Photosynthesis Research **76:** 93–103, 2003.

Minireview

Hydrogen metabolism of green algae: discovery and early research – a tribute to Hans Gaffron and his coworkers *

Peter H. Homann
Department of Biological Science, Florida State University, Tallahassee, FL 32306-4370, USA
(e-mail: homann@bio.fsu.edu; fax: +1-850-644-0481)

Received 15 August 2002; accepted in revised form 24 October 2002

Key words: Norman Bishop, James Franck, Hans Gaffron, green algae, hydrogenase, Erich Kessler, photohydrogen production, photoreduction, Tim Stuart, water photolysis

Abstract

The detection of hydrogen metabolism in green algae more than 60 years ago by Hans Gaffron dispelled the widely accepted dogma at that time that this feature was unique to prokaryotic organisms. Research on this unexpected aspect of algal physiology has continued until today because of its evolutionary implications and possible practical significance. This minireview focuses on the work of Gaffron and his collaborators, whose experiments provided most of the information about the mechanism of hydrogen metabolism in algae during the 35 years following its discovery. It is shown that the emergence of our present mechanistic concepts was closely linked to the changing perception of the process of photosynthetic water oxidation. Whereas the mechanism of 'photoreduction,' i.e., the photoassimilation of carbon dioxide with hydrogen as the electron donor, was well understood already by Gaffron's group as being a reaction mediated by Photosystem I only, a clear concept of the mechanism of light-dependent hydrogen production has been more difficult to establish. Gaffron and his collaborators provided ample evidence, however, that 'photohydrogen' evolution can be fueled by reducing equivalents derived from a photolysis of water as well as by an oxidation of internal and external organic molecules. The presently prevailing view embraces this concept of multiple pathways, but the relative contribution of each of them, and the regulatory mechanisms determining it, remain a matter of debate.

Photoreduction: an unexpected twist in algal photosynthesis and a contentious name

It has been known for about 100 years that molecular hydrogen can be a reactant as well as a product of metabolic reactions in heterotrophic and chemotrophic bacteria. An involvement of hydrogen in photosynthetic processes, however, was not established until more than a quarter of a century later. The stage for the discovery of hydrogen as an electron donor for the photosynthetic reduction of CO_2 was set in 1932 when Cornelis 'Kees' van Niel (1897–1985; for a photograph, see Vernon 2003), a transplanted Dutch scientist at Stanford University, proposed that all photosynthetic processes can be described as a reduction of CO_2 by a hydrogen donor H_2A:

$$CO_2 + 2H_2A \rightarrow (CH_2O) + H_2O + 2A$$

van Niel had recognized that bacterial photosynthesis and photosynthesis by green plants and algae differed only with respect to the source of reducing equivalents, algae being able to use water but bacteria being dependent on the availability other types of reductants ranging from H_2S to organic molecules. Hans Gaffron (1902–1979; shown in Figure 1 with his wife Clara), who had begun his career as an assistant to Otto

* I dedicate this minireview to the memory of Hans Gaffron, one of the pioneers of modern photosynthesis research, my esteemed teacher and mentor, and my friend [see his obituary in Akoyunoglou (1981)].

Warburg (1883–1970) in Berlin, would remark 30 years later that van Niel had 'put research on photosynthesis on the right track for the first time' (Gaffron 1962). Gaffron admitted, however, that he had been a reluctant convert to van Niel's view. He had interpreted his data on bacterial photosynthesis (Gaffron 1935) in a way that prompted van Niel to complain about Gaffron's 'arguments against a unified concept of photosynthesis in green plants and photosynthetic bacteria' (van Niel 1935). The disagreements were amicably resolved shortly thereafter when van Niel visited Gaffron at the Kaiser-Wilhelm-Institut für Biologie in Berlin for some joint experiments.

Hans Gaffron's early work with photosynthetic bacteria not only became instrumental in forging a close friendship with van Niel but would also establish his life-long interest in the role of molecular hydrogen as a metabolite and its significance during the evolution of photosynthetic organisms. Gaffron's entry into the world of hydrogen metabolism occurred when he read that Pieter Roelofsen in Utrecht had discovered molecular hydrogen to support the photosynthetic reduction of carbon dioxide by sulfur bacteria (Roelofsen 1934). Gaffron had been exploring the diversity of reductants that could be used by non-sulfur purple bacteria and promptly found that his organisms were able to make use of molecular hydrogen as well (Gaffron 1935). From this time on, the enzyme hydrogenase, the biological catalyst of hydrogen metabolism described and named by Marjory Stephenson and Leonard Stickland in 1931, had to be reckoned with in research on photosynthesis. In fact, Hans Gaffron would add hydrogenase as a key element to the concept of a fundamental similarity of photosynthesis in bacteria and green plants. He did so after leaving Nazi Germany for the United States in 1937. Initially, Gaffron found refuge as a guest of Professor van Niel in his laboratory at Pacific Grove, California. There Gaffron made the surprising discovery that some green algae can be 'adapted' to perform a bacterial type of photosynthesis with hydrogen as a reductant (Gaffron 1939). After joining the Fels Fund-supported research laboratory of James Franck (1882–1964; 1925 Nobel laureate in physics) at the University of Chicago, he extended his investigations and determined, among other things, that the assimilation of 1 CO_2 was accompanied by a consumption of the expected stoichiometric amount of 2 H_2 (Gaffron 1940a).

Initially, Hans Gaffron referred to this process as 'photoreduction with hydrogen' or as 'anaerobic photosynthesis,' but he then proposed to call it simply 'photoreduction' in order to distinguish it from oxygen-producing 'photosynthesis' (Gaffron 1940a). The term 'photoreduction' established itself in spite of vigorous opposition from Eugene Rabinowitch (1901–1973), who only reluctantly adopted it in Volume I of his compendium on photosynthesis 'as a short substitute for "photoreduction of carbon dioxide by reductants other than water" ' (Rabinowitch 1945). Rabinowitch's unhappiness with the term 'photoreduction' is documented in his correspondence with Hans Gaffron, of which several letters (available from the author) were left behind by Gaffron when he retired from Florida State University in 1973. In one of the letters, Hans Gaffron defended his choice by citing the need for a short expression 'as a matter of practical expediency.' Rather typically, he argued that 'the actual meaning is set down dictatorially by definition and not by scientific analysis' and goes on: 'Once everybody has learned what is meant by this expression, it will be used without hesitation.' Eugene Rabinowitch eventually conceded defeat, accusing Hans Gaffron of 'bullying (him) into the use of "photoreduction" ' and then declaring once more that 'it *is* a bad term.'

It is difficult today to appreciate the conceptual background of this controversy. For Rabinowitch, the photosynthetic nature of the anaerobic photoreduction of carbon dioxide was of paramount importance, whereas for Gaffron the focus was on the reducing action of hydrogen, which he saw as an interference with the process of oxygen production. James Franck and Hans Gaffron in Chicago (shown in Figure 2) had accepted a mechanistic view of photosynthesis proposed independently by van Niel (1935, 1941) and Hirosi Nakamura (1937) according to which all photosynthetic organisms accomplish the light-dependent reduction of carbon dioxide with hydrogens abstracted from water molecules (for today's definition of photosynthesis, see Gest 2002). Rabinowitch, incidentally, had reservations about this concept and suggested in his compendium on photosynthesis (Rabinowitch 1945) what eventually would prove to be correct (see Ke 2001), namely that in photosynthetic bacteria the oxidizing potential of the primary light-generated oxidant is not sufficiently high to oxidize, i.e., dehydrogenate, water. Franck and Gaffron (1941), for their part, concerned themselves with the fates of the 'H' and the 'dehydrogenated water OH' of the reaction mechanism proposed by van Niel. They proposed that OH would form a peroxide-like compound that is either rehydrogenated to water by some reduct-

Figure 1. Hans Gaffron and his wife Clara, whose cheerful personality is remembered fondly by all who got to know her and who contributed to the accomplishments of the Gaffron laboratory by keeping the algae of its culture collection alive. Time and occasion of this photograph are unknown.

ant or decomposed to oxygen if the organism happens to possess an appropriate catalase-like enzyme. For photoreduction to become possible in green algae, molecular hydrogen had to reduce the peroxide to water before oxygen could be produced by the action of their 'photocatalase' (Figure 3). Indeed, when Foster Rieke determined that CO_2 assimilation by oxygen-producing photosynthesis and by photoreduction had essentially identical quantum requirements, the conclusion seemed inevitable that 'photoreduction proceeds through the very same sequence of primary photochemical reactions as does normal photosynthesis' (Rieke 1949).

From work with photosynthetic bacteria it could be inferred that the ability of molecular hydrogen to function as a reductant for the photochemically produced oxygen precursor required the participation of the enzyme hydrogenase. It had also been reported that some bacterial hydrogenases were inactivated rapidly when exposed to molecular oxygen. It was not surprising, therefore, that Gaffron's algae had to be 'adapted' in the dark under anaerobic conditions before they were capable of performing photoreduction. As another consequence of the oxygen sensitivity of the putative algal hydrogenase, it was expected that photoreduction could be maintained only when oxygen-producing normal photosynthesis was shut down, or when the oxygen it generated was

Figure 2. Nobel laureate James Franck on the right with Hans Gaffron, at the University of Chicago in 1951.

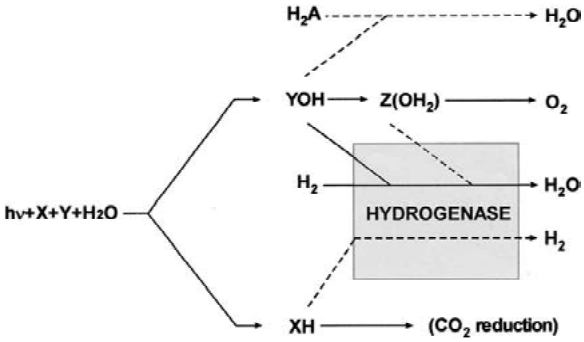

Figure 3. Schematic presentation of the mechanism of the light-dependent hydrogen metabolism in green algae as envisaged by James Franck and Hans Gaffron. In its original form as drawn up by Gaffron (1957), the diagram represented photoreduction only. It has been modified here to show, in addition, reaction sequences proposed by Gaffron and Rubin (1942) for photohydrogen evolution.

scavenged by oxygen-consuming reactions. Hans Gaffron actually had observed that the rate of hydrogen consumption was proportional to the light intensity only up to certain threshold and, beyond it, would be replaced after a few minutes by the evolution of oxygen from regular photosynthesis. Apparently, only at low light intensities were oxygen-scavenging reactions adequate to allow photoreduction to proceed. Aside from cellular respiration, Gaffron (1940a, b) implicated a recombination of hydrogen and oxygen in the water-producing oxy-hydrogen or 'Knallgas' reaction that he knew to occur in hydrogen bacteria.

Because Hans Gaffron saw his green algae in essence as photosynthetic bacteria endowed with a photocatalase, he realized that his discovery of photoreduction had offered him an opportunity to separate in a single organism the oxygen-producing reaction from the transfer of hydrogen to carbon dioxide. To this end, he undertook a study of the effects of various types of known metabolic 'poisons' on the process of photoreduction in algae (Gaffron 1942, 1945) and found that some of them stabilized it because they curtailed specifically the photosynthetic production of inhibitory oxygen. The importance of this investigation was that it revealed how comparative analyses of the responses of photosynthesis and photoreduction to experimental manipulations can identify conditions that selectively impair the oxygen-producing step. Perhaps the most significant early application of this approach was aimed at the role of manganese, which had been shown to be essential for photosynthetic activity by Andre Pirson in Germany (Pirson 1937; see also Pirson's perspective in 1994). In the course

Figure 4. Erich Kessler. The picture was taken at the University of Erlangen, Germany, where Erich Kessler is now professor emeritus. He had served as Professor and Member of the Board of Directors of the Institute of Botany and Pharmaceutical Biology.

of further investigations after World War II, Pirson and his coworkers recognized a similarity between the responses of algal photosynthesis to manganese deficiency and to some of the metabolic poisons used by Hans Gaffron (Pirson et al. 1952). This insight suggested to them that manganese might be required for the mechanism of oxygen production. If so, algae should retain their ability to perform photoreduction when they had been grown in manganese-deficient media. A few years later Erich Kessler (shown in Figure 4), from Pirson's research group, confirmed this prediction during a visit to the Franck–Gaffron laboratory in Chicago. Manganese-deprived algae indeed had an undiminished capacity for photoreduction that was stabilized just as in Gaffron's inhibitor studies (Kessler 1957). These observations provided the first hint at a cofactor function of manganese in the photosynthetic mechanism of oxygen evolution. (For a discussion of the mechanism of water oxidation, see Renger this issue.)

As discussed above, Gaffron and his coworkers assumed the switch from photosynthesis to photoreduction to be a matter of competition between the hydrogenase-catalyzed reduction of the putative 'photoperoxide' by hydrogen and the catalysis of its decomposition to molecular oxygen. However, when

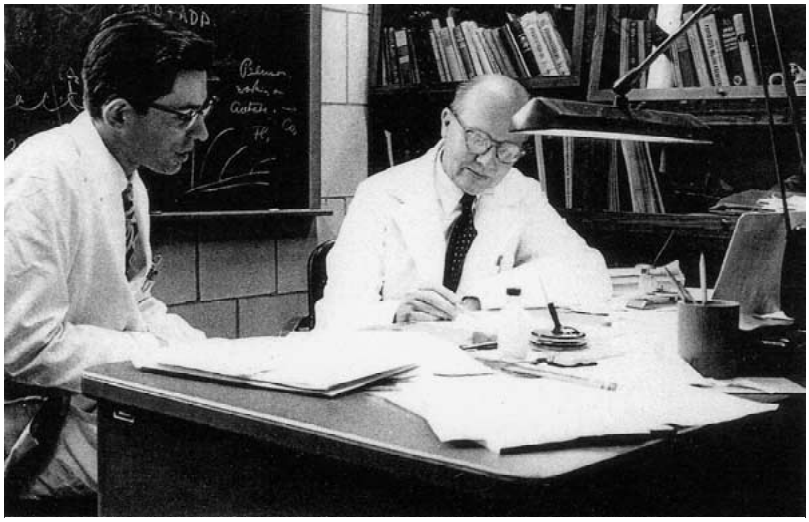

Figure 5. Norman I. Bishop (left) with Hans Gaffron in 1957 at the University of Chicago. Norman Bishop is presently professor emeritus in the Department of Botany and Plant Pathology at Oregon State University.

Robin Hill and Fay Bendall presented their 'Z'-scheme in 1960, the question arose whether the substitution of hydrogen consumption for oxygen evolution during photoreduction might reflect a lack of involvement of the entire water-oxidizing Photosystem II. This possibility was addressed by Norman Bishop (shown in Figure 5), who had joined Hans Gaffron and in 1960 moved with him from the University of Chicago to Florida State University in Tallahassee. It had been established by that time that, of the two cooperating photochemical events observed a few years earlier by Robert Emerson and his coworkers (1957), the one driven by light with wavelengths above about 700 nm was associated with Photosystem I. Norman Bishop made use of this criterion and concluded that photoreduction was indeed supported by Photosystem I alone because, unlike normal photosynthesis, it proceeded very well in far-red light (Bishop and Gaffron 1962; Bishop 1967).

Disposal of hydrogen derived from the photolysis of water and from oxidations of metabolites under anaerobic conditions

The discovery of a hydrogen-dependent photosynthesis in green algae had revealed that these eukaryotic organisms retained traits of their prokaryotic ancestors, but how bacteria-like were they? To be sure, they appeared to be unable to survive, let alone grow, under strictly anaerobic conditions (reviewed by Kessler 1974). Only a few years after the detection of photoreduction, however, Hans Gaffron and Jack Rubin had discovered in hydrogenase-containing algae another metabolic feature known from studies of photosynthetic bacteria. In the dark and after adaptation in a nitrogen atmosphere, these algae would release molecular hydrogen and carbon dioxide. Gaffron and Rubin (1942) predicted that the two gases would be consumed again via photoreduction if the light were turned on and that eventually photosynthesis might take over. To prevent these two CO_2-requiring events from happening, Gaffron and Rubin conducted measurements in a CO_2-free atmosphere and trapped any CO_2 given off by the algae in a well with KOH. Under such conditions, the accumulation of hydrogen in the gas phase not only continued in the light but did so at an up to 10-fold higher rate. Another demonstration of such a 'photohydrogen production' by suspensions of hydrogenase-containing algae without interference from competing reactions turned out to be possible when 2,4-dinitrophenol was added. This compound inhibited photosynthesis and photoreduction as well as hydrogen production in the dark, but in contrast to reports for bacterial systems, it did not affect the light-dependent evolution of hydrogen by the algae.

The pioneering study by Gaffron and Rubin not only uncovered the surprising ability of some green algae to release small but substantial amounts of molecular hydrogen upon illumination but also revealed the complexity of hydrogen metabolism in these organisms. Unraveling it required following the fates of

different gases simultaneously and the ability to assign changes within a set of different gas-producing and gas-consuming reactions that could occur concurrently. In a first attempt to understand the mechanism underlying the photoproduction of hydrogen by green algae, Gaffron and Rubin determined in a series of volumetric experiments that the effect of light was to stimulate the evolution of hydrogen while leaving the extent of carbon dioxide production largely unchanged. This observation suggested to them that the photoproduced hydrogen did not originate in some 'kind of a photofermentation' but was disposed of during the photochemical oxidation of water when the generated 'H' was 'unable to reach' carbon dioxide. The dehydrogenated water 'OH' that was left behind was proposed to be reduced to water by a pool of endogenous hydrogen donors (cf. Figure 3), which, as a stimulation of hydrogen production by added metabolites like glucose revealed, apparently could be augmented from external resources.

Gaffron and Rubin's view of the mechanism of photohydrogen production remained unchallenged for at least another decade. Albert Frenkel, who had collaborated with Hans Gaffron when he studied photoreduction in blue-green algae, turned to the flagellate green alga *Chlamydomonas* (Frenkel 1952) and confirmed the selective stimulation of hydrogen production in the light that Gaffron and Rubin had described for *Scenedesmus*. He also compared the rates of photosynthetic oxygen production and hydrogen evolution at low light intensities and found them consistent with the assumption that hydrogen and oxygen originated from the same oxidoreduction events. He did not, however, invoke a complete oxidation of water to oxygen during photohydrogen production. Indirect evidence for such a possibility was perhaps obtained by Leonard Horwitz and F. L. Allen (1957), who were members of the research team of James Franck and Hans Gaffron at the time. These investigators extended earlier experiments by James Franck and his collaborators (Franck et al. 1945), who had sampled an originally oxygen-free gas phase over illuminated algae and determined its oxygen concentration by a sensitive method based on phosphorescence quenching. Horwitz and Allen confirmed their observation that, in an atmosphere of hydrogen supplemented with 2% CO_2, some oxygen is produced even at very low light intensities. Normal photosynthesis obviously was not shut down completely in adapted algae under conditions conducive to photoreduction. Significantly more oxygen accumulated in the gas phase when hydrogen had been replaced by nitrogen. This result was to be expected because the photoassimilation of carbon dioxide with hydrogen as a reductant no longer competed with normal photosynthesis under these conditions. According to Horwitz and Allen, an important additional reason for the differences in oxygen yield was a consumption of oxygen by the oxy-hydrogen reaction when hydrogen was available. What they did not consider was a contribution to the oxygen balance by the photoproduction of hydrogen that Gaffron and Rubin had shown to be optimal in an atmosphere of nitrogen. A complete photolysis of water resulting in a simultaneous production of hydrogen and oxygen apparently was not yet considered a possibility in Franck and Gaffron's research group. Ironically, this mech-

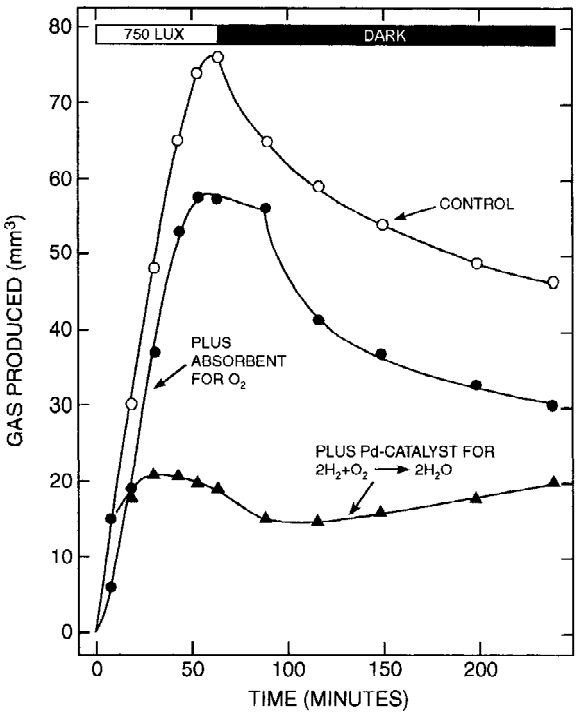

Figure 6. Gas exchange of anaerobically adapted cells of *Scenedesmus* measured manometrically by Norman Bishop in the absence of CO_2 under nitrogen and in a medium containing 10^{-5} M carbonyl-cyanide *m*-chlorophenylhydrazone to prevent photoreduction. The diagram is a modified version of the one published by Bishop and Gaffron (1963). Comparison of the trace obtained in the presence of oxygen-absorbing alkaline pyrogallol with the total gas production of the control allows an estimate of the amount of oxygen formed relative to the amount of hydrogen. Moreover, if all of the photoproduced hydrogen were the result of water splitting, no gas exchange should have been measured in the presence of the Pd catalyst that catalyzes the reconstitution of water. The lower curve therefore suggests that sources other than water supplied reducing equivalents for hydrogen production during the first minutes of illumination.

Figure 7. Tim S. Stuart (right) and Hans Gaffron in the laboratory at Florida State University. Tim Stuart is now Senior Advisor at the US Environmental Protection Agency. (The photograph was printed in *Research in Review* by the Graduate Research Office at Florida State University on the occasion of Professor Gaffron's retirement in May 1973; it is reproduced here with permission.)

anism was addressed at that time on the other side of the Atlantic by C.J.P. Spruit in Wageningen, The Netherlands (Spruit 1958). Using innovative polarographic techniques, he tested whether a quantitative relation might exist between the production of oxygen and hydrogen by adapted algae, but his results were inconclusive. He succeeded, however, in detecting the expected ratio of two hydrogens to one oxygen after he had killed the cells by freezing them in liquid nitrogen.

When the concept of two photosystems took hold in the early 1960s, the focus shifted to the roles played by Photosystem II and Photosystem I in the process of light-dependent hydrogen evolution. A reinterpretation of Gaffron and Rubin's hypothesis, of course, had to invoke a critical role for the water-oxidizing Photosystem II. This contention had already received support by Kessler's observation that photoproduction of hydrogen was severely impaired in his manganese-deficient algae, quite in contrast to photoreduction (Kessler 1957). Definitive proof for an involvement of Photosystem II came a few years later with a new and exciting experimental approach that was initiated in Gaffron's laboratory by Norman Bishop. Bishop had set out to generate mutants of the green alga *Scenedesmus* that could grow heterotrophically and develop normal pigmentation but were incapable of performing photosynthesis. He continued this effort after the laboratory's relocation to Florida State University in Tallahassee and eventually succeeded in identifying among such mutants one that was fully competent in assimilating carbon dioxide via photoreduction and could do so without reverting to oxygen-producing photosynthesis (Bishop 1962). This observation and other measurements indicated that the mutation had affected reactions involved in photosynthetic oxygen evolution without impairing the function of Photosystem I. It turned out that this condition resulted in an inability to photoproduce hydrogen (Bishop and Gaffron 1963). Moreover, in wild-type cells the rate of hydrogen production was increased by a superposition of far-red light on red light (cf. Emerson et al. 1957) in the same synergistic fashion as was the rate of photosynthesis. These revelations left no doubt that hydrogen was liberated in the light as a result of an oxidation of water in Photosystem II and a transfer of the reducing equivalents to hydrogenase via Photosystem I. But there remained the still unanswered question of whether the evolution of hydrogen was really sustained by a reduction of the putative photoperoxide, as had been postulated by Gaffron and Rubin, or perhaps instead by its decomposition to oxygen. Bishop and Gaffron addressed this question by designing an elaborate set of manometric experiments and decided between the two alternatives in favor of a complete

photolysis of water when they detected that oxygen was released simultaneously with the photoproduced hydrogen (Figure 6).

Norman Bishop's investigations should have settled the issue of the mechanism underlying the evolution of hydrogen by illuminated green algae, but this was not to be. In the late 1960s, Hans Gaffron persuaded his postdoctoral associate Heinrich Kaltwasser to revisit the problem. Joined by Tim Stuart, a graduate student, he found conditions under which Bishop's *Scenedesmus* mutant would produce hydrogen in the light at rates that were almost as high as those seen with the wild-type strain (Stuart and Kaltwasser 1970). This study, and extensive further investigations by Tim Stuart (shown in Figure 7), revealed that hydrogen photoproduction by adapted wild-type cells was inhibited only partially when electron flow from Photosystem II was prevented by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and other Photosystem II-impairing chemicals and that far-red light was able to support significant hydrogen-evolving activities (Stuart and Gaffron 1971, 1972a, b). Using mass-spectrophotometric analyses, the authors also failed to detect the simultaneous evolution of oxygen and hydrogen that the manometric experiments of Bishop and Gaffron had revealed (Stuart and Gaffron 1972c; see Figure 8). Furthermore, they observed a stimulation of photohydrogen production by glucose (Gaffron and Rubin 1942) even when an involvement of Photosystem II had been excluded by DCMU, and the stimulation was proportional to the amount of glucose added (Stuart and Gaffron 1971). This latter result suggested that glucose served as a reductant to an electron carrier between the two photosystems. (Norman Bishop has pointed out to me, in a letter, that in some experiments the effect of glucose might at least in part be accounted for by its inhibitory action on photoreduction, which, according to an early publication [Bishop 1961], he attributes to a competition between carbon dioxide assimilation and glucose uptake for light-generated ATP.) In his final publication on the mechanism of hydrogen photoproduction by green algae, Tim Stuart summarized his experimental results in a scheme that shows how reducing equivalents can be supplied for hydrogen evolution with and without involvement of a functional Photosystem II (see Figure 9).

Some of Stuart's results were corroborated independently by Patrick Healey (1970). As Kaltwasser and Stuart as well as Stuart and Gaffron had done in many of their experiments, he used in his assays rela-

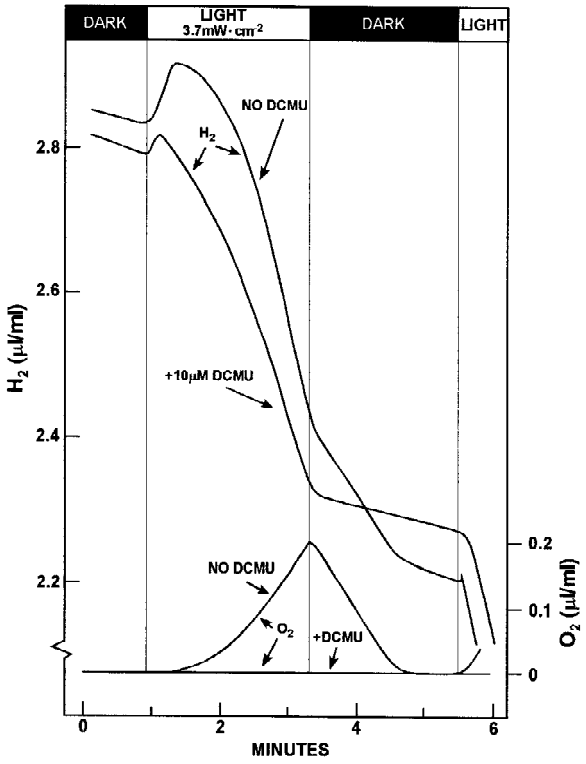

Figure 8. Gas exchange of *Scenedesmus* cells measured by Tim Stuart using mass spectrometry. Gasses were sampled from an atmosphere of hydrogen supplemented with 5% CO_2. The diagram is a modified version of the one published by Stuart and Gaffron (1972). It reveals that an initial burst of hydrogen production was followed by an uptake of hydrogen via photoreduction. No oxygen was detected during the burst of photohydrogen production even though it was partially inhibited by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). The subsequent appearance of oxygen presumably reflected the onset of normal photosynthesis. After the light was turned off, this oxygen was consumed by cellular respiration and by combination with hydrogen in the oxy-hydrogen reaction.

tively high concentrations of the uncoupling agent carbonylcyanide *m*-chlorophenylhydrazone with the aim of fully eliminating hydrogen reabsorption by photoreduction. Healey found, among other things, that under such experimental conditions the action spectrum for photohydrogen production from *Chlamydomonas* had all the characteristics of a process mediated by Photosystem I alone. Meanwhile, Norman Bishop, then at Oregon State University, had substantiated further the requirement for both photosystems in studies with inhibitors and with new algal mutants, some with lesions in Photosystem II and some with an impaired Photosystem I. Using a dual polarographic electrode system, his research team demonstrated again that living adapted algal cells produce oxygen simultaneously

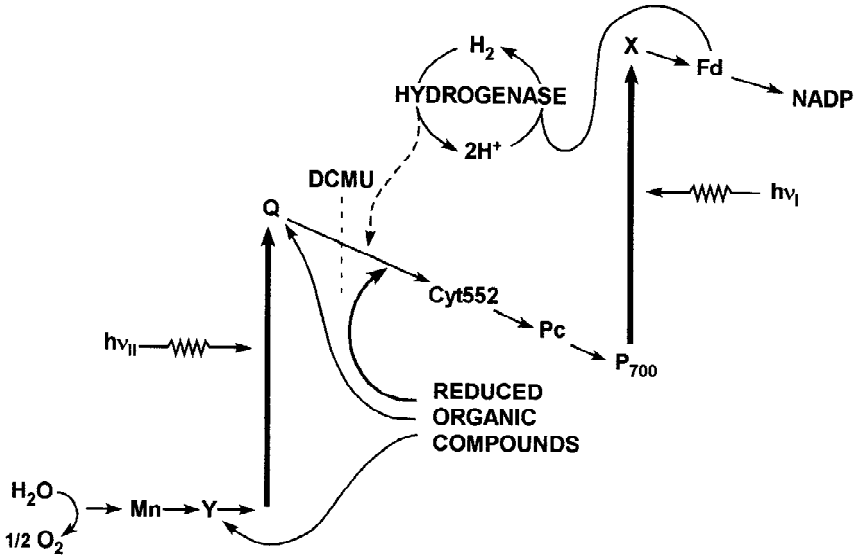

Figure 9. Summary of the conclusions of Tim Stuart and Hans Gaffron regarding the sources of reducing equivalents for photohydrogen production by *Scenedesmus* cells and their sites of entry along the photosynthetic electron transport chain. The diagram is a modified version of the one published by Stuart and Gaffron (1972b).

with hydrogen (Bishop et al. 1977). Yet those studies also revealed, and Bishop confirmed later in collaboration with Horst Senger in Marburg (Senger and Bishop 1979), that a Photosystem II-independent photoproduction of hydrogen can occur to some extent.

Even though some of the contradictory results remain to be reconciled, a consensus appears to have emerged that the photoproduction of hydrogen by green algae can be fueled by a photolysis of water as well as by reducing equivalents delivered from external or internal sources to either the reducing or the oxidizing side of Photosystem II. The controversy has been reduced now to a discussion of the degree to which light-dependent hydrogen production can be supported by Photosystem I alone. Which position one takes depends not only upon the species of alga used and upon the age and growth conditions of the cells but also upon the assay conditions and which kinetic phase of hydrogen production is being analyzed. The data of Bishop and his coworkers (Bishop et al. 1977; Senger and Bishop 1979) as well as those of Stuart and Gaffron (1971, 1972a–c) provide ample evidence for this assertion.

Early evidence for a physiological role of algal hydrogenases

Aside from the significance of the retention of hydrogenase as an evolutionary relic, it has remained a puzzle why it occurred seemingly at random among the eukaryotic algae. Erich Kessler had realized early on that an answer to this puzzle might be found in a comparison of the fitnesses, under conditions of stress, of algae known to have an active hydrogenase under anaerobic conditions and of those which do not. He noticed that the former did not suffer extensive chlorosis when subjected to very high light intensities or when Mn-deficiency had curtailed the assembly of active water-oxidizing complexes. He also concluded from analyses of chlorophyll-*a* fluorescence kinetics that in these algae the plastoquinone pool became more reduced during extended darkness (Kessler 1968, 1970). The remarkable aspect of these observations is that they were made under aerobic conditions, i.e., under conditions that should have rendered hydrogenase inactive. Because no evidence has ever been obtained for an active algal hydrogenase under aerobic conditions, one may ask whether an anaerobic microenvironment is generated in the chloroplasts when light intensities are excessive or when the normal function of Photosystem II is impaired. Such a scenario would allow a constitutive hydrogenase to become active and help divert light-generated reducing and oxidizing power from potentially destructive reactions as has been discussed recently for anaerobic conditions by Röbbe Wünschiers and Rüdiger Schulz from Horst Senger's group (Wünschiers et al. 2001). The

observed entry of reducing equivalents into the reducing side of Photosystem II, furthermore, may reveal that hydrogenase-containing algae do indeed possess an efficient mechanism for funneling electrons from metabolites to the photosynthetic electron-transport chain. This feature may provide a mechanism for a rapid and efficient disposal of any excess of accumulated reducing equivalents under anaerobic conditions, but it apparently contributes little to sustained photohydrogen production which depends on water as its main source of electrons (see Figure 6, Pow and Krasna, 1979, and the up-to-date account by A. Melis and T. Happe to be published in Part 3 of the historical issues).

Acknowledgments

I am grateful for the many helpful comments and suggestions I have received from Dr Norman I. Bishop and Dr Tim S. Stuart, whom I met early in my career as members of Hans Gaffron's group in Tallahassee and whose professional lives took them elsewhere more than 30 years ago. I also wish to thank Barbara Gaffron for sharing with me informative autobiographical notes of her father-in-law and for making available the photographs shown in Figures 1, 2, and 7. I am indebted, furthermore, to Ken Womble for preparing the illustrations, and to Ann Thistle who proofread the manuscript and suggested improvements to the text. Finally, I wish to acknowledge the exchange of ideas with my former graduate student Anastasios Melis, who contributes, together with Thomas Happe, a review of the history of the exploration and practical exploitation of the algal hydrogenase to Part 3 of these historical issues. This paper was edited by Govindjee.

References

Akoyunoglou G (ed) (1981) Photosynthesis, Vol III. Balaban International Science Service, Philadelphia.

Bishop NI (1961) The photometabolism of glucose by hydrogen-adapted algae. Biochim Biophys Acta 51: 323–332

Bishop NI (1962) Separation of the oxygen evolving system of photosynthesis from the photochemistry in a mutant of *Scenedesmus*. Nature (Lond) 195: 55–57

Bishop NI (1967) Comparison of the action spectra and quantum requirements for photosynthesis and photoreduction of *Scenedesmus*. Photochem Photobiol 6: 621–628

Bishop NI and Gaffron H (1962) Photoreduction at λ705 mμ in adapted algae. Biochem Biophys Res Comm 8: 471–476

Bishop NI and Gaffron H (1963) On the interrelation of the mechanisms for oxygen and hydrogen evolution in adapted algae. In: Photosynthetic Mechanisms in Green Plants pp 441–451. Publ 1145. Natl Acad Sci – Natl Res Council, Washington, DC

Bishop NI, Frick M and Jones LW (1977) Photohydrogen production in green algae: water serves as the primary substrate for hydrogen and oxygen production. In: Mitsui A, Miyachi S, San Pietro A and Tamura S (eds) Biological Solar Energy Conversion pp 3-22. Academic Press, New York

Emerson R, Chalmers R and Cederstrand C (1957) Some factors influencing the long wavelength limit of photosynthesis. Proc Natl Acad Sci US 43: 133–143

Franck J and Gaffron H (1941) Photosynthesis, facts and interpretations. Adv Enzymol 1: 199–262

Franck J, Pringsheim P and Lad DT (1945) Oxygen production by anaerobic photosynthesis of algae measured by a new micromethod. Arch Biochem 7: 103–142

Frenkel AW (1952) Hydrogen evolution of the flagellate green alga *Chlamydomonas moewusii*. Arch Biochem Biophys 38: 219–230

Gaffron H (1935) Stoffwechsel der Purpurbakterien. Biochem Zeitschr 275: 301–319

Gaffron H (1939) Reduction of carbon dioxide with molecular hydrogen in green algae. Nature 143: 204

Gaffron H (1940a) Carbon dioxide reduction with molecular hydrogen in green algae. Am J Bot 27: 273–283

Gaffron H (1940b) The oxyhydrogen reaction in green algae and the reduction of carbon dioxide in the dark. Science 91: 529–530

Gaffron H (1942) The effect of specific poisons upon the photoreduction with hydrogen in green algae. J Gen Physiol 26: 195–217

Gaffron H (1945) *o*-phenanthroline and derivatives of vitamin K as stabilizers of photoreduction in *Scenedesmus*. J Gen Physiol 28: 269–285

Gaffron H (1957) Photosynthesis and the origin of life. In: Rudnick D (ed) Rhythmic and Synthetic Processes in Growth, pp 127–154, Princeton University Press, Princeton, New Jersey

Gaffron H (1962) On dating stages in photochemical evolution. In: Kasha M (ed) Horizons in Biochemistry, pp 59–89. Academic Press, New York

Gaffron H and Rubin J (1942) Fermentative and photochemical production of hydrogen in algae. J Gen Physiol 20: 219–240

Gest H (2002) History of the word photosynthesis and evolution of its definition. Photosynth Res 73: 7–10

Healey, FP (1970) The mechanism of hydrogen evolution by *Chlamydomonas moewusii*. Plant Physiol 45: 153–159

Hill R and Bendall F (1960) Function of the two cytochrome components in chloroplasts: a working hypothesis. Nature 186: 136–137

Horwitz L and Allen FL (1957) Oxygen evolution and photoreduction in adapted *Scenedesmus*. Arch Biochem Biophys 66: 45–63

Ke B (2001) Photosynthesis: Photobiochemistry and Photobiophysics. Kluwer Academic Publishers, Dordrecht, The Netherlands

Kessler E (1957) Stoffwechselphysiologische Untersuchungen an Hydrogenase enthaltenden Grünalgen. I. Über die Rolle des Mangans bei Photoreduktion und Photosynthese. Planta 49: 435–454

Kessler E (1968) Effects of manganese deficiency on growth and chlorophyll content of algae with and without hydrogenase. Archiv Mikrobiol 63: 7–10

Kessler E (1970) Photosynthesis, photooxidation of chlorophyll and fluorescence of normal and manganese-deficient *Chlorella* with and without hydrogenase. Planta 92: 222–234

Kessler E (1974) Hydrogenase, photoreduction and anaerobic growth. In: Stewart WDP (ed) Algal Physiology and Biochemistry, pp 456–473, University of California Press, Berkeley

Melis A and Happe T (2001) Hydrogen production. Green algae as a source of energy. Plant Physiol 127: 1–9

Nakamura H (1937) Über die Photosynthese bei der schwefelfreien Purpurbakterie, *Rhodobacillus palustris*. Beiträge zur Stoffwechselphysiologie der Purpurbakterien, I. Acta Phytochimica 9: 189–229

Pirson A (1937) Ernährungs- und Stoffwechselphysiologische Untersuchungen an *Fontinalis* und *Chlorella*. Z Bot 31: 193–267

Pirson A (1994) Sixty years in algal physiology and photosynthesis. Photosynth Res 40: 207–222

Pirson A, Tichy C and Wilhelmi G (1952) Stoffwechsel and Mineralsalzernährung einzelliger Grünalgen. I. Vergleichende Untersuchungen an Mangelkulturen von *Ankistrodesmus*. Planta 40: 199–253

Pow T and Krasna AI (1979) Photoproduction of hydrogen from water in hydrogenase-containing algae. Arch Biochem Biophys 194: 413–421

Rabinowitch EI (1945) Photosynthesis and Related Processes, Vol I, Interscience Publishers, New York, 509 pp.

Senger H and Bishop NI (1979) Observations on the photohydrogen producing activity during the synchronous cell cycle of *Scenedesmus obliquus*. Planta 145: 53–62

Renger G (2003) Apparatus and mechanism of photosynthetic oxygen evolution: a personal perspective. Photosynth Res 76: 269–288 (this issue)

Rieke FF (1949) Quantum efficiencies for photosynthesis and photoreduction in green plants. In: Franck J and Loomis WE (eds) Photosynthesis in Plants, pp 251–272. Iowa State College Press, Iowa City

Roelofsen PA (1934) On the metabolism of the purple sulphur bacteria. Proc R Acad Sci Amsterdam 37: 3–12

Spruit CJP (1958) Simultaneous photoproduction of hydrogen and oxygen by *Chlorella*. Meded Landbouwhogesch Wageningen 58: 1–17

Stephenson M and Stickland LH (1931) Hydrogenase: a bacterial enzyme activating molecular hydrogen. I. The properties of hydrogenase. Biochem J 25: 205–214

Stuart T and Gaffron H (1971) The kinetics of hydrogen photoproduction by adapted *Scenedesmus*. Planta 100: 228–243

Stuart T and Gaffron H (1972a) The mechanism of hydrogen photoproduction by several algae. I. The effects of inhibitors of photophosphorylation. Planta 106: 91–100

Stuart T and Gaffron H (1972b) The mechanism of hydrogen photoproduction by several algae. II. The contribution of Photosystem II. Planta 106: 101–112

Stuart T and Gaffron H (1972c) The gas exchange of hydrogen-adapted algae as followed by mass spectrometry. Plant Physiol 50: 136–140

Stuart T and Kaltwasser H (1970) Photoproduction of hydrogen by Photosystem I of *Scenedesmus*. Planta 91: 302–312

van Niel CB (1932) On the morphology and physiology of purple and green sulfur bacteria. Arch Mikrobiol 3: 1–112

van Niel CB (1935) Photosynthesis of Bacteria. Cold Spring Harbor Symp Quant Biol III: 138–150

van Niel CB (1941) The bacterial photosyntheses and their importance for the general problem of photosynthesis. Adv Enzymol 1: 263–328

Vernon LP (2003) Photosynthesis and the Charles F. Kettering Research Laboratory. Photosynth Res 76: 379–388 (this issue)

Wünschiers R, Senger H and Schulz R (2001) Electron pathways involved in H_2-metabolism in the green alga *Scenedesmus obliquus*. Biochim Biophys Acta 1503: 271–278