THE SUBCELLULAR CONCENTRATION OF IONS AND ELEMENTS IN THIN CRYOSECTIONS OF ONION ROOT MERISTEM CELLS
AN ELECTRON-PROBE EDS STUDY

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SUMMARY
Quantitative electron-probe energy dispersive X-ray microanalysis has, for the first time, been accomplished at a subcellular level in plant tissue using cryofixed and thin freeze-dried cryosections. The subcellular concentrations of Na\(^+\), Cl\(^-\), K\(^+\), P, S, Mg\(^{2+}\) and Ca\(^{2+}\) were measured in mol/kg dry weight in two types of root meristematic cells of the onion, Allium cepa. The cell wall of the meristematic cells had much higher concentrations of K\(^+\) and Ca\(^{2+}\) than was found in the intracellular compartments. Storage granules in the protoderm cells were about 6-12 times lower in P and were about four times higher in S as compared to other intracellular compartments. Comparison between the concentrations of ions and other elements in meristematic plant cells and in mouse cardiac myocytes confirms that major differences in cytoplasmic Na\(^+\) and Cl\(^-\) concentrations do indeed exist between these cell types.

INTRODUCTION
Little is known about the subcellular concentrations of ions in higher plant cells (Harvey, 1983). Such information is needed for an understanding of the uptake and transport of ions and their roles in metabolic processes and water relationships. For this reason ion analysis of plant tissues in the frozen hydrated state using electron-probe energy dispersive X-ray microanalysis (EDS) has been vigorously pursued on bulk scanning electron microscope (SEM) samples (Echlin, Hayes & McKoon, 1983; Preston, 1982; van Steveninck, van Steveninck & Läuchli, 1982a; van Steveninck, van Steveninck, Stelzer & Läuchli, 1982b). These analyses of bulk frozen hydrated specimens of plant material have already provided valuable information on the distribution of Na\(^+\), K\(^+\) and Cl\(^-\) in various cell types and various subcellular locations in several non-halophytes. However, it has not previously proved possible to obtain absolute measures of concentrations of ions and elements using bulk SEM samples, since it is difficult to know whether the excitation is restricted to the intended compartments. Also, a lack of suitable calibration still prevents absolutely quantitative analysis using this technique (van Steveninck et al. 1982). The reports of Marshall

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Key words: ion concentration, electron probe EDS, Allium cepa, cryosections, X-ray microanalysis, onion root meristematic cells, heart myocytes.
(1980, 1982) do, however, suggest that accurate quantitative analysis of elements in localized regions of cells in bulk frozen hydrated specimens may be possible.

It appears that the dearth of accurate quantitative information on the subcellular concentrations of ions and elements in plant material rests partly on the fact that it has previously proved to be technically difficult to cut thin cryosections of cryofixed plant materials. The present study deals with analysis on thin cryosections of appropriately cryofixed plant tissue and it appears to be the first successful application of quantitative electron-probe energy dispersive X-ray microanalysis to such material.

Briefly, we were able to measure the subcellular concentrations of several different ions and elements in meristematic cells and in the cell wall of the onion root tip. The concentrations of these elements were compared with the subcellular concentrations of the same ions and elements in mouse cardiac muscle cells, obtained using the same technique.

**MATERIALS AND METHODS**

**Plant material**

Fresh green onion plants, *Allium cepa*, were purchased from a store. All of the roots were cut off close to the white bulb, which was then immersed in deionized distilled water that was changed each day. The plants were placed in the window for natural light. After 3 days a number of new roots had grown out from the bulb. These roots were also cut off close to the bulb and new roots were again allowed to grow out for 2 more days. The root lengths then ranged from about 0.5 to 1.2 cm with a diameter of about 1 mm. The 0.3 mm root-tip cap portions of the shorter roots were cut off with a razor blade and the next 1.0 mm sections were cut off and further trimmed. A parasaggital longitudinal section was then cut from this 1.0 mm length of root in such a way that the flat cut surface of the larger portion of root could be positioned face down on the flat surface of a small brass freezing stub. Such root specimens were then plunged rapidly into a bath of stirred liquid propane, which was cooled in a bath of liquid nitrogen. The specimens were sectioned to a depth of >150 μm before any of the sections were collected for study. This ensured that we obtained sections through the cortex of the root, which is the region of most rapidly dividing meristematic cells (Esau, 1965; Jensen & Ashton, 1960).

**Animal material**

Female mice, 4 months of age, were used for this study. The animals were killed by cervical dislocation, thus avoiding any potential effect on the distribution of elements in tissue induced by anaesthesia. To obtain heart samples, the chest cavity was opened and the heart, which was still beating, was removed. Using a razor blade, a small sample was cut from the apex of the heart. This sample was mounted, cut-surface down, on a small brass pin. The pin was plunged into liquid propane, which was cooled in liquid nitrogen. Pins with frozen samples were stored in individual vials on canes in liquid nitrogen until the time of sectioning.

**Cryoultramicrotomy**

Frozen tissue samples were sectioned on an LKB V ultramicrotome with a cryokit (LKB Produkter, Bromma, Sweden) that had been extensively modified (Hagler et al. 1980; Seveus, 1980; Smith, Morris, Richter & Cameron, 1983b; Somlyo, Shuman & Somlyo, 1977). A large styrofoam trough was used in the cryokit. The foil heater was placed in the bottom of the trough. A 40° glass knife with a 5° clearance angle was made. Sections were cut at −100°C (knife and specimen), using a manual advance of 0.1 μm and a cutting speed of 1 mm/s. Using an eyelash probe, the frozen sections were transferred to a Formvar film over a 1.5 mm hole in a 3 mm diameter pyrolytic carbon grid (Ted Pella, Inc., Tustin, CA). Each grid rested on a metal post that was part of a metal carousel
Ion concentration in onion cells

of posts, which was placed on top of the knife holder, as close to the back edge of the knife as possible. To flatten the sections and to prevent their loss, they were immediately sandwiched between two layers of Formvar by placing a carbon/Formvar-coated aluminium ring over the grid on the post (Smith, Morris, Richter & Cameron, 1983). As each grid was filled with two or three sections, a new post with grid was rotated into position for placement of the next sections.

When all sections had been cut, the carousel of grids was transferred to a custom-made aluminium freeze-drying chamber housed in the cryokit. A custom-machined teflon O-ring and aluminium lid were placed on the freeze-drying apparatus. The chamber was evacuated with a rotary pump. Pressure was applied to the Teflon O-ring by using hexnut drivers on hex screws in the lid. The applied pressure made it possible to form a good vacuum seal in spite of the low temperature and the rigidity of the O-ring. A trap containing type 4A molecular sieve beads (Fisher Scientific Co., Pittsburgh, PA) was present in the vacuum line. Once a vacuum had been established, liquid nitrogen was added to a Dewar flask around the beads, thus enhancing the vacuum freeze-drying process by cryosorption. Freeze-drying was carried out at —100°C. After 2 h the valve to the pump was closed. Cryosorption pumping continued overnight and the freeze-drying chamber was allowed to warm to 24°C. The valve between the molecular sieve trap and the freeze-drying chamber was then closed, and the specimen chamber was vented with dry nitrogen gas. The grids were removed from the carousel and stored in a plastic grid box in a desiccator. X-ray analysis was performed on the same day, if possible.

X-ray microanalysis

At the time of X-ray microanalysis, a sample in its Formvar sandwich on the carbon slot grid was placed into a special holder fabricated from beryllium and carbon. The tissue was imaged in the scanning transmission electron-microscopic (STEM) mode and X-ray analysis was performed as previously reported (Smith, Stabler, Cameron & Medina, 1981) at 25 kV accelerating voltage.

Ten cells were analysed from each animal. Spectra were collected from the nucleus and the myofibrillar cytoplasm of each cardiac myocyte. Spectra were also collected from the mitochondrially enriched cytoplasm near the nucleus.

Data were analysed statistically by a one-way analysis of variance for each element. The Student-Newman-Keul multiple range test was used to determine which means were significantly different.

RESULTS

Fig. 1 is a photomicrograph of a scanning transmission electron-microscopic image of a 0.1 μm thick freeze-dried cryosection of onion root innermost cortex cells. This non-stained section shows morphology typical of the type that can be obtained by our cryopreparative procedures. Clearly visible are the cell walls that surround each cell. Also apparent is the nucleus and, in most nuclei, a nucleolus can be observed. One of the cells in the micrograph shows condensed chromosomes at mitosis, which assured us that we were in a region of active cell reproduction. Because distinct and rather large areas of condensed chromatin can be discerned in the interphase cells of Allium, it was possible to place the electron-probe raster within an area of condensed chromatin for X-ray microanalysis. No mass loss, as judged by a constant X-ray count rate, could be detected under such microprobe conditions. Reduction of the area rastered to smaller sizes on the condensed chromatin did result in a noticeable loss of mass during the analysis and for this reason the areas rastered for X-ray analysis were kept as large as possible and frequent checks for loss of mass were made.

Fig. 2 shows protoderm cells in a region near the edge of the root. The protoderm cells differ from the innermost cortex cells in that they contain storage granules.
Fig. 1
Table 1 summarizes the subcellular elemental concentration of the onion root-tip innermost cortex cells and of the mouse myocytes expressed in mmol/kg dry weight ± s.e.m. The plant elemental concentration data are presented for the nucleus (including areas of both condensed chromatin and interchromatin), the nucleolus, areas of 'open' cytoplasm free of dark inclusions that we suspect are mitochondria, and areas of primary cell wall including the middle lamella between cells. In the case of

Fig. 1. STEM of a freeze-dried unstained cryosection of innermost cortex cells in the meristematic region of onion root tip. The nucleus in most cells shows a discernible nucleolus. Areas of condensed chromatin are seen within the nuclei. One of the cells is in mitosis (arrowheads). Bar, 5·0 µm; ×3250.

Fig. 2. STEM of a freeze-dried unstained cryosection of protoderm cells in a region near the edge of the onion root tip. Storage granules are indicated by arrowheads. Bar, 5·0 µm; ×3250.
Table 1. Subcellular elemental content of the innermost cortex cell in the onion root tip and in the mouse cardiac myocyte

<table>
<thead>
<tr>
<th>Cell type and compartment</th>
<th>( n^* )</th>
<th>( \text{Na}^+ )</th>
<th>( P )</th>
<th>( S )</th>
<th>( \text{Cl}^- )</th>
<th>( K^+ )</th>
<th>( \text{Mg}^{2+} )</th>
<th>( \text{Ca}^{2+} )</th>
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<tbody>
<tr>
<td>Onion cortex cell</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Nucleus</td>
<td>10</td>
<td>55±12</td>
<td>672±47</td>
<td>176±6</td>
<td>55±6</td>
<td>796±17</td>
<td>139±19</td>
<td>36±9</td>
</tr>
<tr>
<td>Condensed chromatin</td>
<td>9</td>
<td>48±23</td>
<td>743±63</td>
<td>140±8</td>
<td>63±3</td>
<td>780±29</td>
<td>108±30</td>
<td>19±6</td>
</tr>
<tr>
<td>Nucleolus</td>
<td>10</td>
<td>111±35</td>
<td>722±59</td>
<td>174±19</td>
<td>37±9</td>
<td>826±21</td>
<td>146±24</td>
<td>32±7</td>
</tr>
<tr>
<td>Open cytoplasm</td>
<td>10</td>
<td>91±18</td>
<td>688±38</td>
<td>146±10</td>
<td>39±6</td>
<td>728±39</td>
<td>156±30</td>
<td>32±7</td>
</tr>
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<td>Lateral cell wall</td>
<td>4</td>
<td>34±14</td>
<td>170±32</td>
<td>250±31</td>
<td>31±9</td>
<td>1476±43</td>
<td>63±11</td>
<td>107±13</td>
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<tr>
<td>Mouse cardiac myocyte</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Nucleus</td>
<td>5</td>
<td>226±29</td>
<td>357±45</td>
<td>178±19</td>
<td>207±29</td>
<td>333±27</td>
<td>56±12</td>
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<tr>
<td>Cytoplasm</td>
<td>5</td>
<td>214±34</td>
<td>253±33</td>
<td>185±20</td>
<td>191±29</td>
<td>305±28</td>
<td>49±8</td>
<td>20±3</td>
</tr>
<tr>
<td>Mitochondria-rich cytoplasm</td>
<td>5</td>
<td>167±38</td>
<td>463±35</td>
<td>242±33</td>
<td>169±36</td>
<td>253±23</td>
<td>38±3</td>
<td>23±8</td>
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One-way analysis of variance statistics

<table>
<thead>
<tr>
<th>( F ) value</th>
<th>( P ) value</th>
<th>NS†</th>
<th>&lt;0·001</th>
<th>&lt;0·001</th>
<th>&lt;0·001</th>
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<tr>
<td>6·29</td>
<td>&lt;0·001</td>
<td></td>
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<td></td>
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<td>&lt;0·001</td>
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<tr>
<td>11·16</td>
<td>&lt;0·001</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0·001</td>
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<td>115·80</td>
<td>NS†</td>
<td></td>
<td></td>
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<td>&lt;0·001</td>
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<td>3·35</td>
<td>7·67</td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Results of S–N–K multiple range test‡

|        |        |        |        |        |        |        |        |        |
| 34     | 170    | 140    | 31     | 253    | 19     |        |        |        |
| 48     | 253    | 146    | 37     | 305    | 20     |        |        |        |
| 55     | 357    | 174    | 39     | 333    | 23     |        |        |        |
| 91     | 463    | 176    | 55     | 728    | 24     |        |        |        |
| 111    | 672    | 178    | 63     | 780    | 32     |        |        |        |
| 167    | 688    | 185    | 169    | 796    | 32     |        |        |        |
| 214    | 722    | 242    | 191    | 826    | 36     |        |        |        |
| 226    | 743    | 250    | 207    | 1476   | 107    |        |        |        |

\( ^* n \), number of cells measured in the case of the plant, and number of mice measured in the case of the myocytes (10 cells measured per mouse). The myocyte data reported here represent a re-analysis of data previously reported by Roland & Vian (1979).

† NS, not significant.

‡ Values in each column that share a common line are not significantly different, whereas all values that do not share a common line are significantly different at \( P<0·001 \). S–N–K, Student–Newman–Keul.
the mouse myocytes the subcellular areas measured included the centrally located nucleus (free of a nucleolus), cytoplasmic areas containing contractile elements, and areas enriched in mitochondria (see Fig. 3).

Table 1 also presents the results of a one-way analysis of variance for each element to determine whether significant differences could be detected between any of the compartments. In this regard, highly significant differences \((P < 0.001)\) were found for each element except Mg\(^{2+}\). Once it was established that significant differences existed between the elemental concentrations of some compartments, it was necessary to run a Student—Newman—Keul multiple-range test to determine exactly which compartments differed significantly. The results of this analysis are summarized at the bottom of Table 1.

The most striking differences found between the plant cell compartments were between the primary cell wall and the other cellular compartments. Compared to the cellular compartments, the cell wall had a lower concentration of P but higher concentrations of S, K\(^+\) and Ca\(^{2+}\) in mmol/kg dry weight. As can be seen from the data in Table 1, these differences were found to be at least two- to three-fold for each element, but one should remember that these data are expressed on a dry weight basis.

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Fig. 3. STEM of a freeze-dried unstained cryosection of mouse heart. A centrally located nucleus is seen in a cross-section of a cardiac myocyte. Dense accumulations of mitochondria (arrowheads) are seen between the less-dense regions of contractile elements. The smaller size of ice-crystal reticulations seen in the cardiac myocytes compared to the larger size of ice reticulations seen in the plant cells (Figs 1 and 2) is tentatively ascribed to the higher content of water in the plant cells (see Cameron, Hunter, Ord & Fullerton, 1984). Bar, 5.0 \(\mu\)m; \(\times4000\).
Table 2. Subcellular elemental content of onion root protoderm cells

<table>
<thead>
<tr>
<th>Cell compartment</th>
<th>( n^* )</th>
<th>( \text{Na}^+ ) (mmol/kg dry wt ± s.e.m.)</th>
<th>P (mmol/kg dry wt ± s.e.m.)</th>
<th>S (mmol/kg dry wt ± s.e.m.)</th>
<th>Cl(^-) (mmol/kg dry wt ± s.e.m.)</th>
<th>K(^+) (mmol/kg dry wt ± s.e.m.)</th>
<th>Mg(^{2+}) (mmol/kg dry wt ± s.e.m.)</th>
<th>Ca(^{2+}) (mmol/kg dry wt ± s.e.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleus</td>
<td>6</td>
<td>63 ± 13</td>
<td>592 ± 69</td>
<td>81 ± 6</td>
<td>28 ± 8</td>
<td>536 ± 41</td>
<td>61 ± 10</td>
<td>9 ± 3</td>
</tr>
<tr>
<td>Condensed chromatin</td>
<td>5</td>
<td>27 ± 8</td>
<td>603 ± 66</td>
<td>110 ± 9</td>
<td>56 ± 5</td>
<td>608 ± 42</td>
<td>57 ± 14</td>
<td>11 ± 4</td>
</tr>
<tr>
<td>Open cytoplasm</td>
<td>5</td>
<td>37 ± 11</td>
<td>331 ± 22</td>
<td>93 ± 13</td>
<td>34 ± 11</td>
<td>375 ± 20</td>
<td>67 ± 14</td>
<td>16 ± 7</td>
</tr>
<tr>
<td>Storage granules</td>
<td>6</td>
<td>51 ± 19</td>
<td>49 ± 8</td>
<td>406 ± 71</td>
<td>32 ± 7</td>
<td>280 ± 21</td>
<td>79 ± 21</td>
<td>19 ± 7</td>
</tr>
<tr>
<td>Lateral cell wall</td>
<td>5</td>
<td>78 ± 11</td>
<td>57 ± 21</td>
<td>142 ± 16</td>
<td>58 ± 7</td>
<td>882 ± 102</td>
<td>62 ± 11</td>
<td>260 ± 7</td>
</tr>
</tbody>
</table>

Results of one-way analysis of variance

- \( F \) value: 1.718
- \( P \) value: NS†

Results of S–N–K multiple range test‡

<table>
<thead>
<tr>
<th>elemental content</th>
<th>49</th>
<th>57</th>
<th>331</th>
<th>592</th>
<th>603</th>
</tr>
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<td>Na(^+)</td>
<td>81</td>
<td>93</td>
<td>110</td>
<td>142</td>
<td>406</td>
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<tr>
<td>P</td>
<td>280</td>
<td>375</td>
<td>536</td>
<td>608</td>
<td>882</td>
</tr>
<tr>
<td>S</td>
<td>9</td>
<td>11</td>
<td>16</td>
<td>19</td>
<td>260</td>
</tr>
</tbody>
</table>

\( ^* n, \) number of cells measured.

† NS, not significant.

‡ Values in each column that share a common line are not significantly different, whereas all values that do not share a common line are significantly different at \( P<0.001 \).
and that this method of expression probably does not reflect the expected data if the concentrations of ions and elements were expressed on a wet weight basis.

The only significant difference found between intracellular compartments in the plant cortex cells was the higher nucleolar concentration of K$^+$ compared to that in the open cytoplasm.

Several significant differences were shown to exist between the subcellular concentrations of elements in the plant cortex wall compared to the myocyte (Table 1). The intracellular concentrations of P and K$^+$ were significantly higher in all of the subcellular compartments in the plant cortex cell compared to the myocyte. On the other hand, the intracellular concentrations of Cl$^-$ and Na$^+$ were significantly lower in all of the subcellular compartments in the plant cortex cell compared to the myocyte. Indeed, the myocytes showed four to five times the concentration of Na$^+$ and Cl$^-$ compared to the plant cortex cell.

The protoderm cells of the onion root differ from the innermost cortex cells in that they contain storage granules of various sizes (Fig. 2). With regard to the subcellular concentrations of elements, the storage granules differ markedly in elemental content compared to those measured in the other intracellular compartments (Table 2). For comparison, the granules were about six to twelvefold lower in P but were about fourfold higher in S on a dry weight basis. Also, we found the storage granules to have significantly less K$^+$ than the condensed interphase chromatin. The nuclear areas, which contained areas of open nucleoplasm as well as condensed chromatin, and the condensed chromatin areas both had about twofold more P than the open cytoplasm. The condensed chromatin was higher in P than any other cellular structure and also had a significantly higher concentration of K$^+$ than was found in the open cytoplasm. The lateral cell wall of the protoderm was found to be significantly lower in P than all the intracellular compartments except the storage granules. On the other hand, the lateral cell wall was from 10- to 20-fold higher in Ca$^{2+}$ than any intracellular structure and the K$^+$ concentration was also higher in the lateral cell wall than in any other intracellular structure.

**DISCUSSION**

*The subcellular concentrations of ions and elements in the meristematic cells of onion root*

As mentioned in the Introduction, little is known about the subcellular concentrations or distribution of ions in higher plant cells. Most of what we do know has come from rather indirect methods such as via ion profiles using atomic absorption spectroscopy of portions of plant tissues (Jaschke, 1979; Jaschke & Stelter, 1976) or by more direct methods with ion-selective electrodes (Gerson & Pode, 1972). The former technique has not allowed discrimination between extracellular and subcellular compartments, and the latter method has been criticized because the placement of the Cl$^-$ electrode tip may not have been in the cytoplasm proper (Greenway & Munns, 1980). For comparison with our data, Davis & Jaworski (1979) reported that maize roots at 1 mm and 30 mm-NaCl, plus 1 mm-Ca$^{2+}$ on the exterior, showed cytoplasmic
Na\(^{+}\) concentrations of 15 and 50 mm, respectively, on a wet weight basis. Root tips of mung bean at 60 mm-Cl\(^{-}\), plus 0.5 mm-Ca\(^{2+}\) on the exterior, showed a 30 mm-Cl\(^{-}\) concentration by whole-tissue weight analysis (Gerson & Pode, 1972), which agreed with an independent Cl\(^{-}\) electrode measurement. To allow comparison of these findings with our data, we have assumed a cytoplasmic water content of about 90% in our root innermost cortex cells, which were grown in distilled water. From this assumption we estimate that concentrations of about 10 mm-Na\(^{+}\) and 4–5 mm-Cl\(^{-}\) exist in the cytoplasm. All of these ion concentration data would appear to be in reasonable agreement if we assume that the Na\(^{+}\) and Cl\(^{-}\) concentrations in the cytoplasm are increased somewhat, as the NaCl concentration on the exterior of the root was increased in these non-halophytes.

**Elemental concentrations in the wall of meristematic cells**

The extracellular plant cell wall, in comparison to the intracellular regions of the plant cell, had lower concentrations of P but higher concentrations of S, K\(^{+}\) and Ca\(^{2+}\). Logically one may ask if these elements are in a free or bound state. In the case of cell wall, much of the Ca\(^{2+}\) might be expected to be bound to newly deposited pectin molecules and this portion of the Ca\(^{2+}\) would not therefore be free (Alberts et al. 1983; Roland & Vian, 1979). It also seems possible that at least some of the K\(^{+}\) in the cell wall is bound to anionic charge groups and in this regard logical candidates would be sulphate, accounting for the high concentration of S, and carboxyl groups, while the low concentration of P in the cell wall suggests that phosphate groups are less likely candidates for binding of the cations.

Because of the large differences in elemental concentrations observed between the cell wall and the intracellular compartments in the case of K\(^{+}\) and Ca\(^{2+}\), one can appreciate why estimates of the intracellular concentrations of these ions obtained from tissue analysis might be the subject of some criticism.

**Comparison of ion and elemental concentrations in onion meristematic root cells and mouse cardiac myocyte**

One is struck by several of the large differences in elemental concentrations between the intracellular environment of the plant cell and that of the animal cell in this study. The most striking differences are the fourfold higher concentrations of Na\(^{+}\) and Cl\(^{-}\) in the animal cell. Let us briefly examine the extracellular conditions of the plant and animal cells that may be related to these differences. The present study shows that the green onion plant will grow roots when its bulb is immersed in deionized distilled water, whereas cells in the mammalian body require a relatively constant and complex extracellular environment of ions and nutrients for their continued survival. When mammalian cells are exposed to deionized distilled water they swell and rupture, while the cells in the onion root tip survive and thrive under the same deionized distilled water conditions. The mechanism preventing the onion root tip cell from swelling and rupturing is the rigid cellular wall, which is able to withstand a relatively high hydrostatic pressure, and this rigid wall apparently allows the cell to adapt and survive in such an osmotic environment.
As discussed above, the intracellular concentrations of Na\(^+\) and Cl\(^-\) in root cells appear to be determined, in part at least, by the concentrations of these ions in the exterior environment of the root (Davis & Jaworski, 1979; Jaschke, 1979; Jaschke & Stelter, 1976). Thus, the fact that we grew the onion roots in deionized distilled water may help explain their lower intracellular concentrations of Na\(^+\) and Cl\(^-\) in relation to previously reported concentrations of these ions in the roots of plants that were maintained in an environment that had somewhat higher concentrations of Na\(^+\) and Cl\(^-\).

The cardiac myocyte in situ is in an environment where the extracellular Na\(^+\) concentration is several times higher than its intracellular Na\(^+\) concentration. One might therefore ask how it is that the Na\(^+\) concentration does not run down its electrochemical gradient and move into the cell. The fact that the Na\(^+\) concentration in the animal cell does not equilibrate with the extracellular environment has most frequently been attributed to a Na\(^+\),K\(^+\)-ATPase molecular 'pump' in the plasma membrane, which pumps K\(^+\) into the cell and Na\(^+\) out of the cell. As no such pump has been found in the plasma membrane of higher plant cells, one may ask if the intracellular Na\(^+\) concentration of plant cells is simply a reflection of the extracellular Na\(^+\) concentration, or if higher plants (especially those that grow or tolerate high-salt environments, i.e. halophytes) have adopted other mechanisms for Na\(^+\) exclusion (Flowers, Troke & Yeo, 1977). Recent reviews do indeed indicate that halophyte plants have mechanisms for maintaining relatively low concentrations of Na\(^+\) in their cytoplasm without the need for a plasma membrane Na\(^+\),K\(^+\)-ATPase (Flowers et al. 1977; Greenway & Munns, 1980; Harvey, Hall, Flowers & Kent, 1981; Harvey, 1983). Indeed Harvey et al. (1981) have already done electron-probe X-ray microanalysis of ion concentrations in freeze-substituted dry sections to obtain estimates of Na\(^+\), K\(^+\) and Cl\(^-\) concentrations in the cytoplasm, cell wall and vacuoles of leaf mesophyll cells of the halophyte, Suaeda maritima; she makes clear the importance of compatible synthesized solutes, such as glycinebetaine, as osmotic components in the maintenance of subcellular gradients of inorganic ions.

Questions concerning the mechanism(s) of salt tolerance and other questions concerning plant physiology are of both academic and economic importance. The electron-probe EDS methods presented herein provide an important tool to help clarify such questions.

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REFERENCES


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