ELECTRIC POTENTIAL DIFFERENCES IN BEAN ROOTS AND THEIR RELATION TO SALT UPTAKE

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Summary

Reversible diffusion of KCl is shown to occur between an aqueous solution and young bean roots, probably in the protoplasmic phase. Evidence for this is presented from a study of electric potential difference (p.d.) changes and changes in environmental salt concentration in different samples but under comparable conditions.

Characteristic changes of p.d. with time are obtained when a root tip taken from distilled water is dipped in 0.01N KCl followed by 0.0001N KCl, indicating an increase of concentration in the root tissue while in the first solution and then a decrease while in the more dilute solution.

When excised root tips which have been in distilled water are immersed in 0.01N KCl, conductivity measurements show a mean initial uptake of 0.13×10^{-5} g. mol./g. of tissue. An approximately equal amount of salt leaves the roots if they are then transferred to 0.0001N KCl.

The region of the tissue to which the electrolyte has access by diffusion, called the "apparent free space," is calculated to be 13 per cent. for the material used. Approximately 10 of the 13 per cent. is likely to be protoplasm.

The evidence supports the hypothesis that electric p.d.'s between young root tissue and surrounding electrolytes are the result of differential mobility of cations and anions in the protoplasmic phase of the epidermal cells.

The relation of the evidence to theories postulating a high resistance barrier to ion diffusion at the boundary of protoplasm and environment is discussed. It is concluded that the properties of the protoplasm/environment boundary do not include high resistance to ion diffusion. It is possible that protoplasm contains a certain concentration of non-mobile anions resulting in a Donnan distribution of ions between this phase and the surrounding solution. Ways of testing this hypothesis are suggested.

I. Introduction

In an earlier paper (Hope 1951) the results were given of investigations concerning electric potential differences (p.d.) appearing between solutions of electrolytes of various concentrations applied at two loci on broad bean roots. It was shown that these could be interpreted as diffusion p.d.'s between the root phase and the surrounding solution. These p.d.'s were caused by differences of concentration of electrolyte and in mobility of anion and cation in

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diffusion between the environment and what was presumed to be the protoplasmic phase of the root cells. These results were compared with those of Lundegardh (1940) and agreement found in most instances but it was pointed out that Lundegardh's results, which he concluded were consistent with an adsorption of ions to a lipoid-type membrane at the boundary between protoplasm and environment, could equally well be interpreted on the basis of diffusion of ions.

In Section III (*e*) of that paper (Hope 1951) experiments were reported in which potential changes with time seemed to show a reversible change of concentration of the root phase corresponding to changes in concentration in the environment. These experiments have been repeated under more controlled conditions to eliminate spurious p.d. changes at the reference point on the root. Also, evidence is presented from a study of the conductivity changes in solutions containing root tips to show that there is movement of salt into the root phase from a concentrated solution by means of diffusion. This is followed by back leakage of the salt if the root is transferred to a more dilute solution. An estimate is made of the percentage of the root tissue concerned with the free diffusion of salt. The nature of the phases into which salt can diffuse reversibly is discussed. Experiments are suggested with which to test the hypothesis that the root protoplasm contains a bulk distribution of immobile anions.

II. Experimental Method

Broad bean (*Vicia faba* L.) roots were grown as described in the earlier paper. In the present experiments, however, instead of being taken immediately from the moist spaghnum (of unknown ionic composition) the roots when 2-3 cm. long were held in a perspex trough containing aerated distilled water and equilibrated for 17-24 hr. at a fairly constant temperature of $18 \pm 3°C$. In the experiments concerning the uptake of salt by root tissue, a number of tips 5 mm. long (containing the meristematic region and elongating cells) were excised from roots between 2 and 4 cm. long and, with several changes of water, equilibrated for 17-24 hr. in a flask aerated with moist air.

(*a*) *P.D. Experiments*

Reference should be made to Hope (1951) for details of nomenclature and technique. However, the following modifications were made. Tenth-normal calomel half-cells dipping into glass tubes containing 0.1N KCl and 10 g. agar/l. were used to make contact with the various solutions surrounding the root. The p.d.'s were measured with a high input resistance valve electrometer.

The roots were removed from the distilled water and the surface mopped dry of excess moisture. Contact at the *b* region was established 20-25 min. before the time zero of Figure 1. This was done by lowering the root, held at an angle of 20-30° from the horizontal, onto a polystyrene cup holding 0.0001N KCl into which dipped the reference contact tip. If this contact was restricted to a small area of the root, liquid did not tend to travel to the *a* region and the two loci were kept insulated from each other.

(*b*) *Conductivity Experiments*

The method of following salt uptake by measuring the change in conductivity in the electrolyte surrounding the living tissue is substantially the same as that used by Robertson (1941). The tissue : solution ratio was usually 0.7 g. root tips: 10 ml. solution. No significant difference in salt uptake was found between whole and excised root tips, so the cut tips were adopted because of convenience since the tips of whole roots with their large seeds are difficult to arrange in a small volume of solution. In addition, with whole roots a variable volume of tissue (greater than that immersed in the solution) is concerned with absorption and the uptake per gram of tissue is difficult to measure. The excised tips were transferred from distilled water to a small cup containing 10 ml. of KCl, which was aerated gently with moist air, the whole being held in a perspex box through which moist air was flowing. In each experiment the temperature was constant within $\pm 1°C$. Samples of *c.* 0.5 ml. of solution were withdrawn for measurement of conductivity at intervals. Each of these was returned to the experiment just before the next sampling.

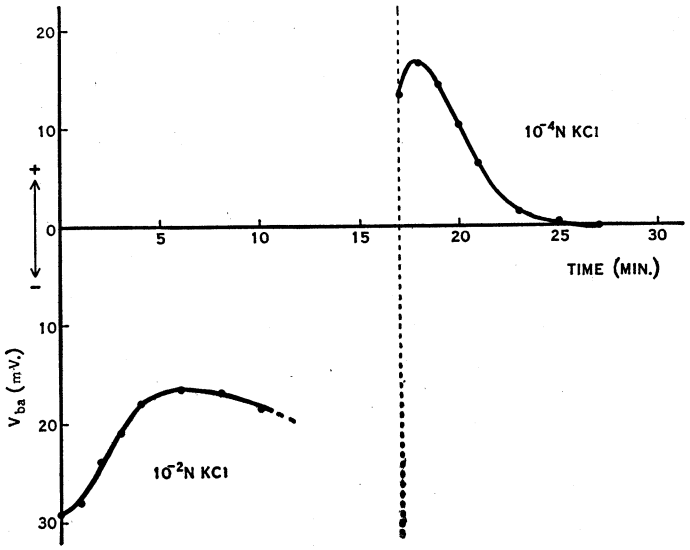

Fig. 1.—Variation of p.d. at the root — solution interface at *a* (tip of root) with time. The roots were immersed first in 0.01N then 0.0001N KCl. The sign of the p.d. is that of the solution at *a* relative to the reference solution at *b* (10-20 mm. from tip). The points plotted are the means for five experiments.

III. Results

(*a*) *Change with Time of the Potential Difference between Root Tips and 0.01N, 0.0001N KCl Solutions*

Figure 1 shows that the potential of a 0.01N KCl solution applied to the region "*a*" at the tip of the root becomes more positive by up to 12 mV. in the

first 5 min. with respect to a reference solution at "*b*" some 10-20 mm. from the *a* region. If the 0.01N solution is then removed and 0.0001N KCl substituted, the characteristic quick change in p.d. is obtained, the solution at *a* immediately becoming some 40 mV. more positive but then drifting *c.* 15 mV. more negative in the following 5 min.

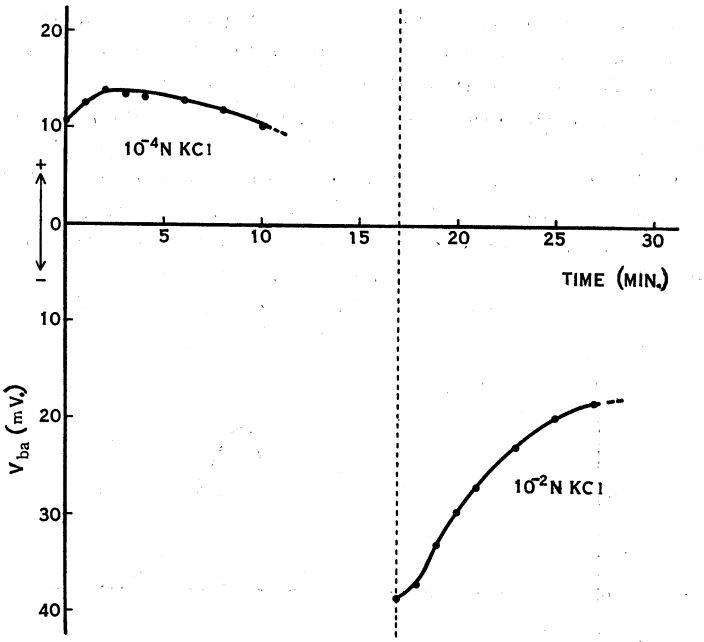

Fig. 2.—Variation of p.d. with time when the root tips are immersed in 0.0001N, then 0.01N KCl. The sign convention is as for Figure 1. Mean of five experiments.

It has previously been shown that for quick changes in salt concentration, the potential difference between the reference point *b* and the solution surrounding *a* could be represented approximately by the equation for diffusion potentials:

$$V_{ba} = k + 58\frac{u-v}{u+v}\log_{10}\frac{c_2}{c_1} \quad .. \quad .. \quad (1)$$

where the p.d. is in mV. at 20°C. and the sign that of *a* relative to *b*; *u*, *v* are the mobilities of the cation and anion in the phase in which they are diffusing; c_2 the inside and c_1 the outside salt concentrations; and *k* a constant. In previous experiments approximately 30 mV. change per tenfold change in [KCl] was obtained, and the ratio u/v was calculated to be approximately 3. In later experiments the change varied between 20 and 25 mV./tenfold concentration change. If V_{ba} changes with time for constant c_1 then *k*, u/v, or c_2 must be a function of time. *k* includes the phase p.d. between the solution at *b* and the root and this must be assumed nearly constant in the time intervals involved

since the contact is made well before the circuit is completed by dipping the tip into solution. This was not the case in the experiments reported earlier in which the reference point at *b* was variable owing to contact being made with 0.1N KCl only some 5-10 min. before that at *a*. Also, u/v must be nearly constant since normal quick changes in p.d. occur with quick changes of c_1, viz. 20-25 mV./tenfold concentration change. Thus the results seem to suggest that c_2 increases as the tip remains in 0.01N KCl and decreases again when in 0.0001N KCl.

Figure 2 shows that if the root tip is taken from distilled water and the first contact at *a* made with 0.0001N KCl then V_{ba} remains approximately constant for 10 min. The application of 0.01N KCl then gives a negative change of 40-50 mV. in V_{ba} followed by a positive drift of nearly 20 mV. This is consistent with the diffusion hypothesis since the root phase in which diffusion can occur is presumably free of diffusable ions after being treated with distilled water. Thus an initial application of 0.0001N KCl causes much less change in c_2 than the subsequent application of 0.01N KCl.

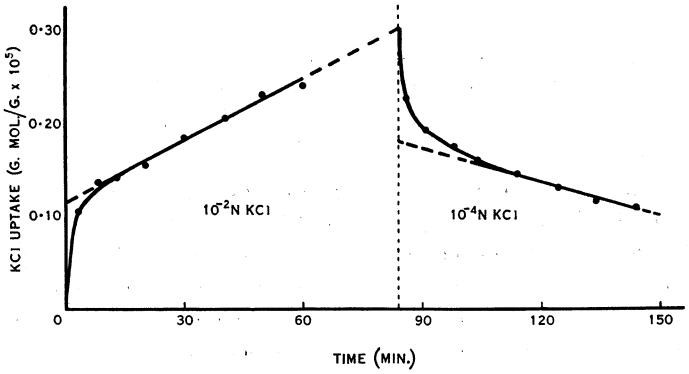

Fig. 3.—Initial uptake and accumulation followed by leakage of KCl by excised root tips immersed in 0.01N and 0.0001N KCl solutions respectively.

(*b*) *Uptake of KCl by Excised Root Tips*

The conductivity method (Robertson 1941) was used to find the changes in concentration that occur in KCl solutions of various concentrations, surrounding root tips. It is shown that when tips of broad bean roots are transferred from distilled water, the surfaces being carefully mopped dry of free liquid, to 0.01N KCl solution, the conductivity of the KCl decreases in a regular and repeatable manner. Figure 3 shows the result of a typical experiment. When in 0.01N KCl, the uptake of salt appears in two stages, an initial uptake (Robertson 1944; Robertson, Turner, and Wilkins 1947) in which usually $0.13 \pm 0.02 \times 10^{-5}$ g.mol./g. tissue leaves the solution in about 20 min., followed by a steady decrease in solution concentration at the rate of $0.14 \pm 0.04 \times 10^{-5}$ g.mol./g./hr. The latter is active accumulation by the respiratory mechanism

(Robertson 1941). If the tips are then transferred to 0.0001N KCl they leak an amount approximately equal to the initial uptake in 0.01N KCl and in a similar time, followed by a slow, steady leakage at the rate of $0.05 \pm 0.02 \times 10^{-5}$ g.mol./g./hr. (Fig. 3).

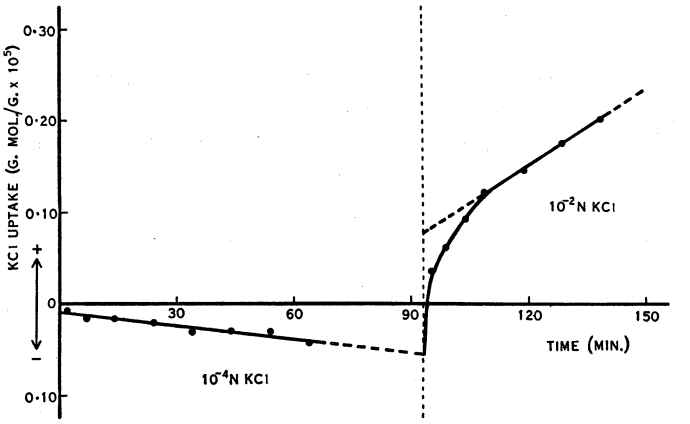

Fig. 4.—Slow leakage followed by initial uptake and accumulation of KCl by excised root tips immersed in 0.0001N and 0.01N KCl respectively.

Figure 4 gives a typical result of placing the equilibrated root tips immediately into 0.0001N KCl for approximately 1 hr. followed by treatment with 0.01N KCl. No marked initial leakage is observed in the dilute solution but an uptake of 0.12×10^{-5} g.mol./g. occurs in the more concentrated one, in about 20 min., followed by a steady uptake at the usual rate. These results should be compared with those obtained with excised sections of barley roots by Milthorpe and Robertson (1948). The accumulation rate for bean root tips is of the same order as that for the barley roots (0.45×10^{-5} g. mol./g./hr.) but the initial uptake in the barley experiments was approximately 1×10^{-5} g. mol./g. compared with $0.13 \pm 0.02 \times 10^{-5}$ g. mol./g. (an average of 15 replicates) for broad bean roots. This disagreement could be due to a difference in pH (see later) or to insufficiently dried roots in the barley experiments.

(*c*) *Distribution of Free Spaces in Bean Root Tissue*

The experiments just described show that there is a reversible diffusion of KCl into bean root tips of average amount 0.13×10^{-5} g. mol./g. when the tips are placed in a 0.01N solution. If that part of the tissue to which the KCl has access is empty of electrolyte to start with, then during the initial uptake 0.13 g. of each g. of tissue reaches a concentration of 0.01N. This proportion has been termed the "apparent free space"* (A.F.S.). The A.F.S. for the root

* This term has been used by G. E. Briggs (unpublished data) to describe the portion of the plant tissue into which substances in solution apparently move by free diffusion.

tissue is thus 13 per cent. It is possible that the KCl can diffuse reversibly into:

(i) Intercellular spaces which are injected with distilled H_2O;

(ii) Spaces in the cellulose wall lattice which likewise contain water with very little dissolved substance in it; and

(iii) The protoplasm of the cells.

Since the salt accumulated by the salt respiration mechanism does not leak out to any extent in very dilute solutions (Robertson and Thorn 1945) it is almost certain that the cell vacuoles are not concerned in the diffusion of KCl in the initial uptake and initial leakage.

(i) The mean intercellular space in the 5 mm. tip of bean roots has been estimated as follows. Transverse microtome sections, fixed and stained with haematoxylin, were placed in a microprojector and the cell outlines drawn on squared paper. The intercellular space was calculated from the difference between total area and area occupied by cells. The root cap, epidermal, and stele cells are closely packed but the cortical cells are more nearly circular in section. From 10 sections cut at various distances from the root cap, the mean intercellular space is 7 per cent. by area, and therefore 7 per cent. by volume. This may be an over-estimation if shrinkage occurred during fixation and staining but a living section, cut free-hand and non-stained, also had 7 per cent. intercellular space. However, nearly all the spaces between cells in the cortex are filled with air and not liquid, as shown in Plate 1, Figure 1, which is a photomicrograph of a transverse section hand-cut and placed in water. The darker spaces around the cells are evidently air bubbles. This air disappears if the section is placed in a vacuum for a short time, as seen in Plate 1, Figure 2. The contribution of the intercellular space to the A.F.S. is therefore small and may be zero if all spaces are filled with air.

(ii) It is only possible to make a rough estimation of the contribution by the cellulose wall spaces to the A.F.S. Wardrop* (personal communication) has suggested the following approach. The dry weight of the 5 mm. tips of broad bean roots is about 12 per cent. of the fresh weight. Brown and Sutcliffe (1950) suggest that about 25 per cent. of the dry weight of comparable sections of barley roots is cellulose. If this figure is applicable to bean roots then cellulose comprises about 3 per cent. by weight of the fresh tissue and less by volume.

If the cellulose network is associated with its own volume of water (a possibility suggested by Robertson†, personal communication) the cellulose walls contribute less than 3 per cent. to the apparent free space.

(iii) It is thought likely that up to 10 per cent. of the tissue, not including intercellular and cell wall spaces, is permeable to KCl. It is almost certain

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that this free space is protoplasm. It is hoped to be able to make an estimate of the amount of protoplasm in a given piece of bean tissue by a staining technique.

IV. Discussion

In experiments reported earlier (Hope 1951) it was strongly indicated that potential differences appearing in broad bean roots as a result of a difference of concentration of electrolyte applied at two insulated loci were due to diffusion p.d.'s set up at the boundaries between the root and solution.

The results given in Section III(*a*) of this paper, and summarized in Figures 1 and 2, support this hypothesis. They have been interpreted to show that the root phase increases in ionic concentration when transferred from distilled water to 0.01N KCl since the surrounding solution drifts positive with respect to a constant reference electrode. The subsequent negative drift when the root is transferred to 0.0001N KCl similarly indicates a decrease in root concentration. Since a 20-25 mV. change in p.d. has been shown to correspond to a tenfold change in outside concentration, the 10-20 mV. drifts in Figures 1 and 2 may be tentatively taken to indicate a 3-10-fold concentration change somewhere in the root.

These changes of concentration have been proved directly by following the changes in conductivity of solutions of KCl surrounding excised root tips. The graphs of Figures 3 and 4 show that about 0.13×10^{-5} g. mol. KCl/g. of root tissue enter the root tips in about 20 min. when they are taken from distilled water and dipped in 0.01N KCl. The same amount of KCl leaves the tips following a transfer from 0.01N KCl to 0.0001N KCl. The A.F.S., or that part of the tissue to which solute has access by diffusion, is calculated to be 13 per cent. by weight (and 13 per cent. also by volume). Consideration of the intercellular spaces indicates that these are not part of the A.F.S. since they are filled with air and, apart from a small contribution by spaces in the cellulose fibres of the cell walls, the A.F.S. must be largely protoplasm.

The ability of ions of an electrolyte to penetrate the protoplasm to more than a few molecular layers is contrary to Lundegardh's (1940) hypothesis that a lipoid-type membrane, containing fixed negative valencies and having a high resistance to ion movement through it, is present at the interface between protoplasm and surrounding medium. According to Lundegardh's ideas the first step in the active accumulation process was a passive adsorption of metallic cations to the membrane in exchange for hydrogen ions. In the light of the present evidence, and contrary to Lundegardh's theory, it seems more likely that ions which are later transported by the accumulation mechanism are able to diffuse without great difficulty into the bulk of the protoplasm. Whatever the nature of the interface between the protoplasm and the environment, its properties do not seem to include a high resistance to diffusion of the ions of KCl.

There have been several suggestions made that protoplasm contains a bulk distribution of negative immobile ions (as opposed to the Lundegardh monolayer; see particularly Blinks 1940; Briggs and Robertson 1948). The facts

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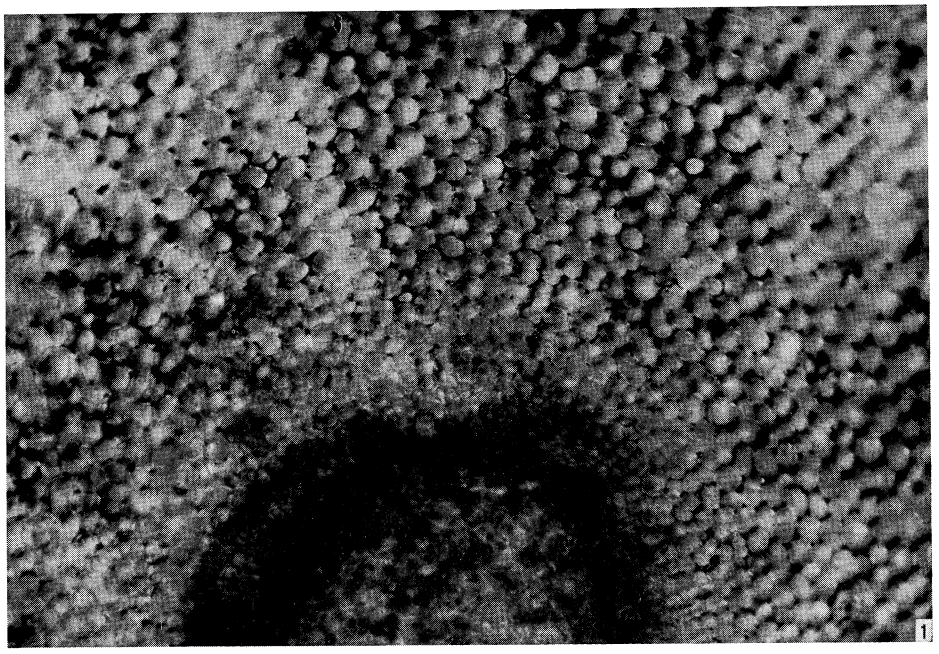

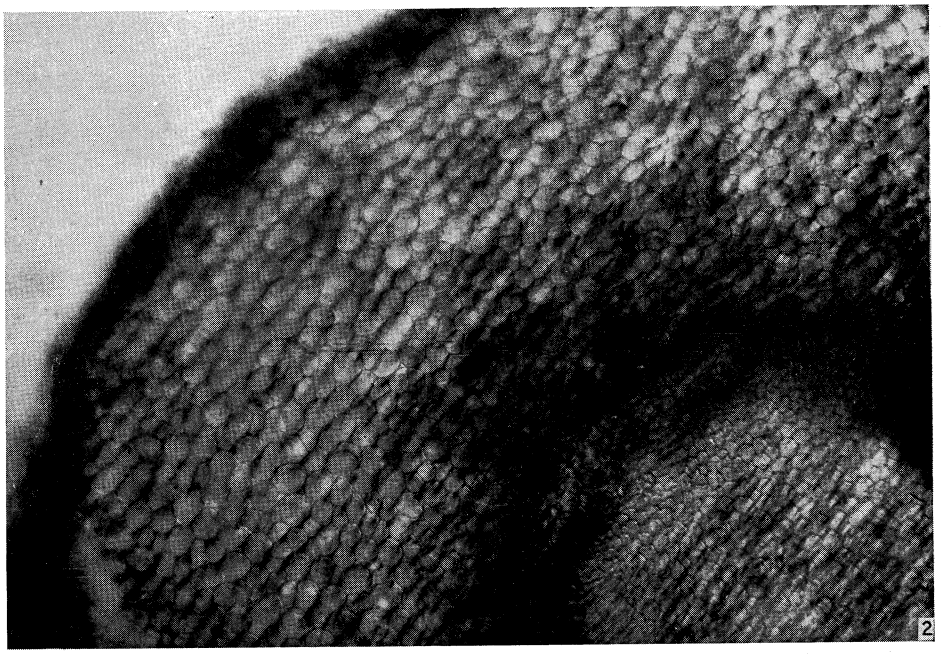

Fig. 1.—Photomicrograph of a fresh, hand-cut section of a bean root 3-5 mm. from the apex, under water. The dark spaces between the cells, particularly in the cortex, are air bubbles.

Fig. 2.—A second section, which was placed in a vacuum for 5 min., showing the absence of the dark spaces between the cells. Both approx. x109.

that the cation/anion mobility ratio for diffusion in protoplasm is increased compared with its value in water, and that the isopotential point of (at least the outside of) protoplasm occurs at a pH of 3-4 (Lundegardh 1940; Hope 1951) are also in accordance with this idea. Such a system would result in a Donnan distribution of ions between the protoplasmic and solution phases. It is proposed to test this by observing the effect on the size of the A.F.S. of varied salt concentration and varied pH. Robertson and Wilkins (unpublished data) have found that the initial uptake in carrot tissue is increased as a result of a decreased pH in the medium. This is consistent with a reduced concentration of immobile anions (due to de-ionization) allowing a larger concentration of mobile ions to be reached in the protoplasm.

Also, as suggested by Briggs and Robertson (1948), it should be possible to distinguish between diffusion in a phase containing immobile ions and that in one containing only mobile ones by modified experiments on the relation between p.d. and salt concentration. The p.d. *versus* salt concentration equation contains terms involving the concentration of immobile ions which reduce to the linear relation for simple diffusion p.d.'s when this concentration is zero. However, when the concentration of the immobile ion is not zero considerable deviations from equation (**1**) should occur.

V. Acknowledgments

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