeabilities throughout the shunted tissues, so modifying one route of upward ion transport.

However, this again would lead to a permanent modification of the potential pattern.

6. From our experiments it is evident that shunting does reduce the rate of transfer of ions from the rooting medium to the leaf but it has been remarked that shunting with deionised water, $MgCl_2$ and $FeCl_3$ produces a greater reduction than with KCl solution.

At 0.1N concentrations the shunting media will have quite a marked osmotic effect. Three times Shive's solution plasmolyses coleoptiles and this is osmotically equivalent to 0.14N KCl, to 0.09N $MgCl_2$ and to 0.07N $FeCl_3$.

There were never any signs of tissue damage after shunting but the shunting medium will remove water from the tissue which will be replaced by water entering the roots, and a water stream, bringing

ions up the shoot may be induced by this exosmosis from the leaves. With deionised water, this effect will not occur.

Although water exchange will be small, for the shoot is covered with a relatively water impermeable cuticle, any water loss that does occur, will be greater, the greater the osmotic pressure of the shunting medium. At 0.1N concentration the relative O.P's are KCl 9.35, $MgCl_2$ 5.1 and $FeCl_3$ 2.6.

With this theory one would expect the greatest uptake with KCl (which we found) and least with deionised water. From our results however it is difficult to place $MgCl_2$, $FeCl_3$ and deionised water shunting solutions in their relative orders.

Unfortunately this interesting theory was not envisaged till after the experiments were completed. It could readily have been checked using mannitol solutions.

Conclusions

The experiments reported here do not lend positive support to the hypothesis that the measured longitudinal electrical potential differences of tissues influence the rates of salt uptake into these tissues. Shunting may lead to both changes in the measured potentials, and to changes in salt uptake, but there is no simple correlation between the two.

The potential changes brought about by shunting vary with the particular ions employed and are not directly correlated with

their relative specific conductances.

They are ascribed to (a) a shorting out of the potential contribution of the shunted tissue to the measured potential.

(b) The setting up of diffusion potentials, in their broadest sense, between the tissue and the shunting media, which contribute to the measured potentials.

The changes in ion uptake reported here are ascribed to

(a) A reduction in transpiration due to the presence of the shunting medium.

(b) Exosmosis from the tissue into the shunting medium leading to an enhancement of water movement up the plant.

CHAPTER 10.

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CHAPTER: 11.

CHAPTER 11

A DISCUSSION OF OSCILLATION IN BIOELECTRIC POTENTIALS AND THE NEGATIVE FEED-BACK CYCLE RESPONSIBLE FOR THEM.

SUMMARY

Brief details of oscillations in potentials that we have observed and the conditions under which they occurred are given. The hypothesis put forward by Scott & Jenkinson (1957 - 1962), to explain the oscillations they found in bean roots, is then briefly described and an attempt is made to apply this to our results. It is suggested that the permeabilities of membranes, upon which depend the measured potentials, are themselves dependent on the auxin concentration gradient existing across them. Changes in auxin supply or utilisation, or changes in membrane permeabilities through some other factor, lead to damped oscillations in the permeability, till a new equilibrium position is attained, which is dependent upon the rate of auxin supply to and utilisation within the cells.

Oscillations are a regular feature of the measured bio-electric potentials of Oat plants. During this work we have found that:-

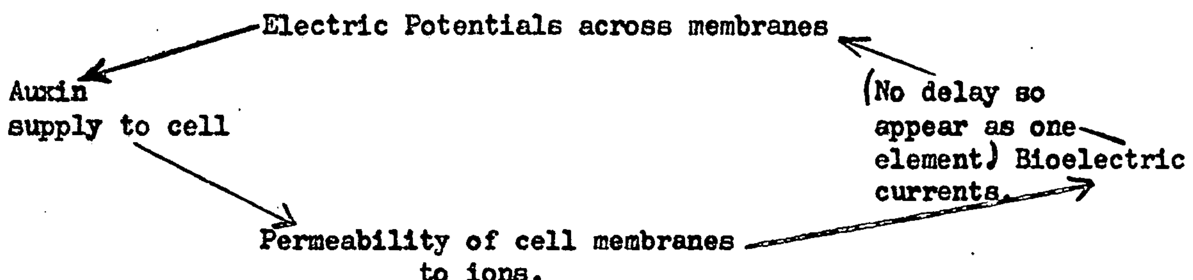
(a) After making contact there are generally damped oscillations in the electrical potentials measured (Figure 4.1.).

(b) Changes in white light intensity induce damped oscillations in the electrical potentials of chlorophyll containing plant tissues. (Figure 5.3.B) and also in coleoptiles. (Figure 7.5.).

(c) Oscillations may arise spontaneously during the course of experiments and may be of a damped or of a regular form.

There are large variations in the amplitude, from oscillations that are hardly detectable, that is fractions of millivolts, up to in one case 25 millivolts. Also the periodicity varies from a few minutes up to 60 minutes.

Scott (1957), Jenkinson & Scott (1961), and Jenkinson (1962), have developed, from experiments with Broad Bean roots, a theory to account for oscillations in bio-electrical potentials. They suggest that a negative feed-back system exists which is on the verge of instability. This is composed of a 4 element loop, namely



The feedback loops gain is available due to the high sensitivity of membrane to auxin action.

We are not in a position to criticize or expand this theory but it seems that some of our results are relevant.

In our work on the light responses of the Oat coleoptile we noted that light immediately induced oscillations or modified pre-existing oscillations in the potentials of the illuminated tissues. In tissues below the illuminated region the changes took place only later. This latter change is interpreted as changes in the level of auxin translocated to the lower region. The immediate changes in the illuminated tissue are interpreted as changes in membrane permeabilities, but this will be in the presence of constant external auxin. Therefore oscillatory changes in potential may arise either due to changes in auxin supply or may take place in the presence of a constant auxin concentration.

We also found that decapitation of coleoptiles leading to a depletion of auxin, led to a disappearance of oscillations.

In another experiment the electrical potential responses of a double decapitated Oat coleoptile to the application of a contact and changes in the intensity of illumination were investigated. The results are shown in Figure 11.1. There were changes in the electrical potential on application of the contact and on turning on a white light but in neither case were there

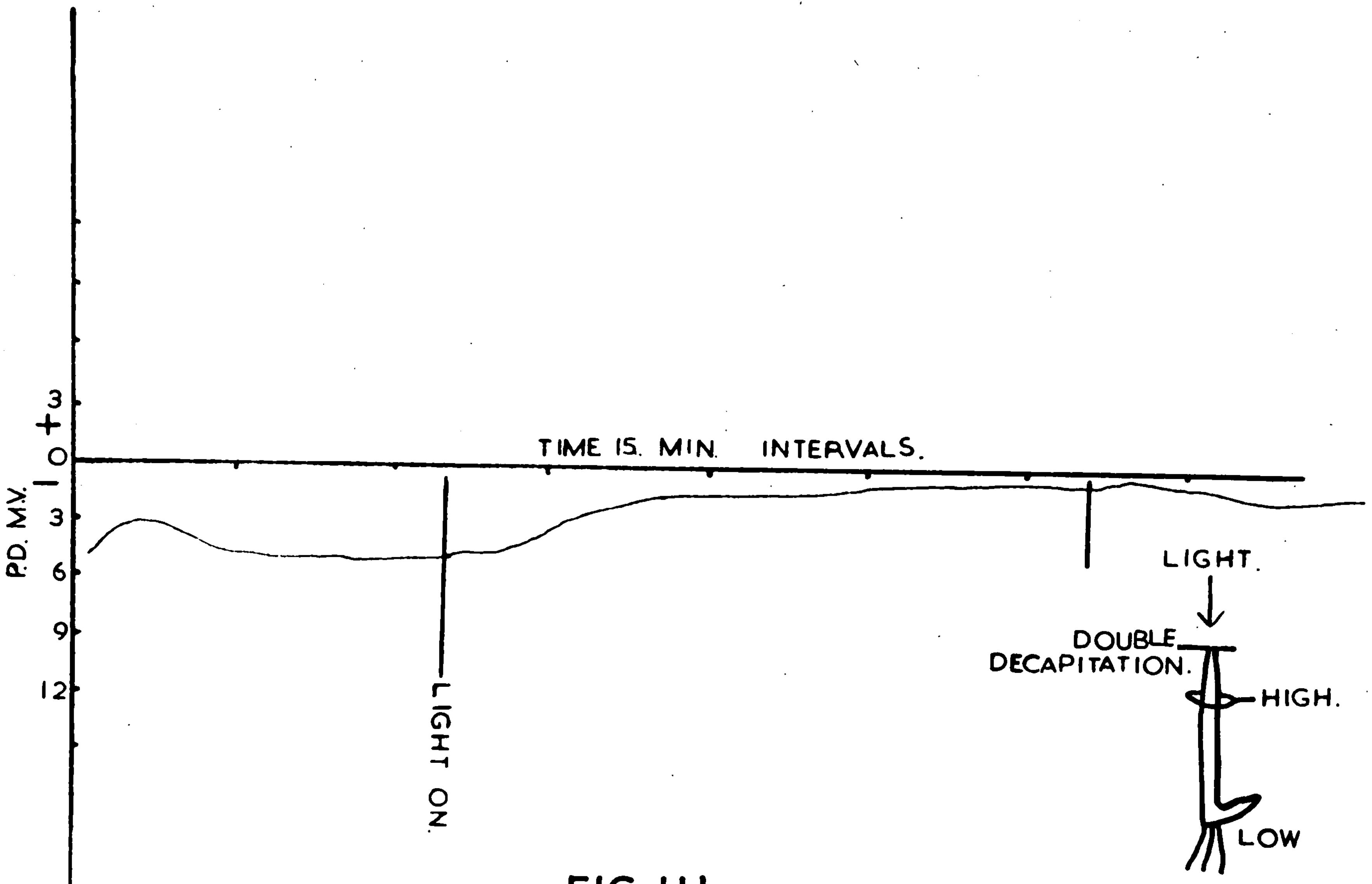
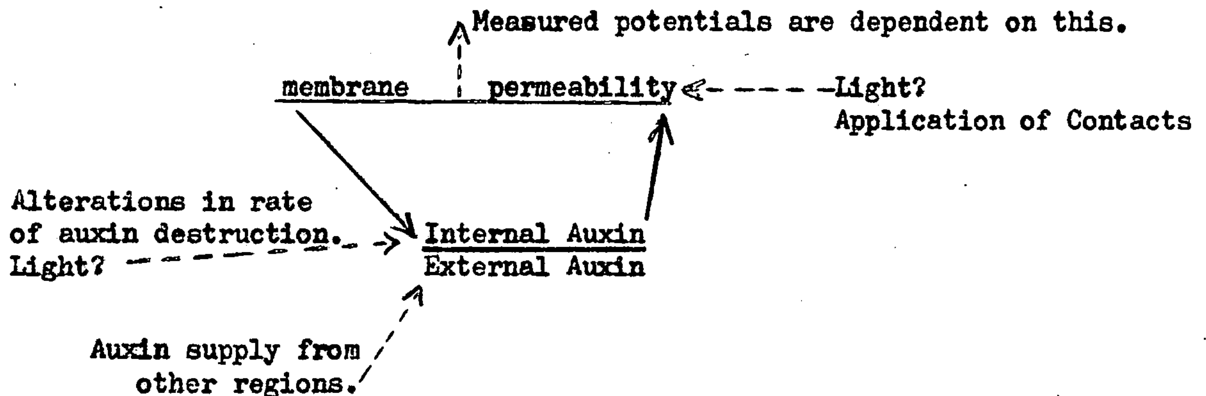


FIG. 11.1.

subsequent damped oscillations. It seems therefore that there can be changes in permeabilities without the presence of auxin but the feed-back mechanism does not function in its absence.

These results are consistent with a feed-back cycle involving auxin and membrane permeabilities. It is furthermore suggested that it is the auxin balance on the two sides of the membrane that influences the permeability of the mebrane. Changes in either the cell internal concentration of auxin (Changes in rate of destruction?) or in external auxin concentration, will set up oscillations in the membrane permeabilities till a new balance is established. Also any factor directly influencing the membrane permeability such as the application of contact media, will similarly set up oscillations. The suggested fee-back cycle is:-



High external or low internal auxin leads to an increase in permeability leading to an increase in auxin entry reducing the ratio.

There will normally be a delicate balance between auxin destruction, entry, and external concentration and therefore of the membrane permeability. The auxin balance change and the permeability change brings about the changed oscillations in the membrane permeabilities.

The above is shown as a two element cycle only, which is quite sufficient to explain the effects but further experimentation would be necessary to determine exactly how many elements are involved.

Scott (1957), claims that there is no correlation between growth and the potential oscillations, though one would have expected there to be if the oscillations reflect changes in auxin balance.

However Clark (1935), found that the potential changes in Oat coleoptiles subsequent to setting up experiments were reflected in growth rate changes. This point seems worthy of further investigation.

In conclusion, our results upon oscillating potentials are compatible with a negative feed-back cycle involving auxin and membrane permeabilities. It is suggested that among other things, the membrane permeability is a function of the auxin concentration difference across itself.

CHAPTER 11

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CHAPTER.12.

CHAPTER 12

THE MEASUREMENT OF ELECTRICAL POTENTIALS IN FUNGI.

SUMMARY

After a brief introduction giving reasons for the measurement of the potentials in fungi, unsuccessful experiments on Basidiomycete fruit bodies collected in the field are described. Later work on Collybia velutipes is then detailed is then detailed. Descriptions of the culture technique used and of the measuring chamber that had to be developed to overcome problems of fruit body dessication are given. The experiments for which the chamber was used are described. Analyses of the results of the experiments show that it is possible to explain changes in P.D's with growth as functions, of differential changes in ion concentrations brought about by differences in growth in the various parts of the fruit body. The potentials measured are ascribed to Donnan potentials with an immobile anion.

This is a description of work upon the electrical potentials of some Basidiomycete fruit bodies. Tropic responses in Badisiomycetes do not in general seem dependent on the redistribution of a growth promoting substance and therefore differ from those of higher plants. The individual parts of the fruit body appear to respond independently and it would be of great interest to investigate changes, if any, in the electrical pattern under tropic responses. If a change could be found it could be studied without the liklihood that it was causally involved in the redistribution of a growth promoting substance regulating the curvature. Also any modifications of the bio-electric potential pattern could be assumed to be independent of a growth substance concentration gradient. (Banbury 1959).

There is only one published reference to work on electrtal potentials in fungi, Colla (1927), who deals with resting and injury potentials of Basidiomycetes. He used calomel electrodes, 0.9% sodium chloride solution as contact medium and a potentio-meter circuit to measure the potentials.

Our first experiments on fungi were on exampled of various species collected in the field. They were brought into the laboratory and the potentials immediately measured. This was before the development of the flowing drop contact. The fungal

fruit body surface was more readily wettable than that of the coleoptile and contact drops could not be formed on the surface. This difficulty was surmounted by cutting small agar rings with cork borers and placing these on the surface of the fruit body. The well of the ring was then filled with the contact medium and into this the contact was placed. Shive's solution was used as contact medium.

After these rather unsatisfactory experiments, the work on fungi was left for some time until the following observations were made.

These new experiments were on Collybia velutipes fruit bodies using flowing drop contacts. In its young stage Collybia shows both photo and geo responses. It is readily grown in culture on 2% Malt agar and as long as the temperature does not exceed 20°C and occasional light is given to the cultures to initiate fruit body formation, no trouble is experienced in producing suitable fruit bodies. After fruit body initiation temperature and illumination are of no consequence. To facilitate experimentation the cultures were grown on 1" specimen tubes completely filled with agar, inside sterile, screw top jars. All measurements of potentials were made in red light and Shive's solution was used as contact medium.

It was decided to use the mid-region of the upper side of the pileus as the reference point and measure all other potentials with reference to a fixed contact there. The apical contact was

a flowing drop contact placed vertically and the other contact a flowing drop contact in the normal horizontal position. In the preliminary experiments measurements were made in the open laboratory but within fifteen minutes of making contact, the fruit body had dried out. A humidity chamber was constructed from a perspex tank lined with moist filter paper but even then dessication rapidly followed.

The cause was now realised to be the rapid air flow into the vacuum lines of the flowing drop contacts, over the fruit body surface. To solve this problem a more refined chamber was made through which air saturated with water was pumped. At the same time the vacuum at the contact tip was reduced to that just sufficient to maintain a true contact drop. The air was saturated with water vapour by just pumping through glass coils in a constant temperature water bath to raise its temperature slightly above ambient; it then passed through a glass column, the air entering at the base through a sintered glass plate and bubbled up through water in the column. Also in the column, almost filling it, were placed loofahs. These gave a very large water to air surface area and hence sufficient saturation of the air. The air then entered the experimental chamber. A themometer in the chamber registered the temperature, which, since the chamber was in a room controlled at $20^{O}C \pm 1$, could itself be controlled by adjustment of the water

bath temperature. It was maintained at 22°C approximately.

Internal condensation on the faces of the perspex tank was a problem which had to be solved as it prevented observation when making contact. A thin lantern slide was stuck to the inside surface by a black "Bostic", rubber adhesive film around its edge, care being taken to ensure a complete seal. This effectively solved the problem. The apparatus used is shown in Plate 12.1.

The first series of results are concerned with comparisons of the resting potentials of Collybia fruit bodies, with Shive's solution as contact medium, at different stages of their growth. These are shown in Figure 12.1.A.

The fruit body is already formed into a head and a stipe when far too small for any electrical measurements to be made. The stipe elongates and the pileus expands but the gills do not really begin to enlarge till the stipe stops growing, then they increase in size and the cap becomes partially turned inside out.

The growth has been divided into two stages, the first with no gill expansion and the second after the gill expansion, but within each group the specimens are arranged according to height. This is more logical than arranging on height alone for in one example the stipe was 25 millimetres long and there was no gill expansion, and yet in another the fruit body was mature with a stipe only 7 millimetres long.

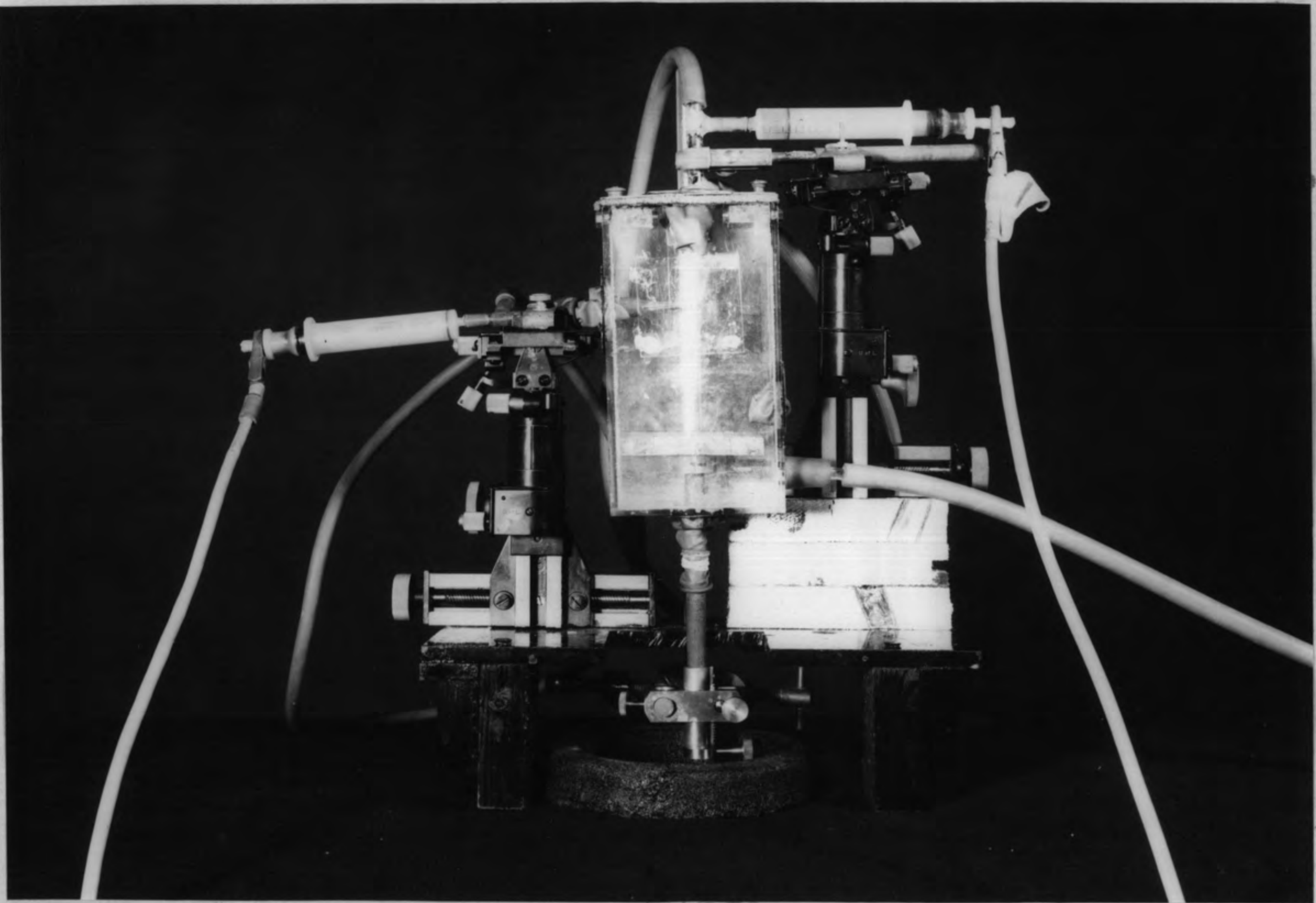
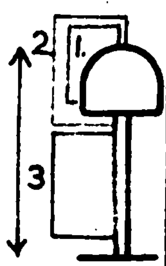


PLATE.12.1.



UPPER CONTACT HIGH. P.D. IN MV

GILLS NOT EXPANDED.				GILLS EXPANDED.			A
HT IN MM.	P.D. 1	P.D. 2	P.D. 3	HT IN MM	P.D 1	P.D 2	P.D 3
5	−1	−4	0	8·5	+1	−3	−10
10	0	−6		14	−1	−8	−17
11	+1	−2	−4	14	−1	−7	−17
11	+1	−2	−8				
28	+9						

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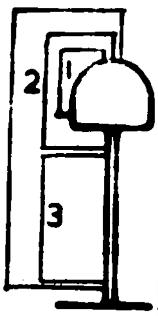


CONTACT MEDIUM SHIVES				CONTACT MEDIUM 10% SHIVES			B
P.D. 1	P.D. 2	P.D. 3	P.D. 4	P.D. 1	P.D. 2	P.D. 3	P.D. 4
RETURNED TO SHIVES AFTER 10%			−18 −16				−27
+1	−2	−8	−10	−1	−10	−10	−20

FIG. 12.1.

An analysis of the results shows:-

1. Potentials existing across the pileus surface are small and vary in polarity from specimen to specimen.

2. In all examples the pileus is negative with respect to the stipe and this negativity tends to be greater in mature specimens.

3. There is a potential gradient up the stipe such that the upper regions are negative with respect to the lower and with increase in size of the stipe this gradient increases. There appears to be an increase in this gradient in mature plants independent of the actual size of the stipe.

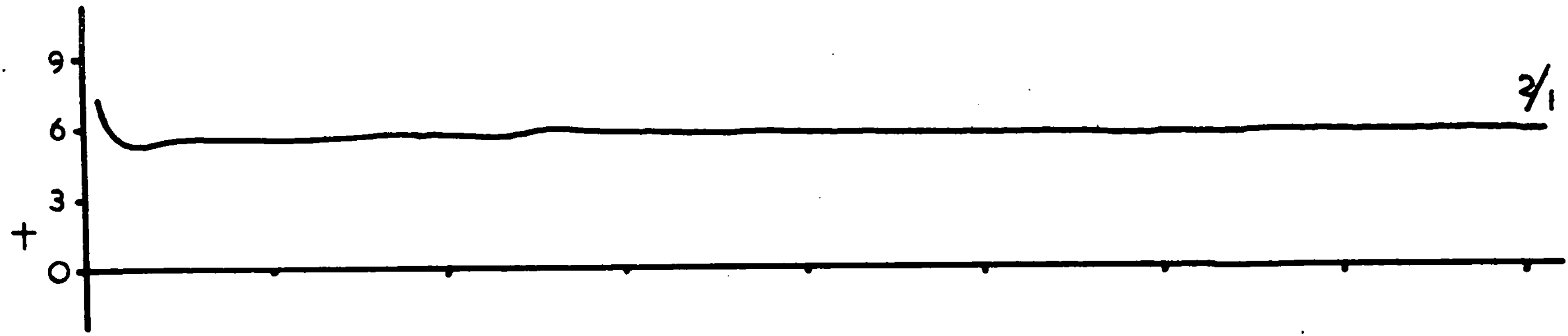
There is disagreement on exactly how growth in Basidiomycete fruit bodies takes place but it seems likely that much of the growth after the initial stage involves little addition of new organic material but presumably some water uptake. Growth may thus lead to some dilution of the cell contents. If the potentials are membrane potentials, this will alter the potential between the tissue and the contact due to the change in concentration of the internal iohs. Even if the potentials are due to Donnan exchanges between the cell wall and contact medium, since the same material, after expansion, is made to go further, the ion exchange capacity will have been reduced, altering the potential. The measured potential will change if the effects at the two contacts are unequal, which will be the case if

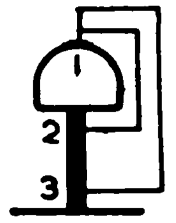
growth differs in different parts of the fruit body.

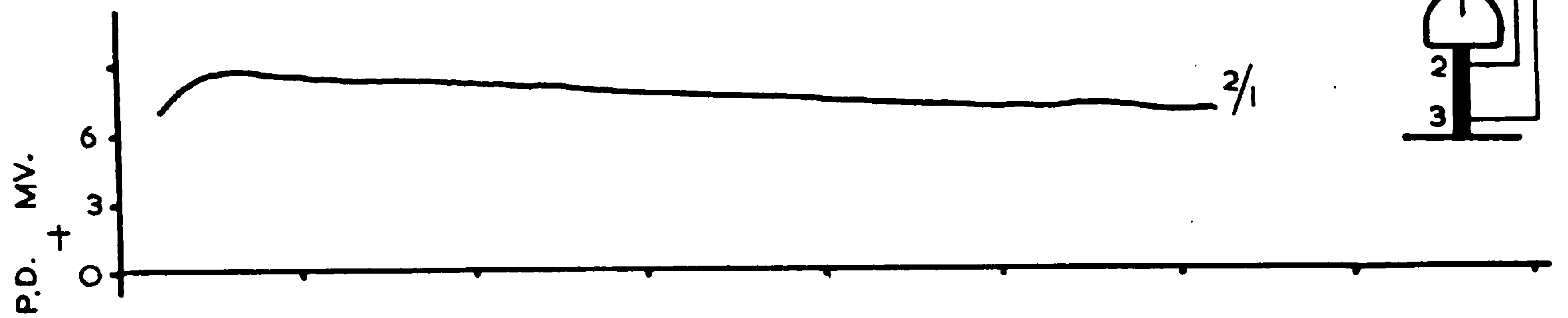
When the pileus is rapidly expanding at the completion of the stipe growth, its potential becomes more negative with respect to the stipe and as the growth of the stipe led to the upper regions becoming more negative with respect to the lower, it is suggested that elongation is greater in the upper regions of the stipe than the lower. This deduction is in agreement with direct observations on extension growth of marked Basidiomycete stipes by Bonner, Kane and Levey (1956).

In the next series of results (Figure 12.1.B.) 10% Shive's solution was compared with Shive's solution, the solution being changed in concentration at both contacts simultaneously. They show that with dilution at both contacts, the contact at the apical regions becomes more negative than that at the basal. A change in potential upon change in contact medium is indicative of a diffusion potential in the broadest sense. (Chapter 3.)

Figure 12.2. shows some typical recorder charts for potentials in Collybia. The most remarkable aspect of these is the constancy of the potentials. There is a gradual increase or decrease in the potential for the first few minutes after making contact, but the potential then established remains relatively constant, showing only very slight increases or decreases, or in some cases no changes at all, over periods of several hours. This result differs markedly







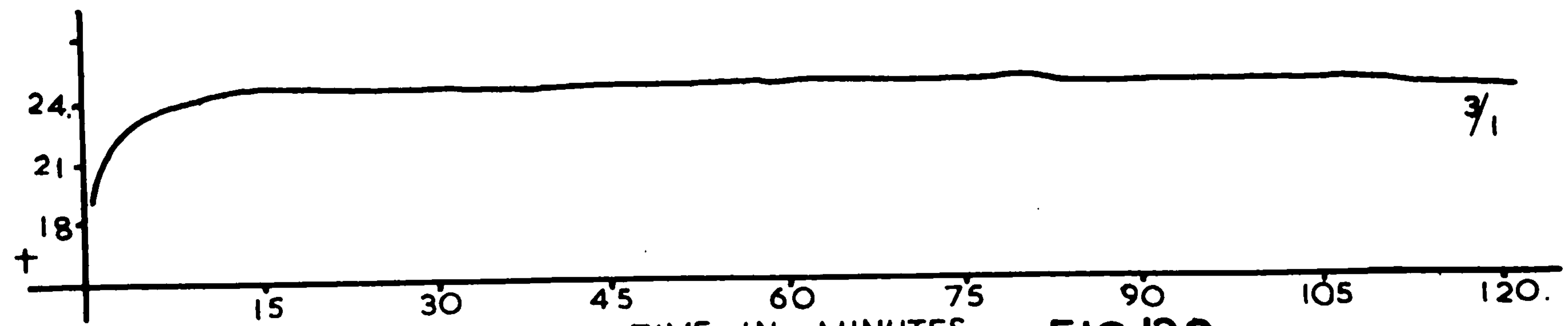


FIG.12.2.

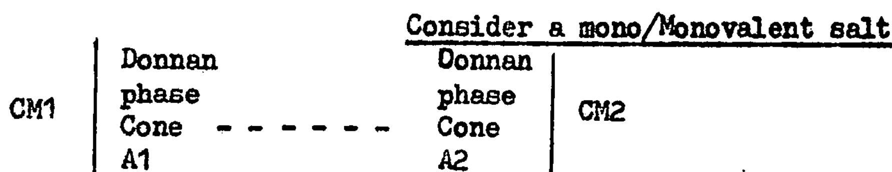
from Avena where oscillations, regular and demped, and apparently random changes in potential are the order of the day.

If the dominant potentials measured are simple membrane potentials, then though the permeabilities at the two points of contact must differ for a potential to exist, as the potentials remain constant, the membrane permeabilities must also remain constant or change in unison. These properties coupled together would produce constant potentials but seem improbable. It is far more likely that the dominant potentials are Donnan potentials with the concentration of the immobile ions differing at the two points of contact. From the usual situation in higher plants it is suggested that the cell membranes are impermeable to anions. It is also possible that ion exchanges between fixed charges in the fungal hyphal walls are involved, which of course will be independent of membrane permeabilities.

From our results, we know that growth leads to an increase in the negativity of growing tissue. We also know that dilution of contact media at both contacts leads to an increase in negativity of the previous negative values, and that the upper regions of a fruit body are negative to the lower.

Consider two membranes impermeable to anions separating from the cell cytoplasm two contact media, CM1 and CM2. Let concentrations of CM1 = CM2 = CM.

Consider a mono/Monovalent salt



∴ Potential Difference of CM1 with respect to CM2, $E\,{}^{CM1}_{CM2}$

$$= \frac{RT}{F}\ \log_n \frac{\sqrt{A_1^2 + 4CM} + A1}{\sqrt{A_2^2 + 4CM} + A2} = X$$

Let A_1 be the upper region of the fruit body

From our results $E\,{}^{CM1}_{CM_2}$ is negative

∴ X is negative.

But this can only apply if A_2 is greater than A_1

∴ The upper Donnan phase concentration is smaller than the lower. When growth takes place, it was suggested that the upper region ion concentrations were diluted. Therefore $E\,{}^{CM_1}_{CM_2}$ should become more negative. This is the observed result.

Let us now consider simultaneous change in contact media at both contacts. Let original contact media concentration be CM1 and the new concentration CM2.

Also let CM1 be greater than CM2.

$$\therefore \text{P.D. change} = \frac{RT}{F}\left(\underbrace{\log_n \frac{\sqrt{A_1^2 + 4CM_1^2} + A1}{\sqrt{A_2^2 + CM_1^2} + A_2}}_{Y} - \underbrace{\log_n \frac{\sqrt{A_1^2 + 4CM_2^2} + A1}{\sqrt{A_2^2 + 4CM_2^2} + A2}}_{Z}\right)$$

but CM1 $>$ CM2 and from the previous result A2 $>$ A1.

$\therefore$ Z more nearly approximates to log $\frac{A1}{A2}$

and Y more nearly approximates to log $\frac{CM1}{CM1}$ i.e. to 0

$\therefore$ Polarity of P.D. charge is - log $\frac{A1}{A2}$ i.e. positive.

In the experiments with the more concentrated contact media, the P.D. of the upper region is slightly negative with respect to the lower (-amv) and with dilution this becomes more negative (-bmv).

$\therefore$ the change is -a - - b mv = b - a mv

But b $>$ a

$\therefore$ Polarity of change is positive. This confirms the theoretical result.

More detailed calculations have not been carried out as:-

1. Insufficient results exist.
2. Shive's solution is a complex mixture of ions.

However, we may conclude that as the potentials measured are small, then there can be little difference in the exchange capacities between the different regions of the fruit body. Also, since contact media concentration change does have a marked influence on the potentials, the concentration of the Donnan phase must be close to the contact media concentration.

No further work has been carried out on Collybia due to the lack of time, though a new humidity chamber which allows a transverse positioning of the electrodes on the fruit body has been constructed, with which, in future, it is hoped to study geo and photo responses.

Conclusions

Constant reproducible potentials can be measured between different parts of the fruit body of Collybia velutipes. These change in magnitude but not in polarity during the period of growth. They show a remarkable constancy and are believed to be Donnan potentials set up due to immobile anions in the fungal tissues. The changes with growth are accounted for by dilution of the Donnan phases in the growing regions.

CHAPTER 12.

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CHAPTER 13

THE DYNAMIC CONDENSER METHOD OF MEASUREMENT OF ELECTRICAL POTENTIAL DIFFERENCES.

SUMMARY

A detailed literature survey of all papers it has been possible to locate upon this method of potential measurement is given. Preliminary unsuccessful experiments making use of the Vibron 33B are described, and then experiments using a telephone earpiece and a loudspeaker as vibrators are detailed. These were also failures.

An original method, making use of a metal plate mounted on the circumference of a rotating disc, perpendicular to the radius, is then described, the amplifier circuit diagram given, and some preliminary results listed. A comparison between results obtained by this method and by the more conventional electrode/liquid contact, is attempted.

If we have two condenser plates connected through an external circuit and charged to different potentials, then when the distance between the plates is altered, a detectable current flows from one and to the other through this circuit.

Volta in 1801 and later Kelvin (1898), used the method to determine the contact potential between different metals and Guyot (1924) and Garrison(1925) to determine the surface potential of films (The physics and Chemistry of Surfaces - Adam).

A very sensitive modification of this method has been described by Zisman (1932), in which one of the plates is made to vibrate rapidly along an axis perpendicular to its plate by means of a tensioned steel wire kept in vibration by an air blast. This periodically alters the distance between the plates. The oscillating current flowing between the plates if they are not at the same potential, is amplified and the output fed to a pair of ear-phones giving an audio signal.

The metals forming the plates are those between which the contact potential is to be determined. By applying a potential by means of a potentiometer, the audio signal can be suppressed when the applied potential is equal but of opposite polarity to the contact potential. Zisman published two other papers in 1939 concerning this method.

Fink & Dehmel (1936), utilised a similar system and Porter (1937), describes a similar method for measurement of contact potentials. He also derived an approximate solution for the signal strength arising from the flow of the current generated, though the resistance of the external circuit. This is the first of several different solutions.

Still in this field of application, Frost & Hurka (1940), describe a vibrating probe for experiments on the electrical properties of absorptive films.

The system can also be used to measure potentials in much the same way as with a galvanometer or valve voltmeter. One of the input leads of the instrument is connected to a metal plate close to which another is vibrated which is connected to the other input lead.

The system was first utilised by Ross-Gunn (1932), who made use of the semi-cylindrical electrodes to which one input lead was attached. Inside these were rotated, by a small electric motor, semi-cylindrical inductors connected by a commutator to the amplifying circuit.

Palevsky, Swank & Grenchik (1947), give a detailed analysis of the design and theory of dynamic condenser electrometers and in the same volume Scherbatskoy, Gilmartin and Swift describe an instrument based on this principle for measuring radio-activity

in oil well holes. Since then several commercial electrometers have appeared of which the Vibron 33B is an example. Since there is no through circuit in these instruments other than across the condenser gap, the input resistance is that of the insulation. The D.C. input signal is converted into an A.C. by means of the vibrating capacitor. This is amplified. After amplification, the output phase and potential which is proportional to the input polarity and potential, is fed to a suitably calibrated phase detecting instrument so that the input polarity and potential may be read off directly. The energy for the A.C. output is derived from the mechanical energy of the capacitor vibrations and the output frequency is that of the vibrations.

In 1950, Blüh & Scott described apparatus based on these principles to measure electrical potentials in plant tissues. The plant is considered as one plate of the condenser. Close to its surface is positioned the other plate which is vibrated by means of a telephone ear-piece. A reversible electrode is inserted in the growth medium and the circuit completed through a high resistance, connected to the vibrating plate, across which the amplifying circuit is placed. Since neither the plate position nor presentation area of the plant tissue are constant, the output signal cannot be made to give a direct measure of the potential. It is necessary to use a null method by applying a potential to the plant with a

potentiometer till the A.C. output is zero. The applied potential is then equal but of opposite polarity to that of the plant surface nearest to the vibrating plate.

Hertz (1960), and Grahm and Hertz (1962), describe a vibrating plate method that they have used for the measurement of geo-electric potentials in plants. They claim to get results which differ from more conventional methods.

Many times during this research a need was felt for a method of measurement of bioelectrical potentials not dependent upon the use of liquid contacts and it was eventually decided to develop such a system.

The very earliest attempts were based on the adaptation of the Vibron instrument as the null detector. The High input lead had a small plate soldered on, and this was held in juxtaposition to the plant. The low lead was connected to a $Zn/ZnSO_4$ electrode in the growth medium, by way of a potentiometer circuit formed from two decade resistances. These allowed the application of $\pm$ 0 to 100 millivolts to the plant. Theoretically the plate close to the plant should have an equal but opposite charge induced on it by the plant potential. This should cause the input plate of the internal vibrating reed to assume the polarity of the plant, giving an indication on the Vibron scale. By application of a potential to the plant this reading should be altered and when reduced to zero,

the applied potential will be equal in magnitude but of opposite polarity to that of the plant.

Such was the theory but in practice no sensible effects could be obtained. Applying large potentials (up to 1 volt), to a plate positioned near to the input plate hardly influenced the potential. However the background potential was very high and more careful shielding might have been beneficial. These experiments were abandoned as there was more pressing business for the Vibron and it was thought advisable to develop an independent system.

There is some evidence that the vibron system could be made to work, for occasionally when an experiment has been left running on the recorder and the contact has broken, there have been changes to new potential values which have remained remarkably constant for an open circuit.

The first design of a vibrating probe was based on that of Blüh and Scott (1950) but used an amplifying circuit designed and assembled by Mr. Lincoln of the Physics Department, Durham College, in the University of Durham. This had a selective amplification of frequencies in the 200 to 300 c.p.s. range. This frequency was chosen to avoid interference from stray A.C. of 50 c.p.s. The motive power for the probe came from a telephone ear piece driven at 250 c.p.s. and the output was displayed on a cathode ray oscillograph (C.R.O.). The circuit diagram is shown in Figure 13.1.

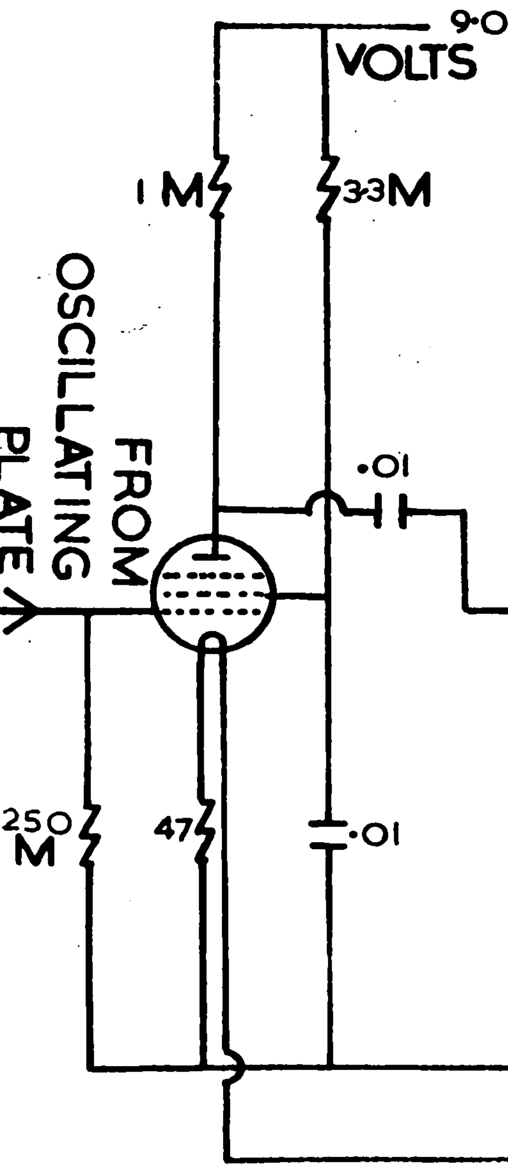


FIG. 13.1.

This was not successful, the failure being ascribed to the very small amplitude of the probe vibrations. Attempts were made to increase this by the use of a loudspeaker to drive the probe, but though at 50 c.p.s. the amplitude was over 0.25 inches at 250 c.p.s. the amplitude was still very small. Using the coil and permanent magnet of the loudspeaker and tuned lengths of various materials, it was still not possible to obtain much larger amplitudes at 250 c.p.s. A reapprisal was obviously necessary and this resulted in a system which is believed to be completely original.

It was reasoned that if the plate was mounted on part of the circumference of a wheel, perpendicular to the radius, and if the wheel was rotated in close proximity to the plant, the plate would be alternately near to and away from the plant. The wave form of the A.C. output would be different from that with perpendicular to and fro.movement of the plate but since a null point method could be used, this would not matter, for when the potential on the plant surface was exactly balanced by the applied potential, the output would be zero.

To give the required frequency rate, a rate of rotation of the plate of at least 15,000 r.p.m. was required, yet one also wanted a driving system which would not give rise to electrical interference. Both these conditions were fulfilled by air turbines.

for which this is a slow speed and which would also give rise to no electrical effects.

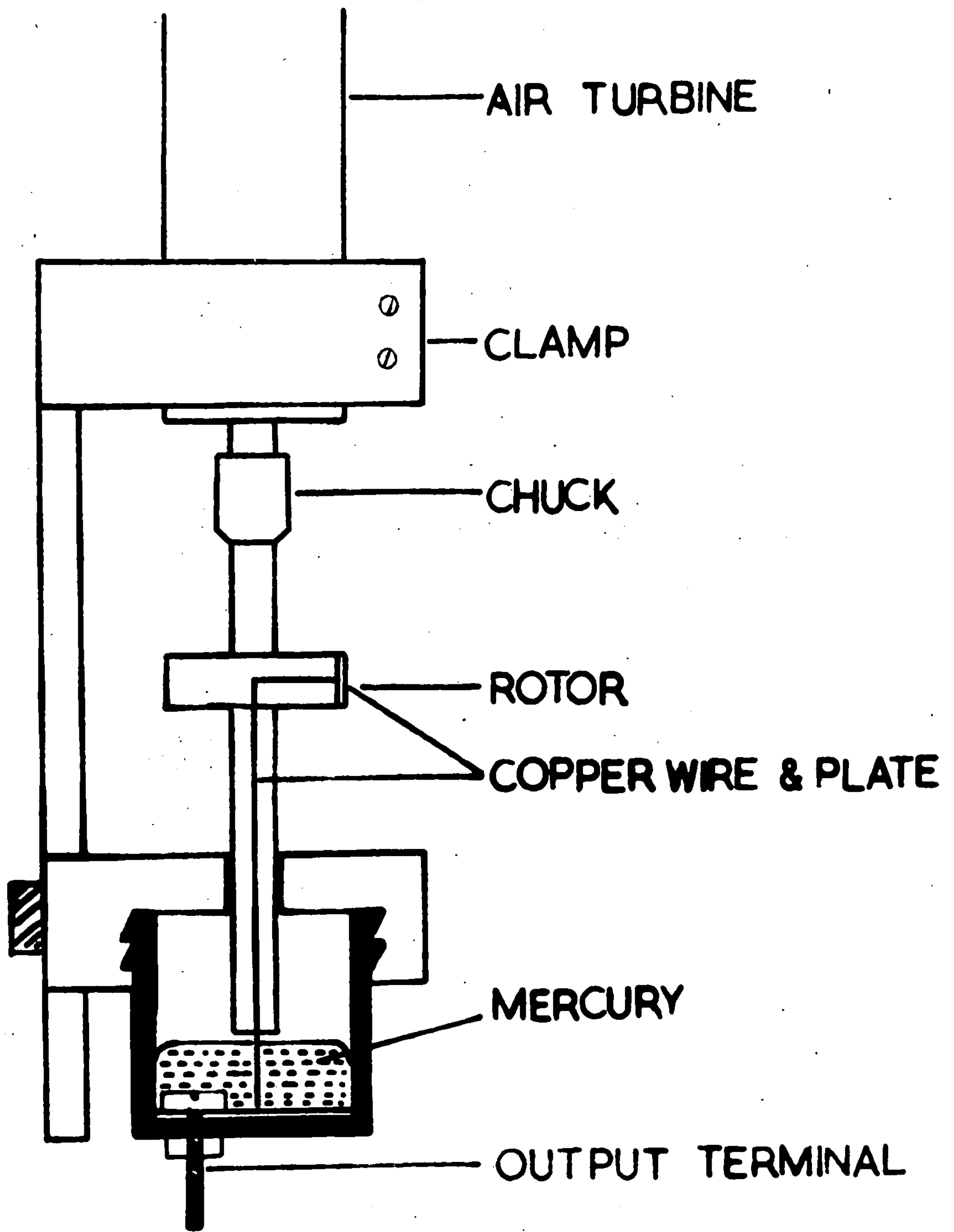
A Desoutter hand tool was obtained. In the standard form the air is discharged on to the rotating head but since the plant would be situated close to this, it had to be modified so that the air was discharged away.

From our design, the Durham Science Laboratories Workshop constructed the plate unit which is shown in Figure 13.2. & Plate 13.1.

The apparatus was completed just before our experimental work ceased and only a preliminary report is possible. The plate unit has only been tested in an open laboratory using another metal plate to simulate the plant. It was found that an alternating current whose magnitude and frequency depended upon the speed of rotation, was generated even when no charge existed on the experimental plate. This, it is suggested, may be due to the rotation of the plate in the earth's magnetic field, though screening has not been tried to test the suggestion.

Hope for the method comes from the fact that application of a charge (1 volt) to the plate resulted in a change in the output which though small, may, by screening and attention to circuit details, be adequate for null point detection.

One question immediately springs to mind when one studies this method. What is the relationship between the electrical



ROTOR, CLAMP, ETC., CONSTRUCTED FROM "TUFNOL."

FIG. 13.2.

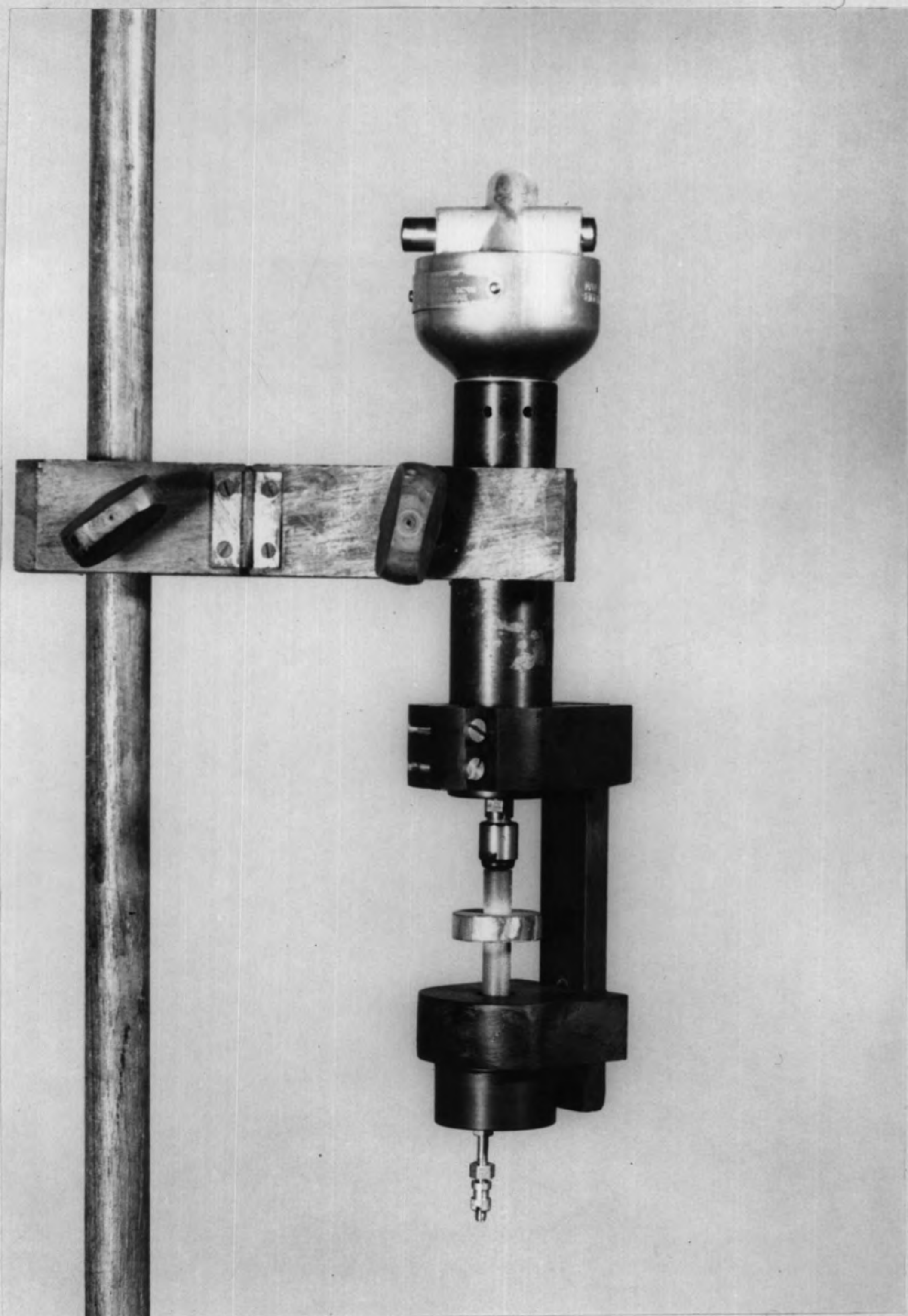


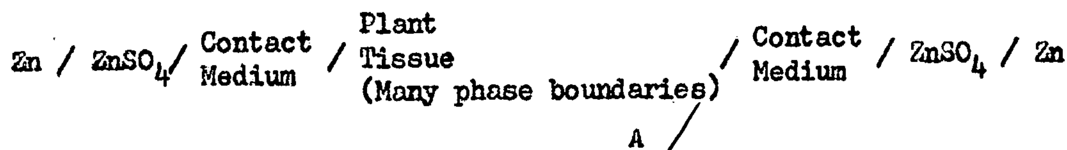
PLATE. 13.1.

potentials measured by conventional electrodes and those measured by this method?

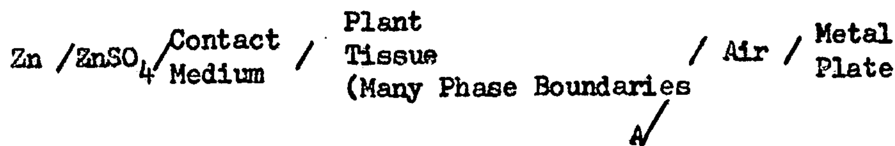
With the vibrating plate system one is measuring the potential that exists between the two parts of the condenser, the vibrating plate being earthed. One measures the surface potential of the plant with respect to earth. With the conventional electrode/contact system one measures the potential existing between the input wires of the measuring instrument.

In both cases, the potential measured is the algebraic sum of all the potentials at the different phase boundaries in the systems. Comparing the two systems, phase boundaries being denoted by, /,

Conventional Electrode



Vibrating Plate



The two systems are similar to the left of phase boundary "A" but there are differences to the right so the values given for the two methods will not necessarily agree. For instance, a $Zn/ZnSO_4$ electrode junction alone has a value of -0.762 volts, and with the

vibrating plate system, this is not counter-balanced. It however will be to some extent opposed by the metal plate/air junction which from Andauers results (1927), also has a value of about -0.7 volts. The values of the Plant/air and Plant/contact medium potentials in the two systems will also differ.

If, however, we are studying changes in potential which are due to changes within the plant tissue, then the two methods should give similar magnitudes of change, even though their absolute values of potential differ.

Application of a vibrating plate system to potential measurements in plant tissues could provide much interesting information especially when the data are compared with those from conventional methods. It is hoped in future to continue the development of the rotating plate electrode, for not only is this a unique method of electrical potential measurement, but it also offers advantages over any other system bases on capacitative methods.

CHAPTER 13.

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EPILOGUE.

EPILOGUE.

Reading through this thesis one could not but notice the number of times the words "further experimentation is necessary", occur. Since one of the objects of the research set out in the introduction, was to cover as wide a range of topics as possible, raising questions about them rather than always finding the answer, it is suggested this is a sign of success.

It is hoped in the future to continue with the work and esoecially by the use of intracellular electrodes confirm that changes ascribed to alterations of membrance permeabilities are in fact due to this.