THE ELECTROCHEMICAL STATE OF CELLS OF BROAD BEAN ROOTS

I. INVESTIGATIONS OF ELONGATING ROOTS OF YOUNG SEEDLINGS

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Summary

An attempt is made to apply the Ussing–Teorell criterion for passive ion movement to root cells of young broad bean seedlings. This requires the estimation of ion concentrations in the cells, ion fluxes between cellular compartments and the external medium, and membrane potential differences.

Ion fluxes were estimated in short-term isotope loading and elution experiments, and it is argued that these estimates are valid for sodium and potassium efflux and for sodium influx. A procedure is described for determining the time course of ion accumulation by root cells from a study of root growth and the spatial distribution of the ion in the root. This is shown to lead to estimates of net sodium flux that are in satisfactory agreement with tracer estimates of influx and efflux.

The interior of cortical cells is at a potential of -130 mV with respect to an external medium containing 1 mM KCl, 1 mM NaCl, and 0·5 mM $CaCl_2$. The potential is the same for meristematic, elongating, and mature cells.

It is concluded that sodium is actively pumped out of cortical cells in all parts of the root. Potassium appears to be close to electrochemical equilibrium although it is actively accumulated by non-vacuolated cells.

Permeability coefficients of the plasmalemma in mature cortical cells to potassium, sodium, and calcium are tentatively estimated to be 22×10^{-8}, 6×10^{-8}, and $0{\cdot}043 \times 10^{-8}$ cm/sec respectively.

I. INTRODUCTION

In recent years there has been an upsurge of interest in problems of ion accumulation by plant cells and particularly in the role of cellular membranes in selectively regulating the entry of ions into the cell. It is easily shown theoretically (Briggs, Hope, and Robertson 1961; Dainty 1962) that electric fields in these membranes play a vital part in this regulation, and must be taken into account when deciding whether an ion is entering the cell actively (i.e. whether metabolic energy is being used to influence the ion movement), or whether it is entering passively.

The electrochemical theory of ion movement has been applied successfully in a number of recent investigations of the coenocytic cells of the large algae (Scott 1967), but little attention has been given to the cells of higher plants which differ from them in a number of ways apart from the obvious one of size. With the higher plants, it is not practicable to measure ion content or ion fluxes directly for individual cells, and these quantities have to be inferred from studies of the bulk properties of

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multicellular systems. It is also more difficult to obtain reliable measurements of the electrical characteristics of their cellular membranes.

In spite of the more formidable difficulties, it is of great importance to extend the electrochemical approach to the cells of higher plants in order to provide a basis for the understanding of mechanisms of salt accumulation in these plants, for it cannot be assumed that the mechanisms involved in the large algae apply generally to all plant cells.

An approach to this problem has been described in several papers by Higinbotham, Etherton, and Foster. Etherton and Higinbotham (1960) measured potential differences across membranes of individual cells of various higher plants, finding internal potentials of -80 to -120 mV with respect to a bathing medium 0·1 mM in potassium. In later work (Etherton 1963; Higinbotham, Etherton, and Foster 1964, 1967), plant tissue was immersed in bathing solutions of various ionic compositions for periods of up to 48 hr, and attempts were made to ascertain the electrochemical status of the ions involved from observations of the ion concentration differences and potential difference between cell interior and external medium.

Recently, Etherton (1967) estimated sodium and potassium effluxes from pea root tissue using ^{22}Na and ^{86}Rb (as a tracer for potassium). He then calculated the net flux of these ions using the Ussing–Teorell equation (i.e. on the assumption that the ions were moving passively). By comparing these calculated net fluxes with those obtained from direct analysis of the ion content of the tissue as a function of time, he was able to confirm the assertion from the earlier work that sodium was actively extruded from root cells. The method was not sufficiently accurate to resolve the electrochemical status of potassium, although earlier work had suggested that this ion was actively accumulated from solutions of low potassium concentration but extruded actively if the external concentration was high.

Apart from a few investigations with storage tissue (Laties, MacDonald, and Dainty 1964; Poole 1966; Macklon and MacDonald 1967), the only other experiments in which ion flux, ion concentration, and membrane potential measurements have all been made on the same higher plant tissue have been recently described by Pitman and Saddler (1967). Excised roots of 6-day-old barley plants were used and at 5°C were approximately in a state of flux equilibrium. These investigators were able to measure the fluxes of sodium and potassium across the tonoplast and plasmalemma and to estimate the cation content of the cytoplasmic phase. Although potassium was close to electrochemical equilibrium at high external potassium concentrations it was concluded from the effects of inhibitors that potassium was actively accumulated, while sodium was pumped out of these cells.

The investigations to be described in this paper represent another attempt to apply electrochemical theory to the cells of a plant root. The ionic behaviour of the root of a young broad bean seedling in a steady state of growth is considered, based on a series of experiments using tracer techniques, microanalyses of tissue extracts, and electrical potential measurements with inserted microelectrodes. Since the non-homogeneous nature of root tissue must inevitably complicate the analysis of its properties, particular attention is given to variations in behaviour in different parts of the root, including its meristematic, elongating, and mature regions. A method of

determining the net uptake of ions by root tissue from knowledge of the ion distribution in the root and the rate of root growth is described. These estimates of net flux are compared with those predicted from tracer experiments.

II. Experimental Methods

The material used in these investigations was a long-pod variety of *Vicia faba* L. Seedlings were grown in running tap water to which a concentrated solution of salts was added automatically to make the growth medium 1 mM in Na^+ and K^+, 0·5 mM in Ca^{2+} and 3 mM in Cl^-. Seeds were soaked for 24 hr in the flowing growth medium, after which the testas were removed and the cotyledons impaled on rods which were suspended above the bath so that the seedling radicles were submerged. Age of the seedlings was reckoned from the time at which they were set up in the growth bath. The growth medium in the 17-litre bath was changed at the rate of 220 ml/min, temperature being held at $25 \pm 0 \cdot 5$°C. Although lighting conditions did not appear to be critical, exposure to strong light was avoided. Two-day-old plants whose roots were about 30 mm long were used in most experiments.

Cation concentrations in the tissue were measured using a Coleman model 21 flame-photometer. For each experiment, 10 roots were selected and excised. They were quickly rinsed and lightly clamped on a Perspex stage. The roots were cut transversely into 1-mm segments with a sharp razor-blade which was mounted so that it could be moved in the direction of the root axis by means of a calibrated screw drive. Each lot was weighed in a separate beaker and soluble substances were removed by boiling with distilled water for 1 hr. The extract was decanted and the residue reboiled with more distilled water for a further 15 min. The second extract was added to the first and analysed for sodium, potassium, and calcium content.

Chloride in the water extract was estimated using a modification of the differential potentiometric method described by Bishop and Dhaneshwar (1962) with acetone as the solvent and added nitric acid (A.R.). Nitric acid sharpens the end-point, possibly by depressing organic acid dissociation.

The radioactive isotopes ^{22}Na, ^{42}K, and ^{36}Cl were used to estimate ion fluxes in seedlings. Short-term tracer experiments were performed for reasons given in Section V(*b*). The validity of these estimates of ion flux will be considered there in detail. For tracer uptake investigations, the roots of a group of 10 seedlings were immersed in about 25 cm^3 of a labelled solution of the same ionic composition as the growth medium, which was stirred and aerated. After loading for 1 hr (for most experiments), the roots were washed in non-radioactive solution for a further hour with the aim of removing isotopes from the short-term compartments. Temperature was maintained at 25°C during these operations. Roots were then excised and sectioned into 1-mm segments, and each lot was weighed and desiccated by heating in an individual planchet. The sample activity was measured using either a Philips electronic counter model 4035 or an EKCO scaler type N683A. Samples of known volume of the radioactive bath were evaporated and counted in order to calibrate the equipment. To allow for ^{42}K decay, all counts were standardized to the same time zero. In some experiments, uptake of sodium and potassium was studied simultaneously on the same roots using a medium labelled with both ^{22}Na and ^{42}K. The samples were counted immediately and again after about 1 week, by which time the ^{42}K activity was negligible.

The tracer efflux from different regions of the intact root was determined using a small Perspex container divided into several chambers by means of equally spaced latex rubber membranes stretched vertically across it at intervals of about 2 mm. Holes of suitable diameter were burnt through the membrane so that they were aligned horizontally. A non-excised root which had been loaded with isotope for 5 hr and washed for a further hour was fitted through the holes so that the membranes formed a firm but gentle seal at each point of contact. The volume of each chamber was about 0·5 cm^3. Solutions in the chambers were replaced by unlabelled medium at 15-min intervals for a period of 90 min. Isotope recovered in the last hour was used as an estimate of efflux. At the end of the experiment, methylene blue was added to alternate compartments to dye the root so that when removed from the chamber it could be

sectioned at the positions of the membrane for weighing and assay of the isotope retained by the tissue. It was felt that neither the pressure of the membranes nor the small size of the chambers affected the behaviour of the root as the total efflux from all compartments was the same as for a root dipping freely in the solution. Furthermore, the bioelectric field in the solution near the root (usually a sensitive indicator of injury — Scott, McAulay, and Jeyes 1955) was unaffected.

Potential differences across cellular membranes in the root were measured by insertion of glass microelectrodes of about $0 \cdot 5\ \mu$ diameter filled with 3M KCl. Balanced calomel half-cells were used to make electrical connections to the electrometer (Keithley model 603). The seedling was placed horizontally in a Perspex trough through which the experimental medium was perfused at 25°C, and the microelectrode was inserted using a screw-type micromanipulator. Insertion into the root was observed with a stereoscopic microscope.

III. Theory

Estimation of Net Fluxes in the Tissue of a Growing Root

As a root develops, the cells of which it is composed increase in size and ion content as they become further from the root tip. In the following analysis, it is shown how the time course of ion accumulation by root tissue or by individual cells can be estimated from consideration of the spatial distribution of ions in the root and from a study of root growth.

Consider a thin transverse segment of the root which at time t is at a distance x from the root tip. The segment has length l and area a. The total amount of an ion in the segment, q, is equal to cla, where c is the mean ionic concentration. Alternatively, if c is expressed as ion content per unit mass, a is the mass per unit length of root. In general, c, l, and a (and hence q) are all functions of x and t. Thus

$$\mathrm{d}q = (\partial q/\partial t)\mathrm{d}t + (\partial q/\partial x)\mathrm{d}x,$$

and the net inward flux into the segment

$$\phi = \mathrm{d}q/\mathrm{d}t = \partial q/\partial t + (\partial q/\partial x)\, v,$$

where v is the rate at which the portion of root below the segment is elongating.

Beyond the meristematic region, the length of the segment varies in proportion to the length of cells in it. It is convenient to choose for consideration a segment whose length l is equal to the mean length of its cortical cells, since these form the bulk of root tissue [nearly 90% in the mature parts of the root — see Section IV(a)]. $(\partial q/\partial t)$ is therefore the rate of change with time of ion content per cortical cell length of the root at a particular distance from the root tip (usually a small contribution to ϕ), and $(\partial q/\partial x)$ is the rate at which ion content per cortical cell length changes along the root.

The partial derivatives are obtained in the following manner. Values of q at different distances from the root tip are calculated from corresponding values of the ion concentration (here expressed in milliequivalents per kilogram — see Figs. 2 and 3), the cortical cell length (see Fig. 1), and the mass per unit length of the root (Table 1). These values are plotted against x for both 2-day and 3-day roots and the derivatives with respect to time and distance read off from the graphs.

The value of v can be obtained directly from measurements of the growth rate of different parts of the root, using, for example, the techniques of Erikson and Goddard (1951). However, in the present experiments, the root was found to be approximately in a steady state of growth [see Section IV(a)], in which case v is proportional to l beyond the meristematic region, the constant of proportion being equal to the number of cortical cells added to a column per unit time. Under these conditions v can be written as lV/L, where L is the length of mature cortical cells and V is the rate of elongation of the whole root. Hence

$$\phi = \partial q/\partial t + (\partial q/\partial x)\, lV/L.$$

IV. Results

(a) *Root Structure and Growth*

Observation of a large number of roots indicated that the rate of elongation after 36 hr of growth in the standard medium (1 mM K^+ and Na^+, 0·5 mM Ca^{2+}, 3 mM Cl^-) of 25°C was approximately constant and remained so for at least the next 48 hr. A small diurnal variation was disregarded, and the mean rate was taken to be 0·8 mm/hr.

Table 1

Data for 2-day-old bean roots grown at 25°C

l and d are length (μ) and mean diameter (μ), respectively, of the cells

Distance from Tip (mm)	Epidermis		Cortex		Pith		No. of Cells across Diameter	Root Density (g/cm³)	Mass per Unit Root Length (mg/mm)
	l	d	l	d	l	d			
2	17	21	20	24	51	21	83	1·11	1·3
4	26	23	31	29	84	25	89	1·01	2·4
6	51	25	68	34		25	84	0·95	2·7
8	83		116					0·92	2·9
10	96		144	39	337			0·91	3·1
12	103	23	165	40	344	23		0·89	3·3
14	107		178	40	400			0·88	3·5
16	111	27	184	41			91	0·88	3·7
18		27	185	40			93	0·87	3·9
20							93	0·87	4·1

Table 1 shows the mean dimensions of cells in a 2-day-old root grown under the standard conditions. These data were obtained from thin longitudinal and transverse sections of wax-embedded roots cut with a microtome, supplemented by observations of fresh roots sectioned by hand. To obtain the mean dimensions of cortical cells, a total of about 500 cells was measured from sections of six roots. The standard deviation of cell length in one region of the root was about 15% of the mean length. Fewer measurements were made of epidermal or pith cells and less accuracy is claimed for their dimensions as given in the table. Root density was estimated from the rates of fall or rise of root segments in water or sucrose solutions of known densities.

No marked differences in cellular dimensions were observed at the same distance from the tip in 2-day and 3-day roots. The root at this age appears to be approximately in a steady-state condition of growth, the size and rate of enlargement of the cells being a function of distance from the root tip but not of time. This simplifies the calculation of net uptake of ions by the root as explained in the previous section.

In this paper, most attention was given to the properties of cortical cells, since these form the bulk of the root tissue. At distances of 7 mm and 15 mm from the root tip, cortical cells occupy 91 and 92% respectively of the root volume, while 80% of tissue at 2 mm is procortical. No evidence was found that pith or epidermal cells have vastly different ionic properties, and it was therefore felt that data obtained on root tissue as a whole could be taken to provide information on cortical cells.

Figure 1 gives the length, volume, and surface area per unit volume of tissue for cortical cells at different distances from the root tip.

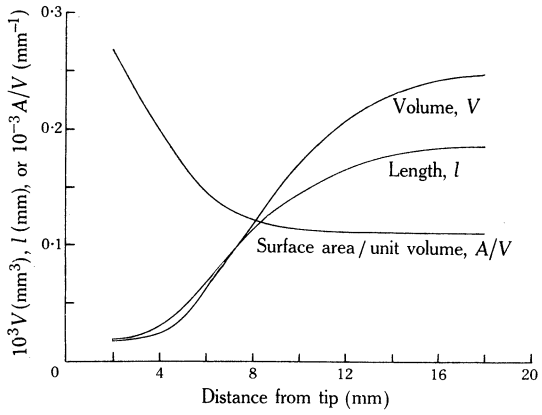

Fig. 1.—Length, l, volume, V, and surface area per unit volume, A/V, for cortical cells and procortical cells at different distances from the root tip.

(*b*) *Analysis of Distribution of Ions in Root Tissue*

Figure 2 shows the distributions of water-extractable cations in 1-mm segments of 2- and 3-day roots, growing at 25°C in the standard medium. Each graph represents the mean of between 5 and 13 separate experiments, each involving 10 roots. Concentrations are expressed as milliequivalents per kilogram fresh weight of tissue and the limits indicate the standard error of the mean. No allowance was made for possible phosphate interference in the determination of Ca^{2+} by flame-photometry, and the values given in Figure 2 may be underestimated. Note that potassium concentrations are higher than for the other ions. The K/Na ratio is particularly high in the tip region (about 18 for 2-day roots) falling to 3·5 in more mature parts of the root.

It is clear that there is no major change in cation content or distribution in the root during the early period of growth, although of course the content of individual cells changes greatly during this period. Even at 8 days, the distribution of potassium in the tip region was almost the same as at 2 days. Sodium levels, however, were about double those for the younger roots.

Analysis of root tissue for chloride is also shown in Figure 2. It is seen that the chloride concentrations in most regions of the root are much greater at 3 days than at 2 days. These concentrations are far from sufficient to balance the sodium and potassium in the root. It seems likely that the remaining anions are largely organic, although Higinbotham, Etherton, and Foster (1967) report high concentrations of sulphate, phosphate, and nitrate in roots growing in media rich in these ions.

Data given in Figure 2, together with data on root growth from Figure 1, are used to calculate net fluxes of sodium and potassium in root tissue by methods described in Section II. These net fluxes at different distances from the root tip are shown in Figures 3 and 4. It is noted that the net fluxes are greatest in the regions of most

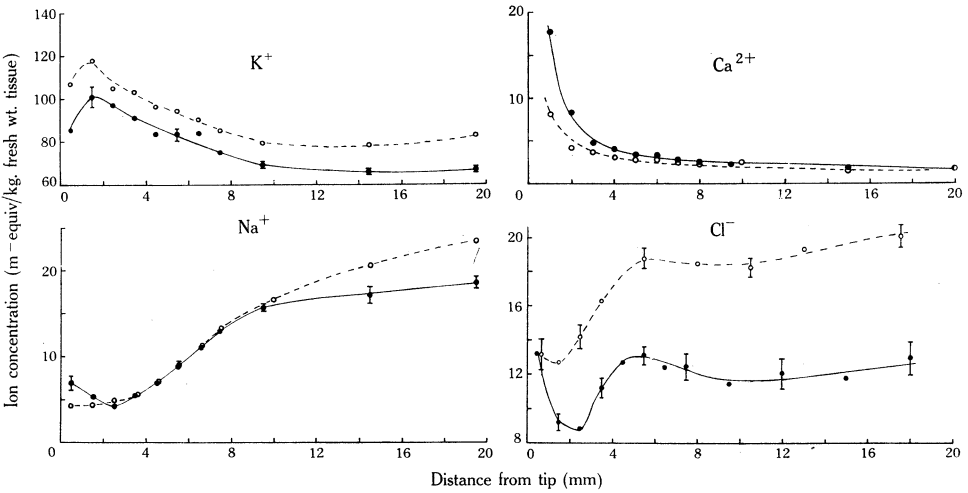

Fig. 2.—Water-extractable Na^+, K^+, Ca^{2+}, and Cl^- in bean roots grown for 2 (●) and 3 days (○) at 25°C in the standard medium (1 mM KCl, 1 mM NaCl, and 0·5 mM $CaCl_2$). The limits indicated for some points are standard errors of the mean value.

rapid cell enlargement (as is to be expected), although the peak for K^+ is about 1 mm closer to the root tip than that for Na^+. Similar estimates of net flux for Ca^{2+} and Cl^- from concentration data in Figure 2 are not shown. At 18 mm from the root tip the estimated net influxes of these ions are 0·019 and 0·70 m-equiv/kg/hr respectively.

(c) *Tracer Estimates of Influx and Efflux*

Figures 3 and 4 also show the influx and efflux of Na^+ and K^+ as functions of distance from the root tip for 2-day-old bean roots in the standard growth medium at 25°C. Fluxes were estimated in short-term loading and elution experiments as previously described, with ^{42}K and ^{22}Na as tracers. The validity of these estimates will be considered in detail in Section V. For estimation of sodium and potassium uptake 100 and 70 plants respectively were used. Elution of sodium was observed on 14 individual roots, and on 19 roots for potassium. Figure 5 shows the influx of

chloride into the root. Efflux measurements were not attempted because of the low specific activity of ^{36}Cl.

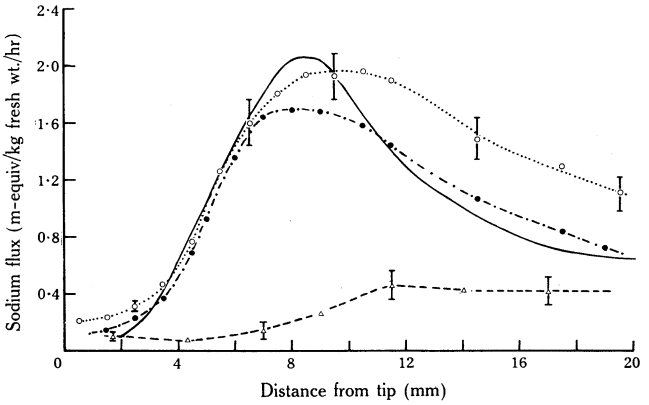

Fig. 3.—Sodium fluxes for 2-day-old bean roots. — Net uptake rate calculated from ion distribution data (Fig. 2) and growth data (Table 1). O Influx estimated in ^{22}Na-uptake experiments. △ Efflux estimated in ^{22}Na-elution experiments. ● Net uptake rate estimated from influx − efflux.

It is seen from these results that sodium influx is very low in the tip region of the root and is much greater for vacuolating (4–12 mm from tip) and mature (>15 mm) cells. The difference between influx and efflux estimated from tracer

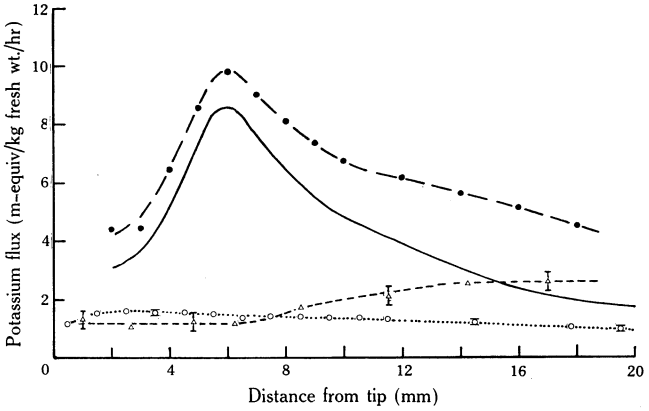

Fig. 4.—Potassium fluxes for 2-day-old bean roots. — Net uptake rate calculated from ion distribution data (Fig. 2) and growth data (Table 1). O Influx estimated in ^{42}K-uptake experiments. △ Efflux estimated in ^{42}K-elution experiments. ● Influx estimated from efflux + net uptake rate.

experiments is in good agreement with the net flux estimated through consideration of the ion changes in the growing root. In the case of potassium, these two estimates of net flux are seriously at variance for reasons discussed in Section V(*b*). Influxes

of chloride determined in tracer experiments also appear to be underestimates, being less than the net flux estimates.

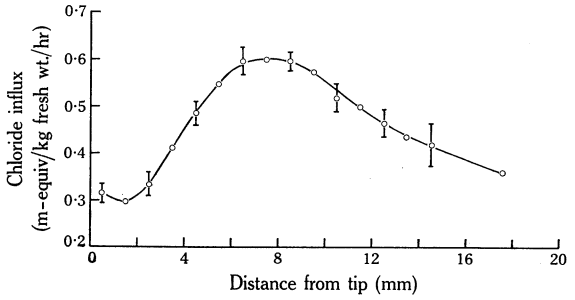

Fig. 5.—Chloride influx for 2-day-old bean roots estimated in ^{36}Cl-uptake experiments.

(*d*) *Potential Differences across Cellular Membranes*

When a microelectrode was inserted into a cell of an intact bean root immersed in the standard medium at 25°C, the internal potential was observed to change abruptly and to continue slowly to become more negative for 3 or 4 min. A reading was regarded as satisfactory if thereafter it remained steady for at least 30 min.

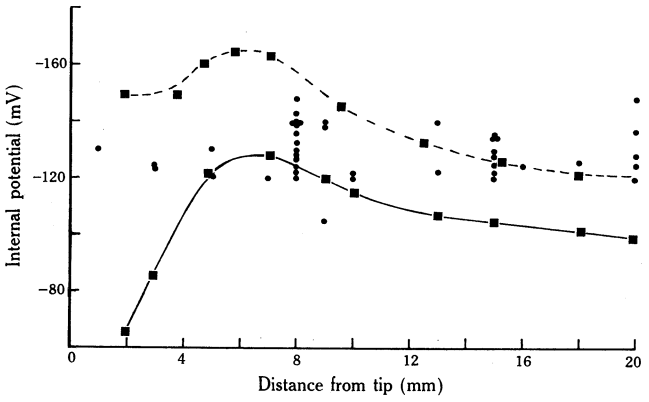

Fig. 6.—Measured values (●) of the potential of the interior of cortical cells of bean roots relative to the external medium (1 mM KCl, 1 mM NaCl, 0·5 mM $CaCl_2$), and values of the potential calculated on the assumption that sodium (■———■) or that potassium (■– – –■) is moving passively, i.e. that the Ussing–Teorell criterion is applicable.

Readings were discarded if the tip potential on withdrawal had changed by more than 5 mV. Values of the potential for cortical cells at different distances from the root tip are plotted in Figure 6. It was not possible to separate the plasmalemma and tonoplast potentials.

Although there is some scatter in the readings, no major variation in the membrane potential along the root was observed, the mean potential being approximately -130 mV.

V. Discussion

(a) *Electrochemical Changes in Growing Cells*

The uniformity along the root of the membrane potentials of cortical cells (-130 mV) is interesting in view of the large and rapid changes in size and ion content of the growing cells. In the tip region the vacuoles are undeveloped and the cellular content is predominantly cytoplasmic. Potentials measured in these cells are almost certainly those across the plasmalemma. The fact that the same potential is observed in mature cells may indicate (1) that the contribution at the tonoplast to the potential between vacuole and external medium is small, as is found for many of the fresh water algae and *Avena* root hair cells (see Scott 1967); and (2) that the ionic composition of the cytoplasm is similar at all stages of cellular growth, being approximately that of tissue in the tip region. In this region, the K/Na ratio is much higher than for mature cells. Discrimination against sodium by cytoplasm has been shown for *Nitella* (Spanswick and Williams 1964; Kishimoto and Tazawa 1965), and for barley root cells (Pitman and Saddler 1967).

(b) *Validity of Tracer Estimates of Influx and Efflux in Short-term Experiments*

The fact that plant tissue cannot in general be regarded as a single compartment in its exchanges of ions with the environment complicates the estimation of ion fluxes by means of isotope tracers. A simple analysis of data obtained in 1 or 2 hr of loading or elution is feasible only if the non-free space (i.e. the vacuolar and cytoplasmic phases) of the tissue approximates to a single compartment. This is the case *either*

(1) if the plasmalemma fluxes are much less than the tonoplast fluxes, in which case the plasmalemma is the main barrier to ion diffusion and the cell interior assumes a near-uniform specific activity, *or*

(2) if the ion content of the cytoplasm is low and its equilibration with the external medium is achieved in a time which is short in comparison with the duration of loading or elution.

In case (1), the estimated fluxes are those across the plasmalemma. In case (2), the estimated fluxes depend on those across both membranes but will approximate to the tonoplast fluxes if this membrane is the main barrier to ion movement.

With growing roots it is not possible to undertake a complete compartmental analysis, leading to evaluation of the separate fluxes across the plasmalemma and the tonoplast, such as has been performed by MacRobbie and Dainty (1958) for *Nitellopsis obtusa*, and by Pitman (1963) for beet disks. These experiments occupy periods of 20–40 hr, during which time the root would have elongated several centimetres and initiated secondary roots. In addition, considerable amounts of ion would have been translocated in the root (Scott and Martin 1962). For these reasons,

the use of isotopes for flux estimations in the present experiments on intact roots was limited to short experiments. It is now necessary to consider whether the observations can be satisfactorily interpreted in terms of the simple model discussed above.

With the large, fresh-water algal cells, the plasmalemma is the main barrier to ion diffusion. This has been shown in tracer experiments with *Nitella* (MacRobbie 1962) and *Chara* (Hope 1963), and are consistent with electrical measurements by Walker (1960), who showed that the major part of the resistance and potential difference between vacuole and external medium occurs across this membrane.

No clear picture has emerged from the few investigations of root cells. The only measurements of the separate resistances of the membranes are by Greenham (1966), who found that the plasmalemma resistance is at least five times that of the tonoplast in root hair cells of maize and oats. It also appears to be the site of the main potential drop (Etherton and Higinbotham 1960). On the other hand, recent tracer studies by Pitman and Saddler (1967) on excised roots of 6-day-old barley plants at 5°C suggest that the potassium flux across the two membranes are comparable and that the sodium flux is about 20 times greater across the plasmalemma than across the tonoplast. Etherton (1967) finds sodium and potassium fluxes across the plasmalemma about five times greater than across the tonoplast in pea roots. The concentration of ions in the external medium was large (20–40 m-equiv/l of cations), and the plasmalemma may assume a more important role in limiting ion movement in a more dilute environment (Epstein 1966; Torii and Laties 1966).

Notwithstanding how the plasmalemma and tonoplast fluxes compare, the single internal compartment approximation appears to be valid in the case of sodium in higher plant cells. This is apparently due to the low capacity of the cytoplasm for this ion. Pitman and Saddler (1967) imply that external sodium equilibrates with the cytoplasm in root tissue in about 12 min at 25°C. In fact, at these temperatures it is often impossible to separate a cytoplasmic component in a full-scale analysis (Pitman 1963).

In the work described in this paper, the good agreement between the net flux of sodium in root tissue estimated from growth data and the separate fluxes estimated from the short-term tracer experiments (Fig. 3) strongly suggests that the latter estimates are valid and that the non-free space of the root is behaving as a single compartment for sodium. It is not possible to conclude from these experiments whether the main barrier to sodium diffusion is at the plasmalemma or at the tonoplast.

In the case of potassium, no such satisfactory agreement is found (Fig. 4). In fact, the tracer experiments suggest that there is a net efflux from the upper part of the plant, although it is clear from growth data that the potassium content of cells in this region must be increasing with time. The major cause for this discrepancy appears to be the diffusion of potassium from the cotyledons. The concentration of potassium in freshly soaked cotyledons is high (151 m-equiv/kg wet weight), falling by about 16% after 2 days of root growth. Since the cotyledons have a mass of about 2 g, they lose about 50 μ-equiv. of potassium during the 48-hr period, an average rate of about 1 μ-equiv/hr. This is more than sufficient to meet the requirements of the root, which at 2 days has a mass of about 80 mg. If the mean

net influx is 5 m-equiv/kg/hr, the rate of increase of potassium in the root is 0·4 μ-equiv/hr. A small net gain of potassium (0·02 μ-equiv/hr/plant) by the bathing solution was observed with young seedlings whose roots (but not cotyledons) were immersed in 1 mM KCl solution, confirming that some of the potassium leakage from the cotyledons occurs via the roots.

It could be argued that influx has been underestimated in these short-term isotope experiments because of loss of isotope from the non-free space during the first hour of washing. The amount lost during this period is not sufficient to account for the large discrepancy (a factor of 3–6), and in any case experiments involving tracer uptake for longer periods confirm the order of magnitude of the rate of accumulation of tracer by the tissue.

Data in Figure 4 are therefore assumed to indicate that only 15–35% of the potassium ions entering the root came from the external solution. The remainder is unlabelled potassium from the cotyledons, and it is envisaged that this enters the cell either by direct non-free space pathways or through the free spaces of the root where it in effect reduces the specific activity of the potassium immediately adjacent to the cells. In either case, the entry of unlabelled ion from the cotyledons would cause influx to be underestimated in tracer-uptake experiments. The low tracer estimates of chloride influx may be accounted for in the same way. By contrast, the sodium concentration in cotyledon cells is low (3·4 m-equiv/kg wet weight), and diffusion from this source does not represent an important factor for sodium flux determinations.

The elution of labelled potassium from the cells should not be affected by leakage of the unlabelled ion from the cotyledons. It remains to consider whether data obtained in short-term ^{42}K wash-out experiments can be used as estimates of potassium efflux.

For potassium, the single internal compartment model can only be justified if the plasmalemma is rate-limiting, because the second alternative of a rapidly equilibrating cytoplasm is not found for this ion (Pitman and Saddler 1967; Pallaghy and Scott, unpublished results). In the excised barley root tissue, Pitman and Saddler find little difference between the potassium fluxes across the two membranes at 5°C in media containing 2·5 mM KNO_3. Pallaghy and Scott, however, find that the tonoplast flux is about eight times greater than the plasmalemma flux in bean root tissue that had been excised for 30 hr in 1 mM KCl, and it appears that the ratio might be even greater in more recently excised tissue, and hence by implication in the root cells of whole plants.

It is therefore felt that the tracer elution data give plausible estimates of efflux of potassium across the plasmalemma. If the assumption of a plasmalemma barrier is not justified, the efflux across it will be overestimated, because the specific activity of the cytoplasm during short-term elution experiments will be greater than that of the cell interior as a whole. Making the assumption that the efflux data are correct, they can be added to the net fluxes obtained from growth data to obtain a more realistic estimate of potassium influxes. These are shown in Figure 4.

The complication of a vacuolar compartment no longer arises in the tip region of the root, and fluxes estimated for cells in this region can be taken to be those across the plasmalemma.

(c) *Application of Ussing–Teorell Equation*

If ions are moving passively and independently across a membrane, the influx, ϕ_{in}, and efflux, ϕ_{out}, are related to the ion activity and electric potential inside (a_i, E_i) and outside (a_o, E_o) the membrane by the well-known Ussing–Teorell equation:

$$E_i - E_o = E = \frac{RT}{zF} \ln \left(\frac{a_o \, \phi_{out}}{a_i \, \phi_{in}} \right) = \frac{58}{z} \log_{10} \left(\frac{a_o \, \phi_{out}}{a_i \, \phi_{in}} \right) \quad \text{mV},$$

at 25°C for an ion of valency z. Application of this equation to the incomplete data available for root tissue is of questionable validity. The influx and efflux across both the plasmalemma and the tonoplast are not known, nor are the ion concentrations in the cytoplasmic phase. The available data must therefore be assumed to apply to a composite membrane separating the vacuole and external medium. Furthermore, an estimate must be made of vacuolar concentrations in the predominant cortical cells from the data in Figure 2 for whole root tissue. These data probably underestimate the vacuolar concentrations by about 10%. A further correction, disregarded by almost all workers, is necessary if activities in the Ussing–Teorell equation are replaced by concentrations. In aqueous solutions having concentrations of 3 and 100 m-equiv/l (approximately those in the present experiments), activity coefficients are about 0·94 and 0·77 respectively. By assuming the activity coefficient ratio to be unity, the estimate of vacuolar potential will be too negative by about 6 mV.

Taking these factors into consideration, the vacuolar potential is calculated for sodium and potassium from the Ussing–Teorell equation (i.e. on the assumption that ion movement is passive and independent). For potassium, the influx data used here are those obtained by adding efflux to the net flux. These calculated potentials are compared in Figure 6 with values measured at different positions along the root.

The Ussing–Teorell equation does not take into account the effects of water drag on ions. There is a large water uptake by the rapidly enlarging cells 5–12 mm from the tip, reaching a maximum value in this region of 6·5 mole/kg/hr. It is to be expected that ions carried in with this water flux would enhance the (ϕ_{in}/ϕ_{out}) ratio, causing calculated values of E to be more negative than would be the case if the ions were moving under forces that are purely electrochemical. This may account for the peaks in the graphs of the calculated potentials for both sodium and potassium in the elongating region.

The predicted potentials for potassium are in agreement with the measured potentials in the mature parts of the root (and also in the elongating region if the more negative values can be attributed to water drag). This suggests that potassium movement to the vacuole can be accounted for without postulation of an active pump. However, if efflux has been overestimated (see previous section), the calculated potentials would be more negative, and an inwardly directed potassium pump might then have to be postulated.

In the case of sodium, the calculated potentials are less negative than observed, and an outwardly directed pump appears to be necessary for this ion. Sodium extrusion is a common feature of all plant systems that have been investigated (Dainty 1962; Gutknecht 1966; Etherton 1967).

In the tip region (0–4 mm) where water drag should not be a major factor, potassium appears to be actively accumulated and sodium actively extruded. These pumps would have to be sited at the plasmalemma of these mainly non-vacuolated cells, the mechanisms possibly still operating in mature cells if the large (K/Na) ratio observed near the tip is characteristic of cytoplasm generally.

The Ussing–Teorell criterion for passive ion movement cannot be examined in the cases of calcium and chloride as effluxes were not measured for these ions.

(*d*) *Calculation of Membrane Permeability Coefficients*

The permeability coefficient, P, of the membrane for sodium and potassium can be calculated from the relationship

$$\phi_{in} = -P\frac{zFE}{RT}\frac{a_o}{1-\exp(zFE/RT)}.$$

This expression is based on the Goldman (1943) model of the membrane which requires the assumption that the influx of the ion, ϕ_{in}, is wholly passive. The membrane for which a_o is known is the plasmalemma and in estimating the permeability coefficient of this membrane, it has been assumed that the measured values of ϕ_{in} and E apply to the plasmalemma also. For these calculations, ϕ_{in} is expressed in moles per second per square centimetre of membrane (mature parts of the root having about $1{\cdot}2\times10^6$ cm^2 of cell surface area per kilogram of fresh tissue) and a_o in moles/cubic centimetre. The values obtained 18 mm from the root tip are quoted in Table 2, where they are compared with values obtained from similar flux measurements on other plant cells. If part of the potassium enters the cells from the cotyledons by non-free space pathways the P_K value will be an overestimate.

Although ϕ_{in} and ϕ_{out} were not determined for calcium, it is probable that the net flux is a good estimate of ϕ_{in}, as the passive efflux of this ion against strong electrochemical forces should be negligible. The net uptake of 0·019 m-equiv/kg/hr at a distance of 18 mm from the tip leads to an estimate of membrane influx of $2{\cdot}2\times10^{-15}$ mole/cm^2/sec, and even this low value may be an overestimate if part of the increase along the root in extractable calcium per cortical cell length is associated with the cell wall. If this value of ϕ_{in} is accepted, P_{Ca} becomes $4{\cdot}3\times10^{-10}$ cm/sec, i.e.

$$P_K : P_{Na} : P_{Ca} = 1 : 0{\cdot}27 : 0{\cdot}002.$$

Bean root cells therefore appear to be relatively less permeable to calcium than *Nitella*, for which $P_{Ca}/P_K = 0{\cdot}09$ (Spanswick and Williams 1965).

In the case of chloride, no estimate of the permeability of the plasmalemma is possible from the available data if the influx has a large active component. If it is assumed that chloride moves passively across the plasmalemma, as is found for potato at 0°C by Laties, MacDonald, and Dainty (1964), P_{Cl} is calculated to be not less than $1 \cdot 43 \times 10^{-6}$ at 18 mm from the tip (assuming ϕ_{in} to be at least equal to the net influx). A ratio $P_{Cl}/P_K \geqslant 6 \cdot 5$ is unlikely in view of the values much less than unity found in other plant cells. Furthermore the chloride concentration in the cytoplasm would have to be extremely low (less than 2×10^{-5}M if the ion is to be distributed passively across the plasmalemma). The chloride content of non-vacuolated cells near the tip is higher than in the external medium (Fig. 2) and although it is possible that most of this could be bound in intracellular bodies such as the nucleus, the cytoplasm is unlikely to have the very low levels of chloride required for passive distribution. It is more probable that there is an inwardly directed chloride pump at the plasmalemma, accounting for most of the observed influx, although this should be confirmed in suitable experiments with inhibitors.

TABLE 2

PLASMALEMMA PERMEABILITY COEFFICIENTS

Tissue	$10^7 P_K$ (cm/sec)	$10^7 P_{Na}$ (cm/sec)	Reference
Broad bean root cortical cells	2·2	0·6	This paper
Nitella axillaris	13	—	Diamond and Solomon (1959)
Chara globularis	3	0·1	Gaffey and Mullins (1958)
Nitella translucens	5	0·9	MacRobbie (1962)
Chaetomorpha Darwinii	3	1·2	Dodd, Pitman, and West (1966)
Pea epicotyls	0·78*	—	Higinbotham, Etherton, and Foster (1967)
Oat coleoptiles	1·9*	—	Higinbotham, Etherton, and Foster (1967)

* Values quoted by Higinbotham, Etherton, and Foster (1967) are too large by a factor of 10^3 if the unit used is cm/sec.

Finally, it should be pointed out that the membrane potential cannot be explained as a diffusion potential determined principally by the passive movement of potassium and sodium, as is the case in many other plant cells. The cell interior is too negative, and in fact it is this large negative potential (-130 mV) which appears to be responsible for potassium having a net influx. It is possible that an outward diffusion of hydrogen ions, arising as a by-product of organic acid synthesis in the developing cell, is largely responsible for the membrane potential (Scott 1967).

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