

Silver as a Disinfectant

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I. Introduction

The antimicrobial effects of silver (Ag) have been recognized for thousands of years. In ancient times, it was used in water containers (Grier 1983) and to prevent putrefaction of liquids and foods. In ancient times in Mexico, water and milk were kept in silver containers (Davis and Etris 1997). Silver was also mentioned in the Roman pharmacopoeia of 69 B.C. (Davis and Etris 1997).

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In 1884, silver nitrate drops were introduced as a prophylactic treatment for the eyes of newborns, and this became a common practice in many countries throughout the world (Wahlberg 1982) to prevent infections caused by *Neisseria gonorrhoeae* transmitted from infected mothers during childbirth (Klueh et al. 2000; Slawson et al. 1992). In 1928, the “Katadyn Process,” based on the use of silver in water at low concentrations, was introduced (Krause 1928).

Silver ions have the highest level of antimicrobial activity of all the heavy metals. Gram-negative bacteria appear to be more sensitive than gram-positive species (Feng et al. 2000; Kawahara et al. 2000; Klueh et al. 2000). Kawahara et al. (2000) posited that some silver binds to the negatively charged peptidoglycan of the bacterial cell wall. Because gram-positive species have a thicker peptidoglycan layer than do gram-negative species, perhaps more of the silver is prevented from entering the cell.

Generally speaking, the observed bactericidal efficacy of silver and its associated ions is through the strong binding with disulfide (S–S) and sulfhydryl (–SH) groups found in the proteins of microbial cell walls. Through this binding event, normal metabolic processes are disrupted, leading to cell death. The antimicrobial metals silver (Ag), copper (Cu), and zinc (Zn) have thus found their way into a number of applications.

II. Applications and Uses

A. Drinking Water

Chlorine has been used as the principal disinfectant for drinking water since the early 1900s. In the 1970s, it was discovered that chlorination caused the formation of numerous chlorinated compounds in water, including trihalo-methanes and other disinfection by-products (DPB), that are known to be hazardous to human health (Moudgal et al. 2000; Von Gunten et al. 2001). There is therefore a need to assess alternative disinfectants (Yahya et al. 1992).

Silver electrochemistry experiments suggest that silver may have potential as a chlorine alternative in drinking water disinfection in applications in which chlorine may be considered too hazardous (Pedahzur et al. 2000). Silver has been used as an effective water disinfectant for many decades (Kim et al. 2004), primarily in Europe (Russell and Hugo 1994). It has also been used to treat recycled water aboard the MIR space station and aboard NASA space shuttles (Butkus et al. 2004; Gupta et al. 1998).

Both the Environmental Protection Agency (EPA) and the World Health Organization (WHO) regard silver as safe for human consumption. Only argyria (irreversible skin discoloration) occurs with the ingestion of gram quantities of silver over several years or by the administration of high concentrations to ill individuals. There have been no reports of argyria or other toxic effects caused by silver in healthy persons (World Health

Organization 1996). Based on epidemiological and pharmacokinetic data, a lifetime limit of 10 grams of silver can be considered a No Observable Adverse Effect Level (NOAEL) for humans (World Health Organization 1996). In the United States, no primary standards exist for silver as a component in drinking water. The EPA recommends a secondary nonenforceable standard of 0.1 mg/L (100 ppb) (Environmental Protection Agency 2002). The World Health Organization (1996) has stated this amount of silver in water disinfection could easily be tolerated because the total absorbed dose would only be half of the NOAEL after 70 years.

Silver has been used as an integral part of EPA- and National Sanitation Foundation (NSF)-approved point-of-use (POU) water filters to prevent bacterial growth. Home water purification units (e.g., faucet-mounted devices and water pitchers) in the United States contain silverized activated carbon filters along with ion-exchange resins (Gupta et al. 1998). Today, some 50 million consumers obtain drinking water from POU devices that utilize silver (Water Quality Association 2001). These products leach silver at low levels (1–50 ppb) with no known observable adverse health effects. Such filters have been shown to prevent the growth of *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* in water supplies (Russell and Hugo 1994); however, several studies have raised questions about their efficacy (Bell 1991). Reasoner et al. (1987) established that bacterial colonization of such devices occurs within a matter of days and may result in a large number of bacteria in the product water.

B. Cooling Towers/Large Building Water Distribution Systems

Cooling towers provide cooling water for air compressors and industrial processes that generate heat (Broadbent 1993). They provide an ideal environment and a suitable balance of nutrients for microbial multiplication (Martinez et al. 2004). Chlorine is a popular method for controlling such bacterial growth, but there are difficulties in maintaining disinfection efficacy, particularly at a high temperature or pH (Kim et al. 2004). Chlorination can also cause corrosion of cooling tower facilities (Kim et al. 2004).

Ag/Cu ionization has been used in cooling towers to control bacterial growth (Lin et al. 2002). In a study by Martinez et al. (2004), an appreciably reduced chlorine concentration of 0.3 parts per million (ppm or mg/L) was combined with 200 ppb Ag and 1.2 ppm Cu. This method had an appreciable impact on levels of coliform bacteria, iron-related bacteria, sulfate-reducing bacteria and slime-forming bacteria in a cooling tower (Martinez et al. 2004).

Large hot water distribution systems in hospitals and hotels have also often been attributed as a source of contaminating bacteria (Kim et al. 2002). Contaminated systems are usually treated by either superheating the water with flushing of the distal sites (heat-flush), by hyperchlorination, or by installing Ag/Cu ionization units (Stout and Yu 1997). Greater bacterial

reductions have been observed with Ag/Cu ionization than with the heat-flush method (Stout et al. 1998). Ag/Cu ionization is known to provide long-term control (Liu et al. 1994; Mietzner et al. 1997) and may be used in older buildings in which the pipes could be damaged by hyperchlorination (Stout and Yu 1997). Such systems are easy to install and maintain, are relatively inexpensive, and do not produce toxic by-products (Liu et al. 1994).

One microorganism that has been commonly isolated from cooling towers is *Legionella pneumophila*, the causative agent of Legionnaires' disease (Fliermans et al. 1981; Landeen et al. 1989). Many outbreaks have been linked to cooling towers (Bentham and Broadbent 1993; Brown et al. 1999; CDC 1994) and evaporative condensers (Breiman et al. 1990). *L. pneumophila* is also commonly isolated from the periphery of hot water systems in large buildings such as hospitals, hotels, and apartment buildings where temperatures tend to be lower (Zacheus and Martikainen 1994). Ag/Cu systems have been in common use in hospitals to control *Legionella* for more than a decade (Stout and Yu 2003). Mietzner et al. (1997) reported that one such ionization system maintained effective control of *L. pneumophila* for at least 22 mon. *Legionella* may develop a tolerance to silver after a period of years, requiring higher concentrations to achieve the same effect (Rohr et al. 1999).

C. Recreational Waters

Bacteria, protozoa, and viruses may occur naturally in recreational waters or be introduced into swimming pools by bathers or through faulty connections between the filtration and sewer systems (Beer et al. 1999). Species carried by bathers include the intestinal *Streptococcus faecalis* and *Escherichia coli*, as well as skin, ear, nose, and throat organisms such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus salivarius*, *Pseudomonas aeruginosa*, and *Mycobacterium marinum* (Singer 1990). Mild to serious illnesses caused by ingestion of or contact with contaminated water (Beer et al. 1999; Craun 1988) can be the result of improperly maintained pools, spas, and hot tubs (Kebabjian 1995).

In recent years, there has been a rapid increase in the number of public, semipublic, and private pools built in Europe and America. Adequate disinfection of such waters is becoming an increasingly important health issue (Singer 1990). Traditionally, chlorine-based products are used for disinfection of swimming pools (Borgmann 2003). Chlorine produces harmful DBPs caused by the halogenation of organic compounds (urine, mucus, skin particles, hair, etc.) released into the water by swimmers (Kim et al. 2002). Thus, there is also a need for alternative disinfectants for recreational waters (Yahya et al. 1992).

Silver (Ag_2SO_4) at a low concentration (10 ppb) has been shown to kill more than 99.9% of heterotrophic bacteria in swimming pools within 30 min

(Albright et al. 1972). Silver has been used commercially in pools, but it is too slow to be used as a primary disinfectant. Regulatory agencies in some countries have recommended its use only in combination with another disinfectant (Anonymous 2006). Electrolytic generation of Ag and Cu ions allows ppb concentrations to be maintained in a convenient and reproducible manner.

D. Food and Dietary Supplements

Silver has been used to treat vinegar, fruit juices, and effervescent drinks and wine (Foegeding and Busta 1991). It is also available in Mexico as colloidal silver in gelatin ('Microdyn') for use as a consumer fruit and vegetable wash and in the U.S. as an alternative health supplement or in silver citrate complexes as food additives (Silver 2003).

E. Medical Applications

Silver has been used in numerous medical applications (Hotta et al. 1998; Yoshida et al. 1999). In dentistry, silver nitrate is effective against a number of oral bacteria including gram-negative periodontal pathogens and gram-positive streptococci that cause periodontitis (Spacciapoli et al. 2001). Dental amalgams contain approximately 35% Ag(0) and 50% Hg(0). It is unclear whether sufficient Ag(0) is released and oxidized to Ag(I) to produce an antimicrobial effect; however, the release of Hg(II) selects for metal-resistant bacteria (Silver 2003). New amalgams have therefore been introduced that contain silver alone (Silver 2003).

Silver salts have traditionally been administered to the eyes of newborn infants to prevent neonatal eye infections (Isenberg 1990). Silver ions are the most commonly used topical antimicrobial agents used in burn wound care in the Western world (Poon and Burd 2004). Both silver nitrate and silver sulphadiazine have also been used as topical antiseptics for cutaneous wounds (Fox and Modak 1974; Gupta et al. 1998; Li et al. 1997; Rosenkranz and Carr 1972). A topical cream containing 1.0% silver sulphadiazine and 0.2% chlorhexidine digluconate has been marketed as Silvazine in the U.S. (Silver 2003).

Silver sulphadiazine has recently been incorporated directly into bandages used on burns and large open wounds (Furr et al. 1994; Innes et al. 2001; Silver 2003). Unlike silver nitrate, silver sulphadiazine does not react with sulfhydryl groups or proteins. Thus, its action is not diminished in the wound (Liau et al. 1997; Modak et al. 1988). Nevertheless, the silver is still the antimicrobial portion of the molecule. Two commercial silver-coated dressings (Acticoat and Silverdin) prevented muscular invasion by *P. aeruginosa* in experimental burns in rats (Ulkur et al. 2005). *P. aeruginosa* and *S. aureus* populations were similarly affected by Silverlon, an FDA-approved wound dressing (Heggers et al. 2005).

Silver has also been used to coat vascular, urinary, and peritoneal catheters (Cicalini et al. 2004; Gentry and Cope 2005), prosthetic heart valve sewing rings (Auer et al. 2001; Ionescu et al. 2003), vascular grafts, sutures, and fracture fixation devices (Blaker et al. 2005; Darouiche 1999). Plastic indwelling catheters coated with silver compounds retard the formation of microbial biofilms (Silver 2003). Manal et al. (1996) determined that the adherence of four strains of *E. coli* was decreased by 50%–99% in comparison to silicone and latex catheters. In two separate clinical studies, 10%–12% of patients with silver-treated catheters developed bacteriuria (>100 microorganisms/mL) versus 34%–37% of patients with standard Foley catheters after 3 d. The onset of bacteriuria was thus delayed in comparison to latex catheters (Liedberg et al. 1990; Lundeborg 1986). Gentry and Cope (2005) also found a 33.5% reduction in catheter-associated urinary tract infections following the introduction of silver-coated catheters.

The complex of silver with antibiotics on the surfaces of polytetrafluoroethylene vascular grafts has been examined in a number of studies. Silver increased the elution and prolonged the duration of ciprofloxacin release in one such study (Darouiche 1999).

F. Antimicrobial Surfaces/Materials

Silver may be added to polymers (Brady et al. 2003) to confer antimicrobial activity. The result is consumer products such as washing machines, refrigerators, and ice machines that have incorporated silver (<http://www.agiontech.com/CorporateOverview.pdf>, retrieved May 30, 2006; <http://www.samsung.com/silvercare/index.htm>, retrieved May 30, 2006). Silver has been added to plastics to produce items such as public telephones and public toilets (in Japan), toys, and infant pacifiers (Silver 2003). Johnson Matthey Chemicals (UK) utilizes an inorganic composite with immobilized slow-release silver as a preservative in their cosmetics (Silver 2003). Synthetic fabrics with silver are popular in items such as sportswear, sleeping bags, bedsheets, and dishcloths (Silver 2003; Takai et al. 2002). These fabrics are believed to reduce the level of bacterial contamination and thus odors (Silver 2003).

Silver may also be added to inorganic ceramics (e.g., zirconium phosphate, zeolite) (Cowan et al. 2003; Galeano et al. 2003; Kim et al. 1998; Kim et al. 2004) that are able to trap metal ions and may then be added to other materials (e.g., paints, plastics, waxes, polyesters) to confer antimicrobial properties (Quintavalla and Vicini 2002; Takai et al. 2002). Zeolite ceramic (sodium aluminosilicate) has a porous three-dimensional crystalline structure in which ions can reside; it has a strong affinity for silver ions and can electrostatically bind up to 40% silver (wt/wt) (Kawahara et al. 2000; Uchida 1995). Zeolites act as ion exchangers, releasing silver into the environment in exchange for other cations (Hotta et al. 1998; Kawahara et al. 2000). The

amount of silver released is dependent upon the concentration of cations in the environment (Kawahara et al. 2000). The bactericidal activity of Ag-zeolite appears to result from both the effect of silver ions (Matsumura et al. 2003) and the generation of reactive oxygen species, under aerated conditions, such as superoxide anions, hydroxyl radicals, hydrogen peroxide, and singlet oxygen (Inoue et al. 2002).

Studies on stainless steel surfaces coated with zeolites containing 2.5% Ag and 14% Zn ions demonstrated significant reductions in *L. pneumophila* (Rusin et al. 2003), *S. aureus* (Bright et al. 2002), *Campylobacter jejuni*, *Salmonella typhimurium*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 (Bright KR, Gerba CP, unpublished data). Vegetative cells of *Bacillus subtilis*, *B. anthracis*, and *B. cereus* were also inactivated by at least three orders of magnitude within 24 hr by a Ag/Zn-zeolite whereas *Bacillus* spores were completely resistant under the same conditions (Galeano et al. 2003).

III. Antimicrobial Efficacy

The antimicrobial effect of silver has been demonstrated in numerous and varied applications against many different types of microorganisms including bacteria, viruses, and protozoa. An overview of the available experimental data on silver disinfection is presented in Table 1.

IV. Antimicrobial Mechanisms

Proposed mechanisms of the antibacterial and antiviral actions of silver are summarized in Table 2.

A. Antibacterial Action

The antibacterial effects of silver are not completely understood. Numerous mechanisms have been proposed. Several are generally accepted:

1. Extracellular binding or precipitation of silver to bacterial cell walls and membranes (Bellantone et al. 2002; Efrima and Bronk 1998; Goddard and Bull 1989; Slawson et al. 1992). Bacterial cell walls contain negatively charged peptidoglycans that will most likely electrostatically bind some Ag⁺ on their own (Thurman and Gerba 1989).

2. Energy-dependent or independent accumulation of silver inside cells (Slawson et al. 1992). Possible active uptake of silver by a transport system for an essential metal with a similar charge or ionic size (Slawson et al. 1992; Solioz and Odermatt 1995).

3. Binding of silver to cellular proteins, including enzymes (Slawson et al. 1992). Silver is known to stain proteins (Slawson et al. 1992). It binds to sulfhydryl (-SH) groups on enzymes, leading to their inactivation (Feng et al. 2000; Liao et al. 1997; Slawson et al. 1992; Thurman and Gerba 1989)

Table 1. Microorganisms for Which Silver Has been Shown to be Effective.

Organism	Treatment	Reference
<i>Hartmannella vermiformis</i> <i>Tetrahymena pyriformis</i> <i>Naegleria fowleri</i>	100ppb Ag + 1,000ppb Cu or 500ppb Ag + 5,000ppb Cu 400ppb Cu + 40ppb Ag or 800ppb Cu + 80ppb Ag, and combined with 1.0ppm free chlorine	Rohr et al. 2000 Cassells et al. 1995
Mouse malaria	Silver sulphadiazine	Davis and Etris 1997
SARS-coronavirus Coronavirus 229E (human) Feline coronavirus Feline calicivirus	Ag/Al ₂ O ₃ wafers Ag/Cu zeolite	Han et al. 2005 Bright KR, Gerba CP, unpublished data
HIV-1 (AIDS)	1.0, 5.0, 10.0, and 20.0ppm of Ag ₄ O ₄	Antelman 1992
HIV-1103	Silver thiosulfate complex encapsulated in silica gel microspheres	Davis and Etris 1997
Poliovirus (type 1 Mahoney)	400ppb Cu + 40ppb Ag or in combination with free chlorine at 0.2 and 0.3ppm	Yahya et al. 1992
Poliovirus (type 1 Mahoney) Papovavirus SV-40, A 426 Adenovirus (prototype 6) Vaccinia (Elstree strain)	Sanosil Super 25 (contains silver and hydrogen peroxide) at a concentration of 0.025% and 0.1%	Kadar et al. 1993
Herpes simplex type 1 Herpes vesicular stomatitis	0.05% Sanosil Super 25 Silver sulphadiazine	Davis and Etris 1997
Bacteriophage MS-2	400ppb Cu + 40ppb Ag or in combination with free chlorine at 0.2 and 0.3ppm	Yahya et al. 1992; Thurman and Gerba 1989
<i>Saccharomyces cerevisiae</i>	Minimum inhibitory concentration of Ag ₄ O ₄ is 1.25ppm	Antelman 1992
<i>Candida albicans</i>	Minimal inhibitory concentration of Ag ₄ O ₄ is 2.5–5.0ppm	Antelman 1992
<i>Escherichia coli</i> <i>Pseudomonas aeruginosa</i> <i>Staphylococcus aureus</i>	AgBC (bioactive glass doped with Ag ₂ O) at concentrations 0.05 to 0.20mg/ml	Bellantone et al. 2002
<i>Escherichia coli</i> <i>Staphylococcus aureus</i>	AgNO ₃	Feng et al. 2000
<i>Escherichia coli</i>	Ceramic balls coated with Ag and Cu at a concentration of 0.05ppm Ag and 0.05ppm Cu	Kim et al. 2004

Table 1. *Continued*

Organism	Treatment	Reference
<i>Pseudomonas aeruginosa</i> <i>Micrococcus lutena</i> <i>Staphylococcus agalactiae</i>	Minimum inhibitory concentration of Ag ₄ O ₄ is 1.25–2.5 ppm	Antelman 1992
<i>Escherichia coli</i> <i>Enterobacter cloacae</i> <i>Staphylococcus pyogenes</i>	Minimum inhibitory concentration of Ag ₄ O ₄ is 2.5 ppm	Antelman 1992
<i>Bacillus subtilis</i> <i>Staphylococcus aureus</i> <i>Staphylococcus faecium</i>	Minimum inhibitory concentration of Ag ₄ O ₄ is 5.0 ppm	Antelman 1992
<i>Staphylococcus epidermidis</i>	Minimum inhibitory concentration of Ag ₄ O ₄ is 0.625 ppm	Antelman 1992
<i>Escherichia coli</i> <i>Pseudomonas aeruginosa</i> <i>S. aureus</i> (MRSA) <i>S. aureus</i> (non-MRSA)	Ag-Zeolite Ag/Zn zeolite	Inoue et al. 2002 Takai et al. 2002;
<i>Listeria monocytogenes</i> <i>Escherichia coli</i> <i>Staphylococcus aureus</i> <i>Pseudomonas aeruginosa</i>		Cowan et al. 2003;
<i>Bacillus anthracis</i> <i>Bacillus subtilis</i> <i>Bacillus cereus</i>		Galeano et al. 2003
<i>Staphylococcus aureus</i> <i>Legionella pneumophila</i> <i>Escherichia coli</i> O157:H7 <i>Campylobacter jejuni</i> <i>Salmonella typhimurium</i> <i>Listeria monocytogenes</i>	2.5% Ag / 14% Zn (wt/wt) zeolite	Bright et al. 2002; Rusin et al. 2003; Bright KR, Gerba CP, unpublished data
<i>Streptococcus mutans</i> <i>Streptococcus mitis</i> <i>Streptococcus salivarius</i> <i>Streptococcus sanguis</i>	Ag-Zn Zeolite and SiO ₂ at ratio concentrations of 5/55, 10/50, 20/40, and 30/30 wt%	Hotta et al. 1998
<i>S. aureus</i> (non-MRSA) <i>S. aureus</i> (MRSA)	0.1 ml of Silvazine (1% silver sulphadiazine + 2% chlorhexidine digluconate)	George et al. 1997
<i>Vibrio cholerae</i>	1.0 and 2.0 ppm of Ag ₄ O ₄ ; low concentration of Ag ⁺	Antelman 1992; Dibrov et al. 2002
<i>Neisseria gonorrhoeae</i> <i>Treponema pallida</i> <i>Trichomonas</i>	Treated with silver sulphadiazine	Davis and Etris 1997
<i>Legionella pneumophila</i>	Treated with Ag + Cu	Davis and Etris 1997

Table 2. Summary of Mechanisms of Inactivation of Bacteria and Viruses Using Silver.

Scientific observation	Reference	Type of microbe
Release of silver into the system	Slawson et al. 1990; Inoue et al. 2002	Bacteria
Oxidative destruction catalyzed by silver	Modak and Fox 1973; Richards 1981	Bacteria
Affinity for sulfhydryl groups	Davis and Etris 1997; Feng et al. 2000	Bacteria and Viruses
Targeting of Na ⁺ -translocating NADH: ubiquinone oxidoreductase (NQR) at low concentration of Ag ⁺	Dibrov et al. 2002	Bacteria
Targeting of membrane proteins	Dibrov et al. 2002	Bacteria
Inhibits oxidative metabolism required by the cells	Davis and Etris 1997; Heining 1993	Bacteria
Inhibits uptake of nutrients	Slawson et al. 1990	Bacteria
Causes metabolite leakage	Slawson et al. 1990	Bacteria
Binds to DNA	Modak and Fox 1973; Richards 1981; Thurman and Gerba 1989	Bacteria and Viruses
Site-specific Fenton mechanism	Thurman and Gerba 1989; Samuni et al. 1984; Yahya et al. 1992	Viruses
Immobilization of the virus to a surface	Thurman and Gerba 1989	Viruses
Blocks or destroys host-cell receptors	Thurman and Gerba 1989	Viruses
Inactivation of the nucleic acid within the viral capsid	Thurman and Gerba 1989	Viruses

and eventually to the inactivation of the bacteria (Liau et al. 1997). Monovalent silver ions bind to these functional groups, resulting in a stable $-S-Ag$ group that inhibits hydrogen transfer, the source of energy transfer (Davis and Etris 1997). Silver also complexes with sulfhydryl groups in the cell membrane that are components of enzymes which participate in transmembrane energy generation and electrolyte transport (Klueh et al. 2000); this may cause the formation of $R-S-S-R$ bonds that block respiration and electron transfer (Davis and Etris 1997; Heining 1993).

4. Binding of silver to deoxyribonucleic acid (DNA) (Thurman and Gerba 1989). Silver displaces the hydrogen bonds between adjacent nitrogens of purine and pyrimidine bases (Klueh et al. 2000; Richards 1981); this may stabilize the DNA helix and prevent replication of the DNA and sub-

sequent cell division (Modak and Fox 1973; Richards 1981; Thurman and Gerba 1989).

5. Binding of silver to electron donor groups (Thurman and Gerba 1989) containing nitrogen, oxygen, and sulfur such as amines, hydroxyls, phosphates, and thiols in cells (Grier 1983; Modak and Fox 1973).

Several observations support these proposed mechanisms. Compounds with thiol groups such as sodium thiosulfate, sodium thioglycollate, and lysozymes are able to neutralize silver activity. Silver binds to the thiol groups on these compounds and is no longer able to bind to proteins (Liau et al. 1997; Richards 1981). In a study by Bellantone et al. (2002), silver was depleted from an aqueous solution over time in the presence of bacteria. This loss was assumed to be the result of silver binding to the cell wall or accumulation inside of cells. In a separate study, silver iodide inside a polymer was able to bind sulfhydryl groups on proteins on bacterial outer membranes. It was then transported intracellularly, where it accumulated until it reached a toxicity threshold, leading to bacterial death (Brady et al. 2003). Silver accumulation has been observed in nongrowing *E. coli* cells because of both binding at the surface and intracellular uptake (Ghandour et al. 1988).

Feng et al. (2000) visualized the fate and action of silver in *E. coli* and *S. aureus* by transmission electron microscopy. In both species, the cytoplasmic membrane shrank and detached from the cell wall. An electron-light region appeared in the central region that contained large amounts of phosphorous, as determined by X-ray microanalysis. It therefore likely contained highly condensed DNA molecules. Numerous electron-dense granules both surrounded the cell wall and were deposited inside cells, surrounding, but not found within, the electron-light central region. The electron-dense granules contained significant amounts of both silver and sulfur, suggesting a combination of silver and proteins. It was proposed that the cells might produce proteins that aggregate around this nuclear region to protect DNA molecules (Feng et al. 2000). A similar mechanism has been found for heat shock proteins (Nover et al. 1983). Condensed DNA is unable to replicate. No cell growth or multiplication was observed during continuous cultivation with fresh liquid nutrient medium during the course of the experiment. Proteins were inactivated after the silver treatment and the cell wall was severely damaged in some cells. The effects were milder in *S. aureus* than in *E. coli*. The thicker cell wall of the gram-positive *S. aureus* protects it to some degree from penetration of silver ions into the cytoplasm (Feng et al. 2000).

Several other potential antibacterial mechanisms have been proposed for silver in recent years. Silver collapses the proton motive force on the cell membrane (Dibrov et al. 2002; Williams et al. 1989). Dibrov et al. (2002) found that there was a total collapse of the respiration-generated transmembrane pH gradient in vesicles and also of the membrane electric

potential (in the absence of added Na^+). Low concentrations of silver ions induced massive leakage of protons (H^+) through the membrane of *Vibrio cholerae*, which resulted in the complete deenergization of the cells and most likely cell death. This effect might have been the result of modified membrane proteins or a modified phospholipid bilayer (Dibrov et al. 2002). Toxicity may also cause leakage of cellular metabolites and intracellular ions such as potassium (Slawson et al. 1992).

Silver blocks the respiratory chain of bacteria in the cytochrome oxidase and NADH-succinate-dehydrogenase region (Klueh et al. 2000). One of the primary targets of Ag^+ ions is the Na^+ -translocating NADH:ubiquinone oxidoreductase (NQR). Submicromolar Ag^+ ions inhibit energy-dependent Na^+ transport in membrane vesicles; this is one of the proposed mechanisms of inactivation at low Ag^+ concentrations (Dibrov et al. 2002).

Silver also inhibits the oxidation of glucose, glycerol, fumarate, succinate, D- and L-lactate, and endogenous substrates of *E. coli* cells by the inhibition of the *b* cytochromes and cytochrome *d* at the site of substrate entry into the respiratory chain and also flavoproteins in the NADH and succinate dehydrogenase regions (Bragg and Rainnie 1973). Schreurs and Rosenberg (1982) described a mechanism specifically for silver nitrate in which it inhibits the uptake of inorganic phosphate and causes efflux of accumulated phosphate; this also induces leakage of mannitol, succinate, glutamine, and proline, causing metabolite leakage (Slawson et al. 1990).

Adsorption of atomic oxygen on the surface of silver provides a reservoir of oxygen. As a result of the catalytic action of silver, oxygen is converted to active oxygen (such as hydroxyl radicals). Silver can thus catalyze the complete destructive oxidation of bacteria (Davis and Etris 1997; Yoshida et al. 1999).

B. Antiviral Action

To date, there have been no detailed studies describing the interaction between silver and viruses. Viruses that contain sulfhydryl termini may bind silver, which might affect their replication cycle (Davis and Etris 1997). One theory is that there is a site-specific Fenton mechanism in which the metal binds to a biological molecule and is reduced by superoxide radicals or other reductants and then reoxidized by hydrogen peroxide. Continuous redox reactions in a cyclic manner result in damage, as radical formation occurs near the target site of the molecule (Samuni et al. 1984; Thurman and Gerba 1989; Yahya et al. 1992).

Tzagoloff and Pratt (1964) proposed that silver modifies the adsorption of viruses to cells. Thurman and Gerba (1989) suggested that the inactivation mechanism should be one that does not require a metabolic process, for example, the immobilization of the virus to a surface, the blocking or destruction of host-cell receptors, or the inactivation of the nucleic acid within the viral capsid.

C. Antiprotozoal Action

The mechanisms by which silver acts against protozoa are not presently understood; nevertheless, many of the mechanisms that have been reported for bacteria most likely play some role against protozoa as well. For instance, silver will most certainly be able to bind to proteins on the cell membrane and, if transported inside the cell, to DNA as well. Binding to DNA could prevent replication, and binding to proteins could inhibit their function. If some of these proteins are transmembrane proteins, this may also inhibit transport and nutrient uptake.

It has been reported that silver and copper inactivate *Tetrahymena pyriformis* more easily than they do amoebas (Rohr et al. 2000). *Hartmannella* is inactivated by a concentration of 100 ppm Ag and 1,000 ppm copper (Rohr et al. 2000). There are also reports of the inactivation of *Naegleria fowleri* by the use of silver, copper, and free chlorine when used in combination (Cassells et al. 1995).

V. Silver Resistance

Rusin and Gerba (2001) defined resistance as the ability of a bacterial population to grow in working concentrations of an active disinfectant. Tolerance was defined as the ability of an organism to survive short-term exposure to a disinfectant or to survive for a longer period of time than more-sensitive bacterial strains. Many papers have been published describing silver resistance that would be considered as mere tolerance following these criteria, making a thorough discussion of silver resistance somewhat problematic. For the purpose of this review, the term “resistance” includes both true silver resistance as well as silver tolerance as the terms are not always discernible based on published descriptions of empirical data.

Some bacteria appear to have natural resistance to silver (Wood 1984). Silver-resistant bacteria are usually found in areas where bacteria are regularly exposed to silver such as in hospital burn wards, hospital water distribution systems, and contaminated soil near silver mines (Silver 2003). Two proposed mechanisms of this resistance are that silver ions are excluded from the cell or mobilized outside the cell (Slawson et al. 1992). These processes are typically performed by membrane proteins that are energy dependent and function as either ATPases or chemiosmotic cation/proton antiporters (Silver 2003). Bioaccumulation or sequestration of silver, although it does exist, is not common, and its relationship to silver resistance is unclear (Silver 2003). Silver-resistant strains of *E. coli* do not accumulate intracellular silver deposits whereas sensitive strains contain dense deposits (Starodub and Trevors 1990). The gram-positive organism *Enterococcus hirae* (formerly *Streptococcus faecalis*) possesses a homeostatic mechanism to manage intracellular copper concentration via an ion pump. The *E. hirae* CopB ATPase in membrane vesicles was found to expel both

Cu^+ and Ag^+ from the cytoplasm, causing an accumulation of Cu^+ and Ag^+ inside native inside-out membrane vesicles (Solioz and Odermatt 1995).

In gram-negative bacteria, plasmid-mediated silver resistance is believed to be the most common and typically involves energy-dependent efflux of silver from the cell. Plasmid-mediated silver resistance in *Salmonella* involves a total of nine genes and is unusual in that it includes three separate types of resistance mechanisms: a periplasmic metal-binding protein (SilE) that binds silver at the cell surface, a chemiosmotic efflux pump, and an ATPase efflux pump (SilCBA and SilP) (Silver 2003). This resistance system is somewhat homologous to the plasmid-mediated *pco* copper resistance system in *E. coli* (Silver 2003).

The *agr* gene cluster (containing genes formerly named *ybdE*, *ylcABCD*, and *ybcZ*) encodes a silver resistance system in *E. coli* that is homologous to the central six genes (*silA* through *silS*) of the *sil* resistance system (Silver 2003). The specific mechanisms of silver resistance have been reviewed in greater detail elsewhere (Chopra 2007; Silver 2003).

VI. Synergism with Other Disinfectants

Synergy between silver ions and other antimicrobials such as potassium permanganate, potassium peroxymonosulfate (Bright KR, Gerba CP, unpublished data), hydrogen peroxide (Armon et al. 2000; Rafter et al. 1999), biguanides (Bright KR, Gerba CP, unpublished data), chlorine (Yahya et al. 1992), chlorite and chlorate (Rafter et al. 1999), and UV light (Butkus et al. 2004) has been observed by a number of investigators against a variety of microbiological species including bacteria, viruses, and oocysts (Table 3). Interestingly, metal ions in many instances enhance the effectiveness of the system well beyond that predicted by the individual components; that is, a synergistic effect is observed. It has been postulated that the oxidizer disrupts the cell wall and effects the rapid penetration of the metallic ions into the cell where irreversible precipitation of the DNA occurs (Armon et al. 2000; Straub et al. 1995; Yahya et al. 1992). Other mechanistic interpretations are, of course, possible. For instance, at higher levels of chlorine, silver is precipitated as AgCl_2^- that actually increases the sensitivity of silver-sensitive bacteria (Silver 2003).

Inactivation of *L. pneumophila* by combined copper and silver has been shown to be relatively slow when compared with that of free chlorine; nonetheless, when they were included in addition to low levels of free chlorine, the inactivation rates of bacterial indicator organisms were greater than those for free chlorine alone (Landeem et al. 1989; Yahya et al. 1990). Beer et al. (1999) found that electrolytically generated copper and silver ions used in swimming pool water along with lower levels of chlorine provided control of total coliform and heterotrophic bacteria equivalent to the control provided by high levels of chlorine. Yahya et al. (1990) demonstrated that adding 400 ppb copper and 40 ppb silver to water systems containing contaminants similar to those in swimming pools allowed the

Table 3. Synergism with Other Disinfectants.

Scientific observation	Reference
Copper and silver metals are capable of inactivating poliovirus and coliphages. This effect is greatly enhanced in the presence of oxidizers.	Yahya et al. 1992
Silver significantly enhances the effectiveness of UV light against MS-2 virus.	Butkus et al. 2004
Synergistic effect between silver, copper, and free chlorine in the inactivation of <i>Naegleria fowleri</i> .	Cassells et al. 1995
Silver shown to be synergistic with chlorite, chlorate, and oxidizers (peroxymonosulfate and hydrogen peroxide).	Rafter et al. 1999
Silver is effective in preventing biofilm formation in water. This effect is enhanced in the presence of hydrogen peroxide.	Armon et al. 2000
Silver exhibits synergistic effect with potassium monoperoxysulfate against <i>Acinetobacter baumannii</i> and <i>Bacillus globigii</i> spores.	Bright KR, Gerba CP, unpublished data
Silver and copper ions shown to have synergistic effect against <i>Salmonella typhimurium</i> and <i>Escherichia coli</i> .	Bright KR, Gerba CP, unpublished data

concentration of free chlorine to be reduced at least threefold (from 0.1 to 0.3 ppm). Enhanced inactivation rates for *E. coli*, *S. aureus*, *L. pneumophila*, *S. faecalis* (Landeem et al. 1989; Yahya et al. 1990), and *P. aeruginosa* (Landeem et al. 1989) were also obtained when water was treated with 400 ppb copper, 40 ppb silver, and 0.2 ppm free chlorine. These studies suggest a synergistic effect upon microorganisms subjected to copper or silver ions in the presence of low levels of chlorine.

Silver has also been shown to have synergistic activity with other metal ions such as copper and zinc. In one study (Lin et al. 1998), both copper and silver ions were found to be effective in inactivating *L. pneumophila*, and the combined effect was greater than the sum of the individual effects when each was administered alone.

In two studies, silver-resistant strains of *Acinetobacter baumannii* were found to accumulate high amounts of silver, most of which was surface bound. This resistance was reduced by the purging of a plasmid (Deshpande and Chopade 1994; Shakibaie et al. 1999). In one experiment, the plasmid was successfully transferred to *E. coli* by conjugation; however, the subsequent increased silver resistance conferred to *E. coli* was the result of the efflux of silver ions from the cell rather than accumulation (Deshpande and Chopade 1994).

If the oxidizing effect of other disinfectants damages outer cellular structure, it may permit silver ions to rapidly penetrate into the cell; this may

bypass the role of silver accumulation on the cell surface as a resistance mechanism.

VII. Conclusions

Both the EPA and the WHO regard silver as safe for human consumption. It does not pose a risk to human health (World Health Organization 1996) and, in contrast to numerous other commonly utilized disinfectants, is not considered a hazardous substance (Ibarluzea et al. 1998; Kim et al. 2002; World Health Organization 1996). Silver inactivates a wide variety of microorganisms such as bacteria, viruses, and protozoa, alone or in combination with other disinfectants (Cassells et al. 1995; Davis and Etris 1997; Inoue et al. 2002), although this effect is not instantaneous.

To date, the development of resistance to silver does not appear to be a concern in real-world applications. Silver has successfully been utilized for centuries (Davis and Etris 1997; Grier 1983) and is still effective against a wide variety of microorganisms (Hotta et al. 1998; Kim et al. 2004; Rohr et al. 2000; Yahya et al. 1992). Resistance does exist in certain microorganisms (Silver 2003); however, this usually occurs in environments with high silver concentrations such as those near silver mines (Silver 2003). Silver tolerance is more likely to develop under more typical circumstances and silver usages. For example, organisms found in hospital wards and hospital water distribution systems are probably only tolerant because silver has been shown to be effective in hospital water distribution systems for several years (Blanc et al. 2005; Liu et al. 1994; Rohr et al. 1999; Stout and Yu 2003).

Further research needs to be undertaken for silver to be accepted as a disinfectant in certain applications by regulatory agencies. This research should provide sufficient information to corroborate real-world observations about the efficacy of silver as a disinfectant and any potential problems related to its use such as the development of microbial resistance.

Summary

Silver has been used as an antimicrobial for thousands of years. Over the past several decades, it has been introduced into numerous new venues such as in the treatment of water, in dietary supplements, in medical applications, and to produce antimicrobial coatings and products. Silver is often used as an alternative disinfectant in applications in which the use of traditional disinfectants such as chlorine may result in the formation of toxic by-products or cause corrosion of surfaces. Silver has also been demonstrated to produce a synergistic effect in combination with several other disinfectants. Many mechanisms of the antibacterial effect of silver have been described, but its antiviral and antiprotozoal mechanisms are not well understood. Both microbial tolerance and resistance to silver have been reported; however, the effect of silver has been observed against a wide variety of

microorganisms over a period of years. Further research is needed to determine the antimicrobial efficacy of silver in these new applications and the effects of its long-term usage.

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