BIOGRAPHICAL MEMOIRS

Irwin Fridovich

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A Biographical Memoir by James A. Imlay

Figure 1 Irwin Fridovich. *Photo courtesy of the Duke University School of Medicine.*

Irwin Fridovich spent his entire scientific career ensconced in the Biochemistry Department at Duke University. He passed away at age ninety in 2019. He is recognized for the discovery of superoxide dismutase, an event that almost single-handedly created the field of oxygen radicals and oxidative stress. A complete accounting of the impact of superoxide and other reactive oxygen species in biology is not yet in hand; but, at minimum, they play important roles in the oxygen sensitivity of organisms, the toxicity of select antimicrobials, and the function—and dysfunction—of the cell-based immune system. Irwin was noted for the chemical sense that he brought to biological problems, a lucid writing style, and the development of the basic tools of his craft. Many remember him as a friendly and willing collaborator, and a few scientific adversaries will recall his unwillingness to shy away from a disagreement.

Early Years

Irwin Fridovich's paternal grandparents fled pogroms in Russia and landed in New York City in the early twentieth century. Irwin was born there, but he never truly turned into a city boy: His stories about his boyhood focused upon things like raising canaries in the small family apartment, exploring rural New York as part of a Boy Scout troop, and spending summers as a teenager working upstate on truck and dairy farms. Those experiences instilled in him a love of the outdoors that never went away—many years later, after winning a scientific prize, he used the award money to purchase a tract of forest in North Carolina.

As a teenager, Irwin attended the Bronx High School of Science, an incubator that famously turned many second-generation kids of immigrants into notable scientists. During Irwin's time the principal was Morris Meister, whose son Alton became a widely recognized biochemist.

Like many young people coming from families of limited means, Irwin went on to attend the City College of New York (CCNY), which charged no tuition and enabled students to live at home. There Irwin encountered a charismatic biochemist, Abraham Mazur, and his native interest in living things grew to appreciate that the roots of biology lay in chemistry.

During this period Irwin worked part-time jobs at the college, and in the course of one of them he met the young

woman who was to become his wife. Mollie and Irwin would raise two daughters, Sharon and Judith, who went on to successful scientific careers. Sharon is a professor of ophthalmology at Duke University, and Judith is a professor of human genetics at Emory University. To his own trainees, Irwin would emphasize that family should be a priority above work; in my own case, after I completed a postdoctoral fellowship in his lab, Irwin considered that I had three young children, and he attempted to give me a raise so generous that the university prohibited it. Of his own family Irwin later wrote, "The love and sense of continuity, provided first by children and now by grandchildren, are ultimately the finest things that life provides."

Upon Irwin's graduation from CCNY, Mazur offered him the chance to work with Mazur at his second job at the New York City branch of Cornell University. Irwin did so for a year and learned the art of protein purification. Mazur recognized talent, and he not only encouraged Irwin to go to graduate school, but he directly guided him to the lab of Mazur's friend, Philip Handler, at Duke. Irwin had never been to North Carolina, but he accepted the advice and headed off to Durham. He never left.

Interestingly, graduate school had not been Irwin's first choice. His childhood jobs with farm animals had inspired the thought that he might become a veterinarian, and he had applied to the Cornell University College of Veterinary Medicine. He was denied admission. Irwin later wrote that the admissions officers likely imagined that a New Yorker would only be interested in ministering to household pets, unaware that in reality Irwin's passion was for a large-animal practice. Privately, however, Irwin suspected a darker truth, telling colleagues that the Cornell veterinary school in that era explicitly limited the admission of Jews. And years later, towards the end of a long, celebrated career, he admitted that his love of outdoor work had never slackened—and that if he had it to do all over again, he would reapply to vet school.

Irwin later described Phil Handler as the most impressive man he had ever met. Handler had a remarkable knowledge of biochemistry, and he served as the president of the National Academy of Sciences from 1969 until his death in 1981. When Irwin joined his lab, Handler asked him to investigate the oxidation of sulfite to sulfate—a terminal step in the catabolism of cysteine. The mammalian liver possesses a dedicated sulfite oxidase; that enzyme was explicated by K. V. Rajagopalan (Raj), a postdoc in the lab who went on to discover and characterize the molybdenum-based cofactor that it contains (and who became Irwin's lifelong friend and lunch partner). Irwin's assignment was to pursue a second, unidentified enzyme that could trigger sulfite-dependent oxygen consumption. Dialysis inactivated this activity, and it could then be reconstituted by the addition of a small molecule from boiled crude extract. Purification of the molecule from 2 kilograms of liver yielded a compound with the spectral characteristics of hypoxanthine, and indeed authentic hypoxanthine was able to re-establish the enzyme activity. It required several years to work through the idiosyncrasies of the chemistry, but by the point of his graduation, Irwin had determined that xanthine oxidase was the factor. Its oxidation of sulfite was not direct, however; instead, xanthine oxidase leaked electrons to oxygen, and the resultant oxygen species in turn oxidized sulfite univalently, thereby initiating a free-radical chain up to 200 events long.

At this point, Irwin encountered disbelief among fellow scientists for the first time. On paper, the transfer of an electron to oxygen should generate superoxide, a radical species that Linus Pauling had calculated would be a stable molecule. But when Irwin and Handler reported their results at a scientific meeting, a member of the audience pushed back on their suggestion that superoxide could be released by the enzyme. The measured univalent reduction potential of oxygen was so low, he said, as to preclude the idea that xanthine oxidase could make it. Accordingly, when Irwin wrote his early papers, he proposed that oxygen sat on the enzyme surface and merely conducted electron flow from an enzyme metal center to sulfite.

Years later, shortly after I arrived in Irwin's lab as a new postdoc, I received a letter from a biophysicist who objected on theoretical grounds to a conclusion that I had drawn in a recent paper. I shared the letter with Irwin, who grew animated: "Tell him that data matter, and theory does not!" He then told me the tale of how an erroneous measurement of the oxygen reduction potential had pushed him off the superoxide trail for years. Unfortunately, I was not yet used to editing Irwin, and in my letter back to the biophysicist I dutifully included Irwin's comment verbatim, alongside my own explanation as to why my model had not violated any principles. Shortly I received a letter back from the biophysicist, thanking me for my own argument, but telling me to pass along his incredulity at Irwin's statement, let alone that Irwin would mentor a trainee in this way.

A New Faculty Member—and the Discovery of Superoxide Dismutase

The concept of in-breeding was apparently not an issue in those days, or it may not have been easy to attract new academic talent off the beaten trail to North Carolina. In any case, Handler freely promoted his outstanding trainees: Henry Kamin, Raj, and Irwin all became faculty members in Handler's biochemistry department. This provenance was still evident thirty years later when I came aboard. In a group meeting I was puzzled when people repeatedly referred to something called "wing buffer," which seemed to be used in

all their assays. I had never heard of it, and thinking "wing" might be the trivial name for a new synthetic compound, I pulled a postdoc aside to ask about it. Oh no, he said. It was just a mix of potassium phosphate and EDTA. All the labs in that wing of the Nanaline Duke building used it as a standard reaction buffer—the PIs all having carried it over from their days in Handler's lab.

As a new assistant professor, Irwin examined the promiscuous behavior of xanthine oxidase in detail, using not only dyes but also cytochrome *c* as convenient electron acceptors, whose reduction could easily be monitored spectroscopically. Irwin suspected that electron transfer from xanthine oxidase to these oxidants occurred from several of its various redox cofactors, with the particular site determining whether one or two electrons were transferred. None of this seemed normal. Textbooks emphasized the extreme specificity of enzymes; yet, here was one going rogue. He also noted that preparations of xanthine oxidase were sometimes contaminated by an inhibitor that could suppress electron transfer to cytochrome *c*; at the time, he suggested it was myoglobin, without having in mind a clear mechanism.

It was in the course of following these leads that a new graduate student, Joe M. McCord, made a puzzling observation. McCord noticed that the kinetics with which xanthine oxidase transferred electrons to cytochrome *c* depended upon the concentration of xanthine oxidase. That made no sense. This observation could not fit within the notion that cytochrome *c* received electrons intimately from the enzyme; instead, it implied that a radical species of oxygen must actually diffuse away from the enzyme before it encountered cytochrome *c*. And that new view immediately suggested that contaminating inhibitors might work by intercepting the presumptive diffusible species—superoxide—and catalytically degrading it.

Irwin and Joe promptly set about purifying the inhibitor. It turned out to be a copper protein that others had named erythrocuprein, without knowing its function. Irwin and Joe confirmed that the homogeneous protein did indeed block xanthine oxidase from reducing cytochrome *c*—and then, in a tour de force of biochemistry, they identified the step at which erythrocuprein interceded. It did not impair xanthine oxidase turnover: urate production was unchanged. After using electrolysis to generate tetrabutylammonium superoxide in dimethylformamide, they infused the product into a solution of cytochrome *c* and showed that erythrocuprein still blocked its reduction—proving that the protein was not working by blocking superoxide formation. Further, by confirming that it also inhibited the ability of xanthine oxidase to reduce both tetranitromethane and oxidize epinephrine, they showed erythrocuprein did not intercede by binding to cytochrome *c*. Instead, it catalytically scavenged superoxide

Figure 2 Faculty of the Duke University Department of Biochemistry, 1969. Irwin stands second from the left. *Photo courtesy of the Duke University School of Medicine.*

in free solution. This beautiful paper [McCord, J, Fridovich I (1969) *J. Biol. Chem.*] leveraged Irwin's skills as a chemist's biochemist, set the stage for all the biology that has followed, and has been cited more than 17,000 times.

Superoxide Dismutase: Years of Drama

Their report of the superoxide dismutase activity of erythrocuprein triggered a two-decade conversation that by turns captivated believers and inspired heated responses from skeptics. SOD catalyzes a simple reaction: A single electron is transferred from one molecule of superoxide to the catalytic Cu(II), releasing oxygen; the electron is then again transferred from the resultant Cu(I) to a second molecule of superoxide, generating hydrogen peroxide. It was immediately apparent that such an activity could simply be the adventitious consequence of solvent exposure of a metal atom that, by chance, possessed an intermediate reduction potential conducive to both reactions. Indeed, histidine-chelated copper exhibits some dismutation activity on its own. Irwin's own work with xanthine oxidase had demonstrated that accidental electron transfers are not rare. This idea—that the dismutation activity of erythrocuprein was adventitious—became the mantra of doubters.

In some ways this objection is ironic. The reason that the SOD proposal received such push-back was that it implied that electron movement in the cell was poorly controlled, to the point that cells have to guard against the consequences of inappropriate chemistry. This need for some "quality control" was novel in biology; the focus at that time was upon unraveling what seemed like an exquisitely coordinated system built upon enzymes of immense specificity. Yet this critique—that dismutation was an

artifactual activity—relied upon the idea that SOD itself was prone to making mistakes.

The argument stretched over two decades. It was sometimes loud. I was a graduate student at the University of California, Berkeley, in the mid-1980s, and its Biochemistry Department offered a short course entitled Controversies in Science—with a particular focus upon the ongoing SOD story. The goal of that course was presumably to persuade students of the value of professional approaches to scientific disagreements, but the actual effect was to reveal how entertaining blunt exchanges could be. At research conferences, conversations about SOD could be tense—and rarely were they resolved by the data that were presented. Irwin recounted that after he found his seat following one back-and-forth with a skeptic, his close colleague Raj leaned over and suggested that the audience would now surely be convinced. Irwin was less certain. So during the lunch that followed, he asked several others at their table whether they had drawn a conclusion. Initially they hesitated to say anything—and then one person volunteered that while it was clear that there had been an important disagreement, they were not able to discern who had the better argument.

Indeed, the scientific community at large is always a poor judge of the merits of a claim; instead, it relies upon the informed opinions of a small cohort of experts. In the early days of any truly ground-breaking work, few people have the requisite expertise.

Careful thinkers were further irked by premature claims being made by what came to be the health-food industry. Antioxidant fervor, stoked by Linus Pauling himself, led to proposals that were clearly unmerited by the data in hand: that superoxide and associated oxygen species drive carcinogenesis, heart disease, aging, and all other manner of ills that befall us.

Is the True Function of Superoxide Dismutase to Disproportionate Superoxide?

The discovery of SOD created an unusual dilemma for Irwin: he and Joe had stumbled across an enzyme with an activity that had never been imagined. Did organisms even contain superoxide? Was it harmful? These are physiological questions, and Irwin was neither by training nor instinct a physiologist. This fact became clear to me when I joined his lab in 1987—almost twenty years after he had begun to use *Escherichia coli* to probe the role of SOD. One day I discovered in the lab refrigerator an enormous bottle of deep purple liquid. "What is this?" I asked one of the long-term postdocs. "It's vitamin B_{12}," he said, and he noted that the lab added it to all of their media. Why? Because in 1970 when Irwin walked next door to get a sample of *E. coli* from Henry Kamin, he had asked Henry whether the strain was able to make all the amino acids and vitamins. Sure, Kamin said, everything but B_{12}—not realizing that Irwin was unaware of the fact that no *E. coli* strain either makes or requires B_{12}. Kamin's off-hand remark would trigger decades of pointless supplementation of media in Irwin's lab.

In the fifteen years after the seminal paper, Irwin tackled the SOD problem in a series of steps that progressively sharpened the claim. He induced his departmental colleagues Jane and Dave Richardson to solve its structure. It revealed a barrel comprised of antiparallel β-strands, with the catalytic copper atom exposed at the bottom of an adjacent channel. Collaborators used pulse radiolysis to determine the kinetic efficiency (second-order rate constant) of the enzyme; the answer—2×10^9 M^{-1} s^{-1}—was stunningly high, matching the diffusion limit. The Richardson lab found that this efficiency was partly due to charged residues near the channel lip that guide the anionic substrate into the barrel. It seemed implausible that such an opportune structure and extreme speed might evolve by accident.

Irwin's lab determined that SOD activity is found throughout the microbial kingdom, which suggested that superoxide might be an inevitable feature of aerobic life. This pattern seemed to complement that of catalases and peroxidases, which decompose hydrogen peroxide (a product of superoxide dismutation) to oxygen and water. Moreover, the metal cofactor for many of the bacterial SODs was manganese or iron rather than copper, which made the adventitious-activity model less likely. Interestingly, the lab determined that eukarya have a manganese enzyme in the mitochondrial matrix that fits with endosymbiotic theory, which held that a bacterium was the precursor of the mitochondrion. The implication was that superoxide stress is ubiquitous and ancient.

A survey of microorganisms revealed that SOD and catalase activities tend to be low among obligate anaerobes and high among aerobes. Textbooks (to this day) cite those data as indicating that anaerobes might be anaerobes *because* they lack scavenging enzymes; but Irwin and McCord were more cautious, noting that if molecular oxygen can directly poison anaerobes, then they would have no need for SOD. The correlation did imply, however, that SOD is especially necessary when cells grow in the presence of oxygen—further supporting the hypothesis that the role of SOD is to rid the cell of superoxide.

The next step was to see whether a single organism might demonstrate the same correlation. By good fortune, *E. coli*, everyone's favorite model organism, is facultative—it can grow both with and without oxygen. *E. coli* was found to possess both manganese- and iron-dependent SODs; the iron enzyme is constitutive, but the manganoenzyme is synthesized only when oxygen is present. Irwin argued that such oxygen regulation supported the dismutase model.

With Hosni Hassan, Irwin took that idea a step further. They reasoned that if superoxide is the authentic substrate of SOD, then perhaps SOD will be most strongly expressed when superoxide levels are high. One way to test this was to expose cells to chemicals that might generate intracellular superoxide at high rates. Several candidate compounds were known. Paraquat, a viologen; juglone, a quinone; and phenazinemethosulfate, a phenazine, were all capable of abstracting an electron from redox enzymes and then transferring it to molecular oxygen. And, indeed, when any of these substances—and numerous others in the same chemical classes—were added to *E. coli* cultures, its titers of manganese SOD rose up to twenty-fold.

At this point the argument that SOD serves to degrade superoxide was strong, but it was wholly circumstantial. A key piece was missing: What threat might superoxide pose, such that organisms required an enzyme to get rid of it? By 1980, this issue had become the crux of the controversy. Because hydroxyl radicals can oxidize pretty much anything, some biologists had anticipated that the superoxide radical might do so as well. But chemical experiments were disappointing: when pulse radiolysis was used to generate superoxide in the presence of various biomolecules, no reactions were observed. The failed candidates included amino acids, carbohydrates, nucleic acids, and lipids. These data were interpreted—correctly—as indicating that superoxide does not react with the basic molecules of which cells are made. The notion of superoxide toxicity began to wobble.

Looking further, Irwin and Charles Beauchamp determined that xanthine oxidase could generate an oxidant powerful enough to generate ethylene from methional. Xanthine oxidase makes a mixture of superoxide and hydrogen peroxide—and, weirdly, SOD and catalase were each able to inhibit ethylene production. They initially suggested that superoxide might generate hydroxyl radicals by transferring an electron directly to the peroxide, but radiation biologists assured them that the kinetics were prohibitive. The model was revised when McCord found that contaminating iron mediated the exchange in vitro, and for a decade the presumptive toxicity of superoxide was ascribed to this reaction. Pushback eventually emerged—this time because physiologists recognized that the cell would surely contain other reductants capable of reducing iron, and these other reductants must be far more abundant than superoxide.

The tipping point finally arrived in 1986. In that year, Daniele Touati of the Institut Jacques Monod managed to clone and delete the genes encoding both the manganese- and iron-dependent cytoplasmic SODs of *E. coli*. Both of the single mutants were healthy, but the double mutant exhibited profound growth defects when it was cultured in a minimal aerobic medium. No defect was exhibited in an anaerobic medium. Another group swiftly demonstrated that heterologous expression of the human copper enzyme restored the ability of the double mutant to tolerate oxygen. And with that, eighteen years after its discovery, it was universally accepted that superoxide dismutase was indeed a superoxide dismutase after all.

But what injuries produced the growth defects in the SOD-deficient cell? Touati's mutant could grow aerobically only if it were supplemented with a mixture of amino acids, including leucine, isoleucine, and valine, suggesting that superoxide stress had poisoned the capacity of the cell to synthesize these. This pattern matched what Olen Brown had observed ten years earlier when he exposed wild-type *E. coli* to hyperbaric oxygen; he had subsequently identified dihydroxyacid dehydratase, an enzyme in the branched-chain biosynthetic pathway, as the specific target of this stress. Using this hint, Irwin and a graduate student, Che-Fu Kuo, showed that the same enzyme was extremely sensitive to superoxide in vitro and that it rapidly lost activity inside paraquat-treated cells. The lab quickly followed up by identifying several other superoxide-sensitive dehydratases: aconitase, fumarase, and others. Damage to these TCA-cycle enzymes jibed with the inability of the SOD mutants to use TCA-cycle substrates, such as acetate, as carbon sources. The special vulnerability of these enzymes was then explained by Dennis Flint: Each of them utilized a solvent-exposed iron-sulfur cluster to coordinate and activate substrate for dehydration. Superoxide destroyed activity by binding and abstracting an electron from the cluster; once oxidized, the cluster became unstable, and the key substrate-binding iron atom dissociated. The rate constant for this event could exceed 10^6 M^{-1} s^{-1}, a tremendous rate for an accidental reaction, which explained why cells require so much SOD activity. At long last, the reality of superoxide stress had been unveiled.

Sequelae: Locating the Impact of Superoxide in Biology

In 1974, Puget and Michelson announced that a copper/zinc SOD was present in *Photobacterium leiognathi*, a symbiont of a fish. This was the sole such enzyme known in bacteria, and the inference was drawn that a trans-kingdom gene transfer had occurred, from host to resident bacterium. This was big news. Subsequently, though, additional sightings were made in *Haemophilus* and others. This situation was puzzling, as it threw the phylogeny of the enzyme into turmoil. It was at this point that Irwin announced that even *E. coli*—*E. coli*, the subject of hundreds of SOD experiments!—also had a copper/zinc isozyme. It had been missed because the enzyme is exclusively induced in stationary phase, a difficult-to-reproduce phase that was anathema to dedicated physiologists. His lab discovered it by letting bacteria grow

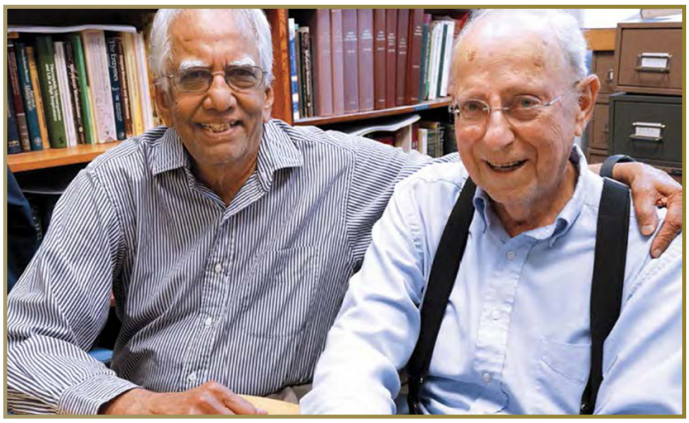

Figure 3 Inseparable colleagues and friends: Raj and Irwin in Irwin's office at Duke, 2016. *Photo courtesy of the Duke University School of Medicine.*

all weekend, out of convenience, and then harvesting them first thing on Monday. Phylogenetic analysis based on this enzyme and partial sequences from other bacterial homologs revealed that copper SODs had evolved, actually, in bacteria and then been transferred to eukarya, almost certainly as a component of the mitochondrial ancestor. In bacteria, the enzyme was located in the periplasm, and in eukarya a substantial fraction resided in the analogous compartment, the mitochondrial inter-membrane space. In both cases its presumptive role is to scavenge superoxide that arises by electron leakage from the respiratory chain.

Further insights emerged. *Salmonella typhimurium*, a pathogenic cousin of *E. coli*, was shown to have two such enzymes: a virtually identical copy of the *E. coli* enzyme, plus a second homolog that was encoded on a resident phage. Ferric Fang's group determined that the latter enzyme is requisite for the virulence of the bacterium. By then, Bernie Babior had discovered that the "oxidative burst" of phagocytes consists of rapid superoxide production; the *Salmonella* enzyme turned out to defend the bacterium from this element of the host immune response. It was induced by a signaling system that sensed an appropriate host environment, and mutants that failed to induce it were incapable of colonizing phagocytes, the normal location of *Salmonella* growth. The whole storyline, starting with Irwin's discovery of the *E. coli* enzyme, had revealed a key aspect of how mammals suppress bacterial infections—and of how professional pathogens evade this defense.

With time it became apparent that the NADPH oxidase that generates phagocytic superoxide is a close relative of the enzymes that plants activate to suppress bacterial invasion and that amoebae use to kill their prey. But it took additional follow-up, to yet another observation made in Irwin's lab, to capture the big picture.

Irwin and Hosni had used redox-cycling natural products to demonstrate SOD induction during superoxide stress. For their purposes these compounds were mere reagents. Subsequently, Bruce Demple and Bernie Weiss tracked down the control of this system to a two-component transcription system they called SoxRS. Much later, my own lab discovered that the inducing stimulus was actually not superoxide but the drugs themselves, which directly activate SoxR by oxidizing its iron-sulfur cluster. The tell-tale sign was that the response was absent in SOD mutants, despite the poisonous levels of superoxide that they contained.

Irwin was upset by our claim—the model whereby superoxide induced the scavenging enzyme had seemed so logical, and for years it had been a key element of the argument for the role of SODs. I soon found myself in the situation in which I had previously observed others: in the midst of a sharp scientific disagreement with Irwin. He penned a letter to the editor of *Free Radical Biology & Medicine*, the house journal of the Society for Redox Biology and Medicine that he had co-founded. Never one to mince words, his letter started, "A recent, apparently paradigm-changing, paper by Gu and Imlay is erroneous. In what follows we sample a few of its shortcomings in the hope of forestalling the confusion it will otherwise sow." I had to laugh at his excess ("*a few* of its shortcomings"), and then he and I settled down to collegial discussions of the nitty-gritty, which focused upon methods that were fairly open to critique. Indeed, it took a few more years to fully persuade him. One morning I received an email from Irwin: "Your arguments supporting the preeminent role of redox cycling compounds, such as the pyocyanines, in eliciting the evolution of the members of the SoxR and S regulons are water tight. My congratulations." I have used this story in classes, to illustrate not only the frustrations that arise when serious people disagree on issues that are important to them, but how they can progress to resolution and concede a point.

The biological upshot was more important: Irwin and Hosni had actually discovered a programmed response to redox-cycling natural products. Indeed, genes under control of the SoxR regulon were subsequently found to encode drug-modifying enzymes and drug exporters. We now recognize that bacteria and plants poison their competitors by assaulting them with agents that will generate massive amounts of internal superoxide. Viewed in context with the NADPH oxidase, it becomes clear that superoxide is a weapon of choice in the endless cross-species warfare within the biological world.

The most consequential example of oxidative stress, though, is the phenomenon of obligate anaerobiosis, which Irwin and McCord had leveraged in arguing that SOD protects life from its aerobic environment. Certain aspects of their argument turned out to be wrong, but they got the big picture right. Recent analysis has shown that anaerobes,

which traffic electrons at especially low potentials, can inadvertently generate superoxide at very high rates when they are perfused with oxygen. They do possess modest titers of scavenging enzymes, but these are insufficient to quell the stress arising from full aeration, and enzyme damage results. As Irwin and Joe had predicted, anaerobes don't trouble themselves to fix the problem by making higher titers of SOD, because molecular oxygen simultaneously oxidizes some specialized enzymes that are key to anaerobic metabolism. This scenario recapitulates both ends of Irwin's original xanthine oxidase phenomenon: the inappropriate oxidation of redox enzymes by oxygen itself, and then downstream injuries by the superoxide that is formed. In the end, this biochemistry drives the diversity and structure of microbial communities, from the soil to the human gut.

Irwin's Retrospective View

In 1992, restriction fragment length polymorphism analysis revealed that the familial form of amyotrophic lateral sclerosis (ALS, or Lou Gehrig's Disease) is caused by missense mutations in the gene encoding copper/zinc SOD—and the buzz was that Irwin was in line for a Nobel Prize. But that prediction faded when it became clear that ALS was more likely a protein-folding disorder, rather than a pathology of oxidative stress resulting from loss of SOD activity. The mutations destabilized the monomer β-barrel, exposing strands that would then form interprotein β-sheets with a second monomer, in turn liberating one of its strands. Resultant SOD aggregates presumably choke neurons. Still, this behavior ultimately arises from the exigencies of SOD chemistry: its tight channel, which filters out compounds that might otherwise react with the copper atom, and its high titer, which is needed to quash steady-state levels of superoxide. In any case, Irwin had always been philosophical about recognition. In his desk was a file labeled "Nobel": He was so frequently nominated by various people, who inevitably contacted him to ask for copies of his most pertinent papers, that he had finally simplified his life by assembling them in a single folder. But he assured colleagues that the award would pass him by, in part because he had been too sharp in his disagreements with some people who carried weight. More importantly, as he reminded trainees, one must derive pleasure from the science itself, rather than depend upon praise from others.

When I joined Irwin's lab, other members sought to capture Irwin's somewhat-unfiltered nature by telling a story. For a period, the postdocs had taken to conveying their frustrations with experimental problems by shouting a particular profanity too raw to be quoted here. Irwin found this amusing. Shortly thereafter, the entire lab attended a national meeting at which Irwin was the featured speaker. In the question-answer session after his talk, a member of the audience inquired why a relevant experiment had failed to provide a clear outcome. Irwin paused, and then smiled. "In our lab, we have a saying when experiments don't work out," he began—and then he stopped, looking with puzzlement at his postdocs in the first row, who were frantically waving and shaking their heads—before he finished with, "Nothing's easy." The point of their anecdote was that it had seemed entirely plausible to them that he would go the other way with his response. But I tell students this story because his actual answer, distilled from a long career, is a concise lesson for those who work on problems that have no precedent.

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