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Annu. Rev. Plant Biol. 2002. 53:1–25
DOI: 10.1146/annurev.arplant.53.091201.142547

PAVING THE PATH

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Key Words Sam Ruben, Rat House, ORL, radiobiochemical exploration, neutron activation

■ **Abstract** Fortuitous preparation and experiences led to the opportunity to use radioactive carbon dioxides to discern the path of carbon in photosynthesis. The search for the CO_2 acceptor led to recognition of the growth stimulatory effect of methanol and its derivatives. With the techniques developed, radiochromatographic exploration led to discovery of major membrane lipids containing phosphorus, sulfur, and arsenic.

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INTRODUCTION

Confluence of events and experience led to successes in discerning the path of carbon in photosynthesis. Enjoying the excitement of understanding how Nature works, coupled with serendipitous good fortune, I find myself describing some of these adventures in the following pages.

Managed from Above

At Caltech, on May 12, 1942, I had just finished describing my synthesis of fluoro-iodo-thyronines, sphingosine analogs, neurobiology of the sea scallop and discussion of my 10 Propositions before my Doctoral Committee, when Linus Pauling asked one more question: "Andy, can you write on the board the differential equation for decay of a radioactive isotope?" Well, I managed to get it down all right, but it was a surprise. I had no idea why he asked that question; it had absolutely nothing to do with my thesis! But, knowing his approach after several years in his classes, I wasn't surprised and proceeded to forget all about that incident. He had embarrassed me one evening in the lab, finding me balancing a 12-liter flask of hot methanol on the edge of my sink and suggested that it might not be necessary for me to go further in his quantum mechanics courses. I had not done at all well in the final exam. So, why did Pauling ask me the question? Sixty years later it dawned on me that Linus Pauling and Wendell Latimer, Dean of the College of Chemistry at U.C. Berkeley, had contrived to provide support in organic chemistry for a young and rising star of the Berkeley faculty, Chemistry Instructor Sam Ruben, who was on the verge of being advanced to Assistant Professor.

Within a week or so, after my exam and the Linus Pauling episode, I received an invitation from Chemistry Department Chairman Joel Hildebrand in U.C. Berkeley to join the faculty of the Department as Instructor. The salary would be $2000.00 per annum. That was nearly $167 a month! It was a stellar opportunity and the beginning of my life within the path of carbon in photosynthesis, eventually leading toward the honor of preparing this chapter.

BACK ON THE FARM

My father delivered me on September 24, 1917, in the wood-shingled Sisters Hospital in Modesto, not far from a similar event, the birth of Osamu Hayaishi in Stockton. Two conflagrations were going on, the war in Europe (World War I) and a distressing fire of the horse barn across the street, not a comforting event for my wonderful mother. Home was in nearby Riverbank, overlooking the Stanislaus River and superb farm land. Carl Bennett Benson had started medicine at the University of Chicago, impressed by lectures of the great physiologist, A.J. Carlson. It was cut short by tuberculosis contracted from a roommate. He chose to recover in Los Angeles at the University of Southern California School of Medicine where a life-long friendship with classmate Montague Cleeves developed. Graduating in 1915 he accepted a position in Riverbank with the Santa Fe Railroad. You can imagine my impressions at the age of five, being in the center of a circle of steam-belching screaming locomotives in the great roundhouse. My father's patient Mrs. Foster phoned my father when her incubators were hatching, asking him to drive me to her farm. There, I was excited to watch the emerging turkey chicks, which she sold to local farmers. Californians will recognize the name, Foster Farms, managed now by her grandchildren who were delivered by my father. They now process two million chickens each week.

Coming from first-generation families of Swedish immigrants, farming in Minnesota, my father soon found a Minnesosta-like site near Modesto on the tree-lined bank of the Tuolumne River with a parallel verdant slough screaming with redwing blackbirds, roving coots, turtles, and, later, huge French frogs (we called them French frogs, though I don't know how French they were as they came from the nearby town of French Camp). He managed to establish an adjacent farm for his parents and brothers and sisters. No place on earth could have been finer for a young boy: pristine Nature and fine uncles for help and advice.

We moved from Riverbank to Escalon where my father established a small hospital and outfitted me with a donkey and small plough to work the vacant lot. Later, when we moved to Modesto, I rode my donkey, a two-day trip, which young reporter Max Foster noted in the Modesto paper as Another Ass Comes to Town. High school there was exciting. The band was highly ranked in the country, finishing second in national competition for two consecutive years. Amos Alonzo Stagg came to cheer our football team. Willie Brown, the chemistry teacher, had been on the Stanford football team. He was big, and everyone enjoyed his contagious enthusiasm. Students feared and loved him. At home a chemistry set enhanced my scientific enthusiasm, and the fifth of July, even more. Collecting unspent fireworks bombs early in the morning, I rebuilt them with new fuses and enjoyed creating mayhem at odd hours. It was hazardous sport like my experiments with my father's portable X-ray apparatus and fluoroscopic screen. I spent evenings studying and grinding and figuring a telescope mirror. Later at Caltech I visited the shop weekly to watch the grinding of the 200-inch mirror. Now, I carry my Caltech Athenaeum membership card signed by Edwin P. Hubble who was Secretary at the time.

UNDERGRADUATE DAYS AT UNIVERSITY OF CALIFORNIA, BERKELEY

As I finished high school in 1935, my father took me to Berkeley for an interview with Wendell Latimer, Chairman of the Department of Chemistry. He was encouraging, and I enrolled. There were only 20 students in chemistry lab sections with Professors Hildebrand and Latimer. Those two semesters were a true privilege. Chemistry classes were exciting, and with this select group of top students, the lab camaraderie was enriching. Daily, we were shooed out of the lab at five o'clock by Carlos, a unique janitor who would attract little attention except for his attitude. His kindly gruff forcefulness rarely failed to clear the lab. Only later did I realize that his forcefulness stemmed from his close relationship with the Dean, who I understand would sometimes need to borrow money from Carlos. With years of collecting postage stamps from the waste baskets of the Dean and Chairman, Carlos Abascal had become a resourceful stamp dealer. We maintained a delightful friendship and exchanged letters for several decades.

Hot summers were spent with Chick Cleeves in the dry yard of our apricot farm, making trays and managing the fruit cutting, sulfuring and then spreading the stacked trays from their wagons on the long rail track. It was hot but productive—nearly five tons of dried fruit at 14¢ a pound. Consequently, I am immune to sulfur dioxide. Tending irrigation levees, I know how to manage a shovel. I enjoyed building a brick house with a master brick mason from Hull, England, and developed sincere respect for master craftsmen as well as some skill at slacking lime and mixing "mud."

In my spare time I enjoyed some nonchemistry courses. I took an optical physics course from Luis Alvarez (later, Nobel Laureate) and remember his arrival for class one morning almost pale with fevered excitement. The word that Lise Meitner and Otto Hahn (Nobel Laureate) had reported nuclear fission was a cataclysmic shock in the Berkeley physics department. The optics lecture was dispensed with. Of course, the fact that Europe was on the verge of conflict enhanced his apprehension. I had been taking a superb course in European history from Professor Kerner who brought day-to-day tension to reality as Hitler took the Sudetenland and Austria. In the other corner of the campus I was enjoying courses in civil, mechanical, chemical, and electrical engineering as well as fluid mechanics. Having survived courses in physics, I found the engineering courses interesting, easy, ultimately useful, and just fun. A few of our lab mates often snuck under the cloud of smoke from the Alhambra Casino Philippine cigar of G.N. Lewis into his Thursday afternoon seminars. At the time I was making trinitrotriphenyl methane for Glenn Seaborg's research with Lewis on the color of molecules.

CLIMBING AT CALTECH: 1939–1942

With help from above, my applications to several schools were accepted. I chose Caltech because of its proximity to home and to the Cleeves family. Arriving together, Dick Noyes and I developed a lasting friendship. We took the first of

several qualifying exams in Pasadena, September, 103°F temperatures. That first evening, Dick broke his ankle at table tennis. He recovered well, and we enjoyed countless expeditions and climbs in the San Gabriels and the Sierra Nevada as well as scaling the wall of Crellin Laboratory early Sunday mornings.

In the Niemann laboratory, directly above Linus Pauling's office, I enjoyed fellowship and advice from superb colleagues. Jim Mead, a year ahead of me, guided the technical aspects of my research on synthesis of fluorinated thyroxine analogs, a 20-step synthesis that started with a gallon of anethole (anise oil) oxidized, in flames, within a flask of hot fuming nitric acid and ended with two grams of difluorodiiodothyronine. Jim was working on the structure of sphingosine, which later became part of my thesis work on periodate and lead tetraacetate degradation of its vicinal amino glycol. This experience led directly to my subsequent work with Al Bassham on sedoheptulose and ribulose degradations. I enjoyed rapport with Dick Lewis, son of Gilbert Newton Lewis; Chuck Wagner; John Hays; Ernie Redeman and Kurt Mislow, postdoctoral associates. Jim Mead led beach expeditions to a rented house in South Laguna where we fished with spear and snorkel for hours till we were blue. My handmade dive mask is now an antique. Mead was a close associate and good friend; I served as best man at his wedding. This association proved invaluable in 1960 when our family moved West and spent a year in Jim's Laboratory of Nuclear Medicine and Radiation Biology of UCLA's School of Medicine before moving to La Jolla and Scripps Institution of Oceanography.

The war in Europe was raging, and I recall clearly, as does everyone, the news of December 7, 1941. Conscription had begun and decisions were considered. Our family supported conscientious objectors and sympathized with the tenets of the Society of Friends. A small group of us at Caltech met frequently at the noon hour with Bob Emerson, descendent of the distinguished Quaker family. I registered as a Conscientious Objector. This later became a problem for my position in the Berkeley chemistry department, which was to become increasingly involved in research for the war effort.

BACK TO BERKELEY

Life in the Rat House

So when I arrived in Berkeley, Latimer directed me to the Rat House where Sam welcomed me to a small office/laboratory and a mass of dirty glassware freshly deserted by Henry Taube (Nobel Laureate, 1983). Sam introduced me to the Warburg Apparatus in the dingy lab upstairs lighted by bare clear light bulbs. He handed me his copy of Manometric Methods by Burris, Stauffer, and Umbreit and explained that he needed to know more about the ratios of photosynthesis to respiration in his *Chlorella*. Needless to say, I did not realize at the time that Bob Burris was but a few years my senior, nor that his son John would later be earning his doctorate in my laboratory, nor that his student Nathan Edward Tolbert (20, 23, 29) would become a close friend and colleague who later would convince the world that photorespiration exists (in spite of my failure to consider it very remarkable).

Ruben & Kamen (28) had observed "reversible fixation" of $C^{11}O_2$[1] forming a water-extractable product possessing a carboxyl group. They had deduced from their early fixation experiments that the product(s) of dark fixation could result from the reversibility of the reaction—$RH + CO_2 \leftrightarrow RCOOH$ (28, 34). van Niel pointed out that in bacterial photosynthesis no O_2 is produced and that bacteria must have access to a reducing agent to provide hydrogen for the reduction of CO_2. (No oxidizing agents present in living things are powerful enough to dehydrogenate water except for the photochemical reaction centers of photosynthetic organisms.)

At each step of these developments Sam Ruben and Martin Kamen proposed cyclic processes for the regeneration of the CO_2 acceptor, R-H. With the imposing influence of the phosphorylation processes being developed by Herman Kalckar and by Fritz Lipmann, Sam Ruben increasingly leaned toward the concept of the "high energy phosphate bond" participating in the process. At this point, I arrived in the Rat House and was immediately influenced by Sam's enthusiasm, as expressed in "Photosynthesis and Phosphorylation" (29). Sam was also influenced by the exciting developments from Otto Warburg and handed me copies of the latest papers from Berlin. I had no idea that in a few years I would be with Otto Warburg in his laboratory in Dahlem or that he would send me and Clint Fuller checks for $1000.00 to attend his lecture in Strassbourg in 1963 (17).

I joined Sam and his students in the many late-night experiments with $C^{11}O_2$ (14). They always began at approximately 8 p.m. because Martin needed the time for the bombardment of his boron target after the physicists on the "37 inch" cyclotron left for supper. On maybe three occasions, Martin called just before the experiment, "Cyc's sick, Sam": a disappointment, but an opportunity for us to go home early. With a bombardment completed though, Martin connected his evacuated "aspirator chamber" to the target and collected its gaseous $C^{11}O_2$ and $C^{11}O$. Owing to the short half-life of C^{11}, there then followed the 100-yard dash from the cyclotron to the Rat House and Sam's waiting arms and his demand to "leave at once" (19). Martin was far too radioactive. The aspirator was coupled to a copper oxide–filled tube within a hot combustion furnace for conversion of the gas mixture to pure $C^{11}O_2$ for the photosynthesis experiments. At first I was a helper while more-experienced Charlie Rice and Mary Belle Allen performed their preplanned duties. Each experiment was meticulously planned and orchestrated by Sam. I recall no experiment that failed because of errors in planning. I attended some planning discussions with Martin and Sam in the empty classroom. I was horrified at the vigor of their arguments, but they always reached agreement on the experimental plan unscathed. Sam managed the stopcocks and transfers from the liquid air-cooled spiral trap for the $C^{11}O_2$ to the waiting algae. Never was there any friction involved in working with Sam Ruben. He was a gentle human who drove himself but did not demand the same of his colleagues. He certainly appreciated

[1]I use the "atomic weight superscript" terminology of the time. I consider it didactically superior to the current practice, which I use in a following page.

their efforts with good humor. Charlie Rice was "Rice Crispies," and Mary Belle Allen was "Madam Curie." Sam revealed little of his family life in the lab (18). With little children to manage it was not at all easy for him or for his talented chemist wife, Helena. Sam was very interested in the Menschutkin Reaction mechanism studies of Denham Harman; I remember he repeatedly called out "Denham!" for some reason or other. Now I enjoy frequent contact with Denham, the father of our widespread concern for free radical involvement in human aging. Those late-night experiments in the Rat House were the last of a long series (28, 34). Though long-lived radiocarbon (C^{14}) was "invented" by Ruben and Kamen (27), they had not applied it for photosynthesis study; its specific activity was too low and its measurement too tedious.

Soon it was 1943 (and World War II), and Sam's efforts were directed toward a meteorological effort related to movement of gas clouds. Martin Kamen, beset by the House on Un-American Activities Committee and the State Department, was engaged by the local shipyard (19). At this point Sam gave me all of the $BaC^{14}O_3$ in the world to follow the path of carbon. Coupled with an appointment in the finest chemistry department in the country, such a golden opportunity now seems nearly unbelievable. At the time I was too embedded in the problems at hand to realize where I trod in the path of science. The path of carbon had been paved, but its end still lay beyond the horizon.

With the $C^{14}O_2$ I carried out many dark fixations with *Chlorella*, following Sam and Martin's concept of reversible reaction with R-H to yield R-COOH. To reduce the respiratory exchange, they were done in nitrogen—not a good idea. Hence, the product of such fixation was a massive accumulation of succinate. (Science is more about failure than success.) Counting C^{14} was a chore, and it was difficult to achieve reproducibility. Sam had some "Libby Screen Wall Counters" in which the weak C^{14} betas could fly through the screen wall into the G-M counting region. Because they required repeated assembly with deKhotinsky sealing compound each time, I made new ones with standard taper joints, which could easily be opened and reused. I prepared my samples on the interior surfaces of glass cylinders rotated on rollers and dried with hot air blown from a small burner. They could slide over, or away from, the screen wall of the G-M counter. Each was filled with counting gas on the high-vacuum line, and its voltage plateau determined. With the low activities, the work was tedious.

I have been asked about the evolution of our "photosynthetic algae illumination vessel," which Melvin Calvin and others called a "Lollipop." It was a result of Sam Ruben's effort to illuminate a dense algal suspension as uniformly and compactly as possible in thin flat circular Warburg flasks having one or two stopcocks and standard taper joints. I merely turned it on its side so that it could be illuminated from both sides of the thin round vessel and mixed by shaking or by an inserted gas stream. With a large bore stopcock on the bottom for rapid collection of experimental samples, it had assumed the outline of a flat lollipop.

To follow the chemical reactivity of the unknown product(s) I converted the fixed activity to derivatives, using diazomethane to methylate carboxyl groups,

and acetic anhyhdride in pyridine to acetylate hydroxyl groups. Evidence for reaction was estimated from behavior of their radioactivity's partition between water and ether or water and ethyl acetate. I could confirm the conclusion of Ruben and Kamen that the product possessed a carboxyl group; the partition coefficient between water and ethyl acetate was 0.14. (Three years later, in ORL, with the daily helpful collaboration of Ed McMillan (Nobel Laureate, 1951), I crystallized the dark fixation product and co-crystallized it with succinic acid to constant specific activity. Little did I realize that he was also involved in crystallizing salts of Neptunium.)

Rat House (Berkeley) Continued

My thesis research under Carl Niemann at Caltech had involved study of the structure of the vicinal aminodihydroxy analogs of sphingosine. I was intent on establishing the location of its amino group in relation to that of its two hydroxyl groups. Clearly, periodate oxidation was a viable approach. During my three years at CalTech I had followed all the carbohydrate chemisty courses delivered by Carl Niemann, former sudent of Karl P. Link in the University of Wisconsin, Madison. All of Niemann's many publications with Link were on the subject of carbohydrate chemistry. I was probably more familiar with carbohydrate chemistry than any other faculty member in the Department of Chemistry when I arrived at U.C. Berkeley as Instructor in July, 1942. My undergraduate classmates, Dick Powell and Bob Connick, came later. The true carbohydrate scholar, of course, was the lovable Zev Hassid with whom I had become well acquainted while working with Sam Ruben in the Rat House. When I was asked by G.N. Lewis to present the Thursday afternoon seminar, I chose the then-novel periodate oxidation as my subject. I had done some of the critical experiments in the Rat House before the seminar, and the sphingosine problem was finally solved. As usual, the seminar began with G.N. striking a big match and waving its flame in front of his famous Philippine cigar. When I was finished, he asked several penetrating questions, as usual. Melvin Calvin was there, but I doubt if he recalled any of the seminar's content; carbohydrate chemistry and periodate oxidation were beyond his interests at that time.

Phosgene and Ruben's Accident

Sam had visited the army's Dugway Proving Ground where tests with goats indicated that phosgene exposure induced lung edema and that the fluid was similarly antigenic to other goats. Having been exposed to the immunological studies of Dan Campbell and Linus Pauling, my theory was that phosgene, a double acid chloride, could couple two proteins or so alter the conformation of one protein to yield a novel antigenic protein. So I developed rapid phosgene synthesis from the 20-minute half-life of $C^{11}O_2$ from Martin Kamen and the 37-inch cyclotron (27a). Sam's technique with the phosgene-exposed rat was to drop the victim into the Waring Blender to produce a protein preparation for measurement of its C-11. For Sam's own phosgene experiments I had fitted small steel bombs with valves

so I could fill them with phosgene from the ancient Kahlbaum ampoules lying in sawdust in back of the chemical store room. Not having a chemical hood I did this carefully with ice-cooled ampoules and vacuum transfer to the steel bombs. I still insist that all students should recognize the odor of phosgene.

Because of my tenuous "conscientious objector" draft status, Dean Latimer was unable to extend my teaching contract, and I left for Civilian Public Service in the Nevada mountains, fighting forest fires, building dams, and logging for Forest Service construction projects. A group of technically skilled members was selected for a photogrammetry group in Reno, producing maps from aerial photographs. This led to field work for the maps in the hot, fire-prone foothills of northern California. Again with help from above, I was transferred to the Stanford Chemistry Department under Professor Francis Bergstrom to make antimalarial drugs. This, again, fostered new friendships, with Murray Luck (founder of Annual Reviews), Dick Ogg, Carl Noller, and Ted R. Norton who returned to Dow after the war. Ted later headed the Agricultural branch of Dow Chemical Company and hired Melvin Calvin as consultant for Dow. The Stanford experience was valuable and delightful. It ended with transfer to the related project at Caltech where I enjoyed exciting visits with Sam Wildman and Art Pardee.

A few weeks after I had departed in July 1943, Sam's phosgene supply became exhausted, and with his broken arm in a sling, he tried to transfer liquid phosgene (b.p. 8°C). Too impatient to cool the ampoule in ice salt, he immersed it in liquid air. The aged soft glass cracked, releasing the deadly liquid into the boiling liquid air, splattering it all over his wool sweater from which it was impossible to escape. It is said that Sam carried the boiling cauldron out of the building to avoid further dangerous exposures, then lay down on the grass by Strawberry Creek. One of his student assistants was hospitalized, and Sam succumbed in a day as his lungs filled with fluid. Only a week before, I had received a thoughtful letter from him. This great tragedy of science left the path of carbon without its real leader and Sam's family without the future it deserved (18).

May of 1946 brought the invitation from Melvin Calvin to join his bio-organic group as Associate Director of the not-yet existent photosynthesis laboratory. This would be a separate branch of his Bio-organic Group, which was developing C-14 techniques and compounds for medical applications in collaboration with John Lawrence, M.D., the brother of Ernest Orlando Lawrence, who had pioneered treatments of leukemia with radioactive phosphate.

ORL

The Old Radiation Laboratory (ORL) was the birthplace of the nuclear age and home of Ernest Lawrence's 37-inch cyclotron in the center of the old maple-floored well-built wood building. Though still functioning, the cyclotron was scheduled for transfer to UCLA's physics department. We occupied the long room just west of the central cyclotron area. Its floor was orange-yellow with uranium residues

and somewhat radioactive. This problem was easily solved by a cover of low-cost brown linoleum.

I was trained in the excellent organic laboratories at Caltech, which contained some features born in European laboratories. So, the chemical hoods, lab benches, and facilities I designed for our space in ORL worked out very well. Full fluorescent fixtures were placed directly over the black hardened plate glass bench tops where tiny vials would not tip over, and if they did, a spill could be quantitatively recovered. The largest white vitreous porcelain sinks with high swing faucets with long flexible extensions were a great success with large equipment (and, later, for my controlling an explosion and fire in our laboratory at Penn State.) For viewing the radioautograms made from the two-dimensional paper chromatograms I designed a large white Formica table where they could be compared and discussed. I designed a vacuum evaporation system, based on one from Caltech, which evaporated solutions faster than the popular Büchi Rotavapor apparatus that came later. Each $C^{14}O_2$ experiment involved evaporation of the algae or leaf extract. There must have been a thousand of them. I always claimed that I could boil water faster than anyone.

That experience was reinforced by my delightful interactions with Hans Schmid from the Chemistry Department of the University of Zürich. I worked with Hans for several months on the vac line, preparing $C^{14}O_2$ and making the necessary measurements of the C^{14} activity that I had developed with Ruben in 1942–1943. After Schmid, came Roger Boissonnas' brief but delightful visit for the same purpose. Soon I had the good fortune of meeting Roger's Geneva classmate Gérard Milhaud and his charming wife, Vera, in Paris in 1952, the beginning of permanent scientific, cultural, and adventurous experiences on many research ships and river boats.

The stream of distinguished visitors passing through ORL included Ernest Lawrence and Philo Farnsworth, working on their three-color gun for television, later the SONY Trinitron; Irène Joliot Curie; Emilio Segré; Ed McMillan; Georg von Hevesy; George Burr; Hiroshi Tamiya; Fredrick C. Bersworth; and countless others.

Hiroshi Tamiya and Kazuo Shibata

Hiroshi and Nobuko Tamiya came to Berkeley from Japan during the months of September to December 1952, staying with Mrs. Kami on University Avenue. They provided a wonderful new insight into Japanese culture for all of us. He had been trained in the laboratory of René Wurmser during its leadership in development of biochemical redox systems. In Melvin Calvin's home he was always the star of the evening. Nobuko was teaching Japanese cooking, though her training at the Cordon Bleu was in French cuisine. She captivated the hearts of everyone. At the end of the 1930s Hiroshi was to begin research with C-11 from the RIKEN cyclotron, one of the four in the world. He often told me of the sad demise of the RIKEN cyclotrons destroyed during the American occupation. With his friend Harry Kelly, scientist with the Supreme Command during the American occupation, Hiroshi, physicist Seiji Kaya, physicist Ryokichi Sagane, and nuclear physicist Yoshio Nishina had led the reorganization of RIKEN (33).

I felt fortunate to be the focus of Hiroshi's interest in photosynthesis. He was anxious to confirm conclusions from his kinetic observations of the effects of oxygen on photosynthesis. We did a number of $C^{14}O_2$ fixation experiments in the hood at the east wall of our first room in ORL, with and without oxygen, but their meaning seemed to be obscure. I regret that we could not reach a definitive conclusion from the experiments. My many experiments showing the great accumulation of glycolic acid during photosynthesis in air as compared to that in air with less than normal oxygen had verified Hiroshi's predictions.

Hiroshi's further contribution to our science was his arrangement for his student Kazuo Shibata, son of Seiho Takeuchi, leading Japanese painter of the turn of the century, to join our group in ORL. That was an important step in the enhancement of our appreciation of Japanese culture and science. He joined us later aboard R.V. Alpha Helix at the Great Barrier Reef where he discovered algae with unique UV-absorbing properties. Kazuo became a friend of everyone and my best friend and adviser until his untimely death.

Paper Chromatography: The Solvents

Bill Stepka, a student in plant nutrition, brought paper chromatography from Rochester where it had been established in F.C. Steward's laboratory by C.E. Dent. Two-dimensional paper chromatography effectively separated amino acids, sugars, and other groups of compounds. Their solvents included noxious and sickening lutidine and collidine as well as phenol; each required separation of the organic phase from the "water phase" before use. The physicists in offices near our Chromatography Room on the second floor of ORL were so sensitive to such odors that several were taken to Cowell Hospital for treatment.

I formulated a phenol-water solvent by using distilled phenol with 40% of its weight of water. This gave a water-saturated phenol solution at room temperature. I selected propionic acid for addition to n-butanol for acidification and for enhancing the water content of the "organic phase" of our solvent. Knowing the necessary amounts of water, butanol, and propionic acid, I prepared two solutions, one of water and butanol and the other of water and propionic acid such that equal volumes of the two solutions would yield a solution identical with the organic phase of that system. This was simple, avoided esterification, and allowed chromatographic separation of a host of components, from lipids to polysaccharides and sugar diphosphates.

Paper chromatographic separations are, in effect, gradient elutions. The solvent loses water to the paper as it travels and becomes more "organic" in composition. Thus the sugar phosphates separated with the water-rich solvent and later the phospholipids and pigments separated in the more organic, less aqueous, solvent. I demonstrated this by analyzing the solvent composition as it traveled on the paper.

Paper chromatographic separations are the result of "partition" between the moving organic phase and a stationary aqueous phase. The partition coefficient for each substance and solvent system is unique; this was the basis of my 1943

study of the products of dark $C^{14}O_2$. I recall two of Glenn Seaborg's seminars on his work defining the actinide series. There it was clear that partition between two immiscible solvents can provide valid information, even when only a few atoms are involved.

The primary attribute of our application of two-dimensional paper chromatography (6) was the result of applying an aliquot portion of the "total extract" of the plants labeled in the experiment. Others had complicated interpretation of their work by chromatographing several kinds of extracts separately. It is absolutely essential that the whole assembly of products be examined in the chromatogram. Nothing can escape. The insoluble proteins and polymers then remain on the origin. They could be measured and treated chemically or enzymatically for further resolution and identification. My students may recall that I urged them to carry a sharp pocket knife for cutting out radioactive spots from the chromatogram for elution. As my father had said, "Without his pocket knife, a man is naked."

"Standardyes" for chromatographic Rf orientation were selected from Mr. Ray's fabulous chemical storeroom, on the basis of their chemical structures and estimated relative water/solvent solubilities, for co-chromatography with labeled compounds. The mobilities of Tropeolin (Orange II) and Ponceau-4R (red) were reasonably reliable for comparison with C14 compounds, the Rf of Tropeolin being the most useful. Hence one could judge the position of a labeled compound by virtue of its mobility relative to those of the standardyes, even after the solvent front had disappeared.

Pattern Recognition

Following his doctorate in ORL, Alex Wilson, as Dean in New Zealand's University of Waikato, hoped to measure "genius" in students. He realized that there are at least two types of genius—those capable in mathematical concepts and those capable in "pattern recognition." The first category includes the peoples from Asia and the Middle East, strong in numerical and business transaction capabilities. The second includes the artists with their strengths in recognition of color or structural relationships. The real geniuses in pattern recognition, Alex said, are the Australian Aboriginal "Black Trackers" whose capabilities for tracing animals or criminals over a boulder patch are well documented. Their powers for observing, storing, and detecting relationships in visual information are phenomenal. Such was the recognition of novel information revealed in the hundreds of two-dimensional paper chromatograms accumulating in our studies of $^{3}H_2O$, $^{14}CO_2$, ^{35}S, ^{32}P, ^{74}As, ^{75}Se, and ^{125}Sb metabolism.

Norway 1951–1952

Melvin Calvin, his wife, Genevieve, and her Norwegian mother visited Norway after his 1949 coronary infarct and recovery, during which I wrote our review for the *Annual Review of Plant Physiology*. At the agricultural college, Professor Lindemann induced Calvin to send a colleague to Norway to establish a laboratory

for radioisotope applications in agriculture. This resulted in my appointment as Fulbright visiting professor and in delightful experiences for my wife, Ruth, and our four children. It also began our friendship with the Helge Larsen family and with pharmacognosy Professor Arnold Nordal and his family. Nordal had generously provided me with his pure sedoheptulose and knowledge of its chemical properties. Later we collaborated in ORL and at Scripps Institution of Oceanography. Several excellent students came from Norway (25).

Invitation from Otto Warburg

While in Europe I was invited to present a paper (4), a comprehensive review of the path of carbon, including the carboxylation of a C_2 from ribulose diphosphate to yield PGA, at the meeting of the Bunsengesellschaft für physikalische Chemie in Lindau, a distinguished group of photochemists, which included Otto Warburg. Sam Ruben had introduced me to Warburg's works, his algae, and his manometry. He was very interested in our results and in an opportunity to recruit our support for his heretic contentions that four quanta were required for fixation of CO_2 and production of O_2. He invited me to Berlin to observe how he grew his special *Chlorella* and made his measurements. It was a delightfully impressive experience to have lunch with him and Herr Heiss in their home. After lunch I walked just down the street to the quiet Dahlem Museum and into a small room where I found myself alone—with *Nefertiti*. That thrilling experience still brings out the goose bumps. I appreciated Warburg's arguments, though they seemed easily interpretable on the basis of my own experiments with preilluminated algae. Later, with the Linderstrom-Langs at the Carlsberg Laboratory in Copenhagen, I presented another seminar, again with Otto Warburg in the audience. (He was visiting Denmark to see his allergy physician.) On a beautiful afternoon I drove him and Herman Kalckar to Hamlet's Castle at Helsingör. Warburg peered through an iron grate into the darkness below, "Ach, it's a perfect place for that Midwest Gang." This, of course, followed Warburg's stay in Urbana where the polemic over quantum requirement of photosynthesis had become heated.

Fraction 1 Protein: Exciting Months in the Laboratory with Jacques Mayaudon, 1954

Jacques Mayaudon came to our ORL with a Fellowship from the Belgium Foundation, I.R.S.I.A., 1954–1955. The project that Melvin Calvin asked Jacques to develop proved less than stimulating, and he came to me in ORL hoping to work on photosynthesis. Our laboratory had discovered most of the important aspects of the path of carbon in photosynthesis (3) by that time. Melvin and the whole laboratory were intently concerned with his exciting theory of the role of thioctic acid (1, 15) in the quantum conversion process of photosynthesis (17). I was anxious to follow the carboxylation process by demonstrating and, hopefully, isolating the enzyme responsible for the process that we called carboxydismutase. Jacques Mayaudon was the ideal collaborator.

Very fortunately I had frequently visited Sam Wildman at Caltech in Pasadena where my wife's family resided. I followed Wildman's (35) progress in isolating and characterizing the major protein of leaves that he named Fraction I Protein. But neither Wildman nor his associates at that time considered its possible function as the critical enzyme in the path of carbon in photosynthesis.

I had developed a procedure for isolating unlabeled ribulose diphosphate from *Scenedesmus* algae using C^{14}-ribulose diphosphate as a marker for selecting the proper stripe area of my one-dimensional paper chromatograms for elution of the unlabled compound. By that time I knew how to enhance the concentration of ribulose diphosphate by withholding CO_2 prior to extraction of the algae and then adding $C^{14}O_2$ to ribulose diphosphate in a buffered solution of the enzyme produced carboxyl-labeled phosphoglycerate, which was a measure of the activity of the enzyme.

Soon it became clear that our carboxylase activity was being concentrated by the same ammonium sulfate precipitation isolation procedures as Wildman had followed with his Fraction 1. It was exciting, and we worked feverishly up to the time I had to leave for Penn State. I consider our discovery that the enzyme catalyzing carboxylation of ribulose diphosphate was the same predominant protein isolated by Sam Wildman one of my most exciting revelations (33). Forty-six years later, Sam Wildman still recalls my phone call with the news. I typed a manuscript and submitted it through Radiation Laboratory channels. Melvin reported the isolation a few weeks later at the Brussels Biochemistry Congress, but the real discovery of its identity with Fraction I protein only appeared in a paper by Mayaudon two years later (23).

LIFE WITH LIPIDS, COPEPODS, SALMON, AND THE SEA

Radiochromatographic Exploration

Several discoveries resulted from recognition of novel products or relationships revealed on film in the radioautographs of the chromatograms, their radiograms. Among the first were the recogntion of glycolate as a product of photorespiration and of ribulose and sedoheptulose, which appeared as a result of phosphatase activity in the *Rhodospirillum rubrum* chromatograms I had prepared with Clint Fuller's photosynthetic bacteria. Later, in chromatograms of P^{32}-labeled spinach I noticed a major novel product that proved to be glycerophosphorylglycerol, GPG, the deacylated derivative of the new major phospholipid, phosphatidylglycerol. In my search for thioctic (lipoic) acid in S^{35}-labeled algae the huge radioactive spot on the chromatogram, which for a time thrilled Melvin, later proved to be the plant sulfolipid, Nature's finest surfactant molecule. Later, novel compounds appeared in radioautographs of algae labeled with sulfur, arsenic, or antimony. A review (8) of these adventures was presented upon receipt of the 1987 Supelco-AOCS Research Award.

Choline Phosphate

For several years our former colleague Nathan Tolbert had reported a fast-moving compound in the xylem of barley seedlings. At Penn State I chromatographed P^{32}-labeled barley and noticed a novel neutral product. I asked Jake Maizel to have a look at it. Jake came back in a week, having recognized it as the zwitterionic choline phosphate. We wrote a manuscript for *Plant Physiology*, added Tolbert's name, and sent it off (22). Feeling that Jake would benefit from a move to Caltech, I arranged for him to move. Thus Jake then went on to Caltech and a successful career. Later, with Tolbert's acumen and energy, the important growth regulator, chlorocholine chloride (CCC) (30) was developed. A similar compound, choline sulfate (25), now recognized as an osmoregulator, appeared in chromatograms of S^{35}-labeled salt-excreting mangrove leaves.

Phosphatidylglycerol

A tantalizing unknown phosphorus compound appeared in the middle of the chromatogram of an extract of P^{32}-labled spinach leaf. I labeled it "Up," for lack of a better name. It had appeared in similar extracts of algae. Often its concentration appeared to exceed those of other phosphate esters identified earlier by explorers like Neuberg, Fischer, Meyerhof, Lohmann, and Leloir. It seemed there could hardly be any more, especially one that was a major phosphorus compound in spinach, algae, and many other plants. It certainly had to be a well-known substance, but all attempts to show that failed. Futile search for the probable solutions to the problem of identity consumed an embarrassing year or more. Only by my good fortune and the wisdom of Hiroshi Tamiya, Assistant Professor Bunji Maruo from the Institute of Applied Microbiology arrived at State College to collaborate in 1956. I had hydrolyzed Up to produce a smaller derivative (greater Rf value on the chromatograms), yet another "unknown." Soon, Bunji had identified it as the well-known glycerophosphate. With C^{14}-labeled Up the hydrolysis product was glycerol-C^{14} and glycerophosphate-C^{14}. What could be simpler and, at the same time, more perplexing? Both were too obvious. It dawned on us that the unknown was GPG or glycerophosphorylglycerol, a frighteningly simple but completely novel combination of glycerol and phosphate. Such glycerophosphoryl esters were well known as skeletons of the glycerolphosphatides. Could GPG be such a skeleton of a phospholipid of plants? We turned our attention to the P^{32}-labeled lipids of algae. With the fatty acids removed, GPG appeared in every instance. It sometimes even exceeded the amounts of the known esters of choline, ethanolamine, and inositol. Phosphate, glycerol, and fatty acids—how could such a simple lipid be a major component of plants without it having been known long ago?

It was more likely that Nature was playing a trick on us than that we could be looking at a new and important member of membranes. Even as I carried the manuscript to the mailbox, it was frightening to consider that Nature could have withheld such a simple secret for so long. Now we know that phosphatidylglycerol

may be a major membrane lipid of living things. Not only plants but bacteria and fungi chose to use it in their membranes. Such simplicity was hard to imagine. Years later, Laurens van Deenen and Eugene Kennedy honored our audacity by republishing our paper in Volume 1000 of *Biochimica et Biophysica Acta* (13). In a scholarly series of publications our student Isao Shibuya has delineated the factors regulating phosphatidylglycerol of the *Escherichia coli* cell membrane.

The Plant Sulfolipid: Nature's Finest Surfactant Molecule

Deacylation of the S^{35}-labeled lipid produced a water-soluble product (11). Acid hydrolysis further yielded a smaller molecule. C^{14}-labeled sulfolipid was isolated from Chlorella and acid hydrolyzed to yield glycerol and a sulfosugar that with the skillful collaboration of Helmut Daniel and Ralph Mumma was identified as the 6-sulfonic acid of 6-deoxyglucose (quinovose). Thus, the sulfolipid was sulfoquinovosyl diglyceride, probably the strongest amphipathic lipid in Nature. This discovery opened a study of sulfocarbohydrate metabolism, a system analogous to the known phosphorylated fermentative metabolism. Having saturated this field, we dropped this research in 1963. For nearly 40 years the field languished for the lack of brilliant ideas or novel approaches. Recently, with the works of Ernst Heinz and Christoph Benning, progress has been made. Benning's work appears to be revealing a biological mechanism for insertion of the $-SO_3H$ group, which closely resembles the free radical addition of SO_2 to a 5,6-glucoseenide as carried out by Jochen Lehmann (21). Using modern genetic tools, Benning has closely approached the true mechanism. Possible utilization of the sulfosugar in novel biodegradable commercial surfactants becomes a possibility.

Neutron Activation Chromatography

Having worked for years in ORL in the slow neutron cloud generated by the 60-inch Crocker Cyclotron, I felt no compunction about utilizing the excellent and convenient slow neutron source of the Penn State nuclear reactor facility. Few others were using the reactor in 1958, and formalities were minimal (Not so anymore, as now one would be burdened by regulations, forms, record keeping, etc.). Our society fails to recognize the possibly beneficial effects of low levels of radiation and radioisotope exposure, which may induce our enzyme systems toward DNA repair. My own early exposures to X-ray radiation from experimenting with my father's portable X-ray Tesla system, X-ray tubes, and fluorescent screen may actually have been helpful in inducing my DNA (thymine dimerization, etc.) repair enzymes. Similarly Martin Kamen's cyclotron experience could have induced his successful radiation damage repair systems. Regions of low radiation exposure from the Chornobyl disaster now appear to develop unexpectedly low cancer incidence.

Neutron Activation Analysis had come to imply the detection and assay of radionuclides produced within a sample upon slow neutron exposure. As a result of neutron capture, the radioactive nuclide ejects a number of gamma rays. The recoil from such emissions drives the victim's nucleus out of any previous chemical

bonds, thus rendering molecular chemical identification impossible. With the Penn State Reactor I succeeded in overcoming this inadequacy and developed the techniques for identifying and assaying the phosphorus compounds and some other groups of organic carbon compounds separable by our methods of two-dimensional paper chromatography. It was a simple, convenient, and reasonably precise analytical method. I think it was a brilliant methodology, but it seems neither known nor used today. It was sometimes criticized as being time consuming. Hardly so, not much time is really consumed, though the time period between the extraction and chromatography and the final result may be a week or two, depending on the analytical precision desired. This was often the criticism suffered by paper chromatography and the final radiogram on the X-ray film. However, this was never a problem; there were always plenty of other projects going on at the same time. The result was worth the wait. In fact, I often exposed two films to the paper chromatogram so that eager folks like Melvin Calvin might have something to think about while the second film was exposed properly for documentation.

Neutron activation chromatography was nicely applied for assay of phosphorylated esters derived from large animals whose size precluded normal radioisotope labeling. We determined relative concentration of the several mitochondrial membrane phospholipids of bovine and sheep liver. The lipids were deacylated and the phosphodiesters chromatographed on paper having 1-μg samples of phosphorus (orthophosphate) as control standards. The paper, rolled and sealed in a polyethylene tube, was lowered to the swimming-pool reactor and exposed to the neutron flux for six hours. Short-lived isotopes were allowed to decay a few days and the paper exposed to X-ray film to reveal the characteristic pattern of deacylated lipid products. Comparing their induced radioactivity with that induced in adjacent 1-μg standards provided for quantitative determination. The recoil from neutron capture destroys the initial bonds of the diesters but hardly distorts their location on the paper. Tatsuhiko Yagi and I followed this with an episode of hot-atom chemistry to understand the vagaries and fate of the recoiling P^{32} atom in various media (32).

Cell Membrane Model

Jim Danielli, a father of the lipid bilayer model for cell membranes, opened the first of the international conferences on Membrane Structure and Function in Frascati, Italy, with, "Ladies and Gentlemen, and—Dr. Benson." I was to present a heretic model for the membrane, completely different from his widely accepted Davson-Danielli bilayer model. With collaboration of Elliot Weier and Tae Hwa Ji in our study of chloroplast lamellae and many bacterial membranes, I was convinced that the requirements for specificity of fatty acyl chains in many systems must indicate specific interaction of membrane proteins with the hydrophobic saturated or unsaturated C_{16} to C_{22} chains in such lipids. Clearly, there were hydrophobic pockets within proteins that specifically accommodate such chains. The electron micrographs of stained membranes appeared to represent layers of such lipoproteins. The work of Tae Ji corroborated observed interactions of carotenoids, chlorophyll,

and lipids consistent with that concept. Hence, I entitled our paper, The Cell Membrane, a Lipoprotein Monolayer (7). That was 34 years ago.

In 1986 Bunji Maruo alerted me to a superb publication in *Science* on the structure of oxidized cytochrome c oxidase, which included the positions of eight phospholipid molecules, three of them phosphatidylglycerol (31). That paper was the first to reveal the positions of the fatty chains of the phospholipids within a membrane protein molecule. I feel that the advent of the intense light sources now available to crystallographers, particularly the powerful SPRING-8 collector ring near Osaka, will produce further evidence supporting my 1967 contention.

The countless publications relating to the position of fatty chains in the lipid bilayer model appeared naïve to me. The two-dimensional representations of such chains seemed unrelated to reality. One of the questions is the disparity of chain lengths in such membranes and the effects of multiple unsaturation. I assembled models of such lipids and concluded that the least uncomfortable arrangements of carbons in the n-3 chains of the α-linolenic (18-3) and docosahexaenoic acid (22:6) must involve helical positioning of the several double bonds. This reduces the length of the chain to nearly that of the C-16 and C-18 saturated fatty acids. This, then, can explain the apparent symmetry of the two lipid layers of the bilayer involving the long polyunsaturated chains. It could also provide for more specific interaction of the polyunsaturated chains with hydrophobic pockets within proteins.

Wax Ester: World's Major Nutritional Energy Source

My long-time colleague Dr. Judd C. Nevenzel had collaborated for years with James F. Mead in the Laboratory of Nuclear Medicine and Radiation Biology at UCLA. Judd is a master gas chromatographer and a guru of lipidometric information. He and Mead gravitated to an interest in lipids of fish, and Judd became intensely interested in the lantern fish, which daily migrates vertically approximately 1000 m. Judd discerned that these fish produced unusual amounts of wax esters, which he investigated in my laboratory at Scripps. Interaction with my graduate student Richard F. Lee and his interest in copepod metabolism led to Nevenzel's recognition of their wax ester content, up to 70% of the dry weight of the animal. As a result we spent considerable time at sea studying the *Calanus plumchrus* population of the Strait of Georgia between Vancouver and Victoria. We hoped to discern relationships between vertical migration capabilities and the compressibility of their liquid wax ester.

Soon we recognized that wax ester is the stored energy source for copepods and most of the pelagic animals of the ocean. We found that there were no specific wax ester lipases and that other lipases could hydrolyze wax ester only one tenth as rapidly as they could hydrolyze ordinary fats. Thus the deep sea animals are forced to conserve their energy supply, allowing them time to find a mate or to capture uncertain food in a dark nutrition void. Wax ester in marine animals, then, serves as Nature's Starvation Insurance.

Later I found that corals exude some wax (cetyl palmitate) in their mucus, which is collected by small reef fish. Lee and I published an article on The Role of Wax in Marine Food Chains (12). The orange roughy, a widely marketed deep sea fish from New Zealand and Australia, became popular in the 1980s. If an orange roughy were broiled whole, the result would be immediate diarrhea because of the high wax ester content of its skin and bones. The commercial fillets, on the other hand, are free of wax ester.

Salmon Research

The wax ester of the British Columbia copepods is avidly consumed by small salmon, arriving from their rivers rich in nutrients from the melting snow and glaciers. The newly nutritious seawater gives rise to a great diatom bloom, the food source for *Calanus plumchrus* and other copepods that conserve the highly unsaturated fatty acids of these phytoplankton in their wax esters, providing energy for the young salmon. Beginning in 1968 I led expeditions to study the mechanisms of the rapid aging of spawned Pacific salmon, which die, totally emaciated, a week after spawning, The 15 medical scientists aboard our RV Alpha Helix each discerned an important process leading to demise of the salmon. A major effort developed from the interests of Gérard Milhaud from the Institut Pasteur in Paris. Having developed the thyroid hormone, calcitonin, for treating osteoporosis in man, he proceeded to study its function in the salmon. Today salmon calcitonin, a 32 amino acid peptide, is an important therapeutic agent for treating osteoporosis. In 1971 our site of research moved to the more accessible village of Alert Bay with its vigorous fishing industry. I designed and equipped four successive laboratories around which our friend and benefactor Bob Peterson, founder of the Jack-in-the-Box chain of drive-in restaurants, built research ships and provided continuous enthusiastic support for our work. Canadian fishery officer Ray Scheck recruited assistance of a skillful salmon fishing family, Lily and Porgie Jolliffe. Members of the native Kwakiutl Tribe and its Nimpkish Band of Alert Bay, the Hunt family has a long and distinguished history. Lily (Kakasolas) Jolliffe, a gorgeous strong-willed lady with great cultural depth, is of the Hunt family.

Franz Boas

I gave a talk on salmon aging to the local Sons of Norway one evening and met a very interested member who was familiar with the salmon fishing industry. Later he presented me with his classic copy of Kaptein Jacobsen's Reiser til Nordamerikas Nordvestkyst. It had engendered the salmon fishery of Alaska. Jacobsen had traveled the coast of British Columbia in 1881, collecting Native American artefacts for Hagenbeck museums in Hamburg, Berlin, and Prague. At a "showing" of a group of coastal peoples in Berlin, young Franz Boas and his friend Sigmund Freud became interested in these unique humans from the Pacific Coast. Boas was excited at the prospect and obtained from Jacobsen a letter of introduction to George Hunt, Chief of the Kwakiutl nation at Fort Rupert on Vancouver Island. Boas arrived

there in 1886 and spent 15 years with Chief Hunt, documenting the language and culture of the Kwakiutl. It was the beginning of scientific anthropology.

Needless to say, reading that Franz Boas of La Jolla High School was a winner in the Westlinghouse Science Talent Search (now called the Intel Science Talent Search) in California was exciting. I called his mother and learned that he was indeed a descendent of Franz Boas. I couldn't resist taking him to Alert Bay to meet Lily and her sister Laura and to experience a night of fishing on a salmon seine boat. He had never met the 65 members of the Boas family who had enjoyed a reunion with the 300-member Hunt family in Alert Bay a few years earlier. With his visit a great success, young Franz Edward went off to Harvard with happy memories.

When I was a freshman student at Berkeley, anthropology Professor Ronald Olson was assigned to be my Adviser. Subsequently I took some of his courses, which included the works of Franz Boas and the culture of the coastal Indians. Thirty years later, I found myself in the midst of these very people whose culture Boas had documented. It has been a great privilege.

Arsenate in the Sea

When it became apparent that the analyses for phosphate in seawater also included arsenate, Johnson and Pilson discovered that tropical surface waters of the Sargasso Sea and the Caribbean could include up to four times as much arsenate as phosphate, both absorbed by the same transport system. Our algae when fed radioarsenate, As-74, produced much arsenolipid and several of its hydrolysis products (16). It was an exciting adventure. Edmonds and Francesconi in Perth had identified arsenobetaine in rock lobster tails of Western Australia, so I suspected the arsenolipid could be the result of production of the arseno analog of phosphatidylcholine. Bob Cooney and Ralph Mumma developed a neat paper electrophoretic method for identification of arsenicals, based on their acid dissociation constants. Later, on the Great Barrier Reef, Roger Summons and I found the highest reported arsenic content, 1000 ppm, in the kidney of the giant clam, *Tridacna maxima*. Cooney and I had been misled by our tracer identifications based on chromatography in various solvents and electrophoresis in several pH buffers. Edmonds and Francesconi successfully identified the arsenical compounds as 5-dimethylarsenoribose derivatives, the lipid being a 3′-arsenoribosylphosphatidylglycerol. We found relatively small amounts of methanearsonate and dimethylarsinate, which many laboratories had identified by chromatography and mass spectrometry as major arsenicals. We interpret this discrepancy as the result of slow accumulation of methylarsonate and dimethylarsinate, whereas the inital products of arsenate metabolism are the arsenosugar and arsenolipid as revealed in our chromatograms.

The long history of toxicity of arsenite and the reputed ability of the "arsenic eaters of Styria" to tolerate up to five lethal doses with lunch suggest careful metabolic adaptions by their gut flora. Such adaptations could as easily develop in communities with excessive arsenic in drinking water. Protective mechanisms may develop, just as with low radiation exposures.

Methanol and Plant Productivity

In 1970 when fuel oil prices soared Arthur Nonomura had isolated an alga that exuded oil droplets. With Fred Wolf in the Calvin Laboratory he had isolated the most productive strain of *Botryococcus braunii*. Trying to improve its growth rate, Arthur asked me for suggestions. "Give them some methanol, Arthur, they'll love it." Of course I wasn't at all confident about this, but Arthur tried methanol and the alga grew twice as fast. That was the beginning of our methanol episode. Thirty years earlier I had investigated the metabolism of C^{14}-methanol in *Scenedesmus* and *Chlorella* while in search of CO_2 fixation intermediates. It was probably the first synthetic C^{14}-methanol and was made by my dear friend Bert Tolbert, brother of Nathan Edward Tolbert.

After his research tenure Arthur started a cotton ranch in Arizona and observed that foliar methanol application prevented afternoon wilting. Without midday depression of photosynthesis associated with wilt, the methanol-treated cotton showed more rapid development and an earlier harvest time than controls. After the boost in growth was verified in a DuPont-supported project, an optimistic publication appeared in the *Proceedings of the National Academy of Sciences* (26). Not surprisingly, some attempts to reproduce the observations without long duration high photorespiratory rates were inconclusive. Arthur continued his efforts, as several convincing confirmations were reported. Finally, with Roland Douce, Richard Bligny, and their capable collaborators, we examined the fate of ^{13}C-methanol in living plant cells as revealed by nuclear magnetic resonance (NMR). Dramatic development of the absorption peak for ^{13}C-methyl glucoside appeared on the screen. Though there was no a priori reason for concluding that it was an important growth factor, Arthur tried applying methyl glucoside solutions to the roots of his test plants. Success was immediate and reproducible. After developing a penetrating foliar solution with methyl glucoside, the metabolite proved far more consistent in activity over a wider range of parameters than simple foliar methanol treatment. Even C-4 plants responded to methyl glucoside applications. Field tests with hybrid corn and canola demonstrated 10% and 15% enhancement by methyl glucoside. Methanol and methyl glucoside reduce the effects of the afternoon drop in photosynthetic activity of field crops. Though there is no clear explanation yet for the observed effects, the treatment is effective and convincing.

Le Metre

The Paris Academy, in their infinitesimal wisdom, decreed the ten-millionth of the Earth's quadrant should be the standard of measure, a "Gift from God" to the countries of Europe that had found their many units of weights and measures complicating trade and communication. Those wealthy "scientists" in their crimson robes had never built a house, baked bread, or prepared meals for a family; nor had they measured tire pressures or the temperatures of concern to humans in Europe as well as the tropics.

The names of the units that people must use should be one syllable, as in inch, foot, pound, mile, and quart. A unit's name should not have to repeatedly inflict

on the writer, speaker, or listener the fact that the unit has one thousand meters in it or that it is a hundredth or a thousandth of some larger unit.

Now, if one plans to devise a measuring system with accuracy sufficient for the majority of the earth's humans, the scale of such measurement should range from 00 to 99, providing one per cent precision. That scale should encompass most of the measurements of interest to most of the people involved, a principal of a "democratic" system of measurement.

Though not planned by Herr Gabriel Daniel Fahrenheit, his temperature scale, from 00° to 99°, includes most of the temperatures to which humans are exposed and which they measure most frequently. It avoids the negative temperatures and imprecision of the Celsius scale.

For our gas, water, and air pressure measurement needs, the scale from 00 to 99 psi provides a range useful for most applications: 3 psi for household gas pressure, 70 psi for household water pressure, 24 to 95 psi for tire pressures. These applications encompass most of the needs of our families, plumbers, and auto mechanics.

The problem is not the decimal nature of the metric system. Thomas Jefferson was hoping to decimalize the foot and the pound; he had had great success in creating our decimalized currency. The head start and European adoption of the Paris Academy's Metric System was the downfall of his plan. This philippic contends that contrivance of the Metre was the greatest disaster of the eighteenth century that plagues less perspicacious societies to this day.

EPILOGUE

I have taken the liberty of the privilege provided by Annual Reviews to describe some of my experiences that provided the skills and background for following the path of carbon in photosynthesis (5, 9, 10) as well as to relate some personal concerns and events of interest beyond the path. Even though the Editors may have expected me to just write about the path of carbon and our many adventures within ORL, the wooden building that was the birthplace of the cyclotron and Carbon-14 and the source of the short-lived Carbon-11 used by Sam Ruben and Martin Kamen in their history-making exploration with radioactive carbon dioxide (1938–1942), I am sure that the photosynthesis story is familiar to most plant physiologists. It has been repeatedly described in elegantly competent works by Dr. J.A. Bassham (2) and most recently by our colleague Dr. R.C. Fuller (17).

Though such autobiography might only be as important as yesterday's newspaper, something only good for wrapping fish, I was enheartened by the words of Joseph Priestly, whose experiments inaugurated the modern era of photosynthesis research. He began his memoirs, published in 1806, with the following modest declaration: "Having thought it right to leave behind me some account of my friends and benefactors, it is in a manner necessary that I also give an account of myself and as the like has been done by many persons and for reasons which posterity has approved I make no apology for following their example."

I have tried to follow my path through fortuitous and serendipitous events, many of which resulted from guidance by my parents, teachers, and and broadly cultured colleagues. Though my own level of culture pales in the shadows of those of my mentors, good fortune and health have been blessings indeed.

Mentors

Father, Carl Bennett Benson, M.D.; Mother, Emma Carolina Alm; Irina Barsegova; Dee Dorgan Benson; Michael D. Berry; Montague Cleeves, M.D.; Helmut Daniel; Robert Emerson; Fritz Goreau; A. Baird Hastings; R. Barry Holtz; Benjamin F. Howell, Jr.; George N. Jolliffe, Jr.; Francis C. Knowles; Mutsuyuki Kochi, M.D.; Helge Larsen; Bunji Maruo; Marie Mathers; James F. Mead; James E. Merlin; Gérard Milhaud, M.D.; Stanley L. Miller; Ralph O. Mumma; Judd C. Nevenzel; Arnold Nordal; Ted R. Norton; Margaret Painter; Stuart Patton; Elizabeth Baker Pelz; Robert Oscar Peterson; Harry Rosenberg; Sam Ruben; Kenji Sakaguchi; Paul D. Saltman; Pete Scholander; Michio Seki; Kazuo Shibata; J. Rudi Strickler; Roger E. Summons; Hiroshi Tamiya; Eduard A. Titlyanov; Bert M. Tolbert; Eberhard G. Trams; Viktor E. Vaskovsky; Ellen C. Weaver; and A.A. Yayanos.

Memorable Experiences

(*a*) Charles Lindbergh at Sacramento, 1927. (*b*) Fireworks, Gagarin Celebration, Moscow, 1961. (*c*) Surtsey eruption, 1964. (*d*) Isla Socorro Sea Bridge, 1979. (*e*) Solar eclipse, 5 min, La Paz, Baja California, 1991. (*f*) Guest at presentation of Enrico Fermi Award to Martin Kamen, 1997.

ACKNOWLEDGMENTS

I am indebted to skillful proofreading of my manuscript by John F. Kern and Carole Mayo.

DEDICATION

In memory of Chris, Bonnie, Kazuo, Juan, and Big Paul.

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Annual Review of Plant Biology
Volume 53, 2002

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