MEMBRANE POTENTIAL DIFFERENCES IN BEAN ROOTS

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Summary

Various theories for the formation of electric potential differences in plant organs are outlined and the present investigation placed in relation to them.

Broad bean root tips behave as hydrogen electrodes in dilute solutions of the mineral acids HCl, HNO_3, and H_2SO_4 in a limited pH range, as shown by the change in p.d. between the root surface and the solution with change in H^+ ion concentration.

When the root tips are placed in neutral salt solutions of concentration between 0.1 and 0.0001N, there is a linear change in potential with logarithmic change in salt concentration. The solution is more positive for smaller concentrations. This is interpreted as a diffusion potential phenomenon, with greater mobility of the cation than the anion through the protoplasmic membrane. The ratios of mobility of cation and anion for four salts are calculated on this assumption.

When bean root tips are placed in buffer solutions with pH's in the range 3-5 and with a constant molar concentration of 0.1, there is no significant change in potential with pH.

There is a significant and nearly normal change in p.d. between root and solution for a change in KCl concentration from 0.01N to 0.1N when the KCl is at pH 3 or 5. Both buffered KCl and KCl + HCl bathing solutions were used.

The hypothesis is advanced that the p.d. between root and solution is the result of the diffusion of inorganic ions through or into the root protoplasmic membrane. The results are discussed in relation to Lundegardh's theory concerning the adsorption and exchange of cations at root surfaces.

I. Introduction

There has been a great deal of controversy in the last 10-20 years as to the origin of the electrical potential differences measured in biological systems. Several distinct theories have emerged which *seem* to be mutually exclusive, and in some cases apparently conflicting experimental results have appeared.

Lund and his co-workers (Lund and Kenyon 1927; Marsh 1928; Lund *et al.* 1947) having done extensive work on the electrical polarity of onion roots, *Avena* coleoptiles, Douglas fir, etc., postulate from the combined evidence that the "polarity potentials," or the main part of them, are oxidation-reduction potentials connected directly with the respiratory mechanism of the plant.

The redox theory has been criticized (Blinks 1949; Lundegardh 1940; Thomas 1939) partly on the grounds of experimental evidence and partly for

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theoretical reasons, it being thought that since noble metal electrodes were not used to measure the p.d. and are not present in the biological materials, a redox mechanism involving sources and sinks of electrons could not be directly responsible for the measured p.d.'s. However, this objection is not serious since it can be *assumed,* not unreasonably (Höber *et al.* 1947), that the plant contains loci that can act as oxidation-reduction electrodes. On the other hand, certain experiments of Thomas (1939) do not seem to conform to the redox hypothesis.

Blinks (1949), Osterhout (1936), and Hill and Osterhout (1937) find that membrane potentials appearing in large single-celled organisms can be explained as diffusion potentials of inorganic salts. The ratio of the mobilities of the cation and anion appear to be modified by the external protoplasmic membrane from the value for an aqueous solution.

Lundegardh (1940) postulates an alternative mechanism for the appearance of the membrane potentials in cereal roots. He has investigated the change in p.d. between root and surrounding solution with change in concentration of acid or salt in the solution and obtains results that he interprets as being due to an adsorption-exchange of cations on the root surface. Williams and Coleman (1950) also report cation exchange processes taking place on root surfaces. However, Lundegardh's results also seem consistent with the assumption of diffusion potentials, except for one particular experiment on the influence of the acidity of the bathing solution on the change of p.d. with concentration.

In view of the unsatisfactory position as regards the theories of bioelectric potentials (redox as opposed to diffusion, diffusion as opposed to adsorption-exchange potentials), it was thought advisable to make a start on the problem of the mechanism for the production of bioelectric potentials by repeating and extending some of Lundegardh's experiments to try to decide between the "diffusion" and "adsorption-exchange" theories. Such a research was also desirable in view of the apparent importance of salt concentration and pH in determining the measured p.d.'s in certain other experiments in this laboratory on plant organization.

The results given below in the main agree with those of Lundegardh but a disagreement has been found in the experiments on the change of p.d. with salt concentration at varied pH's. In consequence of this, the results when taken as a whole seem to be consistent with the assumption of diffusion potentials set up at the boundary between an acid-dissociated membrane and a surrounding solution.

However, it is emphasized that knowledge of the nature of the membrane potentials at any one region of a root surface does not permit conclusions to be drawn about the longitudinal p.d.'s that appear when equimolar contacting solutions are applied at two different locations on the root (Lund *et al.* 1947; Thomas 1939). Further experiments are in progress to elucidate the nature

of these p.d.'s. They are a function of at least two membrane p.d.'s which may themselves be different functions of salt concentration and pH, depending on the location of the two contacts.

Also, the radial p.d.'s between surface and inside of roots and hypocotyls (McAulay, Ford, and Hope, unpublished data) are no doubt composed of several phase boundary potential differences, one of which is that between protoplasmic membrane and contacting solution and is dealt with here. Other p.d.'s probably exist between protoplasm and cell sap and between different cells.

It is possible that all three mechanisms mentioned are concerned in bio-electric potentials, the measured p.d. being a function of one or more of them in single-celled organisms, others in non-liquid absorbing organs, and so on.

II. Apparatus and Materials

Potential differences were measured with a valve millivoltmeter of high input resistance, the grid current being less than 10^{-12} A. The accuracy was about ± 2mV. in the range 0-200mV. The concentration of hydrogen ion in the salt, acid, and buffer solutions was measured with a valve pH meter, accurate to within at least 0.1 pH units.

The electrodes used were small 2N calomel half-cells with a 2N KCl/agar jelly bridge waxed into 6 mm. diameter glass contact tubes filled with 1N KCl/agar. The other ends of these were drawn out to approximately 100 μ diameter. These were moved in the region of the plant roots with screw micro-manipulators. The contact tip + electrode zero error when both tips were placed in any of the solutions used was always less than 1 mV.

The roots were held·in a vertical position in a perspex box saturated with water vapour and with a flow of fresh air bubbling through a water reservoir at the base. The roots were illuminated with a spotlamp and observed with a low power dissecting microscope.

Commercial broad bean (*Vicia faba* L.) roots were used as the experimental material in the experiments to be described. They were germinated by soaking in tap water 24 hours and planting in spaghnum in a glass-house at 20°C. Approximately two days after planting the roots were from 20 to 40 mm. long and were used at this stage. The diameter of the root about 10 mm. from the tip was between 1.5 and 2.5 mm.

The plants were handled by the seed coat in the transfer from spaghnum trough to saturated box and there allowed to stabilize for some minutes. The tip of the root was dipped into a polystyrene cup holding 3 ml. of solution, the cup being filled with an eye-dropper and emptied via a rubber tube connected to its base. During the whole series of experiments the temperature inside the box varied between 18.5°C. and 23.5°C.

III. RESULTS

(a) Response of the Potential of the Root Tip to Changing Concentration of Mineral Acids

It is shown that the root tip of the broad bean behaves as a hydrogen electrode in solutions of certain mineral acids of concentration less than about $10^{-3}N$. The potential of the root tip relative to the acid solution changes by approximately 60 mV. at 21°C. for a ten times change in acid concentration. As Lundegardh has pointed out, the high speed of response of the p.d. on the application of a new concentration points to the boundary between root tip and solution as the site of the changing p.d. Many experimenters assume the site to be the outer protoplasmic membrane of the epidermal cells.
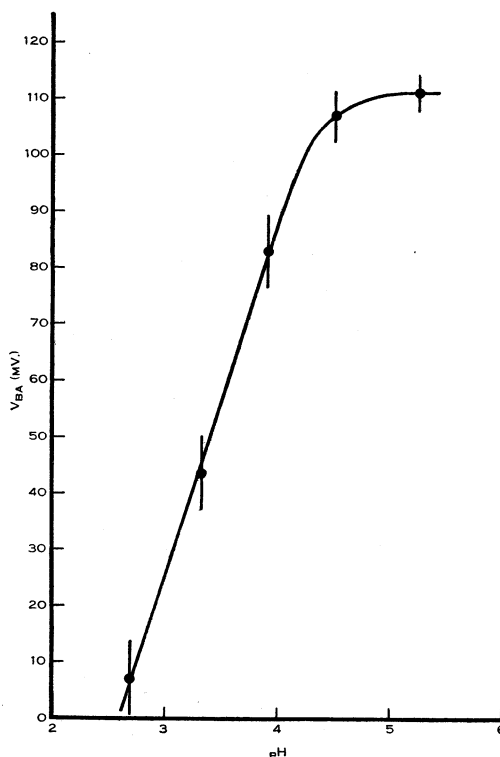

Fig. 1.—Variation of p.d. between KCl/agar contact (at *b*, 10 mm. from the root tip) and solution (*a*) with pH of HCl solution. The sign of the p.d. is that of *a* relative to *b*. The points plotted are the means and standard deviations for five experiments.

The graph of change in p.d. with change in pH for HCl concentrations in the range pH = 2.5-5.5 is shown in Figure 1. The points plotted are the averages and standard deviations for experiments on five roots. The acids

were applied to the root in increasing concentration.   In the pH range 2.5-4.0 the equation to the graph is

$$V_{ba} = -155 - 60 \log_{10} [HCl] \text{ (mV.)},.. \quad .. \quad .. \quad (1)$$

the sign being that of the solution a relative to b, a reference point.   The curving over of the graph after pH 4 could be caused by evolved CO_2 from the root lowering the pH of the applied acid from the measured value.   Equation (1) may be compared with the familiar Nernst equation for electrode potentials:

$$E = E_o - 58 \log_{10} [M^+] \text{ (mV. at } 20°C.) \quad .. \quad .. \quad (2)$$

Contact with a root was made about 10 mm. from the tip at "b" through 0.1N KCl/agar jelly and 1-2 mm. of the root tip was dipped into the cup containing the HCl solutions.   The circuit was completed by dipping the second contact tip into the solution beside the root (contact "a").   Readings were taken at the beginning and end of one-minute intervals, the solution then being changed.   Similar experiments with HNO_3 and H_2SO_4 gave results that are not statistically different from Figure 1.

The x-axis intercept may be thought to give the average pH of the outer layers of the protoplasm of the root tips, but little significance can be attached to the value (2.6) because of an unknown membrane potential between root and KCl/agar at b.   There is, however, evidence that this unknown p.d. is small.

The maximum concentration of the acids applied was limited to a little greater than 10^{-3}N because it was found that acid of about that concentration killed the roots on prolonged immersion.   When strong acid is applied and the concentration decreased, graphs are obtained with an average slope of about 40 mV. per pH unit.   This is possibly due to permanent damage caused by the initial application of acid of pH 2.7.

(b) Response of the Potential of the Root Tip to Changing Concentration of Neutral Salts

The salts KCl, NaCl, KNO_3, and $NaNO_3$ give an approximately linear relation between change in p.d. and log (salt concentration) when applied to the root tip in concentrations between 0.1N and 0.0001N.   The graph of an average of 10 experiments with KCl is shown in Figure 2.   The straight line has the equation

$$V_{ba} = -38 - 32 \log_{10} [KCl] \text{ (mV.)}, \quad .. \quad .. \quad .. \quad ..(3)$$

which, if a diffusion type potential is assumed to be present, may be compared with

$$E = E_o - 58 \frac{u - v}{u + v} \log_{10} [KCl] \text{ (mV. at } 20°C.) .. \quad ..(4)$$

where E is the measured p.d., u, v the mobilities of cation and anion respectively in the protoplasmic membrane, and E_o a constant including the p.d. between the KCl/agar and the root surface at b and also a term containing the concentration of salts in the membrane.   By identifying equations (3)

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and (4), u/v, the ratio of the mobilities of cation and anion in the protoplasmic membrane, may be evaluated.

TABLE 1
RATIOS OF IONIC MOBILITIES

Salt	Av. Slope of Graphs of p.d. vs. log [MA]	u/v Calc.	u/v in Aqueous Solutions at ∞ Dilution
KCl	32 mV./log unit	3.5	0.98
NaCl	28 mV./log unit	2.9	1.04
KNO$_3$	25 mV./log unit	2.5	0.66
NaNO$_3$	31 mV./log unit	3.3	0.70

Table 1 shows the average slopes of graphs for 10 experiments with each of KCl, NaCl, KNO$_3$, and NaNO$_3$ solutions, the calculated values of u/v, and for comparison, the ratios of mobilities in aqueous solutions at infinite dilution.
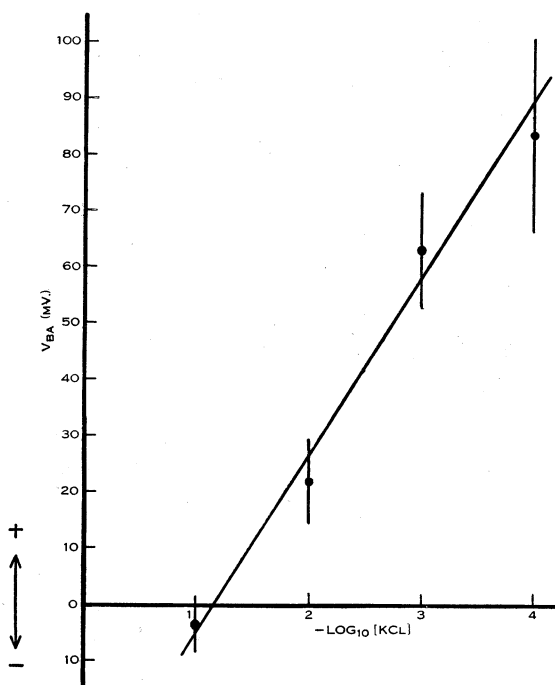

Fig. 2.—Variation of p.d. between root and solution with concentration of KCl, showing the means and standard deviations for 10 experiments. The sign of V_{ba} is that of a relative to b.

It is seen that, if the graphs are interpreted in this way, K$^+$ and Na$^+$ have a similar mobility in the membrane compared with Cl$^-$. In these experiments, there was no statistical difference in the results obtained when solutions were applied in increasing and in decreasing concentration.

(c) Response of the Potential of the Root Tip to Changing pH in Buffer Solutions

Experiments of the type described in Sections III (c) and III (d) were made to find if exchange of cations for H^+ ions (adsorbed to the root surface) takes place when the root is immersed in a salt solution. This process is the basis of Lundegardh's theory of salt adsorption (see Lundegardh 1940 and Section IV). It is obvious that such exchange would be regulated by the pH of the surrounding salt solution, since, for example, if the pH of root surface and solution were the same, H^+ ions would have little tendency to leave the root. Also, the potential difference between root and solution would depend upon the nature of the adsorbed cation (of the strength of the dipoles it forms), and this provides a means of testing the hypothesis.

TABLE 2

CHANGE IN P.D. WITH pH IN BUFFER SOLUTIONS

pH Change	4.9-4.5	4.5-3.9	3.9-3.5	3.5-3.1	3.1-3.5	3.5-3.9	3.9-4.5	4.5-4.9
P.d. change	+4	+4	0	—2	—6	—2	0	0

When buffer solutions* in the pH range 3-5 and with equimolar salt concentrations are applied to the root tip in either increasing or decreasing pH, there is no characteristic change of p.d. with change in pH. There is, however, a general drift of the p.d. with time when the root is in any particular solution and this is enlarged upon in Section III (e). Table 2 records for a typical experiment the change in p.d. between root and solution for the

TABLE 3

CHANGE OF P.D. FOR A CONCENTRATION CHANGE FROM 0.01N TO 0.1N AT DIFFERENT pH'S

(i) Buffered KCl				(ii) KCl and HCl		
Root No.	δV at pH 3 (mV.)	δV at pH 5 (mV.)		Root No.	δV at pH 3 (mV.)	δV at pH 5 (mV.)
1	22	12		1	25	28
2	24	45		2	23	26
3	21	32		3	23	27
4	28	28		4	23	27
5	37	22		5	22	35
Mean	26.4	27.8		Mean	23.2	28.6
S.D.	± 5.8	± 10.8		S.D.	± 1.0	± 3.2

corresponding change in pH. It is seen that the p.d. changes are small and random and that the membrane potential is thus a function of total molar concentration (and time) rather than of hydrogen ion concentration.

* McIlvaine's buffer (citric acid plus disodium hydrogen phosphate) was made up to pH's in the range 3 to 5, the total molar concentration being about 0.1 for each solution.

(*d*) *Response of the Potential of the Root Tip to Change of KCl Concentration*
at Different pH's

It is shown that the change in potential between root tip and solution for a ten-times change in concentration of KCl at pH's of 3 and 5 is very nearly the same. Table 3 shows the results of 10 experiments, five with buffered KCl and a second five with KCl acidified with HCl. The table records the change in V_{ba} for a change in KCl concentration from 0.01N to 0.1N, firstly at pH 3 and secondly at pH 5. In (i) the response is independent of pH while in (ii) the difference may be significant but δV at pH 3 is still large. It is seen that in these experiments the change of p.d. with change in salt concentration is not greatly influenced by the pH of the salt solution.
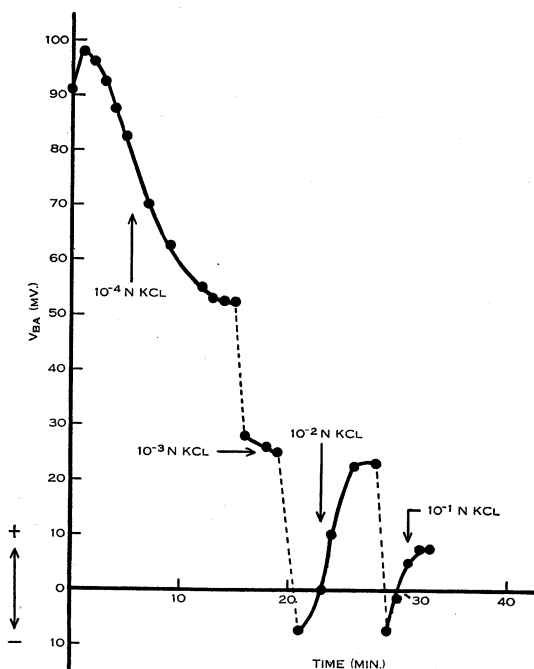

Fig. 3.—Variation of p.d. V_{ba} with time for different concentrations of KCl solution. The sign of the potential is that of *a* relative to *b*.

(*e*) *Change of the Potential of the Root Tip with Time for Various*
Concentrations of KCl

Figure 3 is a typical graph of the time rate of change of V_{ba} for changing KCl solutions of concentration 0.0001, 0.001, 0.01, and 0.1N. The usual changes in p.d. are obtained on changing the concentration of the solution, but while the p.d. with the root tip in 10^{-3}N KCl is practically constant with time, the solution tends to become more negative when the root tip is in 10^{-4}N KCl and more positive when in 10^{-2}N, 10^{-1}N KCl.

From equation (4), if this expression is accepted as describing the p.d.: concentration relation, it is seen that if E is to vary for constant [KCl] then either E_o or u/v must vary with time. Since the changes in p.d. obtained on changing the KCl concentration tenfold are comparable with those of Figure 2, u/v must be assumed constant. On the other hand, E_o contains terms including both the phase boundary p.d. at b and the salt concentration of the root phase. As the 10^{-1}N KCl/agar contact at b was established only some 5-10 min. before the time "zero" of Figure 3, the p.d. at b cannot be assumed constant and it is felt that a detailed interpretation of Figure 3 must be left until further experiments are made. Without consideration of the p.d. at b the p.d. variations in Figure 3 point to diffusion of salt into the root at outside concentrations of 10^{-1} and 10^{-2}N and back diffusion into the bathing solution at 10^{-4}N.

This effect would have to be reconciled with existing theories for the accumulation of salt by root tissue from dilute solutions.

IV. DISCUSSION

The experiments described were made following and extending Lundegardh's (1940) to elucidate the nature of the phase boundary potential differences found between roots and solutions surrounding them. The results on the whole are consistent with the hypothesis of diffusion potentials set up at the interface between the solution and the root outer protoplasmic membrane.

The signs of the potential differences are such as to suggest that the cation has a greater mobility than the anion, with the salts investigated. The results indicate that the protoplasm is acid-dissociated with a pH in the region of 3. The data of certain of the experiments are in agreement with Lundegardh's but the crucial experiments of Sections III (c) and III (d) do not seem to conform to the adsorption mechanism and this is discussed in the following.

According to Lundegardh the potential of a root surface relative to a solution bathing it is derived from a difference in hydrogen ion concentration between the root and solution. If there has been a primary adsorption of hydrogen ions (from acid or pure water) to postulated free negative valencies in a monolayer, then the root will behave as a hydrogen electrode in solutions of pure acids. Also H^+ ions are said to be exchanged for metallic cations when the root is placed in a salt solution. The pH of the root surface, and thus its potential relative to the solution changes with varying concentration of salt.

The results given in Sections III (a) and III (b) are in accordance with this view but also with the hypothesis of a diffusion process in an acid-dissociated membrane. A concentration of free hydrogen ion of about 0.001N in the root phase accounts for the relation between p.d. and pH given in Figure 1 while selective permeability makes reasonable the relation between p.d. and salt concentration when the root tip is placed in a salt solution (Fig. 2 and Table 1).

Further, on the adsorption-exchange theory, the tendency of exchange of H^+ for M^+ in a salt solution should depend to a large degree on the pH of this

solution. Lundegardh reports that at low pH's (3-4) little exchange of H^+ for M^+ occurs, as reflected by the absence of change of p.d. for change in salt concentration at this acidity. However, in the experiments reported (Sections III (c) and III (d)) there was no significant change in p.d. for change in pH at a constant concentration of about 0.1M nor was there much difference in magnitude in the response to a change in KCl concentration from 0.01N to 0.1N at pH 3 and pH 5. This is at variance with Lundegardh's results and theory, and speaks once more for a diffusion type potential difference being present.

Similarly, the evidence given in Section III (e) showing the variation of the root p.d. with time in various concentrations of salt is consistent with a tendency towards the establishment of equilibrium between the ions in bathing solution and in the protoplasm by the mechanism of diffusion.

V. ACKNOWLEDGMENTS

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