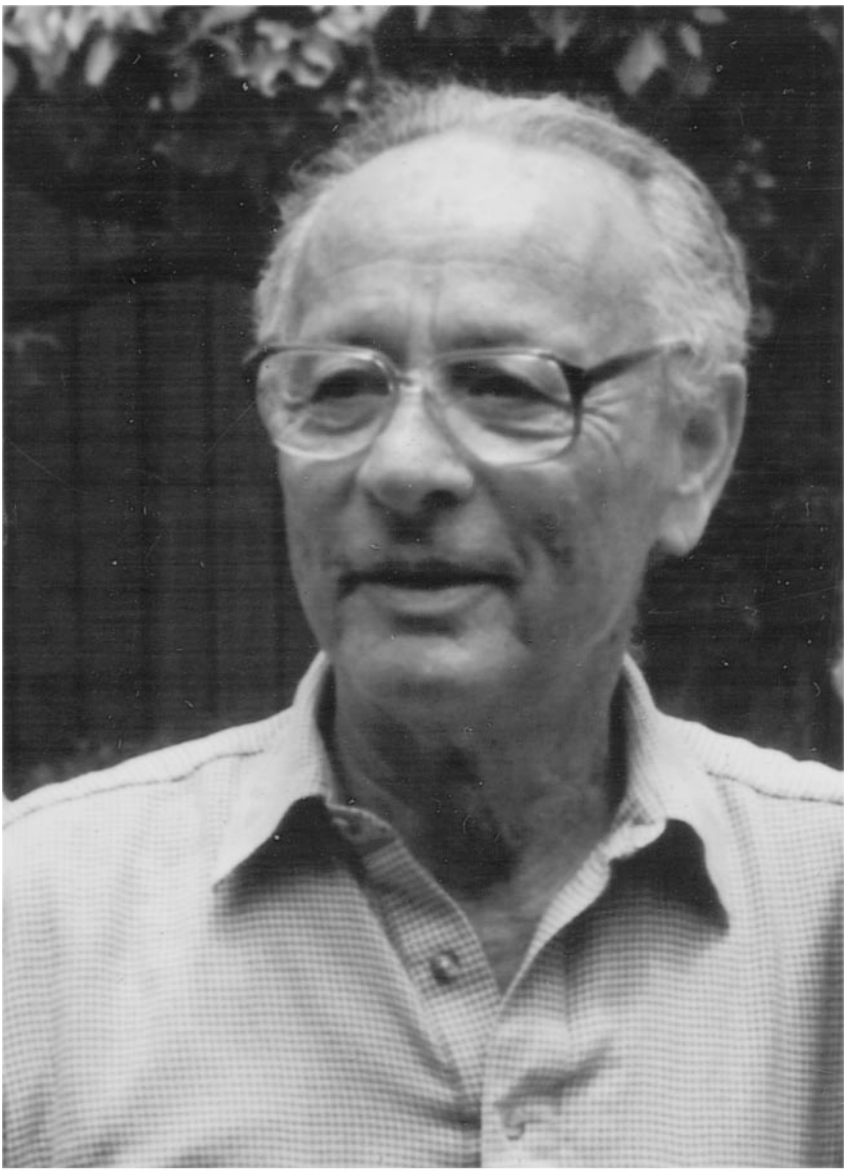

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MY ROAD TO BIOPHYSICS: Picking Flowers on the Way to Photosynthesis

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PROLOGUE

When I was approached by the Editor to write the Prefatory chapter, I felt, of course, honored but was hesitant to accept the task because I had recently finished a personal account of three decades of research in photosynthesis, the first part of which included reminiscences and stories of my past (55). After some thought, however, I decided to accept the challenge. There was life before photosynthesis, after all. During my ∼50 years of research activity, I worked on different topics in "straight" physics, some of which have close connections to the methodologies and approaches used in biophysics. I had also worked on several biophysics problems that were not covered in the previous review. I decided, therefore, to take this opportunity to give an account of this part of my research. To fill out the relatively short shrift that I give here to photosynthesis, the reader is referred to the previous review (55). The Editor asked me to write a personal account, including reminiscences and stories. In trying to follow his advice, some overlap with previously published accounts was unavoidable.

CZECHOSLOVAKIA 1924–1941

I was born in 1924 in Bratislava, a border town close to Austria and Hungary. My father had two passions: business (textiles) and sports (he held for many years the Czechoslovak record in the hammer throw). My mother was more intellectually inclined. She taught in a lyceum for teachers before my sister and I arrived on the scene. From my early childhood I was a tinkerer. I took apart all the clocks and watches in the household and, most of the time, put them back together in working condition. Interspersed with the mechanical activities were chemical experiments. I remember putting a chunk of sodium on a piece of blotting paper and watching it catch fire as it floated on the water surface in the bathtub. Alas, disaster struck when it hit the wall; the bathtub cracked and the entire apartment was flooded. At age 14, I became a radio ham and pursued vigorously what now would be called electronics. In parallel I developed another hobby: growing crystals. My fascination with growing crystals has not abated to this day.

In the meantime, the situation in Slovakia had become grim and perilous. Being a Jew, I was expelled from school in 1938 at age 14. Slovakia, although nominally independent, had become a puppet state of Nazi Germany. Interestingly, we

youngsters, who joined a Zionist organization, saw the writing on the wall more clearly than our parents and their generation, and we were constantly exploring possibilities for an escape.

PALESTINE 1941–1946

In 1941, in the middle of the war, at age 17, I and eight other youngsters from the Zionist movement succeeded in escaping through Hungary, Romania, Turkey, and Lebanon to Palestine (what is now Israel), at the time a British mandate. At the border, we were promptly arrested and interned by the British.[1] A shocking welcome after having barely succeeded in escaping from hell. After a brief internment we were released and joined a kibbutz (communal settlement). Although we had prepared ourselves ideologically for the last three years to live a communal life, some of us found the conformity, drabness, and lack of opportunity for intellectual and professional growth difficult to take. After a year and a half I left for Haifa, where my sister lived and where a technical university, the Technion, was located. I started to work as a radio repairman in the evenings while taking some technical courses during the day in the trade school adjacent to the Technion. One of my teachers was Franz Ollendorff, an internationally recognized authority in electrical engineering, who was also a professor at the Technion. In the fall of 1943, he offered me a position as his laboratory assistant, which I enthusiastically accepted. One of the first challenges that Ollendorff confronted me with was to build an oscilloscope. It is difficult to imagine nowadays that in 1943 there was not a single oscilloscope in the Technion or probably in all of Palestine. From captured German and Italian equipment, I salvaged a cathode-ray tube plus other components and designed and constructed an oscilloscope. Being a patriot, I had the time axis running from right to left in accord with Hebrew writing. Ollendorff was delighted, and when Haim Weizmann (a scientist and one of the great Zionists, who later became the first president of Israel) visited the Technion, he was shown the "Hebrew oscilloscope." He enthusiastically shook my hand and I felt as though I was in Heaven (which in Israel is closer to earth than here).

In addition to repairing radios and being Ollendorff's lab assistant, I also worked for the Haganah (an underground organization whose ultimate goal was the establishment of an independent Jewish state) as an electronics expert. Let me describe a couple of projects as examples of my involvement in "applied research" to give you a feeling of the prevailing times. One dealt with tapping into the direct telephone line between the British High Commissioner in Jerusalem and 10 Downing Street in London and building an unscrambling device to make the conversations intelligible. This work was kept under wraps for over 50 years, and I was not privy to whether it actually worked in the field. An article describing this action appeared

[1]As I write this, I receive a phone call from Eli Bar-On, a fellow escapee, to remind me that this week (April, 2001) it is 60 years that we left Slovakia. He is arranging a get together in Israel of the surviving eight members of the group.

for the first time in an Israeli newspaper in 1992 (1), but a definitive acknowledgment of its working I received only in February 2000 from the Ministry of Defense of the state of Israel (9).

The second project dealt with the invention, in collaboration with a friend, Hanan Myer, of a secret optical signaling device. It worked on the following principle: A neon lamp connected to a 50 Hz power line extinguishes and lights up 100 (i.e. 2×50) times per second. The eye cannot follow such a high frequency and instead sees a steady light. However, when viewed through a telescope whose view is alternately obscured and cleared by a reed vibrating 100 times per second, the lamp is seen either lit or dark, depending on the relative phase of the vibration of the reed with respect to the voltage on the neon lamp. By changing the phase of the voltage feeding it, the neon lamp appears to the viewer either as dark or lit. Thus, Morse code signaling is accomplished by changing the phase. Viewers equipped with a vibrating reed telescope would receive the signal. The unaided eye, being insensitive to the changes in the phase, sees a steady light. The neon lamps were installed on the roofs of hospitals in the form of Stars of David (the Israeli equivalent of the Red Cross), forming a connecting network throughout Israel.

I experienced a flashback of these episodes, combined with strange feelings, when I met John Kendrew at the Board of Governors meeting at the Weizmann Institute a few years ago and he told me that he was stationed as a British officer in Palestine at about the same time I performed these jobs.

Unfortunately, none of the above activities made up for the lack of a high school education, and I could not get accepted into the Technion, which I very much wanted to attend. In 1944, Ollendorff arranged for me to take a special entrance examination. I passed all subjects except the Bible (Old Testament). All of Ollendorff's pleading with the administration did no good. As rector Kaplanski and his admission committee declared, "A Jewish engineer has to know the Bible." I was devastated at that time, although some amusing incidents played out decades later in connection with my failing the exam. One of them occurred in 1975. I was invited to give a set of lectures in connection with the opening of the Solid State Institute at the Technion. After my last talk, an old man with a cane hobbled to the podium, congratulated me on my research and delivery, and remarked that part of the credit for my career should be given to him. "We have never met," he said, "but I was a member of the admission committee in 1944 that refused your entrance to the Technion." And when I received an honorary doctorate from the Hebrew University of Jerusalem in 1994, I could not resist pointing out how much easier it was to obtain that degree than to be accepted to the Technion. Concerning the lack of a high school education, I wonder whether it actually did not have a beneficial effect. The rigidity of the high school system might easily have suppressed any creativity and imagination.

So in 1944, there was nothing left for me but to try to get accepted into a university abroad where the Bible would not be a required subject for admission. I applied to about 50 universities in the United States; only 2 were willing to accept me as a special student: Harvard and the University of California at Berkeley. Harvard was out for financial reasons and so I opted for Berkeley. There was

still the financial problem of the passage to the United States, which was by no means trivial, considering the poor economic situation in Palestine at that time. My previous hobby helped me out.

I remembered the beautiful Rochelle salt crystals I grew in Slovakia. These crystals are piezoelectric. They can therefore be used to construct microphones and phonograph pickups. During the war, none of these items could be imported to Palestine, and there was a dire need to have them for public address systems to entertain Allied troops stationed there. I set up a small production line to manufacture piezoelectric devices, mostly microphones that I sold to the entertainment establishments. By 1946, I had accumulated enough money to embark on the trip to the United States.

UNIVERSITY OF CALIFORNIA, BERKELEY 1947–1954

Undergraduate Studies 1947–1950

I arrived in Berkeley in December 1946, in time for the start of the semester in January 1947. I was excited to get started after an ∼eight-year hiatus in my formal education. I really wanted to study biophysics, having been influenced by Schrödinger's *What Is Life* (142), which I had read while still in Israel. But U.C. Berkeley did not have an undergraduate program in biophysics. Furthermore, the Jewish authorities in Palestine did not look favorably on studying such "useless" fields. Not even physics was condoned. It had to be something more practical like engineering, so I chose the closest field, engineering physics. Like a sponge, I soaked up courses in math, physics, chemistry, astronomy, engineering drawing, and physiology. This was above the allowed limit, but I received permission to do it.

There was a little problem. I had some money to cover tuition and rent for one semester, but there wasn't much left for food. I roomed with an equally poor Israeli, Aaron Gibor, who majored in biology. We took Physiology 1A together, a huge class with laboratory sessions where we dissected frogs. After class, we collected them and boiled the legs for dinner. Fortunately, toward the end of the semester we worked on rabbits, so our diet improved. Somehow, we managed to get through the semester. When summer arrived, I got a job picking fruit in the San Joaquin Valley. I thought my kibbutz experience would come in handy, but I was in for a surprise. The pace of the Mexican fruit pickers was breathtaking (literally). In the kibbutz we discussed ideological issues, like the plight of the suffering proletariat, while picking fruit at a relatively leisurely pace, whereas here I encountered the suffering proletariat that had no time to discuss anything, trying to pick as much fruit as possible because we were paid by the box. It was hard work. The money I made in the summer, together with working for room and board (washing dishes and cleaning rooms), carried me through the fall semester of 1947. Thereafter I became a reader, which meant correcting homework papers and exams at $1.00/h in all courses in which I had received a good grade. In my senior year, I worked part time as an electronic technician in the Electrical Engineering department. In 1950, I graduated with a B.S. in Engineering Physics.

Graduate Studies in Electrical Engineering 1950–1951

In 1949, I married a fellow student and we were expecting a baby. To support the family, I started to work full time as an engineer in the electrical engineering department under the supervision of David Sloan, the inventor of the Resnatron, a high-power microwave tube that was used in England during the war to jam German radar. He continued to build bigger and bigger Resnatrons, and I was given the task of designing the cathode to supply the high currents required by these giant tubes. In parallel, I was able to take some graduate courses in electrical engineering, and fortunately, my work (on thermionic emission of a new dispenser-type cathode) was accepted as a thesis topic. I received a M.S. in electrical engineering in 1951.

Graduate Studies in Physics 1951–1954

Now married to an American citizen, I had the equivalent of a green card and therefore felt less constrained by the desires of the Israeli authorities. Consequently, I switched to physics and joined the solid-state group of A. Kip and C. Kittel. They had a program in electron paramagnetic resonance (EPR), which was a relatively new field, and they needed somebody with an engineering background to design and build EPR spectrometers. My thesis project was electron spin resonance of conduction electrons in metals. But I still had biophysics on my mind, and so on Sundays I came to the lab and put miscellaneous biological materials such as leaves and blood into the microwave cavity. Lo and behold, when the leaves were illuminated a signal appeared. This, incidentally was before the publication of the pioneering paper by Commoner et al. (26) on EPR in biological systems. I also found a signal in blood at $g = 6$, but all hell broke loose when one Sunday Kittel came in and found me doing these frivolous experiments instead of focusing on my thesis project. He threatened to throw me out if he caught me at it again. (Another confrontation with Kittel is discussed in the section on acceptors in silicon.) In addition, he pooh-poohed the signal at $g = 6$, which he was convinced must be an artifact, "Every fool knows that angular momentum is quenched and the g-values cannot deviate far from $g = 2$." It was later that Ingram's group published their work on hemoglobin (14) and that Griffith (77) explained the origin of the $g = 6$ signal. This experience reinforced my belief not to be awed by authority. I wonder now whether these frustrating early experiments had anything to do with my working on heme proteins and photosynthesis decades later. I received a Ph.D. in physics in 1954.

BELL TELEPHONE LABS (BTL) 1954–1960

As I was finishing my Ph.D. thesis, I decided to apply for a job in industry rather than academia. There were essentially two reasons for that decision. One was the academic rat race I witnessed in Berkeley. The stress on assistant professors seemed awesome. I reasoned that by going to a high-caliber industrial research

lab and doing good work, I had a chance to later step into a tenured academic position. The second reason was my intention to return to Israel, in which case the industrial experience would be important because there were no academic research jobs available in Israel at that time.

After receiving my Ph.D. in physics, I joined the research group at Bell Telephone Labs in Murray Hill, New Jersey. I had been interviewed by Bill Shockley of transistor fame, but by the time I arrived, Shockley had left. The reason for his leaving is rather interesting. Shockley argued that the prevalent system for remunerating scientists made no sense. He pointed out that the contributions of scientists differed by orders of magnitude, whereas their salaries differed by only factors of ∼2. He advocated a linear rather than logarithmic relation between contributions and salaries. He circulated a memo in which he proposed this idea and outlined how to measure contributions (e.g., number of patents, papers, talks, and acknowledgments) As an example, he used himself and came up with a yearly salary of one million dollars, which he requested be paid to him. The management balked and Shockley walked.

Systematic Investigation of EPR Spectrometers

Arriving at Bell Labs, I was given complete freedom to pick a project of my own choosing. Arnie Honig, a postdoc at Berkeley during my last year at UCB, had published an exciting, but to me not quite believable, result on EPR in silicon (82). I decided to look into that problem. However, first I wanted to build a good EPR spectrometer. At that time, no commercial EPR spectrometers were available, and everybody was building them haphazardly, making unsubstantiated claims of sensitivities and superiority. I delayed the silicon experiments and instead undertook a detailed, systematic investigation of the sensitivity problems of EPR spectrometers, a topic that I had discussed at length with a fellow graduate student, Alan Portis, when I was still at Berkeley. The outcome of this work was published in an in-house journal (44) after it was rejected by the *Review of Scientific Instruments* for being too long. I must admit to mixed feelings that this, essentially an engineering article, is the most quoted of all my papers. Perhaps it should be a consolation that Jim Hyde, who contributed greatly to the design of the commercial Varian EPR spectrometer, told me that he had used the results of this paper extensively.

Development of the Electron Nuclear Double Resonance (ENDOR) Technique

Honig had found that EPR signals of shallow donors in phosphorous-doped silicon have long relaxation times at helium temperatures (82). Such long spin-lattice relaxation times had never been observed for electrons. Honig therefore interpreted these results in terms of nuclear relaxation times with a concomitant high (∼100%) nuclear polarization (82). This interpretation, which created great excitement in the field, did not feel right to me, and I soon showed that his finding was the result of unexpectedly long electronic (and not nuclear) spin-lattice relaxation times (42).

These long relaxation times made it easy to apply to the system various "spin calisthenics" techniques, among them the inversion of energy levels (15). In P-doped Si, the hyperfine interaction of the P^{31} nucleus with the electron creates a four-level system ($I = {}^1/_2$, $S = {}^1/_2$). By judiciously flipping the populations of the electronic and nuclear levels, a sizable nuclear polarization (equivalent to the electron spin polarization) could be obtained (42). Because we flipped nuclei and monitored their population via the EPR signal, this represented the first electron nuclear double resonance (ENDOR) experiment.

Investigation of Nuclear Structures by ENDOR

DETERMINATION OF NUCLEAR MAGNETIC MOMENTS In ENDOR experiments, one observes nuclear transitions via the electron spin resonance. Because the magnetic moment of the electrons is $\sim 10^3$ larger than that of nuclei, one has an NMR technique many orders of magnitude more sensitive than standard NMR. The method is, therefore, well-suited to determine magnetic moments of radioactive (or rare) nuclei, whose low abundance requires the high sensitivity. We determined the spin and magnetic moment of P^{32} (57). Several other nuclei were subsequently investigated by ENDOR (reviewed in 53).

DETERMINATION OF THE HYPERFINE STRUCTURE ANOMALY The isotropic hyperfine coupling is proportional to the probability of the electron being at the nucleus, i.e., to the square of the electronic wavefunction $|\Psi(0)|^2$ and to the nuclear g-factor (71). One usually assumes the nucleus to be infinitely small and its moment a point dipole. However, the nucleus has a finite extent that has to be taken into account by integrating the wavefunction over the nucleus (90a). Thus, the ratio of the hyperfine couplings of two isotopes will differ from the ratio of their nuclear g-factors. This is a small (fraction of a percent) effect, and consequently, the hyperfine couplings have to be measured with high precision. ENDOR provides a method to do this, and together with J. Eisinger, we determined the hyperfine structure anomaly of the S^{121}, Sb^{123} nuclei in antimony-doped silicon (39). From the results of these experiments, we obtained the distribution of the magnetic moment inside the nucleus.

Determination of the Electronic Wavefunction of Paramagnetic Impurities in Solids by ENDOR

Probably the most important application of ENDOR is the determination of hf interactions when they are not resolved in the EPR spectrum. When an unpaired electron in a solid interacts with many nuclei, the individual hf lines are not resolved, and one obtains a single, so-called inhomogeneously broadened line (129). Using ENDOR, one can resolve these lines, thereby providing a method of mapping the electronic wavefunction of the unpaired electron.

The success of the ENDOR technique is based on the fact that the individual hyperfine lines that make up the inhomogeneously broadened line do not communicate with each other, i.e., they can be saturated in what is now called hole

burning. Phil Anderson, whose office was around the corner from my lab, closely followed the ENDOR experiments. He was puzzled by the lack of diffusion of the spin packets, which led him to develop the now well-known Anderson localization theory (8).

Shortly after ENDOR was used to polarize nuclei (42), it was applied to investigate F-centers in KCl and shallow donors in silicon (43). The silicon work kept me occupied for the next two to three years and culminated in a long publication (45), in which I gave reference to the witch of ENDOR (also spelled Ein Dor) in the Old Testament[2] (137). The editor of the *Physical Review*, S. A. Goudsmit, was upset. He called and bawled me out not only for making frivolous references in a serious journal, but also for neglecting to reference to Mark Twain, who also wrote about the witch of ENDOR. It did not help to point out that one needs to refer only to the first publication; Goudsmit was adamant to delete it. When I finally gave in and pointed out that they would have to renumber the references, he gasped because this was the first reference followed by another 82. But he did it, and the reference to the bible appeared only later in a book by Low (97). In 1957, Wolfgang Pauli visited Bell Labs, and I had the daunting experience of explaining ENDOR to him. It was an interesting visit, which is described elsewhere (53).

The Construction of the Solid-State Maser

The first MASER (microwave amplification of stimulated emission by radiation), the precursor of the LASER, was constructed by Townes and collaborators in 1954 (74). It used a gas, ammonia, as the active element, had a miniscule power output, and filled up an entire room. It was, therefore, not yet a practical device. The search for a more powerful, user-friendly, solid-state maser was on. Bloembergen suggested a scheme in which at least three electron spin resonance levels were used (16). By saturating the outer levels with a strong microwave field, a population inversion (negative temperature) of two levels could, in principle, be obtained.

In collaboration with Scovil and Seidel, we constructed such a system using a single crystal of diluted gadolinium ethyl sulfate with cerium as an impurity. Cerium with its short relaxation time can interact at a given orientation of the magnetic field with two of three gadolinium levels, facilitating the population inversion (68). Although growing the crystal was not a great feat, my previous experience in crystallization came in handy once again. The maser worked (143) and created quite a splash. It was put on the first U.S. satellite, to the great satisfaction of Bell Labs and the U.S. scientific community, which had felt frustrated by Russia's leadership in the space program, as demonstrated by the launching of the first satellite Sputnik. Incidentally, when I visited Israel the following year, there was

[2]Briefly, the story relates how worried Saul went to consult the witch of ENDOR about his fate. When she told it to him, he fainted. The following analogy of this story to ENDOR can be made: Theoreticians calculate wavefunctions that were difficult to determine experimentally prior to the development of ENDOR, so they seldom could be proven wrong. Now, when they compare the experimental ENDOR results with their predictions, some of them faint.

great interest in the maser. The increased sensitivity of the radar, due to the use of the maser, made it possible for the Israeli army to see Cairo.

After the successful operation of the maser, the management of Bell Labs put on some pressure to publish the results as soon as possible, lest we be scooped by Bloembergen. I had no such fears and made a bet with Phil Anderson that nobody but us would have a working maser by the end of the year. Anderson conveyed this later to Bloembergen with the not-too-unexpected result of cooling the relation between us. (I guess the expression "horse-sense" originated from the fact that horses do not bet on the outcome of human affairs.) Nico, if by chance you should read these lines, I hope you will forgive me for the arrogance of my youth.

A different kind of solid-state maser was constructed in collaboration with Jim Gordon in 1957 (58). It was based on the adiabatic fast passage scheme (15) for inversion of two electron spin levels first proposed in connection with a maser by Combrisson et al. (24). We used the EPR transition in phosphorus-doped silicon. To satisfy the maser conditions, we had to reduce the EPR line width. This required removing the isotope Si^{29} ($\sim$5% abundant), whose magnetic moment interacts with the electron spins. To accomplish this, I had to convince the authorities of the isotope separation unit at Oak Ridge of the importance of the project. I convinced them and when we finally received the silicon sample and purified it, the two-level maser worked as predicted (58).

I should add that it was an awe-inspiring experience for me to stand in front of the several-stories-high mass spectrometer (the Calutron) that was used during the Manhattan Project to separate U^{235} from U^{238}. It seemed to me disproportionate that this huge machine would have to work several days exclusively for us to produce the tiny silicon sample, and I almost faltered in my resolve to request the sample.

A Bad Choice of Priorities: Parity Nonconservation

In the fall of 1956, I gave a colloquium at Columbia University on the nuclear polarization scheme. After the colloquium, C. S. Wu and T. D. Lee excitedly tried to persuade me to measure the asymmetry of ß-decay in a polarized sample of donor nuclei in silicon. T. D. Lee and C. N. Yang had circulated a preprint of an article in which they suggested that one of the conservation laws of physics, parity, did not hold in the case of weak interactions (92). This could be critically tested by measuring the asymmetry of ß-decay. I listened politely with limited interest and promised them I would get to it as soon as I finished the ENDOR experiments and the work on the maser. After finishing these at the end of 1956, I took an extended skiing vacation in the West. On the way back I stopped off at the University of Pittsburgh where I gave a colloquium describing the maser and the nuclear polarization scheme. At the conclusion, I mentioned that I would like to test Lee & Yang's hypothesis of parity nonconservation. I saw some blank faces among the faculty sitting in the front row, and it felt as if the temperature of the room had dropped by 10 degrees. Finally, G. C. Wick said, "But don't you

know that parity nonconservation has already been proven by several groups?" (reviewed in 53). Of course, I did not know; I had been skiing for a month. So much for a bad choice of priorities and poor judgment: By not having jumped at the opportunity, I missed participating in one of the major upheavals in modern physics. I do not regret the skiing, but the maser? Who remembers that now? I am glad to see that ENDOR at least is still being used. Upon my return to Bell Labs, as promised, I did the parity nonconservation experiment in ^{32}P-doped silicon. But by that time, this was "old hat," and the results were never submitted for publication.

Speaking of bad judgment, in connection with parity nonconservation, the great W. Pauli also made one. T. D. Lee had sent a preprint of his article to Pauli who replied that he was convinced that parity is conserved and was willing to bet his reputation on it. While his letter was en route, the nonconservation of parity was proven. Everybody anxiously waited to see how Pauli would react to this. A letter finally arrived with Pauli acknowledging that he had been wrong but adding that we must admit how clever he was in betting his reputation of which he had plenty to spare and not betting money of which he had little.

A Brief, Unsuccessful Attempt at a Biophysics Experiment

In 1956, K. S. Cole, a well-known neurophysiologist from the National Institutes of Health (NIH), gave a lecture at Bell Labs describing nerve conduction. He described the unresolved problem of whether the sodium ion flux passing across the membrane is made up of individual ions or clusters of ions. At the time I was concerned with noise problems and suggested to Cole that one could, in principle, resolve this question by measuring the quantal nature of the fluctuations in the current, i.e., the noise amplitude should be proportional to the square root of the number of charged entities crossing the membrane. Cole invited me to spend a week in his laboratory at NIH, which I accepted. There I reacquainted myself with an old friend, the frog sartorius muscle, which had contributed to my diet a decade earlier. The experiments were inconclusive; they floundered because we did not have a sufficiently noise-free amplifier. I was in the middle of developing ENDOR and the solid-state maser and therefore did not pursue this problem further. Later I learned about the elegant experiments of Katz and others involving essentially the same idea of applying quantal analyses to neurotransmission [reviewed in (86)]. The topic of fluctuations and noise always held a special fascination for me, and I later took it up again at the University of California at San Diego.

The Puzzle of Shallow Acceptors in p-type Silicon

In contrast to the extensive work on shallow donors in silicon, several attempts to observe the paramagnetic resonance absorption from shallow acceptors in Si were unsuccessful. This remained a puzzle for several years. I remember in this connection an interaction (if it can be called that) with John Bardeen, the modest, quiet, two-time Nobel Prize winner. We were driving together from a semiconductor conference in Rochester, New York, to Murray Hill, New Jersey. At the start of

the trip, Bardeen asked me whether I was still unsuccessful in observing an EPR resonance from acceptors. After hearing my answer, he puzzled over it and said, "That's strange, let me think about it." The next five hours were spent in complete silence. When he stepped out of the car in Murray Hill, all he said was, "I am sorry, I don't understand it."

The resolution to the puzzle came during a discussion with Walter Kohn. The valence band of silicon (at the wave vector $k = 0$) is degenerate. Local random strains lift the degeneracy by varying amounts, giving rise to a multitude of possible spin transitions with different g-values. The consequent broadening of the resonance line makes its observation difficult. By applying uniaxial stress to the silicon crystal, the degeneracy is lifted in a well-defined way and the resonance should be observable.

We performed the experiment on a single crystal of boron-doped silicon and applied uniaxial stress with a calibrated spring. Upon applying stress, the resonance appeared as if by magic (59). The puzzle of the acceptor was solved.

Acceptors in Si had a special meaning for me; they had almost cost me my Ph.D. years earlier. During the last year of my thesis work, I received a cable from Kittel asking me to stop working on EPR in metals and switch to p-type silicon. Kittel had visited Harvard, where Bloembergen showed him his EPR results on p-type silicon. Bloembergen interpreted these results as arising from acceptors. I considered the use of this information as unethical on Kittel's part and ignored his cable. When Kittel returned and heard my objections, he became furious and asked me to leave the group. It was only through the intervention of my cosupervisor A. F. Kip that I was allowed to return and finish my thesis. The story has an ironic ending. Bloembergen published his EPR results on p-type silicon (156). However, his interpretation was wrong. As I showed later, the resonance did not arise from acceptors but from surface states (45). It is probably the only published mistake that Bloembergen has ever made.

Industry Versus Academia: A Difficult Decision

After a few years at Bell Labs, the time had come to reevaluate my earlier decision to join an industrial lab. The research conditions at Bell Labs were superb. The management was enlightened and farsighted. There was complete freedom in the choice of problems with all the technical and financial support at one's disposal. There were no distractions from research like the ones in academia, e.g., committees, grant writing, and teaching. Yet there were some negative aspects. To start with, the lack of graduate students and postdocs that keep you on your toes and give the satisfaction of seeing them develop (although I did have, as an exception, one graduate student, Don Wilson, who was registered at Rutgers University). Then there were the inherent corporate annoyances. For example, a letter that I wrote to Communist Czechoslovakia in 1959 was opened and returned, stating that there should be no communication with communist countries on Bell Labs stationary. Another example was the company's celebration of the

successful operation of the solid-state maser in a restaurant "For Whites Only." My refusal to participate brought unpleasant repercussions. There were also some rules that I did not like and fought. For instance, technicians were not supposed to be coauthors on papers because "they were paid to do the job." I did, nevertheless, put my excellent technician, Ed Gere, on several papers (e.g., 57–59) in spite of stiff resistance from the management. Finally, Bell Labs did not seem to be the right place to pursue biophysical research, which I ultimately planned to do. Although the management would have supported such efforts (and indeed it subsequently did), the infrastructure (e.g., activities in biology and biochemistry) was missing.

A recurring topic of discussion among us was whether "Bell Labs is a place to age gracefully." The answer to this was provided for me by the following incident. I frequently passed an office in which I saw an elderly gentleman slumped over a desk, reading. I was curious: Who was he? Nobody knew. I finally found out from the director of research: He was the famous J. B. Johnson, whose beautiful and seminal papers on the "Johnson Noise" in the 1920s I had read and admired. How sad, I thought, to see a person of this stature glide into obscurity instead of motivating generations of students at a university.

In 1958, Felix Bloch, the co-inventor of nuclear magnetic resonance (NMR), offered me a tenured position at Stanford. It was a tempting offer, but after much thought I refused in view of my still-existing plan to return to Israel. Bloch understood; he had similar feelings in the 1940s and went to see Einstein to discuss this problem. The great man's reply was that he is first a scientist and second a Jew. This satisfied Bloch, but unfortunately, not me. I never really made peace with not living in Israel.

In 1959 another opportunity arose. The physics department at Columbia University was thinking of starting a program in solid state physics. Furthermore, Charlie Townes, the co-inventor of the maser and laser was leaving and somebody had to take over his students. Having worked both on masers and in solid-state physics, I was approached to apply for the position. Its advantage over Stanford was that I could keep my lab at Bell and not burn my bridges in case the position did not work out.

A JOINT APPOINTMENT BETWEEN BELL LABS AND COLUMBIA UNIVERSITY 1959–1960

Despite the objections of I. I. Rabi, the strongman at Columbia, to a program in solid state physics (for details, see 55), the department decided to go ahead with it, and I was offered a joint appointment between Columbia University and Bell Labs. I inherited about 10 graduate students from Townes, among them Arno Penzias, the future Nobel Laureate and director of Bell Labs, whom at the time no one suspected would reach such heights (for details, see 55). Townes asked me to pay special attention to a promising young student from Argentina, Elsa Rosenvasser. I not

only followed his advise, I went overboard. I essentially neglected the rest of the students and paid exclusive attention to Elsa, who in 1961 became my wife.

A Brief Excursion into High Energy Physics: Muonium Formation in Solids

At Columbia I collaborated with A. Sachs and R. Prepost on an experiment at the Nevis synchrocyclotron combining high-energy physics with solid-state physics. This gave me the opportunity to see how the high-energy half of the physics community lives.

When a spin-polarized μ-meson (muon) beam enters a solid, it may transiently attach itself to an electron, forming a (μ^+e^-) hydrogen-like atom, called muonium. This process can be studied by measuring the depolarization of the muon spin during the formation of muonium. Because the electronic structure of semiconductors was well understood, this system seemed well suited to study muonium formation with the goal of extending its use as a structural probe in less-well-understood materials. The results in silicon and germanium showed that muonium formed a transient shallow donor with a lifetime that depended on the number of free holes and electrons (i.e., impurity concentration) (66). Although the results were interesting, working at an accelerator with a large supporting group and a grueling schedule did not appeal to me. Twenty-five years later I had the same feeling doing extended X-ray absorption fine structure (EXAFS) experiments on reaction centers at the Stanford Linear Accelerator (37).

My association with Columbia was an interesting experience. However, it was not a long-term solution. The joint appointment with Bell Labs made it a neither here nor there situation. In addition, commuting was a pain, I did not like living in New York, and for that matter, I was not enamored of Murray Hill, New Jersey, either. At that point another opportunity presented itself.

UNIVERSITY OF CALIFORNIA, SAN DIEGO 1960–PRESENT

In 1960, Roger Revelle, Director of the Scripps Institute of Oceanography at La Jolla—a fascinating man, a combination of charismatic visionary and con man—convinced the Regents of the University of California to establish a new campus in San Diego (La Jolla). He came to Bell Labs to recruit solid-state physicists and invited three of us for a visit to La Jolla. He promised us that UCSD would remain a graduate school with a light teaching load and an emphasis on research. He also showed me a beautiful lot with an ocean view that I could get if I came. (We later found out that he showed the same lot to all of us). It sounded exciting to be in on the ground floor in building a new campus, where one's ideas can still make a difference. My colleagues at Columbia thought I was crazy to forego a professorship at Columbia and move to La Jolla. They and others thought that it would be impossible to build a first-rate university in an idyllic playground such as

La Jolla. They were wrong, of course. Scientists carry their compulsions, neuroses and talents with them and are, to first order, unaffected by the environment.

I accepted Revelle's offer with the proviso that after establishing an experimental solid-state research program, I would be free to pursue my interest in biophysics. My first experiments at UCSD were essentially continuations of my work at Bell Labs, which I briefly describe. It took the proverbial seven years to switch full-time to biophysics.

Resonance Experiments under Uniaxial Stress

The EPR spectrometer, including the capability of applying uniaxial stress that was developed at Bell Labs, was duplicated at UCSD by my wife Elsa and Roger Isaacson. Roger is an exceptionally capable coworker, part of my group for the past 40 years. During the first couple of years, I also continued to collaborate with my colleagues at Bell Labs.

EXCITED STATES OF SHALLOW DONORS According to band theory, the energy levels of the conduction band in silicon are composed of a ground-state singlet and a doubly and triply degenerate set of excited states. Because the optical transitions from the ground state to these excited states are forbidden, it is difficult to obtain their position directly from optical spectra. However, application of uniaxial stress admixes the excited states into the ground state. We measured the effect of this admixture with Don Wilson, a graduate student at Rutgers who had worked with me at Bell Labs, by determining the shifts in electronic g-values and hyperfine couplings of shallow donors in a silicon crystal subjected to uniaxial stress (160). This allowed us to determine the positions of the excited states.

VALENCE BAND PARAMETERS FROM CYCLOTRON RESONANCE At low temperature, free holes and electrons in a semiconductor crystal in an external magnetic field execute orbital or cyclotron motion at an angular frequency determined by their effective mass. Application of an external microwave field at the angular frequency gives rise to an absorption called cyclotron resonance (30). This technique has been used extensively to study the band structure of silicon and germanium. However, because of the degeneracy of the valence band in silicon (at $k = 0$), the sign of two of the three band parameters could not be determined, giving rise to a theoretical controversy among several groups. This controversy was settled in collaboration with J. Hensel at Bell Labs by measuring the cyclotron resonance in silicon at low temperature (1.2 K) subjected to uniaxial stress (80, 81). This enabled us to determine all band parameters.

COUPLING BETWEEN THE ELECTRON SPIN AND THE LATTICE The EPR signal of a paramagnetic ion or atom embedded in a solid is determined by the strength and details of the interaction of the spin with the surrounding lattice. This interaction is difficult to calculate theoretically but can be determined by applying an external

pressure, which displaces the atoms and ions from their equilibrium values. From the concomitant change (shifts) in the EPR spectra, one can deduce the spin-lattice couplings. These are of particular importance in trying to understand the spin-lattice relaxation time T_1 with which the electron spin system comes to thermal equilibrium with the lattice. The mechanism of this process is a time-varying strain associated with the lattice vibrations (phonons) that, at the right frequency, produces transitions between the electronic levels. The uniaxial stress can be viewed as a zero-frequency phonon.

A detailed analysis of the effect of uniaxial stress on the EPR spectra of Mn^{2+} and Fe^{3+} In MgO was the topic of Elsa's Ph.D. thesis (41): She obtained the spin-lattice coefficients and correlated them with the spin-lattice relaxation time as well as with the line widths, which were caused by random internal strains.

Nuclear Polarization via Hot Electrons

The attainment of nuclear polarization is important in the study of nuclear physics (e.g., see Parity Nonconservation above). Nuclear polarization schemes rely on high-magnetic fields and low temperatures (milli K) or on microwave fields inducing electronic transition. While still at Bell Labs, I thought of an alternate, simple scheme (46) that my first postdoc at UCSD, Gil Clark, set out to verify.

The method is based on the Overhauser effect, the extension of which is the well known nuclear Overhauser effect (NOE) that is used in the structure determination of biomolecules by NMR. Originally, Overhauser showed that an enhanced nuclear polarization in metals may be obtained by saturating the electron spin resonance absorption of the conduction electrons in an external radio frequency field (120). The degree of saturation of the electrons, i.e., the deviation of the ratio of spin-up to spin-down states from the Boltzmann thermal equilibrium can be characterized by the spin temperature T_S. I showed that the relevant parameter for nuclear polarization is not T_S but the difference between T_S and the temperature T_R that characterizes the kinetic energy of the electrons (46). In conductors, the electrons are in equilibrium with the lattice temperature T_L, i.e., $T_R = T_L$. However, in semiconductors at low temperatures, the mean free path of the electrons is large so that in an electric field, the acquired kinetic energy between collisions cannot be entirely gotten rid of during collisions with the lattice. This results in the creation of "hot electrons" (reviewed in 89), i.e., $T_R > T_L$. Analogously, the relaxation mechanisms of the electron spins and their kinetic energy are different, $T_S \neq T_R$, resulting in a nuclear polarization. Thus, by simply passing a direct current through a semiconductor at low temperature, a nuclear polarization is achieved. At the time this idea was floating around, Anatole Abragam, a leader in magnetic resonance, was visiting Bell Labs. Although he could not find a flaw in the argument, the simplicity of the scheme made him skeptical.

Gil Clark embarked on the project to prove the validity of the hot electron polarization scheme (22). We used the semiconductor InSb and measured the nuclear polarizations at 4.2 K of In^{115}, Sb^{121}, and Sb^{123} in the presence of an

electric field. The nuclear relaxation times were several hours, and we ran the experiments uninterrupted for ~24 h. Nuclear polarization enhancements of up to 100 were obtained in agreement with theoretical predictions.

Going over these old experiments, I am struck by two points. One is the leisurely pace at which research was pursued. The original idea was put forth in 1959; it took four years for the experiments to be completed. The other point is more general in nature. It deals with the disparity between one's own evaluation of the scientific and intellectual content of one's work and the reaction by the scientific community. The work described in this section did not make an impact when it was published and was mostly ignored. On the other hand, I value this work considerably more than other publications that received more attention from the scientific community. However, I was recently pleased and surprised when, after nearly 40 years, I came across a reference to this work in connection with the new field of spintronics and quantum computing (138). I should also mention an unexpected benefit derived from this work. In a footnote of our publication (22), we suggested the possibility of using these ideas to construct a d.c.-driven maser. General Dynamics was interested in pursuing this idea and filed for a patent. Their remuneration enabled us to build a nice house in La Jolla.

An Interesting Encounter with Albert Szent-Györgyi

While I was involved in establishing a solid-state program at UCSD, I tried to acquaint myself with biology and biochemistry by reading and attending lectures. One of the books that I came across in 1962 was by Albert Szent-Györgyi (149), the discoverer of vitamin C and for over half a century a colorful personality in science. The main theme of the book was the nature of ordered water around biological structures. He claimed that water is ordered over distances of several microns ($>10^4$ Å). As a solid-state physicist, I had a hard time believing the H_2O would be ordered over $\sim>10^4$ layers. I did a quick, "Friday afternoon" experiment to disprove Szent-Györgyi's contention. By measuring the frequency response of the dielectric constant of a water-soaked millipore filter with 100 Å pore sizes, I showed that H_2O is not ordered even at a distance of 100 Å. Just as I had finished reading the book, I received a letter from Szent-Györgyi inviting me and Elsa to spend a weekend at his home in Woods Hole to discuss EPR as free radicals had become his newest interest. I thought that this would be a good opportunity to raise the question of the ordered H_2O. Besides, I also wanted to meet my childhood hero about whom my mother used to tell bedtime stories. One of them described how Szent-Györgyi, after the establishment of Czechoslovakia in 1918, fearing for his life, built a raft, took his microscope, and floated down the Danube from Bratislava (where he held a professorship) to Budapest. We happily accepted the invitation. Szent-Györgyi was a charming host, a marvelous raconteur, but totally unreceptive when I tried to broach the subject on my mind. Finally, after two days of unreceptiveness, I dropped all diplomacy on the way to the airport and told him that the proposition in his book was wrong; H_2O is not ordered over several microns. He

looked at me quizzically and nonchalantly replied, "Oh, did I say several microns? I am so bad with numbers, don't pay any attention to them." What an anticlimax and blow to the image of my childhood hero! This little vignette of Szent-Györgyi is in perfect accord with Max Perutz's recent description of him (125).

Paraelectrics

PARAELECTRIC RESONANCE OF ELECTRIC DIPOLES The theories of magnetic and electric susceptibility have many features in common, and in fact, in Van Vleck's classic exposition on the subject, both are treated on an equal footing (153). However, in contrast to the large body of work on paramagnetic resonances, no paraelectrical resonance was ever observed despite a great deal of work on paraelectrics (reviewed in 17). The reason for this disparity is that the energy levels of the magnetic moment in an external magnetic field are quantized (i.e., the spins point along specific directions), whereas electric dipoles lacking an angular momentum are not quantized in an electric field. One needs therefore to impose the spatial quantization by embedding the dipole in a crystal that forces the dipole to orient along specific crystallographic axes. The possibility of observing paraelectric resonance in such a system was first mentioned by Kuhn & Lüty (91).

The system that we (Herb Shore, a colleague theorist at UCSD, and Ian Shepherd, a graduate student) picked was a KCl crystal into which OH^- ions were introduced as an impurity. The dipole moments of the OH^- ions orient themselves along the six ⟨100⟩ crystallographic directions (91). The application of an external electric field changes the energies of the different dipolar orientations, and transitions between the energy levels can be induced. The OH^--KCl crystal was introduced into the electric field of a microwave cavity of a conventional EPR spectrometer. Transitions were observed by varying the external electric field. From the spectra the dipole moment of OH^- and the zero-field splitting of the levels were deduced (69). A noteworthy difference between the electric dipole and magnetic dipole transitions is that the coupling of the former to the electromagnetic field is many orders of magnitude stronger, giving rise to large signals.

It was satisfying to finally observe paraelectric resonance. However, because of the special conditions required to observe these transitions, the interest of the scientific community in paraelectric resonance never came close to the interest in paramagnetic resonance.

COOLING BY ADIABATIC DEPOLARIZATION OF OH^- MOLECULES IN KCl The method that had been universally employed for obtaining temperatures below a few tenths of a degree K makes use of the magnetocaloric effect first demonstrated in 1933 (73). The method is based on the change in entropy of an assembly of magnetic dipoles during the adiabatic removal of an externally applied magnetic field.

In analogy with the magnetic system, the removal of an electric field in a paraelectric system should also increase the entropy and cool the system (85).

Such cooling below 1 K was first observed by Ian Shepherd, a graduate student in our lab. Shepherd used the system of OH^- impurities in KCl. Upon removal of an electric field of 75 kV/cm, he reduced the temperature of the KCl crystal from 1.3 K to 0.36 K (144). The advantage of this system is that it does not use an external magnetic field. Therefore, in principle, it can be used in a two-stage tandem depolarization scheme in which the second stage uses adiabatic demagnetization to further reduce the temperature of the system.

The Electronic Structure of Porphyrins

Martin Kamen, who joined the chemistry department at UCSD in 1963, tried hard to get me interested in photosynthesis. He brought the physical chemist David Mauzerall as a visitor from the Rockefeller University to breach the gap between our disciplines. Mauzerall produced, photochemically, porphyrin-free radials whose EPR and optical spectra we investigated in detail (103, 104). The idea was that porphyrins may serve as model compounds for bacteriochlorophyl. But at that time, the connection to photosynthesis remained remote. However, this work stimulated us to do further spectroscopy on porphyrins.

J. R. Platt gave a seminar at UCSD around 1965, claiming that the excited state of porphyrins has the rather remarkably high magnetic quantum number $|M_z|$ of 9 units (128). With Mike Malley, a graduate student, we set out to investigate Platt's theory by measuring the optical absorption in a magnetic field of 10^5 Gauss. Circular polarized light of opposite senses induces transitions to the $+M_z$ and $-M_z$ states, respectively. From the optical splitting between the two polarizations, a value of $|\Delta M_z| = 9 \pm 1$ was indeed found, to our surprise (63), as predicted by Platt (128).

The change in the optical spectra of porphyrins in an applied electric field, i.e., the Stark effect, which determines the difference in polarizability of the excited and ground state, was also investigated (100). These experiments provided a useful experience for later work on the Stark effect in photosynthetic reaction centers (RCs) (95, 96).

A Sabbatical at MIT; Courses at Woods Hole and Cold Spring Harbor 1967–1968

After seven years at UCSD, I still hadn't switched to biophysics as originally planned. It became clear to me that if I seriously wanted to make the switch, I needed to leave the known and cozy environment of the physics department and immerse myself in a more biologically oriented surrounding. The opportunity came with an invitation from Cyrus Levinthal at MIT to spend a year there as a visiting professor in the biology department. Before going to MIT I spent the summer at Woods Hole taking the intense and highly instructive physiology course. One of the sections I took was on bacterial photosynthesis given by Rod Clayton. It greatly appealed to me, but I did not want to commit myself to this topic before exploring other areas during my sabbatical year at MIT.

MIT was quite an experience: the mad hustle and bustle compared to UCSD; the people, in obvious quest for fame and glory, had little time left for a visiting greenhorn. There was one exception, Lisa Steiner, an immunologist whose course I took. We became, and still are, good friends and colleagues. She taught me a lot of biology and protein chemistry, and we started a fruitful collaboration.

I soon came to realize the differences in research approaches pursued by physicists and biologists. This is perhaps best illustrated by an encounter with Salvador Luria at MIT.

"Hi, George, how nice to see you here. What are you doing?" he asked.

"I am sitting in on several biology and biochemistry courses," I answered.

"Yes, but what are you doing?"

"Well, I am going to seminars and talking to people about their work."

"Yes, but what are you doing?" he insisted.

"I am also getting some experience in the laboratory of Lisa Steiner."

"Yes, but what are you doing?"

So it finally dawned on me: Biology is a doer's field. You have to run centrifuges and gels and not spend time in deep thoughts as physicists are prone to do. The challenge for biophysicists is to effectively synthetize the approaches from both disciplines.

The difference between physics and biology may perhaps be best summarized as follows: Physics is difficult but simple (i.e., when you have mastered, often with great difficulty, the basic principles of quantum mechanics, Newton and Maxwell's equations, everything follows logically). Biology, on the other hand, is easy but complex (i.e., no difficult concepts but a great number of facts). This difference is exemplified by Leo Szilard's remark when he turned his interest from physics to biology: "I get my best ideas while taking a bath. But with biology I have problems; I always have to jump out and look up a fact."

After a year at MIT, I spent the summer of 1968 at Cold Spring Harbor taking a course with Max Delbruck. I had previously taken a course in 1964 at Cold Spring Harbor on phage and bacterial genetics, which had been started by Delbruck close to two decades earlier. Although it was a great experience, I did not follow it up as I spent the following sabbatical in Buenos Aires with my wife setting up an EPR program in solid state physics. I did, however, have the good fortune in 1964 to meet Delbruck, with whom I kept close contact until his death in 1981. Delbruck's course in 1968 dealt with phycomyces, a system that Delbruck championed over photosynthetic bacteria. He believed that photosynthesis is too complex a system to make significant progress in. A few years later he changed his mind, and in the late 1970s, he invited me to visit CalTech for a few days and to give two seminars (on photosynthesis and fluctuation spectroscopy). Delbruck was reputed to tell every seminar speaker that his was the worst presentation that Delbruck had ever heard. So I was a little apprehensive and started the first seminar by expressing my delight in being invited to give two seminars as both could not be the worst. Debruck immediately pointed out the fallacy of the argument as the second seminar could be worse than the first. Actually, he was kind to me and offered me a position

at CalTech. I felt honored but saw no reason to leave La Jolla, where I was content at UCSD and where my wife had a position at San Diego State University.

By the end of the summer of 1968, I had made the decision to work on the primary processes in bacterial photosynthesis. The decision was based on several factors. I liked the seeming simplicity of the bacterial system in which, in principle, one can obtain from one bacterium in a few days "an ensemble of $\sim 10^9$/ml identical particles," a concept dear to a physicist's heart. It also had the advantage of the relative ease of genetic manipulation. Additional appealing aspects were the multidisciplinary nature of the field, the small number of people working in it, and the possibility of solving the basic question of the identity of the primary reactants using a technique with which we were familiar (EPR/ENDOR). Thus, photosynthesis has been the main theme of our work since 1968. I recently published a review on it (55) and, therefore, present here only some of the highlights. But before doing so, I want to discuss some other biophysics work performed in parallel with the photosynthesis work.

The Electronic Structure of Hemes

Hemes serve as prosthetic groups in an important class of biomolecules (e.g., myoglobin, hemoglobin, cytochrome). They have been extensively studied, and Mb and Hb were the first proteins whose three-dimensional structures were determined by J. Kendrew and M. F. Perutz (reviewed in 29). The business end of these molecules is the iron in the prosthetic heme group, which ligates to the protein and to which oxygen binds.

The electronic structure of the iron in the heme can be studied by a variety of physical methods, including magnetic resonance. The energy levels of high-spin Fe^{3+}, for example in metmyoglobin, are split in zero magnetic field. The zero-field splitting, D, reflects the strength and symmetry of the ligand field and therefore serves as a sensitive probe of the structure of the environment of the Fe^{3+}. Several methods have been used to determine D (reviewed in 47). The most direct is by the magnetic resonance absorption in the far infrared (submillimeter) region. This approach was taken in collaboration with Paul Richards at Bell Labs on myoglobin (67) and several hemin compounds (134). An alternative method that we used to obtain D in metmyoglobin utilized the temperature dependence of the spin-lattice relaxation time of Fe^{3+} (139). This method is based on the absorption and emission of phonons to and from the excited state (118).

More detailed information of the electronic structure of Fe^{3+} and its environment can be obtained from EPR (reviewed in 47) and ENDOR. The first ENDOR experiment that we performed with Charles Scholes, a postdoc, was on metmyoglobin in frozen solutions (140). Hyperfine couplings of the Fe^{3+} spin with the nuclei ^{14}N, ^{57}Fe and ^{1}H were observed (61). These experiments were subsequently extended to high-spin methemoglobin.

We also studied by ENDOR some of the abnormal (mutant) hemoglobins. For example in Hb-Hyde Park (79), the histidine on the fifth ligand of the β-subunits

is replaced by tyrosine (i.e., α_2-oxy·$\beta_2$92His → Tyr). It had been postulated that the distal histidine takes over the role of the proximal histidine and binds to the iron. We showed by ENDOR that this is not the case (61).

The most intriguing problem is the cooperative oxygenation effect in hemoglobin (reviewed in 127) To study this effect by EPR or ENDOR, the hemes need to be converted into the high-spin met form. Fortunately, there exists a mutant Hb-Milwaukee ($\alpha_2 \cdot \beta_2$67Val → Gln) in which the normal α-subunits can be reversibly oxygenated while monitoring the ENDOR signal of the β-chain met hemes. Hb-Milwaukee undergoes a deoxy to oxy-quaternary structural change that is isomorphous with that of normal Hb (126). We focused on the ENDOR of the ^{14}N peak associated with the histidine of the β-subunit and found that it shifted by 100 kHz when the α-subunits were oxygenated (61). This shows that a primary event at the α-subunit produces a structural change at the β-subunit. To correlate the spectral shift with specific structural changes, more work needs to be done. An alternative approach to studying the cooperative oxygenation effect by EPR/ENDOR uses a nitrosyl (NO•) radical as a ligand to Fe^{2+} (reviewed in 83).

All the work described above was performed on frozen solution in which the molecules are randomly oriented. Consequently, information on the anisotropic contributions to the hyperfine couplings is lost. Several attempts to observe ENDOR in single crystals were unsuccessful (e.g., 38). This failure was due to the close distance between hemes (∼25 Å), which shortens the spin-relaxation processes with detrimental results on the ENDOR signal. To avoid this problem, we prepared mixed crystals of myoglobin in which the majority of the molecules (90%) were in the diamagnetic CO-ligated form. In these crystals, we observed and analyzed in detail the ENDOR signals at different orientations of the crystal with respect to the magnetic field (141).

Fluctuation Spectroscopy

When I took the phage course at Cold Spring Harbor in 1964, Delbruck asked me whether I could think of a direct physical method to demonstrate the dynamics of unzipping and replication of DNA. Because in replicating *E. coli* the rate of breaking base pairs is ∼100 per sec, the first thought that came to mind was that the frequency spectrum of some parameter, e.g., conductivity, should reflect this rate. I kept mulling this over and around 1970 finally decided to try the idea on a simple model system: the dissociation of an electrolyte.

DETERMINATION OF KINETIC PARAMETERS FROM THE NOISE SPECTRUM OF A CHEMICAL REACTION Consider the simple chemical reaction $A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B$. To obtain the rate constants k_1 and k_2, one traditionally monitors the time dependence of the concentration of the reactants after the system has been perturbed. This is the well-known relaxation technique pioneered by Eigen & DeMaeyer (36). There is, however, in principle, an alternative way of obtaining the kinetic parameters without the

application of an external perturbation. The method is based on the basic principle that the concentration of a reactant fluctuates around its equilibrium value. From a spectral analysis of the fluctuations, one should obtain the same kinetic information as from relaxation methods [This follows from the general "fluctuation-dissipation theorem" (132)].

To demonstrate the feasibility and validity of the fluctuation approach, we investigated the association-dissociation of a divalent electrolyte, a system that had been characterized in detail by the relaxation technique (90). An excellent graduate student, Mike Weissman, joined the effort. We measured the conductivity fluctuations of the divalent electrolyte ($Be^{2+} + SO_4^{--} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} BeSO_4$) caused by the concentration fluctuations. The conductivity fluctuations were converted to voltage fluctuations by passing a constant current through the electrolyte (70). The frequency spectrum of the fluctuations is a Lorentzian given by $[1 + (\omega/\Delta\omega)^2]^{-1}$, where $\Delta\omega$ is related to the kinetic parameters by $\Delta\omega = k_1 + k_2$. The values that we obtained agreed with those obtained previously by the relaxation technique (90). For $BeSO_4$, the value of $\Delta\omega$ is in the audio range, and we recorded it on tape. With increasing temperature, $\Delta\omega$ increased. It made a nice lecture demonstration to listen to a chemical reaction and hear the pitch increase as the temperature was raised.

The stage was set to measure the fluctuations in a solution of DNA during the melting process. But, as so often happens, we got sidetracked by other experiments (described below) and never reached our original goal. In retrospect, it is a pity. Wouldn't it be exciting to listen to the replication of *E. coli*? A new form of voyeurism, I suppose.

DETERMINATION OF THE MOLECULAR WEIGHT OF DNA In a seminar given in 1973, Bruno Zimm pointed out the difficulties of obtaining the molecular weight (M) of large macromolecules such as DNA by using classical methods (e.g., light scattering and sedimentation). Since I was tuned to the concept of fluctuations, an obvious solution presented itself to me. The idea is based again on the inherent fluctuations of the concentration of solute molecules in a given volume. If on the average there are N molecules in a volume, there is an inherent uncertainty of $\sqrt{N}$ in their number. Thus, from the fluctuations in concentration, obtained by sampling different equal volumes, the number of molecules N can be determined. By measuring independently the weight per volume of solute molecules W, M is obtained from the relation $M = W/N$.

The two problems that needed to be addressed were the determination of the concentration of DNA and the production of a large number of precisely equal volumes. The work was done in collaboration with Mike Weissman and a postdoc, Hansgeorg Schindler (155). A rotating cylindrical cell was filled with the DNA solution, and a precise volume within it was defined by a beam of laser light. The concentration of DNA was determined from the fluorescence of bound ethidium bromide. As the cell rotates, the beam of light exposes a continuous set of volumes that are precisely equal and statistically independent.

The molecular-weight values obtained for T2 phage DNA (1.14×10^8) and *E. coli* DNA (3.0×10^9) were in agreement with results obtained by classical methods (reviewed in 135). The advantage of the fluctuation method is that it is absolute and that it gives rapid and precise results. (For T_2 phage, $\pm 5\%$ in one minute of data collection).

Fluctuation spectroscopy (FLUSY) has been and is being used on a large variety of different systems (reviewed in 49). It now represents an important part of the tool kit for investigating kinetic processes. In concluding this section, I have to admit that these fluctuation experiments gave me a special thrill. Perhaps it is the satisfaction of obtaining useful information from noise after battling it for so many years.

Systematic Investigation of Protein Crystallization

To determine the structures of macromolecules by X-ray or neutron diffraction, one requires well-ordered single crystals. It seemed rather scandalous that in the 1970s, more than 100 years after the crystallization of the first protein by Hartvig (119), the process of crystallization remained more of an art than a science. Our optimistic goal was to change this state of affairs by embarking on a systematic investigation of the crystallization process.

Zvi Kam, a graduate from the Technion in Israel joined our group in 1971 and undertook the challenge. We were ultimately interested in crystallizing the RC from photosynthetic bacteria, but we decided to start with a simpler, easily crystallizable protein, egg white lysozyme (5), as a model compound. We divided the crystallization process into three temporally distinct phases: (*a*) nucleation, (*b*) postnucleation growth, and (*c*) cessation of growth. (62, 84).

The nucleation of crystals has many similarities with the condensation of droplets from supersaturated vapor, first described by Gibbs in 1875 and later elaborated by Volmer (e.g., 154). Protein molecules attach to each other to form aggregates whose total free energy depends on their size. Above a critical size, the aggregate starts to grow spontaneously. The main question is whether the aggregate is an ordered crystallized entity or a disordered amorphous precipitate. From the size distribution of the aggregate during the prenucleation phase, one can distinguish between the two alternatives. To determine the distribution, we introduced the use of quasi-elastic light scattering (13), which is now used by many protein crystallographers. The advantage of monitoring the prenucleation phase is that one does not need to wait long periods of time to determine whether a change in a parameter favors crystallization over precipitation.

The postnucleation growth involves the attachment of molecules to the aggregate. The kinetics and mechanism of this process were studied by a variety of techniques. In one, the formation of a protein-depletion layer around a growing crystal was measured optically to determine the kinetics of attachment (84). In another technique, crystal growth was measured in a flow system that eliminated the protein-depletion layer. This work was carried out by Steve Durbin (31), a postdoc

who succeeded Kam in our lab. The most direct method of studying the mechanism of growth is to visualize the surface of the crystal using electron microscopy. With this technique, we visualized lattice defects (e.g., dislocations) and showed that they play an important role in postnucleation growth (32). We reproduced the main features of the observed crystal growth of lysozyme by a Monte Carlo simulation (33). The cessation of growth of crystals in the presence of an adequate supply of protein in solution has been attributed to an accumulation of defects at the surface, which makes the attachment of molecules unfavorable (reviewed in 34).

In conclusion, it may be worth pointing out that crystallization can be viewed as an example of a large class of biological problems dealing with recognition on a molecular level. Thus, a better understanding of crystallization may open up vistas in other areas. Finally, how far have we come in understanding crystallization of proteins, i.e., have we reached our original goal? I would say not quite yet, but we and a large number of other groups (including those reviewed in 34, 108) have made significant progress to that end.

Bacterial Photosynthesis

One of the attractions of this field was that so few people worked on bacterial photosynthesis as compared with green plant photosynthesis. It seemed puzzling to me why people did not try to understand the simpler system first, before proceeding to a more complicated one. I mentioned this to Martin Kamen who, being a wise man and having worked in the field a long time, had an answer, "Alas, that's because everybody is an m.c.p." (mammalian chauvinistic pig). People (mammals) work on plants because they feed on plants or on animals that feed on plants. There was another equally poor reason. Many workers in green plant photosynthesis considered bacterial photosynthesis an oddity that has nothing to do with real photosynthesis. I had faith in the parsimony of nature. It seemed to me unlikely that nature would develop two unrelated systems to accomplish the same goal. Indeed, it turned out that green plant photosynthesis has a great deal in common with bacterial photosynthesis, and it is now generally accepted that work on the bacterial system contributed greatly to the understanding of the more complex oxygen-evolving system of green plants.

Bacterial photosynthesis has occupied us for the past three decades. As mentioned in the introduction, this work was covered in detail in a recent review (55). Consequently, only the highlights are covered here.

ISOLATION AND PURIFICATION OF THE REACTION CENTER In photosynthesis, light is converted into chemical energy. The primary event of this process is a photon-induced charge separation that occurs in an integral membrane protein called the reaction center (RC). Its existence was first postulated in 1932 (40), and direct spectral evidence for its presence in bacteria was obtained in 1952 (35). When I returned to UCSD in the fall of 1968, my first goal was to isolate and purify the RC and identify the primary reactant of the charge separation process.

The pioneering work on the isolation of a photosynthetic unit was done by Reed & Clayton in 1968 (130). They obtained a complex having a molecular weight of $\sim 10^6$. Our goal was to reduce the size of the complex and obtain the smallest structural unit capable of performing the charge separation. The critical step in the isolation procedure was the detergent solubilization. After trying a large number of different detergents, we found that LDAO (lauryl dimethyl amine oxide) gave the best results; the purified photosynthetic unit had a molecular weight of $\sim 10^5$, i.e., an order of magnitude smaller than the previously isolated unit. It represents the birth of the RC as we know it today.

The results on the isolation and characterization of the RC were presented at the International Conference on Photosynthesis in Gatlinburg, Tennessee, in May 1970 (48). I was excited about our accomplishment and expected a similar reaction from the audience. But to my surprise and disappointment, a large fraction of the people in the audience were not only unenthusiastic but downright skeptical. They thought that something must be wrong: "How can such a small unit with a molecular weight of $\sim 10^5$ be responsible for that marvelous process of photosynthesis? Those physicists, you must have lost something essential during your purification." Was it mistrust of physicists doing biochemistry, or was it the conservatism of some biochemists, reminiscent of their long opposition to Mitchell's chemiosmotic theory? At any rate, soon thereafter, the results were duplicated (23) and are now universally accepted.

IDENTIFICATION AND CHARACTERIZATION OF THE PRIMARY DONOR AND ACCEPTORS

When we started to work in this field, the chemical identities of the primary reactants were not known. What a scientific incongruity! After $\sim$200 years of research in photosynthesis, the main actors participating in the light-induced charge separation had not been identified. So together with Jim McElroy, a physics graduate student, and David Mauzerall, who spent a sabbatical and several summers at UCSD, we embarked on identifying first the primary donor, D, and later the acceptors, A_1 and A_2.

The technique of choice to investigate the primary reactant was EPR/ENDOR, with which we were very familiar. In the charge-separation process, $DA \overset{h\nu}{\underset{k_{AD}}{\rightleftharpoons}} D^+A^-$, each species has an unpaired electron (or hole) that makes both of them amenable to EPR spectroscopy. The first photo-induced free radicals (the same signals I had observed earlier as a graduate student at UCB) were reported by Commoner et al. (25) and found in bacteria by Sogo et al. (145). These authors, however, did not identify the origin of the signal. The reason is the nature of EPR, which makes it difficult to identify the radical species from an inspection of the spectra. We resorted, therefore, to the model compound approach in which one compares the signal from different radicals, used as model compounds, with the unknown species. As the model compound, we picked the cation of bacteriochlorophyll. A comparison of the EPR signals from $BChl^+$ and D^+ showed their g-values to be identical (2.0026 ± 0.0001) (106). However, the line width of D^+ was $\sim$40% narrower than that of $BChl^+$. This prevented us from claiming that $D \equiv BChl$,

although it was clear that the primary donor had something to do with bacteriochlorophyll. This conclusion was further strengthened by showing that the kinetics of the optical absorbance changes that Duysens associated with a specialized bacteriochlorphyll (35) and of the light-induced EPR signal were the same (107).

The puzzle of the line width was solved by Norris et al. (112) who postulated that the electron on D^+ is shared between two bacteriochlorophylls that form a dimer. This should reduce the EPR line width by $\sqrt{2}$, i.e., ~40%, as was observed. But to base the dimer hypothesis on a single number, i.e., the ratio of line widths, seemed a little shaky. A more definitive proof would be to show that the electron spends only half of the time on one BChl of the dimer, i.e., the square of its wavefunction, $|\Psi(r)|^2$, in the dimer should be half of that in the monomer of the model compound. So with Arnold Hoff, a postdoc in our lab, and Roger Isaacson, we used the ENDOR technique to measure the hyperfine couplings (which are proportional to $|\Psi(r)|^2$) in both the $BChl^+$ monomer and in the donor (60). The results showed that, on the average, the hyperfine couplings are smaller in the donor by a factor of 2. Similar results were obtained by Norris et al. (111). This showed unequivocally that the primary donor is a bacteriochlorophyll dimer.

Having identified the primary donor as a BChl dimer, the next task was to investigate its detailed electronic structure. Using ENDOR, we determined the spin-density distribution of the electron in the bacteriochlorophyll macrocycle. The work was performed on RCs in frozen solution as well as in single crystals. In single crystals, both the isotropic and anisotropic components of the hyperfine couplings could be determined (51, 93).

The identification of the primary acceptor proved to be more difficult. At the 1970 Gatlinburg Conference, we reported the observation of an unusually broad (~500 Gauss) EPR signal (in contrast to the ~10 Gauss line for D^+), which we attributed to the primary acceptor (48). Such a broad line cannot be due to a free radical but is more likely associated with electrons in the unfilled d-shell of transition metals. Indeed, we found by atomic absorption that there is ~1 Fe/RC (48). While we were investigating the broad EPR signal, we heard from Paul Loach that he observed a shift in the g-value of the narrow signal due to D^+, which he attributed to an overlapping unresolved signal from the acceptor (94). We exchanged samples and found that his RCs, which were prepared under rather harsh conditions (6 M urea, pH12), had lost the Fe. We proceeded to develop milder conditions for the removal of the Fe and performed EPR experiments at higher frequencies (35 GHz rather than 9 GHz) to resolve the second line. To determine its chemical identity, we again used the model compound approach as we had done for the primary donor. We showed that the EPR spectrum of ubiquinone (Q) was identical to that of the acceptor A^- (64).

Supporting evidence for the role of quinones came from reconstitution experiments performed by Mel Okamura, a biochemist who joined us as a postdoc in 1970. Mel removed and re-added quinones to the RC and correlated the quinone

content with photochemical activity (115). He showed that two quinones bind to the RC, one more tightly (the primary quinone, Q_A) and one less tightly (the secondary quinone, Q_B). Q_A was shown to have an obligatory role in the photochemical activity; Q_B was assigned the role of the secondary acceptor (115).

The broad EPR line was attributed to the electron on the quinone interacting with the large magnetic moment of the Fe, forming an Fe-quinone magnetic complex. Several techniques were used to elucidate the electronic structure of the complex. These included Mossbauer spectroscopy, static magnetic susceptibility measurements, EPR spectroscopy, and EXAFS (reviewed in 65). The main conclusion was that iron is in the high-spin Fe^{2+} state, irrespective of the reduction state of the quinone: We determined that Fe^{2+} is located approximately equidistantly between Q_A and Q_B but does not form ligands to them.

The electronic structure of the quinones was investigated by ENDOR in RCs in which the Fe was replaced by diamagnetic Zn (27) to reduce the EPR line width. This work (98) was started in 1983 in collaboration with a postdoc, Wolfgang Lubitz, with whom we are continuing to collaborate to this day. A detailed account of the quinone work, including the history of their identification, can be found in (54, 65, 99).

In addition to the single-electron radicals D^+ and Q^-, we also investigated the biradicals $BPhe^{-\cdot}Q_A^{-\cdot}$ (114) and $Q_A^{-\cdot}Q_B^{-\cdot}$ (19), which form intermediate states in the electron transfer path (BPhe is an intermediate acceptor). In these biradicals, one observes a splitting of the EPR spectrum related to the exchange interaction J between the unpaired spins. The value of J is an important parameter in calculating electron transfer rates.

FURTHER CHARACTERIZATION OF THE RC A preliminary characterization of the RC was done in 1970 in conjunction with its initial isolation and purification (48). We determined by SDS-PAGE that the RC has three subunits, which we labeled according to their electrophoretic mobility L, M, H (for light, medium, and heavy). We did not want to assign molecular weight to the subunits because we knew that SDS-PAGE gave reliable, quantitative, molecular-weight values only for water-soluble proteins. Unfortunately, as it turned out later, molecular-weight determinations were even qualitatively off, and H had the lowest molecular weight. But by that time, the nomenclature had already been accepted and it remains to this day. We also sent samples of RCs to Lisa Steiner at MIT who determined the amino acid composition and found the RC to be the most hydrophobic protein thus far reported. We subsequently isolated the three subunits, determined their stoichiometry to be 1:1:1 (117), and Lisa Steiner determined the amino acid composition of each subunit (146).

The determination of the amino acid sequence proved to be more difficult as the isolated subunits were insoluble in aqueous solutions, which at the time were used in sequencing proteins. I remember a few frustrating but interesting weeks spent in John Walker's lab at the MRC in Cambridge, United Kingdom, trying to work out the proper conditions for the liquid-phase sequencer. Lisa finally succeeded

with a postdoc, M. R. Sutton, in sequencing the amino-terminal 25–28 residues of the three subunits (148). These sequences were later used by JoAnn Williams, a talented graduate student with a green thumb for recombinant DNA techniques. JoAnn isolated the genes that encode the L- and M-subunits and determined the sequence of these subunits (158, 159). Each subunit showed five hydrophobic regions that were postulated to form transmembrane helices. The sequence of the H-subunit showed only one transmembrane helix (157). An exciting result of the sequence work was the homology that was found between the L- and M-subunits and the D_1 and D_2 polypeptides found in green plants (159).

The subunit harboring the Q_A-binding site was determined by Tim Marinetti, a postdoc in our lab in 1978–1979. He introduced the photoaffinity label [^{3}H] 2-azidoanthraquinone in the Q_A site. When illuminated with UV light, it photolyzed and became attached to the protein. Analysis of the photolyzed protein by SDS-PAGE revealed that the M-subunit was selectively labeled. This showed that the primary quinone site is located at or close ($\sim$5 Å) to the M-subunit (102). The Q_B site could not be determined by photoaffinity labeling because of the more stringent structural requirement for the binding of the secondary quinone.

The topography of the RC in the bacterial membrane was investigated by Gunars Valkirs, a graduate student, by using indirect immunoferritin labeling (152). Ferritin is an electron-dense molecule, which permits the localization of the binding site by direct electron-microscopic visualization. Anti-M antibodies to which ferritin was attached labeled both sides of the membrane, showing that the M-subunit spans the membrane. Because of the similarity of the amino acid compositions of L and M, the L-subunit was assumed to also span the membrane. Most anti-H preparations labeled the cytoplasmic side, and only one out of six labeled the periplasmic side. From this we concluded that H is asymmetrically oriented with respect to the membrane, most of it being on the cytoplasmic side (152).

CRYSTALLIZATION OF THE RC AND DETERMINATION OF ITS STRUCTURE BY X-RAY DIFFRACTION While pursuing a systematic approach to try to understand the crystallization of proteins, we did occasionally try the nonscientific trial-and-error approach to crystallize RCs. The prevailing dogma at the time was that integral membrane proteins cannot be crystallized because of the presence of randomly oriented detergent molecules. This was brought home to us in the review of our 1978 grant in which we were severely criticized for suggesting the crystallization of a membrane protein [For a verbatim excerpt of the review see (52)]. We did not agree with the criticism, and to keep up hope we posted one of Escher's pictures in our lab showing an ordered array (crystal) of ducks [see Figure 12 in (55)].

By 1980, two membrane proteins, bacteriorhodopsin (109) and porin (72), had been crystallized; in 1982, H. Michel successfully crystallized the RC from the photosynthetic bacterium *Rhodopseudomonas viridis* (110). Shortly thereafter with Jim Allen, a postdoc, we crystallized the RC from *Rhodobacter sphaeroides* as I described in the National Lecture of the Biophysical Society in February 1983 (50).

Following the crystallization, the X-ray structure of the cofactors and proteins were published by Deisenhofer et al. (28). In 1985, we started a collaboration with the crystallography group of Doug Rees at UCLA to determine the structure of the RC from *R. sphaeroides*.

Preliminary phases and structures of the RC from *R. sphaeroides* were obtained by the molecular replacement method using the coordinates of the RC from *R. viridis* (7, 20). By 1987, the structure of the RC from *R. sphaeroides* reached a resolution of 2.8 Å (6) and is now 2.1–2.2 Å (105, 147). It is probably fair to say that the photosynthetic RC had for a long time been the best-characterized membrane-bound protein and served as a good model for this class of proteins (131).

The structure of the cofactors is shown in Figure 1*a*, and of the RC protein with its modeled location in the membrane is shown in Figure 1*b*. Both the cofactors and the L- and M-subunits exhibit an approximate twofold symmetry about a line joining the donor and the Fe. It was gratifying to us that the main features of the structure corroborated our previous findings, i.e., the L- and M-subunits have each five transmembrane helices, and the H-subunit has one (157) with most of it exposed on the cytoplasmic side (152). The cofactors are all associated with the L- and M-subunits (117), the primary donor is a bacteriochlorophyll dimer (51), Q_A is associated with the M-subunit (102), and the Fe is approximately equidistant from Q_A and Q_B (18) [for additional details, see (55)]. In a review article that appeared before the results of the X-ray diffraction were obtained, we proposed a structure that bears a remarkable resemblance to the structure shown in Figure 1 [see Figure 35 in (113)]. What nobody had predicted was the beautiful twofold symmetry. In retrospect, in view of the sequence homology of the L- and M-subunits, a far-sighted person could have predicted the symmetry. But it was not predicted and the result falls, therefore, into the category of "I thought of it, the minute I saw it."

The structure shown in Figure 1 represents the native RC in its ground state. Considerable experimental evidence had accumulated to show that a conformational change occurs during charge separation [reviewed in (75)]. To determine the molecular nature of this change, we trapped the charge-separated state $D^+Q_B^-$ at cryogenic temperatures and determined the structure by X-ray diffraction. We found that Q_B^- had moved 5 Å toward the cytoplasmic side with an accompanying 180 degree propeller twist about the isoprene chain (147). This structural change has important implications on electron transfer. Another important point we addressed is whether there are structural changes accompanying the site-directed mutations introduced to investigate the mechanisms of electron and proton transfers in the RC (21).

Thoughts of Switching Fields Again

With the X-ray determination of the three-dimensional structure of the RC, which corroborated most of the preceding work, the field of bacterial photosynthesis seemed to have reached its apogee. Although knowledge of the structure opened

up new approaches and many questions remained unanswered, the field seemed to me less challenging than when we had started 15 years ago. Perhaps that feeling was engendered by the fact that I had never worked such a long period in one specific field. I felt that at age 60 I was still good for one last switch in my career. Neurobiology seemed to me an exciting field, and in the summer of 1984 I took once more a course in Woods Hole. It was a demanding but exciting experience to work for two months in a new field. The project that I had in mind was to grow nerve cells on a silicon chip and follow electrically their development and connections. Bell Labs, known for its chip technology, offered me a position, and in the Spring of 1985 I visited them to discuss the details of the sabbatical arrangement. Unfortunately, on my return from Newark to Boston I suffered a heart attack on the plane. After listening to the prognosis of the cardiologists (which turned out to be too pessimistic), I did not feel up to investing a few years to get started in a new field, and all previous plans were scrapped. Bacterial photosynthesis was a familiar and comfortable field, in which it was easy for me to direct students and postdocs. Therefore, I opted to stay with it. But the style of my research changed. I had always been involved in the actual lab work, and in addition, I always had my own pet project on the side that was not necessarily connected with photosynthesis. To be honest, I miss these activities. In supervising research rather than doing it oneself, something vital is lost. The best analogy that I can come up with is "kissing through a veil." It is, of course, still fun and I am enthusiastic about our research as I hope comes through in the next sections.

Back to Bacterial Photosynthesis

THE STRUCTURE OF THE RC: CYT C_2 COMPLEX The secondary donor in *R. sphaeroides* is cyt c_2^{2+}, which forms a transient docked structure with the RC in which electron transfer occurs between reduced cyt c_2^{2+} and the oxidized donor, D^+. Several models for the structure of the transient RC-cyt c_2 complex have been proposed (reviewed in 151). They were all based on indirect experimental evidence and theoretical considerations. Our goal was to determine the structure by X-ray diffraction. Although it was generally believed that because of the transient nature of the complex, cocrystals would not form. Noam Adir, who joined us in 1990, succeeded after many trials in obtaining cocrystals that diffracted to 3.5 Å. Unfortunately, the cyt c_2 occupancy in the crystals was low ($\sim$25%), and only a preliminary structure was obtained (4). Herb Axelrod, who had previously determined the structure of cyt c_2 (12) in our lab, continued Adir's work and obtained cocrystals that had an $\sim$80% occupancy of cyt c_2 (10). In collaboration with Doug Rees's group, Herb collected a complete data set at the Berkeley Synchrotron source to a resolution of 2.4 Å. The data are now being processed, and a reliable structure should soon be forthcoming. An important point in this study was to show that the structure of the complex in the crystal is the same as in vivo. This was accomplished by comparing the electron transfer kinetics (which depend critically on the detailed structure) in the crystal and in solutions. They were the same (4).

An alternative approach to study the structure of the complex is to probe the area of contact between the RC and cyt c_2 by selectively mutating residues on the periplasmic side of the RC and measuring the effect of the mutation on the binding affinity and electron transfer. This work was performed by two graduate students, Scott Rongey, who started it (136), and Michelle Tetrault, who completed it (151). The results are in good agreement with the crystal structure.

ELECTRON TRANSFERS: KINETICS AND MECHANISMS The RC catalyzes the reduction of the secondary quinone, Q_B, to quinol, Q_BH_2, by a series of light-induced electron and proton transfers (see Figure 2). The initial electron transfer reaction, which occurs on a picosecond timescale, has been omitted for simplicity (reviewed in 87). We focused on the slower electron transfer reactions, $k_{AB}^{(1)}$, and $k_{AB}^{(2)}$ (steps 2 and 4 in Figure 2).

The transfer of the first electron from $Q_A^{-\cdot}$ to Q_B ($k_{AB}^{(1)}$) has been investigated by several groups, starting with the pioneering work of Parson (124) (reviewed in 116). In 1984, David Kleinfeld, a graduate student in our lab, provided convincing evidence that $k_{AB}^{(1)}$ is associated with a conformational change (88). He observed that electron transfer from Q_A^- to Q_B proceeds at cryogenic temperatures when the RCs were cooled under illumination but did not occur in RCs cooled in the dark. It is clear that different conformations were frozen in depending on whether the RCs

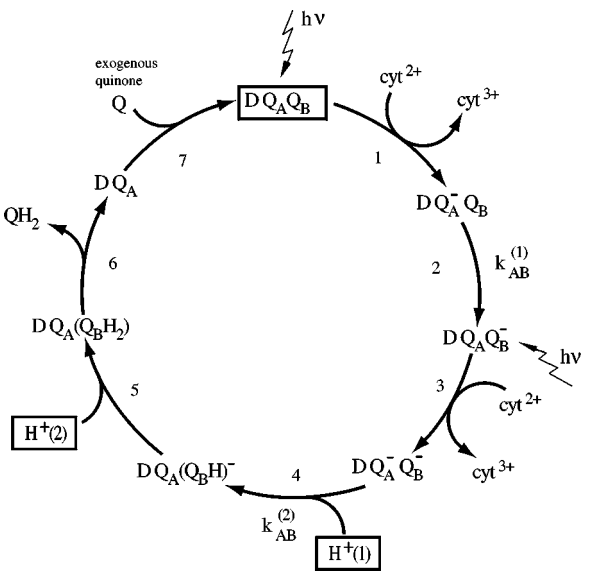

Figure 2 The quinone reduction cycle in bacterial RCs. Q_B is reduced in two one-electron reactions, $k_{AB}^{(1)}$, and $k_{AB}^{(2)}$, and binds two protons, H+(1) and H+(2), The reduced QH_2 leaves the RC and is replaced by an exogenous quinone, thereby resetting the cycle. Time to complete the cycles is ~1 ms (116).

were in the charge-separated state or not. The molecular basis of the conformational change has been determined by X-ray diffraction (147), as discussed in a previous section. Mike Graige, a graduate student, has shown that $k_{AB}^{(1)}$ is a conformationally gated process in which the rate-limiting step is the conformational change and not the intrinsic electron transfer (75). To prove this, Graige used the driving force assay, which he had developed earlier (76). In this assay, quinones with different redox potentials are substituted into the Q_A^- site, thereby changing the driving force for electron transfer. If electron transfer were rate limiting, $k_{AB}^{(1)}$ should increase, according to the Marcus theory, with increasing driving force (101). No dependence of $k_{AB}^{(1)}$ on driving force was found, proving that another step, e.g., a conformational change, is rate limiting. But what parameters determine the kinetics of the rate-limiting step is at present not understood.

The transfer of the second electron, $k_{AB}^{(2)}$, which proceeds on a millisecond timescale, (see step 4 in Figure 2) is coupled to proton transfer as represented by the net reaction [reviewed in (116)]

$$Q_A^- \cdot Q_B^- \cdot + H^+ \overset{k_{AB}^{(2)}}{\longleftrightarrow} Q_A(Q_BH)^- \cdot \qquad (1)$$

Graige, using the driving force assay, showed that this is a two-step process: one involving proton uptake, the other electron transfer. The process involves an intermediate state $Q_A^- \cdot (Q_BH)$. By a detailed analysis, Graige showed that proton transfer precedes rate-limiting electron transfer (76).

PROTON TRANSFERS: MECHANISMS AND PATHWAYS The protonation of Q_B and its release as Q_BH_2 (steps 4–6 in Figure 2) is, from a physiological point of view, the important process in the RC, with electron transfer serving as a prerequisite for protonation. Q_B is located in the interior of the RC protein, which presents a problem for the transport of protons from the aqueous exterior through the protein with a low dielectric constant. The two main problems of protonation are, therefore, the mechanism of proton transfer and the identification of the protein pathway(s).

It is now commonly accepted that proton transfer to $Q_B^- \cdot$ occurs via a donor-acceptor chain of protonatable amino acid residues and/or water molecules (reviewed in 116). To identify the residues involved in the pathway, protonatable residues were replaced with nonprotonatable residues by site-directed mutagenesis, the X-ray structure providing guidance for picking prospective candidates. The first mutant that affected proton transfer was constructed in 1989 by Paddock (123), then a graduate student and now a research associate in our group. This was followed by a large number of mutations performed by several groups (e.g., 150, 116). From the results of these experiments and with the aid of the X-ray crystal structure, we identified at least three possible proton transfer pathways (2). A chance observation by Paddock helped us to identify the predominant pathway. Paddock found that divalent metals (e.g., Zn^{2+}, Cd^{2+}) reduced $k_{AB}^{(2)}$ by an order of magnitude, presumably by blocking proton transfer (122). X-ray structure analysis revealed that these metals bind to His-H126, His-H128, and Asp-H124 (11),

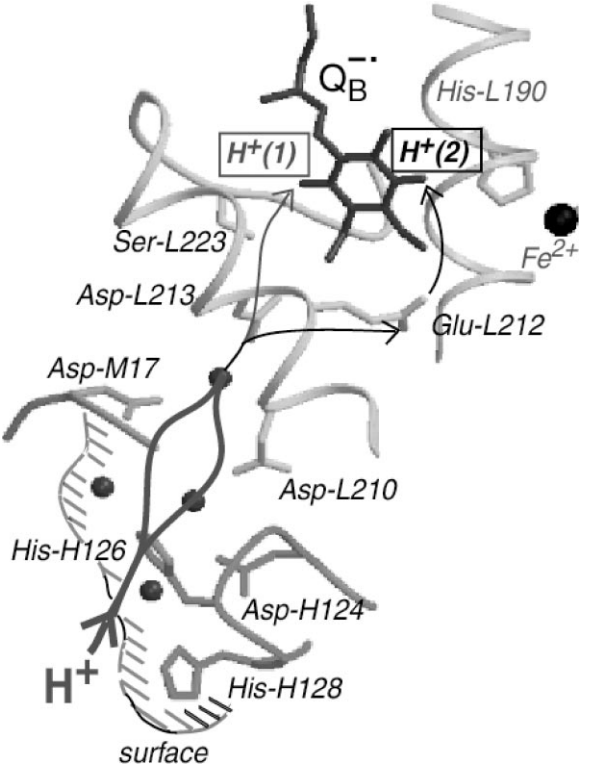

Figure 3 Part of the RC structure showing the region between the protein entry point and $Q_B^{-\cdot}$. The pathways of the two protons are shared up to the bifurcation near Asp L213 (3). The surface of the protein (*stippled*) and a few water molecules (*dots*) are indicated. Modified from (121).

which were therefore identified as being at or near the entry points of both protons (3). From the results of the mutagenesis work and the metal-binding studies, the proton transfer pathway shown in Figure 3 was deduced. The detailed kinetics of each protonation step are not understood and are being investigated at present.

CONCLUDING REMARKS

As I look over five decades of research, wandering from topic to topic, picking a flower here and there, I feel that it has been a wondrous and joyful journey. I really should add a decade or two to include my childhood and adolescent tinkering, which seems to have been as enjoyable, intense, and meaningful as doing research in later years. As time has gone by, the feelings have remained the same; only the budget and the type of questions asked have changed.

A recent prefatory chapter entitled, "Whatever happened to the fun?" (133), disturbed me and made me ponder the question. Part of the loss of fun can, no doubt, be attributed to the unavoidable added responsibilities that accrue during one's career and that detract from one's research activities. But another likely contributor, that one may be loath to admit, is the striving for recognition. I believe it is important to keep this enemy—our ego—in check lest we get lost in the clouds climbing the endless ladder to fame and glory.

Occasionally, I was asked which of my experiments I found most exciting and rewarding. My standard glib answer used to be, "The next one to be performed." I shall now try to answer the question in a more serious and general way. Rather than focus on a specific experiment, I want to explore the features that make a project exciting and satisfying. Aesthetics plays an important role, e.g., the beauty of crystals that made me originally grow them without regard to their usefulness, the beauty of symmetries (e.g., paraelectric resonance/paramagnetic resonance), and the simplicity of concepts (nuclear polarization by hot electrons, molecular weight of DNA by fluctuation spectroscopy). Another feature that attracts me is the combination of techniques and fields. On the technique side, for example, is the development of ENDOR, which resulted from a combination of two well-developed techniques, NMR and EPR. The combination of fields resulted in applying solid-state techniques to nuclear physics (determination of nuclear moments, hyperfine structures anomaly) to high-energy physics (muonium) and electronics (maser). This attraction of combining fields was surely an important component in my choosing research in biophysics, which is truly interdisciplinary. Nature does not care which tools from what discipline are used. The approach in our lab is to pick a relevant problem and use whatever technique is required to solve it.

The choice of bacterial photosynthesis was motivated by a desire to have a theme, to develop a field from its infancy to maturity, in contrast to the series of unconnected experiments that comprised most of my other research. Thus, the photosynthesis work resulted in a change in style. It was not a single experiment that brought the field to maturity but a large number of successive ones, each addressing different problems as they appeared, and in the process using a variety of techniques that we either knew or had to learn.

Speaking of styles and attitudes, there is one point that I want to get off my chest. I dislike the paranoia that leads some researchers to be excessively secretive in communicating with colleagues. Open discussions of one's work, in my opinion, are well worth the risk of being scooped.

Over the past years, I have come to realize the importance of not having been swayed by authority, dogmas, or fads, i.e., of not conforming. I consider conformity the nemesis of research as well as of private and public life. In research, it can destroy originality and creativity. In politics, it can destroy democracy and lead to aberrations like the Holocaust, an event that has haunted me and shaped a significant part of my life.

I wish I could finish with some noble thought, such as saying that my work was motivated by a desire to improve the condition of mankind. After all, understanding

photosynthesis could help solve the energy crisis and the global food problem. But such altruistic motivation has not driven me. I simply enjoy tinkering and doing research; it fulfills an inner need. If it also contributes to something useful, so much the better. It is the icing on the cake.

ACKNOWLEDGMENTS

I remember with pleasure and gratitude the many students, postdocs, collaborators, and colleagues who helped, guided, and accompanied me on parts of my long (50 years) journey in scientific research. There are clearly too many to name them all, but I would like to highlight a few. I wish to express my indebtness to Martin Kamen and Rod Clayton for introducing me to bacterial photosynthesis; Dave Mauzerall for our early collaboration in photosynthesis; Lisa Steiner for teaching me biochemistry and for remaining a valuable colleague, sounding board, and friend; Doug Rees for collaborating and teaching us X-ray crystallography; and foremost Mel Okamura for collaborating on all aspects of photosynthesis and for the pleasure of working with him all these years. I wish to thank Ed Gere for his expert technical help at Bell Labs, Roger Isaacson for his superb and dedicated technical assistance for the past 40 years, and Ed Abresch for his assistance with the biochemical work and for supplying our group with reaction centers for the past ~25 years. I am grateful to the NSF and NIH for supporting our research all these years. Finally and most importantly, I wish to thank my wife, Elsa, for her support and active interest in my work and my daughters for putting up with my long working hours and frequent absences.

LITERATURE CITED

1. Abranovich S. 1992. The Best Kept Secret. *Yediot Ahronot.* 23 April:13–20
2. Abresch EC, Paddock ML, Stowell MHB, McPhillips TM, Axelrod HL, et al. 1998. Identification of proton transfer pathways in the X-ray crystal structure of the bacterial reaction center from *Rhodobacter sphaeroides*. *Photosynth. Res.* 55:119–25
3. Ädelroth P, Paddock ML, Sagle LB, Feher G, Okamura MY. 2000. Identification of the proton pathway in bacterial reaction centers: both protons associated with reduction of Q_B to Q_BH_2 share a common entry point. *Proc. Natl. Acad. Sci. USA* 97:13086–91
4. Adir N, Axelrod HL, Beroza P, Isaacson RA, Rongey SH, et al. 1996. Co-crystallization and characterization of the photosynthetic reaction center-cytochrome c_2 complex from *Rhodobacter sphaeroides*. *Biochemistry* 35:2535–47
5. Alderton G, Fevold HL. 1946. Direct crystallization of lysozyme from egg white and some crystalline salts of lysozyme. *J. Biol. Chem.* 164:1–5
6. Allen JP, Feher G, Yeates TO, Komiya H, Rees DC. 1987. Structure of the reaction center from *Rhodobacter sphaeroides* R-26: II. The protein subunits. *Proc. Natl. Acad. Sci. USA* 84:6162–66
7. Allen JP, Feher G, Yeates TO, Rees DC,

Deisenhofer J, et al. 1986. Structural homology of reaction centers from *R. sphaeroides* and *R. viridis* as determined by X-ray diffraction. *Proc. Natl. Acad. Sci. USA* 83:8589–93

8. Anderson PW. 1978. Local moments and localized states. *Science* 201(4353):307–16
9. Archive of "Haganah" History, Ref. 1193
10. Axelrod HL, Abresch EC, Okamura MY, Feher G, Yeh AP, Rees DC. 1999. X-ray structure determination of the RC-cyt c_2 complex from *Rhodobacter sphaeroides*. *Biophys. J.* 76:A20 (Abstr.)
11. Axelrod HL, Abresch EC, Paddock ML, Okamura MY, Feher G. 2000. Determination of the binding sites of the proton transfer inhibitors Cd^{2+} and Zn^{2+} in bacterial reaction centers. *Proc. Natl. Acad. Sci. USA* 97:1542–47
12. Axelrod HL, Feher G, Allen JP, Chirino AJ, Day MW, et al. 1994. Crystallization and X-ray structure determination of cytochrome c_2 from *Rhodobacter sphaeroides* in three crystal forms. *Acta Crystallogr. D* 50:596–602
13. Benedek GB. 1969. Optical mixing spectroscopy, with applications to problems in physics, chemistry, biology and engineering. In *Polarisation, Matière et Rayonnement, Livre de Jubilé en l'honneur d'Alfred Kastler*, pp. 49–84. Paris: Presses Univ. France
14. Bennet JE, Ingram DJE, George P, Griffith JS. 1955. Paramagnetic resonance absorption of ferrihaemoglobin and ferrimyoglobin derivatives. *Nature* 176:394
15. Bloch F. 1946. Nuclear induction. *Phys. Rev.* 70(7–8):460–74
16. Bloembergen N. 1956. Proposal for a new type solid state maser. *Phys. Rev.* 104(2):324–27
17. Bridges F. 1975. Paraelectric phenomena in CRC. *Crit. Rev. Solid State Phys.* 5:1–88
18. Butler WF, Calvo R, Fredkin DR, Isaacson RA, Okamura MY, Feher G. 1984. The electronic structure of Fe^{2+} in reaction centers from *Rhodopseudomonas sphaeroides* III. EPR measurements of the reduced acceptor complex. *Biophys. J.* 45:947–73
19. Calvo R, Isaacson RA, Paddock ML, Abresch EC, Okamura MY, et al. 2001. EPR study of the semiquinone biradical $Q_A^- \cdot Q_B^-$ in photosynthetic reaction centers from *Rb. sphaeroides* at 326 GHz: determination of the exchange interaction J_0. *J. Phys. Chem. B* 105(19):4053–57
20. Chang CH, Tiede D, Tang J, Smith U, Norris J, Schiffer M. 1986. Structure of *Rodopseudomonas sphaeroides* R-26 reaction center. *FEBS Lett.* 205:82–86
21. Chirino AJ, Lous EJ, Huber M, Allen JP, Schenck CC, et al. 1994. Crystallographic analyses of site-directed mutants of the photosynthetic reaction center from *Rhodobacter sphaeroides*. *Biochemistry* 33:4584–93
22. Clark WG, Feher G. 1963. Nuclear polarization in InSb by a d.c. current. *Phys. Rev. Lett.* 10(4):134–38
23. Clayton RK, Wang RT. 1971. Photochemical reaction centers from *Rhodopseudomonas sphaeroides*. *Methods Enzymol.* 23:696–704
24. Combrisson J, Honig A, Townes CH. 1956. *Compt. Rend.* 242:2451–53
25. Commoner B, Heise JJ, Townsend J. 1956. Light-induced paramagnetism in chloroplasts. *Proc. Natl. Acad. Sci. USA* 42(10):710–18
26. Commoner B, Townsend J, Pake GE. 1954. Free radicals in biological materials. *Nature* 174:689–91
27. Debus RJ, Feher G, Okamura MY. 1986. Iron-depleted reaction centers from *Rhodopseudomonas sphaeroides* R-26.1: characterization and reconstitution with Fe^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}. *Biochemistry* 25(8):2276–87
28. Deisenhofer J, Epp O, Miki K, Huber R, Michel H. 1984. X-ray structure analysis of a membrane protein complex. Electron density map at 3 Å resolution and a model of the chromophores of the photosynthetic

reaction center from *Rhodopseudomonas viridis*. *J. Mol. Biol.* 180(2):385–98
29. Dickerson RE, Geis I. 1969. *The Structure and Action of Proteins*. New York: Harper & Row. 120 pp.
30. Dresselhaus G, Kip AF, Kittel C. 1955. Cyclotron resonance of electrons and holes in silicon and germanium crystals. *Phys. Rev.* 98(2):368–84
31. Durbin SD, Feher G. 1986. Crystal growth studies of lysozyme as a model for protein crystallization. *J. Cryst. Growth* 76:583–92
32. Durbin SD, Feher G. 1990. Studies of crystal growth mechanisms of proteins by electron microscopy. *J. Mol. Biol.* 212(4):763–74
33. Durbin SD, Feher G. 1991. Simulation of lysozyme crystal growth by the Monte Carlo method. *J. Cryst. Growth* 110:41–51
34. Durbin SD, Feher G. 1996. Protein crystallization. *Annu. Rev. Phys. Chem.* 47:171–204
35. Duysens LNM. 1952. *Transfer of excitation energy in photosynthesis*. PhD thesis. Univ. Utrecht, The Netherlands
35a. Ehrenberg A, Malmstrom BG, Vanngard T, eds. 1967. *Magnetic Resonance in Biological Systems*, Vol. 9. New York: Pergamon
36. Eigen M, DeMaeyer L. 1963. Relaxation methods. In *Techniques of Organic Chemistry*, ed. A Weissberger, 8:895–1054. New York: Intersci. Publ.
37. Eisenberger P, Okamura MY, Feher G. 1982. The electronic structure of Fe^{2+} in reaction centers from *Rhodopseudomonas sphaeroides* II. Extended X-ray fine structure studies. *Biophys. J.* 37:523–38
38. Eisenberger P, Pershan PS. 1967. Magnetic resonance studies of met-myoglobin and myoglobin azide. *J. Chem. Phys.* 47(9):3327–33
39. Eisinger J, Feher G. 1958. Hfs anomaly of Sb^{121} and Sb^{123} determined by the electron nuclear double-resonance technique. *Phys. Rev.* 109(4):1172–83
40. Emerson R, Arnold W. 1932. The photochemical reaction in photosynthesis. *J. Gen. Physiol.* 16:191–205
41. Feher ER. 1964. Effect of uniaxial stresses on the paramagnetic spectra of Mn^{3+} and Fe^{3+} in MgO. *Phys. Rev.* 136(1):A145–57
42. Feher G. 1956. Method of polarizing nuclei in paramagnetic substances. *Phys. Rev.* 103(2):500–1
43. Feher G. 1956. Observation of nuclear magnetic resonances via the electron spin resonance line. *Phys. Rev.* 103(3):834–35
44. Feher G. 1957. Sensitivity considerations in microwave paramagnetic resonance absorption techniques. *Bell. Syst. Technol. J.* 36(2):449–84
45. Feher G. 1959. Electron spin resonance experiments on donors in silicon. I. Electronic structure of donors by the electron nuclear double resonance technique. *Phys. Rev.* 114(5):1219–44
46. Feher G. 1959. Nuclear polarization via "hot" conduction electrons. *Phys. Rev. Lett.* 3(3):135–37
47. Feher G. 1970. Determination of the zero field splitting parameter "D". In *Electron Paramagnetic Resonance with Application to Selected Problems in Biology*, ed. C deWitt, J Matricon, pp. 61–64. New York: Gordon & Breach
48. Feher G. 1971. Some chemical and physical properties of a bacterial reaction center particle and its primary photochemical reactants. *Photochem. Photobiol.* 14(3):373–87
49. Feher G. 1978. Fluctuation spectroscopy. *Trends Biochem. Sci.* 3(5):N111–13
50. Feher G. 1983. Primary process in bacterial photosynthesis. Biophys. Soc., Natl. Lect. *Biophys. J.* 41:3
51. Feher G. 1992. Identification and characterization of the primary donor in bacterial photosynthesis: a chronological account of an EPR/ENDOR investigation. *J. Chem. Soc. Perkin Trans.* 2, pp. 1861–74
52. Feher G. 1998. Light reflections III. *Photosynth. Res.* 55(2–3):375–78

53. Feher G. 1998. The development of ENDOR and other reminiscences of the 1950's. In *Foundations of Modern EPR*, ed. GR Eaton, SS Eaton, KM Salikov, 1:548–56. Singapore: World Sci. Publ.
54. Feher G. 1998. The primary and secondary electron acceptors in bacterial photosynthesis: I. A chronological account of their identification by EPR. *Appl. Magn. Reson.* 15:23–38
55. Feher G. 1998. Three decades of research in bacterial photosynthesis and the road leading to it: a personal account. *Photosynth. Res.* 55:1–40
56. Feher G, Allen JP, Okamura MY, Rees DC. 1989. Structure and function of photosynthetic reaction centers. *Nature* 339:111–16
57. Feher G, Fuller CS, Gere EA. 1957. Spin and magnetic moment of P^{22} by the electron nuclear double-resonance technique. *Phys. Rev.* 107(5):1462–64
58. Feher G, Gordon JP, Buehler E, Gere EA, Thurmond CD. 1958. Spontaneous emission of radiation from an electron spin system. *Phys. Rev.* 109(1):221–22
59. Feher G, Hensel JC, Gere EA. 1960. Paramagnetic resonance absorption from acceptors in silicon. *Phys. Rev. Lett.* 5(7):309–11
60. Feher G, Hoff AJ, Isaacson RA, Ackerson LC. 1975. ENDOR experiments on chlorophyll and bacteriochlorophyll in vitro and in the photosynthetic unit. *Ann. NY Acad. Sci.* 244:239–59
61. Feher G, Isaacson RA, Scholes GP, Nagel R. 1973. Electron nuclear double resonance (ENDOR) investigation on myoglobin and hemoglobin. *Ann. NY Acad. Sci.* 222:86–101
62. Feher G, Kam Z. 1985. Nucleation and growth of protein crystals; general principles and assays. *Methods Enzymol.* 114:77–112
63. Feher G, Malley M, Mauzerall D. 1967. Direct observation of the Zeeman splitting of the excited state of porphyrins. See Ref. 35a, pp. 145–57
64. Feher G, Okamura MY, McElroy JD. 1972. Identification of an electron acceptor in reaction centers of *Rhodopseudomonas spheroides* by EPR spectroscopy. *Biochim. Biophys. Acta* 267: 222–26
65. Feher G, Okamura MY. 1999. The primary and secondary acceptors in bacterial photosynthesis: II. The structure of the Fe^{2+}-Q^{-} complex. *Appl. Magn. Reson.* 16:63–100
66. Feher G, Prepost R, Sachs AM. 1960. Muonium formation in semiconductors. *Phys. Rev. Lett.* 5(11):515–17
67. Feher G, Richards PL. 1967. Determination of the zero-field splitting "D" in heme chloride by far-infrared spectroscopy. See Ref. 35a, pp. 141–44
68. Feher G, Scovil HED. 1957. Electron spin relaxation times in gadolinium ethyl sulfate. *Phys. Rev.* 105(2):760–62
69. Feher G, Shepherd IW, Shore HB. 1966. Paraelectric resonance of OH^{-} dipoles in KC1. *Phys. Rev. Lett.* 16(12):500–3
70. Feher G, Weissman M. 1973. Fluctuation spectroscopy: determination of chemical reaction kinetics from the frequency spectrum of fluctuations. *Proc. Natl. Acad. Sci. USA* 70(3):870–75
71. Fermi E. 1930. Über die magnetischen Momente der Atomkerne. *Z. Phys.* 60: 320–33
72. Garavito RM, Rosenbusch JP. 1980. Three-dimensional crystals of an integral membrane protein: an initial X-ray analysis. *J. Cell Biol.* 86(1):327–29
73. Giauque WF, Macdougall DP. 1933. Attainment of temperatures below 1° absolute by demagnetization of $Gd_2(SO_4)_3 \cdot 8H_2O$. *Phys. Rev.* 43(9):768–68
74. Gordon JP, Zeiger HJ, Townes CH. 1954. Molecular microwave oscillator and new hyperfine structure in the microwave spectrum of NH_3. *Phys. Rev.* 95(1):282–84
75. Graige MS, Feher G, Okamura MY. 1998. Conformational gating of the electron transfer reaction $Q_A^{-} Q_B \rightarrow Q_A Q_B^{-}$, in bacterial reaction centers of *Rb.*

sphaeroides determined by a driving force assay. *Proc. Natl. Acad. Sci. USA* 95:11679–84

76. Graige MS, Paddock ML, Bruce JM, Feher G, Okamura MY. 1996. Mechanism of proton-coupled electron transfer for quinone (Q_B) reduction in reaction centers of *Rb. sphaeroides*. *J. Am. Chem. Soc.* 118(38):9005–16
77. Griffith JS. 1956. On the magnetic properties of some haemoglobin complexes. *Proc. R. Soc. London Ser. A* 235:23–36
78. Deleted in proof
79. Heller P. 1966. Hemoglobinopathic dysfunction of the red cell. *Am. J. Med.* 41(5): 799–814
80. Hensel JC, Feher G. 1960. Valence band parameters in silicon from cyclotron resonances in crystals subjected to uniaxial stress. *Phys. Rev. Lett.* 5(7):307–9
81. Hensel JC, Feher G. 1963. Cyclotron resonance experiments in uniaxially stressed silicon: valence band inverse mass parameters and deformation potentials. *Phys. Rev.* 129(3):1041–62
82. Honig A. 1954. Polarization of arsenic nuclei in a silicon semiconductor. *Phys. Rev.* 96(1):234–35
83. Hutterman J. 1993. ENDOR of randomly oriented mononuclear metalloproteins. In *Biological Magnetic Resonance*, ed. LJ Berliner, J Reuben, 13:219–52. New York: Plenum
84. Kam Z, Shore HB, Feher G. 1978. On the crystallization of proteins. *J. Mol. Biol.* 123(4):539–55
85. Känzig W, Hart RH Jr, Roberts S. 1964. Paraelectricity and ferroelectricity due to hydroxyl ions in alkali halides; paraelectric cooling. *Phys. Rev. Lett.* 13(18):543–45
86. Katz B. 1966. Quantal nature of chemical transmission. In *Nerve, Muscle and Synapse*, pp. 129–41. New York: McGraw-Hill
87. Kirmaier C, Holten D. 1993. Electron transfer and charge recombination reactions in wild-type and mutant bacterial reaction centers. In *The Photosynthetic Reaction Center*, ed. J Deisenhofer, JR Norris, 2:49–70. San Diego, CA: Academic
88. Kleinfeld D, Okamura MY, Feher G. 1984. Electron-transfer kinetics in photosynthetic reaction centers cooled to cryogenic temperatures in the charge-separated state: evidence for light-induced structural changes. *Biochemistry* 23: 5780–86
89. Koenig SH. 1959. Hot and warm electrons—a review. *J. Phys. Chem. Solids* 8:227–34
90. Köhler G, Wendt H. 1966. Di Bestimmung von Gleichgewichtskonstanten aus kinetischen messungen. *Ber. Bunsenges. Phys. Chem.* 70:674–81

90a. Kopfermann H. 1956. *Kernmomente*. Frankfurt: Akad. Verlag.

91. Kuhn U, Lüty F. 1965. Paraelectric heating and cooling with OH^--dipoles in alkali halides. *Solid State Commun.* 4:31–33
92. Lee TD, Yang CN. 1956. Question of parity conservation in weak interactions. *Phys. Rev.* 104(1):254–58
93. Lendzian F, Huber M, Isaacson RA, Endeward B, Plato M, et al. 1993. The electronic structure of the primary donor cation radical in *Rhodobacter sphaeroides* R-26: ENDOR and TRIPLE resonance studies in single crystals of reaction centers. *Biochim. Biophys. Acta* 1183:139–60
94. Loach PA, Hall RL. 1972. The question of the primary electron acceptor in bacterial photosynthesis. *Proc. Natl. Acad. Sci. USA* 69(4):786–90
95. Lockhart DJ, Boxer SG. 1987. Magnitude and direction of the change in dipole moment associated with excitation of the primary electron donor in *Rhodopseudomonas sphaeroides* reaction centers. *Biochemistry* 26:664–68
96. Lösche M, Feher G, Okamura MY. 1987. The Stark effect in reaction centers from *Rhodobacter sphaeroides* R-26 and *Rhodopseudomonas viridis*. *Proc. Natl. Acad. Sci. USA* 84:7537–41

97. Low W. 1960. In *Paramagnetic Resonance in Solids*, ed. F Seitz, D Turnbull, p. 67. New York: Academic
98. Lubitz W, Abresch EC, Debus RJ, Isaacson RA, Okamura MY, Feher G. 1985. Electron nuclear double resonance of semiquinones in reaction centers of *Rhodopseudomonas sphaeroides*. *Biochim. Biophys. Acta* 808(3):464–69
99. Lubitz W, Feher G. 1999. The primary and secondary acceptors in bacterial photosynthesis: III characterization of the quinone radicals $Q_A^{-\cdot}$ and $Q_B^{-\cdot}$ by EPR and ENDOR. *Appl. Magn. Reson.* 17:1–48
100. Malley M, Feher G, Mauzerall D. 1968. The Stark effect in porphyrins. *J. Mol. Spectrosc.* 25(4):544–48
101. Marcus RA. 1993. Electron-transfer reactions in chemistry: theory and experiment (Nobel Lecture). *Angew. Chem.* 32:1111–21
102. Marinetti TD, Okamura MY, Feher G. 1979. Localization of the primary quinone binding site in reaction centers from *Rhodopseudomonas sphaeroides* R-26 by photoaffinity labeling. *Biochemistry* 18: 3126–33
103. Mauzerall D, Feher G. 1964. A study of the photoinduced porphyrin free radical by electron spin resonance. *Biochim. Biophys. Acta* 79:430–32
104. Mauzerall D, Feher G. 1964. Optical absorption of the porphyrin free radical formed in a reversible photochemical reaction. *Biochim. Biophys. Acta* 88:658–60
105. McAuley KE, Fyfe PK, Ridge JP, Isaacs NW, Cogdell RJ, Jones MR. 1999. Structural details of an interaction between cardiolipin and an integral membrane protein. *Proc. Natl. Acad. Sci. USA* 96(26): 14706–11
106. McElroy JD, Feher G, David C, Mauzerall DC. 1972. Characterization of primary reactants in bacterial photosynthesis. I. Comparison of the light-induced EPR signal ($g = 2.0026$) with that of a bacteriochlorophyll radical. *Biochim. Biophys. Acta* 267:363–74
107. McElroy JD, Mauzerall DC, Feher G. 1974. Characterization of primary reactants in bacterial photosynthesis II. Kinetic studies of the light-induced signal ($g = 2.0026$) and the optical absorbance changes at cryogenic temperatures. *Biochim. Biophys. Acta* 333:261–78
108. McPherson A. 1999. *Crystallization of Biological Macromolecules*. New York: Cold Spring Harbor Lab. Press. 586 pp.
109. Michel H, Oesterhelt D. 1980. Three-dimensional crystals of membrane proteins: bacteriorhodopsin. *Proc. Natl. Acad. Sci. USA* 77(3):1283–85
110. Michel H. 1982. Three-dimensional crystals of a membrane protein complex: the photosynthetic reaction center from *Rhodopseudomonas viridis*. *J. Mol. Biol.* 158(3):567–72
111. Norris JR, Scheer H, Katz JJ. 1975. Models for antenna and reaction center chlorophylls. *Ann. NY Acad. Sci.* 244:260–80
112. Norris JR, Uphaus RA, Crespi HL, Katz JJ. 1971. Electron spin resonance of chlorophyll and the origin of signal I in photosynthesis. *Proc. Natl. Acad. Sci. USA* 68(3):625–28
113. Okamura MY, Feher G, Nelson N. 1982. Reaction centers. In *Photosynthesis: Energy Conversion by Plants and Bacteria*, ed. Govindjee, 1:195–272. New York: Academic
114. Okamura MY, Fredkin DR, Isaacson RA, Feher G. 1979. Magnetic interactions and electron transfer kinetics of the reduced intermediate acceptor in reaction centers (RCs) of *Rhodopseudomonas sphaeroides* R-26. Evidence for thermally induced tunneling. In *Tunneling in Biological Systems*, ed. B Chance, D DeVault, H Frauenfelder, RA Marcus, JR Schrieffer, N Sutin, pp. 729–43. New York: Academic
115. Okamura MY, Isaacson RA, Feher G. 1975. Primary acceptor in bacterial photosynthesis: the obligatory role of ubiquinone in photoactive reaction centers of *Rhodopseudomonas sphaeroides*.

Proc. Natl. Acad. Sci. USA 72(9):3491–95
116. Okamura MY, Paddock ML, Graige MS, Feher G. 2000. Proton and electron transfer in bacterial reaction centers. *Biochim. Biophys. Acta* 1458:148–63
117. Okamura MY, Steiner LA, Feher G. 1974. Characterization of reaction centers from photosynthetic bacteria. I. Subunit structure of the protein mediating the primary photochemistry in *Rhodopseudomonas sphaeroides* R-26. *Biochemistry* 13(7):1394–403
118. Orbach R. 1961. Spin-lattice relaxation in rare-earth salts. *Proc. R. Soc. London Ser. A* 264:458–84
119. Osborne TB. 1892. Crystallized vegetable proteins. *Am. Chem. J.* 14:662–89
120. Overhauser AW. 1953. Polarization of nuclei in metals. *Phys. Rev.* 92(2):411–15
121. Paddock ML, Ädelroth P, Chang C, Abresch EC, Feher G, Okamura MY. 2001. Identification of the proton pathway in bacterial reaction centers: cooperation between Asp-M17 and Asp-L210 facilitates proton transfer to the secondary quinone (Q_B). *Biochemistry* 40:6893–902
122. Paddock ML, Graige MS, Feher G, Okamura MY. 1999. Identification of the proton pathway in bacterial reaction centers: inhibition of proton transfer by binding of Zn^{2+} or Cd^{2+}. *Proc. Natl. Acad. Sci. USA* 96:6183–88
123. Paddock ML, Rongey SH, Feher G, Okamura MY. 1989. Pathway of proton transfer in bacterial reaction centers: replacement of glutamic acid 212 in the L subunit by glutamine inhibits quinone (secondary acceptor) turnover. *Proc. Natl. Acad. Sci. USA* 86:6602–6
124. Parson WW. 1969. The reaction between primary and secondary electron acceptors in bacterial photosynthesis. *Biochim. Biophys. Acta* 1098:151–58
125. Perutz MF. 1998. *I Wish I Had Made You Angry Earlier*. New York: Cold Spring Harbor Lab. Press
126. Perutz MF, Pulsinelli PD, Ranney HM. 1972. Structure and subunit interaction of haemoglobin M Milwaukee. *Nat. New Biol.* 237(78):259–63
127. Perutz MF, Wilkinson AJ, Paoli M, Dodson GG. 1998. The stereochemical mechanism of the cooperative effects in hemoglobin revisited. *Annu. Rev. Biophys. Biomol. Struct.* 27:1–34
128. Platt JR. 1965. Electronic structure and excitation of polyenes and porphyrins. In *Radiation Biology III*, ed. A Hollaender, 2:71–123. New York: McGraw-Hill
129. Portis AM. 1953. Electronic structure of F centers: saturation of the electron spin resonance. *Phys. Rev.* 91(5):1071–78
130. Reed DW, Clayton RK. 1968. Isolation of a reaction center fraction from *Rhodopseudomonas spheroids*. *Biochem. Biophys. Res. Commun.* 30(5):471–75
131. Rees DC, Komiya H, Yeates TO, Allen JP, Feher G. 1989. The bacterial photosynthetic reaction center as a model for membrane proteins. *Annu. Rev. Biochem.* 58:607–33
132. Reif F. 1965. *Fundamentals of Statistical and Thermal Physics*, ed. EU Condon, Sect. 15:572–73. New York: McGraw-Hill
133. Richards FM. 1997. Whatever happened to the fun? An autobiographical investigation. *Annu. Rev. Biophys. Biomol. Struct.* 26:1–25
134. Richards PL, Caughey WS, Eberspaecher H, Feher G, Malley M. 1967. Determination of the zero-field splitting of Fe^{3+} in several hemin compounds. *J. Chem. Phys.* 47(3):1187–88
135. Roberts TM, Lauer GD, Klotz LC. 1975. Physical studies on DNA from "primitive" eucaryotes. *CRC Crit. Rev. Biochem.* 3(4):349–449
136. Rongey SH, Feher G, Okamura MY. 1995. Investigation of the binding domain in the *Rhodobacter sphaeroides* cyt c_2: reaction center complex by site-directed mutagenesis of Asp-M184 and Asp-L155 to Lys. *Int. Photosynth. Congr., 10th*,

Montpellier, Fr., ed. P Mathis, pp. 635–38. Dordrecht: Kluwer
137. Samuel I. The Old Testament. Chapter 28:7
138. Das Sarma S, Fabian J, Hu XD, Zutic I. 2000. Theoretical perspectives on spintronics and spin-polarized transport. *IEEE Trans. Magn.* 36(5):2821–26
139. Scholes CP, Isaacson RA, Feher G. 1971. Determination of the zero-field splitting of Fe^{3+} in heme proteins from the temperature dependence of the spin-lattice relaxation rate. *Biochim. Biophys. Acta* 244(1):206–10
140. Scholes CP, Isaacson RA, Feher G. 1972. Electron nuclear double resonance studies on heme proteins: determination of the interaction of Fe^{3+} with its ligand nitrogens in metmyoglobin. *Biochim. Biophys. Acta* 263(2):448–52
141. Scholes CP, Lapidot A, Mascarenhas R, Inubushi T, Isaacson RA, Feher G. 1982. Electron nuclear double resonance (ENDOR) from heme and histidine nitrogens in single crystals of aquometmyoglobin. *J. Am. Chem. Soc.* 104(10):2724–35
142. Schrödinger E. 1944. *What is Life*. Cambridge, UK: Cambridge Univ. Press. 92 pp.
143. Scovil HED, Feher G, Seidel H. 1957. Operation of a solid state maser. *Phys. Rev.* 105(2):762–63
144. Shepherd I, Feher G. 1965. Cooling by adiabatic depolarization of OH^- molecules in KCl. *Phys. Rev. Lett.* 15(5): 194–98
145. Sogo P, Jost M, Calvin M. 1959. Evidence for free-radical production in photosynthesizing systems. *Radiat. Res. Suppl.* I:511–18
146. Steiner LA, Okamura MY, Lopes AD, Moskowitz E, Feher G. 1974. Characterization of reaction centers from photosynthetic bacteria. II. Amino acid composition of the reaction center protein and its subunits in *Rhodopseudomonas sphaeroides* R-26. *Biochemistry* 13(7): 1403–10
147. Stowell MHB, McPhillips TM, Rees DC, Soltis SM, Abresch E, Feher G. 1997. Light induced structural changes in photosynthetic reaction centers. *Science* 276:812–16
148. Sutton MR, Rosen D, Feher G, Steiner LA. 1982. Amino-terminal sequences of L, M, and H subunits of reaction centers from photosynthetic bacterium, *Rhodopseudomonas sphaeroides* R-26. *Biochemistry* 21(16):3842–49
149. Szent-Györgyi A. 1957. Excitations and the biological matrix. In *Bioenergetics*, pp. 32–40. New York: Academic
150. Takahashi E, Wraight CA. 1990. A crucial role for AspL213 in the proton transfer pathway to the secondary quinone of reaction centers from *Rhodobacter sphaeroides*. *Biochim. Biophys. Acta* 1020:107–11
151. Tetreault M, Rongey SH, Feher G, Okamura MY. 2001. Interaction between cytochrome c_2 and the photosynthetic reaction center from *Rhodobacter sphaeroides*: effects of charge modifying mutations on binding and electron transfer. *Biochemistry* 40(29):8452–62
152. Valkirs GE, Feher G. 1982. Topography of reaction center subunits in the membrane of the photosynthetic bacterium *Rhodopseudomonas sphaeroides*. *J. Cell Biol.* 95(1):179–88
153. Van Vleck JH. 1932. *The Theory of Electric and Magnetic Susceptibilities*. New York: Oxford Univ. Press. 384 pp.
154. Volmer M. 1939. *Kinetik der Phasenbildung*, ed. KF Bonhoeffer. Dresden: Steinkopff
155. Weissman M, Schindler H, Feher G.1976. Determination of molecular weights by fluctuation spectroscopy; application to DNA. *Proc. Natl. Acad. Sci. USA* 73(8): 2776–80
156. Willenbrock FK, Blombergen N. 1953. Paramagnetic resonance in n- and p-type silicon. *Phys. Rev.* 91(5):1281–81
157. Williams JC, Steiner LA, Feher G. 1986.

Primary structure of the reaction center from *Rhodopseudomonas sphaeroides*. *Proteins* 1(4):312–25

158. Williams JC, Steiner LA, Feher G, Simon MI. 1984. Primary structure of the L subunit of the reaction center from *Rhodopseudomonas sphaeroides*. *Proc. Natl. Acad. Sci. USA* 81(23):7303–7
159. Williams JC, Steiner LA, Ogden RC, Simon MI, Feher G. 1983. Primary structure of the M subunit of the reaction center from *Rhodopseudomonas sphaeroides*. *Proc. Natl. Acad. Sci. USA* 80:6505–9
160. Wilson DK, Feher G. 1961. Electron spin resonance experiments on donors in silicon. III. Investigation of excited states by the application of uniaxial stress and their importance in relaxation processes. *Phys. Rev.* 124(4):1068–83

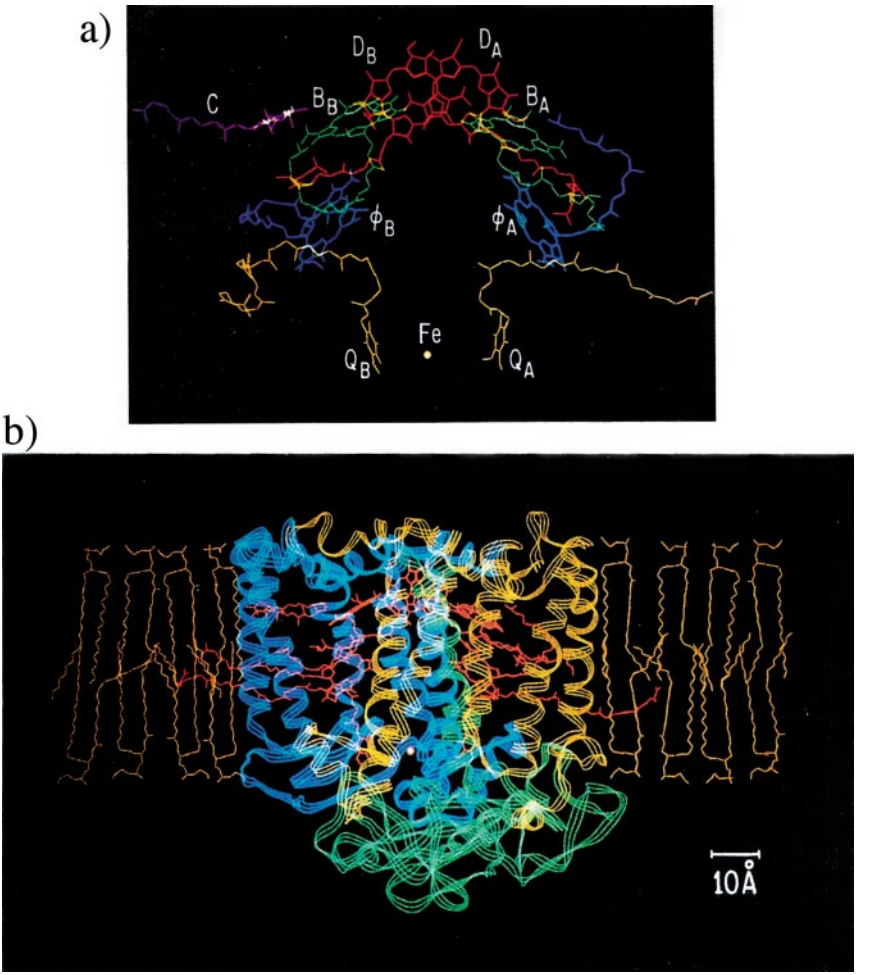

Figure 1 The structure of the reaction center from *Rb. sphaeroides*. (*a*) Cofactor structure. D-Bacteriochlorophyll dimer, B-Bacteriochlorophyll, ϕ-Bacteriopheophytin, Q-Ubiquinone 10, subscripts A and B refer to the two pseudosymmetrical branches. (Electron transfer proceeds preferentially along the A-branch.) (*b*) The structure of the reaction center and its position modeled into a sketch of the lipid bilayer. L-subunit, *yellow*; M-subunit, *blue*; H-subunit, *green*. Cofactors in *red*. The twofold symmetry axis is in the plane of the paper joining the Fe (*dot*) near the cytoplasmic side (*bottom*) with the bacteriochlorophyll dimer near the periplasmic side (*top*) (56).

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