
TIME COURSE OF THE PHOTOSYNTHETIC INDUCTION PERIODS IN CERTAIN HIGHER PLANTS AS RELATED TO CHANGES IN DEGREE OF STOMATAL OPENING¹

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The so-called "induction period" of photosynthesis usually refers to the interval of time required for the photosynthetic process to establish a constant velocity after the plant has been exposed to light. An induction experiment thus involves subjecting the plant to darkness for a given time interval and then recording the changes in the photosynthetic rate during the following period of illumination. The length of this induction period and the characteristic changes in induction curves have been extensively studied in both algae and higher plants by using CO₂ absorption or O₂ evolution as indices of photosynthesis. Van der Veen (1950), McAlister and Myers (1940), and certain others have used the absorption and evolution of CO₂ and O₂ as indices of the photosynthetic mechanism itself in higher plants such as *Holcus lanatus* and *Triticum vulgare* respectively. The results of these experiments with algae and higher plants have been compared as indices of the course of photosynthesis in such organisms (Rabinowich, 1956; Van der Veen, 1950).

However it has also been found that the diffusion of gases in the leaves of higher plants is often limited by the degree of stomatal opening. The extent to which changes of stomatal width modify the diffusion of gases during the first minutes of illumination must be known before gas evolution and absorption studies can be used as valid indices of photosynthesis or of photosynthetic induction patterns. It has been noted by several workers that stomata rapidly close in the darkness if the leaf is surrounded by normal air, but remain open for long periods of time if the leaf is supplied a gas stream deficient in O₂ (Scarth et al., 1933); deficient in CO₂ (Linsbauer, 1917; Freudenberger, 1940; Heath 1948, 1949, 1951, 1959); or deficient in both O₂ and CO₂ (Howe, 1959). Stålfelt (1932) demon-

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strated that the diffusive capacity of the stomata increased with increasing stomatal width (using stomatal transpiration as an index).

In the present work photosynthetic induction was studied while stomata were apparently open and while they were closed. The leaves of several greenhouse angiosperms were subjected (upon turning off the lights) to modified air streams in which stomata have been found to remain open in the darkness. The induction curves of photosynthesis after these dark pretreatments with modified air streams and after dark pretreatments in normal air have been recorded. It has thereby been possible to estimate the role that stomatal change or "stomatal induction" might play in modifying the photosynthetic induction period.

MATERIALS AND METHODS

The five species of plants used were *Phaseolus vulgaris* L. var. Black Valentine, *Coleus blumei* Benth., *Ricinus communis* L., *Pelargonium zonale* Ait., and *Kalanchoë pinnata* Pers.

A particular plant or group of plants was selected for each experiment and transferred from the greenhouse to the laboratory. An attached leaf of the experimental plant was inserted in a leaf chamber of the type used by Böhning and Burnside (1956) and was sealed in place with mortite.

The apparatus used in measuring "true photosynthesis" consisted of a cylinder of compressed gas connected by tygon tubing to a plexiglas leaf chamber, a drying tube, a dust filter, a model 15 Liston-Becker infrared gas analyzer, and a Sargent wet test gas flow meter.

The desired light intensity was obtained by placing the lights at various distances from the leaf chamber. Before experimentation, the illumination was measured with a Weston illumination meter by placing the photocell on the upper surface of the leaf chamber. Values of illumination thus obtained were slightly higher than the light intensity at the leaf surface which was about one inch lower than the top surface of the chamber. The light intensity used in all experiments was 2200 ft-c. An analysis of the transmission spectrum of a 10-cm thick CuSO_4 solution layer is given by Cressman (1957) who has adapted certain data of Withrow (1953). No significant portion of the visible spectrum is removed by the filter, while most radiation beyond 750 $\text{m}\mu$ is removed. The leaf was darkened by switching the flood lamps off and covering the chamber with a black cloth.

The following gases: (1) "CO₂-deficient, O₂-deficient air" (technical grade N₂, no measurable CO₂ and low O₂ content); (2) "CO₂-deficient air" (technical grade bottled air passed through a column containing 0.2 N NaOH solution yielding a gas stream containing approximately 20 percent O₂ and no measurable CO₂ content); (3) "O₂-deficient air" (technical grade CO₂ blended with technical grade N₂ yielding a gas stream of about 0.03 percent CO₂ and low O₂ content); and (4) "normal air" (supplied from a tank of technical grade water-pumped compressed air—0.03 percent CO₂ and 20 percent O₂ content) were supplied at the instant the leaf was darkened. Gas analysis was by volume percent. The effect of reduced CO₂ concentration, reduced O₂ concentration, or reduced O₂ and CO₂ concentration together, of the gas supplied during the dark period upon subsequent photosynthetic induction in air (79 percent N₂, 20 percent O₂, and 0.03 percent CO₂) was thus measured. A flow rate of 22.6 liters/hr was used with all gases.

A continuous record of the microampere readings during each experiment was obtained with an Esterline-Angus recorder. The microampere readings were converted to parts per million of CO₂ by using a standard curve for the analyzer.

To calculate the rate of "true photosynthesis" the CO₂ content of the gas passing over the illuminated leaf was substrated from the CO₂ content of the gas passing over the darkened leaf. This quantity (ΔCO_2) was represented in milligrams of CO₂ per square decimeter of leaf area per hour—CO₂ absorbed $\text{mg}(\text{dm})^{-2}(\text{hr})^{-1}$. The area of only one leaf surface was used in all calculations.

Points used in graphing were chosen from the recorder graph (which had a curved ordinate), replotted on perpendicular axes, and connected by a smooth curve since the data were continuous. Such replotting enables simultaneous viewing and comparison of groups.

At the end of the experiment the leaf was traced and the leaf area was estimated by measuring the area of the tracing with a compensating polar planimeter. At the conclusion of certain experiments the experimental leaf was given a final dark treatment in one of the aforementioned atmospheres. At the end of the treatment the leaf was rapidly removed from the leaf chamber and benzene applied to determine the aggregate condition of the stomata. The benzene infiltration technique of Molisch (1912) has been adequately studied. Both its advantages and disadvantages have been clarified. Although liquids of various surface tensions can be used to find the relative degree of stomatal opening (Bonner and Galston, 1952), it was presently decided to use benzene in all such tests. Upon occasion the results of such benzene tests were compared to the results of direct microscopic stomatal analysis. It was found that benzene infiltration occurred readily when stomata were open but occurred slowly or not at all when stomata were closed.

RESULTS AND DISCUSSION

Two typical experiments are presented for discussion since a complete analysis of all experiments is given in Howe (1959). By comparing the induction curves in figure 1 it can be seen that following a dark period in CO₂-deficient air or in CO₂-deficient, O₂-deficient air, the rate of photosynthesis was high during the first minutes of light. But following dark intervals in normal air, photosynthetic rates were low during the induction period.

Figure 2 demonstrates the effect of O₂-deficient air upon induction. After 30 min darkness in either CO₂-deficient air, O₂-deficient air, or in CO₂-deficient, O₂-deficient air, greater rates of photosynthesis are evident during the induction than following pretreatment in normal air.

In examining these two figures it is seen that true photosynthesis in figure 2 is significantly higher than true photosynthesis in figure 1. In table 1 the relationship between true photosynthesis, apparent photosynthesis, and respiration rate is demonstrated for both figures. It was found in the other experiments (Howe, 1959) that the rate of respiration was always small and rather uniform as seen here; therefore true photosynthetic data were used for simplicity. The difference between maximum true photosynthesis between these two figures is thus probably attributable to differences between individual plants, growing season, etc., and not to gross differences in respiration rate.

After a dark interval in air of low CO₂ and/or low O₂ content the stomata were generally open and the rate of photosynthesis was high during the first minutes of illumination. Following a similar dark interval in normal air the stomata were generally closed and photosynthetic rates were low during the induction period. In Black Valentine bean and geranium this correlation was frequently but not always evident. In *Coleus* the results were similar to those in bean and geranium although not as pronounced.

In other experiments (Howe, 1959) it was found that stomatal opening in Black Valentine bean after a protracted period of darkness in normal air required 8 to 14 min for completion. It was also found that stomata were generally open after treatments in the modified air streams mentioned. More studies of stomatal condition are needed. Such stomatal-induction correlations were not consistently evident in castor bean cotyledons.

In their studies of photosynthesis McAlister and Myers (1940) simultaneously plotted CO₂ absorption and fluorescence during the induction period of photosynthesis in wheat leaves. They found that the rate of photosynthesis was

accelerated more rapidly when the O₂ concentration of the gas stream was reduced. Van der Veen reported in a series of papers that in leaves of *Holcus lanatus* (1949a, 1949b) and other higher plants (1950) lag periods and fluctuations occurred in the rate of the photosynthetic process during induction (as measured by

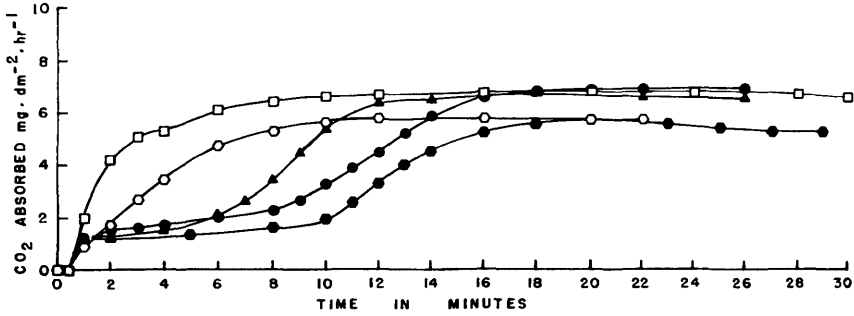


FIGURE 1. Time course of photosynthesis in Black Valentine Bean following dark periods in CO₂-deficient, O₂-deficient air; CO₂-deficient air; and normal air. The primary leaf of a plant was used 18 days after planting date. Date: December 9, 1958. Leaf Area: 0.49 dm². Light intensity: 2200 ft-c.

Pretreatments:

- In darkness without CO₂
- ◊ In darkness without O₂ or CO₂
- ▲ In darkness with air (CO₂ and O₂)
- In darkness with air (CO₂ and O₂)
- In darkness with air (CO₂ and O₂)

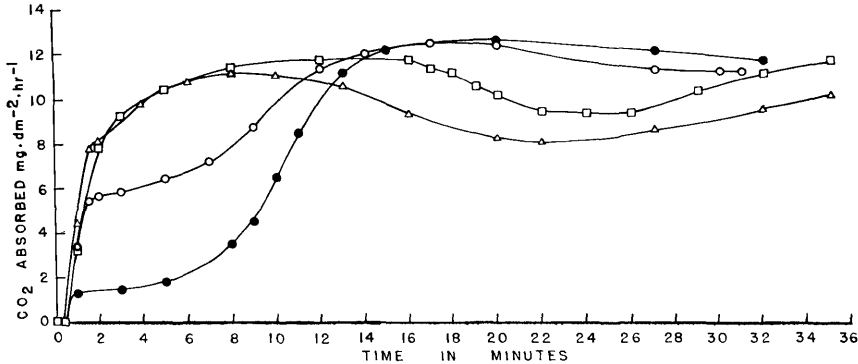


FIGURE 2. Time course of photosynthesis in Black Valentine Bean after dark periods in CO₂-deficient air, O₂-deficient air, N₂, and normal air. The primary leaf of a plant was used 19 days after planting. Date: March 5, 1959. Leaf Area: 0.38 dm². Light intensity: 2200 ft-c.

Pretreatments:

- △ In darkness without O₂
- In darkness without O₂ or CO₂
- In darkness without CO₂
- In darkness with air (CO₂ and O₂)

At the conclusion, the experimental leaf was exposed to a gas stream of N₂ for 30 min in the darkness while the opposite primary leaf was in the air (also in darkness). Benzene was applied to the under surface of each with the following result:

Time in seconds after application of benzene:

	0	75
Experimental leaf (in N ₂)	complete infiltration	complete infiltration
Check leaf (in air)	no infiltration	slight infiltration

diaphragm techniques.) He also found that pretreating the plants in air of low O_2 content partially overcame the lag in photosynthesis. He explained this effect of low O_2 content in terms of what he called an "adaptation factor" (1949b) in photosynthesis. He explained crests and troughs in the photosynthetic induction curves in terms of the photosynthetic mechanism itself. Thus certain workers have moved directly from induction curves to inferences concerning the biochemistry of photosynthesis.

On the basis of the present results it appears that the "lag" or long induction of photosynthesis found in higher plants could well be attributable simply to the slow opening of stomata. It is thereby understandable why such lag periods and continued fluctuations were not encountered by Van der Veen (1950) when a morphologically simple species such as *Chlorella* was used. It is also possible

TABLE 1
The relationship between the rate of true photosynthesis, respiration, and apparent photosynthesis in Black Valentine bean leaves for the maximum values of figures 1 and 2

	True photo. CO ₂ absorbed: mg (dm) ⁻² (hr) ⁻¹	Avg. dark resp. CO ₂ evolved: mg (dm) ⁻² (hr) ⁻¹	Apparent photo. CO ₂ absorbed: mg (dm) ⁻² (hr) ⁻¹
Fig. 1	8.09	1.53	6.56
Fig. 2	13.5	1.70	11.8

TABLE 2
Rate of CO₂ evolution in Black Valentine bean leaves during a 2-min interval of darkness in normal air, following dark pretreatment with certain gas streams (data from same experiment shown in figure 1)

Previous dark pretreatment	CO ₂ evolution: mg (dm) ⁻² (hr) ⁻¹
CO ₂ -deficient, O ₂ -deficient air	0.65
CO ₂ -deficient air	0.65
Normal air	1.53

that the crests and troughs of the curves published by Van der Veen (1949b) are attributable to stomatal changes rather than to inhibition or acceleration of photosynthesis at the level of the chloroplast.

It could be possible at this point that the enhancement effect of low O_2 and/or CO_2 concentration upon subsequent induction in air is a direct enhancement of photosynthesis at the level of the chloroplast. In fact, the enhancement following low O_2 pretreatment in particular could be attributed to the well-known enhancement of photosynthesis at low O_2 partial pressures (McAlister and Myers, 1940). However, the enhancement of photosynthesis after CO_2 -deficient air pretreatment is not easily explained by attributing the enhancement to a direct effect upon mesophyll chloroplasts. Rather it would seem that 15 to 60 min exposure to CO_2 -deficient air would effectively decrease the concentration of CO_2 within the mesophyll and would thereby lead to a reduced rate of photosynthesis upon illumination, rather than the greatly enhanced rates that were measured.

It might be reasoned also that the pretreatment with either low O_2 or low CO_2

causes an abundance of ribulose diphosphate (potential CO_2 acceptor) to arise in the cells. This would result from the low level of CO_2 in the tissues which either pretreatment would induce. Then, when CO_2 is supplied, a rapid uptake of CO_2 which results would simply reflect absorption of CO_2 by this abundance of CO_2 acceptor, while true photosynthesis would be much slower than the rate of CO_2 uptake. This hypothesis has been tested in each experiment by virtue of the experimental design. After every pretreatment in either low O_2 , low CO_2 , or low O_2 and CO_2 , the plant in the darkness was subjected to 1 or 2 min of normal air before illumination. If the above hypothesis were correct, one would expect a vigorous CO_2 uptake in the darkness during the 2-min interval of air before illumination. In actuality however, there was frequently an evolution of CO_2 during that time, as seen in the data of table 2 which relate to figure 1 of this article. Comparable rates of CO_2 evolution or very slight uptake were noted in other experiments, but in no case was there a rapid uptake of CO_2 after such pretreatments.

Such enhancement finds ready explanation if it is attributed to a stomatal mechanism. No major treatment of stomatal mechanism will be attempted as several adequate ones are now available (Kettelapper, 1959; Stålfelt, 1956). Low O_2 content in the gas streams presently used would lead to a reduced respiratory rate of the mesophyll and would thereby lead to reduced CO_2 production by these cells, effectively reducing the CO_2 concentration in the vicinity of the guard cells. Circulating air of low CO_2 content would also reduce the CO_2 concentration in the vicinity of the mesophyll and guard cells, also contributing to keeping stomata open. Use of normal air would correspondingly contribute to stomatal closure.

Although these data are by no means conclusive proof for either of the above views, it has been suggested how reduced CO_2 pretreatment would lead to open stomata and thereby afford a ready diffusion pathway during the subsequent induction period. However, there is no other apparent mechanism by which a reduction of CO_2 content during the dark period would greatly enhance the rate of photosynthesis at the level of the chloroplast.

On the basis of these experiments it becomes evident that direct inferences concerning the biochemistry of photosynthesis per se cannot be reasonably drawn on the basis of changes in shape of the photosynthetic induction curves measured in higher plants unless the degree of stomatal opening and stomatal change is also known. If the stomata are closed at the onset of an induction experiment, the induction curve of stomatal opening in light will be superposed upon the induction curve of photosynthesis. The lags and fluctuations found in gas evolution and uptake studies using higher plants may have their basis in the changing diffusive capacity of the stomata. The induction period of stomatal opening in light could thereby mask the induction period of the photosynthetic process as it occurs at the level of the chloroplast.

SUMMARY

Comparisons of the induction curves of true photosynthesis in the attached leaves of Black Valentine bean, *Coleus*, and geranium reveal correlations between the type of gas supplied during the darkness and the rate of photosynthesis during subsequent illumination in normal air. After dark intervals in air of low CO_2 and/or of low O_2 content the stomata were generally open and the rate of photosynthesis was high during the first minutes of illumination. Following similar dark intervals in normal air the stomata were generally closed and photosynthetic rates were low during the induction period.

In Black Valentine bean and geranium (but not in castor bean cotyledons) such changes in stomatal diffusive capacity were accompanied by profound modifications in the time course curves of photosynthetic induction. It is reasoned

that if stomatal closure occurs at the onset of an induction experiment, an induction curve of stomatal opening in light will modify the induction curve of the photosynthetic process itself. More studies of stomatal condition are needed.

Evidence is presented that certain of the lag periods and fluctuations reported in the photosynthetic induction curves for higher plants might be due to changes in the stomatal diffusive capacity. Further studies in this area are necessary. The condition of the stomata must be known before valid inferences concerning the process of photosynthesis can be drawn on the basis of induction curves in higher plants.

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