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PERSONAL PERSPECTIVE

Memories: from protein synthesis to photosynthesis

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Abstract Emphasis herein is on the early years of my scientific life, primarily in graduate school and at the McCollum-Pratt Institute, Johns Hopkins University, as techniques learned and research performed then became the basis for future scientific endeavors. Studies on the mechanism of conversion of light energy into chemical free energy were a logical consequence of earlier investigations on enzyme-catalyzed hydrogen transfer reactions and pyridine nucleotide coenzyme biochemistry. Identification of several protein factors involved in pyridine nucleotide reduction by illuminated chloroplasts is described and, hopefully, adequately and honestly referenced to complementary research in other laboratories. Coupled with progress were changes in nomenclature of the protein factors and are so noted. In particular, David Wharton proposed the descriptive name, ferredoxin, for the non-heme iron and labile sulfide-containing proteins which serve as redox cofactors in a variety of energy conserving reactions. The inclusion of "*Lessons*" is adapted from Efraim Racker (1976, *A new look at mechanisms in bioenergetics*. Academic Press, NY). They are lessons that I learned and are included herein solely for graduate students.

Keywords Ferredoxin · Ferredoxin–NADP oxidoreductase · Non-Heme Iron Proteins · Photosynthesis · Robert (Robin) Hill · David Shemin · Erwin Chargaff

Anthony (Tony) San Pietro, the author

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Early remembrances

I was delivered into this world by Caesarean Section at St. Catherine's Hospital, Brooklyn, New York, on April 22, 1922. My parents, Rimetrio (James) and Maria (nee Cibelli) had been married for eight years before my appearance; an old world family-arranged marriage with no

time alone prior to the taking of marriage vows. At the time of their marriage, my mother was seventeen years old and my father was twenty-two years old. Neither of my parents was allowed much early education but rather each had to work to provide needed financial support for their families. My mother left school after the ninth grade and began piece employment as a buttonhole seamstress for men's shirts. Her salary was related directly to how many shirts she finished and not the hours she worked. She continued to be employed during the beginning years of their marriage and, much to my father's chagrin, often proclaimed that she was earning more than him. My father finished only the early grades of elementary school before being forced to leave school to help full time my grandfather who was a junk collector. The eldest son Joseph ran away from home (and this chore) and, though under age, joined the US Navy. Thus, in old world fashion, my father became the heir apparent to help my grandfather. As soon as he was able, my father sought and obtained employment in a jute mill by proclaiming (with confidence but with some exaggeration) to have the mechanical skills needed to maintain the mill machinery in working condition.

I was educated in the public school system in New York where progress was accelerated as I was encouraged to complete both the 2nd and 3rd and the 7th and 8th grades each in one year. As an only child, this pleased my parents greatly but did little to advance my maturity especially as I lived a very sheltered life at home through graduation from college in the spring of 1942 and thereafter during graduate school. I began graduate study in the Department of Biochemistry, College of Physicians and Surgeons, Columbia University, in the summer of 1942 but soon thereafter was given a five-year leave of absence for military service. In October 1942, I was inducted into the U.S. Army—the first extended time away from home and where maturity began almost overnight and continued all through military service.

Throughout my education my father exerted the greatest influence on my studies—my mother was essentially silent in this regard. She was a typical Italian wife and mother whose major responsibility was the home, all else was left to my father. Despite little formal schooling, my father continued his self-education throughout his lifetime and became a designer and builder of intricate machinery to provide a beveled edge on glass of any shape. He built the wooden molds, had them cast at the metal foundry, machined the castings, and assembled the machine. During my early years, my father taught me three very important things:

(1) *Self-Reliance*—to have always confidence in my ability;
(2) *Work/Study Ethic*—with hard work and study I could accomplish any goal I set for myself; and
(3) *Common Sense*—good down-to-earth common sense is important and should never be overlooked.

On a practical level, my father taught me most, if not all, of the basic craft and manual skills. He would never make something for me but rather would teach me the proper use of the tools necessary to make it. It was a team effort—he the teacher and I the worker. But he was always there to be sure that I was using the tools correctly and that there was never any danger to me. I still have some of his tools—especially steel tempered pliers—which to this day serve to remind me of how much he did for me and I hope that I have done as much for our three children, James Robert, Laura Ellen, and Judith Alane. My greatest joy was his knowing that I was made a Distinguished Professor—he died one month later in May 1975 at age 82. I wish he could have been alive when I was elected to the National Academy of Sciences in 1983. Following his death, my mother remained in Bloomington, Indiana, where my wife and I attended to her well being until her death in February 1991 at age 94.

Military service

I did my basic training at Camp (now Fort) Campbell in Kentucky with the small tank 92nd Reconnaissance Battalion of the 12th Armored Division. Following basic training, some 15% of the Division was allowed furloughs; I was one of the lucky ones as about half of the Division was reassigned as replacements to the 1st Armored Division in North Africa. Soon after my return from furlough, the Battalion Commander selected me and one other person—each of us having completed undergraduate schooling—to take the examination for the Army Specialized Training Program (ASTP) which we both passed. I selected the Biochemistry program offered at Ohio State University since my college majors were Biology and Chemistry. This program was soon eliminated and I was transferred to the Engineering Program at Pennsylvania State College (now University) where I chose to study Electrical Engineering rather than Civil or Mechanical Engineering. It was a *serendipitous choice* as both Civil and Mechanical Engineering were soon eliminated and only those of us in Electrical Engineering were allowed to complete the program. I began study on September 13, 1943 and continued through December 2, 1944. During this period, I learned the mathematical skills that were so important in my later graduate studies. I am, and always will be, deeply indebted to Professors Fred and Helen Owens who provided both excellent mathematical training and kind and warm hospitality; their home was open always to us students in the military and was our home away from home. Shortly after our marriage in 1953 my wife and I visited with them—a visit I cherish to this day; unfortunately, both passed away shortly thereafter.

Upon completion of the ASTP program, I spent the remainder of my military service at the Manhattan Project, Los Alamos, New Mexico, as a member of the Biochemistry Group under the direction of Wright Langham, a civilian employee. During this period, our group investigated the effect of citrate ion concentration, valence state, and various other factors on the body distribution and excretion of plutonium following its intravenous administration. During my 14-month stay at Los Alamos (1944–1946), I was most fortunate to take courses in Organic Chemistry (David Lipkin), Physical Chemistry (Irwin B. Johns), and Radiochemistry (Joseph Kennedy). At the conclusion of the latter course, I was honored to be asked by Dr. Joseph Kennedy, then Head of Chemistry and Metallurgy, if I would consider doing graduate study with him. Upon my discharge from the Army I did visit with him at Washington University in St. Louis but declined his kind offer and returned to graduate study at Columbia. Further, I was allowed to attend seminars given by the scientific staff—the most memorable by Enrico Fermi dressed in blue jeans, a blue denim shirt, and a leather jacket.

In retrospect, my military service was overseen by an unknown "*Guardian Angel,*" perhaps reasonably so given saintly first and family names!

Graduate study—Columbia University

I was discharged honorably from the Army in February 1946 but did not return to graduate school until the following summer. The first two years of graduate study (1946-1948) were spent in the Chemistry Department where my fellow graduate students and I amassed about 70 graduate credit hours. Hans T. Clarke, then Chairman of Biochemistry, had previously been Chief Research Chemist at Eastman Kodak and insisted that graduate students in Biochemistry should have a broad knowledge of Chemistry. In addition, we were expected to learn the art of glass blowing, an ability I have unfortunately long since lost.

As I recall this period, the course entitled "Natural Products Laboratory" is particularly memorable for its content but more so because the teaching assistant was Isaac Asimov, then a chemistry graduate student. As known to one and all, Isaac Asimov was later to become the foremost writer of Science Fiction and a most imaginative interpreter of scientific subjects.

In addition to graduate study I served as a Teaching Assistant in an evening section of "Organic Chemistry Laboratory" during the period February 1947 through June 1948. Travel time between my parent's home and the University was one and one-half hours using two subways and a bus. The Laboratory was scheduled twice weekly; on these days I would arrive home at midnight and had to return for class early the following morning.

The academic year 1948–1949 was a particularly intense period as I began a three-year graduate student residence in the Biochemistry Department and, in addition, accepted the appointment of Instructor in Chemistry at Hofstra College, Hempstead, Long Island, to provide for financial self-sufficiency. The GI Bill provided for the early years of graduate study; now at age 26, I no longer wished to impose financially on my parents. Thus, on Tuesdays and Thursdays throughout the year, I taught the lecture sections of Inorganic, Organic, and Physical Chemistry at Hofstra College. This teaching experience was an excellent preparation for the qualifying examinations to be taken at midyear in the Biochemistry Department.

During the fall semester of 1948, I attended the Medical School Biochemistry course lectures on the three alternate weekdays of Monday, Wednesday, and Friday. Even with our graduate chemistry background, Hans T. Clarke only reluctantly absolved us from the laboratory section provided we agreed to take the weekly 10 min laboratory quizzes. We happily agreed! At the beginning of the spring semester of 1949, I took the qualifying examinations for admission to candidacy for the Doctoral Degree—a grueling two consecutive days of morning and afternoon three-hour written examinations covering Biochemistry, Inorganic, Organic, and Physical Chemistry. Having passed the qualifying examinations, and soon thereafter the language examinations in French and German, I was formally accepted for graduate study in the laboratory of David Rittenberg where I spent the next two and one-half years. I was fortunate to receive a Life Insurance Medical Research Pre-Doctoral Fellowship for the two-year period of 1949–1951 which allowed me to devote full time to graduate study.

David Rittenberg proposed a graduate program whereby I would gain research experience by investigating a variety of problems and thereafter, at the appropriate time, select a problem for the graduate thesis. Thus, three projects were undertaken prior to beginning my thesis research. They were: (1) Isolation of the enzyme hydrogenase from *Proteus vulgaris*; (2) Uptake of ^{15}N-labeled amino acids by rat liver slices; and (3) Synthesis of Aspartic Acid-2,3^{14}C–^{15}N and its conversion to Uric Acid in the pigeon. The results of the last project appeared in my first solely authored publication (San Pietro 1952).

A study of the "Rate of Protein Synthesis in Humans" was the project chosen for the graduate thesis (San Pietro and Rittenberg 1953a, b). Two aspects of the thesis research are noteworthy. First and foremost, the experimental subjects were David Rittenberg and I. We received singly by intravenous injection one of three ^{15}N-labeled compounds, namely, Urea, Glycine, or Aspartic Acid. To determine the size of the Urea Pool and the Urea Space, we received

intravenously ^{15}N-Urea in the brachial vein in one arm, and blood samples were removed from the brachial vein in the other arm at 10-min intervals for a period of one hour. To determine the rate of Protein Synthesis and the size of the Metabolic Pool—the appearance of isotope in the urine was measured over a period of two days following intravenous administration of either ^{15}N-labeled Glycine or Aspartic Acid. Secondly, mathematical skill was a necessity to solve the equations describing the kinetic interrelationships between the amino acids and protein metabolism proposed in the theoretical model. The thesis was 44 pages in total length of which some 14 pages were devoted to theoretical considerations, solution of mathematical expressions, and sample calculations. Clearly, the mathematics learned during the military-supported ASTP program was of utmost importance. Unfortunately, this is another ability which has diminished with the passage of time.

The thesis defense was presented in a conference room in the Lowe library at the 116th Street campus of Columbia University. David Rittenberg and I rode the subway from the Biochemistry Department at 168th street to the Lowe library. During the subway ride, he mentioned that the questions he planned to ask were written on the card in the breast pocket of his jacket. I believe that my eyesight focused on his jacket breast pocket from that moment until our arrival at the Lowe library. Fortunately, the questions proved answerable, the thesis defense was judged favorably by the committee and the Doctoral degree was awarded in May 1951.

During graduate study I learned what the correct way to read scientific literature is.

> *Lesson 1:* Not everything that is written is necessarily correct. To read properly a scientific publication, you should evaluate the data presented and draw your own conclusions there from *prior* to reading the interpretations offered by the author. If your interpretation differs from that of the author, you should ask *"Why the difference?"* and then attempt to resolve the difference in interpretation.

Thereafter, I was appointed a Research Associate and spent one additional year with David Rittenberg during which time I attempted again the isolation of the hydrogenase enzyme from *Proteus vulgaris*. Unfortunately, biochemical research was limited as most of the year was devoted to fabrication of a glass vacuum system, and establishing the enzyme assay method based on the difference in thermal conductivities of the *ortho* and *para* forms of hydrogen. Following my departure in the summer of 1952, the project was continued by Alvin Krasna, then a graduate student and later an Emeritus Professor in the Department of Biochemistry.

I was privileged to be in residence at Columbia during the exciting and notable years of seminal research by the faculty in Biochemistry. In particular, I note the contributions of David Shemin and Erwin Chargaff. The pathway of porphyrin biosynthesis in animal tissues was first revealed by the isotope labeling and enzyme experiments by David Shemin and his colleagues. This pathway is especially important because of the central role of the porphyrin nucleus in the cytochromes, in hemoglobin, and in chlorophyll. The base compositions of DNAs from a variety of species were determined by Erwin Chargaff and his colleagues. They found that the ratios of adenine to thymine and of guanine to cytosine were nearly one in all species studied. The significance of the base pairing specificity became apparent in the DNA model proposed by James Watson and Francis Crick (1953).

Johns Hopkins

During my last year at Columbia, I was awarded an NRC (National Research Council) Post-Doctoral Fellowship to pursue research with Professor William C. Rose, a noted nutritionist and biochemist, at the University of Illinois. *Serendipity Enters Once Again!* Although I was only an *adopted* member of David Shemin's group, he concerned himself with my future scientific development—a concern he very kindly and unselfishly assumed until his death in 1991. He proposed that I decline the NRC Fellowship and that I learn modern biochemistry in the laboratory of Sidney P. Colowick and Nathan O. Kaplan at the recently established McCollum-Pratt Institute, Johns Hopkins University, Baltimore. This was a momentous decision that affected positively my personal life and redirected my research endeavors. He phoned William D. McElroy, Director of the Institute, and arranged for me to visit and interview for a Post-Doctoral position. Sidney P. Colowick and Nathan O. Kaplan accepted me into their laboratory and I was requested to apply for an external Post-Doctoral Fellowship; if unsuccessful, I would receive a McCollum-Pratt Fellowship. Fortunately, I received a two year Fellowship from the National (Polio) Foundation beginning on July 1, 1952.

Further, I was allowed to spend the summer of 1952 away from Johns Hopkins and was privileged to be accepted into the Microbiology course offered by Professor Cornelis B. Van Niel at the Hopkins Marine Station of Stanford University, Pacific Grove, California. This was my first exposure to microbiology and the information gained would prove useful in my study of bacterial transhydrogenase.

Pyridine nucleotide biochemistry

I began my residence at Johns Hopkins in September 1952 and undertook collaborative research with Sid Colowick and Nate Kaplan on the mechanism and stereo specificity of the reaction catalyzed by the transhydrogenase enzyme

from *Pseudomonas fluorescens*. This choice of the problem was excellent. I learned a great deal of protein chemistry and enzymology from them, and I brought to this research, knowledge of the application and mass spectrometric measurement of stable isotopes.

Using deuterium as the tracer, we demonstrated that the transhydrogenase reaction involved direct hydrogen transfer rather than electron transfer. In addition, the stereo specificity, exhibited by the enzyme, was shown to be opposite to that of the yeast alcohol dehydrogenase. The opposite stereo specificities exhibited by transhydrogenase, and yeast alcohol dehydrogenase, suggested that two stereoisomers of reduced deuteriopyridine nucleotides are possible, but only one of which is formed by enzyme-catalyzed direct hydrogen transfer (Fig. 1).

Although Sid Colowick and Nate Kaplan collaborated extensively at that time on problems of mutual interest, they each pursued individually other research problems with their students and Post-Doctoral Fellows. While my major project was collaborative with both of them, they each invited me to participate in their individual research programs. Our collaboration continued even after my appointment to the faculty in 1954 and for eight years thereafter.

Elucidation of the structure of the reduced pyridine nucleotide, NADH, was undertaken with Sid Colowick and his student, Maynard E. Pullman. It had earlier been proposed that one of the two ortho positions of the nicotinamide ring was the site of reduction. Again, using deuterium as the tracer, we demonstrated that neither ortho position is involved in the reduction of NAD and proposed that the para position of the nicotinamide ring is the actual site of reduction (Pullman et al. 1954; Fig. 1). The experimental technique used did not rule out unequivocally either ortho position of the nicotinamide moiety (adjacent to the ring Nitrogen) as the site of reduction. However, independent evidence ruling out the meta positions was provided by other investigators (Birgit Vennesland, personal communication).

In light of the finding of Pullman et al. (1954), I undertook an investigation to elucidate the structure of the

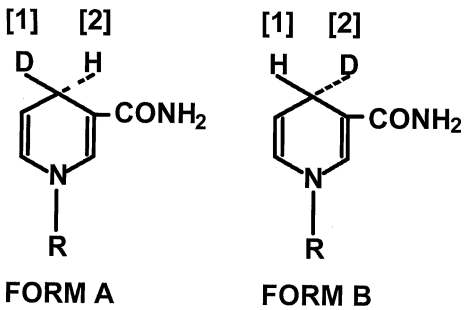

Fig. 1 Stereoisomers of reduced deuteriopyridine nucleotides (Pullman et al. 1954). The numbering in the ring starts at "N" (1); going counterclockwise, the next position is 2, 3 and 4 (at the very top), 5 and then 6. Ortho is for positions 2 and 6, and para is for position 4

Nicotinamide Adenine Dinucleotide Cyanide (NAD-CN) complex which had an absorption spectrum resembling that of NADH but shifted 15 nm toward the ultraviolet. It had earlier been shown that the ability to form reversibly a complex with cyanide is a general property of N^1-substituted nicotinamide compounds. Complex formation occurs at high (1M) cyanide concentration; complex dissociation at low (1mM) cyanide concentration results in the regeneration of NAD. Because of the instability of the complex, we made no attempt to isolate it.

It was possible, however, to elucidate indirectly the structure of the NAD-CN complex since the hydrogen on the carbon atom to which the cyanide group was attached would undergo ionization and exchange with deuterium in the medium. Thus, complex formation in the presence of D_2O yielded NAD labeled with deuterium at the site of cyanide addition. Using a procedure analogous to that of Pullman et al. (1954), the position of deuterium, and thereby the site of cyanide addition, was ascertained to be the para position of the nicotinamide moiety. This result was verified by demonstrating that the deuterium in the NAD was on the same carbon at which oxidation–reduction occurs (San Pietro 1955a).

In light of the basic nature of KCN, it was important to determine whether or not deuterium would be incorporated into NAD under solely alkaline conditions. Deuterium exchange with NAD did occur. The surprising finding, however, was that under mildly alkaline conditions deuterium is introduced into only one of the two ortho positions, namely carbon 2, rather than at the para position (carbon 4) as is the case with the NAD-CN complex. Under the mildly alkaline conditions used, only the hydrogen attached to carbon 2 of the nicotinamide moiety would be sufficiently activated—by virtue of being adjacent to the ring nitrogen and beta to the carboxamide group—to undergo ionization and exchange with the medium (San Pietro 1955b).

As an aside, I should note here that Ronald Breslow (1957) had a similar and striking discovery that the aromatic hydrogen at position 2 of the thiazolium ring, in thiamine, readily undergoes non-enzymatic ionization and exchange with solvent deuterium. Deuterium exchange at this position would be favored by virtue of its being adjacent to both the nitrogen and sulfur atoms of the thiazolium ring. This finding provided the first clue as to the active site in thiamine pyrophosphate which serves as a coenzyme in a variety of enzymatic reactions.

The collaboration with Nate Kaplan, and his students and Post-Doctoral Fellows, involved four different but interrelated studies. The first was concerned with the preparation of Pyridine Nucleotide Analogs by the Carbonyl Addition Reaction. The product of the addition reaction of NAD with carbonyl compounds is at the same oxidation level as NADH and exhibits properties similar to NADH. In this

study dihydroxy acetone, glyceraldehyde, and acetone addition products were prepared; isolated as the barium salt; and their properties elucidated (Burton et al. 1957).

A second study focused on the mechanism of Flavoprotein-Catalyzed Pyridine Nucleotide Transfer Reactions. Using deuterium as a tracer, the mechanism of reduction of acetyl pyridine NAD by NADH, catalyzed by pig heart diaphorase, was shown to involve electron transfer rather than direct hydrogen transfer. In addition, the enzyme catalyzed an exchange between the hydrogen of NADH or acetyl pyridine NADH and D_2O. Thirdly, a mechanism was proposed to explain the direct and stereospecific hydrogen transfer between the substrate and coenzyme catalyzed by the alcohol dehydrogenase type enzymatic reactions. The last study focused on the purification and properties of pig spleen pyridine transglycosidase. The purified enzyme exhibited both transglycosidase activity, when a suitable pyridinium compound is present, as well as hydrolase activity—in the absence of the pyridinium compound—with either NAD or NADP as substrate. The transglycosidase activity allowed for the synthesis of various analogs of the pyridine nucleotides.

I learned from Nate Kaplan a very important principle/lesson concerning proper experimental technique. In general, in their studies the experimental approach involved coupling the reaction under investigation with one or more established enzyme-catalyzed reactions. When told by a student or Post-Doc that the reaction did not work, Nate immediately asked "Did you check the activity of each of the coupling enzymes?" Often the answer was an embarrassing "No"!

> *Lesson 2*: Freezing does not necessarily assure retention of activity. Never assume that just because an enzyme preparation has been stored in the freezer that it will be active when thawed and used in a reaction.

In 1954, I joined the faculty of Johns Hopkins University as an Assistant Professor in Biology and the McCollum-Pratt Institute. In addition to initiating an independent research program and preparing lectures on nucleic acid biochemistry, I collaborated during 1954–56 with Robert Ballantine on building a mass spectrometer. The spectrometer tube was purchased commercially, and the associated electronics and glass gas handling system were fabricated in house (Fig. 2).

Photosynthetic pyridine nucleotide reduction

As an extension of my interest in hydrogen transfer reactions in biological systems, I began an investigation on the reduction of pyridine nucleotides by illuminated grana (thylakoids) because of their important and well-known physiological function as coenzymes in numerous enzyme-catalyzed reactions. This photobiological activity was previously demonstrated (Arnon 1951, Tolmach 1951, and Vishniac and Ochoa 1951)—but only indirectly—by coupling the light reaction with a suitable dehydrogenase and assaying the product thereof. In light of this information, my initial approach, also indirect, was to measure the turnover of the coenzyme, i.e., photoreduction followed by dark oxidation, assayed as deuterium incorporation from the medium. In this manner, deuterium would be incorporated into the oxidized form of the coenzyme provided the stereospecificities of the reduction and oxidation processes were not identical. *An unexpected, but very important, finding ensued.* As expected, the oxidized form of the coenzyme did contain deuterium indicative of turnover. The unexpected result was the finding that, at high grana (chlorophyll) concentration, there was a small accumulation of the reduced form of the coenzyme. I remember vividly to this day the excitement Helga Lang and I felt when we noted a small—but meaningful—decrease in absorbance at 340 nm upon the addition of alcohol dehydrogenase and acetaldehyde to the reaction mixture.

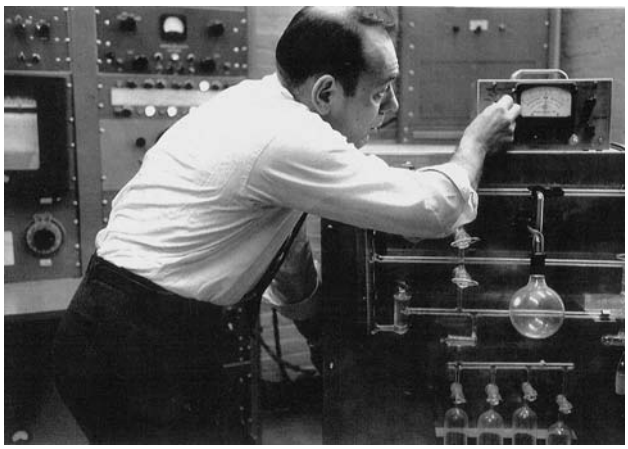

Fig. 2 Tony San Pietro assaying a sample on the Johns Hopkins mass spectrometer; photograph, ~1956

Inasmuch as the accumulation of the reduced coenzyme was detected only at high—but not at low grana concentration—our interpretation was that the grana provided not solely the photolytic system but, in addition, one or more unknown factors required for the reduction of the coenzyme. Vishniac and Ochoa (1951) had earlier invoked a similar interpretation to explain the low rate of pyridine nucleotide reduction observed in their experiments. *Serendipity Enters Once Again!* Fortunately for us, they did not attempt to identify the unknown factor(s). Our interpretation and theirs were validated by the demonstration of pyridine nucleotide reduction at low grana concentration, provided the reaction mixture was supplemented with a soluble extract of chloroplasts (San Pietro and Lang[1] 1956, 1958, Arnon et al. 1957).

[1] Our 1958 paper was designated a Classic Paper in Biochemistry—a series of papers reprinted to celebrate the centenary of the Journal of Biochemistry (JBC Vol. 280, No. 51, December 23, p. e48, 2005). An unexpected honor.

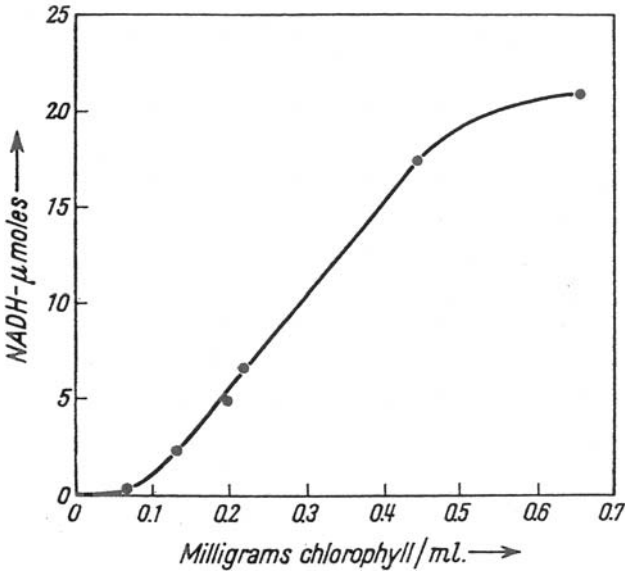

Fig. 3 Relationship between Chlorophyll concentration and accumulation of NADH. (San Pietro and Lang 1956)

This finding allowed for the direct and rapid measurement of the initial rate of the reduction of the pyridine nucleotides since the reduction could be assayed spectrophotometrically by the increase in absorbance at 340 nm of the reaction mixture without the prior removal of the grana. The availability of a rapid assay procedure and the easy accessibility of large amounts of starting material provided for a biochemical approach to the elucidation of the nature and function of the unknown factor(s) as well as the mechanism of the photobiological coenzyme reduction process (Fig. 3).

The reduction of NADP[2] by illuminated chloroplasts requires both ferredoxin and ferredoxin–NADP oxidoreductase. Independent investigations leading to the isolation and characterization of these two proteins have extended over a number of years and in a number of different laboratories. A detailed account of these investigations is available elsewhere (Hill and San Pietro 1963).

The first demonstration that a soluble factor can be added back to chloroplasts to reconstitute their overall electron transport reaction must be attributed to Robert (but known as Robin) Hill and his colleagues (see Bendall 1994 for its description). They found that untreated chloroplast suspensions from many plants were capable of reducing muscle methemoglobin in light. When the chloroplast suspension was diluted, the activity fell off in a way indicative of the presence of a soluble factor. The washed chloroplasts were incapable of reducing methemoglobin in light, but addition of the soluble fraction of the leaf juice restored the activity. The soluble methemoglobin reduction factor (MRF) was thermolabile.

We were unaware of their findings when we reported on the restoration of pyridine nucleotide reduction under the conditions of low grana concentration provided that a soluble extract (protein) from chloroplasts was added (San Pietro and Lang 1958). The soluble protein was purified about 35-fold and its role in the reduction of pyridine nucleotides by illuminated chloroplasts investigated. At that time, it was assumed that the activity exhibited by the protein was enzymatic in nature and the functional name Photosynthetic Pyridine Nucleotide Reductase (PPNR) was proposed. As noted below, this assumption was invalidated later when the redox property of the protein was elucidated.

In this same time era, Daniel Arnon and his collaborators reported the presence of a TPN[3]-reducing factor in an extract of chloroplasts and noted that it was not required for the reduction of ferricyanide (see Arnon et al. 1957). They had earlier shown that the addition of an aqueous extract of whole chloroplasts to washed green particles restored to the particles the capacity for photosynthetic phosphorylation. We also noted a similar effect of PPNR on photosynthetic phosphorylation. Thus, these two factors appeared to be identical.

The seeming identity of all three factors noted above was confirmed when MRF was shown to catalyze the reduction of NADP by illuminated chloroplasts. At the suggestion of H. E. Davenport, the ability of PPNR to catalyze the reduction of Cytochrome c by illuminated chloroplasts was examined and it was found to catalyze this reaction (Lazzarini and San Pietro 1962).

By 1962, it was known that these four proteins exhibited similar activities, had similar absorption spectra in the visible region, contained non-heme iron, had redox potentials close to the hydrogen electrode at neutrality, and were devoid of flavin. In that same year, Fry and San Pietro (1962) reported the presence in PPNR of labile sulfide, equimolar with the non-heme iron, which is liberated as hydrogen sulfide upon acidification. This finding was confirmed subsequently for the other proteins by several other investigators. As noted above, the four proteins were known by various functional names for a number of years until they were collectively christened *Ferredoxin* by Tagawa and Arnon (1962).

My personal recollection of the genesis of the descriptive name *Ferredoxin* is worthy of note. Following the discovery and isolation of the protein from *Clostridium pasteurianum*, there was a contest at DuPont to provide a name for this new bacterial redox factor. The winner of the

[2] The use of NAD rather than NADP in our experiments was dictated by price. My recollection is that the cost of NADP was about $1000 per gram; in contrast, the cost of NAD was only about $50 per gram.

[3] NAD, DPN, and Coenzyme I are one and the same as are NADP, TPN, and Coenzyme II.

contest was David Wharton and the basis for his derivation of *Ferredoxin* was:

fer	redox	in
iron-containing	oxidation-reduction	protein

Thereafter, ferredoxin became the generic name for this new group of non-heme iron, and labile sulfide-containing, low potential redox cofactors.

Although the ferredoxin terminology was universally accepted, most investigators were unaware of the Wharton derivation. Piqued by curiosity, Harold Davenport, in typical scholarly fashion, sought help from the dictionary and mentioned to me personally in 1965, clearly with tongue in cheek, an alternative derivation; namely,

ferre	doxin(doxy)
iron	woman of low morals

A bit of levity is always most welcome, even in science.

As noted earlier, the reduction of NADP by illuminated chloroplasts requires both ferredoxin and a flavoprotein known presently as ferredoxin–NADP oxidoreductase. Here also, as with ferredoxin, the flavoprotein paraded under a variety of names for a period of years until its role in photosynthetic electron transport was elucidated by Tagawa and Arnon (1962).

The flavoprotein was isolated and purified initially from spinach chloroplasts by Avron and Jagendorf (1956) as an NADPH specific Diaphorase. Several years later during the purification of PPNR, an FAD-containing flavoprotein was separated and partially purified by Keister et al. (1960, 1962) as a Pyridine Nucleotide Transhydrogenase.

The nomenclature confusion was clarified in 1962 when Tagawa and Arnon presented definitive evidence that the chloroplastic flavoprotein functions to link ferredoxin to NADP reduction and proposed the name ferredoxin–TPN reductase (known presently as ferredoxin–NADP oxidoreductase). A year later, the crystallization of the chloroplast flavoprotein was reported and that it exhibited the properties reported earlier for the NADP specific Diaphorase, discovered by Avron and Jagendorf (1956) as well as for the Pyridine Nucleotide Transhydrogenase, purified by Donald Keister in my laboratory.

In **Appendix 1**, I have provided some personal and general comments on 'A role of non-heme iron proteins in energy conversion.'

Although not discussed further here, the discovery, in 1954, of photophosphorylation, independently in the laboratories of Daniel Arnon (in chloroplasts) and Albert Frenkel (in bacterial 'chromatophores'), served also to stimulate markedly the biochemical study of photosynthesis (Arnon et al. 1954a, b; Frenkel 1954). For a number of years thereafter, seemingly contradictory reports appeared concerning the effect of a phosphate acceptor system on the rate of NADP photoreduction by illuminated chloroplasts, the ATP/2e ratio, and cyclic versus non-cyclic photophosphorylation.

> *Lesson 3:* The results of an experiment are valid but only for the experimental conditions specified. Failure to confirm these results does not mean that the earlier results were incorrect but rather that the original experimental conditions were neither faithfully nor accurately duplicated.

Remembrances of Johns Hopkins

The decade at the McCollum-Pratt Institute, Johns Hopkins University, was most memorable both scientifically and personally for a variety of reasons. The scientific environment included a daily journal Club, a monthly dinner/lecture meeting of the Enzyme Club with colleagues at the National Institutes of Health, the annual McCollum-Pratt Symposium, seminars by invited speakers, many Post-Doctoral Fellows from within the country, as well as from abroad, and numerous graduate students. Even the hours spent shooting pool in the Faculty Club at lunch time were interspersed with discussions of ongoing research.

Beyond the science, the family oriented, caring relationship, shared equally with all members of the Institute, was most important. We were thoughtful of each other, shared a commonality of purpose, shared each other's joys and sorrows, argued scientifically among ourselves but were totally united against outside intrusions. We respected each other both as scientists and individuals. Friendships established then continued throughout our lives.

Several specific recollections are worthy of note. Sid Colowick was one of the most brilliant scientists with whom it was my good fortune to be associated with. While mostly advantageous, his brilliance was on occasion a disadvantage as his breath of knowledge sometimes precluded pursuing an investigation. Another lesson learned (quoted from Racker (1976)).

> *Lesson 4:* "Progress is made by young scientists who carry out experiments older scientists said wouldn't work. (F. Westheimer)"

While equally brilliant, Nate Kaplan's overwhelmingly and enthusiastic infectious approach to research is my unique remembrance of him. I recall performing enzyme activity assays using the Beckman DU Spectrophotometer. If the enzyme exhibited activity the meter needle would move from the null position and the reading taken afterwards. I can still see Nate peeking over my shoulder and saying "It's going, it's going!" Occasionally the enzyme preparation was inactive and, although the needle remained

stationary, Nate had seemingly willed the needle to appear to move by tilting his head away from the vertical. His enthusiasm was a sight to behold!

I began a long term collaboration and friendship with Howard Gest when he came to Johns Hopkins with his student, Harry D. Peck, to study the mechanism of hydrogenase action using the isotope exchange reaction between water and molecular hydrogen (Peck et al. 1956). We collaborated again at the Kettering Laboratory when we co-organized and co-edited with Leo P. Vernon a symposium on Bacterial Photosynthesis in 1963. As noted below, Howard Gest was instrumental in my departure from the Kettering Laboratory and my acceptance of a position at Indiana University in 1968.

Professor Britton Chance kindly invited me to come to his laboratory at the Johnson Research Foundation, University of Pennsylvania, to study the kinetics of PPNR reduction in the light and reoxidation in the dark with or without the addition of TPN (Chance and San Pietro 1963). In the absence of TPN, the initial rate of reduction of PPNR was approximately twice the rate of CO_2 reduction under similar conditions. As expected, the presence of TPN markedly reduced the kinetics of PPNR reduction as well as the dark reoxidation rate. Beyond the science, I am indebted to Professor Chance for his gracious hospitality as an overnight guest in his home during my two visits to his laboratory.

During the Johns Hopkins period, I acknowledge with sincere thanks the significant contributions of Ettore Appella, Keelin Fry, John Giovanelli, Donald Keister, and Robert Lazzarini as well as the technical expertise provided by Ms. Helga Lang and Mr. Francis Stolzenbach.

Kettering Research Laboratory

In 1961, Leo Vernon assumed the Directorship of the Charles F. Kettering Research Laboratory and soon thereafter invited me to join the laboratory as a Senior Investigator (see Vernon 2005 for a history of the Kettering Laboratory). When I discussed this offer with Bill McElroy he—with my best interest in mind—offered the following advice. At the Kettering Laboratory I would be intimately involved in the creation of a first-rate laboratory in Photosynthesis and Nitrogen Fixation Research. If I chose to remain at Hopkins, he predicted my eventual promotion to Full Professor but noted the absence of a building opportunity which comes along infrequently. After much serious consideration, I accepted Leo's offer and moved to the Kettering Research Laboratory in Yellow Springs, OH, on July 1, 1962. I was most fortunate to have three colleagues (Keelin T. Fry, Donald Keister, and Robert Lazzarini) accompany me and to have an opportunity to teach annually a one semester Biochemistry course in the Chemistry Department of the nearby Antioch College.

During the early years of my six-year tenure, my decision to leave Johns Hopkins and to move to the Kettering Laboratory seemed well founded and correct. The Kettering research staff was excellent and included Bill Bulen, Rod Clayton, Bacon Ke, Berger Mayne, Gil Seely, and Leo Vernon as colleagues (see Vernon 2005). The more than adequate financial support—provided in part by the Kettering Foundation and supplemented by Federal grants—allowed for numerous Post-Doctoral appointments and for invitations to established and eminent scientists. The research facilities and technical support were first rate and provided an environment conducive for creative and significant research. The appointment of a Scientific Advisory Committee with a great breadth of scientific expertise supplemented well the two areas of research—Photosynthesis and Nitrogen Fixation.

I was very fortunate to collaborate with a large number of scientists during my six year tenure in the Kettering Laboratory. During this period my colleagues and I published about 40 articles in scientific and refereed journals. It is my sincere pleasure to acknowledge the excellent and significant contributions of Clanton C. Black, Peter Böger, Richard A. Dilley, Peter Ellyard, William. Evans, Keelin T. Fry, Howard Gest, Elizabeth Gross, Robin Hill, Takekazu Horio, Sakae Katoh, Robert A. Lazzarini, Noun Shavit, Masateru Shin, Atusi Takamiya, Anders Thore, and Leo P. Vernon.

I was the Organizer and Editor of one Symposium entitled Non-Heme Iron Proteins: Role in Energy Conversion, convened in 1965. Additionally, I was the Co-Organizer and Co-Editor for two other Symposia—one with Howard Gest and Leo P. Vernon, and a second with M. Lamborg and F. T. Kenney. All three were convened at the Kettering Laboratory. The idea of convening scientific symposia was a carry over from my time at Johns Hopkins where Symposia focused on a timely area of research were convened and published annually. [Several selected titles[4] are: A Symposium on Light and Life, The Chemical Basis of Heredity, Phosphorous Metabolism I and II, Inorganic Nitrogen Metabolism, and Mechanism of Enzyme Action. These Symposia contributed greatly to the reputation of The McCollum Pratt Institute, Johns Hopkins University, as a premier Biochemistry Department. It was envisaged that the Kettering Symposia would serve likewise.]

I recall here only a select few of the highlights—as well as the truly tragic occurrence—experienced during my tenure at the Kettering Laboratory. A most memorable highlight was the visit of Robin Hill who kindly invited me to co-author a paper with him (Hill and San Pietro 1963).

[4] For a list of titles of other conferences, see Govindjee (2005).

Fig. 4 Robin Hill visiting my parents in their home; photograph, ~1963

During his visit he met my parents and interacted very well with my father despite the great disparity in their educational backgrounds. I enjoy still today looking at the picture of Robin Hill visiting with my parents in their home in Yellow Springs, Ohio (Fig. 4). My family and I enjoyed a return visit with Robin and Priscilla Hill in Cambridge and were kindly invited to stay overnight in their 300-year-old home. My young son, James, enjoyed especially being invited into Robin's study tucked away in the attic under the eaves.

Following my initial visit to Japan in 1967, I made additional 13 visits to Japan prior to my retirement in 1988. During this time I established relations with a number of Japanese scientists some of whom came to do research in my laboratory either in Yellow Springs or in Bloomington, Indiana. I recall below briefly the visits of Atusi Takamiya and Sakae Katoh. I did not realize how large the number of different beers available in Southwestern Ohio was until

Fig. 5 Hiroshi Tamiya, Tony San Pietro, and Atusi Takamiya. Photograph was taken, in 1969, when San Pietro was returning home via Tokyo from a one month US National Science Foundation sponsored lecture tour in India

Fig. 6 Akira Mitsui, Masateru Shin, Shigehiro Morita, Atusi Takamiya, Tony San Pietro, and Sakae Katoh; photograph, taken in 1969

Professor Atusi Takamiya's visit (Fig. 5). He adorned totally the shelf along the wall at the back of the laboratory bench with at least one empty bottle of each of the beers he had sampled.

The arrival of Sakae Katoh and his wife, Masako, was the second highlight of my tenure at the Kettering Laboratory (Fig. 6). Sakae was a dedicated scientist, an excellent experimentalist and very knowledgeable about the research he pursued at the Kettering Laboratory. My older daughter, Laura, received a wonderful surprise when Masako gave her one of the kimonos she wore before her marriage but could no longer wear now that she was married. I am honored to have had a long standing friendship with Sakae and Masako. An honor I will cherish for the remainder of my life.

Prior to coming to the Kettering Laboratory, my family and I spent several summers on Long Island at the Brookhaven National Laboratory. During one of our Sunday excursions, we came across an antique cradle which my wife purchased even though our children were beyond the cradle age. However, Clanton and Betty Black made excellent use of the cradle with each of their children—initially in Yellow Springs and later in Athens, GA.

Two tragic events occurred during my tenure at the Kettering Laboratory—the assassination of President John F. Kennedy and the untimely demise of Photosynthesis Research. The announcement of President Kennedy's assassination came over the loud speaker at the Laboratory as I was walking to my office on the third floor. I felt as though I had been punched in the solar plexus and just leaned back against the wall where I stayed for an undetermined amount of time.

Even to this day it is troubling to recall the demise of Photosynthesis Research at the Kettering Laboratory. As the newly appointed Director of the Kettering Laboratory, Leo Vernon had brought together there a group of scientists

whose prior accomplishments in Photosynthesis Research were very well established. Almost overnight Leo had transformed successfully the Kettering Laboratory into a predominant force in Photosynthesis Research. In a very short time he had successfully discharged the challenge set forth by the Kettering Foundation Board at the time of his appointment.

During the six-year period, 1962–1968, Photosynthesis Research and Nitrogen Fixation were the two major areas of research at the Kettering Laboratory. Toward the end of this period, there was a dramatic change in the profile of the Kettering Board membership. The focus of the Kettering Foundation changed from academic research to mission-oriented research. Nitrogen Fixation remained since it was conceived as a potentially financially rewarding endeavor by the Kettering Foundation Board whereas Photosynthesis Research was not so conceived. Further, newly instituted endeavors in Education and Civic and Community Programs by the Kettering Foundation were funded at the expense of Photosynthesis Research but not the mission-oriented Nitrogen Fixation program. *The almighty dollar reared its ugly head*. Fortunately for me, I received in early 1968 an invitation from Howard Gest to present a seminar at Indiana University and to consider a possible position there. The visit went well, a position at Indiana University was offered and my family and I moved to Bloomington, IN, in the summer of 1968.

Just to give a glimpse of some of the work done at the Kettering Laboratory, I have included a few examples of research done there in **Appendix 2**.

Indiana University

On July 1, 1968 I succeeded Marcus M. Rhoades, an eminent maize geneticist, as Chairman of the Botany Department at Indiana University. The research expertise of the 13 faculty members was varied and diverse; e. g., Taxonomy, Morphology, Physiology, Algology (Phycology), and Biochemistry. Despite the diversity of interests, the faculty interacted very well both scientifically and socially. Soon thereafter, the faculty agreed to change the Departmental name from Botany to Plant Sciences. At that time, the Plant Sciences Department, together with the Microbiology and Zoology Departments, comprised the Division of Biological Sciences. The Division Chairman was the well-known biochemist Frank. W. Putman. I remained as Chairman of the Plant Sciences Department until 1977 at which time the Division was replaced by a Department of Biology with almost 45 members and a single Chairman.

As noted earlier one condition for going to the Kettering Laboratory was an opportunity to teach an undergraduate biochemistry course at Antioch College. I have always enjoyed teaching and the opportunity to return to a University Position was most gratifying. During my two-decade tenure at Indiana University I taught annually an undergraduate sophomore level biochemistry course. In the later years of my tenure some former students who had gone on to Medical School would tell me how much my undergraduate course helped them with the Medical School biochemistry course. Interestingly I saw some of these students at Art Fairs in Indianapolis where my wife, Alice, was displaying her watercolor paintings. So many nice remembrances!

In 1997, I underwent a quadruple bypass heart surgery and one of the doctors who treated me was a young lady who had taken my biochemistry course the last year I taught it. The tables were now turned and she was telling me what I had to do to recover successfully from the surgery. Another nice remembrance!

The willingness of many colleagues to come to do research in my laboratory at Indiana University was most rewarding both scientifically and socially. I learned a great deal from each and every one of these colleagues who have distinguished themselves as excellent researchers and teachers. Prior to my tenure at Indiana University all the scientists with whom I collaborated were at the Post-Doctoral level or beyond. I was most fortunate to have four graduate students—Dan Brune, John Golbeck, James Siedow, and Charles Yocum—do their research in my laboratory and receive their Doctoral Degrees from Indiana University. The remaining collaborators had received their training elsewhere and included Assunta Baccarini-Melandri, Peter Boger, Jerry Brand, Gerhardt Bookjans, Virginia Curtis (With Robert Togasaki), Dan Davis, Giorgio Forti, Howard Gest, Yorinao Inoue, Brigitte Klemme, Jobst Klemme, David Krogmann, Steve Lien, Charles McBride, Andrea Melandri, Akira Mitsui, Dov Pasternak, Hidehiro Sakurai, Noun Shavit, Elisha Tel-Or, Robert Togasaki, and Jon Weinstein. Although my research interest remained focused on the mechanism of conversion of photon energy into chemical free energy in plant and algal photosynthesis[5], a much broader spectrum of research was pursued at Indiana University. For example, photosynthetic research was expanded to include bacterial photosynthetic systems as well as consideration of biosaline based agriculture for countries bordering saline water and with high solar insolation but a paucity of fresh water. I had co-organized two additional international conferences on this topic—one in La Paz, Mexico, and a second in Karachi and Islamabad in Pakistan.

[5] For a Timeline of research in oxygenic photosynthesis, see Govindjee and Krogmann (2005).

Just to give a glimpse of some of the work done at Indiana University, I have included a few examples of research done there in **Appendix 3**.

My wife, Alice, was very pleased with my decision to leave the Kettering laboratory in Yellow Springs, OH, and move to Indiana University in Bloomington, IN. Although not trained in fine arts, she pursued a successful avocation as a watercolor artist. The availability of numerous art galleries in Bloomington, Indianapolis and Nashville, IN, the Bloomington Area Arts Council and the splendid Fine Arts Department at the University provided support for the move to Indiana University. In later years my wife tutored Rae Starker, the wife of the world renowned cellist Janos Starker, in watercolor painting techniques.

Several events are worthy of note here. In 1969, I became a Charter Member of the Johns Hopkins Society of Scholars. In 1975, I was honored to be given the title of Distinguished Professor of Plant Biochemistry. I was elected to the National Academy of Sciences in 1983. Lastly I was given an Honorary Doctor of Science Degree by Purdue University in 1992.

Emeritus status

I retired officially from Indiana University on June 30, 1992 at age 70. Thereafter I focused my attention on providing mentoring and tutoring support to needy students. On the Bloomington campus I participated in the Faculty and Staff for Student Excellence (FASE) mentoring program and was the mentor for one or two undergraduate students annually until 2003 when I retired from the program. I participated also in a similar—but more broadly based—program at the University's Indianapolis commuter campus under the leadership of Professor Herman J. Blake, Vice-Chancellor for Undergraduate Education. He was instrumental in creating an Alliance for Minority Participation in Science, Mathematics, and Engineering. The Alliance was a cooperative and interactive effort between five Colleges/Universities in Indiana (DePauw University, Earlham College, Indiana University—Purdue University at Indianapolis (IUPUI), Manchester College, and Wabash College) and five historically Black Colleges/Universities (Alcorn State University, Bennett College, Bethune-Cookman College, Oakwood College, and Shaw University). I was pleased to serve as a member of the Steering Committee for the Alliance and interacted with students and faculty by personal visits to the historically Black Colleges/Universities. Another program of note under the direction of Dr. Regina Turner in the Office of the Vice Chancellor for Undergraduate Education (IUPUI) was an outreach community program with several black churches where elementary students were tutored in mathematics on Saturday mornings. I participated on many Saturday mornings and focused on the translation of the written word into an algebraic equation which was the major difficulty for most of the young students.

I was a consultant to the Solar Energy Research Institute (later National Renewable Energy Laboratory, NREL) in Golden, CO, from 1978–1982 and was present when former President Jimmy Carter and Prime Minister Fukuda of Japan celebrated SUN day. In 1995, I returned as a consultant to the biological production of hydrogen program at NREL, supported by the US Department of Energy until 2006 when funding was drastically reduced. I began a consulting agreement with COOK Biotechnology, Inc., in West Lafayette, IN, in 1997, which continues to the present. The company prepares and markets a material entitled Small Intestine Submucosa (SIS) prepared from porcine small intestine which has been used in a multiplicity of surgical applications; e.g., several types of hernia repair, as a Dural substitute, as a Pubovaginal sling to reduce or eliminate urinary incontinence.

Finally, I audited the first two years of medical school classes provided by the Medical Sciences Program in Bloomington, Indiana. It was a most unique experience to attend classes where the other 25 students were each about 56–57 years younger than me. The medical information gained helped me to understand better the nature of the medical problems my wife and I suffered as well as providing a sound medical basis for my consultancy with Cook Biotechnology.

Concluding remark

It is often stated that "Behind every successful man is a woman." This is particularly true in my case. If I have achieved any measure of success, it is because I was blessed with a loving wife who was very supportive of my scientific endeavors. She unselfishly accepted many family responsibilities during the first 25 years of our marriage, while I pursued a career in science. It was only when our youngest child, Judith, was in college that she began to express artistically her long held interest in watercolor painting. Although her formal training was in X-ray technology at Johns Hopkins, she became a successful watercolor artist (Fig. 7). It was now my turn to be the supportive husband, who would help her at numerous Arts and Crafts Fairs and gallery exhibits in Indiana and surrounding states. I did so for the next 23 years or so until she was diagnosed with stomach cancer in early 2002. After 3 major surgeries and chemotherapy, she passed way on March 20, 2004 at age 73. I continue to live in the house we purchased in 1968 in Bloomington, IN, where her loving spirit is ever present.

Fig. 7 Floral watercolor by Alice San Pietro

Acknowledgment It is my sincere pleasure to recall the many fine contributions of the students and scientists identified herein with whom I have collaborated during my scientific career. I have learned from each and every one of them and hope they feel they have benefited from our collaboration. I also thank Clanton C. Black and Bob Buchanan for reading this perspective, and Govindjee for inviting and editing this manuscript.

Appendix 1

A role of non-heme iron proteins in energy conversion

The ubiquity of the non-heme iron, labile sulfide-containing proteins in biological systems, and their role in energy conversion processes was only beginning to be realized in the mid-1960s. The initial discovery of the bacterial and plant ferredoxins in the 1950s focused primarily on their role in electron transport. The bacterial and plant ferredoxins are relatively simple proteins of low molecular weight (<20,000) and have no other detectable cofactors. The discovery of more complex non-heme iron proteins with higher molecular weights (>20,000) and which contain cofactors, such as flavins, and Coenzyme Q, was coincident with their role in energy conserving processes. By the mid-1960s, non-heme iron proteins were shown to function in mitochondrial electron transport and nitrogen fixation as well as photosynthetic electron transport. Clearly, the importance of non-heme iron proteins was beginning to approach that of the heme proteins. I believe the current view is that they are equally important.

I was fortunate to convene a symposium in 1965 entitled "Non-Heme Iron Proteins: Role in Energy Conversion." Prior to the time of this symposium the problem of energy conversion was hardly even mentioned mainly because the focus earlier (1950s) had been on the simple bacterial and plant proteins. The more recent (early 1960s) research dealt with complex non-heme iron proteins such as aldehyde oxidase, which contains ubiquinone-Coenzyme Q, flavin, iron, labile sulfide, and molybdenum. This enzyme was proposed as a model for the electron transport chain in mitochondria because its susceptibility to inhibitors such as amytal, antimycin, and oligomycin is reminiscent of oxidative phosphorylation in mitochondria.

The late Professor Efraim (Ef) Racker, a pre-eminent biochemist, chaired the last session of the symposium and ended his Chairman's Remarks as follows: "This morning we shall hear from Dr. Butow that there is a non-heme iron linked electron transport system in mitochondria which appears to be closely linked to the process of oxidative phosphorylation. Indeed, it begins to look as if non-heme iron (NHI) may become almost as important to studies of energy conversions as NIH. The remarkable vision of the organizer of this symposium who selected the title before a direct relationship between non-heme iron proteins and energy conversion was established must be admired." As the sole organizer of that symposium, I was, and will always be, grateful to Ef Racker for his kind and gracious remark!

Appendix 2

Examples of some publications from the Kettering Research Laboratory (arranged chronologically) are:

Gest H, San Pietro A, Vernon LP (eds) (1963) Bacterial Photosynthesis. Antoioch Press, Yellow Springs, Ohio

Fry KT, Lazzarini RA, San Pietro A (1963) The photoreduction of iron in photosynthetic pyridine nucleotide reductase. Proc Natl Acad Sci USA 50:652–657

Black C C, San Pietro A, Norris G, Limbach D (1964) Photosynthetic phosphorylation in the presence of spinach phosphodoxin. Plant Physiol 39:279–283

San Pietro A (ed) (1965) Non-heme iron proteins: Role in energy conversion. Antioch Press, Yellow Springs, OH

San Pietro A, Black CC (1965) Enzymology of energy conversion in photosynthesis. Ann Rev Plant Physiol 16:155–174

Evans WR, San Pietro A (1966) Phosphorolysis of adenosine diphosphoribose. Arch Biochem Biophys 113:236–244

Katoh S, San Pietro A (1966) Activities of chloroplast fragments: I. Hill reaction and ascorbate-indophenol photoreduction. J Biol Chem 241:3575–3581

Shavit N, San Pietro A (1967) K^+-Dependent uncoupling of photophosphorylation by nigericin. Biochem Biophys Res Commun 28:277–283

Gross E, Shavit N, San Pietro A (1968) An inhibitor of energy transfer in chloroplasts. Arch Biochem Biophys 127:224–228

Shin M, San Pietro A (1968) Complex formation between ferredoxin and ferredoxin-NADP reductase. Biochem Biophys Res Commun 33:38–42

Appendix 3

Examples of some publications from Indiana University (arranged chronologically) are:

Yocum CF, San Pietro A (1969) Ferredoxin reducing substance from spinach Biochem Biophys Res Commun 36:614–620

Klemme B, Klemme JH, San Pietro A (1971) PPase, ATPase and photophosphorylation in chromatophores of *Rhodospirillum rubrum*: Inactivation by phospholipase and reconstitution by phospholipids. Arch Biochem Biophys 144: 339–342

Brand J, San Pietro A, Mayne BC (1972) Site of polylysine inhibition of photosystem I in spinach chloroplasts. Arch Biochem Biophys 152:426–428

Siedow JN, Curtis VA, San Pietro A (1973) Studies on Photosystem I: 1 Relationship of plastocyanin, *Cytochrome f* and P700. Arch Biochem Biophys 158:889–897

Siedow JN, Yocum, CF, San Pietro A (1973) The reducing side of photosystem I. In: Sanadi DR, Packer L (eds) Current Topics in Bioenergetics, pp 107–123, Academic Press, NY

Lien S, San Pietro A (1975) An inquiry into biophotolysis of water to produce hydrogen. pp 1–50, Publication Prepared for US NSF (National Science Foundation)

Golbeck JH, Lien S, San Pietro A (1976) Quantitation of labile sulfide content and P700 photochemistry in spinach photosystem I particles. Biochem Biophys Res Commun 71:452–458

Davis DJ, San Pietro A (1977) Chemical modification of ferredoxin: evidence for a complex between ferredoxin and ferredoxin-NADP oxidoreductase in NADP photoreduction. Biochem Biophys Res Commun 74:33–40

McBride, AC, Lien S, Togasaki RK, San Pietro A (1977) Mutational analysis of *Chlamydomonas reinhardi* in chloroplast membranes: Application to biological solar energy conversion. In: Mitsui A, Miyachi S, San Pietro A, Tamura S (eds) Biological Solar Energy Conversion, pp 77–86, Academic Press, New York

Shavit N, Lien S, San Pietro A (1977) On the role of membrane-bound ADP and ATP in photophosphorylation in chloroplast membranes. FEBS Letters 73:55–58

Bookjans G, San Pietro A, Boger P (1978) Resolution and reconstitution of spinach ferredoxin-NADP oxidoreductase. Biochim Biophys Acta 80:759–765.

Lien S, San Pietro A. (1981) Effect of uncouplers on anaerobic adaptation hydrogenase activity in *C. reinhardtii.* Biochem Biophys Res Commun 103:139–147

Sakurai H, San Pietro A (1985) Association of Fe-S center(s) with the large subunits of photosystem I particles. J Biochem (Japan) 98:69–76

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Arnon DI, Allen MB, Whaley FR (1954a) Photosynthesis by isolated chloroplasts. Nature 174:394–396

Arnon DI, Allen MB, Whatley FR (1954b) Photosynthesis by isolated chloroplasts. II Photophosphorylation, the conversion of light into phosphate bond energy. J Am Chem Soc 76:6324–6329

Arnon DI, Whatley FR, Allen MB (1957) Triphosphopyridine nucleotide as a catalyst of photosynthetic phosphorylation. Nature 180:182–185

Avron M, Jagendorf AT (1956) A TPNH diaphorase from chloroplasts. Arch Biochem Biophys 65:475–490

Bendall D (1994) Robert Hill. Biographical Memoirs of fellows of the Royal Society 40:141–171

Breslow R (1957) Rapid deuterium exchange in thiazolium salts. J Am Chem Soc 79:1762–1762

Burton RM, San Pietro A, Kaplan NO (1957) Preparation of pyridine nucleotide analogs by the carbonyl addition reaction. Arch Biochem Biophys 70:87–106

Chance B, San Pietro A (1963) On the light-induced bleaching of photosynthetic pyridine nucleotide reductase in the presence of chloroplasts. Proc Natl Acad Sci USA 49:633–638

Frenkel A (1954) Light-induced phosphorylation by cell free preparations of photosynthetic bacteria. J Am Chem Soc 76:5568–5569

Fry KT, San Pietro A (1962) Studies on photosynthetic pyridine nucleotide reductase. Biochem Biophys Res Commun 9:218–222

Govindjee (2005) A list of photosynthesis conferences and of edited books in photosynthesis. In: Govindjee, Beatty JT, Gest H, Allen

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Keister DL, San Pietro A, Stolzenbach FE (1962) Pyridine nucleotide transhydrogenase from spinach, II. Requirement of enzyme for photochemical accumulation of reduced pyridine nucleotides. Arch Biochem Biophys 98:235–244

Lazzarini RA, San Pietro A (1962) The reduction of Cytochrome *c* by photosynthetic pyridine nucleotide reductase and transhydrogenase. Biochem Biophys Acta 62:417–420

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Pullman ME, San Pietro A, Colowick SP (1954) On the structure of reduced diphospho-pyridine nucleotide. J Biol Chem 206:129–141

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San Pietro A (1955b) Base catalyzed deuterium exchange with diphosphopyridine nucleotide. J Biol Chem 217:589–593

San Pietro A, Lang HM (1956) Accumulation of reduced pyridine nucleotides by illuminated grana. Science 124:118–119

San Pietro A, Lang HM (1958) Photosynthetic pyridine nucleotide reductase. I. Partial purification and properties of the enzyme from spinach. J Biol Chem 231:211–229

San Pietro A, Rittenberg D (1953a) Study of the rate of protein synthesis in humans: I measurement of the urea pool and urea space. J Biol Chem 201:445–456

San Pietro A, Rittenberg D (1953b) Study of the rate of protein synthesis in humans; II Measurement of the metabolic pool and the rate of protein synthesis. J Biol Chem 201:457–473

Tagawa K, Arnon DI (1962) Ferredoxins as electron carriers in photosynthesis and in the biological production and consumption of hydrogen gas. Nature 195:537–543

Tolmach LJ (1951) Effect of triphosphopyridine nucleotide upon oxygen evolution and carbon dioxide fixation by illuminated chloroplasts. Nature 167:946–948

Vernon LP (2005) Photosynthesis and the Charles F. Kettering research laboratory. In: Govindjee, Beatty JT, Gest H, Allen JF (eds) Discoveries in photosynthesis. Springer, Dordrecht, pp 1133–1142

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Watson JD, Crick FHC (1953) A structure for deoxyribose nucleic acid. Nature 171:737–738