

INHIBITION OF STOMATAL OPENING BY DL-GLYCERALDEHYDE, AN INHIBITOR OF CALVIN CYCLE

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ABSTRACT

DL-glyceraldehyde, an inhibitor of Calvin cycle was fed to the leaves of *Commelina bengalensis* L. This treatment inhibited light-dependent oxygen evolution and stomatal opening. However, it did not cause stomatal closure in isolated epidermal strips. By contrast, application of 3-(3,4-dichlorophenyl)-1,1-dimethyl urea caused stomatal closure to both the leaves and the isolated epidermal strips. Taken together, these observations suggest that (a) the mechanism controlling the stomatal closure in intact leaves is different from that in isolated epidermal strips and (b) DL-glyceraldehyde can be used as a tool to study the stomatal function.

INTRODUCTION

DL-GLYCERALDEHYDE is an inhibitor of Calvin cycle and it does not affect the photochemical events in photosynthesis¹. This inhibitor, when fed to the leaves through transpiration stream, can bring about total inhibition of carbon assimilation as revealed by changes in the slow secondary fluorescence kinetics of intact leaves². In this paper we describe the effect of this Calvin cycle inhibitor on stomatal opening both in intact leaves and in isolated epidermal strips.

EXPERIMENTAL

Leaves of *Commelina bengalensis* L were collected fresh from the field grown plants. The leaves were floated on distilled water under a bank of fluorescent lamps (10,000 lux) for 2 hr. After this pre-illumination, leaves were kept in various experimental solutions with their petioles dipped. Pure distilled water served as the control and DL-glyceraldehyde (Sigma, USA) and DCMU (Dupont, USA) were used at the concentration of 20 mM and 50 μ M respectively. At different times the epidermis was removed and the pore width of the stomata was measured using a light microscope fitted with a precalibrated oculo-meter.

Photosynthesis was measured as the light-dependent rate of oxygen evolution from the leaf bits (1 mm²) using a Clark type oxygen electrode (Yellow Spring Instruments, USA) connected to a locally made amplifier and a strip-chart recorder (Toshniwal Bros, India). During the measurement of oxygen exchange, the leaf bits were kept stirred in the respective experimental solution.

RESULTS AND DISCUSSION

When the cut ends of the leaves were kept dipped in 20 mM solution of DL-glyceraldehyde, complete stomatal closure occurred in about 90 min (figure 1). After this period, the photosynthetic capacity of the leaves was also found completely inhibited (table 1). However, DL-glyceraldehyde could not cause stomatal closure in isolated epidermal strips (figure 2).

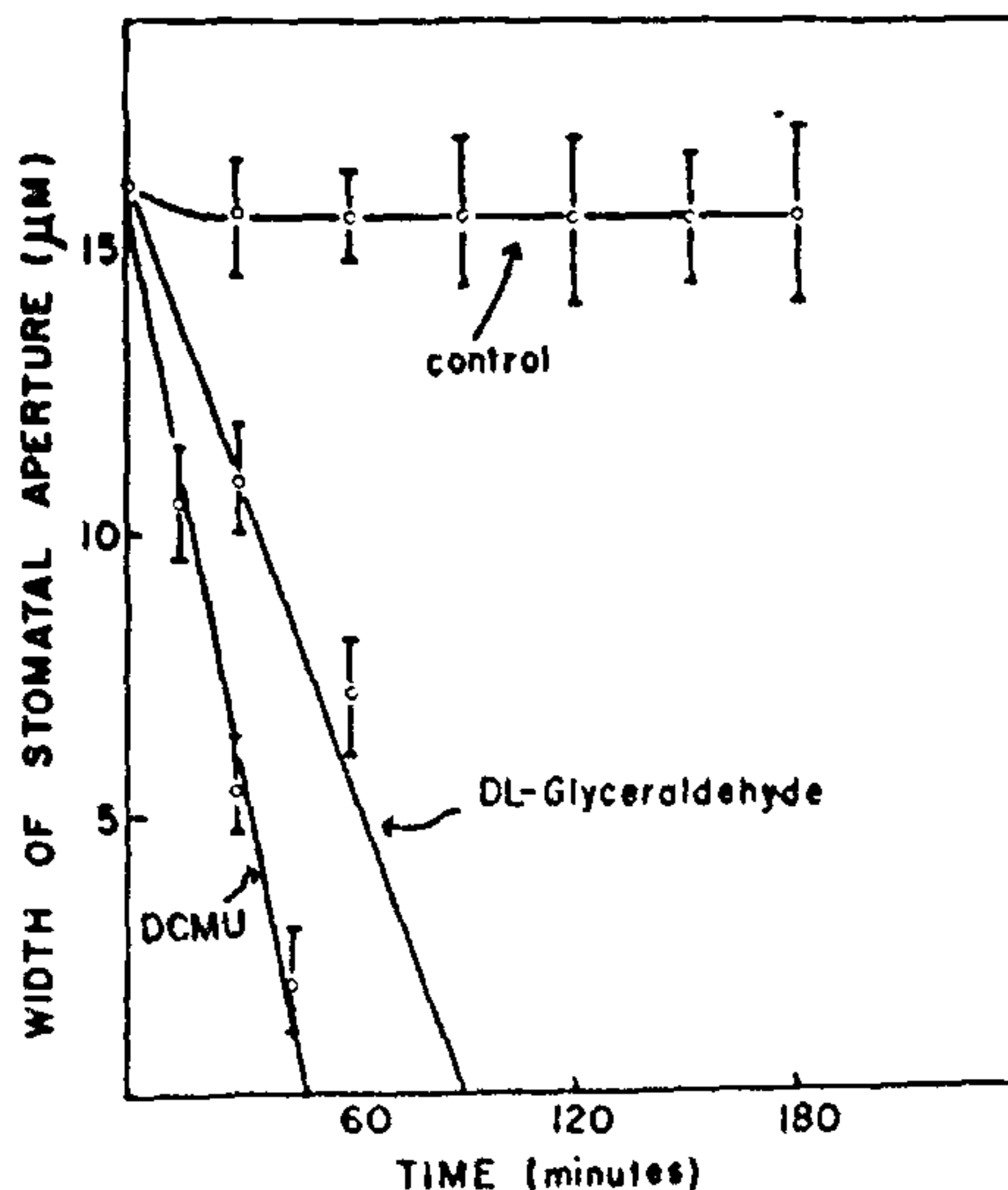
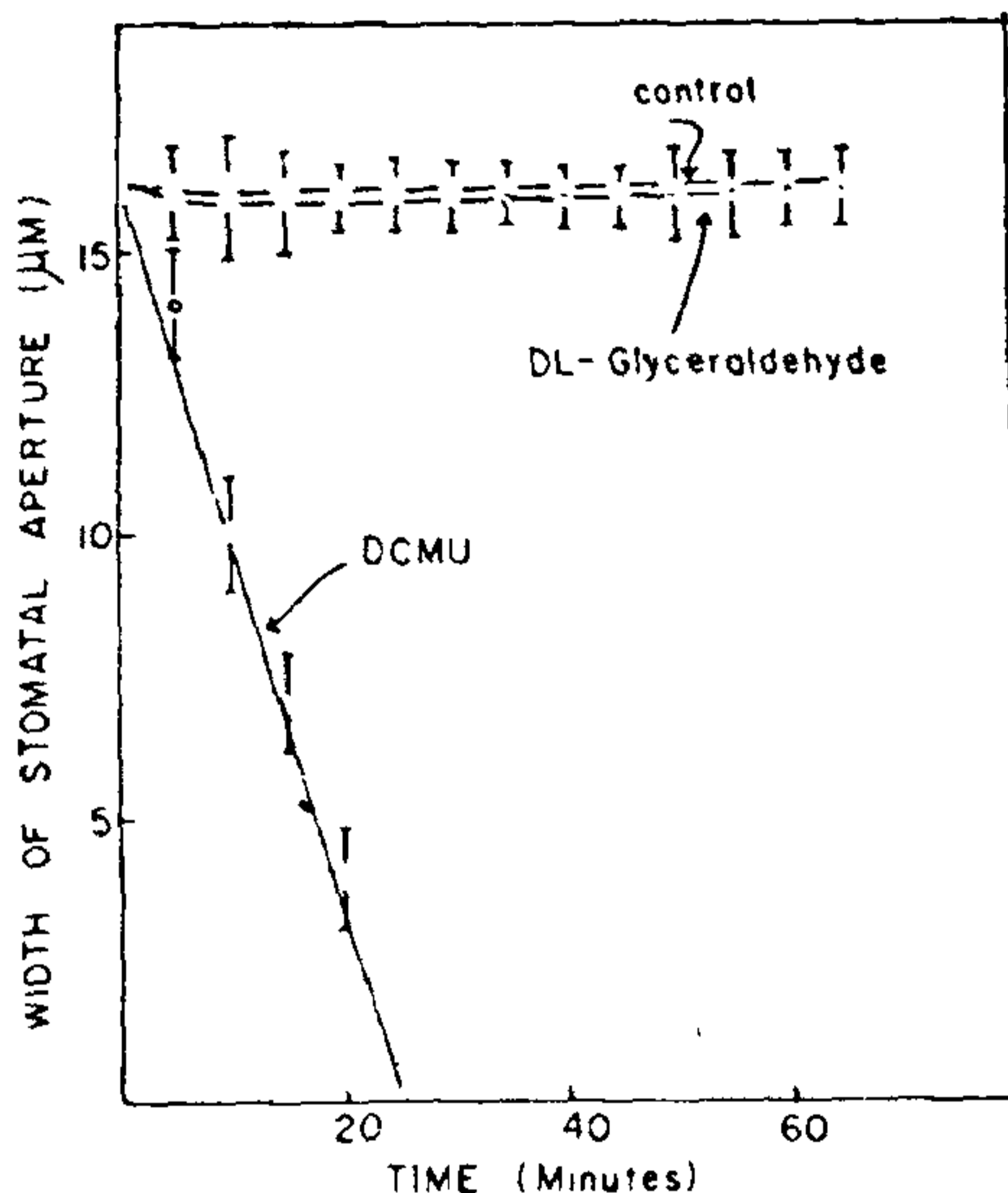


Figure 1. Effect of DCMU and DL-glyceraldehyde on stomatal opening in intact leaves.

Table 1 Effect of DL-glyceraldehyde and DCMU on photosynthesis and respiration

	Respiration $\mu\text{mol O}_2$ consumed/mg Chl/hr	Photosynthesis $\mu\text{mol O}_2$ evolved/mg Chl/hr
Control	23.3	21.3
DL-glyceraldehyde	22.9	Not detectable
DCMU	23.2	Not detectable

**Figure 2.** Effect of DCMU and DL-glyceraldehyde on stomatal opening in isolated epidermal strips.

For comparison, the effect of DCMU, a specific inhibitor of non-cyclic photosynthetic electron transport, on stomatal opening and photosynthesis was followed. DCMU was found to be more potent than DL-glyceraldehyde in inducing the stomatal closure. DCMU could bring about stomatal closure in intact leaves within 30 min while it took about 90 min for DL-glyceraldehyde (figure 1). Further, DCMU could bring about stomatal closure even in the isolated epidermal strips (figure 2).

Earlier experiments have shown that stomatal opening in isolated epidermal strips is driven by a common pool of ATP contributed from oxidative and photo-

phosphorylation³. Recent observations have indicated that the guard cell chloroplasts contain a functional photosystem II and operate a DCMU sensitive electron transport⁴⁻⁸. In the light of these information, our observation on the effect of DCMU in the isolated epidermal strips indicates the significance of non-cyclic photosynthetic electron transport in maintaining the stomatal opening.

The inability of DL-glyceraldehyde in inducing the stomatal closure in isolated epidermal strips could be attributed to the fact that the Calvin cycle is not operative in the guard cell chloroplasts⁴. Both micro-histochemical⁹ and immunological¹⁰ approaches have shown that the guard cell chloroplasts lack the key enzymes of Calvin cycle.

One could explain the stomatal closure induced by DL-glyceraldehyde in intact leaves in terms of a possible correlation existing between mesophyll photosynthesis and stomatal conductance. Wong *et al*¹¹ suggested that stomata respond to a metabolite of photosynthesis in the leaf mesophyll tissue. They have shown that when the capacity of the leaves to fix CO₂ is altered by various means, the diffusive conductance of the epidermis is also changed in nearly the same proportion as the rate of CO₂ assimilation. However it should be pointed out that the approaches of Wong *et al*¹¹ to modulate the CO₂ fixation capacity of the mesophyll were not specific to mesophyll. All their three methods to modulate CO₂ fixation in mesophyll namely, DCMU treatment, different levels of irradiance and difference in water potential and nutrient status are known to bring about stomatal closure even in isolated epidermal strips^{12,13}. In contrast, in our experiments, the application of DL-glyceraldehyde specifically inhibited the mesophyll photosynthesis without having any effect on the chloroplasts of guard cells. Thus our observation lends support to the suggestions of Wong *et al*¹¹ more than their own experimental observation and also stands as a new method to specifically manipulate the CO₂ fixation capacity of the mesophyll cells in the study of stomatal function.

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