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Gunny, germs, and iron-57

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In the summer of 1967, I was writing up my thesis at the Technische Hochschule Darmstadt when I received a letter from Hans Frauenfelder. He inquired whether I would be interested in coming to Urbana to study enzymes with Mössbauer spectroscopy. My wife, Hilde, and I contemplated the matter for a few days and decided to accept. After 2 years (that was the idea) we would return to Germany, and I would try to get a job at Siemens. My background was in nuclear physics, and my plan was to work on reactor safety or perhaps semiconductors, but certainly not on P450.

Soon after my arrival in Urbana, I was introduced to various colleagues in biochemistry who were supplying the physics group with cytochrome *c*, putidaredoxin, and, a little later, P450. The physics groups of Hans Frauenfelder and Peter Debrunner (my wonderful postdoctoral advisor) had extensive collaborations with Gunny, known to outsiders as Professor I.C. Gunsalus. Early on, stories filtered down to me that painted the picture of someone who was both a superb scientist and, simultaneously, a real character (to understate the case). Of course, with my German background I was a bit anxious at the prospect of meeting a man who had placed the UV/vis in the ladies' room in order to impress upon the dean that there was a lack of research space. Nevertheless, shortly after my arrival I went to my first appointment with the man who, as it would turn out, was going to decisively redirect my scientific career.

I knew fiddlesticks about biochemistry and my English was confined to what had stuck from my gymnasium education. During our first meeting, Gunny awed me with a 45-minute discourse on microbial degradation, telling me everything known to man. I learned that *Pseudomonas putida* needs CPKNSCaFeMg (Chopkins coffee mighty good) for a decent living. I don't know how the bug, supported by NIH, got hold of the camphor it was degrading all the time. Gunny awed me with a dissertation on NAD-something and *ptdrdxn*, pronounced precisely as *ptdrdxn* (by Gunny), and he explained the camphor degradation pathway in every detail. I remember vaguely that *ptdrdxn* was tossing around reducing equivalents, two to be exact, and the action took place in different segments of the P450 cycle; the second toss occurred in the lower left of Gunny's notepad after oxygen had joined the melee. Never did I did suspect then that oxygen activation would become an integral part of my scientific life. After returning to the Physics Department, I lost Gunny's notepad. It was recovered by a grad student who announced that he had found a Xerox copy of the original version of the Gilgamesh epos. Of course, the writing was not cuneiform but gunneiform, and *ptdrdxn* was not a god of Uruk but an electron transfer protein and effector in a bug from Urbana.

Did I mention that we had at least two parties per week? In 1972, Hilde and I had been advised to buy a house. But since we were naive Germans used to paying in cash, we were not considered credit-worthy by the bank. During one weekend party, Gunny inquired how our house-buying was doing, and Hilde told him that the bank had turned us down. Gunny said some unprintable stuff and then directed our attention to the quality of the wine. On Monday morning a bank employee called and told us that the loan had been approved after they received a persuasive phone call from Professor Gunsalus.

When the collaborations between the Urbana physicists and biochemists became more extensive, Gunny, Hans Frauenfelder, and Peter Debrunner started the Micro-colloquium. This was a great opportunity for physics and biochemistry students to exchange ideas. We learned a lot from each other and got new ideas that probably could not have been formulated in any other

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environment. In a seminal experiment spawned during one of my Micro-colloquium talks, Vince Marshall, a microbiology postdoc in Gunny's lab, used a flashlight to see whether a P450–CO sample was properly positioned in a glass helium dewar. In the process he rediscovered flash(light) photolysis and redirected Hans Frauenfelder's career from time reversal to CO reversal. Gunny was always proud of the "boys in physics" (Laura Eisenstein and Shirley Chan included), and he made sure that his students and postdocs had plenty of physics exposure. I once gave a Micro-colloquium talk about the high- and low-spin forms of P450 and chloroperoxidase. After the talk, Gunny said, "Eckard, the biochemistry kids know the difference between high-spin and low-spin, but they don't know what a spin is. You should teach a course about spins." That suggestion gave rise to "Spins for Biochemists," which I taught twice in Urbana and then for 15 years in Minnesota. It also redirected my thoughts from Siemens towards an academic career. As the Micro-colloquium was a roaring success, we introduced the idea later in Minnesota as MPIG, the MetalloProtein Interest Group, which is still running strong.

Gunny's lectures were always meticulously prepared. I particularly remember a brilliant talk at the "Metals in Biology" Gordon Research Conference at the Miramar in Santa Barbara (Metals in Biology was made in heaven; that's a fact). He was invited to speak about P450 and putidaredoxin. Just before Gunny started, John Lipscomb whispered something into my ear that caused me to freeze. "No, he will not do that!" Five minutes into his talk Gunny told the audience that "John and Eckard will now give you the details" and left the podium. That was my first speaking exposure to the many people who would become dear friends and close collaborators.

In 1972 Bill Orme-Johnson came to Urbana to deliver a seminar about nitrogenase. He showed an EPR signal with g values at 4.3, 3.7, and 2.0 and emphasized that it was not known whether the signal originated from molybdenum and/or from iron. Iron, of course, caught my attention, but when Bill disclosed that the protein contained between 14 and 36 irons, I thought that this was a Mössbauer project for the 21st century, rather than one for the Age of Aquarius and *ptdrdxn*. In the evening Gunny called me at home: "I am having dinner with Bill in about 30 minutes. Bill is an extremely bright guy, but you should sort out the iron for him. Please, join us for a good bottle of wine." During dinner Bill and I scribbled Mo and Fe, in mM and mg/cm^2, and a bunch of question marks on a napkin, and I agreed to study a sample of nitrogenase. I told Bill that I would have a look, and a quick one at that, that I would report whether iron was part of the EPR-active center (that was the easy part), and that then I would send, pronto, the sample back to Wisconsin, because no respectable Mössbauer spectroscopist would study such a miserable mess. Despite all the talk about French restaurants and great wines, nothing, to tell the truth, touches the heart of a spectroscopist more than a highly resolved spectrum. So, after inspecting the first spectrum, I called Bill and told him that there were at least four irons in the EPR-active center and that he could forget about the return of the sample and instead grow more bugs on CPKNSCa^{57}FeMg. I said something like, "Bill, I think this is the beginning of a beautiful collaboration." Indeed, it was the beginning of a beautiful collaboration that lasted 20 years. It was also the beginning of a friendship that has lasted to this day. I once talked with Bill about Gunny's letters of recommendation. We agreed that they were masterpieces of composition and insight; Bill referred to them as Faust III. Nitrogenase has been a major part of my career (we have just published a lengthy paper on the electronic structure of the FeMo cofactor), and Bill Orme-Johnson has been a significant part of my life. I am grateful to Gunny for initiating both experiences.

In 1974, I accepted a faculty position at the Freshwater Biological Institute at the University of Minnesota. John Lipscomb had just finished his Ph.D. in Gunny's lab and John Wood, the Director of the Institute, and I talked to Gunny about the possibility of having John join us as a postdoc. I felt that it would be better for John to go to an established place because the Freshwater Institute had just opened; we owned a few pencils, but our only regular visitors were two mallards and a bunch of chipmunks. However, Gunny strongly felt that the Institute would be a great place for John, and "by the way, John's presence will be good for you guys." It was amazingly good for us! John was interested in moving away from the oxygenase field to study nitrate reductase, and my first postdoc, Larry Que, wanted to model the P-clusters of nitrogenase. These are both interesting systems, but they were a little beyond the instrumentation we had at the Freshwater in the beginning. Three months after my arrival in Minnesota we recorded our first 4.2 K Mössbauer spectrum but had to wait for another year to get a computer. While we were getting up to speed, we decided to do something "simple." It had been suggested in the literature that resting protocatechuate 3,4-dioxygenase had a single ferric ion coordinated by four cysteinyl residues. We could check this coordination readily by recording a Mössbauer spectrum and plotting the result with a teletype (the spectrum was seven feet along the abscissa, which caused an astute reviewer to suggest shortening the manuscript). John grew the bugs and purified and crystallized the enzyme, and his career was irreversibly headed back into the biochemistry of oxygen activation. The ferric dioxygenase was red, and because Larry is fatally attracted to anything with an absorption band in the visible, he

joined the fray. The absence of a computer thus has enriched the field of oxygen activation with a great biochemist and a superb chemist. Protocatechuate 3,4-dioxygenase turned out not to have any cysteine, and it was anything but simple, but it spawned a whole research area on the biophysics and the mechanism of non-heme dioxygenases. My NSF grant on nitrogenase and my NIH grant on oxygen activation have since been funded for 28 years; what would I have done without Gunny's guidance?

I often tell my students about Gunny, in particular about his advice to a visiting undergraduate: "My son, it does not matter how much stuff you throw up the wall; all that matters is how much you make stick." In our lab, this great statement on life is known as Gunny's law of diminishing returns. Sometimes you can't tell what sticks until you look back over your shoulder after traveling away a few steps. I realize now that that many of the things that still seem to be clinging to the wall had their origins with Gunny.

As I was sitting in front of my computer screen and closing the document *Gunny*, Word asked "Do you want to save the changes to *Gunny*?" I responded "No changes to Gunny, pleeese."