# Silver Pharmacology: Past, Present and Questions for the Future

**Michael W. Whitehouse**

Disclaimer: The author declares no conflict of financial interest with any commercial organisations.

Dedicated to the memory of Douglas Perrin (1922–1998), a physical chemist from New Zealand, with a passionate interest in using metals in medicine.

**Abstract** Silver pharmacology is at the cross-roads. It has a long history as a chemosterilent but is currently denigrated by some vested interests and other 'knowledge monopolies'. It deserves better—particularly in these critical times of ever mounting incidence of antibiotic resistance. This reappraisal outlines some approaches to a dispassionate debate as to why we should, or should not, be reconsidering silver as an addition to (not a substitute for) other antibiotics at the front line of medicine. This will require more understanding about (i) the chemistry of silver in a biological environment; (ii) the different physical and bio-reactive properties of ionised silver (Ag(I)) and nanoparticulate metallic silver ($Ag^o$); (iii) the antibiotic potential of both Ag(I) and $Ag^o$; and (iv) establishing objective Quality Controls for potential silver therapies. Six appendices (A–F) provide some technical data and focus further upon the need to clearly define (a) procedures for manufacturing nanoparticulate metallic silver (NMS); and (b) the purity and properties of NMS preparations—especially stability, antibiotic efficacy and safety of products offered for clinical evaluation. A further appendix (G) deals with some political considerations currently impeding impartial clinical research on silver therapeutics.

## Abbreviations

| | |
|---|---|
| [ ] | Either concentration usually in water or indicating a metallo-complex |
| $Ag^0$ | Zerovalent metallic silver |
| Ag(I) | Monovalent silver, oxidation No. 1 |
| Ag(III) | Unstable trivalent silver, powerful oxidant |
| AgAc | Silver acetate |
| $AgNO_3$ | Silver nitrate |
| ATP | Adenosine 5′-triphosphate |

M.W. Whitehouse (✉)
Schools of Medicine (Gold Coast) and Natural Sciences (Brisbane),
Griffith University, PO Box 68 Stones Corner, Brisbane, QLD 4120, Australia
e-mail: whitehousemd@spin.net.au



K.D. Rainsford et al. (eds.), *Novel Natural Products: Therapeutic Effects in Pain, Arthritis and Gastro-intestinal Diseases*,
Progress in Drug Research 70, DOI 10.1007/978-3-0348-0927-6_7

| | |
|---|---|
| A.W. | Atomic weight |
| BBF | Bacterial biofilm |
| $Cu^0$ | Metallic copper |
| Cu(II) | Cupric ions, a stable solute |
| DIY | Do-it-yourself (e.g. home preparation of 'colloidal silver') |
| LED | Light- emitting diode |
| MBC | Minimum bactericidal concentration to kill a particular organism |
| MIC | Minimum inhibitory concentration, as an antimicrobial |
| nm | Nanometre i.e. $10^{-9}$ m |
| NPS | Nanoparticulate zerovalent (metallic) silver clusters |
| $p[Ag^+]$ | Negative (base 10) logarithm of the silver cation concentration |
| pH | The negative (base 10) logarithm of the hydronium ion concentration |
| RA | Rheumatoid arthritis |
| SERS | Surface-enhanced Raman spectroscopy |
| WWW | World Wide Web i.e. electronic sources of information |

'There are many lies told in every society today about what and what does not have value, or can or cannot transform our lives. The challenge is to identify them.'

Mary Evans 2013 (Teacher and Theologian, Ethiopia)

## 1 Prologue

This chapter discusses some of the chemical and physical properties of silver that might help us to (a) better understand its interactions with biological organisms and ecosystems and (b) separate the hard facts and science from the commercialism, 'politics', ignorance and hype surrounding this 'hot topic' of silver as a medicinal. But why is it so hot? Largely because of expectations that so-called 'colloidal silver' (CS) will be the healing remedy for many of our ills; particularly as an antibacterial, antifungal and anti-protozoal agent, able to combat many antibiotic-resistant organisms (especially virulent bacteria and protozoa). It is also cheap and available without prescription.

A flood of reports, speculations, sales promotions and rather unfounded optimism about CS assails us almost daily via the World Wide Web (WWW). What is the reality behind this phenomenon? We really should know this to sift the facts from the fantasies. Also we need to examine/question present government regulations, virtually excluding it as a legitimate drug—lest we make a terrible mistake, erroneously pre-judging the future and value of any *well-characterised* medicinal silver preparations.

For many health professionals, it is rather a surprise that silver even has a pharmacology. It is rarely mentioned in standard medical texts beyond occasional allusions to its value as an adjunct for promoting wound healing. Yet it is widely known for its medicinal potential as an antiseptic. As a noble or 'coinage' metal it is

classed together with copper and gold in Group 11 (formerly 1B) of the periodic table of the chemical elements. Their long history of use in coinage for jewellery and for making mirrors also reflects (sic) their chemical stability, durability and resistance to corrosion.

Copper and gold have long been recognised for their bio-utility (Whitehouse and Walker 1978; Shaw 1999; Whitehouse 2008) and see Table 1. Copper is also an essential micronutrient, being a constituent of many enzymatic redox systems. As an *endobiotic*, it strongly binds to several defensive proteins to combat the toxicity of dioxygen and its metabolites. Copper also has an essential anabolic role being involved in the biogenesis of the extracellular matrix and connective tissues in animals. (Frausto da Silva and Williams 1991).

Silver and gold are *xenobiotic* and therefore suspected toxins. In fact, their toxicity may be considerably less than that of copper, a notable irritant (unless strongly complexed).

**Table 1** Synopsis of noble (coinage) metal pharmacology

| Metal | As | Use | References |
|---|---|---|---|
| Copper<br>Atomic<br>No = 29 | $Cu^0$ | a. Antifouling, marine vessels<br>b. Bracelets/bangle for arthritis<br>(a) and (b) = slow-release depots for reactive Cu(II) | Walker and Keats (1976) |
| | Cu<br>(II) | Algicide, molluscicide,<br>Schistosomaticidal<br>Anti-inflammatory (transdermal)<br>CuO (copper bullet) = oral supplement for livestock | Walker et al. (1980)<br>Costigan and Ellis (1980) |
| Silver<br>At. No. = 47 | $Ag^0$ | Nanoparticles:<br>Antibiotic<br>Matrix for drug delivery | |
| | Ag<br>(I) | Antiseptic<br>Accelerated wound healing | Higginbottom (1826)<br>Spadaro et al. (1974), Becker and Selden (1985) |
| Gold<br>At. No. = 79 | $Au^0$ | Microparticulate *Swarma bhasma* (Ayurvedic medicine)<br>Colloidal gold:<br>Oral anti-inflammatory ($Aurasol^R$)<br>Parenteral anti-arthritic (rats) | Brown et al. (2007)<br>Abraham and Himmel (1997)<br>Brown et al. (2008) |
| | Au<br>(I) | Injected aurothiolates (AuSR) and oral Auranofin<br>Anti-arthritic<br>anti-tumour<br>anti-parasitic<br>Aurocyanide—a pharmaco-active metabolite of AuSR | Kean and Kean (2008)<br>Tiekink (2008)<br>Madiera et al. (2012)<br>Berners-Price and Filipovska (2011)<br>Graham et al. (2008) |

*Note* This is only an outline, indicating some recent advances

The chemistry of silver appears deceptively simple at least in vitro and geologically (Stwertka 2012; Browne 2013). But it is another story to discuss silver's interactions with, and transformations by, living systems; the consequences of which may be expressed over time periods ranging from seconds to centuries. With organic pharmaceuticals, there is relative certainty that they will ultimately be inactivated, being degraded somewhere within the biosphere.

Silver, unique even among inorganic pharmaceuticals (think lithium, bismuth, platinum) remains intrinsically bio-reactive until finally captured by detoxification with sulphide anions. Seems scary? But we have learned to live with—and beneficially harness other scary entities ranging from oxygen (atomic number 8) to uranium (At. No. 92). With its atomic number being 47, silver lies almost halfway between these extremes. Just as animals and mankind evolved to coexist with and harness oxygen to utilise foodstuffs first and fossil fuels later, so we have had to learn how to control uranium as another source of energy (nuclear fuel). Having met these challenges, unlocking and controlling the power of silver pharmaceuticals ought then to be relatively simple—but at present it is not. Which is why this area where xeno chemistry meets physiological chemistry is just as fascinating today as it was to Paracelsus and his fellow alchemists 500 years ago.

Silver perhaps has/should have an important para-pharmaceutical role, e.g. when used as a conductor of electricity within biocontexts favouring healing and tissue repair i.e. regenerative medicine. Some aspects of this may be better understood in terms of physics, rather than chemistry.

The first English Nobel Laureate for Literature, in 1907, Rudyard Kipling (1865–1936) wrote these pithy comments:

> 'Gold is for the mistress,
> Silver for the maid,
> Copper for the craftsman cunning at his trade.'

It is the maid who serves the wider numbers and more frequently. In a pharmaceutical context, will this ever legitimately be said about silver?

This survey is only an introduction, not a detailed review. It focuses first on some essential background information and *then* tries to help clarify what might be the essential Quality Controls for medicinal silver preceding any significant clinical studies, either as a therapeutic agent or a preventive medication—both now and in the foreseeable future.

## 2 Some History (and More Commentary)

Silver is one of the few metals occurring naturally and was known to the Chaldeans in the Fourth Century BCE. It is mentioned several times in the Christian Bible (Second Century BCE onwards). Table 2 highlights some notable advances in our understanding of silver pharmacology. Some of them were made not by physicians but by the military and by surgeons. Whilst they may not have understood the

**Table 2** A brief history of silver medication/disinfection

| |
|---|
| • Both Cyrus the Elder, founder of the Persian Empire (600–529 BCE), and Alexander III ('The Great'), a Greek general (356–323 BCE), were each reputed to have owed their military success to carrying and disinfecting the drinking water for their troops, using silver containers |
| • Specific uses in medicine are known to the eminent Arabic physician Gerber, the 'Father of Chemistry', and his pupils (Mahomaden School 702–765 CE) |
| • Argyria, a side-effect affecting the skin (see later) noted by Avicenna (980 CE) (Hill and Pillsbury 1939) |
| • Silver cutlery and tableware adopted by those who could afford it, as an antidote to microbial contamination e.g. prevent putrifaction |
| • Surgical insertion of thin silver plates to cover/close battle injuries especially to the scalp was routinely practised by the Knight of St John at their hospital in Valetta, Malta (16C). This procedure was reported to both diminish mortality and increase rates of wound healing compared with other treatments then available |
| • John Higginbottom MRCS (1826), Nottingham UK advocates application of 'lunar caustic' aka silver nitrate 'in the cure of certain wounds and ulcers' |
| • Small silver articles e.g. US$1 coins inserted into water, milk and other beverages for preservation during long voyages e.g. Mormon migration across the American Prairies from Illinois to Utah (1861–1888) |
| • The Merck Index First Edition (1889) lists at least 18 silver salts for pharmaceutical use |
| • Henry Crooks (1914) introduced electrical methods for preparing metallic silver colloidal particles (hydrosols), then shown to be germicidal against the typhoid bacillus (Simpson and Hewlett 1914) |
| • Treatment of 'trench fever' in World War I, caused by lice carrying *B.quintana*, using injected collogol a silver-protein colloid (Sweet and Wilmer 1919). |
| • Antibacterial activities of silver preparations against defined microbes documented in the British Medical Journal during 1920s—and a large number of reports published elsewhere since the 1950s |
| • Treatment of 'chronic arthritis' in Vienna with a colloidal silver preparation collagel (Loewenstein and Fee 1928) |
| • Early Twentieth Century: Mandatory introduction in many states of the USA of 'colloidal silver' = Ag(I)-impregnated small proteins to prevent blindness in the newborn, associated with *Chlamydia* and other micro-organisms in the birth canal |
| • Robert Becker an orthopaedic surgeon (Syracuse NY) discusses the 'silver wand' i.e. metallic silver rods inserted into wounds (Becker and Selden 1985) and electrically stimulated to produce ionic silver locally that greatly |

(continued)

**Table 2** (continued)

| |
|---|
| accelerate healing of bone fractures (Spadaro et al. 1974)—possibly by inducing de-differentiation of connective tissue cells[a] |
| • Re-epithelialisation of skin grafts increased by locally released silver ions |
| • Proving superior to standard antibiotic treatment (e.g. neomycin and polymyxin) (Demling and De Santi 2002) |

[a]For these and other original contributions contravening 'politically correct' dogmas, Becker was dismissed from the Veterans Administration Hospital and repeatedly denied further funding from the US government (Becker and Selden 1985)

mechanisms underlying their discoveries, they nonetheless realised their immense practical benefits.

There are some thoughtful short discussions of the historical use of silver and some particular formulations (Hill and Pilsbury 1939; Gibbs 1999; Jefferson 2003; Lansdown 2010; Hancock 2011; Browne 2013). Several others are available on the WWW, including those originating from

silvergenesis.com (South Africa); with an excellent bibliography,
cwahealth.com,
Eric J. Rentze via Immunogenic Research Foundation (oligodynamic.com/history.html), and
Colloidal-silver.com.au (Australia) to cite just a few.

Much of the recent science/technology of silver has centred on preparing (very) *stable* nanoparticulate silver for various industrial applications. New methods of manufacture, stabilisation, characterisation and application appear at almost weekly intervals. By contrast, there is far less progress being reported in establishing its medical applications; not surprising in view of the conservatism of some parts of the medical profession and its manipulators at the present time.

By contrast, many medicinal properties of nanoparticulate silver are better understood in terms of its *instability* in a bio-environment e.g. being a slow-release source of bio-reactive oxidised silver, Ag(I); the particle serving as a drug/toxin precursor—often described by pharmacologists as either a 'latent' drug (Harper 1958) or a pro-drug (Albert 1979).

Small is not only beautiful but may also be more effective. Silver nanoparticles, 20 nm diameter, are approximately 50 times smaller than many bacteria (1–2 μm diameter) allowing many particles to become attached to a single planktonic bacterium. So these present times with technologies for reproducibly preparing nanosilver particles, have enabled a new range of studies probing the future of silver in medicine, particularly as an antimicrobial agent.

## 3 Physical Properties of Silver ($Ag^0$)

The colour of metallic silver may be 'silver', grey/black or yellow according to particle size. For many centuries, it has been used as a yellow pigment in stained glass. It is physically soft and highly malleable. For daily use, it must be hardened by forming alloys e.g. with up to 20 % copper in jewellery and bracelets. [Sterling silver contains 7 % added copper] It forms an amalgam, dissolving in mercury, formerly much used in dental fillings.

The optical properties of metallic silver set it apart from other metals. It has a high lustre, efficiently reflecting visible light at frequencies above 420 nm. It absorbs light at 417 nm (necessitating the use of powdered aluminium instead in some astronomical telescopes). Silver ions absorb electromagnetic radiation (EMR) most efficiently below 420 nm allowing their photo-reduction by hydrated electrons to generate atomic silver particles (Laroo 2013).

Molten silver absorbs molecular oxygen, which is liberated—sometimes explosively—when the metal resolidifies upon cooling. This compatibility/affinity with oxygen without concomitant oxidation underlies the catalytic—and possibly some of the bio-reactive—properties of finely divided metallic silver.

The physical properties of nano-sized silver particles are being intensively researched especially for applications in novel micro-devices e.g. for electrical (bio) sensing, electronic switching, etc. as well as potential therapeutic uses.

The *stability* of bulk silver metal ($Ag^0$) and its remarkable physical properties ensured its ever-increasing commercial importance for nearly 3 millenia. Metallic silver is no longer used in popular coinage, now being replaced by the cheaper and lighter alloys. Despite being superceded as an essential component of the photographic process, it is still in high demand for jewellery, dental amalgam, manufacturing mirrors and electronic devices. Fine films of metallic silver are being increasingly used prophylactically as an anti-infective aid for coating catheters, especially those inserted into blood vessels, lymphatics, ureters, etc. to minimise introduced infections.

A very practical use of nanoparticulate copper, silver and gold is facilitating physicochemical analyses using surface-enhanced Raman (infrared) spectroscopy (SERS); silver being particularly useful. This technique has the potential for indicating the specificity of a molecular fingerprint combined with the sensitivity for detecting single molecules (Cialla et al. 2012).

## 4 Silver Chemistry: An Outline

Useful discussions are to be found in texts by Mellor (1923), Thompson (1973), and the monumental compendium of inorganic chemistry (Gmelin 1971) written in German and some English. This section mainly focuses on transformations that may occur under physiological conditions i.e. at relatively low temperatures, in

saline media, amidst an abundance of ligands for ionic Ag(I) and of supramolecular structures able to interact with nano-sized metal particles, $Ag^0$.

Silver metal is sufficiently inert to cause no/minimal irritation when inserted into/under the skin e.g. nose rings and other body-piercing jewellery. This is in marked contrast to other metals e.g. copper, aluminium and even stainless steel.

Table 3 indicates some characteristics of four different states of silver, each with distinctive properties; some of which may overlap under certain conditions in vivo.

The redox potential ($E^0$) for Ag(I) aq → $Ag^0$ is +0.80 V, while that for Ag (III) → Ag(I) is 1.9 V; indicating that Ag(I) may be readily reduced to $Ag^0$ and that the argentic ion Ag(III) is a more powerful oxidant, akin to trivalent gold (for Au

**Table 3** Biostatic/biocidal mechanisms of silver species

| *I. As silver metal, $Ag^0$* |
|---|
| E.g. containers, cutlery, (undebased) coinage, germicidal coatings, surgical inserts, acting:<br>As 'hostile' surfaces for microbial colonisation,<br>By sorption of microbial toxins on container surfaces,<br>By *local* oxidation when contaminated → diffusible bio-reactive Ag(I) |
| *Note* These surfaces are not always totally inert e.g. tarnishing with soluble or volatile sulphides |
| *II. As nanoparticulate metallic silver ($Ag^0$), 2–50 nm diameter possessing* |
| Very high sorptive activity involving:<br>(a) van der Waal's bonding to bacteria/other bio-substrates<br>(b) Coulombic interactions with anions e.g. $HCO_3^-$, acidic biofilm proteins and polysaccharides<br>Catalytic activity<br>(a) Well characterised for commercial oxidative syntheses e.g. methanol → formaldehyde at 500 °C<br>(b) Less certain in physiological contexts e.g. at interfaces between biophases or on sorptive macromolecules: (but see Huang et al. 2014.)<br>Lability, releasing bio-active (toxic) silver ions (Kittler et al. 2010; Liu et al. 2010) |
| *III. As oxidised/ionic silver* |
| (a) as univalent (Argentous) Ag(I),rarely ionic in vivo but rapidly interacting with a broad range of bioligands (Table 4) inactivating enzymes, denaturing proteins and polyphosphates and destabilising membranes<br>(b) As trivalent (argentic) Ag(III), usually tightly complexed e.g. stabilised as the mixed valency complex $Ag(I)_2Ag(III)_20_4$ (Tetrasil) used as a germicide in ointments; also claimed as a treatment (Imusil) for AIDS (Antelman 1997) |
| *Notes*<br>Some of these are well-established, others less so<br>$Ag_4O_4$ was formerly described as 'silver peroxide, $Ag^0$'. It is not a peroxide because it does *not* liberate hydrogen peroxide when acidified |

(III) → $Au^0$,$E^0$ = 1.5 V). Ag(III) is only likely to exist transiently but might possibly be generated in a highly oxidant environment (Fischer and Jansen 1995). For reviews of Ag(III) chemistry, see McMillan (1962) and Tudela (2008).

The reactivity of *metallic* silver metal as nano-sized particles contrasts with that of the bulk metal. Indeed some physical chemists have deemed it another state of matter. This is because nano-sized materials are intermediate between the domain of quantum mechanics (describing the behaviour of lone molecules) and classical physics, governing the behaviour of bulk materials (Burcham 2010). These particles have a very large surface area relative to mass, which enormously increases their sorptivity, reactivity, etc. For example, silver does not normally react with hydrochloric acid, which dissolves nanoparticulate silver with the evolution of hydrogen (Li and Zhu 2006).

By contrast, oxidised/ionic silver (Ag(I)) is intrinsically unstable, being reduced to black metallic silver by light (the basis of photography) and also by a very large number of electron donors e.g. aldehydes qualitatively detected by forming a silver mirror. The black stain appearing on the skin after handling solutions of silver salts is another example of its bio-reduction.[1] This demonstrates both the reactivity of silver ions and the reducing i.e. electron-donating properties of many bio-substrates (also see Sect. 7).

A number of bio-ligands (L) can form complexes with Ag(I) in vivo, of varying stability and bioavailability (Table 4). The solubilities of some (model) silver complexes in water listed in Appendix A provide some guide to the relative stabilities of individual Ag-L bonds and comparative bioavailability of silver after forming these complexes. The actual *strength* of the Ag-L bond is indicated by their dissociation constants, $K_m$ (Perrin 1979; Hogfeldt 1982). For the Ag-S bond, the $K_m$ is $10^{-54}$, which is considerably smaller in magnitude than the solubility of $Ag_2S$ in water ($1.4 \times 10^{-4}$ M). Each of these figures indicates the very high affinity of silver and propensity to form Ag-S(R) bonds. Such measurements help us to understand more about the likely transit and distribution of Ag(I) not only within the body but also within the external bio-environment. For example, resolving the question of whether the low solubility of silver chloride might be selectively increased in the stomach by either local interaction with hydrochloric acid; perhaps forming the hydrated $[AgCl_2]^-$ anion (Forbes 1911; Forbes and Cole 1921) or binding with other gastric ligands from the diet or within the mucosal lining.

The bio-reactivities of both $Ag^0$ and Ag(I) are further discussed in Sect. 7 and 8.

[1]The author A.C. Doyle, a physician himself, has his character Sherlock Holmes identify Watson, his future associate, as a physician at his first meeting, by the black stain on his fingers after using silver nitrate as a sterilant.

**Table 4** Some silver bioligands

| Ligand | References |
|---|---|
| Thiols | Webb (1966), Jocelyn (1972) |
| Selenols | |
| Disulphides | Jocelyn (1972) |
| Oligophosphates e.g.<br>• NAD(P)<br>• Teichoic acids[a] | Kornberg and Pricer (1953) |
| Halides and thiocyanate ($SCN^-$)<br>$I^- > SCN^- > Br^- > Cl^-$ | |
| Phosphates<br>Phospholipids and nucleic acids | |
| Carboxylates | |
| Nitrogenous ligands<br>• Nucleobases: cytosine > thymine<br>• Amines<br>• Imidazole | Shukla and Sastry (2009),<br>Czoik et al. (2008) |
| Unsaturated (ethenoid) fatty acids | Stahl (1965), Adlof and Emken (1985) |

These are bioconstituents that bind Ag(I) listed in approximate order of diminishing affinity
[a]In cell walls of Gram-positive bacteria

## 5 Misapprehensions About Silver 'Biology'

Before attempting any rational discussion about silver pharmacology, we should be aware of four widely circulating 'untruths' concerning the properties of silver in various biosystems.

1. Silver is not a *trace* element, as the term is understood by agricultural and nutritional scientists. So far as we know today, silver is not an essential constituent of any biosystems; using the term 'essential' to imply loss or disruption of some biological function in its absence.
   Not so long ago boron, silicon, vanadium, molybdenum, arsenic (and a slowly increasing list of other elements) were not considered functional bioconstituents. Today we know otherwise. However, much nonsense is currently being circulated that we ourselves, our livestock, foodstuffs and the agri-environment are suffering silver depletion and therefore becoming 'sicker'. It is extremely doubtful that silver was ever widely distributed to be retained in biosystems. To continue to call it a 'trace element' implying it is a missing nutrient, totally obscures its utility for medicine as an xenobiotic (as most drugs are today).
   However, we should not ignore the biodynamics of metallic silver ($Ag^0$) as an oxidisable material in the natural environment, subject to metabolic transformations, just as any other xenobiotic.
2. Silver is often categorised as a *heavy* metal, an inaccurate generalisation considering its atomic weight (AW) is 108 i.e. much less than that of heavier toxic

metals such as mercury (AW 201), lead (207) and uranium (238); all justifiably classified as unselective poisons.
Silver is much less toxic than any of these heavy metals, being very rarely lethal. Nevertheless, silver does suffer 'guilt by association' when misclassified as 'heavy'—being implied to be much more toxic than it really is. [The presence and compatibility of other chemical elements in biosystems such as strontium, atomic weight (87), molybdenum (95), iodine (126), of similar atomic mass is usually accepted without comment.]

3. Describing a product as 'colloidal silver' is sufficient to 'say it all'. In fact, this is such an ambiguous, even misleading, appellation that it ought to be abandoned when and wherever possible. At least two different types of silver products have been/are being sold for pharmaceutical use under this unspecific designation. In general, they have somewhat different biological properties, particularly with regard to efficacies and in engendering toxic responses.

   (i) Historically the description 'colloidal' was used to specify water-soluble silver-impregnated macromolecules, particularly those classified as colloids (mid Nineteenth Century onwards). They were often prepared from casein (milk), serum proteins or some of their derived polypeptides, by interaction with silver salts. The products were either Ag(I)-polypeptides, or very fine $Ag^0$ particles embedded in a polypeptide coating—or a mixture of both valencies. In these products, silver was complexed chemically (or physically) and not precipitated by chloride, phosphate and other silver-insolubilising anions in physiological fluids (eyes, stomach, etc.).

   (ii) More recently (mid Twentieth Century), processes to manufacture extremely fine dispersible nanoparticles (NP)[2] made silver available as $Ag^0$ with much increased bio-reactivities compared to the bulk metal; able to act as a slow-release source of oxidised silver (Ag(I)) = a latent 'toxin'. The properties of these metallic nanoparticles are heavily influenced by their methods of production that determine their size, ionic character ('charge'), stability *and* content of impurities. Unless these preparative methods or the physical character (and chemical reactivities) of the NP products are clearly specified, it is both meaningless and foolish to imply/accept that they are somehow equivalent i.e. would have very similar bio-regulant properties in a particular context. This is one reason why it has been so difficult to interest drug regulators and medical practitioners in accepting the legitimacy of silver medications.

4. 'Silver will turn you blue'. This appalling half-truth is vigorously perpetuated by those who should know better. It turns up repeatedly (out of all proportion to its significance) when seeking information about colloidal silver on the WWW. There are two reasons for this; one honest and the other not so.

[2]Nano = $10^{-9}$, 1-millionth of a mm.

(i) When 'colloidal silver' preparations became available in the late Nineteenth century, they usually were Ag(I)-impregnated proteins/polypeptides to be used topically e.g. as optical disinfectants to combat neonatal gonococcal infections. Although they were not prescribed, they were also often consumed for alimentary disinfection, nearly always being used at much higher silver dosage. A few people acquired a blue or grey skin pigment, Argyria. This was not surprising considering the photosensitivity of silver salts exposed to light. The effect was cosmetic, but fortunately infrequent. A careful analysis of over 600 clinical (and anatomical) reports (Hill and Pilsbury 1939) failed to establish any malfunction of an essential organ, but noted that Argyria was a consequence of

   (a) long-term, often non-prescription use;
   (b) the 'colloidal silver' products were nearly always poorly characterised patent medicines;
   (c) silver nitrate caused far higher incidence of Argyria (>50 %) than many colloidal silver preparations (6 %) or silver oxide (0.5 %) (Hill and Pillsbury 1939); and
   (d) the cumulative dose of silver consumed was of the order of grams—truly an overdose for sensible internal disinfection.

(ii) Various well-funded organisations, including the Food & Drug Administration USA (FDA), the Therapeutic Goods Administration Australia (TGA) and some less than honest private organisations e.g. QuackWatch, and Friends of Science in Medicine, have all repeatedly declared or implied that Argyria is a common side-effect of silver medication. Not only do they ignore the real incidence and general lack of harm from Argyria, but they also try to outlaw the current use of silver medications for healing; implying they are almost certainly serious health hazards.[3]

For some years, the FDA and TGA have maintained embargoes on claims for medicinal activity and therefore the sale (even clinical trials) of any form of colloidal silver as a medicine. Nevertheless both these government regulators concede that it may be used to sterilise water; surely a medicinal property!!

This simple fact was well recognised long before the TGA and FDA existed and came to adopt such unscientific postures as seemingly not wanting to know what is safe *and* what may be beneficial if it happens to concern silver; particularly if it is not patentable. Currently, the official attitude seems to be that the public must be shielded by outright bans from using, advertising (even researching), any form of silver for medicinal purposes.[4] This has undoubtedly helped the proprietary antibiotic industry and continues to do so—to the detriment of the very much larger

[3]These regulatory agencies ignore the far greater health hazards, including mortality caused by heavily promoted drugs they had approved e.g. the NSAIDs, Vioxx$^{R}$ and Prexige$^{R}$ that ultimately had to be withdrawn. By contrast, no one has died from silver medications.

[4]The legal penalties for breach of these regulations are truly an eye-opener.

number of potential beneficiaries, particularly the sick, the poor and the ailing if they happen to be infected with antibiotic-resistant, but possibly silver-responsive, micro-organisms (Also see Appendix G).

## 6 An overview of Silver Pharmacology (also See Sect. 7)

As discussed in textbooks, this is a minute topic: often totally ignored in many of the standard texts. When mentioned, it is nearly always in the context of treating burns and other superficial wounds; usually with the silver salt of sulphadiazine (an acidic sulpha drug dating from the 1940s). This is essentially an Ag(I) pharmaceutical, of defined composition and approved as a legitimate drug under various trade names (e.g. Silvadene, Flamazine, etc.). It is also biocidal to *Candida* and *Trepona palladum* (syphilis).

However, other silver preparations have many, already proven, uses in different biological contexts. These include antimicrobial, restorative and other aids for improving health or dealing specifically with chronic disease or dysfunctional wound healing. For many purposes, short-term therapy may be sufficient, e.g. as a germicide—with clear endpoints for determining efficacy and accepted methodologies to determine dosage and duration of activity. For other purposes, such as exploring the nexus between a prior infection and chronic debilitating diseases e.g. rheumatoid arthritis (RA), anklyosing spondylitis (AS) or multiple sclerosis (MS), see Ebringer's three books (2012, 2013, 2015), longer microbial eradication programs may be all-important. Sometimes these might be more effective and probably less harmful when used intermittently e.g. as a 'purge'. Chronic treatments necessitate using the safest possible yet effective doses. It is here that the concept of metallic silver as a slow-release toxin may be quite helpful.

The antibacterial activities of nanosilver preparations are often less pronounced against Gram-positive organisms e.g. Staphylococci, Streptococci. Gram-positive bacteria are distinguished by their content of anionic polyphosphates (teichoic acids) in their cell walls. The more silver-susceptible Gram-negative organisms (*E. coli; Proteus)* have a significant proportion of lipopolysaccharides and lipoprotein in their cell walls.

Bacteria with numerous flagellae e.g. *Proteus* also present an exocellular target for potential immobilisation by silver particles and Ag(I) released therefrom.

Shifting the focus back in time from *treating* established infections/inflammatory disorders i.e. therapeutic use; to using anti-infective silver preparations for *preventing* disease i.e. as prophylactics, then raises new concerns about long-term toxicities (See Sects. 8 and 9). Questions about (a) efficacy such as how much to use, or for how long (?); and (b) what are safe limits for extended use will have to be asked. Careful consideration must be given to preventing targeted microbes acquiring silver resistance by over- exposure. Suitable guidelines developed by consensus and some revision of present drug regulations will also be needed.

In the natural world, there are adaptive mechanisms whereby a potent metal cation may be detoxified by reduction to conglomerated metal. This bioreduction has been exploited to prepare nanosilver using *Enterobacteria* (Shahverdi et al. 2007), cyanobacteria (Legke et al. 2007), *Shewanella oneidensis* (Suresh et al. 2010) and many other micro-organisms. It is believed to also account for the geological formation of some gold deposits (Legke et al. 2006).

These examples of facilitated transformations within the biosphere indicate the relative safety of metallic silver and metallic gold as being 'desirable' end-products of reductive detoxication. They also suggest possibilities for reactivating metals by reversing this reductive detoxication process—or to put it more plainly—enhancing intoxication by controlled delivery by either (a) facilitated oxidations of inert metal → pharmaco-reactive cation(s) or (b) inhibiting the inactivating reductases e.g. with piperitone(an inhibitor of nitro-reductases) that potentiates nitro-furantoin antibiotics (Shahverdi et al. 2007) .

These considerations of safety versus efficacy show the need to clearly *distinguish* biological properties of $Ag^0$ as bulk metal or nanoparticles from those of its oxidation products, Ag(I) and even Ag(III); while also recognising the biodynamics of transition from one oxidation state to another. This suggests possibilities for controlled recycling i.e. inert → active entity and also the reverse transition, within particular pathological contexts (e.g. inflammatory loci). A clearer understanding of these contexts should open new vistas for silver as a medicinal, either as a prime drug or a therapeutic adjunct.

One example of this adjunctive role is interference with the protective biofilms created by chronically invasive bacteria such as *Pseudomonas* or *Enterococci* etc. These 'films' impede the antibacterial action of many antibiotics that effectively suppress populations of free-floating/planktonic micro-organisms. Disruptions of these biofilms, either by (a) adhesion of nano/micro-particulate silver ($Ag^0$) or (b) complexation of cationic silver (Ag(I)) with anionic protein/polysaccharide constituents of the bacterial biofilms (BBF), theoretically allows a double-pronged therapy—especially if adherent/trapped $Ag^0$ is then locally oxidised to intoxicant Ag(I) by electron withdrawal, either controlled or spontaneous (Table 5). Anti-BBF activities of silver preparations have been described (Chaw et al. 2005). These will surely be followed by many more (we hope) critical studies in coming years.

By contrast, the two million or more reports about medicinal silver on the Web are nearly all concerned with the antibacterial properties of metal silver usually in the form of nanoparticulate silver dispersions (NPS). In theory, this is an entirely different 'drug' from germicidal Ag(I); except that so many NPS preparations of medicinal $Ag^0$ may be contaminated with Ag(I); the degree of which is rarely specified but almost certainly determines their stability, efficacy *and* toxicity.

Currently, this is the core problem of nano-silver pharmacology; the reality being it is often that of a multi-component mixture. This is not unlike describing the medicinal properties of such traditional pharmaca as opium, cardiotonic glycosides, coffee, ephedra, etc.; all of which are composite drugs whose potency may be greater than that of any individual components (Weil 1996).

**Table 5** Bacterial biofilms (BBF) and silver: some facts and speculations

| *Facts* |
|---|
| (i) Silver-impregnated surfaces usually defy bacterial colonisation<br>(ii) BBF contain a range of micro-organisms, requiring a broad-spectrum microbicide<br>(iii) Most antibiotics mainly control replicating bacteria. But 'hibernating' bacteria lodged in BBF may still be Ag-susceptible<br>(iv) BBF present 'sticky' substrates for $Ag^0$ attachment, as NPS<br>(v) BBF contain many potential argentophilic 'docking' sites for Ag(I) including the 'extracellular polymeric substances'(EPS) composed of:<br>– Exocellular DNA<br>– Anionic glycans e.g. polysaccharide intercellular adhesin (PIA)<br>– Anionic glycolipids<br>– Proteins from host (fibrin, etc.) and colonising bacteria<br>– (Esterified) unsaturated fatty acids in phospholipids etc. |
| *Speculations* |
| (a) The presence of both (i) non-acylated D-glucosamine and (ii) anionic phosphate or hemisuccinate groups in the PIA of staphylococcal biofilms (Mack et al. 2009) may each provide exocellular 'docking sites' for silver to facilitate biofilm disruption.<br>(b) A quorum-sensing molecule = pyocyanine (aphenazine), a virulence factor in Ps. *aeruginosa*) (Lau et al. 2004), might be a target for silver deactivation<br>(c) Silver pharmaceuticals may augment the action of cationic agents used for wound debridement e.g. polihexanide<br>(d) Crafted Ag(I) complexes and appropriate delivery systems may confine/potentiate disinfection—*and* stimulate local healing (Becker and Selden 1985; Becker et al. 1998) of wounds, ulcers and other localised targets 'blanketed' with BBF |

Under some circumstances, ionic Ag(I) 'impurities' in $Ag^0$ preparations may physically stabilise the metallic nanoparticles when adsorbed, so ensuring maximal ionic repulsion between the particles and minimising their spontaneous cohesion/precipitation. Another modification of the surface of nanoparticles may involve oxygen as an 'impurity' by forming Ag-O bonds i.e. an oxide skin (Roy et al. 2007), in essence diminishing the overall cationic character of the particles. So the impurities perhaps become a potential stabiliser, even an activator. But if we reverse the perspective, the NPS may also carry the more bio-reactive 'adsorbed' ionic Ag(I) or bound oxygen. [This is discussed further in Appendix F—speculation (iii).]

Summarising this dualism: $Ag^0$ as NPS *without* Ag(I) as cation or oxide = a potentially unstable package perhaps not widely bio-distributed; but when coated *with* Ag(I) = a stabilised, more dispersible, system combining a bio-reactive surface coating with a far less reactive supportive reservoir of $Ag^0$.

## 7 Mechanisms of Action of Silver Pharmaceuticals (Table 3)

These can be broadly categorised as properties of:

(a) readily available Ag(I);
(b) also of Ag(I) but only expressed after delayed delivery e.g. by local oxidation of $Ag^0$;
(c) unoxidised silver $Ag^0$, rapidly manifested by presenting surfaces inimical to bacterial growth e.g. silver-tipped catheters, silver amalgams used in dentistry, silver-impregnated bandages/underwear, etc.; and
(d) very small silver particles trapped in bacterial biofilms or ingested by phagocytic parasites as discussed (Sect. 6).

The significance of some reports about the pharmaco-activity of Ag(I), as studied in vitro, is difficult to assess. For example, treatment with silver salts (nitrate, sulphate) degranulates rat leukemic mast cells in vitro, a potentially noxious property (Suzuki et al. 2002). But is it likely to occur in vivo, particularly in the context of so many competitive ligands for Ag(I), ranging from plasma chloride and albumen to endo-cellular thiols? It might—but would it be significant i.e. with long-term consequences?

The main potential of current silver pharmaceuticals may lie with their composite character; for example being delivered as relatively inert micro $Ag^0$ particles but activated by local inflammation or other compartmentalised physico-chemical processes that might deliver 'payloads' of toxic Ag(I). An analogy here may be the antibiotic action of oral lactoperoxidase (LPO), which oxidises thiocyanate ($SCN^-$) actively secreted into saliva; *but* only when hydrogen peroxide becomes available from proliferating oral bacteria. In one sense, silver can replace this LPO-$SCN^-$ combination, provided that the peroxide (or other oxidants) can be sourced from either a) the targeted bacteria in situ or b) delivered as an adjunct supplement. This scenario presents Ag(I) as a substitute for $SCN^-$ but in fact it may reduce thiocyanate's availability and incipient toxicity [It is a precursor of cyanide.] by forming the highly insoluble AgSCN.

A fundamental biological property of free silver (I) ions is their interaction with, and usually inactivation of, bioregulant thiols both large and small e.g. enzymes, glutathione. The proton gradient established across energised biomembranes (mitochondria, chloroplasts) that sustains ATP synthesis (Mitchell 1966) is destroyed not only by lipophilic anions e.g. free fatty acids, many acidic NSAIDs but also by several thiolphilic reagents (Whitehouse and Leader 1966). Silver ions can interfere with mitochondrial ATP synthesis in vitro (Chappell and Greville 1954) and de-energise vital membranes of *Vibrio cholera* (Dibrov et al. 2002).

The question is: will the concentration of Ag(I) that poisons ATP production in vitro ever be attained in vivo i.e. in the presence of so many endocellular or exocellular silver bioligands e.g. adenosine nucleotides, chloride ions etc.? There may be two answers to this question—because ATP biogenesis in (a) prokaryocytes

is topographically more exposed to ambient poisons (and fewer detoxicants) than (b) eukaryocytes, where ATP is generated within endocellular mitochondria surrounded by all the intracellular constituents able to capture, bind, and detoxify Ag (I). This may be one of the keys to the relative selective toxicity of silver, being more damaging to vital energy production in invading bacteria than it is to that in the invaded host.

In summary: silver therapies may be (a) pluripotent—with several modes of action, (b) involve more than one silver species, and (c) act over different time spans ranging from rapid antibiosis to facilitating tissue repair (and ?regeneration).

## 8 Toxicity of Silver Pharmaceuticals

Three quotations help summarise this topic.

- "All things are poisons. It is only a matter of the dose".
  Paracelsus (1493–1541) Swiss physician and alchemist.[5]
- "Silver is an old problem and nanosilver is a new challenge".
  (Luoma 2008)
- "Silver is not carcinogenic……and should be placed in a No Risk category".
  Lansdown (2010)

So there are at least three questions we might ask:

1. Why is silver apparently such a feeble toxin; as compared to other metals of similar mass e.g. cadmium(=AW112) or thiophilic character e.g. lead and mercury?
2. Why are so many microbes so much more silver-sensitive than their host's epithelia/endothelia/blood cells? Glib phrases such as 'silver bullets' or 'selective toxicity' may describe, but do not explain, the host's relative insensitivity. Some comparative anatomy may help here: (a nuclear) prokaryocytes have relatively exposed DNA but eukaryotes do not. Moreover, their DNA is not usually associated with histone proteins, i.e. less protected.
3. Why is one relatively *benign* but much publicised side-effect of ingested silver, namely Argyria, officially considered so much more noxious—than self-imposed debilities and *mortality* from ingesting high doses of alcohol, salt, sugar or tobacco smoke? None of these are banned but using silver as an antibiotic, is! This is not a very enlightened attitude in the present circumstances of increasing antibiotic resistance.

It is difficult to find convincing records of silver being lethal, other than in (a) exceptional industrial circumstances (e.g. a worker falling into a vat of silver

[5]This is an apt quotation because Paracelsus himself experimented with and used silver in his medical practice and apparently was an ardent proponent of silver medications.

cyanide used for 'silver plating') or (b) after exposure to chronic inhalation of silver dust or fumes e.g. Bolivian miners working under bad conditions, extracting silver ores that may also contain lead, cadmium, etc. In other contexts, it is not considered to be an industrial hazard (Browning 1968; Venugopal and Luckey 1978 or a carcinogen.

Surveying the affinities of silver ions for so many bioligands (see Table 4 and Appendix A), it is remarkable that it appears so unreactive i.e. non-poisonous in many biosystems. One explanation is the ubiquitous distribution of chloride ions, rendering Ag(I) largely insoluble [the solubility of AgCl is normally about $2 \times 10^{-3}$g/L i.e. 2 ppm]. Another is the wide extracellular distribution of organic deactivators such as (a) endogenous bio-reductants e.g. ascorbate, methylglyoxal, glutathione, and (b) thiols both in the circulation, notably albumen and macro-globulins or in the liver and several other tissues e.g. glutathione and the metal-lothioneins (Vasak 2011). Metallothioneins in animals can be induced/increased with some metals (Zn or Cd) but whether this also occurs with silver is not clear at present. They are also present in some bacteria; notably M *tuberculosis*, some *Pseudomonas*, some aproteo bacteria and *Staphylococcos epidermidis* (Blindauer 2011, 2014).

These natural antidotes to silver intoxication are expended in much lesser quantities than for example the glutathione needed to help detoxify paracetamol/acetaminophen, used in doses greater than 1 g/day (Whitehouse and Butters 2014).

So we might anticipate cationic silver intoxication being a *serious* health hazard, as opposed to causing a cosmetic side-effect (Argyria), perhaps *only* when (a) these natural antidotes are severely depleted—e.g. after overdosing with paracetamol—or (b) as effects of very small silver particles ($Ag^0$); if and when these particles are taken up by non-phagocytic mammalian cells. The prevailing surface charge on the particles will largely determine their cellular attachment, subsequent retention and local membrane interactions or pinocytosis. In fact silver phagocytosis may be sometimes therapeutic if it helps 'quench' over-active inflammogenic leukocytes [a Trojan horse therapy] as the ingested $Ag^0$ slowly disrupts cellular function. Nevertheless we cannot afford to be complacent until such hazards as potential genotoxicity (AshaRani et al. 2009) have been properly evaluated for risk, reversibility etc. in vivo, not just in vitro—where detoxicant mechanisms may be absent from the test systems.

A number of exogenous thiols e.g. N-acetylcysteine, NMEA mesna (coenzyme M), D-penicillamine have been used to minimise intoxication by acrolein, cyclo-phosphamide, lead and other thiol-depleting xenobiotics. Supplementation with selenium is also recommended to overcome Ag(I) intoxication of essential seleno proteins e.g. glutathione peroxidase and thyroid peroxidases containing seleno-cysteine at their catalytic centres.

Thioredoxin reductases (TrxR) are another class of seleno-enzymes that regulate the redox homeostasis in animal cells, controlling the balance between oxidised and reduced forms of regulatory thiols e.g. glutathione (Fig. 1).

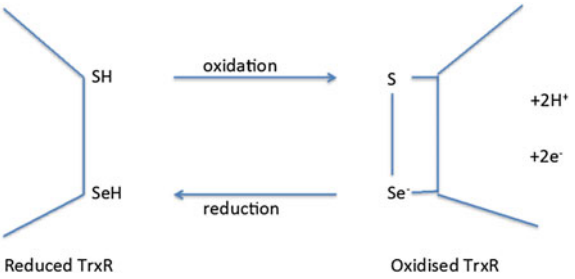


**Fig. 1** The TrxR enzyme with adjacent thiol and selenol functions that participate in reversible 'redox shuttling' (*e* = electron)

These enzymes are a target for certain metallo-drugs notably anti-cancer agents containing gold (Omatu et al. 2006) or platinum (Arner et al. 2001). However, their silver sensitivity is not clear at present. This enzyme appears to be absent from many bacteria but may be present in other parasitic organisms.

So in the presently unlikely situation of physiological silver overload developing, there are some useful leads for detoxification and antidotal strategies.

## 9 Silver and the Environment

Sometimes this seems to be a topic with rather more theories and scare stories than 'hard' facts. A thoughtful review by Luoma (2008) provides a more reasonable perspective.

Much data have been generated concerning the toxicity of Ag(I) to fresh water fish and other organisms living in an aqueous environments with a low chloride content. One concern has been the irreversible poisoning of the fish's gills. This is not a problem unique to soluble silver, being replicated with other waste products from mining industries e.g. arsenic, tin, etc. Another problem is how valid are conclusions from *one* well-designed laboratory study of the effect of *one* type of NPS on *one* test organism, often separated from its natural environment—for understanding how *various* silver products might interact with *various* natural environments over *various* time spans.

There are no easy answers here. Nevertheless scientific studies conducted with silver-responsive organisms (e.g. zebra fish embryos) in the 'abnormal' environment of a laboratory can still provide insights for examining the nature and potency of silver detoxicants within the environment e.g. citrate and fulvic acids (Osborne et al. 2012).

By contrast, the potential eco-toxicity of nanosilver preparations in soil samples seems remarkably low, as the particles clump together spontaneously forming large aggregates—not so different from some native silver deposits (Maass 2014). Another natural detoxicant mechanism is oxysulphidation transforming nanosilver

particles into very insoluble silver sulphide after aerobic oxidation i.e. $Ag^0 \rightarrow Ag(I)$ (Liu et al. 2011).

The great debate is now, or certainly should be, about toxicities to 'good' bacteria present in the gastrointestinal tract or those constituting the essential microflora in sewage processing plants. If they are less susceptible to silver antibiotics than other microbiota, then why is this so? Does it imply they have already acquired silver resistance, particularly enzymic capacities to bio-reduce $Ag(I) \rightarrow Ag^0$? If so, is this transferable? This could present serious problems for human/animal therapeutics if silver were to join the very many organic antibiotics that are becoming evermore useless. Alternatively, if these good/beneficial bacterial are also silver-susceptible, how can they be protected from over-zealous use of NPS? The answers to these queries will be rather critical for determining the future acceptability of silver pharmaceuticals, beyond being used in crisis therapy e.g. to help treat super-infections.

## 10 Looking to the Future of Silver in Medicine

Again, there are a number of questions needing acceptable answers, sooner rather than later. For example:

(i) What are the basic 'truths' about silver pharmaceuticals (SP) that need to be *continually* re-evaluated e.g. its credible history, safety and efficacy?
(ii) What are the new opportunities for SP—perhaps seeming crazy/unrealistic today—but allowing further advances in health care tomorrow, particularly in the area of preventive medicine?
(iii) What are the real legal difficulties and other obstacles to researching SP and bringing them to clinical trials? Are these impediments still acceptable *today* or are they just *inherited* baggage from the past? (Maybe too much politics, too little science and commonsense.) The legal and financial disincentives currently hindering SP research and development need to be either justified—or *changed.* Retaining the *status quo* is indefensible, particularly at a time of ever-increasing microbial resistance to organic antibiotics.

Complementing these queries, Table 6 presents some other strategies for further research, some being more practical than others, at this time.

A useful guideline for changing attitudes about using silver pharmaca would be to adopt (or adapt) the Utilitarian Principle, originally proposed by the English philosopher, Jeremy Bentham (1748–1832), posing the question: 'What brings the greatest good/happiness for the greatest number?' This ought at all times to over-ride vested interests and negative mindsets, currently impeding honest enquiries about the future of silver as legitimate silver pharmaceuticals, even life-saving medicinal aids.

**Table 6** Silver pharmaceuticals: exploring further applications

| This brief list indicates that more research is needed, for example into: |
|---|
| (1) Better methods to dry, store and redisperse pharmaco-active nano-silver preparations *without* irreversible aggregation and/or significant losses of potency<br>(2) Using $Ag^0$ or Ag(I) as supplements to other antibiotic and anabolic/restorative therapies i.e. in an Adjunctive role[1]<br>(3) Producing silver nano- and micro-formulations of $Ag^0$ (or even Ag(I)?) designed to be slow-release/targeted delivery systems for Ag(I), either alone or together with other bio-regulants[2]<br>(4) $Ag^0$ providing 'platforms' of controlled size, charge etc. to physically or chemically *carry*/deliver pre-bound pharmaca[3] e.g. as inserts into wounds or for controlling unhealthy intestinal microbes and parasites e.g. *Proteus*, *protozoa*, nematodes etc.<br>(5) Continuing development for other pharmaceutical applications beyond human usage e.g. improved water purification, hydroponic horticulture, controlling protozoa and other parasites in farm animals, etc.<br>(6) The presently unanticipated/unavailable new delivery systems that may be highly valued tomorrow e.g. beneficially harnessing some of silver's remarkable physical properties (light absorption, high thermal and electrical conductivities) as micro-thin inserts or using other bio-compatible technologies |

[1]Perhaps patterned after silver sulphdiazine for burns or using NPS with membrane-permeating antibiotics e.g. polymyxin B (Ruden et al. 2009) or β-lactam antibiotics (Li et al. 2005)

[2]Furthering the well-tested tradition of 'drug latention', (Harper 1958) exemplified by cyclophosphamide, leflunamide, even aspirin

[3]Perhaps following the example of using thioligands to alter the physical characteristics, bio-distribution and reactivity of colloidal gold particles (Brust et al. 1994)

## 11 Postscript; on a More Personal Note

As an ignorant biochemist, 50 years ago, I wrote a chapter for Progress in Drug Research (Vol. 8, 1964) surveying drugs for inflammatory disease. It discussed gold but alas, ignored silver (including a seminal publication by Chappell and Greville 1954). This omission reflected first my ignorance that the noble metals were more than coinage and the basis for much fine craftsmanship—and secondly, my then lack of 'hands-on' experience with animal assays.

The literature as best as I could survey it, with the help of Chemical Abstracts (published by the American Chemical Society) was also pretty secretive about the history and potential of silver in medicine. The Bodleian Scientific Library in Oxford, was a treasure-trove but failed to diminish my ignorance about so-called natural medicines and their continuing importance for 'modern medicine', then just discovering auto-immune disease(s). A few journeys to London to investigate the

splendid library of the Royal Society of Medicine rewarded me with much information about the complexities of the inflammatory response and its regulation by patented pharmaceuticals. But metal pharmacology (other than haphazard reports about the efficacy of antimonials, bismuth preparations and 'gold salts') was definitely not on the radar at that time.

When at last (some 20 years later). I pondered the obvious question: why had other areas of medicine e.g. endocrinology, genetic disorders, etc. surged forward so dramatically but many chronic diseases including the whole auto-immune family still lacked a clear aetiology *and* an even clearer program for treatment/eradication, perhaps based on neutralising/eliminating defined causative agents. Didn't they exist? Meanwhile during the 1970–1980s at least, the treatment of chronic inflammation was frankly a mess—nearly always palliative, rarely curative and abounding in horrible side-effects e.g. gastric bleeding from poisonous NSAIDs and osteoporosis from the corticosteroids—not to mention the wretched consequences of aggressive immunosuppression, all too often employed as a treatment of last resort.

So it was encouraging to discover that eminent pioneers of modern medicine such as William Osler (1849–1919) and contemporary mavericks like Alan Ebringer (1938) were willing to consider infection as a prime cause of rheumatoid arthritis and ankylosing disease. They were of course derided for having no real evidence since ex vivo microbial analyses (applying Koch's postulates) had continually failed to unambiguously identify disease-causing agents. Historically the focus was upon cultivating viable pathogens from joints, certainly not upon extra-articular microbes establishing immune responses initially *outside* the joint, which then adversely affected joint physiology and function. Koch's original postulates certainly transformed medical microbiology but they also very much limited the search for etiological agents to those present and viable within the diseased tissue; ignoring any which had been and gone but left a memory of their presence. These were serious constraints if the search for causative agents focussed wholly on the wrong anatomical compartment.

Finally it all seemed to come together with the realisation that silver therapeutics needed to be re-assessed with an open mind, without prejudice in the context of a chronic inflammatory disease. There were historic precedents sufficient at least to ask the simple question: why not?

Animal responses were amazing, if silver was tested in the appropriate (slow-release) formulation and at the right stage of disease development. Nanosilver given orally prevented arthritis development at extraordinarily low doses but soluble silver salts and silver (I) oxide, given orally, were not anti-arthritic (Whitehouse et al. 2013).

But what was the relevance for RA etc. of these responses to NPS in rats? A preliminary study indicated that (arthritigenic) *Proteus* might be eliminated from the lower bowel and urinary bladder by a short course of ingesting an NPS preparation previously shown to be a powerful antibiotic against two species of *Proteus* in vitro (Disaanayake et al. 2014). An interesting finding maybe; but how would patients with RA respond? Might the silver prophylaxis be too late (the pessimistic

view)? Alternatively, if the RA pathogenesis needs to be restimulated intermittently, then this late silver therapy might be beneficial (the optimistic view).

Extrapolating from animal studies, however well-designed and carefully conducted, is always difficult. The sooner one can measure responses, both beneficial and/or toxic, in *real* patients with *real* diseases over *realistic* time frames, then the sooner will silver therapeutics be either vindicated *or* should be abandoned. The impediments to undertaking such an important clinical study should be clearly recognised and surmounted as soon as possible.

To quote Osler:

> 'The value of experience is not in seeing much but in seeing wisely.'
> (Peskett 2014)

In this survey of silver pharmacology we have seen much, mainly laboratory findings—but without more approvals for verifiable clinical studies, how do we become much wiser?

**Acknowledgments** This chapter owes much to (i) the enthusiasm of Gerald Hancock, Hans Laroo, John Petty, Ross Stevens, and Alan White who each taught me much about the practicalities of silver research, both within and outside the laboratory; and (ii) Desley Butters whose skills and patience ensured a typescript fit for publication.

## Appendix A: Solubilities of Some Silver Salts/Complexes in Water

Section A, with physiological anions; Section B with some xenobiotic anions
$S$ = solubility (gm/L), temperature ($T$) = 25 °C except as noted.

| | Anion | $S$ (g/L) | $T$ |
|---|---|---|---|
| A. | Nitrate | $2.57 \times 10^{3}$ | |
| | Acetate | 11.1 | |
| | Nitrite | 4.2 | |
| | Sulphate | 2.2 | |
| | Carbonate | $3.3 \times 10^{-2}$ | |
| | Phosphate | $6.4 \times 10^{-3}$ | |
| | Chloride | $1.9 \times 10^{-3}$ | |
| | Chloride | $2.1 \times 10^{-1}$ | 100 |
| | Stearate | $6.5 \times 10^{-4}$ | 20 |
| | Oxide ($Ag_20$) | $2 \times 10^{-4}$ | |
| | Sulphite | $1.4 \times 10^{-4}$ | |
| | Bromide | $1.35 \times 10^{-4}$ | |
| | Iodide | $2.6 \times 10^{-6}$ | |
| | Cyanide | $2.3 \times 10^{-6}$ | |

(continued)

(continued)

| | Anion | *S* (g/L) | *T* |
|---|---|---|---|
| | Oxalate | $3.6 \times 10^{-11}$ | |
| | Thiocyanate | $1.0 \times 10^{-12}$ | |
| | Sulphide | $1.5 \times 10^{-14}$ | |
| B. | Perchlorate | $5.57 \times 10^{3}$ | |
| | Fluoride | $>10^{3}$ | |
| | Thiosulphate | $7 \times 10^{2}$ | 20 |
| | Bromate | 1.6 | |
| | Salicylate | 0.95 | |
| | Iodate | 0.44 | |
| | Chromate | $3.5 \times 10^{-2}$ | |
| | Azide | $2.0 \times 10^{-8}$ | |
| | Arsenate | $1.0 \times 10^{-22}$ | |

*Notes*
(i) Silver nitrate, sulphate and acetate are much more water-soluble than silver nitrite, sulphite, carbonate or oxalate.
(ii) Stability constants for Ag-L bonding are recorded by Hogfeldt (1982) where L is inorganic and by Perrin (1979) for some organic ligands. These indicate strength of the bonds i.e. dissociation (affinity) constants for Ag-L [Solubility data only provide some indications of bond formation but do not quantify the strength of this bond.]

## Appendix B: Reproducible Production of Nano $Ag^0$ for Medicinal Use

(also see Laroo 2013)

Some variables that should be recognised *and* carefully controlled include as follows:

- Cleanliness of glassware, etc.
- Purity of the silver metal ($Ag^0$) or silver salts (Ag(I)) which should always be of the highest grade available (>99 %). DIY products using cheaper grades of metallic silver, coinage, silver buckles, etc. will generate *toxic* products—either containing lead and cadmium (from incomplete refining) or the metals added as hardeners, e.g. copper, nickel, etc.
- Purity of water or other solvents, including content of oxygen and carbon dioxide. Removing chloride ions is essential.
- Physical procedures involved in manufacture. If using
  - Radiant energy (light, gamma rays), define bandwidth and intensity of radiation, duration of exposure, etc.;
  - Microwave energy, define wattage and duration;

  - Heat, define temperature and conditions for cooling;
  - Sonic energy, define key variables;
  - Electro-chemical procedures, define current, voltage, purity of DC supply, temperature control and degree of illumination.

- For chemical methods producing NPS from Ag(I) salts, also define
  - Purities of essential reagents used as reductants, dispersants and stabilisers
  - Methods to remove by-products and/or excess reductants: some of these may be physiologically unacceptable e.g. borate or formate or oxalate after using borohydride or formaldehyde or ethylene glycol respectively as the reducing agents. [The much cited Carey-Lea method includes ferrous ions in the mix (Frens and Overboek 1969.]
  - Qualities of essential 'extras' e.g. stabilisers/preservatives
  - Operational procedures for any further physical manipulations to separate impurities, enhance potency or lengthen shelf-life (e.g. ultra centrifugation, dialysis, etc.)
- Identify optimal storage conditions: specifying temperature, light sensitivity, nature of containers (plastic, borosilicate or soda glass, etc.)

*Note* Many methodologies described in the literature focus on NPS production for industrial uses *not* medicinal purposes. They should be adopted only *after* recognising the more stringent purities and stability needed for approved medications.

## Appendix C: Quality Controls for NPS Products

'The important thing about knowledge is discrimination, not quantity'.
Peskett (2014)

*Note* These quality controls can be just as important for understanding toxicities of nano-products. (Warheit et al. 2008) as well as their beneficial pharmaco-activities.

I. Physico-chemical

- Analysis for total silver content and purity[6] and proportion of ionic Ag(I); measure p[Ag].
- Definition of NPS product by determining:

[6]Atomic absorption spectrometry (AAS) is not always suitable or accurate for determining silver content; sometimes being influenced by extraneous components ('matrix effect').

- Range of particle sizes
- Particle charge (volts) and polarity (+ or −) i.e. Zeta potential
- Particle shape, if transmission electron-microscopy is accessible
- Acidity/alkalinity i.e. pH
- Electrical conductivity
- Light scattering properties and light absorption (400–450 nm)
- Sorption properties e.g. using fluorescent/fluorogenic indicators
- Flocculation by anions/cations and acidity[7]
- By-products present, if not previously removed
- Additives e.g. stabilisers, anti-flocculants, pharmaceutical adjuncts
- Light sensitivity
- Shelf life before obvious deterioration
- Optimal conditions for storage e.g. dark bottles? and temperature?.

II. Biological properties in vitro (for quick testing)

- Stability in physiological media = saline, artificial gastric juice, urine
- Compatibility with plasma proteins, etc.
- Toxicity to selected microbes (bacteria,[8] yeast, protozoa), plant tissues, e.g. germinating radish seeds, to cultured animal cells and whole organisms e.g. brine shrimps[9]

## Appendix D: 'Colloidal Silver' (CS): Some Facts and Fallacies

(*Note* These are items for continuing discussion, not dogmatic truths)

**Fallacies**

I. A CS preparation can be a universal treatment for nearly all microbial infections—but where is the detailed evidenced?
II. It is a 'good' medicine because it has traditional origins, 'mystique', etc.—but so did apricot kernels (Laetrile) for treating cancer.
III. It is cheap and easy to manufacture and therefore suitable as a home-made remedy—but so are many poisons e.g. methanol from wood, insecticidal nicotine from tobacco leaves.
IV. 'It must be good', receiving so much attention on the Web—but much of this is advertising and too little is unbiased commentary.

[7]Use acetic or sulphuric acids; avoid nitric acid (oxidant) and hydrochloric acid (precipitates Ag (I)).

[8]Caution: certain microbiological assays e.g. using agar gels (negatively charged) may alter the proportion of Ag(I) to $Ag^0$ and bias test results.

[9]See Cock et al. (2012).

V. If it were useless, why would the medical establishment want to 'suppress' it? [This is a tendentious matter, based more on expediency than principle.]
VI. 'It must be safe', considering the widespread use of silver coinage, cutlery, containers, etc. in our domestic environment—but only rarely are we using unvarnished bulk silver in medicine.

**Facts**

(i) Unless defined by composition e.g. purity, particle size, shape and charge, and stability; a CS preparation cannot be expected to successfully interact *consistently* with a chosen pathogen/other targeted biosystem.
(ii) Current silver pharmaceuticals are remarkably non-toxic, in contrast to many inorganic drugs that were once 'in-vogue'. These latter medications were notoriously toxic with quite dreadful therapeutic indices; *vide* historic use of mercurials, antimonials, arsenicals and more recently the platinum carcinostats that are all still being used in orthodox/allopathic medicine.
(iii) Cost is misleading and many qualities may be sacrificed by using (a) impure metallic silver sources, (b) haphazard control of preparative procedures, (c) inadequate checks for impurities and (d) failure to control other essential qualities e.g. stability ex vivo (light, heat, etc.).
(iv) Drug regulators and the medical fraternity are rightfully concerned to prevent abuse and harm from any antibiotics to both the consumer *and* the modern environment e.g. the essential microbial population of sewage sludge. Current regulatory procedures for CS and other silver pharmaceuticals seem particularly oppressive (prohibition, etc.); especially when contrasted with the minimal management of *proven* health risks affecting the whole community, such as alcohol, tobacco, and the over-consumption of sugar, salt, (w6)unsaturated fats; all of which are largely unregulated.
(v) The safety of (bulk) metallic silver, as in coinage and domestic silverware, is largely irrelevant when considering latent toxicities of ingestible, cationic and nano-metallic silver preparations.

## Appendix E: A Summation About NPS: The Good, the Bad, and the Ugly

### Good

- Long-term stability without refrigeration
- Potential to adhere to/penetrate bacterial biofilms
- Adjunct to use with planktonic antibiotics, either for synergy or 'back-up' therapy
- Cheap. Possible to prepare near/on-site for appropriate infection control
- Restricted diffusion, for maximal topical efficacy (e.g. on skin/gastric ulcers)

- Slow-release source of pharmaco-active Ag(I) = selective toxin?
- Potential for using NPS particles as carriers/delivery systems for other pharmaca (attached covalently or by Coulombic interactions)

**Not So Good, often quite Bad in fact**

- Inadequate quality controls for safety, purity, stability, antibiotic activities, etc. and risk of adverse reactions.
- Flood of unproven claims on the WWW for pharmaco-efficacy; often misleading and some being nonsense
- Very limited funding for research in an area with poor prospects for high financial returns e.g. from exclusive patents
- Lack of vision by health authorities to help side-step this impasse and also support more studies of silver pharmaceuticals for controlling antibiotic-resistant microbes

**Ugly**

- Claims that very small silver particles (<2 nm) penetrate the brain, causing firing of the neurones
- Poorly controlled targeting for systemic use
- Ill-founded arguments about environmental toxicities (with much propaganda but too little science)
- Political interference and suppressive tactics to suit vested/misguided interests, rather than patient's welfare
- Polemics: regulation favouring *status quo* (basically 'ban silver') to the detriment of *independent* trials to (i) ascertain possible long-term medicinal benefits and (ii) understand safe limits for both individuals *and* the environment. [Certainly not good science and rather a discredit to Modern Medicine, manipulated or otherwise.]

*Note* This table is far from complete

## Appendix F: Antibiotic Potency, Stability and/or Bio-transformation of Nanoparticulate Silver (NPS): Some Facts and Speculations

### Facts

1. "One size fits all" is clearly untrue in describing antibiotic activities of NPS products, however carefully prepared to minimise heterogeneity (size, charge density, shape, etc.). So for example, the type of preparations that may be anti-protozoal may have little value as an antiviral.

2. NPS ($Ag^0$) prepared by chemical procedures, particularly reduction of soluble Ag(I) salts or complexes, such as $[Ag(NH_3)_2]^+$, will have their anionic/cationic character largely determined by the preparative conditions.

   Eg $AgNO_3$ and tribasic citrate salts produce anionic NPS hydrosols, readily aggregated by lanthanum (III) ions i.e. their 'superficial' charge is negative.

3. By contrast NPS preparations derived without added chemicals e.g. by photo-induced reduction of electrolytically generated Ag(I) cations (Laroo 2013) will most likely be cationic i.e. not aggregated by La(III).
4. The charge densities of NPS particles would largely determine their stability ex vivo, i.e. shelf-life. In theory, the smaller the particles, the greater their ionic character relative to total mass: consequently they will be less likely to spontaneously aggregate. But their shelf life may be no indication of their utility for combating infections in vivo, if they need first to be transformed/bioactivated in vivo (see Item iii below).
5. Many reports about the antibiotic potencies of NPS preparations provide few details of how disperse (i.e. size variation) and stable they are. For example, NPS-citrate preparations are intrinsically unstable. Commercial NPS samples, stabilised with citrate and used as reference 'standards' for sizing, can aggregate over time with distinctive changes in colour, opacity—and very often biopotency ex vivo. Such deterioration may not always be a negative feature, if it reflects spontaneous transformations that might be accelerated in vivo when used medicinally or ex vivo within the environment after excretion.

**Speculations**

(i) Should we be constructing a working concept of NPS 'metabolism', considering not only these spontaneous transformations in vitro (see item 5 above) but *also* the effects of the bio-environment upon an administered polysilver-X NPS complex? This perspective questions the tacit assumption that silver pharmacology is primarily all about the effects of $Ag^0$/Ag(I) *upon* targeted bio-systems; while usually ignoring the reverse interaction i.e. how the internal environment may act upon an NPS preparation to determine pharmaco-activity.

(ii) If so, the nature of factor(s) X may profoundly influence not only the physico-chemical *stability* of an NPS preparation ex vivo but also its potential *instability* (and pharmaceutical value) within the various biosystems it may encounter.

(iii) Concerning the nature of this factor X: if it is either a potentiator or an inactivator may then determine the medicinal value of an NPS preparation. For example:

- If X is oxygen, will this oxide/dioxygen 'film' be diminished by 'scrubbing' with acids in vivo or amplified by $H_2O_2$ and other bio-oxidants?
- If X is citrate, will this dissociate and be catabolised in vivo, generating a silver particle more likely to spontaneously aggregate (after losing a

particle-stabilising negative charge); perhaps providing a less efficient 'pay load' of bio-reactive Ag(I)?

- If X is ionic Ag(I), will a mobile NPS that is cationic in vitro now be neutralised, even aggregated, by chloride or phosphate ions in vivo?
- If X is an argentophilic bioligand (Table 4) captured in vivo, will this always immobilise or destroy the value of an NPS product? In some contexts it might possibly increase the hydrophilicity of an NPS preparation, altering its bio-distribution, so changing its efficacy.
- If X is a particle 'stabiliser', may this protective coating be so stable as to isolate a reactive NPS from its targeted biosubstrate? Generalisations about the inefficacy of NPS preparations as an antibiotic in vitro e.g. where X equals PEG or PVP (Xiu et al. 2012) or oleate, may be true in their context but not necessarily valid where X is readily dissociated or metabolised in vivo.

iv. So how might the nature of X further determine antibiotic activity? As an illustration of this question, here are two examples:

(a) The *acidic* environment of the stomach may determine the anti-*Helicobacter pylori* activity of an NPS-oxide preparation, to help control gastric ulceration; but the anti-ulcerant effect may not be the same in a different compartment such as within the more *alkaline* environment of the proximal duodenum. [Even though *Helicobacter* provokes both stomach and duodenal ulceration.]

(b) Some ligands such as esterified ethenoid (unsaturated) fatty acids might reversibly trap some types of NPS at strategic loci, so enhancing their local potency in vivo e.g. within lipid membranes.

v. Statements that NPS particles are less effective antibiotics for Gram-positive (GP) bacteria than Gram-negative (GN) species—as determined from standard ex vivo bioassays—sometimes may have little meaning for understanding how they might behave in vivo. For example, cationic NPS species might be expected to bind to the anionic polyphosphate teichoic acids within the outer cell wall of GP bacteria. But several reports describe the lesser microbial activity of NPS preparations upon these teichoate-coated bacteria ex vivo. This would not be surprising if the NPS preparations being tested ex vivo were themselves anionic e.g. citrate-coated. But to extrapolate from this hard data to predicting that all NPS preparations would be less useful for treating infections by GP bacteria—without thoroughly evaluating the potency of *cationic* NPS preparations—may perpetuate a grave error in our perceptions of what are the more useful formulations with which to conduct clinical trials.

vi. By contrast, GN bacteria carrying a relatively high content of lipids within their cell coating, may either preferentially take up and bond with hydrophobic NPS particles (with low charge density) or 'capture' cationic NPS particles that may disrupt the stabilising effects of their anionic membrane phospholipids.

**In Conclusion**

Beware of simplistic generalisations about potencies of antimicrobial NPS preparations which implicitly assume that they are always 'naked' silver particles. In fact they are more likely to be complex entities, represented here as NPS-X. So in the absence of essential details about (a) their bio-evaluation ex vivo (e.g. with or without using agar plates) and (b) their physical stability, chemical constitution (noting what factor X might be) and natural lability—we may be dealing with 'rogue products'. This is certainly no basis for a scientifically grounded antibiotic revolution, as yet.

## Appendix G: Political Considerations

This ought not to be part of any scientific enquiry into the merits or hazards of any drug, new or old. The facts, the theories, the clinical evaluations, etc. should all be dispassionately considered—without interference or censorship from either vested interests or poorly informed government regulators. At present, silver therapeutics is not allowed this freedom. There are several reasons for this situation. Here are three:

1. Governmental regulations concerning use of, and claims for, medicinal silver are presented in the following e-publications.
   http://www.fda.gov/ohrms/dockets/98fr/081799a.txt
   http://www.gpo.gov/fdsys/pkg/FR-1999-08-17/pdf/99-21253.pdf
   http://nccam.nih.gov/health/silver and for Australia https://www.tga.gov.au/colloidal-silver-related-products
   They are dogmatic, deny medicinal claims and even punitive if you disagree. This is not a good state of affairs for progressing the healing arts, especially those with traceable histories of their benefits exceeding possible harm *and* supported by unambiguous scientific observations. But wisely considered, these current regulations concerning the medical use of silver should catalyse a genuine debate about how to unshackle laboratory and clinical research on silver pharmaceuticals—to move beyond a negative risk-averse mindset (i.e. 'thou shalt not….'). This needs to be replaced by a climate of open *and* honest enquiries about what may be beneficial to humankind (and animals) and about what may not be so (especially for the environment).
   Recently, we have seen other traditional therapies decriminalised e.g. cannabis for cancer pain. So why not silver therapeutics, especially if they might save lives from infections that are currently antibiotic-resistant? [But how will we ever know this under the present suppressant regulations?]
2. The power of industrial lobbyists (Angell 2004; Goldacre 2012).
   The influence of pharmaceutical companies upon the 'education' of physicians and operations of various drug-regulatory agencies has inverted some of the

functions of these agencies [They were originally established to prevent harm to the public, not to seemingly protect monopolistic interests at the expense of public wellbeing.]

We are left with the unsatisfactory status of alternative/traditional therapies, like those using silver and without patent protection. They are the subject of considerable misinformation and rarely funded to provide the evidence demanded by these lobbyists, insisting they be treated as 'new drugs'—even when historically shown to be much more acceptable and rather cheaper than many licensed/patented alternates. This is another aspect of the 'rhetoric versus reality' (Angell 2004) as practised by Big Pharma to destroy its competition.

3. 'Consumer protection'

In theory, this is a very good thing but so often it is captured by vested interests and debased in practise.[10] It is naive to believe official dogmas that such alternate/traditional therapies are nearly always either unproven(!) (but see Bone and Mills 2013) or too dangerous to be licensed—even when Quality Assured. Agencies minding our health need to do their own homework; if necessary sponsor and publish their own research and (for a change) listen to non-industrial advocates sometimes.

## References

## Recommended Further Reading

(i) A short overview of colloidal nanosilver—production, properties, standards and bio-efficacy (Laroo 2013). [This is an open access article, distributed under the Creative Commons.]
(ii) An extensive review of 'Nanobiosilver ' and its uses in medicine with over 600 references (Eckhardt et al. 2013)
(iii) A comprehensive survey entitled "Nanosilver", sponsored by the US Environmental Protection Agency, with over 500 references (Varner et al. 2010). [Ironically, this arm of the US government considers it to be 'safe', in contrast to the FDA which does not seem to agree with this conclusion.]
(iv) A valuable review of silver disposition, toxicity and utility for preventing infection, with over 160 references (Lansdown 2010) [This is also an open access article, distributed under the Creative Commons.]
(v) A valuable bibliography particularly relating to the healing potential of silver colloids and electrically generated silver ions, with over 180 references (Flick 2009)

[10]An astonishing development in some parts of Europe is that sales of vitamins, formerly freely available OTC, are being regulated—apparently being deemed unsafe without a prescription. The beneficiaries seem to be those pharmaceutical companies which have taken over former suppliers or created new subsidiaries to provide the now licensed nutritional supplements. Not surprisingly, costs to consumers have risen quite considerably, a corollary of over-ruling a free market.

(vi) A short review covering silver's history and silver nanoparticles as antimicrobials (Rai et al 2009)
(vii) A timely survey of silver nanotechnologies and the environment, with over 100 references (Luoma, 2008)
(viii) Challenging insight from Material Scientists about the complexities of aqueous nanosilver systems—including altered states of water and oxide 'skins' being formed around the nanoparticles (Roy et al 2007)
(ix) The phenomenon/problem of microbial silver resistance, reviewed by (Simon) Silver* (2003) (*Not a typographical error: this is the author's surname.)
(x) Two wider perspectives on selective toxicity (but not discussing silver) by the late Adrian Albert AO (1907–1989), the Australian National University, Canberra (Albert 1979, 1987)

Abraham GE, Himmel PB (1997) Management of rheumatoid arthritis: rationale for the use of colloidal metallic gold. J Nutr Environ Med 7:295–305
Adlof RO, Emken EA (1985) The isolation of omega-3 polyunsaturated fatty acids and methyl esters of fish oils by silver resin chromatography. J Oil Fat Ind 62:1592–1595
Albert A (1979) Selective toxicity: the physico-chemical basis of therapy, 6th edn. Chapman & Hall, London 662 pp
Albert A (1987) Xeniobiosis: foods, drugs, and poisons in the human body. Chapman and Hall, London 367 pp
Angell M (2004) The truth about the drug companies. How they deceived us and what to do about it. Random House, New York, 304 pp
Antelman MS (1997) Methods of curing AIDS with tetra-silver tetroxide molecular crystal devices. US Patent 5,676,977 (Oct. 14, 1997. C1.424/618)
Arner ESJ, Nakamura H, Sasada T, Yodoi J (2001) Analysis of the inhibition of mammalian thioredoxin, thioredoxin reductase and glutaredoxin by cisdiamminedichloroplatinum (II) and its major metabolite. Free Radical Biol Med 31:1170–1178
AshaRani PV, Mun GLK, Hande MP, Valiyaveettil S (2009) Cytotoxicity and genotoicity of silver nanoparticles in human cells. ACS Nano 3:279–290
Becker AJ, Becker RO, Flick AB (1998) Iontophoregic system for stimulation of tissue healing and regeneration. US Patent 5814094, 27 Sept 1998
Becker RO, Selden G (1985) The body electric. Electromagnetism and the fountain of life. Harper, New York, 364 pp. [especially Chapter 8, "The silver wand", pp 163–180 and the Postscript "Political Science" pp 330–348]
Berners-Price SJ, Filipovska A (2011) Gold compounds as therapeutic agents for human diseases. Metallomics 3:863–873
Blindauer C (2011) Bacterial metallothioneins: past, present and questions for the future. J Biol Inorg Chem 16:1011–1024
Blindauer C (2014) personal communication
Bone K, Mills S (2013) Principles and practise of phytotherapy. Modern herbal medicine. Elsevier, Edinburgh, 1051 pp
Brown C, Bushell G, Whitehouse MW, Agrawal DS, Tupe KM, Paknakar KM, Tiekink ERT (2007) Nanogold pharmaceutics II Characterisation of the gold in *Swarma Bhasma*, a microparticulate used in Indian traditional medicine. Gold Bull 40:245–250
Brown CL, Whitehouse MW, Tiekink ERT, Bushell GR (2008) Colloidal metallic gold is not bio-inert. Inflammopharmacol. 167:133–137
Browne J. (2013) Silver In: Seven elements that have changed the world. London. Weidenfeldt & Nicolson, pp 105–125.
Browning E (1968) Silver in toxicity of industrial metals, 2nd edn. Butterworth, London, pp. 296–301
Brust M, Walker M, Bethell D, Schiffrin DJ, Whyman R (1994) Synthesis of thiol-derivatised gold nanoparticles in a two-phase liquid-liquid system. J Chem Soc Chem Commun 801–802
Burcham P (2010) Nanotoxicology: a primer for chemists. Chem Aust 77:18–22
Chappell JB, Greville GD (1954) Effect of silver ions on mitochondrial adenosine triphosphatase. Nature 174:930–931

Chaw KC, Manimaran M, Tay FEH (2005) Role of silver in destabilisation of intermolecular adhesion forces in *Streptococcus epidermis* biofilms. Antimicrob Agents Chemotherap 49:4853–4859

Cialla D, Bohme R, Theil F, Weber K, Schmitt M, Popp J (2012) Surface-enhanced Raman spectroscopy (SERS): progress and trends. Anal Bioanal Chem 403:27–54

Cock I, Mohanty S, White A, Whitehouse M (2012) Colloidal silver (CS) as an antiseptic: two opposing viewpoints. Pharmacog Comm 2:47–56

Costigan P, Ellis KJ (1980) Retention of copper oxide needles in cattle. Proc Aust Soc Animal Prod 6:637–642

Czoik R, Heintz A, John E, Marczak W (2008) Complexes of silver with histidine and imidazole investigated by the calorimetric and potentiometric methods. Acta Phys Pol, A 14:A52–A56

Demling RH, DeSanti L (2002) The rate of re-epithelialization across masked skin grafts is increased with exposure to silver. Burns 28:264–266

Dibrov P, Dzioba J, Gosink KK, Hase C (2002) Chemiosmotic mechanism of antimicrobial activity of $Ag^+$ in *Vibrio cholera*. Antimicrob Agents Chemotherap 46:2668–2670

Disaanayake DMBT, Faoagali J, Laroo H, Hancock G, Whitehouse M (2014) Efficacy of some colloidal silver preparations and silver salts against *Proteus* bacteria, one possible cause of rheumatoid arthritis. Inflammopharmacol 22:73–77

Ebringer A (2012) Rheumatoid arthritis and *Proteus*. Springer, London, 223 pp

Ebringer A (2013) Ankylosing spondylitis and *Klebsiella*. Springer, London 256 pp

Ebringer A (2015) Multiple sclerosis, mad cow disease and *Acinetobacter*. Springer, London, 200 pp

Eckhardt S, Brunetto PS, Gagnon J, Prieve M, Geise B, Fromm KM (2013) Nanobio Silver: its interactions with peptides and bacteria, and its uses in medicine. Chem Rev 113:4708–4754

Evans M (2013) Encounter with god. Scripture Union, Milton Keynes, p 90

Fischer P, Jansen M (1995) Electrochemical syntheses of binary silver oxides. Inorg Synth 30:50–55

Flick AB (2009) Silver colloid bibliography, 183 references reproduced on the website "The Bigger Picture and the Quest for Truth" under the heading re colloidal silver "Isn't it time the FDA went and ……?" http://www.911forum.org.uk/board/viewtopic.php?p=134716&sid=aecf964695bebd62b053876d0f5bb877 Retrieved 4 April 2014

Forbes G (1911) The solubility of silver chloride in chloride solutions and the existence of complex argentichloride ions. J Am Chem Soc 33:1737–1946

Forbes GS, Cole HI (1921) The solubility of silver chloride in dilute chloride solutions and the existence of complex argentichloride ions. II. J Am Chem Soc 43:2492–2497

Frausto da Silva JJR, Williams RJP (1991) The biological chemistry of the elements. Clarendon Press, Oxford 561 pp

Frens G, Overboek G (1969) Carey Lea's colloidal silver. Kolloid-Zeitschrift und Zeitschrift für Polymere 223:922–929

Gibbs RJ (1999) Silver-colloids—do they work? http://www.silver-colloids.com. Accessed 8 April 2014

Gmelin M (1971) Silber In: Gmelin's Handbuch der Anorganische Chemie, 8 Aulflage Nr 6. Verlag Chemie, Weinheim

Goldacre B (2012) Bad pharma: how drug companies mislead doctors and harm patients. Faber & Faber, London, 448 pp

Graham GG, Whitehouse MW, Bushell GR (2008) Aurocyanide, dicyanoaurate (I), a pharmacologically active metabolite of medicinal gold complexes. Inflammopharmacol. 16:126–132

Hancock GL (2011) Colloidal metallic silver, 2nd edn. Kalaya Publications, Kalbar. Accessed http://www.kalayaproducts.com.au]

Harper N (1958) Drug latentiation. J Med Chem 1:467–500

Higginbottom J (1826) An essay on the application of the lunar caustic: in the cure of certain wounds and ulcers. Longman Rees Orme Brown & Green, London, 147 pp. (Kessinger Publishing: http://www.kessinger.net)

Hill WR, Pillsbury DM (1939) Argyria. The pharmacology of silver. Williams & Wilkins Co, Baltimore, 172 pp
Huang YF, Zhang M, Zhao LB, Feng JM, Wu DY, Ren B, Tian ZQ (2014) Activation of oxygen on gold and silver nanoparticles assisted by surface plasmon resonances. Angew Chem Int Ed Engl 53:2353–2357
Jefferson W (2003) Colloidal silver today. The all-natural wide-spectrum germ killer. Healthy Living Publications, Summertown, 63 pp
Jocelyn P (1972) Biochemistry of the SH group. Academic Press, London
Kalishwaralal K1, Barathmanikanth S, Pandian SR, Deepak V, Gurdinathan S (2010) Silver nanoparticles impede the biofilm formation of *Pseudomonas aeruginosa* and *Streptococcus epidermis*. Colloid Surf B Biointerfaces 79:340-344
Kean WF, Kean IR (2008) Clinical pharmacology of gold. Inflammopharmacol. 16:112–125
Kittler S, Greulich C, Diendorf J, Koller M, Epple M (2010) Toxicity of silver nanoparticles increases because of slow dissolution under release of silver ions. Chem Mater 22:4548–4554
Kornberg A, Pricer WE (1953) Isolation of diphosphopyridine nucleotide. Biochem Prep (ed. EE Snell) 3:20–24
Lansdown ABG (2010) A pharmacological and toxicological profile of silver as an antimicrobial agent in medical devices. Adv Pharmacol Sci. doi:10.1155/2010/910686
Laroo H (2013) Colloidal nanosilver—its production, methods, properties, standards and its bio-efficacy as an inorganic antibiotic. J Phys Chem Biophys 3(5):100130
Lau GW, Hassett DJ, Ran H, Kong F (2004) The role of pyocyanin in *Pseudomonas aeruginosa* infection. Trends Mol Med 10:599–606
Legke MF, Ravel B, Fleet ME, Wanger G, Gordon RA, Southam G (2006) Mechanisms of gold bioaccumulation by filamentous cyanobacteria from gold (III)-chloride complex. Environ Sci Technol 40:6304–6309
Legke MF, Fleet ME, Southam G (2007) Biosynthesis of silver nanoparticles by filamentous cyanobacteria from a silver (I) nitrate complex. Langmuir 23:2694–2699
Li L, Zhu Y-J (2006) High chemical reactivity of silver nanoparticles towards hydrochloric acid. J Col Interface Sci 303:415–418
Li P, Li J, Wu C, Wu Q, Li J (2005) Synergistic antibacterial effects of β-lactam antibiotic combined with silver nanoparticles. Nanotechnol 16:1912–1917
Liu J, Sunshine DA, Shervani S, Hurt RH (2010) Controlled release of biologically active silver from nanosilver surfaces. ACS Nano 4:6903–6913
Liu J, Pennell KG, Hurt RH (2011) Kinetics and mechanism of nanosilver oxysulfidation. Environ Sci Technol. doi:10.1021es 2015 539 s
Loewenstein W, Fee K (1928) Uber kollargolbehandlung des chronischen gelenkrheumatismus. Klin Woch (Berlin) 39:1860–1864
Luoma SN (2008) Silver nanotechnologies and the environment: old problems or new challenges. Project on emerging technologies. (Pen) 15. Woodrow Wilson International Centre for Scholars, Washington DC
Maass GJ (2014) Dietary supplement silver nanoparticles: no threat to the environment. Pharmacog Comm
Mack D, Davies AP, Harris LG, Knobloch JKM, Rohde H (2009) *Staphylococcus epidermidis* biofilms: functional molecules, relation to virulence, and vaccine potential. Top Curr Chem 288:157–182
Madiera JM, Gibson DL, Kean WF, Klegeris A (2012) The biological activity of auranofin. Inflammopharmacol. 20:297–306
McMillan JA (1962) Higher oxidation state of silver. Chem Revs. 62:65–80
Mellor JW (1923) Comprehensive treatise on inorganic and physical chemistry, vol III. Longman Green, London, pp 295–490
Mitchell P (1966) Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. Biol Revs 41:445–502
Nesbitt RU Jr, Sandmann BJ (1977) Solubility studies of silver sulfadiazine. J Pharm Sci. 66:519–522

Nesbitt RU Jr, Sandmann BJ (1978) Solubility studies of silver sulfadiazine. J Pharm Sci 67:1012–1017

Omatu Y, Folan M, Shaw M, Messer RL, Lockwood PE, Hobbs D et al (2006) Sublethal concentrations of diverse gold compounds inhibit cytosolic thioredoxin reductases (TrxR1). Toxicol In Vitro 20:882–890

Osborne OJ, Johnston BD, Moger J, Balousha M, Lead JR, Kudoh T, Tyler CR (2012) Effects of particle size and coating on nanoscale Ag and $Ti0_2$ exposure in zebrafish (*Daniio rerio)* embryos. Nanotoxicology 1743–5404. doi:10.3109/17435390.2012.737484

Perrin DD (1979) Stability constants of metal ion complexes. Organic ligands, 2nd edn. Pergamon Press, Oxford, pp 1263

Peskett H (2014) Commentary on proverbs (Holy Bible) in Encounter with God. Scripture Union, Milton Keynes, UK p. 108

Rai M, Yadav A, Gade A (2009) Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv 27:76–83

Roy R, Hoover MR, Bhalla AS, Slawecki T, Dey S, Cao W et al (2007) Ultradilute Ag aquasols with extraordinary bactericidal properties: role of the system Ag-0-$H_2O$. Mater Res Innov 11:3–18

Ruden S, Hilpert K, Berditsch M, Wadhwani P, Ulrich AS (2009) Synergistic interactions between silver nanoparticles and membrane-permeabilising antimicrobial peptides. Antimicrob Agents Chem Ther 53:3538–3540

Shahverdi AR, Minacian S, Shahverdi HR, Jamalifar H, Lohi A-A (2007) Rapid synthesis of silver nanoparticles using culture supernatants of *Enterobacteria*: a novel biological approach. Process Biochem 42:919–923

Shaw CF III (1999) Gold-based therapeutic agents. Chem Rev 99:2589–2600

Shukla S, Sastry M (2009) Probing differential $Ag^+$-nucleobase interactions with isothermal titration calorimetry (IT): toward patterned DNA metallization. Nanoscale 1:122–127

Silver S (2003) Bacterial silver resistance: molecular biology and uses and misuses of silver compounds. FEMS Microbiol Rev 27:341–353

Simpson WJ, Hewlett RT (1914) Experiments on the germicidal action of colloidal silver. Lancet 184:1359

Spadaro JA, Berger TJ, Barranco SD, Chapin SE, Becker RO (1974) Antibacterial effects of silver electrodes with weak direct current. AntimicrobAgents Chem Ther 6:637–642

Stahl P (ed) (1965) Thin layer chromatography., A laboratory handbook Springer, Berlin, pp 178–179

Stillman MJ, Presta A, Gui Z, Jiang DT (1994) Spectroscopy of copper, silver and gold—metallothioneins. Met-Based Drugs 1:375–394

Stwertka A (2012) A guide to the elements, 3rd edn. Oxford University Press, New York, pp 127–129

Suresh AK, Pelletier DA, Wang W, Moon J-W, G B, Mortensen NP et al (2010) Silver nanocrystallites: biofabrication using *Shewanella oneidensis*, and an evaluation of their comparative toxicity on Gram-negative and Gram-positive bacteria. Environ Sci Technol. 44:5210–5215

Suzuki Y, Yoshimaru T, Matsui T, Ra C (2002) Silver activates calcium signals in rat basophilic leukemia-2H3 mast cells. J Immunol 169:3954–3962

Sweet JE, Wilmer HP (1919) A treatment for trench fever. Lancet 252–254

Thompson NR (1973) Silver. In: Bailor JC, Emeleus HJ, Nyholm R, Trotman-Dickenson AF (eds) Comprehensive Inorganic Chemistry. Pergamon Press, Oxford, pp 79–128 (with 352 refs.)

Tiekink ERT (2008) Anti-cancer potential of gold complexes. Inflammopharmacol. 16:138–142

Tudela D (2008) Silver (II) oxide or silver (I, III) oxide? J Chem Educ 85:863–865

Varner K, El-Badaway A, Feldhake D, Venkatapathy R (2010) State of the science literature review: everything nanosilver and more. US Environmental Protetion Agency, Washington DC, 363 pp

Vasak M (2011) Metallothioneins: chemical and biological challenges. J Biol Inorg Chem 16:975–976 (An introduction to several mini reviews, pp 977–1134)
Venugopal B, Luckey TD (1978) Silver (Ag). In: Metal toxicity in mammals, vol 2. Chemical toxicity of metals and metalloids. Plenum Press, New York, pp 32–36
Walker WR, Beveridge SJ, Whitehouse MW (1980) Anti-inflammatory activity of a dermally applied copper salicylate preparation (Alcusal). Agents Actions 10:38–47
Walker WR, Keats DM (1976) An investigation of the therapeutic value of the 'copper bracelet'. Agents Actions 6:454–459
Warheit DB, Sayes CM, Reed KL, Swain A (2008) How meaningful are the results of nanotoxicity studies in the absence of adequate material characterisation? Toxicol Sci 191:183–185
Webb JL (1966) Enzyme and metabolic inhibitors, vols III and V. Academic Press, New York
Weil A (1996) Materia-medica. In: Health and healing. Warner Books, London, Chap. 9, pp. 96–111
Whitehouse MW (2009) Therapeutic gold: is it due for a comeback? Inflammopharmacology 16:107–109
Whitehouse MW, Butters DE (2014) Paracetamol (acetaminophen): a blessing or a hidden curse. Inflammopharmacology 22:63–65
Whitehouse M, Butters D, Vernon-Roberts B (2013) Conditional pharmacology/toxicology V: ambivalent effects of thiocyanate upon the development and the inhibition of experimental arthritis in rats by aurothiomalate (Myocrysin®) and metallic silver. 21:291–300
Whitehouse MW, Leader JE (1966) Uncoupling of oxidative phosporylation and inhibition of a thiol enzyme (papain) by some cyclic Beta-diones and ninhydrin. Biochem Pharmacol 16:537–551
Whitehouse MW, Walker WR (1978) Copper and inflammation. Agents Actions 8:85–90
Xiu Z, Zhang Q, Puppala HL, Colvin VL, Alvarez PJJ (2012) Negligible particle-specific antibacterial activity of silver nanoparticles. Nano Lett. 12:4271–4275