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Ion Distribution in Roots of Barley Seedlings Measured by Electron Probe X-Ray Microanalysis[1]

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ABSTRACT

The distribution of ions, particularly K and Na, was studied in roots of barley seedlings grown on various ionic solutions. Analyses were made by means of electron probe x-ray microanalysis using frozen, fractured bulk specimens. By this technique, it was demonstrated that there can be variability in the ratio K/Na measured in the vacuoles of cortical cells, with this ratio often being lower in epidermal cells of the root than in the inner cortex. A sharp difference in the K/Na ratio was also found between cells of the endodermis and those of the adjacent cortex, and generally higher ratios of K/Na occurred in the stele than in the cortex. Estimation of the concentrations in the cytoplasm was at the limit of resolution of this technique, but it can be shown that the K/Na ratio in the cytoplasm was higher than that in the vacuole. In low salt roots, the K concentration in the cytoplasm was higher than that in the vacuoles. The results with the x-ray microprobe confirm other measurements based on flux analysis or analysis of small samples of the root.

Studies with Characean cells have established that, in cells at flux equilibrium, the ratio of K/Na in the cytoplasm is higher than that in the vacuole. In these large cells, it was possible to use direct analysis of separated cytoplasm and vacuole to confirm estimates from flux analysis. For example, results from MacRobbie (13, 14) and Spanswick and Williams (19), using *Nitella translucens,* showed that K/Na was 8.5 and 1.15 in the cytoplasm and vacuole, respectively, compared with 0.1 in the solution. For *Nitella flexilis,* Kishimoto and Tazawa (6) showed that K/Na was 25 and 2.9 in these corresponding sites, while in the solution it was 0.5.

Studies with higher plant cells (roots) have shown some resemblances to the Characean cells, as the electrochemical potential of K in the vacuole of cells at flux equilibrium was either higher or the same as that in solution; however, Na was substantially lower than in the solution in studies on pea (1, 2), barley (17), bean (15), and onion (12). Flux analysis based on tracer exchange with barley roots (17) estimated the ratio K/Na in the cytoplasm at 11 and in the vacuoles at 2.9, compared with 0.33 in the solution. Similar measurements with onion roots led to estimated ratios of K/Na of 10.6 in the cytoplasm and 1.9 in the vacuole at a given ratio of 1.0 in the solution (12). Although these results were consistent with models of ion transport in the cells proposed by workers with Characean cells, the use of efflux analysis with roots has been open to criticism because it represented the overall effect of cells in the stele and cortex. In barley roots, the ratio of K/Na in the stele could be shown to be higher than that in solution by analysis of the isolated stele and cortex (16). Although the basic model for ion transport in higher plant cells has been generally supported, this distribution of K and Na within the cell remains probable but hypothetical and lacks the test of direct analysis available with Characean cells. The availability of the electron probe x-ray microanalyzer offered a way of testing this ratio directly on frozen hydrated material.

One other prediction that has been made about K^+ distribution in barley root cells is that, in low-salt roots, the average K^+ content of about 15 to 30 μmol/g fresh weight is largely in the cytoplasm, so that the K^+ concentration in the cytoplasm should be larger than that in the vacuoles. The prediction was based on K requirements for pyruvate kinase activity (22) and, more recently, on comparative biochemical data (23).

Electron probe x-ray microanalysis also allows comparisons to be made between groups of cells, as between cortex and stele or between the inner and outer parts of the cortex.

This paper describes experiments with barley roots that confirm the general proposition about K and Na distribution in the cells of the root. The various methods of compartmental analysis and the results obtained on ion distribution in roots have been critically reviewed by Läuchli and Pflüger (11).

MATERIALS AND METHODS

Seeds of barley (*Hordeum vulgare* cv. Aramir) were germinated in the dark on aerated 0.5 mM $CaSO_4$ solution and then transferred to various solutions in the light. Plants were used when about 7 days old. The solutions used were: (a), 0.5 mM $CaSO_4$; (b), full nutrient solution containing (in mM concentration) 2.5 K, 7.5 Na, 10 Cl, 3 Mg, 2 Ca, 3 SO_4, 4 NO_3, and 0.8 phosphate (pH 5.5); (c), 0.5 mM KCl + 4.5 mM NaCl + 0.5 mM $CaSO_4$; and (d), 0.5 mM $CaSO_4$, transferred to 5 mM NaCl + 0.5 mM $CaSO_4$ 24 h before measurements.

Prior to analyses, roots were excised and washed for 2 min in 0.5 mM $CaSO_4$. The content of K and Na in the roots was determined by flame photometry; Cl was determined by electrometric titration. The ion contents in the roots of barley grown in various ionic solutions are given in Table I.

Electron Probe X-Ray Microanalysis. Small lengths of root (about 1.5 cm long) were cut about 4 to 5 cm from the apex of the root and mounted in holes on a copper carrier for the electron probe (ETEC Autoscan with a Kevex-Tracor energy-dispersive x-ray analyzing system). The carrier and roots were then frozen in liquid N_2, and transverse surfaces of the roots were prepared by fracturing them with cooled surgical scissors under liquid N_2. When transferred to the cryostage of the electron probe (which was at −150 C), the condensed surface water was carefully sublimed by controlled warming to about −100 C, and the specimen was observed with the scanning electron microscope mode of the

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Table I. *Ion Contents of Barley Grown in Various Ionic Solutions*

Ionic Solution	Ion Content		
	K	Na	Cl
	μmol/g fresh wt ± SE		
$CaSO_4$ (0.5 mM)	12.8 ± 1.4	16.4 ± 1.7	16.7 ± 2.2
Full nutrient solution containing K (2.5 mM), Na (7.5 mM), and Cl (10 mM)	99.5 ± 3.5	23.3 ± 0.2	29.0 ± 1.9
KCl (0.5 mM), NaCl (4.5 mM), $CaSO_4$ (0.5 mM)	59.7 ± 0.5	53.0 ± 0.9	84.3 ± 0.4
NaCl (5 mM), $CaSO_4$ (0.5 mM)	7.6	34.0	53.6

electron probe.

After freezing in liquid N_2, the structural preservation of the root cells was satisfactory. Initial freezing in Freon and rapid transfer into liquid N_2 gave improved results. Representative scanning electron micrographs of frozen barley roots after initial freezing in Freon are presented in Figures 1 and 2. A survey micrograph featuring part of the cortex and the entire stele is represented in Figure 1. A higher magnification micrograph (Fig. 2), with portions of the inner cortical parenchyma, endodermis, and stele, permits a better distinction between vacuolar and cytoplasmic compartments. The coarse, crystalline regions of the cells represent vacuoles, whereas the more densely structured, reticulate areas represent cytoplasm, and nuclei are often evident. In this study, the specimens for x-ray microanalysis were frozen in liquid N_2. A more detailed study on the use of various freezing methods for x-ray microanalysis will be published elsewhere (Markhart and Läuchli, manuscript in preparation).

The electron probe was operated at an accelerating voltage of 10 kv, with a specimen current of about 10^{-9} Å. The distance between specimen surface and detector was 10 mm. At this accelerating voltage, the depth of electron penetration was estimated to be about 2 to 3 μm, assuming a specimen density of 1.0 (9). When a static, narrow electron beam is used (spot analysis), the spatial resolution can be estimated using the method of Hall (3). The range of resolutions that can be obtained for K and Na when the accelerating voltage is varied is shown in Figure 3. The lowest voltages satisfactory for analysis of Na and K are 3 kv and 8 kv, respectively. Under these conditions, the best resolution attainable is about 0.5 μm for Na analysis and about 2 μm for analysis of K. At 10 kv (routinely used in this study), the resolution was estimated to be about 2 to 2.5 μm for analysis of K (and other important elements of similar atomic number) and 2.5 to 3 μm for Na analysis (Fig. 3). Alternatively, small regions (about 3 × 4 μm) can be probed using a scanning beam. Hence, the vacuole can be analyzed easily using this technique. Analysis of the cytoplasm in vacuolated cells (thickness range about 0.5–5 μm) is at the limit of the method, but useful information can still be obtained. Occasionally, the fracture occurs across the cell wall/cytoplasm interface, and more substantial areas of cytoplasm are then accessible to the probe beam. The more usual approach was to focus the beam on the common cell wall and the adjacent cytoplasms of

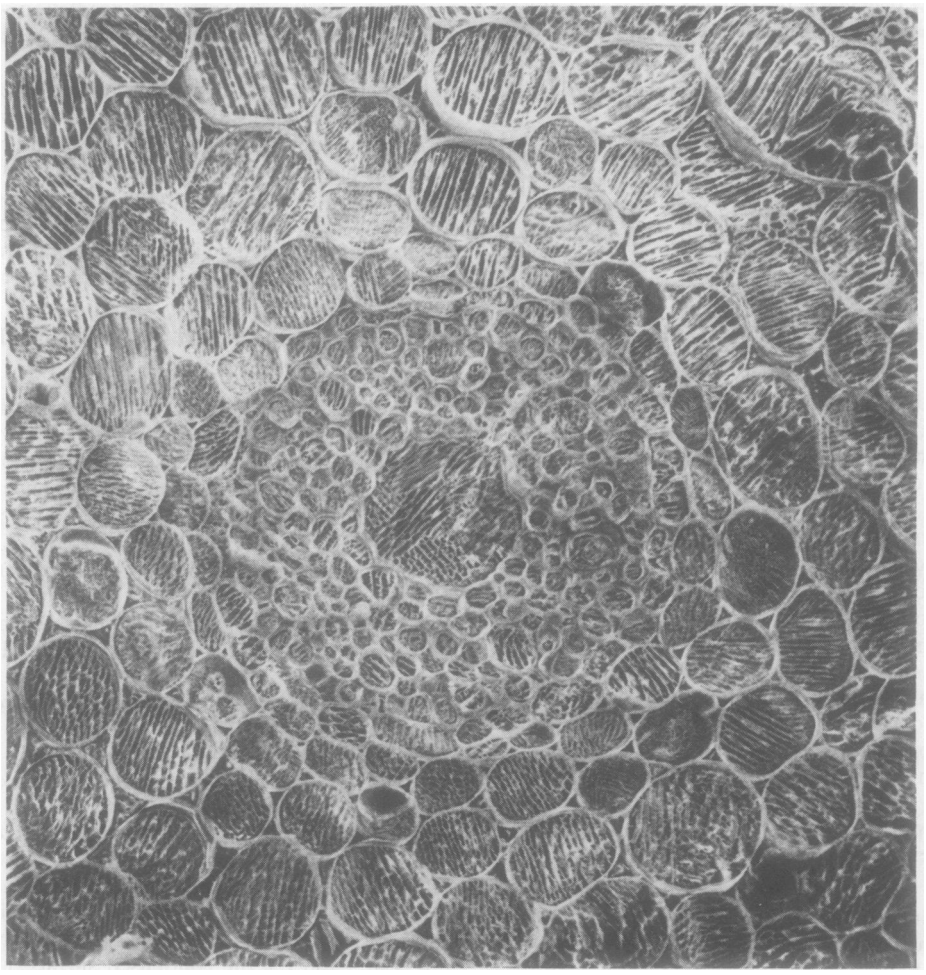

FIG. 1. Scanning electron micrograph of frozen, transversely fractured barley root about 4 to 5 cm from the apex, featuring the stele and part of the cortex. Ice crystals are visible in the cell vacuoles and in the vessels.

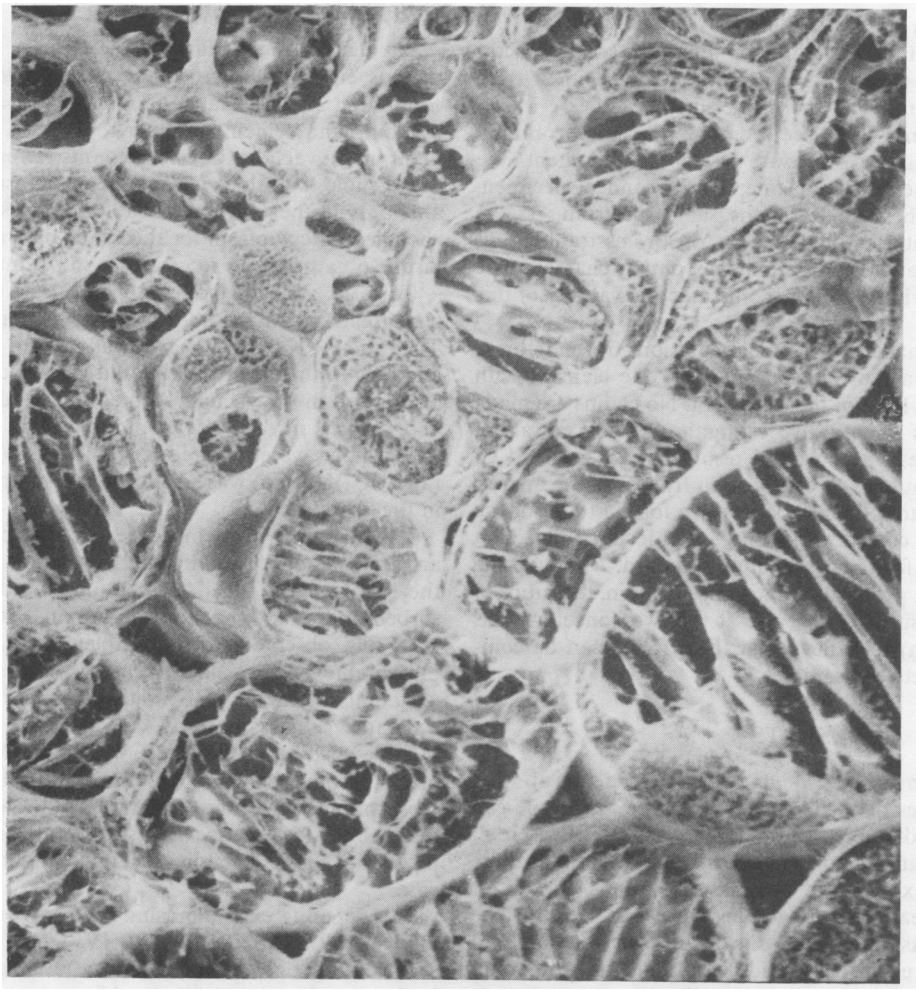

FIG. 2. Scanning electron micrograph from the region cortical parenchyma-endodermis-stele, showing vacuolar and cytoplasmic compartments.

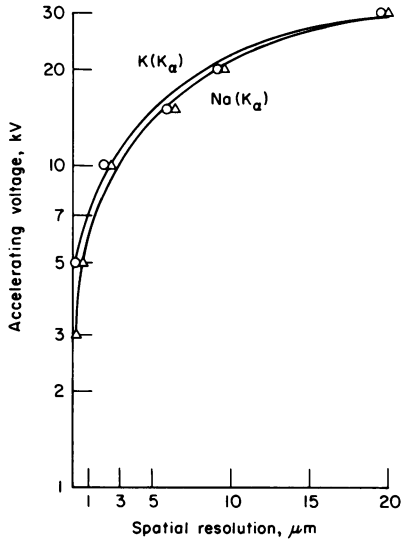

FIG. 3. Estimated spatial resolution of x-ray microanalysis as a function of accelerating voltage, assuming a specimen density of 1.0 (using the method of Hall [3]).

two neighboring cells. The area analyzed sometimes included a small amount of vacuolar region. The relevance of this type of measurement will be discussed later.

An example of an energy-dispersive x-ray spectrum (Fig. 4)

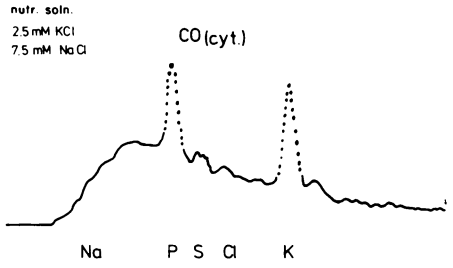

FIG. 4. X-ray spectrum from the cytoplasm of a root cortical cell (CO[cyt.]). Barley seedlings were grown in complete nutrient solution containing 2.5 mM KCl and 7.5 mM NaCl.

from the exposed cytoplasm of a cortical cell shows that the elements K and P predominate and that little Na and Cl are present when the seedlings were grown on full nutrient solution also containing 2.5 mM KCl and 7.5 mM NaCl. The spectrum also shows the normal 'Bremsstrahlung' distribution (24); spectra with an atypical Bremsstrahlung were rejected.

The reading used was the total count in channels between limits set at the base of the peaks, less the background count interpolated between the channels at the peak limits. Measurement of Ca was affected by the $K(K_\beta)$ emission and was not reliable at high K concentrations. The absolute reading from a scan depends on a number of geometrical factors as well as on the ionic content of the samples. For example, the angle of the beam with the sample, possible absorption of emitted x-rays by a cell wall projecting above the sample or other rough surface features, and interfering

emission from the Cu of the holder (particularly with Na) all affected the actual output. Doubtful or uncharacteristic spectra were disregarded, and measurement sites on the specimen were selected to be representative of the whole surface.

The 'peak-background' was preferred to the more common 'peak/background ratio' as a measure of elemental content. We wished to determine the ratios of elements in a scanned specimen, a comparison that is best made on the same spectrum. As the background varies with emitted energy of Bremsstrahlung, it seemed more appropriate to compare the net output characteristic of the element. Tests with standards confirmed the validity of this approach.

Comparisons were made with standard solutions frozen in loose cotton wool to confirm the relative values of one element with another and to test the limit of measurement. No claims are made that the method gives quantitative estimation of concentration, but Table II shows peak-background results for K, Na, and Cl with two different solutions. As can be seen from this, the analytical system is about 4 to 5 times less sensitive for Na than for the heavier elements. We consider that the lower level of detection was about that shown for the more dilute standard. Although the actual count rate is not proportional to concentration, the ratios of K/Na and K/Cl are sufficiently close at the two concentrations (allowing for variation) to believe that these ratios can be compared meaningfully between samples.

RESULTS

The scanning beam of 3 × 4 μm was suitable for probing sections of the vacuoles of cortical cells, well away from the cell wall, inasmuch as the diameters of the larger cortical cells were in the range of 20 to 30 μm. In some cases, it was evident that there could have been an exposure of cytoplasmic strands or even of the nucleus to the beam. Such samples were not used for the comparison below. As described, this beam was too large to sample the cytoplasm exclusively, and cytoplasm survey refers to the overall result from the common cell wall region, two adjacent layers of cytoplasm, and possibly some vacuole. The cytoplasm appeared to occupy from one-third to two-thirds of the whole field. Attempts to estimate the contribution of the cytoplasm to such cytoplasmic surveys are discussed later.

Three particular questions are examined here. (a), Does the ratio of K/Na in the cytoplasm differ from that in the vacuole, as predicted from flux measurements? (b), Is there evidence that the K concentration in the cytoplasm is higher than that in the vacuoles of low salt roots? (c), To what extent does vacuolar content vary between cells in the cortex and between stele and cortex?

Table II. *Calibration using Solutions Frozen on Loose Cotton Wool*
All counts given are peak-background.

Solution	Counts	Comparative Efficiency[a]	
		K/Na	K/Cl
mM	*per 60 s*		
K			
50	5,600 ± 610	5.1	0.95
1	400 ± 35	4.6	1.0
Na			
50	1,090 ± 140	5.1	
5	440 ± 80	4.6	
Cl			
50	5,900 ± 600		0.95
1	410 ± 80		1.0

[a] Based on equal concentrations of K, Na, and Cl.

Ion Distribution within the Cell. Figure 5 shows the counts for various elements obtained for roots grown in nutrient solution containing 2.5 mM K and 7.5 mM Na. Whereas K and P were the predominant elements in the cytoplasm, K and Na were predominant in the vacuole. Cl and S counts were significantly greater in the vacuole than they were in the cytoplasm, and Mg was detectable only in the cytoplasm. Table III gives the ratio of K/Na for vacuole and cytoplasm for this set of data and also for equivalent results obtained with roots grown in 0.5 mM KCl + 4.5 mM NaCl + 0.5 mM $CaSO_4$. In both cases, K/Na in the cytoplasmic survey was greater than for equivalent measurements in the vacuoles. The ratio P/(K + Na) (Fig. 5) was 0.56 for the cytoplasmic survey and 0.24 in the vacuole, as would be expected, although the level of P and S in the vacuole seemed high and may be due to high S and P levels in the culture solution. High concentrations of P in the vacuoles were reported for lupin roots (21). Note, too, that the ratio Cl/(K + Na) was smaller in the cytoplasm (0.05) than it was in the vacuoles (0.10).

The possibility was considered that the cytoplasmic survey may artificially reduce radiation by absorption in the cell walls when the lower energy Na(K_α) radiation would be more strongly absorbed than would the K(K_α) radiation, hence, increasing the ratio of K/Na observed. For example, Table IV shows a series of measurements on adjacent cells in which there is appreciable variation among the K/Na ratios from the cytoplasmic surveys of the same cells (samples 2, 4, 5, and 7). The sketch shows the relative position of the beam, which was 3 μm × 4 μm. Sample 5 has a particularly high K/Na ratio due to the low Na content. Absorption of the kind contemplated, however, should also have reduced the low energy Bremsstrahlung, producing a background profile with a depleted low-energy component, which was not the case.

In a separate example using roots grown on 2.5 mM K and 7.5 mM Na culture solution (Table IV) where the fracture revealed a surface of cytoplasm, the K/Na ratio in the vacuole was 2.3, and in the cytoplasmic area it was 4.8.

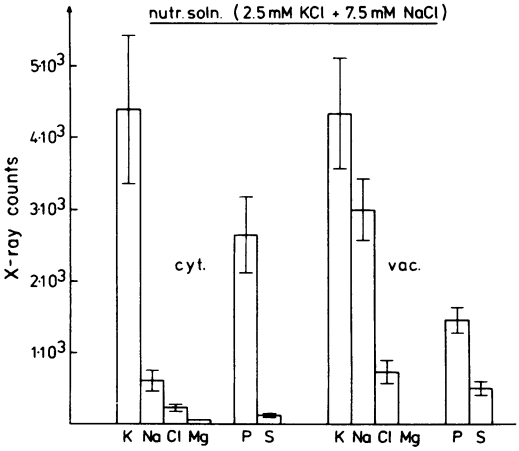

FIG. 5. Distribution of six elements in cytoplasm and vacuole of root cortical cells. Barley seedlings grown in complete nutrient solution containing 2.5 mM KCl and 7.5 mM NaCl.

Table III. *Comparison of X-Ray Count Ratios of K/Na in Vacuole and Cytoplasm of Cortical Cells of Barley Roots*

Solution	Vacuole	Ratio of K/Na in Cytoplasm	Solution
K (2.5 mM) + Na (7.5 mM)	1.5 ± 0.14 (10)	9.1 ± 2.2 (10)	0.33
K (0.5 mM) + Na (4.5 mM)	0.07 ± 0.01 (11)	0.79 ± 0.22 (11)	0.11

Table IV. *X-Ray Counts for a Series of Measurements in Cortical Cells of Barley Roots Grown in Nutrient Solution Containing 2.5 mM K + 7.5 mM Na*

Cell No.	K Counts	Na Counts	K/Na
1 (vac)	11,460	7,600	1.5
2 (cyt)	16,600	7,190	2.3
3 (vac)	5,380	3,070	1.7
4 (cyt)	12,470	2,980	4.2
5 (cyt)	4,430	320	13.8
6 (vac)	9,160	10,750	0.85
7 (cyt)	9,890	5,570	1.8

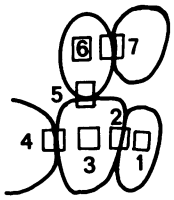

Table V. *X-Ray Counts for Various Elements in Cytoplasm and Vacuoles of Barley Roots Grown in 0.5 mM $CaSO_4$ Solution*

P is the probability of obtaining these differences from the same population.

Element	Vacuole	Cytoplasm	*P*
K	1,280 ± 300	4,150 ± 400	<0.001
Mg	210 ± 100	520 ± 90	0.06
P	1,150 ± 410	4,250 ± 350	<0.001
S	410 ± 190	1,125 ± 245	0.06

K Distribution in Cells of $CaSO_4$-Grown Roots. The data in Table I show that the average K concentration in low-salt, *i.e.* grown in 0.5 mM $CaSO_4$ alone, is about 13 μmol g^{-1} fresh weight, though it ranges up to about 20 μmol g^{-1} fresh weight. A survey of count rates for various elements in the vacuole and cytoplasm of roots grown in 0.5 mM $CaSO_4$ are given in Table V. There was a clear difference in K and P counts between cytoplasm and the vacuole. These estimates were based on a number of fields selected to have different relationships to the cell wall in the cytoplasmic survey and in different cells of the cortex. As will be discussed below, these values are considered to be due to greater K concentration in the cytoplasmic survey than to that in the vacuoles.

Distribution of K and Na across the Root. Measurements of the ratio K/Na in cell vacuoles varied from 0.85 to 1.7 (Table IV). Such differences from cell to cell were more pronounced near the epidermis (Table VI), where there seemed to be an increase in Na content at sites nearer the epidermis; to the extent that these counts represent concentrations, there also seemed to be little difference in K concentration. The change in K/Na from cell to cell in the vacuoles obscures the cytoplasmic K/Na, which is greater than one of the adjacent vacuoles but less than that of the other vacuole. Variation in vacuolar content of cortical cells has also been reported for lupin (21).

Probe measurements were used to compare areas in the cortex with areas in the stele (Table VII), sampling areas of about 30 × 20 μm. In the cortex, samples were taken halfway between epidermis and endodermis and distributed equally around the root. In the stele, samples were taken at the radius of the metaxylem vessels, and they included xylem parenchyma and, presumably, some parts of the phloem. It was also possible to measure the content of the central vessel (Table VII). Consistent differences were evident in the data between the stele and the cortex, although, in the nutrient solution containing 2.5 mM K + 7.5 mM Na, it was surprising to find no detectable Na in any of the areas sampled in the stele. For this reason, a further comparison was made using

Table VI. *Series of X-Ray Counts from the Center of the Cortex (Sample No. 1) to the Epidermis of the Root*

This is the same tissue as in Table IV but a different sample.

Sample No.	K Counts	Na Counts	K/Na
1 (vac)	13,870	4,310	3.2
2 (cyt)	6,625	1,495	4.4
3 (vac + nucleus)[a]	14,480	2,095	6.9
4 (cyt)	7,925	3,250	2.4
5 (vac)	11,860	6,875	1.75
6 (cyt)	8,120	11,000	0.73
7 (vac)	11,010	18,600	0.60

[a] Nucleus was in the center of the field.

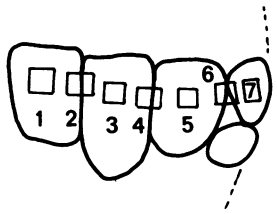

Table VII. *Relative Concentrations of K and Na as Determined by X-Ray Counts of the Cortex and Stele*

Solution	K/Na Ratio		
	Cortex	Stele	Central Vessel
mM			
K (2.5) + Na (7.5) (nutrient solution)	3.3 ± 0.1 (5)	ND[a]	11.0 ± 3.0 (8)
KCl (0.5) + NaCl (4.5)	0.23 ± 0.07	ND	ND
$CaSO_4$ (0.5), to NaCl (5) + $CaSO_4$ (0.5)	0.05 ± 0.01 (6)	0.87 ± 0.27 (3)	0.68 ± 0.06 (3)

[a] ND, Not detectable (Na ~ 0).

roots of seedlings grown on 5 mM NaCl following 0.5 mM $CaSO_4$. In this case, Na clearly penetrated to the stele, but the K/Na ratio was higher in the stele than it was in the cortex, and the ratio in the central vessel was lower than that of the average cell of the stele.

It is possible that the differences between stele and cortex reflected the smaller size of the cells in the stele (Fig. 2) and, therefore, more cytoplasm occurred in the probe field. To test the difference between stele and cortex in another way, measurements were made of the vacuoles of endodermal cells and the adjacent cortical cells. In the plants grown on 5 mM NaCl, the ratio of K/Na in the endodermis was 1.05 ± 0.25 and 0.10 in the innermost cortical cell. Clearly, the endodermis acts as an efficient barrier to Na.

DISCUSSION

Published values for K and Na distribution in barley seedlings grown on solutions containing 2.5 mM K and 7.5 mM Na are summarized in Table VIII. The present data from Table III and Figure 5 support the flux analysis data, showing that the ratio of K to Na is higher in the cytoplasm than it is in the vacuoles, and are consistent with the conclusion by Jeschke (4) that Na in barley root cells is predominantly localized in the vacuole. The ratio of K/Na in the vacuole (mean, 1.5; Table III) is lower than the average in the root (4.3), as would be expected. Inasmuch as the cytoplasm survey included some vacuolar component, the ratio of

Table VIII. *Distribution of K and Na in Barley Seedlings Grown on Solutions Containing 2.5 mM K and 7.5 mM Na*

	Plant Part								
			Root						
	Shoot	Exudate from cut root	Isolated stele	Cortex	Average			Cytoplasmic phase[a]	Vacuole[b]
	μmol/g fresh wt	*mM*			*mM*	*μmol/g fresh wt*			
Concentration									
K	215	30			80[c]	62[d]	99.5	92	79
Na	27	4			23	22	23.3	8	24
Ratio K/Na	8/1	7/1	4.1	2.1	3.5	2.8	4.3	11	3.3

[a] Flux analysis, from (17).
[b] Assumes 5% cytoplasm.
[c] This column, from (17).
[d] This column, from (16).

K/Na in the cytoplasm should be larger than the ratio of 9.1 (Table III) and possibly larger than the ratio of about 11 found from flux analysis (Table VIII).

Data from Table VII also are in agreement with the observations that the stele contains more K relative to Na than does the cortex and that the ratio of K/Na in the exudate from the root (*i.e.* in the vessels) is higher than the average ratio for the root (*i.e.* mainly cortex).

Though the general trend is confirmed by the data presented here, there is a larger difference between vacuolar K and Na and cytoplasmic or stelar K/Na than that predicted in Table VIII. Comparison of Tables III, IV, and V also reveals that there can be considerable variability among roots and among cells of the same root. X-ray microanalyses from roots of other plant species also demonstrated variation in the vacuolar contents of individual cells (21) or a gradient in the ratio of K/Na across the cortex (20). The variations that have been observed are clearly not consistent with the homogeneous distribution assumed in flux analysis (17). From the results shown in Table V, we inferred that there is more K in the cytoplasm of low-salt roots (grown in $CaSO_4$) than there is in the vacuoles. Although the variable surface geometry of the freeze-fractured material makes it difficult to convert the peak counts to concentrations, the comparison of adjacent areas of the section should be indicative of the partition of K between the two phases. The average K concentration from analysis of the roots was 20 μmol g^{-1} fresh weight, and the ratio of K counts of the cytoplasm to the vacuoles is 3.24. Observation of the scanned fields suggested that cell wall was about 20% of the field. If it is assumed that there is about 5% cytoplasm in the barley root and 80% vacuole, then the concentration of K in the vacuole and cytoplasm can be estimated for varied percentage areas of cytoplasm in the probe field (A) as:

$$K_{vac} = A/(0.009 + 0.0654\,A)$$

and

$$K_{cyt} = K_{vac}\,(2.44 + A)/A$$

While the resolution of the method clearly permits the analyses of the vacuoles, separate analyses of the cytoplasm in vacuolated cells are possible only at the resolution limit of the method, as demonstrated in this study. A point source beam gives the best resolution; it has been used to obtain separate analyses of cytoplasm, plastid, and vacuoles in the intertidal red alga *Porphyra* (Wiencke and Läuchli, manuscript in preparation). In the study described here, both a point source beam and a scanning beam, probing small regions, have been applied, and similar results were obtained. Results are given in Table IX. If the volume of cytoplasm were as high as 10%, then the concentration in the cytoplasm and vacuole would be 71 and 8 mM for 30% of the beam covering cytoplasmic areas.

Table IX. *Effect of Differences in the Percentage of Cytoplasm in the Scanning Beam on the Estimation of K Concentration in the Cytoplasm and Vacuoles*

Percentage of Beam as Cytoplasm	Estimated K Concentration	
	Cytoplasm	Vacuole
%	*mM*	
20	119	9
30	95	10
40	81	11
50	71	12

The estimated K concentrations compare well with those obtained by Jeschke and Stelter (5) using flameless atomic absorption spectroscopy, *i.e.* 110 mM in the cytoplasm and 20 mM in the vacuole. The ability of the cell to concentrate K in the cytoplasm may be important under conditions of K deficiency, and, as it occurs in the presence of Na, it may explain the sparing effect of Na on K deficiency as a release of K by Na from the vacuoles to the cytoplasm.

The scanning beam method has the advantage of providing averaging values due to the larger area or volume excited by the beam, and it improves the signal to background ratio, thus increasing the sensitivity for K and Na. Sensitivity is particularly important for Na measurements, as there are several potentially competing x-ray peaks (*e.g.* Cu, Mg), and the background increases strongly with decreasing x-ray energy in this region of the spectrum (*cf.* Fig. 4).

Through the method used in this study, very clear distinctions can be made from cell to cell in the root (Tables IV and VI). Two particular problems which are not amenable to other types of study can be tackled with this approach. One is the differences found between endodermal cells and adjacent cortical cells. This has particular relevance to the role of the symplast and development of the endodermis (10, 18). The other is the difference between cells of the epidermis and underlying cortex. The results here show that there can be larger proportions of Na in the epidermis than there are in the adjacent cortical cells, and it has been shown in other studies that the epidermis of various roots

can have different structures and properties than do the cortical cells, *e.g.* in *Atriplex* (7), in agreement with earlier studies using microautoradiography and x-ray microanalysis (8). This ability to compare distribution across the root has considerable value in interpreting how roots are able to control the uptake of K, Na, and Cl, and it gives a new dimension to information on the accumulation and distribution of ions as compared with analyses of the gross or average content of the root.

In conclusion, the results provide direct evidence for K/Na distribution in the cytoplasm and vacuole of root cells that supports earlier predictions based on flux analysis. The method has considerable potential, especially for study of salinity effects on plants and ionic relations of plants as affected by various nutritional regimes. Conversion of the relative data to absolute concentrations by means of suitable standards should add substantially to the potential of this method.

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