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BioMetals: a historical and personal perspective

Simon Silver

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Abstract Understanding of BioMetals developed basically from a starting point about 60 years ago to current mechanistic understanding of the biological behavior of many metal ions from protein structural and functional studies. Figure 1 shows a Biochemical Periodic Table, element by element, with requirements, roles and biochemistry of the specific ions indicated. With few exceptions, the biology is of the ions formed and not of the elemental state of each. Early BioMetals efforts defined nutritional growth needs for animals, plants and microbes for inorganic "macro-nutrients" such as magnesium, calcium, potassium, sodium, and phosphate and of "micronutrients" such as copper, iron, manganese and zinc. Surprises came early with regard to microbes, for example the finding that Escherichia coli (then and now the standard microbial model) grows happily in the apparent total absence of calcium, sodium, and chloride, which are certainly major animal nutrients. Some elements such as mercury and arsenic are never required by living cells, but are always toxic, often at very low levels. Therefore, the division into nutrient elements and toxic elements came soon. For most inorganic nutrients, excessive amounts can be toxic as well, for example for copper and iron.

S. Silver (✉)
University of Illinois, Chicago, IL, USA
e-mail: simon@uic.edu

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Understanding of BioMetals developed basically from a starting point about 60 years ago to current mechanistic understanding of the biological behavior of many metal ions from protein structural and functional studies. Figure 1 shows a Biochemical Periodic Table, element by element, with requirements, roles and biochemistry of the specific ions indicated. With few exceptions, the biology is of the ions formed and not of the elemental state of each. Early BioMetals efforts defined nutritional growth needs for animals, plants and microbes for inorganic "macro-nutrients" such as magnesium, calcium, potassium, sodium, and phosphate and of "micronutrients" such as copper, iron, manganese and zinc. Surprises came early with regard to microbes, for example the finding that *Escherichia coli* (then and now the standard microbial model) grows happily in the apparent total absence of calcium, sodium, and chloride, which are certainly major animal nutrients. Some elements such as mercury and arsenic are never required by living cells, but are always toxic, often at very low levels. Therefore, the division into nutrient elements and toxic elements came soon. For most inorganic nutrients, excessive amounts can be toxic as well, for example for copper and iron.

When I rotated as a beginning PhD student in Bert Vallee's metal spectroscopy laboratory at Harvard

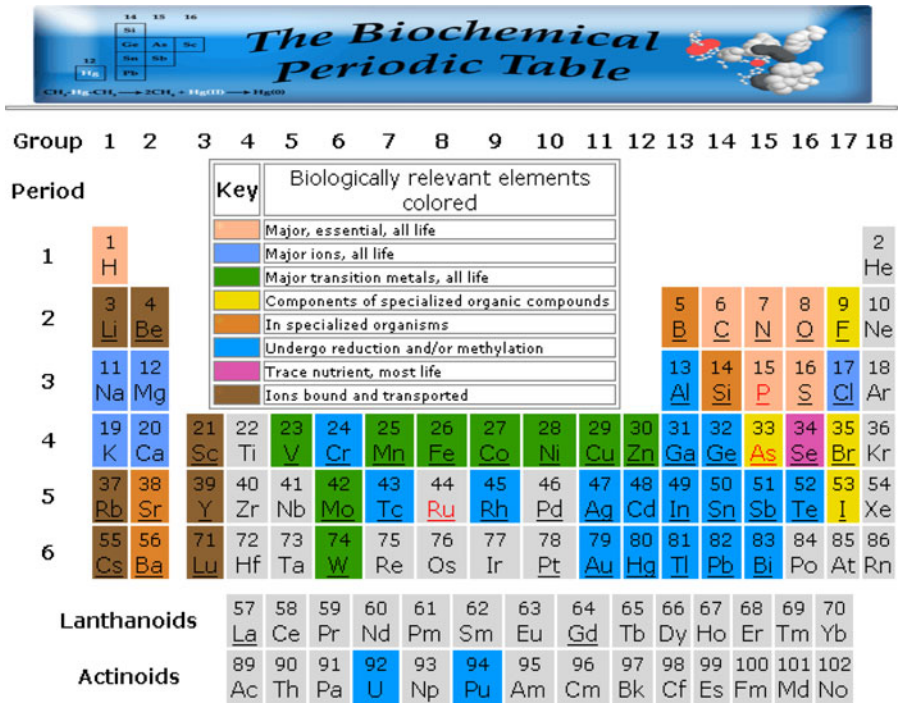

Fig. 1 A Biochemical Periodic Table. Courtesy of L. Wackett, University of Minnesota, with permission

Medical School, we never imagined the soon-to-be-discovered metallothionein binding protein or cation-specific P-type ATPase transport systems. Vallee died (age 90) only a month before the BioMetals 2010 meeting; if he wrote a personal history of BioMetals, it would have been quite different.

The broad subject matter of BioMetals has been addressed in reviews and monographs, most notably in the book series edited by Sigel and Sigel (1973–2005). Single monographs edited by Weinberg (1977) (in which we published what I believe were the first ever summaries of microbial Mg^{2+}, Mn^{2+} and Ca^{2+}; Silver and Jasper 1977; Jasper and Silver 1977; Silver 1977), Rosen (1978), Rosen and Silver (1987), Silver and Walden (1997) and Nies and Silver (2007) are some in which I have participated. *Annual Reviews of Microbiology* articles also covered many topics including toxic elements (e.g. Summers and Silver 1978; Silver and Misra 1988; Silver and Phung 1996); and there were numerous brief reviews in other journals and monographs.

The Editors requested a personal as well as scientific historic perspective over the last 50 years, so I will start with my serendipitous entrance to the subject, and move back and forth element by element, between initial discovery research and current understanding. Surprisingly (and once counter-intuitive for chemists), genetic analysis became the key entrance to bio-understanding of metals. This remains the case today. Chemists need to learn genetics, as much as biologists need to learn more chemistry (much as many biologists do not wish to).

At the start of the Molecular Biology revolution, different researchers undertook understanding of structure and function for basic components of all living cells, often using *E. coli* as the most productive model organism. Some studied DNA, others RNA, and still others protein synthesis, structure and function. By chance, I came to a life's effort studying "A bacterial view of the chemical Periodic Table" (see Fig. 1) and with the famous molecular biology bias that if one wants to understand all life, including humans and the African elephant, then one should study *E. coli* (since as J. Monod said the elephant and *E. coli* are basically the same; Fig. 2). This lesson has been remarkably useful for BioMetals, where similarities are frequent among mechanisms common to all living cells, including P-type cation membrane transport ATPases (Silver et al. 1993) and more recently arsenic methylation, shared from bacteria to humans (Qin et al. 2006).

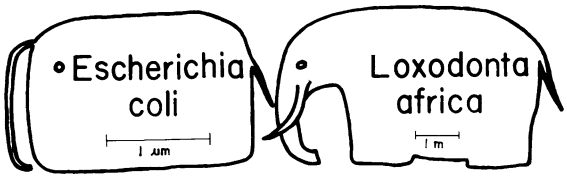

Fig. 2 *E. coli*, the elephant (S. Silver, unpublished; see e.g. Struhl 2002)

Quickly, it became apparent that bacteria have genes specific for transport of, metabolism of, or resistance to almost every element, 1 through 83, specifically, one by one. Genes become the basis for studying metabolism and sometimes toxicity, as there is microbial redox chemistry even for some higher-numbered actinides. One can argue about minor points, such as whether cadmium is used by rare marine microbes and that arsenic and antimony share genes, biochemistry and mechanism of toxicity, but the overall conclusion is that there is a "biology" of each of the first 84 elements (except for the Group 18 noble gases).

As a young postdoc, I wanted to find the physiological and biochemical basis for the rII mutations in bacteriophage T4, then the first genes to be mapped in great detail. Still today, we do not understand the basic mechanism of rII gene function. Mg^{2+} enabled rII mutant phage to grow (Garen 1961), which did not otherwise happen, especially in the presence of moderate levels of Na^{+}. In London UK, I hesitated to purchase $^{28}Mg^{2+}$, since it has a 21 h t1/2 and Amersham (UK) would not quote a price until they first made some. A couple of years later in Berkeley California (USA), money seemed less restricting and I found nothing about rII gene function, but instead found that *E. coli* has highly specific Mg^{2+} cation transport. The initial manuscript (later published as Silver 1969) was rejected, with the editorial comment that magnesium transport was not needed since everyone knew that magnesium freely passed across biological membranes. That was not my first journal manuscript rejection, which had been from Sydney Brenner (then Editor of *J. Mol. Biol.*), who accepted my accompanying manuscript on bacteriophage acridine resistance (Silver 1967), but turned down the first *E. coli* bacterial manuscript on what we now call the Acr (acridine and multidrug) efflux system as "not really the sort of stuff we want." Brenner suggested that I look for "a more appropriate journal such as *J. Bacteriol.*" So I went to the library to see that unfamiliar journal and a year later the report was published (Silver et al. 1968). Acr has since become the best understood of the 3-polypeptide (inner membrane, outer membrane, and periplasmic space spanning) chemiosmotic efflux pumps called RND for Resistance, Nodulation and Development. Our laboratory continued with Acr for a while, for example mapping the gene for the outer membrane protein TolC (Whitney 1971). Much later Nies et al. (1989b) completed the DNA sequence for the first of the RND cation efflux systems, CzcCBA (for Cd^{2+}, Zn^{2+}, and Co^{2+} resistance). Brenner was earlier and has remained a valued teacher. However on the wall, under glass above my desk, there is the anonymous saying:

> An Editor is a low form of Life
> Below the Viruses,
> And just above Academic Deans.

We were happily studying a new transport system and found it everywhere (in other bacteria, including Gram positives, in mammalian cells in tissue culture, and in intact *Arabidopsis* plants). The only cell that lacked Mg^{2+} transport was the mature human red blood cell (unpublished). It appears that Mg^{2+} transport shuts down during red blood cell maturation. This early work on *E. coli* Mg^{2+} transport benefitted from the collaborative competition with the laboratory of E. P. Kennedy at Harvard, initially with graduate student Joan Lusk and later with postdoc David Nelson (now better known as principle author of the "Lehninger Principles of Biochemistry" textbook). I acknowledge also human lessons learned from Gene Kennedy, who started science as a streetcar-commuting student near where I now live in Chicago. Later, the cation transport community and I benefitted greatly from Mike Maguire picking up the problem of Mg^{2+} transport, bringing it to the current elegant level of understanding (a small part of which he presented at BioMetals 2010).

Brookhaven National Laboratory dis-assembled the reactor in which they made $^{28}Mg^{2+}$. I started the manuscript that became Silver (1969) writing that I had found specific magnesium transport but that the experiments could not be reproduced since the radionuclide was not longer available—anywhere. Fortunately, reactor-produced $^{28}Mg^{2+}$ was replaced by $^{28}Mg^{2+}$ produced by triton bombardment on a

tandem Van der Graaf generator. Brookhaven produced $^{28}Mg^{2+}$ whenever two groups (Kennedy's and my own) ordered it for the same day (again a 21 h t1/2), so our two labs did basically the same experiments, on the same days, sometimes 6 times a year. Dave Nelson and I would exchange photo-copied raw data the following week. As Mike Maguire frequently reminds us, when the $^{28}Mg^{2+}$ price was increased from hundreds of dollars per shipment to perhaps $30,000, experimental work with $^{28}Mg^{2+}$ stopped. One more story of the informal cooperation of those days: once I received a phone call in my Berkeley lab from the Oakland CA airport that a $^{28}Mg^{2+}$ shipment was waiting for me to pick it up. I phoned Brookhaven since I had not ordered any and there was an airline strike at that time. Brookhaven said they had learned of an airplane coming to Oakland so they made some. If I did not want it, I need not pay. Can you imagine?

Mn^{2+} specific transport was next discovered serendipitously when $^{28}Mg^{2+}$ was not available. Mary Lou Kralovic suggested that Mn^{2+} was often considered a substitute for Mg^{2+} in activating enzymes and since $^{54}Mn^{2+}$ has a t1/2 of over 300 days, that might work. We bought some and had our first experience with gamma-emitters. We found a high-affinity, high-specificity Mn^{2+} transporter in *E. coli*, quite unrelated to Mg^{2+} transport (Silver and Kralovic 1969). Mn^{2+} transport is rather universal in all cells and now called NRAMP (Natural resistance-associated macrophage protein) in animal cells and MntH in bacteria (Richer et al. 2003). How lucky can one get?

Mn^{2+} was the first cleanly chemiosmotic-coupled cation transport system identified as functioning in subcellular membrane vesicles in *E. coli* (Bhattacharyya 1970). Pinaki Bhattacharyya was the first of several postdocs from India in our laboratory; sadly he died suddenly in June 2010. There was another memorable manuscript rejection (three times, as I recall) by Paul Boyer (then Editor of *Biochem. Biophys. Res. Commun.*), when I explained energy-inhibition patterns as consistent with Peter Mitchell's chemiosmotic coupling ideas. Boyer, who was then extremely anti-chemiosmosis, turned down our report as unsound, since "if the conceptual background make no sense, then the data supporting it do not matter" (an approximate paraphrase, now after 40 years). These were exciting times for BioMetals (protons are cations). Mitchell refused to attend a New York Academy of Science Symposium intended to celebrate his "victory in the proton wars" and Jennifer Moyle one afternoon in their laboratory in Cornwall (UK) in summer 1970 strongly criticized me as not being sufficiently chemiosmotic in our explanations of our cation transport results. We wish our younger people such excitement.

We moved on to Ca^{2+} transport and were frustrated that *E. coli* did not bio-accumulate significant levels of calcium. Instead *E. coli* appeared to efflux Ca^{2+} to lower intracellular amounts (Silver et al. 1975). The spore-forming Gram positive bacterium *Bacillus subtilis*, however, does accumulate massive amounts of Ca^{2+} in the spores, as part of the process of water dehydration and developing heat resistance. We lacked a good assay for efflux pumps, and at the 1974 American Society of Microbiology meeting in Chicago, after Kathy Toth presented preliminary data, Barry Rosen came up to her (as he had a good assay—inside-out membrane vesicle preparations—seeking a reasonable substrate). That night in a hotel room, Barry and I first met. He started work on bacterial calcium transport (Rosen and McClees 1974); beginning an interlab and personal relationship that has now lasted over 35 years.

Later in 1974, I started my first sabbatical leave, with Frank Gibson, Graeme Cox and Ian Young (although Ian was already dissociating himself) in Canberra Australia, in what could be considered the best iron siderophore lab in the world. Supporters of Joe Neilands (in Berkeley, California) and later Volkmar Braun and Klaus Hantke (in Tűbingen, Germany) may disagree. Gibson and coworkers had discovered, worked out the structures, and then the genetic basis for synthesis and transport of enterochelin (called enterobactin elsewhere) and aerobactin. Gibson and Cox had moved on to study H^+-driven ATP synthesis and had isolated the genes for each subunit of the F1F0 complex, thus persuading most mitochondrial worker skeptics that ATP synthesis was the same in bacteria, mitochondria and chloroplasts. I spent a happy productive year studying the β subunit of the ATP synthase F1 complex—the 1st of now nine times in Australia; I like the place. Barry Rosen was also studying *E. coli* ATP synthase in the USA, but he soon left that field, as it was highly competitive and really not cooperative.

Down the hall in Canberra, Harry Rosenberg was learning about Pit (inorganic phosphate transport) and

Pst (phosphate specific transport) in *E. coli* (see Cox et al. 1981; Torriani-Gorini et al. 1994). As Harry's mutants were either "Pitted" or Pst, when I soon needed to name the arsenic resistance genes, I selected *ars*, as it also sounds rather Aussie when spoken quickly with an Australian New South Wales accent. I tried to continue this pattern with *aso* for Le Phung's later arsenite oxidase genes (Silver and Phung 2005); however, that name did not last. I was the caricature Yankee, thinking "out of the box" (in the Yank baseball meaning of the phrase, which is quite different from that in Commonwealth cricket). I remember drawing F1F0 with a big black "H^+ ↑↓" on the lab frig door with an unerasable marker pen, at a time when Gibson and Cox still refused to think of the ATP synthase as being proton-driven.

About this time, and with some overlap between efforts on "normal" nutrient cations, we became involved with toxic heavy metal cations. It started when Anne Summers became enthusiastic about bacterial plasmids. As she was bored with the next stages of *E. coli* magnesium research, she suggested looking at the newly reported plasmid resistance to Ni^{2+}. After all, we had shown that Ni^{2+} was a competitive inhibitor of $^{28}Mg^{2+}$ uptake and $^{63}Ni^{2+}$a substrate for the system. So the plasmid arrived in the mail from David Smith, along with a note saying that Ni^{2+} resistance was not quantitatively striking, but that the plasmid had a determinant for strong resistance to Hg^{2+}. That began our interest in toxic metal resistance systems on plasmids. We bought our first $^{203}Hg^{2+}$ and had a completely wrong hypothesis that (if we were inventing resistance to mercury) a cellular polythiol would make sense. I was specifically thinking of a polythiol version of valinomycin, the polyhydroxyl small peptide that discriminates between K^+ and Na^+. Wrong ideas do not last long. Anne came down the hall quickly from her first $^{203}Hg^{2+}$ transport measurements with the very disquieting result that the background of the low-background planchet Geiger counter has gone up 1000×, from 3 to 3,000 cpm. We took the toy apart and it was clearly not electronic noise but radioactive contamination. It was not in the window of the gas flow counter, but in the actual walls of the counting chamber.

Anne and I went home distressed; and both of us came in the next morning with the same hypothesis: that *E. coli* has given $^{203}Hg^{2+}$ the ability to fly (like the flying elephant in the Walt Disney Dumbo films—again *E. coli*, the elephant!) and to amalgam into the counter tube wall. [The contaminated tube was placed in a drawer for a couple of years; >10 half-lifes.] It took Anne 6 months, clever thinking, and a friend in the Psychiatry Department (which had the first gas chromatogram/mass spec then at Washington University), to show convincingly that the product was Hg^0, volatile mercury gas (Summers and Silver 1972). This paper was also initially rejected for publication, on the advice of reviewers who understood the absurdity of bacteria making mercury gas. A range of confusing artifacts and additional experiments that might take years were suggested as needed before publication. In my opinion and experience, reviewers are often limited, when handling novel results for which there really is no peer knowledge. Actually, two other laboratories (Tonomura et al. 1968; Komura and Izaki 1971; by chance both in Japan) had discovered Hg^0 volatilization independently and earlier. But in those pre-Internet days, we did not know about their work until late in the writing-up stage. Being third is not always bad; claiming to be first is often a matter of opinion. I later became friends with both Tonomura and Izaki during visits to Japan, most recently in 2010 when Izaki (who has now retired) came to a celebration party at Tohoku University, where he had found mercury volatilization and where I now teach. Izaki says he stopped the mercury research, as just not able to maintain the pace of Anne Summers, whom they did not realize was a PhD student. Another message is that a great and independently thinking graduate student often can trump a more-senior less-focused group.

The work on mercury resistance took a different and very productive direction when Tapan Misra brought DNA sequencing to our laboratory, and indeed to our university campus; and we started collaborating with Nigel L. Brown in the UK. Misra et al. (1984) was the first of about a dozen primarily DNA sequence reports. It is not possible now to imagine the time back then when it took Tapan more than 6 months to generate the first kilobase pair of DNA sequence data. At one time the Misra lab at the University of Illinois—Chicago was one of four independent labs in Chicago working on the molecular biology of bacterial mercury resistance. Now there are none.

Cadmium (and later arsenic) research in my laboratory started a little as had mercury work, when Anne Summers was "tired of" magnesium. This time Alison Weiss, who did a lovely job starting *Staphylococcus* mercury work (Weiss et al. 1977), announced herself bored with mercury; and since arsenic resistance and cadmium resistance were on the same plasmid, pI258 (about 40 genes), why not study them?—and determine their basis for functioning. Her first report on cadmium resistance (Weiss et al. 1978) soon was followed by a series of papers from our laboratory on the mechanism of cadmium resistance, before Tynecka et al. (1981) demonstrated that the mechanism was energy-dependent efflux and not some form of reduced uptake. Zofia Tynecka arrived from Lublin Poland for a sabbatical year in our USA lab (on the recommendation of the same Frank Harold who had over a decade earlier forced my unwilling mind to take Peter Mitchell's chemiosmosis seriously). Zofia arrived with a manuscript with over 100 figures on radioactive cation transport, intended for *J. Bacteriol.* It took the better part of the year of hard writing to reduce her "life opus" to two *J. Bacteriol.* manuscripts (Tynecka et al. 1981). I am quite proud of the 22 publications by people working in my laboratory that have been published without my being listed as co-author, but generally mostly written by me. These two by Tynecka were a good example; Bhattacharyya (1970) and Whitney (1971) were others. It was a better standard that I grew up with: when the head of the laboratory did not put his name on all published reports. Now we have an inferior standard with counting numbers like McDonald's hamburgers. Numbers count but they are not a measure of quality. I worked with two PhD mentors and then two postdoc mentors, without ever having their co-authorship of any of the dozen papers I published from their laboratories. S.E. Luria (not me) submitted my first PhD thesis report, published as an article in *Nature*. However, he was not listed as co-author. He dominated the writing, as he was famous for doing. A few years later, when I asked Don Glaser to communicate my first *PNAS* paper, I gave him the draft manuscript with his name listed as co-author, as I was concerned about never having the head of the laboratory where I worked as co-author again and again. Don brought the manuscript back to the lab the next day, said he would communicate it; and the only change that he made was to remove his name as co-author (Silver 1965). He said "I wondered what you have been doing for the last year." Think about it: It is Watson and Crick (1953) and not Watson, Crick, Perutz, Kendrew and Bragg. It is Meselson and Stahl (1958) and not Meselson, Stahl, Delbruck, Pauling and Vinograd. If we could get back to those more romantic and optimistic patterns, our BioMetals research would benefit.

Our studies of bacterial arsenic resistance started when Bob Hedges and I went as we sometimes did to a West End London theatre at Christmas time in the late 1970s. I had brought him Christmas presents from the St. Louis (USA) sister to deliver to a Hammersmith Hospital (London, UK) endocrinologist neighbor of his. Bob said "Enough mercury" (we had recently published together in *Nature* the first solid report on plasmid-gene degradation and volatilization of mercury from organomercurials; (Schottel et al. 1974). Bob said he had just found plasmid resistance to arsenic and "you should explain it." I last saw Hedges (a wonderful hunter of new and novel bacterial plasmids in Gram negative bacteria) when we went together to see Jan van Eyck's altarpiece "The Adoration of the Lamb of God" in Ghent, shortly before he killed himself. Alison Weiss' name was not on the paradigm-changing arsenic report (Silver et al. 1981). It would have been nice to add it and then claim a minyan and ask for one figure per author.

There are a number of interesting stories associated with this publication, which was my only one as co-author with Gail Willsky (on the *BioMetals* Editorial Board). I claim that this report (Silver et al. 1981) started modern microbial arsenic research. All three "peer" reviewers wrote that the manuscript should not be published. One said that he or she was not technically qualified to review the details; and since everyone potentially a peer was already an author, then the manuscript could not be reviewed and thus could not be published. Another wrote that there was no purpose to publishing this paper, since all potential readers were already authors. We had nine authors in four countries on three continents; and the data and thinking were obviously mine. But before starting the experiments, I had the unpublished preliminary results from Gail Willsky (in Boston, USA) and Bill Shaw (in Leicester, UK). They pointed the right direction. Mike Malamy, who had been Gail Willsky's PhD mentor at

Tufts, wanted to remove his name as co-author, since the writing did not reflect his thinking. The conclusions did. We needed his Department chair to persuade Mike to leave his name as author, so that her name could stay—as she was happy with how solid a story this had become. Mike preferred three back-to-back preliminary notes to one solid paper.

I added Richard Novick (who did not need this) as co-author, realizing that we had published earlier on the basis of mercury and cadmium resistances in *S. aureus* with benefit of his mutant isolates and thinking, but without his co-authorship. It just seemed nice. And Richard's primary suggestion was to go to a different journal (*Plasmid*) where he was Editor in Chief, rather than the journal *J. Bacteriol.*, where S. Silver was Editor in Chief. You should have fun. Reviewers and subsequent readers noted that we had *E. coli* and *S. aureus* together in a single paper, rather than divided into two parallel papers. [A little later, for Nakahara et al. (1985) I wrote one mercury resistance paper with *Streptococcus* and *Streptomyces* in the title instead of two, and a reviewer commented that I probably did not know the difference.] In a McDonald's or White Castle world, numbers count rather than quality. Certainly university promotions committees and national grant proposal panels everywhere now count numbers. The key experiments in Silver et al. (1981) were pipetted with Harry Rosenberg during my second sabbatical leave in Canberra. Harry pipetted (as was his custom) and I was relegated to washing the radioactive filters and counting the samples. When I left Canberra, I rang Harry from the Sydney airport and told him that I had left a new experiment in the scintillation counter for his analysis. I do not know what he thought; they really were old vials being recounted just for fun. Please note that this long paragraph on what is considered a major paper concerns only the human aspects, and not the technical findings—which were major and several.

Two other human stories concern the entrance of Barry Rosen to microbial arsenic metabolism, on which he is undoubtedly the most important and productive contributor over the last three decades. Barry and I were sitting in the Washington National Airport, waiting for my plane to depart, when I was grouching about metabolic inhibitor studies of our then-new efforts on energy-coupling for arsenic efflux from *E. coli.* Barry immediately said "it is an ATPase" and soon later asked how I would feel if he started working on that system. It must have been my next trip to the Washington area, when I carried with me the needed bacterial strains and the first sample of radioactive $^{74}AsO_4^{3-}$. My middle son Andrew remembers today that as we approached the X-ray luggage screening device in the airport, I removed the heavy lead container from my carry-on luggage, removed the small plastic tube with maybe 0.1 ml liquid radioactive arsenate solution, and put the tube in my pocket, and the empty lead back in the suitcase. The then-13 year old child shook his head with understanding when maybe 3 m beyond the X-ray machine, I placed the tube back in the lead container for the rest of the trip. In those days (30 years ago) we transported bacterial strains and radioactivity more relaxed than now. When I moved from St. Louis to Chicago in 1986, the Washington University Radiation and Chemical Safety officer approved of and basically required me to carry in the trunk of my personal car those materials that a moving company would not carry: all the lab supply of radionuclides, with gamma emitters showing an allowed radiation level at the surfaces of the boxes, plus the sulfuric and nitric acid, and the benzene, toluene and other volatile solvents. The goal was to facilitate good science. Today we would call this a "dirty car bomb."

Another remarkable student Ji discovered the enzyme arsenate reductase (Ji and Silver 1992b), as the basis for arsenate oxyanion resistance and as the function of the *arsC* gene that we had much earlier sequenced (Chen et al. 1986). Two human stories are worth putting on paper here: Ji became involved in arsenic resistance after two dinners at the ASM meeting in New Orleans. Fritz Götz from Tübingen, Germany tried to persuade Barry Rosen to study the *S. aureus* arsenic resistance system, as his laboratory had sequenced a gene cluster from a different *Staphylococcus* species and editors would not publish the results. Barry was not interested, as it was not *E. coli*, so the next night they persuaded me to do it; and a few days later I asked Ji to take it over. Barry and I were skeptical of the Götz results, as Fritz' sequence lacked the *arsA* and *arsD* genes of our first *E. coli* plasmid (Chen et al. 1986). We thought something must be wrong with the Götz results, but instead we were wrong as Ji found the same three genes *arsR*, *arsB* and arsC (Rosenstein et al. 1992; Ji and Silver

1992a) rather than five genes. I was visiting Tübingen on a day when Fritz needed to go to the Rathaus for a debate on whether to make Tübingen a "recombinant DNA-free city." By the time he returned that evening from a hopelessly frustrating day, Andreas Peschel and I had completed the draft of their manuscript and the two manuscripts were submitted and published back to back. Cooperation is better than aggression.

A little later, Ji waited until I left for a trip to Japan to test his hypothesis that the bacteria might convert arsenate to arsenite (using the thin layer chromatography assays he had adapted to prepare radioactive arsenite for membrane transport studies). If he had asked me, I would have said it was a foolish hypothesis for bacteria to convert less toxic arsenate to more toxic arsenite. When I returned, he showed me the data; and within an hour, we had the hypothesis that it would be thioredoxin-linked; and within a few weeks, Ji had advanced from whole cell assays to cell-free enzyme assays and demonstrated the activity in vitro (Ji and Silver 1992b). We flew on a snowy winter day to Detroit and told the Rosen lab group these new results. ArsC was an enzyme. And since that time its crystal structure has been solved many times (initially by the Rosen lab) and its detailed mechanism understood (Silver and Phung 1996; Mukhopadhyay et al. 2002; Messens and Silver 2006).

A new story of arsenic enzymology, and one I consider to have a "brilliant" future (as the British slang use the word) is arsenic methylation by *S*-adenosyl methionine arsenite methyltransferase, which is a relatively small soluble enzyme found widely in living cells, from a few bacteria (Qin et al. 2006; but not *E. coli*) and Archaea, to many fungi, and mammals, including humans (Thomas et al. 2007). The enzyme and the series of reactions seem quite the same, even to the requirements of *S*-adenosyl methionine as methyl donor and probably glutathione as electron donor for the sequence of reactions (Mukhopadhyay et al. 2002; Thomas et al. 2007; Qin et al. 2006): arsenate [As(V)] + $2e^-$ → arsenite [As(III)] + CH_3^+ → monomethylarsonic acid [MAs(V)] + $2e^-$ → monomethylarsonous acid [MAs(III)] + CH_3^+ → dimethylarsinic acid [DMAs(V)] + $2e^-$ → dimethylarsinous acid [DMAs(III)] + CH_3^+ → trimethylarsine oxide [TMAsO(V)] + $2e^-$ → trimethylarsine [TMAs(III)] gas. Here was an example of "The elephant, *E. coli*", as identification of the bacterial gene and protein were dependent on earlier sequences from the mammalian enzyme (Qin et al. 2006).

I continued Johnny Appleseed-like to wander across the Periodic Table, occasionally planting an element that sprouted into a new subject for useful research in microbiology laboratories (as Anne Summers once nicely phrased it when introducing my talk at a Gordon Conference). I appreciate that. Anne Summers was the first "modern" worker on the *mer* mercury resistance operon in North American and remains four decades later the strongest person in this area. Carlos Cervantes wrote from Mexico that he wanted to come and work in Chicago on chromate. He did that and eventually obtained a UNAM (Mexico City) PhD based on Chicago work, when he was already an Associate Professor at Morelia. Carlos started studies of chromate resistance and efflux, and remains two decades later the strongest worker on this toxic oxyanion. Ohtake, more recently doing polyphosphate work in Osaka Japan, worked with Cervantes on chromate reduction and DNA sequencing (Cervantes et al. 1990). Dietrich and Anke Nies shared lab space with Carlos, and so they tested their *Cupriavidus metallidurans* strain CH34 (it had four earlier Genus and species names before the current one) for chromate resistance, efflux and the DNA gene sequence (Nies et al. 1989a, 1990).

My mercury colleague Nigel Brown once rang from the UK that he was going to Melbourne Australia to visit family. Could I suggest a lab to where he might escape on some days. That started Brown's very productive exchanges with the Melbourne University "copper mob", then led by B.T.O. Lee and Jim Camakaris (also at BioMetals 2010), which included extensive visits to Birmingham UK by Barry Lee, Duncan Rouch and Jill Williams, all from Melbourne. I never got deeply into copper work myself, but I helped write a couple of early papers, and hopefully was somewhat useful at the edges. I worked parts of 1992–1994 with the copper group at Melbourne Uni; and Le Phung worked with Julian Mercer at the Murdoch Institute at Royal Children's Hospital in Melbourne, a short walk from the University Genetics Department where I worked. Dame Elisabeth Murdoch (who is still around, age 101) had provided the endowment for the Murdoch Institute; and was then only in her 80s, Dame Elisabeth came to the lab occasionally to learn about the research progress being made. She is best known

as the mother of Australian-American newspaper and television mogul Rupert Murdoch.

Working with Julian made sense, since he was the molecular geneticist working on Menkes Syndrome disease, at the best place for this disease in the world. The head of the unit, David Danks, a gentlemanly Australian pediatrician, had been first to realize the connection between copper deficiency and the horrible lethal X-linked condition, Menkes Syndrome. That is another long story, but I did not understand at that time why Jim Camakaris pushed me across his patio to talk with David at a barbie at the Camakaris home. I did not understand why until the next year when Chris Vulpe (who had a poster and student at BioMetals 2010, but did not come himself) phoned me in Chicago from UCSF, where he was an MD/PhD student. We had maybe the most wonderful phone call of my life. Chris started "I have isolated a cDNA for a candidate gene for an obscure disease you never heard of" … "and most of the GenBank hits for related sequences come from your lab." I responded foolishly "Oh yes, I have heard of Menkes and I had a similar phone call 2 weeks ago." Chris correctly deduced that one of his three-possible competitors had also been successful using the same logic to isolate the gene defective in Menkes boys. That was Tony Monaco (in Oxford, UK) who first phoned me. But Tony had only approximately 2 kbp of the 5′ of the Menkes gene cDNA, encoding the N-terminal 6 metal-binding domains similar to that on our bacterial Cd^{2+} efflux ATPase (Nucifora et al. 1989) and our MerP small periplasmic mercury-binding protein (Silver et al. 1993). Chris had the whole 4.5 kbp thing. I had advised Tony that he undoubtedly had a gene for a P-type ATPase and that if he continued a few hundred more base pairs, he would leave the determinant of the cation binding domain for the ATPase and transport domain. Of course, I could not tell Chris Vulpe who the earlier phone call had been from, but he knew the possibilities. When Chris told me point by point what he was thinking, I happily agreed that he had already himself come to all the interesting conclusions.

This was a great surprise, since when we were last in Melbourne, no one had a clue as to what the biochemical basis was for the copper nutrition defect in Menkes. I thought it might have something to do with metallothionein, a binding protein, and certainly not a membrane transport ATPase. I told Chris Vulpe that I had been with the Melbourne copper mob the year before—actually the bacterial group and not the mammalian copper group—and that I would soon return. He hesitated again as to whether he should have called me and what to say. But it was an easy call, as both of us understood there was a race. A couple of days later, I got a FAX from Jim Camakaris as to whether Le and I would take the flat they had tentatively rented for our visit. I responded "Yes, and does Julian know the recent Menkes excitement?" That was all I needed to say, as Julian Mercer had spent the previous year on sabbatical in Ann Arbor Michigan, where the needed cell line from translocated female X-chromosome mutation (broken in the middle of the Menkes gene) had originated. All four competing labs apparently were using the same Ann Arbor female patient's chromosomes to FISH (fluorescence in situ hybridization) with candidate Menkes gene cDNAs as probes. If I remember correctly (and I was not in Melbourne), Julian had just returned from Ann Arbor with the method working but without isolating a candidate cDNA. They had used an inappropriate (poor but commercially available and easy) cDNA library and therefore had not been successful. David Danks received my message, as Julian was not there; David and Jim persuaded Julian to keep trying and it was only a matter of days, knowing that the logic and approach were correct, for him to have the Menkes cDNA. I never found out who was the fourth group seeking to FISH Menkes cDNA; in Italy, I believe. They apparently got a false positive, the wrong cDNA, which is not hard to imagine. When writing up for publication, when three groups had the same cDNA, and it made beautiful sense, but the fourth was different, they fell by the side of the road. My final role here was to push hard for three back-to-back reports in *Nature Genetics* (1993), as the best way for interested readers to learn about the genetic basis of Menkes. A rush to press in different journals would have been ugly.

A further related story was that Le Phung in Chicago had just found one of the first bacterial copper transport ATPase genes in a cyanobacteria strain (Phung et al. 1994), basically as a PCR artifact. What she found was unrelated to (but more interesting than) what she had been seeking. That meant it was sensible for her to work in the Menkes lab in Melbourne that year. The scientific world is now larger than 50 years ago, but it still is surprisingly

small. When Le's new (and then partial) gene for a P-type ATPase looked closest to one from Marc Solioz (a speaker on copper at BioMetals 2010) in Switzerland, I asked Wolf Epstein, our local expert of these systems. Wolf told us that Marc's ATPase was for copper, but had on reasonable basis been misidentified as probably for potassium. That was quickly corrected. Le found another surprise gene (the two were back to back on the chromosome) and came home one evening and said "What should I do? I have this new sequence which when translated is homologous to an unknown gene product from an unfamiliar bacterium with the PI J.I. Rood." I laughed and suggested a FAX from her to Julian Rood at Monash University (also in Melbourne, 25 km apart) saying "Dear Julian, I am glad that we are to come to your house for dinner in 2 weeks…. I just found a gene … can you explain it?" I still do not know the function of that gene.

Our most recent contribution to a molecular biology of toxic metal cations began when K. Matsui undertook as a thesis topic isolating the genes for plasmid resistance to Ag^+ in *E. coli*. As earlier, a determined graduate student can succeed (Gupta et al. 1999), where in this case I had previously failed in a more biochemical and microbiological approach. Matsui found nine contiguous genes involved in silver resistance. This was our first effort at machine rather than film sequencing, and our longest at that time. Progress has been reviewed by Silver (2003) and Silver et al. (2006). The nine genes are transcribed into three mRNAs and encode a novel Ag^+ P-type ATPase, a three polypeptide SilCBA chemiosmotic efflux system (similar to our earlier Cd^{2+}, Zn^{2+}, Co^{2+} CzcCBA system; Nies et al. 1989b), a two-component sensor kinase/responder SilRS system, and two periplasmic proteins, SilE (an unusual 10 histidine small polypeptide that binds 5 Ag^+ cations cooperatively) and SilF (a component of the SilCFBA system, which we initially missed as a gene, since the size was so small, but that was identified in the subsequently found *E. coli* chromosomal homolog, now called CusCFBA). The editor M. McEvoy solved the structure of CusF. SilE was purified and studied in detail in an unpublished PhD thesis of J.F. Lo, another excellent graduate student; and the results summarized by Silver (2003) and Silver et al. (2006).

In summary, from a starting point when Life was thought to be just about the biochemistry of C, H, N, and O (maybe with some P and S) incorporated into bio-molecules, we have over ½ a century learned the biology of essentially all of the elements up through #83 or 84 (Fig. 1). The killing of a former Russian "Security Agent" with polonium (#84) in 2006, maybe extended interest still one more element. The Russian's spokesman before and after his death was Alex Goldfarb, a researcher on *E. coli* RNA polymerase, and the son of my phage T4 acridine resistance friend, David Goldfarb. Each element, as its ionized cations and anions, can be understood as playing useful biological functions or showing toxicity that calls for bio-resistance mechanisms. Each of these has a specific genetic and biochemical basis, which often is best understood in terms of function/structure relationships of the proteins responsible.

References

Bhattacharyya P (1970) Active transport of manganese in isolated membranes of *Escherichia coli*. J Bacteriol 104:1307–1311

Cervantes C, Ohtake H, Chu L, Misra TK, Silver S (1990) Cloning, nucleotide sequence and expression of the chromate resistance determinant of *Pseudomonas aeruginosa* plasmid pUM505. J Bacteriol 172:287–291

Chen C-M, Misra TK, Silver S, Rosen BP (1986) Nucleotide sequence of the structural genes for an anion pump. The plasmid-encoded arsenical resistance operon. J Biol Chem 261:15030–15038

Cox GB, Rosenberg H, Downie JA, Silver S (1981) Genetic analysis of mutants affected in the Pst inorganic phosphate transport system. J Bacteriol 148:1–9

Garen A (1961) Physiological effects of rII mutations in bacteriophage T4. Virology 14:151–163

Gupta A, Matsui K, Lo JF, Silver S (1999) Molecular basis for resistance to silver cations in *Salmonella*. Nat Med 5:183–188

Jasper P, Silver S (1977) Magnesium transport in microorganisms. In: Weinberg ED (ed) Micro-organisms and minerals. Marcel Dekker Publishers, New York, pp 7–47

Ji G, Silver S (1992a) Regulation and expression of the arsenic resistance operon from *Staphylococcus aureus* plasmid pI258. J Bacteriol 174:3684–3694

Ji G, Silver S (1992b) Reduction of arsenate to arsenite by the ArsC protein of the arsenic resistance operon of *Staphylococcus aureus* plasmid pI258. Proc Natl Acad Sci USA 89:9474–9478

Komura I, Izaki K (1971) Mechanism of mercuric chloride resistance in microorganisms. I. Vaporization of a mercury compound from mercuric chloride by multiple drug resistant strains of *Escherichia coli*. J Biochem 70:885–893

Messens J, Silver S (2006) Arsenate reduction: thiol cascade chemistry with convergent evolution. J Mol Biol 362:1–17

Misra TK, Brown NL, Fritzinger DC, Pridmore RD, Barnes WM, Haberstroh L, Silver S (1984) Mercuric ion-resistance operons of plasmid R100 and transposon Tn501: the beginning of the operon including the regulatory region and the first two structural genes. Proc Natl Acad Sci USA 81:5975–5979

Mukhopadhyay R, Rosen BP, Phung LT, Silver S (2002) Microbial arsenic: from geocycles to genes. FEMS Microbiol Rev 26:311–325

Nakahara H, Schottel J, Yamada T, Harville J, Silver S (1985) Mercuric reductase enzymes from B group *Streptococcus* and from *Streptomyces* species. J Gen Microbiol 131: 1053–1059

Nies A, Nies DH, Silver S (1989a) Cloning and expression of plasmid genes encoding resistances to chromate and cobalt in *Alcaligenes eutrophus*. J Bacteriol 171:5065–5070

Nies A, Nies DH, Silver S (1990) Nucleotide sequence and expression of a plasmid-encoded chromate resistance determinant from *Alcaligenes eutrophus*. J Biol Chem 265:5648–5653

Nies DH, Nies A, Chu L, Silver S (1989b) Expression and nucleotide sequence of a plasmid-determined divalent cation efflux system from *Alcaligenes eutrophus*. Proc Natl Acad Sci USA 86:7351–7355

Nies DH, Silver S (eds) (2007) Molecular microbiology of heavy metals. Springer, Heidelberg, 460 pp. ISBN 978-3-540-69770-1

Nucifora G, Chu L, Misra TK, Silver S (1989) Cadmium resistance from *Staphylococcus aureus* plasmid pI258 *cadA* gene results from a cadmium-efflux ATPase. Proc Natl Acad Sci USA 86:3544–3548

Phung LT, Ajlani G, Haselkorn R (1994) Cloning and sequencing of the gene for a putative copper-transporting P-type ATPase from *Synechococcus* PCC 7942 related to the human Menkes and Wilson disease products. Proc Natl Acad Sci USA 91:9651–9654

Qin J, Rosen BP, Zhang Y, Wang G, Franke S, Rensing C (2006) Arsenic detoxification and evolution of trimethylarsine gas by a microbial arsenite *S*-adenosylmethionine methyltransferase. Proc Natl Acad Sci USA 103:2075–2080

Richer E, Courville P, Bergevin I, Cellier MFM (2003) Horizontal gene transfer of "prototype" Nramp in bacteria. J Mol Evol 57:363–376

Rosen BP (ed) (1978) Bacterial transport. Marcel Dekker Publishers, New York

Rosen BP, McClees JS (1974) Active transport of calcium in inverted membrane vesicles of *Escherichia coli*. Proc Natl Acad Sci USA 71:5042–5046

Rosen BP, Silver S (eds) (1987) Ion transport in prokaryotes. Academic Press, San Diego, 332 pp

Rosenstein R, Peschel A, Wieland B, Götz F (1992) Expression and regulation of the antimonite, arsenite, and arsenate resistance operon of *Staphylococcus xylosus* plasmid pSX267. J Bacteriol 174:3676–3683

Schottel J, Mandal A, Clark D, Silver S, Hedges RW (1974) Volatilisation of mercury and organomercurials determined by inducible R-factor systems in enteric bacteria. Nature 251:335–337

Sigel H, Sigel A (eds) (1973–2005) Metal ions in biological systems, vols 1–44; then Metal Ions in Life Sciences (2006–2009, 5 vols to date). Wiley, Chichester, UK, then The Royal Society of Chemistry, Cambridge

Silver S (1965) Acriflavine resistance: a bacteriophage mutation affecting the uptake of dye by the infected bacterial cells. Proc Natl Acad Sci USA 53:24–30

Silver S (1967) Acridine sensitivity of bacteriophage T2: a virus gene affecting cell permeability. J Mol Biol 29: 191–202

Silver S (1969) Active transport of magnesium in *Escherichia coli*. Proc Natl Acad USA 62:764–771

Silver S (1977) Calcium transport in microorganisms. In: Weinberg ED (ed) Microorganisms and minerals. Marcel Dekker Publishers, New York, pp 49–103

Silver S (2003) Bacterial silver resistance: molecular biology and uses and misuses of silver compounds. FEMS Microbiol Rev 27:341–354

Silver S, Budd K, Leahy KM, Shaw WV, Hammond D, Novick RP, Willsky GR, Malamy MH, Rosenberg H (1981) Inducible plasmid-determined resistance to arsenate, arsenite and antimony(III) in *Escherichia coli* and *Staphylococcus aureus*. J Bacteriol 146:983–996

Silver S, Jasper P (1977) Manganese transport in microorganisms. In: Weinberg ED (ed) Micro-organisms and minerals. Marcel Dekker Publishers, New York, pp 105–149

Silver S, Kralovic ML (1969) Manganese accumulation by *Escherichia coli:* evidence for a specific transport system. Biochem Biophys Res Commun 34:640–645

Silver S, Levine E, Spielman PM (1968) Acridine binding by *Escherichia coli*: pH dependency and strain differences. J Bacteriol 95:333–339

Silver S, Misra TK (1988) Plasmid-mediated heavy metal resistance. Annu Rev Microbiol 42:717–743

Silver S, Nucifora G, Phung LT (1993) Human Menkes X chromosome disease and the staphylococcal cadmium resistance ATPase: a remarkable similarity in protein sequences. Mol Microbiol 10:7–12

Silver S, Phung LT (1996) Bacterial heavy metal resistance: new surprises. Annu Rev Microbiol 50:753–789

Silver S, Phung LT (2005) Oxidation and reduction: the microbiology of inorganic arsenic. Appl Environ Microbiol 71:599–608

Silver S, Phung LT, Silver G (2006) Silver as biocides in burn and wound dressings and bacterial resistance to silver compounds. J Ind Microbiol Biotechnol 33:627–634

Silver S, Toth K, Scribner H (1975) Facilitated transport of calcium by cells and subcellular membranes of *Bacillus subtilis* and *Escherichia coli*. J Bacteriol 122:880–885

Silver S, Walden W (eds) (1997) Metal regulation of gene activity in bacteria, plants and animals. Kluwer, New York, 473 pp

Struhl K (2002) From *E. coli* to elephants. Nature 417:22–23

Summers AO, Silver S (1972) Mercury resistance in a plasmid-bearing strain of *Escherichia coli*. J Bacteriol 112: 1228–1236

Summers AO, Silver S (1978) Microbial transformations of metals. Annu Rev Microbiol 32:637–672

Thomas DJ, Li J, Waters SB, Xing W, Adair BM, Drobna Z, Devesa V, Styblo M (2007) Arsenic (+3 oxidation state) methyltransferase and the methylation of arsenicals. Exp Biol Med 232:3–13

Tonomura K, Maeda K, Futai F, Nakagami T, Yamada M (1968) Stimulative vaporization of phenylmercuric acetate by mercury-resistant bacteria. Nature 217:644–646

Torriani-Gorini A, Yagil E, Silver S (eds) (1994) Cellular and molecular biology of phosphate and phosphorylated compounds in microorganisms. American Society for Microbiology, Washington, DC, 431 pp

Tynecka Z, Gos Z, Zajac J (1981) Energy-dependent efflux of cadmium coded by a plasmid resistance determinant in *Staphylococcus aureus*. J Bacteriol 147:313–319

Weinberg ED (ed) (1977) Microorganisms and minerals. Marcel Dekker, New York

Weiss AA, Murphy SD, Silver S (1977) Mercury and organomercurial resistances determined by plasmids in *Staphylococcus aureus*. J Bacteriol 132:197–208

Weiss AA, Silver S, Kinscherf TG (1978) Cation transport alteration associated with plasmid-determined resistance to cadmium in *Staphylococcus aureus*. Antimicrob Agents Chemother 14:856–865

Whitney EN (1971) The *tolC* locus in *Escherichia coli* K-12. Genetics 67:39–53

With apologies, as this was requested to be a "personal history", so the references are mostly those demonstrating the interests and involvement of the author. A more balanced list would need to be much too long.