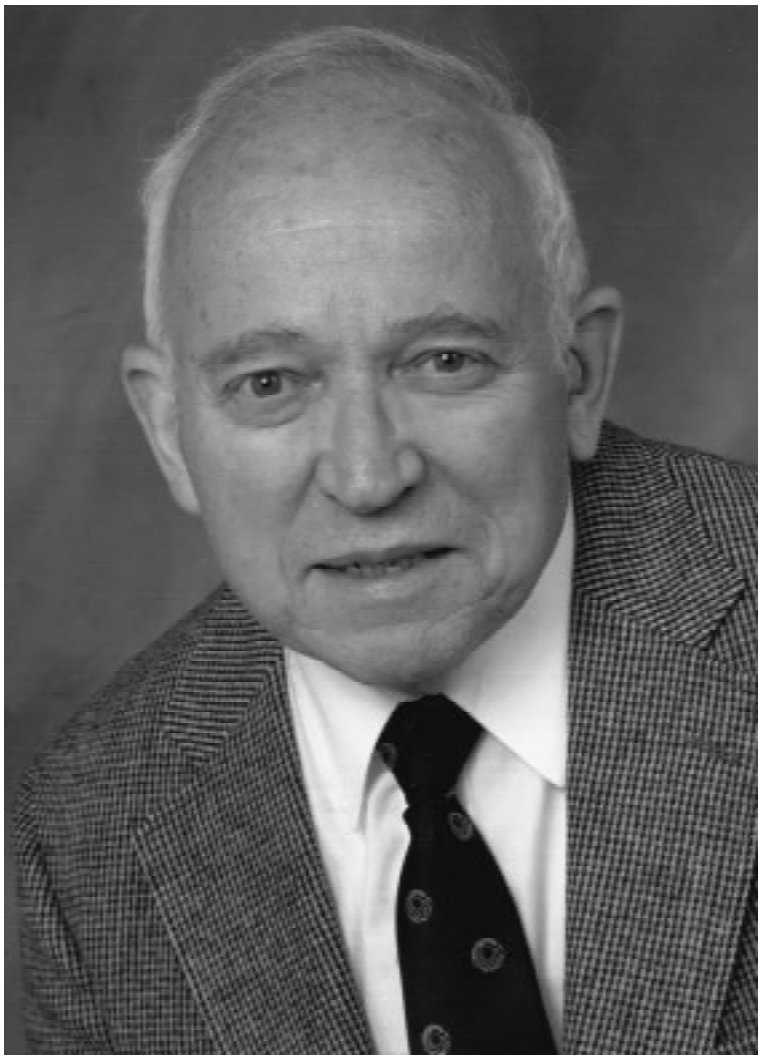

D.W. Krogmann

Photosynthesis Research **63:** 109–121, 2000.

Personal perspective

The golden age of biochemical research in photosynthesis *

David W. Krogmann
Biochemistry Department, Purdue University, West Lafayette, IN 47907, USA

Received 24 October 1999; accepted in revised form 12 January 2000

Key words: carotenoid protein, cyanobacteria, cytochromes, photosynthesis

Abstract

The perspectives and enthusiasms recorded in this review describe the events I witnessed and, in small ways, contributed to. Two great rewards emerged from my experiences – the pleasure of doing experiments and the great wealth of friendships with students and colleagues. As a graduate student, phenomena appeared at the bench before me which clarified the coupling of electron transport to ATP synthesis. My first PhD graduate student measured concentrations of pyridine nucleotides in chloroplasts and his results have been often confirmed and well used. All of the many graduate students who followed contributed to our understanding of photosynthesis. I have taken much pleasure from documenting the details of photosynthetic phosphorylation and electron transport in cyanobacteria. Studies of the '*c*' type cytochromes in these organisms continue to fascinate me. My experiences in government in its efforts to promote research are unusual, perhaps unique. A rare event outside the laboratory – a natural bloom of cyanobacteria – stimulated new thoughts and special opportunities for laboratory science. Photosynthesis seems magisterial in its shaping of our planet and its biology and in the details of its cleverness that were revealed in the time of my witness.

Beginnings, Washington, DC

In the 1930s and 1940s, I was born, raised and had traveled extensively in Washington, DC. My parents, Rudolph Francis Krogmann and Cecelia Mary O'Dea Krogmann, were American-born children of immigrants from Germany and Ireland. They placed high value on education and on reading literature and history. These lessons were a fine legacy. At Gonzaga High School, a biology teacher, Jesuit Father J. Dougherty, dedicated to both his subject matter and the instruction of his students, nudged me toward biology. At the Catholic University, several similarly dedicated teachers reinforced my enthusiasm. Dr H.C. Hanson taught plant physiology and his reverence for the then scarce facts of photosynthesis was touching. Near the end of my studies, I took a course in biochemistry from Dr Keith Laidler and this convinced me that there were opportunities in this new branch of science. In the Spring of 1953, Dr Andy Benson came to our university and gave an engaging lecture on the work he was doing with Melvin Calvin and Sam Aronoff on the path of photosynthetic carbon dioxide fixation in *Chlorella*. The obvious power of the techniques used and the clarity and enthusiasm of the speaker were irresistible. There were other, more subtle influences toward science in Washington, DC. I had visited the museums and the National Zoo many times. I was aware of the Department of Agriculture (USDA) Laboratories in Beltsville, the forensic laboratories of the Federal Bureau of Investigation, the laboratories of the National Bureau of Standards and the then rapidly growing National Institutes of Health (NIH) in Bethesda. These might be places of future employment. A story was then current of how President Roosevelt had selected the site for the NIH. On a Sunday afternoon, the President was driven to several possible locations for the new laboratories and, on reaching a site in the suburb of Bethesda, Maryland, he was delighted by his recollection of Bethesda's old significance in the Bible. Christ had cured a sick man

* Written at the invitation of Govindjee.

at the ‘well of Bethesda’ in Jerusalem. The President chose the new Bethesda as a site of union of research with the relief of human suffering. The subsequent, enormous growth in government support of research in biological sciences was often justified by the promise of relief from suffering through better medical and nutritional support for humankind. There has been much progress but the goal is still distant.

Graduate studies, Baltimore

In the Fall of 1953, I began graduate studies in the Biology Department of Johns Hopkins University with support from work on a research project on mineral nutrition in animals. In retrospect, I would later realize the stunning good luck in arriving at that place at that time. The Department was invigorated by W.D. McElroy who headed the McCollum Pratt Institute and who would become the Chairman of the Department. There was a large group of faculty interested in biochemistry, many postdoctoral fellows and a few dozen graduate students. In their first year of graduate study, students interested in biochemistry attended two 1-hour lectures at the beginning of each day, 6 days a week. These classes were taught in stints of varying duration by many faculty members and the goal was to impart a high professional level of knowledge in every area of biochemistry. Two prominent teachers were Sidney Colowick and Nathan Kaplan and both were encyclopedists. Dr Colowick once remarked at dinner that he thought he knew all of the practicing biochemists in the world. The science was young then and now that feat seems no longer possible. The huge series of volumes called ‘*Methods in Enzymology’* was launched by these two scholarly men. Graduate students were obliged to attend Journal Club that met three times a week nearly every week of the year. All attendees brought a bag lunch and heard an oral review of a journal paper presented by a faculty member, post doc or grad student. Questions were punishing and the standards were high. Beyond that, the lights and centrifuges burned late every night all week. It was a wonderful introduction to research.

Coincident with my arrival in Baltimore, André Jagendorf (Figure 1) joined the faculty there and I was allowed to join his lab. I found and read an extraordinary book entitled ‘Photosynthesis’ by Eugene Rabinowitch (1945) (Figure 1). This book reviewed all the research on photosynthesis since the beginning of science. It was clearly written and a joy to read. Then and for some years to follow, it was widely assumed by casual observers that Melvin Calvin and his colleagues had solved the problem of photosynthesis by their elucidation of the path of carbon fixation. This work won the Nobel prize in 1961. Of course, this casual assumption was wrong. The best was yet to come. The discovery of cyclic photophosphorylation in bacterial chromatophores by Albert Frankel (1954) and the recognition of similar activity in higher plant chloroplasts by Dan Arnon et al. (1954) opened a new world of bioenergetics research. The Calvin–Benson cycle of photosynthetic carbon dioxide fixation had given specific emphasis to the widely perceived requirements for ATP and reduced NADP in biosynthetic processes. Photophosophorylation provided a source of ATP synthesized by illuminated chloroplasts supplemented with low molecular weight cofactors. Years earlier, R. Hill (1939) had demonstrated that isolated chloroplasts could simultaneously produce oxygen and reduce a high potential electron acceptor like ferricyanide in the light. Later, H.E. Davenport and R. Hill found photoreduction of the relatively low potential metmyoglobin by chloroplasts supplemented with a water soluble protein factor (Davenport 1960). Mordhay Avron (Figure 1) came to André Jagendorf’s lab and purified a chloroplast NADPH diaphorase which would later be identified as the ferredoxin NADPH oxidoreductase (Avron and Jagendorf 1956). At this time, Anthony San Pietro (Figure 1) joined the Biology faculty at Johns Hopkins and identified a protein factor – ultimately named ferredoxin – which enabled chloroplasts to reduce pyridine nucleotides (San Pietro and Lang 1956). A long wait was imposed for the technology to develop for amino acid sequencing and crystal structure determinations of these two proteins. In any case, the illuminated chloroplast was surely capable of supplying ATP and NADPH for carbon dioxide fixation. Now the interesting question was ‘How?’.

The excitement of these times was exhilarating. Johns Hopkins enjoyed a constant flow of distinguished visitors and symposia on bioenergetics and photobiology brought the great luminaries to that campus. There were many memorable seminars. Martin Kamen’s erudition and staccato delivery made cytochromes an irresistible object for research. Sterling Hendrick’s account of phytochrome research was a marvel. In addition I began to meet young scientists from England – Francis Whatley, David Hall; Spain – Manuel Losada; Germany – Achim Trebst; Italy – Giorgio Forti and Israel – Mord Avron who would

Figure 1. Participants at the Brookhaven Symposium in Biology 'Energy Conversion by the Photosynthetic Apparatus'. June 6–9, 1966. *Back row:* W. Menke, W. Robinson, T. Punnett, J. Friend, R. Levine, *H. Witt,* R. McCarty, *A. Jagendorf, N. Good,* C. Fuller, G. Forti, *B. Kok,* D. Keister, R. Bachofen. *Center row:* R. Park, P. Joliot, K. Sauer, S. Izawa, S. Miyachi, W. Arnold, T. Weier, *L. Duysens,* G. Cheniae, H.. Linschitz, *A. San Pietro,* M. Nishimura, B. Mayne, W. Parson, W. Vredenberg, J. Olson, *R. Dilley,* M. Schwarz. *Front row:* H. Lyman, R. Clayton, R. Pearlstein, *E. Rabinowitch, Govindjee,* D. Fork, *M. Avron,* M. Baltsheffsky, B. Chance, L. Packer, R. Olson, O. Owens, E. Gantt, B. Ke, G. Hind. (The italicized names are cited in the text.)

create centers of photosynthesis research in their countries. They became treasured friends. Baltimore was close to Atlantic City where the biochemistry society held its annual meeting in the Spring. From the start of graduate school, I was able to attend these meetings. All presentations were oral – full lectures by the most accomplished and ten-min talks by both veterans and neophytes. It was immediately obvious that good oral presentations were an essential part of a career in research. The bioenergetics sessions invariably began with amusing and good-natured sparring among the great men who then proceeded to draw and quarter the postdoctoral fellows of their rivals who presented new data. Nobel prizes were destined for bioenergetics and the competition was evident at these meetings. André encouraged me to attend meetings of the American Society of Plant Physiologists where there were rich photosynthesis sessions.

I was slow and uncertain in beginning research, but I received two very important lessons from André. I saw him doing an experiment virtually every day. Most professors were obliged to forsake the bench and divide their time between counseling post docs, technicians and students and writing papers and grant requests. I have persisted in following André's example. Doing experiments is an enduring pleasure. When I found the courage to ask what he was doing, André would, in three sentences, tell me the question asked, the technique used and the goal. This lesson set for me the scale of research – to concentrate on the effort of the day and let the results lead you on from there. Spectrophotometric assays were the main tool of research and André suggested I develop an assay that would enhance the sensitivity of measurement of ferricyanide reduction by illuminated chloroplasts (Krogmann and Jagendorf 1957). The assay worked easily and enhanced the sensitivity of the measurement by an order of magnitude. The assay allowed us to observe effects on ferricyanide reduction by illuminated chloroplasts indicating coupling of ATP synthesis and its uncoupling by osmotic shock treatment and by ammonium ions (Krogmann et al. 1959; Avron et al. 1959). Ammonium ions were the first uncoupler for photophosphorylation and quickly became a popular tool in studies of this process (Krogmann 1985). These results provided vivid evidence for the similarity of chloroplast phosphorylation to the oxidative phosphorylation of mitochondria. At the time, the mechanism of the coupling phenomenon was mysterious. Later, Mord Avron would demonstrate that an essential coupling factor protein complex could be reversibly removed from the chloroplast membrane (Avron 1963). This began a long journey toward the still incomplete structure of the ATP synthesizing complex and the molecular mechanism of its function. André would do the 'chloroplast acid bath phosphorylation' experiment which provided compelling evidence for the Mitchell hypothesis that a proton gradient provided the energy to drive both chloroplast and mitochondrial ATP synthesis (Jagendorf and Uribe 1966). This was such a simple and elegant experiment that the conclusion was invincible. It is one of my great favorites.

Postdoctoral research, Chicago

As I finished my thesis work, I began to wonder about using a new organism to study photosynthesis. It seemed that spinach chloroplasts were attracting hordes of devotees. There was some work on algae – *Scenedesmus, Chlamydomonas, Euglena* – which promised much via application of the techniques of microbial genetics. Just then, Maurice Margulies came to André's lab as a post doc. Maurice had done graduate work at Yale where there was still a class in algology and he urged me to consider blue-green algae since they were the most primitive of oxygen producing photosynthesizers. If you are going to escape from spinach chloroplasts, you might as well go all the way. This was good advice.

In 1957, I completed my degree work and spent the following year in André's lab as a post doc. I had presented a paper in Journal Club on the work of Birgit Vennesland on the stereospecific transfer of hydrogen in and out of pyridine nucleotide coenzymes. This so fascinated me that I sought and received a post doctoral position in her laboratory at the University of Chicago. While I did one deuterium experiment which gave a result of beautiful clarity, that area of research had been mastered and Dr Vennesland's enthusiasm was directed to chloroplast reactions. She was intensely interested in every experiment done and her care and thoughtfulness in preparing manuscripts for publication were outstanding. Our papers were published in the *Journal of Biochemistry* with very rare, minor revisions requested. Dr Vennesland gave me fine instruction in the preparation of research grant proposals and in other professional matters that helped greatly in the years to come.

In 1957, Robert Emerson published experiments on the red drop in quantum yield of photosynthesis

and the enhancement of efficiency of far red light utilization by shorter-wavelength light (Emerson et al. l957; see a review by Myers 1971). This began an enormous clarification of our thinking about photosynthesis. Govindjee and Rabinowitch (1960) (Figure 1) found spectroscopic evidence for different forms of chlorophyll *a in vivo* and for their distinct photochemical functions. Robin Hill and Dereck Bendall (1960) published a hypothesis that the redox responses of cytochromes b_6 and *f* to light and the gap in redox potential between these two cytochromes suggested distinct chemical responses of two photosystems. A year later, L.N.M. Duysens (Figure 1) published elegant action spectra for the oxidation and reduction of cytochrome *f* to confirm and elaborate on this hypothesis (Duysens 1989). Bessel Kok (Figure 1) crafted a clear and elegant description of the Photosystem 1 reaction center (Kok and Hoch 1961). I attended 'A Symposium on Light and Life' at Johns Hopkins in 1961 and marveled at the convergence in the presentations of these works (McElroy and Glass 1961). A Photosystem I particle was soon described and now, nearly 40 years later, we see a crystal structure emerging for this (Klukas et al. 1999) (see H.T. Witt in Figure 1) and other supra molecular complexes. I well remember a Gordon Conference in the early 1960s, in Tilton, New Hampshire where there was heated discussion of whether the arrows depicting photoacts should point up or down and whether to rotate the Z clockwise or counterclockwise in what came to be known as the Z scheme. It seemed grand to reconcile the measured requirement of eight quanta per carbon dioxide molecule fixed and the efficiency of energy conversion in oxidative phosphorylation with the passage of four electrons through two photoacts to generate NADPH and ATP. Physics had provided a powerful insight into the biochemical machinery.

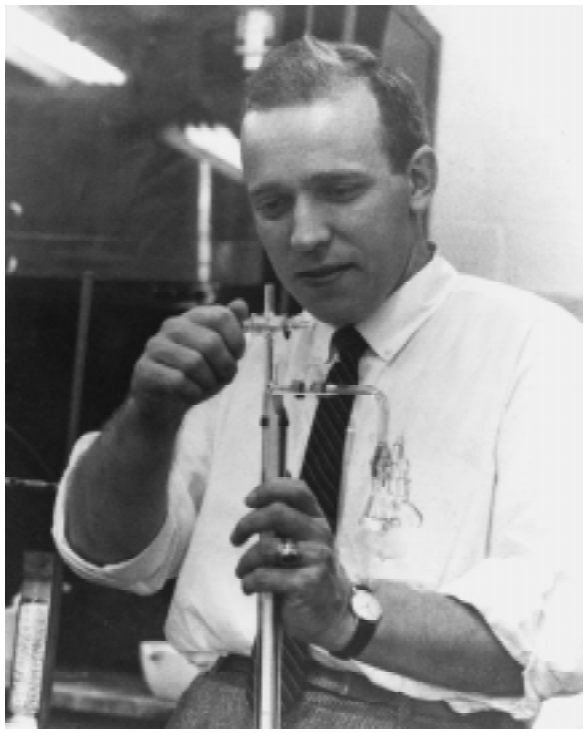

Figure 2. Author (D.W. Krogmann) holding a Warburg manometer – an elegant but now antique device for measuring changes in gas pressure induced by whole cell photosynthesis or by the Hill reaction of illuminated chloroplasts. Photo taken in 1961.

Academic ascent – Detroit, then West Lafayette

In 1960, I joined the faculty of the Chemistry Department at Wayne State University in Detroit, Michigan. I was fortunate to obtain immediately a research grant from NIH to equip a lab and support students in their graduate research. (Figure 2). Bill Ogren appeared among the first group of students. He was a fine experimentalist and his thesis and later accomplishments were outstanding. In that period, graduate students were earnest and spoke of 'making a contribution' in science. I taught introductory biochemistry to a large class at night and there were many working people – autoworkers, school teachers and others of humble stations who were well motivated, respectful of knowledge, and a pleasure to know. Now I could begin work on blue-green algae which has, ever after, been rewarding. In the early 1960s, blue-green-algae were recognized as prokaryotes and so became cyanobacteria. My newly elevated professional status brought the great scientific and personal benefit of more travel to scientific meetings. Gordon Conferences occasioned regular and exceedingly beneficial visits to New Hampshire. Triennial visits abroad came for the International Photosynthesis Congresses and for meetings on photosynthetic prokaryotes. My research was informed by all of the publications of Professor Jack Myers and especially those describing the work of his student Ray Holton. In 1963, Holton and Myers published descriptions of soluble electron transfer catalysts in cyanobacteria (Holton and Myers 1963, 1967a, b) which have instructed much of my life's work. Both of these authors have been exemplars of fine experimental work and kindness. The work in Detroit made it clear that while cyanobacteria differed

radically from eukaryotic algae in cell structure and in antenna pigment composition, the photosynthetic apparatus was virtually identical to that in all eukaryotes including higher plants. This was less exciting than I had hoped but, in the long run, offered a great advantage in understanding oxygenic photosynthesis. When Gregorieva and Shestakov (1982) discovered the high frequency transformation of *Synechocystis* 6803, an organism that can grow photoheterotrophically on glucose, a marvelous door opened in photosynthesis research. The dissection of cyanobacterial photosynthesis by methods of molecular biology is a dazzling accomplishment, especially since it provides much instruction about photosynthesis in all eukaryotes.

In 1967, I was contacted by Dr Barney Axelrod of Purdue University and offered an opportunity to join the Biochemistry Department that he chaired there. I had much admired Dr Axelrod and had several other good friends at Purdue so it was a welcome opportunity. In a stroke, I had escaped urban traffic jams and found an excellent research environment. I would gain as colleagues Fred Crane, Dick Dilley (Figure 1), Bill Cramer and later Lou Sherman who provided intellectual stimulus, support and friendship that could hardly be equaled. As in Detroit, I would work with graduate students who would become colleagues and friends of inestimable value.

In 1976, I attended a photosynthesis meeting at the Brookhaven National Laboratory and met a Mexican scientist – Carlos Gómez Lojero from the Departamento de Bioquimica, Centro de Investigacion y de Estudios Avanzados del Instituto Politecnico Nacional in Mexico City. I had read of a company in Mexico City that was producing the cyanobacterium *Spirulina maxima* on a commercial scale. I asked Carlos to help me contact this company so that I might buy a large sample of cyanobacteria for research. Growing and harvesting large quantities of cyanobacteria in the laboratory is a daunting task. Carlos found the company, became fascinated with the organism and then arranged for me to visit his laboratory. I was able to ship home several kilos of frozen cell paste and to exchange research experiences with Carlos and his students. Thus, I found a dear friend and a life long collaborator. Since then, I have visited Mexico often and enjoyed the hospitality and enthusiasm of many Mexican scientist friends.

André Jagendorf (1998) began his Personal Perspective by reflecting on the role of chance in shaping a career. I suspect that my choice of photosynthesis as a research area weighted the chance that I would come to the Midwest. The state universities here seem to acknowledge the importance of our research by hiring people to study this process which is at the base of the region's agricultural prosperity. In 1974, Tony San Pietro, who had come to chair the Botany Department at Indiana University, organized the first Midwest Photosynthesis Meeting and his neighbors are forever grateful. About 80 participants gather at a central location – Turkey Run State Park in Indiana - and discuss fine science. The name of the park refers not to the activities of the conference participants but to a cleft in the great plains where turkeys escape the cold winds of Winter. At this meeting, graduate students present their thesis work and gain entrance to the professional world. Another joy of my location has been the opportunity to visit neighbours and sometimes do experiments with them. It was a short and pleasant drive to Bloomington, Indiana and Tony San Pietro, to Champaign Urbana, Illinois and Bill Ogren, John Whitmarsh, Don Ort, and Govindjee, to East Lansing, Michigan and Norm Good (Figure 1) and Lee MacIntosh, to Columbus, Ohio and Liz Gross, to Oshkosh, Wisconsin and Toivo Kallas – to name a few of many fine neighbours.

Government service

Just prior to my move to Purdue, I was invited to serve for 1 year as a rotating Program Director for Molecular Biology at the National Science Foundation (NSF) in 1966. The Molecular Biology Program, supporting biophysics and bioenergetics research, predated the modern meaning of its title and owed its origin to the Cambridge crystallographers who had labored long to solve the structures of biological macromolecules. I accepted this assignment gladly since I felt fondness for the city of my birth and since my parents had worked in government with gratitude as this rescued them from the Great Depression. Photosynthesis research was a part of the NSF program so I had a meager qualification for the assignment. This was a wonderful year. I learned a great deal about a wide breadth of science and worked with people dedicated to intelligent service of scientific research.

In 1977, Dr Joe Key of the University of Georgia was asked to organize a new office at the Department of Agriculture which would make grants for basic research in support of agriculture. In turn, Joe asked me to direct the photosynthesis research grants program. My prior experience at NSF was an asset and many

of the NSF procedures were adapted to the new programs at the USDA office. I succeeded Joe as head of the office in 1979. On arriving for duty, a senior civil servant told me that I would need much coaching before testifying before Congress and my instruction was arranged. In fact, this was of little use. I found my correspondence was being read (for policy compliance) and was being corrected, none too expertly, for imagined errors in style and grammar. After years of freedom in universities, bureaucracy's grip seemed amazing. I was immediately assigned the role of responder to all comments and queries concerning a 'Golden Fleece Award' from a very powerful, senior Senator. The award had been bestowed on a grant made by our office. Clearly, I was to be a sacrificial lamb. The grant had been made by our Human Nutrition program and had been identified by the Senator's aid as worthy of ridicule and a waste of the taxpayers dollars. My reading of the grant showed it perfectly reasonable research. I composed a brief defense of the science in the grant and gave it by phone to a number of radio talk show hosts who said our conversation was being broadcast to large audiences in Chicago, San Francisco, St. Louis, etc. The hosts seemed to find this explanation satisfactory and in a few days that dubious celebrity passed. Next came letters to the Secretary of Agriculture and very intense phone calls to me from lobbyists who apparently wished to construe in our grant a subversive plot to advocate vegetarianism. Their efforts would justify the handsome salaries they obtained from organizations of meat producers and processors. Finally there were calls and letters of a humble and genuinely interested tone from vegetarians about the substance of the proposed research. I recalled an old suggestion that carnivory might be associated with aggressive behavior and herbivory associated with the opposite.

'Seven Days in May'

In 1981, I returned to USDA on an intermittent appointment to fill in for a Head of Office who had to cancel at the last minute. Early in this term, I was told by my superiors that review panelists might have to undergo a clearance procedure. I objected in the strongest terms that panelists were selected for their scientific expertise and no other clearance was warranted. All the enormous work of preparing a huge number of proposals for evaluation and arranging for the panel meetings went smoothly until 2 weeks before the panel meetings were to begin. Then came a fateful Monday morning phone call, never documented in writing, ordering that all the panels should be postponed until the panelists could be cleared. I received no communication from any one in the chain of command between the lofty office originating this order and my own. A reporter from *Science* magazine called and asked about a rumor he had heard of panelist security clearance and I gave him the phone number of the responsible party in the office of the Secretary of Agriculture. *Science* promptly published a story (Marshall 1982) indicating that this official wanted panelists to be cleared on the basis of their political suitability. I had been given to understand the clearance would be a low level FBI check and was appalled to learn that it would be done by party officials. Shortly after, Dan Rather expressed his indignation at this on the CBS nightly news and an editorial in the Sunday New York Times (Editorial NYT 1982) decried Lysenkoism in the Department of Agriculture. On the following Monday, another call from the Secretary's office came saying that our panel meetings could be held as scheduled without further screening of panelists. The bright light of public exposure had a photosynthesis-like effect of clearing the air. On rare occasions, high drama can accompany government service.

By the end of that year at USDA, I was sure that my service to that agency was forever done. I remember, with much gratitude, the help and support of my administrative assistant, Ms Holly Schauer, who had, in a long career at both NSF and USDA, given fine service to the scientific community.

The formation of this grants program in the USDA had a special beneficial effect that I can give unique witness to. In 1975, a meeting of scientists working on the biochemistry and physiology of plants was held at Boyne Mountain, Michigan to discuss and identify research areas of rich promise if new funds could be made available for basic research to use emerging techniques. Prior to the 1976 presidential election, a possible drawdown in world grain reserves raised the prospect of televised famine and this later prompted the Carter administration to seek funding for new basic research in support of agricultural productivity. The research areas agreed on at the Boyne Mountain meeting appeared in the legislation as targets for funding in the new programs to be administered by the USDA. Just at this time, the new techniques of gene recognition, sequencing and manipulation broke on the scientific horizon with revolutionizing force. The

extant system of agricultural research seemed lethargic and it had often neglected basic research in favour of practical technology. The new grants program in the USDA stimulated the agricultural research community to compete for funds that would support use of the new molecular biology techniques to achieve understanding and control of crop productivity by manipulation of genes. With the help of the new grants program, the pace of agricultural science was accelerated and the basic science content of the work was increased.

Cyanobacteria – from natural blooms to protein crystals

In August of 1983, nature and a series of rare, chance events led to some science that was great fun. I was visiting my sister and her family in Bethesda, Maryland and found an article in the *Washington Post* describing a noxious green slime that had blanketed the Potomac River just south of Mount Vernon, Virginia. I recalled having seen a photo in the *National Geographic* Magazine in the 1960s that identified a great raft of cyanobacteria floating in the Potomac at this same location. I went to the site of the reported bloom at Pohick Bay Regional Park and took samples to examine under the microscope. All the samples showed virtually pure *Microcystis aeruginosa*. This seemed a rare opportunity to get a generous supply of a unicellular cyanobacterium. Gas vacuoles propel the cells to the surface of the water to harvest light energy and the cells accumulate there in a thick layer. Skimming the surface scum and draining some of the water allowed the collection of kilos of cell paste. My avarice was prompted by experience with NMR spectra of cytochromes and plastocyanin which required very large amounts of pure protein (Ulrich et al. 1982). This material provided fuel for lots of interesting research for several years to come. While collecting the *Microcystis,* I chatted with people who worked at the park and learned interesting things. Some residents had blamed the bloom on nutrients released from the very modern sewage treatment plant up river and surely the ammonium level in the water was high. We found that the cells had no nitrate or nitrite reductase. Some eco-activists had raised the possibility of crisis since *Microcystis* is known to produce a toxin. While *Microcystis* blooms in the mid and far west occasionally kill farm animals that drink from overgrown ponds, strains of this organism from east of the Appalachians usually are far less toxic. We measured very low toxin levels in the Potomac bloom. A park employee told me that she remembered rare but similar blooms at this site in her childhood in the 1920s. I was prompted to recall newspaper reports of algae nuisances from my childhood in the 1940s. Most significant was the realization that 1983 was a year of severe drought in the Potomac River valley. I set out to find records of algal blooms on the Potomac and the first reference found for a bloom in 1930 noted that that was a year of severe drought. A series of references for blooms in subsequent years turned up in the Washington newspapers and showed a perfect correlation between occurrence of blooms and very low water flow in the Potomac (Krogmann et al. 1986). At the time of this writing, there has been a severe drought in the Potomac water shed and Pohick Bay turned green on or about July 23, 1999. A scum began to appear in mid August, but heavy rains in late August and early September purged the bay and prevented collection of new material for protein isolations. The larger question of why blooms occur is still open. There are data available that were reconstructed from tree ring measurements which identify the July–August–September streamflow of the Potomac River since 1730 (Cook and Jacoby 1983). I found evidence here for a drought in the 1860s which correlated with a letter from a soldier in the Union Army camped on the banks of the Potomac River. He wrote to his parents that he could not bathe in the river since it was ‘like pea soup’ which matches the condition I saw in 1983. I hope to search the plantation owners’ diaries and the colonial newspapers in drought years for reports of blooms that might have occurred before the age of pollution.

The large quantity of *Microcystis aeruginosa* yielded many new insights. The organism is easily broken by freezing and thawing and is unique among cyanobacteria in that the phycobilisome pigments are not released as small, soluble subunits in the aqueous extract. The green membrane fragments are easily removed by filtration through cloth and all of the blue pigment proteins are removed by filtration through a 100 kiloDalton membrane filter. These blue antenna proteins can be fifty percent of the total cell protein, and so are an enormous burden when one is seeking catalytic proteins of much lower abundance. With the antenna protein burden removed, the concentrated extract is red in color due to cytochromes and ferredoxin. Anion exchange chromatography promptly revealed proteins that had been buried in the blue fraction. A unique water soluble, orange carotenoid protein turned up. This has been found in several cyanobacteria (Holt

and Krogmann 1981; Diversé-Pierluissi and Krogmann 1988) and its characterization has been rewarding (Wu and Krogmann 1997; Kerfeld et al. 1997). The orange carotenoid protein is unique in its solubility in water and in its amino acid sequence. I have fractionated protein extracts from a great number of plants and algae and have seen nothing like it. Perhaps the orange carotenoid protein is a transport vehicle in cyanobacteria used to move carotenoids from their site of synthesis in the photosynthetic membranes to the periphery of the cell where carotenoids accumulate in response to high intensity light. The crystal structure promises new insights into the relation of hydrophobic interactions to spectroscopic properties.

The low potential cytochrome *c*, which is found only in cyanobacteria and planktonic algae, was found in generous amount that led to determination of its amino acid sequence, isolation of its gene and its crystallization (Kang et al. 1994). The fact that this cytochrome is autooxidizable suggests that its redox function is related to some anaerobic process. In the temperate zone, cyanobacteria and algae must spend the winter in anaerobic sediments and maintain themselves by fermentation. The Potomac *Microcystis* were collected from a dense scum on the river and most of the cells were in a dark, anaerobic state. There is fossil evidence for cyanobacteria of great antiquity. It seems they were present more than three billion years ago (Schoff 1999). The cyanobacteria may be the source of oxygen in the earth's atmosphere, which, only one billion years ago, reached a concentration that permitted the appearance of aerobic life forms. I sense we will be wiser when we understand the anaerobic processes which sustained life for many a night and long Winter and for the several billion years that preceded aerobic times, and which still sustains the cyanobacteria and many algae. The photosynthetic machinery of cyanobacteria is surely linked to hydrogen metabolism and is likely linked to other arcane anaerobic processes as well. Thus far we have studied cyanobacteria grown photoautotrophically under intensely aerobic conditions so we may expect there is more to learn about growth and survival in other circumstances.

Concluding remarks

To realize that one has lived in a Golden Age is humbling. Thanks are surely due to my teachers and students – both have given much help and instruction. Gratitude is due to fellow citizens and their civil servants who provided funds – a unique advantage of the last half century in comparison to the longer history of science. Golden Ages have endings and occasionally I hear remarks indicating that photosynthesis research is finished. I think this conclusion is in error and I know that, in science policy circles, this type of conclusion is often motivated by partisan designs to shift funds to more glamorous alternatives. There is evidence of much more to be understood about photosynthesis. The New York Times has recently published two articles describing new experiments that reveal intriguing properties of quanta. One (Brown 1999) tells of experiments of Hau et al. (1999) who have been able to reduce the speed of light. The other Times article (Brown 1997) describes the work of Nicolas Gisin and his colleagues in Geneva on the long connections between quantum events. These experiments suggest the possibility of greatly improved understanding of quanta, of their capture and their transformation in photosynthesis.

Schopf (1999) has extended the history of photosynthesis far back into the Precambian past by finding fossils of cyanobacteria in rock formed 3.5 billion years ago. Recently, Summons et al. (1999) have given us a chemical marker for these fossils. We will gain a much better history of life and of photosynthesis as these discoveries are expanded over the fossil record. The discoveries of *Prochloron*, a cyanobacterium with chlorophyll *a* and *b*, by Lewin (1975) and of *Acaryochloris marina*, a cyanobacterium with chlorophyll *d*, by Hu et al. (1999) suggest that a more careful search for small, fastidious photosynthesizers in exotic niches will provide valuable insights into the evolution of photosynthesis. The great surge in DNA sequencing found photosynthesis in the forefront again with the Kazusa Institute completing the sequencing of the genome of *Synechocystis* 6803. There are many unidentified genes here whose function will be ferreted out. This promises complete instruction in the generation, assembly and function of the photosynthetic apparatus. Genomes of anoxygenic photosynthetic bacteria and of eukaryotes from *Arabidopsis* to crop plants are being sequenced. Advances in X ray diffraction techniques and the current structure-function work suggests that a high resolution structure of the photosynthetic apparatus is in prospect. Add to these the unanticipated discoveries that accompany experimentation. The future looks bright.

Acknowledgements

Govindjee (Figure 1), Bill Ogren and André Jagendorf all gave encouragement to write this and I am grateful to them. Thanks are also due to Geoff Hind for the photo of the participants of the 1966 Brookhaven Symposium. The photo is a favorite of mine. Govindjee gave extraordinary help in preparing this manuscript as well. I would like to thank all of the 33 graduate students (see 'Appendix') who have done theses with me and the others who have worked in my lab. Memories of their progress, their achievements, and their friendship are priceless. My wife Loretta and children, Michele, Pat and Paul, have read and edited this manuscript. Their presence and support has been wonderful.

Appendix

Graduate students of Dr David Krogmann

PhD	Year
William L. Ogren	1966
James J. Lightbody	1966
Walter A. Susor	1966
Shaw Shan Lee	1967
Richard C. Honeycutt	1971
Jerry Brand	1971
Richard Schneeman	1974
Steven Paul Berg	1975
William Lee Ellefson	1977
Eldon Lloyd Ulrich	1978
Kwok Ki Ho	1979
Paula Kay Evans	1982
Jawed Alam	1983
James Russel Sprinkle	1985
Larry Z. Morand	1989
Duane D. Culler	1991
Charmaine Kang Grant	1994
Yi Pyng Wu	1996

MS	Year
Lorraine K. Hallenga	1962
Edwin Olivero	1963
Maria A. Meskauskas	1963
Warren C. Duane	1964
Edward A. Mendelin	1966
Charles A. Sigmund	1967
A. Michael Young	1967
Flora Tang	1972
Thomas K. Holt	1980
Mary Patricia Padgett	1985
Cathleen Lindsay Overholt	1987
Richard Anthony Whitaker	1987
Maria de Los Angeles Diverse-Pierluissi	1987
Coral Felecia Harper	1988
Robbin K. Knutson	1998

References

Arnon DI, Whatley FR and Allen MB (1954) Photosynthesis by isolated chloroplasts. II Photosynthetic phosphorylation, the conversion of light energy into phosphate bond energy. J Am Chem Soc 76: 6324–6329

Avron M (1963) A coupling factor in photophosphorylation. Biochim Biophys Acta 77: 699–702

Avron (Abramsky) M and Jagendorf AT (1956) A TPNH diaphorase from chloroplasts. Arch Biochem Biophys 65: 475–490

Avron M, Krogmann DW and Jagendorf AT (1959) The relation of photosynthetic phosphorylation to the Hill reaction. Biochim Biophys Acta 1000: 383–393

Brown MW (1997) Signal travels faster than light. New York Times, July 22, p C2

Brown MW (1999) Lene Westergaard Hau; she put the breaks on light. New York Times, March 30, p F2

Cook ER and Jacoby GC (1983) Potomac river stream flow since 1730 as reconstructed by tree rings. J Clim Appl Meteorol 22: 1659–1672

Davenport HE (1960) A protein from leaves catalysing the reduction of metmyoglobin and triphospho-pyridine nucleotide in illuminated chloroplasts. Biochem J 77: 471–477

Diversé-Pierluissi M and Krogmann DW (1988) A zeaxanthine protein from *Anacystis nidulans*. Biochim Biophys Acta 933: 372–377

Duysens LNM (1989) The discovery of the two photosystems: A personal account. Photosynth Res 21: 61–80

Editorial NYT (1982) Mr Block on the Lysenko trail. New York Times, May 9, p 20E

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

Frankel A (1954) Light induced phosphorylation by cell-free preparations of photosynthetic bacteria. J Am Chem Soc 76: 5568–5570

Govindjee and Rabinowitch E (1960) Two forms of chlorophyll *a in vivo* with distinct photochemical functions. Science 132: 159

Gregorieva G and Shestakov S (1982) Transformation in the cyanobacterium *Synechocystis* sp. 6803. FEMS Microbiol Lett 13: 367–370

Hau LV, Harris SE, Dutton Z and Behroozi CH (1999) Light speed reduction to 17 m per second in an ultracold atomic gas. Nature 397: 594–598

Hill R (1939) Oxygen production by isolated chloroplasts. Proc R Soc London Ser B 127: 192–210

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

Holt TK and Krogmann DW (1981) A carotenoid protein from cyanobacteria. Biochim Biophys Acta 637: 408–414

Holton RW and Myers J (1963) Cytochromes of blue-green algae: Extraction of *c*-type with strongly negative redox potential. Science 142: 234–235

Holton RW and Myers J (1967a) Water soluble cytochromes from a blue-green alga. I Extraction, purification and spectral properties of cytochromes *c* (549, 552, and 554) of *Anacystis nidulans*. Biochim Biophys Acta 131: 362–374

Holton RW and Myers J (1967b) Water soluble cytochromes from blue green algae. II Physicochemical properties and quantitative relationships of cytochromes *c*. Biochim Biophys Acta 131: 375–381

Hu Q, Marquardt J, Iwasaki I, Miyashita H, Kurano N, Mörschel E and Miyachi S (1999) Molecular structure, localization and function of biliproteins in the chlorophyll *a/d* containing oxygenic photosynthetic prokaryote *Acaryochloris marina*. Biochim Biophys Acta 1412: 250–261

Jagendorf AT (1998) Chance, luck and photosynthesis research: An inside story. Photosynth Res 57: 215–229

Jagendorf AT and Uribe E (1966) ATP formation caused by acid-base transition of spinach chloroplasts. Proc Natl Acad Sci (USA) 55: 170–177

Kang C, Chitnis P, Smith S and Krogmann DW (1994) Cloning and sequence analysis of the gene encoding the low potential cytochrome *c* of *Synechocystis* PCC 6803. FEBS Lett 344: 5–9

Kerfeld C, Wu YP, Chan C, Krogmann DW and Yates TO (1997) Crystals of the carotenoid protein from *Arthrospira maxima* containing uniformly oriented pigment molecules. Acta Cryst D 53: 720–723

Klukas O, Shubert W-D, Jordan P, Kraus N, Fromme P, Witt H T and Saenger W (1999) Localization of two phylloquinones, Qk and Qk′, in an improved electron density map of Photosystem I at 4-A resolution. J Biol Chem 274: 7361–7367

Kok B and Hoch G (1961) Spectral changes in photosynthesis. In: McElroy WD and Glass B (eds) 'A Symposium on Light and Life', pp 397–461. The Johns Hopkins University Press, Baltimore, Maryland

Krogmann DW (1985) Citation Classic: Uncouplers of spinach chloroplast photosynthetic phosphorylation. Current Contents 16: 14

Krogmann DW (1991) The low potential cytochrome *c* of cyanobacteria and algae. Biochim Biophys Acta 1058: 35–37

Krogmann DW and Jagendorf AT (1957) A spectrophotometric assay of the Hill reaction with ferricyanide. Plant Physiol 32: 373–374

Krogmann DW, Jagendorf AT and Avron M (1959) Uncouplers of spinach chloroplast photosynthetic phosphorylation. Plant Physiol 34: 272–277

Krogmann DW, Butalla R and Sprinkle J (1986) Blooms of cyanobacteria on the Potomac River. Plant Physiol 80: 667–671

Lewin R and Withers NW (1975) Extraordinary pigment composition of a prokaryotic alga. Nature 256: 735–737

McElroy WD and Glass B (1961) A Symposium: Light and Life, pp 1–924. Johns Hopkins Press, Baltimore, Maryland

Marshall E (1982a) Security checks on USDA reviewers. Science 216: 600

Marshall E (1982b) USDA official defends loyalty checks. Science 216: 1391

Myers J (1971) Enhancement studies in photosynthesis. Ann Rev Plant Physiol 22: 289–312

Rabinowitch E (1945) Photosynthesis, pp 1–599. Interscience Publishers, New York

San Pietro A and Lang HM (1956) Accumulation of reduced pyridine nucleotides by illuminated grana. Science 124: 118–119

Schopf W (1999) Cradle of Life, pp 1–367. Princeton University Press, Princeton, New Jersey

Summons RE, Jahnke LJ, Hope JM and Logan GA (1999) 2-Methylpropanoids as biomarkers for cyanobacterial oxygenic photosynthesis. Nature 400: 554–557

Ulrich E, Krogmann DW and Markley JL (1982) Structure and heme environment of ferrocytochrome c553 from H-NMR studies. J Biol Chem 257: 9356–9364

Wu YP and Krogmann DW (1997) The orange carotenoid protein of *Synechocystis* PCC 6803. Biochim Biophys Acta 1322: 1–7