







Personal perspective

## In one era and out the other

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### Abstract

A guided tour through much of photosynthesis research as I saw it, 1936–2001, is presented here. For earlier perspectives, see Myers 1974 (*Plant Physiol* 54: 420–426) and 1996 (*Photosynth Res* 50: 195–208).

### Introduction

I use the title with apologies to Sam Levinson that I lifted it from his light biographical essay of 1973. It accurately describes what I have to say on my course through two eras of photosynthesis research. When Govindjee invited reflections from one of the oldest *photosynthetikers* around, he surely expected a scholarly article. The problem is one of timing. It's just too late. I have too little mastery of the literature of the past five years, the only part that counts in current science. So I shall rely on old notes and my memory, which has lost sufficient resolution that it will not obscure history with details.

### Beginnings

In 1936, I arrived at the University of Minnesota as a graduate student in botany. I had spent two years at Montana State College after a tour to do a Master's Degree in chemistry. I was learning some biology, which at that time was a distinctly different discipline. Microbiology had become my first inclination. But offers for graduate assistance were not numerous and when one came from Minnesota for work on plant physiology and photosynthesis, it became overriding. My introduction to graduate research came from Joe Beeber, the senior graduate student in plant physiology, who was studying photosynthesis in pot-

ted geraniums. I watched him make gas analyses with a Haldane-Carpenter apparatus that measured CO<sub>2</sub> to 0.03% and O<sub>2</sub> to 20.94%. It had been developed by the Benedict group of physiologists to study animal metabolism via respiration rates.

The machine was powered by the operator who held a reservoir of mercury to pump the gas sample through pipettes containing KOH and pyrogallol. It was precise and unequivocal, but it consumed a 20 ml gas sample, was slow, and technically demanding. Joe finally let me use his machine after long observation. Watching the laborious progress of his study led me to wonder if there could be better ways to learn about photosynthesis. My first year was consumed by courses. I had thermodynamics after a repeat of physical chemistry and then biochemistry from R. A. Gortner. I remember a lucky strike when I challenged a current hypothesis that all protein molecular weights could be reduced to multiples of a few 'prime' numbers. It turned out that Prof. Gortner also did not believe that. In bacteriology courses, A.T. Henrici led me to microbes beyond the bacteria, even to the green algae studied by Martinus Beijerinck. Required reading of German led me to Otto Warburg, partly because his short sentences made biochemistry a joy to read. In plant physiology, George Burr taught me what was known about photosynthesis – not much because it was still enclosed in a green box. The best we could do was to measure what went in and came out and maybe

nibble a little at the edges of the box. That was the hallmark of the era I entered.

Then it became time to discuss a dissertation with my professor. Though George Burr likely had his own project in mind, he listened critically but sympathetically to mine. The green algae were anomalous (compared to higher plants) in producing chlorophyll (Chl) in darkness. They had been used by Warburg in studying photosynthesis. They had the advantage that a measurable sample contained large numbers and would eliminate the individual variation seen in work with geraniums. I knew how to grow algae such as *Chlorella*. I would study their Chls and their photosynthesis in dark *versus* light-grown cells. We had a brand-new photoelectric spectroscopy laboratory that would be great for looking at absorption spectra of the Chls. But how would I study their photosynthesis? I would build a Warburg apparatus. With our limited budget, how would I get the gear? Fortunately, I had a plan worked out to beg, borrow, or build the components. After a long hesitation, Burr approved. For that and our developing relationship I have held him in high esteem. Indeed, his contributions to C-4 photosynthesis are well known. He let me take off on my own with minimal but helpful guidance, checking week-by-week ensuring that I ran into no roadblocks. It was an era when a graduate student could run on a very loose rein (also see Myers 1996). I have been fortunate ever since in following a path, often tortuous, on which I pursued the anomalies, the ideas that seemed not to fit. Since that path seldom led to a frontal edge, my own history is less informative than thoughts on the pivotal ideas of my time, a few of which I shall sketch out as I saw them.

### Maximum quantum yield of photosynthesis

The first big event of the era was the maximum quantum yield (maximum number of oxygen molecules evolved per quantum absorbed) controversy, usually phrased in terms of its reciprocal, the minimum number of quanta needed to produce one oxygen molecule. Otto Warburg (see Warburg and Neglein 1923) had presented detailed evidence for a number close to four, actually a measured 4.3 quanta absorbed per O<sub>2</sub> produced. That number stayed on the books unquestioned for 15 years. Who wanted to challenge Warburg and such an elegant result? Four electrons are needed for conversion of CO<sub>2</sub> to (CH<sub>2</sub>O). So the process seemed to be thermodynamically near-

perfect. There was no room at the inn for the stable intermediates needed for biochemistry. Of course, if the number were much higher, then stable intermediates could be scattered along the route and the quantum number would have little predictive value. So the question symbolically became ‘four or eight?’ The controversy began when the photochemist, Farrington Daniels (1938) and his colleagues at Wisconsin (see Manning et al. 1938), challenged Warburg’s number. Using absorption gas analysis in longer-term experiments with the green alga *Chlorella*, they found quantum numbers much greater than four. In 1941 and the following years, Robert Emerson, with his associate Charleton M. Lewis, focused in on the quantum yield number, using the same manometric methods he had once learned from Warburg and had since become a critical experimentalist (see e.g., Emerson and Lewis 1943). In bicarbonate–carbonate buffer, in which virtually all gas exchange is O<sub>2</sub>, Emerson and his colleagues observed numbers like 8–10. In ‘physiological media’ where CO<sub>2</sub> also is freely exchanged and the CO<sub>2</sub>/O<sub>2</sub> exchange ratio must be assumed, lower quantum numbers, as observed by Warburg, depended on the time protocol of light and dark periods, even to the timing of pressure readings. My friend, Govindjee (1999, 2000), who became one of the players, regards the resulting ‘manometric debate’ as the guts of the controversy from his close perspective. Aside from light–dark transients that contributed uncertainty to manometry, the timing protocol (minutes to hours) was nontrivial. As in other microbes, the normal business of *Chlorella* is in making more cells, rich in protein, poor in carbohydrate. So under truly steady-state (growth) conditions, as much as 20% of the energy balance goes to nitrate reduction and the overall product is more reduced than carbohydrate. A quantum number to be attributed to carbohydrate synthesis must be obtained in short-time experiments with carbohydrate-starved cells. The ratio of quanta/O<sub>2</sub> is deceptively simple. Its meaning depends on the actual photochemical product which is the subject of experimental manipulation. The controversy generated a lot of heat and lined up *photosynthetikers* on two sides, led by protagonists Otto Warburg and Robert Emerson, with a widely billed showdown between them in Urbana in 1948. Later that year, Warburg was given a scientific hearing at Woods Hole. Measurements of quantum number continued for a number of years. I understand from others that Warburg never wavered from his belief that quantum number 4 described real photosynthesis. Emerson turned to the

more interesting question of wavelength effects that led to his discovery of enhancement, known today as the Emerson enhancement effect (Emerson et al. 1957; Emerson and Rabinowitch 1960). (A photograph of Robert Emerson appears in the Editorial of this special issue, whereas a photograph of Otto Warburg appears in the paper by P. Homann, this issue.)

Bessel Kok (1948) had the temerity to make his photosynthetic debut with his dissertation on quantum yield in *Chlorella*. He tackled the difficult task of manometric measurement in optically thin suspensions which required attendant measurement of the fraction of light absorbed. In thin suspensions, he could resolve the effects of very low intensities that are buried in use of thick suspensions. Kok showed that, at least for his strain of *Chlorella*, at very low light the light intensity curve has a break to a higher slope (Kok effect). I thought it a great achievement. But Kok came late to the party. With increasing knowledge of dark reactions and stable intermediates, there was no way to envision four quanta driving oxygenic photosynthesis. The controversy just fizzled out.

### CO<sub>2</sub> fixation

Another research controversy flashed briefly in 1947–1948 until quenched by hard data. The question was what to look for and how to do experiments with the newly available C-14 (invented by Sam Ruben and Martin Kamen). On one side was the Chicago group of Brown et al. (1948) with a strategy based on faulty expectations from the previous heroic but fragmentary work with C-11 (Ruben et al. 1939) and the belief of James Franck that intermediates of carbon reduction would be found on a Chl-protein. On the other side was the Berkeley group led by chemists Andy Benson and Melvin Calvin (see Benson and Calvin 1947; Benson et al. 1949) who were learning about photosynthesis by on-the-job training. Initially, they looked for and found labeling in small molecules such as succinic, malic, and aspartic acids. I remember a height of the argument at the J. Franck and W.E. Loomis symposium of 1948 when I was just getting into photosynthesis big time. I was pushing the idea that in algae nitrogen reduction should be included with carbon reduction in the energetic balance sheet of photosynthesis. I recall commiserating with Andy Benson backstage when it became evident that our ideas were not audience-friendly. Everyone knew that photosynthesis was carbon assimilation and that

it could not follow anything like a reversed path of respiration. The argument cooled in 1949 when the Berkeley group found early-labeled phosphoglyceric acid, PGA (see Benson and Calvin 1950). By 1954, we had a C-3 path of carbon (see A. Benson, this issue) that could be driven by ATP, NADPH, and H<sup>+</sup>s, though it took a little longer to understand the C-4 and related pathways (see M.D. Hatch, this issue).

### Two light reactions and two photosystems

The second era of my career began with the recognition of two light reactions with action spectra that identified two different pigment systems. We had lived with the simplest assumption of a single photochemical event. General acceptance of that change in paradigm occurred in the years 1960–1961. The change came like the break in a dam that could no longer hold back the accumulated anomalies unexplainable by a single photoact. I reckon the dawn of this era as the ‘Light and Life’ Symposium held in March, 1960. And I take as pivotal a paper by Bessel Kok and George Hoch (1961). In it they said out loud: ‘Is Photosynthesis Driven by Two Light Reactions?’ Then they made their claim:

The observations discussed in the above sections strongly indicate the occurrence of two different light reactions: the first sensitized by chlorophyll *a* and a direct bleaching of ‘P700’; the second sensitized by accessory pigments acting indirectly via the mediation of dark steps and restoring ‘P700’.

Also presented at the symposium was a suggestion by R. Hill and W.D. Bonner on roles for the chloroplast cytochromes. This was followed by the detailed proposal of R. Hill and F. Bendall (1960) that located cytochromes in the dark reactions between the photoacts and gave rise to the Z-scheme. By the time of the symposium publication, Duysens et al. (1961) had demonstrated reversible oxidation of Cyt *f*, labeled Photosystems 1 and 2, and explained the Emerson enhancement effect in terms of two photoacts operating in series.

Designating a first in science, as I have done, is far less certain than establishing a patent. There are no legally written ground rules so there is question whether any claim is defensible. My own criteria lie in the literature and my notes on the Symposium. At that time, both Duysens and Hill must have been thinking of schemes with a second photoact. At Urbana, Illinois, E. Rabinowitch must have invoked another

light reaction to explain Emerson's enhancement phenomenon (Emerson and Rabinowitch 1960; Govindjee and Rabinowitch 1960). Myers and French (1960) had extended understanding of enhancement. At the 'Light and Life Symposium' [McElroy and Glass (eds) 1961], Stacy presented our later work to show enhancement in alternated light beams implying an interaction with lifetimes measured in seconds. Stacy even showed a scheme with two light events. But, as with most contemporary reports, we had only rational suggestions without substantive backing. I shall reflect a little on the notion that some part of the success of both Duysens and Kok lay in a common aspect of their strategies. They chose red alga *Porphyridium* and cyanobacterium (blue-green alga) *Anacystis* in which the accessory pigments allow action spectra for Photosystems 1 and 2 to be more easily separated than for the Chl *a/b* systems of the green alga *Chlorella*. An extension of these thoughts leads me to be thankful for the way in which Nature chose up sides for the photosynthetic pigments. I see no principle saying that the two photosystems had to have different action spectra though it was a great help that they do. My course through the two eras followed by own biophysical approach. I leave the exponential development on the biochemical side to David Krogman's (2000) great play-by-play account of his career. The two-light-reaction paradigm provided a scaffold and a particular task for dark reactions: electron transfer between the photoacts and to the reduction of carbon. Amenable experimental material was provided by spinach chloroplasts. Chloroplastology progressed from production of  $0.3 \mu\text{l}$  of  $\text{O}_2$  (Hill and Scarisbrick 1940) to the announcement that the chloroplast is a complete photosynthetic unit (Arnon 1955) and the 'golden age of biochemical research on photosynthesis' (Krogman 2000).

### My laboratory in Texas

I was never a serious player in use of chloroplasts. It was easier for me to behave like a microbiologist and use experimental material provided by steady-state cultures of algae, first the *Chlorellas* and then the blue-greens that became cyanobacteria. In my lab, the 1960s and 1970s became an especially productive period for my students. With *Chlorella*, James Eley extended our observations of enhancement and Jim Pickett clearly ruled out past suggestions that the light-saturated rate might be wavelength depend-



Figure 1. Stacy French.

ent. Also in *Chlorella*, Celia Bonaventura found the light-1/light-2 phenomenon of slow changes in energy distribution to the two reaction centers (see Bonaventura and Myers 1969). We also had among us a friendly stranger, James Walker, a biochemist who convinced his Prof. R.J. Williams that *Chlorella* should be a rich source of biochemical machinery. Then my great 'blue-greeners,' William Kratz, Larry Jones, Chase Van Baalen, Catherine Stevens, and Richard Wang, showed how much could be learned from the cyanobacteria. All of us were aided by Jo-Ruth Graham, a steady technician, a lab manager, and a self-appointed mother to all.

### Reflections, collaborators and heroes

Besides the regenerating give-and-take with students, my scientific course was shaped by two great collaborators. One was Stacy French, Director of the Carnegie Plant Biology Laboratory at Stanford (Figure 1). Stacy had a long history of contributions to spectroscopy of the photosynthetic pigments. He was also an inventor, remembered for building his own optical gear from surplus bombsights. When I arrived at Stanford, as a visiting researcher, in 1959, he had already learned from Francis Haxo the technology of the platinum oxygen electrode. A thin layer of *Chlorella* cells



Figure 2. William Arnold.

settled to provide a thin layer on a polarized platinum surface provided rate measurements of oxygen exchange. Stacy had the optics and shutters for two actinic light beams already in place. For the next several months, I watched recordings of the polarographic current signal of oxygen rate displaying the interaction of two monochromatic light beams. Those months of working with Stacy were the most productive of my career. In 1964, Yoshihiko Fujita came to my lab in Texas as a post-doc. He was one of four young scientists of my 'Japanese Connection' proselyted for me by their mentor, Hiroshi Tamiya. They made their contributions to other areas of algal physiology. Yoshi Fujita (1964) zeroed in on biochemical problems in photosynthesis of cyanobacteria and is still engaged after an early Japanese retirement. Our first productive two years together has continued ever since, including a few months with him in Japan when he was creating a new lab in the Basic Biology Research Institute in Okazaki. He instructed me in what he called 'the Japanese way.' My tribute to Yoshi is that he was once my student, later my teacher. My career has been shaped also by two heroes, men whom I hoped to emulate. The first was William (Bill) Arnold, whom I revered because he thought crisply like a physicist and always started a discussion from first principles. I often visited Bill at Oak Ridge just to get psyched up (Figure 2). He was a practical *theoretiker* who



Figure 3. Bessel Kok.

thought extra long and hard before ever planning an experiment. I was pleased to write a tribute to him and Robert Emerson (Myers 1994) for their 1932 experiments in an unexplored field of very short light flashes, so far ahead of their time. My second hero was Bessel Kok, a man younger but more gifted than I (Figure 3). Our paths crossed often, first on mass culture of algae, then on flashing light, then again when Ann and Pierre Joliot joined him for their magnificent joint work at Baltimore, Maryland (Joliot et al. 1968). Bessel and I often came to the same common result, but he almost always got there first and with better evidence. I recall one session together sharing our thoughts on the frustratingly low yields of *Chlorella* in sunlit mass cultures. We both had come to realize that the low intensities for light saturation were caused by a too-large Chl antenna. Both of us had thought about managing cells for a lower Chl. But we also knew that, in a dense culture, higher Chl cells would always win in the competition for light. So our discovered idea died right there, though happily – for Bessel was a great drinking buddy. I was able to write an appreciation of Bessel in a biographical memoir (Myers 1987). My research career ended a few years ago. Productivity was no longer great enough to justify use of university lab space. My lab was cleaned out for use by a younger scientist. I watched with some mental trauma as a spectrophotometer, monochromators, standard lamps, thermopiles,

and galvanometers were hauled away – mostly to junk – for who wants the instruments of a previous era. So I end reflections on a career in two eras of photosynthesis research. It has been rewarding in fulfilling the romantic expectations with which I started in 1936.

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