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Biochemical and Biophysical Research Communications 312 (2003) 85–89
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A creek runs through it

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Received 25 September 2003

Each of us, no matter how old, can look back to one or more significant events that shaped our careers. In my own case it was a meeting between Stan Dagley and Gunny on Tennis Court Road in Cambridge that occurred sometime prior to 1961. I am not sure about the date because I did not learn about the meeting until 1993. I do know, however, that the discussions between Dag and Gunny initiated a chain of events that sent Peter Chapman, Peter Trudgill, and Dag himself (for a sabbatical leave) to Gunny's laboratory in the Division of Biochemistry at the University of Illinois. From my own perspective in 1961, as an undergraduate in the Department of Biochemistry at the University of Leeds, Peter Chapman's departure to pursue postdoctoral studies with Gunny opened up a graduate student desk in Dag's laboratory. Two years later, Dag left for Urbana and John Wood and I were left to share the Leeds laboratory. We were, however, far from being isolated. Dag corresponded regularly with each of us. Those letters from America were clearly hand written on fragile, blue, prepaid air mail envelopes. They were a window on graduate studies in the US and often contained suggestions in response to our progress reports. In my own case, progress in 1963 had foundered on the fate of 2-oxo-4-hydroxyvalerate in the catechol *meta*-fission pathway. The solution came in one of Dag's eagerly awaited letters which described his isolation of a pseudomonad that could grow with *m*-cresol as its sole source of carbon and energy. I could sense from his letter that he was delighted to say that the source of the organism was 'Boneyard Creek,' which ran close to his house on the Urbana campus. The extent of his delight can be gauged in the following paragraph, taken from Dag's paper in *Experiences in Biochemical Perception*, a volume honoring Gunny on his seventieth birthday:

> In those days, a topographical feature of the city of Urbana was the Boneyard Creek, and between its muddy banks there meandered a stream of legendary pollution. Such was its fame that I once read an article in (of all journals) the *Transactions of the Leeds Literary and Philosophical Society*, where mention was made of the last date when a live fish had been discovered in its water. The writer believed that the finder was the victim of a hoax. But whatever the status of higher forms of life, there was no doubt that the Boneyard supported a rich and catabolically versatile microbial flora, and that I had the advantage of unimpeded access. We lived in a house built upon a concrete raft that spanned the creek with the rich microbial habitat below; the house has since been demolished, and the stream enclosed for most of its length. Under those favorable circumstances, I soon isolated *Pseudomonas* U, an organism that eventually proved useful to investigators in laboratories ranging from Melbourne, Australia to Bangor, Wales. [1]

Pseudomonas U did indeed go on to achieve international status, beginning in 1963 when it arrived in Leeds harboring a godsend in the form of an enantiospecific aldolase that converted 2-oxo-4-hydroxyvalerate to pyruvate and acetaldehyde. This resulted in the first edition of the hydrolytic branch of the *meta*-fission pathway for the metabolism of catechol [2] and a predictive pathway for the metabolism of substituted catechols, which explained Peter Chapman's seminal identification of succinate in the degradation of phenylpropionate [3]. Dag returned to Leeds in 1964, and the autumn of that year saw John Wood and me head to the US for postdoctoral studies: John to the laboratory of Ralph Wolfe in the Microbiology Department at the University of Illinois and me to the College of Pharmacy at the University of Wisconsin to work with Charlie Sih. In one year with Charlie I learned the value of analytical chemistry and this was to serve me well when, in the fall of 1965 as if moved by some inexorable force, I found myself on the Urbana campus, not in the Division of

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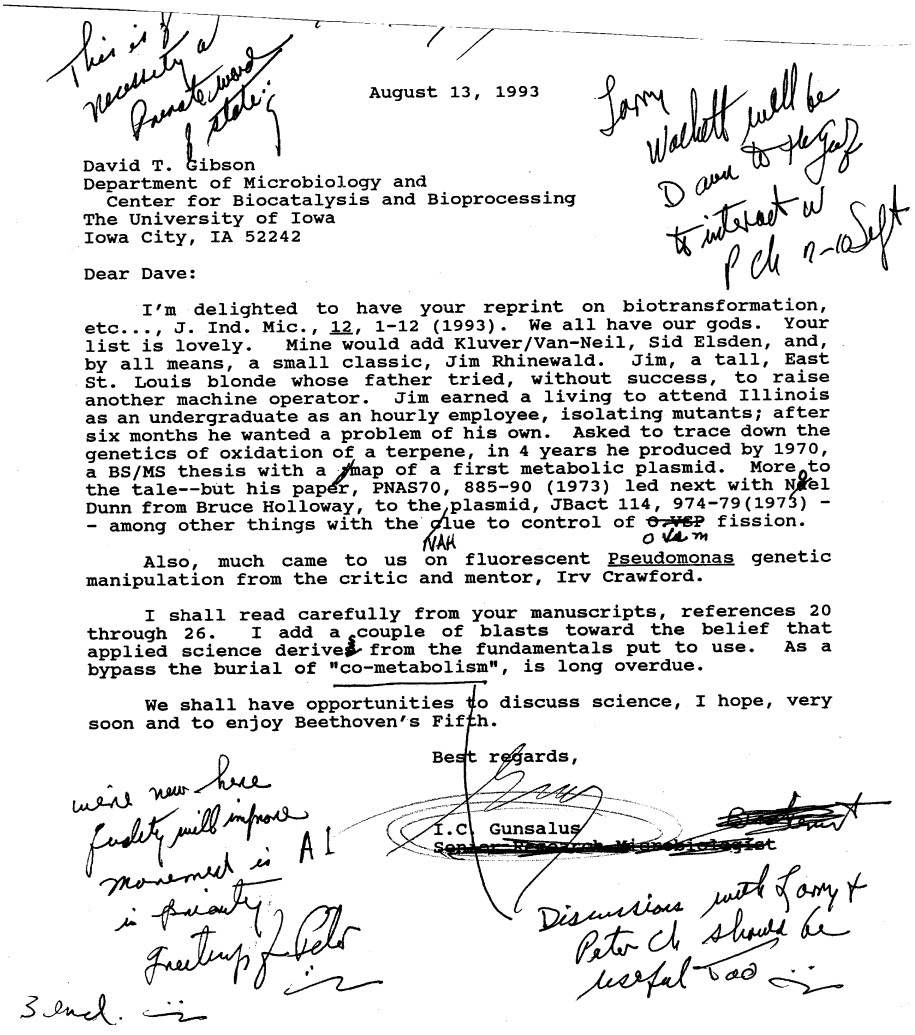
doi:10.1016/j.bbrc.2003.09.182

Biochemistry, but close by in Burrill Hall, which housed the Department of Microbiology. There I began postdoctoral studies with Reino Kallio, who had moved from The University of Iowa to take the position of director of life sciences. Reino's only directive to me was to equip his laboratory and do research on the metabolism of hydrocarbons. It was an exciting time to be in the Department of Microbiology. Leon Campbell was the new editor of the *Journal of Bacteriology*, Sol Spiegelman had daily bulletins outside his laboratory on the replication of a fragment of DNA, Ralph Wolfe had established conditions for the large-scale culture of *Methanobacillus omelianskii* and was making groundbreaking progress on the biochemistry of methanogenesis, and Carl Woese was sitting with his feet on his desk (I never saw him in any other position), thinking about the Third Kingdom. Exciting things were also happening outside of Burrill Hall. Up the road in the Division of Biochemistry, Gunny and his students were dissecting the camphor methylene hydroxylase system from (+)-camphor-grown cells of *Pseudomonas putida* C1 [4,5]. It was hard for me to believe that in the midst of all this excitement I had been given the opportunity to develop a research project on the bacterial oxidation of hydrocarbons. The choice of a project was not difficult. At the time it was known that catechol, and protocatechuic acid were central intermediates in the metabolism of many aromatic compounds. Little was known, however, about the reactions leading to these ring-fission substrates. This was especially true for aromatic hydrocarbons, which clearly, in my mind, fell within the sphere of Reino's directive. In 1965, thanks to the pioneering studies of Eric Boyland and his colleagues at the Chester Beatty Research Institute in London, it was known that mammals oxidize aromatic hydrocarbons to optically active *trans*-dihydrodiols [6]. A literature search revealed that *trans*-dihydrodiols were also postulated intermediates in the bacterial oxidation of benzene and naphthalene [7,8]. Thus the stage was set for a project that would provide convincing evidence for the involvement of *trans*-dihydrodiols in aromatic hydrocarbon metabolism. I made the obligatory pilgrimage to Boneyard Creek, which was in spate due to recent rains. A sample of the mud taken from the side of the creek was brought back to Burrill Hall and used as the inoculum for the eventual isolation of a strain of *Pseudomonas putida* that would grow with ethylbenzene and toluene as its sole source of carbon and energy. *trans*- and *cis*-Benzene dihydrodiols were synthesized and a Warburg experiment was conducted with toluene-grown cells. Benzene, catechol, and *cis*-benzene dihydrodiol were rapidly oxidized. *trans*-Benzene dihydrodiol, the expected intermediate, was not. In the months ahead I was able to show that benzene oxidation to *cis*-benzene dihydrodiol required two protein fractions, NADH and ferrous iron. Attempts to purify either fraction resulted in the loss of activity [9]. The similarity of these results to Gunny's early work with Jens Hedegaard [4] on the (+)-camphor methylene hydroxylase and David Cushman's identification of a ferroprotein (putidaredoxin) component of the hydroxylase [5] seemed obvious and I called Gunny to see if he had any suggestions that could jumpstart our benzene oxygenase studies. This led to our first face-to-face conversation. I was overwhelmed. I should have known more about the ongoing studies with the camphor methylene hydroxylase, especially about the purification and properties of cytochrome $P450_{CAM}$ that catalyzed the hydroxylation reaction [10]. Gunny quickly moved past these developments into the latest EPR studies on putidaredoxin. I was clearly out of my depth. Gunny was thinking about bringing physicists on board to study $P450_{CAM}$ and I realized that the putative benzene oxygenase would only be a poor second fiddle if it turned out to be another bacterial cytochrome P450. I returned to Burrill Hall with a sample of putidaredoxin in my pocket and a list of experiments suggested by Gunny. Putidaredoxin failed to stimulate benzene oxidation, all attempts to show the presence of a P450 spectrum in either protein fraction failed, and heme-reactive reagents did not inhibit benzene oxidation.

My 2 years on the Urbana campus passed all too quickly. Janet and I had a memorable dinner with Gunny and Carol before we started packing for the long drive to Texas, where I was to begin as an assistant professor in the Department of Microbiology at The University of Texas at Austin. Two weeks after our arrival I found that Waller Creek, which passed close to the department, had all of the attributes assigned by Dag to Boneyard Creek in Urbana. This Texas repository of waste from a number of university departments, including the Chemistry Department, yielded a variety of useful strains before it became the victim of a campus-wide beautification project. Strains included *Pseudomonas* Pxy, which oxidized *p*- and *m*-xylene through 4- and 3-methylcatechol, respectively [11]. Studies on the genes encoding enzymes for xylene degradation were conducted in a collaboration with Al Chakrabarty, who had left Gunny's laboratory for the bright lights of General Electric's Research and Development Center in Schenectady. Al and his group were able to show that the genes encoding all of the enzymes required for growth with *p*- and *m*-xylene are located on a nonconjugative plasmid called XYL. Transfer to the well-known *P. putida* G1 strain (PpG1) required a transfer plasmid designated factor K [12]. XYL could not compete with TOL [13] and has thus slipped well below the horizon of most modern literature retrieval systems. Be that as it may, the collaboration with Al Chakrabarty led to a friendship that continues today. Other strains of note from Waller Creek were *Pseudomonas* sp. strain NP and *Beijerinckia* sp. strain B1 (later known as *Sphingomonas yanoikuyae* B1), which yielded chiral *cis*-arene

diols from naphthalene [14] and biphenyl [15], respectively. These strains, together with *Pseudomonas putida* F1 from Urbana, almost challenge the concept of enzyme specificity. Together they are known to oxidize more than 300 aromatic substrates and many of the products are chiral arene *cis*-diols [16,17]. In the 1970s we continued work on toluene dioxygenase from toluene-grown cells of *P. putida* F1. Progress was slow. During the same time period Gunny melded the forces of physics, organic chemistry, and biochemistry to focus on $P450_{CAM}$ [18]. The results blistered the pages of *PNAS*, *Biochemistry*, and *BBRC*. The orphan status of toluene dioxygenase led me to meetings that focused on oxygenases. Here I was able to renew my conversations with Gunny and to observe first hand the role he played in the evolution of the P450 field. The 1973 Stockholm meeting of the International Union of Biochemistry was a classic example. The discussions at the end of a packed P450 symposium were lively to say the least and brought back memories of a day in London spent listening to a debate in the House of Commons. I should note, however, that the combative exchanges on that afternoon in Stockholm seemed soon forgotten in a post-conference boat trip to the Swedish archipelago. On the flight home I was left to marvel at the advances in all fronts of the P450 field. Gunny's influence at this and other oxygenase meetings was palpable and undiminished at a 1981 meeting in Hakone, Japan. The occasion was a symposium in honor of Osamu Hayaishi for his contributions to the fields of oxygenases and oxygen metabolism. It was a wonderful meeting in a venue with Mount Fuji in the background. More reflective interactions with Gunny that come to mind include a delightful brunch with Gunny and Dorothy in San Francisco at the time of a 1972 National Academy of Sciences meeting on the biodegradation of organic molecules in the Biosphere [19]. Peter Chapman and Douglas Ribbons were also present and afterwards we each concluded that we had never seen Gunny more happy and that for the first time we felt that we had met someone who could more than match him word for word. In 1987 a symposium was held at the Gray



August 13, 1993

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Dear Dave:

I'm delighted to have your reprint on biotransformation, etc..., J. Ind. Mic., 12, 1-12 (1993). We all have our gods. Your list is lovely. Mine would add Kluver/Van-Neil, Sid Elsden, and, by all means, a small classic, Jim Rhinewald. Jim, a tall, East St. Louis blonde whose father tried, without success, to raise another machine operator. Jim earned a living to attend Illinois as an undergraduate as an hourly employee, isolating mutants; after six months he wanted a problem of his own. Asked to trace down the genetics of oxidation of a terpene, in 4 years he produced by 1970, a BS/MS thesis with a map of a first metabolic plasmid. More to the tale--but his paper, PNAS70, 885-90 (1973) led next with Nigel Dunn from Bruce Holloway, to the plasmid, JBact 114, 974-79(1973) -- among other things with the clue to control of ~~O-VEP~~ fission.

Also, much came to us on fluorescent Pseudomonas genetic manipulation from the critic and mentor, Irv Crawford.

I shall read carefully from your manuscripts, references 20 through 26. I add a couple of blasts toward the belief that applied science derives from the fundamentals put to use. As a bypass the burial of "co-metabolism", is long overdue.

We shall have opportunities to discuss science, I hope, very soon and to enjoy Beethoven's Fifth.

Best regards,

I.C. Gunsalus

~~Senior Research Microbiologist~~

Fig. 1.

Freshwater Biological Institute in Navarre, Minnesota, to honor Stanley Dagley for his contributions to our understanding of microbial metabolism. Dag's "microcrobial world" revolved around the carbon cycle and this was the central theme of the symposium [20]. Dag was a gentle giant, soft spoken, and yet passionate about teaching and research; he motivated and stimulated students and colleagues alike. Gunny was the keynote speaker at the banquet and as we listened to his presentation it was clear that the meeting on Tennis Court Road more than two decades previously had resulted in a lasting friendship based on a love of literature, scholarship, and mutual respect. It was a shock and a great loss to all of us when Dag passed away later that year.

After 21 years in Texas, Janet and I returned to the Midwest to join Irving Crawford in the Department of Microbiology at the University of Iowa. Irv was a quiet scholar, with his love for music, *Pseudomonas* genetics, and orchids evident to all. His admiration for Gunny knew no bounds, beginning in 1966 when Gunny came to Irv's lab at Scripps with a collection of *P. putida* tryptophan auxotrophs and set in motion a series of elegant studies on the regulation of the *P. putida* tryptophan biosynthetic pathway [21]. My interactions with Gunny became a little more frequent after we became settled in Iowa City. This was a result of Gunny's taking up residence at the EPA's Gulf Breeze Research Laboratory on Sabine Island. This situation led to his attendance at meetings with some emphasis on environmental microbiology. Locations included Chicago for the 1989 *Pseudomonas* meeting; Lake Tahoe in 1995 for a Keystone Symposium on Environmental Technology, memorable because of more than 12 inches of snow; sunny Mallorca in 1996 for a Ken Timmis meeting on the Biodegradation of Organic Pollutants, where we celebrated Gunny's 84th birthday at lunch; New Orleans in 1996 for a memorable ASM meeting; and Urbana in 2000 to celebrate the endowment of the I.C. Gunsalus Professorship in Biochemistry at the University of Illinois. From these all too brief conversations with Gunny I learned how to listen and the conversations led to correspondence. In a paper commemorating Gunny's 70th birthday, Nick Ornston stated that "Gunny's mind operates simultaneously on several circuits" [22]. The same might also be said of his letters. I show the one in Fig. 1, however, because it reveals a warm reflective tribute to Jim Rhinewald and Irv Crawford.

A long letter from Gunny after the 1996 ASM meeting contained several 'Gunnyisms' that stand alone without interpretation or context. For example, on Dagley: '. . .later the smaller graduate family felt the man undivided. Ratios are important.' On teaching: 'Always said I could be excited in lecture, if only I could find two pairs of bright eyes to pace me, to tell when I lost the thread, as told by their eyes dulling, telling me to recycle a little, and see if I could pick up the broken pieces.' On memories: 'The van Neil thing too was real, when I saw his excitement at "rusty water" along the edge of Enfield Glen spring creek. The little man jumped from the path 4 feet down, whipped out a botanists 8 power, on his knees examined the mass, explained the content—which I did not understand at all.' On meetings: 'On the globe traveling circus telling lies, or half lies, to the same half scientists mixed in with a few old-timers, not a matter of age, who do care.' On the new phylogeny: 'am puzzled if 16Srna, or gyrase, can dig deeper than the phenotypic variance that floored Roger (Stanier) and Kees (van Neil), and the latter with Kluyver as late as mid 50's.'

Gunny also has an inexhaustible supply of quotations and I once asked him the source of 'I was born not knowing and have had only a little time to change that here and there.' The answer came in the form of a book, *Genius*, *the Life and Science of Richard Feynman*, signed by the author, James Gleick. Gunny's admonition, 'start with the epilogue, a little thick but thought and time cure indigestion,' was mentoring at its best from someone I have come to know as an advocate and a friend. This latest stimulus package has not caused me to abandon my reductionist upbringing or Stephen Hawkin and the principles of the 'grand unified theory (GUT).' Gleick's narrative, however, does provide a scaffold for learning and perhaps understanding how to live with 'chaos.' As for toluene dioxygenase, it turned out to be a member of the Rieske non-heme iron dioxygenase family and that is a story for another day.

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