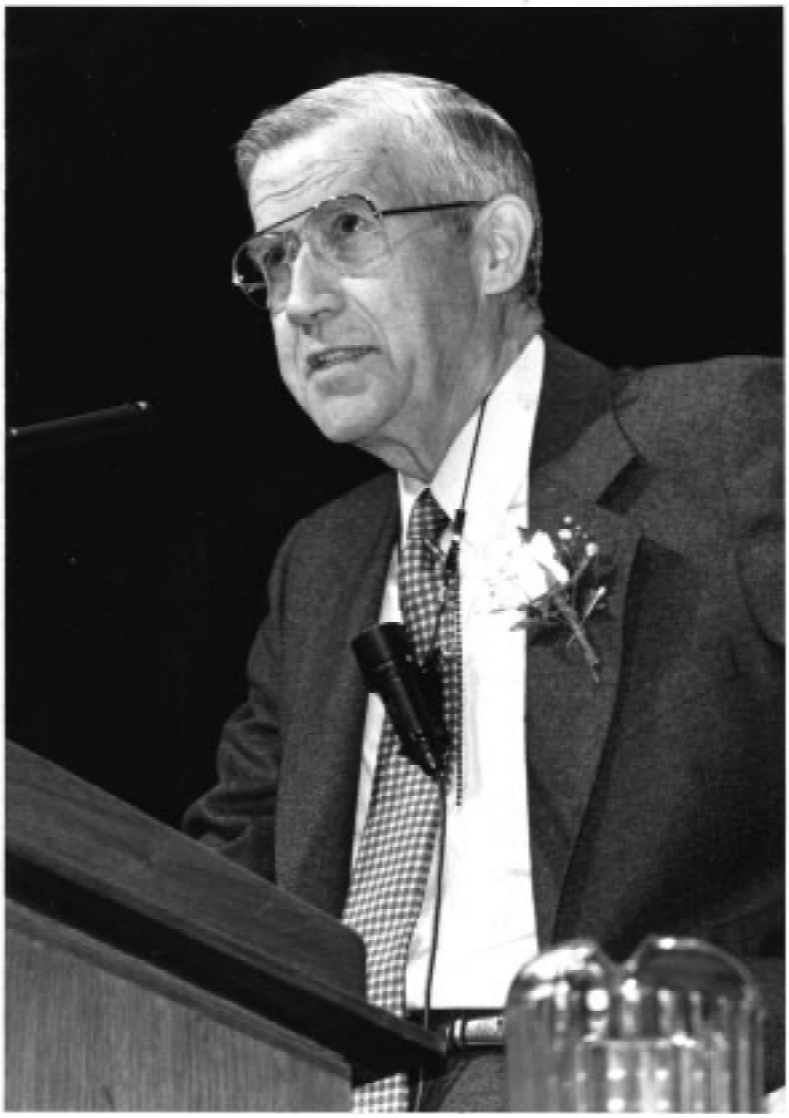

R. Clinton Fuller

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Personal perspective

Forty years of microbial photosynthesis research: Where it came from and what it led to *

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Abstract

What follows is a very personal account of my professional life and the early years that preceded it. I have described the social and economic conditions in America and how the nineteen twenties and thirties nurtured our scientific future. The description of the early part of post-World War II research covers my experience in the areas of nutritional biochemistry, biochemical genetics and proceeds to photosynthesis. The latter era lasted around 35 years. For me the most memorable research accomplishments in which I was a participant during this period was the first demonstration of the primary carboxylation enzyme in an *in vitro* system in algal and higher plants as well to show that it was structurally associated with the chloroplast.Our group while at Oak Ridge and the University of Massachusetts assembled data that described the complete macromolecular assembly of the photosynthetic apparatus of the unusual photosynthetic green bacterium *Chloroflexus aurantiacus* and created a model of that system which differed greatly from the chomatophore system for the purple bacteria. For the last decade, my scientific journey, with numerous new colleagues has turned to the exciting area of biomaterials.We characterized and modeled the completely new bacterial intracellular inclusions responsible for the synthesis and degredation of biosynthetic, biodegradable and biocompatible bacterial polyesters in the cytoplasm of *Pseudomonads.*

Abbreviations: ATP – adenosine 5′triphosphate; NAD and NADP (formerly DPN and TPN) – nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate, respectively; RUBP – (formerly abbreviated as RUDP) ribulose 1,5 bis-phosphate

Introduction

When Govindjee asked me to write my personal perspective article for the 'Historical Corner', I hesitated over how to compile a readable paper about my half-century involvement in science. Reading a dozen or so previous perspectives concerning photosynthesis research that had been written for the 'Historical Corner' showed me a wide variety of approaches. All of the articles were excellent pieces of scholarship. However, I was impressed particularly by the approaches taken by my former colleagues David Walker (1997), Howard Gest (1994), George Feher (1998) and Gerhart Drews (1996); their papers seemed to fit so well with their personalities. Encouraged, I decided to let myself be driven by my own personality – come what may! I have been very fortunate in my colleagues over the years. The prolific, scholarly writings of Howard Gest (1992) on the role of serendipity in both the general area of science and, specifically, in photosynthesis as well as Royston Robert's *Serendipity – Accidental Discoveries in Science: A Micro-history of the Phenomenon* (Roberts 1989), have greatly influenced me in writing this personal perspective. These authors em-

* Written at the invitation of Govindjee, Editor of the Historical Corner.

phasize that chance happenings play a tremendous role in the advances of science and in scientific careers. They also profess that the scientist must be able to grasp the event created by a serendipitous situation and proceed accordingly. With the above in mind, I believe that unexpected events in science lead to eventual progress. Taking advantage of chance circumstances requires not only solid training in the area of endeavor, but also the selection of people with the inherent instinct to take advantage of these unplanned happenings. Roberts quotes two famous nineteenth century scientists, Louis Pasteur and Joseph Henry, both of whom became famous for their discoveries in chemistry and physics respectivly due to a series of unexpected events. Pasteur was originally trained as a chemist, but also influenced the world through his work in both microbiology and immunology. Even more importantly, he discovered chirality or isomers in organic molecules. His discovery occurred through a series of chance observations: when crystals of tartaric acid and 'racemic' acid (identical molecules but the former a synthetic product) were examined under a microscope, they formed crystals that formed 'left hand' and 'right hand' structures. These experiments confirmed their observations using polarized light analysis. Pasteur summarized the importance of training: "In the field of observation, chance favors only the carefully prepared mind." Joseph Henry, the distinguished American physicist, expressed the same principle: "The seeds of great discoveries are constantly floating around us but only take root in minds well-prepared to receive them." Success in science is a truly Darwinian process.

The beginnings: March 5, 1925 and December 7, 1941: Two days that will live in infamy

My birth coincided with the giddy excesses of the mid 1920s when jazz and bootlegged liquor numbed our society to social and economic reality. From 1925–1932, laissez-faire government maintained a sharp split between the wealthy and the disadvantaged. A wildly rising stock market masked conditions that were becoming increasingly depressed. Banks folded. Agriculture and industries from mining to textiles collapsed. Cities teemed with unassimilated immigrants. Our culture largely stagnated with some exceptions in literature. F. Scott Fitzgerald described the desperate self-indulgences of the wealthy in a spiritually vapid time. The most important literary contributions, however, probably emerged from writers fueled by a new realism. Theodore Dreiser, Sinclair Lewis and John Steinbeck all pitted the gritty realities of poverty and injustices of the social structure against the American Dream. It would take the devastating turmoil of the Great Depression and World War II to redirect our social, intellectual and, particularly, scientific priorities. Refugees from the persecutions in Europe flooded our country; many of these or their children became the distinguished scholars of the next few decades. Graduates from US colleges, unable to find jobs, turned to graduate studies. For instance, City College of New York requiring no tuition, sent their students on to the best institutions for a PhD education even while 25% of our population lacked work. My generation of scientists found some of its greatest teachers among these graduate students.

My own initiation into science was interwoven with the progressive post-Depression federal programs that funded our public health and natural resources as well as the arts and humanities. One such program was the Public Health Service, the embryonic source of the National Institutes of Health. Agendas for forestry, as well as natural resource preservation, were set up by the Department of the Interior. The Department of Agriculture not only boosted agricultural research for our own nation by strengthening the United States Department of Agriculture Research Laboratories, but also deepened our commitment to other countries by including world food production in its agenda. President Franklin Delano Roosevelt, with the urging and participation of his wife, Eleanor, also funded the arts and humanities under the Works Progress Administration. Science, during this period, received most of its support from the private foundations such as Rockefeller, Carnegie and Ford.

An introduction to science: 1943–1952

When the Japanese bombed Pearl Harbor on December 7, 1941, I was a 16-year-old high school junior. My age group had little choice about the future since the draft only exempted those under the age of 18 or those with physical disabilities (including flat feet!). Under the Roosevelt administration policy, induction into the armed forces was delayed until an 18-year-old had graduated from high school; at that point he either would be drafted into the Army or could volunteer for the Navy or the Marines. War veterans were able to enter college and receive 4 years of government-supported education as a result of another

forward-looking policy of the Roosevelt and the Harry S. Truman administrations.

Choosing the Navy turned out to be a lucky event affecting my career in science. The Navy had set up the so-called V-12 Program which could be entered if a highly competitive exam were passed. The program, much broader in terms of professional choice than a similar one set up by the Army, allowed all enlisted personnel with high school diplomas to be sent on active duty to a university for the training to become a lawyer, doctor, engineer, teacher, scientist or other professional. I was accepted into the V-12 Program and assigned to Brown University in July of 1943.

Since I had expressed interest in the life sciences, my assignment was to major as a pre-med student. I remember my mother exclaiming with joy, "Eureka, my son the doctor!" I simply groaned over having to take a lot of chemistry that I nearly had flunked in high school. With less than impressive motivation, I started the very demanding Navy program. For every single month of the year, we students took six courses a semester in order to attain the Bachelor's degree in two years. Courses in 'Leadership' diluted our intellectual pursuits (reminiscent of our current ones in 'Cultural Diversity'?). Two miles of formation running, marching to meals, various military formations and drills punctuated each day. Many of us became disciplined and healthy despite ourselves. Nonetheless, we all looked for ways to reduce the heat of this rigorous schedule.

Just as with the students of today, we tried to fit one or two 'gut courses' into our schedules. I chose Music 101 that looked like a breeze to me as classical music had filled my upbringing. In this class I met the woman who was to become my wife and wonderful, lifelong partner. Music 101, taught by a great musician, inspired us beyond the marching drill beats to the highly skilled but delicate rhythms of Mozart and to the heady jazz rhythms of Benny Goodman, Tommy Dorsey and Glenn Miller. Carol, my future wife, sat next to me. Soon we were comparing notes on English, her major, and science that left her cold. However, we overcame the barriers before long and exchanged our interests. While I enrolled in a Restoration Drama class, Carol took Biology 101 taught by Professor George Kidder who had inspired me in a biochemistry course and, later, would launch my career. In more ways than one, Music 101 and Biology 101 were fortuitous events in our lives.

Soon circumstances beyond anyone's control again transformed my life. Due to graduate in September1945, I had expected to be off to the Far East and the invasion of Japan. Instead, the Allies bombed Hiroshima and Nagasaki. With the sudden end of World War II in August, I found myself discharged into civilian life as an unemployed college graduate. However, I would be able to continue research with George Kidder at his invitation. I had taken several of his courses and spent time (mostly washing dishes) in his lab. More importantly, I had become involved in discussions about biochemistry. Indeed, I had become very interested in his research.

At this time George was studying the comparative biochemistry of nutrition in the Protozoan ciliate *Tetrahymena geleii*. His contention was that all animals not only had the same nutritional requirements for amino acids as well as various vitamins, but also the same precursors and pathways for synthesizing proteins, nucleic acids and carbohydrates. It was indeed a perfect model system for studying animal biochemistry. His first contribution was the successful growing of *Tetrahymena* on a defined media of the 18 essential amino acids, glucose and cofactors from yeast extract or liver. In a liver extract, an element that was not present in yeast was identified as being required for growth; it was designated 'liver factor II'. When purified and characterized, this factor turned out to be folic acid. This discovery confirmed for George that *Tetrahymena*, like all animals, required this vitamin. My first scientific paper, published 53 years ago, concerned this work (Kidder and Fuller 1946). In 1947, George presented this material at a symposium of the American Society of Biological Chemistry in Atlantic City, New Jersey. An enthusiastic lecturer, he had emphasized his belief that the Protozoan *Tetrahymena* was a "nutritional and biochemical animal". André Lwoff of the Institute Pasteur opened the ensuing discussion with, as I recall, the following question: "George, in your examination of this animal, have you discovered any other characteristics that might suggest an even more highly evolved animal? Have you, for instance, ever observed that *T. geleii* has hair or mammary glands?" George quickly replied, "Hair, yes, it is, of course, a protozoan ciliate. But mammary glands? Not *yet*. But we're looking!"

Besides being a superb teacher and world-recognized biochemist, George was ambitious for his students. Soon after I had started to work for him as a PhD student at Brown, Amherst College offered him a position. So my new wife, the converted English major, and I began to anticipate a move. George warned me that Amherst College did not give a PhD. However,

I could obtain an MA degree through a cooperative effort among Amherst, Mt. Holyoke and Smith Colleges as well as the newly named University of Massachusetts. Then he would see that I entered a first class PhD program elsewhere.

So, in the fall of 1946, Carol and I – newly married – trekked north to Amherst where time passed rapidly. When my MS was in hand, George again helped by urging me to involve one of two eminent scientists for my PhD work. He suggested either E.L.Tatum, then at Yale or George Beadle at Cal Tech. (that both of these scientists would win Nobel prizes was obvious to George). Applying to both places, I received immediate acceptance by Yale but not by Cal Tech! California, however, was not out of the picture since another serendipitous event cropped up. George returned from a meeting around Christmas, 1945, and exclaimed, "Clint, you're in trouble. Ed Tatum is leaving Yale and going to Stanford. You better go down to New Haven and talk to him." By the end of the day, I had the option of staying with David Bonner at Yale or joining Tatum at Stanford with full support and a tuition waiver. It was a tough decision for me. Joshua Lederberg, one of Tatum's senior students, indeed did finish at Yale; eventually sharing the 1960 Nobel Prize with Beadle and Tatum; he later became the president of Rockefeller University. For me, however, the West won out. I joined ranks with Tatum, his associates and students. Carol and I stuffed all of our belongings into a used 1938 Ford and headed for Palo Alto in the late spring of 1948.

The first task for the Tatum team was to build a couple of constant temperature rooms and 200 handmade test tube racks; we hoped that our stipends for this work would be handsome. For the next 2 months, one-inch turkey wire, cutters and pliers bloodied our fingers as we formed the racks. At the end of the summer, Ed presented each graduate student with $100. Overwhelmed by the enormity of our reward, we decided to head for Yosemite National Park for a week of camping before school started. Together with our families, we rapidly blew our stipends on steaks and good California wine ($1.00 a gallon for Gallo's best). We all got to know each other very well that summer!

Exciting and important, the years at Stanford (1948–1952) passed all too quickly. The era heralded the field of molecular genetics. James Watson and Francis Crick had not yet appeared on the scene. The work of George Beadle, a geneticist, and Ed Tatum, biochemist, held center stage in the gene expression beginnings. "One gene – one enzyme" was the cry of the lab at Stanford. For the next 4 years, we all were involved. My PhD thesis gave me a terrific background in *Neurospora* genetics. My research also introduced me to cell biology and to the biochemistry of cellular structure and function at the macromolecular level. These research areas would be the ones that I would be involved in for the rest of my career in both photosynthesis and biomaterial sciences. My PhD thesis involved a mutant of *Neurospora crassa* that required the vitamin inositol for growth. When grown on limiting amounts of this vitamin requirement, the organism showed a slow growth rate and small colonies on agar rather than fast-spreading mycelial growth. I also discovered that wild-type *Neurospora* grown on hexachlorocyclohexane (an inhibitor of inositol metabolism) induced the wild-type organism to behave similarly, in both growth rate and morphology, to the inositol requiring mutant. At this time the biochemical role of inositol was unknown. It was later shown that it was incorporated as a phospholipid in mitochondrial membranes and that its absence caused the cells to be respiration deficient, which accounted for their retarded growth and morphological changes. My thesis resulted in two publications and a little bit of the intellectual glory of the Tatum, Beadle and Lederberg Nobel Prize in 1960 (Fuller et al. 1956; Fuller and Tatum 1956)

By May of 1952, I was finishing my degree and found myself short-listed for several academic jobs but without any sure employment prospects. (In those days post-doc positions were not a requirement for starting positions at universities.) A little desperation crept into our family life, now supplemented by two infants. Again serendipity came to the rescue. Ed Tatum was the faculty head of the Sigma Xi Honorary Society at Stanford. As such he had invited a chemist, Melvin Calvin from the University of California at Berkeley, to give the annual Sigma Xi lecture, entitled "The Path of Carbon in Photosynthesis". Ed, as always, involved his students in social events centered upon distinguished visitors. So we all were invited to the Tatum house for a barbecue and to meet Calvin. When I was introduced, he remarked, "Well, young man, I understand that you're just finishing up your degree – what do you plan to do with the rest of your life?" Undaunted, I quickly answered that I was looking for a job. When he asked if I considered myself a microbiologist, I immediately replied in the affirmative. (At that point I would have said yes to anything!) Then he asked me if I could come up to Berkeley where they were having a lot of trouble with

Figure 1. Picture of the Calvin photosynthetic group in 1954 outside of the Old Radiation Laboratory in Berkeley. Left to right: Ning Pon; J. A. Bassham; Jean Bourdon, Paris, France; R. C. Fuller; Rodney Quayle, later Vice-chancellor of Bath University, England; Hans Kornberg, Oxford, later Professor of Biochemistry, Cambridge University (now Commonwealth Professor Sir Hans Kornberg at Boston University); Hans Griesbach, late Professor and Head of Botanical Institute, Albert Ludwig's University, Freiburg; Alice Holtham, Department secretary and artist; Malcolm Thain, England; Melvin Calvin; Paul Hayes, Laboratory Manager; Jacques Mayoudon, post-doctoral student from Belgium; and late Professor Kazuo Shibata, Riken, Tokyo, Japan. This was a typical group during the 1940s, 1950s and 1960s. Missing in the picture is Andrew A. Benson, who took the picture. Reprinted from Calvin (1961). Photograph is a courtesy of Lawrence Radiation Laboratory, University of California at Berkeley, California.

yeast growing in their algal cultures and needed a "microbiologist to clean up the situation".

Thus I gained a position as Research Associate in Melvin Calvin's laboratory. Showing the lab to me, he commented that he hoped I liked it as 60–70 h of my week would be spent there for the next few years. He was so right! With PhD in hand, I moved my family to Berkeley on July 1, 1952. My salary was a princely $6000 a year! Melvin introduced me around to the other staff, visitors and students associated with photosynthesis. (Melvin had an equally large group involved in animal cell biochemistry.) Figure 1 is a photograph of the photosynthesis group, which was taken in 1954 outside of the 'Old Radiation Laboratory' on the Berkeley campus. This picture was taken by A.A. Benson.

The Calvin years: 1952–1955

My research with the Calvin group at the Old Radiation Laboratory (ORL) was one of the most exciting learning experiences in my career. For the next 35 years, I was launched into the area of the cell biochemistry and comparative structure related to function in microbial photosynthesis. Worldwide research in photosynthesis concentrated in the following areas at that time: the biophysics of solar energy capture; the conversion to chemical-reducing power in the form of reduced pyridine nuceotide; the production of ATP; and the path of carbon after CO_2 fixation. This research surged tremendously during the 1950s and 1960s. The discovery and use of ^{14}C by Sam Ruben of the Chemistry Department at Berkeley and Martin Kamen at the Radiation Laboratory in the late thirties, opened

the way for studying the new (for those days) radioactive tracer technology and the mechanism of photosynthetic CO_2 fixation. The giants of these areas of photosynthesis research included: Eugene Rabinowitch, Otto Warburg, James Franck, Robert Emerson, William Arnold, Cornelis B. van Niel, Hans Gaffron, Daniel Arnon, Jack Myers, Melvin Calvin and his two long-time associates, Andrew A. Benson and J. Alan Bassham, as well as many others. These scientists actively produced theories, probed and obtained experimental results that hopefully backed their proposals. I was fortunate to have had various interactions with all of them. The conflicting ideas sometimes generated bitter arguments that polarized we younger participants behind our leaders and their respective research philosophies and concepts. In the literature these sharp exchanges were subdued by the reviewers to far more civility than thought necessary at the podiums or on the floors of many memorable meeting places. At Gattlinburg in the Tennessee Smoky Mountains, Brookhaven National Laboratory and at several universities that hosted meetings of the American Society of Plant Physiologists, the arguments could flair up into brutal attacks. The depth of the personal enmity among the giants shocked us younger participants (Seaborg and Benson 1997). With somewhat less responsibility for our profession than our truly great mentors, we got to know each other's research, different backgrounds and approaches and in time we appreciated how much healthy competition, education and stimulation actually resulted from our leaders' conflicts. Many of us became good friends and colleagues over the years.

Two major scientific thrusts filled the early 1950s: one involved the path of carbon; the other involved the primary photochemical act of energy capture and its conversion from light to chemical energy. I entered the Calvin group when about 20 staff along with graduate students and post-docs were working on the photosynthesis project. In addition many visitors, selected carefully on the basis of their abilities to enhance both areas of photosynthesis research, also contributed important work. Initially, Andy Benson used the new anionic and cationic resins from Dow Chemical Co. as a purification media for the isolation of $^{14}CO_2$ photosynthetic fixation products. (Calvin was a long time consultant for Dow.) Subsequently, Bill Stepka, also at Berkeley, introduced the lab to paper chromatography and radioautography as analytical techniques at the ultrasensitive tracer level. Gaffron's group made many discoveries,particularly in energetics, that kept the Calvin group on the right path (Gaffron 1960).

I cannot emphasize enough the importance of these technologies either utilized or designed by those people involved in the Calvin years. The imaginative staff together with intelligent students and visitors designed new instrumentation and technology for the analysis of the photosynthetic process. All of the initial work used $^{14}CO_2$ and algae, as the plant of choice, to trace the path of carbon; this work depended upon a simple piece of glassware invented by Andy Benson and designated, for its obvious shape, as the 'lollipop' (see Figure 2).

After turning on the lights, one simply held the lollipop with one hand and injected ^{14}C-labelled bicarbonate solution into the vessel with the other hand. Then one opened the stopcock as rapidly as possible in order to kill the algal suspension in boiling 95% ethanol. Proficiency with this process meant that the $^{14}CO_2$ fixation could be stopped after about 5 s in the light. This type of procedure produced chromatograms with many labeled sugar phosphates and phosphogyeric acid (PGA). The latter was indicated as an early if not primary fixation product. The only difficult aspects of the procedure were that the experiments had to be carried out at room temperature and that, needless to say, timing the formation of fixation products was not reproducible. Further, we were not really able to study the kinetics of the reactions, and the amount of material ^{14}C-labeled compounds was so small as to make analysis difficult. One instance of chemical instrumentation and engineering involved the 'algal steady-state apparatus' designed by Alex Wilson (a graduate student from New Zealand) under the direction of Al Bassham and Andy Benson. Experiments in this apparatus could be carried out at low temperatures so that the enzymatic reactions were slowed down (Calvin et al. 1954). The *Journal of the American Chemical Society* published, as part of Alex's thesis, a detailed diagram of that apparatus together with its function and capabilities for illuminating the path of carbon work.

Both the diagram and legend to Figure 3 evoke historical interest since the drawing must be the most complicated to ever pass reviewers and then be reduced to one column of fine print. Several of us tried to talk Melvin out of publishing this unintelligible masterpiece without further simplification. We failed. However, just prior to submission of the manuscript, several staff members (who will remain unnamed) colluded with the departmental secretary and artist-in-

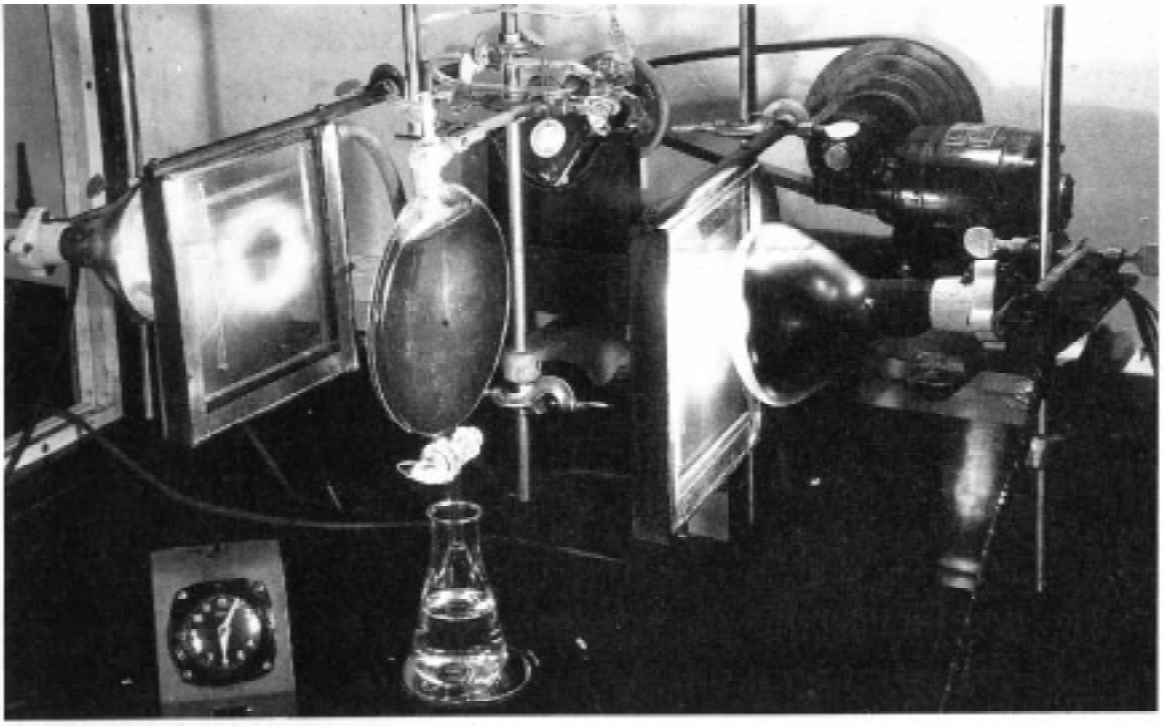

Figure 2. All of the early experiments from 1947–1953 with $^{14}CO_2$ and algae were carried out with this apparatus, the 'lollipop' as described in the text. Reprinted from Calvin (1991). Courtesy of Lawrence Radiation Laboratory, University of California at Berkeley, California.

residence, Alice (Holtham) Lauber, to add a simplifying entity to the figure. That entity, reproduced in Figure 3 between n and A in the center of the algal suspension tank, shows a simpler way to fish for data. Melvin missed our addition to the drawing. The reviewers and publishers also missed it. But the *Journal of the American Chemical Society* published our added simplification which still survives in the archive sections of most science libraries (Wilson and Calvin 1955).

Visitors also made essential contributions to our work. Arnold Nordal, a Norwegian of worldwide stature in plant synthesis and storage of sugars as well as sugar phosphates, together with Gerard Mihaud, from the Institute Pasteur, contributed vitally in the areas of biochemistry, metabolism and enzymology in microorganisms. Other visitors, some of them long-term, included Rod Quayle, a recent PhD graduate from London and later Vice-chancellor of Bath University; Hans Kornberg, later Sir Hans and Professor and Chair of Biochemistry at Cambridge University; and Hans Griesbach, later Professor and Chair of Botany at Albert Ludwig University in Freiburg, Germany. This wonderful group, under the daily supervision and mentorship of Andy Benson and Al Bassham, plunged into the path of carbon research. John Baltrop, a Professor of Chemistry at Balliol College, Oxford, was recruited to enhance the second major thrust of the lab, which was the primary photochemical act of energy capture and conversion from light to chemical energy. As a physical organic chemist, he interacted well with Melvin's creativity and intuition.

This young, vigorous group of associates, which gathered under one scientific roof to work on a major project, formed an early example of the interdisciplinary approach to the life sciences. Similar approaches existed in the Manhattan Project and at places like Chicago and Oak Ridge. But the focus on such a small area as photosynthesis with a great team of biologists, chemists and physicists was certainly Melvin's creation. Along with both Andy Benson and Al Bassham, who was with Melvin for his whole career, we all greatly benefited from that interdisciplinary approach in our future careers.

I would like at this point to express a personal note that represents my own feelings and the recollections of many of the scientists who with me experienced the research years at the ORL in Berkeley on photosyn-

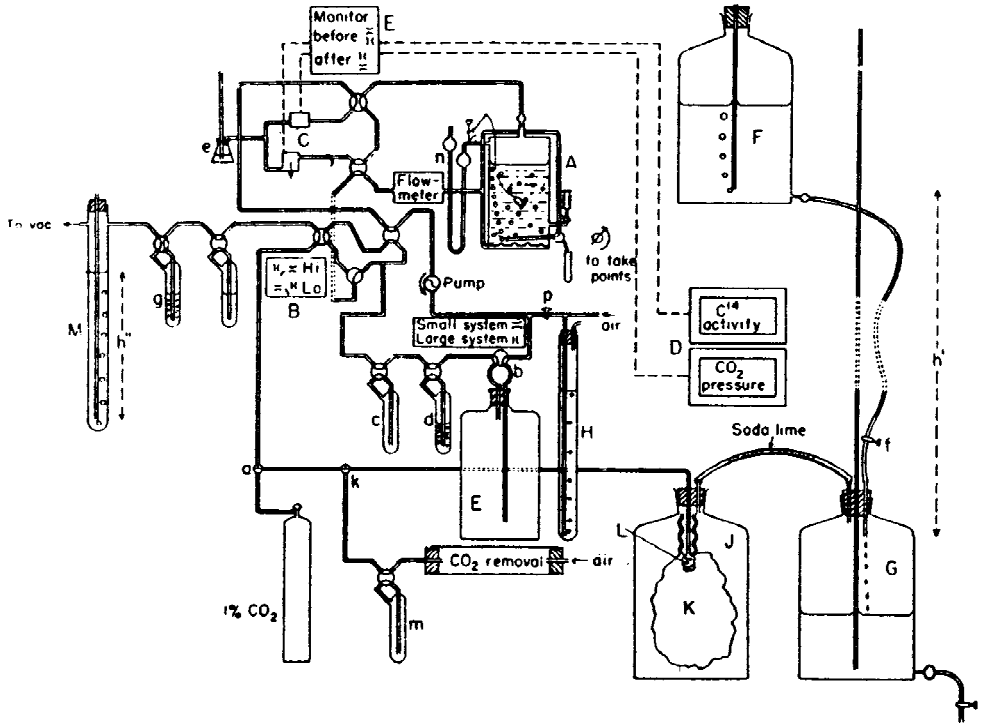

Figure 3. Diagram of the apparatus for measuring transient phenomena. The apparatus itself consists of an illumination vessel 'A' and three gas systems. One is a recycling system for high partial pressure of CO_2; one is a non-recycling system for $^{12}CO_2$, and the third is a non-recycling system for $^{14}CO_2$. It is possible to switch between the recycling system and either of the non-recycling systems by turning three stopcocks on the control panel 'B', and also to switch between the non-recycling systems by turning stopcock 'a'. The gas is continuously monitored for CO_2 partial pressure and radioactivity by an infrared gas analyzer and an ionization chamber, respectively, 'C'. The data are recorded continuously and automatically on chart recorders, 'D'. The monitors may be placed 'before' and 'after' the illumination vessel by turning two stopcocks on a control panel ('E' shows how the stopcocks located below should be turned). When the 'high' recycling system is in operation the gas passes through a large reservoir 'E' (5 l). This reservoir can be bypassed by turning stopcock 'b', leaving only a very small volume recycling. Under these conditions the rate of photosynthesis or respiration can be read directly from the recorders. This feature is invaluable for obtaining the necessary data for designing experiments where certain optimum conditions are required. Trap 'c' is used to introduce the $^{14}CO_2$ at the end of a run. The constant head device in 'F' and 'G' enabled one setting of the screw clamp 'f' to maintain the flow constant throughout a run. The balloon 'K' is filled through stopcock '.' The tube from 'F' to 'G' is clamped off and the water is run out of 'G' pulling air from 'J' into 'G' which pulls the balloon open sucking CO_2-free air into 'K.' The $^{14}CO_2$ is added via trap 'm'. Since 0.003% CO_2 was being handled, and since it was important that its specific activity be maintained, a soda-lime absorber was placed between 'G' and 'J'. This absorber was considered necessary in the light of the solubility of CO_2 in rubber. At first much trouble was encountered with the balloon bursting while it was being evacuated, but this problem was solved by means of device 'L' which is a small perforated glass bulb through which the gases are removed from the balloon. The many holes and irregular shape of this glass bulb prevent the outlet from being blocked by the collapsed balloon before the balloon is completely empty. Samples representative of the algal suspension can be taken by turning the stopcock on the illumination vessel in a clockwise direction. The figure and the legend are essentially reprinted from Wilson and Calvin (1955), by the permission of the American Chemical Society.

thesis. Calvin's autobiography, *Following the Trail of Light* (Calvin 1992), represents an extremely singular view of the research carried on in the laboratory particularly in the area of the path of carbon for which he received the Nobel Prize. In all the 175 pages of his autobiography there is not one sign of Andy Benson or a mention of him. There is not one picture of Andy in a book that contains 51 photographs ranging from graduate students to the King of Sweden. There is not the citation of a single paper with Benson as an author or co-author in an extensive bibliography of over 150 references. Benson's name appears nowhere in the text and consequently is absent in the 12-page index. This appears to be an undeserved slight to a great scientist both personally and professionally who had contributed in a major way to all of Calvin's research and technology in the field of photosynthesis. Andy was a real leader in the laboratory both intellectually and experimentally. He should have been a partner in The Nobel Prize. Al Bassham's contribu-

tions are also understated, although he is pictured and cited through the text. I know that all of us who were colleagues at Berkeley agree that it was Andy and Al who contributed greatly to our own success in future endeavors. I have no idea what may have caused this unfortunate event, but I think that history should record that the contribution of Andy Benson is not properly recognized in Calvin's autobiography.

My immediate task upon arriving in Berkeley, besides cleaning up algal cultures, plunged me into the path of carbon experiments; these included the examination of other photosynthetic microorganisms (besides algae). Working closely with Al Bassham and Andy Benson, I learned the ^{14}C labeling, chromatography and radioautograhy technology. By the late forties, Andy and Melvin (Calvin and Benson 1948) had pretty well established that 3-phosphoglyceric acid (PGA) was an early (<5 s) CO_2 fixation product. In addition the four-carbon acids of the Krebs cycle were labeled heavily with ^{14}C when algal cells were pre-illuminated and then exposed to $^{14}CO_2$ in the dark. Benson suggested that during pre-illumination experiments in *Chlorella*, light produced reducing power in the form of NADPH or NADH accounting for the formation of malate, succinate, and citrate after the light was turned off. PGA was also formed, but its synthesis greatly increased with continuous exposure to light during $^{14}CO_2$ fixation (Benson and Calvin 1947). In 1939 Robin Hill at Cambridge University demonstrated the light-dependent evolution of O_2 from isolated chloroplasts, now known as the Hill reaction (Hill 1939). So, by the late forties O_2 evolution and NADP reduction were known to be involved in carboxylations in both oxidation reactions and photosynthesis. Andy Benson had always strongly stressed the importance of the synthesis of sugar monophosphates and diphosphates during the photosynthetic reduction of CO_2. Professor Arnold Nordal, an expert on sedoheptulose (C-7) and related sugars had arrived at the lab from Norway. Severo Ochoa (1948), Harland Wood (1946) and others worked on respiratory reductive carboxylations. Transkelotase and transaldolase enzyme reactions had been clarified in the synthesis mechanisms of C-5 and C-7 sugar phosphates. Martin Gibbs and colleagues at Brookhaven National Laboratories observed the asymmetric labeling of the sugar phosphates in short-term photosynthesis (Horecker et al. 1954; Gibbs 1996). As a result of these elegant but puzzling results, Calvin suggested the following: that β-carboxylation of RUBP could yield an unstable six-carbon β-keto acid as an intermediate; and that this acid, when hydrolyzed, spontaneously split into two molecules of PGA with the primary $^{14}CO_2$ labeling the β position of the six-carbon unstable intermediate. His chemical deductions were simply brilliant. This carboxylation at the β position of ribulose formed the basis of the asymmetric ^{14}C labeling in the formation of the sugar phosphates. The missing link – of the elusive two-carbon acceptor – proved to be the top two carbons of RUBP. In other words Calvin's new carboxylation turned out to be $C_1 + C_5 = C_6 \div 2 = 2$ three-carbon PGAs as shown in Figure 4.

From that point on, the reductive pentose phosphate or Calvin Cycle fell together. The path of carbon was solved thanks to the interdisciplinary staff, visitors, and students, led by both Andy and Al and of course Melvin's leadership (Calvin et al. 1954).

Melvin received the 1961 Nobel Prize in Chemistry for the above work. Later he received an invitation to the White House. As a gesture of recognition to Nobel Prize winners, President John F. Kennedy, and his wife Jackie, wined and dined the current as well as past American Laureates including Ed Tatum and Melvin together with his wife, Genevieve. This event inspired Kennedy's now famous quote: "this is the most extraordinary collection of talent and human knowledge gathered at the White House, with the possible exception of when Thomas Jefferson dined here alone."

J. Rodney Quayle (Rod) had joined Melvin's lab in 1953 as a visiting scientist. Rod and the rest of us collaborated with Andy on the first *in vitro* demonstration of RUBP carboxylase enzyme in a cell-free system (Quayle et al. 1954). This system was crude. No source of ribulose 1,5-bisphosphate for a substrate existed, only having been characterized as a 'radioactive spot' on paper chromatograms. However, by eluting enough of these spots from paper chromatograms, we did isolate enough of the proposed substrate to carry out a cell-free enzyme assay with $^{14}CO_2$. The radioautograph of that experiment showed one major compound, ^{14}C-labeled PGA. The data demonstrated the first evidence of an *in vitro* enzymatic reaction of the ribulose 1,5-bisphosphate carboxylase. In all fairness, Weisbach, Smyrniotis and Horecker substantiated our conclusion. In their paper published in the same issue of the *Journal of the American Chemical Society*, Weisbach et al. (1954) showed a carboxylation using ribose-5-PO_4(R5P) as a substrate and demonstrated that a soluble extract of spinach leaves carried out a carboxylation that formed a carboxy ^{14}C-labeled PGA. This preparation, how-

ever, was a 'multienzyme' system. The reaction also required energy and reducing-power in the form of ATP and NADPH. In retrospect we know that these authors had missed the CO_2 fixation reaction mechanism by two enzymatic steps – a phosphorylation and an epimerase reaction that yielded RUBP from ribose 5-phosphate, then followed by the carboxylation.

However, a few less than impressive events also occurred during my stay at Melvin's lab. Andy Benson chronicled one such event in 1995. Since his note so wonderfully describes life in that lab and involved me, I quote it at length (Benson 1995):

Saga of a great theory of photosynthesis

> "The most exciting idea which I and many of my colleagues experienced was Melvin Calvin's 'Thioctic acid Mechanism of Photosynthesis', a superb concatenation of information, ideas and experimental evidence which appeared to fit with all we knew of photochemical energy conversion in the chloroplast. It developed at the time thioctic acid (lipoic acid) and its function had just been discovered. It is a yellow compound, with the proper absorption at 330 mμ to accept the energy from an excited chlorophyll molecule. Melvin Calvin and G.N. Lewis were highly respected in photochemical circles and the absorption of energy by thioctic acid seemed logical. The product, a dithiyl radical [R–S...S–R] was consistent with the plethora of sulfur radicals detected in photosynthetic tissues with the then-novel paramagnetic resonance spectrometers. John Barltrop, who had come from Oxford University's chemistry department, proceeded to develop experimental support for the theory. He and Calvin collected convincing evidence for the reaction of such radicals with water or alcohols. Thus photolysis of the strained disulfide ring could yield both R-SH and R-SOH, a sulfenic acid, on the same thioctic molecule. One, a reducing agent, and the other, a sulfur analog of an alkyl hydroperoxide capable of yielding oxygen. Thus the energy of the quantum absorbed by chlorophyll could yield the chemical essentials of photosynthesis.
>
> Finally, Balthrop and Calvin tested the hypothesis in Scenedesmus treated with added thioctic acid; oxygen production increased 50%. The plausibility of the theory was elegantly developed in over forty pages of ensuing publications documenting the experimental evidence. Had Nature overlooked this opportunity it would have made a mistake. The quality of the research was superb, as one can appreciate from the meticulous publications. For a year the whole effort was exhilarating. It was truly a Nobel idea.
>
> The high point of this saga was Melvin's lecture in a 1954 national meeting in San Francisco. Beginning with his usual hesitant manner and leading to a magnificent crescendo of convincing evidence for the mechanism of the quantum conversion of photosynthesis, the audience was totally impressed. The great C.B. van Neil jumped from his seat in the front row with tears in his eyes to congratulate Melvin. It seemed a consummation of his own decades of thought and effort dedicated to understanding photosynthesis.
>
> Final proof lay in identification of thioctic acid in the chloroplast; but the assay was tedious and required microbiological experience. Clint Fuller, with a new PhD from the Stanford laboratory of Beadle and Tatum, was recruited to assay thioctic acid. I grew some *Chlorella* in sulfate-S^{35}, chromatographed the extract and prepared the radioautograph of my paper chromatogram. With Melvin and the others standing around the great white table, I laid the film on the paper. There, was a huge black spot, right in the position we expected. Melvin's eyes just about dropped out onto the film. It was a breathtaking moment.
>
> The S^{35} radioactivity had to be proved to be thioctic acid. Try as he would, Clint Fuller and his *Streptococcus fecaelis* bacterial assay couldn't find a trace of thioctic acid that coincided with the radioactive ^{35}S spot. One by one, the evidence for the several critical steps weakened and the thioctic theory quietly evaporated. The massive effort, the elegant chemistry and photochemistry produced impressive publications which no longer attract attention. Yes, the theory was in ashes but we must see a 'take home lesson' in this saga. One can survive a failed effort; even one which had involved man-years of work and excitement."

We all learned.

At this time Andre O.M. Stoppani, a visitor from Argentina, joined Calvin's lab. Andre was a microbiologist who later became President of the Argentinian Academy of Science. His presence gave us all the opportunity to begin working on the path of carbon in prokaryotic photosynthetic bacteria. The presence of Roger Stanier and Mike Doudoroff, previ-

ous PhD students of van Niel at Stanford and members of the Microbiology Department at Berkeley, further reinforced our interest. How the prokaryotic organism managed and coordinated energy capture, ATP production, pyridine nucleotide reduction and CO_2 fixation in the unstructured, non-compartmentalized prokaryotic cell was not known.

Daniel I. Arnon and his collaborators had demonstrated that all of these processes took place in the eukaryotic chloroplast, structurally segregated from respiratory and other biosynthetic activities of the cell. We wanted to investigate the organization of these processes in prokaryotic systems and determine the structures that exist for the separation of the competing respiratory as well as synthetic metabolism. We published several papers, referred to in the next section, on our findings. Although we gathered interesting information on the cellular biochemistry in both *Rhodopseudomonas* and *Chromatium* (Stoppani et al. 1955), it had become clear that this research would not remain part of the Calvin mission in the long run.

In running his lab, Melvin had a not particularly subtle, yet gentle, way for separating personnel from the group. For instance, he would call me into his office and tell me that he had recommended me to present a seminar at 'x' or 'y' academic institution since he was unable to go. I gave several of these seminars in 1954 and even received job offers at some less than exciting places. With no forewarning, I heard about the job possibilities only after my arrivals at the various institutions. In the fall of that year, I again responded to a call from Melvin's office, expecting more of the same. But he inadvertently presented me with a real opportunity this time. Melvin had been invited by The American Society of Plant Physiologists to give a symposium paper at their annual meeting to be held in Gainesville, Florida. Due to what he called 'previous commitments', Melvin now could not attend the meeting. However, he already had submitted an abstract, co-authored collectively by me, Andy and Rod Quayle, to be presented at the meeting. Well, none of us had been aware of such a submission! Pointing out the 'good exposure' for me, he asked if I could go in his place! Clearly, pressure for my independence was growing; I jumped at the chance. Then the very human side of Melvin emerged. "Clint", he asked, "doesn't your family live back East, and wouldn't you like to visit them on the way home?" I suggested that detouring to Providence, Rhode Island on my way from Florida to Berkeley would be a pretty expensive divergence. Reflecting, he suggested sending me, with all expenses paid, to Brookhaven National Laboratory (BNL) on Long Island; after my stay as a consultant there, I could visit my parents on my way home. After we remembered that Martin (Marty) Gibbs who was working on asymmetric ^{14}C labeling and degredation of sugars in photosynthetic $^{14}CO_2$ fixation was there, Melvin said that he could arrange everything.

So I attended the meeting of the American Society of Plant Physiologists in Gainesville and continued on to BNL. I gave my seminar there and met with Martin Gibbs for a lengthy conversation about the path of carbon. On that evening some of the staff joined me for a terrific Atlantic seafood dinner; this social event would influence my future plans. On the next morning, Marty informed me that the Chair of the Biology Division wanted to talk with me about an available position for a plant biochemist. Howard Curtis, the Chair, and I had a long, very memorable talk. I also recall long discussions with Dan and Marian Koshland as well as ones with several other plant scientists including Seymour Shapiro and Otto Stein whom I eventually would follow to the University of Massachusetts. In different ways at different times in my career each one of these BNL scientists had a tremendous influence on my career both at Brookhaven and at future institutions. All convinced me that Brookhaven, on both personal and professional levels, would be a great place to be in the 1950s.

Thanks to the intervention and enthusiasm of Marty Gibbs, my stay at Brookhaven culminated in a job offer on the spot. The mid-1950s coincided with explosive advances in science and wonderful financial support for photosynthesis research. The position offered to me was that of plant physiologist on the staff in the Biology Division of BNL. The job would include a generous operating budget, equipment as needed, the salaries for a technician as well as postdoctoral positions and travel expenses. There was no excuse for not succeeding! Those were the days! Upon returning to Berkeley, I announced my new job to Melvin who, along with me, had not known that the position was available. Serendipity again! The fortuitous job opening certainly coincided with Melvin's generosity in providing a way for me to visit my family in Rhode Island. His thoughtfulness and Marty's support combined to move me into the next stage of my career during an era that would enthusiastically support photosynthesis through government agencies for another quarter of a century.

On my own: Brookhaven National Laboratory, 1955–1960

My family increased to five, and still growing, moved back East in March 1955. I set up my lab, and the facilities were superb. I developed my own research program on microbial photosynthesis. With Marty Gibbs, I started a collaborative project that resulted in several published papers about the regulation of RUBP carboxylase synthesis in dark and light-grown *Englena*. We demonstrated that the enzyme was synthesized only in the chloroplast of light-grown chlorophyll-containing cells (Fuller and Gibbs 1956, 1959). Specifically, our work showed the *in vivo* regulation of enzyme synthesis correlated with the structural development of the chloroplast. During the previous summers, Marty had collaborated with several visiting scientists including Bernie Horecker, Irwin Gunsalus and others who had studied both the enzymes of sugar biosynthesis and the assymetric $^{14}CO_2$ label of sugars (Gibbs 1996). Joining Marty's group for a year, Otto Kandler, the well-known microbiologist from Munich, expanded upon the degredation studies of $^{14}CO_2$ fixation products. Our lab work taught us all a great deal about microbiology and enzymology. Post-docs and visiting scientists also began to appear in my own lab. Stuart Tannenbaum, with a PhD directed by David Shemin at Columbia and a post-doc with Tatum while I was at Stanford, spent several summers with me. Bob Smillie, from Canada and Australia, Irwin Anderson, John Bergeron and Sam Conti were among the outstanding post-docs who worked with me at BNL. I was fortunate enough not only to start in-depth studies on the comparative biochemistry of the carboxylase system (Smillie and Fuller 1959) but also research on the structure of the photochemical apparatus of a variety of photosynthetic microbes.

At this time the consortium of universities which operated Brookhaven National Laboratory allowed sabbatical leaves. Thanks to Rod Quayle and Hans Kornberg, associates from my days in Calvin's lab, I received an invitation from Professor Sir Hans Krebs to spend a year in Oxford. The National Science Foundation awarded me a Senior Research Fellowship. (Five years beyond the PhD turned one into a 'Senior.') Thus I and my family, now increased to six members, arrived in Oxford during the fall of 1960. Our new home belonged to Oriel College and was built in the 13th century. Serving as a hospital for lepers at one time, the house stood just outside the walls of Oxford. It was absolutely charming even though the lack of central heat guaranteed some chilly days and nights.

At Oxford I worked closely with Hans Kornberg, studying photosynthetic CO_2 fixation in the obligate anaerobic bacterium *Chromatium vinosum* (Fuller et al. 1961). My own work with Stoppani at Berkeley and Brookhaven had shown that Krebs cycle intermediates (Stoppani 1955), particularly malate and succinate, were labeled heavily by $^{14}CO_2$ in both *Rhodopseudomonas* and *Chromatium* at very short times. Both Krebs and Kornberg suggested that we examine the enzymes of this obligate anaerobe to see if these enzymes might be involved only in the protein and/or fatty acid synthesis rather than the energy-generating citric acid cycle. That the RUBP carboxylase was the initial photosynthetic CO_2 fixation enzyme in several species of purple photosynthetic bacteria had been demonstrated earlier by us (as referenced above). Early experiments at Oxford had indicated an apparent absence of both malic acid dehydrogenase and α-ketoglutaric acid dehydrogenase. By the time of my sabbatical at Oxford, Krebs had described the citric acid cycle as the major source of ATP, through oxidative phosphorylation. He mostly worked with pigeon heart miochondria. Krebs thought that all cells must contain these two enzymes for their roles in biosynthesis as well as oxidative energy production.

Krebs simply did not believe that my data showed that the purple bacteria, even being obligate anaerobes dependent on photophosphorylation**,** could synthesize the necessary bacteriochlorophyll without these enzymes. Wanting to watch me do the experiment, Krebs pronounced, "Clint, there must be some error in your techniques." The assays for these two enzymes were carried out spectrophotometrically on the then new Cary model 11 spectrophotometer. I made up cell-free preparations of *Chromatium* and *Rhodospirillum rubrum*. The latter, capable of growing aerobically in the dark through the use of an intact citric acid cycle, functioned as a positive control for these two missing enzymes. Using a cuvette with oxaloacetate as the substrate, NADH and buffer provided the assay for malic acid dehydrogenase. With *R. rubrum* as the control, the malic dehydrogenase source would reduce the oxalic acid to malate with the concurrent oxidation of NADH to NAD and the loss of its absorption in the ultraviolet. With Krebs and Kornberg watching over my shoulder, I added a drop of the cell-free *Chromatium* extract to the cuvette. No change occurred in optical density. Nothing happened to indicate the presence of

malic dehydrogenase activity. Then I added a similar amount of identically prepared extract of the *R. rubrum* (containing the identical amount of protein) to the same cuvette. In 10 s the NADH was oxidized. In this way Krebs and Kornberg became reluctant believers of my data.

However, Howard Gest and J.T. Beatty later demonstrated that the missing two enzymes actually did exist in *Chromatium* (Beatty and Gest 1981). Because of their tight binding to the cytoplasmic membrane particles, both enzymes in my Oxford experiments, apparently, had been spun out in the centrifugation of the enzyme preparation. So much for premature speculation lacking the proper controls such as looking for activity in all the cell factions. My only excuse might be that photosynthetic prokaryotes, in terms of cell biology as well as membrane-bound enzyme activity in both photosynthesis and respiration, involved a relatively unknown area of science in 1960. Today we know that the difference between *Chromatium* and *R. rubrum* could well be a simple variation in the density of the two cytoplasmic membranes. In my case, failed efforts become paths of learning. I was pushed onto newer and even more exciting areas.

A diversion to medical education: 1961–1968

Ending my Oxford sabbatical, I accepted a position at Dartmouth Medical School as Chair of a new Department of Microbiology. Marsh Tenney, the Dean and a distinguished mammalian physiologist, hired me. Primarily, I accepted the position because of the outstanding cast of characters hired by him to rebuild the Medical School. Shinya Inoue, a cell biologist from Princeton, headed cytology and anatomy. Inoue had hired Kenneth Cooper and Andrew Szent-Gyorgi. Biochemistry, originally headed by Manuel Morales, had hired Melvin Simpson, Peter von Hippel, Ed Westhead, Lucille Smith, Walter Englander and Lafayette Noda. Kurt Benirschke, a distinguished cytogeneticist, headed the Pathology Department. From The Massachusetts Institute of Technology, Walter Stockmeyer had just arrived to chair the Chemistry Department in the college. As of yet the Microbiology Department had not been developed. During the decision-making time, I was concerned that I neither had much academic or administrative experience nor was working in a research area directly related to medical education. I asked Marsh if he had any reservations about an inexperienced 35-year-old, who worked in photosynthesis. After all no one ever died of photosynthesis! Glaring at me, he replied, “How old do you think I am?” (He was 39!). Marsh was interested in hiring outstanding basic scientists who were oriented towards research and capable of interacting with the college science faculties at the undergraduate level to develop a medical and premedical program of distinction. He wanted me to do the same for the Microbiology Department, and I did. I hired S.F. Conti, a real microbiologist from Cornell who also had been a post-doc with me at BNL. I also recruited Lawrence Kilham, a senior medical virologist from the National Institutes of Health (NIH). Already at Dartmouth, Philip O. Nice, who had real experience in medical microbiology, together with several younger associates completed the team. These scholars well complemented the undergraduate biology department as well as the medical school’s departments of Biochemistry and Cytology. Our work on photosynthetic prokaryotes flourished.

Visitors to my lab included David Hughes initially from Oxford and then from Cardiff University in Wales where he was Professor of Microbiology (Hughes et al. 1963). Rod Clayton arrived to take a skiing and science sabbatical with us. From my lab and that of Sam Conti, post-docs initiated our joint work on *R. rubrum* and the green photosynthetic bacteria; they included Louise Anderson (Anderson and Fuller 1967a–d; Anderson et al. 1968), Stanley Holt (Holt et al. 1966; Holt and Fuller 1966), Elisabeth Gantt and Sam Conti (Gantt and Conti 1965, 1966). While we studied photosynthetic prokaryotes, those in Sam’s lab worked on the structure of the photosynthetic apparatus of the eukaryotic red algae as well as the prokaryote cyanobacteria.

During the period I was at Dartmouth important research carried out by Jessup Shively at Clemson University had a major impact on our knowledge of the structure, assembly and function of the RUBP carboxylase enzyme in photosynthetic prokaryotes. Started in the 1960s, this research was pursued during the decade, and led to the discovery of the carboxysome – a prokaryotic cellular inclusion consisting of the enzyme and six other proteins. The photochemical apparatus and photochemical electron transport system were organized inside the cell on the lipoprotein bilayered cytoplasmic membrane. The carboxysome was organized as a paracrystaline array of the enzyme and six glycoproteins and attached to the cytoplasmic membrane by the small subunit peptide of the RUBP carboxylase. This protein assembly in prokaryotes allows for rapid mobilization and regulation for

photosynthetic carbon dioxide fixation and subsequent carbon metabolism (Shively et al. 1988).

Interactions with Otto Warburg

During my stay at Dartmouth, another historically interesting event took place in photosynthetic research. The event involved Otto Warburg, the physician and giant of German biochemistry who had won a Nobel Prize in Physiology and Medicine in 1931. He also won a second Nobel Prize in 1944; however an order from Hitler intervened which forbade the acceptance by Germans of the Prize. Warburg belonged to a Jewish family, that had attained wealth and fame in the field of banking and the support of art as well as distinction in the fields of medicine and science. His father (Emil Warburg) was a famous physicist who was a Professor at Freiburg and Berlin. Although most of his family escaped from the Nazis during the 1930s and established their banking empire in London, Otto Warburg remained in Germany as head of the Kaiser Wilhelm Institute (changed after the war to the Max Planck Institute at Berlin-Dahlem). It is of historical interest to note that Warburg was not harassed personally or professionally by the Nazis. One reason was that his mother's family was not Jewish which made him only one half Jewish. Another reason was that Reichsmarshall Goering had made a declaration that "I decide who is a Jew" and arranged for a reexamination of Warburg's ancestry and ruled that he was only a quarter-Jew. During the war years another highly placed Nazi Reichsleiter Bouhler, Chief of Hitler's chancellery, personally protected Warburg when he was indeed critical of the regime. Warburg's willingness to let his Jewish blood be diluted and thus make a pact with the Nazis incensed both his family in England and his colleagues outside Germany. It is also worthy to note that both Hitler and Goering were hypochondriacs and terrified of cancer. Warburg was sure that his oxidation-reduction research was going to lead to a cure for cancer, a thought that he promoted widely. The above information, including the involvement of the Nazi hierarchy in Warburg's safety, is well documented by Professor Sir Hans Krebs in his biography of Warburg (Krebs 1981). Krebs spent 4 years as an associate of Warburg in Berlin before escaping to England (he was 100% Jewish). Krebs passed this information on to me personally as well as to others, prior to his publication of the biography while I was spending a year's sabbatical with him at Oxford.

After World War II, Warburg visited the United States, at the invitation of Robert Emerson and Eugene Rabinowitch at the University of Illinois (see Figure 5). Warburg was disputing the quantum requirement of photosynthetic O_2 evolution as observed by Robert Emerson and Carleton Lewis (1943a,b) and William Arnold (1949). In essence their disagreement boiled down to whether photosynthetic O_2 evolution required only three to four quanta of light or the eight to 12 quanta clearly demonstrated by Emerson, Arnold and others. Lasting for years, this difference in opinion never became completely resolved. Govindjee (1999) has reviewed this controversy and its background in an article for the Historical Corner in Photosynthesis Research; he explained the dilemma with a fascinating analysis of the Emerson and Arnold experiments versus the ones by Warburg. The dream of photosynthesis being perfect in nature and 100% energy-efficient drove Warburg. Of course, like everything else in nature, photosynthesis neither was nor is perfect.

That Warburg had a brilliant mind and intellect and was experimentally creative no one can deny. It was his conclusions not his experiments that created the great controversy. The distinguished biochemist Ephraim Racker (Racker 1972) stated this contradiction in Warburg's research. "Few will challenge the statement that Warburg was one of the great biochemists. His experimental approach was monumental and ingenious. Yet Warburg's views on two vital areas of his research interests, cancer and photosynthesis, are now almost universally dismissed as erroneous and naive."

I became involved with a Warburg controversy in photosynthesis research in the area of CO_2 fixation and the RUBP carboxylase *in vitro* and *in vivo* enzymatic experiments carried out by myself, Marty Gibbs, Andy Benson and our associates from 1954 to 1964. Warburg's interpretation of his results involved a hypothetical chemical reaction of carbonic acid with chlorophyll to form a complex that he referred to as 'phytolyte'. This complex required the oxidation of reduced carbohydate from respiration to form carbonic acid and the subsequent 'carboxylation' of chlorophyll to yield O_2 and reduced carbon; this process only required a low quantum requirementof 3 according to his results and calculations. This, of course did not agree with all the path of carbon research from the laboratories of Calvin and his associates on both the RUBP carboxylase system and the formation of PGA as the primary reduced fixation product from carbon dioxide.

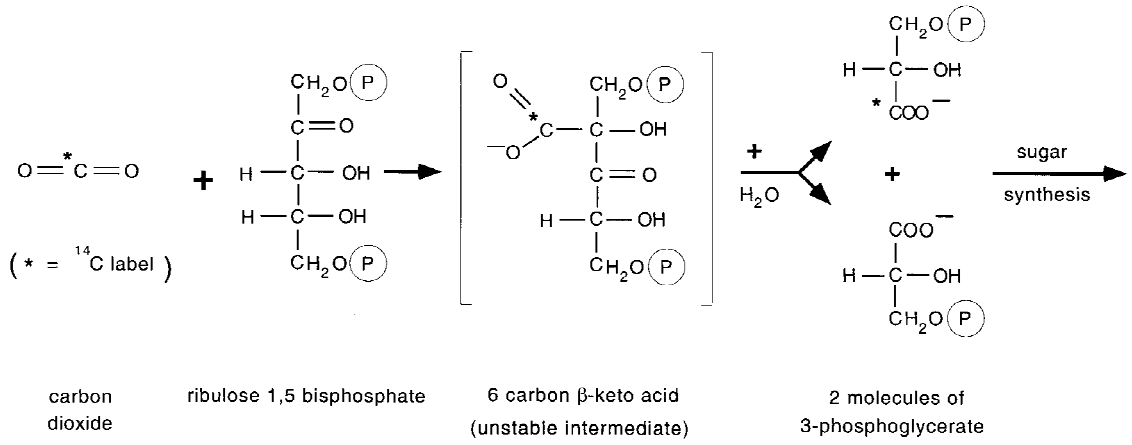

Figure 4. The reaction mechanism for the primary carboxylation and the formation of phosphoglyceric acid (see text).

In 1965 Andy Benson and I each received a letter from the Max Planck Institute in Berlin. (While I was educating Dartmouth medical students about energetics, Andy had gone to the University of California Marine Biology Laboratory in La Jolla.) My letter saluted me as 'Dear Professor Benson' and invited Andy to be a discussant of Warburg's symposium paper at a joint meeting among the Swiss, French and German Biochemical Societies to be held in Strasbourg. But Warburg's enclosed personal check for 4000 Deutchmarks was written out to *me*. When I got on the phone to Andy, he told me that his letter had accompanied a check for him, but the invitation had greeted Professor Fuller. In any case Warburg seemed to mix up a lot of things in addition to quantum requirements for O_2 evolution and CO_2 fixation in photosynthesis. I still have a photocopy of that check in fond memory of a great man who could be so very wrong. We subsequently found out that a third American scientist, Harlan Wood at Western Reserve University also had been invited. When we discussed the situation among ourselves, all of us expressed puzzlement over being chosen and some discomfort with what 'discussing' Warburg's presentation might mean. Nonetheless, we accepted, hoping that the Biochemical Societies would know what was going on.

Since 4000 Deutsch marks equaled about $1000 in those days, I was able to take my wife and enjoy a vacation with her after the Warburg symposium. When Andy, Harlan Wood and I arrived in Strasbourg, we received a note to us from Professor Helmut Holzer, a well-known biochemist from Freiburg who would be chairing the meeting on the following morning. His note directed us each to speak for 30 min after Warburg had made a presentation for an hour. We were still in the dark. Assuming that Warburg knew very well that all of our data and ideas completely contradicted his own, we had no idea what he expected. So we asked Professor Holzer what was going on. He explained that the symposium was to be a "Hanging Party, as you Americans say." In July of 1962 Warburg had attended a meeting in Gif-sur-Yvette, France, where he had expounded on his 'photolyte' hypothesis. (Warburg 1963). At the meeting, Calvin had responded to Warburg's talk with unusual restraint by simply sitting on his hands – in silence. Warburg had interpreted this reaction as an act of submission! As followers of the Calvin CO_2 fixation cycle, we three were to surrender publicly (I assumed) to Warburg in our discussions of his paper. Professor Holzer knew, of course, what we actually would do. Returning home that night, we all were very glad that we had cashed those checks!

The Warburg symposium was quite an event. On the following morning, most of the attendees waited on the steps of the convention hall to greet Warburg

Figure 5. Photograph of the Nobel laureate Otto Warburg in Robert Emerson's Laboratory at the University of Illinois taken in 1948. Standing with him is the then Assistant Professor of Botany at Illinois, Dr. Oswald Tippo who later became Chancellor of the University of Massachusetts at Amherst. In 1971 he hired the author of this Perspective as the Head of the Biochemistry Department at that institution.

upon his arrival. His long, black Mercedes-Benz with a license plate reading MP-B1 (Max Planck–Berlin One!) pulled up, and a liveried chauffeur opened the passenger door. Warburg stepped out wearing a suit, tie and 10-gallon Cowboy hat (perhaps from his Illinois days in the 1940s?). Jacob Heiss, his constant companion since World War I, scientific associate, laboratory manager and cook (Krebs 1981) followed him. The three of us discussants each were introduced. Looking me up and down, he shook my hand and exclaimed, "Herr Fuller, you are much too young!" A complement, I assumed!

His paper presented nothing new, simply the photolyte story with three to four quantum requirement, carbonic acid from respiration and O_2 evolution, all resulting from the 'carboxylation' of chlorophyll. As first discussant, Andy followed Warburg, giving a scholarly and elegant presentation of the whole path of carbon story from PGA through the Calvin cycle using radioactive $^{14}CO_2$ tracer. Warburg had rejected all the work that used isotopes as artifacts. Following Andy, I presented my work on the RUBP carboxylase enzyme including the regulation of its synthesis in prokaryotes and eukaryotic chloroplasts along with its absence in organisms containing none of the structural components of the phtosynthetic or the autrotrophic bacteria. Both Andy and I simply ignored Warburg's presentation. Harland Wood, however, did not. He was the world expert in the biochemistry and chemistry of carboxylation reaction mechanisms. Later Harland would discover the role of biotin in these types of reactions in mammalian and microbial systems. Presenting convincing descriptions of the biochemical and chemical mechanisms, Harland ended his talk with the following remark: "*Professor Warburg, I have presented a review of carboxylation mechanisms. From studying all such reactions known in biochemistry, and many possible ones in chemistry, I know of no known mechanism that would support your photolyte hypothesis.*" Then he sat down. This was no hanging party for us Americans! After the symposium not a word was spoken about the path of carbon. Warburg and the mayor of Strasbourg hosted an elaborate dinner and reception. In fact Warburg invited the three of us to his Institute in Berlin. Although my wife and I took off for a short holiday in Switzerland, Andy did visit the Institute. I assume that he became educated on the necessity of growing *Chlorella* in the light from a North-facing window in the lab and on the use of angle-held centrifuges to spin out the cells in order to repeat Warburg's experiments. Warburg, as Govindjee (1999) has pointed out, never did surrender. He died in 1970.

A transition

In 1966 I, along with three other department heads and many of the basic science faculty, left Dartmouth. The mass departures resulted from academic and, inevitably, political reasons. Since the story is long and complicated, I will not go into many details here. In short we molecular biologists not only had raised a lot of grant money but also had started a vigorous PhD program. We also were proceeding rapidly (too rapidly for the college administration) towards establishing a basic science medical program which was right on the cutting edge and should have met Marsh Tenny's early expectations. Our work, I believe,

played an important role in how Dartmouth Medical School evolved into the 4-year top-notch medical, research and teaching center for central New England that distinguishes it today. The event leading to our departure is a rather poignant, and somewhat amusing, example of academic disagreement. Sixteen of us (all full Professors) had written a proposal for the development of graduate programs that would form a graduate school, or its equivalent, for our Molecular and Cellular Biology PhD program; our proposal also included the appointment of a graduate Dean, instead of the Medical School Dean, for this program. After sending our proposal to the President, John Dickey, we asked for a meeting with him. Our meeting took place just before Christmas in 1965. We had delegated Andrew Szent-Georgyi as our advocate since he presented himself as being soft-spoken and cool-headed. I, on the other hand, had been ordered to keep my mouth shut despite my position as Principal Investigator of a large NIH Training Grant that had been awarded to run our proposed program. But I agreed happily and let Andrew do the talking. President Dickey told us that he, indeed, had read our proposal, which he called the 'Fuller document'. I cringed but didn't say a word. After some discussion, the exasperated-looking President gave us our answer. "Lady and Gentlemen", he began referring to Professor Lucille Smith and the rest of us, "Dartmouth is about to enter its third century. It has gotten along very well for the past 200 years without Molecular and Cellular Biology, and I perceive it doing very well in its third century with or without Molecular and Cell Biology. Good evening and Merry Christmas." On the way out, Shinya Inuoe quietly remarked that we really hadn't expected any presents this Christmas. Despite this last instance, my Dartmouth years were a positive personal and professional time. Microbial photosynthesis flourished.

An event that occurred in Calvin's scientific travels happened a number of years after I had left Berkeley and was on the faculty of Dartmouth Medical School. It was so typical of Melvin's quick mind and wit, topped with his inevitable dash of arrogance, that it is worth repeating here as it was heard by hundreds but as far as I can determine was never noted in print.

The year was 1963 and Melvin had been asked to give a Sigma Xi lecture at my old Alma Mater Amherst College. George Kidder my mentor at Amherst asked me down from Hanover to Amherst for the lecture and associated festivities. The lecture was titled 'Chemical Evolution and the Origin of Life' an area of interest to Calvin for a long time and lately a subject of research in the laboratory. The talk was to be given in Johnson Chapel a late 18th century church on the campus that was also used as a lecture hall for distinguished visitors. It was a convincing exposition. He described his work and that of others in the field, that organic acids, amino acids as well as pyrimidines and purines could be formed when an artificial environment of methane, nitrogen, ammonia and water were exposed to an electric discharge. There was a question period and a gentleman dressed in clerical cloth stood up and congratulated Melvin for his superb and convincing lecture. He continued: "Professor Calvin you have told us how living processes and life itself could start from these simple chemicals on the primitive Earth but please tell us what have you left for God to do?" Without a moments hesitation Calvin replied "God sent me here to tell you about it." A true Prophet! It brought down the house.

Oak Ridge National Laboratory (ORNL): 1967–1971

Later becoming the Department of Energy, the US Atomic Energy Commission (AEC) was the governing body of the USAEC National Laboratories in the mid 1960s. I had worked at two of these institutions: the Berkeley Radiation Lab at the University of California and Brookhaven National Laboratory. In 1967 I was offered a position as the director of a new enterprise that would become the University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences. This new and unique educational development happened after an annual scientific review meeting hosted by the AEC. The US Atomic Energy Commissioners, the heads of various Divisions of the ORNL and senior administrators from the University of Tennessee (UT) all gathered socially. Dr Dixie Lee Ray, a plant scientist and marine ecologist was the chairperson of the AEC. She later became governor of Washington. In a conversation with Dr Alvin Weinburg, Director of ORNL, she called all of us working at the National Labs 'the eunuchs of science'. She meant that we carried out all sorts of research in physics, chemistry and the biomedical sciences without reproducing ourselves! Dr Alex Hollander, Chairman of the Biology Division at ORNL, and Dr Andy Holt, President of the UT at Knoxville, also took part in this conversation, that would result in the idea of a new graduate school. In order to reproduce our knowledge, they came up with

the concept of the University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences. Using the research laboratories and scientific staff of the Biology Division ORNL, the school would train PhD graduate students for careers in the biomedical or life sciences. Students would be awarded degrees from the University of Tennessee at Knoxville. (Though only 20 miles from Knoxville, Oak Ridge was hidden away in the foothills of the Cumberland Mountains for security purposes during the war.) In 1967 I was appointed Director of the new school. We received handsome appropriation from the Tennessee legislature, gifts of old military housing in Oak Ridge for the students and the funding for several new academic appointments from outside of the Biology Division. I offered these positions to Drs Don and Ada Olin, cell biologists from Dartmouth Medical School as well as to Dr Frank Hamilton, a microbiologist-geneticist from Purdue University and the first African-American professor to have a tenure-track position at UT Knoxville. The four of us were full-time tenured professors at UT. Adjunct professorial positions from the Biology Division made up the rest of the faculty. Placing a national laboratory within the education business at a state university was a wonderful idea and has been copied at other institutions since then.

Following several distinguished colleagues in photosynthesis research to Oak Ridge also delighted me. The late Ed Tolbert had preceded me at both Berkeley and Oak Ridge. Bill Arnold, of course, was at Oak Ridge when I arrived. He contributed to studies on the concept of the 'photosynthetic unit'; the maximum quantum yield of oxygen evolution by calorimitry; solid state model of photosynthesis; primary photochemical reactions at extremely low temperatures; excitation energy transfer in photosynthesis and emission of light by plants (delayed fluorescence and thermoluminescence) (see e.g., a special issue of Photosynthesis Research honoring his achievements (Govindjee et al. 1996)).

Early on in my stay at ORNL, a memorable moment occurred with Bill Arnold. I was discussing my love for the simplicity of working with the primitive Green Bacteria. Bill paced around his office, cluttered with years of unopened mail, and ended up at the blackboard. He drew the perfect model for the mechanism of solar energy conversion to chemical energy as visualized by Bill and remembered by me (see Figure 6).

Then he exclaimed that I, as a biochemist, only needed to discover how the electron is made available. I have used his scheme in my teaching for years. Although Bill would have been a magnificent teacher, his love for science drove him to pursue a research career. His career enhanced the field of photosynthesis in many ways (Duysens 1996).

Our research lab at Oak Ridge was everything you could expect at an AEC National Laboratory. Two colleagues in the Division, Stan Carson and Fred Hartman, were particularly great as staff members who supported photosynthesis. While Stan was a former van Niel student, Fred was a protein biochemist who helped to unravel the primary and secondary molecular structure of the RUBP carboxylase enzyme. Postdoctoral students and visitors advanced our studies on the structure and function of the Green Photosynthetic Bacteria. During those years I spent too much time on administrative duties.

Amherst again: The University of Massachusetts, 1971–present

In the spring of 1971, I received an offer from the University of Massachusetts at Amherst to develop and chair a new Department of Biochemistry. Biochemistry had been sequestered in the Chemistry Department of the university since the 1950s; only two or three professors taught a few courses. No undergraduate major and no graduate program had existed. The biochemists had not flourished there. The newly appointed Dean of Science offered a position to Professor Ed Westhead so that the area of modern biochemistry at the University would be strengthened. An enzymologist, Ed had trained as a physical protein chemist. He was young but developing a national reputation in his area of research. He also was a fellow refugee of mine from Dartmouth. Before taking the position, Ed set down the condition that the University establish a new Department of Biochemistry. When the university agreed, he accepted, traveling down the Connecticut River from Hanover, New Hampshire to Amherst Massachusetts. His mission was to recruit a new head from outside of the University. Ed liked my work in microbiology at Dartmouth and wanted me to do the same in biochemistry at Amherst. Fortunately the timing was right as I wanted to step down from my Deanship at Oak Ridge to a job requiring less administrative duties. Heading a small department with only four to six new positions to fill and having more time for research seemed very attractive. As it turned out, I could do more research but could not

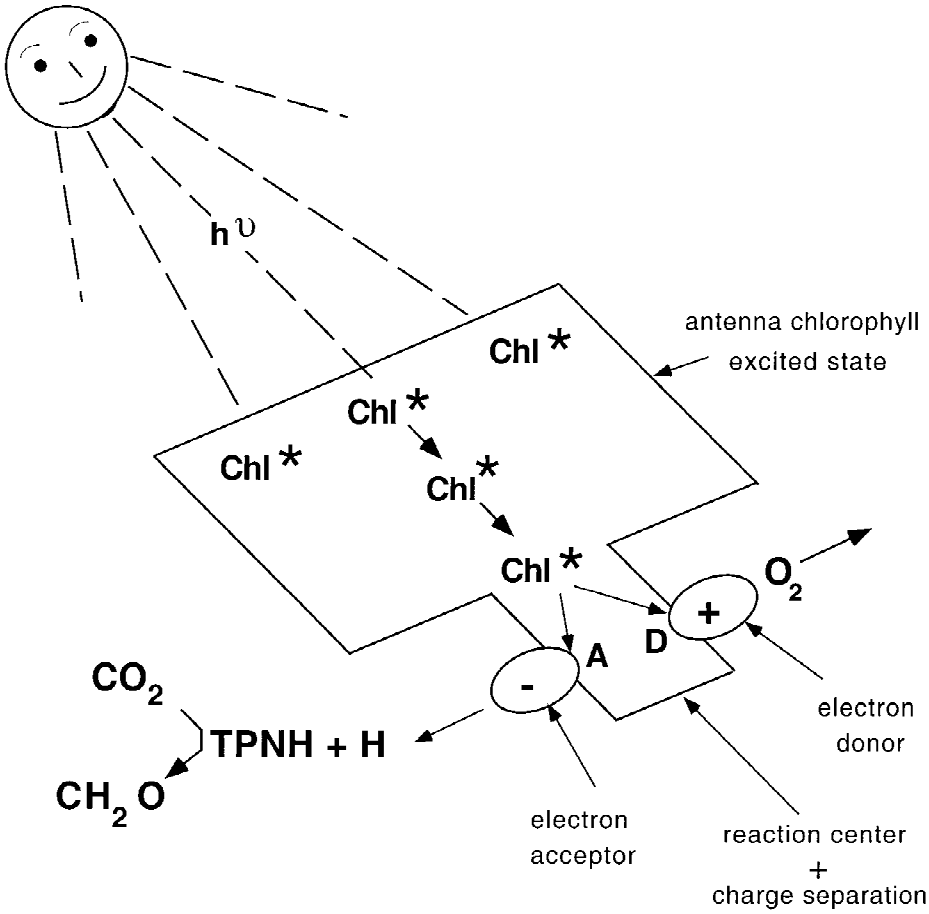

Figure 6. A rendition from a blackboard sketch by William Arnold at the Oak Ridge National Laboratory, given to the author to demonstrate the simplicity of photosynthesis.

leave the administrative functions behind. Serendipity again had come into play. Who knows where I, or our research, would have been if Ed had not left Dartmouth for Amherst in 1968. In any case, in the fall of 1971, I again moved to Amherst where 28 years would be filled with research, mentoring and teaching in two apparently unrelated fields of research: microbial photosynthesis and biomaterials together, creating for me an exciting future in microbial biochemistry and polymer chemistry. This perspective will conclude by describing the research carried out by myself and my colleagues at the University of Massachusetts on the green photosynthetic bacteria and in the area of biomaterials.

The photosynthetic green bacteria

At both Dartmouth and Oak Ridge, I had initiated work on the anaerobic green sulphur bacteria, several species of *Chlorobium* and *Chloropseudomonas ethylicum*, a new organism discovered by Elena Kondratieva at Moscow State University (Shaposhnikov et al. 1960). Since John Olson (1994) has written in detail about the history and science of this latter organism I will not repeat the information here. Brian Gray, one of my students at Oak Ridge, had demonstrated that *Choropseudomonas ethylicium* was a symbiotic consortium of a sulfate-reducing anaerobe (*Desulfovibrio*) and *Chrobium thiosulfitophilum* (Gray et al. 1973).

We also had obtained a culture of the newly discovered facultative non-sulfur filamentous green bacteria *Chloroflexus aurantiacus*. This wonderful new

discovery gave rise to a whole new family of facultative green bacteria, the Chloroflexiaceae. I feel that Beverley Pierson, Dick Castenholz and Bill Sistrom, all from Oregon State University at Eugene, derserve the credit for finding this organism. As I interpret Beverley's elegant description of their work, serendipity played a great role in their discovery that actually started in Yellowstone National Park (Pierson and Castenholz 1974; Pierson 1994). On a field trip to that park, she found a moderate thermophilic, filamentous organism which was nicknamed 'Orange Tuffy' (OT) by her. It was an orange, almost golden-brown organism. In love with this bug, Beverley proposed to Dick Castenholz that her thesis research be on it. He refused because the project would be too risky and difficult for her as a graduate student. However, she prepared a research plan and worked closely with Bill Sistrom to prove that OT really might be a photosynthetic green bacterium. The rest is history. Bill insisted that Beverley prove that OT had bacteriochlorophyll *a* as well as *c*. She did. Dick, as thesis director, enthusiastically jumped on board. This triumvirate had made history by isolating, culturing and characterizing the pigment systems of *Chloroflexus*. I believe that Beverley's enthusiasm, careful work and creative science combined in her to fit the definition by Louis Pasteur and Joseph Henry of 'a carefully prepared mind' capable of receiving and putting together serendipitous scientific events (Roberts 1989). That she chose to spend a 1981–1982 sabbatical year in Philip Thornber's laboratory at the University of California at Los Angeles was no accident. She and Thornber described in detail the reaction center of *Chloroflexus* (Pierson and Thornber 1983).

Our research in Amherst on *Chloroflexus* resulted in part from Bob Blankenship (Chief Editor of this Journal until December 1999), arriving in 1979 at the Chemistry Department of Amherst College, our neighboring institution. Across town from each other, both of our labs shared post-doctoral, graduate and undergraduate students who all worked at first on *Chloroflexus* and later on *Heliobacterium chlorum* (Gest and Flavinger 1983). The latter organism we obtained from Howard Gest. He has reviewed the research on this organism elsewhere (Gest 1994). Our considerable work with electron microscopy has been reviewed several times over the years (Sprague and Fuller 1990). We also described and characterized in detail the isolated purified photosynthetic apparatus of *Chloroflexus aurantiacus*. The OT thermophile indeed

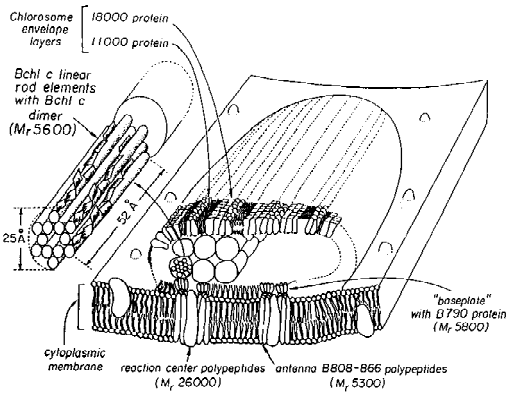

Figure 7. The green bacterial chlorosome as described in text. Reprinted from Feick and Fuller (1984), by permission of the authors. © American Chemical Society.

turned out to be a facultative green photosynthetic bacterium.

Our Amherst research covered the spectrum of the photosynthetic activities of this organism: energy capture; electron transport; carbon metabolism; molecular genetics; and macromolecular modeling. The joint research resulted in over 30 publications; I will note only a few that depended upon both the North American and European laboratories with which we collaborated: Blakenship and Fuller 1982; Bruce et al. 1982; Feick et al. 1982; Fuller 1989; Oelze and Fuller 1987; Redlinger et al. 1990; Sprague et al. 1981; Staehlin et al. 1978; Zannoni and Fuller 1988. This work in my laboratory involved students, post-docs and visiting scientists as well as collaborations with David Knaff in Texas and Davide Zannoni in Bologna, Italy. Two sabbatical leaves from the University of Massachusetts proved that one, indeed, could be intellectually revived at any age. My visits to the Institut für Mikrobiologie at Freiburg with Gerhart Drews for the year of 1977–1978 and to the Institute für Molecular Biologie at the Federal Technical University in Zurich with Herbert Zuber in 1985–1986 opened new avenues for me during my 'Chloroflexus years' (Wechsler et al. 1985).

My research with Reiner Feick, a former PhD student from the laboratory of Gerhart Drews, and who did post-doctoral work with me in Amherst, best summarizes the above work. Feick's work culminated with the development of a macromolecular model of the *Chloroflexus* cellular photosynthetic system shown in Figure 7 (Feick and Fuller 1984).

The importance of this model is that it demonstrates the separation of the antenna bacteriochloro-

phyll *c* (Bchl *c*) structure, the chlorosome, from the energy-producing cytoplasmic membrane. All the green bacteria contain the chlorosome as a light-harvesting, cellular inclusion. The chlorosome does not have the classical membrane structure of a lipoprotein bilayer as found in all electron transport energy-producing systems that either oxidatively or photochemically produce ATP. The lipoprotein bilayer is an essential structure for these latter activities in all eukaryotes (chloroplasts and mitochondria). In all photosynthetic prokaryote electron transport, ATP production occurs either within or across the cytoplasmic membrane, or on intracellular lipoprotein derivatives from them, such as the 'chromatophore' membrane system of the purple bacteria. With a few exceptions, all non-oxygenic photosynthetic prokaryotes use bacteriochlophyll *a* as both antenna and reaction center pigments. Only the green bacteria have developed the intracellular inclusion containing Bchl *c* as a primary antenna, shuttling the exited state to the energy-producing cytoplasmic membrane as depicted by the model presented in Figure 7.

Biomaterials: A new adventure in research – serendipity at its best

Along with the Chemistry and Polymer Science and Engineering Departments, our department had moved into a new research tower on the campus in 1973. Four elevators, designed to service the laboratories, were controlled by a computer program which must have been designed in the Neanderthal era. Perhaps members of the Mathematics Department, one of the largest undergraduate departments, suffered the most since their department occupied the sixteenth floor. One member, while waiting in the lobby, had enough time to calculate that close to 3 months out of the next 25 years in his career would be spent waiting for an elevator. The only advantage to cooling our heels in the lobby was that faculties of different departments could get to know each other, both professionally and personally.

One of my acquaintances from waiting was Professor R.W. (Bob) Lenz, a distinguished organic polymer chemist. We exchanged information on our areas of research interest; I described microbial and cellular photosynthesis while he explained the organic chemistry of polymerization. One day while waiting in the lobby, he told me that the Office of Naval Research (ONR) in Washington, DC, had just phoned him. The ONR, which had supported his research for years through its Chemistry Division, described to him a new program jointly funded with the Chemistry and Molecular Biology Program. The ensuing conversation between Bob and and myself would prove fateful. "Clint", he asked rhetorically, "you work with those crazy bugs that make polyesters, don't you?" Doing a double-take, I replied that out of all the 'crazy bugs' that had been involved in my research, I knew of not one that could make a polyester, or "material like this cheap shirt I'm wearing", as I put it. So much for my chemistry! We both laughed. But I was curious. When I asked what kind of polyesters he was working on, he answered compounds like polyhydroxybutyrate (PHB). I was astonished since I had thought of PHB as a lipid that had been messing up my cell fractionation studies in *Rhodosprillum rubrum* for years. Bob asked me, "What was a *Rhodospillum rubrum*?" So much for his microbiology. Bob referred me to the excellent review by John Anderson and Eddie Dawes fron which I learned both the basics and a great deal about biopolymers (Anderson and Dawes 1990). Thus we were launched into a decade of wonderful, exciting and productive collaborative research. On the spot we composed a reply to the ONR. We missed the elevator but got the grant. The ONR had a long history of supporting cutting edge basic research. Kenneth Wynne of the Chemistry program and Michael Marron of the Molecular Biology program deserve credit for supporting several groups around the country including Bob Lenz and myself in this new interdisciplinary area of biomaterials. In 1990, The North Atlantic Treaty Organization supported the first International Symposium on Microbial Polymers which attracted about 40 scientists from around the world. The symposium takes place every 2 years, and the last one in 1998 attracted over 300 participants to its meeting in Japan (Fuller 1999). This new field of endeavor is still on a roll! Serendipity enhanced the science and careers of two scientists blessed with open, well trained minds and slow elevators!

In 1925 at the Institute Pasteur, Lemoigne discovered lipid-like inclusions in cells of *Bacillus*. In the following year, he identified these inclusions as poly-β-hydroxybutyric acid, PHB (Lemoigne 1925, 1926) Although only a few successors of Lemoigne carried out research on these PHB inclusions over the next three decades, Mike Doudoroff and Roger Stanier at Berkeley rediscovered this work in the late fifties (Doudoroff and Stanier 1959). The first biochemical, intracellular studies on these isolated 'granules', now correctly referred to as PHA inclusions (Fuller 1990),

has been reviewed by Joe Merrick (1978). At the Massachusetts Institute of Technology Anthony Sinsky and Oliver Peoples as well as Alex Steinbuchel and Hans Schlgel at Göettingen had initiated research on the molecular genetics and physiology of *Alcaligenes eutrophus* (People and Sinsky 1989; Steinbucheel and Schlegel 1991). Bernard Witholt and his associates initiated ultrastructural, biosynthetic and molecular genetic studies using *Pseudomonas oleovorans* as the experimental system (Huisman et al. 1992; Lageveen et al. l988). In a most important contribution, the laboratory group of Douglas Dennis at James Madison University was the first to demonstrate gene transfer and expression of the PHA synthesis genes from *A. eutrophus* into *E.coli* (Kidwell et al. 1995; Slater et al. 1982, 1983).

Over the years research on the biochemistry of the PHA synthesis and degredation in a wide variety of prokaryotes including the photosynthetic bacteria has slowly emerged. These advances have been reviewed by Yoshiharu Doi at RIKEN in Japan (Doi 1990, 1995). Our own work, starting in 1987, concentrated primarily on Pseudomonads (Brandl et al. 1988; Foster et al. 1999; Fritzsche et al. 1990a–c; Lenz et al. 1992) as well as on several photosynthetic bacteria: *R. rubrum*, to *Rhodobacter spheroides* and *Chromatium vinosum* (Brandl et al. 1988, 1989, 1991). Our research group consisting of up to 30 associates at any one time, ranging from undergraduates and graduate students to post-docs and visiting scientists, completed studies on both the intra- and extra-enzymatic degredation of polyesters (Foster et al 1994, 1996, 1999; Gilmore et al. 1992). With the information gathered in the last decade or so, we can conclude that PHAs are found in a wide variety of free-living bacteria – never in eukaryotic cells or organisms. These polyesters are formed as copolymers with a variety of chain length repeating units (Doi et al. 1987, 1988). The polymers can be 'functionalized' by feeding the organisms on carbon substrates containing unsaturated double bonds, phenyl groups, etc. (Curley et al. 1996, 1997; Hazer et al. 1995; Kim et al. 1991, 1992). In summary microbial polyesters are biosynthetic, biodegradable and biocompatible thermoplastic elestomers of great potential for industrial, environmental and medical use.

My own most recent interest (Griebel et al. 1968) harks back to the studies by Merrick and Doudoroff (1961, 1964). These scientists first showed that isolated PHA inclusions were associated with enzyme activity for the synthesis and degredation of the polymer. In our laboratories, the research using *P. oleovorans* grown on octanoic acid as a substrate has established protein association with the polyester inclusion. In this system the PHA is a random copolymer of C-6, C-8, C-10 repeating monomer units. In addition the purified inclusion contains four major proteins that have been purified and identified as the polymerase and depolymase enzymes and two structural proteins. Using the immunogold antibody label of these proteins and electron microscopic examination, we could construct a macromolecular model of the PHA inclusion that is shown in Figure 8 (Fuller 1995, 1999).

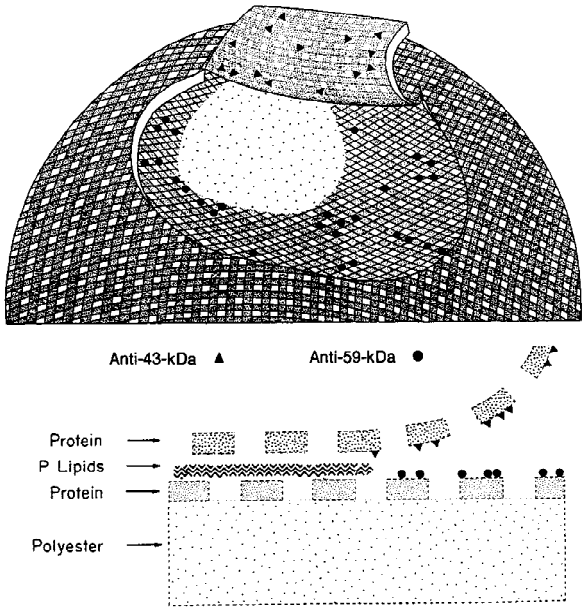

Figure 8. The polyhydroxyalkanoate cellular inclusion in Pseudomonads as described in the text. Reprinted by permission of the authors (Stuart et al. 1995), and the Canadian Society of Microbiology.

The organization of this prokaryote inclusion resembles that of the chlorosome in the green photosynthetic bacteria. Both are partitioned from the cytoplasm by a lipoprotein monolayer boundary, and both are organized as independent structures in the cell. Neither the PHA prokaryote inclusion nor the chlorosome in the green bacteria is structured as a classical membrane lipoprotein bilayer, which separates two water-containing compartments as in eukaryotes. Rather, both separate a water-based cytoplasm from a lipid-containing area. Although cost may prevent these biomaterials from being commonly used for awhile, we have made great strides in our knowledge of the

Figure 9. Down in the dumps? Not a bit of it. Clint Fuller and Bob Lenz, Professors Emeriti, in their most recent laboratory. Photograph from the University of Massachusetts, Amherst.

prokaryotic structure and function, as well as in a whole new era of material science in the last 10 years.

Bob Lenz and I now are retired Professors Emeriti who both stay active in the field. Our laboratories are closing down as the dollars rightfully flow to our younger colleagues, many of whom we have trained. As shown in Figure 9, we occasionally visit our field station in Amherst so that we remind ourselves of the horrible world of non- degradable plastic waste in the 20th century and dream of a future in biodegradable plastics in the 21st century.

This wonderful 50-year journey in science ranges from Protozoan nutrition via biochemical genetics and photosynthetic carbon metabolism, cell structure and function in photosynthetic prokaryotes to microbial biomaterials. This research has produced 225 publications and many honors. Of the latter, I most treasured the Chancellor's Medal from the University of Massachusetts in 1988, given to a faculty member for distinguished research and scholarship, for my work on photosynthesis. The award included an invitation to give a university-wide lecture; mine I entitled 'Light and Life'. It's nice to be so honored at home! In 1997 I received an honorary ScD, from Moscow State University in Russia. Very honored, I dedicated my award lecture to the late Professor Elena Kondratieva (Olson et al. 1996), a distinguished microbiologist who was my friend and colleague. She had trained many young students and had collaborated with many of us in work on microbial photosynthesis.

Ending this perspective, I would like to reflect a little more on the people who have traveled this road with me. I have cited many of my mentors, associates and students. My aging memory alone is responsible for any persons who may have been omitted inadvertently. Special recognition goes to those scientific colleagues who have been with me from the beginnings in the 1950s and are still be with me today. My thanks for being there at the right time go to Andy Benson, Martin Gibbs, Howard Gest, Sam Conti, Gerhard Drews, Herbert Zuber and, of course, Bob Lenz, my current colleague and partner. Serendipity and suc-

cess couldn't have happened if you all hadn't been there at the right moment. Clearly I owe a debt of gratitude to two administrators, along the path of this career: namley Marsh Tenney at Dartmouth, who hired a young and rather inexperienced person to help rebuild a school where I learned a lot and hope that I added some; and Oswald Tippo, Chancellor at the University of Massachusetts, who hired me in 1971 and was a valued colleague and friend until his death at 87 in the summer of 1999. He is pictured in 1948 with Otto Warburg at the University of Illinois in Figure 5 of this paper.

On a personal level, I am forever grateful to my wife and life-long partner Carol, who along with my now grown children, David, Kathy, Lynn and Jon, all joined my peripatetic career from 1946–1971. Living in California, Long Island, England and Europe was terrific. Being at Dartmouth, Oak Ridge and the University of Massachusetts, Amherst, where my career began and will end, were joyous times. My family had wonderful travels, camping trips, and expeditions to museums and concerts. Now my four children with our nine grandchildren are off on their own journeys.

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