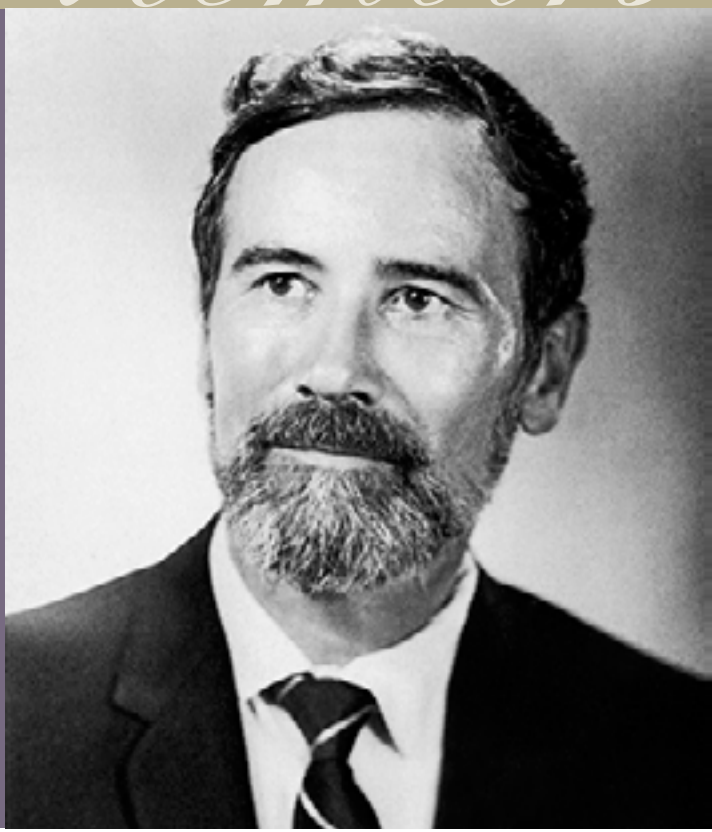
Roderick K. Clayton

1922–2011

BIOGRAPHICAL *Memoirs*



A Biographical Memoir by
Maarten J. Chrispeels
and Colin A. Wraight

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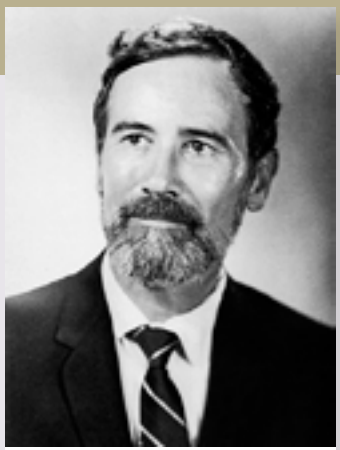
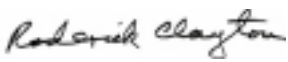
RODERICK KEENER **CLAYTON**

March 29, 1922–October 23, 2011

Elected to the NAS, 1977





By Maarten J. Chrispeels and Colin A. Wraight

During his research career at three different institutions in the United States, Roderick K. "Rod" Clayton made seminal contributions to our understanding of phototaxis—the movement of an organism stimulated by light—and the photosynthetic reaction center in bacteria. He identified P870 in the spectrum of partially purified reaction centers of *Rhodobacter (Rba) sphaeroides* as a specialized bacteriochlorophyll (BChl). Later, in 1971, he was able to purify the minimal reaction center for photosynthesis at the same time as George Feher's laboratory was doing the same thing. This allowed a detailed characterization of this important paradigm of biological energy conversion, including measurement of its very high quantum yield of charge separation.

Rod wrote two accounts of his life and his research, published in *Photosynthesis Research*. These cover his work on reaction centers (Clayton, 2002) and also provide insights into his personal life (Clayton, 1988). In addition, one of us (Colin Wraight) has written a detailed account of Rod's life and work for a 2014 issue of *Photosynthesis Research* (Wraight, 2014). This issue is dedicated to three pioneers in the field—Clayton, Feher, and Louis Duysens.

Youth and wartime service in the Air Corps

Rod was born in Tallinn, Estonia, on March 29, 1922, the son of John H. Clayton and Helena Mullerstein. His father was an American reporter working for the Hearst newspaper group. His mother, an Estonian, worked at the American embassy in Talinn at the time. She was the daughter of a Lutheran minister and had attended the University of St. Petersburg, in Russia. When the revolution broke out in 1917, she fled to Finland and eventually returned to Estonia. The Clayton family—Rod had an older brother, Dale—spent time in places all over Europe.

When Rod was six the family moved back to the States and settled near Chicago, where John Clayton worked for the *Chicago Tribune*. Rod attended the Todd Seminary for Boys until the stock market crashed and the Great Depression set in. In 1935, when he was 13, the Claytons moved to Pasadena, California, where Rod attended high school. He loved the new environment, excelled in high school, and then enrolled in courses at Pasadena Junior College, which was combined with the high school. The move to Pasadena greatly expanded his horizons, and he showed a keen interest in academics, gymnastics, and butterfly collecting.

Rod's parents moved back to Chicago, but he had his sights set on the California Institute of Technology (Cal Tech), where he enrolled and majored in chemistry. He did very well for two years, but "enticed by the beach, poker and girls," he failed his third year and was advised to take a year off to "grow up." The United States had entered World War II by that time, and Rod enlisted in the Army Air Corps in the summer of 1943. He trained at the Blytheville Army Airfield in Arkansas, was posted to Guam, and flew sorties over Japan. At Blytheville he met his future wife, Betty Jean "BJ" Compton, who remained his life's companion and steady compass until her untimely death in 1981.

Graduate study and postdoctoral research

After receiving his discharge from the army, Rod returned to Cal Tech, accompanied by BJ and a baby son, Rick, to finish his undergraduate degree with a major in physics. He entered the Cal Tech graduate school and was accepted into the laboratory of Max Delbrück. Delbrück was a renowned physicist (elected to the NAS in 1949) who had made the transition from physics to biology and had pioneered research on phage genetics and replication.

Rod's research focused on photo- and chemotaxis in the photosynthetic bacterium *Rhodospirillum (Rsp.) rubrum*, a large bacterium visible under the light microscope. He refined our understanding of the action spectrum of phototaxis and firmly established the relationship between phototaxis and photosynthesis. His work was marked by its quantitative rigor, which allowed him to develop a thorough experimental and theoretical description of the "step down" response, in which *Rsp. rubrum* reverses course several times when the light intensity is suddenly decreased. He showed that the phototactic response is highly dependent on the metabolic state of the cells, including light levels, oxygen tension, and carbon source. He finished his dissertation in 1951 and received his

degree *summa cum laude* in the presence of his young family, which now included his one-year-old daughter, Ann.

Rod spent the following year in the laboratory of Cornelis B. van Niel (Elected to the NAS in 1945) at the Hopkins Marine Station and then spent four years (1952-1956) at the U.S. Naval Postgraduate School in Monterey, California. At both places he extended some of his unpublished doctoral work on the relationship between phototaxis and metabolism. Rod had devised a way to measure photosynthesis and respiration simultaneously to monitor the competition between anaerobic (phototrophic) and aerobic metabolism and their effects on taxis. His research and extensive reading on how nerve impulses are generated led him to propose that the positive phototactic response of *Rsp. rubrum* is an example of a general excitatory system. He carried out elegant experiments to support his theoretical description of phototaxis. In Monterey he also taught physics to military officers.

Killing the catalase hypothesis

Rod applied for and received a National Science Foundation Senior Postdoctoral Fellowship to join the microbiology unit at Oxford University, headed by Professor Donald D. Woods. He proposed to study the presumed preventive role of catalase in the photooxidative killing of phototrophic bacteria, which occurs when both light and oxygen are present. He chose to work with *Rhodobacter (Rba) sphaeroides*—then called *Rhodopseudomonas spheroides*—because he had discovered that this species gave a much bigger "fizz" when hydrogen peroxide was added to the culture. He measured catalase levels and the induction of catalase, and he soon showed that, contrary to expectations, catalase did not have a protective role in photooxidative killing. Part of this work was carried out during a 6-month stay at Helge Larsen's laboratory at the Norwegian Institute of Technology in Trondheim.

Rod and his family returned to the United States in 1958, where he joined the biological division, headed by Alexander Hollaender, at the Oak Ridge National Laboratory(ORNL), in Tennessee. He started screening for mutants of *Rba. sphaeroides* that had high catalase activity and lacked colored carotenoids. When such mutants were kept in the light for several weeks they lost their BChl and became blue/pink. These mutants were highly susceptible to photooxidative killing, despite high levels of catalase. He continued to work on this topic and put the last nail in the coffin of the hydrogen peroxide hypothesis.

A peripatetic researcher and photosynthesis

Serendipity plays a big role in scientific discovery. The blue/pink mutants that lacked carotenoids proved to be important for all of Rod's subsequent work on photosynthesis. This work started at the ORNL, where he began a collaboration with William "Bill" Arnold (elected to the NAS in 1962). As an undergraduate Arnold had done one of the seminal experiments in photosynthesis in the laboratory of Robert Emerson at Cal Tech (Emerson and Arnold, 1932). The experiment demonstrated the existence of the "photosynthetic unit," a conceptual precursor of the reaction center.

Arnold now wanted to know if photosynthetic events occurred at liquid helium temperatures. Together, Rod and Arnold showed that the spectroscopic signatures of light-induced activity in chromatophores were unchanged down to 1.3 K. They interpreted their results as showing excitation followed by migration of electrons and concluded that "the first step in photosynthesis appears to be the separation of an electron and a hole in a chlorophyll semiconductor." Drawing on earlier work by Louis Duysens, Rod proposed in *Bacteriological Reviews* in 1962 the possibility of a localized site of energy trapping by charge transfer.

He showed that the major light-induced changes in chromatophore membranes were of bacteriochlorophyll (BChl) origin and were similar in different species of bacteria. Were these spectral changes caused by small changes in many molecules or a big change in a small subset of molecules of BChl? He was able to show that the changes were caused by the photooxidation of BChl and that it was a special component, similar to the P700 proposed by Bessel Kok (Kok, 1956) for oxygenic photosynthesis. Rod coined the term "photosynthetic reaction center," slightly modifying the term "reaction center" used by Duysens in his doctoral dissertation at the University of Leiden. Rod obtained excellent confirmation of his proposal by working with his carotenoid-less mutants. The blue/pink cultures had lost nearly all their BChl, leaving only the photoactive component (P870), which Rod calculated to represent 2 to 5 percent of the original amount of BChl.

In 1961 Rod moved to the Dartmouth Medical School in Hanover, New Hampshire, at the invitation of Clinton Fuller. Some star basic scientists were working there at the time. He continued his research, focusing on the use of Triton X-100 to dissolve chromatophores from his pink, carotenoid-less cultures of *Rba. sphaeroides*. A detailed spectroscopic analysis showed that P870 was a specialized BChl associated with bacteriopheophytin (BPhe).

Discord between the basic scientists and the clinicians at the medical school prompted Rod in 1962 to move for the fourth time in six years, now to the Charles F. Kettering Research Laboratory in Yellow Springs, Ohio. In his new venue he continued to use Triton X-100, which yielded (partially) purified reaction centers, and he was able to show unequivocally that the spectrum had three peaks: P800 and P870 representing BChl, and a peak at 760 nanometers (nm) representing BPhe. He thought the ratio of P800 to P870 to be 2:1—though it was later shown to be 1:1—but he correctly found the ratio of BPhe to BChl to be 1:2. The BPhe in the complex was later confirmed to be a functional component by Rod's postdoc Hon Yau (Yau, 1971). Rod also examined the relationship between fluorescence and photochemistry and confirmed the suggestion by Duysens that they were competitive. Careful analysis allowed him also to conclude that energy transfer was from the singlet state, not the triplet state.

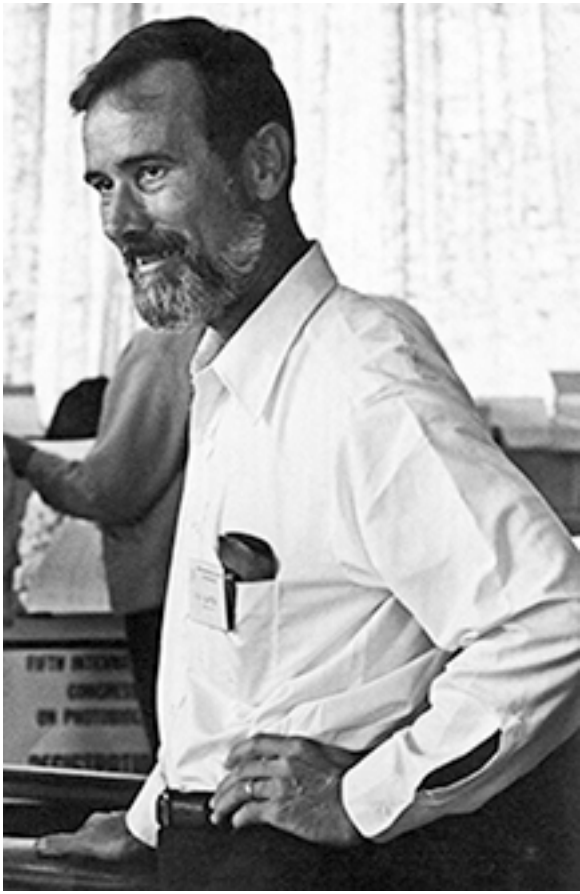
When they moved to Yellow Springs in 1962, and the children were growing up—Rick was 14 and Ann was 12 at that time—BJ had agreed to let Rod train her to work in the laboratory. She became the "lab mother," who kept everyone in check, trained them in laboratory procedures, and maintained the bacterial cultures.

A home at Cornell

Rod's work on the photosynthetic reaction center was certainly noticed by scientists at universities building larger faculties in the 1960s and '70s. In 1966 he chose to accept an offer from Cornell University in Ithaca, New York, where he received a dual appointment in the Departments of Biological Sciences and Applied Physics Although not trained as a biochemist, he focused on the purification of reaction centers. Purification of membrane proteins was in its infancy, and few researchers appreciated that different detergents would produce different results because they differed in the way they solubilized membranes.

Rod stuck with Triton X-100 until George Feher passed on a tip received from Bob Bartsch. Bartsch suggested that he should use lauryl-dimethylamine-N-oxide (LDAO) to solubilize the membranes. Using this detergent, Feher (Feher, 1971) and Rod (Clayton and Wang, 1971) published at the same time the purification of the minimal reaction center complex. They found its molecular mass to be 70,000, although the true value is 105,000.

Rod pursued efforts to measure the quantum yields of photochemical activities and fluorescence emission. The climax of this work was Ken Zankel's determination in Rod's laboratory of the absolute fluorescence yields from the reaction center, a tiny number that allowed them to calculate the rate of the primary photochemical transfer event. The value they obtained was 7 picoseconds, in close agreement with later direct measurements of 3-4 ps (Zankel, et al., 1968).



Clayton at the Second International Congress on Photosynthetic Research, Stresa, Italy, 1971. (Photography courtesy of Colin Wraight.)

Were there other things in Rod's life besides work? Well, not many. By his own admission, he let few things interfere with his absolute dedication to science. At Cornell, as elsewhere, his lab was always small, usually with no more than one or two grad students, postdocs, or visitors. He worked alone or in collaboration with BJ, who had become an accomplished research technician and lab manager in spite of her lack of formal training in science. When they moved to Yellow Springs in 1962, and the children were growing up—Rick was 14 and Ann was 12 at that time—BJ had agreed to let Rod train her to work in the laboratory. She became the "lab mother," who kept everyone in check, trained them in laboratory procedures, and maintained the bacterial cultures. Rod, himself, was potentially a serious risk taker in the lab, and BJ saw as part of her spousal role the need to keep Rod focused and on a tight rein.

Rod did enjoy skiing and butterfly collecting. Colin Wraight remembers them going skiing when he was a postdoc in Rod's lab in the early 1970s. Rod was a good skier but

certainly no expert. Nevertheless, he was intrepid and attacked the moguls with gusto and always muddled through. His other extra-curricular pursuit, as an avid butterfly collector, was more peaceful. Dan Lindsley, a *Drosophila* geneticist (elected to the Academy in 1974) who had overlapped with Rod at Cal Tech as a grad student and again at Oak Ridge, was also a keen butterfly collector and remembers going on collecting trips with an enthusiastic Rod.

Woods Hole and the photosynthetic unit

Rod was thrilled to be invited to be a guest lecturer in the physiology course at Woods Hole. He felt that this was the sign that he had "arrived," and he was offered and accepted a concurrent position there as a part-time instructor. Every summer from 1967 to 1971 he would dismantle half the electro-optical equipment in his Cornell laboratory and transport it to Woods Hole for use by the students. He loved the intellectual excitement of those summer discussions.

Robert Haselkorn (elected to the NAS in 1991) recalled:

> *Rod was one of the directors of the Physiology course in the summer of 1971. He had three assistants: BJ, Lou Sherman and myself. I had just adapted the slab gel system developed by Bill Studier for protein electrophoresis, and used it first for phage T4 proteins and later for the proteins of cyanobacteria. During that summer, Rod and BJ put together a system for partial purification of reaction centers from a number of photosynthetic bacteria. I had a quick look at the preps on an SDS slab gel and was delighted to see three major bands, which we named H, M and L for heavy, middle and light. There was a remarkable feature that characterized these three bands: they bound SDS differently from all the other polypeptides. As a result, they fluoresced after staining with Coomassie Blue, so they stood out, enough to be seen in the crudest extracts.*

The work was published in the *Journal of Molecular Biology* (Clayton and Haselkorn, 1972).

While isolating and characterizing reaction centers in his Cornell lab, Rod also worked on the photosynthetic unit, composed of a well-defined trap and associated light-harvesting antennae. The tool to study this was fluorescence emission that came from the bulk pigments but was controlled by the activity of the reaction center. The first description of energy transfer needed for the excitation of the reaction center was

provided by Duysens. Rod described prompt fluorescence and delayed light emission from the bulk pigments in a variety of species of bacteria and algae. He also embraced the idea of dead fluorescence coming from pigments that were not functionally connected to a trap.

Colin Wraight, who is one of the authors of this memoir, joined Rod's lab in 1972 after spending a year in the Duysens lab in Leiden. After discussions with Rod, Wraight decided to try to measure as accurately as possible the quantum yield of charge separation in bacterial photosynthetic reaction centers. Much previous work with various preparations had yielded variable results ranging from 0.4 to 1.0. Wraight performed the experiment and, using the newly refined extinction coefficients for reaction centers established by Sue Straley in Rod's lab, Wraight concluded that the quantum efficiency was 1.02. This confirmed the higher values obtained in previous work by Rod and by Paul Loach, and placed important constraints on the relationship between fluorescence and photochemistry.

As early as 1962, Rod had observed light-induced reduction of ubiquinone (UQ) in chromatophores in more or less stoichiometric proportion to the photooxidation of BChl. This indicated that UQ might correspond to the primary electron acceptor. In 1970 Sue Straley discovered the absorbance band at 450 nm, which we now know to be characteristic of ubisemiquinones. Just two years later several groups including Rod's published results showing that a ubisemiquinone anion radical was the reduced primary acceptor. This was later firmly established by Richard Cogdell, who arrived as a postdoc in 1973, by extracting reaction centers with dry organic solvents. The reaction centers were now inactive, and activity could be restored by adding ubiquinone back.

At the end of 1973, Rod went on sabbatical to Bill Parson's lab at the University of Washington, taking Cogdell with him. Parson was starting nanosecond kinetic studies, and their joint work led to the discovery of two short-lived intermediary states, one of which was shown to be on the path of electron transfer.

In 1975 Wraight, who had taken a job at the University of Illinois but was spending time in Les Dutton's laboratory at the Johnson Foundation because he did not yet have the necessary equipment at the U of I, discovered the characteristic binary oscillations of semiquinones in the reaction centers. André Verméglio, a new postdoc in Rod's lab, discovered the same phenomenon at the same time. They both concluded that two ubiquinones acted in series as a two-electron gate. Rod brokered an agreement that they

would publish back-to-back papers in the same issue of *Biochimica et Biophysica Acta* (Verméglio, 1977; Wraight, 1977).

The '70s also brought Rod honors, of which he said "…of course I wanted (them) and felt good when they came." In 1975 he was elected to the American Academy of Arts and Sciences and in 1977 to the U.S. National Academy of Sciences, and in 1982 he and George Feher (elected to the NAS in 1975) shared the first annual Prize in Biological Physics of the American Physics Society.

Decline, fall, and resurrection

In 1980 BJ was diagnosed with lung cancer, and in the fall of 1981 she passed away in their country house in Thetford, Vermont. Rod was devastated. His life companion and the compass that helped him chart a steady course were gone. He rapidly drifted into a downward spiral of drugs and alcohol. Rod wrote about these dark days himself, and we refer the reader to the account in his own words (Clayton 1988). His former associates, and colleagues who visited him, were dismayed by the changes in Rod's life. Abrupt events in 1984 shook him awake. Cornell University could not ignore the situation and gracefully negotiated Rod's retirement as the Liberty Hyde Bailey Professor Emeritus in the Division of Plant Biology.

Rod's slow recovery began in 1985, and in 1988 he moved to California to be closer to his two children. He continued to collect butterflies, and his sizeable collection was donated after his death to the Texas Lepidoptera Survey in Houston. He also discovered he had considerable artistic talents. He bought a gas-fired pottery kiln and started experimenting with ceramics and glazes, which he developed to great effect. In 1999 he moved to Santa Rosa, California, and became involved with a local group that trained service dogs. In addition to ceramics, he increasingly turned to drawing people and animals.

In 1988 Rod attended the Gordon Research Conference on Physical Aspects of Photosynthesis, at the Holderness School in New Hampshire, at the invitation of the organizer, Colin Wraight. The X-ray structures of the bacterial reaction centers of two species had just been published, and Rod was very excited about reconnecting with science. The participants, but especially his former coworkers, were gratified to see him resurrected, even if his scientific career had come to an end.

A full life

In spite of the traumatic events that changed the course of Rod's life immediately after BJ's death, his life's experience was a rich one. The excellence of his science was recog-

nized by his peers through the honors that accrued to him. The young people in his lab experienced him as a challenging, creative, and stimulating mentor. His scientific career ended unnecessarily early and in a messy way, but as he was recovering, he eagerly applied his creativity to new endeavors, making new friends and immersing himself in a new environment.

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