TIME COURSE OF PHOTOSYNTHETIC RHYTHMS IN *PHASEOLUS VULGARIS* L. AS RELATED TO CHANGES IN DEGREE OF STOMATAL OPENING[1, 2]

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During the course of photosynthetic induction experiments previously reported (Howe, 1959) rhythmic fluctuations in the rate of photosynthesis in bean leaves were encountered. Continued fluctuations in photosynthetic rate were found upon two occasions to correlate with the degree of stomatal opening. In the present paper these data are reported and literature pertinent to the problem of photosynthetic and stomatal rhythms is reviewed. It is argued that rhythmic fluctuations of guard cells cause concurrent fluctuations in the rate of photosynthesis.

MATERIALS AND METHODS

The same experimental method was employed in these experiments as in Howe (1962). Plants of *Phaseolus vulgaris* L. var. Black Valentine were used. "True photosynthesis" was measured in attached leaves by using cylinders 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 wet test gas flow meter. A gas flow rate of 22.6 liters per hour was used. In certain phases of these experiments, the leaf was subjected to special air mixtures deficient in O_2, CO_2, or both O_2 and CO_2. These gas mixtures were used since they have been demonstrated to affect the degree of stomatal opening and time course of photosynthesis. 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 high during the first minutes of illumination (Howe, 1962). Following similar dark intervals in normal air the stomata were generally closed and photosynthetic rates were low during the induction period.

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Experimental leaves were subjected to a light intensity of 2200 ft-c filtered through a 10-cm thick $CuSO_4$ solution in a plastic bath. (No significant portion of the visible spectrum is removed by the filter, while most radiation beyond 750 mμ is removed.) The microampere readings were monitored on an Esterline-Angus recorder. The recorder curves themselves have been mounted on time scales and photographed Since the recorder curves are in milliampere units, and not in actual photosynthetic units, a decrease in CO_2 content of the gas stream has been labeled "relative photosynthesis" The actual rates of photosynthesis at various phases of these recorder curves have been calculated and are included in the respective figure captions to make the photographs of more quantitative value. Also included in one figure is the rate of dark respiration—CO_2 evolved: mg $(dm)^{-2}(hr)^{-1}$.

To calculate the rate of "true photosynthesis" for the captions, the CO_2 content of the gas passing over the illuminated leaf was subtracted from the CO_2 content of the gas passing over the darkened leaf. This quantity (ΔCO_2) was represented in milligrams of CO_2 per square decimeter of leaf area per hour—CO_2 absorbed mg $(dm)^{-2}(hr)^{-1}$. The area of only one leaf surface was used in calculations.

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 rapidly removed from the leaf chamber and benzene applied to determine the aggregate condition of the stomata. The benzene infiltration technique of Molisch (1912) was thus used as in Howe (1962).

RESULTS AND DISCUSSION

Diminishing Rhythms

In certain experiments, the rate of photosynthesis fluctuated between high and low levels for 30 min or more after the onset of illumination. These fluctuations will presently be called "diminishing rhythms" since they vanished after some time in the light after which a steady rate of photosynthesis prevailed. Such a diminishing photosynthetic rhythm can be seen in figure 1. In this experiment such rhythms resulted after previous dark treatment in either commercial N_2 (O_2 and CO_2 deficient air) or in O_2 deficient air. Peaks 1 and 2 occurred at times 10 and 35 min respectively, while trough number 1 occurred at 21 to 24 min after the onset of illumination.

In figure 2 such a rhythm also appeared during illumination following 30 min of darkness in normal air. At times 24, 61, and 93 min respectively the three peaks appeared, with intervals between the peaks of 37 and 32 min duration.

Such diminishing rhythms have been noted in the photosynthetic rates of higher plants by at least two other workers. Van der Veen (1949a) (1949b) measured the changes in CO_2 content of a gas stream passing over leaves of tobacco, *Sciadopitys*, and *Holcus lanatus* during the induction period of photosynthesis. He used the diaferometer (heat conductivity) method of Aufdemgarten and a stream of air which was saturated with water vapor and had a CO_2 concentration of 3 per cent. After illuminating leaves of tobacco or *Holcus lanatus* a series of fluctuations in the rate of photosynthesis was apparent for several minutes. Van der Veen applied names to various stages of these fluctuations, and studied the effects of temperature, length of preceding dark period, content of air stream, and intensity of light upon the fluctuations. The amplitude of the fluctuations he found was greater with increasing temperatures. As the dark period became longer, the time of the appearance of each particular phase of the rhythm became later. At 300 lux (about 279 ft-c) no fluctuations were present, while at 30,000 lux (2790 ft-c) they were distinctly evident. Van der Veen (1949a) found that

the steady-state of photosynthesis ensued after about 6 or 7 min of illumination of tobacco leaves at 20 C. During this 6-min induction period as many as 2 peaks and 2 troughs were evident. Van der Veen (1949b) found that at 2790 ft-c a steady-state of photosynthesis prevailed in the leaves of *Holcus lanatus* after about 7 or 8 min of illumination at 2790 ft-c after a dark period of 1 min. During the first 8 min as many as three peaks and three troughs in the rate of CO_2 absorption were evident. Van der Veen attempted to explain the crests and troughs in the photosynthetic induction curves in terms of the photosynthetic mechanism itself. He theorized concerning the interaction of an "inhibitor" and a photosynthetic "activator." However, Van der Veen's data for induction in *Chlorella* (1950) show no diminishing rhythms.

McAlister and Myers (1940) report the existence of diminishing rhythms in the induction period of CO_2 absorption in wheat leaves. They found that the fluctuations were apparent following 10 min darkness in air or nitrogen of 0.36 per cent CO_2. The rhythms were not apparent after similar treatments in air or N_2 of 0.03 per cent CO_2. When the rhythm was present, the first peak was reached at about 1 min after illumination, and the trough occurred after about 2 min of illumination. After one such peak and trough a steady-state prevailed from 3 min after the onset of illumination. Their particular experiments were performed at a "high light intensity." Their induction curves for *Chlorella*, however, show no diminishing rhythms. One single deflection in photosynthetic rate is apparent in this plant.

Thus rhythms have existed at 0.03 per cent CO_2 (in the present work), 0.36 per cent CO_2 (McAlister and Myers, 1940), and at 3 per cent CO_2 (Van der Veen) in different higher plants. The diminishing rhythms were not present (Van der Veen) at low light intensities.

If, as Van der Veen suggests (1949b), the fluctuations in photosynthetic rate of wheat leaves can be explained solely on the basis of the chemical activation or inhibition of CO_2 fixation, one would expect to find such fluctuations commonly extant in all photosynthetic organisms, including the algae.

It is characteristic of the induction curves of *Stichococcus* (Aufdemgarten, 1939), and *Chlorella* (McAlister and Myers, 1940), and (Van der Veen, 1950), that illumination after a dark interval is followed (depending on temperature, light intensity, and nutrition) by an immediate uptake of CO_2 for the first 20 to 30 sec of illumination. This "CO_2-gulp" is followed by a long or short (again depending on the aforementioned factors) period of inhibition of photosynthesis. However, no secondary peaks have been reported in the induction of algae as are found in higher plants. The first gulp is commonly found in higher plants and algae, but the continued oscillation is peculiar to higher plants.

The possibility that these latter fluctuations are due to rhythmic changes in the degree of stomatal opening is considered later in the discussion.

Undiminishing Rhythms

In some experiments it was found that the rhythmic fluctuation in the rate of photosynthesis in bean leaves continued unabated for many hours. Such rhythms will be presently called "undiminishing rhythms." In figure 3 an undiminishing rhythm occurred in which the peaks came at about 25, 55, 86, and 117 min after the time of illumination. Thus the times from peak to peak were 30, 31, and 31 min respectively.

The undiminished rhythm established in figure 4 was of a different shape than that of figure 3. In the case of the former, the light was turned on at 0 on the time scale after 30 min of darkness in air. From 38 to 41 on the time scale the lights were turned off. It appears that this 3-min dark period changed the shape of the rhythm that immediately followed, and caused the hump found at the end of each peak. The time intervals between peaks in this rhythm were 37,

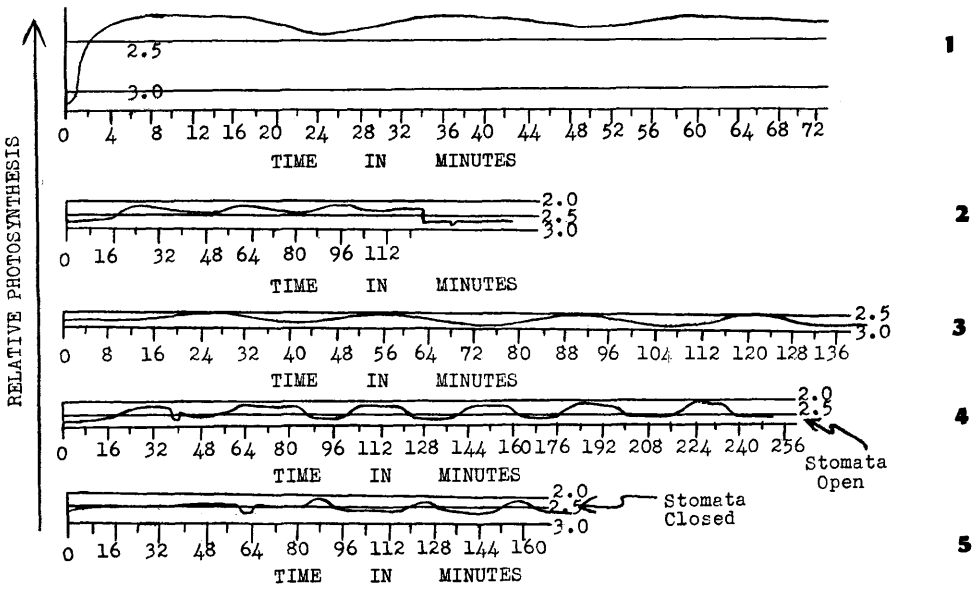

FIGURE 1. Curve formed during the time course of photosynthesis in a primary leaf of Black Valentine Bean. The plant was used 19 days after planting. Date: March 5, 1959. Leaf Area: 0.38 dm^2. Light intensity: 2200 ft-c. After a 30 min period of darkness (27 min, 30 sec in "N_2; 2 min, 30 sec in air), the light was turned on for 2 hr, 8 min. Rate of photosynthesis at time 12 min: 11.8 mg $(dm)^{-2}(hr)^{-1}$ CO_2 absorbed. Avg. dark respiration: 1.53 mg $(dm)^{-2}(hr)^{-1}$ CO_2 evolved.

FIGURE 2. Curve formed during the time course of photosynthesis in a primary leaf of Black Valentine Bean. The plant was used 25 days after planting. Date: March 11, 1959. Leaf Area: 0.25 dm^2. Light intensity: 2200 ft-c. After a 30 min period of darkness in normal air, this 2 hr, 5 min light period is shown. Rate of photosynthesis at the first "trough" of the rhythm: 6.44 mg $(dm)^{-2}(hr)^{-1}$ CO_2 absorbed. Rate of photosynthesis at the first "peak" of rhythm: 11.5 mg $(dm)^{-2}(hr)^{-1}$ CO_2 absorbed.

FIGURE 3. Curve formed during the time course of photosynthesis in a primary leaf of Black Valentine Bean. The plant was used 13 days after planting date. Date: September 6, 1958. Leaf Area: 0.37 dm^2. Light intensity: 2200 ft-c. Following a 15 min period of darkness (13 mm, 30 sec in CO_2-deficient air; 1 min. 30 sec in normal air) the light was turned on for the 3 hr, 28 min period shown. Rate of photosynthesis in the trough of this rhythm was as low as 0.54 mg $(dm)^{-2}(hr)^{-1}$ CO_2 absorbed. The rate of photosynthesis in the peak of the rhythm was as high as 4.13 mg $(dm)^{-2}(hr)^{-1}$ CO_2 absorbed.

FIGURE 4. Curve formed during the time course of photosynthesis in a primary leaf of Black Valentine Bean. The plant was used 27 days after planting. Date: March 13, 1959. Leaf Area: 0.28 dm^2. Light intensity 2200 ft-c. After 30 min of darkness in normal air, the light was turned on at time "O". From time 37 to 40 the leaf was subjected to a 3-min dark period. From time 40 onwards a 4 hr, 26 min light period is shown. At the peak of the rhythm (time 266 min) the leaf chamber was opened, and benzene immediately applied to the under surface of the leaf. Infiltration occurred immediately. Rate of photosynthesis at the peak of rhythm: 10.3 mg $(dm)^{-2}(hr)^{-1}$ CO_2 absorbed. Rate of photosynthesis at the trough of rhythm: 2.68 mg $(dm)^{-2}(hr)^{-1}$ CO_2 absorbed.

FIGURE 5. Curve formed during the time course of photosynthesis in a primary leaf of Black Valentine Bean. (Opposite leaf of same plant cited in figure 4.) After 1 hr of light (in normal air) the leaf was subjected to a 4-min dark interal (time 60 to 64.) During the following 1 hr and 50 min an undiminishing rhythm is apparent. At time 172 min the leaf chamber was opened and benzene applied to the under surface of the leaf during the trough stage of the rhythm. No infiltration of benzene occurred. Rate of photosynthesis at peaks of rhythm: 7.85 mg $(dm)^{-2}(hr)^{-1}$ CO_2 absorbed. Rate of photosynthesis at the trough of rhythm: 1.33 mg $(dm)^{-2}$ $(hr)^{-1}$ CO_2 absorbed.

42, 40, 39, and 39 min. At the crest of the seventh peak the leaf chamber was quickly opened and benzene was applied to the experimental leaf. Complete infiltration occurred immediately.

In figure 5 an undiminishing rhythm of photosynthesis was manifest in the opposite primary leaf of the same bean plant that had been used in figure 4. The leaf was illuminated during the usual "night" period of the plant. During the 60 min of continuous light that followed, no fluctuations in the rate of photosynthesis were apparent. Then the light was turned off for 4 min (60 to 64 on the time scale.) From 64 to 173 on the time scale the leaf was under continuous light at a uniform flow rate of air, and the undiminishing fluctuations resulted. From the onset of the 4-min dark period to the crest of the first peak was 25 min, and the time periods between subsequent peaks were 36 and 33 min. After 173 min benzene was applied to the experimental leaf while the rhythm was at a trough phase. No infiltration was apparent.

Thus a wide stomatal aperture correlated with the peak phase of the photosynthetic rhythm and a narrow aperture with the trough phase of photosynthesis during the course of the rhythm. It would appear that the rhythmic fluctuation of the guard cells in these experiments was causing a concurrent fluctuation in photosynthetic rate. It appears from a study of figure 5 that the 4-min dark period interrupting the light induced concurrent rhythms in guard cells and photosynthesis.

Undiminishing rhythms of stomatal fluctuation were reported by Stålfelt (1929a) in needles of *Picea excelsa* and leaves of *Vicia faba* by using a direct observation technique. He found that the amplitude and time length of stomatal fluctuations increased as the temperature increased. The time interval between the maximum openings was about 20 min. He said he found by using extensive research material that such periodic pulsations of guard cells lasted incessantly. Stålfelt (1929b) again dealt with certain features of the recurring pulsations.

Gregory and Pearse (1937) found the fluctuations of stomatal diffusive capacity evident in leaves of *Pelargonium zonale* by means of the resistance porometer. Went (1944) recorded a periodicity of stomatal fluctuation in tomato leaves with the duration of the opening period lasting 40 to 50 min. There was a period of about 40 min between maximum openings in the rhythm.

It should be noted that the time intervals reported for stomatal rhythms by these workers are similar to the intervals in the photosynthetic rhythms of figures 2 to 5.

Maximow and Krasnosselsky-Maximow (1928) reported undiminishing rhythms in the photosynthesis of buckwheat, barley, soybean, and millet plants. They then suggested that the basis of these rhythmic fluctuations lies in the undiminishing fluctuations of stomatal opening reported by other workers.

If an explanation of photosynthetic fluctuations is attempted on the basis of the stomatal rhythms, rhythmic fluctuation in the rate of transpiration would also be expected. Such fluctuations have indeed been noticed by Boresch (1933) in the transpiration rate of oat leaves. He used a hygroscopic technique in conjunction with a recording device. On one of the charts there were about 30 pulsations in the rate of transpiration per hour.

In a recent review of the literature concerning stomatal changes, Stålfelt (1956) visualizes two sequences of reactions which contribute to stomatal opening, and another pair of sequences which cause stomatal closure. In one opening sequence, photosynthesis takes place in the guard cells and an uptake of CO_2 causes a pH rise which affects certain enzyme systems causing osmotically inactive carbohydrates (starch) to change into osmotically active ones (sugar). A slow opening of stomata results. He visualizes another opening sequence in which change of acid concentration causes a change in the nature of the charge of plasma-colloids. Such colloidal changes supposedly modify the viscosity, resistance, permeability, and plasma solution of the cell—thereby causing a rapid stomatal

opening. A closing sequence he outlines, operative upon the onset of darkness, involves a reversal of the two opening mechanisms mentioned. Another series of closing reactions supposedly occurs in the light. As the stomata are open, moderate water deficits build up due to transpiration water loss. When these water deficits develop, enzyme systems are affected and the change of sugar to starch follows with the slow closure of stomata resulting.

Stålfelt (1956) then applied his theory to stomatal fluctuations and visualized these as resulting from an interaction of a "hydroactive closing" movement, and a "photoactive opening" movement of stomata. Accordingly the light contributes to stomatal opening as suggested. However, the stomata open to such a great extent that a water deficit soon develops within the cells. When this water deficit reaches sufficient magnitude the stomata begin to close. They subsequently close to such a degree that the water deficit vanishes and internal CO_2 concentrations are concomitantly decreased by continuing photosynthesis. This in turn causes increased pH, carbohydrate transformations, and a photoactive opening of stomata. Stålfelt thus pictures an "automatisches Hin und Her" or back and forth of stomatal change that ensues.

If the fluctuations of stomatal opening such as those reported by Stålfelt (1927) (1928) (1929a) and Went (1944) do indeed cause the rhythms of photosynthetic rate and transpiration rate as recorded by Boresch (1933), Stålfelt (1932) (1935b), Maximow and Krasnosselsky-Maximow (1928), and in this paper figures 2 to 5, it would be expected that the photosynthetic rhythms would be of the same irregular appearance as the stomatal and transpirational rhythms. The photosynthetic rhythms, however, show a greater degree of regularity than either the stomatal or transpirational fluctuations. The basis of this disparity seems to stem from the experimental procedures used in the two sets of experiments. In the work of Stålfelt (1927) (1928) (1929a), Went (1944), and Boresch (1933), the leaf was not exposed to a flowing air stream. In work of Boresch and Went leaves were exposed to the open air of the laboratory, while in the work of Stålfelt leaves were usually placed on moist filter paper in petri dishes. Stålfelt (1935a) mentions the effect of so called "ruhiger Luft" (still air) upon transpiration. He found (1932, 1935b) that stomatal transpiration changed 11 to 35 per cent upon opening the door of the analytical balance used for measurement. Thus flow rates of air around the leaf in so called "still air" will at best be irregular and likewise rhythms of transpiration or stomatal change would be expected to be irregular.

In the present work and that of Maximow and Krasnosselsky-Maximow (1928) the leaf or entire plant was enclosed in a chamber through which air was circulated at an unchanging flow rate. The fluctuations in water deficit under such conditions would be expected to appear with greater regularity of duration and amplitude.

The question posed by Boresch's results (why the rhythms did not consistently occur on all the days of experimentation) remains unanswered. Nor is it understood in the present paper why the fluctuations in photosynthesis are sometimes of a diminishing nature and other times are undiminishing.

In figure 5, after the 4-min dark period interrupting the light, a rhythm was fully established. It is not known what relationship existed between the rhythm and the dark period, but future research may reveal the extent and nature of the causality.

SUMMARY

The rate of "true photosynthesis" in leaves of intact bean plants was measured by means of infrared gas analysis. In certain experiments a rhythmic fluctuation of photosynthesis occurred. The "peak" and "trough" of such rhythms were about 20 to 30 min apart. In some cases the fluctuation soon leveled off after one or two cycles ("diminishing rhythms"). However, in other experiments the rhythm persisted unabated for numerous cycles ("undiminishing rhythms").