RESEARCH IN PHOTOBIOLOGY AND PHOTOCHEMISTRY

FINAL REPORT

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1964 - 1973

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Introduction

This final report covering nine years of NASA supported research begins with a list of all our publications during the period, which permits at a glance to see what a significant part the funds granted by NASA have meant for our scientific advances. The respective papers have been marked by asterisks. Because the NASA grant was the last to be terminated, the stars prevail at the end of the list, corresponding to the papers which are going to have the dateline 1973.

After the list of publications I have collocated several summarizing descriptions of the research involved, to indicate what the various staff members who are mentioned not only as authors, but as payees of NASA, have contributed individually. As predicted in my latest proposal of 4/25/72, my laboratory has been closed definitely on June 28th, 1973. Dr. Paschinger, last of the post-doctorals, kept working to this very date, since the research, as had been hoped for, brought forth an agreeable crop of new observations.

All of the personnel mentioned in the next section have found suitable employment, mainly however because the majority of them are now going to continue with their scientific research abroad. This is the outcome of my policy during the last years to engage promising young foreign scientists, since it was virtually impossible to secure for gifted American post-doctoral a satisfactory permanent position.
Publications, 1966-1973

1966


1967


1968


* Papers supported entirely or in part by NASA grant
Publications (continued)

1968


1969


1970


1971


1972


Publications (continued)


Papers in preparation (supported entirely or in part by NASA grant).

* H. Paschinger, J. Paschinger and H. Gaffron. Photochemical disproportionation of sulfur into sulfide and sulfate by *Chlorobium limicola* forma *thiosulfatophilum*. (Summary attached)

* H. Paschinger and H. Gaffron. A changed nitrogenase activity by substituting tungsten for molybdenum. (Summary attached)

* T. S. Stuart and H. Gaffron. Photochemical evolution of hydrogen and fixation of nitrogen in *Rhodospirillum rubrum* as followed by mass spectrometry.

* Erich Kessler. Effect of anaerobiosis on photosynthetic reactions and nitrogen metabolism of algae with and without hydrogenase. (Summary attached)

Abstracts supported entirely or in part by NASA grant.


Review and Evaluation of Major Research Topics

Blue Light (Dr. Kowallik's research)

Even before the chemical constitution of the chlorophyll molecule was established, it had become common knowledge that plants contain in their chloroplasts pigments other than just chlorophyll with quite different light absorption characteristics, such as carotenes, which absorb blue light only, and phycobilins, which absorb mainly the light in the green gap of the chlorophyll spectrum. Careful experiments in many laboratories, which began in the 1930s, established that these pigments did not work independently of chlorophyll but transferred their absorbed light energy to chlorophyll. Therefore they received the name "accessory pigments", meaning that the actual mechanism of photosynthesis remained unchanged regardless of how much of the incident light might be absorbed by such accessory dyestuffs. About twenty years ago some Russians reported differences in growth of plants grown in either blue or red light and later they reported even noticeable differences in the products of photosynthesis. These publications were first ignored by American scientists because it is a rule in physics that a pigment like chlorophyll will have the same photochemical response regardless of the wavelength of the light it has absorbed. But then came the recognition of at least two separate photosynthetic systems (I and II) and with that some doubts whether chlorophylls adsorbed to proteins might deviate from the behavior established by physicists for the extracted pure pigment. The question arose whether other pigments, not bound properly to the chlorophyll system, might influence, even when present in very small quantities, the direction of the synthetic growth processes following the chlorophyll photochemistry.

When Dr. W. Kowallik at Göttingen told us that he was able to confirm the Russian claims that several hours of blue illumination led to a proportionately greater production of proteins compared with that of carbohydrate, I invited him to our laboratory to find out whether the method involving hours of illumination could not be replaced by experiments where unknown influences of blue light could be ascertained in a matter of minutes after "light on". We were successful in discovering such an effect, which Dr. Kowallik first reported in 1966 and at the Brookhaven Symposium of the same year. A summary of our results was published in Nature in 1967. At the present time (1973) the literature is full of partly contradictory reports on blue light effects. Our original measurements showed that this was an effect on the respiratory and fermentation mechanism in
plants, not on photosynthesis, and was mediated by small amounts of blue-absorbing pigments such as cis-carotenes or more likely flavins, and not connected with chlorophyll. As can be ascertained by the subsequent publications from Dr. W. Kowallik's and Dr. G. Schmid's laboratories (Cologne), the study of blue light effects has merged with the problems of photorespiration and glycolate metabolism. In other words, these lines of investigations belong among the research on flavin photochemistry which can be found throughout the living world, ranging from fungi (phycomyces) to chicken embryo hearts. Since these observations are everything but photosynthetic reactions, we dropped this topic after Dr. Kowallik left our laboratory.

**Color Effects in Chloroplast Reactions and the Role of Accessory Pigments**

*(Dr. Harnischfeger's Research)*

Time and again it has happened in the course of investigations of photosynthesis that a surprising observation was made which could have been made a decade or two earlier. One of these is the blue-red effect in the reactions of chloroplasts when studied in the course of several hours. Dr. Harnischfeger has described them in several publications starting with a note in 1969.

It was well known that no matter how carefully chloroplasts are extracted from the leaves of higher plants their capacity to evolve oxygen in the light (Hill reaction) diminishes with time. It was generally assumed that this decay was due to a deterioration of enzymes in the photosynthetic system and therefore ought to be quite independent of the wavelength of light used in these experiments. We found that this old assumption held true roughly during the first hour of the aging of chloroplast preparations and also when the same preparations were tested again several hours later, and the rates for the chloroplast reaction had declined to about one-fifth of the original optimal rates. But during the period in between the reaction rates measured in blue light and in red light do not stay the same. The decay of the water splitting reaction in photosystem II is much faster when followed in blue light than in red light, to the point that a switch from blue to red restores the rate of the reaction to the value it had had at the time when the results of the illuminations in the blue and in the red were indistinguishable. By comparing the trend of the reaction in time with the outward appearance of the aging chloroplasts in a scanning electron microscope, Harnischfeger guessed that somehow the blue-red effect was caused by osmotic forces which interfered with the arrangement of different pigments in the photosynthetic system. The role of osmotic pressure could be proved by the (temporary) restoration of decayed blue light rates simply by adding sucrose to the deteriorating chloroplasts.
The sum of a host of further observations has forced us to assume that the photosynthetic chlorophyll system is so structured that the accessory (and mainly blue-absorbing) pigments are the first to be physically detached from the main chlorophyll reaction center. What becomes osmotically disturbed is the ability of accessory pigments to transfer their absorbed light energy to chlorophyll, while the latter is still able to make use of the red light which it receives directly. Pigments which are disconnected from the normal photosynthetic electron transfer path continue to absorb light—and it is well known that in this case the consequence of light excitation results in unspecific photooxidations which in turn may inactivate the enzymes of the photosynthetic electron transfer chain. A puzzle which needs further attention is the sturdiness of the core of the photosystem II reaction which, after having sustained so much damage, continues to function at a diminished rate but overall quite normally.

**Role of Hydrogenase in Aerobic Green Algae (Professor Kessler's research)**

In 1954 E. Kessler, then a post-doctoral in our Chicago laboratory, compared the known need for manganese in photosynthesis (Pirson, 1937), with its role after the same organisms had been adapted to photoreduction of carbon dioxide with hydrogen (Gaffron, 1942). The complete insensitivity of the latter process toward lack of manganese in the culture medium, i.e., toward the resulting manganese deficiency in the cells themselves, proved that manganese is part of the system needed to evolve oxygen from water, that is of photosystem II.

Though the involvement of hydrogenase with the photochemical reactions of photosystem I has been explored by us off and on since 1942, particularly in comparison with the hydrogen metabolism of the photochemical, anaerobic purple bacteria, it has remained unclear until quite recently whether the existence of a hydrogenase in normally aerobic organisms was only an evolutionary relic, which had lost its function, or whether the presence of hydrogenase conveyed some advantage to our aerobic microorganisms. There is the peculiar situation that hydrogenase-containing algae have so far, despite many attempts, never been shown to grow under such anaerobic conditions in which the purple and green bacteria flourish. They grow only by way of respiration with oxygen, which, under illumination, they produce themselves. And traces of free oxygen are sufficient to inactivate the hydrogenase and consequently also to suppress the typical anaerobic evolution of hydrogen gas or photoreduction of carbon.
dioxide. Following earlier experiments by Allen published from our laboratory, who compared the kinetics of recovery from anaerobic inhibition of Chlorella with that of Scenedesmus, Kessler has now in a series of measurements of fluorescence, pigment changes and rapid recordings of gas exchange, found that possession of a hydrogenase conveys a sort of protection against suffocation during prolonged periods of anaerobiosis in the dark. The summary of his latest work, finished during the past winter, is attached to this report; the longer paper is in press.

The Photoproduction of Molecular Hydrogen (Dr. Schick's and Dr. Stuart's research).

We had found in 1942 a peculiar capacity of hydrogen-adapted algae. When illuminated under nitrogen in the absence of carbon dioxide they released small amounts of free hydrogen gas. This reaction has kept our attention for thirty years. The question was whether it could be a direct consequence of the dehydrogenation of water in photosystem II whenever the normal carbon dioxide reduction in photosystem I was interfered with. In succession Drs. N. Bishop, H. Kaltwasser and T. Stuart have tackled this problem from 1963 to the present. The more we worked on this problem the more ramified the course of electron transport reactions via photosystem I seemed to become. Finally around 1969 we decided that the manometric method, which so far had sufficed to give us new and easily understandable observations, ought to be replaced with a much more sensitive and specific one in order to be sure that our deductions from a great variety of manometric observations were correct. We embarked on developing a small mass spectrograph for rapid kinetic analyses, by which we could follow the movements of O₂, H₂, CO₂ and N₂ without any doubt about the nature of the gases absorbed or developed. With this instrument we were able to prove that the puzzling low rates of hydrogen evolution in the light were only the remaining balance between hydrogen expulsion and internal hydrogen utilization. The moment all synthetic photoreactions which require ATP were stopped by specific inhibitors of photophosphorylation, the evolution of free hydrogen assumed the long-hoped for high rates of a typical light reaction catalyzed by the chlorophyll pigment system. Moreover the question of the possible connection between hydrogen photoproduction and the decomposition of water in system II could be answered in an unexpected way. The electrons leading to hydrogen evolution via photosystem I originate in small preformed pools of endogenous hydrogen.
donors - some of them even connected with photosystem II. But a continuous evolution of hydrogen concomitant with the normal dehydrogenation of water in photosystem II could not be demonstrated.

The Hydrogen and Nitrogen Metabolism in Purple Bacteria (Dr. Schick's and Dr. Stuart's research).

A few years after the discovery of hydrogenase in green algae and of the photoproduction of free hydrogen, a similar but seemingly much more vigorous reaction was discovered by Gest and Kamen in purple bacteria. This light-induced reaction depended very much on the composition of the culture media in which the bacteria had been grown. To clarify the difference between the mechanism of the photochemical release of hydrogen in green algae and in purple bacteria, we invited Dr. H.-J. Schick, who had had some experience with photosynthetic bacteria, to investigate this particular issue. His subsequent publications on the metabolism of *Rhodospirillum rubrum* gave us a number of quantitative relationships between the photoassimilation of malate, the photoreduction of carbon dioxide with hydrogen, and, to our surprise, the amazingly high rate of reduction of free nitrogen, provided the bacteria had been grown so as to accumulate a very active nitrogenase. Dr. Schick found that an efficient photoreduction depended stoichiometrically on the amount of reduced nitrogen available; and in turn the reduction of nitrogen with hydrogen could continue only if organic compounds were (or became) available which would take care of newly assimilated nitrogen.

Dr. Tim Stuart has now used the mass spectrograph to check again on Dr. Schick's results. They could easily be confirmed and expanded to the point where we can summarize the results of several years of work as follows. The difference between hydrogen evolution in algae and bacteria is that in algae hydrogen evolution is most pronounced when phosphorylations are not functioning, while in purple bacteria also the evolution of hydrogen, just like all other photochemical reactions known, depends on cyclic phosphorylation.

Photochemical Evolution of Hydrogen Gas in Green Algae and in Purple Bacteria (Dr. Paschinger's research)

The sensitivity of the mass spectrograph technique in dealing with the question of hydrogenase in green algae and purple bacteria soon showed that at the moment of transition from darkness to light the very first move of electrons is that from the pigments to ferredoxin and then to hydrogenase. The release of hydrogen gas is the very first photochemical reaction and seems to precede, if
only for a few seconds, any one of the long known events in the photosynthetic metabolism such as carbon dioxide reduction, oxygen evolution or photophosphorylation, the latter being the consequence of internal back reactions along a complex cycle of enzymes between the primary reduced and oxidized photochemical reaction products.

Nitrogen fixation via the photochemical release of hydrogen appears to be the secondary consequence of photophosphorylation which, together with a photochemically mobilized source of hydrogen donors, either organic substances like malate or hydrogen gas, proceeds purely as an enzymatic dark reaction via the enzyme nitrogenase. These observations, which will be the content of Dr. Paschinger's two publications, one on the photochemical disproportionation of sulfur into sulfide and sulfate in the green sulfur bacterium Chlorobium, the other on a changed nitrogenase activity by substituting tungsten for molybdenum.

These observations were the result of systematic comparisons of photochemical productions of hydrogen gas by three types of organisms - our hydrogenase-containing green algae, the well-studied purple bacterium Rhodospirillum rubrum and the green Chlorobium. Summaries of the proposed publications are attached. The overall results can be condensed into the statement that each type of microorganism has its own specific way to utilize electrons mobilized by their photochemical responses.

**Origin of Life (H. Gaffron)**

We have not officially worked on the origin of life as a research title, but the problem of photobiology inevitably comes to the fore in all discussions of the role of light for the appearance of life on earth, or in the modern version of "exobiology". Because I commented on this theme on various occasions, I am still asked to survey the status of the relationship of photosynthesis to this topic. Furthermore some years ago NASA invited me to take part in some of their Symposia, and so it might be appropriate to summarize my views as they have developed, particularly in the recent two years. It might also be of interest to NASA that the Russians have sent me an invitation to chair a session on the same topic at the International Botany meeting in Leningrad, 1975.

We have excellent proof that for millions of years photosynthesis has been the main source of free energy for the support of life on earth. As soon as this role of photosynthesis had been clearly recognized at the end of the 19th century, the Darwinian ideas of evolution led to the notion that photosynthesis
not only supported life as we know it at the present time, but might have been the decisive factor to cause the spontaneous appearance of living things on earth. The Russian and American space projects brought about a renewed interest in the role of light in evolution. I intend to present at the forthcoming meeting in Leningrad the following thesis: Photosynthesis, as it occurs all around us in the green world of the present era, did not exist at the time of the assumed "origin of life" because life presupposes the existence of enzyme systems and of the genetic code. Therefore photosynthesis as we know it developed later as part of the Darwinian evolution. We believe this happened in three recognizable steps, while assuming a greater and greater complexity: photophosphorylation, followed by photoreduction, followed by the photoxidation of water and the evolution of oxygen. Each set of the tripartite mechanism of photosynthesis depended on the appearance of the right set of enzymes and their blueprints in the genetic code. It follows that conclusions on the role of light during the origin of life cannot be based on present day specific knowledge of living photosynthetic reactions, until we know more about the origin of enzymes and of nucleic acids. All that we have learned during the last half century concerning the problem of life's origin can be summarized by stating that the problem has to be postponed and later reformulated in view of the enormous expansion of our understanding of the details in Darwinian evolution.

Quite different from "origin of life" is the question of origin of organic substances, particularly of those which have to exist in abundance before we can speak about the self-assembly of enzymes, of membranes, and of polynucleotides. This problem, known as the Oparin-Haldane theory, has been solved by the experiments of the last decade. There are now sufficient NASA publications to convince us that further affirmations are hardly necessary - though of course being far from exhausted, the question of spontaneous appearance of organic molecules under primitive earth conditions will continue to be a favorite research topic. But it is a serious defect in logic to assume that primeval organic chemistry is going to facilitate greatly the truly big problem, namely the spontaneous appearance of self-reproducing organic complexes.

All the basic amino acids, nucleic acids and vitamins have been standing on the chemists' shelves. It was not necessary to wait for the triumph of the Oparin hypothesis in order to begin to "make life" in the test tube. Essential
was the knowledge of the geochemists and in recent years that of the planetary chemists about the environmental conditions which dictated the outcome of any synthetic efforts. Primeval chemistry is a going project - but primeval self-assembly are words without any orderly hypothesis to direct our efforts, often used only to cover up the total lack of a basic new conception.

Principal Investigator, Professor H. Gaffron, 1/12 time 1965-1972.

Post-doctorals:

Dr. Wolfgang Kowallik, April 1, 1965, to April 30, 1967; now Professor at Botany Institute of University of Cologne, Germany. (full-time)

Dr. Hans-Jürgen Schick, October 1968 to July 1970, now an industrial chemist in Marburg, Germany (full-time)

Dr. Götz Harnischfeger, October and November, 1970, now Research Associate at Plant Physiology Institute of University of Göttingen, Germany. (full-time)

Dr. Hubert Paschinger, April, 1972, to June, 1973; now Research Associate at Physical Chemistry Institute of University of Vienna, Austria (full-time).

Graduate Students:

Mr. Robert Cunningham, 1/2 time, May, 1967 to August, 1967.

Mr. Götz Harnischfeger, 1/2 time, May, 1967 to August, 1967 and January, 1968 to October, 1970 (also a post-doctoral for a short time)


Technical assistants:

Mr. John Stoutamire, May, 1965 to August, 1965. (full time)

Mrs. Patricia Hayward, September, 1965 to November 1970. (full time)

Mrs. Cecile Taylor, December 1970, to December 1972. (full time)


Mr. E. Walter Herold, 1965 to 1968 and 1972, 2/12 time 1970 and 1971, 1/12 time
### Equipment purchased (items over $100)

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Photochemical Disproportionation of Sulfur into Sulfide and Sulfate

by Chlorobium limicola forma thiosulfatophilum

H. Paschinger, J. Paschinger and H. Gaffron

Institute of Molecular Biophysics
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Tallahassee, Florida, 32306, USA

Received

Summary. The anaerobic bacterium Chlorobium assimilates carbon dioxide in the light with various sulfur compounds as electron donors. The well-known metabolic pathway proceeds from the oxidation of sulfide via sulfur to sulfate. In the dark the reaction is partially reversed when sulfur is reduced to hydrogen sulfide. The fermenting cells thereby release an excess of reductant. We have now found a hydrogen sulfide production from sulfur which is light-dependent. It is more than ten times faster than the dark reaction. To see this, one has to illuminate the cell suspension in the absence of carbon dioxide and flush it continuously with hydrogen, helium or argon. The $\text{H}_2\text{S}$ is trapped with $\text{ZnCl}_2$ and the $\text{S}^-$ titrated with iodine. The amount of $\text{H}_2\text{S}$ evolved in the light increases proportionally with the amount of sulfur added, and the yield is about one-half of the added sulfur when compared on a molecular basis. Another part of the metabolized sulfur appears at the same time as sulfate, but all the sulfur oxidized to sulfate does not account for the larger amount of sulfur reduced to hydrogen sulfide. Very likely other unanalyzed oxidized sulfur compounds must also have been produced.

Use of hydrogen instead of argon as the anaerobic gas phase does not increase the amount of $\text{H}_2\text{S}$ produced, nor does the addition of thiosulfate, which suggests that sulfur itself is the preferred electron donor for the sulfur reduction.
Up to a light intensity of 10,000 ergs cm$^{-2}$ sec$^{-1}$ there is no difference in $\text{H}_2\text{S}$ production whether $\text{CO}_2$ is present or not. Without $\text{CO}_2$, saturation of the light-dependent evolution of $\text{H}_2\text{S}$ is reached at about 40,000 ergs cm$^{-2}$ sec$^{-1}$. In contrast, presence of $\text{CO}_2$ at this light intensity makes the sulfide production disappear completely. But if the gas exchange upon illumination at high light intensity is traced with a mass spectrometer, a $\text{H}_2\text{S}$ gush appears during the first 3 minutes, followed by a $\text{CO}_2$ fixation while simultaneously $\text{H}_2\text{S}$ is now taken up, functioning as electron donor for $\text{CO}_2$ assimilation.

In comparison with *Chlorobium*, it is interesting that *Rhodospirillum rubrum* has a known $\text{H}_2\text{S}$ production which is not light-dependent. Indeed light has no direct effect on this reaction, which proceeds without the production of sulfate. However, with the addition of malate, the rate of $\text{H}_2\text{S}$ evolution in the light does increase, since the cells use malate as an electron donor during their photo-chemical metabolism.
A Changed Nitrogenase Activity by Substituting Tungsten for Molybdenum

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Received

Summary. We have studied the activity of the tungsten analog to the molybdenum-containing nitrogenase of *Rhodospirillum rubrum*. For this purpose we cultured, besides the normal cells, molybdenum-deficient ones and cells replete with tungsten in place of molybdenum. The growth rate of the tungsten cells and of the molybdenum-deficient cells was about one-fourth that of the normal cells. A 100-fold increase of the tungsten concentration in the medium did not suppress the growth rate any further. The rate of nitrogen fixation found in Mo-deficient and W cells was about one-fourth of that in normal, molybdenum-containing cells. The hydrogen evolution via nitrogenase with tungsten cells dropped to only 2/3, while the molybdenum-deficient cells showed only 1/4 of the hydrogen evolution rate of normal cells. This suggests that substituting W for Mo inhibits $N_2$-fixation far more than $H_2$ evolution via the nitrogenase. The reduced activity of the nitrogenase in W cells was caused by tungsten and not by any remaining traces of Mo. The addition of tungsten to Mo-deficient cells after harvesting (i.e., after the growth in a molybdenum-free culture medium) caused an increase both in the hydrogen evolution rate and in the rate of nitrogen fixation. There was a difference, however, between the effect of adding traces ($10^{-5}$M) of tungsten or of molybdenum to molybdenum-deficient cells in respect to hydrogen evolution and to nitrogen fixation. Both metals, W or Mo, increased the rates of hydrogen evolution in the light by the same amount.
On the other hand, the increase of the rate of nitrogen fixation after adding tungsten was only one-half of the effect which could be achieved by adding molybdenum. This might be explained by a lesser affinity ($1/2$) of nitrogen to the tungsten enzyme than to the molybdenum enzyme. At least such differences appeared again when we measured the respective rates in relation to varied nitrogen partial pressures.
Effect of Anaerobiosis on Photosynthetic Reactions and Nitrogen Metabolism of Algae With and Without Hydrogenase

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Received

Summary. The inhibition by anaerobic incubation of photosynthetic O₂ evolution is much stronger in algae without hydrogenase than it is in algae with hydrogenase. The effect of anaerobiosis is most pronounced at rather low light intensity (about 1,000 lux), in acid medium (pH 4), and after prolonged anaerobic incubation in the dark (about 20 hours). These results indicate that the presence of hydrogenase might be ecologically advantageous for algae under certain conditions.

Chlorophyll fluorescence showed the fastest response to anaerobic incubation, and the most pronounced difference between algae with and without hydrogenase. After only 30 min under N₂ + CO₂, fluorescence in algae with hydrogenase starts with a peak and decreases within 12 to 20 sec to a rather low steady-state level which is only slightly higher than that found under aerobic conditions. In algae without hydrogenase, fluorescence is rather low during the first 1 to 2 sec and then rises to a higher steady-state level which is much higher than that of the aerobic controls. This indicates an inhibition due to anaerobiosis of photosystem II in algae without hydrogenase.

Algae with hydrogenase can react in different ways during the first minutes of illumination. In some cases there is an immediate photoproduction of H₂, which is followed after a few minutes by photosynthetic O₂ evolution; in other
Kessler

algae there is a simultaneous production of $\text{H}_2$ and $\text{O}_2$ from the very beginning; in a few experiments there was no photoproduction of $\text{H}_2$ at all, and in this case there was no photosynthetic $\text{O}_2$ evolution either. Thus photoproduction of $\text{H}_2$ seems to be the process which normally enables algae with hydrogenase to oxidize and thereby activate their photosynthetic electron transport system after anaerobic incubation.