Plant Physiol. (1970) 46, 483–487

Light-dependent Influx and Efflux of Potassium of Guard Cells during Stomatal Opening and Closing[1]

Received for publication February 18, 1970

G. D. HUMBLE[2] AND THEODORE C. HSIAO
Laboratory of Plant-Water Relations, Department of Water Science and Engineering, University of California, Davis, California 95616

ABSTRACT

Stomata in epidermal strips of *Vicia faba* opened in light and closed in darkness when floated on dilute K^+ solutions. Opening and closing, respectively, paralleled the fluxes of labeled K^+ into and out of the strips. The gain and loss of K^+ by the strips were shown by colbaltinitrite stain to be centered at guard cells. Intact epidermal cells, however, appeared to take up K^+, complicating interpretation of the data.

The specific requirement of K^+ for stomatal opening in light appeared to be related to the specific uptake of K^+. There was little or no light stimulation of opening in strips on Na^+, nor was there stimulation of Na^+ uptake. The marked light stimulation of opening on K^+ was generally matched by stimulation of K^+ uptake.

Anaerobiosis markedly reduced opening in leaf discs but not in strips. Under anaerobic conditions, opening in strips was not appreciably affected by 3(3,4-dichlorophenyl)-1,1-dimethylurea (diuron) but was completely inhibited by carbonyl cyanide *m*-chlorophenylhydrazone plus diuron. Inhibition of opening was generally correlated with inhibition of K^+ uptake by the strips. Also stomata in strips opened well under far red light (>700 nanometers). These data suggest that photosystem I and cyclic electron flow can supply the necessary energy for K^+ uptake and stomatal opening.

Recent evidence indicates that the mechanism underlying stomatal opening is uptake of K^+ in osmotic amounts by guard cells. Fujino (6) and Fischer and Hsiao (5) independently reached this conclusion from studies with epidermal strips containing guard cells shown to behave similarly to those in leaf discs (4). Further, we have shown that, of the various ions tested, K^+ is specifically required for the light-activated opening of stomata of *Vicia faba* L. (8). Data of Fischer and Hsiao indicate that absorption of K^+ and the associated increase in anions may account for the increase in solute concentration in guard cells associated with opening. This was supported very recently by data obtained by Sawhney and Zelitch (18) with tobacco guard cells by an electron microprobe.

A corollary of this proposed mechanism of opening is that a net efflux of K^+ from guard cells underlies closing. We now report on the influx and efflux of K^+ from epidermal strips during stomatal opening and closing, the location of K^+ in the strips, and a probable source of energy for K^+ uptake.

MATERIALS AND METHODS

The plant material (*V. faba* L., var. Long Pod) and basic method were those of Fischer (4) as modified by us (8). Briefly, the procedure was as follows. Epidermal strips were taken from leaves of plants grown 3 to 5 weeks at 26 C and 3000 ft-c (light period), floated in darkness so stomata were closed, transferred to test solutions of KCl or NaCl buffered at pH 6 with 0.5 mM tris-maleate containing 0.2 meq/liter of Ca^{2+} (4), and placed in a chamber in either light (2200–2400 ft-c) or darkness with humidified Co_2-free air passing continuously over the solutions. The test solution was maintained at 30 to 34 C as monitored with thermocouples. After floating for 3 hr (unless otherwise stated), strips were removed for microscope measurement of stomatal aperture (8). Five or six replicates were used in an experiment.

Strips pulled slowly off leaves contained a high proportion of intact epidermal cells. To obtain strips low in intact epidermal cells, leaves were floated on water for 15 min. Strips were then pulled off very rapidly at a sharp angle from the turgid leaves.

As mentioned previously (8), with some plant material stomata opened substantially on buffer alone and did not close readily in the dark. Data obtained with such material were disregarded.

Isotopic labeling was used to estimate K^+ and Na^+ uptake. Test solutions of KCl and NaCl were labeled with carrier-free $^{86}RbCl$ and $^{22}NaCl$ to a specific radioactivity of 15×10^3 to 15×10^4 cpm/μmole of KCl or NaCl. After the uptake period, the strips were rinsed by floating briefly on ice-cold 0.1 mM $CaCl_2$ and then transferred to ice-cold 50 mM $CaCl_2$ buffered at pH 6 and moved about for 4 min to remove exchangeable $^{86}Rb^+$ or $^{22}Na^+$. Finally, the strips were washed again in 0.1 mM $CaCl_2$. Although desorption and washing were carried out in the light of the laboratory (about 100 ft-c), the low temperature should have prevented any significant metabolic reaction. Washed strips were arranged in the center of planchets, dried with a heat lamp, and counted in a gas flow counter. Usually four strips, each 14 mm^2 in area, constituted a replicate for counting. Aliquots of standard solutions were similarly placed, dried, and counted. In estimating K^+ uptake, it was assumed that $^{86}Rb^+$ and K^+ moved identically, as shown in other higher plant systems (*e.g.*, 16), and because we found (8) that K^+ and Rb^+ had the same specific effect on stomatal opening.

Unless otherwise noted, all strips used in ion uptake experiments had a low percentage of intact epidermal cells (generally less than 25%) to minimize complication from uptake by these cells. Stomatal apertures were measured on strips from the same

[1] Supported in part by Grant B-029-CAL from the Office of Water Resources Research, United States Department of the Interior, and by a grant from the Water Resources Center, University of California.

[2] Present address: Michigan State University-Atomic Energy Commission Plant Research Laboratory, East Lansing, Michigan 48823.

leaf simultaneously given identical treatments except that the solutions were not radioactive.

The location of K^+ in epidermal strips was ascertained by staining with a method similar to that of Macallum's (12) employed by Fujino (6). Exchangeable K^+ was desorbed from the strips in the same way as when K^+ uptake was measured with $^{86}Rb^+$. Then the strips were floated for 2 min on freshly prepared sodium cobaltinitrite reagent [$Co(NO_2)_3 \cdot 6H_2O$, 4 g; $NaNO_2$, 7 g; CH_3COOH, 2 ml; and H_2O, 13 ml]. Excess reagent was washed off with ice-cold water. The strips were mounted in immersion oil and examined for yellow crystals, marking the location of K^+.

Anaerobic condition was obtained by passing nitrogen instead of CO_2-free air over the strips on test solutions. Nitrogen was bubbled through the solution to remove dissolved oxygen prior to use. When metabolic inhibitors were used, the test solutions in some cases contained up to 2% ethanol, which was necessary to dissolve the inhibitor. Stomatal opening on KCl is not affected by 2 or 5% ethanol (3). When ethanol was used, the same amount was added to the treatments not receiving the inhibitor.

The effect of far red light was also studied. A plastic filter (type FRF 700, Westlakes Plastics, Lenni Mills, Pa.) was used which transmits no light at wave lengths shorter than 600 nm, less than 0.3% at 680 nm, and 0.6% at 690 nm. At 700 nm the transmittance rises sharply as wave length increases to 40% at 720 nm and 80% at 750 nm (15). Precautions were taken to exclude all stray light.

RESULTS

Influx and Efflux of K^+. Fischer and Hsiao (5) suggested that net K^+ efflux occurs when stomata close, though they were unable to demonstrate that conclusively. The efflux of K^+ from epidermal strips during stomatal closing was demonstrated in this study (Table I). Strips on 1 mM KCl opened in light while taking up K^+, and lost K^+ on closing in the dark. Strips placed on nonradioactive KCl in the dark lost slightly more radioactivity than strips left on the radioactive solution, especially strips with a high percentage of intact epidermal cells.

Table I. *Effect of Darkness Following Light on Stomatal Aperture and Labeled K^+ Content in Epidermal Strips with High and Low Proportion of Intact Epidermal Cells*

Means of four experiments, each consisting of five replicates. Total amounts of K^+ taken up varied considerably among experiments, but the effects of the treatment variables were consistent in all experiments. For the strips low and high in intact epidermal cells, respectively, the initial apertures averaged 1.5 and 0.3 μ, and the apertures in strips floated on buffer without K^+ for 3 hr in light averaged 3.3 and 2.8 μ.

Proportion of Intact Epidermal Cells[1]	3 hr in light on labeled K^+	3 hr in light on labeled K^+, then 2 hr in darkness on:	
		Labeled K^+	Unlabeled K^+
	stomatal aperture (μ)		
Low	8.6	5.4	...
High	8.8	3.4	...
	relative radioactivity in strip[2]		
Low	1.00	0.70	0.53
High	1.26	0.89	0.63

[1] The proportion of intact epidermal cells varied considerably from strip to strip and averaged 18 and 61%, respectively, for the "low" and "high" strips.

[2] Radioactivity in cpm per cm^2 of strip area is expressed relative to that of the strips low in epidermal cells floated for 3 hr in light on labeled K^+.

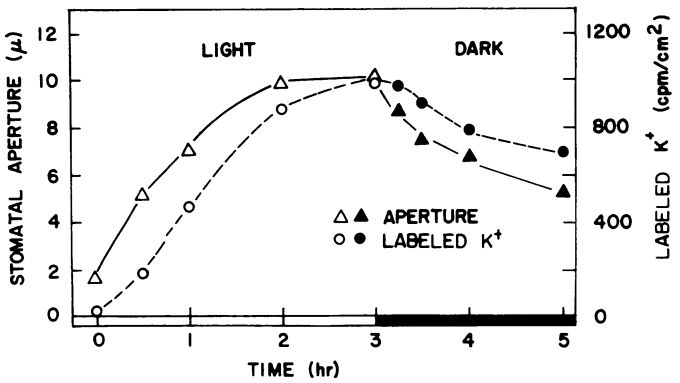

FIG. 1. Stomatal aperture and $^{86}Rb^+$-labeled K^+ in epidermal strips in response to consecutive light (open symbols) and dark (closed symbols). Epidermal strips were placed under light and CO_2-free air on 1 mM KCl labeled with $^{86}Rb^+$ at time zero. The strips remained on labeled KCl under CO_2-free air throughout. Intact epidermal cells in the strips averaged 23%.

The data in Table I also show that the percentage of intact epidermal cells affects K^+ uptake by epidermal strips. In all treatments on 1 mM KCl, strips with a high percentage of intact cells contained more label than those with a low percentage.

Figure 1 shows the time course for labeled K^+ content and stomatal aperture in light followed by darknesss. In light, stomatal aperture increased for about 2 hr and then leveled off. The increase in K^+ content paralleled closely the increase in aperture. Other experiments have shown that there is little or no further increase in uptake or aperture even after 6 hr in light.

Upon transfer to darkness after 3 hr in light, the stomata began to close almost immediately. Radioactivity in the strips decreased as the stomata closed, though it was higher than during opening when comparison was at the same aperture. Presumably this is the result, at least partially, of dilution of the label.

The stomata represented in Figure 1 did not close in the dark as much as in the previous work (8), probably because of the low percentage of intact epidermal cells. A low percentage of intact epidermal cells appeared to have little influence on the opening of stomata in strips in light, but definitely hindered closing (Table I). After 2 hr in the dark, the mean stomatal aperture was greater in strips low in intact epidermal cells than in strips high in intact cells.

Location of K^+ in Strips. It is implied that the K^+ taken up during opening is substantially localized in guard cells. This was substantiated by staining with cobaltinitrite. When floated on 1 mM KCl, stomata in strips kept in the dark did not show noticeable staining for K^+ (Fig. 2a). In contrast, strips in light had open stomata (though closed by the staining process) and guard cells with pronounced stains (Fig. 2b). There was no marked difference between light and dark treatments in the staining of epidermal cells. Thus, the greater K^+ content in the strip in light (Table I and Fig. 1) must be largely centered in guard cells. When strips in light were transferred to darkness, stomata closed and there was no preferential nor marked staining in guard cells. This suggests that the efflux of K^+ in darkness was mainly from guard cells. Although the changes in K^+ with light and dark were only obvious in guard cells, probably epidermal cells also take up some K^+ (see "Discussion").

Influx of K^+ vs Influx of Na^+. We have shown (8) that several monovalent cations other than K^+ also induce stomatal opening, particularly at high concentrations. The effect of K^+ and Rb^+, however, was greatly accentuated by light whereas the effects of other ions were not. If the uptake of ions is responsible for opening, then there should be little or no light stimulation of uptake of ions such as Na^+, in contrast to a pronounced stimulation of K^+ uptake. The data (Fig. 3) supported this deduction. There was

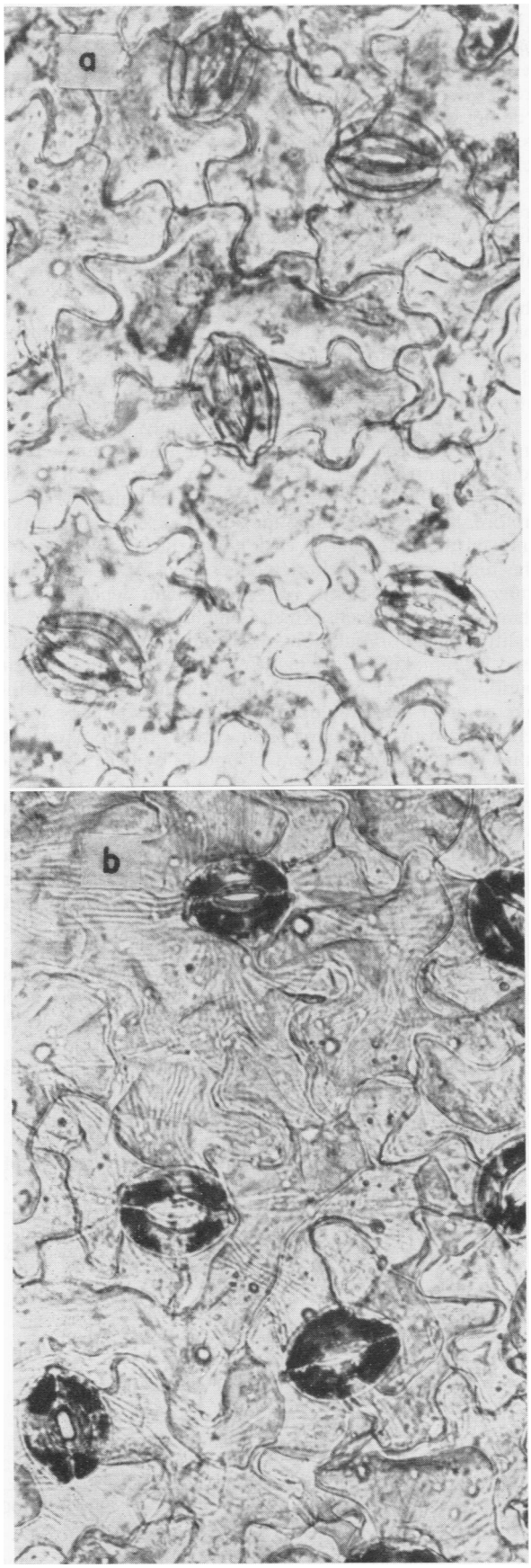

FIG. 2. The location of K^+ in epidermal strips floated for 3 hr on buffered 1 mM KCl as shown by colbaltinitrite stain. For clarity in photographs, the yellow stain marking the location of K^+ was turned black by treating the stained strips for a few seconds with 1% $(NH_4)_2S$ (7). a: Strips floated in darkness. Mean stomatal aperture was about 3 μ. b: Strips floated in light. Mean stomatal aperture was about 10 μ. Staining caused stomata to close.

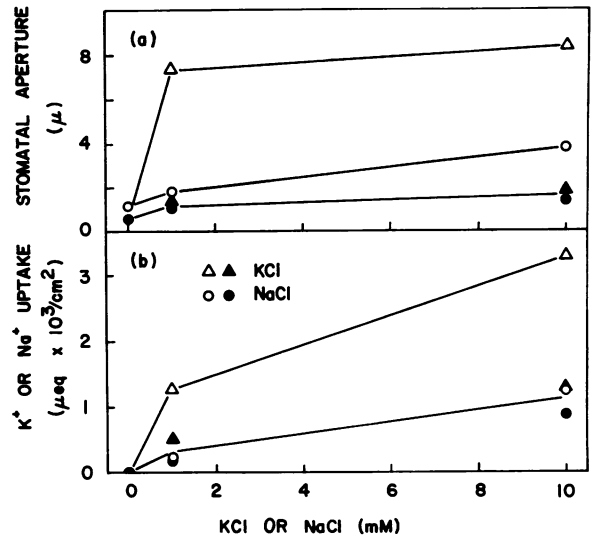

FIG. 3. Stomatal opening in epidermal strips in response to K^+ and Na^+ and the related K^+ and Na^+ uptake, in light (open symbols) and darkness (closed symbols). The experimental period was 2 hr. Intact epidermal cells in the strips averaged 15%. a: Stomatal aperture. The difference in aperture between light and dark on 10 mM NaCl was not generally observed in other experiments. b: Uptake of K^+ and Na^+ as estimated with $^{86}Rb^+$ and $^{22}Na^+$, respectively.

little or no light stimulation of stomatal opening in strips on Na^+; and there was no apparent light stimulation of Na^+ uptake. The marked light stimulation of stomatal opening on K^+, on the other hand, was generally matched by the light stimulation of K^+ uptake.

Metabolic Energy for K^+ Uptake and Stomatal Opening. The probable metabolic energy for K^+ uptake and stomatal opening was determined by inhibiting conditions and chemicals to successively eliminate the various sources of energy. The isolated epidermal strip system was particularly suited for this because it avoids possible effects via the mesophyll. Placing strips in a nitrogen atmosphere reduced stomatal opening only slightly below that of strips in CO_2-free air (Fig. 4). External oxygen apparently is not essential for stomatal opening. The opening of stomata in leaf discs, however, was markedly inhibited under nitrogen (Table II). Stomata on discs kept under nitrogen opened normally when returned to CO_2-free air for 2 hr, showing that the tissue was not permanently damaged. Oxygen is thought to be necessary for the opening of stomata in leaves (11, 19). There is indication, however, that anaerobic condition can maintain stomatal opening (19) or even cause further opening (17).

Diuron[3] inhibits oxygen evolution and net electron flow in photosynthesis (9). Diuron at 1 μM and a nitrogen atmosphere together caused only a small reduction in stomatal aperture (Fig. 5). K^+ uptake appeared to be reduced more than aperture. In other tests, increasing the concentration of diuron to 5 or 10 μM had no further effect on stomatal aperture. Presumably, stomata can open in light, at least partially, without oxidative phosphorylation and without photosynthetic noncyclic electron flow.

[3] Common name and abbreviations: diuron (DCMU):3(3,4-dichlorophenyl)-1,1-dimethylurea; Cl-CCP: carbonyl cyanide *m*-chlorophenylhydrazone; Ψ total water potential; ψ_s: solute potential; ψ_p: pressure potential.

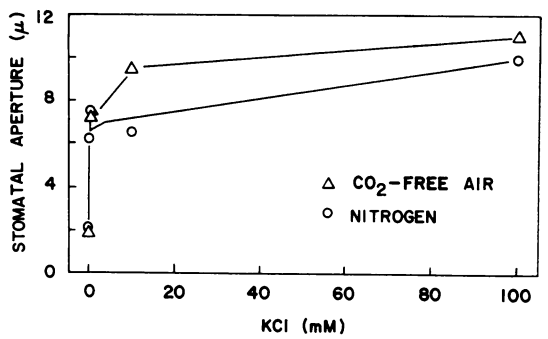

FIG. 4. Stomatal opening in epidermal strips in light on KCl as affected by a nitrogen atmosphere. The experimental period was 2 hr. The lowest KCl concentration used was 0.1 mM.

Table II. *Stomatal Opening in Leaf Discs in Light as Affected by a Nitrogen Atmosphere*

Leaf discs were floated on water under the specified atmosphere. The experiment was carried out simultaneously and with the same leaves as the experiment presented in Figure 4.

Stomatal Aperture			
CO_2-free air, 2 hr	N_2, 2 hr	CO_2-free air, 4 hr	N_2, 2 hr followed by CO_2-free air, 2 hr
μ			
9.8	3.1	11.0	9.6

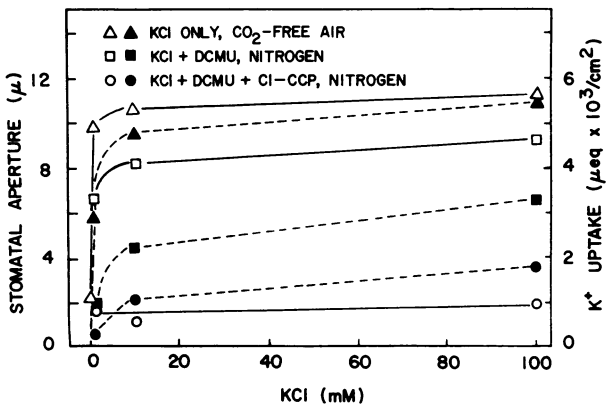

FIG. 5. Stomatal opening (open symbols and solid line) and K^+ uptake (closed symbols and broken line) in light in response to: KCl under CO_2-free air; KCl + 1 μM diuron under N_2; KCl + 1 μM diuron + 1 μM Cl-CCP under N_2. The lowest KCl concentration in all series was 1 mM. Buffer without KCl as a treatment was included only in the "KCl under CO_2-free air" series.

When Cl-CCP, an uncoupler of photophosphorylation, was also present at 1 μM in the test solution, there was virtually no stomatal opening and K^+ uptake was reduced markedly (Fig. 5).

Cyclic electron flow is sustained by photosystem I, which absorbs light of wave lengths (*e.g.*, > 700 nm) not absorbed by photosystem II. Stomata opened as wide in light with wave length greater than 700 nm as in white light (Fig. 6). Also in far red light, as in white light, they did not open without K^+ being supplied.

DISCUSSION

The results, obtained with several different approaches, strengthen the previous proposal (5, 8) that the specific uptake of K^+ by guard cells in amounts sufficient to lower the solute potential substantially is the mechanism of stomatal opening in light. The corollary of this proposal is that an efflux of K^+ from guard cells underlies stomatal closing in the dark. Such an efflux was demonstrated. Staining showed the K^+ taken up on opening to be largely concentrated in the guard cells, substantiating the conclusion reached previously from theoretical considerations (5).

Previous data indicated that the K^+ taken up by strips was not readily exchangeable (5). When labeled strips were floated on unlabeled KCl, the loss of label in 30 min was negligible. However, when the strips were floated on unlabeled KCl for 2 or 3 hr in the present study, slight exchange of K^+ between epidermal strips and external medium became evident. Labeled strips lost more radioactivity in the dark floating on unlabeled K^+ than on labeled K^+ (Table I). Strips high in intact epidermal cells showed greater exchange in the dark than low ones (Table I), suggesting that the exchange might be confined mainly to epidermal cells.

The loss of radioactivity from the strip when stomata closed in the dark cannot be readily converted to net loss of K^+, because the label was diluted by the original K^+ content of the cells. Therefore, the loss of each count during closing represents more K^+ moved than the gain of each count during opening.

The highly specific light-activated effect of K^+ on stomatal opening (8) is apparently due to a light-dependent uptake mechanism in guard cells which is highly specific for K^+. Light gave little or no stimulation of Na^+ uptake but markedly increased K^+ uptake. In contrast to the difference in light, similar amounts of K^+ and Na^+ were taken up in the dark. Although uptake of other ions was not determined, it would be logical to conclude from the effects of other cations on stomatal opening (8) that the large enhancement of uptake by light is confined to K^+ (and Rb^+).

Willmer and Mansfield (20) found no significant difference between Na^+ and K^+ at 66 meq/liter in effects on stomatal opening in *Vicia*. At this concentration, which is higher than may be expected in the apoplast of the leaf, the difference between K^+ and Na^+ should be minimal (8). They also concluded that epidermal strips of *Vicia* are not suitable for studies of light responses of stomata. However, we have shown that, at the high concentrations they used, opening in the dark is pronounced and response to light is therefore small (8). Also Ca^{2+} was not added to their solutions. Exogenous Ca^{2+} is essential for the integrity of the selective ion absorption mechanism of roots (2). Ca^{2+} must be ubiquitous in the apoplastic solutions of plants. Pallaghy (personal communication) has observed that the specific effect of K^+ over Na^+ in the opening of *Vicia* stomata in light is evident only when Ca^{2+} is present.

The present data indicate that the energy derived from photo-

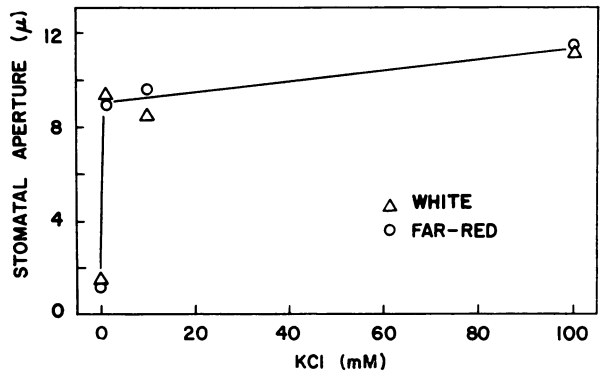

FIG. 6. Stomatal opening on KCl solutions in response to white and far red light (>700 nm). Far red light was obtained by passing a part of the white light (2200–2400 ft-c) through the filter.

synthetic cyclic electron flow can be sufficient and possibly necessary for K^+ uptake and stomatal opening in light. Anaerobic condition plus diuron caused a small reduction in stomatal aperture and a somewhat larger reduction in K^+ uptake. Under these conditions photosynthetic cyclic electron flow should continue. When the high energy state resulting from cyclic electron flow was dissipated by Cl-CCP, however, there was virtually no stomatal opening and very little K^+ uptake. The results are consistent with the interpretation that stomatal opening in strips does not require net electron flow in large part and can depend on cyclic electron flow.

Opening in strips appeared to be the same under light with wave length greater than 700 nm as under white light. Since photosystem II does not absorb light of wave length of 700 nm or longer (13), only photosystem I, and therefore only cyclic electron flow, could proceed under this condition. Thus, the results obtained with this independent approach fully support the conclusion based on the effects of inhibitors. In a study with inhibitors, Rains (16) concluded that the energy for K^+ uptake by leaf tissue in light comes partly from cyclic photophosphorylation.

Diuron had little effect on the opening of stomata in strips in light under CO_2-free air in this and a previous study (3), but caused marked closing in strips under normal air (10). Allaway and Mansfield (1) suggested that the effect of an inhibitor of the diuron type on stomata in leaves, marked only in normal air, is related to increases in CO_2 level resulting from the inhibition of photosynthetic CO_2 fixation.

Red light is considered to be less effective than blue light in inducing stomatal opening in leaves (14). Light of 700 to 720 nm, however, maintains the opening of stomata in epidermal strips, though not as effectively as light of 675 or 432 nm (10). The opening of stomata (in contrast to maintenance of opening) in epidermal strips in light of wave length greater than 700 nm is demonstrated for the first time.

There is good evidence (5, 18) indicating that sufficient K^+ is transported when stomata open and close to serve directly as the osmotic agent (in contrast to a catalytic role). However, data in Figures 3 and 5, when compared at two widely different external K^+ concentrations, show instances where equal or greater uptake of K^+ resulted in smaller apertures. For example, in Figure 3, K^+ uptake in the dark on 10 mM KCl is almost as great as that in light on 1 mM, though the apertures were much greater in light. The apparent disparity possibly is caused by intact epidermal cells, which probably take up some K^+, as suggested by the greater uptake when more epidermal cells remained intact in a strip (Table I). Although compared to guard cells, concentration of K^+ in epidermal cells must be low (Fig. 2), total uptake by epidermal cells, particularly at high external concentrations, may still be significant because they constitute by far the majority of the viable cells in the strip (7% of area for guard cells *versus* a low limit of 15 to 20% of area for intact epidermal cells). There is also the possibility that externally adsorbed radioactivity was not completely removed by the desorption procedure. This error would also be more serious at high external concentrations. These complications may account for the aforementioned anomaly of narrower aperture at apparently higher K^+ uptake.

Another aspect is also related to the question of the movement of K^+ in osmotic amounts. A unit of K^+ taken up brought about a greater increase in aperture in the earlier part of the opening process than in the later part (Fig. 1). This suggests that K^+ is not acting catalytically in causing opening, in which case opening should lag behind the uptake of K^+, and possibly continue after K^+ content has ceased to increase. Instead, the data are consistent with the proposal that K^+ acts directly as an osmotic agent. An increment of K^+ in the guard cell would lower the water potential (Ψ) by lowering the solute potential (ψ_s). The volume of water taken up to re-establish equilibrium of Ψ with the outside is variable, depending on the turgor and volume-pressure relationship of the cell. If turgor is low and the cell flaccid, a large amount of water would be taken up since there is little increase in pressure potential (ψ_p) associated with an increment in volume and the only main increase in Ψ is due to dilution of solutes (increase in ψ_s). If turgor is high, with the cell wall stretched taut, the amount of water taken up would be small since an increment in volume greatly increases ψ_p in addition to ψ_s. Therefore, an increment in K^+ is expected to be more effective in causing opening when the stomata are closed or slightly open than when they are nearly fully open. This would also explain the results of the inhibitor experiments (Fig. 5), which show a small reduction in aperture corresponding to a large reduction in K^+ uptake when stomata are wide open, but a large reduction in aperture corresponding to a small reduction in K^+ uptake when stomata are only partially open.

How K^+ moves out of guard cells during closing is an intriguing aspect that requires further investigation.

Implicit in our work is the assumption that the basic mechanism of stomatal movement is the same in leaves as in isolated strips. Evidence supporting this will be presented elsewhere.

Acknowledgments—We thank Drs. C. R. Stocking and D. W. Rains for advice. The approach used in the inhibitor experiment was suggested by Dr. Rains, who also provided us with the $^{22}Na^+$. Dr. R. Huffaker kindly made the far red filter available. We are grateful to Miss Jean Koskela for her excellent technical assistance.

LITERATURE CITED

1. Allaway, W. G. and T. A. Mansfield. 1967. Stomatal responses to changes in carbon dioxide concentration in leaves treated with 3-(4-chlorophenyl)-1,1-dimethylurea. New Phytol. 66: 57–63.
2. Epstein, E. 1961. The essential role of calcium in selective cation transport by plant cells. Plant Physiol. 36: 437–444.
3. Fischer, R. A. 1967. Stomatal physiology with particular reference to the after effect of water stress and to behaviour in epidermal strips. Ph.D. thesis. University of California, Davis.
4. Fischer, R. A. 1968. Stomatal opening in isolated epidermal strips of *Vicia faba*. I. Response to light and to CO_2-free air. Plant Physiol. 43: 1947–1952.
5. Fischer, R. A. and T. C. Hsiao. 1968. Stomatal opening in isolated epidermal strips of *Vicia faba*. II. Response to KCl concentration and the role of potassium absorption. Plant Physiol. 43: 1953–1958.
6. Fujino, M. 1967. Role of adenosinetriphosphate and adenosinetriphosphatase in stomatal movement. Sci. Bull. Fac. Educa. Nagasaki Univ. 18: 1–47.
7. Gomori, G. 1952. Microscopic Histochemistry; Principles and Practice. University of Chicago Press, Chicago.
8. Humble, G. D. and T. C. Hsiao. 1969. Specific requirement of potassium for light-activated opening of stomata in epidermal strips. Plant Physiol. 44: 230–234.
9. Kok, B. 1965. Photosynthesis: the path of energy. *In*: J. Bonner and J. E. Varner, eds., Plant Biochemistry. Academic Press, Inc., New York. pp. 903–960.
10. Kuiper, P. J. C. 1964. Dependence upon wavelength of stomatal movement in epidermal tissue of *Senecio odoris*. Plant Physiol. 39: 952–955.
11. Louguet, P. 1968. Influence de l'anaérobiose sur le mouvement des stomata à l'obscurité chez le *Pelargonium x hortorum*. Physiol. Vég. 6: 83–92.
12. Macallum, A. B. 1905. On the distribution of potassium in animal and vegetable cells. J. Physiol. 32: 95–128.
13. MacRobbie, E. A. C. 1965. The nature of the coupling between light energy and active ion transport in *Nitella translucens*. Biochim. Biophys. Acta 94: 64–73.
14. Mansfield, T. A. and H. Meidner. 1966. Stomatal opening in light of different wavelengths: effects of blue light independent of carbon dioxide concentration. J. Exp. Bot. 17: 510–521.
15. Obendorf, R. L. 1966. Light stimulation and distribution of photosynthetic carboxylative phase enzymes in greening barley leaves. Ph.D. thesis. University of California, Davis.
16. Rains, D. W. 1968. Kinetics and energetics of light-enhanced potassium absorption by corn leaf tissue. Plant Physiol. 43: 394–400.
17. Raschke, K. 1967. Erweiterung der Spaltöffnungen von *Vicia faba* durch Stickstoff. Naturwissenschaften 54: 73–74.
18. Sawhney, B. L. and I. Zelitch. 1969. Direct determination of potassium ion accumulation in guard cells in relation to stomatal opening in light. Plant Physiol. 44: 1350–1354.
19. Walker, D. A. and I. Zelitch. 1963. Some effects of metabolic inhibitors, temperature and anaerobic conditions on stomatal movement. Plant Physiol. 39: 390–396.
20. Willmer, C. M. and T. A. Mansfield. 1969. A critical examination of the use of detached epidermis in studies of stomatal physiology. New Phytol. 68: 363–375.