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Lighting the path: a tribute to Robert Emerson (1903-1959)

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Abstract. The concepts of the Photosynthetic Unit, and the quantum efficiency emerged in the early quantitative studies on the "light reaction". Determination of the minimum number of quanta (ϕ^{-1}) needed for the release of one O_2 molecule had a long and bitter controversy. According to Otto Warburg, ϕ^{-1} per O_2 was between 2.8 and 4. The experiments of Robert Emerson, on the other hand, gave values of 8 to 12. A number of important discoveries led to support the two light reaction/ two photosystem scheme of photosynthesis that we now use as the basis of design of further research. Several kinds of experimental evidence reinforced this scheme: the Emerson enhancement effect, and the two-light effects studied by Bessel Kok, Lou Duysens, Jan Amesz and Horst Witt were particularly significant. The theoretical ideas of Hans Gaffron, James Franck and Eugene Rabinowitch, the experiments of William Arnold, Lawrence Blinks, Jack Myers and C. Stacy French, and the challenges of Daniel Arnon added much spice to the development of our current concepts. It was, however, the biochemical insights of Robin Hill that rationalized the diverse kinds of evidence that accounted for the experimental facts in terms of the two-light/two-photosystem mechanism, popularly known as the "Z' scheme. In this paper, I present my personal views of events related to the development of the current picture of the two-light reaction/two-pigment system scheme.

Introduction

The idea that photosynthesis consists of a light and a dark phase, the latter being dependent upon [CO_2] and temperature, was clearly shown by Blackman (1905) through measurements of "light curves" of photosynthesis. At low light intensities, light limited the rates of photosynthesis, whereas at high light intensities, the dark reactions limited the rates of photosynthesis (see Andy Benson, "Paving the Path", these proceedings). Without light, there is no photosynthesis. Light is essential to provide the energy for photosynthesis. It is needed for the uphill transfer of 4 electrons from H_2O to CO_2, producing O_2 and (CH_2O): 1.2 eV/electron transferred since the redox potential of H_2O/O_2 is +0.8 eV, and that of $CO_2/(CH_2O)$ is -0.4 eV. With 23 kcal/eV, the minimum ΔG needed is 112 kcal. With red photons (40 kcal/mol), the minimum number of photons needed per O_2 (ϕ^{-1}) will be 2.8 to 3. According to Einstein's law of photochemical equivalency, ϕ^{-1} will be 4 photons/ O_2 since 4 electrons are transferred from H_2O to CO_2. Warburg and Negelein (1923) indeed measured the ϕ^{-1} to be close to 4 for one O_2 in *Chlorella*.

The concept of photosynthetic unit

It was at the Kerckhoff Laboratories, California Institute of Technology, Pasadena, CA, that the concept of "photosynthetic unit" originated. Assistant Professor of Biophysics Robert Emerson (see Rabinowitch 1961) and an undergraduate student William Arnold (see Myers 1994) discovered it. Emerson and Arnold (1932; Arnold 1935, pp. 3-15) observed that under the most optimal condition of photosynthesis (single turn-over and saturating flashes of light, with optimal dark times between them), a maximum of only one O_2 molecule was evolved per about 2,400 chlorophyll (Chl) molecules present in *Chlorella* (Fig. 1, top left) although the maximum quantum yield of O_2 evolution must have been very high. Gaffron and Wohl (1936) provided the interpretation and the concept: light absorbed by most of the Chl molecules (now called the antenna) in the "photosynthetic unit " is transferred to a few molecules, the "photo- enzyme "(in today's terms, reaction centers, RCs) for chemistry by resonance migration of excitation energy. At RCs, photochemistry takes place: conversion of excitons into redox chemical energy. The experiments of Emerson and Arnold provided still another conclusion: the bottle-neck dark reaction is ~10 ms.

The quantum yield controversy

The inverse of the maximum quantum yield (ϕ_{max}) is the minimum quantum requirement per molecule of O_2 evolved ($\phi_{min}{}^{-1}$, written as ϕ^{-1} in this paper). This number was the subject of an intense and long-standing controversy between Warburg (3-4 quanta) and Emerson (8-10 quanta) (see photographs, Fig. 1). The first scientist to obtain a different result from that of Warburg was Arnold (PhD thesis in 1935 at Harvard University, Cambridge, MA, pp. 35-38 &Table XI). In *Vallisneria*, red light of 420 ergs/cm^2/s, at a temperature of 22 C, gave the efficiency of 35% (equivalent to 8 photons/ O_2). Arnold published this only in 1949 at the insistence of Hans Gaffron. However, the real challenge appeared when Emerson and Lewis (1941,1943) checked the observations of 16 quanta per O_2 by Manning et al. (1938) and obtained ϕ^{-1} of 8-12 photons/ O_2. In 1950, Warburg et al. note in the Appendix " *We can confirm Emerson's finding that in the carbonate-bicarbonate mixtures, the quantum requirement is 10 to 12, but we cannot confirm that the same quantum efficiency is obtained in the acid culture medium*." In the acid culture medium, they obtained a ϕ^{-1} of 4. Emerson and Chalmers (1955) made a thorough investigation of this problem and showed that there was a O_2 transient in acid culture medium and ϕ^{-1} was ~9 (when calculated at steady state), but only if the transient changes were included, it would be ~4.8. Warburg (1958), however, proposed that young synchronous cultures must be used, 5% CO_2 , and blue catalytic light must be provided to obtain the low ϕ^{-1} . Thus, in 1968, Rajni Govindjee et al. redid the experiments using the new conditions specified by Warburg. They confirmed Emerson's, not Warburg's values. Finally, Warburg et al. (1969) published a measured ϕ^{-1} of 12 per O_2 molecule released at a low light intensity. The importance of this paper is that it is the last and the final paper on this topic by Warburg before his death in 1970. This value of 12 quanta per O_2, if extrapolated to zero light intensity, gives a value of 8 quanta per O_2 . *The measured ϕ^{-1} of 12 is in agreement with Emerson's measured values.* However, Warburg did not believe that these high numbers are correct, and, presented calculated values of 3 to 4, using the unproven and outdated concept of an intermediate called "*photolyte*". In view of the fact that both Warburg and Emerson were ideal experimentalists, the ‘resolution’ of the measured values brings relief to us (Govindjee 1999). As Warburg believed, photosynthesis does follow Einstein’s law of photochemical equivalency, i.e., one absorbed photon performs one photoact. It is just that we need 8 photons/ O_2 because 4 electrons must be transferred twice from H_2O to evolve 1 O_2.

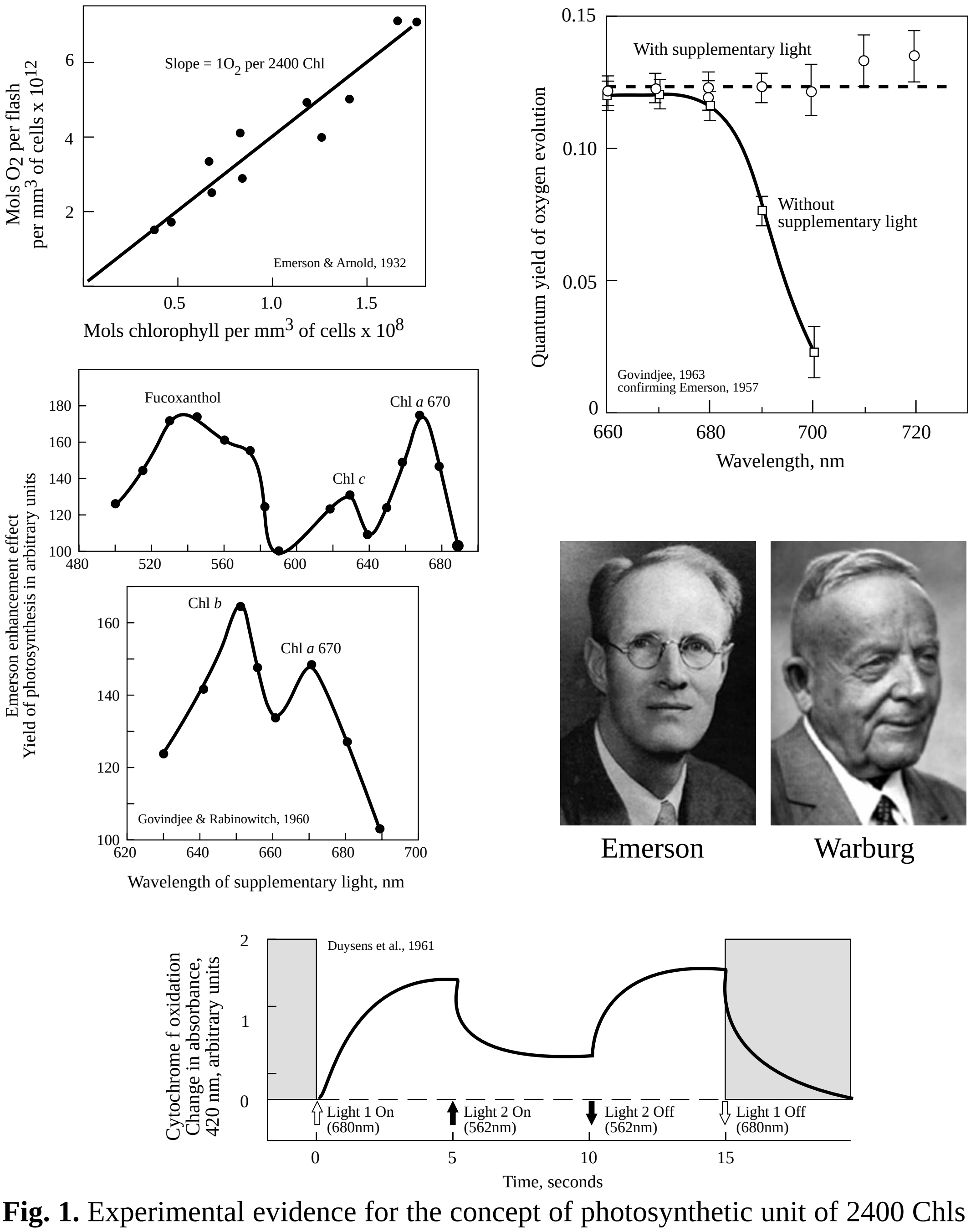

Fig. 1. Experimental evidence for the concept of photosynthetic unit of 2400 Chls per O_2 evolved (top left), for the "Red drop" and the Emerson Enhancement Effect (top right), for the presence of Chl *a* along with Chl *b* (in green algae), or fucoxanthin (in diatoms) in the short-wavelength system of Emerson (middle left), and the antagonistic effect of two lights on the redox state of Cyt *f*, proving the series scheme of photosynthesis (bottom). Also shown are photographs of Robert Emerson and Otto Warburg. See text, and Govindjee (2000) for references and further details.

Discoveries that led to the concept of two-light reaction two-pigment system scheme

The Emerson enhancement effect. The discovery that the low yield of photosynthesis in the "Red Drop" region (Emerson and Lewis 1943) can be enhanced when the photosynthetic system is simultaneously exposed to far red light and short -wavelength light (Fig.1, top right; Emerson et al. 1956, 1957; Emerson 1957, 1958, Emerson and Chalmers 1958, Emerson and Rabinowitch 1960) was responsible for the concept of two light reactions and two photosystems. The concept of the two-light reactions requires a minimum ϕ^{-1} of 8, as was already known (Rabinowitch 1945). Since net O_2 exchange was measured, these results could have been easily due to effects on respiration, as was suggested by Blinks (1957) for his two-light effect transients. The following proved that the effect was in photosynthesis: (1) discovery of the Emerson enhancement effect first in the quinone Hill reaction (Hill 1937) in cells by R. Govindjee et al. (1961), and then in $NADP^+$ reduction in chloroplasts (R. Govindjee et al. 1962, 1964); (2) discovery that the effects are indeed in photosynthesis by the use of ^{18}O experiments using labeled $H_2{}^{18}O$ (see Govindjee et al. 1963, and references therein).

Emerson and Chalmers (1958) implied that one of the light reactions was sensitized by Chl *a,* and the other by Chl *b* or other accessory pigments. This idea contradicted Duysens (1952) who showed that Chl *b* transferred 100% of energy to Chl *a*. Govindjee and Rabinowitch (1960) showed a Chl *a* peak at 670 nm, along with other peaks in the action spectrum of the Emerson effect (Fig. 1, middle left). Thus, both systems were sensitized by Chl *a*, although with different spectral peaks (Albers and Knorr 1937, French 1958). Other significant contributions were: (1) Myers and French (1960) showed that the two lights could be given with some time delay indicating that the interaction between the two systems is through chemical intermediates. (2) Govindjee et al. (1960) showed the two-light effect through quenching of blue-excited Chl *a* fluorescence by far-red light, whereas Kautsky et al. (1960) suggested two light reactions in the same system, through analysis of fluorescence measurements.

The experimental evidence. Kok (1959) showed an opposite effect of two different λs of light on the redox state of P700, that he had discovered earlier (1956). In *Anacystis nidulans*, red light oxidized P700, and orange light reduced the $P700^+$. The key experiment on the antagonistic effect of two different λs of light on Cyt *f* was shown by Duysens et al. (1961); they named the system that oxidized Cyt *f* , system 1, and the one that reduced Cyt f^+, system 2 (Fig. 1, bottom). This experiment established the series nature of the two-light reaction/ two-photosystem scheme. (Rabinowitch (1956) had already predicted this particular result.) Elegant kinetic measurements using single-turn over flashes followed from Witt et al. (1961) establishing the two-light reaction scheme of photosynthesis. Other significant and critical experiments responsible for the acceptance of the scheme were: (1) Chemical "surgery" (partial reactions; use of inhibitors; artificial electron acceptors; and donors) by several research groups (see e.g., a review by Trebst 1974) (2) Physical separation of the system 1 and 2 activities (Boardman and Anderson 1964). (3) Use of mutants of *Chlamydomonas reinhardtii* by Levine and coworkers (Gorman and Levine 1966; see Levine 1968) in confirming the two light reaction scheme.

Theoretical Schemes. Rabinowitch (1945) presented a speculative two-light reaction scheme, based on the ideas of Franck and Herzfeld (1941), to explain the $\phi^{-1}$of 8 quanta/O_2. This scheme is similar to the "Z" scheme except that the identity of the intermediates were unknown. Surprisingly, Franck (1958) presented an untenable physical scheme of up-conversion in one Chl molecule by two photons to explain the Emerson enhancement effect. Hill and Bendall (1960) presented their famous "Z" Scheme the year Emerson and Rabinowitch (1960) presented a two-light/two pigment system scheme. This was based on the concept of the redox potentials, the desire to explain the source of energy for the formation of ATP, and to provide roles to two cytochromes (also see Hill 1965). Although the idea of two reaction centers was implicit in the scheme of Duysens et al. (1961) and "P680" was already speculated by Rabinowitch and Govindjee (1965), it was not discovered until 1969 by Döring et al. in Witt's laboratory.

The Challenges. Arnon has made our lives spicier by taking several Bengazi gallops. In 1961, it was two light reactions, with Chl *b* running system 2, and Chl *a*, system 1 (Losada et al. 1961). In 1965, it became a single light reaction (Arnon et al. 1965). In 1970, Arnon et al. had 3 light reactions, ferredoxin being reduced by two systems II (a and b) working in series. Finally in 1992, a highly complicated 3 light reaction scheme was presented, with two systems 2, one using pheophytin, and the other Q_A. Greenbaum et al. (1995) and Lee et al. (1996) claimed from their work with PS1- minus *Chlamydomonas* mutants that PS2 alone is enough to do photosynthesis. However, oxygenic CO_2 fixation does not occur in the absence of PS1. Nevertheless, the search for alternate pathways need not be abandoned (see Redding and Peltier 1998).

For other alternate hypotheses, see Govindjee and R. Govindjee (1975) and Wild and Ball (1997). I end this paper with two quotations: (1) from Myers (1974) "*One reaches the conclusion that some of the concepts we cherish today will perish tomorrow*". I would like to modify it by changing the last "will" to "may". (2) from Hill (1965) "*In the end, when everything is settled, few of us perhaps will really desire to look back on it all.*"

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