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WARREN LEE BUTLER

1925—1984

A Biographical Memoir by

ANDREW A. BENSON

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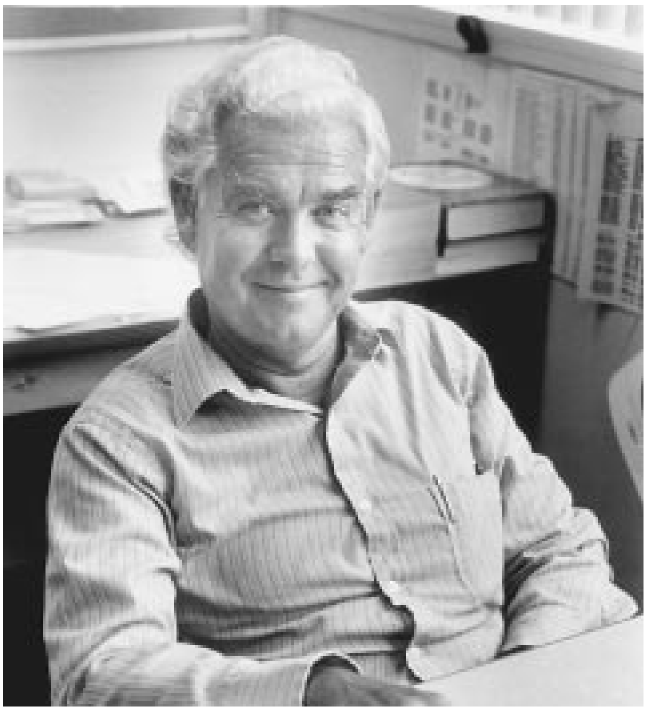

WARREN LEE BUTLER

January 28, 1925 – June 21, 1984

BY ANDREW A. BENSON

UNDERSTANDING THE MECHANISMS adopted by plants for detecting changes in day length and season was to become one of Warren Butler's crowning achievements. Last student of Nobel physicist James Franck, Butler chose to understand the photochemical adaptations and pigment systems of plants and developed important concepts and understanding of basic photometabolic processes.

Warren Butler, only son of Orval L. and Lois Jordan Butler, was born January 28, 1925, in Yakima, Washington. His mother was a daughter of a Methodist minister. The family moved to Portland, where Warren and his younger sister, Connie, graduated from high school and his father was successful in an auto parts business. Young Butler enrolled in the Army Specialized Training Corps for training in physics. During training in Texas he was conscripted into the infantry and was shipped overseas.

After one day in England, the eighteen-year-old Butler left for France before Christmas in 1944. On patrol searching for enemy soldiers said to have penetrated behind the lines over snow-covered terrain, the group of four or six men detonated a land mine. One was killed; Butler was seriously wounded. Because of inadequate medical support during the Battle of the Bulge, Butler ended up with an amputated left hand and left leg removed above the knee. Recovery in Utah and development of prostheses was time consuming. Because of his immense will power, he survived the severe wounds, determined to live, learn, and lead a normal life.

With the skill and ingenuity of an engineer, Warren went through his exceptionally productive life with a prosthetic left arm and leg. His sense of humor prevailed as he showed students he could handle hot flasks and equipment with his mechanical hand. Winslow Briggs recalls a macabre scene illuminated by a green light source in the laboratory: Butler with his arm up to the elbow in a huge dewar of liquid nitrogen, vapor swirling around him, one bushy eyebrow raised, and a Machiavellian expression on his face. In the words of Jon Singer, "As to Warren's personal boldness, again all of us who knew him have many examples to cite. His most remarkable expression of his boldness was the absolute indifference he seemed to show to his physical handicaps, and his simple refusal to let them interfere with his life and work. He scaled such obstacles in his life as would have defeated many of us."

Back in Portland, Warren entered the physics program at Reed College, where he met Lila Bowen; they were married in 1951. With a B.A. thesis entitled "An Investigation of the Use of Ultrasonics in an Optical Shutter Arrangement" in 1949, he sought graduate training in biophysics. Yale and the University of Chicago accepted him. He visited Yale, but he decided to return to Chicago, which admitted thirty-six applicants; after a year, however, only a third were accepted for the doctorate program.

Warren had not considered photosynthesis as an option until he met James Franck, and was accepted as his last graduate student. Butler often expressed admiration, warmth, affection, and great appreciation for his distinguished mentor. With his 1955 doctoral thesis ("Measurements of Photosynthetic Rates and Gas Exchange Quotients During Induction Periods") finished, he sought a position at the University of Maryland, but decided against it and accepted a position with the U.S. Department of Agriculture at Beltsville, having been attracted by Sterling Hendricks. His work with Hendricks and Borthwick continued from 1956 to 1964.

As S. J. Singer pointed out at the memorial service for Butler:

> The interactions of radiation with matter were quite well understood in the '30s and it seemed likely that Franck, fascinated by the problem of photosynthesis and Delbrück by the effect of X-rays in producing genetic mutations, found that radiation provided the means to introduce the elegance of Quantum Physics into the major problems of biology. However, biology being a complex science is more directly related to chemistry than to physics. These pioneers treated Biology as a sub-discipline of Physics. It was left for the next generation of physicists, now known as biophysicists, to take the plunge into the chemical world of the cell and the organism. This required the elegance of the physicist to be coupled to the boldness of the biological chemist, and in Warren Butler this combination of qualities shone forth.

Understanding plants' recognition of the changes in day length and season was to become one of Warren Butler's crowning achievements. The problem, long recognized by plant physiologists, came into focus at the Beltsville laboratories of the U.S. Department of Agriculture with the studies of botanist Harry A. Borthwick and physiologist Marion W. Parker, which implicated a specific on-off photopigment system controlling flowering in a soybean plant. A single flash at night could trigger the process. With the foresight of F. G. Cottrell, they were joined in 1940 by chemical physicist Sterling B. Hendricks, head of the Mineral Nutrition Laboratory, who had actually worked on USDA projects since 1922, and was determined to study the "action spectra" for the process. Resuming their strategy after the war with the first large spectral illumination culture system in which whole plants grew in light of specific wavelengths, they discovered essentiality of red light for floral initiation. Their work with the more rapid lettuce-seed germination system of Vivian and Eben H. Toole led to recognition of photoreversibility of a "red" and "far-red" form of the pigment. Contemporary science, though, did not believe that plant physiological experiments could ever reveal molecular details of their pigmentation.

Biophysicist Warren Butler joined the group in 1956 and recognized their problems in demonstrating the reality of their "pigment of the imagination." He credited Hendricks with "probably the boldest and most brilliant single stroke in the history of plant physiology."

Butler had approached Alan Mehler for a job, and Mehler called Siegelman, who talked to Karl H. Norris in the Poultry Division. Norris had been to the University of Chicago and got along very well with Butler. Though he was never an official part of the USDA plant physiology division and worked under Norris in the Poultry Division, he adapted the novel and powerful dual monochromator spectrophotometer of Norris to examine small, highly scattering plant tissue samples. His outstanding capabilities as a photophysicist led to important contributions to both theory and technology. Hendricks and Siegelman, whom he and Borthwick had recruited from the pioneering Research Laboratory, brought their samples to Butler for examination. None revealed detectable red-far red pigment.

In the middle of June 1959 the breakthrough came. In Butler's words:

> Hendricks appeared in my lab one day with several Petri dishes of dark-grown turnip seedlings . . . We removed the cotyledons and pressed them loosely into the cuvette and measured the absorption spectrum of the sample after irradiation with red and far-red light. To our amazement and delight,

mixed with skepticism, we found that the difference spectrum between the red and far-red irradiated sample was precisely that predicted for phytochrome by the physiological action spectra.

Lila recalls Warren's elation that evening, when he said, "Lila, I think we've hit on something big."

At last the Beltsville group had the stuff in a bottle and the stuff needed a name. According to Harry Borthwick, Warren Butler one day "half jokingly" suggested the term phytochrome, from the Greek words for plant and color. Upon purification, the red-absorbing form of phytochrome proved to be blue in color and the far-red absorbing form green.

Butler, Norris, Siegelman, and Hendricks published their landmark paper ("Detection, Assay, and Preliminary Purification of the Pigment Controlling Photoresponsive Development of Plants") in the December 1959 issue of *Proceedings of the National Academy of Sciences.* That paper reported: "This work supplies the three needed elements for further progress: A source of the pigment, a method of assay, and a system for separation." The paper ended with: "There would seem to be no essential barrier to finding the nature of the enzymatic action of the pigment P735, which constitutes the limited pacemaker or bottleneck of control evident in plant development, and to elaborating physiological and biochemical aspects of its action."

Butler found a simple and elegant way to carry out accurate light absorption studies on samples of living plants, despite their opacity due to light scattering. He used light as a simple non-destructive analytical tool to follow chemical events in the plant. To someone other than a physicist, the problem of making such measurements on opaque materials must appear to be intrinsically insoluble. The technique Warren Butler employed and the instrument he devised were highly successful. With agricultural engineer Karl

Norris, Butler had developed a single beam spectrophotometer to measure absorption spectra of fruit, vegetables, eggs, dry seeds, and even a two-by-four. By placing the sample directly on top of an end-on phototube, they obtained useful absorption spectra of dense light-scattering samples like intact tissues and thick homogenates. Butler published a review article on light scattering and its utility in studying biological systems. After a paper by Butler and Siegelman at an AIBS meeting at Stanford, James Bonner remarked, "It sounds like you guys have a new Erector Set."

With this single-beam methodology, Warren, together with Sterling Hendricks, was able to demonstrate the existence of a minute amount of a pigment in living etiolated turnip and maize seedlings that exhibited the predicted reversible light absorption behavior of the agents responsible for photoperiodism. These absorption characteristics provided the indispensable criteria that enabled the protein pigment to be isolated in a pure state. A more convenient dual-wavelength difference photometer was developed for assaying phytochrome. This made possible the purification and isolation of phytochrome.

The first public announcement of the detection of phytochrome was a legendary fiasco, but it led to an important discovery. Hendricks had been invited to speak at the Ninth International Botanical Congress in Montreal in August 1959. Norris had produced a simple, easily transportable photometer in which absorbance of short segments of corn seedlings could be measured following red and far-red light. The instrument had a large circular meter, easily seen by an audience. Borthwick, Hendricks, Siegelman, and Butler drove to Montreal with the instrument and several clear plastic boxes of corn seedlings in their car trunk. On several occasions when stopping for gas, someone would open the trunk to see how everything was riding.

I clearly remember helping Warren carry his box and papers from the living quarters of the university to the lecture hall. I had been totally unaware of his physical handicap and, at first, was surprised when a perfectly healthy young man asked me for help in carrying his materials. Only then did I notice that he had only one arm; later I realized he had only one leg.

Word had spread that this remarkable, even mystical pigment was finally to be demonstrated. The lecture hall was full. Hendricks talked for thirty minutes giving background material to prepare the audience for the demonstration. Butler described what the instrument was going to measure; irradiated their seedlings with red light; and obtained the first reading, setting the meter at “9 o’clock.” Then he irradiated the plants with far-red light, expecting the meter to move to 3 o’clock as regularly observed. The meter never moved. He rapidly prepared another sample, but again the meter refused to move. The audience was kind, but the failure was a complete mystery.

On their return to Beltsville, Butler and Siegelman salvaged an important lesson from that failure. They found that the far-red form of phytochrome is not stable; once generated by exposure to red light, it is slowly destroyed. Every time the trunk was opened on the way to Montreal, the red-absorbing form was converted to the far-red form, some of which then disappeared. Discovery of the instability of the far-red form was the key to the subsequent purification of the pigment. Siegelman, however, felt that the “loss of signal is still not really understood.”

The 1959 Montreal congress was the first opportunity for western scientists to meet their counterparts from the Soviet Union. An afternoon discussion group included A. A. Krasnovsky and E. V. Evstigneev, along with Hiroshi Tamiya (Japan), C. S. French, N. I. Bishop, Mary Belle Allen, M.

Gibbs, H. Gaffron, James C. Smith, A. A. Benson, and Warren Butler.

When computers arrived on the scene, Karl Norris immediately adopted the PDP-8 and helped Butler get into programming. Soon Butler recognized the mathematical simplicity of plotting the fourth derivatives of spectral curves. His "fourth derivative spectrometry" was put to good use in resolving spectrally vicinal components. His last two phytochrome papers published in 1980 and 1982 were concerned with subcellular distribution and localization of the two forms.

Butler cooperated with many colleagues and was interested in numerous aspects of photosynthesis, including chloroplast development, development of PS I and PS II in light, dependence of pigment absorption maxima on chloroplast structure, relationships of structure and energy transfer, changes and lifetimes of the long-wavelength chlorophyll fluorescence in vivo and in vitro, orientation of chlorophyll in vivo, the position of cytochromes b-559 and b-563 relative to the other components of the photosynthetic electron transport chain, and restoration of electron transport in tris-washed chloroplasts by electron donors to PS II. He and his coworkers subfractionated chloroplast membranes into functional pigment-protein complexes and sought for changes of P-680, of C-550 during irradiation at low temperature.

The primary photochemistry of photosystem II of photosynthesis was a major objective of Butler's later research. Of the two chlorophyll light-absorption systems, photosystem II, the oxygen-liberating system, has been a major challenge. Absorption of a quantum of light by an array of several hundred "light-harvesting" chlorophyll molecules cascades to a reaction center where P_{680}^{+} and Q^{-} are produced. In the process, oxygen is liberated. Butler and Erixon developed a method for reducing Q^{-} and thereby isolating the photochemical process for spectrophotometric measurement.

Butler was able, with his fourth derivative spectrometry, to reveal the absorption changes of cytochrome b-559, which became reduced under extremely high light intensities, binding a proton strongly in the reduced form when photoinhibition of PS II would occur. Thus, the low potential form can accept electrons directly from the reduced primary acceptor, pheophytin, the reaction center of PS II, binding a proton strongly when in the reduced form. Cytochrome b-559 fascinated him for years. It possessed a potential difference of 300 mV between the reduced and oxidized forms. It was assumed and later confirmed that the high potential form was closely associated with photoinhibition. Observations were greatly simplified by making measurements at -196° where the PS I system did not interfere. Butler's classic review, "Fluorescence Yield in Photosynthetic Systems and Its Relation to Electron Transport" (1966) defined his interests and concerns during the last part of his career. From his measurements of fluorescence yields, Butler became interested in energy distribution and utilization in the photosynthetic systems. He used his tripartite model of the plant's photochemical apparatus first in 1974 to describe the energy partitioning among photosynthetic units, PS I, PS II, and a third system, LHC, light-harvesting Chl a/b complexes. He introduced terminology for measurements of energy transfer among the three. With Masao Kitajima, Butler enjoyed the excitement of their improved understanding of low temperature fluorescence. It made possible the measurement of distribution of energy absorbed by photosystems I and II and their light-harvesting complexes, which he described as a tripartite model system.

The blue light responses attracted Butler's imagination. Butler was interested in the blue light phenomenon, long recognized by plant photophysiologists. He analyzed the destructive effect of blue light on mitochondrial cytochromes, the Soret bands that serve as photoreceptors, and consequent inhibition of respiratory activity. Starting with the typical biophysicist's concept, namely that absorbed light is likely to induce a change in the photoreceptor molecules that might be detected spectrophotometrically, Butler determinedly searched for such blue light-induced absorbance changes (LIACs) in fungal cells, presumably expecting changes in the 440-480 nm and 560 (557) nm regions, after irradiating the cells with blue light that corresponded to a photoreduction of a non-mitochondrial b-type cytochrome. An action spectrum for this LIAC in *Neurospora* cells demonstrated that the photoreceptor was a flavin, which, upon irradiation, emitted an electron, reducing the b-type cytochrome. Thus, Butler speculated that the cytochrome b-557 might be a component in the signal transduction chain very close to the primary photoreceptor flavin.

After the hemoflavoprotein nitrate reductase had been proposed as the photoreceptor for light-stimulated conidiation of the fungus *Neurospora crassa,* Butler and his coworkers found in a partially purified enzyme preparation, which had been inactivated by the reduction of the internal molybdenum cofactor, that blue light could reactivate the nitrate reductase by reoxidizing the cofactor. His action spectrum revealed again a flavin as photoreceptor for this reaction. Interestingly, as we now know, a flavin coacts with a second pigment molecule; more recently, it was discovered that the molybdenum of the cofactor is bound to a special pterin. In one of his earliest studies (1973), they identified a photoreceptor for phototaxis in the slime mold *Dictyostelium.* The absorbance changes that Butler and his collaborators observed corresponded in the fungi to a photoreduction of a b-type cytochrome; the action spectrum for the response revealed a flavin as photoreceptor.

That concept developed into a concern for the function of a hemoflavoprotein nitrate reductase and Butler's action spectrum indicated that a flavin was a photoreceptor chromophore.

The achievements in blue light research of Warren Butler and his group included application of the biophysical approach to the search for blue light photoreceptors, the technical refinement that allowed detection of small absorbance changes in live, dense samples, and consequently the identification of flavin and cytochrome b-557 cooperating in the photoreception/signal transduction in several blue light-regulated responses.

Warren Butler's skills in teaching were superb; his seminars and his undergraduate teaching were models of clarity and enthusiasm. Jon Singer recalls asking Warren to give a lecture on phytochrome for his undergraduate chemistry class. "An unforgettable lecture, with several striking demonstrations that he had prepared, he proceeded to give a lecture that for its carefully chosen level of exposition, its brilliant clarity, its obvious significance, and its simple elegance was the best single undergraduate lecture I have ever heard. He had every one of the 250 or so students riveted to their seats throughout, and received an astonishing and spontaneous storm of applause at its end."

Jon Singer, who with Martin Kamen had recruited Warren Butler for the fledgling Department of Biology at the University of California, San Diego, spoke of it as "one of the brightest events of my term as chairman. Warren's work was matched to and clearly stemmed from his personal qualities. His elegance was evident in countless ways, in his handsome face, the striking shock of silver hair, and in his marvelous smile."

The Butler children—Alison, Hillary, Laird, and Leslie—enjoyed a busy schedule in La Jolla, spiced with frequent adventures in Baja California and the California mountains. With the capable strengths of their mother Lila, Warren's potential for accomplishment seemed unlimited. Engineering being his forte, Warren Butler enjoyed the challenges of navigating the impossible roads of Baja California. His counterpart at the Scripps Institution, Professor John D. Isaacs, provided experienced encouragement and appreciation of the problems and delights of such adventures. Resourcefulness and ingenuity were clearly major attributes displayed in all aspects of Warren Butler's life. A dominant characteristic of Warren Butler was his spirit of adventure. He felt at home in the palm oases of the desert, on sand dunes at the ocean, and often in the forests. In Baja California and other places, sleeping on the ground and driving over non-existent roads often included fearless attacks of technical and mechanical challenges. Warren Butler's response to challenges of nature and science ignited the enthusiasm of his students and colleagues, whose appreciation grows with time.

I AM INDEBTED to the many thoughtful statements by speakers at a memorial service honoring Warren Butler on June 28, 1984, the obituary memorials published by Professor Helga Ninnemann, and the tribute volume published by "The Japanese Students of Professor Warren Butler," Hideyuki Matsuda and Kimikyuki Satoh, editors, for much of the material presented here. Discussions with Lila Butler, Helga Ninnemann, H. W. Siegelman, Jonathan Singer, and Winslow Briggs provided personal reflections on many aspects of Butler's life and work.

CHRONOLOGY

1925	Born in Yakima, Washington, on January 28
1943-46	U.S. Army Infantry
1949	B.A., physics, Reed College, Portland, Oregon
1951	Married to Lila Brown in Portland, Oregon, on September 1
1955	Ph.D., biophysics, University of Chicago
1955-56	Research associate, University of Chicago
1956-64	Biophysicist, Instrumentation Research Laboratory, U.S. Department of Agriculture
1964-65	Visiting professor, Johnson Foundation, University of Pennsylvania
1964-84	Professor of biology, University of California, San Diego
1975-77	Professor and chairman, Department of Biology, University of California, San Diego
1984	Died of cancer in La Jolla, California, on June 21

AWARDS AND HONORS

1976	Elected to the National Academy of Sciences
1978	Elected to the American Academy of Arts and Sciences
1981	Elected a foreign associate of the French Academy of Sciences

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