ILLINOIS YEARS—1975-1986

The beginning of the final period of my appointment at the University of Illinois had no particular marker other than that the "family" at 606 West Indiana Avenue consisted of Nell, Malu, and me. Ken, Marcia, Jim and David were on their own; they will have to tell their stories at some other time. Malu, a purebred Norwegian elkhound, had been obtained in 1971 and had been named by David. When she was mature enough, she had been bred to a champion male ("King") when we were in Snowmass Village. Eight puppies had been born ("A"—"H"), and she practically exhausted herself feeding them. Even when we gave them supplementary milk, Malu insisted on making a major contribution and would seek out each one of them to nurse, no matter whether we would make-believe hide one. Her motherly instinct was most impressive. Nell had taken Malu through several training programs, with the result that she was satisfactorily obedient and responded to conversational directions. She gave us affection, play and companionship as long as she was with us, and she enjoyed her three houses: Urbana, Manistee, and Snowmass Village. While the puppies were developing, they lived on our screened-in porch where they had a house, a litter box, and large play area. When I came home from the laboratory, I would arrive by the porch door, settle down in the stairwell, and say, "Time for loving." At that signal, they would swarm into my arms and roly-poly all over me. It was a joyous experience. When A-H, each with a different personality, had had their shots, registration as purebreds, and had reached about four months of age, Nell sold them to carefully selected buyers. With the proceeds, she bought an airline ticket to accompany me on a chemistry trip to Europe. That was her wish and decision.

A mature Malu, deep in thought, Snowmass

There were other trips derived from chemistry which we could enjoy

together now that the children were away from home. While the major events are covered in separate sections that appear later, several that occurred during the 1975-1986 period are worthy of mention at this stage in the writing. In 1975, I received the Edgar Fahs Smith Award of the Philadelphia Section of the American Chemical Society and the University of Pennsylvania and gave the required Memorial Lecture. Edgar Smith had been one of the founders of *Chemical Abstracts*. Privately, I believe that the award selection might have had something to do with three of my former students having been important members of that A.C.S. Section. In any case, the banquet was a highly agreeable social occasion, with all the wives in attendance. Nell had been treated to a tour of Philadelphia's historic sites and museums during the day, and she did not have to listen to my lecture that afternoon.

When I was inducted into foreign membership of the Polish Academy of Science in 1977, we did not have to journey to Poland, but only to Chicago, where a brief ceremony at the Polish Consulate accomplished the process. The staff members of the consulate appeared pleased when Nell and I accepted their invitation to a celebratory meal at Ambassador West, one of Chicago's famous restaurants. They seemed equally pleased when we accepted their further invitation to have a few drinks before the meal. We surmised that staff members of the Consulate were perhaps not always accorded the honor of participating in such banqueting. On this occasion the Consul had excused himself because of other commitments. I must say that we were all in a very happy and friendly mood as the meal progressed.

Those words also describe our feelings when Nell and I attended the Fifth Symposium on the Chemistry of Nucleic Acid Components that was held at Bechyně Castle in Czechoslovakia (Bohemia) in September of 1981. Looking through the correspondence with the late Jiri Beránek, who was the organizer of the symposium, I find that each of us was aware of the purpose of the symposium: to bring Czech students and internationally known professors together in a congenial and beautiful setting. Please recall that Czechoslovakia was still a Communist country, although it was not nearly as restrictive as it had been at the time of my first visit in 1962. I was being offered some special treatment because I had been one of the originators of that particular symposium series. Nell and I settled for a night of rest and a pre-symposium walking tour of Prague. Pertinent excerpts of my letter of March 31, 1981, to Jiri Beránek follow:

> *"Thank you, also, for explaining the philosophy of the meeting that encourages participation of as many young people as possible, which I strongly endorse. I will be happy to be a part of it, and please do not give me more time than you give the others whom you have invited.*

"I am pleased that you are willing to make my wife a participant in the meeting so that she can accompany me."

I also indicated that I would prepare a ready-for-print manuscript and would send in the symposium fee. Jiri's reply in the next month indicated that I had caught the intended spirit of the Symposium.

"Your generosity and understanding of our efforts and organizing work are very much appreciated."

We thoroughly enjoyed the Symposium at Bechyně Castle in all its aspects: the daily chemical discussions, seeing international friends and colleagues each day, and seeing new scenery that we had not imagined that we would ever see. There were bus tours to attractive locations in Bohemia. Nell was very happy to find that there were many wives in attendance, two-thirds of whom she knew from Australia, Switzerland, France, Germany, and the United States. We returned home via the Netherlands, where we could visit with all of the Dutch families.

We caught up with our Western U.S. families in June of 1982 after a trip across the U.S. and Canada in our old Buick via a route that treated us to the National Parks: Snowmass Village, CO - Denver - Craig, CO - Jackson Lake, WY - Colter Bay - Yellowstone (Old Faithful Inn) - Yellowstone (Mammoth Hot Springs Hotel) - Whitefish, Montana - Glacier-Waterton Lakes Park (Prince of Wales Hotel, Canada) - Banff, Alberta - Kamloops, British Columbia - Vancouver - Seattle. There, in Seattle, we visited David and Elena and James and Patty plus Corinne, who had been born when we were in Yellowstone. For the return, we dropped down to Oregon, traversed Idaho into Utah and Western Colorado, through Denver and then home via our well-practiced route. The car was still holding together after the 6000-mile journey. There were no chemistry lecture-stops on that tightly scheduled trip. The substitute was a greatly enhanced appreciation of Nature and the wonder of a third generation on the way.

In 1984, I began attending symposia in honor of chemists of my own generation. I was together with my good friends, the late Stan Tarbell of Vanderbilt University at a Symposium in Honor of Professor Norman H. Cromwell, University of Nebraska-Lincoln, and Stan wrote to me (May 25, 1984) after the Symposium:

"While I was listening to the presentation of your elegant work, I recalled how we heard Art Cope's talk at the Marvel Symposium in Tucson in 1961. Art was talking about his work on some nitrogen rearrangements; we looked at each other and said almost together,

'This is really beautiful,' and it was. I'm not sure that all of your audience at Lincoln quite appreciated what they were hearing, although Chris Michejda did."

From a usually reticent New Englander (Stan), this was high praise indeed. By way of explanation, Chris had been at the University of Rochester (Stan's former location) and the University of Illinois.

Josef (Gus) Fried

Also, in 1984, I attended and spoke at a Symposium Honoring Professor Josef Fried at the University of Chicago. Gus Fried, Elkan Blout, and I formed our long-lasting friendship triumvirate when we were graduate students at Columbia University. Bristol-Meyers Squibb and the University of Chicago launched in 1990 the first of a series of *annual* Josef Fried Symposia of Bioorganic Chemistry. Elkan Blout was also honored at a Symposium commemorating his 65th birthday at the Harvard Medical School in Boston, and a Symposium Tribute to Stanley Cristol of the University of Colorado, Boulder took place in 1985. These were all such good friends that I enjoyed participating in their symposia and adding a few personal comments to each of my invited lectures. The year 1985 was also the time of an unusual event, namely, my induction into the Mount Vernon High Schools Hall of Fame. Marcia and Tom very kindly chauffeured us when Nell and I presented ourselves on that occasion. The now-unified high schools are in the northeast corner of the city. When Morton Sultzer, my old friend and mentor, was on the Mount Vernon School Board, he convinced the other members and the city government that expansions at the old sites were impossible and that the Wartburg Estate was the only location on which to build the combined high schools, previously named A.B. Davis and Edison (the technical high school). The only deficit, i.e., bussing to the distant location, became a positive factor when bussing became a legally mandated requirement for all high schools. I thought of Morton Sultzer and of all the excellent teachers I had had, and of special friends from those years (1930-1933), during the induction ceremony, which was conducted in an appropriately friendly but formal manner. The original estate possessed a lake on which we had played a pick-up form of ice hockey when we were growing up.

Research

It takes time for some discoveries to be recognized or appreciated, especially if a scientist moves from one field of endeavor to another. However, any recognition or appreciation is always welcome. When Nell and I journeyed to Lyon, France, in 1984, where I was a participant in the conference on 'Role of Cyclic Nucleic Acid Adducts in Carcinogenesis/ Mutagenesis" at the International Agency for Research on Cancer, it was good to hear Helmut Bartsch say in his keynote address that "the real renaissance of cyclic nucleic base adducts began in 1972" with our work. Concerning the field of cytokinin discovery and research, one finds in the obituary of Folke K. Skoog (1908-2001) that appeared in the *Plant Molecular Biology Reporter* [**19**, 109-112 (2001)] the following statement:

> **"For more than 20 years, Skoog's group also collaborated with Nelson J. Leonard, a chemist at the University of Illinois, in synthesizing and testing hundreds of possible cytokinins and antagonists, and in establishing the principles governing their structure-activity relationships."**

The period of time mentioned actually included the years 1962-1984. Local recognition (monetary) occurred again in a new Illinois title for the period 1981-1986: Reynold C. Fuson Professor of Chemistry, Professor of Biochemistry, and Member, Center for Advanced Study, University of Illinois at Urbana-Champaign. Moreover, I was able to relax my teaching to half-time in my final semester before retirement by a "leave" for the other half into the Center. Coming late in my career, but nevertheless well appreciated, were Awards for Excellence in Teaching, 1980 and 1984, given by the School of Chemical Sciences, University of Illinois. I had finally found out—after 40 years—how to teach! Jim Leonard voiced the opinion that those two awards were the most important of the many good things that had come my way during long years of teaching and research.

How and when do novel research ideas occur to the scientist? In the case of this scientist, the sources of inspiration ran the gamut of possibilities. Spatial probes for adenine, adenosine, and adenosine phosphates had been developed from a 3-substituted adenine that was found in nature. Fluorescent probes for nucleic acid bases and their phosphate derivatives had been developed as part of an initial purpose to render all of the natural

nucleosides fluorescent, as I have already discussed. In fluorescent ATP and its congeners, the N1 hydrogen acceptor and N^6-hydrogen donor positions are blocked. Because these positions may be involved in enzyme or protein interactions in some systems, I felt I had to devise an improved type of probe in which analogous loci would remain free while the property of fluorescence would be retained by the inclusion of a third ring in the adenine system. "Why not try a central 'benzo' ring between the terminal pyrimidine and imidazole rings?" The idea occurred to me while I was shaving one morning. I had to ask my image in the mirror: "Why haven't you thought of that before? You have had all of the separate bits of information available for two years or more and yet you haven't put it all together until this moment." The image in the mirror was chagrined; however, it was not too late. No one else had as yet conceived of the idea. The answer was found in the *linear*-benzoadenine series.

The fact that the new, nitrogen-containing ring system had to be synthesized *de novo*, which was done first by Alan Morrice and Dr. Mark Sprecker (publication in 1975), along with two angular benzoadenines, gave us some respite so that we could do ample experimentation before the compounds would have any off-the-shelf availability. We shortened the synthetic methodology for the *lin*-benzopurines in general and made the desired ribosyl and phosphoribosyl derivatives. Armed with the knowledge of the similarity in behavior of *lin*-benzo-ATP and ATP with a representative group of kinases and convinced of the desirability of relating the nucleotide names, I approached Dr. Waldo E. Cohn for approval of the nomenclature. Waldo was the "czar" of biochemical nomenclature. It took some argumentation, but the conviviality of a summer Gordon Research Conference in New Hampshire helped in gaining his imprimatur. Research on the fluorescent dimensional probes moved forward at a brisk pace at the University of Illinois when Dr. Jorge R. Barrio returned to us from an interim teaching position in his native Argentina where dangerous political fracturing of the country was in progress. We found usefulness in the fluorescence properties of the *lin*-benzoadenine nucleotides in studies of static and dynamic behavior, which yielded information concerning coenzyme-enzyme binding sites. Another postdoctorate, David I.C. Scopes, who joined the group had an interesting background in that he had most recently worked with Gus Fried's younger brother, John, at the Syntex Corporation in California. I found willing collaborators in other laboratories as I had during our investigation of the etheno-bridged probes.

> Evaluation of the fluorescent, dimensional probes: *lin*-benzoadenosine 5'-triphosphate, 5'-diphosphate, and 3',5'-

monophosphate with respect to binding and activity as enzyme cofactors.

The fluorescent "stretched-out" analogs, *lin*-benzoadenosine and *lin*-benzoadenosine 3',5'-monophosphate, are able to interact with AMF-dependent protein kinase, a pivotal enzyme involved in many hormonal responses (with Dr. M. J. Schmidt, Eli Lilly and Company).

Interaction of *lin*-benzoadenosine 5'-di-and triphosphate with mitochondrial ATP synthetase, purified ATPase, and the adenine nucleotide carrier (with Henry A. Lardy, University of Wisconsin).

Incorporation of modified nucleoside 3',5'-biphosphates into oligoribonucleotides with T4 RNA ligase (with Professor Olke C. Uhlenbeck).

Utility of the spectroscopic responses of *lin*-benzoadenine nucleotides for determining divalent metal ion association constants and interaction with positively charged micelles and with representative enzymes.

Allosteric activation of aspartate transcarbamylase with *lin*-benzo-ATP.

Dimensional probing of the ATP binding site on firefly luciferase (with Dr. Marlene DaLuca, University of California, San Diego).

A fluorescence displacement titration technique for characterizing the nucleotide binding site on the catalytic subunit of protein kinases (with Dr. R. Roskowski, Jr., Louisiana State University Medical Center).

All of these findings seemed to have been hoped for in the blurb that appeared under "Monitor" in the *New Scientist* of January 25, 1979, a portion of which follows.

"Detailed pictures of the active sites of many enzymes, even when they have not been crystallized and studied by

> X-ray diffraction, may come out of some beautiful synthetic chemistry by Nelson Leonard, Jorge Barrio, and their colleagues at the University of Illinois . . . In its most recent researches, Leonard's group has made and studied analogues of the adenine nucleotides which are longer by about 2.4 Ångstroms than the natural compounds. These lin-benzoadenosine derivatives retain all the features of the natural molecules but they contain an additional internal benzene ring; the Illinois group regards them as 'dimensional probes' of active sites. Since so many biochemical processes involve ATP, NAD, cyclic AMP and related compounds, this series of molecules can be used to study an immense range of enzymes. They are also fluorescent, so their fluorescence emission conveys information about the sites to which they are bound.
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> "But the real power of this approach is demonstrated by its application for the synthesis of ATP in mitochondria, the main system for the conservation of energy in cells. At present we have no information about active sites in this system. The analogue approach has been tried in the past, but in most cases the analogues used have been synthesized directly from ADP or ATP and the possibility of contamination of the analogues with free ATP or ADP has made the results difficult to interpret. Because of their route of synthesis, Leonard's lin-benzoadenosine derivatives are absolutely guaranteed free of such contaminants."

The underlying concept has been effective in assessment of the space available for the adenine portion of adenosine triphosphate (ATP) with various enzymes and transport proteins. The importance of the problem lies in the fact that fully one-sixth of all presently known enzymes require ATP as a substrate or cofactor. The fluorescence properties of the coenzyme analogues have also been used to define limiting equilibria between stacked and unfolded conformations of these alone and in contact with their receptor proteins. We made other compounds containing a fused benzene ring in the center, including benzologs of enzyme inhibitors.

In work going on elsewhere, the original concept has been followed and has also been altered by formal scission of the benzene ring to create analogues that possess terminal pyrimidine and imidazole rings, or other

heterocyclic ring pairs, with different tethers between the rings. The interest in these analogues lies, *inter alia*, in their potentiality as anti-cancer drugs. In designing and synthesizing these "split" nucleoside analogs, Katherine L. Seley of the Georgia Institute of Technology, in particular, has been most appreciative of our "pioneering work" in the *lin*-benzo series. She introduced a new class of shape-modified nucleosides which she refers to as "fleximers." Others who derived initial guidance from our work, according to their publications, include my friends Stewart Schneller and Vasu Nair.

At one of the Aspenyl Chemistry Meetings in Snowmass, Jack Roberts' description of his pathfinding work on ^{15}N NMR and my contributions to nucleic acid chemistry led to a decision to collaborate in an analysis of 5-azacytidine ^{15}N resonances in neutral and protonated form. In another year and after a number of downhill runs and ski-lift rides together, we collaborated in a study of the tautomers and locus of protonation of adenine and its derivatives by ^{15}N NMR spectroscopy. This was followed by our synthesis of (+)-[1-^{15}N]biotin, which lead to the assignments of the ^{15}N NMR resonances of biotin, thereby providing another probe for following the biological carboxylation and transcarboxylation of biotin and for investigating the phenomenal interaction between biotin and avidin. The final collaborative paper was concerned with the ^{15}N NMR assignments in a systematic series of azacycl[3.3.3]azines of varying nitrogen content. Years later, at a dinner in Pasadena, CA, to celebrate Jack Roberts' 70th birthday, I had the pleasure of describing his contributions to chemistry, universities, foundations, and friends. When I mentioned that I could probably pinpoint his four major contributions to science as being our collaborative papers, the audience groaned, appreciating the hyperbole. On that occasion, Jack and Edith Roberts introduced me to one Peggy Phelps, whom I was to marry in 1992.

One of the azacycl[3.3.3]azines we made, named tri-*s*-triazine (and, earlier, cyamelurine), was of particular theoretical interest. The unsubstituted nucleus was common to some heat-stable compounds that had been made in Germany more than 150 years earlier and to which Linus Pauling had assigned the three-fused-rings structure. The physical and spectroscopic properties and the structure, established by x-ray crystallography, satisfied most of the theoretical predictions related to the 12π-electron periphery of tri-*s*-triazine. Our communication elicited a letter from Linus Pauling, reproduced here, which had a very stimulating effect on my young coworkers, Mitchell Rossman and Dr. Ram Hosmane.

NELSON J. LEONARD

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5 November 1982

Professor N. J. Leonard
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Dear Professor Leonard:

I am surely pleased that you have succeeded in synthesizing the compound cyamelurine.

I must say that I was also pleased, nearly 50 years ago, when I realized that I had thought of a sensible structure for cyameluric acid and hydromelonic acid. Professor Franklin at Stanford had given me nicely crystallized samples of some of the compounds, and I had been trying to think of a sensible structure - the ones that he had written for the compounds and that other early chemists had written did not seem to me to be sensible.

Sincerely,

Linus Pauling

LP:dm

Our two-step synthesis of tri-*s*-triazine was particularly satisfying to me because I knew that many others had tried to make it and had failed. Furthermore, I had suggested to Mitchell and Ram the successful conditions for carrying out the second step. They enjoyed teasing me about this, saying something to the effect: "We tried your idea, *but* it worked!" We were lucky in those years to be blessed with both success and good humor in the laboratory. The cooperative spirit of my research

colleagues, tempered by much kidding along with competition, endowed this period with special pleasure.

We achieved entry into another N-heterocyclic system via a remarkably simple two-step procedure. Ken Cruickshank, a bagpipe player from Scotland, and Kunihiro Sumoto, from Japan, showed us how to achieve access to the rare 10π 1,3,4,6-tetraazapentalene ring system, which opened a final, major chapter of research on covalently liked DNA/RNA cross sections.

In the spring semester, 1986, my organic chemistry colleagues at the U. of I. invited a series of speakers, who appeared almost every week on the campus, to talk about their research. They were a carefully selected group: all were former students and postdoctorates or close friends who had connections with Illinois. I greatly appreciated and thoroughly enjoyed the thoughtful gift, presaging my retirement.