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TRIBUTE

Berger C. Mayne (1920–2011): a friend and his contributions to photosynthesis research

Darrell Fleischman · Gerald E. Edwards · Govindjee · Leland Mayne · Vijai Tyagi · Karen Jacobsen-Mispagel

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Abstract We provide here insights on the life and work of Berger C. Mayne (1920—2011). We remember and honor Berger, whose study of photosynthesis began with the most basic processes of intersystem electron transport and oxygen evolution, continued with application of fluorescence techniques to the study of photophosphorylation and the unique features of photosystems in specialized cells, and concluded with collaborative study of photosynthesis in certain nitrogen fixing symbioses. Berger loved the outdoors and was dedicated to preserving the environment and to social justice, and was a wonderful friend.

Keywords C_4 plants · Chlorophyll · Prompt and delayed fluorescence · Hill reaction · Emerson enhancement effect · Nitrogen fixation · Photophosphorylation · Photosystem I · Photosystem II

Darrell Fleischman was invited to contribute a Tribute to Berger C. Mayne by Govindjee, Founding Historical Corner Editor of Photosynthesis Research. Several others joined Fleischman in honoring Berger Mayne.

D. Fleischman (✉)
Department of Biochemistry and Molecular Biology, Wright State University, Dayton, OH 45435-0001, USA
e-mail: darrell.fleischman@wright.edu

G. E. Edwards
School of Biological Sciences, Washington State University, Pullman, WA 99164-4236, USA
e-mail: edwardsg@wsu.edu

Govindjee
Department of Plant Biology, University of Illinois at Urbana–Champaign, Urbana, IL 61801, USA
e-mail: gov@illinois.edu

L. Mayne
Department of Biochemistry and Biophysics, E. R. Johnson Foundation, University of Pennsylvania, Philadelphia, PA 19104, USA
e-mail: mayne@mail.med.upenn.edu

V. Tyagi
G. E. Healthcare Life Sciences, Head Office, 800 Centennial Ave., Piscataway, NJ 08854, USA
e-mail: tyagi.vijai@gmail.com

K. Jacobsen-Mispagel
University of Georgia College of Veterinary Medicine, Athens, GA, USA
e-mail: Kljvet@gmail.com

Early life

Berger Mayne was born on July 10, 1920, in the small settlement of Towner, in eastern Colorado, USA. His love of nature found expression in hunting and fishing, and sometimes even in adopting local wildlife. During World War II, he served at an army hospital in Hawaii. In 1947, Berger graduated from Western State College in Gunnison, Colorado, with an A. B. degree in Biology. A formative experience occurred while he was dissecting a shark during a biology laboratory, when he accidentally dragged his necktie through a puddle of blood. Subsequently, he only wore bow ties (Fig. 1).

Berger attended graduate school at the University of Utah, and received his Ph.D. in Experimental Biology in 1958. Working with John Spikes and Rufus Lumry, he examined the relationship between chlorophyll *a* fluorescence yield and Hill reaction velocities in chloroplasts and the green alga *Chlorella* (Mayne 1958; Lumry et al. 1959; Spikes and Mayne 1960). Throughout his career, Berger would continue to use his expertise with chlorophyll fluorescence measurement to address a diversity of questions.

Fig. 1 Berger C. Mayne (undated; wearing a bow-tie); photo provided by Leland Mayne (see text)

While in graduate school, he met another graduate student, Yolande (Yolie) Carter; they were married in 1956. Their first son, Leland (a coauthor of this tribute), was born in Salt Lake City in 1958. Yolie shared Berger's love of camping, hiking and skiing, and they passed that enthusiasm on to their children and grandchildren, and even to foreign visitors to the Charles F. Kettering Laboratory at Yellow Springs, Ohio, where Berger was to spend the majority of his career.

After graduate school

The Maynes then moved to the University of Minnesota, where Berger took up a position as Research Associate. A second son, Walter, was born in 1959. At Minnesota, Berger continued fluorescence studies, and began using a recording mass spectrometer to measure oxygen exchange during photophosphorylation, and he demonstrated the simultaneous production and consumption of oxygen during photosynthesis (Nakamoto et al. 1960; Krall et al. 1961). By using labeled oxygen, the Minnesota group was able to clarify an anomalous stimulation of photophosphorylation by CO_2 (Ables et al. 1961). Berger Mayne and Alan Brown collaborated in a study of the enhancement of the Hill reaction in far red light by light of shorter wavelengths (the second Emerson effect) (Mayne and Brown 1963). See further discussion on this topic by one of us (Govindjee) under "On the two-light effect (the Emerson enhancement effect)".

In 1962, Berger joined Roderick Clayton's group at the Charles F. Kettering Research Laboratory, Yellow Springs, Ohio, as a Senior Postdoctoral Fellow. Under the guidance of Eugene Kettering, the C. F. Kettering Foundation had decided to build a strong photosynthesis group at the laboratory, and in 1961 appointed Leo P. Vernon its Director. Vernon chose Rod Clayton (1922–2011) to lead biophysics research, and Berger was selected to participate in that program. He was to remain on the staff until shortly after the Laboratory was transferred to the Battelle Memorial Institute in 1984 (Vernon 2003). Berger was on the editorial board of Plant Physiology (1983). His final publication from the Kettering Laboratory was a review chapter in which he summarized the basic processes of photosynthesis and nitrogen fixation and speculated about how they might be coupled (Mayne 1984).

The Kettering Foundation gave the Laboratory to the Battelle Memorial Institute, which closed it a few years later. Berger and Yolie both entered the Peace Corps and served in Liberia. Afterward, they continued to participate in outdoor activities and promoted environmental causes. Yolie preceded Berger in death in 2005. Berger's death in November 2011 was caused by a head injury during a bicycle accident. He was 91, and scarcely slowing down. He had attended a photosynthesis seminar at Wright State University only a few weeks earlier.

Berger Mayne's initial research at the Kettering laboratory

Berger worked on photosynthetic bacteria and showed that quenching of bacteriochlorophyll fluorescence of the anoxygenic photosynthetic bacteria by light was an oxidative effect. He found that when the bacteria contained colored carotenoids, they were protected from fluorescence quenching by far red light (Mayne 1965). At this time, the idea that a pigment, P700, discovered by Bessel Kok (Kok 1956, 1957), might be the reaction center of Photosystem I (PSI) in plants was being discussed. Following earlier studies with bacteria by Clayton, Berger and Dan Rubinstein demonstrated that light-induced P700 bleaching was approximately half reversible in cyanobacteria at liquid nitrogen temperature (for a detailed discussion on P700, see Ke 2001). These experiments supported the idea that, analogous to "P870" in photosynthetic bacteria, P700 might be the primary electron donor of PSI (Mayne and Rubinstein 1966).

Connection between delayed light emission (delayed fluorescence) and the chemiosmotic hypothesis (by Darrell Fleischman)

Berger Mayne and Rod Clayton began a detailed study of delayed fluorescence (DF), or delayed light emission (DLE) in chloroplasts (for a review on DLE, see Govindjee and Jursinic 1979). Mayne and Clayton (1967) examined the effects of a variety of electron transport and phosphorylation inhibitors and phosphorylation uncouplers on DLE and found that, under a variety of conditions, the

intensity of DLE mirrored the predicted magnitude of the so-called high-energy phosphorylation intermediate. DLE increased when Hill reaction electron acceptors were added, and was inhibited by PSII inhibitors such as DCMU [3-(3,4-dichlorophenyl) 1,1 dimethylurea] and by phosphorylation uncouplers. DLE was also inhibited by phosphorylation cofactors (which would consume the intermediate during ATP formation), but the intensity was restored by "energy transfer inhibitors" such as phlorizin.

At about this time, Jagendorf and Uribe (1966) reported that chloroplasts could form ATP without illumination if they were incubated briefly in a low pH medium (acid) followed by quick addition of a base. The acid–base transition was believed to have created a proton concentration difference across the thylakoid membrane. This "proton gradient" would be the concentration part of the protonmotive force (pmf) postulated to be the "high energy intermediate" in Peter Mitchell's chemiosmotic hypothesis (Mitchell 1961). Mayne and Clayton (1967) reasoned that if the high energy intermediate were the precursor of delayed fluorescence, and if it could be generated by an acid–base transition, it should be possible to produce light emission by an acid–base transition—in effect a reversal of the light-driven formation of the proton gradient. They subjected chloroplasts to a similar acid–base transition in front of a photomultiplier, and found that a burst of light was indeed emitted when the base was injected (Mayne 1966; Mayne and Clayton 1966). It was soon realized that a pH difference of the magnitude generated by the acid–base transition would be inadequate to create excited singlet states of chlorophyll. Mayne (1968, 1969) then demonstrated that pre-illumination was essential for acid–base luminescence. An electron had to be placed in a low potential acceptor before the generation of the proton gradient. It was now possible to vary the conditions—temperature, delay between illumination and base injection—in order to obtain new information about the coupling between light absorption, electron transport and phosphorylation, and about the stability of the high energy intermediate. Such experiments contributed to the acceptance of the chemiosmotic hypothesis.

Mayne then explored the possibility of inducing luminescence by other chemical treatments, and found that injection of salts, hydrosulfite, benzoate or benzoic acid would also induce light emission. (Also see Mar et al. 1974 for effects of benzoate and chloride ions.) When the chloroplasts were preilluminated with a series of short flashes, Berger found that the intensity of salt- or benzoate-induced luminescence displayed a flash number dependence, as had been found for oxygen evolution and delayed fluorescence. Mayne and Hobbs first presented the results of this research in 1971 at a conference (see Hardt and Malkin 1973; Fleischman and Mayne 1973). These observations provided information on the S-state (of the Oxygen Evolving Complex, OEC) that was the probable precursor of the chemically induced luminescence. Goltsev et al. (2009) have reviewed the current ideas about the relation of delayed fluorescence to the redox states of the chloroplast donors and acceptors.

During this time, and for years afterward, I shared a laboratory with Berger. We had an ideal relationship. We rarely collaborated in the strict sense, but we worked on parallel projects. While Berger was discovering the effect of uncouplers on chloroplast DLE, I was finding parallel effects on the light-induced red shift of the carotenoid absorption bands in photosynthetic bacteria. Rod Clayton suggested that I do similar studies with delayed fluorescence in the bacteria. For the next few years, we performed similar experiments with delayed fluorescence and chemically and physically induced luminescence. Since Berger usually studied chloroplasts and I studied bacteria, we freely exchanged ideas and helped each other (he most frequently helping me) without feeling that we were stealing ideas or competing. It was an ideal synergism. When we weren't working, he would sometimes take me on skiing or hunting trips—and tease me incessantly.

Berger and Yolie were wonderful hosts for visitors to the laboratory and for students who were working there, inviting them to great meals and even taking them skiing and fishing. Many of them remained lifelong friends.

On the two-light effect (the Emerson enhancement effect): putting Berger's work in context of our work; our paths crossed (by Govindjee)

In 1957, Robert Emerson and co-workers discovered that, in several types of algae and in a cyanobacterium, the rate of photosynthesis, as measured by oxygen evolution, was higher in two beams of light given together (one being in the far-red end of the spectrum, >700 nm, and the other in the short wavelength region), than the sum of the rates in the two beams of light given separately (see a perspective in Govindjee and Björn 2012). This two-light effect was the precursor of the concept of the two-light reaction two-pigment system hypothesis. The problem was that the methods used (manometry) could not distinguish between effects of light on respiration (oxygen uptake) and photosynthesis (oxygen evolution). Thus, mass spectroscopy was the only way to know the truth.

Our research path and that of Berger crossed here: Using the green alga *Chlorella*, Mayne and Brown (1963), and Govindjee et al. (1963) showed that the Emerson enhancement effect was in photosynthetic oxygen evolution in spite of the effect of light on respiration. Another method to check if the two-light effect was in photosynthesis or respiration was to examine this effect in the Hill

reaction, where no respiration occurred. Rajni Govindjee et al. (1960) showed clearly the existence of the two-light effect in the quinone-Hill reaction in *Chlorella* cells. However, Mayne and Brown (1963) could not confirm it; in addition, they did not find a two-light effect in the ferricyanide Hill reaction in chloroplasts, and, thus concluded that ferricyanide and quinone Hill reactions require only a one light reaction. Govindjee and Bazzaz (1967) were able to reconcile the apparently different results by showing that, depending upon the experimental conditions, ferricyanide can accept electrons from PSII (one light reaction) or from PSI (two light reactions). A similar situation must exist for the quinone Hill reaction, although it is well established that the $NADP^+$-Hill reaction has the two-light effect.

Berger was a humble and peaceful person. He was also very quiet. We know this from several encounters with Berger, including my one visit to his home in Yellow Springs for lunch. One incident that I recall well is the following. At a major conference (International Botanical Congress) in Seattle, Washington, in the 1960 s, Daniel Arnon gave a major plenary lecture where he declared that the $NADP^+$-Hill reaction does not have a two-light effect. When I raised my hand and said that we (my wife Rajni and I) have clearly shown such an effect in collaboration with George Hoch (R. Govindjee et al. 1962, 1964), Daniel Arnon put me down by saying, "You must be using wrong experimental conditions." I turned to Berger and asked what he thought. He said I see two-light effects all the time in the $NADP^+$-Hill reaction. I requested him to stand up and say that. He said "Govindjee, relax; it is not worth arguing in public; the truth will come out." *He was quiet and peaceful, and he was right.*

In about 1969, Berger began a series of studies of differences in spectral and electron transport characteristics of mesophyll and bundle sheath cells isolated from plants that fix carbon via the 4-carbon dicarboxylic acid cycle, initially in collaboration with Clanton Black and Gerry Edwards. As you will see below, my path crossed, although tangentially, his once more.

On the photochemical differences in mesophyll and bundle sheath cells of C_4 plants (by Gerry Edwards)

Early in the studies on C_4 plants, Berger Mayne made significant contributions to the understanding of photochemistry in the two photosynthetic cell types, mesophyll and bundle sheath, which are required for the functioning of C_4 plants; see Raghavendra and Sage (2011) for a book on C_4 photosynthesis. In the 1960s, biochemist Clanton Black (1931–2011; see a minireview by Black and Osmond 2005) and an agronomist Harold Brown at the University of Georgia had an interest in knowing differences in the efficiency of photosynthesis in crops and weedy species. They published a paper in *Weed Science* on the competitive ability of plants with respect to photosynthesis, based on reported differences in physiological features and emerging information on plants having a C_4 cycle (Black et al. 1969). Clanton Black then teamed up with Berger at the Charles F. Kettering Research Laboratory (see Vernon 2003, for the history and the people and their research in this Lab). They published a paper in *Plant Physiology* in 1970 showing that leaves of several C_4 species have a higher ratio of the reaction center of PSI (P700) to chlorophyll (Chl), and a higher Chl *a*/*b* ratio, than the C_3 species (Black and Mayne 1970). They suggested that cyclic photophosphorylation should be quite active to support the high photosynthetic capacity of C_4 plants, and to meet the additional ATP requirement in C_4 photosynthesis.

During postdoctoral studies with Clanton Black, I developed a method to mechanically separate intact mesophyll cells from bundle sheath cells of the weedy species *Digitaria sanguinalis*, and joined Berger to also characterize photochemical features of these chloroplasts (Mayne et al. 1971a); this work was also presented in the memorable Symposium on *Photosynthesis and Photorespiration* at the Australian National University, Canberra, Australia, in 1970 (Mayne et al. 1971b). Taking Berger's lead, Bazzaz and Govindjee (1973) extended this work by studying several photochemical and spectral properties of maize (*Zea mays*) bundle sheath and mesophyll chloroplasts, focusing on the different spectral forms of Chl and their orientation, differences in variable to constant Chl fluorescence, and in the yield of Chl fluorescence. Bundle-sheath chloroplasts contained, relative to short wavelength absorbing Chl *a* forms, more long wavelength Chl *a* forms (Chl *a* 693 and Chl *a* 705) and less Chl *b*. Although the entire electron transport chain was present in both types of chloroplasts, there were other differences confirming Mayne's excellent work.

At the University of Wisconsin, Ryuzi Kanai and I developed an enzymatic procedure to separate mesophyll protoplasts from bundle sheath strands of different types of C_4 grasses. With these preparations, Berger defined the differences in the relative levels of PSI and PSII between the mesophyll and the bundle sheath cells of the three known biochemical types of C_4 plants which operate different C_4 cycles, utilizing different C_4 decarboxylases: (1) NADP-malic enzyme (NADP-ME); (2) NAD-malic enzyme (NAD-ME); and (3) phosphoenolpyruvate carboxykinase (PEP-CK) type (Mayne et al. 1974); also see Edwards and Walker (1983). This work included analysis of the two types of chloroplasts by absorption spectra and fluorescence emission spectra at liquid nitrogen temperature (77 K),

delayed light emission (delayed fluorescence), reversible light-induced absorption changes in P700, total P700/chlorophyll, and Chl *a*/*b* ratios. Berger showed that bundle sheath chloroplasts in NADP-ME type C_4 grasses are deficient in PSII, and enriched in P700 content. However, the degree of PSII deficiency in bundle sheath chloroplasts was species dependent (which subsequently has been correlated with the degree of grana development and occurrence of phosphoenolpyruvate carboxykinase (PEP-CK) as a secondary decarboxylase). Berger's evidence supporting enriched PSI content in bundle sheath chloroplasts, and enriched PSII and linear electron transport in mesophyll chloroplasts in NADP-malic enzyme (NADP-ME) type C_4 species, and the reverse partitioning in NAD-malic enzyme (NAD-ME) type C_4 plants, provided information on how the energy requirements in these different systems are met. Results supported a malate-C_4 cycle in NADP-ME type plants with cyclic reaction in PSI supporting high ATP requirement in bundle sheath chloroplasts, and an aspartate-C_4 cycle in NAD-ME types with cyclic photophosphorylation supporting the high ATP requirement in mesophyll chloroplasts. A summary of this work was presented in a symposium organized at the University of Wisconsin in 1975 by Bob Burris and Clanton Black; this symposium included many leading scientists in the field who shared emerging insights on the mechanisms of C_4, CAM, and photorespiration (Edwards et al. 1976). Berger's research on relative levels of PSI and PSII in mesophyll versus bundle sheath chloroplasts was important towards understanding how the photochemical provision of energy (ATP and NADPH) is coordinated with the reactions of carbon assimilation in different types of C_4 species, and is now a part of established textbook illustrations of C_4 photosynthesis. During this research with Berger Mayne in the 1970s, I was able to visit him several times at the Kettering lab, and have fond memories of my interactions with him and of Berger and Yolie's gracious hospitality (especially the time I visited with my wife and our newborn son).

From September 1974 until August 1975, Berger was a Visiting Lecturer in the Botany Department at the University of Georgia. There, he conducted further studies on the pathways of carbon fixation in C_4 plants in collaboration with the group led by Clanton Black. They examined the relationship of plant metabolism to leaf and cell morphology (Black et al. 1975), biochemical components of the CO_2 compensation point of higher plants (Kestler et al. 1975) and presented evidence that showed that the major photosynthetic CO_2 assimilation pathway is C_4 in *Panicum* species, with some species having characteristics intermediate between those of C_3 and C_4 plants (Goldstein et al. 1976). While at the University of Georgia, Mayne taught a plant physiology course, assisted in advising undergraduate and graduate students, and hunted quail with Clanton Black.

Berger Mayne collaborates with Gerald Peters on a symbiotic relationship

After Eugene Kettering's death in 1969, The Kettering Foundation decided to discontinue photosynthesis research at the Laboratory and emphasize nitrogen fixation. The Kettering laboratory was chosen to participate in the Indo-US Program in Science and Technology Cooperation, administered by the United States Agency for International Development (USAID). Workers at the Laboratory would collaborate with Indian scientists in the development of biological nitrogen fertilizers (green manures) to circumvent the use of expensive and polluting chemical nitrogen fertilizers. Berger collaborated with Gerald Peters and his group on studies pertaining to photosynthesis in the *Azolla-Anabaena azollae* symbiosis. (*Azolla* is an aquatic fern that carries the heterocystous cyanobacterium *Anabaena azollae* in leaf cavities.) It had been used as a green manure in rice fields in North Korea and Thailand (Moore 1969). The Kettering studies encompassed photochemical activities of PSI and PSII, P700 content and delayed fluorescence in the fern and the endophytic cyanobacterium (Peters and Mayne 1974a, b; Ray et al. 1978; Peters et al. 1979, 1980) as well as characterization of the endophyte's phycobiliproteins (Tyagi et al. 1980, 1981). The pathways of carbon dioxide fixation in the fern and endophyte were also elucidated using pulse-chase studies (Ray et al. 1979).

Recollections of my time with Berger Mayne (by Vijai Tyagi)

I went to the Kettering Lab (1978–1980) to work with Jerry Peters and Berger Mayne on their project on the growth of the nitrogen fixing *Azolla*. I was supposed to work on the *Azolla* project; however, my interest shifted towards study of the very bright proteins, the biliproteins in the endophyte cyanobacterium *Anabaena,* a project funded by another of Peters' grants. Jerry, Berger and Bill Evans (Peters et al. 1980) had shown previously that *Anabaena* was the nitrogen fixing organism living in the cavities inside *Azolla* leaves. We purified the phycocyanin and phycoerythrin from this endobacterium, which was a first for this species. The fact that these proteins harvested energy for use in the nitrogen fixation process was suspected, but was not quite confirmed. This became my project and I devoted more than a year to it. Berger introduced me to the characterization of these proteins using fluorescence spectroscopy. The very first emission spectra of the phycocyanin that I ever made were in Berger's lab. I was quite intrigued with the plots, but it took me some time to figure out what was going on. However, Berger was always ready to help me understand by

explaining things in his very clear, but short, sentences. This work was published in Archives of Microbiology (Tyagi et al. 1980), accepted without any criticism from the editors or the referees. The overlap of the excitation spectra of the cyanin biliproteins with the emission spectra of phycoerythrins convinced us that these proteins do the same job in harvesting light inside the *Azolla* plant as they do in those species that are 'free-living' (not symbiotic). By this time, our work was getting rather interesting. The next thing we did was to show that the energy harvested by these proteins was actually used in the nitrogen fixation reaction. This was done by showing that the action spectra of the nitrogenase reaction and the absorption spectra of these proteins had quite a significant overlap. While this was indirect evidence, nonetheless it was convincing, and was published in *Plant Physiology* (Tyagi et al. 1981). Berger was always guiding me through his insightful comments, as were Jerry Peters and Bill Evans.

I could tell Berger was an outdoor person at heart because he was one of us who completed a 5 K "fun run" in the summer of 1979. I believe Darrell Fleischman was in it as well, as were Marvin Lamborg and Bill Evans. When the run was over, tired as we were, we all sat under the shade of a tree on the northeast side of the Kettering Laboratory with cans of cold beer and soda (see Fig. 2).

The time I spent at Kettering was a very exciting time in my life. I had just landed in a new country, all the way from India, and was learning new things all the time. I have never again felt that kind of excitement. Berger was an unforgettable part in it; he will live in my memory. My wife and I have two boys who are now grown, and the older one remembers Berger quite well, since Berger invited us all to parties at his house. Once, we borrowed his canoe for a trip on the Little Miami River and almost had an accident. Berger had forewarned us to watch out for fallen trees in the river and forced us to wear life jackets. As it turned out, the life jackets he gave us were of great help when our canoe did actually hit a fallen tree in the river. I live in Indianapolis now, but had lived for 25 years in Urbana (until 2009) where I came to be friends with Govindjee, one of the coauthors of this Tribute.

William (Bill) Outlaw recalls his collaboration with Berger on guard cell photochemistry

It had been thought that guard cells lack PSII on the basis of their morphology and the absence of a critical Calvin-Benson cycle enzyme. In collaboration with William Outlaw and others, Berger Mayne used measurements of delayed and prompt fluorescence and P700 content to demonstrate that both photosystems are present there (Outlaw et al. 1981). (Also see Ogawa et al. 1982 for a fluorescence study on guard cells.) They postulated that the photosystems are present not to fix carbon, but as light sensors which cause stomata to remain open in the light.

Bill Outlaw notes: "At the time of our work, some studies indicated that guard cells lacked PSII. Chloroplast structure (lack of large granum stacks) was taken as supportive (though the areas of membrane appression were extensive). Anyhow, Berger was set up to make the requisite measurements and I had developed a means of isolating relatively large quantities of guard-cell protoplasts. So, the "fit" was natural, and was facilitated by Clanton Black, a mutual friend. Berger opened his home to me and I took residence in an upstairs room that had been his son's bedroom. Berger was gracious beyond need and I came and went as I pleased. I am a morning person and walked to the lab before the crack of dawn and would have the preps ready when Berger arrived. It really was an ideal and economical means of quickly establishing that guard cells have PS II."

Later, William Outlaw set up a sensitive microscope fluorometer and by the use of chlorophyll fluorescence induction kinetics confirmed that guard cells have PSII, i.e., guard cells that had not been protoplasted. He contacted Eduardo Zeiger with his results and it turned out he had also worked on the same problem. He requested the Editor Martin Gibbs (1922–2006) to hold up their paper and publish it back to back with Eduardo's (which was submitted after theirs), which he did. They were published in the January 1981 issue (Outlaw et al. 1981; Zeiger et al. 1981). Somehow, the offprints of Zeiger's were misdated to 1980, so one might read that Berger and Outlaw confirmed Zeiger's findings. Odd how things work out! Of course, the journal itself was correct." Berger also applied his expertise in the use of light emission and absorption techniques to help other workers at the Kettering Laboratory characterize the photosystems in subchloroplast particles (see Vernon et al. 1971; Mohanty et al. 1977).

Eulogy by Karen Jacobsen-Mispagel

The following is a perfectly evocative description of Berger from a eulogy presented at Berger's memorial service by Karen Jacobsen-Mispagel, who worked at the Kettering Laboratory after graduating from Antioch College.

Karen first met Berger Mayne over 39 years ago (in 1973). After graduating from Antioch College, she worked at the Charles Kettering Lab in Yellow Springs for Darrell Fleischman for a year before going on to veterinary school in Georgia. She wrote:

My first memories of Berger:

- At the Kettering Lab: teeth clattering as Berger came down the hallway to the lab he shared with Darrell Fleischman.
- Chili suppers at Berger and Yolie's house on Marshall Street, where it always seemed that they took quiet delight

at serving chili so hot it made your eyes water– chili that Burger &Yolie didn't seem to think was hot at all.
- Berger making pancakes for breakfast, with blueberry syrup. Whenever I would come by to visit, on my way to or from Georgia or Michigan (where I later went to graduate school), it was predictable we would have pancakes for breakfast.
- Quail suppers at the Marshall Street house: where you were warned you may have to pick the pellets out of the birds as you ate.

The importance of family & friends:

- Berger and Yolie always had a way of keeping in touch with people they considered "special." Not sure why but I was fortunate to be one of those people.
- If our yearly family Christmas letter was late (as it often was), we would get a phone call, usually from Berger, in January or so, to say "just checking up on you."
- Berger & Yolie "never missed a wedding or a funeral." I know how much it meant to me 30 years ago for Berger and Yolie to come up to Michigan to celebrate my marriage to Michael Mispagel.

Quietly living by example:

- Berger had an unassuming manner. He was always thinking & analyzing the world around him, setting an example for the rest of us –

Berger, the Environmentalist:

- *Quotes from Berger*:
- "I don't need any more light. I can see alright with just this skylight."
- "If its cold, put a sweater on – we don't need to turn the heat up."
- "I don't know why people think they have to shop at big chain stores instead of shopping locally."
- Berger and Yolie always drove a Ford, when the rest of us were switching to Toyotas.
- Part of the ritual at the Mayne house was setting the table and putting out the napkin rings.
- Always cloth napkins at the Mayne house. Why waste trees by using paper?

Berger was an outdoorsman:

- He loved camping, canoeing, cycling, and quail hunting
- For Berger, dogs were for hunting. His dogs lived outside or in the garage. They were not the "family members", like they are for many of the rest of us.

A story I recall:

One time Berger had 2 hunting dogs (hounds) that Clanton Black, Berger's fellow hunting buddy, had decided he wanted down in Georgia. Since I was driving that way, Berger arranged for me to take these 2 hunting hounds in my little Toyota from Yellow Springs, Ohio to Athens, Georgia. Now, I was a vet student at the time, so one would think that would be no problem….but by the time I got to Georgia with these 2 unruly, smelly, barking, non-house-trained, hunting dogs, I was not a happy camper. So, Clanton, never one to let a favor go unrewarded, paid me handsomely for my work with a gallon of hand-picked blueberries from his bushes.

A role model for the rest of us:

Berger still rode 15+ miles a day on his bicycle at age 91 years young!

A story from *The Okefenokee Swamp Trip in April, 2007*:

- Berger had always wanted to go back to the Okefenokee Swamp, where he and his boys had canoed years earlier. So, we planned a joint family camping/canoeing trip. My husband, Michael, our son, Ben & I, Berger's son, Leland, daughter-in-law, Lynn, and grandkids, Peter, & Eleanor went with Berger to the Okefenokee Swamp in April, 2007. Now, we were in South Georgia, in a spot bordering Florida. But it was unseasonably COLD, COLD, COLD! We woke up in our tents to 26°F, wind blowing, and whistling around us. Berger at this time was 87, almost 88. None of us younger folks wanted to rouse from our sleeping bags or tents in this blustery weather. So, here was Berger, 87 year old, at 7am, up and at the picnic table, starting the Coleman stove to make the coffee! You know, he always did have a way of putting you in your place,….. as if to say, "You wimps!"

Importance of trying to make a difference, trying to improve the lives of others:

- The "annual reports" we received yearly from Berger & Yolie were a testimony to their active, and meaningful lives. A special treat was receiving The "Liberian Lines" while they were in the Peace Corps.

Here are some of my favorite Bergerisms:

- "Things are tough all over."
- "This thing suffers from improvement"
- "I'd like to get my hands on the engineer who designed this thing!"
- "The price of gas just isn't high enough yet, is it?"
- "Oh Drat!"

In closing, I want to share a quote from Ashley Montague, "The goal is to die young….as late as possible." Berger did that, and showed us all how.

And lastly, my mental picture of Berger:

- Standing there, peering through his glasses, with his classic white goatee and a sly smile, his hands in his pockets.

Fig. 2 Berger C. Mayne (1979; photo by Steve Dunbar)

We end this tribute with a picture of Berger Mayne that many of us would want to remember him with, a jovial and thoughtful friend (see Fig. 2).

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References

Ables FB, Brown AH, Mayne BC (1961) Stimulation of the Hill reaction by carbon dioxide. Plant Physiol 36:202–207

Bazzaz MB, Govindjee (1973) Photochemical properties of mesophyll and bundle sheath chloroplasts of maize. Plant Physiol 52:257–262

Black CC, Mayne BC (1970) P_{700} activity and chlorophyll content of plants with different photosynthetic carbon dioxide fixation cycles. Plant Physiol 45:738–741

Black CC, Osmond B (2005) Crassulacean acid metabolism photosynthesis: 'working in the night shift'. In: Govindjee, Beatty JT, Gest H, Allen JF (eds) Discoveries in photosynthesis. Springer, Dordrecht, pp 881–893

Black CC, Chen TM, Brown RH (1969) Biochemical basis for plant competition. Weed Sci 17:338–344

Black CC, Goldstein LD, Ray TB, Kestler DP, Mayne BC (1975) The relationship of plant metabolism to internal leaf and cell morphology and to the efficiency of CO_2 assimilation. In: Black CC, Burris RH (eds) CO_2 metabolism and productivity of plants. University Park Press, Baltimore, pp 113–139

Edwards GE, Walker D (1983) C3, C4: mechanisms, and cellular and environmental regulation, of photosynthesis. Blackwell Scientific, Oxford

Edwards GE, Huber SC, Ku MSB, Gutierrez M, Rathnam CK, Mayne BC (1976) Variation in photochemical activities in C_4 plants in relation to CO_2 fixation. In: Burris RH, Black CC (eds) CO_2 metabolism and productivity of plants. University Park Press Baltimore, MD, pp 83–112

Fleischman DE, Mayne BC (1973) Chemiluminescence as a probe of photosynthetic mechanisms. In: Rao Sanadi D (ed) Current topics in bioenergetics, vol V. Academic Press, New York, pp 77–105

Goldstein LD, Ray TB, Kestler DP, Mayne BC, Brown RH, Black CC (1976) Biochemical characterization of Panicum species which are intermediate between C_3 and C_4 photosynthesis plants. Plant Sci Lett 6:85–90

Goltsev V, Zaharieva I, Chernev P, Strasser RJ (2009) Delayed fluorescence in photosynthesis. Photosynth Res 101:217–232

Govindjee, Bazzaz M (1967) On the Emerson enhancement effect in the ferricyanide Hill reaction in chloroplast fragments. Photochem Photobiol 6:885–894

Govindjee, Björn LO (2012) Dissecting photosynthesis: The evolution of the "Z"-scheme for thylakoid reactions, In: Itoh S, Mohanty P, Guruprasad KN (eds) Photosynthesis: overviews on recent progress & future perspective. I.K. Publishers, New Delhi, pp 1–27

Govindjee R, Govindjee, Hoch G (1962) The Emerson enhancement effect in TPN-photoreduction by spinach chloroplasts. Biochem Biophys Res Commun 9:222–225

Govindjee R, Govindjee, Hoch G (1964) Emerson enhancement effect in chloroplast reactions. Plant Physiol 39:10–14

Govindjee, Jursinic PA (1979) Photosynthesis and fast changes in light emission by green plants. In: Smith KC (ed) Photochemical and Photobiological Reviews, vol 4. The Plenum Press, NY, pp 125–205

Govindjee R, Thomas JB, Rabinowitch E (1960) Second Emerson effect in the Hill reaction of Chlorella cells with quinone as oxidant. Science 132:421

Govindjee, Owens OvH, Hoch G (1963) A mass-spectroscopic study of the Emerson enhancement effect. Biochim Biophys Acta 75: 281–284

Hardt H, Malkin S (1973) Oscillations of the triggered luminescences of isolated chloroplasts preilluminated by short flashes. Photochem Photobiol 17:433–440

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

Ke B (2001) Photosynthesis, photobiochemistry and photobiophysics. Advances in photosynthesis, vol 10 (series ed. Govindjee) Kluwer Academic Publishers, Dordrecht

Kestler DP, Mayne BC, Ray TB, Goldstein LD, Brown RB, Black CC (1975) Biochemical components of the photosynthetic CO_2 compensation point of higher plants. Biochem Biophys Res Commun 66:1439–1448

Kok B (1956) On the reversible absorption change at 705 nm in photosynthetic organisms. Biochim Biophys Acta 22:399–401

Kok B (1957) Absorption changes induced by the photochemical reaction of photosynthesis. Nature 179:583–584

Krall AR, Good NE, Mayne BC (1961) Cyclic and noncyclic photophosphorylation in chloroplasts distinguished by use of labeled oxygen. Plant Physiol 36:44–47

Lumry R, Mayne B, Spikes JD (1959) Fluorescence yield against velocity relationships in the Hill reaction of chloroplast fragments. Discussions, Faraday Society 27:149–160

Mar T, Roy G, Govindjee (1974) Effect of chloride and benzoate anions on the delayed light emission in DCMU-treated spinach chloroplasts. Photochem Photobiol 20:501–504

Mayne BC (1958) The fluorescence of chloroplasts and Chlorella in relation to their photochemical activity (Doctoral thesis, University of Utah, Salt Lake City, Utah)

Mayne BC (1965) The formation of a quencher of the fluorescence of chromatophores from photosynthetic bacteria. Biochim Biophys Acta 109:59–66

Mayne BC (1966) Chemiluminescence of chloroplasts. Brookhaven Symp Biol 19:460–466

Mayne BC (1968) The light requirement of acid–base transition induced luminescence of chloroplasts. Photochem Photobiol 8:107–113

Mayne BC (1969) The light requirement for the chemiluminescence of chloroplasts. In: Metzner H (ed) Progress in photosynthesis research, vol II, pp 947–951

Mayne BC (1984) Photosynthesis and the biochemistry of nitrogen fixation. In: Alexander M (ed) Nitrogen fixation and its ecological basis. Plenum Publishing Corporation, pp 225–242

Mayne BC, Brown AH (1963) A comparison of the Emerson two light effect in photosynthesis and the Hill Reaction. In: Ashida J (ed) Microalgae and photosynthetic bacteria and the Japanese society of plant physiologists. The University of Tokyo Press, Tokyo

Mayne BC, Clayton RK (1966) Luminescence of chlorophyll in spinach chloroplasts induced by an acid-base transition. Proc Natl Acad Sci USA 56:494–499

Mayne BC, Clayton RK (1967) The effect of inhibitors and uncouplers of photosynthetic phosphorylation on delayed light emission of chloroplasts. Photochem Photobiol 6:3–8

Mayne BC, Rubinstein D (1966) Absorption changes in blue-green algae at the temperature of liquid nitrogen. Nature 210:734–735

Mayne BC, Edwards GE, Black CC (1971a) Spectral, physical, and electron transport activities in the photosynthetic apparatus of mesophyll cells and bundle sheath cells of *Digitaria sanguinalis* (L). Scop. Plant Physiol 47:600–605

Mayne BC, Edwards GE, Black CC (1971b) Light reactions in C_4 photosynthesis. In: Hatch MD, Osmond CB, Slatyer RO (eds) Photosynthesis and photorespiration. Wiley, New York, pp 361–371

Mayne BC, Dee AM, Edwards GE (1974) Photosynthesis in mesophyll protoplasts and bundle sheath cells of various type of C_4 plants. III. Fluorescence emission spectra, delayed light emission, and P700 content. Z Pflanzenphysiol 74:275–291

Mitchell P (1961) Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. Nature 191:144–148

Mohanty P, Mayne BC, Ke B (1977) Further characterization of a photosystem II particle isolated from spinach chloroplasts by triton treatment. Biochim Biophys Acta 545:285–295

Moore AW (1969) *Azolla*: biology and agronomic significance. Bot Rev 35:17–34

Nakamoto T, Krogman D, Mayne BC (1960) Oxygen exchange catalyzed by phosphorylating chloroplasts. J Biol Chem 235: 1843–1845

Ogawa T, Grantz D, Boyer J, Govindjee (1982) Effects of cations and abscisic acid on chlorophyll *a* fluorescence in guard cells of *Vicia faba*. Plant Physiol 69:1140–1144

Outlaw WH Jr, Mayne BC, Zenger VE, Manchester J (1981) Presence of both photosystems in guard cells of *Vicia faba* L. implications for environmental signal processing. Plant Physiol 67:12–16

Peters GA, Mayne BC (1974a) The *Azolla, Anabaena azollae* relationship: I. Initial characterization of the association. Plant Physiol 53:813–819

Peters GA, Mayne BC (1974b) *Azolla, Anabaena azollae* relationship. II. Localization of nitrogenase activity as assayed by acetylene reduction. Plant Physiol 53:820–824

Peters GA, Mayne BC, Ray TB, Toia RE Jr (1979) Physiology and biochemistry of *Azolla Anabaena* symbiosis. In: Nitrogen and rice. International Rice Research Institute, Los Banos

Peters GA, Toia RE Jr, Evans WR, Crist DK, Mayne BC, Poole RE (1980) Characterization and comparisons of N_2-fixing *Azolla Anabaena* associations. I. Optimization of growth conditions for biomass increase and N content in a controlled environment. Plant Cell Environ 3:261–269

Raghavendra AS, Sage RF (eds) (2011) C_4 photosynthesis and related CO_2 concentrating mechanisms, advances in photosynthesis and respiration, vol 32. Springer, Dordrecht

Ray TB, Peters GA, Toia RE Jr, Mayne BC (1978) *Azolla, Anabaena azollae* relationship. VII. Distribution of ammonia assimilating enzymes, protein and chlorophyll between host and symbiont. Plant Physiol 62:463–467

Ray TB, Mayne BC, Toia RE Jr, Peters GA (1979) *Azolla, Anabaena azollae* relationship. VIII. Photosynthetic characterization of the association and individual partners. Plant Physiol 64:791–795

Spikes JD, Mayne BC (1960) Photosynthesis. Ann Rev Phys Chem 11:501–530

Tyagi VVS, Mayne BC, Peters GA (1980) Purification and initial characterization of phycobiliproteins from the endophytic cyanobacterium of *Azolla*. Arch Microbiol 124:41–44

Tyagi VVS, Ray TB, Mayne BC, Peters GA (1981) The *Azolla Anabaena azollae* relationship. XI. Phycobiliproteins in the action spectrum for nitrogenase-catalyzed acetylene reduction. Plant Physiol 68:1479–1494

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

Vernon LP, Klein S, White FG, Shaw ER, Mayne BC (1971) Properties of a small photosystem2 particle obtained from spinach chloroplasts. In: Second international congress on photosynthesis, vol 1, pp 801–811

Zeiger E, Armond P, Melis A (1981) Fluorescence properties of guard cell chloroplasts. Evidence for linear electron transport and light-harvesting pigments of photosystems I and II. Plant Physiol 67:17–20