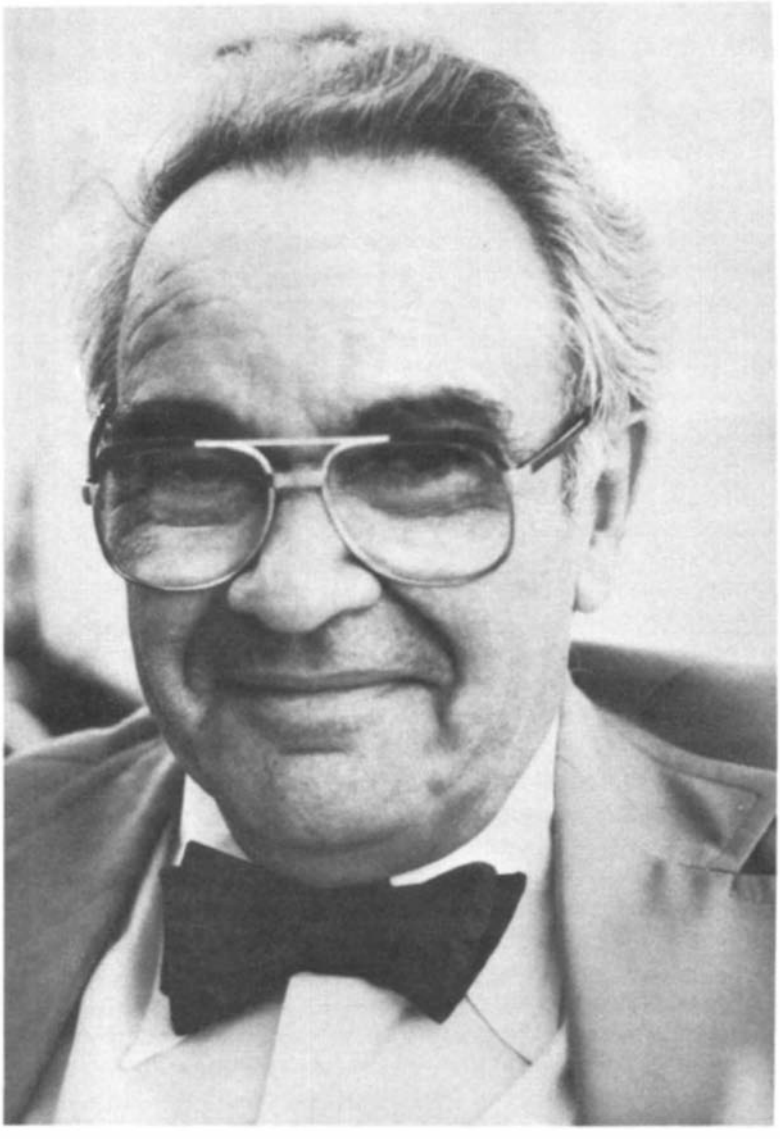

Martin D. Kamen

Ann. Rev. Biochem. 1986. 55:1–34

A CUPFUL OF LUCK, A PINCH OF SAGACITY

Martin D. Kamen

University of California, San Diego, La Jolla, California 92093

CONTENTS

As the Twig is Bent 1
Nuclear Euphoria 4
Photosynthesis and CO_2 Fixation 5
Isotopy and Long-Lived Carbon (^{14}C) 8
Good Science and Bad Politics 12
A Major Diversion 18
Some Relevant Asides 21
Big Questions and Small Answers 22
L'Envoi 30

> This discovery indeed is almost of that kind which I call *serendipity*, a very expressive word, which as I have nothing better to tell you, I shall endeavour to explain to you: you will understand it better by the derivation than by the definition. I once read a silly fairy tale, called *The Three Princes of Serendip:* as their highnesses travelled, they were always making discoveries, by accidents and sagacity, of things which they were not in quest of: for instance, one of them discovered that a mule blind of the right eye had travelled the same road lately, because the grass was eaten only on the left side, where it was worse than on the right—now do you understand *serendipity?*
>
> Horace Walpole. 1754. (1a,b)

As the Twig is Bent . . .

The mention of the word "serendipity" to describe the combination of chance and sagacity as a crucial determinant of scientific creativity (2) is a well-worn cliché, but I can think of no better word to apply to the history of my half-century in research.

0066-4154/86/0701-0001$02.00

That I even got into research at all, and especially biochemical research, is amazing considering the circumstances of my upbringing. My parents were poor Russian Jewish immigrants who met and married in Toronto, where I was born August 27, 1913. My father had learned photography in Canada and opened a studio in Chicago. It was located in Hyde Park, near the campus of the University of Chicago, and we saw many of the faculty who came for portraits, as well as some distinguished visitors like E. Benes, the former prime minister of Czechoslovakia, and Fanny Bloomfield Zeisler, a leading pianist of the time. I showed considerable musical talent and got into the fix of being acclaimed as a child prodigy. The traumas attendant on this experience left me with a permanent aversion to center stage and conditioned me all through my scientific career to downplay achievement.

Throughout my youth, I had many friends who displayed a fervid interest in manipulating toy steam engines, crystal sets, and the like, but I took no interest in things mechanical. Indeed, I thought those who did a bit bizarre. As an occasional helper in our studio darkroom, I encountered some of the smells and arcane processes of a kind of chemistry but other than this had no experience with what might be called science. Entering the University of Chicago as a freshman in the spring quarter of 1930, I declared a major in English literature, probably because it was as good as any subject to expand my considerable background of readings in the classics.

The shock of the Great Depression took effect on the family fortune that summer and suddenly there was need to cast about for a means whereby I could make a living after graduation. My father suggested I try chemistry and as a dutiful son, I somewhat grudgingly agreed. That fall, I switched my major. So much for vocational guidance!

The vagaries of academic organization had assigned an eminent organic chemist, J. W. E. Glattfeld, to be my advisor the previous quarter, and he had labored mightily to fashion a curriculum that disposed of the general science requirement expeditiously. Now he was somewhat taken aback to find his ward opting for a science major. He would have been astounded to be told that inside ten years I would be writing him for advice on the chemistry of C_4-saccharinic acids, his specialty, in connection with my efforts to devise fast efficient syntheses of erythronic acids labeled with the short-lived carbon isotope, ^{11}C.

My skills as a musician helped support my studies in science. I received a partial scholarship for playing first viola in the university orchestra, and picked up bits of cash here and there as a jazz fiddler in the numerous havens of alcoholic refreshment to be found in the university area. Meanwhile I was discovering the excitement and fascination of science. By my junior year, I had become a complete convert. However, I retained a deep love for music and this was to be a source of happiness in later years, particularly as I had

the good fortune to enjoy musical activity without having to make a living from it.

A stubborn tendency to impracticality persisted and resulted in my accumulating almost as many credits in mathematics and physics as in chemistry. I remained resistive to the arguments that the only practical areas were synthetic organic or inorganic chemistry and graduated with honors in physical chemistry after a hectic three years of undergraduate study. The research project in which I had participated to earn the "cum laude" was a determination of ammonia gas emission spectra excited by electrodeless discharge—part of a general program under the aegis of the senior professor, Dr. W. D. Harkins, to study products of ergosterol using radiations other than ultraviolet. The routine lab operations, as well as the implementation of the professor's research program—in the two completely disparate areas of nuclear physics and surface chemistry—were entrusted to a hardworking, imaginative, and much exploited young assistant professor, Dr. D. M. Gans. I became closely attached to him and even somewhat resentful of the pressures that he endured in his efforts to achieve tenure. I carried away from this experience a conviction never to be repressive or demanding of graduate students and fellows who might be under my supervision, if ever I attained the lofty status of a senior professor.

With the Depression still raging in the early 1930s, I had little incentive to leave the university and eagerly accepted Gans's suggestion I stay on and work for a doctorate with Harkins. Inheriting a cloud chamber apparatus that had been built by my predecessor, Henry W. Newson, and by Gans, I dreamed up an overly ambitious thesis research on the angular distribution of protons after collision with fast neutrons. I hoped from the results obtained to deduce something about the nature of intranuclear forces. The means at hand were inadequate, as it turned out. For one thing, the neutron sources were too weak, being made up of a few millicuries of thoria mixed with beryllium powder, However, three years of arduous effort allowed the accumulation of data on some 700 recoils which I used to support a thesis that earned me a PhD in the winter of 1936. A paper was written and published with, as customary, the professor as senior author (3).

Conditions in the outer world were still grim. Gans suggested I spend some time at the famed Radiation Laboratory in Berkeley, California, where the charismatic Ernest O. Lawrence had developed the cyclotron as the most powerful tool of nuclear physics. Harkins intended to build a cyclotron in Chicago, and I could expect to be taken on as a research assistant provided I could claim some expertise as a cyclotroneer. The Radiation Laboratory was Mecca for all aspiring young nuclear physicists, and I was intrigued at the prospect of going there. Working as a photographer, and saving proceeds of various engagements as a jazz fiddler over the Christmas season, I left for Berkeley just as the new year of 1937 was dawning.

Nuclear Euphoria

Although I had not written for permission to work with Lawrence and his group, I received a most hospitable welcome and was particularly happy to see that Franz N. D. Kurie, who had published results (4) similar to mine prior to the appearance of our paper, was on the laboratory staff and eager to press forward on the problem of neutron-proton scattering. I was stunned to see such a profusion of neutrons as emerged from the cyclotron. Each expansion of the cloud chamber Kurie had built in Berkeley showed literally thousands of recoil protons, whereas I had been used to seeing one or two every ten expansions. However, our joy was short-lived. We could not cope with the sheer abundance of neutrons that came from every direction and made accurate definition of the scattering angles impossible. However, serendipity began its entry into my life at this point.

We noticed when the chamber was filled with N_2 gas that almost every expansion showed one or two stubby tracks of recoil protons with a knob on the end. They were homogeneous in range (~1 cm), so that they could only come from neutrons moving so slowly that the recoils produced took up only the reaction energy, there being no excess kinetic energy imparted by the neutrons. There was good evidence (5) that slow neutrons were captured by nitrogen nuclei (^{14}N) to produce a carbon isotope, presumably of mass 14. We could assume that the knobs at the beginning of the stubby tracks were the relatively heavy and slow ^{14}C recoils and that we were observing the reaction: $^{14}N + {}^{1}n \rightarrow {}^{14}C + {}^{1}H$. I filed this observation away in the back of my head along with a mass of data I would be accumulating as the laboratory radiochemist on integral yields of radioisotope prediction. It would be crucial in later encouragement to proceed with the search for long-lived radiocarbon.

Serendipity continued its sway, as events moved forward. I joined with E. M. ("Ed") McMillan, a young laboratory staff member who was already a leader in the field of nuclear physics, to straighten out some anomalous results Lawrence had reported on platinum transmutation by deuterons. We welcomed a young graduate student from the department of chemistry, Samuel Ruben, to help with the difficult platinum metal radiochemistry needed to sort out the activities observed when platinum was bombarded by highly energetic deuterons (up to 8 MeV). We soon found that the anomalies in yields of radioactivity were the results of light element contamination. However, the authentic radioactivities that we discovered provided fresh anomalies in that there were more radioactivities observed than there were isotopes available for assignment. We had to conclude that we were making many nuclear isomers, thereby establishing for the first time the phenomenon of nuclear isomerism as a general occurrence in the heavy elements of the periodic system (6). The serendipitous aspect was not only this discovery but also the circumstance that

Ruben and I were thrown together. We became aware that we could form a fruitful partnership based on a strong mutual respect and friendship and of the fact we would have access to both the considerable facilities of the Radiation Laboratory and the department of chemistry. We reached an understanding to collaborate, with seniority for production and characterization of cyclotron products assigned to me and that for chemical applications to Ruben.

The particular benefit in this arrangement for me lay in that my time in the Radiation Laboratory was largely taken up in monitoring cyclotron productions of radioactive materials, and such productions were assuming increasing magnitude as Lawrence pressed his campaign to encourage biologists and medical researchers to use the cyclotron and its products. By working with Ruben, I could gain more time for our mutually conceived researches. For Sam, the advantages of ready access to the cyclotron were obvious. For him, too, there were growing commitments of time for departmental teaching assignments.

Being at the center of Radiation Laboratory isotope distribution, I was becoming well known to many researchers worldwide, some of whom became good friends later. One was George Hevesy, to whom on one occasion during World War II I sent ^{32}P in three separate batches to minimize loss at sea because of the submarine menace. He wrote a charming letter in reply in which he remarked that if any of the samples failed to reach Denmark, it would be necessary to advise workers on the radioactive content of seawater to be careful with the figures obtained. With others I entered into active collaboration, an example being research with Dr. G. A. Whipple and his group at Rochester on excretion of iron in dogs maintained in various stages of iron depletion.

Photosynthesis and CO_2 Fixation

The fruits of our partnership materialized swiftly. Sam had pressed vigorously his own campaign to interest biologists on campus in using radioisotopes. It should be noted that biochemists, and biologists in general, were low in the pecking order on the Berkeley campus and it took considerable courage for a young graduate student hoping to advance to faculty status in chemistry to risk his future on biochemical research. My first intimation of Sam's campaign came in a request from Lawrence to help I. L. Chaikoff, a young assistant professor in physiology, obtain samples of CO_2 labeled with 21 min ^{11}C. When I inquired what he wanted to do with such short-lived material, Lawrence told me of Chaikoff's ingenious plan to synthesize D-glucose from $^{11}CO_2$ expeditiously and specifically, using the photosynthetic ability of green plants, which everyone supposed produced D-glucose from CO_2. With the ^{11}C–uniformly labeled glucose so obtained, Chaikoff would study glucose metabolism in rats in short-term experiments. He had calculated he would have sufficient radioactive glucose to do a significant series of studies on glucose assimilation and excretion as CO_2.

When I told Sam about this scheme, he reacted angrily. He claimed it had been his idea, which he had convinced Chaikoff would work. The idea certainly had all the boldness and imagination one had come to expect from Sam. I learned that Dr. W. Zev Hassid had been recruited by Chaikoff to help in the isolation and purification of the labeled glucose expected to be formed by feeding $^{11}CO_2$ to barley, wheat, and sunflower plants. I already knew Zev because I played in chamber music sessions at his house organized by his wife, Leila, who was an accomplished violinist.

After many weeks of fruitless effort in which appreciable quantities of labeled glucose were not formed, Sam suddenly realized that it might be possible to solve the age-old problem of the primary CO_2 fixation product in green plant photosynthesis. He proposed I join him and Zev to mount a strong effort to isolate and characterize the labeled material actually formed from $^{11}CO_2$ in short-term experiments. It seemed simple enough. After exposure to the labeled CO_2, the plants could be cut up and placed in acidified boiling water to which carrier unlabeled bicarbonate was added to remove unreacted labeled CO_2. The water-soluble radioactive material could then be examined chemically to see in what compounds it had appeared. The identification could be performed adding carrier for the various compounds in which we guessed the radioactivity was contained. I agreed enthusiastically to give the project my full-time attention for the few months we optimistically expected would be required to solve the problem.

Some hundreds of experiments and three years later, we were still in the dark about the nature of the CO_2 fixation product, but we had learned much about the photosynthetic process. Elsewhere I have summarized the results we obtained (7), which were published in several papers in 1940 (8–10). The main results were to establish fixation in (a) compound(s) which contained hydroxyl and carboxyl moieties, the process being initiated by a reversible carboxylation of some CO_2 acceptor in the dark, followed by photoreduction to carbohydrate and other plant products. A fundamental mystery that was posed by our findings was how the plants obtained the free energy needed to drive the carboxylation reaction. All model reactions we knew of in vitro were strongly endothermic ($\Delta G_0' \sim 10$ kcal), whereas the photosynthetic "dark" carboxylation was exothermic ($\Delta G_0'$ estimated as ~ -2 kcal). Neither we nor anyone else then knew about phosphate, or more relevantly, ATP, or about photophosphorylation, which would be discovered over a decade later.

Our announcement of a dark CO_2 fixation met with complete disbelief among our biochemical friends in the Life Sciences building across the campus. Remarks like "you can't have photosynthesis without light" were heard. We persisted, generalizing the CO_2 uptake to a variety of living systems, including bacteria, fungi, protozoa, plant roots, and chopped rat liver. We observed, using a procedure based on thermal decarboxylation of the precipitates pro-

duced by addition of barium ions to carrier amounts of organic acids, that carboxylation was a general phenomenon (11). The reaction to our results at a center of microbiological research, the Hopkins Marine Station of Stanford University, was diametrically opposed to that of our colleagues in Berkeley. C. B. van Niel, who had been preaching the doctrine of CO_2 as a general metabolite for years, was delighted with our results. Some of his associates and disciples, such as H. A. Barker, S. F. Carson, and J. W. Foster, came to work with us. Together we decisively demonstrated authentic CO_2 fixation in anaerobic fermentations of methane bacteria (12), and oxidative metabolism of propionic acid bacteria and fungi. These researches extended and confirmed the discovery made by Harland G. Wood just a few years earlier, of heterotrophic CO_2 fixation in glycerol fermentations by propionic acid bacteria (13). We summarized our findings in a position paper, written in collaboration with our associates at Pacific Grove (14).

Being out of the mainstream of biochemistry, we were unaware that some biochemists, notably Hans Krebs, had become interested in pursuing experiments like ours with $^{11}CO_2$. Among the group working with A. Baird Hastings at Harvard on incorporation of ^{11}C-labeled lactate into glycogen in animal systems, there was Birgit Vennesland, who guessed correctly that CO_2 might be incorporated, and prevailed on Hastings to include control experiments with $^{11}CO_2$ that demonstrated CO_2 fixation (an excellent summary of the early history of CO_2 fixation is given in Ref. 15). The decisive data were reported by Earl A. Evans, Jr. and Louis Slotin at Chicago using the newly built cyclotron there. They showed that pigeon liver preparations incubated with $^{11}CO_2$ fixed radioactivity in the carboxyl groups of alpha-ketoglutarate (16). (Ironically, Slotin had gone to Chicago and accepted the appointment I had thought I might eventually fill.)

Undoubtedly, our discovery of general CO_2 fixation was one of the major positive contributions of the early work with isotopic tracers. Another important finding from our laboratory was the demonstration, using the rare stable oxygen isotope ^{18}O, that the oxygen evolved in photosynthesis originated from water oxygen and not from CO_2 (17). Our claims depended on the nonparticipation of exchange reactions that equilibrated oxygen from CO_2, molecular oxygen, and water, and that were favored at low pH. Although our experiments were performed at very alkaline pH ($\sim$10), there was no certainty that the internal cellular pH at the chloroplast was not in the acid range. It was also known that atmospheric oxygen reflected the ^{18}O content of atmospheric CO_2, rather than water (the so-called "Dole" effect), but in an article published a few years later, Barker and I discussed this anomaly and showed that it did not refute the conclusion that all atmospheric oxygen originated by photosynthetic photooxidation of water (18). Still later, our results incurred the ire of none other than Otto Warburg, assuredly one of the great figures in biochemistry in

this century, who stubbornly clung to the notion that photosynthetic oxygen came from CO_2 (19). In this, as in his persistent claim that photosynthesis was nearly 100% efficient, he was wrong. More work from other laboratories decisively substantiated our conclusions. Thus, for example, Holt & French found (20), using isolated chloroplasts, and Dole & Jencks showed, using the small difference in ^{18}O content of naturally isotopically equilibrated water and oxygen (21), results consistent with those we reported originally.

Isotopy and Long-Lived Carbon (^{14}C)

The serendipitous route to ^{14}C was not at all clear in the winter of 1939 as Sam and I sat gloomily in the "Rat House," an old frame shack of a building in the chemistry complex where we did our photosynthesis experiments, assessing our chances of solving our original problem—the chemical nature of the first CO_2 fixation product in photosynthesis. Clearly we needed a long-lived carbon isotope. I recalled the stubby tracks I had seen a year previously in the nitrogen disintegration experiments with Franz Kurie. I knew that ^{14}C existed but that in all likelihood it was not long-lived. Nowadays, when ^{14}C is so common an item in nuclear catalogs, it may be difficult to imagine a time when its existence as a long-lived isotope was in doubt.

Ed McMillan had tried making some ^{14}C by exposing a pound bottle of ammonium nitrate crystals to the stray neutrons of the 37-inch cyclotron, but the bottle had been accidentally broken and he had not attempted to repeat the trial. Moreover, Sam Ruben had looked at some graphite targets that had received many microampere-hours of deuteron bombardment in connection with some experiments we had performed using 10 min radionitrogen (^{13}N) and found no activity that could be released by combustion to CO_2. Hence the reaction of deuteron capture in ^{13}C, followed by proton ejection to make ^{14}C, had been ineffectual, or else the half-life of the ^{14}C expected would have had to be greater than several years to escape detection. Theoretically, with ^{14}C having a nuclear spin of zero and ^{14}N, its product in beta decay, a spin of one, the degree of spin forbiddenness was simply not great enough to give ^{14}C a half-life of more than a few days, according to calculations made by J. R. Oppenheimer's group. I knew from my original observations in the cloud chamber that the energy upper limit for the postulated decay of ^{14}C to ^{14}N was no more than a few hundred kilovolt-equivalents. So all indications were that with this energy and the failure to see ^{14}C in the bombarded graphite, the radioactivity to be expected was probably quite short-lived, on the order of seconds.

However, a new development in cyclotron technology had occurred. Robert R. Wilson had confirmed a prediction of Lawrence that huge currents of high energy deuterons circulated in the interior of the cyclotron that were not being

collected at the external target. Working with Wilson in the early morning hours from midnight to six AM, I had shown that highly infusible materials like ferrous phosphide could be soldered to the copper backing of probes Wilson had designed and these could be cooled well enough with water to withstand the great power input of the internal beams (on the order of kilowatts). I had been able to produce large quantities of ^{32}P and ^{59}Fe using such internal targets while the external beam was essentially untouched. Thus, the cyclotron could begin to keep up with the drastically increased demands for fast neutrons needed in radiotherapy and radioactive isotopes required for tracer research. In a paper that Wilson and I published on the internal target technique (22), I wrote prophetically, "The method of internal targets should find its most important application in the preparation of radioisotopes which are long-lived and difficult of activation, as well as in the demonstration of the existence of many radioisotopes as yet undiscovered."

I explained to Sam that it would be marvelous if I could reserve the cyclotron for a week or so to try the bombardment of graphite on an internal target, using all the beam available circulating inside the cyclotron. Such a procedure would raise the total activation several orders of magnitude above those ever tried before. But it was foolish to suppose I could ever get such a dispensation, particularly in view of the pessimism regarding the existence of ^{14}C as a long-lived isotope.

I had barely left Sam and returned to the Radiation Laboratory when I was called to Lawrence's office and told to begin an intensive search for ^{14}C or any other possible long-lived isotope among the elements of primary biological importance. It appeared that Harold Urey had been promoting vigorously the use of the rare stable isotopes (^{2}H, ^{13}C, ^{15}N, and ^{18}O) as tracers in biological research for which no adequate competing long-lived unstable isotopes were available.

The rest of the story is history (23). In a very short time, ^{14}C was discovered (24), as well as ^{3}H (25). As the account I wrote of the dramatic circumstances attending the birth of ^{14}C is buried in an obscure journal (26), I exhume it to quote:

> The weather in Berkeley during the winter months can be rugged. February of 1940 was no exception—as I was painfully aware while sitting at the controls of the ailing 37-inch cyclotron in the old Radiation Laboratory on the University campus. I had been there more or less continuously for three days and nights. As the operation drew to an end in the early morning hours of February 15, there was an extraordinary fanfare of driving rain on the tin roof, punctuated by the blasts of high voltage discharges in the bowels of the machine. Added to the general cacophony were occasional howls, screams and gutteral growls emanating from some recordings of French who-dunnits—a consequence of the activities of language classes which occasionally occupied the lab mezzanine in the upper reaches of the building. Bone-tired and red-eyed, I shut down the machine, rescued the remaining fragments of carbon target, which resembled so many bits of intensely radioactive bird gravel, and

shambled over to the ramshackle hut in which Dr. Samuel Ruben, my collaborator, worked and would be appearing shortly. These precious bits of discouraged graphite hopefully contained evidence for the existence of a long-lived radioactive form of carbon.

Indeed they did! Thus, the most valuable single tool in the nuclear armamentarium, ^{14}C, was revealed. It would contribute immeasurably to the study of life processes, as well as to those of death (as elaborated in the ^{14}C-dating technique invented by Willard C. Libby). Its full potential was realized when the uranium piles produced neutrons in weighable amounts (27a). In addition to its impact on all natural science, as well as archaeology, it provided direct proof (27b) through its anomalously long half-life (5700 years), apparently unique among beta-ray emitters, of the participation in nuclear beta decay of mesons and other particles smaller than neutrons and protons.

As the fall of 1940 neared, our prospects could not have been brighter. Plans were being made to create a future company under Research Corporation auspices to construct and operate a battery of cyclotrons dedicated to ^{14}C production, with an expansion of the whole radioisotope program centered at the Laboratory. In chemistry, Sam's position was growing stronger as his talents were being better perceived; and even some appreciation, however dim, of the worth of his collaborations with biologists was growing. Graduate students were eager to work with him on isotope tracer studies of organic reaction mechanisms and publications in this area were emerging from his group.

But our luck changed as the international situation worsened. Many months before Pearl Harbor, the laboratory had to go on a war footing. All non–war related research was halted. The ammonium nitrate tanks that had been installed to make ^{14}C were removed. I was assigned to head a program of isotope production and Sam was drawn off to work on various projects in chemical warfare. Our expectations of using ^{14}C to solve at last the problem that had started our researches in photosynthesis some three years previously were not to be realized. The minute quantities of ^{14}C remaining from our initial discovery were available for use in some pilot studies on the primary fixation product, as well as in a few researches on exchange reactions and mechanisms of fatty acid oxidation, a few of which were reported. Incidental to my full-time pre-occupations with the wartime isotope program, I crowded in a research on the production and radiochemistry (28) of the long-lived sulfur isotope, ^{35}S, in which isotopically enriched materials (namely AgCl with excess amounts of the ^{35}Cl isotope) were used for the first time to make an isotopic assignment of the radioactivity, in this case to the isotope ^{35}S.

Beset by the frustration of not being able to get on with the photosynthesis research, now that we had the tool, ^{14}C, at hand, we were wholly unprepared for the catastrophes that befell us next. Sam was killed in a laboratory accident in

late 1943, and shortly thereafter in early 1944, I was separated from the Manhattan Project as an alleged security risk. This action taken by Army Intelligence, as I discovered many years later, was predicated solely on the need to prevent possible leaks of information incidental to my extensive social contacts resulting from my musical activities. Army pressures were exerted to close all opportunities for my employment as a scientist in the San Francisco Bay Area, but I managed to hang on working as a marine inspector at the Kaiser shipyard during the day. At night, I contrived to do some research with H. Albert ("Nook") Barker, who graciously offered me a haven in his laboratory. Nook, aided by Tom Norris, a former graduate student of Sam's, had obtained a small quantity (a few microcuries-equivalent) of ^{14}C from the ammonium nitrate tanks still sitting in the "Rat House" and proposed we examine the fermentations of glucose and xylose by the thermophile *Clostridium thermoaceticum,* which was anomalous in producing acetic acid in excess of the expected two moles per mole of carbohydrate dissimilated. It was possible that the excess acetic acid arose by complete synthesis from two moles of CO_2 as in a reaction scheme:

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + \text{"8H"}$$

$$2CO_2 + \text{"8H"} \rightarrow CH_3COOH + 2H_2O$$

This suggestion was eminently testable by tracer procedures. Conducting the fermentation in the presence of $^{14}CO_2$, we showed that the appearance of labeled carbon in the acetate and dilution of the pool of $^{14}CO_2$ occurred in the amount expected, indicating that the two carbons of CO_2 could be used in the synthesis of one mole of acetic acid (29). We also performed other experiments on the agenda of the program we had previously considered and which had been prevented by the war effort. Thus, we examined an anaerobic fermentation of lactate and found a conversion of CO_2 to acetate and butyrate (30), as well as a process in *Clostridium kluyveri* that revealed that during conversion of ethanol and acetate to butyrate and caproate there was an intermediary participation of acetate in the production of the C_4 and C_6 fatty acids with addition of a C_2 unit to the carboxyl carbon of butyrate formed initially (31). These experiments, performed with weak samples of ^{14}C-labeled barium carbonate (a few microcuries–equivalent total in 30–800 milligrams), were the first biological experiments with ^{14}C reported. A major consequence of the work on *C. thermoaceticum* was the initiation of three decades of intensive investigations by Harland Wood, L. C. Ljungdahl, and their associates (32a,b), which elaborated elegantly the existence of a new pathway for synthesis of acetate from CO_2 through catalysis by the correnoid system of B-12.

Early in 1945, the efforts of the Radiation Laboratory administration to have me placed somewhere outside the Bay Area were successful. I was offered an appointment in the Washington University Medical School in St. Louis. The opportunity to begin a new career was welcomed eagerly, although I left Berkeley most reluctantly.

Good Science and Bad Politics

My duties in my new assignment required me to supervise the operation of the Washington University cyclotron which, with the diversion of the Berkeley cyclotrons to war-related research, had become the major source of radioisotope production in the country. It was the property of the Mallinckrodt Institute of Radiology, but its avuncular director, Dr. Sherwood Moore, was happy to see it used for the support of a general program of isotope research at the medical school and on the campus. For help in cyclotron maintenance and in building a mass spectrometer to extend researches to the use of rare, stable isotopes, I could rely on the cooperation of Professor Arthur L. Hughes, the chairman of the physics department, and two excellent technical associates, A. Schulke and H. Huth. A number of investigators in the medical school were anxious to begin or expand projects involving isotopic tracers and materials in basic biomedical research and clinical applications. I had only an empty laboratory to start with but had been allocated ample space in the institute at the medical school and had a modest budget to begin accumulation of laboratory facilities and the employment of technical help.

There was no possibility, at least for some time, of renewing the researches Sam and I had expected to continue. Moreover, two major research groups were already engaged in such an effort, one at Berkeley where Melvin Calvin was taking up the continuation of our original researches in photosynthesis, with Andrew A. Benson providing some continuity, and the other at Chicago under the direction of Hans Gaffron. While I was slowly building my own research effort, I had ample opportunity to collaborate in numerous projects at the medical school. A group, led by Dr. E. V. Cowdry in the department of anatomy, was embarking on an ambitious program in cancer research at the associated Barnard Skin and Cancer Hospital. It received the first shipment of ^{14}C to be made at Oak Ridge. I was a coauthor of a number of papers with these investigators, including studies in calcium metabolism in rotifers, as well as on the effects of phosphate metabolism in human tissues dosed with carcinogens.

Another research of more basic character involved the study of blood dyscrasias in collaboration with Dr. Carl V. Moore in hematology. Dr. Moise Grinstein, a noted porphyrin chemist visiting from Argentina, proposed to use ^{14}C-glycine to study hemoglobin synthesis in various clinical applications. David Shemin and David Rittenberg had shown that ^{15}N-glycine was an efficient precursor of heme and had suggested that the whole molecule was

incorporated into the porphyrin moiety as well as into the globin (33). Accordingly, I proposed that we use ^{14}C-carboxyl-labeled glycine, available from Dr. R. B. Loftfield at the Massachusetts Institute of Technology, for these studies. In preliminary experiments with a dog and a rat, we were surprised to find that carboxyl carbon of glycine entered the globin but not the heme (34). This finding was a key to the eventual elegant unraveling of the details of heme biosynthesis by Shemin (35).

One day, J. W. ("Joe") Kennedy, the newly arrived chairman of the chemistry department, phoned me to inquire if anyone was interested in how viruses multiply. I replied that a free trip, lodging, and a state dinner in Stockholm awaited the successful solver of this problem, whereupon Kennedy proposed an experiment he thought might provide the answer. It involved labeling with high specific ^{32}P activity, the phosphorus of tobacco mosaic virus, or some other suitable virus, using it to infect tobacco leaves, and assaying the viral progeny for ^{32}P content. If none was found, this would indicate the parent virus molecule acted simply as a template. If the ^{32}P were distributed at random through the progeny, it would follow that the parent was fragmented into conserved structures used to resynthesize new virus. I suggested that a better system was *Escherichia coli* bacteriophage because it was not possible to quantify infective units in the case of tobacco mosaic virus and also because we had in residence an outstanding expert on the physiology and manipulation of bacteriophage. He was Alfred V. Hershey in the department of bacteriology. The experiments that resulted included the participation of my first graduate student, Howard Gest, and produced data that were clearly interpretable as a consequence of the fragmentation hypothesis. Moreover, they showed that the recoil process occurring in the beta decay of ^{32}P to ^{32}S denatured the viral molecule, a kind of "suicide." The paper we published (36) was the basis for the development in later years of a thriving area of bacteriophage research based on the "suicide" phenomenon.

Most of my collaborative efforts were expended in a series of productive researches with Sol Spiegelman, then at the beginning of his brilliant career. We studied phosphorus metabolism in yeast. We found differing pool sizes for various yeast phosphates and nucleotides, including a very metabolically active fraction of metaphosphate not in equilibrium with the major metaphosphate pool. We designed experiments to obtain information on phosphate flux in and out of the various intracellular phosphate components. Information so obtained could be related to mechanisms of enzyme formation with particular reference to energy requirements. The results we reported (37) were suggestive with respect to the genetic regulation of enzyme and protein synthesis. We proposed a concept of gene action in which partial replicas of genes were continually produced in the nucleus and exported to the cytoplasm, there to exist as self-duplicating units in a competition for protein which eventually determined

the enzymic composition of the cell. We used the term "plasmagene," coined previously by others, for these self-duplicating units. This term might be said to have prefigured "messenger RNA." (This article got us a lot of notoriety because *Time* magazine wrote a report based on it, titled "Tempest in the Cells.")

The tempo of tracer activity nationwide was accelerating. I found myself attending numerous conferences and seminars as well as writing review articles. The first of these, which provided a respite from the dreaded St. Louis summer in the air-conditioned facilities of the medical school library, was a response to a request from Professor G. Evelyn Hutchison at Yale to assess the status of isotope biochemistry in geochemistry, particularly with respect to the suggestion by the reknowned Russian, W. I. Vernadsky, that anomalies in isotope ratios of various elements in the biosphere could result mainly from intervention by living systems. I had to review all the data then existent on isotope effects and provide a critique of the experimental findings. The article I wrote (38) was a pioneer effort in biogeochemistry. I concluded that natural physicochemical effects could account for all the variations in isotope ratios reported.

The next summer I attended the symposium at Cold Spring Harbor on cytoplasmic factors in heredity and stayed on to write a full-scale text on radioactive tracers in biology for Academic Press (39). I was working under extreme pressure to finish the book before returning to the trenches in St. Louis, and my failure to appear in the outdoors occasioned much comment and concern among the symposium participants. One day I did emerge, blinking in the sun, like a troglodyte coming out of a cave and clad only in swimming trunks, to be waylaid by Max Delbruck and his wife Manny, who led me to the pier where I was deposited in their boat and moved out a half-mile onto the Sound. As I was lolling languidly in the bow, Max suddenly turned and ordered me into the water, saying the swim back would do me good as I needed the exercise. This experience helped my resolve to stay indoors!

As Kurt Jacoby, the fatherly publisher of Academic Press, had surmised, the timing for such a book was perfect. A large, pent-up demand for a modern text on tracer isotope methodology existed and sales were phenomenal for such a technical treatise. At the Press, they called it "Our *Gone with the Wind*." I was particularly gratified with the favorable reviews it received, especially from one of my early heros, Fritz Paneth. Hevesy also wrote a glowing letter, as did another idol, A. J. Kluyver. The only sour note was a brief review in Russia which complimented the translator but complained there were too few references to Russian work. The book went into three editions and many reprintings before the increasing burden of keeping it current forced me to discontinue further production.

Shortly after the arrival in 1947 of my first graduate student, Howard Gest, who had heard about me from friends at Oak Ridge, like Charley Coryell, Harrison Davies, and Waldo Cohn, I acquired two more graduate students, Jack Siegel—also from Oak Ridge—and Herta Bregoff. I had been brooding about the many discussions Sam Ruben and I had had concerning the possibility that phosphorylation in some way made ATP in the light and could be the driving force in the photosynthetic dark CO_2 uptake. Sam had actually published a note (40) on some of his speculations, basing a model scheme on the Warburg-Christian enol phosphopyruvate reaction. My level of sophistication about phosphate metabolism had risen markedly since then, as I sweated at the bench learning to be a biochemist by isolating and purifying enzymes and phosphate esters of the glycolytic pathway and synthesizing various phosphorylated substrates needed in the studies on yeast metabolism.

I had suggested to Howard that he do his thesis work on the effects in algae of illumination on phosphorus turnover. To aid his efforts, I had arranged to have him attend van Niel's famous summer course. He came back enthusiastic about phototrophic bacteria as additional systems for study. I became similarly affected. Siegel was assigned as a thesis subject a reinvestigation, using isotopic carbon, of the claim by J. W. Foster that certain strains of *Pseudomonas* effected a photooxidation of isopropanol that involved simple dehydrogenation of substrate to acetone with simultaneous reduction of CO_2 to cell material, according to the reaction:

$$2\ \begin{array}{l} CH_3 \\ | \\ H\text{-}C\text{-}OH \\ | \\ CH_3 \end{array} + CO_2 \rightarrow 2\ \begin{array}{l} CH_3 \\ | \\ C{=}O \\ | \\ CH_3 \end{array} + (CH_2O) + H_2O$$

It soon became apparent that the phototrophic bacteria were potentially better objects than algae for study, primarily because they might be more amenable to genetic manipulation, if needed, and because they lacked the oxygen-evolving capacity of algae and so might provide an easier access to isolation of the precursors of oxygen. An amazing sequence of serendipitous happenings, beginning with the inadvertent use of glutamate rather than ammonia as a nitrogen source in growth experiments, related in detail elsewhere (41), led Gest and me to the important discoveries of hydrogen photoevolution (42) and nitrogen fixation (43) in the nonsulfur, purple photosynthetic bacteria. These phenomena underscored a basic relation between hydrogen and nitrogen metabolism that opened new areas of research of significance in later years relative to enzymic mechanisms of nitrogen fixation and storage of bioenergy. As an example, a whole field of investigation has developed centered on the mechanisms whereby nitrogenase effects hydrogen evolution.

The demonstration that so large and varied a family of microorganisms as the photosynthetic bacteria included nitrogen fixers shocked the workers in the field and thereby introduced us to new friends among the microbiologists, especially Perry Wilson and his associates, as well as Robert Burris at the University of Wisconsin—a profitable connection as it turned out very shortly.

During his summer in van Niel's laboratory, Siegel found that the original Foster strains no longer existed. He obtained other strains by enrichment cultures, which could perform the dehydrogenation of isopropanol to acetone and beyond. He also extended our observations on photohydrogen production, showing it to be a general phenomenon among phototrophic bacteria and not confined to *Rhodospirillum rubrum,* the original strain investigated. Herta Bregoff established the stoichiometry of the reaction with malate and other substrates and showed that hydrogen evolution began only after complete exhaustion of ammonia in the culture medium.

All of this frenetic and highly productive activity was superimposed on an intense social and musical life that brought me into intimate contact with leading intellectual and musical circles in the city. Unfortunately, it also stirred Security, now the F.B.I., to renewed surveillance because of my many new friends whose liberal politics reinforced suspicion.

Soon after my arrival in St. Louis, Chancellor Arthur H. Compton had taken office and among his first acts was an audience with me in which he attempted to allay my fears of further reprisals stemming from the affair in Berkeley. He had been consulted at the time of my dismissal and had regretfully agreed to it solely on the grounds that it was a precautionary action. He was determined that no further injustice be done, a decision which laid a considerable burden on him in the next decade as he withstood pressures brought to bear by trustees and some others swayed by the campaign of vilification which attended the F.B.I. investigations. From my own reading of history and from the early news reports, it was clear that there was no lack of those who would exploit the mindless hysteria in the wake of the Cold War and attempt to turn prophylaxis into radical surgery.

My fears were soon realized. In response to my application for a passport to attend a symposium in France to which I had been invited, as well as to give lectures in Israel and Australia, federal agents raided the travel office in New York and seized the passport that had been issued a few days previously. For the next seven years I was embroiled in a struggle with the passport division of the Department of State, as well as in a libel action against the *Chicago Tribune* and its associated paper, the *Washington Times-Herald.* One is reminded of Cesare Sterbini's famous lines in the celebrated Calumny aria from Rossini's *Barber of Seville* wherein the elderly Don Bartolo is instructed in the gentle art of calumny by the cynical music master, Don Basilio:

. . . Oh! calumny is like the sigh
Of gentlest zephyrs breathing by;
How softly sweet, along the ground
Its first shrill voice is heard around:
So soft, that, sighing 'mid the bowers,
It scarcely fans the drooping flowers.

Thus will the voice of calumny,
More subtle than the plaintive sigh,
In many a serpent-writhing, find
Its secret passage to the mind,—
The heart's most inmost feelings gain,
Bedim the sense, and fire the brain.

Then passing on from tongue to tongue,
It gains new strength, it sweeps along
In giddier whirl from place to place,
And gains fresh vigor in its race;

Till, like the sounds of tempests deep,
That through the woods in murmurs sweep
And howl amid their caverns drear,
It shakes the trembling soul with fear.

At length the fury of the storm
Assumes its wildest, fiercest form,—
In one loud crash of thunder roars,
And, like an earthquake, rocks the shores.
While all the frowning vault of heaven,
With many a fiery bolt is riven.

Thus calumny, a simple breath,
Engenders ruin, wreck and death;
And sinks the wretched man forlorn,
Beneath the lash of slander torn,
The victim of public scorn.

The storm first broke early in the summer of 1948 in the middle of the exciting discoveries of photohydrogen evolution. Summoned by subpoena to Washington to testify at hearings of the House UnAmerican Activities Committee (HUAC), I turned to Edward U. Condon for help. Condon, the head of the Bureau of Standards, was a prime target for HUAC in its attempt to exploit the Cold War hysteria to bring discredit on the memory of Franklin D. Roosevelt. Condon, writing to me at the time, remarked wryly, "They have the verdict; now all they need is the evidence," and arranged legal help from the office of Arnold, Porter, and Fortas, which was already submerged in appeals for aid from a legion of victims in and out of the government employ.

The press in St. Louis rallied to my cause, encouraged by strong statements of support from Chancellor Compton. The storm passed, not without considerable prejudice to my chances of academic advancement as the notoriety associated with me because of the libel and the passport difficulties effectively choked off offers I would have otherwise received as my career in science prospered. A new and much more formidable threat than the HUAC, Ruth B. Shipley, the chief of the passport division, remained as a barrier to be overcome. The resulting battle to establish the rights of free citizens to a passport that could not be withheld by an arbitrary and capricious bureaucrat consumed another four years. I was fortunate to find legal counsel, ethically motivated and supremely competent and unafraid of professional reprisals, in the persons of Nathan H. David and Alexander E. Boskoff. Taking on the McCormick enterprises, they helped me win the war with a unanimous jury verdict against the *Washington Times-Herald,* upholding our contention I had been libeled, and awarding damages. In a most fortunate development, the McCormick organization, through its powerful connections among various federal agencies, had attempted to bolster its allegation by producing hitherto highly secret files that contained obvious lies, misstatements, and the raw unevaluated data that had formed the basis for the actions taken by the Army in 1945. Without those efforts by Col. McCormick's informants we would have never known the true character of the fabrications that had caused me such trouble for a decade. They would have remained safely concealed, always available to foment continued persecution and harassment.

A Major Diversion

While the grim counterpoint of federal harassment to the main tune of scientific progress continued as the 1950s began, my three graduate students finished and left, leaving no prospects of new recruits because I still had no formal appointment in an academic unit empowered to engage in graduate studies. I was an employee of the department of radiology and had been given the dispensation of supervising doctoral thesis work only because the students involved could qualify for support by the departments of bacteriology or chemistry. It seemed most expedient to follow the practice of other investigators in the medical school and rely on help from postdoctoral fellows. Never having had one before, I wrote for advice to Burris and Wilson at Wisconsin and through them learned that Leo Vernon was available. He had finished work on the characterization of mitochondrial cytochrome *c* reductase and had the experience with redox enzymes I thought essential to implement ideas I had generated on the mechanism of energy bioconversion in photosynthetic bacteria. I had corresponded with Fritz Lipmann and thereby had sharpened my own perceptions of how ATP might be involved. A beginning required elaborating the

enzyme composition of the bacterial chromatophores. Influenced by van Niel's general theory of photosynthesis, I thought the chromatophores should exhibit a reaction with free energy storage analogous to the Hill reaction in chloroplasts. The difficulty in documenting such a process lay in the fact that the oxidizing component of the primary photosynthetic act (customarily schematized as the photoproduction of "OH" from water) was not eliminated by evolution as oxygen, as in green plant photosynthesis. Rather, it would accumulate and back-react with the reductants produced simultaneously, so that only heat would result with no storage of isolated reductants and oxidants. It was clear that enzymic mechanisms prevented such back-reactions in vivo, so I reasoned that by determining what these enzymes were one could devise suitable reagent species to show the Hill analogue of the chloroplast reaction in the anaerobic chromatophores.

Meanwhile, the main line of endeavor remained the elucidation of the light effects on metabolism in the phototrophic bacteria. Research fellows came to aid in this effort—John Glover and Stanley Ranson from England, H. Van Genderen from Holland, and R. Collet from Switzerland—as did Sam Ajl, a newly arrived junior faculty member in the department of bacteriology. The major finding was that dark metabolism of substrates followed the course of the Krebs cycle but that light suppressed cycling of substrate carbon and diverted it almost completely to cell synthesis. As an example, the methyl carbon of acetate appeared quickly in CO_2 in the dark, but not in the light. Equilibration of acetate carbon through the Krebs cycle could be demonstrated by analysis of the label content in cycle intermediates.

On Leo's arrival, we discussed what enzymes to start looking for, and Leo suggested we begin by examining extracts for redox components of the cytochrome system. I was dubious because I was still under the influence of the dogma, then current, that cytochromes were present only in respiratory systems. Leo reminded me he had experience only with the aerobic enzymic components of mitochondrial respiration and coupled ATP synthesis. On his first trial, he found to our surprise that there were relatively large amounts of a heme pigment practically identical spectroscopically with mitochondrial cytochrome *c!*

I wondered how such a feature of *R. rubrum* extracts had escaped previous observation in all the years (at least a half century) this bacterium had been studied as the prototype of bacterial photosynthesis. Writing to Roger Stanier, an authority on bacterial metabolism and general microbiology as well as an old friend, I inquired whether he had ever noticed anything peculiar about *R. rubrum* extracts, particularly the pink color. He replied that it was well known that such extracts were pink, probably owing to the presence of bacteriochlorophyll decomposition products. It appeared no one had ever looked at the spectrum!

It may well be asked why photohydrogen evolution and nitrogen fixation, two such remarkable metabolic features of *R. rubrum,* had also eluded detection through the years. The answer lay in the circumstance that the accepted nitrogen source had always been ammonia, an inhibitor of photohydrogen evolution. Nitrogen fixation had thereby been missed. An irony of history can be remarked here. The noted Dutch microbiologist, M. W. Beijerinck, had originally thought that the free-living nitrogen-fixer, *Azotobacter,* should be called *Para-chromatium,* in view of its strong morphological resemblance to *Chromatium,* the phototrophic sulfur bacterium, but had decided against this because he thought *Chromatium* did not fix nitrogen. Half a century later, we showed that Beijerinck's intuition had been correct!

The news that *R. rubrum* chromatophores contained cytochrome *c* met with the expected disbelief in many quarters. In England, Sidney Elsden repeated our experiments and convinced himself that indeed *R. rubrum* harbored a cytochrome *c*. He went further and did some experiments we had postponed until the fall because Leo and I were to be in van Niel's laboratory that summer. Elsden found that the new cytochrome *c* differed from its mitochondrial analogue in having an isoelectric point near neutral pH and being totally unreactive with the mitochondrial cytochrome *c* oxidase, although, as we had found, it reacted well with the reductase. We jointly published a short note (44), announcing the existence of the new cytochrome, termed later "cytochrome c_2" as the names "*c*" and "c_1" were already assigned to mitochondrial cytochromes. This unexpected diversion from the main objective of our research—from definition of the mechanisms of conversion of light energy in chromatophores to a study of structure and function of cytochromes *c*—eventually developed over the next quarter century into the new field of comparative biochemistry of iron proteins.

Further studies on cytochrome content and associated enzymology in chromatophores revealed a new kind of C-type cytochrome, which exhibited the anomaly of high-spin spectra like those of hemoglobins and catalases, while maintaining the characteristic covalent binding of the heme moiety through thioether linkage to cysteinyl residues as in cytochromes *c* and c_2 (45a).

Adapting an approach by Alan Mehler using oxygen as a "Hill oxidant," we were also able to demonstrate photoreactions in chromatophores and chloroplasts sequestering oxidants and reductants. We observed stoichiometric yields of oxygen uptake and acetaldehyde formation consistent with the oxidation of ascorbate through 2,6-dichlorophenyl indophenol coupled photochemically with catalase-mediated peroxidation of ethanol. In both spinach chloroplasts and *R. rubrum* chromatophores, we could conclude that a Hill system, with molecular oxygen as a Hill oxidant, was operative (45b).

Our next problem was to show that a Hill system was generated under anaerobic conditions. While we were struggling to find an uphill process such

as simultaneous reduction of a low-potential H-donor, like NAD, and oxidation of a high-potential acceptor, like cytochrome *c*, I received a letter from Albert Frenkel, who had worked with Sam Ruben and me as a graduate student in plant physiology back in Berkeley on magnesium ion exchange reactions in chlorophylls and chlorophyll derivatives. He had been on a sabbatical fellowship in Fritz Lipmann's laboratory. Following a suggestion by Lipmann that he look at the reactions in chromatophores that were our interest in St. Louis, he had proceeded to do the simple and straightforward experiment of incubating *R. rubrum* chromatophores anaerobically in the light with ADP and inorganic phosphate. Incredulously, I read that he had found ATP formation in amounts far beyond what might have resulted from utilization of trace amounts of oxygen that might have been present. He had discovered photophosphorylation in chromatophores, the reaction needed to complete the scheme of coupled photochemical energy conversion (46). Independently, Arnon and his associates in Berkeley found the same reaction in green plant chloroplasts (47). Vernon and I could note ruefully that we had missed this discovery by too much cogitation when the phenomenon was readily demonstrable with a simple, direct experiment.

Our work on chromatophore photooxidations and cytochrome composition had been supported partially by a fellowship grant from the National Institutes of Health (NIH) to Vernon. The Institute officials had been quite eager to make this award, but—suddenly reneging on an implied commitment to continue support—they terminated the award, with no time allowed for us to find other employment opportunities for Vernon, who was in no way at fault. This was but one example of many that characterized the political interference in research stemming from Cold War pressures. Fortunately, Leo found employment soon at Brigham Young University and went on to a distinguished career, including many years as research director at the C. F. Kettering Foundation.

With funds from the National Science Foundation, thanks to William Consolazio and Estella Engel, and the Kettering Foundation, as well as from my modest budget, I found it possible to bring Jack Newton to St. Louis. He confirmed an observation I had made that a C-type cytochrome existed in extracts from the photoanaerobic *Chromatium,* characterized it further, and went on to show such extracts could carry out photophosphorylation (48). Meanwhile, I spent much time in the trenches prosecuting the war with the Establishment. Its successful outcome, with victory in the libel action in 1955, the subsequent issuance of the passport, and the decision of the Justice Department to clear my record, brought a resumption of funding from the NIH.

Some Relevant Asides

In the next quarter century, our researches on the metabolism of phototrophic bacteria intensified. At steady state, the number of investigators in our group

was well over two dozen, distributed more or less evenly among postdoctoral fellows, graduate students, and technical support personnel, creating a heavy administrative burden for me. Our efforts involved collaborations with a half dozen research organizations in this country and abroad.

Serendipity continued to play its role in a substantial record of achievement, which included not only opening new research areas, such as the comparative biochemistry of cytochromes and structure-function relations in bacterial iron proteins, but also adventures in academe, as I participated with Nate Kaplan in the creation of a new department of graduate biochemistry at Brandeis University, and with others in founding a new campus of the University of California at La Jolla, and a new academic unit in molecular biology at the University of Southern California. However, I have given most attention in this prefatory chapter to the first quarter century of my career, because it is the story that uniquely records the effect of societal impact.

Recall the legend of Damocles fated to sit at a sumptuous banquet with a sword over his head. I, too, had a scientific banquet spread before me, owing to the fantastic metabolic versatility of the phototrophic bacteria, but the sword of the Federal Establishment dangled overhead. Fortunately, the sword was eventually removed and I could partake of the banquet freely, as I have done since. Only a very brief summary of my experiences, wholly inadequate in chronicling the individual contributions of many colleagues and students, can be offered in the following section.

Big Questions and Small Answers

The phototrophic bacteria exhibit the ability to grow utilizing every known metabolic pattern except oxygenic photosynthesis. Their complexity of cellular organization is probably unsurpassed in living organisms. For example, both anaerobic photophosphorylation and aerobic phosphorylation often exist side by side in their membrane systems (chromatophores) whereas in green algae and plants they are neatly separated and packaged in cellular organelles (chloroplasts and mitochondria). I can cite an interesting case in point—the primitive animal or protozoan *Euglena gracilis*—in which the functional C-type cytochrome ("cytochrome *c*-552") is localized in the chloroplast, whereas a mitochondrial C-type cytochrome ("cytochrome *c*-556") functions as the substrate of a cytochrome *c*-oxidase outside the chloroplast in the respiratory apparatus (49).

I first became interested in this aspect of bacterial metabolism in following up the original observations of H. Nakamura on inhibition of oxygen uptake by light (50). Many investigations on the respiratory systems of *R. rubrum* and related bacteria in our laboratory (see Refs. 51–53 as examples) and elsewhere have failed to resolve wholly the fundamental mechanism of this phenomenon,

but serendipity has entered often in the discovery of new heme proteins and their functional variability.

The ability to travel freely broadened my base of expertise on structure-function relationships of redox enzymes and systems. I worked in Sweden, Germany, England, Japan, and Australia with outstanding researchers like A. H. Theorell, G. Drews, R. Hill, K. Okunuki, F. Egami, and R. Lemberg and their associates. I still remember one exciting afternoon spent with David Keilin and Robin Hill in Cambridge, when Hill and I stood at the bench feeding reagents and protein samples to Keilin who, peering into his hand visual spectroscope, explored the ligand behavior of the heme protein Vernon and I had just discovered and which came to be called cytochrome *c'*. At the end of the day, having learned more cytochrome chemistry than I had known previously, I had the satisfaction of hearing Keilin remark that he would not have believed such a heme protein could exist.

Indeed, he was not alone in this belief. The anomalous behavior of cytochrome *c'* has intrigued and mystified us and others for several decades. It exhibits absorption spectra and magnetic properties usually associated with "unsaturated" high-spin heme proteins, like myoglobins and peroxidases, but a redox behavior, prosthetic group, and mode of binding like that of the "saturated" cytochromes *c*. Its ligand reactions (54) are not typical of either of these in that it reacts only with the small neutral ligands, CO in the reduced state, and NO in both the reduced and oxidized states. It is highly autooxidizable, encouraging the notion it might function as an oxidase, but no convincing data in support of this idea were ever obtained (54, 55). The oxidase function in *R. rubrum* and other phototrophic bacteria is served mainly by an ubiquitous B-type "cytochrome *o*" (54).

The magnetic properties of cytochrome *c'* posed more difficulties. Originally, Anders Ehrenberg and I found (56) a magnetic moment for the oxidized *Chromatium* protein much lower than expected for a pure high-spin heme compound. We suggested the protein might exist as a thermal mixture of high- and low-spin states. An alternative view, based on later EPR studies, proposed cytochromes *c'* to be examples of intermediate spin states with quantum mechanical admixtures of high-spin (57). A trail of investigation eventually led me to the campus of the University of Southern California where P. J. Stephens had a unique magnetic circular dichroism determination apparatus wherewith this question could be probed further. We concluded (58) that the data available could be rationalized by the assumption that cytochrome *c'* was essentially a high-spin heme protein, a conclusion also reached by others (59). The question still remains open. Very probably the explanation is that the ligand field strength that stabilizes the intermediate spin state is so close to that of the upper state that very small changes in the ligand environment can change resultant spin moments (60).

Attempts to establish the function of this ubiquitous protein have been fruitless so far. It does not act as an oxygen-transport heme protein, as in myoglobins. Cyril Appleby and I looked at spectra of cytochrome *c'* down to extremely low oxygen pressures permitted by "NAK" alloy without seeing formation of an oxygen adduct. No evidence for peroxidase activity has been found.

It seemed possible that cytochrome *c'* might be an artifact of isolation procedures and that it existed in vivo as a cytochrome of the B- or C-type. This notion was encouraged by finding of a stable low-spin cytochrome *c* in a number of phototrophic bacteria ("cytochromes *c*-556") homologous with cytochromes *c'*. However, this rationalization seems to have been laid to rest decisively by the recent demonstration of F. R. Salemme, P. Weber, and their associates that the three-dimensional structure of *Rhodospirillum molischianum* resembles not that of a mitochondrial-type cytochrome *c* but those of cytochrome *b*-562, hemerythrin, apoferritin monomer, and tobacco mosaic coat protein (61). These resemblances to so many functionally dissimilar proteins provide no clues at all to functions. The tertiary structure provides a basis for understanding most of the anomalous properties of cytochromes *c'*, including the kinetics of CO binding, studied by M. A. Cusanovich and Q. H. Gibson (62), which indicate, as originally noted (63), the reactions to be complex and with limited ligand accessibility.

Relief from the troubles occasioned by cytochromes *c'* was afforded by the family of mitochondrial-type cytochromes c_2, which we found to be distributed widely among phototrophic bacteria, as well as in at least one nonphotosynthetic bacterium, the nitrate-reducer, *Paracoccus denitrificans*. These cytochromes, structurally and functionally, comprise a group that overlaps continuously in homology with the eukaryotic mitochondrial cytochromes *c*. The first sequence determination of a bacterial cytochrome was accomplished in our laboratory by Karl Dus and Knut Sleiten—that of the prototype cytochrome c_2, from *R. rubrum* (64)—and was followed by many others in the laboratory of R. P. Ambler.

It is amusing to reflect on the fact that this first sequence determination required an expensive, complex, and cumbersome setup that cost the American taxpayer about $1000 per residue, whereas the rapid development of the Sequenator by Per Edman and advances in peptide analysis soon reduced this cost by three orders of magnitude!

Many studies on the physicochemical properties of these heme proteins have been reported but I note just one—that on pH dependence of redox potentials of cytochrome c_2 (65)—because to my knowledge the paper in which we reported our results was the only one among several hundred I submitted over the years in which the reviewers not only recommended its acceptance without revision but urged accelerated publication!

Our interest in functional expression paralleled researches on structure. Kinetic experiments on the structural basis for the inability of cytochromes c_2 to react well with eukaryotic mitochondrial cytochrome *c* oxidase, while exhibiting good reactivity with the reductase—a phenomenon, it will be recalled, which delayed our original recognition of the unique character of the bacterial cytochrome c_2—have promoted insights into the comparative biochemistry of cytochromes *c*. B. Errede in our laboratory performed extensive kinetic analyses of the reactions with mitochondrial oxidase using a selected group of cytochromes c_2. She found that the lysine residues effective in promoting binding and reactivity with the oxidase were the same (66) as those determined by direct chemical modifications carried out by E. Margoliash and his associates at Northwestern University and by F. Millett and his group at the University of Arkansas. A finding—provocative from the evolutionary standpoint—was the demonstration that the eukaryotic cytochrome *c* from the primitive protozoan, *Tetrahymena pyriformis*, reacted as predicted hardly at all with the beef heart oxidase because it lacked all the lysines required for productive binding. It is pertinent that this primitive animal possesses a prokaryotic D-type oxidase, rather than a eukaryotic cytochrome *c* oxidase.

A special project to probe structure-function relationships in cytochromes *c* was the basis for a thesis proposed by Arthur Robinson. He set up a Merrifield machine to make selected heme peptides that could be used to establish parameters for study of the intact protein. One might also use them to study ligand reactions as compared with those of cytochrome *c*. The main outcome was a refinement by John Sharp and Robinson of synthetic techniques, which were calibrated by use in producing an active lysozymelike polypeptide in association with John Rupley and his group at the University of Arizona. The final preparation gave quite a good specific activity, nearly 2–3% of the native protein (67). Later, Robinson used his apparatus to develop the suggestion that controlled deamidation of peptides in proteins might be a basis for biological timing in the aging process (68).

The three-dimensional structure of the *R. rubrum* cytochrome c_2 determined by F. R. Salemme in Professor J. Kraut's laboratory was among the earliest for cytochromes *c* and provided the basis for deductions on structure-function relationships (69), a basis further elaborated by Gary Smith using NMR spectroscopy (70). A serendipitous aspect of this work was his discovery with G. W. Pettigrew of a new N-terminal blocking group derived from *N,N*-dimethylproline in the mitochondrial cytochrome *c* of the insect parasite *Crithidia oncopelti* (71). This amino acid had not been reported as existing in proteins but only in the free form.

The researches on the bacterial cytochromes resulted in wholly new perspectives on potential variabilities in structure and function of the heme group in relation to protein. We showed that at least 12 subgroups of the cytochromes

c exist, based mainly on homologies determined by R. P. Ambler and his associates, using samples supplied largely by R. G. Bartsch and T. E. Meyer in our laboratory. A summary of the present states of knowledge can be found in recent reviews (72, 73).

Another round of serendipity heralded the discovery of yet another kind of bacterial iron protein. Robert Bartsch, just beginning his fruitful career, turned his attention to a reexamination of *Chromatium* extracts. He used DEAE columns, rather than the carboxymethyl cellulose resins we had used previously. Under the conditions required for DEAE chromatography, there appeared a new, readily eluted fraction that we had missed. It contained a greenish-brown protein that proved reversibly oxidizable and exhibited a high positive redox potential. The absorption spectra of both redox forms showed little structure, only a general absorption with a maximum in the near ultraviolet somewhat more pronounced in the reduced form (74). Karl Dus determined its primary structure (75). Later, Henk de Klerk found a similar protein in *Rhodopseudomas gelatinosa,* thereby showing it was distributed not only in the sulfur, but also nonsulfur, purple phototrophic bacteria (76). Subsequently its occurrence in other purple phototrophic bacteria as well as in at least one nonphotosynthetic microorganism has been established. Amino acid sequences from at least seven examples at this time, as determined by S. Tedro and T. E. Meyer, support classification in a group separate from other nonheme proteins, such as ferredoxins and rubredoxin. The sobriquet "HIPIP" (acronym for "High Potential Iron Protein") has been applied, referring to its major distinguishing characteristic, the high positive redox potential, although recently some examples of intermediate potential have been found.

Although ferredoxins and HIPIPs have the same 4Fe-4S cluster as a prosthetic group, the range of redox potentials determined by the protein component extends over 800 mV. An attempt to rationalize this fact has been made (77) based on the proposal that the identical 4Fe-4S clusters are in different diastereomeric environments and also exploiting suggestions on hydrogen bonding deduced from high resolution X-ray structure determinations (78a). These effect nonidentical interactions of a tyrosine residue (Tyr-19) with the inorganic sulfurs in the cluster. However, the expected correlations between circular dichroism spectra and redox potentials are not found (78b). Furthermore, the tyrosine originally supposed to be conserved is not present in *Rhodospirillum tenue* (79). An understanding of the structural basis for HIPIP redox potentials still eludes us. Its attainment should help provide a rationale for structure-function relationships in important enzymes, like hydrogenase and nitrogenase.

In 1957 the first international symposium on enzymes held in Japan afforded the opportunity to meet Professors Okunuki and Egami and their associates and students. Thereby, a long series of important collaborations began with T. Horio, S. Taniguchi, T. Yamanaka, J. Yamashita, and T. Kakuno, all of whom

contributed to the successful prosecution of many researches too numerous to record here. In the course of these collaborations, I became familiar with the extensive Japanese literature on heme protein research and microbiology, and found that H. Iwasaki in the laboratory of Professor T. Mori in Nagoya had anticipated our discovery of cytochrome *c'* (80) as had K. Hori in the same laboratory with HIPIP (81). These observations could not be carried further at Nagoya where neither the procedures for bulk production of pure bacterial cultures nor the collaborating laboratories needed for X-ray structure determinations or for application of the many existent spectroscopies were available. Such requirements were fully met during my tenure at Brandeis and at La Jolla, so that the full significance of the qualitative observations at Nagoya could be appreciated.

While our interest in structure and function of iron proteins took center stage, our experiments were always interdigitated with researches on the photosynthetic apparatus in bacteria. We examined model systems such as one in which Richard Kassner showed that in the obligatory presence of coproporphyrin, ferredoxin could be photoreduced by ascorbate or other electron donors whereas the back-oxidation with oxygen did not require such a catalyst (82).

A major question, stemming from the early work on phosphate nutrition, was the minimal composition of the photosynthetic system in bacteria. In 1967 I was asked to direct a new biochemical unit at the laboratory of photosynthesis in Gif-sur-Yvette where J. Lavorel had just become the new director of research. This opportunity, largely made possible through the efforts of Jacques Monod, to exploit a nutritional approach to the definition of a minimal photosynthetic apparatus, resulted in the creation of a small but vigorously active group under the able direction of Françoise Reiss-Husson. I also hoped to begin a search for a good transducing phage to facilitate the use of powerful methods of nuclear bacterial genetics and molecular biology.

The demonstration by Kassner and me that manganese was not essential in bacterial photosynthetic growth (83), however important it might be in oxygenic photosynthesis, influenced our decision to focus on the iron requirement. At Gif, we used a special strain of *Rhodopseudomonas spheroides* to establish that with minimal iron there were correlations at steady state growth between low levels of ATP and photopigment synthesis, with some indications that low ATP levels preceeded onset of bacteriochlorophyll appearance (84, 85). It was certain that photopigment synthesis accompanied low levels of ATP not only in *Rps. spheroides,* but also in *Rhodospirillum rubrum,* as Jurgen Oelze and I found in Freiburg (86, 87), and Greg Schmidt and I found with *Chromatium vinosum* in La Jolla (88). At Gif, another group led by J. Maroc showed that in the strictly anaerobic sulfate-reducers, vitamins K were normal components, as well as in the phototrophic bacteria (90). Other researches at Gif, reflecting the biophysical approach there, probed fluorescence yields, both variable and

constant, to establish correlations with aging (91a). Oelze and I also showed that photoanaerobic and dark aerobic transport chains had common compartments in *R. rubrum,* but that the inhibitor, 2-hydroxy diphenyl, which acted between the ubiquinone pool and cytochrome *b,* could distinguish the two pathways, inhibiting maximally only the phototrophic NADH-dependent reactions in light-grown bacteria, as compared with the succinate-dependent processes (87).

Some forays into the sulfur metabolism of both phototrophes and sulfate reducers should be mentioned, as they present some unfinished business. A collaboration with Kassner and G. C. Wagner established redox potentials for the couples postulated as involved in sulfite reduction in *Desulfovibrio* strains (91b). One of these couples appeared capable of mediating reductive disproportionation of trithionite to sulfite and thiosulfite with a menaquinone (MK-6). We suggested looking for the associated enzyme. Later, Harry Peck came to La Jolla and collaborated with Siv Tedro and me in demonstrating the general existence of a soluble sulfite reductase in phototrophes (91c). Harry, it should be recalled, has had a distinguished career, beginning as a graduate student with Howard Gest.

The molecular biology program was not developed during my time at Gif largely because of difficulties in recruiting investigators, who showed understandable reluctance to leave the safe confines of the *E. coli* bacteriophage system to embark on the uncertain search for transduction in new systems, even though a strong center of molecular genetics, headed by Boris Ephrussi and Piotr Slonimski, was close by in the next building. We did some preliminary experiments at La Jolla to answer the question of whether a special messenger RNA was required to effect transitions from dark aerobic nonpigment synthesis to photosynthetic anaerobic bacteriochlorophyll production. In *Rps apheroides* we saw no difference in RNA composition comparing bleached cells grown under high oxygen peressure and those grown under normal anaerobic light conditions (92). In other experiments in *R. rubrum* (93) using RNA pulse-labeled with tritiated uracil, no evidence for control of photosynthetic protein synthesis at the transcriptional level was found.

It is apparent that the modern biochemical genetic approach is coming into its own. Reports from the laboratories of G. Drews, H. Gest, B. Marrs, S. Kaplan, and D. I. Friedman already describe substantial progress in isolation of various gene transfer agents and temperate phages. Very recently I have heard of researches by Dr. Fevzi Daldal, who has constructed photosynthetically active mutants of phototrophic bacteria that lack the gene for synthesis of cytochrome c_2. My own experience in relatively recent times has been confined to some interesting collaborative efforts with A. F. Garcia and G. Drews using mutant strains of *Rps. capsulata.* In these experiments, membranes capable only of oxidative phosphorylation were isolated from a strain lacking the ability to synthesize photopigments. These were incubated with reaction centers from a

photosynthetically revertant strain. A complete reconstitution of a reaction system was achieved, functional in that it could, on actinic illumination, couple ATP synthesis to photosynthetic electron transport as evidenced by concurrent photoreduction of endogenous cytochrome *b,* photooxidation of cytochrome *c*, and bleaching of reaction center bacteriochlorophyll (94, 95). These experimental results, wholly consistent with our early observations as well as those of others (96), suggest that the two electron transport chains in phototrophic bacteria—one associated with anaerobic photophosphorylation, the other with aerobic oxidative phosphorylation—have at least some shared components.

An important set of graduate thesis experiments to define light-induced electron transport in *Chromatium* sp. was performed by M. A. Cusanovich using chromatophores in which photoinduced changes in cytochrome content were studied as a function of imposed redox potential (97, 98). Two pathways appeared to be functional, one involving a pigment-heme complex coupled to a "cytochrome *c*-555," and another coupled to a low-potential component and a "cytochrome *c*-552." An optimum potential for coupling of photoinduced electron transport to phosphorylation occurred at 50–100 mV, much like the value found earlier (99) for *R. rubrum.* These results were further extended in thesis work by S. Kennel (100), who also noted that the functional cytochrome *c* complex was a membrane-bound "cytochrome *c*-556,552" with one component at high potential, the other at low, and that the solubilized protein showed an amino acid composition totally different from the soluble cytochrome *c*-553 previously isolated (101). These results point the way to future investigations, emphasizing the fact that soluble components, as isolated, may not be the truly functional proteins in photoinduced bioenergy conversion. In this connection, F. Daldal's experiments, cited above, should be noted. Such considerations also bear obviously on speculations about the evolution of bioenergetic mechanisms.

A diversion in recent times, inspired by the energy crisis of the late 1970s, brought our attention back to an old interest, the hydrogenases. In collaboration with N. O. Kaplan, John Benemann, and Jeffrey Berenson, we demonstrated that spinach chloroplast preparations could be coupled photochemically to clostridial hydrogenase to produce hydrogen and oxygen by a splitting of water (102). This much-quoted experiment indicated that if the problems of chloroplast and hydrogenase stability could be solved, a reactor to use cheap solar energy could be devised. Our further studies, based largely on the ideas and considerable skills of Alexander Klibanov, were directed toward simple means of stabilizing the most reactive as well as least air-stable hydrogenase, that from *C. kluyveri.* An attractive and elegant solution involving simple adsorption of the enzyme on solid supports treated with polycations was demonstrated (103). We wrote a review of our studies indicating there is no substantial difficulty in production of stable, active hydrogenases (104a).

Our results on the bacterial cytochromes *c* have led to proposals in some quarters that "phylogenetic trees" intended to show divergence of eukaryotic mitochondrial cytochromes *c* might be extended to include the prokaryotic cytochromes *c*. However, in addition to the difficulties introduced by functional heterogeneity of the prokaryotic proteins, some showing multifunctional behavior as in *Rps. spheroides* cytochrome c_2, and others with no known functionality, as in other cytochromes from the same microorganism, there occurs a "saturation" phenomenon, by which is meant at a certain limit of change there is no further divergence. Instead convergence or back-mutation may become dominant. This is probably the case for cytochromes c_2, which overlap in homology with the most extremely divergent cytochromes *c* from fungi and protozoa in eukaryotes. Hence available data do not as yet permit construction of all-inclusive phylogenetic trees, although they may be quite valid for the unique case of the eukaryotic cytochromes *c*, constrained by the mitochondrial organization to the single function of mediation of coupled electron transport between the two terminal complexes of the respiratory chain. Terry Meyer and I have had a paper on this subject written for some time with the happy title "Only God Can Make a Tree." It is now in press (104b).

As I have mentioned, my childhood experiences conditioned me to avoid crowds, with a consequent disinclination for "hot" areas of research. I was most happy opening doors through which not only I but others could pass and prosper. This was reflected in a Festschrift (105) consisting of papers published at a symposium to mark my survival to age 65. Success in organizing the meeting and seeing the proceedings through to publication, a colossal task, was due to the devoted efforts of Nathan and Goldie Kaplan and Arthur Robinson.

L'Envoi

The banquet has only begun. Many more discoveries await the investigator hardy enough to meet the challenges posed by the phototrophic bacteria. Just recently, a remarkable repeat of history has been reported, from the laboratory of Howard Gest who, it will be recalled, inadvertently substituted glutamate for ammonia in a culture medium for *R. rubrum*, thereby setting the stage for the discoveries of photoevolution of hydrogen and nitrogen fixation in phototrophic bacteria. He has published a report thirty-five years later in which similar inadvertence, this time a substitution of ammonium sulfate for ammonium chloride, created conditions for the emergence of a new genus. Gest has called it "Heliobacterium chlorum" (106)—a fastidious phototrophic microorganism harboring a new bacteriochlorophyll (107). It could grow because in the soil samples used the enrichment cultures included sulfate-reducing bacteria that produced sulfide, thereby discouraging the overgrowth of more commonly encountered phototrophic forms. This episode underscores the importance of greater sophistication in future researches about nutrition fac-

tors, especially manipulations of nutrients. The metabolic versatility of phototrophic bacteria requires close control of environmental conditions and dictates more concern about culture history than has been exercised in the past in some quarters. The variety of cellular responses to small changes in growth parameters, like temperature, pH, trace metal composition, etc, can be well appreciated from a reading of the doctoral thesis of Paul Weaver (108). [See also a review by Drews & Oelze (109).]

As I enter my sixth decade of research activity, I hope to remain a guest at the banquet table, joining in the excitement of chance discovery whether by others or myself and hoping I can even display some sagacity. For the opportunities to experience the fascination of research fun and games in these last five decades I note the generous support given by the National Institutes of Health and the National Science Foundation. In early times, the officials responsible included especially Dr. William Consolazio and Dr. George Meader. Later, Dr. Marvin Cassman helped at a crucial time, while Ms. Estella K. Engel was a constant source of encouragement and support. When the increasing burdens of academic commitment and grant administration drew me away from the bench, Dr. R. G. Bartsch filled the gap, giving added guidance and counsel to many generations of graduate students. Dr. T. E. Meyer is owed special mention for years of arduous and demanding effort in advancing our studies on the many new forms of bacterial iron proteins, as well as helping to organize the inchoate masses of data accumulated.

I am indebted to Profesor Z. Gromet-Elhanan, whose invitation to participate as a consultant and coinvestigator on a US Israel Binational Grant has kept me au courant on continuing progress in the understanding of reaction center biochemistry. In this connection I should not neglect to mention the stimulus given me by the researches of George Feher, Mel Okumura, and their associates at La Jolla. It is possible a most important contribution on my part was enticing George Feher to apply his considerable skills and expertise in solid-state science to the study of primary photoreactions in bacterial photometabolism.

Finally I record my gratitude to the members of the Editorial Board for the recognition implicit in their invitation to write this prefatory chapter.

ACKNOWLEDGMENTS

Leaders of laboratories who collaborated either personally or by sending postdoctoral fellows may be listed: R. P. Ambler, H. and M. Baltscheffsky, B. Chance, R. E. Dickerson, G. Drews, A. Ehrenberg, F. Egami, H. B. Gray, J. Falk, T. Flatmark, Q. H. Gibson, Y. Hatefi, R. Hill, T. Horio, R. J. Kassner, J. Kraut, N. O. Kaplan, J. Lavorel, R. Lemberg, R. K. Morton, K. Okunuki, S. Sano, R. Sato, J. Schiff, P. Slonimski, and A. H. Theorell. Valuable technical help was provided, particularly by Ms. S. Tedro, and by Mrs. H. Smith, Mrs. R. Smith, S. Lindroth, and R. Pabst.

Literature Cited

1a. Remer, T. C., ed. 1965. *Serendipity and the Three Princes*, p. 6. Norman, Okla: Univ. Okla. Press
1b. Rosenau, M. J. 1935. *J. Bacteriol.* 29:91
2. Cannon, W. B. 1945. *The Way of the Investigator*. New York: W. W. Publisher
3. Harkins, W. D., Gans, D. M., Kamen, M. D., Newson, H. W. 1935. *Phys. Rev.* 47:52
4. Kurie, F. N. D. 1933. *Phys. Rev.* 44:461
5. Burcham, W. E., Goldhaber, M. 1936. *Proc. Cambridge Philos. Soc.* 32:632
6. McMillan, E. M., Kamen, M. D., Ruben, S. 1937. *Phys. Rev.* 52:375
7. Kamen, M. D. 1949. *Photosynthesis in Plants*, a symposium, ed. J. Franck, W. E. Loonis, p. 365. Ames: Iowa State College Press
8. Ruben, S., Kamen, M. D., Hassid, W. Z. 1940. *J. Am. Chem. Soc.* 62:3443
9. Ruben, S., Kamen, M. D., Perry, L. H. 1940. *J. Am. Chem. Soc.* 62:3450
10. Ruben, S., Kamen, M. D. 1940. *J. Am. Chem. Soc.* 62:3451
11. Ruben, S., Kamen, M. D. 1940. *Proc. Natl. Acad. Sci. USA* 26:418
12. Barker, H. A., Ruben, S., Kamen, M. D. 1940. *Proc. Natl. Acad. Sci. USA* 26:426
13. Wood, H. G., Werkman, C. H. 1936. *Biochem. J.* 30:48
14. van Niel, C. B., Ruben, S., Carson, S. F., Kamen, M. D., Foster, J. W. 1942. *Proc. Natl. Acad. Sci. USA* 28:8
15. See 1974. *Mol. Cell Biol.* 5:79
16. Evans, E. A. Jr., Slotin, L. 1940. *J. Biol. Chem.* 136:301
17. Ruben, S., Randall, M., Kamen, M. D., Hyde, J. L. 1941. *J. Am. Chem. Soc.* 63:877
18. Kamen, M. D., Barker, H. A. 1945. *Proc. Natl. Acad. Sci. USA* 31:8
19. Warburg, O. 1958. *Science* 128:68
20. Holt, A. S., French, C. S. 1948. *Arch. Biochem. Biophys.* 19:429
21. Dole, M., Jencks, G. 1944. *Science* 100:409
22. Wilson, R. R., Kamen, M. D. 1938. *Phys. Rev.* 54:1031
23. Kamen, M. D. 1963. *Science* 140:584
24. Ruben, S., Kamen, M. D. 1940. *Phys. Rev.* 57:549
25. Alvarez, L., Cornog, R. 1939. *Phys. Rev.* 56:613
26. Kamen, M. D. 1972. *Environ. South West* 448:11
27a. Snell, A. H. 1976. *Oak Ridge Natl. Lab. Rev.* Fall:45
27b. Primakoff, H. 1982. See Ref. 105, p. 45
28. Kamen, M. D. 1941. *Phys. Rev.* 60:537; 1942. 62:303
29. Barker, H. A., Kamen, M. D. 1945. *Proc. Natl. Acad. Sci. USA* 31:219
30. Barker, H. A., Kamen, M. D., Haas, V. 1945. *Proc. Natl. Acad. Sci. USA* 31:355
31. Barker, H. A., Kamen, M. D., Bornstein, B. T. 1945. *Proc. Natl. Acad. Sci. USA* 31:373
32a. Wood, H. G., Drake, H. L., Hu, S.-I. *Biochemistry Symposium*. ed. E. E. Snell, p. 29. Palo Alto, Calif: Annual Reviews
32b. Wood, H. G. 1982. See Ref. 105, p. 99
33. Shemin, D., Rittenberg, D. 1946. *J. Biol. Chem.* 166:621
34. Grinstein, M., Kamen, M. D., Moore, C. V. 1948. *J. Biol. Chem.* 174:767
35. Shemin, D. 1982. See Ref. 105, p. 117
36. Hershey, A. V., Kamen, M. D., Kennedy, J. W., Gest, H. 1951. *J. Gen. Physiol.* 34:305
37. Spiegelman, S., Kamen, M. D. 1946. *Science* 104:581
38. Kamen, M. D. 1946. *Bull. Am. Mus. Nat. Hist.* 87:107
39. Kamen, M. D. 1947. *Radioactive Tracers in Biology*. New York: Academic. 281 pp.
40. Ruben, S. 1943. *J. Am. Chem. Soc.* 65:279
41. Kamen, M. D., Gest, H. 1952. *Phosphorus Metabolism*. ed. W. D. McElroy, B. Glass. Baltimore, Md: Johns Hopkins
42. Gest, H., Kamen, M. D. 1948. *Science* 109:558
43. Kamen, M. D., Gest, H. 1948. *Science* 109:560
44. Elsden, S. R., Kamen, M. D., Vernon, L. P. 1953. *J. Am. Chem. Soc.* 43:492
45a. Vernon, L. P., Kamen, M. D. 1954. *J. Biol. Chem.* 211:643, 663
45b. Vernon, L. P., Kamen, M. D. 1954. *Arch. Biochem. Biophys.* 51:122
46. Frenkel, A. 1954. *J. Am. Chem. Soc.* 76:5568
47. Arnon, D. I., Whatley, F. R., Allen, M. B. 1954. *J. Am. Chem. Soc.* 76:6724
48. Newton, J., Kamen, M. D. 1956. *Biochim. Biophys. Acta* 21:74; 1957. 25:462
49. Perini, F., Kamen, M. D., Schiff, J. A. 1964. *Biochim. Biophys. Acta* 88:88, 91
50. Nakamura, H. 1937. *Acta Phytochim. Tokyo* 9:189
51. Horio, T., Kamen, M. D. 1962. *Biochemistry* 1:1141
52. Taniguchi, S., Kamen, M. D. 1963. *Biochim. Biophys. Acta* 74:438

53. Yamashita, J., Kamen, M. D., Horio, T. 1969. *Arch. Mikrobiol.* 60:304
54. Taniguchi, S., Kamen, M. D. 1963. *Biochim. Biophys. Acta* 112:1
55. Chance, B., Horio, T., Kamen, M. D., Taniguchi, S. 1966. *Biochim. Biophys. Acta* 112:1
56. Ehrenberg, A., Kamen, M. D. 1965. *Biochim. Biophys. Acta* 102:333
57. Maltempo, M. M., Moss, T. H. 1976. *Q. Rev. Biophys.* 9:181
58. Rawlings, J., Stephens, P. J., Nafie, L. A., Kamen, M. D. 1977. *Biochemistry* 16:1725
59. Emptage, M. H., Zimmerman, R., Que, L. Jr., Munck, E., Hamilton, W. D., Orme-Johnston, W. H. 1977. *Biochim. Biophys. Acta* 495:12
60. Williams, R. J. P. 1971. *Cold Spring Harbor Symp. Quant. Biol.* 36:53
61. Weber, P. C., Bartsch, R. G., Cusanovich, M. A., Hamlin, R. C., Howard, A., et al. 1980. *Nature* 286:302
62. Cusanovich, M. A., Gibson, Q. H. 1973. *J. Biol. Chem.* 248:822
63. Gibson, Q. H., Kamen, M. D. 1966. *J. Biol. Chem.* 241:1969
64. Dus, K., Sletten, K., Kamen, M. D. 1968. *J. Biol. Chem.* 243:5507
65. Pettigrew, G. W., Meyer, T. E., Bartsch, R. G., Kamen, M. D. 1975. *Biochim. Biophys. Acta* 430:197
66. Errede, B., Kamen, M. D. 1978. *Biochemistry* 17:1015
67. Sharp, J. J., Robinson, A. B., Kamen, M. D. 1975. *J. Am. Chem. Soc.* 95:6097
68. Robinson, A. B., McKerrow, J. H., Cary, P. 1970. *Proc. Natl. Acad. Sci. USA* 66:753
69. Salemme, F. R., Kraut, J., Kamen, M. D. 1973. *J. Biol. Chem.* 248:7701
70. Smith, G. M. 1979. *Biochemistry* 18: 1628
71. Pettigrew, G. W., Smith, G. M. 1977. *Nature* 265:661
72. Meyer, T. E., Kamen, M. D. 1982. *Adv. Protein Chem.* 35:105
73. Kamen, M. D. 1983. *Fed. Proc.* 42:2815
74. Bartsch, R. G. 1963. *Bacterial Photosynthesis,* ed. H. Gest, A. San Pietro, L. Vernon, p. 315. Yellow Springs, Ohio: Antioch
75. Dus, K. 1973. *J. Biol. Chem.* 248:7318
76. de Klerk, H., Kamen, M. D. 1966. *Biochim. Biophys. Acta* 112:175
77. Carter, C. W. Jr. 1977. *J. Biol. Chem.* 252:7802
78a. Carter, C. W. Jr., Kraut, J., Freer, S. T., Xuong, Ng., Alden, R. A., Bartsch, R. G. 1974. *J. Biol. Chem.* 249:4212
78b. Przysiecki, C. T., Meyer, T. E., Cusanovich, M. A. 1985. *Biochemistry* In press
79. Tedro, S. M., Meyer, T. E., Kamen, M. D. 1977. *J. Biol. Chem.* 254:1495
80. Iwasaki, H. 1960. *J. Biochem. (Tokyo)* 47:174
81. Hori, K. 1961. *J. Biochem. (Tokyo)* 50: 481
82. Kassner, R. J., Kamen, M. D. 1967. *Proc. Natl. Acad. Sci. USA* 58:2445
83. Kassner, R. J., Kamen, M. D. 1968. *Biochim. Biophys. Acta* 153:270
84. Jolchine, G., Reiss-Husson, F., Kamen, M. D. 1969. *Proc. Natl. Acad. Sci. USA* 64:650
85. Fanica-Gaignier, M., Clement-Metral, J., Kamen, M. D. 1971. *Biochim. Biophys. Acta* 226:135
86. Oelze, J., Kamen, M. D. 1971. *Biochim. Biophys. Acta* 234:137
87. Oelze, J., Kamen, M. D. 1975. *Biochim. Biophys. Acta* 287:1
88. Schmidt, G. L., Kamen, M. D. 1970. *Arch. Microbiol.* 76:151
89. Maroc, J., de Klerk, H., Kamen, M. D. 1968. *Biochim. Biophys. Acta* 102: 621
90. Maroc, J., Azerad, R., Kamen, M. D., Le Gall, J. 1970. *Biochim. Biophys. Acta* 197:87
91a. de Klerk, H., Govindjee, Kamen, M. D., Lavorel, J. 1969. *Proc. Natl. Acad. Sci. USA* 62:972
91b. Wagner, G. C., Kassner, R. J., Kamen, M. D. 1974. *Proc. Natl. Acad. Sci. USA* 71:253
91c. Peck, H. D. Jr., Tedro, S., Kamen, M. D. 1974. *Proc. Natl. Acad. Sci. USA* 71:2404
92. Borda, L., Green, M. H., Kamen, M. D. 1969. *J. Gen. Microbiol.* 56:345
93. Yamashita, J., Kamen, M. D. 1968. *Biochim. Biophys. Acta* 161:162; 1969. 182:322
94. Garcia, A. F., Drews, G., Kamen, M. D. 1974. *Proc. Natl. Acad. Sci. USA* 71:4213
95. Garcia, A. F., Drews, G., Kamen, M. D. 1974. *Biochim. Biophys. Acta* 387:129
96. Thore, A., Keister, D. L., San Pietro, A. 1969. *Arch. Mikrobiol.* 67:378
97. Cusanovich, M. A., Bartsch, R. G., Kamen, M. D. 1968. *Biochim. Biophys. Acta* 153:397
98. Cusanovich, M. A., Kamen, M. D. 1968. *Biochim. Biophys. Acta* 153:376, 418
99. Horio, T., Kamen, M. D. 1962. *Biochemistry* 1:144
100. Kennel, S. J., Bartsch, R. G., Kamen, M. D. 1972. *Biophys. J.* 12:802
101. Kennel, S. J., Kamen, M. D. 1971. *Biochim. Biophys. Acta* 234:485; 253: 153
102. Benemann, J. R., Berenson, J. A., Kap-

lan, N. O., Kamen, M. D. 1973. *Proc. Natl. Acad. Sci. USA* 70:2317
103. Klibanov, A. M., Kaplan, N. O., Kamen, M. D. 1978. *Proc. Natl. Acad. Sci. USA* 75:3640
104a. Klibanov, A. M., Kaplan, N. O., Kamen, M. D. 1980. *Enzyme Engineering*, ed. H. Weetal, G. P. Royer, 5:135 Plenum
104b. Meyer, T., Kamen, M. 1985. *Proc. Natl. Acad. Sci. USA*. In press
105. Kaplan, N. O., Robinson, A. B., eds. 1982. *From Cyclotrons to Cytochromes*. New York: Academic
106. Gest, H., Favinger, J. L. 1983. *Arch. Microbiol.* 136:11
107. Brockman, H. J., Lipinski, A. 1983. *Arch. Microbiol.* 136:17
108. Weaver, P. 1975. *Environmental and mutational variations in the photosynthetic apparatus of* Rhodospirillum rubrum. PhD thesis. Univ. Calif., San Diego
109. Drews, G., Oelze, J. 1981. *Advances in Microbial Physiology*, ed. A. H. Rose, J. G. Morris, 22:1–92. New York: Academic