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Teaching a monkey to listen

Vincent P. Marshall

Kalamazoo, MI 49006, USA

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Postdoctoral studies in the laboratory of Professor I.C. Gunsalus were unique among my educational experiences. Gunny is a man of many interests, with science being the greatest of these, but exposure to his avocations has broadened many of us. His students and colleagues often have developed strong interests in music, art, history, travel, and wine as a result of this influence. There is always a sense of Gunny's creativity and leadership, and at a given moment he is likely to inspire, teach, challenge, frustrate, or lend confidence to those around him. Gunny on occasion would inform a new graduate student or postdoc of his expectations, which were usually well beyond the scope of that individual's previous experiences. A typical Gunsalus remark was "What one monkey can do another can learn." Gunny spoke also of listening as a powerful tool underused by many, and considered a "how to" course on the subject to be needed. Gunny's quest for excellence continues to define him. It was not usual in 1970 for graduate students and postdocs to address their professors using nicknames. I. C. Gunsalus made known that his friends, students, and colleagues usually called him Gunny; however, there was an unspoken convention that his first name was never used. Perhaps, his mother was the last of those referring to him as Irwin.

I came to Urbana in 1970 as recommended by Jack Sokatch, who was my major professor at the University of Oklahoma Health Sciences Center and a former Gunsalus graduate student. My first thoughts as a microbiologist were of learning *Pseudomonas* genetics from Gunny and Al Chakrabarty, but was surprised when he gave me lab space with his biochemistry group. The biochemists were working on cytochromes and iron sulfur proteins as part of Gunny's association with Hans Frauenfelder and Peter Debrunner. Gunny's biochemists and Hans' and Peter's physicists attended two weekly events to further collaboration. These were the Wednesday luncheon and the micro-colloquium. The meetings were occasions for open discussion of ideas by all attending including new postdocs and graduate students. I was surprised to discover that the physicists treated me as if I might be able to do something useful and were interested in working together on projects.

John Tsibris and M.J. Namtvedt had prepared ^{57}Fe-enriched putidaredoxin for use in Mossbauer studies by replacement of the natural abundance iron with ^{57}Fe, but had not done the same with the cytochrome P450-containing camphor hydroxylase (P450cam). The small amount of ^{57}Fe (2.2% natural abundance) in a molecule as large as P450cam necessitated ^{57}Fe enrichment for Mossbauer studies, but John Tsibris was leaving for the University of Florida at a time when Peter, Hans, and Gunny wanted isotopically enriched material. Gunny had mentioned to the physicists that the new "boys in the lab" could be of some help in obtaining it. We knew that all previous attempts of enrichment by physical replacement of the natural abundance iron had resulted in the formation of inactive enzyme, and considered doing the project by means of biosynthetic enrichment. John Lipscomb had recently passed his Preliminary exams and was becoming the lab's principal purifier of P450cam. John and I realized that our collaboration could provide the means necessary for obtaining ^{57}Fe-enriched enzyme and started working toward that end immediately. Ed Conrad had developed a fermentation process for production of P450cam using an inorganic salts medium with camphor as the sole carbon source; however, as camphor is not very soluble in water, there were novel techniques required for its use in this manner. Once learned, we were ready to do the enrichment fermentation. Mike Sharrock from Peter Debrunner's lab had ordered our source of ^{57}Fe from Oak Ridge National Laboratory, but it arrived in the form of iron oxide and not as the water-soluble salt hoped for. I had little experience in inorganic chemistry, so John took the

E-mail address: vince3795@aol.com.

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sample of enriched Fe_2O_3 to an inorganic chemist friend in the Alpha Chi Sigma chemistry fraternity, who converted it to $FeCl_3$. We repaid his kindness by steaming lobsters for a fraternity banquet using Gunny's large autoclave. Growing the enriched *P. putida* turned out to be the easy part, and the real work began with enzyme purification and preparation of the enriched P450cam in various states at concentrations often greater than 3 mM. Later, as Mossbauer studies were carried out in the Debrunner laboratory using the ^{57}Fe-enriched P450cam, Gunny gave one of his highest complements by letting us know that we had "made sense" [1,2]. He had long recognized that students learn much from one another, and encouraged such interactions. Gunny considered that the proper positioning of individuals in laboratories would ensure useful collaborations. He knew the critical elements which would be needed for the solution of a problem well ahead of others and situated us to that end. Gunny assumed that we would make sense if allowed to, and never liked giving specific directions. I recall also working in a similar way with Ralph Meeks and Karl Dus in the enrichment of *P. putida* cytochrome *c* with seleno-L-methionine as a part of Ralph's thesis research, but will not discuss those studies here. I was little aware in 1973 that a large portion of my research activities would continue to be related to these enrichment techniques through the late 1990s until my retirement from Pharmacia and Upjohn in 2000 [3–7].

Hans, Peter, and Gunny would often travel to Europe or other distant sites during the summer months to pursue studies with colleagues. At the last Wednesday luncheon before their departure in 1972, several of the graduate students and postdocs discussed plans to do Mossbauer and esr studies of P450cam at 4.2 K. No one had mentioned doing the corresponding spectrophotometric studies, so I considered doing them as a late summer project. The physicists thought that these data might have some comparative value and agreed to help me in this effort. Someone in either the physics or biochemistry group had done such optical studies at the temperature of liquid nitrogen, so there was a Dewar flask setup with optical ports suitable for spectrophotometry. However, to carry out these studies at 4.2 K, it was necessary to design such a system with two chambers, the outer being for liquid nitrogen and the inner for liquid helium. Bob Austin arranged for a craftsman in physics to build this apparatus and brought it to our lab before going on his summer vacation. Bob had demonstrated the thermistor which detected the condensation of helium at 4.2 K, but I had forgotten how to use it, and Bob was gone. It was an easy matter to see nitrogen condensing in the outer Dewar, but much more difficult to see helium condensing in the inner chamber unless a bright flashlight was used. I recall preparing P450cam in various states in aqueous glycerol and seeing nothing unusual at any temperature until the CO adduct was studied. The expected was observed at room temperature and at the temperature of liquid nitrogen, but at the temperature of liquid helium the Soret maximum of the CO-treated reduced preparation had shifted from 447 to 408 nm, which is the Soret maximum of reduced P450cam lacking CO. When the preparation was brought back to room temperature, its Soret maximum returned to 447 nm. I did not know what if any significance these observations held, but repeated the experiment knowing that Gunny would want to see data. Eckard Munck offered a quantum mechanical first approximation in explanation, but suggested waiting a week or two until Hans and Gunny returned. In the meantime, my flashlight photolysis studies were put on hold. When Hans heard the story from Eckard, he called Gunny and came directly over to East Chemistry with Bob Austin, Eli Greenbaum, Laura Eisenstein, and Ken Beeson to see what this was all about. My first thoughts were of being in trouble, as so much attention was being given to the flashlight experiment. Gunny wanted to see data as expected, and I was relieved when he indicated that good data had been collected. Hans, Bob, Eli, Ken, and Laura began to study this phenomenon in a major research effort which would be continued for many years in Hans' laboratory. Within a year or two, their work had led to the development of new thinking about the dynamics of CO binding by heme proteins and activation energy spectra of biomolecules [8,9]. It is quite possible that if a thermistor had been employed to detect the condensation of helium at 4.2 K, that no subsequent discoveries would have been made. These events would appear to be a good example of the role of serendipity in science.

My time in Urbana ended in 1973 after accepting a position as a research scientist in the Fermentations Products department of the Upjohn Company. Soon, I was fermenting cultures on the 150,000-liter scale, but had many good memories of doing science in Gunny's lab. After about a year in the production area, I transferred to the research division, where I remained for another 26 years in pursuit of one of Gunny's imperatives to "know the bugs." His multidisciplinary team approach to science had prepared me well for a career in industrial research.

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