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Introduction

Irwin C. Gunsalus, versatile and creative scientist

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Early years

Irwin Clyde Gunsalus, later known to his colleagues in the scientific world as Gunny, was born in a prairie farm cottage near Blunt in Sully County, South Dakota, on June 29, 1912. His paternal ancestors, originally from the Atlantic coast of Europe, had arrived in Philadelphia in the 1700s and migrated westward. His maternal grandparents, of British descent, had arrived in Illinois and Iowa and then homesteaded in South Dakota. Young Irwin credited his experiences in farm life, his parents' interest in education, and challenging public school teachers, one of them a disciplinarian who required "Everyone up to potential or else," for the stimulation that led him to a life in learning [1,2]. After 2 years as a chemistry major at South Dakota State College in Brookings, South Dakota, he enrolled in Cornell University as a junior and found that his background had provided an education equal to or superior to that of many of his new colleagues.

Intrigued by his undergraduate exposure to the sciences, Gunny then pursued graduate study in bacteriology at Cornell under the guidance of Dr. J.M. Sherman. After what he described as unsupervised learning from the lactic acid bacteria and from his advisor "Uncle Jimmy," he was awarded the Ph.D. in 1940. His doctoral thesis was titled "The Chemical Nature of the Enterococcus Group Antigen." Gunny was then invited to join the faculty of the department, where he served with distinction for 7 years. In this capacity and beyond at other institutions he would be a prime mover of "bacteriology" into modern "microbiobiology" in a growing overlap with biochemistry, the physical sciences, molecular biology, and genetics.

Move to Indiana and to Illinois

In the fall of 1947, Gunsalus moved to Indiana University, also as a professor of bacteriology. This change provided him with the opportunity to learn modern genetics from some of the field's pioneers through seminars, lectures, and conversations. Significantly, this began a lifelong friendship with the talented Salvador Luria. Three years later, Gunny was enticed into moving to the newly developing Department of Microbiology at the University of Illinois in Urbana, and in 1955 he became head of the Biochemistry Division in the Department of Chemistry. He has commented that, in succeeding the 30-year chairmanship and the mountain of accomplishments of William C. Rose, what seemed a reasonable 5-year challenge required a decade of service in recruiting appropriate faculty to the Division, establishing new courses and facilities, and at the same time growing new skills in research and teaching [2]. This location provided a permanent and stimulating environment for his scientific work for the next 30 years. Some highlights of his career are presented below.

Chemistry and enzymology: reactions of coenzymes with amino and keto acids; bacterial growth factors leading to the discovery of pyridoxal phosphate and lipoic acid

As summarized in a Harvey Lecture presented in 1949 [3], Gunny's previous studies had established a role for pyridoxine in amino acid metabolism. A more active form of vitamin B_6, termed "pseudopyridoxine," had been discovered by Esmond Snell and traced to the 4-aldehyde, pyridoxal, and the 4-amine, pyridoxamine [4]. The important finding that ATP was required as well as the aldehyde for activation of apotyrosine decarboxylase suggested that the coenzyme was a phosphate ester [5], and soon thereafter pyridoxal 5-monophosphate was

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identified as the form active in the reaction [6,7]. Subsequent studies in Gunny's and other laboratories established the general involvement of the cofactor in amino acid formation and catabolism.

In his Harvey Lecture, Gunsalus also summarized evidence for the involvement of a new cofactor called POF in pyruvate oxidation, which in lactic acid bacteria yields acetate, CO_2, and hydrogen peroxide [1]. Later experiments by several investigators showed the occurrence of enzyme complexes containing the factor in keto acid conversions throughout the animal and plant kingdoms. POF was partially purified from yeast extracts [8] and obtained in crystalline form from liver in an effort in which the Illinois group, Lester Reed's group working on the "acetate-replacing factor" for Lactobacilli, and the Eli Lilly company joined forces [9].

Chemical synthesis of the compound provided proof of the structure, and it was named α-lipoic acid [10]. Thus, studies on a previously unknown bacterial growth factor led to characterization of a crucial component in intermediary metabolism [11]. The reaction sequence in α-ketoacid oxidation involving the reductive acylation of the cyclic disulfide form of lipoate to yield the thiol ester, like the role of pyridoxal phosphate in amino acid metabolism, is now standard textbook biochemistry.

Hydrocarbon metabolism, dioxygen activation, and hydroxylase chemistry, and enzymology

A report from the Gunsalus group in 1959 on the degradation of camphor by a pseudomonad culture [12] foreshadowed a major interest of the laboratory that would continue for several decades. Their investigations would lead to the identification of cytochrome P450 as the terpenoid oxygenating catalyst and attract associates and collaborators with expertise in genetics, chemistry, biochemistry, and biophysics. Some of the major findings and experimental approaches are summarized.

As evident from his early research on pyridoxal and lipoate, Gunny always realized the importance of organic chemistry as a tool for revealing biological pathways and metabolic events. This was coupled with his belief that microbes offered the key to understanding the diverse processes by which Nature could transform substrates to products in harvesting metabolic potential and energy coupling. The presence of E.J. Corey in the Illinois Chemistry Department was critical in opening a major new avenue for the Gunsalus laboratory. E.J.'s enthusiasm for terpenoid synthesis offered a chance to define the intermediates and enzymes in the plethora of specialized *Pseudomonas* metabolic pathways and, as much as anything else, cemented Gunny's choice of this organism as the provider of new enzymes for study. Using a strain of *Pseudomonas*, shared with Roger Stanier, Gunny focused attention on the degradation of camphor, which this organism could use as a sole source of carbon and energy. There are many stories in the scientific community as to the origins of this strain. Perhaps the most logical is the suggestion that the bug found its eco-niche as a soil microbe growing under a camphor tree. This would certainly make sense, since Bradshaw's first isolate was reported to have come from the soil around pine trees, where pinene can serve as a sole source of carbon and energy for growth. Another idea, popular in Champaign-Urbana, is that this strain of *Pseudomonas* was pulled by enrichment from the Boneyard, an open ditch that runs through the Urbana campus. Numerous other exciting organisms have since been isolated by microbiologists from this small stream.

The first steps in elucidating the camphor degradation pathway focused on ways to open the cyclic ring system of the [2.2.1] monoterpene to yield intermediates that could enter into the main pathways of metabolism. In the early 1960s Ed Conrad initiated studies of ring cleavage reactions that appeared to follow classic Bayer–Villager chemistry [13]. Investigations into the iron-containing ketolactonase reactions continued throughout the decade [14]. Ed completed his association with the Gunsalus laboratory and remained in the Illinois Biochemistry Department throughout his professorial career.

During this time, Gunny realized that it was critical to bring the genetics of the *Pseudomonas* under control, and the laboratory expanded to include critical phage experiments by Niblack and Al Chakrabarty [15]. This was part of a serious broadening in Gunny's enterprise in searches for new phage and microbial fertility factors, as well as efforts to understand the mechanisms of gene exchange and metabolic pathway topology.

One critical discovery emerging from the prolific Gunsalus laboratory occurred in 1965 with the documentation by Jens Hedegaard of an inducible methylene hydroxylase [16]. Gunny concluded that not only was the application of organic chemistry needed to unravel Nature's secrets of intermediary metabolism, but also critical input from physics was required. Here, as is often in science, the sequence in the study of protein components was dictated by technology, as well as the interests of laboratory personnel and collaborators. Thus, in the late 1960s, Gunny formed important collaborations with the powerful electron spin resonance expertise of Helmut Beinert (who writes in this issue) and Bill Orme-Johnson. The inducible methylene hydroxylase system discovered by Hedegaard had several components, including a small ferredoxin-like protein with a two-iron, two-sulfur center that was initially purified by Cushman and Tsai and further characterized by Tsibris [17,18]. Meanwhile, Katagiri was working on a heme-containing component later found to be the first bacterial cytochrome P450 [19]. The molecular characterization of the microbial diversity of the cytochrome

P450 superfamily was thus launched. Both Tsibris and Katagiri provide insights elsewhere in this issue.

To move further and faster, local expertise was needed. Vitally important to the future mechanistic studies in the Gunsalus laboratory was the formation of a close interaction and collaboration with Hans Frauenfelder, Peter Debrunner, and Eckard Munck in the Physics Department. This all started when a Biochemistry Department faculty member, Charles Todd, attended a seminar given by Roger Cooke, then a graduate student in physics. Here the first Mossbauer spectra of iron in a biological complex (cytochrome c) were reported. Gunny immediately realized that the physical parameters revealed by this form of spectroscopy could be of great importance to understanding the function of the iron proteins being isolated in biochemistry. A sense of the excitement during this time can be gained by reading the articles by Munck and Frauenfelder in this issue. While the quest to understand Nature's secrets certainly paved the road between biochemistry and physics, it is important to realize that these collaborations were aided by a mutual admiration for excellent fruits of the vine. This love and expertise in viniculture were passed on to the students and postdoctoral associates in the laboratory.

Note that a close collaborative relationship between physicists and biochemists was an unusual event in the early 1970s. The term "biophysics" was not well defined and usually meant the study of ion transport or other physiological processes. The areas of scientific acumen are very different for physicists, biologists, and chemists. But the most serious hindrance to effective collaboration is simply language. To overcome the hurdles of communication between these groups, "micro" colloquia were held weekly, "nano" colloquia were convened daily, and "pico" colloquia would be held hourly if some topic needed in-depth attention. The operating principle of all these meetings rested on the saying: "In physics if you really understand something you should be able to explain it to anyone." More than once, one camp would present sophisticated data and fail to convey a basic idea to the others. The meeting did not end until all questions were answered, and new ideas and data, which so often came from these get-togethers, were understood and enthusiastically embraced by all.

Critical progress in understanding camphor metabolism continued to be made on the biochemistry front. The cytochrome P450 system involving the first committed step in the catabolic breakdown by *Pseudomonas putida* was revealed to have three components, with Katagiri and Ganguli defining the hemoprotein component as cytochrome P450cam [20]. The three enzymes that together catalyzed the 5-exo-hydroxylation as the first step in catabolism to form citrate and isobutyrate were an NADH-linked dehydrogenase containing a single flavin group, a small two-iron two-sulfur redox transfer protein termed putidaredoxin, and the heme-containing cytochrome P450. By the late 1960s, the ketolactonase was also revealed to be a three-component system through the efforts of Yu [21]. The first publication from the Illinois physics–biochemistry collaboration focused on the iron–sulfur protein, putidaredoxin [22]. The year 1970 was a banner one in the definition of the P450 components. The collaborations involving physical methods with Peisach, Blumberg, Orme-Johnson, and Beinert aided Tsai and Yu resulted in characterizing the electronic changes at the heme center of P450cam upon substrate binding [23]. Tyson and Tsai identified some of the first intermediate states in the reaction cycle [24], and Yu crystallized the P450 hemoprotein [25].

At this time the Gunsalus laboratories occupied a corner complex on the fourth floor of the "old" Roger Adams Laboratory. Gunny's immense office included space for the several secretaries that conducted various duties for his large operation. This corner provided an internal pathway of communication between laboratories running to the west, which were occupied by the physically and chemically orientated enzymologists, and those toward the south, which housed the microbiologists and geneticists. In the latter group, Richard Hartline focused on induction and repression [26] and Al Chakrabarty [27] and Rheinwald [28] on the diversity of plasmid transfer in the pseudomonads. Later, Bill Toscano, who was guided to the laboratory by Hartline, investigated the transport process. These scientists share their stories in this issue.

Down a hallway, the physical biochemistry of the enzymes involved in pseudomonad camphor metabolism was under intensive study. John Lipscomb, a new graduate student in biochemistry, began the detailed characterization of P450cam turnover and the interactions between redox partners [29,30] and, with Tsibris [31], formed the biochemistry end of the bridge to the Physics Department. The anchor across Green Street was provided by Mossbauer and EPR studies of iron centers by physics graduate students Sharrock [32] and Champion [33] working with Debrunner as their mentor. Eckard Munck was a visiting professor and critical collaborator. Another lane in this biochemistry–physics superhighway was Gunny's involvement with Frauenfelder's flash photolysis studies examining electron transfer and ligand dissociation in heme protein systems [34].

With 1973 came an added twist to the collaborations between the Physics and Biochemistry Departments. Steve Sligar, a physics graduate student operating under the freedom of an NSF Fellowship, wandered from resonance spectroscopy through theoretical astrophysics before finding a Ph.D. research home under Peter Debrunner. In a fateful meeting with Gunsalus, Debrunner, and Gregorio Weber, it was decided that he would formally migrate to a desk in the Gunsalus laboratory and begin biophysical characterizations in the Biochemistry Department. With Lipscomb's patience in teaching a

physicist how to handle valuable biological samples, a new venture in friendship and productivity emerged [35–39]. In physics, a force is manifested by an exchange of particles. With this twist of a physics graduate student operating in biochemistry and vice versa, new approaches and methodologies could be tried and successfully utilized to reveal details of enzyme function.

Gunny was not one to favor idleness. It was expected that all in the laboratory would put in full 12-h days, and he himself was always busy with something. The time to talk with him was reserved for early Saturday morning—no problem since this was expected to be a full working day. With the telephones not ringing so frequently, one found Gunny in a most agreeable mood to discuss science. Many, but not most, in the laboratory realized this and took advantage of the time to exchange science with the Master. Often these discussions would extend throughout the morning, and Gunny would graciously invite those present to join him for lunch at the Urbana-Lincoln hotel. Since no meal was complete without wine, these outings would usually extend until mid-afternoon. With Gunny's abilities at metabolism, he would return to the laboratory full of energy to complete the day's experiments. On one occasion, in his enthusiasm to join one of these lunch outings, a postdoctoral fellow rushed out without looking at the road map for the experiments for the remainder of the afternoon. Coming back to the laboratory after the multi-hour lunch, Gunny offered to help him with the laborious separation that had to be conducted at −80 °C. "What's the next step?" Gunny asked. "Cool in acetone for two hours," came the reply. Oops, maybe this should have been done during lunch! The others in the laboratory exited amidst the tirade.

With the same farsightedness that Gunny used to bring organic chemists, inorganic spectroscopists, and physicists to apply their tools to unravel the secrets of metalloprotein function, he was always pushing to obtain the three-dimensional structure of the oxidative enzymes involved in *Pseudomonas* metabolism. Indeed, in the early 1970s, Lipscomb and Sligar shipped literally grams of highly purified P450 and putidaredoxin to several well-respected crystallography laboratories. However, no diffraction quality crystals were obtained. By 1977 Lipscomb was recruited to a postdoctoral position at the Gray Freshwater Institute in Minnesota by Munck, who had left earlier for a faculty position there, and Sligar took an assistant professorship in molecular biophysics and biochemistry at Yale. Management of the Gunsalus physical biochemistry subgroup fell to Gerry Wagner, a talented bioinorganic postdoctoral associate. Among other research accomplishments, Gerry streamlined the high-throughput production of *Pseudomonas* P450cam, putidaredoxin, and flavoprotein, and continued the quest for crystals. Tom Poulos describes in this issue his success with Gerry in growing diffraction quality crystals of P450cam and the solution of the first three-dimensional structure of a heme monooxygenase. By then many P450 systems were known to catalyze important biotransformations, and the world was anxiously awaiting the publication of the P450cam structure. Gunny provided an immense and critical effort to resolve all the competing personal and commercial interests in this structure, which was finally published in 1985 [40].

Thus, in only a little over a decade, Gunny and his associates were able to isolate a new microbial organism, develop a genetic system for its study, define a class of extrachromosomal elements that gave rise to diversity in the utilization of growth substrates, dissect an 11-step pathway for terpene breakdown, find the first three-component microbial P450 system involved in this pathway, and purify and crystallize the hemoprotein component. During this process, he taught a group of graduate students and postdoctoral associates the fun of science and discovery. Additionally, he launched them on the never-ending quest for the perfect bottle of wine. The following photograph (Fig. 1) captures Gunny sitting at his home in Urbana, doing what he enjoyed most.

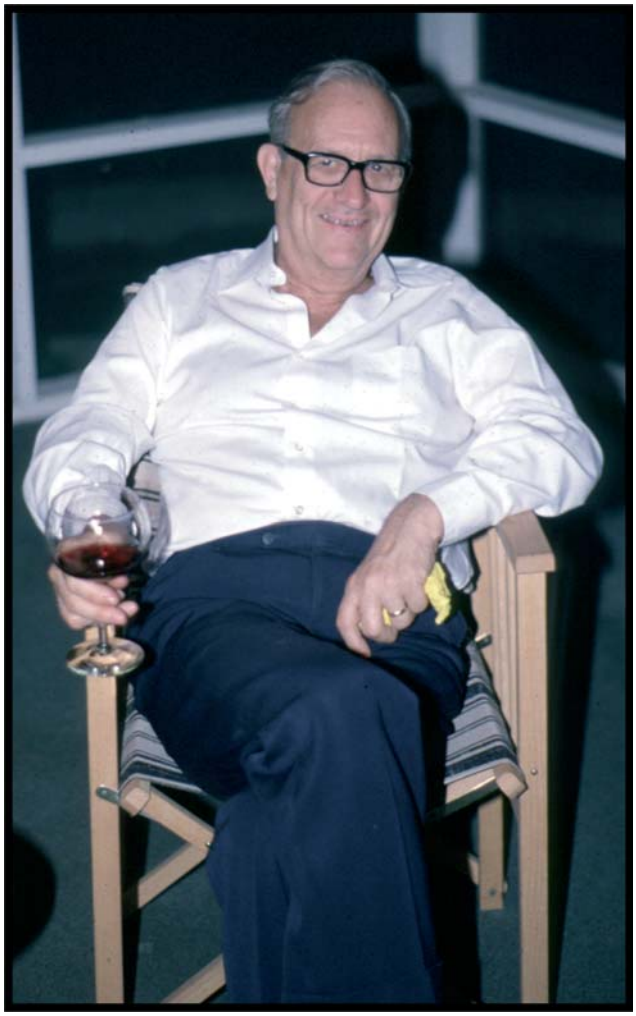

Fig. 1. Gunny in his home.

Emeritus years

With Gunny's move to emeritus status, the laboratory began to contract. His interest in the process of scientific discovery did not wane, however. W.A. Wood, who writes in this issue, had moved to the Salk Institute of Biology Industrial Associates (SIBIA) and attracted Gunny to move to La Jolla. The Gunsalus teaching method expanded beyond science. Typical was the invitation extended to Lipscomb and Sligar to visit after a Metals in Biology Gordon Conference, wherein Gunny provided first-hand education in the purvey of fine fish and the synthesis of bouillabaisse. The commercial aspects of the SIBIA enterprise required some secrecy, but Gunny was back directing a laboratory to discover a microbial process that would reproduce the flavor and aroma of rare mushrooms.

The next Gunsalus adventure came with the charge to build a vehicle to establish scientific education, and the discovery and solution of biological problems in the third world. This came with the official title of Assistant Secretary General of the United Nations, organized through the United Nations Industrial Development Organization. He was very enthused with this challenge to provide some immediate benefit to the problems affecting humankind, and he was immensely successful in establishing centers for biotechnology in Italy and India.

One might think that with two positions beyond his professional career at Illinois it would be time to retire, but not Gunny. It was common to meet colleagues at the Chicago O'Hare airport departure gates waiting to take the small planes to Urbana. Stephen Sligar, dozing after an all-night flight from Asia, was waiting for the connecting flight when he was startled by a shout from a large man madly waving around a paper. "Steve, Steve ... look at this ... I got a new job!" Sure enough, Gunny had a formal offer from the Environmental Protection Agency to assume a position at its research center in Gulf Breeze, Florida. Sligar asked what this job really entailed, to which Gunny replied: "Well, all they want me to do is a little politicking in Washington, but I am going to start a group to look at microbial nitro-aromatic metabolism!" As of late, Gunny has "retired" from the Gulf-Breeze adventure and we can only wait to see what the next challenge will be! Thus, having recruited, trained, and influenced several generations of scientists, he continues to be sought after for his advice on problems ranging from biotechnology to environmental protection.

Honors and awards

Gunsalus has been recognized with numerous honors and awards, including election to the American Academy of Arts and Sciences, the American Academy of Microbiology, the National Academy of Sciences, the Mead Johnson Award in Biochemistry (co-recipient with E. E. Snell), the Selman Waksman Award, and the William C. Rose Award in Biochemistry and Molecular Biology. He holds foreign membership in the French Académie des Sciences and honorary memberships in the Harvey Society of New York and the Japanese Biochemical Society. He has served as president of the American Society for Biochemistry and Molecular Biology and the Federation of American Societies for Experimental Biology. In addition, acknowledgments by many lectureships, visiting professorships, and meetings held in his honor attest to the impact of his research investigations on modern microbiology and biochemistry.

Gunsalus is widely viewed as one of the world's foremost scientists. The accompanying articles in this issue indicate that throughout his long career he has also been an unusually stimulating teacher and a loyal and generous friend to his many colleagues.

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