THE REGULATION OF STOMATAL APERTURE IN TOBACCO LEAF EPIDERMAL STRIPS

IV.* THE EFFECT OF BICARBONATE/CARBON DIOXIDE†

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Abstract

Stepwise decreases in the stomatal aperture of tobacco leaf epidermal strips followed stepwise increases in the concentration of $KHCO_3$ added to bathing solutions. Removal of $KHCO_3$ from the bathing solution resulted in a rapid increase in aperture. The reduction in aperture caused by $KHCO_3$, both in the light and dark, can be reversed by the addition of ATP or phosphoenol pyruvate to the bathing solution. The stomatal opening, supported by a NaCl bathing medium, is reduced by the addition of $NaHCO_3$. From the results it is suggested that HCO_3^-/CO_2† increases the permeability of guard cell membranes causing a net efflux of water or ions or both from the guard cells.

I. Introduction

The main environmental conditions which have been found to regulate the extent of stomatal opening are light intensity, the availability of water, and the concentration of CO_2 in the atmosphere surrounding the leaves.

High ambient CO_2 concentrations reduce stomatal aperture and lowering the concentration causes the aperture to increase. Why and how this occurs is not well understood. This paper reports work that was aimed at trying to understand how HCO_3^-/CO_2 could affect stomatal aperture.

Recent studies in whole plants (Sawhney and Zelitch 1969) and on leaf epidermal strips (Thomas 1970*a*, 1970*b*, 1971) suggest that stomata in tobacco leaves open in the light in response to a light-stimulated accumulation of K^+ in the guard cells. Similar results have been obtained in *Commelina communis* (Fujino 1967) and *Vicia faba* (Fischer and Hsiao 1968).

It is considered that the accumulation of K^+ reduces the guard cell water potential with respect to that of the surrounding water, and this leads to an influx of water, guard cell swelling, and stomatal opening.

The reduction in aperture caused by the presence of the cardiac glycoside ouabain (Thomas 1970*b*) and the stimulation of stomatal opening in the dark and light which occurs when epidermal strip material is left in contact with a stationary

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† As it is uncertain whether guard cells take up and metabolize CO_2 as CO_2 or the HCO_3^- ion the terminology HCO_3^-/CO_2 has been used.

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bathing solution containing K^+ and ATP (Thomas 1971) suggests that the accumulation of K^+ in tobacco guard cells is brought about, at least in part, by a transport ATPase system. In the light, photosynthetic phosphorylation might increase the supply of ATP to the K^+ accumulation mechanism and stimulate the processes by which the stomata open.

If guard cell swelling and stomatal opening is brought about by a net influx of ions and water, closing should occur when there is a net efflux. Ways in which the presence of CO_2 could increase the efflux relative to the influx might be by:

(1) Blocking the influx of ions or water or both, leaving the efflux unchanged.
(2) Changing the characteristics of the guard cell membranes so that they become leaky and increase the efflux of ions and water, or increase the efflux by some other means.
(3) Diverting a limited supply of ATP in the guard cells to carbon fixation and away from ion accumulation and other metabolic processes (e.g. those of the guard cell walls and membranes) which may be required to keep stomata open.
(4) Changing the ion-binding characteristics of the guard cell cytoplasm so that ions are lost from the cell.

This work considers more specifically alternatives 2 and 3.

II. Materials and Methods

These have been described by Thomas (1970*a*, 1970*b*, 1971). The method essentially monitors stomatal aperture by measuring the rate of flow of solution through the stomatal pores on isolated tobacco epidermal strip material in a solution-flow porometer. All bathing solutions were made using distilled freshly deionized water. Unless stated otherwise the pH of the bathing solution was adjusted to 6·4. HCO_3^-/CO_2 was supplied to the bathing medium by the addition of $KHCO_3$ and the concentration of CO_2 in solution was calculated from the equation $pH = 6{\cdot}4 - \log([HCO_3^-]/[CO_2])$ (Smith 1967). In most cases $KHCO_3$ was added at a concentration of $1{\cdot}4 \times 10^{-5}$M which gave a concentration of CO_2 in solution of 7 μM which is approximately the concentration of CO_2 found in water at equilibrium with normal air. ATP, ADP, and PEP were added to bathing solutions as sodium salts.

III. Experimental

(*a*) *Effect of Added $KHCO_3$*

Figure 1 shows the effect of adding an increasing concentration of $KHCO_3$ to the 10 mM KCl bathing solution in which the stomata of tobacco were opened in the light. It shows that with each increase in concentration the stomatal aperture is reduced. Over the concentration range tested the reduction in aperture is approximately linear with increasing concentration. This is similar to the effect of increasing the concentration of CO_2 in the atmosphere surrounding leaves (e.g. Heath and Milthorpe 1950). Flushing with and returning to a $KHCO_3$-free 10 mM KCl solution resulted in a rapid recovery in aperture. This suggests that the reduction in aperture caused by the presence of $KHCO_3$ is only transitory and causes no long-lasting impairment of the stomatal-opening mechanism. Stomata on leaves also show rapid and reversible change in aperture in response to decreases and increases in the ambient CO_2 concentration (Raschke 1966). In unpublished results and in Figure 4 it was

seen that similar effects can be obtained when $1 \cdot 4 \times 10^{-5}$M $KHCO_3$ was added to a 17·6 mM KH_2PO_4–Na_2HPO_4 buffer solution, pH 8·0, in which the stomata were opened in the light. At pH 8·0 the concentration of HCO_3^- would be approximately 40 times that in the KCl bathing solution and suggests that HCO_3^- is also effective in reducing stomatal aperture. As the addition of $KHCO_3$ to buffered bathing solutions results in a reduction in aperture, it seems unlikely that this reduction is caused by changes in the pH of the bathing solution.

Similar results were obtained when *Vicia faba* epidermal strips were used, or the bathing solution consisted of 5 mM K_2SO_4.

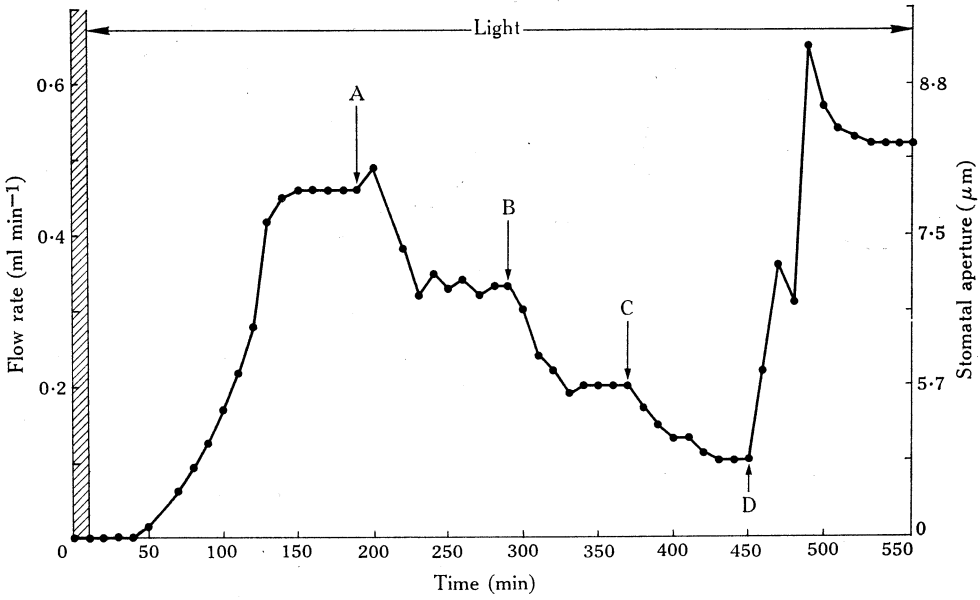

Fig. 1.—Effect of adding increasing concentrations of $KHCO_3$ (A, $0 \cdot 47 \times 10^{-5}$M; B, $0 \cdot 94 \times 10^{-5}$M; C, $1 \cdot 4 \times 10^{-5}$M) and its subsequent removal (D) on stomatal aperture. Basal bathing solution 10 mM KCl.

(*b*) *Effect of $KHCO_3$ in the Absence and Presence of ATP*

Figure 2 shows that the addition of 0·1 mM ATP can reverse the reduction in aperture caused by the presence of $KHCO_3$. In this experiment the stomata were opened in the light while bathed in a 10 mM KCl solution. When the stomata had reached an equilibrium opening the bathing solution was changed to one consisting of 10 mM KCl+$1 \cdot 4 \times 10^{-5}$M $KHCO_3$. This reduced the stomatal aperture. When the stomatal aperture had reached an equilibrium opening in the presence of the added $KHCO_3$, the flow of solution to the porometer was stopped and the epidermal strip allowed to remain in contact with the 10 mM KCl+$1 \cdot 4 \times 10^{-5}$M $KHCO_3$ solution remaining in the porometer for 2 hr. In this way the effect of any reduction in the concentration HCO_3^-/CO_2 by photosynthetic fixation in the guard cells might be followed. This treatment resulted in a partial recovery in the stomatal aperture. Subsequently 0·1 mM ATP was added to the bathing solution. The initial effect of

adding ATP was to cause a reduction in aperture. This effect has been described by Thomas (1971). It was considered that initially the addition of ATP caused a partial blockage along a common path of entry into the guard cells which was shared by ATP, K^+, or water or all three, and that it was not until ATP reached the inside of

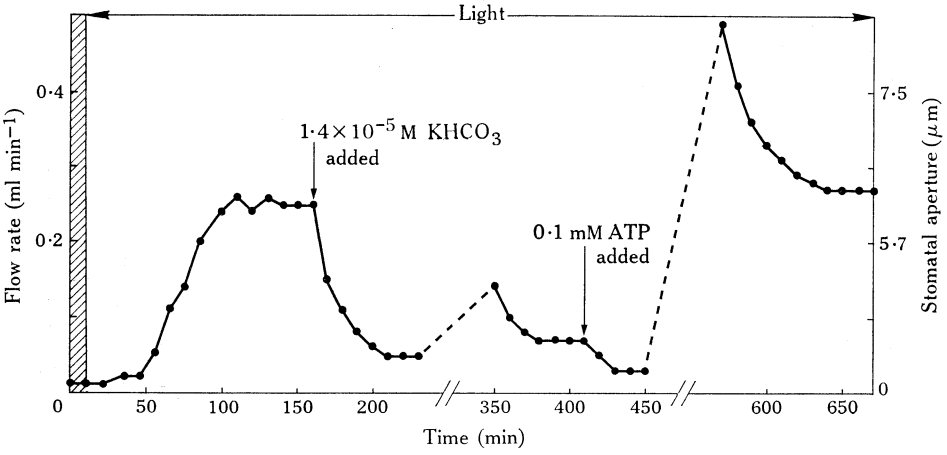

Fig. 2.—Effect of ATP on the stomatal closure caused by the presence of $KHCO_3$ in the light. Basal bathing solution 10 mM KCl. Periods during which flow was stopped shown by dashed lines.

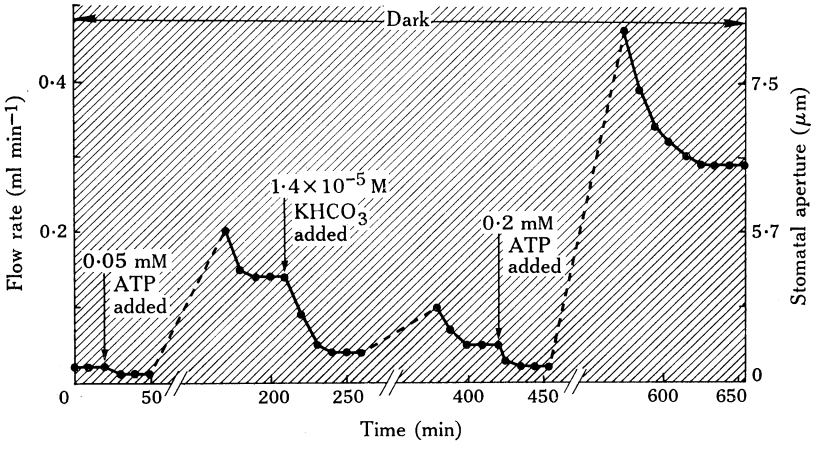

Fig. 3.—Effect of $KHCO_3$ and ATP concentration on stomatal aperture in the dark. Basal bathing solution 10 mM KCl.

the guard cell that it could stimulate stomatal opening. Stopping the continued flow of the 10 mM KCl+$1{\cdot}4\times10^{-5}$M $KHCO_3$+0·1 mM ATP solution for 2 hr resulted in the characteristic increase in aperture which is found in the presence of ATP (Thomas 1971).

Figure 3 shows the result of a similar experiment carried out in the dark. Initially the stomata were opened in the dark by the addition of a relatively low concentration, 0·05 mM, of ATP. After this treatment had resulted in an opening of the stomata, $1{\cdot}4\times10^{-5}$M $KHCO_3$ was added to the 10 mM KCl+0·05 mM ATP bathing solution. Stopping the continued flow of this solution to the epidermal strip for 2 hr resulted in only a small increase in aperture. Following this treatment the concentration of ATP was increased to 0·2 mM and the continued flow of the bathing solution stopped for 2 hr. This resulted in a much larger increase in aperture.

Hence it seems that the addition of ATP at adequate concentrations can reverse the stomatal closing caused by the presence of HCO_3^-/CO_2.

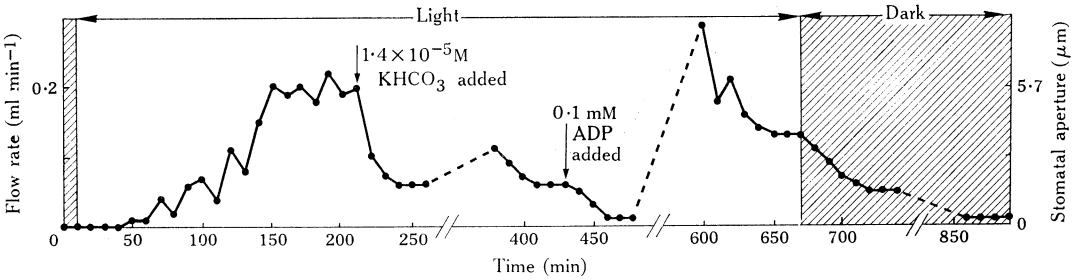

Fig. 4.—Effect of $KHCO_3$ and ADP on stomatal aperture in the light and dark. Basal bathing solution 17·6 mM KH_2PO_4–Na_2HPO_4 buffer, pH 8·0.

Figure 4 shows that the addition of ADP in the light is also effective in increasing stomatal aperture after the addition of $KHCO_3$ to a KH_2PO_4–Na_2HPO_4 buffer solution, pH 8·0, in which the stomata were opened, though ADP is not effective in opening the stomata in the dark. This is similar to the results obtained in the light and dark on the addition of ADP in the absence of HCO_3^-/CO_2 (Thomas 1971). The experiment suggests that under conditions in which more ATP might be synthesized, i.e. the addition of ADP in the light, the aperture can be increased even in the presence of HCO_3^-.

(*c*) *Effect of Phosphoenol Pyruvate (PEP) on HCO_3^-/CO_2-induced Closing*

In addition to ATP it has been found that the presence of PEP in the bathing solution can stimulate stomatal opening in the dark (Thomas 1971). In the experiment shown in Figure 5 the stomata were opened by the addition of 0·05 mM ATP to the 10 mM KCl bathing solution. After the treatment had caused stomatal opening and the stomata had reached an equilibrium opening $1{\cdot}4\times10^{-5}$M $KHCO_3$ was added to the bathing solution and this reduced the stomatal aperture. Stopping the continued flow of the bathing solution only caused a small increase in aperture (cf. Fig. 2) but with the subsequent addition of 0·1 mM PEP a much larger increase was obtained. Hence it seems that the addition of PEP in adequate amounts can reverse the stomatal closure caused by the presence of HCO_3^-.

(*d*) *Effect of $NaHCO_3$ Addition on Na^+-induced Stomatal Opening*

Previous work (Thomas 1970*a*, 1971) has shown that the opening of stomata in a NaCl bathing solution is different from that found when the medium contains K^+.

The stomatal opening which occurs in the presence of NaCl alone, particularly in the dark, was considered to be mainly due to a passive influx of Na^+ into the guard cells. It was also considered that there was an active Na^+ efflux mechanism present in the guard cells as the dark Na^+-supported opening could be reversed by exposure to light and in the presence of ATP, Na^+ could neither initiate nor maintain stomatal opening.

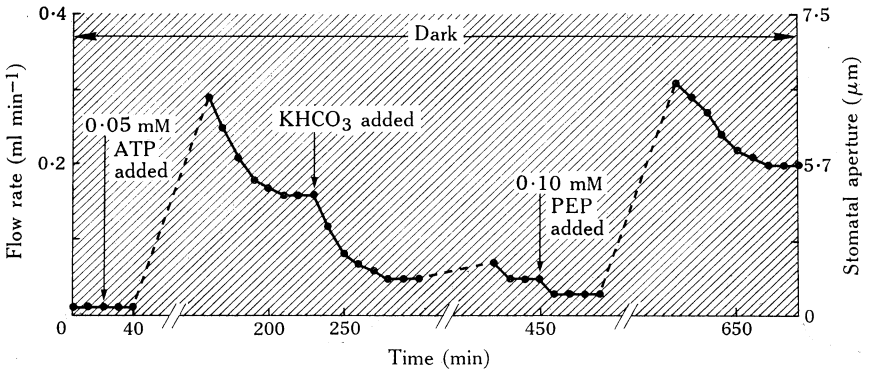

Fig. 5.—Effect of ATP, $KHCO_3$, and PEP addition on stomatal opening in the dark. Basal bathing solution 10 mM KCl.

Figure 6 shows the effect of adding $1 \cdot 4 \times 10^{-5}$M $NaHCO_3$ to the stomatal opening which occurs when epidermal strips are bathed in a 10 mM NaCl solution in the dark; its addition causes a partial closure of the stomata.

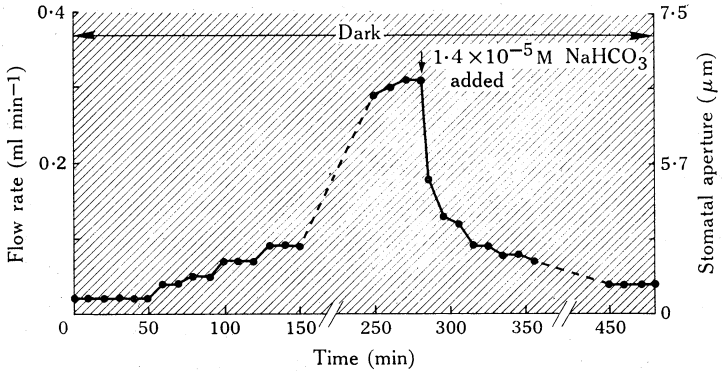

Fig. 6.—Effect of $NaHCO_3$ addition on stomata opened in the dark while bathed in a 10 mM NaCl solution.

$NaHCO_3$ was used in the experiment because it has been found (Thomas 1970*a*) that the addition of K^+ to stomata that have opened in a NaCl bathing solution causes stomatal closure.

The experiment indicates that, though there are considerable differences between K^+- and Na^+-supported stomatal opening, the effect of adding HCO_3^-/CO_2 was to cause a reduction in aperture.

IV. Discussion

The experiments shown in Figures 3–5 might be taken as evidence to show that the stomatal closure caused by the presence of HCO_3^-/CO_2 can be reversed by the addition of adequate amounts of "high energy" substrates such as ATP and PEP. This might be expected if, in the guard cells, ion-uptake processes competed with carbon fixation for a common source of ATP, particularly if the supply of ATP to carbon fixation was favoured with respect to that of ion accumulation. The fixation of CO_2 has a larger ATP and energy requirement, 114–115 kcal mole^{-1} CO_2 reduced, than ion-uptake processes. Raven (1967) gives estimates of 15–16 kcal mole^{-1} of ions accumulated. With this hypothesis it might be considered that while carbon was being fixed ATP is not supplied to the K^+-accumulation process in amounts that are adequate to maintain the K^+ accumulation at the same rate as in the absence of HCO_3^-/CO_2. This might lead to a net efflux of K^+ until a new equilibrium level between influx and efflux was reached. However, this hypothesis does not explain why the presence of HCO_3^-/CO_2 causes a reduction in the stomatal opening which occurs in a NaCl bathing solution (Fig. 6). As ATP neither initiates nor maintains stomatal opening when Na^+ is the only cation in the bathing medium (Thomas 1971) it seems unlikely that competition between carbon fixation and ion accumulation for a common source of ATP could explain the observed reduction in stomatal aperture. In fact if the presence of HCO_3^-/CO_2 drew ATP away from a Na^+-efflux mechanism (Thomas 1971), it might be expected that more Na^+ might accumulate in the guard cells and cause an increase in aperture rather than the observed decrease.

At any time the extent of stomatal opening can be controlled by the net influx of ions and of water into the guard cells. The effect of HCO_3^-/CO_2 is, apparently, to reduce this net influx. An increased supply of energy substrates could increase the net influx and the carbon fixation and reverse the effects of additions of HCO_3^-/CO_2.

It has been found by Glinka and Reinhold (1964) and Reinhold and Glinka (1966) that solutions saturated with CO_2 can change the hydraulic permeability of plant cell membranes and cause an efflux of water. They consider that these changes are brought about by alterations in the cell wall matrix. If guard cell membranes were similarly affected by lower concentrations of CO_2 it might explain the reduction in aperture caused by CO_2.

The presence of CO_2, although it reduces the extent of light opening of stomata on *Commelina communis* epidermal strips, did not greatly reduce the K^+ content of the guard cells (Willmer and Mansfield 1970). This suggests that the effect of CO_2 might be to increase the hydraulic permeability of the guard cells.

Work on theoretical grounds, e.g. by Weiss (1969), considers that on their passage through membranes solute and solvent ions and molecules can cause many changes to occur within the membrane. They could change the elastic properties and the permeability of the membrane by altering their matrix structure. Perhaps changes such as these may take place in guard cell membranes in the presence of HCO_3^-/CO_2.

For guard cell swelling to occur configurational changes may be required in the cell wall and membranes and these changes may require ATP (Thomas 1971). ATP may also be required to reverse the effects of HCO_3^-/CO_2 on membranes.

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