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Potassium Content and Aperture in "Intact" Stomatal and Epidermal Cells of *Commelina communis* L.

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Summary. Measurements of potassium activity with a potassium-sensitive microelectrode have been made in the cells of the stomatal complex, and in epidermal cells, of *Commelina communis* L., as a function of stomatal aperture. The estimated osmotic effects of the changing accumulation of potassium salts in the guard cell have been compared with the previous estimates of the osmotic changes required to open/close the pore. The results suggest that a significant fraction of the osmotic pressure of the guard cells, particularly when closed, is contributed by solutes other than potassium salts. The degree of potassium accumulation may determine the aperture of wide-open stomata, but the potassium changes in the early stages of opening are much too small to account for the osmotic changes required. The difference in potassium contents of "intact" and "isolated" guard cells is close to that required to overcome the previously estimated effect of subsidiary cell turgor on the water relations of the guard cell. In some tissue (but not in all) much more K is lost from epidermal cells than appears in other cells of the complex as the stomata open, and extracellular storage would be required.

Opening of stomatal pores is associated with considerable accumulations of potassium salts, and it is generally accepted that such accumulation is responsible for the turgor changes leading to changes in aperture. However, quantitative estimates of potassium concentrations in guard cells are available for only a few species and are incomplete. There is a need for a detailed study of potassium changes over the whole range of aperture, rather than simply in the open and closed states, and for comparison of such figures with the estimates of the osmotic changes required to change the aperture.

The work reported in this paper forms part of a study of the stomata of *Commelina communis* L., in an attempt to establish the nature of the changes in the ionic state of the guard cell associated with changes in aperture; such a description should include not only concentration figures but also flux and potential data, and this represents the overall aim of the project. The first aim was to measure concentration changes over a range of apertures, in cells in "intact" detached epidermal strips in which guard cells, subsidiary and epidermal cells are all alive, and in "isolated" guard cells, in strips in which all cells other than guard cells have been killed by a treatment at low pH (Squire & Mansfield, 1972). Measurements of the osmotic changes required to open/close the pore in both "intact" and "isolated" epidermal strips were reported in a previous paper (MacRobbie, 1980), and a second paper (MacRobbie & Lettau, 1980) compared these figures with estimates of the changes in ion content in "isolated" guard cells. In "isolated" guard cells potassium may be estimated by the use of ion-sensitive microelectrodes or by tracer measurements, and the agreement between the two methods was reasonable. The conclusion was that for "isolated" guard cells, opened by floating on KCl, potassium changes are too small to account for the changes in aperture, and some other process must also be involved.

In the present paper measurements of potassium activity (K_{ac}) in the cells of "intact" detached epidermal strips are reported, made by the insertion of a double-barrelled K-sensitive electrode, with Corning potassium liquid ion-exchanger (477317) in one barrel. Estimates of the osmotic contribution of the measured changes in potassium salts are then made and compared with the osmotic changes required. The conclusion is that, although potassium accumulation determines the aperture of wide-open stomata, the potassium changes in the early stages of opening are

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too small to account for the osmotic changes, and again some other process must also be involved.

Materials and Methods

Methods have been described in the previous paper (MacRobbie & Lettau, 1980). Plants of *Commelina communis* L., about 6 weeks old, were used; leaves were left for about 3 hr in light or dark, on wet filter paper in a closed petri dish, to open or close the stomata. Epidermal strips were mounted mesophyll-side down on coarse plastic mesh for support. A Research Instruments TVC 500 micromanipulator was used for the insertion of the electrodes, observed at 320× magnification with a Dyson long working distance objective. Potassium activity (K_{ac}) was measured with double-barrelled K-sensitive electrodes, prepared as described by Zeuthen, Hiam, and Silver (1974), with Corning liquid ion exchanger (477317) in one barrel; the tips were about 1 μm. The electrode barrels were connected by means of chlorided silver wires to a solid state, unity gain voltage follower (input impedance $> 10^{10}$ Ω), designed by Mr. P.L. Joyce of the Physiological Laboratory, Cambridge. The output from this was registered on a Tekman recorder. A number of solutions were tried as filling solutions in the reference barrel and there was no evidence of large differences (3 and 0.1 M KCl, 1 and 0.1 M NH_4NO_3). Electrodes were calibrated KCl solutions before and after insertion in cells and had slopes of about 55 mV for a 10-fold change in K-activity over the concentration range 10 mM to 1 M. Results were only used if the calibration after insertion in the cell was comparable with that obtained before insertion. Readings were taken immediately after insertion, and were steady for several minutes. Prolonged readings were not attempted in this work, nor were simultaneous potential measurements recorded.

Results

Measurements of Potassium Activity

Potassium activity (K_{ac}) was measured in the cells of the stomatal complex, at different stomatal apertures (A). Figure 1 shows the arrangement of cells in the complex.

Two sets of measurements were done, on tissue grown under rather different conditions; set *II* tissue was grown with a capillary mat under the pots and therefore had a better water supply. As the results were rather different, they have been separated in the diagrams. Figure 2*A–B* shows the results of K_{ac} measurements in guard cells, epidermal cells, and terminal subsidiary cells for set *I*. As the stomata open large increases in K_{ac} in the guard cells and large decreases of K_{ac} in epidermal and terminal subsidiary cells are observed; in guard cells K_{ac} increases from about 70 mM when near closed, to 300–400 mM when open, while K_{ac} in epidermal cells and terminal subsidiary cells falls from 180–300 mM when near closed to about 80 mM when open. These are similar to the figures obtained by Penny and Bowling (1974), using a K-sensitive electrode based on a sodium cobaltinitrite crystal in the tip.

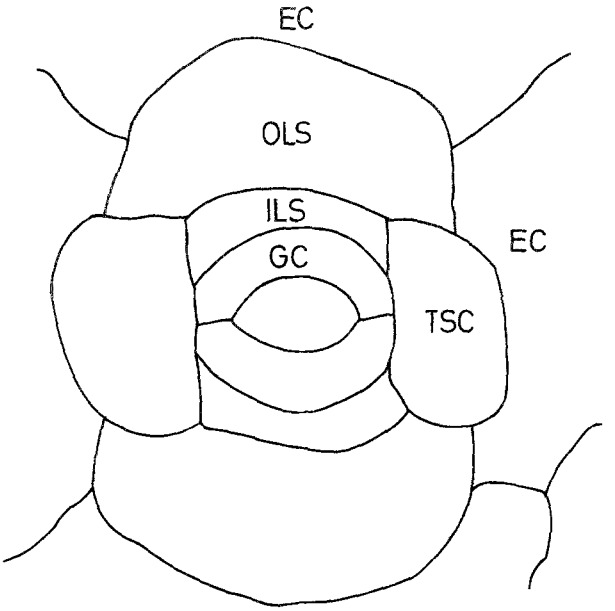

Fig. 1. Arrangement of cells in the stomatal complex of *Commelina*. *GC*, guard cell; *ILS*, inner lateral subsidiary cell; *OLS*, outer lateral subsidiary cell; *TSC*, terminal subsidiary cell; *EC*, epidermal cell

The results of the second set of measurements, set *II*, are shown in Fig. 3A–B. For guard cells the figures for K_{ac} in open stomata are rather lower than in set *I*, rising to only about 250 mM, but the main difference is that the values of K_{ac} in epidermal cells do not change with aperture, with an average of 66 ± 5 (14) mM below 2 μm and 73 ± 5 (7) μm in the range 8–11 μm.

The reason for the discrepancy is not clear, but is thought to be a genuine difference in the tissue, rather than an experimental error. In set *I* the reference barrel contained 3 M KCl, whereas in set *II* measurements were made with both 3 and 0.1 M KCl in the reference barrel; although there appeared to be more scatter in the results with 3 M KCl, there was no marked difference or indication that this was the cause of differences in the results. One possibility considered was that the proportion of potassium balanced by chloride rather than malate differed in the two series. For a given potassium concentration the electrode records a lower value of potassium activity when malate forms a higher proportion of the balancing anion than is recorded in the presence of only chloride, but the effects are not big enough to change the pattern of behavior.

There appeared to be little change with aperture of potassium activity in either inner lateral or outer lateral subsidiary cells. In outer lateral cells K_{ac} had a value of 105 ± 5 (9) mM. In inner lateral subsidiary

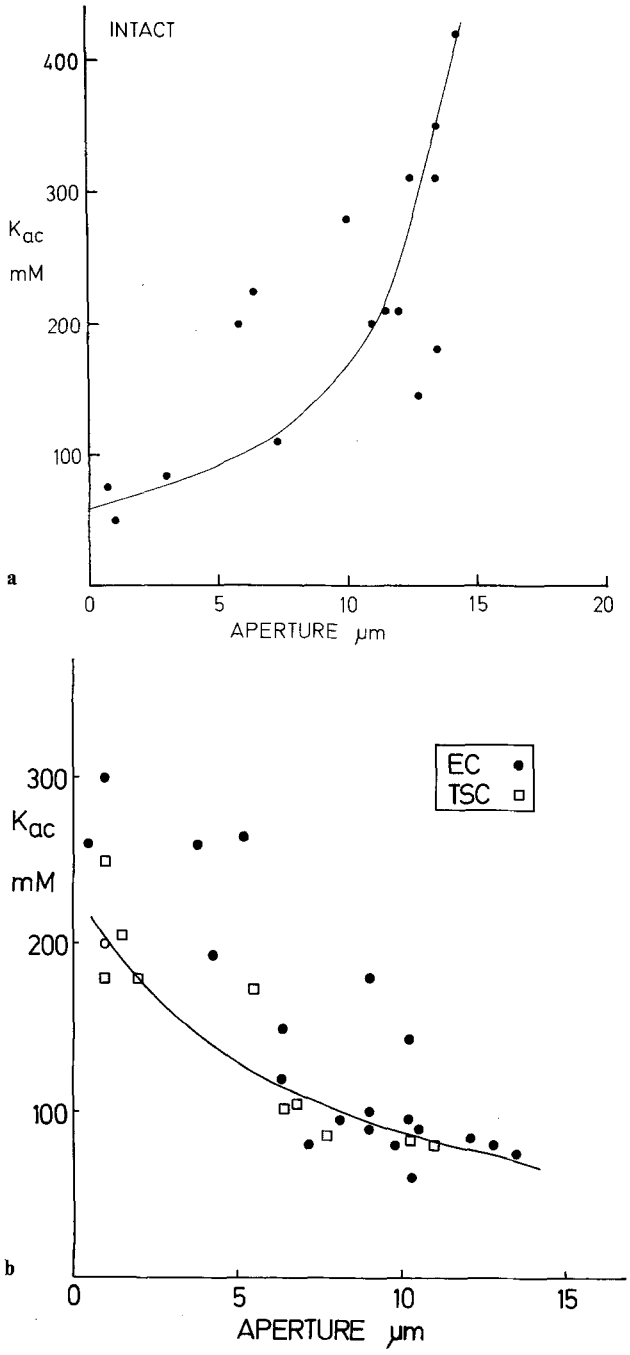

Fig. 2. Measurement of potassium in cells of "intact" epidermis; tissue, set *I*. (*A*): Guard cells. (*B*): ●, Epidermal cells; □, terminal subsidiary cells

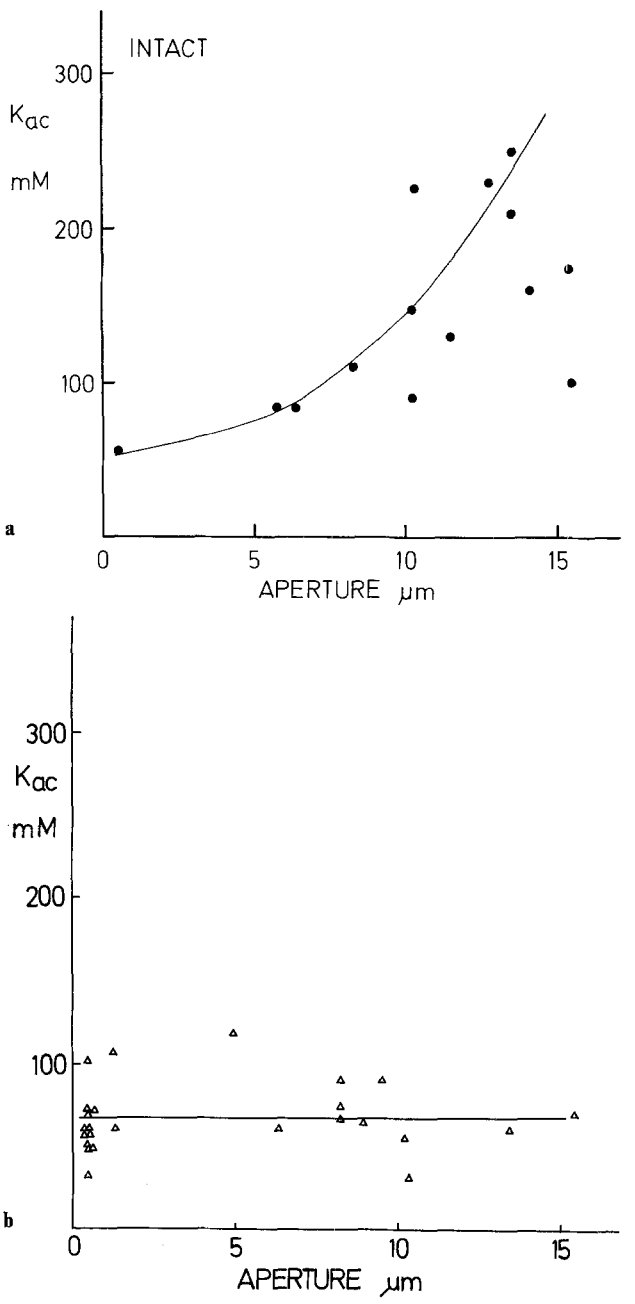

Fig. 3. Measurement of K_{ac} in cells of "intact" epidermis; tissue, set II. (*A*): Guard cells. (*B*): Epidermal cells

cells the mean value was 169 ± 16 (6) mM. In neither case was there a significant correlation with aperture.

Conversion of K_{ac} to Potassium Concentration (K_c): Estimation of the Osmotic Contribution

For the estimation of the osmotic contribution of the changes in potassium activity it was first assumed that the balancing anion was chloride, and the values of K_{ac} were converted to concentration (K_c) on this basis. The osmotic values of KCl solutions (π_{KCl}) were then used to estimate the contribution to the increasing osmotic pressure. In view of the preceding discussion of the effects of malate on K_{ac} measurements, this may seem a doubtful procedure, but in fact the presence of a proportion of malate as balancing anion

Table 1. Determination of K in extracts of epidermal tissue

Method	Pre-treatment	K content (nmol·mm^{-2})
A. Flame photometry (April)	Light	2.72±0.16 (14)
	Dark	2.62±0.25 (16)
B. Electrode (May)	Light	2.54±0.10 (17)
C. Flame photometry (Aug.)	Dark	2.96±0.24 (16)
D. Electrode (Aug.)	Light	3.97±0.33 (19)
		1.08±0.22 (16)
		3.25±0.31 (18)

Tissue A: The ratio of contents in paired samples of epidermis from the same leaf in light or dark before stripping was 1.08±0.11 (7).

Table 2. Potassium activity and aperture in "intact" guard cells

Aperture	K_{ac} (mM)	K_c (mM)	π_{KCl} (mosmol kg^{-1})	$d\pi_{KCl}/dA$ (mosmol kg^{-1} μm^{-1})
2	60	75	135	15
5	76	99	180	18
8	110	150	275	43
11	167	238	440	73
14	256	385	690	100

K_c and π_{KCl} are estimated from K_{ac} by assuming that the balancing anion is chloride. K_{ac} values are read off the smooth curve in Fig. 3*A*.

will produce two differences which tend to cancel each other; the use of the KCl calibration to calculate concentration in a mixture will underestimate K_c, but potassium malate will be osmotically less effective that KCl, and π_{KCl} will be an underestimate by about 25%; with two-thirds balanced by malate then the underestimate will be 50–60%. Thus an accurate comparison with the osmotic changes required will not be possible, but large discrepancies will be detectable.

As a further check on the electrode measurements, potassium activity was estimated in extracts of epidermal strips, and K_c was calculated on the basis of the KCl calibration. These figures are given in Table 1, together with flame photometric determinations of potassium in such extracts. The conclusion is that the potassium concentrations estimated from K_{ac} measurements are not seriously in error.

Table 2 shows the values for K_c, π_{KCl}, and the rate of change of π_{KCl} with aperture for set *II*, for comparison with osmotic measurements on tissue grown under the same conditions. The osmotic changes required, for opening of "intact" guard cells against their own cell walls and also the resistance to expansion of turgid subsidiary cells, were estimated as 116 mosmol kg^{-1}μm^{-1} below 10 μm, 170 mosmol kg^{-1}μm^{-1} in the range 10–15 μm, and 230 mosmol kg^{-1}μm^{-1} above 15 μm (MacRobbie, 1980). Even allowing for the uncertainty in the calculation of π_{KCl}, it is clear that in the early stages of opening, below about 10 μm aperture, the estimates of $d\pi_{KCl}/dA$ are much smaller than the osmotic changes required.

The scatter in points at intermediate apertures in Fig. 2*A*, for set *I*, makes it difficult to present similar calculations for this tissue. In nearly closed stomata K_c is about 90 mM, and π_{KCl} is about 160 mosmol kg^{-1}; at 13–14 μm K_c is about 500–600 mM and π_{KCl} is 900–1100 mosmol kg^{-1}. If the lower estimates of the intermediate values are taken, then the slope $d\pi_{KCl}/dA$ is much less than $d\pi/dA$ below 10 μm; if the highest estimate of K_{ac} at 6 μm is chosen, then the slope below 6 μm is high, but that above 6 μm is correspondingly reduced. Overall, the change in π_{KCl} is much less than the change in π required by the osmotic measurements.

In both sets therefore the conclusion is that, even allowing uncertainties in estimating osmotic changes from the activity measurements, the whole change in internal osmotic value cannot be attributed to the accumulation of potassium salts.

Amounts of Potassium in the Epidermis

An attempt was made to construct a balance sheet for the potassium changes within the cells of the epidermis. Cell volumes were estimated as described previously (MacRobbie, 1980) by microscopic observations. It was difficult to make accurate depth measurements, as the cells are not flat, and the values are therefore approximate – although no more so than previous estimates made. From such measurements the relation between the volume of a single guard cell (V, pl) and the stomatal aperture (A, μm) was obtained. For the purposes of calculation the relation used was $V=4.0+0.2A$. Each guard cell was associated with two epidermal cells, each of volume 56 pl, a terminal subsidiary cell of volume 9 pl, an outer lateral subsidiary cell of volume 19 pl, and an inner lateral subsidiary cell of volume 10.5 pl when the pore is closed, falling to 8.7 pl as the pore opens and the guard cell expands into, and compresses the inner lateral cell.

For set *I* the smooth curves drawn in Fig. 2*A–B* were used to construct a balance sheet. Figure 4 shows the results of these calculations giving the contents, in nmol mm^{-2} or in pmol (guard cell)$^{-1}$, for the total in the epidermis, for epidermal plus terminal subsidiary cells, and for the guard cell. The most

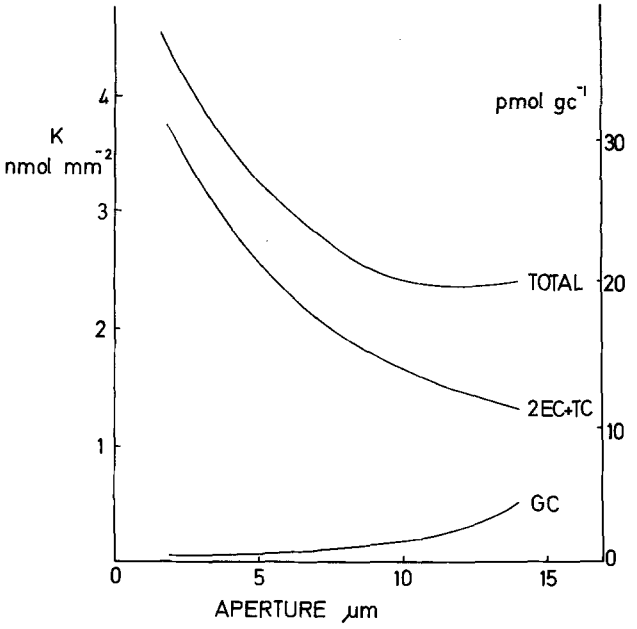

Fig. 4. Potassium content of epidermis, estimated from K_{ac} measurements on cell of epidermis, set *I*, as a function of aperture. K_{ac} converted to K_c by calibration in KCl. Total is $GC+ILS+OLS+TSC+2EC$. Volumes used: *EC*, 56 pl; *TSC*, 9 pl; *OLS*, 19 pl; *ILS*, 10.5 pl (closed) – 8.7 pl (open); *GC* given by V (pl) = $4.0+0.2\ A$ (μm). Frequency, 60 stomates · mm^{-2}.

striking feature is the very large drop in calculated content between closed and about 9 μm open; above 9 μm the loss from epidermal and terminal subsidiary cells is balanced by the gain of potassium by the guard cell. The method of conversion of K_{ac} to K_c means that the calculations must be approximate, but the large change in activity in the large epidermal cells as the stomata open means that, whatever activity coefficient is used for the conversion, there is much more potassium lost from the epidermal cells than appears in any other cells of the complex. As calculated, the figures suggest that opening is associated with a fall in K content in epidermal cells by 2.2 nmol mm^{-2}, and in terminal subsidiary cells by 0.18 nmol mm^{-2}; the K content in the guard cells rises by 0.45 nmol mm^{-2}. Even using the values of K_{ac} directly, with no correction for the activity coefficient, we would have a figure for the underestimated fall in the epidermal and terminal subsidiary cells of 1.6 nmol mm^{-2}, much larger than the increase in the guard cell.

By contrast, the results for set *II* in Fig. 3*A–B* would not show this marked discrepancy between the amount disappearing from epidermal cells and that appearing in other cells. In this instance the increase of 0.28 nmol mm^{-2} in the guard cells is comparable with the changes in subsidiary cells, and the epidermal cells showed little change. The total K content of the epidermis in set *II* is calculated as about 2.3 nmol mm^{-2}, which compares reasonably with the figures in Table 1 from flame photometry. The question of the difference between the two sets and the balance sheet in set *I* will be considered in the Discussion.

Table 3. Differences in potassium content in "intact" and "isolated" guard cells

A (μm)	π_{KCl} (mosmol kg^{-1})		$\Delta\pi_{KCl}$ (mosmol kg^{-1})	P_{gs}[b] (mosmol kg^{-1})
	Intact	Isolated[a]		
6	200	90	110	89
10	375	165	210	222
14	690	270	420	355

[a] The figure is taken from MacRobbie and Lettau (1980) and is the average of estimates from tracer and electrode measurements.
[b] P_{gs}, taken from MacRobbie (1980), is the measure of the contribution of the subsidiary cell turgor to the water relations of the guard cell.

Comparison of "Intact" and "Isolated" Guard Cells

As might be expected, "intact" guard cells, opening against the resistance to expansion of both the subsidiary cell turgor and the guard cell turgor, have much higher potassium contents for any given aperture than do "isolated" guard cells. The difference between the estimates of π_{KCl} in "intact" guard cells in this paper and the estimates of π_{KCl} in "isolated" guard cells (MacRobbie & Lettau, 1980) may be compared with the estimate of P_{gs}, the contribution of the subsidiary cell turgor to the water relations of the guard cell (MacRobbie, 1980). (P_{gs} was estimated as the osmotic pressure of sucrose which had to be added the external solution to bring the cells again to the initial aperture after release of the subsidiary cell turgor.) The results are shown in Table 3, where it is clear that difference in potassium contents in "intact" and "isolated" guard cells is close to the estimate of the extra solute required to overcome the resistance to guard cell expansion provided by the turgor of the subsidiary cells.

Discussion

The most important conclusion from this work is that in the early stages of opening the changes in potassium are too small to account for the osmotic changes required to open the pore. Between 10–15 μm the previous osmotic measurements showed that the solute content of guard cells had to be increased, at constant water potential, by 170 mosmol kg^{-1} μm^{-1}

to open the pore, of which 129 mosmol kg^{-1} μm^{-1} was required to overcome the resistance to expansion of the guard cell wall, and the rest to overcome the resistance of the subsidiary cell turgor. The estimates of the osmotic contributions of the changes in potassium salts in Table 2 are less than this, but rise steeply with aperture. Given the uncertainties in the conversions from K_{ac} to π_{KCl}, it is feasible that the potassium changes are large enough, certainly to provide the major fraction. However, below 10 μm the discrepancy is too large to be explained in these terms and must be a real one. The estimate of 43 mosmol kg^{-1} μm^{-1} for $d\pi_{KCl}/dA$ at 8 μm is difficult to reconcile with the slope of 116 mosmol kg^{-1} μm^{-1} for $d\pi/dA$ below 10 μm, and the value of 18 mosmol kg^{-1} μm^{-1} for $d\pi_{KCl}/dA$ at 5 μm cannot provide the osmotic changes required. Therefore some other process must also be involved in the early stages of opening. A similar conclusion was reached from measurements on "isolated" guard cells in the previous paper (Mac-Robbie & Lettau, 1980).

Although it has been argued that potassium changes are adequate to account for the osmotic changes in *Vicia faba* (Humble & Raschke, 1971), there are considerable uncertainties in the figures on which this conclusion was based. The osmotic changes required were estimated by plasmolysis in K^+-free sucrose solutions, after 30 min exposures to the plasmolysing solutions. Raschke (1979) shows that such treatment will lead to considerable loss of solute, with a half-time of 10 min, and must therefore underestimate the osmotic pressure. If the osmotic change with aperture has a slope of 4.8 atm μm^{-1} quoted by Raschke (1979), rather than the value of 1.6 atm μm^{-1} used by Humble and Raschke (1971), then the measured K changes would not be adequate. Thus, although it is clearly established that in *Vicia* potassium salt accumulation makes a major contribution to the increased osmotic value of guard cells as the pore opens, the figures do not clearly establish that the amounts of potassium are quantitatively adequate.

There are also other indications in the literature consistent with the view that potassium is not the whole story. Using the very elegant methods of microanalysis of single guard cell pairs dissected from frozen dried tissue, Outlaw and co-workers have provided a balance sheet for the solute changes in *Vicia faba* (Outlaw & Lowry, 1977; Outlaw & Kennedy, 1978; Outlaw & Manchester, 1979). Their figures for the changes per guard cell associated with the opening to 10 μm are increases of 1.6 pmol K^+, 0.23 pmol malate, 0.145 pmol citrate, 0.42–0.74 pmol hexose, and 0.14 pmol sucrose, together with a decrease in starch of 0.25 pmol (hexose equivalents). What is striking about these results is the import of material to the guard cells on opening; the disappearance of starch can account for the synthesis of organic acid anions, but there are also considerable increases in both hexose and sucrose. This import is also seen in the increase in dry weight of the guard cells on opening, quoted as 0.7 ng per guard cell pair. Their results show that opening is associated with solute accumulation, not all of which is potassium salt. This might suggest that transport of solutes to the vacuole, including but not exclusively potassium salts, is the critical process in promoting stomatal opening. It may be that the relative contributions of potassium salts and other solutes, such as sugars, vary throughout the aperture range with the degree of potassium accumulation determining the final aperture in wide-open stomata, but being less important in the early stages of opening. In this context it is worth noting that Outlaw & Kennedy (1978) suggest that little malate is synthesized in the initial stages of stomatal opening. It may also be noted that Rogers, Powell and Sharpe (1979), using the qualitative staining method for potassium estimation, reported that in some conditions potassium content in guard cells of *Vicia faba* declined without a corresponding decrease in aperture.

The discrepancy between the osmotic contribution of the potassium salts and the osmotic pressure of the guard cells can also be seen by comparison of the estimates of π_{KCl} in Table 2, with the estimates of π by plasmolytic measurements. These were not done accurately, using closely spaced concentrations of sucrose, but rough estimates can be obtained from wider steps in sucrose concentration. Even in closed guard cells 200–300 mosmol kg^{-1} sucrose had to be added to the (75 mM KCl + 10 mM buffer) bathing solution before the cells plasmolysed; thus the estimates of π of 350–450 mosmol kg^{-1} are much greater than the estimates of π_{KCl} of 135 mosmol kg^{-1} from Table 2. At higher apertures the estimates of π were much greater; with pores of 10–12 μm, guard cells did not plasmolyse in 1300 mosmol kg^{-1} outside and with very wide-open stomata (17–20 μm) cells did not plasmolyse at 2200 mosmol kg^{-1}. Therefore a significant fraction of the osmotic pressure must be contributed by solutes other than potassium salts.

The second main point of interest in the present results is the question of changes in the potassium content of epidermal cells. The results of set *I*, in Fig. 2*B*, suggest that the potassium concentration in the epidermal cells falls from 250–450 mM when closed to about 100 mM when open. These figures are in fact very similar to the values obtained by Penny and Bowling (1974), using a different K-sensitive electrode, based on sodium cobaltinitrite in the

tip; their figures were 448 mM when stomata were closed, falling to 73 mM when open. The following arguments are put forward in support of the view that the measurements reflect a real difference between the two sets of tissue and are reasonable estimates:

a) the agreement between set *I* and Penny and Bowling's results using a different sensing system,

b) the fact that the activity coefficient is likely to be overestimated by using KCl as the calibration standard, but is very unlikely to be underestimated,

c) the fact that the pattern of guard cell behavior is very similar in Figs. 2*A* and 3*A* and that it is hard to imagine a systematic overestimation only in the epidermal cells in strips with closed stomata.

If this is accepted then there are two problems. The first is the question of where the potassium goes which leaves the epidermal cells of set *I*, and the second is why the two patterns of behavior should be so different, with the epidermal cells in set *II* showing no change with aperture, having a value of about 90 mM (K_c). The total K calculated from the determinations in set *II* (2.3 nmol mm^{-2}) compares reasonably with the flame photometric determinations in this tissue. The flame photometry of paired samples from the same leaf after treatment in light or dark (Table 1) shows that the total content of potassium in the epidermis does not change with aperture. The total K calculated from the cell contents in set *I*, for closed stomata, is considerably higher than this, about 4.4 nmol mm^{-2}. The value is, however, similar to the value calculated from Bowling's flame photometric measurements on extracts of epidermis; if the volume of the epidermis is 30–40 nl mm^{-2}, then his figures give K contents of 4.3–5.8 nmol mm^{-2}, independent of aperture. Pearson (1975) found that the epidermal potassium content in *Commelina cyanea* was 3.5 nmol mm^{-2}, and again did not change with aperture.

In set *I*, and in Penny and Bowling's results, the loss of potassium from the large epidermal cells is much greater than the amount which appears in the other cells of the complex. Since the total in the epidermis does not change, the figures require extracellular storage of potassium, presumably within the guard cell walls, in amounts which depend on aperture. In this connection the stomatal sacs in *Commelina* reported by Stevens and Martin (1977) might be important as potassium storage sites, but the results imply that considerable extracellular ion exchange (of K^+ for Ca^{2+} perhaps) is involved in opening. This would suggest that the ionic conditions in the pectin-rich cell wall may be important in determining its elasticity and in the control of stomatal aperture. If so, it would appear not to be essential in all conditions; in set *II*, in which concentrations are rather lower in both guard cells and epidermal cells, in tissue grown with a more abundant water supply, the need for extracellular storage does not appear. No explanation can be offered for the difference.

The comparison of P_{gs} with the difference of the estimates of π_{KCl} in "intact" and "isolated" guard cells suggest that the extra potassium content of "intact" guard cells is appropriate to overcome the extra resistance to expansion provided by turgid subsidiary cells. In view of the uncertainties in the estimation of π_{KCl} from K_{ac}, it was surprising that the agreement is as close, that the various results are as self-consistent.

Overall, the results suggest that guard cells in the intermediate stages of opening would repay study in more detail. It will be important to establish the nature of the changes in the state of guard cells in the early stages of opening and to include study of both potassium salts and other solutes. We may be concerned with the initiation and stimulation of vacuolar solute accumulation, of which potassium salts form a major fraction but not the whole. The most striking structural feature of closed guard cells is the absence of a large central vacuole and the presence, instead, of many small vacuoles. It is this feature of closed guard cells which distinguishes them from other cells, not their ability to accumulate salts in the open condition. It may be, therefore, that attention should be given to the role of transport processes at the tonoplast in the control of stomatal aperture.

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