Stomatal Opening Quantitatively Related to Potassium Transport

EVIDENCE FROM ELECTRON PROBE ANALYSIS

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ABSTRACT

When stomata of *Vicia faba* opened (from a stomatal aperture of about 2 micrometers to one of 12 micrometers) the solute content of the guard cells increased by $4.8 \times 10^{-12}$ osmoles per stoma. During the same time an average of $4.0 \times 10^{-12}$ gram equivalents of $\text{K}^+$ were transported into each pair of guard cells. This amount of $\text{K}^+$, if associated with dibasic anions, is sufficient to produce the changes in guard cell volume and osmotic pressure associated with stomatal opening. Analysis of Cl, P, and S showed that these elements were not transported in significant amounts during stomatal opening. This finding suggests that the anions balancing $\text{K}^+$ were predominantly organic. $\text{K}^+$ was specifically required because no other elements, likely to be present as cations, were found to accumulate in appreciable quantities in guard cells of open stomata.

Unfortunately their procedure contains errors; therefore we are presenting, together with our other results, details of our measuring and calibration technique.

The electron probe microanalyzer can be used for the analysis of many elements, not just K. Thus it provides a means of testing whether the specificity of the stomatal system for K, which was established by Humble and Hsiao (12) in isolated epidermal strips, occurs in intact plants. Accordingly we have measured not only K but also other elements which are likely to be present in quantity as cations in soils and plants, namely Na, Ca, and Mg. We also measured Cl, S, and P to determine which inorganic anions, if any, accompanied the cations taken up during stomatal opening.

MATERIALS AND METHODS

Growth of Plant Material. *Vicia faba* (var. Long Pod) plants were grown in a potting mixture for 3 to 4 weeks in a growth chamber with a daily light period of 16 hr and an irradiance of 8.5 mw cm$^{-2}$ from fluorescent tubes. The temperature was 27°C during the day and 23°C at night, and the relative humidity was about 85%, day and night.

Preparation of Plant Material. Leaves were always taken from the growth chambers about 6 hr after the beginning of the light period so that any possible effects of a diurnal rhythm or previous light treatments on stomatal behavior were the same in all experiments. One of the top four fully expanded leaves was removed from the plant and cut into pieces of about 1 cm $\times$ 2 cm. These sections were floated upper surface down on distilled water for 15 min in the dark. Virtually all the stomata were closed at the end of this treatment. After the dark treatment half the leaf pieces were illuminated for 3 hr by General Electric mercury lamps (type H400 RDX 33-1) producing 8.5 mw cm$^{-2}$ below a 5-cm water filter. The temperature during the light treatment was 25 to 28°C and during the dark it was 23 to 24°C.

Epidermal strips (approximately 3 mm $\times$ 5 mm) were taken from the lower leaf surface at the end of both the light and dark treatments. The strips were pulled off rapidly at an obtuse angle to the exposed mesophyll so that at least 80% of the ordinary epidermal cells were ruptured while the guard cells remained intact (3). The sets of epidermal strips taken after the light and dark treatments were each divided into three groups immediately after removal. One group was used for measurements of the guard cell volume, the second group was used for determination of guard cell osmotic pressure, and the third group of strips was prepared for electron probe analysis.

Volume Measurements. The depth of guard cells was measured by focusing a microscope first on the external cuticular ledges of the stomata and then on the bottom of the guard cells where they met with their neighboring epidermal cells.

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The distance between the two planes of focus was computed from the readings of the micrometer screw of the microscope. Simultaneously a microphotograph was taken of each stomata thus measured. The projected area and width of each stomatal apparatus were determined on an enlargement of the photograph. These measurements were used to compute the guard cell volumes under the assumption of elliptical cross sections for these cells. These values were reduced by 22% in the case of open stomata and 32% in the case of closed ones to correct for deviations from the idealized shape and the volumes occupied by the cell walls. The volumes of the guard cells from the light were further reduced by 2.4% to account for the volumes of the nuclei and by 8% to account for the space occupied by the chloroplasts. The corresponding corrections applied to those from the dark were 3.5 and 12%. These corrections were obtained from measurements on serial sections of guard cells (K. Raschke and M. R. Dickerson, unpublished data). "Guard cell volumes" calculated according to the procedure just described constitute estimates of the combined cytoplasmic and vacuolar space of a stomatal apparatus consisting of two guard cells.

**Osmotic Pressure Measurements.** Epidermal strips were floated cuticle up for 30 min on sucrose solutions, as suggested by Crafts et al. (2) and Fischer (3), at 0.1 M concentration intervals. Then the guard cells were examined for plasmolysis under the microscope.

![Graph](image)

**Fig. 1.** Probability plot of guard cells plasmolyzed after 30 min on graded sucrose solutions. Open symbols: open stomata from light treatment; closed symbols: closed stomata from dark. Each point is the mean of determinations on at least 40 stomata from two or more epidermal strips. Curves are least squares fits to the data.

**Electron Probe Microanalyzer Measurements.** Broken cell contents were removed from the epidermal strips by an initial rinsing and exchangeable ions were then desorbed from the free space by floating the strips for 3 min on ice-cold 50 mM Ca(NO₃)₂. Excess Ca²⁺ was removed by a final rinse in 0.1 mM Ca(NO₃)₂. In earlier investigations CaCl₂ had been used for this purpose (13). We chose Ca(NO₃)₂ instead to avoid contamination with Cl which would have invalidated measurements of the Cl content of the guard cells. The washed strips were placed cuticle up on pure silica slides (Amersil Inc., Hillside, N. J.), blotted into position, and rapidly frozen by dropping the slides into liquid N₂. Preliminary studies showed that there was no advantage in using isopentane as a freezing medium. No adhesive was required to hold the strips onto the slides during freezing or during the subsequent freeze-drying. Neither the desorption nor the freeze-drying had any effect on stomatal apertures. The freeze-dried strips were given a very thin carbon film coating in a Varian model VEIO vacuum evaporator. This prevented charging by the electron beam in the microanalyzer.

The strips were examined in an Applied Research Laboratories model EMX-SM electron probe microanalyzer.

**Electron Probe Microanalyzer Calibration.** One of the greatest difficulties in the absolute calibration of an electron probe microanalyzer is the preparation of adequate standards because of the very small size required. In this work it was found that the standards had to contain in the order of 10⁻⁶ g of the elements studied. We finally chose crystals as standards because they are chemically homogeneous and regularly shaped and could be produced in the very small but measurable sizes required.

Crystals of potassium oxalate, potassium chloride, and sodium chloride were grown on pure silica microscope slides as there was a minimal contamination of the elements under study in this supporting medium. Silica was used rather than a pure metal or carbon support because it allowed the crystals to be examined with a transmitted light microscope. The crystals were shaded with carbon from a point source at a known angle to the slide surface in a Bendix-Balzers high vacuum freeze etch unit. This resulted in shadows of the crystals as non-carbon coated areas. Photomicrograph negatives of the crystals, and of a stage micrometer were projected on a screen so that the surface area of the crystals could be measured directly by comparison with the micrometer. The lengths of the shadows formed by the carbon coating were also measured and the crystal depths were calculated by trigonometry. The weight of each element in the individual crystals was then calculated from published densities and compositions (28).

The instrument was operated at a beam voltage of 20 keV and sample current of 0.02 microampere as measured on the silica slide. The x-rays resulting from excitation of K shell electrons, that is, the Kα x-rays, were used for measurement in all cases. For obtaining absolute quantitative results the shape of the electron beam cross section at the sample was made into a 50-× 0.5-μm line whereas for relative data the conventional method of a 0.5-μm-diameter flying spot beam was used. Samples were scanned by moving the line 40 μm at right angles to its length in 10 sec. The 50-× 40-μm rectangle scanned just encompasses a single stomatal apparatus of *V. faba*.

The crystals and the sample stomata were scanned in the same run and under exactly the same conditions because it was found that the calibration curve was not constant from day to day due to insufficient reproducibility of the microanalyzer adjustments. Background measurements were made for the crystals by scanning a clear area of the slide and for the epidermal samples by scanning an area clear of stomata.
The electron probe microanalyzer technique is discussed in detail in the "Appendix."

RESULTS

Volume Changes in Guard Cells. Volumes of guard cells were measured in 10 open and 10 closed stomata, all from the same leaf as the one from which the epidermal samples were prepared for the electron probe. The average guard cell volumes (as defined in "Materials and Methods") were $4.8 \times 10^{-18}$ liters per stomatal apparatus in stomata from the light and $2.6 \times 10^{-18}$ liters for the ones from the dark.

Changes in Osmotic Pressure. Figure 1 is a plot of the percentages of guard cells plasmolyzed by sucrose solutions of various concentrations. The osmotic pressures of the solutions are those given by Stadelmann (27). By convention, incipient plasmolysis is assumed to occur if 50% of the cells are plasmolyzed. In Figure 1 the osmotic pressure is 19 bars for the plasmolyzed guard cells of closed stomata and 35 bars for open stomata and so the change in osmotic pressure with opening was 16 bars. In a replicate of the experiment the difference was 15 bars although the osmotic pressures in both the closed and open states were smaller, at 16 and 31 bars, respectively.

Generally there was greater variation in the plasmolysis results of guard cells from the light treatment than from the dark.

Relative K Contents of Guard Cells. The relative amounts of K in representative open and closed stomata are shown in Figure 2. The photographs showing the stomata are secondary electron images from the electron probe and the corresponding distribution and concentration of K in these stomata are shown by the scintillation photographs below. The guard cells of the open stoma can be seen to contain much more K than those of the closed stoma. The contents of most of the epidermal cells surrounding the guard cells were removed during preparation of the epidermal strips and virtually no K can be seen there. The concentrations of K near the closed stoma are in mesophyll residues which can be seen in the secondary electron image. Sawhney and Zelitch (26) have published similar photographs of tobacco stomatal apparatuses.

Profiles across Guard Cells of K, Cl, and P. A more accurate measure of the distribution and amounts of elements was obtained by graphically recording the x-rays produced during the movement of a circular electron beam 0.5 um in diameter across the samples. Figure 3 presents results of such scans made across the stomata shown in Figure 2. The K x-ray counts are reduced by a factor of 3 compared to those of Cl.

Fig. 2. Secondary electron images (upper) and corresponding K x-ray images (lower) of (a) closed and (b) open V. faba stomata. The concentration and distribution of K are shown in the lower photographs by the white spots. Beam voltage, 20 kev; current on silica slide, 0.02 microampere. Exposure time for x-ray images, 360 sec.
Fig. 3. Profiles of relative amounts of K, Cl, and P across an open and a closed stoma. The traces are the result of scanning a 0.5-μm-diameter beam across the stomata shown diagrammatically below the traces. In order to indicate the profile scanned, the images of the stomata have been cut off in this diagram where the beam crossed the guard cells. These are the same stomata as those shown in Figure 2. Beam voltage, 20 kev; current on silica slide, 0.02 microampere.

Fig. 4. Examples of calibration curves obtained with potassium chloride and potassium oxalate crystals. Each point is an average of three scans of the same crystals or group of crystals. The lines are least squares fits to the data. A beam with a cross section of 0.5 × 50-μm was swept over a distance of 40 μm in 10 sec for each scan. Beam voltage, 20 kev; current, 0.02 microampere on silica slide.

and P. Relative quantitative evaluations can be made for any particular element from these scans but, of course, comparisons can only be made between elements after application of calibration factors. Thus the K contents of guard cells of the open and closed stomata can be compared. As well as more K, there is more Cl in the open stoma than in the closed ones but the differences are not as great. The P contents, on the other hand, are almost the same. Comparison of the traces and stomata indicates, as might be expected, that the P peaks coincide with the nuclei.

Calibration of the Electron Probe Microanalyzer. Figure 4 is a K calibration curve made from crystals under conditions identical with the ones used for scanning stomata. Crystals of potassium oxalate were measured as well as those of potassium chloride because Cl is known to absorb the Kα x-rays from K (10). The slope of the potassium chloride curve decreased more than that of potassium oxalate at high K levels, presumably as a result of absorption by Cl.

K, Na, and Cl Content of Guard Cells. The 50-μm long beam was used to obtain the average counts from K, Na, Cl, and S in open and closed stomata from the same leaf (Table I). The standard deviations were large because the gross sample counts were often of equal magnitude with those of the background.

There were significant differences between open and closed stomata at the 5% level of the t test for both K and Na but not for Cl. Although the differences in Na were statistically significant, the counts obtained, in contrast to those for K, were very low and very close to the background level. There were not sufficient data on S to test for significance of the results.
DISCUSSION

The electron probe microanalyzer can be used to measure kinds and quantities of elements but normally not their bonding. However, in plants K does not occur in nonionic form and so we assume that it was present as K\(^+\).

Thus our application of the electron probe has produced further, more detailed evidence supporting the previous contentions (4, 26) that with stomatal opening K\(^+\) moves into the guard cells in amounts sufficient to account for the changes in osmotic pressure and that movements of Cl, P, and S are not important in stomatal opening. This work also confirms that K\(^+\) is the specific ion involved in stomatal opening in intact V. juba leaves as was found in isolated epidermal strips by Humble and Hsiao (12). Although a statistically significant difference was found between the Na\(^+\) x-ray counts of open and closed stomata (Table I) the total amount present was less than the resolution of the calibration (Table II). Willmer and Mansfield (29) found that Na\(^+\) had a slightly greater effect than K\(^+\) in stimulating stomatal opening in detached epidermis of Commmelina communis and so K\(^+\) may not be specific for all plants. However Pallaghys (23) has observed that the specificity of cations for light-dependent stomatal opening in isolated epidermal strips of V. juba may be controlled by Ca\(^+\). Stomata opened on both 10 mm KCl and 10 mm NaCl in light when Ca\(^+\) was absent but only on 10 mm KCl when 1 mm CaCl\(_2\) was added. Willmer and Mansfield did not include Ca\(^+\) in their experiments.

The other cations which might be present in sufficient quantities to be responsible for opening, Mg\(^+\) and Ca\(^+\), were not measured but one of us (K. K.) in a preliminary electron probe study, did not find any difference in Ca\(^+\) or Mg\(^+\) between the guard cells of open and closed Zea stomata. Also, neither ion produced opening in isolated epidermal strips of V. juba (4, 12) or C. communis (8).

The K\(^+\) uptake into the guard cells was apparently not balanced by concurrent uptake of inorganic anions. The only three elements likely to be present in quantity as anion constituents, Cl, P, and S, were measured with the electron probe. There was not a statistically significant difference between the Cl content of the guard cells of open and closed stomata (Table I). In one replicate, however, there was a significant difference but the amount present was only a small fraction of the K present. A difference in Cl content can also be seen in Figure 3 but again the total content is small in comparison to K\(^+\). There was little apparent difference in S content, nor was measurable S present (Table I). In Figure 3 the P level is approximately the same in both open and closed stomata. This element appears to be concentrated in the nuclei. Satter et al. (25) found that in Albizzia pulvini, which cause the leaflet pairs to open and close by K transport, the P level remained so constant that it could be used as a relative standard in the electron probe analysis of K\(^+\) fluxes. Thus it is unlikely that P movement is important in balancing K\(^+\) in turgor mechanisms.

Carbonate and bicarbonate were not measured as this is, of course, not possible with the electron probe when an organic matrix is present. Jacobson and Ordin (16) found that little HCO\(_3^-\) but much K\(^+\) was taken up from KHCO\(_3\) by barley roots. Ionic balance was maintained by production of organic acids, particularly malic acid. Also, Jackson and Adams (15) have concluded that K\(^+\) absorption rates of barley roots are independent of the identities, concentrations, and rates of absorption of the associated anions, including bicarbonate. However, it has been postulated (11), from the indirect evidence of potential differences, that in Chara australis an active pump accumulates bicarbonate ions and that K\(^+\) follows passively. So bicarbonate cannot be ruled out although its involvement seems unlikely. The possibility of OH\(^-\) balancing K\(^+\) is remote because if this were the case the pH of the guard cells would rise during stomatal opening to values between 13 and 14 which, of course, have never been observed. If no anions were produced in the guard cells or imported during the accumulation of K\(^+\) the membrane potential of open stomata would be of the order of 100 V. This seems unlikely.

Presumably the excess positive charge produced by K\(^+\) import during stomatal opening is balanced largely by organic acid anions. These may be imported from other parts of the leaf or, more likely, produced in the guard cells. It is possible that the starch breakdown which normally takes place when stomata open may supply anions. It is interesting that Mott and Steward's concept (21) of the initial stages of cell growth involves a similar cation-anion relationship. They found that there was an uptake of K\(^+\) balanced by an "internal secretion" of organic anions. Potassium was the preferred cation and was not accompanied by inorganic anions.

Table I. The Average Number of Counts per Scan (with Standard Deviations) from Various Elements in Open and Closed Stomata from the Same Leaf

In most cases each number is the mean of 3 scans, each of 10 stomata.

<table>
<thead>
<tr>
<th>Stomata</th>
<th>Average Net Counts per 10-sec Scan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K</td>
</tr>
<tr>
<td>Open</td>
<td>1954 ± 552</td>
</tr>
<tr>
<td>Closed</td>
<td>679 ± 76</td>
</tr>
</tbody>
</table>

1 Significant differences, at 5\% level by the t test, between open and closed stomata.

2 Data insufficient for a statistical test.

Table II. The Amounts of Various Elements, Measured with the Electron Probe, in Open and Closed Guard Cells, the Guard Cell Volumes, and the Changes in Stomatal Aperture and Osmotic Pressure

<table>
<thead>
<tr>
<th>Amounts of Elements per Stoma as Measured by Electron Probe</th>
<th>Stomatal Aperture</th>
<th>Guard Cell Volume per Stoma</th>
<th>Guard Cell Osmotic Pressure from Incipient Plasmolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>Na</td>
<td>Cl</td>
<td>µm</td>
</tr>
<tr>
<td>-------------------</td>
<td>-----------------</td>
<td>----------------</td>
<td>----------</td>
</tr>
<tr>
<td>Open stomata</td>
<td>424</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>Closed stomata</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Difference between open and closed stomata</td>
<td>404</td>
<td>0</td>
<td>22</td>
</tr>
</tbody>
</table>

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Table III. The Calculated Amount of Osmotica Required to Produce the Stomatal Opening and the Measured Change in K using Data from Table II

The osmotic pressures of the K salts were determined from cryoscopic data.

<table>
<thead>
<tr>
<th>Osmoles required to increase guard cell volume and osmotic pressure for stomatal opening</th>
<th>480 × 10^{-14}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount of K (g eq) required in various salts to give 480 × 10^{-14} osmotes</td>
<td></td>
</tr>
<tr>
<td>KCl</td>
<td>270 × 10^{-11}</td>
</tr>
<tr>
<td>K malate</td>
<td>360 × 10^{-11}</td>
</tr>
<tr>
<td>K citrate</td>
<td>540 × 10^{-11}</td>
</tr>
<tr>
<td>Measured change in K with opening (g eq)</td>
<td>400 × 10^{-11}</td>
</tr>
</tbody>
</table>

In preliminary tests it became clear that a 30-min period was necessary for completion of plasmolysis, although this is many times longer than the expected half-time for the exchange of water of a plant cell. Similar times have been used previously (2, 4). The data were plotted on probability paper because they were in the form of frequencies of occurrence expressed as percentages. The nonlinearity of the plots indicates that the results obtained were not distributed normally. Also, the method may not have been sufficiently precise, although recent determinations of guard cell osmotic pressure (18) have been made by almost exactly the same experimental method but with more closely spaced concentrations than those used here. We did not find any difference between the results obtained with sucrose or polyethylene glycol 600 solutions.

There is some uncertainty about the guard cell volumes used, not only from the method of calculation, but also about which compartments should be included in the solution-filled intracellular space. It appears from Figure 2 that K+ is distributed throughout the open guard cells but it is difficult to tell the exact distribution without examining thin sections with the electron probe. Observations with a light microscope suggested that in open guard cells most of the volume was vacuole with only a very thin layer of protoplasm around the walls. We have simply assumed that the only parts of the intracellular space not participating in the turgor regulation of the guard cells were the chloroplasts and the nucleus.

Data from the electron probe, osmotic pressure, and volume measurements are summarized in Table II. From these data it can be estimated that the K+ concentration was very high, at least 0.9 eq liter⁻¹, in the guard cells from open stomata. The changes in osmotic pressure and volume were used to calculate the amount of osmotica required to produce opening under the assumption that the basic level of osmotic motor did not change during opening. The osmotes needed are listed in Table III together with the amount of K+ required, if in various salts, to equal the osmotes. This table shows that the amounts of K+ absorbed by the guard cells during opening were sufficient to account for opening if the K+ was associated with a divalent organic anion such as malate. The osmotic pressure in closed stomata appears to be produced largely by organic compounds.

Although the K+ concentrations measured in open stomata may seem very high at first sight, the rates of K+ uptake producing them are similar to those estimated for guard cells by Fischer and Hsiao (4). They are, however, higher than those found previously for leaf tissue. The rate for guard cells, averaged over the 3-hr light period, is approximately 200 μmoles g⁻¹ hr⁻¹ whereas Rains (24) found 3 μmoles g⁻¹ hr⁻¹ for sliced corn leaf and Nobel (22) found 10 μmoles g⁻¹ hr⁻¹ for pea leaf fragments. However, the leaf pieces would contain nonabsorbing tissue and also the very specialized guard cells might be expected to have higher rates than the rest of the leaf.

It should be noted that if the precursor of the anion is already in the guard cells, the problem of whether K+ uptake or anion production initiates opening requires further study.

APPENDIX

Electron Probe Microanalyzer Technique. Since the electron probe has been little used for quantitative studies in biology and this work includes a calibration, details of our techniques will be discussed here, separately from the results.

Development of the method was necessary because the ARL instrument available had no provision for measuring white radiation; therefore Hall's method (9) for biological material could not be used. The method of Sawhney and Zelitch (26) was not suitable as it appears to have errors. Their standards were KClempreggeln filters of approximately the same density as the freeze-dried epidermal strips studied. Assuming the density to have been between 0.4 and 0.5 the 20-kev beam that they used would have penetrated between 19 and 24 μm (1). Thus, the beam would presumably have passed completely through the freeze-dried guard cells but not through the 89-μm thick Gelman filters. Therefore Sawhney and Zelitch probably underestimated the amounts of K+ present in guard cells.

The basis of our technique is the use of (a) a line-shaped electron beam for scanning and (b) crystals for calibration standards. One criticism that could be made against the use of crystals is that there are differences between the standards and the samples of plant material. The crystals were of uniform thickness and presented smooth surfaces at right angles to the electron beam, whereas the freeze-dried epidermal strips to be measured were not uniform in thickness nor did they have a plane surface. In addition, the strips had an organic matrix. However, the attenuation of electrons having a kinetic energy of 20 kev was computed to have been approximately 4 kev in the case of the thickest crystal used for calibration (thickness, 2.2 μm; density, 2.0 g cm⁻³) and approximately 2 kev in the epidermal samples (maximal thickness, 5.5 μm; density, 0.4–0.5 g cm⁻³). Therefore, neither in the standards nor in the epidermal samples were the electrons decelerated below the critical excitation potential for the production of the Kα x-rays. The highest critical excitation potential of the elements measured was that of the Kα radiation of K (3.606 kev [17]; for a further discussion of the relative intensities of x-ray emission from samples and standards see Ref. 1). Complete penetration of both crystals and guard cells was confirmed by observations of excitation by the electron beam of the silicon of the supporting slide beneath the crystals and strips. Therefore the beam integrated totally for K in both the crystals and guard cells. Absorption of the emerging x-rays by corruptions of the strips' surface was minimized by the high take-off angle (32.5°) of the ARL model SM-X instrument. Variations in topography of the surface of the epidermis could have been removed by embedding and sectioning but there would probably have been a loss of K during embedding (1). Sectioning also would have necessitated estimation of the total K of the guard cell from a thin section, and so the errors introduced would probably have been greater than those resulting from the corruptions of the samples.

The amounts of K, Cl, and Na in the guard cells were determined directly from calibration curves, similar to the one shown in Figure 4. The standards for K used, potassium chloride and potassium oxalate, should have been representative of the K in the guard cells because the absorption of Kα x-rays of K by Cl was corrected for the amount of Cl present by use.
of the KCl curve. The remainder of the K was calculated from the potassium oxalate curve. Sawhney and Zelitch (26) did not allow for the absorption of the Kα radiation of K by Cl in their calibration. We made no other corrections for absorption by the organic matrix since the two heaviest main constituents, C and O, have very low mass absorption coefficients for x-rays emitted by K and Cl (10).

The line form beam has a number of advantages for quantitative work. The integration of counts over an area of variable concentration, such as a guard cell, is made possible and so a line gives higher precision than a moving or stationary spot. Also, the moving line is superior to a small stationary spot in that it produces less heat damage in the samples and is superior to a large spot because it gave greater x-ray production per unit of time. Sawhney and Zelitch (26) report that the 1- to 2-μm-diameter beam they used noticeably decreased the K content of plant tissue by volatilization if exposures were longer than 50 sec. We did not find measurable differences in x-ray production after repeated scannings of our samples by the line beam. Similarly, we found that crystals of potassium oxalate were disintegrated by continued exposure to a 0.5-μm-long beam. The differences in size between the crystals and the 50-μm-long electron beam did not seem to introduce any error because when two small crystals were scanned at the same time the results still fell on the same calibration curve. One point in Figure 4 was obtained in this way.

The minimal amount of an element detectable by the instrument used approaches 10⁻¹⁸ g. The results show that this sensitivity was quite adequate for our investigation.

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LITERATURE CITED