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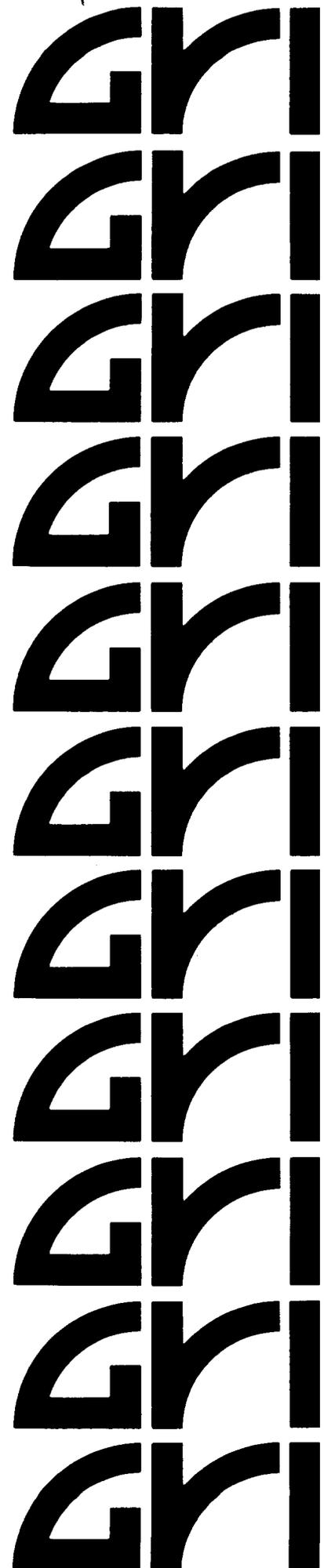
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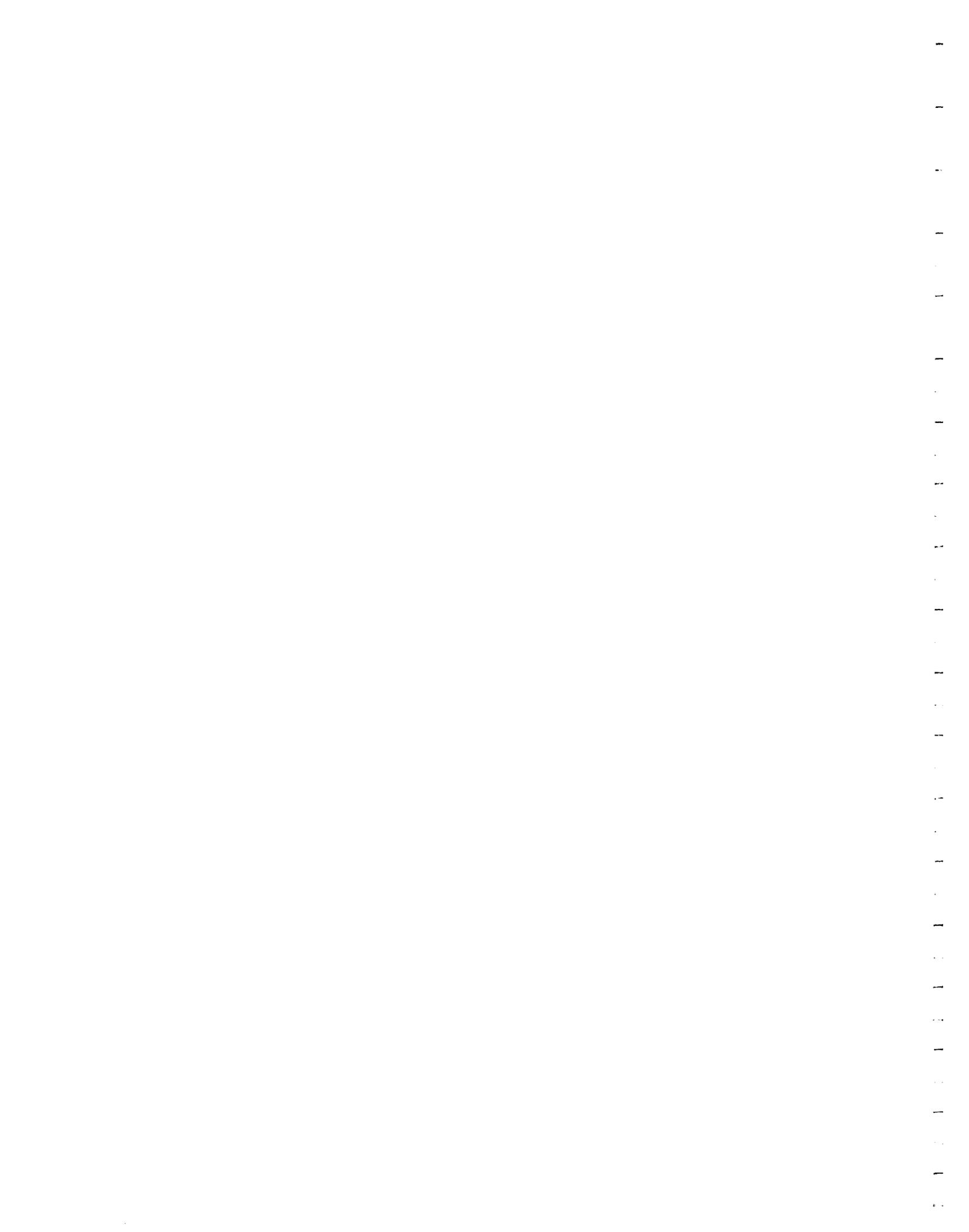
PHOTOSYNTHETIC WATER SPLITTING

1987 ANNUAL REPORT

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PHOTOSYNTHETIC WATER SPLITTING

1987 ANNUAL REPORT

Prepared by

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Chemical Technology Division
OAK RIDGE NATIONAL LABORATORY
Oak Ridge, Tennessee 37831
operated by
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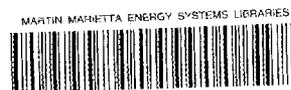
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Photochemistry, Basic Research Division

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16. Abstract (Limit: 200 words) This document is an annual report prepared for the Gas Research Institute dealing with the basic physics and chemistry of photosynthetic water splitting for the production of hydrogen and oxygen. Two key advances have been made during the current reporting period. First, it has been discovered that, contrary to conventional belief, unicellular green algae are capable of evolving molecular hydrogen in the presence of carbon dioxide. The controlling factors that determine hydrogen evolution are either temperature or light intensity. In addition, it has been discovered that certain mutants of the green alga <i>Chlamydomonas</i> are capable of evolving hydrogen in the presence of carbon dioxide. The significance of these discoveries is that the presence of carbon dioxide (or bicarbonate) is a key factor in determining the activity of the Photosystem II water splitting complex. Second, a new advance in oxygen sensor technology has been made that, for the first time, allows the absolute measurement of photosynthetically evolved oxygen from a <u>single</u> colony of microalgae growing on a solidified agar medium. The key aspect of this electrochemical sensor is the utilization of ultra-pure potassium hydroxide as the electrolyte and a recognition of the role that electrolyte impurities play in contributing to base line noise. A tool that allows the absolute measurement of photosynthetic rates from single colonies will be extremely useful in applying the techniques of molecular biology and genetic engineering to the photosynthetic water splitting problem.				
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RESEARCH SUMMARY

Title Photosynthetic Water Splitting

Contractor Oak Ridge National Laboratory

 GRI Contract Number: 5083-260-0880

Principal Investigator E. Greenbaum

Report Period 1987 Annual Report

Objective To understand the basic physics and chemistry of hydrogen and oxygen photoproduction by the photosynthetic process.

Technical Perspective Photosynthetic water splitting is a promising approach to the production of hydrogen as a renewable gaseous fuel. In essence, a biological solar energy conversion and storage system is contemplated in which the energy-rich product is molecular hydrogen. Hydrogen has potential applications as a possible addition to or substitute for natural gas.

Results Initial success in this mission-oriented basic research program was achieved by developing physical instrumentation for performing kinetic and mechanistic studies of the simultaneous photoproduction of molecular hydrogen and oxygen by anaerobically adapted green algae. Two key advances have been made during the current reporting period. First, it has been discovered that, contrary to conventional belief, unicellular green algae are capable of evolving molecular hydrogen in the presence of carbon dioxide. The controlling factors that determine hydrogen evolution are either temperature or light intensity. In addition, it has been discovered that certain mutants of the green alga Chlamydomonas are capable of evolving hydrogen in the presence of carbon dioxide at elevated temperatures and light intensity. The significance of these discoveries is that the presence of carbon dioxide (or bicarbonate) is a key factor in determining the activity of the Photosystem II water splitting complex. Second, a new advance in oxygen sensor technology has been made that, for the first time, allows the absolute measurement of photosynthetically evolved oxygen from a single colony of microalgae growing on a solidified agar medium. The key aspect of this electrochemical sensor is the utilization of ultra-pure potassium hydroxide as the electrolyte and a recognition of the role that electrolyte impurities play in contributing to base line noise. A tool that allows the absolute measurement of

Results
(contd.)

photosynthetic rates from single colonies will be extremely useful in applying the techniques of molecular biology and genetic engineering to the photosynthetic water splitting problem.

Technical
Approach

These results were obtained by developing a novel apparatus for measuring the simultaneous photoproduction of hydrogen and oxygen. The apparatus consists of a computer-controlled laser beam and a flow system in which the hydrogen and oxygen sensors are located downstream from the photosynthetic reactor. Physical removal of the sensors from the locus of gas production has the important technical advantage of eliminating interfering light-induced artifacts in the sensors. The hydrogen sensor is a Taguchi gas-sensitive semiconductor. The oxygen sensor is a Hersch electrogalvanic cell. Using an electrolysis cell and Faraday's Law of Electrochemical Equivalence, calibration curves for the two sensors are prepared using a least-squares fitting routine in conjunction with an HP-85 laboratory microcomputer.

Project
Implications

During the previous year, this research was continued to advance the possibility of effective biophotolytic production of H₂ from water. The finding that light intensity, temperature, or genetic mutants can be appropriately chosen to allow H₂ production in the presence of CO₂ is significant because algal H₂ photoproduction systems may not have to exclude CO₂, and CO₂ appears to accelerate the photosynthetic process. Another result, a method for measuring photosynthetic oxygen evolution directly from isolated colonies, should be a powerful tool in genetic and molecular investigations of photosynthetic processes, including those that lead to H₂ production. GRI plans to continue this research, which is now attempting to improve algal H₂ production at higher light intensities.

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PHOTOSYNTHETIC WATER SPLITTING

GRI CONTRACT 5083-260-0880

1.0 EXECUTIVE SUMMARY

Key progress in developing a biotechnological process for the synthesis of gaseous fuel from inorganic resources has been made in this GRI-supported program. Previous advances include a measurement of absolute conversion efficiencies of approximately 10% in the linear region of the light saturation curve of photosynthesis. Within the stated light range, this efficiency compares very favorably with electricity generation by silicon photovoltaic cells. Based on this and other GRI-supported work, it is clear that the concept of gaseous fuel synthesis by microalgal photosynthesis is technically sound. Additional progress in reaching the stated goal has been made during the current reporting period and is summarized as follows:

The Control of Photosynthetic Reductant. It has been conventionally believed that the presence of carbon dioxide in the atmosphere of photosynthesizing microalgae suppresses their ability to photoproduce hydrogen. During this reporting period, it has been discovered that this is not true in general. This is an important discovery because the ability to control the fate of photosynthetic reductant lies at the heart of hydrogen fuel synthesis by microalgae. The ability to setup an effective kinetic competition for photosynthetic reductant between the Calvin cycle pathway for carbon dioxide reduction and the hydrogenase pathway represents a breakthrough in the

field of hydrogen production by microalgae. This control has been achieved in three ways: temperature, light intensity, and the use of genetic mutants of Chlamydomonas reinhardtii. These mutants were obtained from Professor Gregory Schmidt of the University of Georgia. Experimental results are indicated in Figures 2 and 3 and additional information on experimental methodology is given in the body of the report.

Single Colony Assays. The first measurements on the absolute photosynthetic activity of single colonies of microalgae have been performed. Preliminary experimental results on hydrogen evolution from single colonies were presented in last year's report. These experiments have been completed and have been submitted for publication. GRI has also filed a patent application based on these results. During the current reporting period the ability to assay oxygen from single colonies of microalgae has been achieved. The experimental advance made in the oxygen assay was the utilization of ultra-pure potassium hydroxide in the electrochemical sensor that is used for the assay. This ultra-pure electrolyte greatly improved the signal-to-noise ratio in the sensor and allowed the detection of oxygen evolution from single microalgal colonies.

The results obtained during the present reporting period as well as previous ones indicate that the present GRI approach to gaseous fuel synthesis by photosynthetic water splitting is technically sound. Moreover, it should be noted that these results have been achieved without the aid of the powerful tools of molecular biology and genetic engineering since no suitable transformation system by which foreign

DNA can be inserted and expressed has been available for this approach in the past. That situation is, however, rapidly changing. It is indeed intriguing that the first alga for which a transformation system will become available is Chlamydomonas, one of the best hydrogen producers that we have studied.

2.0 ANNUAL REPORT

The experimental research that was performed during the current period was a logical extension of the promising results that have been previously obtained. The key focus of this work was a further understanding of the limiting aspects of light-activated hydrogen production by photosynthetic water splitting. In particular, a significant discovery has been made with respect to the control of photosynthetic reductant and the role of carbon dioxide in determining the pathway of flow of electrons that are generated by the light reactions of photosynthesis. New gas sensor technology and instrumentation development has also been performed. For the first time it is now possible to measure the absolute rates of photosynthesis based either on hydrogen or oxygen production. The motivation for developing this new methodology is to apply the techniques of molecular biology and genetic engineering to the problem of light-activated hydrogen production by unicellular algae.

2.1 Hydrogen Fuel Synthesis in the Presence of Carbon Dioxide.

One of the well established ideas in the field of hydrogen production by microalgae is that the presence of carbon dioxide in the

surrounding atmosphere suppresses the evolution of molecular hydrogen. The reason for this suppression is illustrated in Fig. 1, where it is shown that there are branching pathways for electrons that emerge from the reducing end of Photosystem I. It has been generally thought that the kinetic competition for photogenerated electrons between the two possible pathways, the Calvin cycle or hydrogenase, is such that the Calvin cycle effectively serves as a complete sink for all of the electrons and that hydrogen cannot be photoproduced in the presence of carbon dioxide. This is, in fact, usually the case. However, during this reporting period we have found three important exceptions to this rule.

It has been discovered that temperature, light intensity, and a mutant strain of algae can be appropriately chosen such that sustained hydrogen photoproduction occurs in the presence of an atmosphere containing carbon dioxide. Figure 2. illustrates sustained hydrogen evolution by the green alga Chlamydomonas reinhardtii at 1°C. Unlike room temperature measurements where this is no sustained hydrogen evolution, these experiments indicate that the hydrogenase/hydrogen pathway can favorably compete with the carbon dioxide pathway at lower temperatures. These results imply that the Calvin cycle pathway is more sensitive to temperature than is the hydrogenase/hydrogen pathway. Moreover, a similar phenomenon has been observed at low light intensities. At room temperature and low light intensities of less than 10 W/m² sustained hydrogen evolution can be observed.

The question of controlling the fate of photosynthetic reductant has also been addressed using mutant strains of microalgae. Professor

Gregory W. Schmidt of the University of Georgia has provided us with the mutant strain Chlamydomonas reinhardtii GE. The data of Fig. 3 suggest that this alga has an impaired pathway for carbon dioxide fixation because even at room temperature and relatively high light intensities, sustained hydrogen photoevolution can be measured.

The significance of these findings is illustrated by further reference to Fig. 1. It can be seen in Fig. 1 that in addition to serving as a possible electron acceptor of Photosystem I, carbon dioxide (probably in the form of bicarbonate) also binds to components of the electron transport chain that are closely associated with Photosystem II. This binding is necessary for optimal rates of electron transport and, in our opinion, is the key limiting factor when hydrogen is evolved in an inert atmosphere.

2.2 Single Colony Gas Evolution Assays.

2.2.1 Hydrogen

An apparatus was constructed which allowed automated screening of individual microalgal colonies for sustained ability to photoevolve hydrogen during anaerobic photosynthesis. The main components of this apparatus were a microcomputer, a helium-neon laser mounted on a computer-controlled X-Y translation stage, a flow-through chamber which contained an agar plate of colonies, and a downstream hydrogen detector which interfaced with the microcomputer for data collection.

Photoevolution rates of less than $1 \text{ nmol H}_2 \text{ h}^{-1}$ were measurable with the gas analyzer. Figure 4 shows typical calibration data and the hydrogen produced by three individual colonies. The integrated experimental yield of hydrogen during calibration is in close agreement

with the theoretical yield of 336 nmol calculated using $0.00518 \text{ nmol H}_2 \text{ s}^{-1} \text{ uamp}^{-1}$. Integrated yields from the calibration data were always within 5% of the theoretical yield, indicating the accuracy of this method of analysis. Another significant feature of the measurement system reflected in the data shown in Fig. 4 is the low-noise level. The shape of the hydrogen evolution profiles accentuate the difference between these measurements and those made with amperometric electrode techniques (1,2). The ability to examine hydrogen evolution for extended time periods gives sustained photohydrogen evolution rates rather than transient rates determined by short-term experiments using amperometric electrodes.

A systematic study revealed that the hydrogen yields from individual colonies varied with the effect of scattered light on adjacent colonies, stability of the colonies under anaerobiosis, and the age, chlorophyll content, and size of colonies.

Scattered Light

The effect of scattered light on stimulating hydrogen evolution from adjacent colonies was examined to determine the minimum separation between colonies so that neighboring colonies would not contribute significantly to the hydrogen yield of the irradiated colony. A plate containing a single colony was placed in the apparatus, and the translation stage controller was programmed to increment the laser beam toward the colony. Results indicated that the colonies should be separated by $>1 \text{ cm}$ when using the unattenuated laser beam in order to avoid stimulating neighboring colonies to produce hydrogen.

Stability of Colonies

The stability of hydrogen evolution from a single colony was investigated so that colonies would not be screened after their hydrogen-producing ability had begun to decline significantly. A single 14-d-old colony was studied in response to 1-h light-on and 1-h light-off cycles for over 100 h (Fig. 5). Both the dark time prior to the first irradiation and the dark time between successive irradiations of the same colony influenced the response of a colony. Figure 6 suggests that the first irradiation should occur within the first 20 to 24 h of dark anaerobiosis. This conclusion was consistent with the Data in Fig. 5. Figure 6 also indicates that the dark time prior to the first irradiation has a minimal effect on hydrogen photoevolution if it occurs within the first 20 to 24 h of dark anaerobiosis. The decline in yields seen after 20 to 24 h appears to be a reflection of the biological stability of the colonies rather than a consequence of the dark time prior to the first irradiation.

Colony Age

Fourteen-day-old colonies, relative to the time of plating, reproducibly gave the best response with regard to H₂ per mass of chlorophyll at multiple time points.

Colony Diameter and Chlorophyll Content

Several 14-d-old colonies were analyzed in order to determine chlorophyll content and diameter. Results showed that colony size was roughly correlated with the amount of chlorophyll per colony and the total H₂ yield generally increased with diameter. Increased total hydrogen yield in wider colonies may reflect the better light absorption configuration of the colonies.

Table I shows the total hydrogen yield, yield per mass of chlorophyll, and chlorophyll per colony for several 14-d-old colonies.

Survivability and Cloning of Screened Colonies

Only 40% or less of the colonies typically survived screening. There was no obvious correlation between colony survival and hydrogen production. Survivors were propagated, and colonies were screened for anaerobic tolerance (i.e., increased survivability following screening) and hydrogen production. They showed no apparent differences when compared with unselected colonies.

Based on the data obtained from control experiments, only ~14-d-old colonies were used to examine variability among members of a population of algal cells. The unattenuated laser beam was used to irradiate the colonies; and the experiments were designed to screen all colonies within 24 h, with a maximum of 10 colonies being screened per experiment. Chlorophyll was extracted following screening, and hydrogen photoevolution by the colonies was compared on the basis of H₂ per unit of chlorophyll. Under these prescribed conditions, approximately 200 colonies were screened for inherent variability in hydrogen photoevolution. A factor of two was the greatest variability for all of the colonies screened during the same experiment.

The results of these experiments demonstrate that photostimulated hydrogen evolution from single algal colonies can be measured. The yields are influenced by a number of key experimental parameters, the control of which was important for correct interpretation of the data. Colonies grown under the same conditions and on the same plate varied in hydrogen production by no more than twofold. Therefore, the value

of twofold was taken as the normal range of variability for C. reinhardtii under our experimental conditions.

The development of an apparatus with the sensitivity for detecting photogenerated hydrogen from a single colony has a number of pertinent applications. At a rate of 10 colonies per day, the apparatus can be used to screen natural populations of algae for hydrogen photoevolution. This approach for making putative identification of interesting new strains is much more efficient than isolating pure cultures and screening them individually. Similarly, the apparatus can be used as a secondary screen to test putative mutants altered in their hydrogen-producing ability and hydrogenase.

In conclusion, the apparatus is a powerful and quantitative tool which should find specific applications in a number of areas involving anaerobic photosynthesis and hydrogen photoevolution. It exceeds other detection methods in sensitivity and long-term stability and is uniquely suited for measuring steady-state hydrogen photoevolution for extended periods of time. Combining point irradiation by means of a laser with the semiconductor sensor has produced a novel method for efficiently examining hydrogen photoevolution from individual clones of an algal population.

2.2.2 Oxygen

Photosynthetically generated oxygen was measured from single algal colonies in a helium atmosphere using an enhanced Hersch galvanic cell placed in the colony screening system described for hydrogen. Previous quantitative study of the photosynthetic capacity of colonies has been largely limited to mutant selection based on either chlorophyll

fluorescence (3,4) or $^{14}\text{CO}_2$ fixation (5). Although both approaches are powerful tools for identifying mutants, neither is particularly useful for studying steady-state photosynthesis in colonies. Therefore, measuring photosynthetic oxygen evolution directly from isolated colonies is a significant accomplishment that should find utility in genetic and molecular investigations of photosynthesis.

The critical feature of the oxygen measuring system was a sensor of sufficient sensitivity to detect the oxygen photoevolved by a single colony. A number of methods are available for the quantitative detection of oxygen; however, very few are satisfactory for extended use under continuous gas flow conditions. The Hersch galvanic cell (6) provided a stable, sensitive alternative to other methods. The oxygen luminometer (7) was the only other system which would reliably detect oxygen at levels comparable to the galvanic cell and also function under continuous gas flow conditions for extended time periods. The galvanic cell had two advantages which made it easier to use and more appropriate for this application: (1) The response of the galvanic cell was measured with a remote picoammeter to the cell whereas the luminometer required complete darkness and a highly sensitive photomultiplier as an integral part of the apparatus. (2) The gas stream was unaffected by the galvanic cell with the exception of the removal of a small fraction of the total oxygen due to the chemical reaction between the electrodes. The luminometer also consumed oxygen but, more significantly, the stream was contaminated with DMSO vapors, the solvent for luminol, making the detection of other gaseous products of algal photosynthesis, such as hydrogen, more difficult.

The performance of the galvanic cell was enhanced to allow the quantitative detection of changes in oxygen concentration of 500 fmol/s. At this level, the signal to noise ratio was about 2. When oxygen production was measured starting at a baseline concentration of zero, production rates of 1 pmol/s were reproducibly detectable. The performance of the cell was improved by using ultra-pure lead foil for the anode and ultra-pure KOH in the electrolyte solution. Critical requirements of the cell are that the electrodes and the electrolyte do not react with each other, the cathode is depolarized only by O₂, and the anode supplies all the electrons for O₂ reduction. The major contaminants in analytical grade KOH, NH₄OH, K₂CO₃, PO₄, and Na⁺, appeared to interfere with the primary reactions or cause side reactions. The use of ultra-pure components served to reduce background noise not caused by inboard O₂ leaks. Also, the functional life of the cell was extended because oxidative side reactions between the anode and the electrolyte were diminished as evidenced by reduced corrosion on the lead anode and the lack of discoloration of the electrolyte solution.

Oxygen production was quantitated using calibration curves generated by electrolyzing water with direct current. The linearity of response of the galvanic cell is shown in the calibration curve (Fig. 7). Table II demonstrates the performance characteristics of the galvanic cell for measuring oxygen production.

Figure 8 shows oxygen photoevolution/time profiles of single Chlamydomonas reinhardtii colonies. In the presence of 300 ppm carbon dioxide in a helium carrier, colonies containing 0.2 ug of chlorophyll

photoevolved sufficient oxygen for detection with the galvanic cell. Under these conditions, oxygen photoevolution was stimulated by the bicarbonate effect on Photosystem II (8) and by the stimulation of RuBP Carboxylase (9). These conditions appear to support normal photosynthesis with no photorespiration. In the absence of carbon dioxide, light driven oxygen photoproduction was reduced by about tenfold. Oxygen was still detectable from colonies in the absence of carbon dioxide but colonies with higher chlorophyll contents, i.e., older and larger colonies, were required to generate detectable levels of oxygen.

The greatest difficulty associated with the use of the galvanic cell for measuring ultra-low levels of oxygen was providing an absolutely leak-free gas flow system. Very small leaks increased the background noise level of the signal and the spikes seen in Fig. 8 were probably due to sporadic inboard oxygen leaks from the atmosphere. Nevertheless, these spikes were not so severe that they greatly diminished the utility of the galvanic cell for measuring oxygen photoevolution from colonies. This apparatus opens a new area for photosynthetic research at the colonial level.

The enhanced galvanic cell, therefore, provides the sensitivity to study photosynthesis at the level of single algal colonies for the first time. The influence of colonial growth on the photosynthetic ability and characteristics of colonies can now be investigated. The galvanic cell coupled with the colony scanning apparatus also allows putative algal mutants and natural isolates to be quantitatively screened for photosynthetic oxygen evolution.

3.0 FUTURE RESEARCH

The accumulated evidence of the experimental results that have been obtained from this GRI-supported research project indicate unambiguously that the underlying concept of using unicellular microalgae for photosynthetically splitting water to molecular hydrogen and oxygen is technically sound. Favorable efficiencies of 10% have been obtained at low-light intensities and further research is needed to understand and remove the limiting aspects of energy conversion efficiencies at higher light intensities. There is a logical strategy that can be employed to accomplish this objective: search for a low chlorophyll mutant of Chlamydomonas that has no other impairment. We will continue to strengthen our ties to the algal photosynthesis community to take advantage of the important advances that are being made in the field of algal genetics and molecular biology. It is felt that our expertise in physical instrumentation and quantitative measurement combined with a clear focus on GRI goals in supporting this mission-oriented research will lead to a practical solution of this important problem.

4.0 PUBLICATIONS AND PATENTS

The following publications were based on GRI-supported research:

1. E. Greenbaum, "Hydrogen Production by Algal Water Splitting," in Algae and Human Affairs, C. A. Lembi and J. R. Waaland (Eds.), Cambridge University Press, 1988 (in press).

2. D. A. Graves, M. E. Reeves, and E. Greenbaum, "Establishment of Control Parameters for in situ, Automated Screening of Sustained Hydrogen Photoproduction by Individual Algal Colonies," submitted for publication (1987).
3. M. Reeves and E. Greenbaum, "Stoichiometry of Photosynthetic Reaction Centers in Chlamydomonas," submitted for publication (1987).
4. E. Greenbaum, "Energetic Efficiency of Hydrogen Photoevolution by Algal Water Splitting," submitted for publication (1988).
5. D. A. Graves and E. Greenbaum, "A Simple Apparatus for Screening Absolute Photosynthetic Rates of Single Algal Colonies in an Anoxic Atmosphere," submitted for publication (1988).
6. E. Greenbaum and M. E. Reeves, "Photosynthetic Water Splitting: A Biotechnological Approach to Gaseous Fuel Synthesis," in Proceedings of the 1986 International Gas Research Conference, Vol IV, pp. 49-54.

The following patent application is pending:

1. Apparatus for Rapid Screening of Single Algal Clones for Gaseous Fuel Synthesis," assigned to the Gas Research Institute, application pending (1987).

5.0 SEMINARS AND INVITED PAPERS

The following presentations were based either totally or partially on GRI-funded research:

E. Greenbaum, "Chlamydomonas Under Stress: Patterns of Hydrogen Evolution and Chlorophyll Retention," Genetics and Photosynthesis Research Group, Department of Cellular and Developmental Biology, Harvard University, Cambridge, MA, December 1, 1986.

E. Greenbaum, "Photosynthesis," four lectures to advanced placement biology students, Oak Ridge High School, Oak Ridge, TN, November 20, 1986.

E. Greenbaum, "Physics and Chemistry of Photosynthetic Water Splitting", lecture topic in the American Physical Society short course An Introduction to Current Research in Biological Physics, New York, NY, March 13-14, 1987.

E. Greenbaum, "Hydrogen Production by Microalgal Water Splitting" The University of Pittsburgh, Pittsburgh, PA, March 25, 1987.

D. A. Graves, and E. Greenbaum, "Automated Colony Screening for Hydrogen Photoevolution from Green Algae", Ninth Symposium on Biotechnology for Fuels and Chemicals, Boulder, CO, May 4-8, 1987.

D. A. Graves and E. Greenbaum, "Photoevolution of Hydrogen from Chlamydomonas sp. in the Presence of Carbon Dioxide," annual meeting of the American Society of Plant Physiologists, St. Louis, MO, July 19-23, 1987.

E. Greenbaum, "Photobiological Hydrogen Production by Microalgae", Gas Research Institute Contractors' Meeting on Gaseous Fuel Synthesis from Inorganic Materials, October 7-8, 1987, Chicago, Illinois.

E. Greenbaum, "Hydrogen Production by Microalgal Water Splitting", Institute of Botany, Academia Sinica, November 4, 1987, Beijing, The People's Republic of China.

E. Greenbaum, "Hydrogen Production by Microalgal Water Splitting", Institute of Biology, Academia Sinica, November 11, 1987, Chengdu, The People's Republic of China.

E. Greenbaum, "Hydrogen Production by Microalgal Water Splitting", Institute of Energy Conversion, November 18, 1987, Guangzhou, The People's Republic of China.

E. Greenbaum, "Hydrogen Production by Microalgal Water Splitting",

Institute of Plant Physiology, November 21, 1987, Shanghai, The People's Republic of China.

6.0 UNIVERSITY INTERACTIONS

This research program is supported by the Botany Department, University of Tennessee under a subcontract with the Oak Ridge National Laboratory. Dr. Duane A. Graves, Post-Doctoral Research Associate, is on assignment to ORNL for this project on a full-time basis.

7.0 SERVICE TO PROFESSIONAL SOCIETIES AND COLLABORATIVE RESEARCH

7.1 Professional Societies

The principal investigator is a member of the Executive Committee of the Division of Biological Physics of the American Physical Society and has been elected Secretary-Treasurer of the Division. He is also a member of the Organizing Committee, Tenth Symposium on Biotechnology for Fuels and Chemicals, May 16-20, 1988, Gatlinburg, TN.

7.2 Collaborative Research

Professor Gregory W. Schmidt, of the Botany Department, University of Georgia, has isolated mutants of Chlamydomonas with low chlorophyll content and the ability to grow exclusively on inorganic substrates, using carbon dioxide as the sole carbon source. This class of mutants is of potential interest with respect to the absolute size of the optical cross section for the water-splitting reaction. We plan to continue collaborating with Professor Schmidt in characterizing the light-response properties of these and other algae.

8.0 SPECIAL RECOGNITION

Based in part on GRI-supported research, the principal investigator was selected by the National Academy of Sciences to participate in the Visiting Scholar's Exchange Program with the People's Republic of China. While in China, he was the guest of the Chinese Academy of Sciences. He visited five cities, and presented seminars on photosynthetic water splitting research which were, in part, supported by GRI.

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Table I. Hydrogen yields and chlorophyll contents of ten 14-d-old colonies

Colony	Total H ₂ (nmol)	Chl/colony (ug)	H ₂ /Chl (nmol/ug)
1	16.2	1.10	15
2	19	0.78	24.4
3	21.6	0.95	23
4	23.5	1.26	19
5	25.5	0.97	26
6	26.2	1.45	18
7	25.5	1.62	15.9
8	25.7	1.44	17
9	21	1.01	20.8
10	15.4	0.99	15.5

Table II. Performance characteristics of the enhanced galvanic cell¹

Current	Theoretical rate ² pmol/s	Measured rate ³ pmol/s	Ratio of Measured to theoretical
1	2.59	2.65	1.023
2	5.18	5.31	1.025
3	7.77	7.45	0.959
4	10.36	11.12	1.073
5	12.95	13.58	1.049
6	15.54	15.73	1.012
10	25.90	27.36	1.056

¹Data represents individual measurements, these are representative of the performance of the galvanic cell. Using the mean of multiple measurements for each current improved the measured/theoretical correlation but in actual applications it is often not feasible to make multiple measurements of the same sample; therefore, values given indicate the level of error associated with single data points.

²Calculated using Faraday's law of electrochemical equivalence (1 uA = 2.59×10^{-12} mol O₂/s).

³Data were collected under experimental conditions. All measurements were made under a He + 300 ppm CO₂ atmosphere. The electrolysis cell was placed immediately upstream of the galvanic cell. The currents shown encompass the typical range of calibration currents used in colony measurements. At currents less than 1 uA, the accuracy of the measurements declined.

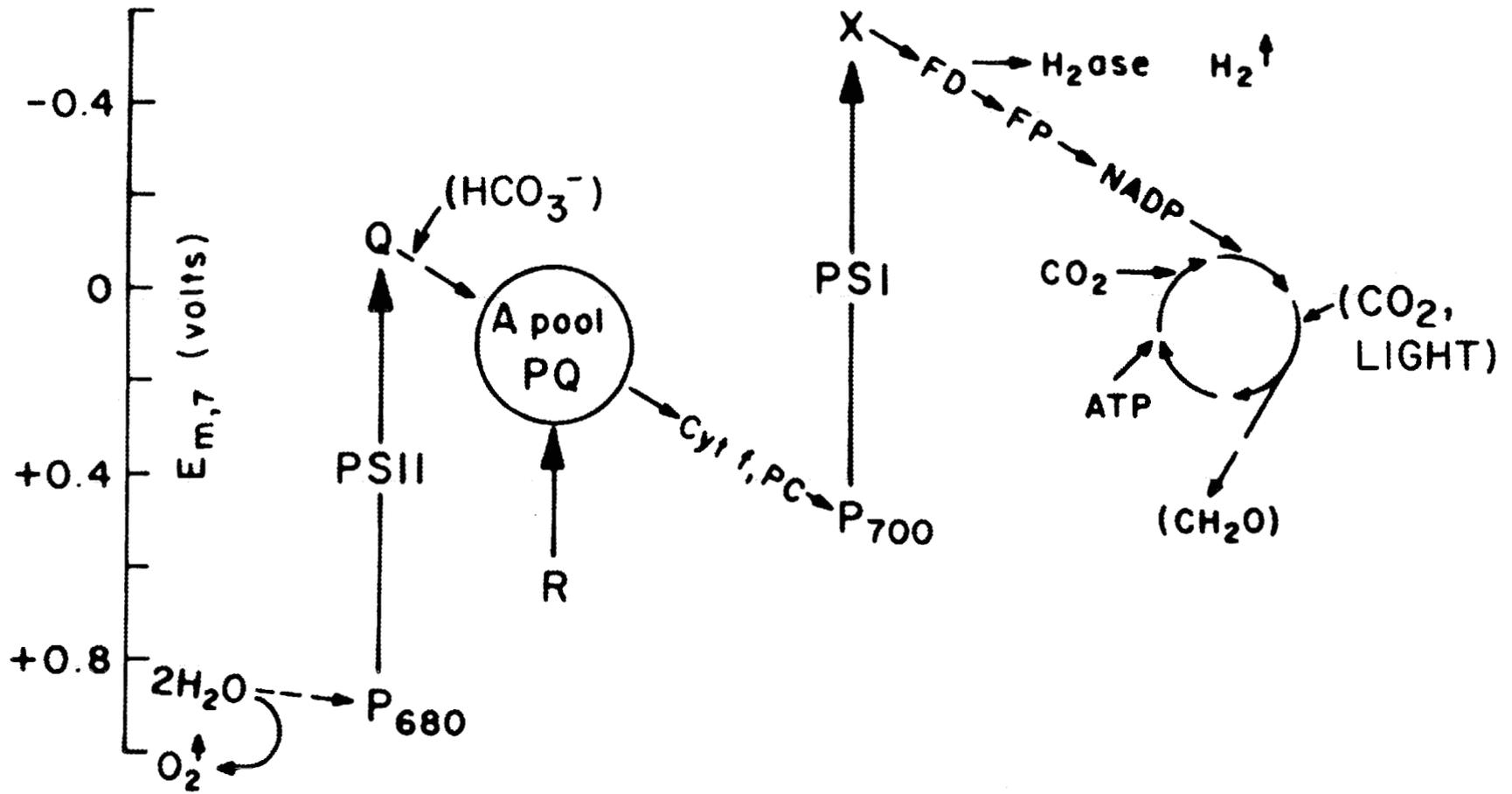
10. FIGURE LEGENDS

- Figure 1 The Z-scheme of photosynthesis illustrating the hydrogen evolution pathway and the points of regulation by carbon dioxide and light.
- Figure 2 Hydrogen photoproduction by Chlamydomonas moewusii in an atmosphere consisting of 0.05% CO₂ in Helium. Temperature = 1°C. At room temperature hydrogen production consists of a brief transient lasting for only a few minutes with a decline to zero.
- Figure 3 Hydrogen photoproduction by the Schmidt mutant, Chlamydomonas reinhardtii GE. In this unusual alga hydrogen production persists at elevated light intensities and temperature even in the presence of carbon dioxide. These results suggest that this mutant has an impairment in the carbon dioxide fixation pathway.
- Figure 4 Typical calibration data and data from three individual colonies. Calibration (time 0-4 h) was performed with 9, 7, 5, and 3 uamp, respectively, for 45 min at each current. The yield of hydrogen during calibration was 336 nmol based on 0.00519 nmol s⁻¹ uamp⁻¹. Starting at 4 h, the laser was on a single colony for 1 h followed by 1 h of darkness before the next colony was irradiated. The inset shows expanded plots and integrated yields for each colony. Colonies were 14 d old.
- Figure 5 Longevity of hydrogen photoevolution in a single 14-d-old colony multiply irradiated at 1-h intervals. Points indicate integrated yields of hydrogen and are plotted at the beginning of the irradiation periods.
- Figure 6 Hydrogen yield time profiles from nine individual colonies, each experiencing different dark times prior to the first irradiation. The yield of hydrogen from the first irradiation of each colony varies from the others by a factor less than two. First-irradiation yields were taken as 100%, with yields from subsequent irradiations being normalized to the first. Each symbol represents a different colony.
- Figure 7 Calibration data for oxygen production generated by electrolyzing water. Direct current was supplied via platinum leads to an inline electrolysis cell containing 1 mM ultra-pure KOH. The current density

was incremented at 30 min intervals to generate quantitative rates of oxygen production. Current was supplied using a Keithley, Model 220, programmable current source. The data indicate that the response of the galvanic cell was linear over the entire range of current densities tested.

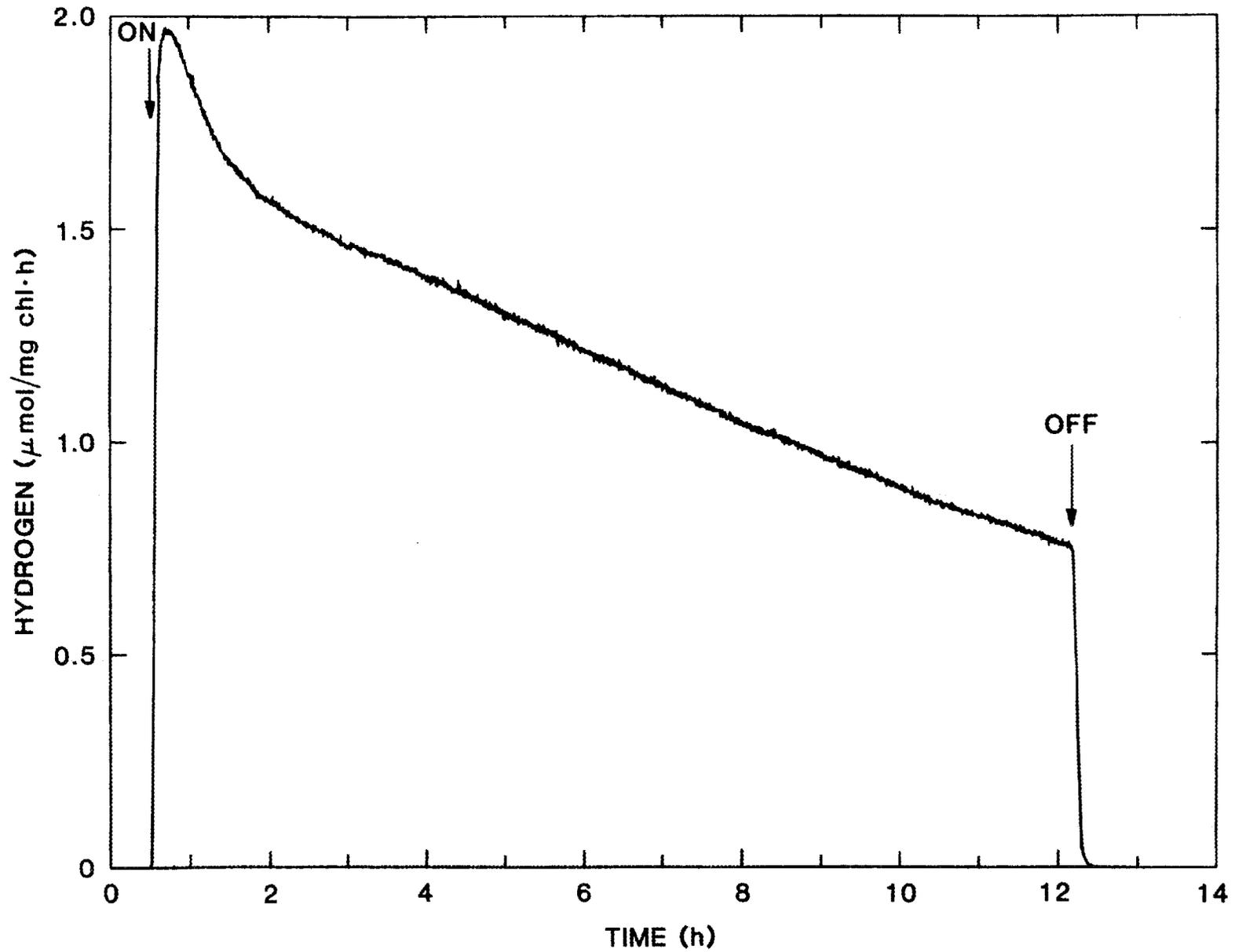
Figure 8

Quantitative measurement of photosynthetic oxygen evolution by single algal colonies. Each panel shows the O_2 /time profile for an individual colony and the chlorophyll content for that colony.



THE "Z"-SCHEME OF PHOTOSYNTHESIS WITH THE H_2 PATHWAY AND POINTS OF REGULATION BY CO_2 AND LIGHT

FIGURE 1



TEMPERATURE = 1°C
HYDROGEN PRODUCTION BY *Chlamydomonas moewusii*
HELIUM + 0.05% CARBON DIOXIDE ATMOSPHERE.

C. reinhardtii, mutant GE

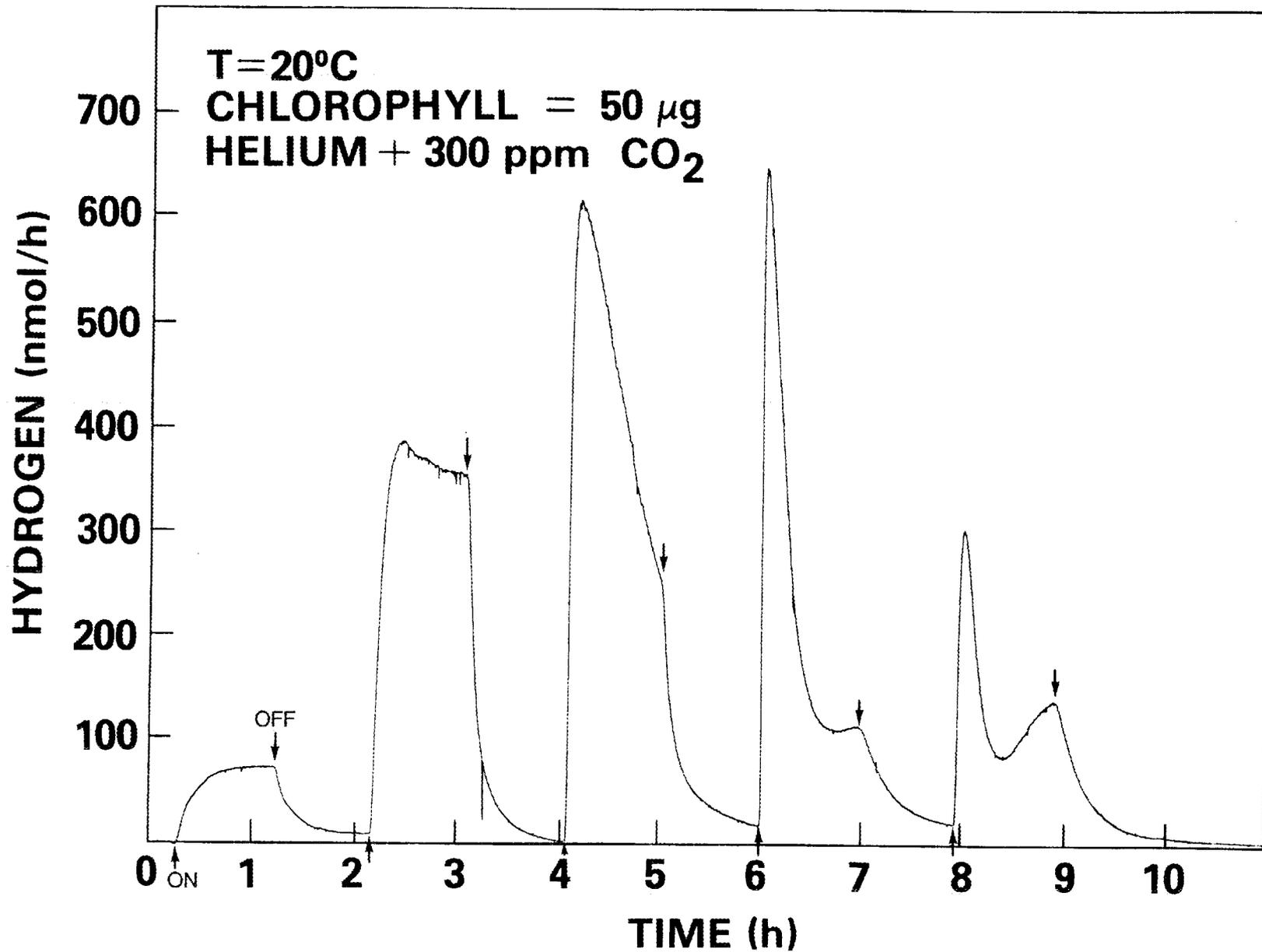


FIGURE 3

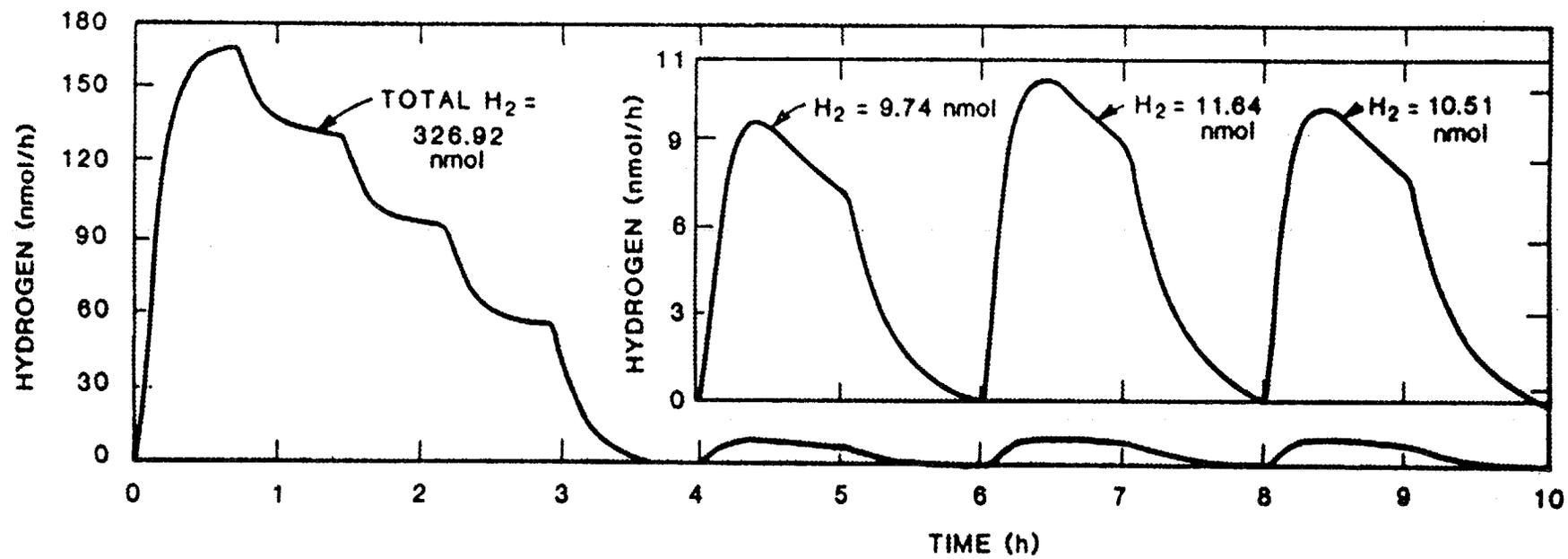


FIGURE 4

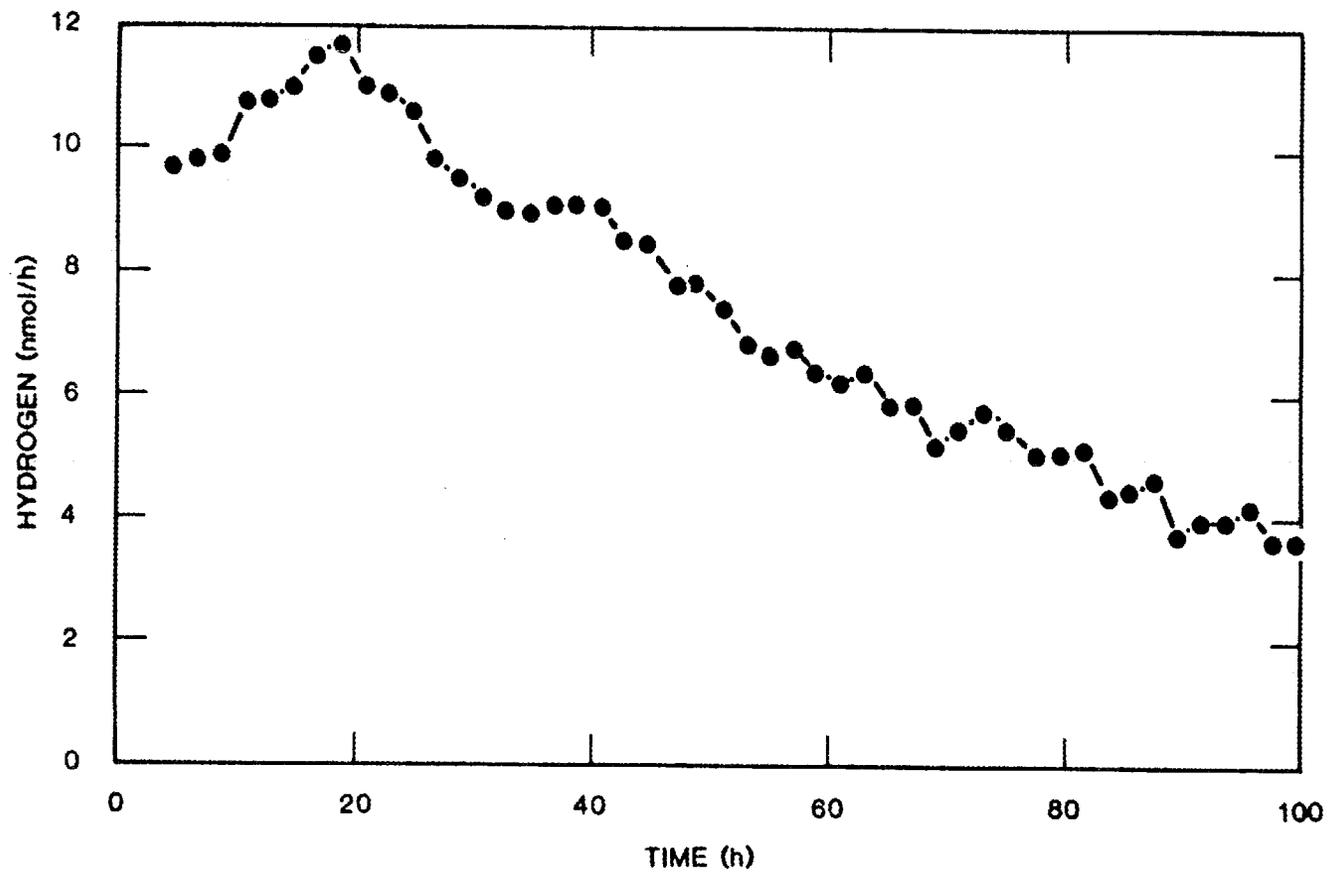


FIGURE 5

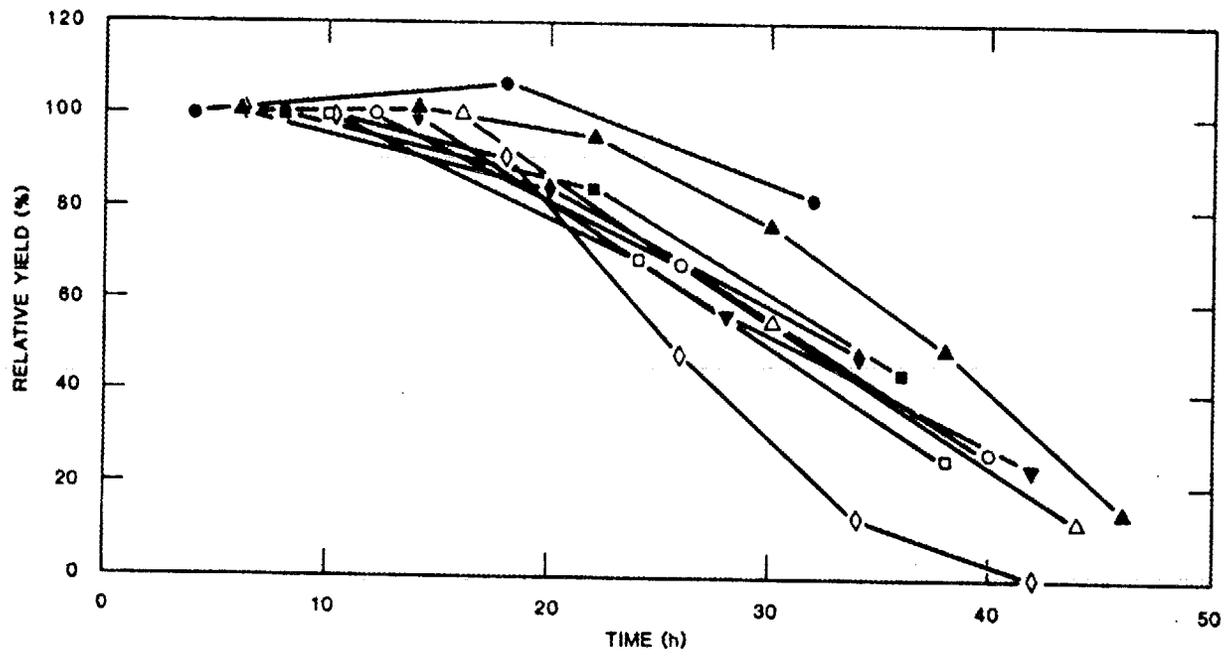


FIGURE 6

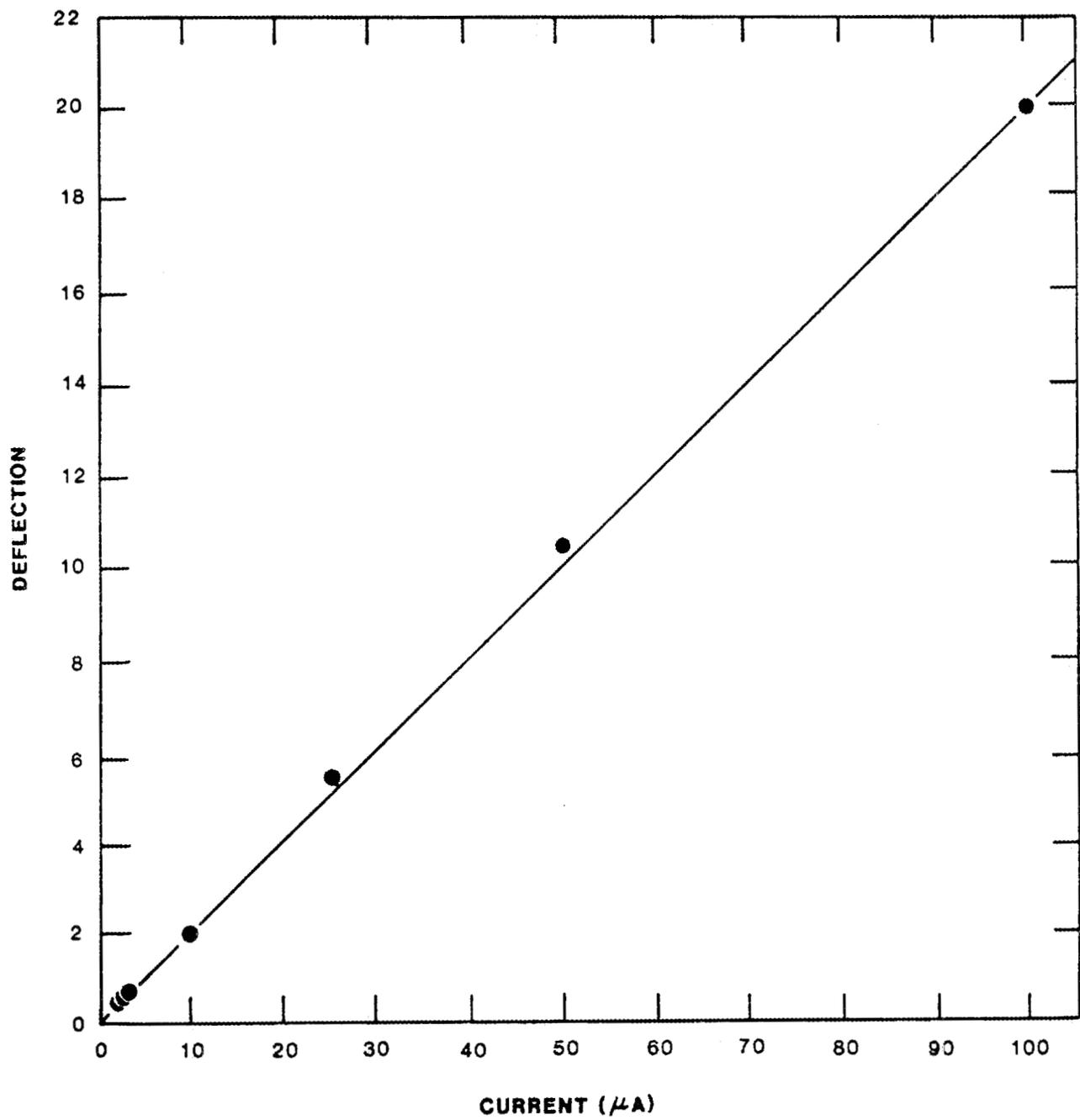


FIGURE 7

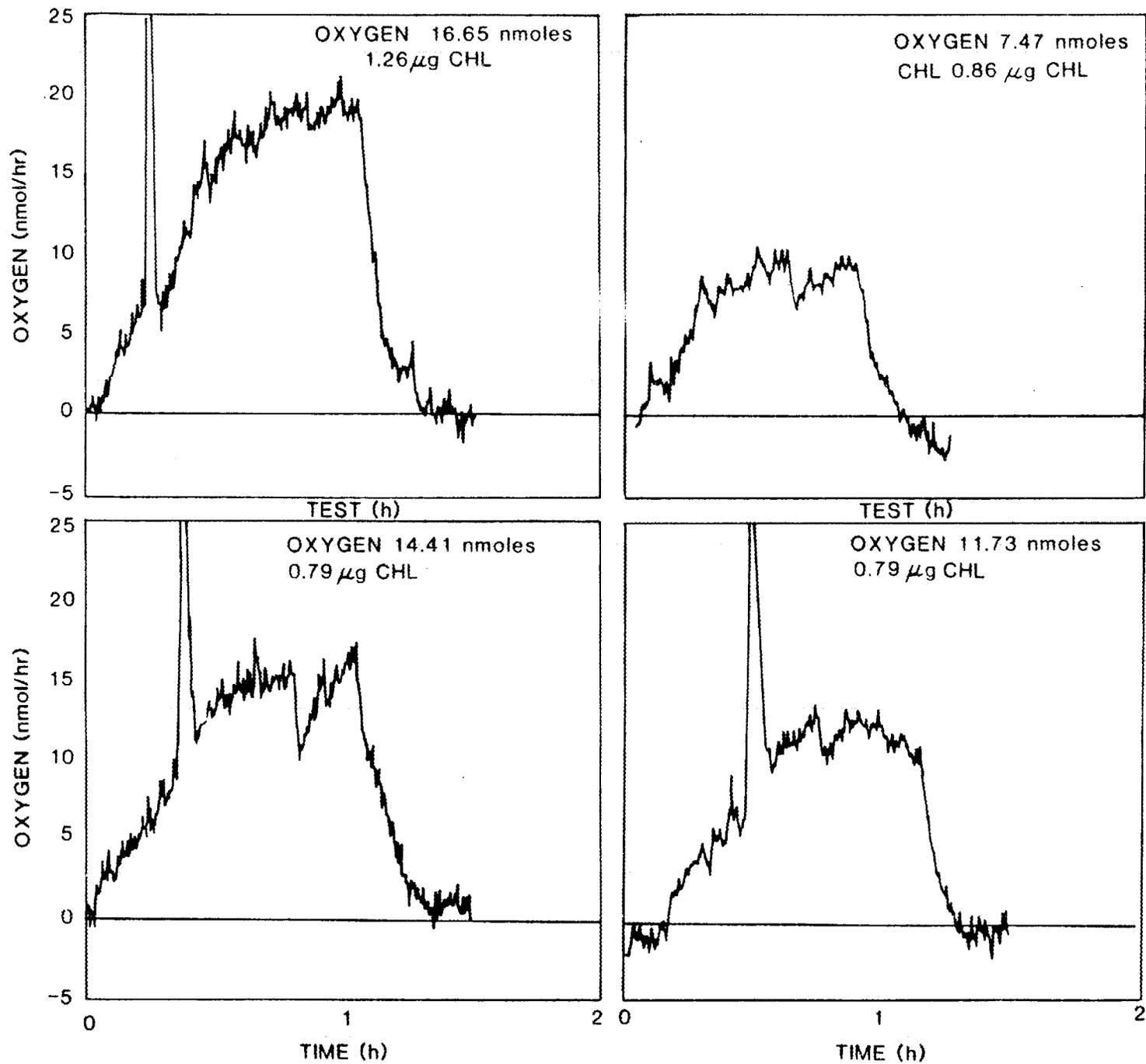


FIGURE 8

