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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.

Govindjee acknowledges the support of the National Science Foundation (NSF GB 1610) and a Carnegie Institution of Washington travel grant.

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

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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.

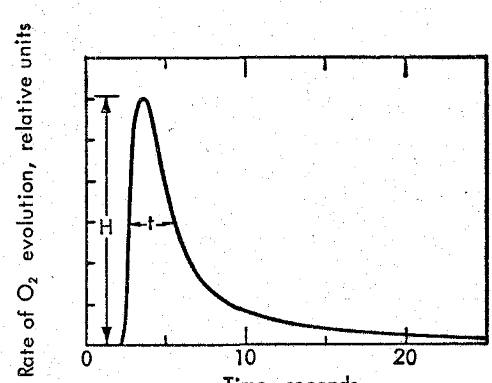

Fig. 45. Rate of O_2 evolution in *Porphyridium* induced by 50-millisecond red (694 mμ) flashes as a function of time; 22°C, 5 per cent CO_2 in air. Flashes given at 30-second intervals.

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Red flashes on green background light. With increasing intensity of the background light two effects on the rate of O_2 evolution are observed. The maximum rate increases to an optimum (fig. 46, middle and bottom). The period of O_2 evolution is prolonged (top curve fig. 46, and fig. 47). At still higher background intensity both effects decrease from their maxima.

Two comparable experiments were done with flash intensities I_1 and I_2. Strangely enough, the higher intensity I_2, which produced a larger maximum rate H, gave a smaller increase in the half-time of O_2 evolution t than did the lower intensity I_1. In a separate series of measurements shown in figure 47 the shape of the curve relating the half-time to the green background intensity was studied in more detail. Similar results were observed under both aerobic and

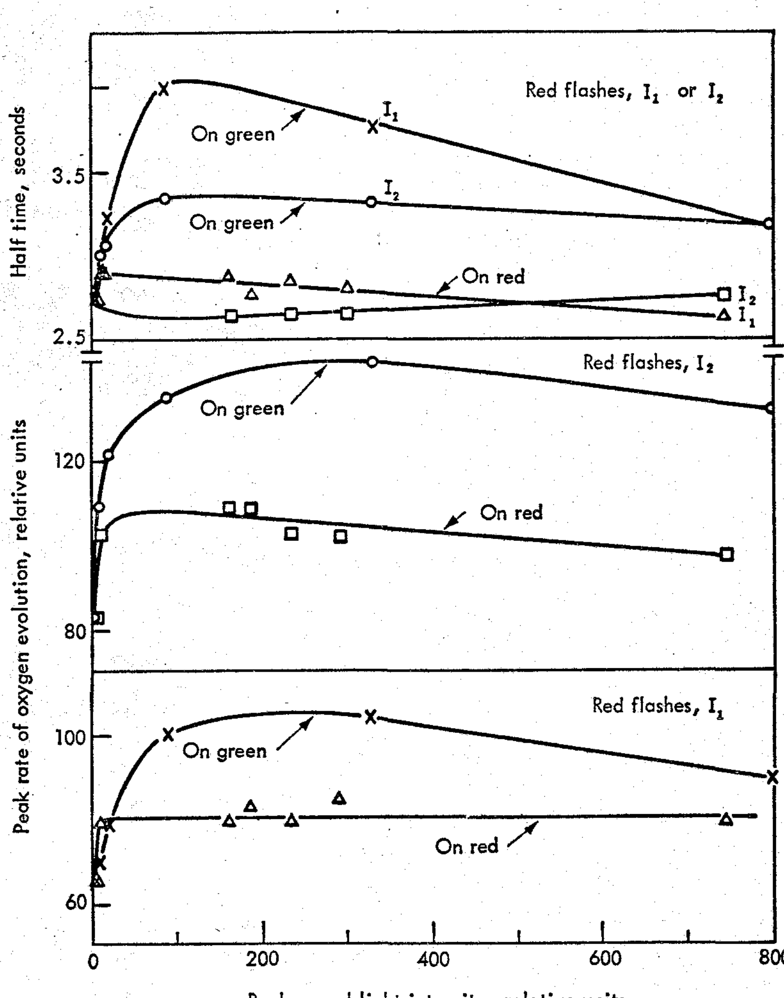

Fig. 46. Half-time, t, of decay of O_2 evolution, and peak rate of O_2 evolution, H, from red flashes (about 694 $m\mu$) of two intensities I_1 and I_2 ($I_2 > I_1$) as a function of the rate of photosynthesis in green (about 550 $m\mu$) background light; 22°C, 5 per cent CO_2 in air. When red flashes are given on different intensities of green background light, enhancement is seen (*a*) by prolongation of the time of O_2 evolution in the top curves; and (*b*) by the increase in peak rate in the lower curves.

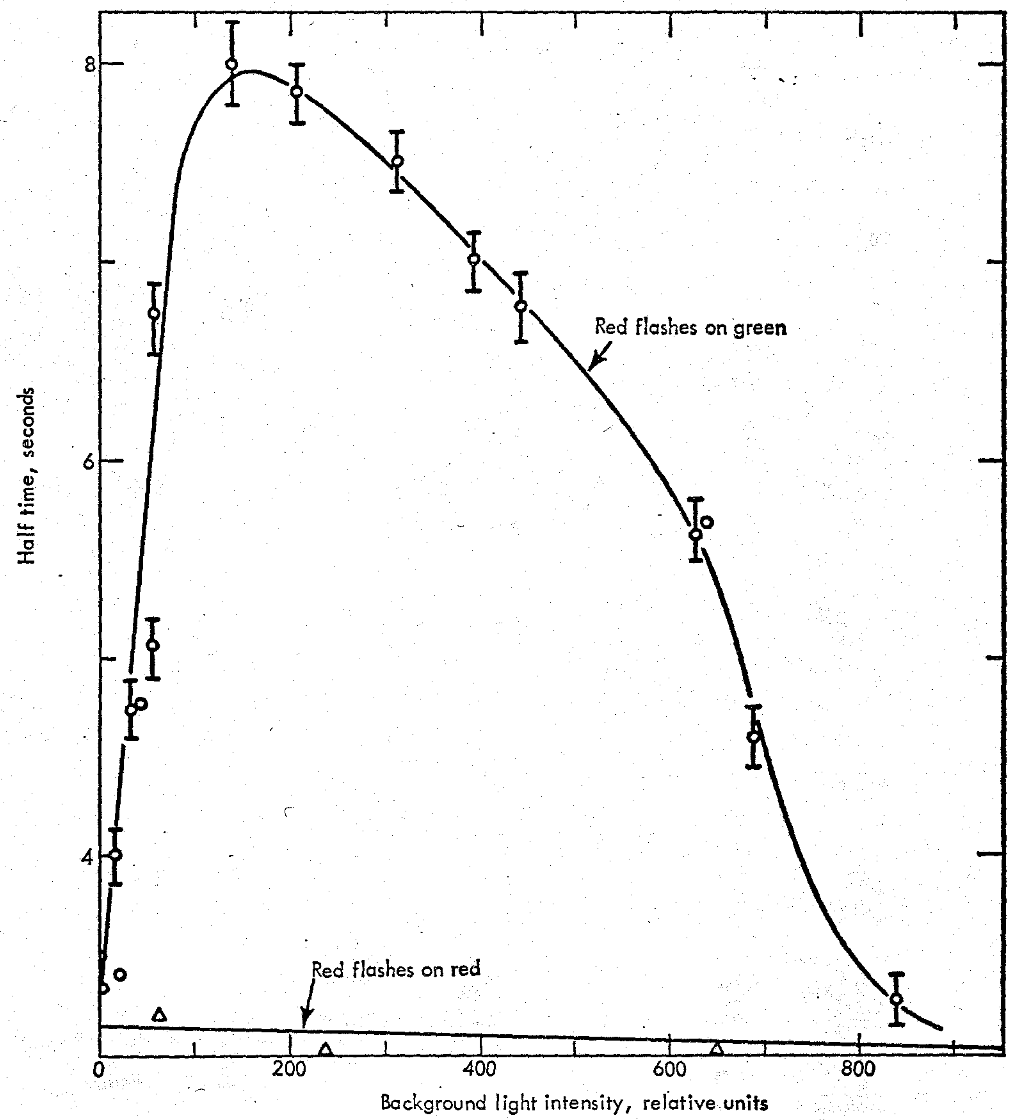

Fig. 47. Half-time *t* of decay of O_2 evolution by red flashes as a function of the intensity of background light; 20°C, 5 per cent CO_2 in air.

anaerobic conditions, except that in the latter some additional (spurious) enhancement arose as the result of the S shape of light curves of photosynthesis in a nitrogen atmosphere. A very small spurious enhancement due to nonlinearity at low intensity was noticed even when 5 per cent CO_2 + 95 per cent air was bubbled through the culture medium that was constantly flowing over the alga.

Green flashes on red background light. We found that the peak rate of O_2 evolution from a green flash on a red background first increases (enhancement) with an increase in the background light intensity, and then attains saturation (bottom curve fig. 48). In contrast to the effect of red flashes on a green background, no prolongation of the O_2 evolution period was noticed at any intensity

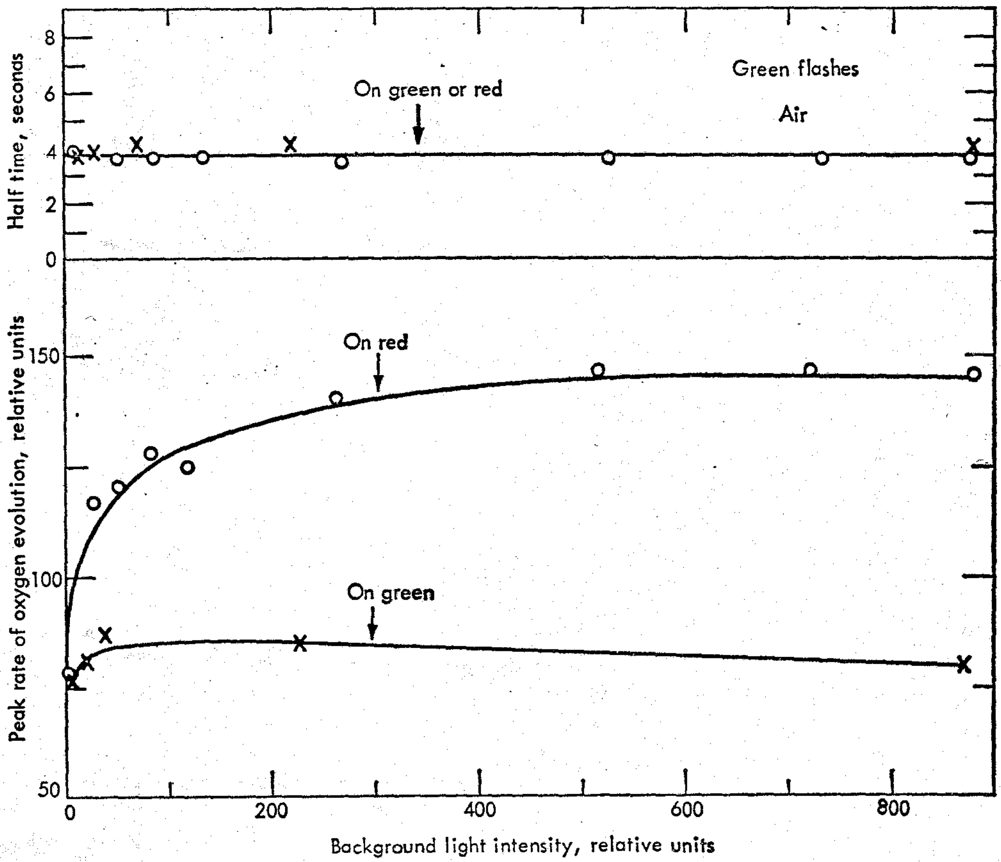

Fig. 48. Half-time *t* of decay of O_2 evolution, and rate of O_2 evolution, *H*, by green flashes as a function of either red or green background light intensity; 22°C, 5 per cent CO_2 in air.

of the red background light used with the green flashes (top curve fig. 48). Results similar to those in air were observed under anaerobic conditions, figure 49, except for some spurious enhancement superimposed on the real enhancement under nitrogen.

Interpretations. The main effects found are summarized in figure 50. The prolongation of O_2 evolution due to a red flash, if it is given on a background of green light, and the dependence of this on intensity of background light, can be interpreted in the two-light reaction scheme of photosynthesis, either in terms of the "spillover" or the "separate package" mechanism (Myers and Graham, *Plant Physiol.*, *38*, 105, 1963, discuss the definition and origin of the two mechanisms).

Spillover model. In both the spillover and the separate package models, we incorporate the idea that the product of light reaction I is long-lived (half-life of the order of seconds). No assumption is needed about the lifetime of the photoproduct of reaction II. When *Porphyridium* is illuminated with green background light, which is mainly absorbed in system II, a part of the energy is transferred to system I (energy "spills over" from system II to system I), leading to a balanced excitation of the two systems. Just how this comes about is uncertain. When a red flash is given on green background light, the green quanta are dis-

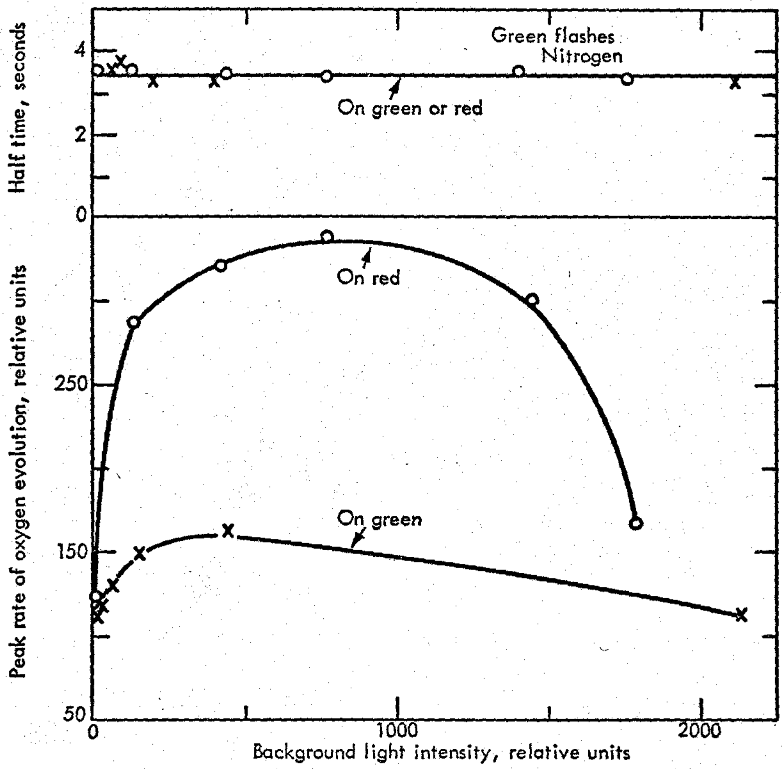

Fig. 49. Half-time t of decay of O_2 evolution, and rate of O_2 evolution, H, by green flashes as a function of background light intensity under anaerobic conditions. Note the spurious effect due to nonlinear light curves from the green flashes given on green background; 22°C, 5 per cent CO_2 in N_2.

tributed during the flash differently from before (more of them can now stay in system II), thus causing enhancement. Depending on the relative intensities of green background light and the red flash, we can anticipate a larger or a smaller enhancement in O_2 evolution by the flash. If the red-light product (product I) is not used up entirely by green light during the red flash, as it will be when the intensity of green background light is low, the green light arriving after the flash can continue producing excess O_2 until all the long-lived product I has been used up. Therefore the enhancement effect will continue for some time after the red flash is over. This explains the prolongation of O_2 evolution observed when a red flash is given on green background light. With green background light of higher intensity, the excess red-light product will be used up more quickly, explaining why the prolongation of O_2 evolution becomes shorter and ultimately disappears. Nevertheless, enhancement will still be revealed by a greater rate of O_2 evolution.

In the other set of experiments the red background light creates a photostationary excess of long-lived red-light product. If a green flash is now added, the quanta of the flash will utilize this product, and an enhancement of the initial rate of O_2 evolution will result. The extent of the enhancement will depend on the intensity of the red background light. There can be no prolongation of O_2 evolution due to a green flash, because as soon as the flash ends the photostationary state is reestablished. No intermediate photoproduct survives a green flash, whether it be long-lived or short-lived, because it is all used up during the flash.

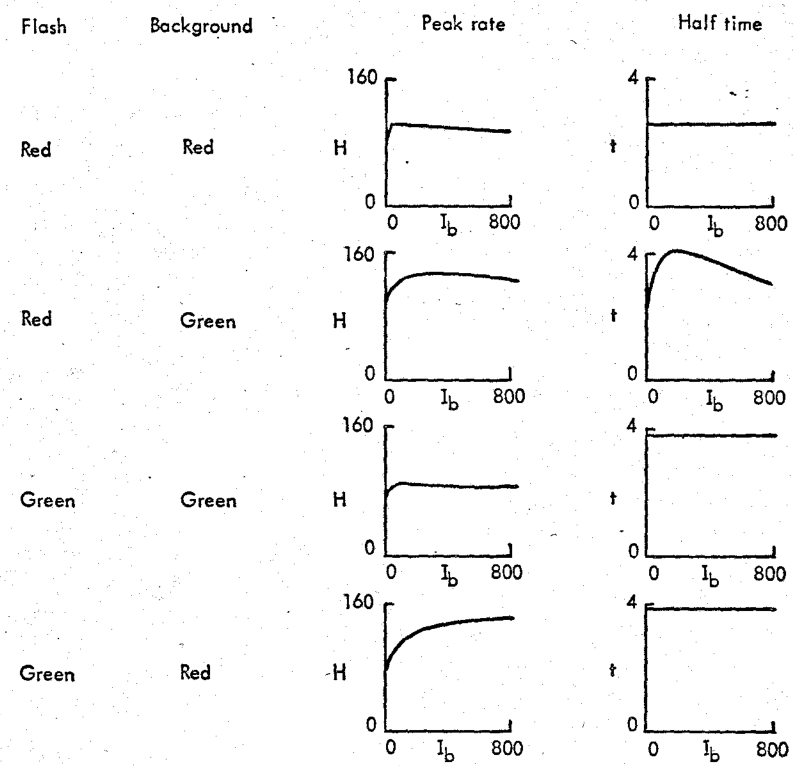

Fig. 50. The types of curves relating the peak rates H, and the half-times t, from single flashes given on different background intensities, may show as an increase of the peak height, as a prolongation of the time during which O_2 is evolved, or as a combination of both effects. Peak height in recorder deflection, t in seconds, I_b in relative units.

Separate package model. In this model we also incorporate the ideas (based on French's observations) that photoproduct I is long-lived, and that photoproduct II is short-lived (half-life less than 4 milliseconds). According to this model, light quanta absorbed in one system are not transferred to the other, although the two systems contain the same assortment of pigments, even if in different proportions. Here also the prolongation of O_2 evolution when red flashes are given on green background is due to the long life of product I, making possible its utilization by excess product of green light. In the reverse experiment, green-light quanta are used more efficiently when the flash is given on a red background, because product II formed in the green flash can react with product I provided by the red background. As soon as the green flash ends, no further reaction is possible, owing to the very short life of product II, so that no prolongation of O_2 evolution is observed.

The flashing-light experiments described above can thus be interpreted in terms of both spillover and separate package models.

Govindjee acknowledges the support from the National Science Foundation (GB 1610) and a Carnegie Institution of Washington travel grant.

SOME CHARACTERISTICS OF PHOTOSYNTHETIC O_2 EVOLUTION BY SYNCHRONIZED *Chlorella* CELLS

Carl J. Soeder

Most of the experiments on photosynthesis of unicellular algae have been carried out with material containing a mixture of all developmental stages. It is