



## HISTORY & BIOGRAPHY

### LETTER TO THE EDITOR

# On the discovery of the two-light effect on chlorophyll *a* fluorescence: Quenching of chlorophyll *a* fluorescence of Photosystem II by Photosystem I light

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## Introduction

I present here my personal recollection and a story of the very first observation (Govindjee *et al.* 1960) of the quenching, by far-red light, of the chlorophyll (Chl) *a* fluorescence excited by blue (or red) light in the green alga *Chlorella*. This two-light and two-pigment system effect, through Chl fluorescence, had followed the discovery of the Emerson enhancement effect in photosynthetic oxygen evolution (see e.g., Emerson *et al.* 1957, Emerson and Rabinowitch 1960, Govindjee and Rabinowitch 1960a,b). The above-mentioned two-light effect, through Chl *a* fluorescence, was soon thereafter investigated thoroughly by Butler (1962), and then by Duysens and Sweers (1963), who explained it by the “Q” hypothesis: Photosystem II (PSII; blue or red light) reducing a Chl *a* fluorescence quencher (Q) and far-red (PSI) light oxidizing its reduced form (QH) back to Q<sup>•</sup> leading to the observed quenching effect by PSI light on PSII fluorescence.

## The rationale

Govindjee (2023) has reviewed the history of the two-light and two-pigment concept in photosynthesis, but only very briefly mentioned the work on chlorophyll *a* fluorescence. The current Letter is to expand a discussion of the early work, initiated in my lab (Govindjee *et al.* 1960). The rationale for doing the fluorescence experiments was the “unsuspected complexity” already known and observed in the rates of photosynthesis in the far-red light alone and the presence of supplementary light of shorter wavelengths (see e.g., Emerson *et al.* 1957, Emerson and Chalmers 1958, Emerson and Rabinowitch 1960,

Govindjee and Rabinowitch 1960a,b). In today’s language, it was the “Emerson Enhancement Effect” that led to the “two light reactions two pigment system” concept (see e.g., Govindjee 2023). However, soon after that, Govindjee *et al.* (1960) considered the existence of different spectral forms of chlorophyll *a* whose excitations were cited for the relationship of photosynthesis to Chl *a* fluorescence—giving the rationale for doing experiments on Chl *a* fluorescence. Govindjee *et al.* (1960) wrote “It seemed natural to look for similar (related to Emerson Enhancement Effect in photosynthesis) complications also in the action spectrum of the excitation of chlorophyll *a* fluorescence *in vivo*.” Then, they stated “It is known that the quantum yield of fluorescence in *Chlorella* drops when excitation is achieved by light above 680 nm.” [Note: they wrongly cited Foster and Livingston (1952); for a discussion of “red drop” in Chl *a* fluorescence, see e.g., a review by Govindjee *et al.* (1967), and a paper by Das and Govindjee (1967).] Then, it was an open question whether this drop was due to dimerization, as in chlorophyll solutions (Lavorel 1957, Weber 1960 – personal communication to Govindjee), or to some other reasons, related to those responsible for the decline of the quantum yield of photosynthesis in the far red. [Alternatively, the latter decline, too, could have been ascribed to the dimerization of chlorophyll *in vivo* as was suggested by Brody (1958)].

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**Dedication:** Dedicated to my teacher the Late Gregorio Weber (4 July 1916–18 July 1997) – the Grand Pioneer of Fluorescence in Biochemistry and Biophysics.

**Conflict of interest:** The author declares no conflict of interest.

All of this was followed by a statement by Govindjee *et al.* (1960) that they had attempted a systematic study of the intensity and spectrum of Chl *a* fluorescence *in vivo* excited by monochromatic light of different wavelengths, and that they noted unexpectedly an effect opposite in sign to the “Emerson Enhancement Effect” in photosynthesis.

What did they discover: By adding a far-red light beam ( $700 \pm 15$  nm) to a beam of shorter wavelength ( $465 \pm 20$  nm or  $670 \pm 10$  nm) light, there was a *decline* (quenching – rather than an enhancement) in the yield of fluorescence, compared to that produced by the sum of the two beams given separately. They did thirteen experiments, using the green alga *Chlorella pyrenoidosa*, and summarized them in their Table 1. For details of their growth conditions, and experimental conditions, see Govindjee *et al.* (1960). What is important to note is that when the same spot on the *Chlorella* suspension was hit with blue 436 nm ( $\pm 20$  nm) exciting light (Photosystem II) and with far-red 700 nm ( $\pm 15$  nm) light (Photosystem I), the former led to fluorescence, but the latter to mostly scattering. [In these experiments, Chl *a* fluorescence was measured at 685 nm ( $\pm 15$  nm) using a photomultiplier RCA 6217 connected to a mirror galvanometer.]

Below, we show data from four of the thirteen experiments obtained upon excitation by light of the same intensity. For other data, see Table 1 in Govindjee *et al.* (1960).

|                                       | A<br>436 nm | B<br>700 nm | C<br>Both | D<br>(A + B) – C | D/A [% decrease]<br>Percent quenching |
|---------------------------------------|-------------|-------------|-----------|------------------|---------------------------------------|
| (1)                                   | 9.4         | 12.0        | 17.5      | 3.9              | 41%                                   |
| (2)                                   | 15.0        | 13.0        | 19.4      | 8.6              | 57%                                   |
| (3)                                   | 18.8        | 29.0        | 37.9      | 9.9              | 52%                                   |
| (4)                                   | 27.0        | 35.7        | 42.5      | 20.2             | 75%                                   |
| Average<br>of the four<br>experiments | 17.6        | 22.4        | 29.3      | 10.7             | 61%                                   |

The above data clearly demonstrate the quenching effect of far-red (PSI) light on the Chl *a* fluorescence excited by blue light (PSII). Govindjee *et al.* (1960) did not attempt to explain this new observation, leaving the options open clearly.

These 1960 experiments were followed by two detailed research papers (Butler 1962, Duysens and Sweers 1963), the first one by the Late Warren Butler (1925–1984; see Benson 1998) and the second by the Late Louis N.M. Duysens (1921–2015; see Govindjee and Pulles 2016).

### What did Butler (1962) write about the Govindjee *et al.* (1960) paper?

After confirming and extensively extending the Govindjee *et al.* (1960) observations, Butler (1962) stated “The results reported here [made on bean leaves] relate to some recent fluorescence studies by Govindjee *et al.* (1960). They found that the intensity of fluorescence (plus scatter) emitted by *Chlorella*, when illuminated with

670- and 700-nm light simultaneously, was less than the sum of the intensities obtained with the 670- and 700-nm beams separately. Their interpretation that the 700-nm beam decreased the yield of fluorescence excited by the 670-nm beam is confirmed in the results reported here.” [We note that Govindjee *et al.* (1960) had used not only red but also blue light for what we now call Photosystem II light.]

### Duysens and Sweers (1963) on the findings of Govindjee *et al.* (1960)

Duysens and Sweers (1963) wrote: “The first direct experimental suggestion of a different effect on [chlorophyll] fluorescence of two light beams of different colors was obtained by Govindjee *et al.* (1960). These authors concluded from experiments with *Chlorella* that the total fluorescence caused by a far-red and red beam was smaller than the sum of the fluorescence intensities in each beam. We call the fluorescence quenching substance “Q”, and the reduced form, which does not quench the chlorophyll *a2* fluorescence, QH. Then excitation of [photo] system 2 [PSII] reduces Q to QH, and excitation of system 1 [PSI] reoxidizes QH to Q. This explains the increases and decreases in chlorophyll *a* fluorescence upon illumination with light 2 and 1, respectively.” [Here again, we note that Govindjee *et al.* (1960) not only used red light, but blue light for exciting what we now call Photosystem II.]. Further, Duysens and Sweers (1963) extended these Chl *a* fluorescence studies on the two-light two-pigment system effects to many more oxygenic photosynthesizers, such as the red algae (*Porphyridium cruentum* and *Porphyra* sp.), spinach leaf and its chloroplasts.

### Concluding remarks

The above letter to the editor is written to bring back to the attention of “photosynthetikers”, as the Late Jack Myers [1913–2006; Brand *et al.* (2008), also see Myers (1996)] may have said, the earliest observation on the presence of two-light reactions and two photosystems through chlorophyll fluorescence – the process that competes with photochemistry – just as does the process of “internal conversion”.

I would like to mention that the preliminary observation in Govindjee *et al.* (1960) was made just before I had to leave for India to fulfill the requirement of my Fulbright Travel Grant from India to USA; it was followed only ten years later in my lab by Mohanty *et al.* (1970) – still using the green alga *Chlorella*.

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