mounted about 2 mm above the opening, forms a chamber which, when the cover is in place, is open to the platinum electrode on the bottom and has a transparent window on top. Light passing through the window impinges directly onto the electrode. Gas inlet and outlet ports connect with the chamber over the electrode.

To measure O_2 exchange rate, the reservoir is filled with the electroconductive medium. Then the Ag|AgCl electrode is placed in the reservoir and the plug is tightened. Sea water is used as the electrolyte with a marine alga. With chloroplast preparations or unicellular algae, a liquid with an ionic strength of 0.01 *M* or more is used. For a marine alga, a section of thallus of exactly the same surface dimensions as the platinum electrode is placed in contact with it. A piece of moist cellophane over the alga holds it against the electrode. A piece of Teflon about 6.5 microns thick covers the cellophane, and a thin rubber gasket is between the bottom surface of the cover and the Teflon. The internal opening of the gasket is slightly larger than the electrode. Then the cover is mounted in place. With a suspension, a similar procedure is followed except that the material is previously filtered onto a Millipore membrane disk from which a section is cut and placed with the preparation directly in contact with the platinum electrode. The method of recording O_2 exchange is as described in *Year Book 61*, pages 343–344.

Gas is supplied through standard regulator valves from commercial cylinders. Brooks Rotameters serve to measure gas flow. Two or more flowmeters in conjunction with a mixing chamber make it possible to supply gas mixtures of almost any composition to the assembly. The usual flow rate is about 2.8 l/hr. If a rapid change in the gas composition is desired, the flow of the new atmosphere may be increased severalfold for flushing the system and then later reduced to the desired rate. As this system is open to the atmosphere, there is no increase over atmospheric pressure in the electrode assembly. Since the gas flow remains constant and the volume is far greater than the gas exchange of the photosynthetic preparation, it is assumed that any photosynthetic gas exchange has a negligible effect on the gas passing through the system. Before entering the electrode cover, the gas is bubbled through a water column. Moistening the gas prevents desiccation of the sample.

Marine algal sections have been used in the apparatus with a gas mixture flowing over them continuously for four or five days without drying or losing their capacity to carry out photosynthesis. With this new system, O_2 evolution rates can be determined in one gas mixture, the atmosphere changed rapidly to an entirely different gas composition and new rate determinations made, the original atmospheric composition replaced, and the rates in the original gas mixture redetermined, all within 5 to 10 minutes.

Induction Transients in O_2 Evolution by *Porphyridium cruentum* in Monochromatic Light

Govindjee and Rajni Govindjee

The following is a progress report on our observations on the induction transients in the red alga *Porphyridium cruentum.*

The pre-a spike. In several algae, the rate of O_2 evolution first increases rapidly upon illumination, reaches a maximum (referred to as the *a* spike by Vidaver, in *Photosynthetic Mechanisms of Green Plants*, p. 726), decreases (*b* slope), increases again slowly (*c* slope), and finally reaches a steady state as in figure 40. In some algae, a pre-*a* transient during the rise of the *a* spike has been observed (Blinks, Vidaver). A pre-*a* transient was never observed in the red alga *Porphyra.* However, by providing a long dark period from 15 minutes to several hours and low temperature (1°–5°C), we have been able to observe a pre-*a* transient in

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another red alga, *Porphyridium cruentum*. A dark period of approximately 30 minutes was long enough to separate clearly the pre-*a* and *a* spikes. The pre-*a* spike was over in less than 5 seconds. The *a* spike itself reached its maximum at about 30 seconds after the start of illumination, figures 41 and 42. Upon repeated exposures (fig. 42, trace 2) the pre-*a* spike is no longer evident, because it probably merges with the *a* spike. Note the time at the peaks of *a* spikes. In trace 1 it is at 30 seconds, and in trace 2 it is at about 10 seconds, showing that the *a* spike is a complex of *a* and pre-*a* spikes. This *a* spike grows in height, trace 3, and after 4–5 exposures it becomes constant. With reduction of the dark interval, the time to attain the peak height lessened as shown in figure 43.

Another interpretation of these curves is that what has disappeared is not the pre-*a* spike but the *a* spike. This idea is suggested by the fact that after repeated

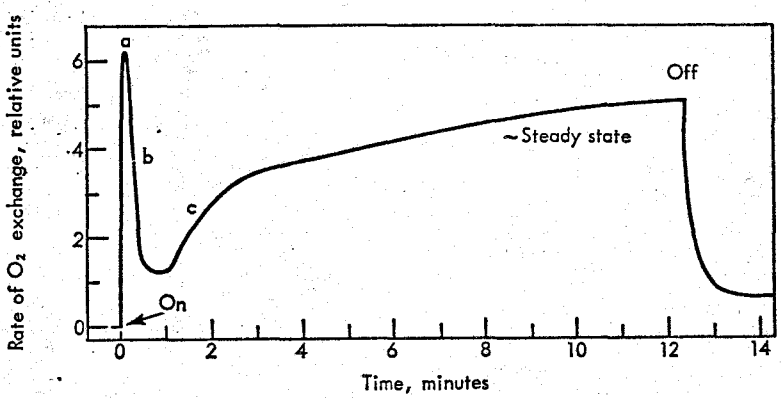

Fig. 40. Course of O_2 evolution in *Porphyridium cruentum* exposed to 540-mμ (green) light at 3°C. Note the O_2 spike (the *a* spike), the *b* slope, the *c* slope, and the steady-state rate. This is a typical time-course curve for *Porphyridium* at 3°C, without a long dark period between exposures.

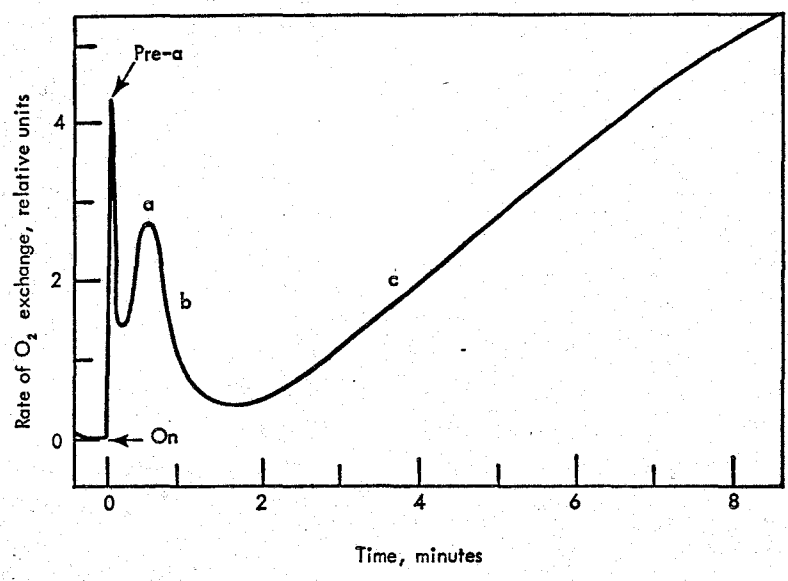

Fig. 41. The course of O_2 evolution in *Porphyridium cruentum* exposed to 540-mμ (green) light at 3°C after 12 hours' dark pretreatment. Two O_2 spikes appear: the pre-*a* and the *a* spike.

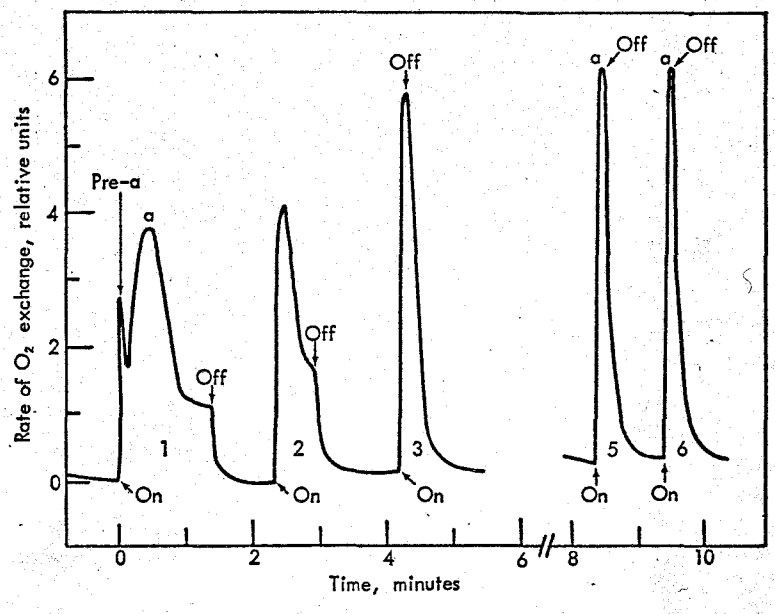

Fig. 42. The pre-*a* spike appears in cells that were in darkness for 30 minutes, then exposed to 694-mμ light. It is not evident in curves made by repeated exposures.

illuminations the position of the spike is about the same as that of the pre-*a* spike and the whole effect is over at 30 seconds where the *a* spike normally peaks.

The pre-*a* spike was observed with both green (540-mμ) and red (694-mμ) light. The need of a long dark period for the production of the pre-*a* spike shows that the accumulation of a product of a slow dark reaction is required for its formation.

The initial transients do not seem to represent the same phenomenon as that observed in green algae. In the green algae the pre-*a* spike can be reproduced every minute or even in less time. It appears to be caused by O_2 uptake by the long-wavelength chlorophyll system temporarily interfering with the rapid initial increase in rate of O_2 evolution. By contrast, the spike of *Porphyridium cruentum* appears to be a true O_2 burst, which after a long dark interval stands out from the more slowly light-induced continuing O_2 evolution. It may also be that differences in rate constants of the dark reactions that make material from which light gives the O_2 burst can explain the differences between red and green algae.

The a spike. The *a* spike is also affected by various regimes of darkness

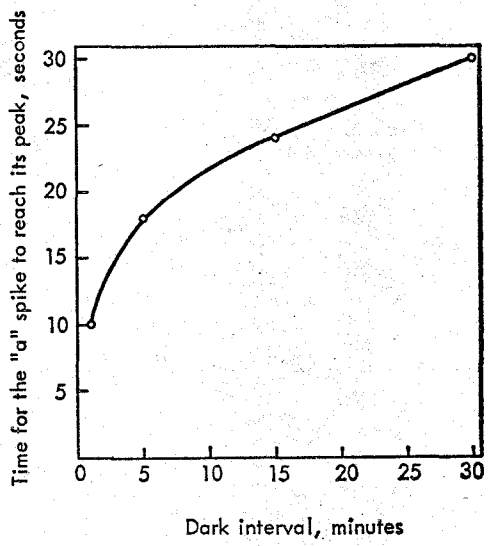

Fig. 43. The time of illumination required for the *a* spike to reach its peak in a series of exposures given after different dark intervals.

and light. Its height increases with repeated exposures to light, and after several exposures it reaches a constant value. However, the total amount of O_2 evolved during the *a* spike may be about the same, since the increase in height is correlated with a more rapid decline, i.e., a steeper *b* slope. With a constant light exposure of 3 minutes followed by dark intervals ranging from 1 minute to 8 minutes, almost identical heights are obtained for the *a* spike. A constant dark interval of 1 minute preceded by light exposures ranging from 1 minute to 8 minutes also gave almost the same heights. Dark intervals shorter than 1 minute, however, did not give reproducible curves. To measure the effects of intensity and wavelength, exposures were therefore given at 1-minute dark intervals and the light was turned off as soon as the peak of the *a* spike was reached. A standard reference exposure was interposed between varied experimental exposures (*Year Book 61*, pp. 336–337).

In either 545- or 694-mμ light the height of the *a* spike increases with increasing intensity and then becomes saturated, as does steady-state photosynthesis.

The a spike at different wavelengths. A crude action spectrum was made, using certain selected points to cover the maxima and minima of phycoerythrin and chlorophyll-*a* absorption peaks. The wavelengths were 500 mμ, 545 mμ (peak of phycoerythrin), 590 mμ, 680 mμ (peak of chlorophyll *a*), 690 mμ, and 700 mμ. Figure 44 shows the results. By comparison with the absorption spectrum of *Porphyridium* (*Year Book 60*, p. 353, fig. 3), it is evident that the *a* spike is lower in the chlorophyll-*a* absorption band than in the phycoerythrin absorption band. Qualitatively, this is in accordance with the results generally obtained for steady-state photosynthesis (Haxo and Blinks; Brody and Emerson; French and Fork).

Discussion. These similarities in behavior of the O_2 spike and of steady-state photosynthesis suggest that the spike production is not a separate effect; both may involve the same reactions except for one reaction that is responsible for the *b* slope. The *b* slope may be due to an oxidation of primary reductant by molecular oxygen as discussed by de Kouchkovsky and Briantais in *Photosynthetic Mechanisms of Green Plants*, page 362, or it could as easily be the exhaustion of a pool of an electron acceptor which becomes depleted then regenerates slowly. In steady-state photosynthesis, the utilization by one system of a product made by the other system competes successfully with its oxidation by molecular oxygen.

To explain the separation in time of pre-*a* and *a* spikes after a long dark period, we may speculate that in darkness some intermediate, which is necessary for photosynthesis, is oxidized by O_2. Upon illumination, this oxidized complex is decomposed, liberating O_2, the intermediate is regenerated, and photosyn-

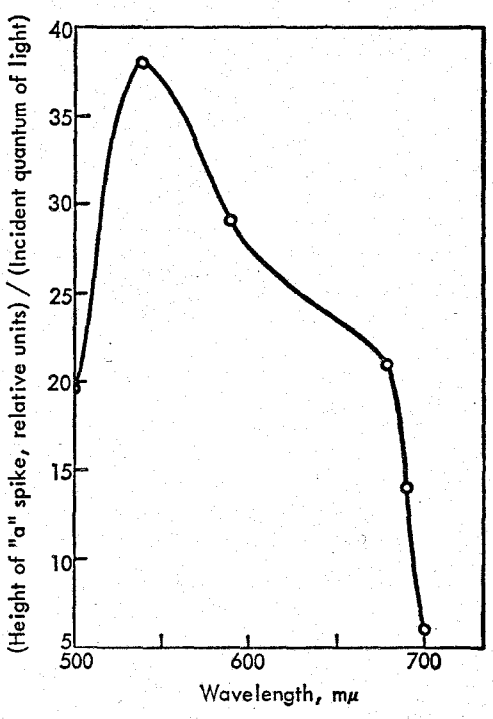

Fig. 44. Action spectrum of the *a* spike for a few selected points.

thesis begins. An alternative explanation for a pre-*a* spike in *Ulva* is discussed by Vidaver elsewhere in this report.

The CO_2 *burst* found by Emerson during the first moments of illumination was also dependent upon a pretreatment to darkness. It may be that the CO_2 burst and the initial O_2 transient phenomena are different manifestations of the same events.

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Oxygen Evolution from a Red Alga Exposed to Monochromatic Light Flashes with Background Light of Different Wavelengths and Intensities

Govindjee and Rajni Govindjee

Since Robert Emerson's discovery that photosynthesis requires two light reactions, the nature of these reactions has been studied intensively. Except for the recent work of Whittingham and of French (*Year Book 62*, pp. 349–352), no study of the kinetics of O_2 evolution in flashing monochromatic light has been made. This method will undoubtedly continue to contribute significantly to our understanding of the photochemistry of photosynthesis. We report here a systematic study of the O_2 evolution induced in the red alga *Porphyridium cruentum* by green-light flashes imposed on continuous red-light background and by red-light flashes on a continuous green-light background.

The rates of O_2 exchanges were measured with a platinum electrode polarograph (*Year Book 60*, p. 362). The electrical signal generated as a consequence of O_2 evolution or uptake by the alga was amplified and recorded.

In confirmation of French's observations, we found two types of enhancement in the photosynthesis of *P. cruentum*: (*a*) an increase in the initial rate of O_2 evolution without prolonged O_2 production caused by a 50-millisecond flash of green light imposed on a red-light background; (*b*) an increase in the initial rate of O_2 evolution combined with prolonged O_2 evolution caused by red-light flashes when imposed on a green-light background. The green light is absorbed primarily by phycoerythrin, and the red light by chlorophyll *a*.

First, *Porphyridium* was exposed to red-light flashes without any background light. A typical recording of O_2 evolution from a single 50-millisecond flash is shown in figure 45. The height H is a measure of the maximum rate of O_2 evolution in the flash. The time t is the width of the trace at one-half of the height H, and is referred to as the half-time. Its duration is partly due to the instrument lag, but any increase in half-time results from a prolongation of O_2 evolution after the flash. After at least three exposures to flashes without background light, continuous background of complementary color was turned on. Then, light flashes were superimposed on the background illumination. The experiments were repeated with various intensities of background light. To check for possible artifacts due to nonlinearity of light curves, red flashes were also superimposed on red background light, and green flashes on green light of different intensities.

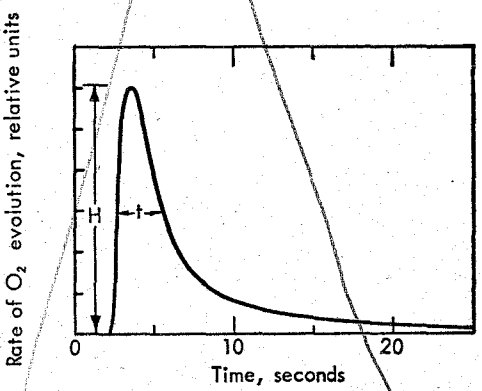

Fig. 45. Rate of O_2 evolution in *Porphyridium* induced by 50-millisecond red (694 mμ) flashes as a function of time; 22°C, 5 per cent CO_2 in air. Flashes given at 30-second intervals.