Mineral Ion Contents and Cell Transmembrane Electropotentials of Pea and Oat Seedling Tissue[1]

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Summary. The relationships of concentration gradients to electropotential gradients resulting from passive diffusion processes, after equilibration, are described by the Nernst equation. The primary criterion for the hypothesis that any given ion is actively transported is to establish that it is not diffusing passively. A test was made of how closely the Nernst equation describes the electrochemical equilibrium in seedling tissues. Segments of roots and epicotyl internodes of pea (*Pisum sativum* var. Alaska) and of roots and coleoptiles of oat (*Avena sativa* var. Victory) seedlings were immersed and shaken in defined nutrient solutions containing eight major nutrients (K^+, Na^+, Ca^{2+}, Mg^{2+}, Cl^-, NO_3^-, $H_2PO_4^-$ and SO_4^{2-}) at 1-fold and 10-fold concentrations. The tissue content of each ion was assayed at 0, 8, 24, and 48 hours. A near-equilibrium condition was approached by roots for most ions; however, the segments of shoot tissue generally continued to show a net accumulation of some ions, mainly K^+ and NO_3^-. Only K^+ approached a reasonable fit to the Nernst equation and this was true for the 1-fold concentration but not the 10-fold. The data suggest that for Na^+, Mg^{2+}, and Ca^{2+} the electrochemical gradient is from the external solution to the cell interior; thus passive diffusion should be in an inward direction. Consequently, some mechanism must exist in plant tissue either to exclude these cations or to extrude them (e.g., by an active efflux pump). For each of the anions the electrochemical gradient is from the tissue to the solution; thus an active influx pump for anions seems required. Root segments approach ionic equilibrium with the solution concentration in which the seedlings were grown. Segments of shoot tissue, however, are far removed from such equilibration. Thus in the intact seedling the extracellular (wall space) fluid must be very different from that of the nutrient solution bathing the segments; it would appear that the root is the site of regulation of ion uptake in the intact plant although other correlative mechanisms may be involved.

The significance of cell electropotentials, PDs, as an important force having definite relationships to ionic gradients in plant cells has been discussed recently by Dainty (4) and by Briggs, Hope, and Robertson (3). The existence of cell transmembrane PDs in higher plants has been established (6, 7, 10, 11, 12, 20) and evidence has been obtained showing that, under certain conditions at least, the PD is closely related in magnitude to the transmembrane concentration gradient of K^+ (cell interior to exterior) (11) as in muscle fibers (13) and Chara cells (15). Whether or not this is true for K^+ or other major nutrient ions in cells of higher plants at ionic equilibrium (or homeostasis) is the subject of this study.

An ionic species which is free to diffuse through the cell membrane, that is, one which is not actively transported in a process requiring metabolic energy, when at equilibrium should show a definite relationship between the activity and the electrical gradients. For cells in a solution containing K^+ and Cl^- the relationship of concentration and potential, if distributed by diffusion only, would be expected at equilibrium to conform to the Nernst equation, e.g.:

$$E_{io} = \frac{RT}{zF} \ln \frac{[K^+_o]}{[K^+_i]} = \frac{RT}{zF} \ln \frac{[Cl^-_i]}{[Cl^-_o]} \qquad \text{I}$$

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where E_{io} is the electropotential between inside and outside the cell; R, the gas constant in joules degree $^{-1}$ equiv. $^{-1}$; T, absolute temperature; z, the valence of the ion; F, the faraday in coulombs/equivalent; and the brackets indicating the concentration of the ion concerned in the inner or outer phases. Similar equations can be written for each of the several ions. For each ion the following derivation is useful (21, 22):

$$\frac{\emptyset_i}{\emptyset_o} = \frac{[C_o]}{[C_i] \exp(zFE_{io}/RT)} \qquad \text{II}$$

where C represents the concentration inside or outside as indicated by the subscript, and E_{io}, the transmembrane PD. Thus the external concentration $[C_o]$ is in electrochemical equilibrium with the internal concentration when (at 25°, and in mv):

$$[C_i] = [C_o] \exp. \pm [z\,(0.039)\,E_{io}] \qquad \text{III}$$

where the plus or minus sign of the exponent refers to the cation or anion, respectively.

It was the purpose of this study to grow and treat seedling tissues under defined conditions and to ascertain experimentally the electrochemical activities between the tissue and external solution of K^+, Na^+, Ca^{2+}, Mg^{2+}, Cl^-, NO_3^-, SO_4^{2-}, and $H_2PO_4^-$. The electropotential gradients between the vacuole and the external medium under these conditions have been reported previously (6). Evidence suggests that there is little or no difference in PD between the cytoplasm and vacuole (7).

Materials and Methods

The plants used were peas, *Pisum sativum* var. Alaska, and oats, *Avena sativa* var. Victory. Seeds were soaked 2 hours prior to planting. For segments of pea epicotyl and oat coleoptile seeds were planted in vermiculite well rinsed with nutrient solution (as specified below) and placed in an incubator in the dark at 25°. Peas were grown for 7 days in darkness; oats were grown for 90 to 96 hours in the dark but were given a 2-hour exposure to red light in the 72- to 76-hour interval. For experiments using root segments, seeds were germinated on glass beads in nutrient solution then transferred to a cheese-cloth net with roots immersed in aerated nutrient solution at a controlled temperature of 25°.

At the times indicated, tissue segments were excised and placed in deionized water. Samples of about 1 g each were blotted and weighed, one being taken for the 0-time interval, the others being divided into 0.2 g samples and placed in 50 ml of nutrient solution in 250 ml Erlenmeyer flasks and shaken for periods as given for each experiment. At the proper time, samples were removed, rinsed in deionized water, blotted, weighed, and frozen for subsequent chemical assay procedures. The

usual proportion of tissue weight to solution volume was thus 4 g/liter; all samples were duplicated.

Root segments 1 to 2 cm long were excised from the terminal 2.5 cm portion after discarding the terminal 0.5 cm. With oat coleoptiles and the third internode of pea epicotyls the uppermost cm portion was discarded and the next 2 cm segments were used. The hollow coleoptile segments were filled with solution prior to immersion.

The nutrient solution had the same salts in the same proportion as that used previously and referred to as 1X nutrient (11). Its composition in mmoles per liter was: KCl, 1.0; $Ca(NO_3)_2 \cdot 4H_2O$, 1.0; $MgSO_4 \cdot 7H_2O$, 0.25; $NaH_2PO_4 \cdot H_2O$, 0.904, plus Na_2HPO_4, 0.048. The pH value was 5.5 to 5.8. In some experiments this solution was used at 10-fold concentration and, as such, is referred to as 10X nutrient.

Chemical Assay Methods. Tissue samples harvested for assay were placed in about 10 ml of deionized water and frozen until assays could be made. For assay these samples were thawed, boiled in deionized water for 15 minutes and the resulting hot solution filtered (using Whatman no. 1 filter paper) into a 25 ml volumetric flask. The residue was boiled a second time in 5 ml H_2O and this solution was added to the first extract. This is referred to as the hot water extract. The combined extracts were brought to volume and aliquots subsequently assayed for K^+, Na^+, Ca^{2+}, Mg^{2+}, Cl^-, NO_3^-, SO_4^{2-}, and $H_2PO_4^-$. These assays are assumed, arbitrarily, to approximate the minimal activities of each of the ions within the tissue. The authors recognize that this assumption cannot be completely correct since several compartments for ions may exist in intact tissue (3, 19) and measurement of ionic partitioning between these requires special methods. Breaking down the membrane barriers may appreciably alter the partitioning. However, it seemed to us that assays of the hot water extractible fraction would provide us with a reasonable estimate of the minimal average ionic activity in the tissue and this combined with the acid digest of the tissue residue, described below, would provide estimates of total ion content as an upper limit of average electrochemical activity within the protoplasm.

The tissue residue, representing the hot water insoluble materials, was removed from the filter paper and subsequently solubilized in 10 ml of concentrated HNO_3. The residue was allowed to stand for 1 day in cold HNO_3 then warmed without boiling 2 to 3 times driving off HNO_3 but not allowing the sample to dry completely. The acid digest was then transferred to a 25 ml volumetric flask and brought to volume for assay of K^+, Na^+, Ca^{2+}, Mg^{2+}, SO_4^{2-}, and $H_2PO_4^-$.

Potassium and Sodium. Potassium and sodium were determined by flame photometry using a Beckman DU spectrophotometer with photomultiplier and flame attachments. The solution used for

standardizing was made up in 0.1 N HCl and was composed as follows in mM: KCl, 8.0; NaCl, 2.0; CaNO$_3$, 1.0; and MgSO$_4$, 1.0. Dilutions as required were also in 0.1 N HCl solution. Assays were made using the 766.5 mμ wavelength for potassium and the 589.3 mμ wavelength for sodium.

Calcium and Magnesium. Calcium and magnesium were assayed by flame photometry using additions of NH$_4$EDTA according to the method of Greweling (8). The standard solution used was as follows in mM: 1.25 MgSO$_4$ · 7H$_2$O; 1.25 CaNO$_3$; 12.5 KCl; and 25 EDTA to which NH$_4$OH had been added as specified in the procedure. Dilutions made for producing standard curves were also made in the NH$_4$EDTA solution. The wavelength used for Ca^{2+} was 422 mμ and, for Mg^{2+}, 285.2 mμ. Aliquots of the water extract were brought to the proper mixture by addition of an equal volume of 50 mM NH$_4$EDTA. Aliquots of the acid extract were gently heated to dryness after addition of one-third volume of 30 % H$_2$O$_2$ and then the residue dissolved in 25 mM NH$_4$EDTA.

Chloride. Chloride was determined electrolytically using a chloride titrator.

Nitrate. Nitrate was assayed colorimetrically by the method described by Morris and Gonzales-Mas (18).

Phosphate. For phosphate assays the colorimetric method of Griswold et al. was used (9). The values are reported as H$_2$PO$_4^-$ although phosphate also occurs as HPO$_4^{2-}$ in the tissue, the proportion depending upon pH.

Sulfate. Sulfate was assayed by a method using colorimetry in the ultraviolet range (wavelength 350 mμ) and chloranilic acid as indicator (1, 17). Aliquots of solution containing water extracts or acid digests were passed through a column of Dowex 50 (X 8, 50 to 100 mesh) to remove cations. To the effluent was added 2 drops of concentrated HNO$_3$ and about one-half volume of 30 % H$_2$O$_2$. This solution was evaporated to dryness at 100°. The residue was taken up into solution with 1 ml of the indicator suspension brought to volume and assayed using a Beckman DU spectrophotometer.

Results

Hot Water Extractible Ions of Pea Root Tissue; Cations. Pea root and epicotyl segments were assayed for ion content after the following 3 regimes: 1) seedlings were grown in nutrient solution of 1× concentration and segments treated for periods up to 48 hours in 1× solution; 2) seedlings were grown in 10× solution and segments treated in 1× solution and 3) seedlings were grown in 10× solution and segments treated in 10× solution.

The results showed quite clearly that in root segments the concentrations of the 4 major cations are essentially constant with respect to time over the

48-hour period in regimes 1 (fig 1), and 3 (fig 2). In regime 2 involving a change from 10× nutrient solution during growth to 1× nutrient during segment immersion there was some loss of K$^+$ and Na$^+$, but equilibration was approached. Since the results of regime 1 closely resembled those for regime 2 in all cases data for the latter are not reported here.

Pea Roots; Anions. In contrast to the pattern of cation uptake some of the anions showed an appreciable amount of net accumulation initially in 10× solution (fig 4) and in the 1× solutions (fig 3); however, no great net change of the anions as a whole took place. Also, there appeared to be a reasonably close approach in 10× solution to a steady state after the first 8 hours for tissue content of SO$_4^{2-}$ and NO$_3^-$. However, the root segments continued to gain H$_2$PO$_4^-$ and lose Cl$^-$ over the 48-hour period. A surprisingly high accumulation of SO$_4^{2-}$ occurred.

Pea Epicotyl; Cations. In segments of pea epicotyl under regimes 1 and 3 only K$^+$ was accumulated in appreciable amounts; with the other cations, Na$^+$, Ca^{2+}, and Mg^{2+} there was a small net uptake (figs 5, 6). There was relatively little difference in the initial K$^+$ content whether the seedlings were grown in 1× or 10× solutions. However, segments treated in 10× solution gained much more K$^+$ than those in 1× solution.

Pea Epicotyl; Anions. Under both regimes 1 and 3 there was a net increase of anions during the 48-hour period (figs 7, 8). The order of these during the first 24 hours was: NO$_3^-$ > H$_2$PO$_4^-$ > SO$_4^{2-}$ > Cl$^-$. In both regimes NO$_3^-$ showed a loss during the 24- to 48-hour interval but in view of the experimental error this may not be significant.

It is apparent that stem segments were not in equilibrium with the concentrations of any of the 8 ions in the external medium, although there was a wide range in the rates and amounts of net uptake. For several ions, however, the rates of net change in tissue content were negligible and the data allow for some evaluations in terms of electrochemical activities. These are dealt with in a later section.

Oat Root; Cations. As in the case of pea roots, oat root segments in regimes 1 and 3 (figs 9, 10) showed little net change in content of most cations. In 1× solution there was some loss of K$^+$ but in 10× solution there was a slight gain. However, the fact that roots grown in 1× solution had a higher initial K$^+$ content than those grown in 10× solution is unexpected and this would appear to be either a result of biological variability or experimental error.

Oat Root; Anions. In regimes 1 and 3 little change in content occurred except for NO$_3^-$. This ion showed a loss in 1× solution corresponding approximately to the loss found in K$^+$ (fig 9,
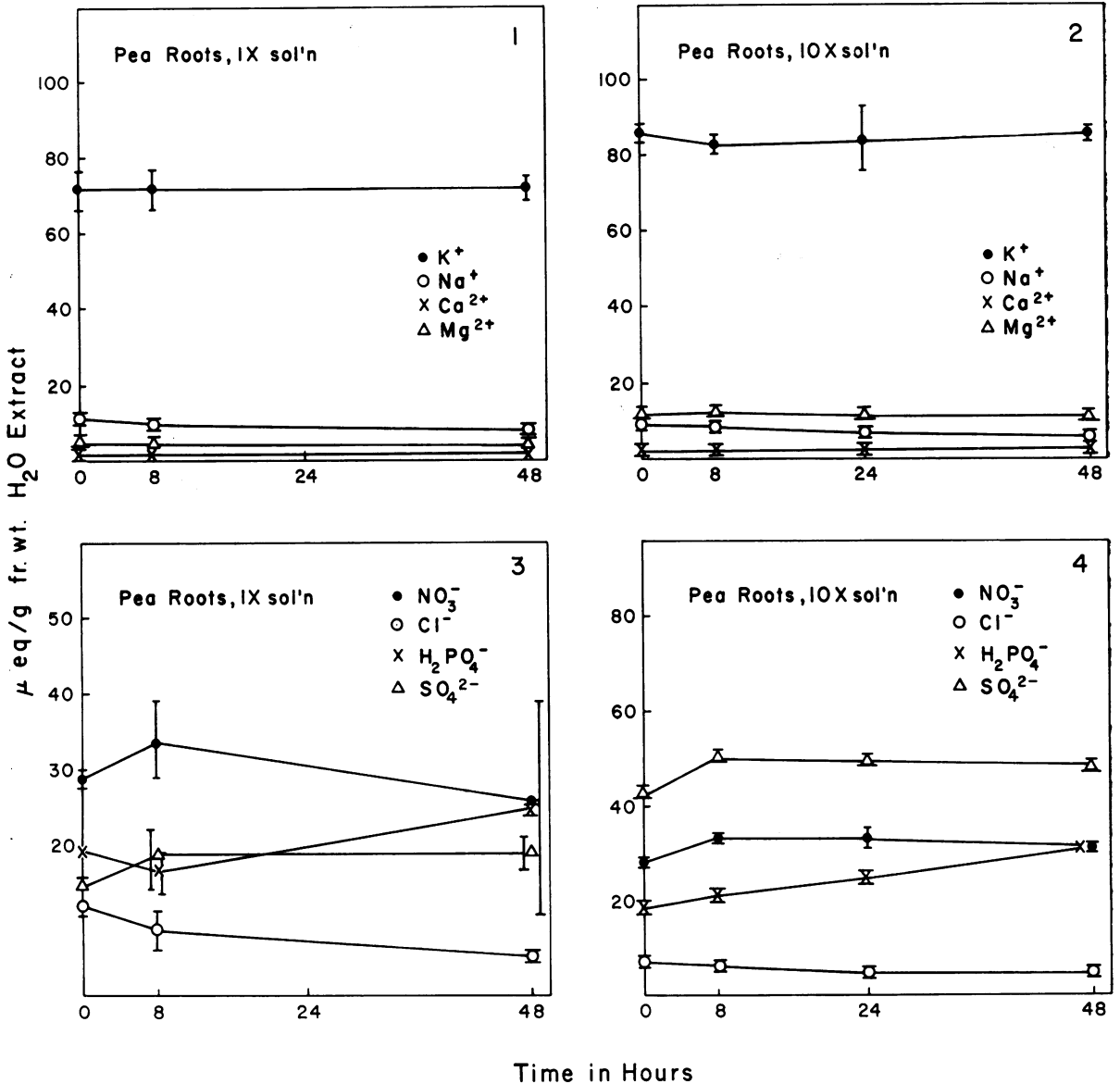
FIGS. 1 to 4. The time course of ion content of pea root segments of cations and anions in solutions as indicated in each figure. The vertical lines at each point show the mean ± SD.

Table I. *Ion Accumulation Ratios at 48 Hours*

The analyses were obtained with hot water soluble extract.

Tissue regime*		Cations				Anions			
		K$^+$	Na$^+$	Mg^{2+}	Ca^{2+}	NO$_3^-$	Cl$^-$	SO$_4^{2-}$	H$_2$PO$_4^-$
Pea roots	1	72	8.0	6	0.80	13	5.3	38	25
	3	8.6	0.6	4.7	0.16	1.6	0.5	9.6	3.2
Pea epicotyls	1	59	1.9	5.8	1.3	14	4.4	22	19
	3	9.5	0.34	1.0	0.17	1.3	0.7	3.5	3.7
Oat roots	1	57	4.7	33	1.6	23	3.5	9.0	19
	3	6.5	0.3	4.2	0.14	1.5	0.5	2.2	1.2
Oat coleoptiles	1	63	5.2	24	1.2	12	21	9.6	15
	3	10.1	2.7	4.1	0.6	4.6	5.3	2.0	2.6

* For an explanation of these treatments see the text (paragraph 1 under Results).

same tissue as in the data of fig 11). Tissues grown and treated in 10× solution showed a similar loss of NO_3^- (fig 12) in this case not matched by a loss of K^+ (fig 10, data from the same tissue as in fig 12). As in the case of the cation content there is higher content of certain ions, e.g., NO_3^- and $H_2PO_4^-$, in the 1× solution than in 10× solution.

Oat Coleoptile; Cations. The net changes in cation content of oat coleoptiles in regimes 1 and 3 are shown in figures 13 and 14. As in pea epicotyl segments the coleoptile tissue was not in a near-equilibrium state with the external solution

and a marked gain of K^+ occurred in 48 hours. Appreciable increases in other cations also took place; however, the final content of Na^+, Mg^{2+}, and Ca^{2+} was still markedly below that found for K^+.

Oat Coleoptile; Anions. Appreciable net gains of anions occurred in regimes 1 and 3 during the 48-hour period (figs 15, 16). Only SO_4^{2-} appeared to approach equilibrium.

Ion Accumulation Ratios and Comparisons of Ions and of Tissues. In table I a summary is presented of the ion accumulation ratios (in μeq g^{-1} fr wt of tissue/μeq ml^{-1} of external solution)
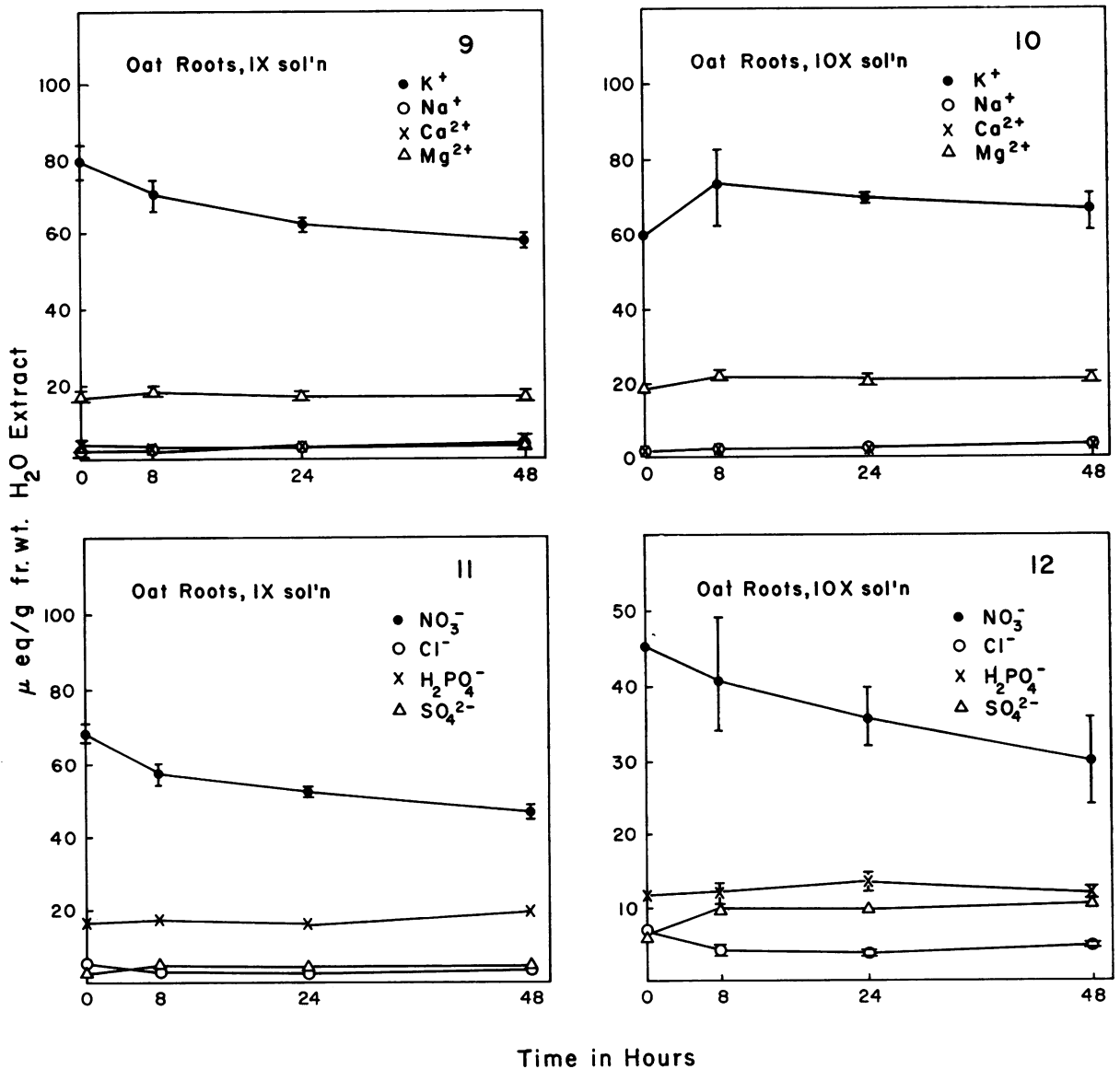

Time in Hours
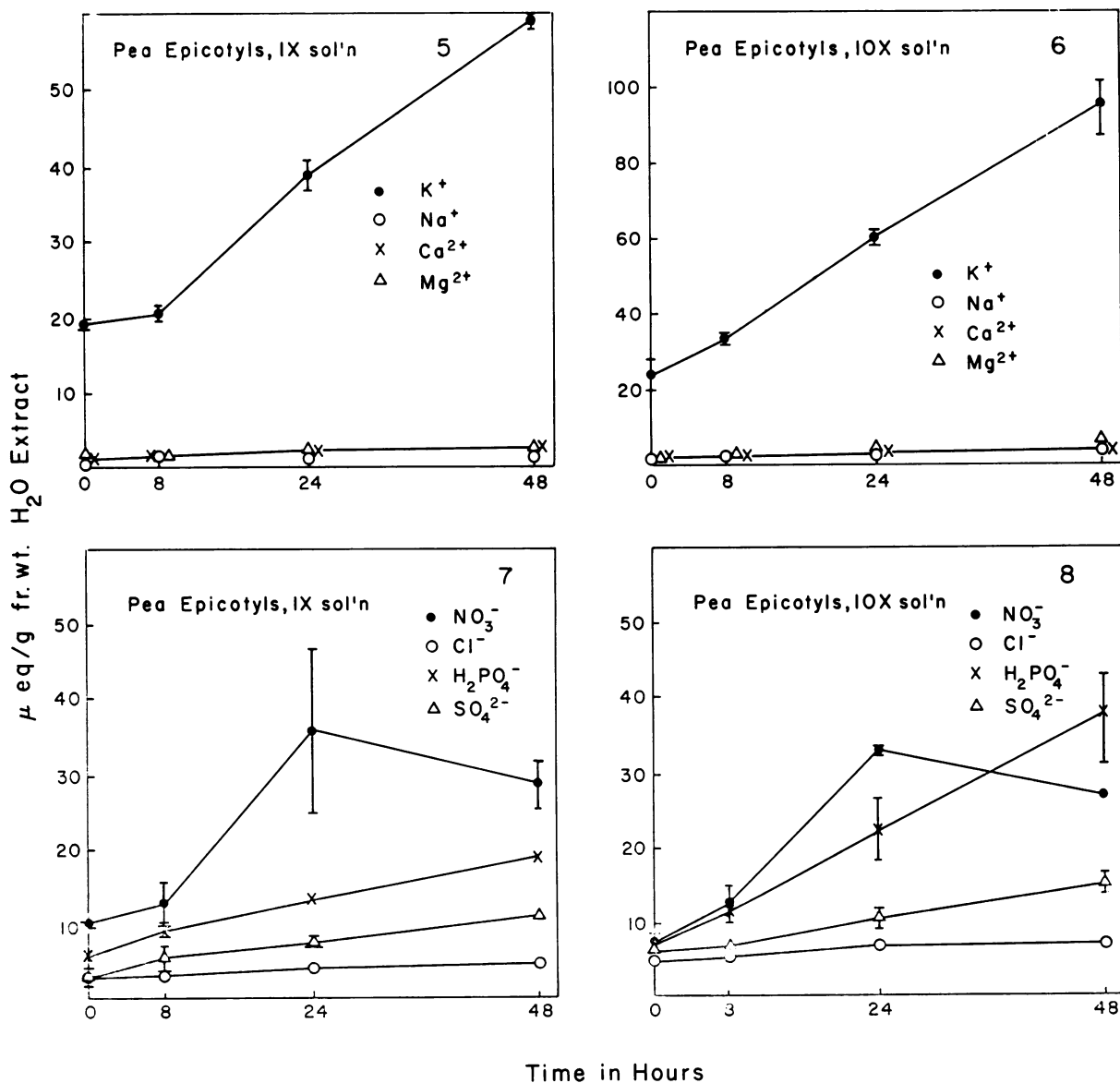
FIGS. 5 to 8. The time course of ion content of pea epicotyl segments of cations and anions in solutions as indicated in each figure. The vertical lines at each point show the mean ± SD. Points lacking vertical bars had smaller SDs than could be shown (excepting in fig 8 in which the 48-hour point for NO_3^- is for 1 sample).

Table II. *Assay of Ions in the Acid-digested Residue of Tissue from Hot Water Extractions*
Values are reported from the 48-hour period only.

Tissue regime*		Cations μeq/g fresh wt				Anions μeq/g fresh wt	
		K^+	Na^+	Mg^{2+}	Ca^{2+}	SO_4^{2-}	$H_2PO_4^-$
Pea roots	1	6.0 ± 0.0	1.8 ± 0.2	4.7 ± 0.4	4.7 ± 0.6	3.8 ± 2.4	0.6 ± 0.0
	3	4.7 ± 0.9	3.4 ± 0.9	6.6 ± 0.9	4.5 ± 0.3	3.9 ± 0.7	8.8 ± 1.3
Pea epicotyls	1	3.4 ± 0.6	1.4 ± 0.0	4.0 ± 0.3	7.1 ± 0.5	0.8 ± 1.0	1.0 ± 0.5
	3	1.9 ± 0.3	3.0 ± 1.2	3.1 ± 0.1	8.2 ± 0.3	...	...
Oat roots	1	6.4 ± 0.5	1.9 ± 0.4	3.0 ± 0.5	1.3 ± 0.0	4.2 ± 1.3	0.4 ± 0.1
	3	5.8 ± 1.6	2.0 ± 0.4	5.3 ± 0.3	2.1 ± 0.1	1.6 ± 0.2	6.3 ± 1.5
Oat coleoptiles	1	17.5 ± 1.3	3.4 ± 1.1	5.4 ± 1.6	5.0 ± 0.1	1.6 ± 0.1	29.0 ± 0.6
	3	16.1 ± 0.2	7.6 ± 0.7	9.3 ± 0.4	10.7 ± 0.2	5.0 ± 1.2	23.1 ± 0.3

* As in table I.

FIGS. 9 to 12. The time course of ion content of oat root segments of cations and anions in solutions as indicated in each figure. The vertical lines at each point show the mean ±SD.

of the hot water extractible ions. In all cases the cations were in the following order $K^+ > Mg^{2+} > Na^+ > Ca^{2+}$, with K^+ accumulation greatly exceeding all the others. The anions, however, show no definite pattern except that NO_3^- accumulation exceeded that for Cl^-. In general, the order was the same as that of the initial seedling content.

Assays of HNO_3 Digest of Residue from Hot Water Extracted Tissue. The assays of ions from the acid digest of the 48-hour samples are given in table II. These values are similar to those of the shorter time periods (not reported). In the case of K^+, SO_4^{2-}, and $H_2PO_4^-$ the fraction remaining in the residue was small compared to that of the hot water extractible fraction. However, in other cases, notably Na^+, Mg^{2+}, and Ca^{2+}, the bound fraction is quite large, sometimes exceeding the hot water extracted portion (compare figs 1-16).

Relationship of Ion Concentration Gradients to Cell Electropotentials. Equation I defines the diffusional relationships of ion concentrations and electropotentials at equilibrium. Cell electropotential measurements have been made for each of the tissues under conditions essentially identical to those obtaining for the ion accumulation study. These have been reported previously in part (6, 11) and additional measurements made since tend to confirm the reliability of these values within an error of about 10 %. (Of course with multicellular tissue it is impossible to measure electropotentials and accumulation ratios with the same cell as in the case of the giant-celled algae.) Having these values we may now estimate the electrochemical activity gradient between the cells and the ambient solutions. It is quite apparent from figures 1 to 16 that the tissue contents are not static; nevertheless, as a first approximation it is meaningful to compare the ion content found by assay with that predicted from measured cell electropotentials using equation II (and assuming that an

Table III. *Concentration of Ions ($\mu eq/g$ Water Content) of Pea and Oat Seedling Tissues*

The ion concentration values are those found experimentally and as predicted from experimentally measured cell potentials in 1X solution at 24 hours. The H_2O content was calculated as 95 % of fresh weight. Actual values ranged from 94.2 % to 95.7 %. The values for ion content are those for the H_2O extraction. The predicted diffusion equilibrium values calculated from equation II assuming equilibration exists) using measurements of cell electropotentials (E_{io}) from Etherton (6), namely, -110, -119, -84, and -105 mv (interior negative) for pea roots, pea epicotyls, oat roots and oat coleoptiles, respectively.
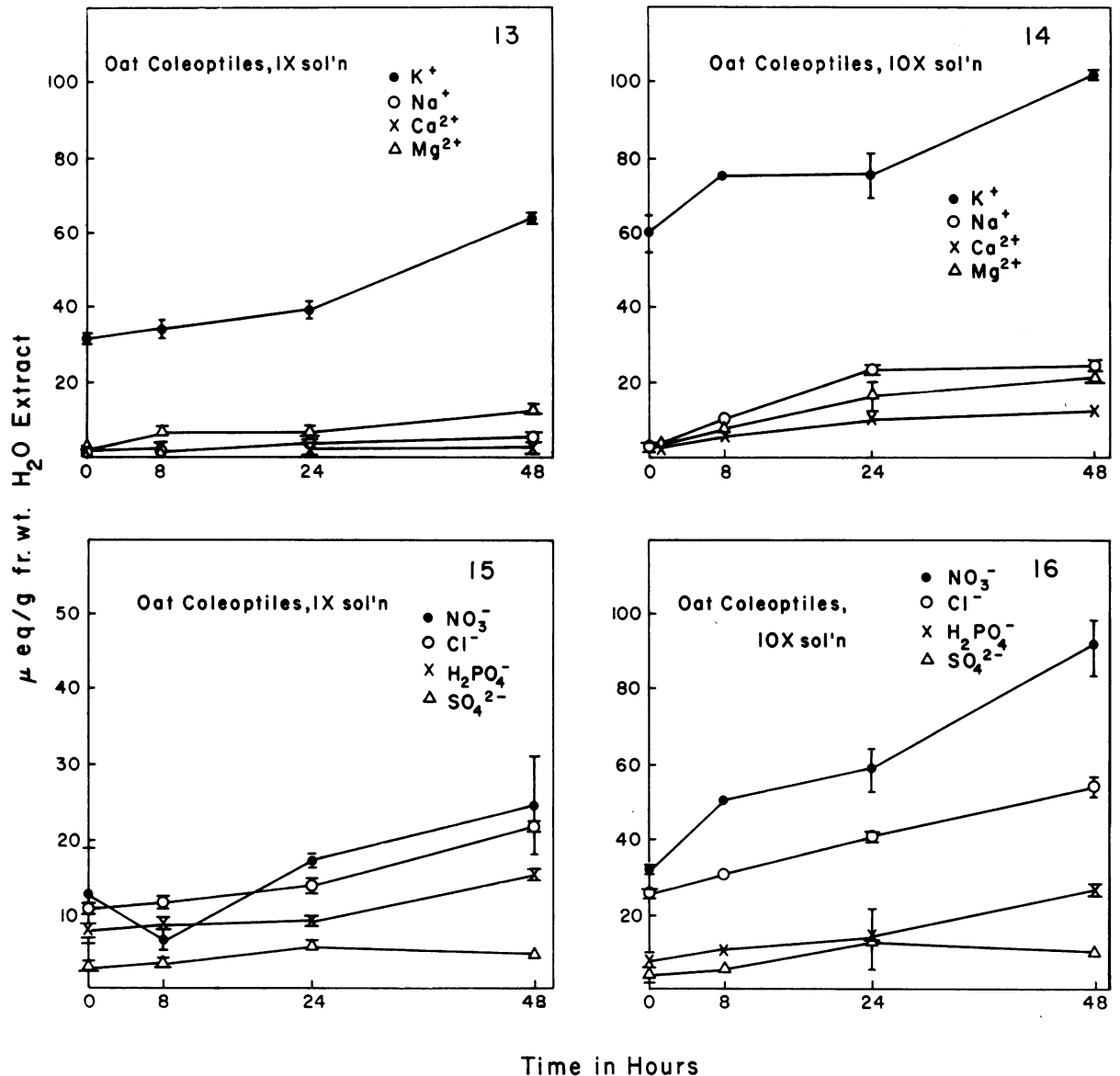
	Pea				Oat			
	Roots		Epicotyls		Roots		Coleoptiles	
Ion	Exptl.*	Predicted	Exptl.*	Predicted	Exptl.*	Predicted	Exptl.*	Predicted
K^+	75	74	41	104	66	27	42	60
Na^+	8	74	2	104	3	27	4	60
Mg^{2+}	3	2700	3	5400	17	350	7	1800
Ca^{2+}	2	10,800	3	21,600	3	1400	2	7200
NO_3^-	28	0.0272	38	0.0192	56	0.0756	18	0.0332
Cl^-	7	0.0136	4	0.0096	3	0.0378	15	0.0166
$H_2PO_4^-$	21	0.0136	14	0.0096	17	0.0378	9	0.0166
SO_4^{2-}	19	0.000094	8	0.000048	4	0.00071	5	0.000138

* Values interpolated from time course curves. Experimental values at 24 hours in a separate experiment, not reported, are not greatly different.

Table IV. *Concentrations of Ions ($\mu eq/g$ Water Content) of Pea and Oat Seedling Tissues*

The ion concentration values are those found experimentally and as predicted from measured cell potentials in 10X solution at 24 hours. H_2O content calculated as 95 % of fresh weight. Actual values ranged from 94.2 % to 95.7 %. The values for ion content are those for the H_2O extraction. The predicted diffusion equilibrium values calculated from equation III using measurements of cell electropotential ($=E_{io}$) from Etherton (6), namely, -108, -119, -71, and -102 mv for pea roots, pea epicotyls, oat roots and oat coleoptiles, respectively.

	Pea				Oat			
	Roots		Epicotyls		Roots		Coleoptiles	
Ion	Exptl.	Predicted	Exptl.	Predicted	Exptl.	Predicted	Exptl.	Predicted
K^+	89	680	64	1040	73	159	79	535
Na^+	7	680	3	1040	3	159	24	535
Mg^{2+}	12	22 750	3	54,000	22	1275	17	14,250
Ca^{2+}	3	91,000	2	216 000	3	5100	10	59 000
NO_3^-	35	0.294	35	0.192	38	1.250	61	0.372
Cl^-	6	0.147	7	0.096	4	0.625	42	0.186
$H_2PO_4^-$	26	0.147	24	0.096	14	0.625	19	0.186
SO_4^{2-}	52	0.00109	11	0.00049	11	0.0197	15	0.00175

13 Oat Coleoptiles, IX sol'n
● K⁺ ○ Na⁺ × Ca²⁺ △ Mg²⁺

14 Oat Coleoptiles, IOX sol'n
● K⁺ ○ Na⁺ × Ca²⁺ △ Mg²⁺

15 Oat Coleoptiles, IX sol'n
● NO₃⁻ ○ Cl⁻ × H₂PO₄⁻ △ SO₄²⁻

16 Oat Coleoptiles, IOX sol'n
● NO₃⁻ ○ Cl⁻ × H₂PO₄⁻ △ SO₄²⁻

μ eq/g fr. wt. H₂O Extract

Time in Hours

FIGS. 13 to 16. The time course of ion content of oat coleoptile segments of cations and anions in solutions as indicated in each figure. The vertical lines at each point show the mean ± SD.

Table V. *Estimates of K^+ Flux Rates and Permeability Coefficients, P_K, over the 24 to 48 Hour Period*

Tissue	$[K_o^+]$*	$[K_i^+]$**	E_{io}	$\varnothing_i/\varnothing_o$	$\varnothing_K$***	$\varnothing_o$	$\varnothing_i$	$P_K \times 10^5$
Pea roots	1	72	—110	1.04	...	...	...	...
	10	84	—108	8.03	...	...	...	...
Pea epicotyls	1	39	—119	2.69	2.32	1.37	3.69	7.83
	10	61	—119	17.20	3.93	0.24	4.17	0.89
Oat roots	1	...	— 84	...	...	...	...	...
	10	70	— 71	2.26	—0.58	...	...	...
Oat coleoptiles	1	40	—105	1.50	2.66	5.32	7.98	19.0
	10	75	—102	7.14	3.01	0.49	3.50	0.86

* $[K_o^+]$ is the external potassium ion concentration times 10^6 eq ml⁻¹.
** $[K_i^+]$ is the potassium ion concentration in the tissue times 10^6 eq (g fr wt)⁻¹ at $t = 24$ hours. This is approximately the time at which E (PD) was measured.
*** $\varnothing_K$ is the net influx in units of 10^{-10} eq g⁻¹ sec⁻¹. It applies to the 24 to 48 hour interval.

equilibrium exists). These values (table III) with 1× nutrient solutions suggest that among the 8 ions studied only K⁺ approaches the relationship predicted for diffusion. Of the ions other than K⁺ only Na⁺ is within 1 order of magnitude of the tissue concentration predicted and some ions, e.g., Ca^{2+} and SO_4^{2-}, may be several thousand-fold removed.

At a 10× concentration of nutrient solution, the electrochemical activities of none of the ions are close to the predicted diffusional equilibrium values (table IV). Again K⁺ approaches electrochemical equilibrium more closely than any other ion. In shoot tissues, at least, net accumulation of K⁺ is proceeding during immersion; thus, in accord with equation II, the flux ratio would have to be known before it could be concluded that active transport of K⁺ is involved.

Calculations of Flux Values. Equation II permits the calculation of diffusional flux ratios for tissue samples not in equilibrium. Since: $\phi = \phi_i - \phi_o$ (where ϕ is the net flux, ϕ_i, the influx, and ϕ_o the efflux) each rate may be estimated. Also, since for K⁺ (4):

$$\phi = -P_x \frac{EF/RT \; [(K_o^+) - (K_i^+)(Ki^+)e^{EF/RT}]}{1-e^{EF/RT}} \qquad IV$$

the permeability coefficient of K⁺ may be calculated. These estimates are summarized in table V. Small experimental errors lead to quite large errors in the estimate unless ϕ_i is relatively large and the ϕ_i/ϕ_o ratio is appreciably removed from unity; for this reason several calculations have been omitted.

The flux ratios show quite clearly that a discrepancy exists between diffusion theory and the experimental measurements. For example, in pea roots at 1× the flux ratio approaches 1 as predicted; however, at 10× the flux ratio, predicted from cell potential and the concentration gradient, is 8.0 despite the fact that the data showed no significant net uptake. We feel that the data showing no net uptake are reliable and that either the measured potential value is in large error, or K⁺ is not diffusing independently. While the potential measurement may be subject to some error the deviation required, −55 mv as compared to the measured potential of −71 mv, is too great to seem likely. Larger deviations in PD would be required in the other tissues. Consequently, the suggestion of some other mechanism, such as an anion pump, appears necessary to explain the various points of evidence in a more logical way.

The flux rates, calculated for those cases in which the data are suitable, give values within a reasonable order of magnitude. With cells of 35 × 200 μ the cell surface area per gram of tissue is about 1000 cm², thus the flux rates are in the range of 0.04 to 2.5 × 10⁻¹² eq cm⁻² sec⁻¹ as in the case of similar measurements of other plants (4, 19). It is not reasonable, however, as these estimates indicate, to expect the ϕ_o to decrease with increasing internal concentration.

Discussion

The overall conclusion from this study using the Nernst equation as a criterion is that none of the 8 major ions is in electrochemical equilibrium in pea or oat seedling tissues although K⁺ approaches it; thus none appear to move passively. However, estimates of the electrochemical gradients provided by this study suggest that for Na⁺, Mg^{2+}, and Ca^{2+} the energy gradient is from the external solution to cell interior. Thus passive diffusion would be from outside to inside and any active transport of these ions, if it occurs, would represent an efflux pumping process. This finding seems to be in distinct contrast to the view that in higher plants any ion which is accumulated (against the chemical gradient) by metabolically active tissue is energetically transported inward.

Each of the anions was accumulated against the energy gradient. The disparity from electrochemical equilibrium was much greater at 1× than at 10× concentrations. It may be inferred that metabolic energy is required for accumulation of each anion. However, as noted by Briggs, Hope, and Robertson (3), the energy of transport may be necessary for only 1 co-ion of a pair, the other co-ion may then diffuse obligatorily because of the requirement for electroneutrality. In view of the fact that cation inward movement is downhill in the electrochemical gradient, we are left with the conclusion that only the anion accumulation process needs obviously to be the active influx transport. Furthermore, evidence that inhibitors such as dinitrophenol may rapidly depress the cell PD suggests that cell potentials are dependent upon metabolism and that ion transport may likely be electrogenic (7, 10, 14), and thus generate the cell PD. In this case cation (particularly K⁺) diffusion would occur as a result of the PD rather than the PD being a consequence of such diffusion. However, once accumulation has occurred, blocking the metabolic pump would prevent the pump PD but then would permit a diffusion potential to occur in accord with the Nernst equation.

The argument may be raised that cell electropotentials are not closely related to ion movement through cell membranes. If ions were transferred in a combined form, e.g., with a co-ion or organic carrier complex, and, thus, in an uncharged state, then they would neither be influenced by the electropotential gradient nor would diffusion of such carrier complexes generate a diffusion potential. Some evidence that K⁺ and Cl⁻ may move inwardly in an electroneutral transport system in beet root tissue has been reported (20). Also the conclusion in the past of other workers from research based on the carrier hypothesis (5) seems to lead to a model of ion uptake completely mediated by electrically neutral carriers. However, on theoretical grounds, it appears very unlikely that any differentially permeable membrane is perfect in the sense implied, i.e., with no ions passing in a charged

state. Rather, the assumptions of some leakage in the bulk phase seems more reasonable, e.g., through pores, and, in this case, ions should influence or be influenced by the electropotential field. Furthermore, there has appeared a considerable body of evidence in support of the view that ion movement is related in some way to cell PDs (2, 3, 4, 6, 7, 10, 11, 12, 13, 14, 16, 20). An excellent example of inwardly directed Cl⁻ passive diffusion in potato disks, quantitatively predictable from electrochemical theory, has been reported by Laties, McDonald, and Dainty (16). In *Nitella translucens* Walker and Hope (23) have clearly demonstrated a marked effect of cell potential on flux rates of K^+, Na^+, and Cl^-.

An interesting aspect of ion regulatory activity of intact plants was revealed in this study. Root tissues approached equilibration in the seedling growth solution; thus excised segments showed a relatively constant ion content in the 48-hour immersion period. In contrast the initial mineral content of top portions was quite independent of the concentrations in the external growth solution. A relatively rapid net uptake by excised epicotyl or coleoptile segments occurred during immersion in 1× and 10× solutions. It seems apparent that the mineral concentrations of the extracellular (wall space) fluid bathing cells of the tops is either drastically different (generally lower) in concentration or that excision strongly modifies ion absorption characteristics. Unfortunately there appears to be little or no information on free space ion content of shoot tissues. The site of the regulatory mechanism would appear to reside in the root system. Further evidence for this conclusion is the fact that in 10× solution the ion content of roots was about the same as in 1× solution.

Acknowledgments

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