event indicate that the line of sight intersected Neptune's equatorial plane at $3 \pm 1\ R_N$ at the time of the occultation (*8*). The duration of 8.1 ± 0.3 seconds gives a chord length of 180 ± 7 km if the occulting object moved with Neptune's motion and a minimum diameter of 100 km if it was moving in a direct, circular, equatorial orbit. The abruptness of the change in signal level eliminates the possibility of a grazing contact and limits the maximum diameter to less than ten times these values.

Such an object would have a stellar magnitude of 16 to 20 for an albedo of 0.6 and would have eluded earlier detection because of its proximity to Neptune. Previous examinations of the Neptune system at the University of Arizona reached a limit of $m_V = 18$ to within about 6 arc seconds of Neptune without result. The new object will be difficult to image from ground-based telescopes unless the observed chord is much smaller than the diameter or the object reaches a much greater distance from Neptune than it had when observed by this occultation.

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4. Two weeks before this event, three University of Arizona teams in the Tucson area observed the 10 May 1981 close approach of Neptune to a star. A fourth team collaborated with scientists from the University of Mexico at the Mexican National Observatory at San Pedro Martir, Baja California [*Int. Astron. Union Circ. 3605* (1981)]. Details of these observations are given in W. B. Hubbard *et al.*, *Bull. Am. Astron. Soc.* **13**, 728 (1981).
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Photosynthetic Hydrogen and Oxygen Production: Kinetic Studies

Abstract. *Steady-state turnover times for simultaneous photosynthetic production of hydrogen and oxygen have been measured for two systems: the in vitro system comprised of isolated chloroplasts, ferredoxin, and hydrogenase, and the anaerobically adapted green alga* Chlamydomonas reinhardtii *[137c(+) mating type]. In both systems, the simultaneous photoproduction of hydrogen and oxygen was measured by driving the systems into the steady state with repetitive, single-turnover, flash illumination. The turnover times for production of both oxygen and hydrogen in photosynthetic water splitting are in milliseconds and are equal to or less than the turnover time for carbon dioxide reduction in intact algal cells. The oxygen and hydrogen turnover times are therefore compatible with each other and partially compatible with the excitation rate of the photosynthetic reaction centers under conditions of solar irradiation.*

A promising approach to the biological production of renewable energy and chemical feedstocks is through splitting of water molecules to produce molecular hydrogen and oxygen by photosynthesis (*1*, *2*):

$$2\ H_2O \xrightarrow[\text{light}]{\text{visible}} 2\ H_2 + O_2$$

The important aspect of this reaction is that it stores energy; the available chemical energy of the products is greater than the available energy of the substrate. In biological systems this photoreaction can be driven by light quanta from the visible portion of the electromagnetic spectrum (400 to 700 nm), a range that includes almost 50 percent of the power radiated in the solar emission spectrum. The only known biological systems for direct hydrogen and oxygen production are green algae and blue-green algae. A third, artificial, system derived from these consists of isolated higher plant chloroplasts with associated electron carriers and catalysts such as ferredoxin and hydrogenase. These three systems are known to produce hydrogen and oxygen with visible light (*3*–*5*) through the reduction of hydrogen ions just as CO_2 is reduced in normal photosynthesis.

Emerson and Arnold (*6*) were the first to measure photosynthesis turnover time, the characteristic time during which biochemistry occurs within a photosynthetic reaction center in preparation for profitable utilization of a second excitation. In the work reported here, the steady-state turnover times for simultaneous light-driven hydrogen and oxygen production were measured by the repetitive flash technique of Emerson and Arnold. These experiments were performed to determine whether the hydrogen and oxygen photoreactions under steady-state conditions are kinetically compatible with each other and with the rate of excitation of photosynthetic reaction centers under normal solar insolation.

Experimental results are presented in Fig. 1. Figure 1A shows data for the in vitro system comprised of isolated chloroplasts, ferredoxin, and hydrogenase [the CFH system (*5*, *7*, *8*)]. The data in Fig. 1B are for anaerobically adapted *Chlamydomonas reinhardtii*, 137c(+) mating type. [The phenomenon of hydrogen production in *Scenedesmus* was discovered by Gaffron and Rubin (*9*).] The two sets of data shown in Fig. 1 resulted from similar experiments, except that a 3-hour adaptation period of darkness was needed for *Chlamydomonas* (for hydrogenase synthesis) before illumination. Each data point was obtained by driving the CFH system or algae into the steady state through repetitive, single-turnover, flash illumination. The frequency of flashing is indicated on the abscissa of each graph, while the ordinate for each is the absolute yield of hydrogen or oxygen per mole of chlorophyll per flash of light.

The data in Fig. 1 provide kinetic information on photosynthetic water splitting by illustrating the simultaneous photoproduction of hydrogen and oxygen from isolated spinach chloroplasts coupled to clostridial hydrogenase through ferredoxin. If the frequency response of the reactions were linear, both hydrogen and oxygen would reach a constant yield per flash (when normalized to the flash rate). For the data of Fig. 1A, this is approximately true in the frequency range 50 to 150 Hz, although yields decrease at both lower and higher flash frequencies.

Solar insolation values can vary from very low to ~ 1 kW/m^2 (*10*). In higher plants, almost 50 percent of the solar spectrum, from ~ 400 to 700 nm, can be used for photosynthesis. The pigment principally responsible for the capture and conversion of this light energy into chemical energy is chlorophyll, a mole-

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cule with an optical cross section of several square angstroms (*11*). This means that a chlorophyll molecule is hit an average of ten times per second at air mass (AM) 1. Assuming 200 chlorophyll molecules per reaction center, the highest rate of excitation under natural conditions would be in the range of 2000 times per second. If the data of Fig. 1 are analyzed on the basis of this excitation rate, the decreasing oxygen and hydrogen yields at high frequencies may be interpreted as inability of the overall reactions to keep pace with the steadily increasing rate of flashing. Operationally, the turnover time is the reciprocal of the frequency at which the absolute yield of hydrogen or oxygen has decreased to 50 percent of the maximum yield. Since the full width at half-maximum (FWHM) for the xenon flash lamps used was < 1 μsec (*12*), only one photoexcitation per flash occurred at each reaction center. The technique of repetitive, single-turnover flashes allows direct measurement of the kinetic limitations for the photoreactions imposed by subsequent dark biochemistry. In Fig. 1A, the turnover time for photosynthetic production of both hydrogen and oxygen for the CFH system is ~ 3 msec, a value less than the turnover time for CO_2 reduction in algal photosynthesis (*13*). Therefore, for AM 1 the CFH system can keep pace with ~ 33 percent of the incident light quanta. The results shown in Fig. 1B for *Chlamydomonas* are more complex, since two turnover times are observed: 9 msec for oxygen and 6 msec for hydrogen. An earlier measurement of the turnover time for photosynthetic hydrogen production in anaerobically adapted *Chlamydomonas* has been reported (*14*); however, in that measurement the algae were not driven into the steady state and oxygen production was deliberately inhibited with 3-(3,4-dichlorophenyl)-1,1-dimethylurea. The steady-state results have direct bearing on the interpretation of oxygen and hydrogen yields from continuous-wave illumination. From a kinetic point of view, the turnover times for photosynthetic hydrogen and oxygen production are reasonably fast and can partially keep pace with the rate of excitation under conditions of natural insolation.

Table 1. Steady-state turnover time for hydrogen and oxygen production in two photosynthetic systems.

System	Turnover time (msec)	
	Hydrogen	Oxygen
CFH	3	3
Chlamydomonas	6	9

Data in Fig. 1 suggest that the major limiting factor in hydrogen and oxygen production is the number of functional photosynthetic units (PSU's). The original experiment of Emerson and Arnold's showed that there are approximately 2400 chlorophyll molecules per molecule of evolved oxygen (coupled to CO_2 reduction). This study indicates that, under steady-state conditions of hydrogen and oxygen photoevolution, there are fewer functional PSU's (fewer O_2 and H_2 molecules produced per molecule of chlorophyll). Since the flashes used were ~ 10 percent saturating, ~ 2 percent of the Emerson and Arnold value was obtained for the CFH system and ~ 10 percent for the *Chlamydomonas* system (Fig. 1). This is believed, at least in *Chlamydomonas*, to be due to subsequent steady-state loss reactions rather than inherently nonfunctional units, since absolute yields of hydrogen or oxygen for individually resolved flashes give PSU sizes very near the Emerson and Arnold value (*15*).

In a study of limiting reactions in the CFH system, Rosen and Krasna (*8*) measured hydrogen evolution under a wide variety of experimental conditions. They concluded that hydrogen production in a chloroplast-hydrogenase system is limited by the steady-state level of reduced electron carrier (such as methyl viologen or ferredoxin) achievable anaerobically by the photosynthetic component. Our experiments with intermittent light imply that the origin of this low constant level is the relatively low number of functional PSU's available for the process, rather than any intrinsic kinetic limitation. This study shows (Table 1) that turnover rate for photosynthetic hydrogen and oxygen production by the CFH system is approximately twice that measured for normal photosynthesis in intact algal cells.

Over the low-fequency range (Fig. 1) the reaction centers were excited at a correspondingly lower rate, causing decreased yields of hydrogen and oxygen. This is interpreted as due to loss of photogenerated, metastable intermediates to adventitious dark reactions (during the correspondingly longer dark times between flashes). According to the model of Kok *et al.* (*16*) for photosynthetic oxygen evolution, four oxidizing equivalents must accumulate in a photosystem II reaction center before the evolution of one molecule of oxygen. Kok *et al.* concluded that these oxidizing equivalents accumulate through a linear, sequential flash illumination, by which each metastable state is more oxidized than its predecessor by one equivalent. Long dark times between flashes allow these metastable intermediates to decay before oxygen evolution, with a concomitant loss in yield. Diner and Mauzerall (*17*) showed that enodgenous reductants can cause a decrease of oxygen yield per flash at low flash frequencies. That such endogenous reductants are present in the clostridial extract CFH system is supported by anaerobic, flash-yield studies of oxygen evolution from chloroplasts as a function of temperature and ferricyanide concentration (*18*).

A final aspect of the experimental data is the stoichiometric ratio of H_2 to O_2;

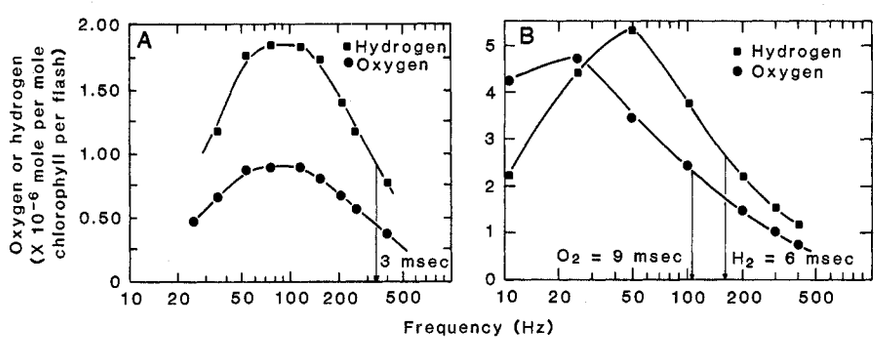

Fig. 1. Simultaneous steady-state photoproduction of hydrogen and oxygen under repetitive, single-turnover, flash illumination. (A) System composed of isolated spinach chloroplasts, ferredoxin, and hydrogenase. (B) *Chlamydomonas reinhardtii*, anaerobically adapted in the dark for 3 hours before illumination. Oxygen inactivation of the hydrogenase was prevented by using a specially constructed flow system to purge the reactor cuvette. In the absence of this purge gas, the increase in partial pressure of photosynthetically produced oxygen will eventually inactivate the hydrogenase enzyme. The physiological status of the alga was carefully controlled with respect to temperature and anaerobiosis. A water-jacketed cell connected to a constant-temperature circulator maintained the temperature at 20°C. Anaerobiosis was maintained by the helium purge and the use of O-ring connectors throughout. The latter helped maintain a minimum of inboard O_2 leakage from the atmosphere. For further experimental details, including the technique for measurement of hydrogen and oxygen, see (*5*, *19*).

for the CFH system (Fig. 1A) the ratio is ~ 2. This photoreaction can be interpreted as an analog of photosynthesis; the reduced compound is molecular hydrogen and the source of electrons is water. However, the stoichiometric ratio of the CFH system can vary widely, depending on reaction conditions, and the ratio of H_2 to O_2 can greatly exceed 2 (*19*). Conversely, Fig. 1B shows that the ratio of H_2 to O_2 for steady-state yields in *Chlamydomonas* is generally < 2. Evidently, under these experimental conditions, not all reducing equivalents are taken up by hydrogenase and evolved as molecular hydrogen (*15*).

Measurements of the simultaneous photoproduction of hydrogen and oxygen have been relatively few compared to those of hydrogen production alone. Spruit (*3*) developed a novel two-electrode polarographic technique for the simultaneous measurement of photoproduced hydrogen and oxygen by *Chlorella*. His principal conclusion was that hydrogen and oxygen metabolisms are closely related and both gases are ultimately given off during illumination from the same source, water. Bishop and Gaffron (*20*) found that the light-dependent evolution of hydrogen appeared to require both photosystems. Gaffron and Rubin (*9*) postulated that the substrate was an organic donor, since addition of glucose caused an increase in the amount of hydrogen evolved [see also (*21, 22*)]. Bishop *et al.* (*23*) used a two-electrode polarographic technique to measure the amount of gas produced in a confined volume; they concluded that water is the primary substrate for hydrogen and oxygen production.

Formidable scientific and engineering development problems remain to be addressed before energy-related applications of photosynthetic hydrogen and oxygen production are possible. These include prevention of wasteful back reactions and development of methods of prolonging the life of the functional components. Moreover, as pointed out by Shinnar (*24*), serious engineering limitations must be dealt with, such as the irreversibilities associated with the production and separation process. The irreversibility loss can be no smaller than the entropy change associated with the process, multiplied by the ambient temperature. Real-world processes will have to cope with fundamental thermodynamic constraints such as these.

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Prenatal Exposure to the Herbicide 2,4-Dichlorophenyl-*p*-Nitrophenyl Ether Destroys the Rodent Harderian Gland

Abstract. *Exposure of mice to the herbicide 2,4-dichlorophenyl-*p*-nitrophenyl ether during gestation produces abnormalities that are not readily apparent at birth but become obvious as the pups mature. By 2 weeks after birth there are severe intraorbital defects resulting from destruction of the Harderian glands behind the eyes. This effect is noticeable only postnatally because the Harderian gland does not grow or function until after birth.*

The preemergence herbicide 2,4-dichlorophenyl-*p*-nitrophenyl ether (TOK) (*1*) is used in the cultivation of grain and vegetable crops. Although this compound is relatively nontoxic to adult rats, exposure during gestation to doses two to three orders of magnitude below the median lethal dose (~ 1 g/kg) reduces survival after birth and causes abnormal lung and heart development and diaphragmatic hernias (*2*). Mice surviving such exposure develop a variety of abnormalities, including hydrocephaly, hyperactivity, malocclusion of the jaws, and reduced palpebral fissures (*3*). In addition, they appear to be micro- or anophthalmic (*3*). However, while TOK produces abnormalities and death in mice postnatally, it does not kill fetuses or give them noticeable orbital defects; nor does it induce many obvious abnormalities in neonates (*4*).

In most animal studies of chemical teratogenicity, the dams are killed just before parturition and the fetuses are removed and examined. We suggest that this procedure cannot provide a complete and accurate assessment of the teratogenicity of chemicals like TOK, since they exert their strongest effects postnatally. The present study was designed to examine the apparent micro- and anophthalmic effects of TOK and to determine how such effects elude detection by standard teratological testing procedures.

Primiparous CD-1 mice were randomly assigned to control or experimental

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