Reflections of a Fortunate Biochemist

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While contemplating the writing of this article I reread some of my early publications and in so doing was appalled at my profound ignorance. That, of course, is the advantage of hindsight. It is also an indication of how much has been learned by me and by others during the intervening half-century. That is the beauty of science; it is a collaborative work in progress that builds knowledge and understanding of the real world. I hope it may be interesting (for readers concerned with the process as well as with the results) to recount how we progressed from abysmal naivete to our current informed view of the biology of oxidation stress. The goal of modesty is served by allowing for the probability that our successors 5 decades hence will consider the knowledge we have achieved pitifully incomplete.

Sulfite Oxidation

Abraham Mazur introduced me to the wonders of biochemistry in an undergraduate course and then during a year spent working in his laboratory at Cornell Medical School. At the end of that year he recommended graduate work and sent me to Duke Medical School to work with Philip Handler, the chairman of the Department of Biochemistry. Handler was the most impressive person I have ever met. He was blessed with a photographic memory, an incomparable mastery of language, and excellent judgment. He assigned me to work with Murray Heimberg, who was then a senior graduate student. He was older than the usual graduate student, having devoted several years to fighting World War II. At one time during this conflict Murray had lost half of his body weight because of malnutrition while a prisoner of war, but he had entirely recovered when I knew him.

We worked long hours, measuring O$_2$ uptake with Warburg microrespirometers and methylene blue bleaching in evacuated Thunberg tubes with a Coleman colorimeter. We found that $\alpha$-hydroxysulfonic acids dissociated to carbonyl compounds plus sulfite and that sulfite was then oxidized to sulfate in the liver extracts we were studying (1). Thus began my long infatuation with sulfite oxidation.

It had already been established that sulfite was readily oxidized by a free radical chain mechanism, but I did not know this and had to discover it for myself. Thus I found that sulfite could reduce cytochrome c and, of course, at the same time cytochrome c could oxidize sulfite. When this was done anaerobically the stoichiometry was 2 cytochrome c reduced per sulfite oxidized, as expected. However, in the presence of dissolved O$_2$ thousands of sulfites were oxidized to sulfate in the liver extracts we were studying (1). Thus began my long infatuation with sulfite oxidation.

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\begin{align*}
\text{SO}_3^{2-} + \text{Fe(III)} & \rightarrow \text{SO}_4^{2-} + \text{Fe(II)} \quad \text{(initiation)} \\
\text{SO}_3^{2-} + \text{O}_2 & \rightarrow \text{SO}_5^{2-} \quad \text{(Eq. 1)} \\
\text{SO}_5^{2-} + \text{SO}_3^{2-} & \rightarrow \text{SO}_5^{-} + \text{SO}_5^{2-} \quad \text{(Eq. 2)} \\
\text{SO}_5^{-} + \text{H}_2\text{O} & \rightarrow \text{SO}_4^{2-} + \text{H}_2\text{O}_2 \quad \text{(formation of products)} \\
\end{align*}
\]
The truly impressive amplification provided by this chain reaction fascinated me and so I played with it to see what it could be used for. Sulfite oxidation ultimately provided: an ultrasensitive manometric assay for xanthine oxidase (2); a method for detecting long lived flavin radicals generated photochemically (3); a method for ultrasensitive manometric activity (4); and much more besides.

**Xanthine Oxidase**

It was sulfite oxidation that led to xanthine oxidase, which in turn led to superoxide and to the superoxide dismutases. Thus a soluble fraction of liver, able to catalyze the oxidation of sulfite, lost this activity upon dialysis, and adding back the concentrated dialysate restored that activity. A search for the dialyzable cofactor of sulfite oxidation yielded hypoxanthine (5). Because hypoxanthine is a substrate for xanthine oxidase we were led to that enzyme. In time we realized that xanthine oxidase, when acting on its substrates aerobically, could initiate the oxidation of sulfite (2). This should have told me that xanthine oxidase was generating a radical from O\(_2\), and that radical (O\(_2^+\)) was initiating the oxidation of sulfite. However, I was woefully slow and that illumination was not to be achieved for several more years.

**Cytochrome c Reduction**

Xanthine oxidase was a fascinating enzyme in its own right with several prosthetic groups and broad specificity with regard to both electron donors and acceptors. One of its intriguing properties was the ability to cause the O\(_2\)-dependent reduction of cytochrome c (6). It had been suggested that H\(_2\)O\(_2\), produced from O\(_2\), was the reductant of cytochrome c, and this explained the O\(_2\) dependence of cytochrome c reduction. That was easily shown not to be the case. While studying this cytochrome c reduction I noted that some preparations of cytochrome c were rapidly reduced whereas others were not. Moreover the samples of cytochrome c that could not be reduced by xanthine oxidase inhibited the reduction of those that could be reduced and did so without interfering with the reduction of O\(_2\) by the xanthine oxidase. This inhibition, which was incorrectly attributed to a myoglobin impurity (7), was actually because of superoxide dismutase present in some of the preparations of cytochrome c.

The oxygen requirement for cytochrome c reduction was explored, and the \(K_m\) O\(_2\) for cytochrome c reduction was found to be 30 times greater than was \(K_m\) O\(_2\) for O\(_2\) reduction (8). This apparent discrepancy would be understood years later (9) as the increase in the proportion of univalent over divalent reduction of O\(_2\) as the pO\(_2\) was increased.

There were other indications that the reduction of cytochrome c was different in kind than the reduction of O\(_2\). Thus a catechol disulfonate (Tiron) was found to inhibit cytochrome c reduction by xanthine oxidase while having no effect on net O\(_2\) reduction (8). This is now understood as being due to the scavenging of O\(_2^+\) by catechols. At the time, O\(_2\) was conceived of as facilitating the reduction of cytochrome c by serving as an electron transporting bridge between the enzyme and the cytochrome. Free O\(_2^+\) could not easily be proposed because its redox potential was thought to be very negative making its production thermodynamically unfavorable (10).

**Chemiluminescence**

The xanthine oxidase reaction had been reported by John Totter (11) to elicit luminescence from lucigenin, and oxygen radicals had been proposed to play a role. When L. Greenlee (12) joined the laboratory he was asked to explore this lucigenin luminescence. Greenlee used an old Farrand fluorimeter with its light turned off as a photometer, and he worked in a darkened room so that he could observe light emission while making additions to the reaction mixtures. O\(_2\) was found to be essential for luminescence, but the intensity of the light produced was greatest when the reaction mixtures were equilibrated with \(\sim 2\%\) O\(_2\). Either more or less O\(_2\) diminished light production. This, with additional data, was taken to mean that light resulted when O\(_2^+\) reacted with reduced lucigenin (12).

**A Diversion to Acetoacetic Decarboxylase**

Work with xanthine oxidase was interrupted by a year long sabbatical spent with F. H. Westheimer in the Department of Chemistry at Harvard University. At our first meeting he invited me to work on anything I chose to do, but I wanted to explore some of his interests, which included decarboxylation. Consequently I started to work on acetoacetic decarboxylase. Frank Westheimer is a superb chemist and a wonderful teacher and I learned a lot during that year.

The department was well equipped for organic synthesis but did not contain a single
spectrophotometer. Roberta Colman, who was also working on the acetoacetate decarboxylase, was using Warburg microrespirometers to assay the enzyme. I was determined to avoid that laborious method and envisioned a spectrophotometric assay. When I brought this thought to Frank he doubted the feasibility of a spectrophotometric assay but nevertheless purchased a very good spectrophotometer for me that very day. Before the week was out I had a workable and convenient assay (13). One of the things I noticed at the outset was the inhibition of acetoacetic decarboxylase by monovalent anions, such as the halides. By happenstance Roberta Colman had been working in a buffer that contained enough chloride to very substantially inhibit the enzyme. When I was introduced to her husband some weeks later he said “you’re the monster who made Roberta redo all of her kinetic studies on the decarboxylase.”

More Diversions

When I returned to Duke Medical School, Philip Handler indicated that I should quit working on xanthine oxidase and branch out into other areas. Hence during the early 1960s I followed up on several chance observations I had made previously. One of these that intrigues me to this day is the ability of amines to markedly extend the range of pH over which horseradish peroxidase is optimally active (14). I am still hoping that someone will reinvestigate this and provide a mechanism.

Steady state kinetic studies of enzyme action were then fashionable, and I had noticed that urea and guanidinium could competitively inhibit xanthine oxidase and could do so at concentrations far below those needed for unfolding proteins (15). The inhibitory power of guanidinium salts was found to vary markedly from lot to lot. I then isolated the symmetrical triazine that was responsible for this variability (16). While working with triazines I found some that were powerful inhibitors of uricase (17) and was pleased to see this information applied to raising the urate level of mice by feeding them my uricase inhibitor (18).

Acetoacetic decarboxylase was not entirely abandoned, and we found that the activity of the isolated enzyme could be irreversibly doubled by mild heating (19). We could offer no explanation for this thermal activation, but it now seems possible that self-splicing was involved. This process, in which a sequence of amino acids is removed from a protein, has been reported several times. The piece removed in this self-splicing is termed an intein. An incident that reveals the stature of Frank Westheimer deals with this autoactivation of acetoacetic decarboxylase. After exploring the phenomenon I submitted a descriptive manuscript to the Journal of Biological Chemistry. The editor (John Edsall) sent it to Westheimer for review, who called me to say that he had the manuscript; that he had noticed the same autoactivation; that I was somewhat ahead on this project; and that he was recommending publication without revision. Would that everyone was as honest and generous as Frank Westheimer.

On to Superoxide Dismutase

All the foregoing pales to insignificance beside the discovery of SOD.¹ That xanthine oxidase might univalently reduce O₂ had been enunciated, and the differences between the reduction of O₂ to H₂O₂ and the roles of O₂ in mediating cytochrome c reduction, initiating sulfite oxidation, and eliciting lucigenin luminescence had been noted. However, such were the misconceptions among radiation chemists and physical chemists concerning the properties of O₂ that it seemed foolhardy to propose free O₂ as a product of the xanthine oxidase reaction. A fallback position that could still explain our many observations was to propose bound O₂. In that case cytochrome c would have to bind to xanthine oxidase, as would the protein competitive inhibitors of cytochrome c reduction.

At this point Joe M. McCord joined my laboratory as a graduate student and was asked to measure that presumed binding. After several heroic efforts provided no evidence for binding he decided to prove that there was no binding. He asked me whether Km was independent of enzyme concentration. I responded that it was, so long as substrate concentration exceeded enzyme concentration. With that assurance he produced kinetic data showing that Km xanthine was, as expected, independent of the concentration of xanthine oxidase. In contrast Km cytochrome c or Kᵢ for the inhibitors of cytochrome c reductions was very much a function of the concentration of xanthine oxidase.

To this day I recall the stunning impact of that data. Previous misconceptions were swept away, and it was immediately clear that xanthine oxidase was releasing O₂ into free solution, where it could reduce cytochrome c, initiate sulfite oxidation, or be intercepted by the protein.

¹ The abbreviation used is: SOD, superoxide dismutase.
inhibitors of cytochrome c reduction. It was also clear that those inhibitors of cytochrome c reduction must be acting catalytically, and the only feasible way they could do so was by dismutating $O_2^-$ into $H_2O_2 + O_2$ (20). Bravo Joe McCord!

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$  \hspace{1cm} (Eq. 5)

Now we knew exactly what to do, and the SOD activity in bovine erythrocytes proved to be abundant and stable and it was soon purified (21). Moreover its activity was demonstrated using electrochemically generated $O_2^-$ in place of the flux of $O_2^-$ made by xanthine oxidase and using tetranitromethane in place of cytochrome c. Joe and I felt like kids in a toy shop. We could and did use the newfound SOD to explore the role of $O_2^-$ in diverse reactions (22, 23) and to measure the effect of reaction conditions on the proportion of univalent over divalent $O_2$ reduction by xanthine oxidase (9). We recognized that our Cu,Zn-SOD had long been studied by others as an abundant cuproprotein of unknown function and given the names hepatocuprein, cerebrocuprein, hemocuprein, etc. We had found the biological activity of the “cupreins,” and I could sympathize with those protein chemists who must have felt cheated. A similar situation arose when J. M. McCord and B. B. Keele, Jr. isolated the SOD from Escherichia coli (25) and Richard Weisiger isolated a very similar SOD from mitochondria (26). These SODs contained Mn(III) and were undeniably the same as the avimanganin that had been isolated and studied by Michael Scrutton, again as a metalloprotein of unknown function.

Knowledge of the sequence and of the structure of proteins has long been seen as useful for deducing evolutionary relationships and mechanisms of action. The sequences of the Cu,Zn-SOD and of the Mn-SOD (to be discussed below) were determined by the laborious methods then available (27), and the homologies in sequence that bespoke a common evolutionary origin for the mitochondrial and bacterial SODs were determined (28). A sample of the Cu,Zn-SOD was given to our in-house crystallographers, David and Jane Richardson. They and their students determined its structure by methods that are still “black box” to me (29, 30). I revealed my complete ignorance of the methods to the Richardsons by asking for the structure only 3 weeks after I had given them the protein sample. They informed me that crystallization was proceeding at its own stately pace and it would take a bit longer. It took a few years longer.

### Physiological Function of SODs

If $O_2^-$ could be made in quantity by xanthine oxidase, it seemed obvious that it would be made by other enzymes as well, and if it could readily cause the oxidation of sulfite and of epinephrine, it could surely cause unwanted oxidations within cells. If SODs were so active and abundant, it seemed to us that they must serve as a defense against $O_2^-$, much as catalases defend against $H_2O_2$. Those views demanded support, which was soon forthcoming. Thus a survey of microorganisms revealed that aerobes contained abundant SOD, whereas obligate anaerobes contained little or none (31). In addition SOD was seen to be induced by aerobic growth (32), and the induced level of SOD protected against the lethality of hyperbaric $O_2$ (33). More definitive evidence was provided by the phenotypic deficits of the SOD-null mutants produced by Danielle Touati (34, 35). In the fullness of time SOD-null mutants were prepared in a variety of prokaryotes, in yeast, and then in mice.

### Pulse Radiolysis

Pulse radiolysis seemed to be the ideal method for directly assaying the activity of SOD and for measuring the rate constant for the SOD/$O_2^-$ reaction. Perusal of the literature of pulse radiolysis suggested that Joseph Rabani might be a willing collaborator. He was working at the Hebrew University in Jerusalem but made periodic trips to the United States to collaborate with someone at Johns Hopkins University. We arranged a meeting and he was willing to study SOD by pulse radiolysis. However this was the time when terrorists were sending letter bombs, and Israelis were particular targets. Rabani advised me that my shipment of SOD would be held up unless I could make it look innocuous, even unimportant. I achieved these criteria by injecting a concentrated solution of Cu,Zn-SOD into a 10-foot length of 1-mm polyethylene tubing. The ends were then sealed with 5-min epoxy, and the tubing, formed into a flat coil, was taped into a reprint. This reprint, in an unsealed envelope, was delivered to Rabani’s laboratory without incident. Given that the enzyme had not been refrigerated for over a week while enroute, I have always regarded the rate constant of $2.3 \times 10^9 \text{M}^{-1} \text{s}^{-1}$ (36) as a lower limit because the Cu,Zn-SOD, albeit a stable protein, must have suffered some loss of
activity enroute to Jerusalem. Moreover, the specific activity of the sample sent to Rabani was 3,200 units/mg, and we have since prepared samples with an activity of 5,000 units/mg.

**An Iron-containing SOD**

We had seen the blue-green Cu,Zn-SOD from erythrocytes and the reddish Mn-SOD from *E. coli* and from liver mitochondria. Yet it was clear that there was another SOD in *E. coli*. This was revealed by the activity stain devised by Charles Beauchamp (37). Fred Yost launched into the isolation of this new *E. coli* SOD. As he approached the successful isolation of this SOD I noticed that the normally outgoing and friendly Fred Yost was going out of his way to avoid me. When I asked his good friend Mick Gregory about this I was told that Fred’s SOD had the wrong color and he was afraid I would not like this result. I was delighted with this new SOD, which proved to contain iron (38). In *E. coli* the Fe-SOD proved to be constitutive and was present in aerobic or anaerobic cultures. The Mn-SOD, in contrast, was expressed only in aerobic cultures and was subsequently shown to be regulated as part of the soxRS regulon (39, 40).

**Paraquat**

During 1975 I had the good fortune of encountering Hosni M. Hassan. He proved to be unusually insightful and productive, and we published many excellent papers together. The one that stands out in my memory dealt with the effect of paraquat on *E. coli* (41). Hosni showed that this widely used herbicide caused a marked induction of Mn-SOD, which rendered the cells resistant toward the toxicities of O₂ and of the quinone streptonigrin. Because paraquat also increased cyanide-resistant respiration it seemed obvious that paraquat engaged in a cycle of alternate reduction and autoxidation within *E. coli*. This would increase the production O₂ while providing a cyanide-insensitive route for electron flow to O₂.

A wide variety of quinones and dyes were subsequently shown to behave similarly. Enhanced sensitivity to paraquat has become a routinely observed phenotypic marker for SOD-null mutants. If the toxicity of paraquat is largely a reflection of the O₂ whose production it mediates, then paraquat toxicity should be dependent upon O₂, and that is the case. Moreover, preventing the up-regulation of Mn-SOD with puromycin should greatly increase that toxicity, and that too is the case (42).

**Two Catalases in *E. coli***

Having described the SODs in *E. coli* that eliminate O₂, it seemed incumbent upon us to examine the catalase that disposes of the H₂O₂. This was undertaken by Al Claiborne who soon found that *E. coli* makes two catalases. One of these was only a catalase whereas the other was a dual catalase/peroxidase (43, 44). We referred to them as hydroperoxidases I and II, and they were subsequently studied in detail by Peter Loewen and his associates.

**A Non-enzymic SOD**

Fred Archibald came to my lab in 1980. He was a very fine microbiologist and could repair anything from automobiles to spectrophotometers. We had previously seen that *Lactobacillus plantarum* lacked SOD yet was aerotolerant (45). Fred was asked to examine this organism. Fortunately he was new to the laboratory and was not familiar with our usual practices. Thus, after only a few days of work he asked me why I had asserted that *L. plantarum* lacked SOD. He had found abundant SOD activity in the extracts of this organism. Ever mindful of possible interference by metal contaminants, I asked him whether he had dialyzed those extracts and included EDTA in the assay buffer. Fred’s response was negative on both counts.

It quickly developed that *L. plantarum* contained a dialyzable, heat-stable, and EDTA-sensitive SOD activity. Given that this organism grows best on manganese-rich medium, it did not take long to show that manganese was accumulated to an intracellular concentration of ~25 mM and that non-enzymic Mn(II) was, in fact, serving as the functional replacement for SOD (46, 47). The stability of the activity to boiling had ruled out a Mn-SOD with a dissociable manganese.

**An Ultrasensitive Assay for SOD**

We were asked to examine both mycoplasmas and methanogens for their content of SOD. It was apparent that we would need an assay more sensitive than any we had previously devised. The usual assays depended upon competition between SOD and some indicating scavenger of O₂. If the indicating scavenger had a low rate of reaction with O₂ the assay was more sensitive. Suppose that the indicating scavenger could be omitted? The assay finally devised used the
xanthine oxidase reaction to establish a steady state level of $O_2^-$ and only then to add cytochrome $c$ as the indicating scavenger. There was a sudden increase in $A_{550\text{ nm}}$ that reflected the steady state level of $O_2^-$. SOD revealed its activity by decreasing the sudden increase in $A_{550\text{ nm}}$. This strategy provided an $\sim$100-fold increase in sensitivity (48).

**Lactobacillus plantarum Again**

In the 1960s E. A. Delwiche working at Cornell University reported that *L. plantarum* contained a CN$^-$-sensitive catalase when grown on heme-enriched medium but produced a CN$^-$-insensitive catalase when grown in the absence of heme. Delwiche named the latter enzyme pseudocatalase. We isolated it and found a manganese-containing catalase (49) that was essential for the survival of this organism in aerobic stationary phase (50). This enzyme was subsequently studied by x-ray crystallography and shown to contain a binuclear cluster of manganese atoms at its active site.

**An Old Bottle of Glycerol**

During the summer of 1980 my daughter Sharon was working in my laboratory. Her project was to isolate an SOD from plant mitochondria. Each morning on the way to the laboratory we stopped at a market and purchased a head of cauliflower. Because Sharon’s goal was the mitochondrial Mn-SOD, she included CN$^-$ in the assay mixtures to suppress activity due to the Cu,Zn-SOD. After a while she found that the plant Mn-SOD was unstable and I suggested that 10% glycerol might stabilize it. Sharon soon reported that CN$^-$ plus glycerol could reduce cytochrome $c$. The only possible explanation seemed to be an impurity in the glycerol, which had been on the shelf for over 10 years. Glycerol from a fresh bottle did not support the reduction of cytochrome $c$ so we tried an oxidation product of glycerol, namely glyceraldehyde. CN$^-$ plus glyceraldehyde or a variety of other $\alpha$-hydroxycarbonyls could reduce cytochrome $c$ or $O_2$ (51). This seemed bizarre because CN$^-$ had been used with such compounds to synthesize sugars for over a century. Had no one noticed the oxidation? Perusal of old literature on the Fischer-Kiliani synthesis, in which aldose sugars were incubated with CN$^-$ yielding a cyanohydrin that could then be hydrolyzed and then reduced to the isomeric pair of longer chain sugars, finally led to a paper in which the author specified filling the flask to the top and sealing it tightly with a rubber stopper before incubating it overnight. He must have noticed a decrease in yield when these simple means of excluding oxygen were not used. Subsequent study of the mechanism of this cyanide-catalyzed oxidation of sugars revealed a role for enediol tautomers and radical intermediates (52, 53). This chemistry is now pertinent to the process of non-enzymic glycation thought to be important in diabetes mellitus and in aging.

**How Super Is Superoxide?**

Increasing intracellular production of $O_2$ by raising $pO_2$ or by addition of redox cycling compounds or by mutational deletion of SOD imposes nutritional auxotrophies, such as the need for branched chain amino acids. O. R. Brown suggested (54) that this was because of inactivation of the dihydroxyacid dehydratase that catalyzes the penultimate step in the relevant biosynthetic pathway. The possibility that $O_2^-$ could directly inactivate this dehydratase was exciting to us because there were still those who were asserting that $O_2^-$ was a biologically benign species. We found that $O_2^-$ did inactivate this enzyme (55) and other [4Fe-4S] cluster-containing dehydratases, such as the 6-phosphogluconate dehydratase (56) and aconitase (57).

Because oxidation of the [4Fe-4S] clusters of dehydratases causes loss of iron and because “free” iron can react with $H_2O_2$ to produce $HO_2^-$, that could lead to widespread damage to DNA, proteins, and membranes. This would moreover provide an explanation for the oft reported damaging synergism between $O_2^-$ and $H_2O_2$. Soon after we proposed this (58) it was supported by experimental data (24). There is an interesting difference between the *in vitro* and *in vivo* ways by which $O_2^-$ and $H_2O_2$ interact to produce $HO_2^-$. Thus, in the test tube, $O_2^-$ served as a reductant for adventitious Fe(III) or Cu(II), and the resultant Fe(II) or Cu(I) then reduced $H_2O_2$ to $HO^- + HO^-$ Such a mechanism was simply not plausible in the reducing environment of the cell, which would keep any free iron or copper in their reduced states. This problem was solved by the finding that *in vivo* $O_2^-$ serves to provide free Fe(II) through its oxidative attack on the [4Fe-4S]-containing dehydratases.
Motivation and Funding

I would like to stress that the work that led to the discovery of the superoxide dismutases did not seem, at the time it was being pursued, to have any relevance to human health or disease. It was merely interesting, and we were motivated purely by curiosity. At the time, that was enough justification and one could get funding from the National Institutes of Health to support such work. Alas that is no longer enough and one has to envision health relevance or not get funded.

Curiosity about the chemistry of life continues as do the experiments to satisfy that curiosity. In recent years we have been: exploring the role of oxidative stress in heat shock and stationary phase death; finding a Cu,Zn-SOD in the periplasm of E. coli; clarifying the subcellular distribution of SODs in liver cells; making low molecular weight catalysts of the dismutation reaction that may be useful as pharmaceuticals for treating reperfusion injuries and inflammations; exploring the role of oxygen-derived radicals in adaptive mutagenesis; explaining the nutritional auxotrophies imposed by lack of SOD activity; studying the basis of the oxygen-dependent toxicity of short chain sugars; adding to the list of enzymes that are known to be controlled by the soxRS regulon; and wondering whether the univalent oxidation of bicarbonate to the carbonate monoanion radical is a factor in oxidative stress. Curiosity remains undiminished at age 72 years and so there will by more—if health and strength allow.

Summary

Each of my published papers ignites a flood of memories of the students and postdoctoral fellows I worked with, of the competitors we contended with, and of the pleasures of achieving an understanding of something formerly hidden from view. I have here recorded only a small fraction of those memories, but that is enough to give the reader a feeling for what we did and how we did it. Hence I close now with gratitude to those co-workers whose efforts have been mentioned herein and with apology to those whose equally meritorious accomplishments were not. I should add that many talented scientists have been drawn to the study of the biology of superoxide, because it has proven to have relevance to diverse physiological and pathological processes. At the time of this writing there are several journals devoted to this field and a PubMed search of the term superoxide dismutase pulled up 20,067 references.

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