Potash Review

Monthly communications by the International Potash Institute, Berne (Switzerland)

Subject 3

Biology
44th suite

October 1972

The Role of Potassium in Stomatal Opening

R. A. Fischer[1]
International Maize and Wheat Improvement Center, Londres, Mexico

Functional stomata are present on the surfaces of all green terrestrial plants. The aperture of stomata in leaves is sensitive to a number of environmental factors including light, carbon dioxide concentration, temperature, humidity and, a recent addition to the list, atmospheric pollutants such as sulphur dioxide. In most species (an important exception are certain succulents, especially *Crassulaceae)* stomata are generally open during the day and closed at night under optimal growing conditions. This is largely the result of opening and closing movements in response to illumination and darkness respectively, with the possible assistance of an endogenous rhythm in stomatal activity. Stomatal opening under such conditions is associated with an increase, usually substantial, in turgor pressure of the stomatal guard cells, resulting from an increase in the concentration of solutes or osmotic pressure of the contents of these cells; closing in darkness is the reverse process. These aspects of stomatal physiology have been extensively reviewed by *Meidner* and *Mansfield* (1968).

Considerable uncertainty existed in the literature as to the exact nature of the solutes causing the rise in osmotic pressure in guard cells following illumination. The starch sugar hypothesis of *Lloyd* (1908) was widely held for many years. It relied largely on the observation that starch, which is often present in large amounts in guard cells chloroplasts, is reduced in amount as stomata open; it was proposed that small molecular weight sugars were formed from the starch, leading to the net increase in osmotic pressure. However the hypothesis eventually lost favour mainly because of the failure to demonstrate the proposed large quantities of sugars in guard cells. No satisfactory hypothesis replaced the starch sugar one.

Some early work had demonstrated the effect of external cations on the behaviour of stomata in isolated epidermal strips *(Iljin,* 1922; *Imamura,* 1943). Potassium and sometimes other group I cations stimulated opening; a catalytic role for potassium was suggested. Later several reviewers had pointed to the possibility that the solute build up in guard cells with opening was the result of the massive accumulation of external solutes, perhaps ions such as K *(Ketallapper,* 1963; *Hanson,* 1963). It was this possibility in particular which stimulated the studies on the mechanism of stomatal movement which are reported here. The initial work was carried out in the plant water relations laboratory of Dr. *T. C. Hsiao* at the University of California, Davis.

[1] Winner of the 4th International Potash Institut Competition for Young Research Workers.

The first step was to obtain functional isolated stomata. The epidermal strip system had been used by previous workers but with little attention as to whether stomata were alive and functioning *(Heath,* 1959). Abaxial epidermal strips from leaves of *Vicia faba* were found to give the most satisfactory strips. Neutral red uptake and protoplasmatic streaming indicated that the guard cells were living; on the other hand most epidermal cells were ruptured. The ability of stomata in floating epidermal strips to respond normally was tested by exposure to light plus CO_2-free air with control strips being kept in dark plus normal air. The former condition was used to simulate the environment of stomata in illuminated leaves, where it had been shown that the reduction in intercellular space CO_2 concentration by mesophyll photosynthesis was a major component causing opening upon illumination *(Meidner* and *Mansfield,* 1968). Floating epidermal strips on KCl solutions permitted a normal stomatal opening response according to several criteria (Table 1). Of the solution used in Table 1 it was easily demonstrated that the only essential component was the K. These results were reported in *Fischer* (1968a, 1968b) and *Fischer* and *Hsiao* (1968).

Table 1 Effect of 3 hours exposure to light plus CO_2-free air (L) or to dark plus normal air (D) on stomatal aperture, guard cell solute potential and guard cell starch score (scale 0 to 7) in leaf discs on water and isolated epidermal strips floating on KCl solution. Initially stomata in dark with aperture of 2 to 4 microns and starch scores of 5.8 (leaf discs) and 5.5 (strips); from *Fischer* (1968b).

Conditions	Leaf disc	Epidermal strip	LSD 5%
	Experiment 1		
	Stomatal aperture, microns		
L	10,0	11,0	2,0
D	1,6	3,8	2,0
	Guard cell solute potential, bars		
L	−16,2	−16,2	6,1
D	− 8,2	− 6,3	1,9
	Experiment 2		
	Stomatal aperture, microns		
L	10,5	11,5	1,2
D	3,5	6,7	1,2
	Guard cell starch score		
L	3,9	2,9	1,1
D	4,9	4,5	1,1

The sensitivity of illuminated isolated stomata in the *Vicia faba* system to external K concentration (Fig. 1) pointed to uptake of K by the guard cells. *Fujino* (1967) working independently in Japan, and no doubt following up the earlier work on ions by *Imamura* (1943), had demonstrated not only a stimulatory effect of K in opening of isolated stomata of *Commelina communis* and *Allium cepa* but also substantial accumulation of K in the guard cells. This accumulation was demonstrated using a K specific stain, cobalt sodium nitrite. Uptake of K during opening had been established; the important question was, quantitatively, how much K was being accumulated?

...

Potash Review

Monthly communications by the International Potash Institute, Berne (Switzerland)

3/44

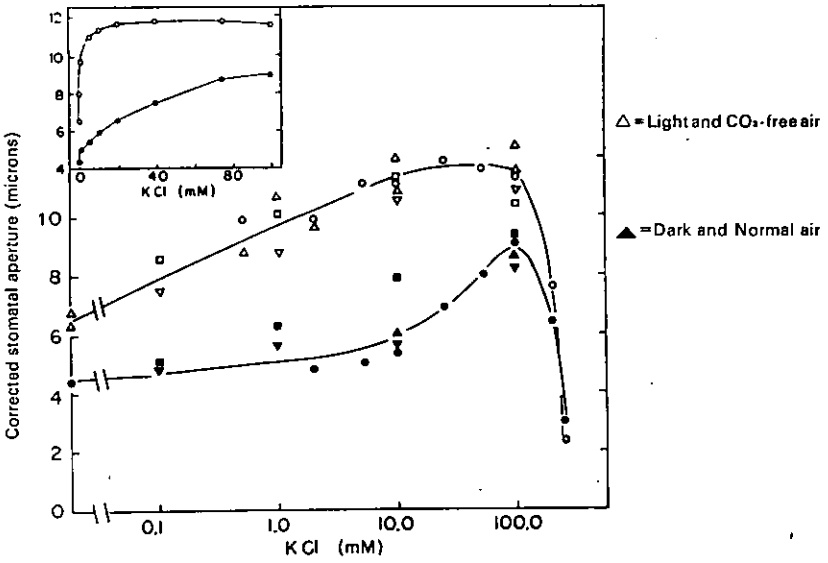

Fig. 1. Stomatal opening in response to KCl concentration in dark+normal air and light+CO_2-free air. Results are from various experiments, each designated by a different symbol (solid, dark+normal air; open, light+CO_2-free air) and involving 5 to 16 replicates. Results were corrected in each experiment to give identical mean apertures in dark+normal air at zero KCl (i.e. 4,4 microns). Insert shows the mean response to KCl on a linear scale, which was plotted from arbitrary points taken off the two composite curves drawn in the main figure (from *Fischer* and *Hsiao,* 1968).

Radioactive tracers, Rb^{86} and later with similar results K^{42}, were used to measure K accumulation by guard cells in *Vicia faba (Fischer* and *Hsiao,* 1968; *Humble* and *Hsiao,* 1970; *Fischer,* 1972). The methodology was similar to that used in studies of ion accumulation by other plant tissues. It was necessary to work with epidermal strips in which all or most epidermal cells were broken, otherwise uptake of tracers by these cells would obscure uptake by guard cells. Early results *(Fischer* and *Hsiao,* 1968) indicated an increase in guard cell K concentration of approximately 300 mM with stomatal opening in light plus CO_2-free air in strips floating on 10 mM KCl. Assuming the uptake or internal formation of a corresponding concentration of anion the calculated increase in guard cell osmotic pressure would be 12 bars, about equal to that measured earlier (Table 1). Thus it was unnecessary to invoke sugar formation from starch or the production of other (unknown) solutes to explain the rise in guard cell osmotic pressure. Starch degradation was observed in the *Vicia* guard cells, and it paralleled stomatal opening and K uptake. It was tentatively suggested by *Fischer* and *Hsiao* (1968) that this starch breakdown lead to organic acid anion synthesis in the guard cells.

The above results and those of *Fujino* (1967) resulted in renewed interest in the role of ions in stomatal opening. With the *Vicia faba* system *Humble* and *Hsiao* (1969) demonstrated a highly-specific requirement of K for the light-activated opening of stomata. They later showed efflux of K when stomata closed, little or no light stimulation of Na uptake, and the dependence of light stimulated K influx on energy provided by photosystem I and cyclic electron flow *(Humble* and *Hsiao,* 1970). The results of *Pallaghy* (1970) suggested that the specific requirement of K in stomatal opening of *Vicia faba* was only manifest in the presence of 1 mM Ca; in the absence of Ca, Na stimulated opening to the same extent as K. *Willmer* and *Mansfield* (1969) showed that stomatal opening in light in epidermal strips of *Commelina communis* was stimulated to a greater extent by Na than by K; in the absence of external ions no stomatal opening took place. *Thomas* (1970) also working with epidermal strips showed that light opening of tobacco stomata was greatly stimulated by K; however dark opening of stomata of *Kalanchoe marmorata,* a succulent species, appears to involve Na accumulation.

Several aspects of the original work demonstrating the role of K in stomatal opening in *Vicia faba* in epidermal strips had been criticised *(Levitt,* 1969; *Milthorpe,* 1969). In particular it remained to be established that K played the same role in non-isolated stomata in leaves. This and other aspects of the system were examined in the laboratory of Professor *R. O. Slatyer* at the Australian National University *(Fischer,* 1971; *Fischer,* 1972).

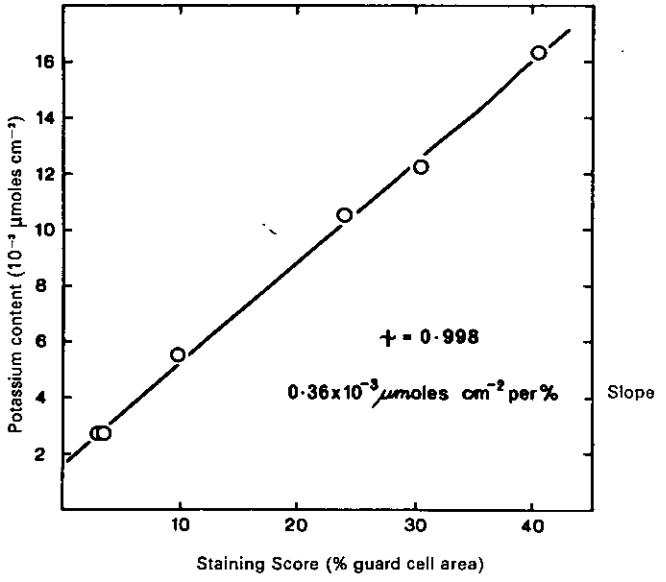

Fig. 2. The relationship between potassium content estimated with ^{86}Rb and guard cell potassium stain for isolated epidermal strips *(Fischer,* 1971). Potassium content is expressed per unit area of epidermal strip. The correlation coefficient (r) of the linear regression is significant at P less than 0,01.

Potash Review

Monthly communications by the International Potash Institute, Berne (Switzerland)

3/44

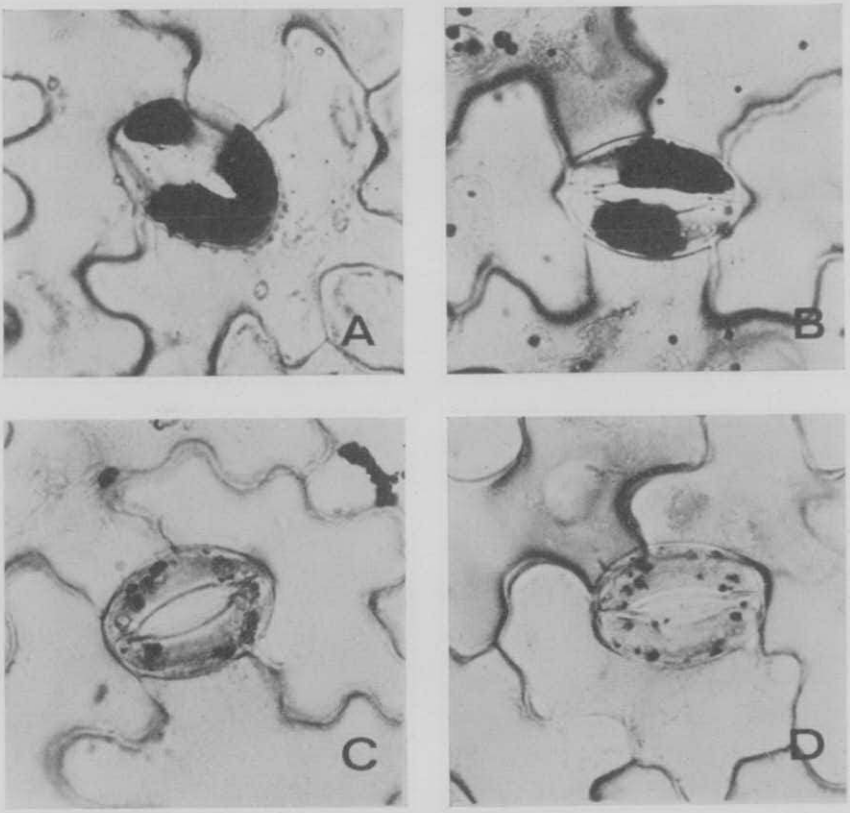

Fig. 3. Typical potassium staining in guard cells of leaf pieces (A) and isolated epidermal strips (B) illuminated in CO_2-free air for 3 hours *(Fischer,* 1971). Staining immediately prior to illumination is shown in C (leaf pieces) and D (isolated strips). Leaf pieces were floated abaxial surface uppermost on distilled water; isolated epidermal strips were floated on 10mM KCl. Note all stomata appear closed after contact with the concentrated staining solution.

Circumstantial evidence pointing to the importance of K for normal stomatal function in leaves were the studies of K deficient maize and alfalfa plants *(Peaslee* and *Moss,* 1966; *Cooper, Blazer* and *Brown,* 1967). *Sawhney* and *Zelitch* (1969) used the electron microprobe to analyze K in individual guard cells on tobacco leaves; massive accumulations were shown in light open stomata. In the case of *Vicia faba Fischer* (1971) employed the K staining technique to obtain quantitative estimates of K concentration in stomata in leaves. With isolated epidermal strips the

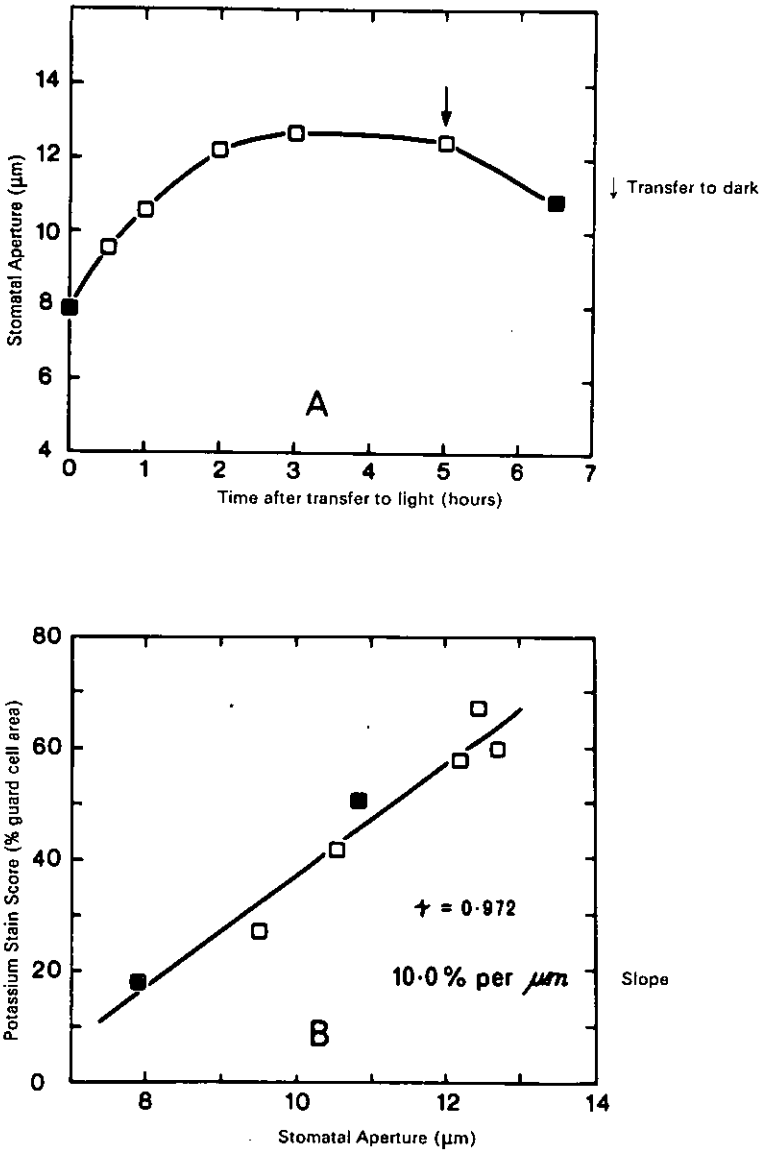

Fig. 4. A. Changes in aperture upon transfer to light plus CO_2-free air then later to dark plus normal air of stomata of leaf pieces floated on distilled water *(Fischer,* 1971). B. Relationship of the degree of potassium staining in the guard cells to stomatal aperture for the aperture changes shown in A. The correlation coefficient (r) of the linear regression is significant at P less than 0,01.

intensity of the K staining reaction in guard cells was calibrated against their content of K as measured with ^{86}Rb (Fig. 2). Stomata, opened on leaf pieces floating on water in light were removed and immediately stained for K content. From the staining intensity of guard cells thus treated, it was estimated that guard cell K concentration in non-isolated stomata was as great as in isolated stomata (Fig. 3). K concentration increased linearly with increase in aperture (Fig. 4), the slope of the relationship being 65 mM K μm^{-1}. Again this is more than sufficient K to explain the observed increases in guard cell osmotic pressure.

The problem of the origin of this K in the leaf has not been solved although it is suggested that K arriving in the vicinity of the guard cells via the transpiration stream could be a major source. Alternatively epidermal cells could loose K to guard cells during stomatal opening as in maize *(Raschke* and *Fellows,* 1971).

It should be pointed out that the above study with *Vicia* refers to aperture in the absence of epidermal cell back pressure. Decrease in epidermal cell turgor in the normal leaf situation contributes a considerable passive component to stomatal opening upon illumination in *Vicia faba (Fischer,* unpublished) and probably in many other species *(Meidner* and *Mansfield,* 1968). Absence of epidermal cell back pressure explains why stomata in darkened epidermal strips of *Vicia faba* are partly open.

The above results with *Vicia faba* were confirmed by a very thorough study involving electron probe analysis of leaf-opened *Vicia faba* stomata: K was accumulated in quantities equivalent to the increase in osmotic pressure while Na, Cl, P, and S were not accumulated to any significant extent during opening *(Humble* and *Raschke,* 1971). This group of workers has also established that K accumulates in guard cells for open stomata in leaves of corn, *Zea mays (Pallaghy,* 1971; *Raschke* and *Fellows,* 1971). *Graham* and *Ulrich* (1972) recently published indirect evidence supporting the involvement of K in stomatal opening of sugar beet leaves. Thus the role of inorganic solute accumulation, in particular K accumulation, by guard cells during light opening of stomata seems to be well established in a wide range of species.

More recently other aspects of K accumulation by *Vicia faba* guard cells in epidermal strips have been studied using radioactively labelled K *(Fischer,* 1972). The main results may be summarized as follows:

1. Intact epidermal cells of epidermal strips do participate in K tracer uptake. If all epidermal cells were intact in a given area of epidermis, they would contain 4 to 6 times the K contained in illuminated guard cells in the same area. Thus numbers of intact epidermal cells must be kept low ($< 5\%$) for precise studies of K fluxes in guard cells.

2. Stomatal opening reaches maximum values after 300 to 500 minutes in the light; the half time for these processes is however about 100 minutes. Since accumulation of K label is linearly related to aperture soon after the simultaneous initiation of illumination and exposure to labelled K solutions, the initial guard cell pool of K (darkened stomata) must be small relative to initial influxes in the light. Influx and efflux measurements after the attainment of a steady state aperture and K content in light suggest that this steady state is due to a fall in the initially high influx of K rather than a rise in the efflux. Maximum net fluxes (influxes) were at least 16×10^{-12} moles cm^{-2} sec^{-1}, somewhat higher than recorded in most plant systems.

3. The presence of external Ca reduces stomatal opening in *Vicia faba (Pallaghy,* 1970). This was closely related to reduced accumulation of K by guard cells. Similarly aperture changes in response to external K concentration were closely related to K accumulation. In the presence and absence of small concentrations of Ca, aperture was linearly related to the logarithm of the external K concentration.

4. Light and CO_2-free air have independent stimulatory effects on stomatal aperture and .K accumulation; given together their effects are usually synergistic *(Fischer* and *Pallaghy,* unpublished).

5. In all situations where content of radioactive tracer measured K content of the guard cells (i.e. after exposure to label for at least 100 minutes), this content was linearly related to stomatal aperture (free of epidermal cell back pressure) with considerable precision and regardless of the factors used to vary aperture (time, light, CO_2, K concentration, Ca, metabolic inhibitors). In the majority of the experiments more than 80% of the variation in stomatal aperture was linearly related to changes in K content of guard cells. The mean of the linear regression slopes of 16 experiments was $2{,}6\times10^{-3}$ µmoles of K per cm^2 of epidermis per µm change in aperture; the standard error of this mean was $0{,}1\times10^{-3}$ µmoles.

6. The slope parameter in 5. represents an increase in guard cell K concentration of 40 mM per µm increase in aperture (2×6300 guard cells cm^{-2}, 5×10^{-9} cm^3 per guard cell). This value, close to earlier estimates of K concentration changes, is however somewhat more accurate. Results of double-labelling experiments with ^{36}Cl and ^{42}K suggest that Cl uptake by guard cells in epidermal trips is usually about one quarter of equivalent K uptake *(Fischer* and *Pallaghy,* unpublished), with organic acid anions, internally-generated from starch breakdown, implicated as the other source of counter ions. If the latter were divalent malate, the osmotic equivalent of the K accumulation would be approximately 1,5 bars μm^{-1}. This agrees reasonably well with recent more accurate measurements of changes in guard cell osmotic pressure *(Fischer,* unpublished); the mean figure was 2,0 bars μm^{-1} for *Vicia* stomata *in* epidermal strips and free of epidermal cell back pressure, and the relationship aperture-osmotic pressure was linear.

Several new aspects of K accumulation by guard cells have been reported recently. *Mansfield* and *Jones* (1971) have shown that the inhibition of stomatal opening in *Commelina communis* by the plant hormone abscisic acid was associated with reduced K accumulation. Secondly the well known stimulatory effect of low intensity blue light on stomata *(Meidner* and *Mansfield,* 1968) appears, in the case of *Vicia faba* at least, to be quantitatively related to stimulated K uptake by guard cells *(Hsiao, Allaway* and *Evans,* 1972).

Many intriguing questions remain to be answered. The postulated appearance upon opening of massive quantities of malate or related anions in the guard cells has yet to be demonstrated; in fact it is difficult from known metabolic pathways to see how organic acid synthesis can proceed in the absence of external CO_2. Little is known about the exact nature of the coupling of K uptake to factors stimulating stomatal opening such as light, blue light and low CO_2 concentration, or the importance of chloroplasts compared to mitochondria as energy sources in the process. Thus although the K uptake hypothesis appears to have answered one long-disputed question regarding stomatal opening, we are still far from a complete understanding of the metabolic bases of this process.

Potash Review

Monthly communications by the International Potash Institute, Berne (Switzerland)

3/44

Acknowledgements

My work was carried out in laboratories of the Department of Water Science and Engineering at the University of California, Davis, and the Research School of Biological Sciences at the Australian National University. I wish to thank their respective leaders, Dr. *T. C. Hsiao* and Professor *R. O. Slatyer* for research opportunities provided. Also Drs. *Hsiao, Pallaghy* and *Allaway* have been associated closely with various aspects of results reported above; their advice and assistance is gratefully acknowledged. This work was supported by grant B-029-CAL from the Office of Water Resources Research, U.S. Department of Interior, and by a Queen Elizabeth II Fellowship to the author.

Bibliography

1. *Cooper R.B., Blaser R.E., Brown R.H.:* Potassium nutrition effects on net photosynthesis and morphology of alfalfa. Soil Sci. Soc. Am. Proc. *31,* 231–235 (1967).
2. *Fischer R.A.:* Stomatal opening: role of potassium uptake by guard cells. Science *160,* 784–785 (1968a).
3. *Fischer R.A.:* Stomatal opening isolated epidermal strips of *Vicia faba.* I. Response to light and to CO_2-free air, Pl. Physiol. Lancaster *43,* 1947–1952 (1968b).
4. *Fischer R.A.:* Role of potassium in stomatal opening in the leaf of *Vicia faba.* Pl. Physiol. Lancaster, *47,* 555–558 (1971).
5. *Fischer R.A.:* Aspects of potassium accumulation by stomata of *Vicia faba.* Aust. J. biol. Sci. *25,* In press (1972).
6. *Fischer R.A., Hsiao T.C.:* Stomatal opening in isolated epidermal strips of *Vicia faba.* II. Responses to KCl concentration and the role of potassium absorption. Pl. Physiol. Lancaster *43,* 1953–1958 (1968).
7. *Fujino M.:* Adenosinetriphosphate and adenosinetriphosphatase in stomatal movement. Sci. Bull. Fac. Educ. Nagasaki Univ. *18,* 1–47 (1967).
8. *Graham L.R.D., Ulrich A.:* Potassium deficiency-induced changes in stomatal behaviour, leaf water potentials, and root system permeability in *Beta vulgaris.* Pl. Physiol. Lancaster *49,* 105–109 (1972).
9. *Hanson K.R.:* Active and inactive transport across cell membranes. In 'Stomata and Water Relations of Plants', Ed. Zelitch, Bull. 664, Connecticut Agricultural Experiment Station, 43–57 (1963).
10. *Heath O.V.S.:* The water relations of stomatal cells and the mechanisms of stomatal movement. In 'Plant Physiology' Vol. 2 (F.C. Steward, ed.). pp 193–250. Academic Press, New York (1959).
11. *Hsiao T.C., Allaway W.G., Evans L.T.:* Action spectrum for guard cell Rb^+ uptake and stomatal opening in *Vicia faba.* Pl. Physiol. Lancaster *49* supp., 63 (1972).
12. *Humble G.D., Hsiao T.C.:* Specific requirement of potassium for light-activated opening of stomata in epidermal strips. Pl. Physiol. Lancaster *44,* 230–234 (1969).
13. *Humble G.D., Hsiao T.C.:* Light dependent influx and efflux of potassium in guard cells during stomatal opening and closing. Pl. Physiol. Lancaster *46,* 483–487 (1970).
14. *Humble G.D., Raschke K.:* Stomatal opening quantitatively related to potassium transport. Evidence from electron probe analysis. Plant Physiol. Lancaster *48,* 447–453 (1971).
15. *Iljin W.S.:* Physiologischer Pflanzenschutz gegen schädliche Wirkung von Salzen. Biochem. Z. *132,* 526–542 (1922).

16. *Imamura S.:* Untersuchungen über den Mechanismus der Turgorschwankung der Schliesszellen der Spaltöffnungen. Jap. Jour. Bot. *12,* 251–346 (1943).

17. *Ketellapper H.J.:* Stomatal physiology. Ann. Rev. Plant Physiol. *14,* 249–270 (1963).

18. *Levitt J.:* Stomatal opening: role of potassium. Science. *163,* 493–494 (1969).

19. *Lloyd F.E.:* The physiology of stomata. Publ. Carnegie Instn., Wash., *82,* 1–142 (1908).

20. *Mansfield T.A., Jones R.J.:* Effects of abscisic acid on potassium uptake and starch content of stomatal guard cells. Planta *101,* 147–158 (1971).

21. *Meidner H., Mansfield T.A.:* 'Physiology of stomata'. McGraw-Hill Book Company, London, 179 pp. (1968).

22. *Milthorpe F.L.:* The significance and mechanism of stomatal movement. Australian J. Science *32,* 31–35 (1969).

23. *Pallaghy C.K.:* The effect of Ca^{++} on the ion specificity of stomatal opening in epidermal strips of *Vicia faba.* Z. Pflanzenphysiol. *62,* 58–62 (1970).

24. *Pallaghy C.K.:* Stomatal movement and potassium transport in epidermal strips of *Zea mays:* the effect of CO_2. Planta *101,* 287–295 (1971).

25. *Peaslee D.E., Moss D.N.:* Photosynthesis in K- und Mg-deficient maize *(Zea mays L.)* leaves. Soil Sci. Soc., Am. Proc. *30,* 220–223 (1966).

26. *Raschke K., Fellows M.P.:* Stomatal movement in *Zea mays:* shuttle of potassium and chloride between guard cells and subsidiary cells. Planta *101,* 296–316 (1971).

27. *Sawhney B.L., Zelitch I.:* Direct determination of potassium ion accumulation in guard cells in relation to stomatal opening in light. Pl. Physiol. Lancaster *44,* 1350–1354 (1969).

28. *Thomas D.A.:* The regulation of stomatal aperture in tobacco leaf epidermal strips. I. The effect of ions. Aust. J. biol. Sci. *23,* 961–979 (1970).

29. *Willmer C.M., Mansfield T.A.:* Active cation transport and stomatal opening: possible physiological role of sodium ions. Z. Pflanzenphysiol. *61,* 398–400 (1969).