ANNEXURE –I
LITERATURE REFERENCES

Medical uses of silver

The **medical uses of silver** include its incorporation into wound dressings to treat external infections, and its use as an antiseptic and disinfectant in medical appliances. Silver is also promoted within alternative medicine in the form of *colloidal silver*, although this particular application has not yet been proved to be either effective or safe.

The silver ion (Ag^+) is bioactive and in sufficient concentration readily kills bacteria *in vitro*. Silver also kills bacteria in external wounds in living tissue, so physicians use wound dressings containing silver sulfadiazine (Ag-SD) or silver nanomaterials to treat external infections.[1] [2] [3] [4] [5] Wound dressings containing silver are increasing in importance due to the recent increase of antibiotic-resistant bacteria, such as MRSA.[6] The disinfectant properties of silver are used in medical applications, such as urinary catheters and endotracheal breathing tubes, where the silver content is effective in reducing incidences of catheter-related urinary tract infections and ventilator-associated pneumonia (VAP), respectively.[7] [8] [9] [10] Silver is also used in bone prostheses, reconstructive orthopaedic surgery and cardiac devices,[11] as well as on surfaces and fabrics to reduce the spread of infection.[12] [13]

Since the 1990s, "colloidal silver", a liquid suspension of microscopic silver particles, has been marketed as an alternative medicine, often claiming impressive "cure-all" qualities. The effectiveness of these products has never been scientifically proven, and in some jurisdictions, it is currently illegal to include such claims in product advertisements.[14] Medical authorities and publications advise against the ingestion of colloidal silver preparations, because of their lack of proven effectiveness and because of the risk of adverse side effects, such as argyria.[2] [15] [16] [17] Historically, colloidal silver was also used as an internal medication to treat a variety of diseases. Their use was largely discontinued in the 1940s, due to the development of safe and effective modern antibiotics and concern about adverse side effects.[17] [18]

Historically

Silver has had some medicinal uses going back for centuries. The Phoenicians are said to have stored water, wine, and vinegar in silver bottles to prevent spoiling.[19] In the early 1900s, people would put silver coins in milk bottles to prolong the milk's freshness.[19] Hippocrates, the "father of medicine",[20] wrote that silver had beneficial healing and antidisease properties.[19] In the early 1900s, silver gained regulatory approval as an antimicrobial agent. Prior to the introduction of antibiotics, colloidal silver was used as a germicide and disinfectant.[21] Physicians used it as an eyedrop for ophthalmic problems,[22] for various infections,[23] [24] and sometimes internally for diseases such as tropical sprue, epilepsy, gonorrhea, and the common cold.[2] [16] [25] Colloidal silver preparations (CSP) were used to treat or prevent gonorrhea and gonorrheal conjunctivitis.[17] Although "silver products were infrequently promoted for oral use, benefits have been even more questionable."[17] With the introduction of antibiotics in the 1940s, the use of silver as an antimicrobial agent diminished.[6] One well known, highly successful, brand name, silver colloid product in the period before 1940 was Argyrol.

Antiseptic

Silver and most silver compounds have an oligodynamic effect and are toxic for bacteria, algae, and fungi *in vitro*. The oligodynamic effect is typical for heavy metals, such as lead and mercury, but, among the elements that have this effect, silver is the least toxic for humans. The antibacterial action of silver is dependent on the silver ion.[12] The effectiveness of silver compounds as an antiseptic is based on the ability of the biologically active silver ion (Ag+) to irreversibly damage key enzyme systems in the cell membranes of pathogens.[12] The antibacterial action of silver has long been known to be enhanced by the presence of an electric field. Applying a few volts of electricity across silver electrodes drastically enhances the rate bacteria in solution are killed. The antibacterial action of silver electrodes is greatly improved if the electrodes are covered with silver nanorods.[26]

Disinfectant

Electrolytically-dissolved silver has been used as a water disinfecting agent, for example, the drinking water supplies of the Russian Mir orbital station and the International Space Station.[27] The World Health Organization includes silver in a colloidal state produced by electrolysis of silver electrodes in water, and colloidal silver in water filters as two of a number of water disinfection methods specified to provide safe drinking water in developing countries.[28] Along these lines, a ceramic filtration system coated with silver particles has been created by Ron Rivera of Potters for Peace and used in developing countries for water disinfection.[29] [30] [31]

External infections

In World War I, before the advent of antibiotics, silver compounds were used to prevent and treat infections. Silver compounds continue to be used in external preparations as antiseptics,[16] including silver nitrate, which can be used in dilute solution as eyedrops to prevent conjunctivitis in newborn babies. Silver nitrate is also sometimes used in dermatology in solid stick form as a caustic ("lunar caustic") to treat certain skin conditions, such as corns and warts.[2]

According to Atiyeh et al. (2007), "The gold standard in topical burn treatment is silver sulfadiazine (Ag-SD), a useful antibacterial agent for burn wound treatment". They do note, however, that silver-impregnated dressings do sometimes result in a slower healing process.[1] Silver sulfadiazine cream (SSD Cream) replaced colloidal silver as the most common delivery system for using silver on the surface of burn wounds to control infection in the 1970s.[1] [2]

The US Food and Drug Administration has approved the use of a range of different silver-impregnated wound dressings.[32]

Laboratory studies at the Biochemical Materials Research and Development Center of Jiaxing College, China, have shown silver-containing alginate fibres provide a sustained release of silver ions when in contact with wound exudates, and are “highly effective against bacteria”.[3] A study administered by the Hull York Medical School found an antimicrobial barrier dressing containing silver provided “a highly effective and reliable barrier to the spread of MRSA into the wider hospital.”[4] [33]

More recently, dressings incorporating nanocrystalline silver or activated silver-impregnated substances have become available,[1] which deliver higher concentrations of the active silver ion.[12] As of 2006, more "than 10 dressings containing pure silver" were available.[5] In particular, silver is being used with alginate, a naturally occurring biopolymer derived from seaweed, in a range of products designed to prevent infections as part of wound management procedures, particularly applicable to burn victims.[5]

Wound dressings containing silver are increasing in importance due to the increase of antibiotic-resistant bacteria, which has imposed clinical limits on the use of antibiotics. Chopra[34] states topical silver is regaining popularity in the management of open wounds, “due largely to the spread of methicillin-resistant Staphylococcus aureus and the resultant reduction in first-line antibiotic prescribing”, and “[s]ome silver-based dressings appear to provide an effective alternative to antibiotics in the management of wound infection.”[6] Silver has proven broad-spectrum antimicrobial activity that includes antibiotic-resistant bacteria, with minimal toxicity toward mammalian cells at low concentrations, and has a less likely tendency than antibiotics to induce resistance due to its activity at multiple bacterial target sites.[6] [35] [36] [37] [38]

However, some sources still hold that the evidence for the effectiveness of silver-treated dressings is mixed, as the evidence is marred by the poor quality of the trials used to assess these products.[39] Consequently, a systematic review by the Cochrane Collaboration found insufficient evidence to recommend the use of silver-treated dressings to treat infected wounds.[40]

In medical appliances

The disinfectant properties of silver are used in some other medical applications, such as catheters and endotracheal breathing tubes.[41] [42] A study on the use of silver-alloy catheters by the University of Michigan School of Medicine concluded "The data supporting the use of silver alloy urinary catheters to reduce urinary catheter-related bacteriuria is reasonably strong."[7] The study also concluded silver alloy catheters are more effective than standard catheters for reducing bacteriuria in adults in hospital having short-term catheterization, and, although they cost about $6 more than standard urinary catheters, they may be worth the extra cost, since catheter-related infection is a common cause of nosocomial infection and bacteremia. Related meta-analysis also clarified discrepant results among earlier trials of silver-coated urinary catheters by revealing silver alloy catheters are significantly more effective in preventing urinary tract infections than are silver oxide catheters.[8] These conclusions are supported by, among others, studies by the University Hospitals Leuven, Belgium[12] and the University Hospital for Anesthesiology and Surgical Intensive Care, Halle, Germany.[43]

In 2007, AGC Flat Glass Europe introduced the first antibacterial glass to fight hospital-acquired infection; it is covered with a thin layer of silver.[44] Ionizable silver is also incorporated into fabrics to reduce the spread of bacteria.[12]

Ventilator-associated pneumonia (VAP) causes substantial morbidity. A 2008 study by Kollef et al. concluded, "Patients receiving a silver-coated endotracheal tube had a statistically significant reduction in the incidence of VAP and delayed time to VAP occurrence compared with those receiving a similar, uncoated tube."[9] In addition, the U.S. Food and Drug Administration (FDA) has recently approved an endotracheal tube with a fine coat of silver for use in mechanical ventilation, after studies found it reduced the risk of ventilator-associated pneumonia.[10]

The use of these devices is contraindicated for persons who are allergic to silver,[12] and although they are widely used in hospitals, no thorough testing and standardization of these products has yet been undertaken.[6]

Effectiveness

A meta-analysis of 26 studies by the Cochrane Collaboration found that, while most were small and of poor quality, there was not enough evidence to support the use of silver-containing dressings or creams, as generally these treatments did not promote wound healing or prevent wound infections. Some evidence suggested that silver sulphadiazine had no effect on infection, and actually slowed healing.[45]

Alternative medicine

Since about 1990, there has been a resurgence of the promotion of colloidal silver as an alternative medicine treatment, marketed with claims of it being an essential mineral supplement, or that it can prevent or treat numerous diseases, such as cancer, diabetes, HIV/AIDS, and herpes,[17] as well as tuberculosis.[2] [18] [46] Silver is not an essential mineral in humans; there is no dietary requirement for silver, and no such thing as a silver "deficiency".[2] No medical evidence supports colloidal silver as being effective for any of these claimed indications.[47] [48]

The commercial product referred to as "colloidal silver", includes solutions that contain various concentrations of ionic silver compounds, silver colloids or silver compounds bound to proteins in water. Such products with concentrations of 30 parts per million (ppm) or less are typically manufactured using an electrolysis process, whereas those with higher concentrations of 50 ppm or more are usually silver compounds that have been bound with a protein. Colloidal silver preparations primarily deliver inactive metallic silver, rather than the active microbicidal silver ion.[49]

No scientific evidence supports the effectiveness of colloidal silver *in vivo*.[2] Some *in vitro* studies demonstrate an antibacterial effect of colloidal silver,[50] although one study in 2004 of a colloidal silver solution marketed on the Internet showed no such antimicrobial activity.[51] No clinical studies in humans demonstrate effectiveness, and a few report toxicity.[16] The U.S. National Center for Complementary and Alternative Medicine has issued an

advisory indicating the marketing claims made about colloidal silver are scientifically unsupported, the silver content of marketed supplements varies widely, and colloidal silver products can have serious side effects to the consumer, including "argyria,... neurologic problems (such as seizures), kidney damage, stomach distress, headaches, fatigue, and skin irritation. Colloidal silver may interfere with the body's absorption of some drugs, such as penacillamine, quinolones, tetracyclines, and thyroxine."[2]

Although colloidal silver products are legally available at health food stores in the United States and Australia and are marketed over the Internet as a dietary supplement, it is illegal in the U.S. and Australia for marketers to make claims of medical effectiveness for colloidal silver. Ingestion of colloidal silver may result in argyria.[52] [53] [54] [55] [56]

Adverse health effects

Further information: Silver and Argyria

According to Lansdown, the risk expected due to clinical exposure to silver is "minimal", as only chronic ingestion or inhalation of silver preparations leads to an accumulation of silver in the human body that can cause argyria, argyrosis (accumulation of silver in the eye), and other conditions.[12] Silver-based products are contraindicated for people who are allergic to silver.[12] The reference dose, published by the United States Environmental Protection Agency in 1991, which recommends the estimated daily exposure that is unlikely to incur an appreciable risk of deleterious effects during a lifetime, is 5 μg/kg/d; meaning 5 micrograms of silver per kilo of weight per person each day – about 1 liter of 10 ppm colloidal silver per month for a 66 kg person.[17] An article from the National Center for Complementary and Alternative Medicine points out silver nitrate and silver sulfadiazine can have negative side effects, and they must be applied to the body externally and not taken internally.[2]

The chronic intake of silver products and the silver buildup from colloidal silver can result in an accumulation of silver or silver sulfide particles in the hair, skin, kidneys, liver, heart and muscles due to high methionine-containing proteins, such as keratin, myosin, tropomyosin, troponin, and key dipeptide glutathione. Serious neurologic (such as seizures), renal, or hepatic complications, as well as headaches, stomach distress, fatigue, and skin irritation have been reported.[57] [58] if ingested, colloidal silver may react with certain drugs, such as Penicillamine, thyroxine, quinolones, and tetracyclines.[59] One death has been reported in the medical literature which the authors felt was due to silver toxicity resulting from repeated oral ingestion of colloidal silver.[60] Colloidal silver can reduce the absorption of some medications, including tetracycline and quinolone antibiotics and can bind to penicillamine, thereby reducing the effectiveness of those medications.[16]

As in photography (where silver is used due to its reactivity with light), silver particles in the skin darken with exposure to sunlight, resulting in a blue or gray discoloration of the skin. This condition is known as argyria, which is a dermatological condition characterized by grayish-blue pigmentation of the skin, nails, gums, and deep tissues; and, in similar manner, it can lead to silver in the eye (argyrosis) and in other organs.[12] Localized argyria can occur as a result of topical use of substances containing silver, while generalized argyria results from the chronic ingestion of such substances.[57] Argyria was long believed to be irreversible,[49] but recently, laser therapy has been used to treat it with satisfactory cosmetic results.[61] [62] [63] The Agency for Toxic Substances and Disease Registry (ATSDR) describes argyria as a "cosmetic problem",[64] although some people consider it to be socially debilitating.[65] [66]

Regulation

In August 1999, the FDA banned colloidal silver sellers from claiming any therapeutic or preventive value for the product, noting colloidal silver was being marketed for numerous diseases without evidence of safety or effectiveness.[48] As a result, the product now has the status of a dietary supplement in the US; it can be promoted with general "structure-function" claims, but cannot be marketed as preventing or treating any illness.[48] Following this ruling, the FDA has issued numerous Warning Letters to Internet sites that have continued to promote colloidal silver as an antibiotic or for other medical purposes.[67] [68]

In 2002, the Australian Therapeutic Goods Administration (TGA) found there were no legitimate medical uses for colloidal silver and no evidence to support its marketing claims. Given the associated safety risks, the TGA concluded "efforts should be made to curb the illegal availability of colloidal silver products, which is a significant public health issue."[69]

Environmental effects

Silver that enters the environment from discarded medical sources can have detrimental effects on micro-organisms and animals (including humans).[70]

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External links

- Colloidal Silver information (http://nccam.nih.gov/health/silver/) from the National Center for Complementary and Alternative Medicine
- Final Rule on Colloidal Silver Drug Products (http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=1999_register&docid=fr17au99-6.pdf) - U.S. Food and Drug Administration
- "Complementary/Integrative Medicine Therapies: Colloidal Silver" (http://google.com/search?q=cache:jzq3x02xQhEJ:www.mdanderson.org/departments/cimer/display.cfm?id=ca255d3c-2ca8-46ed-a19011aa9f45cd23&method=displayfull&pn=5ac57a83-0f8d-4a3f-b743a0cdf23f193c+colloidal+silver+site:www.mdanderson.org&cd=1&hl=en&ct=clnk&gl=us). M. D. Anderson Cancer Center.
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THE USE OF SILVER NITRATE AS A VITAL STAIN, AND ITS DISTRIBUTION IN SEVERAL MAMMALIAN TISSUES AS STUDIED WITH THE ELECTRON MICROSCOPE*

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Plates 31 to 36

(Received for publication, November 1, 1954)

INTRODUCTION

Dyestuffs have been administered to living animals for many reasons. Ehrlich (1885), using dyes which change color upon oxidation or reduction, attempted to determine oxidative potentials in animal tissues. Basic dyes are, in general, quite toxic for reasons not clearly understood. A group of acid, colloidal dyes, such as pyrrol blue, trypan blue, and carmine, were shown by Goldmann (1909), Kiyono (1914), and Evans and Scott (1921), to be segregated by phagocytosis into a class of scavenger cells, the macrophages. These and related dyes, as well as numerous opaque or colored particulate materials (India ink, thorotrast, manganese dioxide, colloidal gold, etc.), have been used in many studies designed to investigate the phagocytic activity of cells and the passage of fluid across cellular membranes, and to mark cells in order to follow their subsequent migration by the colored inclusions.

The introduction of silver salts into animals causes a deposition of metallic silver in many tissues and organs. Clinically, this phenomenon is called argyria, which often follows accidental ingestion of silver from solutions such as argyrol, or the absorption of silver as an industrial hazard. In argyria, the skin, mucous membranes, and many organs become darkened and discolored because of their content of finely dispersed granules of silver metal. The location of the silver has been studied carefully by microscopical methods, so that quite complete accounts of the histopathological lesions in argyria are available (Hill and Pillsbury, 1939). More recently, Gatz (1949) has shown that the administration of very dilute solutions of silver nitrate in drinking water is tolerated with no visible difficulty by rats, and that after many months of such treatment the tissues become heavily laden with silver deposits. This procedure, which permits segregation of the silver without its

* This investigation has been aided in part by grants (B-425, C-2174, and G-3298) from the United States Public Health Service, National Institutes of Health.

reaching toxic concentrations, has been exploited by Wislocki and Leduc (1952), who found that observation of brain sections with both daylight and darkfield microscopes permitted a quite precise delimitation of the blood-brain barrier at the margins of those areas of the brain in which vital staining occurs. Their success with darkfield illumination, which reveals silver particles too small for visualization by ordinary microscopy, prompted us to study the distribution of silver in tissues examined with the electron microscope. We hoped the greater resolution afforded by this instrument would permit more precise localization of the silver deposits. The following passages will illustrate that this hope was well founded, and will describe the ultramicroscopic distribution of silver in the cells and their associated membranes in a number of tissues.

Materials and Methods

Two Swiss albino mice, four albino rats, and two guinea pigs were used. The mice and rats received 1 gm. of $AgNO_3$ per liter in their drinking water for 6 to 12 months. The guinea pigs were given 0.01 per cent $AgNO_3$ for 1 and 2 months, respectively. At the time of autopsy all animals appeared to be healthy. The skin and lips were discolored, and the internal organs were variably darkened by their silver deposits.

The animals were killed by decapitation, the tissues were removed rapidly and small pieces were immersed in Palade's (1952 *a*) buffered osmic acid mixture. After fixation at room temperature for 4 hours the tissues were dehydrated, infiltrated with a 3:1 mixture of butyl and methyl methacrylates, and finally embedded in this mixture of plastics by polymerization catalyzed by benzoyl peroxide. Sections were prepared as described by Dempsey and Lansing (1953) and examined by an RCA electron microscope model EMU at initial magnifications of one to six thousand diameters. The negatives obtained were enlarged photographically as desired. The tissues employed in this study were the kidney, pancreas, liver, and thyroid gland.

RESULTS

Kidney.—The deposits of silver, as visualized in electron micrographs, appeared as dense, granular particles of irregular shapes and sizes. In places, where heavy deposition had occurred, the aggregated granules had diameters up to a few microns; in others, where slight amounts of silver were present, the granules were smaller. Indeed, in our best micrographs, the smallest discernible particles were as small as the limits of resolution of the microscope (in the order of 20 to 30 A). The particles, of somewhat larger size, were easily identifiable because of their extreme density and their sharp angular outlines. No structures even remotely resembling them have ever been seen in several thousand micrographs from various mammalian tissues prepared identically except that silver had not been administered.

The heaviest deposits of silver were located in the basement membranes of the kidney. The glomerular basement membranes were most heavily infiltrated with silver; next in order were the membranes surrounding the

proximal convoluted tubules. The basement membranes of the distal convoluted tubules contained only occasional granules (Figs. 1 and 2).

The localization of the granules in the basement membranes was quite precise. In the glomerulus, silver was not detectable in the endothelial cells lining the capillaries and only rarely in the epithelium forming the visceral layer of Bowman's capsule. The latter observation is interesting, because the epithelial pericytes exhibited curious foot-like processes directed toward the glomerular basement membrane upon which they rested. Between these extensions minute spaces occurred, which communicated with the lumen of the glomerular cavity. These foot-like cytoplasmic extensions have been recognized only in electron micrographs, and in the earlier pictures made from thick sections with poor resolution there was some doubt concerning their exact relationships to the pericytes and basement membranes (Pease and Baker, 1950). Others (*cf.* Rinehart *et al.*, 1953) have traced these processes to the pericytes. In our current pictures (Fig. 3), where a sharp localization of silver is visible in the glomerular basement membrane but not in the end-feet, the conclusion that the end-feet are processes of the pericytes is inescapable.

The cells of the parietal layer of Bowman's capsule were devoid of silver, but the basement membranes associated with them contained moderate numbers of small granules (Fig. 1).

In the proximal convoluted tubules, identifiable by their characteristic brush border at the luminal surface, a moderate amount of silver was deposited in the basement membrane. Figs. 2 and 4 illustrate this arrangement, showing the interesting relationship between the basement membrane and the cellular plasma membrane. This basement membrane apparently forms an essentially smooth, flat sheet enclosing the renal tubule (Fig. 4). The bases of the epithelial cells are composed of a series of complex cytoplasmic evaginations or invaginations, the contours of which are visualized by the denser shadows of the cellular plasma membrane (Figs. 2 and 4). Thus, between the folds of the plasma membrane, there are extracellular pockets into which the basement membrane does not appear to extend. The granules of silver are located in the basement membrane and not in the numerous pockets between the folds of the cell membranes (Fig. 4).

The mitochondria of the cells comprising the proximal convoluted tubules were relatively large cylindrical, or filamentous structures. In high resolution micrographs they were seen to be bounded by an outer membrane, closely apposed to an inner membrane which was often reflected into folds projecting into the interior of the mitochondrion. A rather dense, homogeneous material filled the central cavity between the folds. This appearance was entirely similar to that described by Palade (1952 *b*, 1953), Sjöstrand and Rhodin (1953), and Rhodin (1954). However, in our animals which

stored silver nitrate, many (approximately one-fourth) of the mitochondria exhibited one or more dense, spherical granules embedded in the central matrix. These granules were ordinarily solitary and had a diameter of about 0.1 μ (Figs. 2 and 4). Since the ratio of diameters of granules and mitochondria is about 1:4, it would seem likely that each mitochondrion contains at least one such granule; those not exhibiting a granule in our pictures would be explained by the plane of section missing the granule. Mitochondrial granules have been reported by Palade (1952 *b*) and Rhodin (1954) but the frequency and the density of those encountered here appear to us to be greater than are encountered in the tissues from untreated animals.

In the distal convoluted tubules, silver was encountered only in small amounts in the basement membranes (Fig. 1), and no mitochondrial granules were observed.

Silver was encountered in occasional macrophages in the peritubular stroma, in the form of silver granules of varying size and irregular shape within cytoplasmic vacuoles. It also occurred in the basement membranes of the intertubular capillaries, but none was visible in the endothelium.

Pancreas.—Figs. 5, 6, 7, and 10 illustrate the localization of silver in the pancreatic acinar cells, basement membranes, blood vessels, and intralobular ducts. We have not encountered islands of Langerhans nor the larger ducts and so have no observations to offer concerning these structures.

The heaviest concentrations of silver granules were found in the basement membranes underlying the endothelium of small arterioles. The basement membranes of capillaries (Fig. 6) and small intralobular ducts (Fig. 5) also exhibited numerous silver granules. Occasional macrophages were encountered in the interlobular connective tissue; these contained large segregation vacuoles filled with granular deposits of silver (Fig. 11).

Perhaps the most interesting locus in which silver was found in the pancreas consisted of occasional deposits within the acinar cells. These deposits appeared as rather sizable aggregates of fine granules, the largest groups of which measured 2 to 3 μ in diameter. These were encountered in all parts of the cell, but occurred most frequently in the zone just apical to the nucleus (Fig. 7). In some of these aggregates, the silver was packed so tightly that no other structure could be seen. In others, however, the silver granules were located inside a spheroidal structure bounded by a dark membrane and containing, besides the granules, a homogeneous substance the density of which was comparable to that of mitochondria. In still others, in which only small silver granules occurred sparsely, the spheroidal structures exhibited the internal folds characteristic of mitochondria (Figs. 9 and 10).

Liver.—Silver deposits were encountered in the parenchymatous cells of the liver and in the lining cells of the hepatic sinusoids (Kupffer cells). We

have not investigated the vessels, ducts, and connective tissues located in the portal canals.

The Kupffer cells exhibited vacuoles containing granular aggregates similar to those described in preceding sections for macrophages (Figs. 8 and 11). Our specimens illustrate, incidentally, that the hepatic sinusoids are incompletely lined by the Kupffer cells, a phenomenon previously mentioned by Fawcett (1953). Here and there are locations in which the hepatic cells seem to be bathed directly by the blood plasma within the sinusoid. This disposition would seem to be a true one and not the result of a mechanical artefact, since the Kupffer cells show no evidence indicative of shrinkage and small irregular surface projections of the hepatic cells dip into the lumen of the sinusoid. These appearances suggest that an incomplete layer of lining cells is interposed between the blood stream and the hepatic cells.

The hepatic cells exhibited occasional aggregates of granules contained within vacuolated structures (Fig. 9). These intracellular aggregates occurred more frequently than did the similar ones described for the pancreas. Like those in the pancreas, those with small amounts of silver often showed internal folds, the double membranes of which had dimensions equal to those of mitochondria. In favorable places, the limiting outer membrane could also be resolved into a double layer similar to that of mitochondria. In bodies with slight deposits, the silver was located on the internal membranes, whereas in ones with heavier deposits there were vesicles filled with granules of varying sizes.

Thyroid Gland.—Silver deposits occurred in the basement membranes of the thyroid follicules, and in the similar membrane supporting the endothelial cells of the capillaries. The depiction of the follicular basement membrane is interesting, because the existence of such a membrane has been denied by many histologists. It appears, however, that the thyroid cells rest upon a structure identical in appearance and ability to attract silver granules with that forming the basement membrane of other epithelia. Occasional macrophages, with their segregation vacuoles, were encountered in the interfollicular connective tissue. Silver was not encountered elsewhere in the thyroid gland.

DISCUSSION

The discovery that certain acid, colloidal dyes could be administered to animals with minimal toxicity led to the investigation of the phagocytic activity of cells of the body, to the recognition of the free and fixed macrophages, and to some understanding of the mechanisms whereby cells segregate or otherwise handle unassimilable colloidal materials (Goldmann, 1909, 1912; Kiyono, 1914; Evans and Scott, 1921). The present study, dealing with the distribution of silver when used as a vital dye, introduces the electron micro-

scope as a tool for studying these and similar problems. Because of the greater resolving power of the electron microscope, more definite localization of the silver granules is possible than was the case with light microscopy. Thus, when formerly, with light microscopy, silver deposition was reported as occurring primarily in the glomerular endothelium, it is revealed with electron microscopy to be solely located in basement membrane (Gatz, 1949; Shaver and Mason, 1951). Furthermore, the distribution of silver in basement membranes and in the mitochondria of some cells can now be regarded as certain, when formerly one could say only that silver occurred within cells or apparently beneath their bases. Although the question was not thoroughly explored in the present investigation, it is evidently possible by means of this instrument to detect silver and other heavy metals in traces much beyond the resolution of the light microscope. The future promise of electron microscopy in these respects is great.

The visualization of silver after administering its salts also opens another field for investigation. Silver is a heavy metal, and, like other heavy metals is poisonous if toxic concentrations are reached. The density of the heavy metals renders them ideally suited for studies with the electron microscope. Detailed studies utilizing proper dosages, tissues, and heavy metal poisons should provide information as to the site at which these agents damage the cell. Our present findings, although exploratory only, are suggestive in that the mitochondria appear to be vulnerable to the heavy metal. The appearance of mitochondrial granules in the kidney and the deposit of silver upon the bounding and internal membranes of mitochondria from the kidney, pancreas, and liver suggest that these organelles are the targets of the heavy metal. Damage to mitochondria would provide a ready explanation for the toxicity of silver, since the mitochondria are now regarded as the sites of important oxidative activities.

The segregation of large amounts of silver in basement membranes in many locations, especially in pancreas, thyroid, salivary glands, Harderian glands, renal glomeruli, proximal convoluted tubules, urinary bladder, and chorioid plexuses (Hill and Pillsbury, 1939) suggests a possible function for these structures. Basement membranes, in general, are argyrophilic when sections of fixed tissues are exposed to solutions of silver salts. This argyrophilia, their positive reaction with the periodic acid–Schiff reagents, and the scanty information available from their analyses all point toward the presence of a polysaccharide in their composition. Since the silver administered in the present experiment was introduced in the ionic form and was reduced to metallic silver before its deposition, it is necessary to invoke some agent which has an adequate reducing activity. The carbohydrate moiety of basement membranes would appear to fulfill this requirement. Moreover, its location, being interposed between the blood stream and the parenchymatous

tissues, is so situated as to protect the tissues from exposure to toxic quantities of reducible substances, since these substances would be trapped and filtered out by the barrier of the membrane.

An alternative possibility is that the administered silver is quickly reduced in the gastrointestinal tract or in the blood stream, and that ultramicroscopic particles of colloidal silver are trapped in the basement membranes by a simple filtering action as fluids are transported across them. This possibility, although superficially simple, requires accessory explanations to account for the passage of particulate silver across the endothelial layer, for the aggregation of the small particles into larger and larger ones, and for the failure of silver to accumulate in extracellular spaces contiguous to the basement membrane such as the ones which we have noted in the renal proximal convoluted tubule. In any event, the observation that silver segregation occurs in sharply localized areas offers a challenge to discover the biophysical and biochemical mechanisms responsible, and provides a tool whereby basement membranes, mitochondria, and segregation vacuoles can be altered experimentally as a means of studying their biological significance.

SUMMARY

After chronic administration of a dilute solution of silver nitrate in drinking water to rats, mice, and guinea pigs, granular deposits of metallic silver were detected in electron micrographs of the kidney, liver, thyroid, and pancreas. The silver deposits were in the form of extremely dense, angular particles with sharp outlines. They varied from aggregates a few microns in diameter down to granules at the limit of resolution of the electron microscope.

The principal sites of deposition were (1) basement membranes, especially those of the renal glomeruli, proximal convoluted tubules, and various glands, and those associated with vascular endothelium, and (2) the cytoplasm of fixed and free macrophages. Both in Kupffer cells lining hepatic sinusoids and in the wandering macrophages of other tissues, the silver was segregated in discrete vacuoles. In addition, granular deposits were observed in occasional vesicular structures in the proximal convoluted tubules of the kidney, the hepatic cells, and the pancreatic acinar cell. These structures, in favorable preparations, contained an outer double layered membrane and internal folds similar to those of mitochondria, from which they appear to have been derived. The significance of these findings in heavy metal poisoning and in cellular physiology is briefly discussed.

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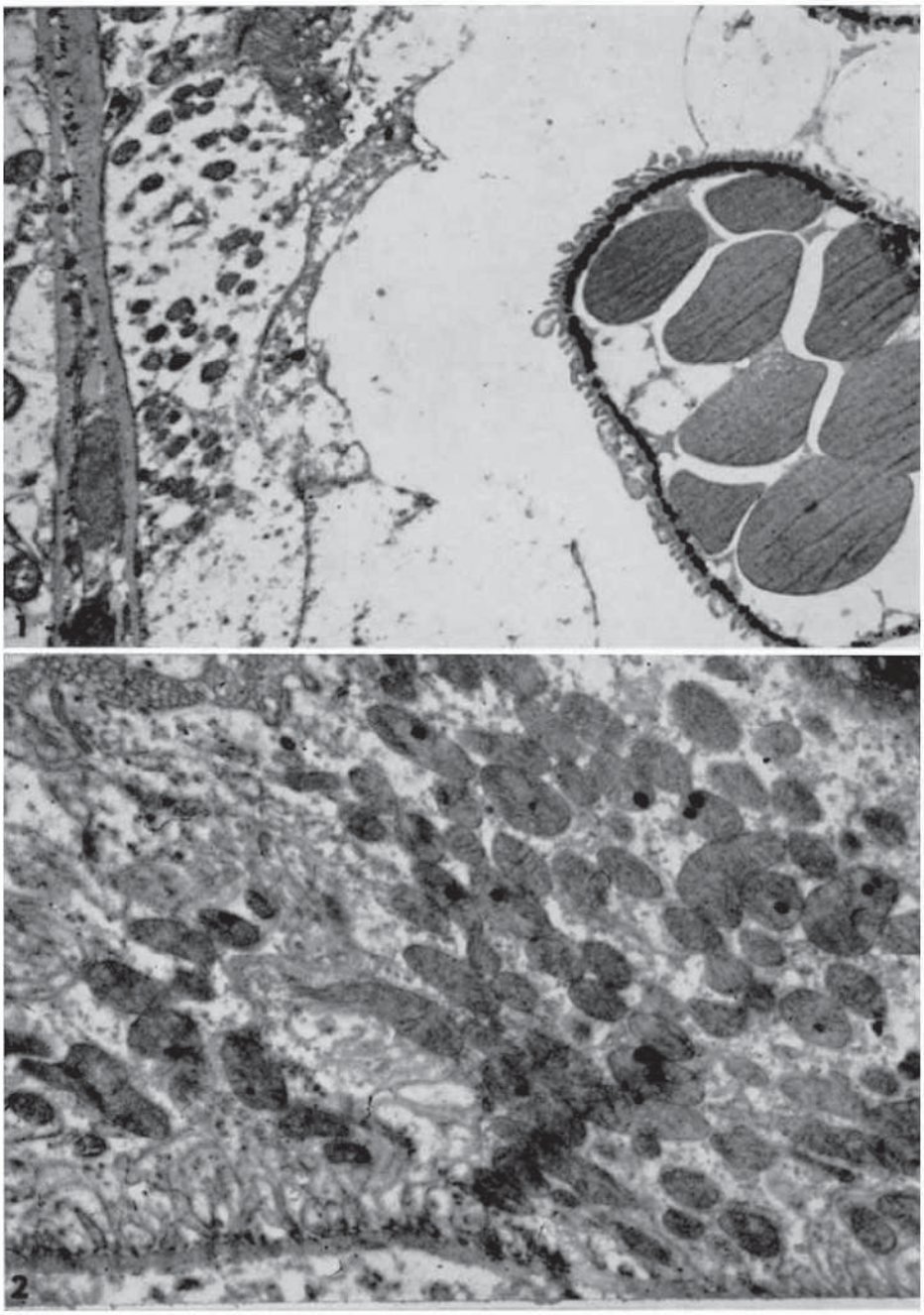
EXPLANATION OF PLATES

PLATE 31

FIG. 1. Section through the wall of Bowman's capsule and one loop of a capillary tuft from the renal glomerulus of a mouse which had received 0.0015 per cent $AgNO_3$ in its drinking water for 6 months. Heavy deposits of silver outline the basement membrane of the capillary on the right, and lesser deposits are seen in the basement membranes of Bowman's capsule and of a distal convoluted tubule on the left. Radiating into the glomerular lumen, some of the end-feet of the pericytes constituting the visceral layer of Bowman's capsule can be seen. × 5,000.

FIG. 2. Section through a proximal convoluted tubule from the kidney of a guinea pig which had received 0.01 per cent $AgNO_3$ in its drinking water for 6 weeks. The small amount of granular silver apparent in the basement membrane at the bottom of the figure is caused by the short time silver was administered. At the top left corner, a typical brush border can be seen. Dark granules occur in many of the mitochondria. At the base of the cell, many reflections of the plasma membrane upward into the cell's substance can be seen. The extracellular channels formed by these reflections do not exhibit silver deposits. × 15,000.

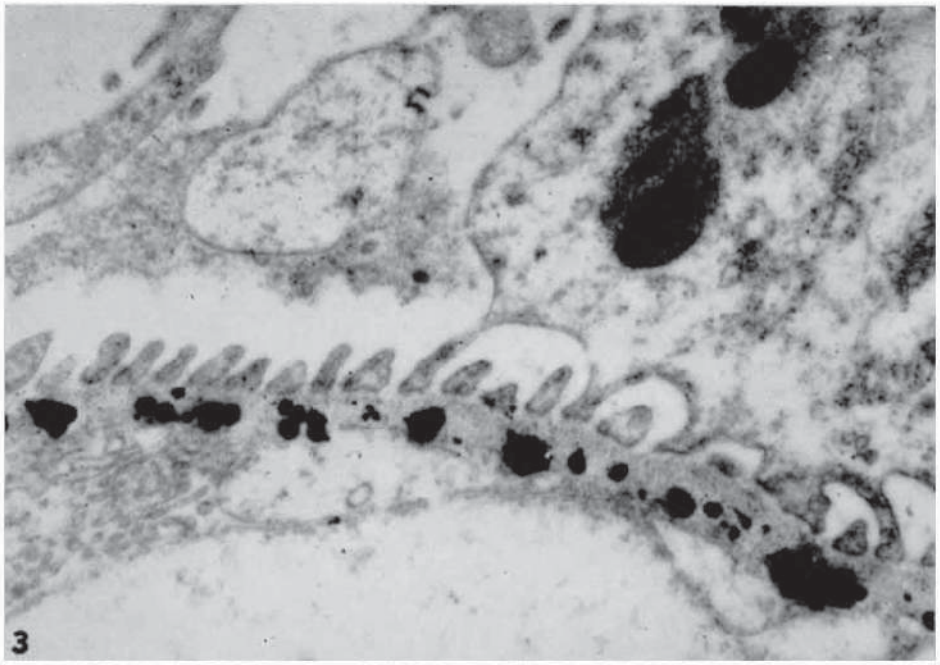


(Dempsey and Wislocki: Electron microscopy of argyria)

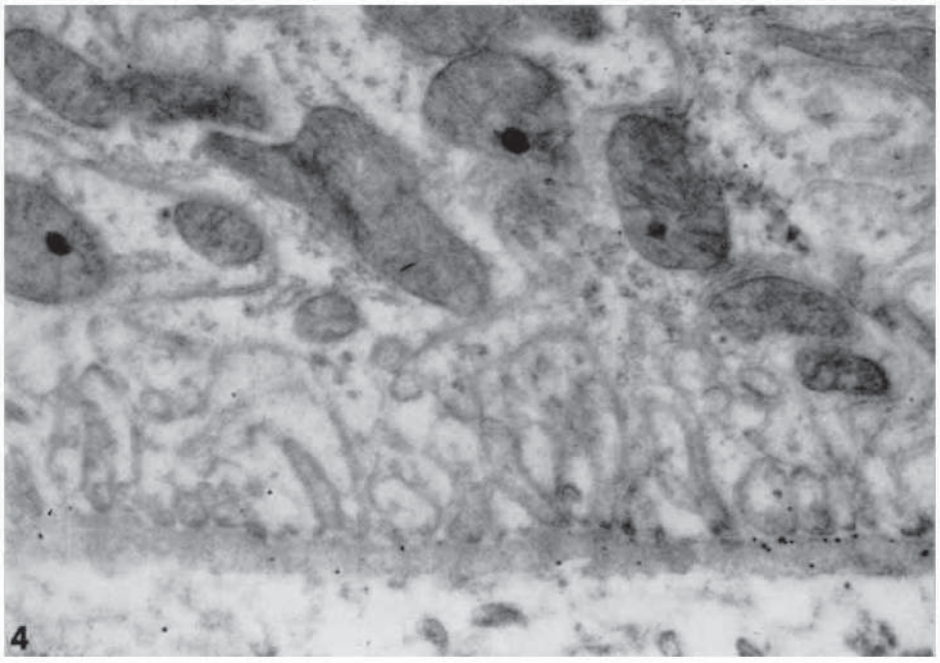
PLATE 32

FIG. 3. Section through a glomerular capillary and its associated pericyte from a mouse given silver for 6 months. The capillary lumen is at the bottom of the figure. Just above the endothelium is the capillary basement membrane, which contains silver granules ranging in size down to the limits of the electron microscope. At the top of the picture are portions of pericytes with end-feet resting upon the basement membrane. The pericyte at the right contains some silver deposits in spheroidal structures located toward the top of the picture. × 50,000.

FIG. 4. Portion of the base of a renal proximal convoluted tubule from a guinea pig which had received silver for 6 weeks. Granules can be seen in the mitochondria and in the basement membrane but not in the extracellular channels created by the recurving plasma membrane. × 25,000.



3



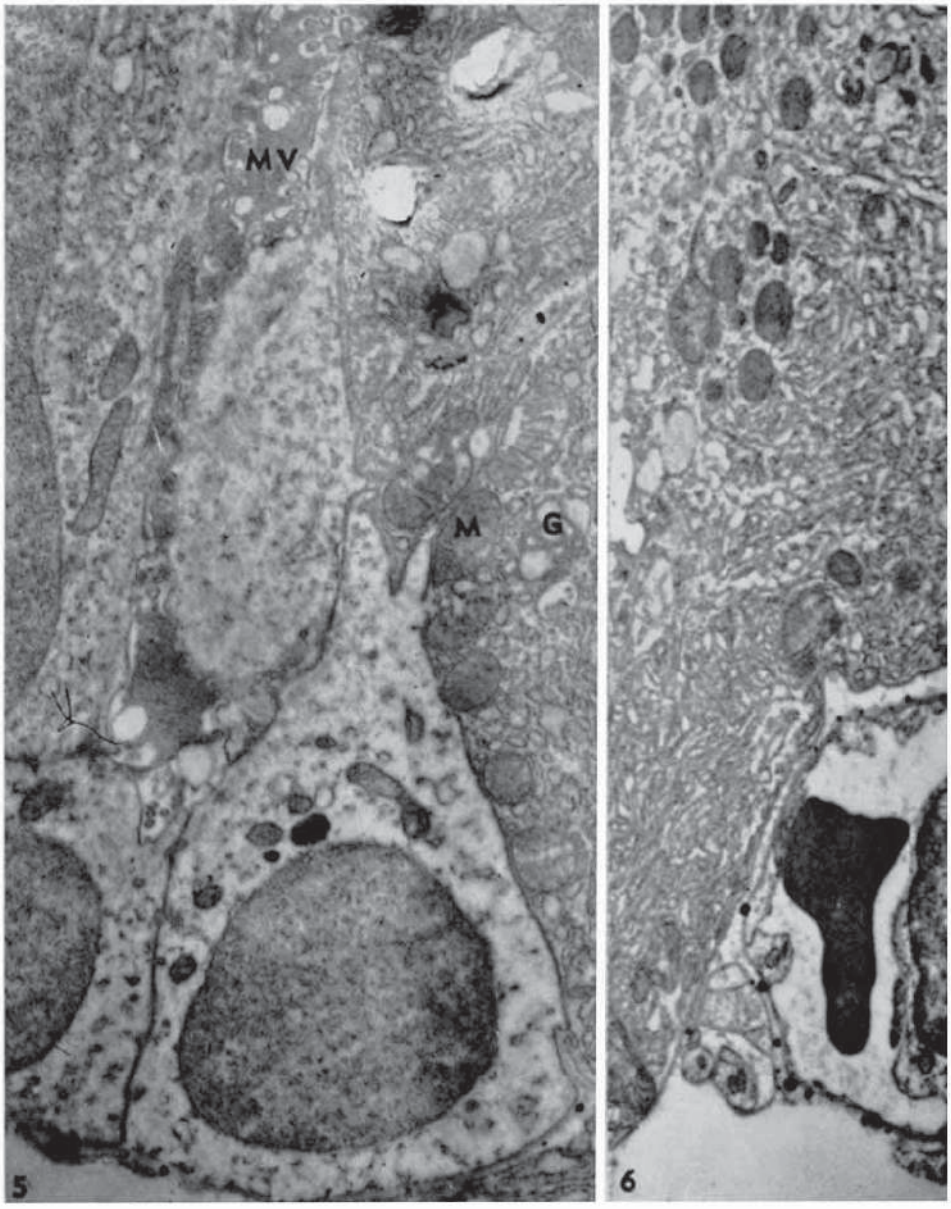
4

(Dempsey and Wislocki: Electron microscopy of argyria)

PLATE 33

FIG. 5. Section through the pancreas of a rat which had received $AgNO_3$ in its drinking water for 10 months. Running from the top center downward is the lumen of an acinus passing into the lumen of an intralobular duct. The apices of two acinar cells are seen along the right margin of the picture. Deposits of silver occur in the basement membrane of the duct (bottom of picture) and in bodies which resemble mitochondria located in one of the duct cells. Mitochondria (*M*), the Golgi region (*G*), microvilli projecting into the lumen of the duct (*MV*), and zymogen granules (*Z*) are illustrated. The granular outlines of the slightly dilated ergastoplasmic sacs can be seen in the acinar cells. × 15,000.

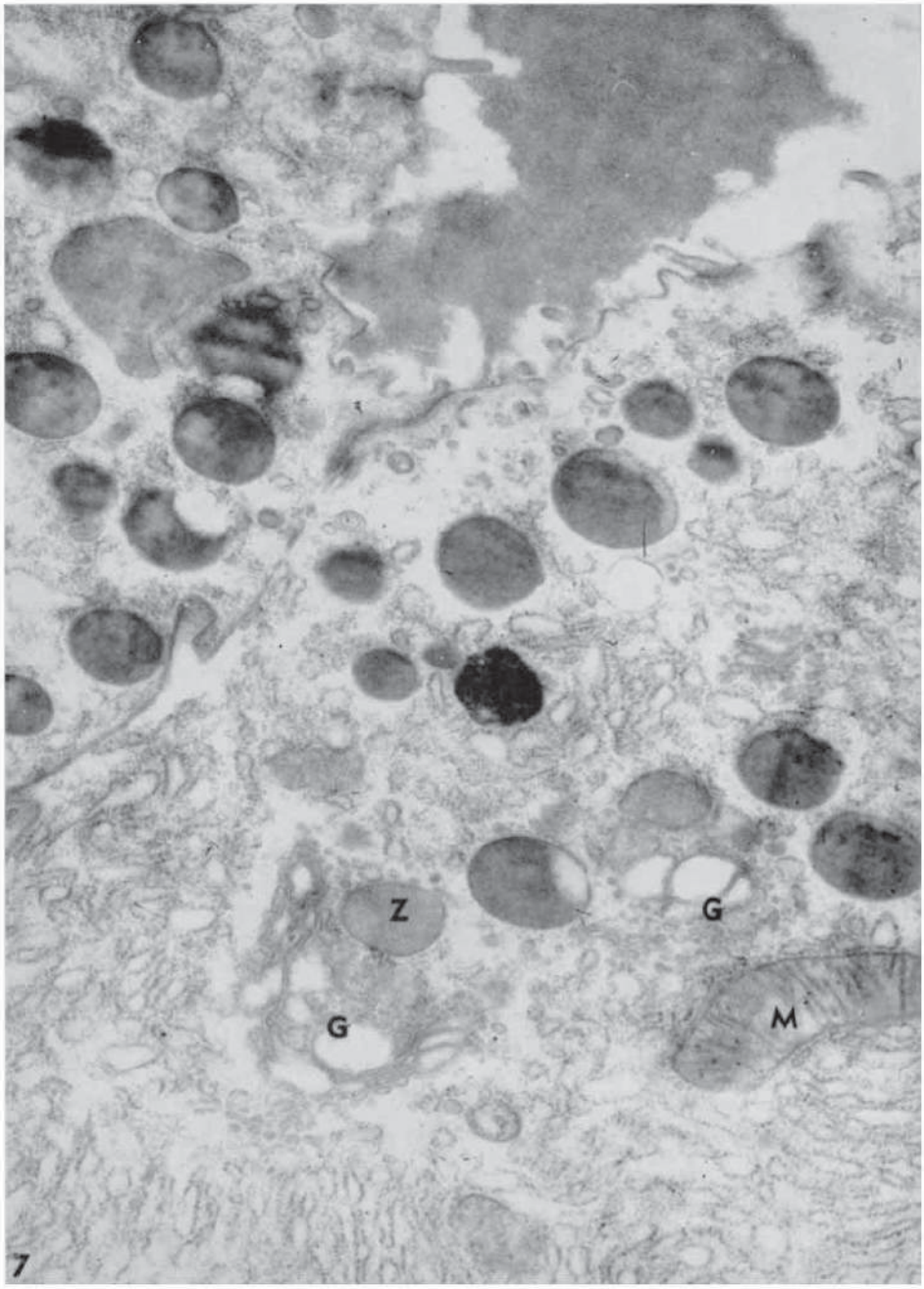
FIG. 6. Section through the base of an acinar cell from the same pancreas as illustrated in Fig. 5. A capillary with a single erythrocyte is shown at the bottom right. Several silver granules can be seen in the basement membrane of the endothelium. × 10,000.



(Dempsey and Wislocki: Electron microscopy of argyria)

PLATE 34

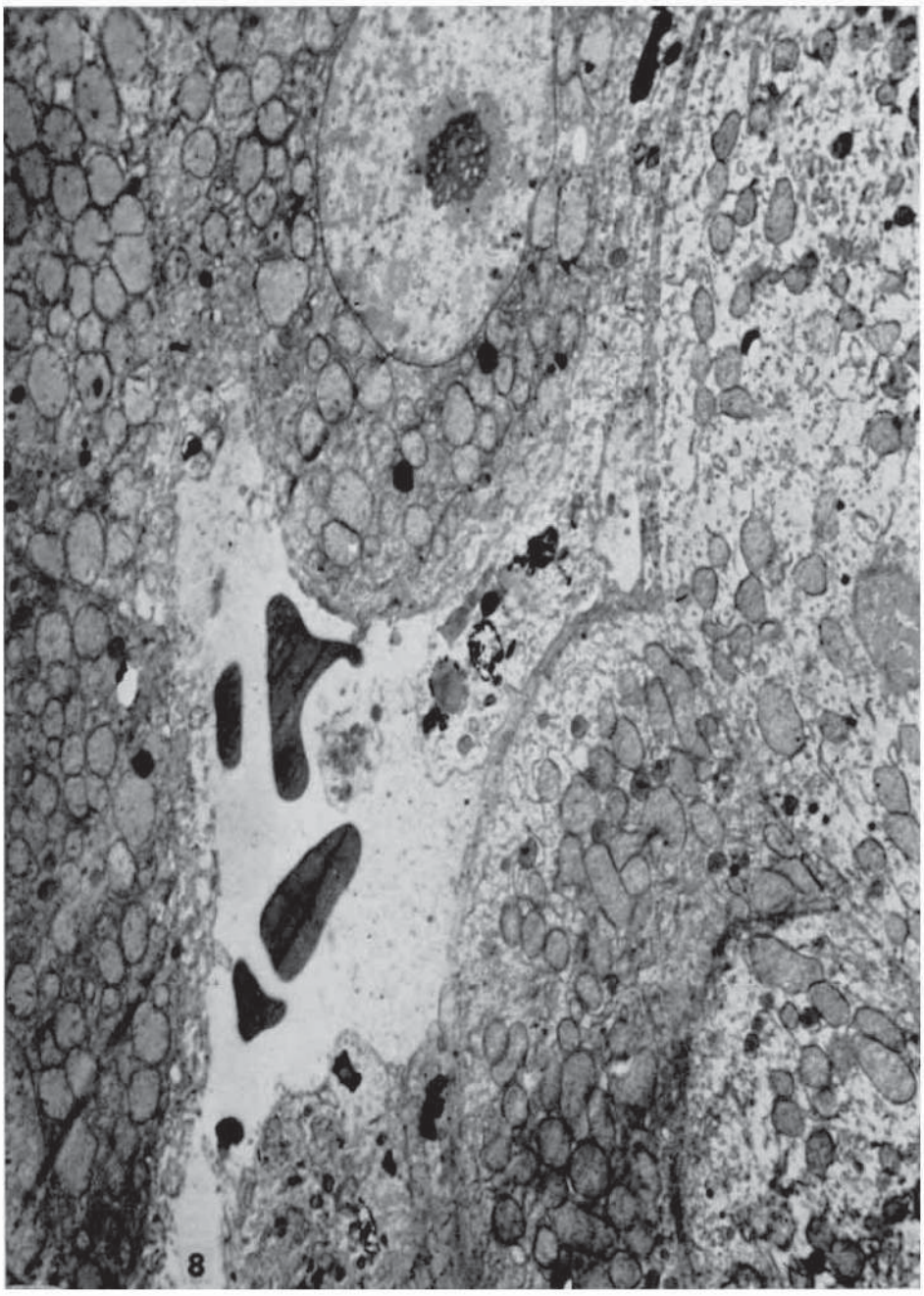
FIG. 7. Section through the apical parts of two acinar cells from the rat utilized for Figs. 5 and 6. At the top right is the acinar lumen, with microvilli projecting into it. Several dark, spherical zymogen granules (*Z*) can be seen. A few mitochondria (*M*) with their internal folds occur in the lower cell. At the lower center, two regions with small tubules and vesicles associated with larger spherical spaces exhibit the appearance Dalton has ascribed to the Golgi apparatus (*G*). At the center of the figure a spheroidal accumulation of silver granules can be seen. × 30,000.



(Dempsey and Wislocki: Electron microscopy of argyria)

PLATE 35

FIG. 8. Section through hepatic sinusoid and hepatic cells from a rat fed silver for 10 months. Projecting into the sinusoid are portions of three Kupffer cells, each of which contains vacuoles filled with silver granules. Dark granules can be seen in some of the hepatic mitochondria. × 5,000.



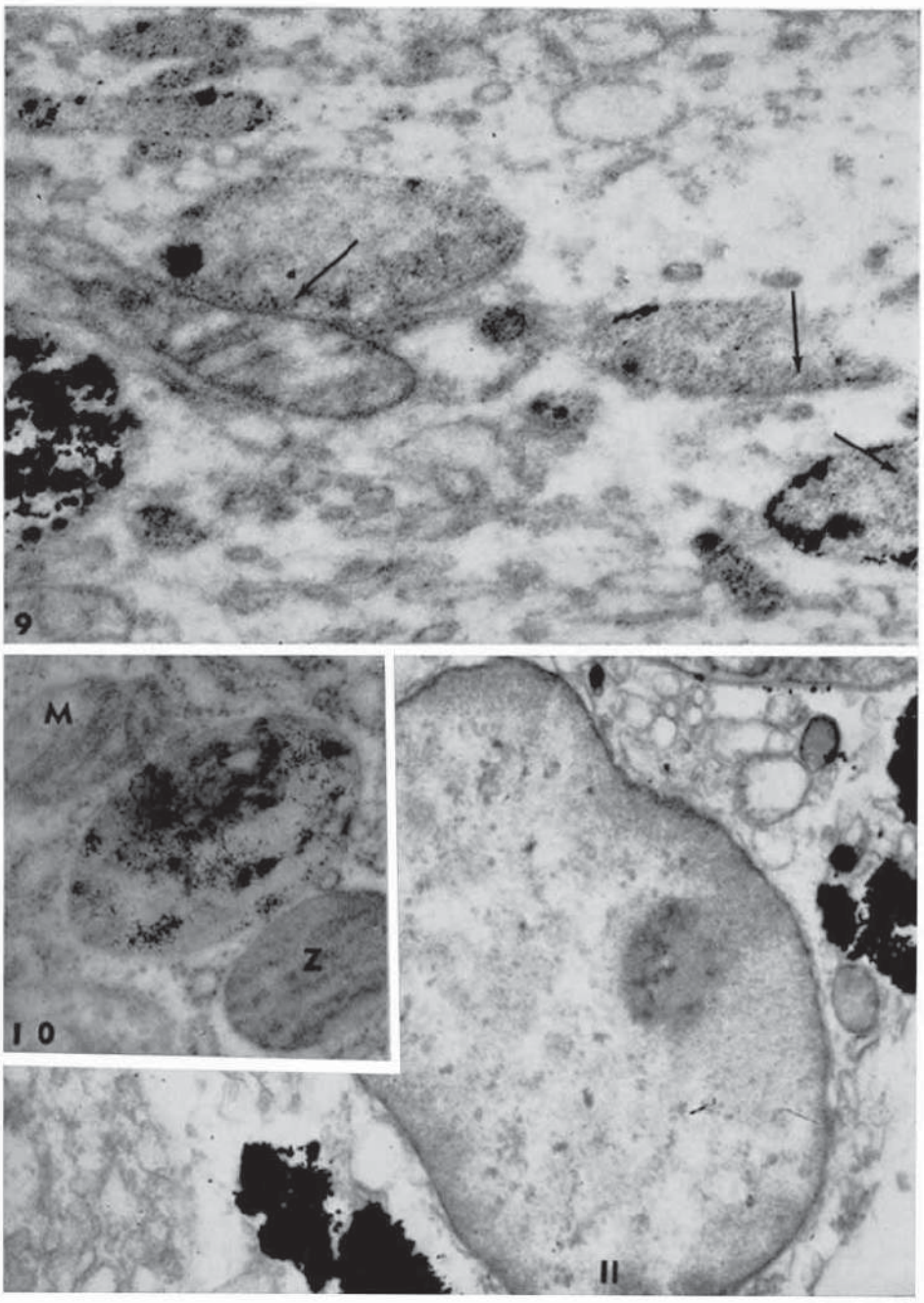
(Dempsey and Wislocki: Electron microscopy of argyria)

PLATE 36

FIG. 9. An area from an hepatic cell from a rat given silver for 10 months. Two large structures containing considerable amounts of silver can be seen at the extreme left and right An elongated mitochondrion with no silver occupies the left center of the picture. Above it, and to the right are structures containing slight silver deposits in which the double outer membrane and fragmentary internal folds (arrows) characteristic of mitochondria can be seen. Smaller structures containing silver and other matrix material are scattered throughout the field. × 25,000.

FIG. 10. Section through the pancreas of a rat given silver nitrate for 10 months. A mitochondrion (*M*) is shown at the upper left, a zymogen granule (*Z*) at the lower right. In the center is a spheroidal body containing granules of silver and a clumped mass of membranous material which resembles the internal folds of mitochondria. × 30,000.

FIG. 11. Section through the pancreas of a rat given silver nitrate for 10 months. The edge of an acinus appears at the extreme top right, and exhibits some small silver granules located in its basement membrane. The rest of the figure illustrates a macrophage located in the interacinar connective tissue space. It contains large segregation vacuoles filled with granules of silver. × 15,000.



(Dempsey and Wislocki: Electron microscopy of argyria)

SILVER FOR HUMAN HEALTH

SILVER FOR HUMAN HEALTH

"Silver fell out of favor. The reason was Argyria—a skin discoloration that results when hundreds of times the proper amount of silver compounds are injected or taken orally." *Science Digest, March 1978.*

"Thanks to eye opening research, silver is emerging as a wonder of modern medicine. An antibiotic kills perhaps half-dozen different disease organisms, but silver kills some 650. Resistant strains fail to develop... silver is the best all around germ-fighter we have." *Dr. Harry Margraf, St. Louis, Missouri, in Science Digest, March 1978.*

"In twelve of the four-teen patients, treatment was considered successful and in all fourteen patients (including the failure) treatment resulted in markedly reduced bacterial flora in the wound as shown by sequential colony count. In no case were any undesirable side effects of the silver treatment apparent." *Dr. Robert O.Becker, M.D. and Dr. Joseph A. Spadaro, Ph.D., "Treatment of Orthopedic Infections with Electrically Generated Silver Ions," The Journal of Bone and Joint Surgery, Vol. 60-A, No.7, Oct. 1978. Research team from the Veterans' Administration Hospital and the Department of Orthopedic Surgery, State University of New York, Upstate Medical Center, Syracuse.*

... even tiny amounts of silver wipe out huge quantities of disease organ-isms in water." *Dr. Charles Fox, Columbia University (inventor of FDA approved sulfadiazine).*

Quotations like these prompted the investigation of current interest in research and the documentation of available information concerning the various uses of silver and silver compounds.

This is a report of the Environmental Health Foundation's review of the recorded data.

HISTORICAL USE

Silver vessels and receptacles for storing and trans-porting water have been found in royal tombs dating back to 4000 B.C. Persian history records the practice of keeping drinking water in copper and silver vessels. In the record of Herodotus' (Rawlinson's translation) we find the following example:

"The Great King, when he goes to the wars, is always supplied with provisions carefully prepared at home, and with cattle of his own. Water too, from the river Choaspes, which flows by Susa is taken with him for his drink, as that is the only water which Kings of Persia taste. Wherever he travels, he is attended by a number of four-wheeled carts drawn by mules, in which Choaspes water, ready boiled for use, and stored in flagons of silver is moved with him from place to place."

History also records that the ancient Babylonian and Greek civilizations were aware of silver's ability to disinfect. They used silver containers to hold and transport water for their royalty as well.

In the book *Antiseptics, Disinfectants, Fungicides and Chemical and Physical Sterilization,* I.B.

Romans reports that the use of silver compounds for medical treatment has been recorded since the eighth century A.D. Thus, silver compounds have been used medicinally in the treatment of various maladies and diseases for more than one thousand years.

LITERATURE REVIEW

More than 200 pages of information on human uses of silver were found by the research team. The collected body of information can generally be reported as follows:

Use of Silver In Recent History

- As reported in *Science Digest,* March 1978, more than half of the world's airlines use silver treated water as the method of choice for protecting airline passengers from water-carried diseases such as dysentery.
- *Science Digest* went on to state that the government of Switzerland approved silver water filters for home and office use throughout the country.
- One report from *Science Digest* included data on the use of silver treated water versus conventional chlorine
- treatment. Fifty gallons of "raw" sewage were dumped into a swimming pool and tested for E. coli, a dangerous pathogen found in the
- human digestive track. Initially, the colony count was 7,000 E. coli per milliliter of water.
- After three hours of pumping raw sewage through silver electrodes, the water was retested and found to be free of E. coli.
- In addition to water treatment, Dr. G.A. Krause produced a colloidal silver pow-der used for dressing wounds, a spray for tonsillitis, and a wet pack for treating burns and abrasions. His goal in producing multiple colloidal products was to limit or totally eliminate pathogenic bacteria from wounds, inflamed mucous membranes and drinking water.

Scientific Research

From an historical perspective, the most progress in the field of silver compounds and human use has occurred during the past 100 years.

For example, it was not until the latter part of the nineteenth century that Karl Sigmund Franz Crede (1884) introduced the use of silver nitrate for the prevention of ophthalmia neonatorum.

At approximately the same time, Raulin (1869), von Behring (1890) and von Nageli (1893) were studying the effects of small quantities of silver and silver nitrate on microbial life such as bacteria and molds.

In 1897, Dr. Benno C. Crede's antiseptic (powdered silver citrate), and his ointment (colloidal silver in an ointment base) were both used in the treatment of wounds and skin

diseases. It is reported that Crede received his inspiration for the creation and use of these silver compounds from the use of silver foil as an anti-infection wound dressing at Johns Hopkins University.

In 1928, G.A. Krause reintroduced the principle of using silver as a coating in filtration systems in order to sterilize water for domestic use.

Schweizer (1929) con-firmed the research data of Krause, reporting that all pathogens, including E. coli, were killed when water was treated with Katadyn Silver. Schweizer also determined that this silver treatment did not damage or eliminate beneficial air or water microorganisms.

Mallmann's (1937) research verified that Electro-Katadyn Silver killed E. coli in treated swimming pools while having no negative effect on useful microbial life.

Sallman (1943) conducted testing on the use of silver in the treatment of nervous diseases and lunacy.

Goodman and Gill (1943) treated vaginitis with silver compound suppositories.

Greenberg (1953) continued silver compound studies, researching the use of silver nitrates applied to the eyes of newborn babies.

Dr. A.C. Barnes (1957) developed a form of mild silver protein known as "Argyrol," which served as an effective local anti-infective.

Silver compounds used today fall within two general groups:

1) The soluble silver salts, such as nitrates and citrates, and the less soluble compounds referred to as the oxides, halogen salts (chlorides and iodides).

2) The proteinates which are not water soluble but exist as colloidal sus-pensions.

SILVER NITRATE COLLOIDAL

Silver Nitrate

Of the soluble silver salts, nitrate is the most common. When combined, nitrate and silver function as an astringent, irritant, or as a caustic, depending on the strength of the solution applied and the duration of use. Silver nitrate is commonly applied as a solution of varying concentrations or by a silver nitrate stick or pencil. Both forms may be used to remove warts or to treat ulcers and granulations. A one-percent solution of silver nitrate is instilled routinely into the conjunctival sac of newborn children to prevent ophthalmia neonatorum (a junctivitis affecting infants born to mothers infected with gonorrhea). This treatment is not without risk since cauterization of the cornea of the eye and blindness may occur.

Colloidal Silver

Colloidal silver suspensions are various insoluble compounds of silver and other elements in a solution. They include the iodides, chlorides and oxides. This insoluble compound is precipitated in the presence of protective colloids. For example, gelatins can suspend small particles that do not settle out but remain in suspension. The miniature particles of silver compound serve as reservoirs, releasing silver ions (the active substances of the compounds) in a controlled manner and in minimal quantities.

These suspended particles often include the attributes of true solutions. Colloidal silver compounds are divided into three classes: strong silver proteins, mild silver proteins and silver halides.

OLIGODYNAMIC EFFECT

Some metals (i.e., silver, copper, brass and tin) are lethal in large doses, but in miniscule quantities, they have the ability to kill bacteria and fungi. This is described as the oligodynamic effect.

Many researchers have attempted to determine and explain how the oligodynamic effect actually works. However, a definitive answer has not been found.

The word "oligodynamic" is a combination of two Greek words: "oligas" meaning little, and "dynamis" meaning power. The definition of this word is "effective in small quantities."

Because silver has this oligodynamic effect, it has been used for centuries to purify water, and through the ages creative people have tried to harness this property for the benefit of mankind. More recently, scientists have discovered that by adding small amounts of a second metal, one lower in the electro-potential series than silver, the oligodynamic effect could be intensified.

In 1928, Krause developed "Katadyn Silver" which has a much stronger oligodynamic effect than pure silver due to the addition of an activating second metal. He further increased the oligodynamic effect by coating other materials, such as sand, or lining water containers with "Katadyn Silver." Because a greater surface area of silver contacted the material to be sterilized, the process could then be completed in a shorter period of time with less silver. Numerous tests verify that even at very low concentrations silver kills the E. coli bacteria within 2 to 24 hours, depending on the quantity of bacteria present.

SILVER PROTEINS

Strong Silver Proteins

The strong silver proteins contain only small amounts of silver. This minimal amount of silver gains its antiseptic benefit because the silver and protein combination compound is largely ionized. Because of the increased ionization, strong silver proteins are more irritant. On the other hand, strong silver proteins tend to alter with storage. This silver compound becomes more strongly bonded to the protein property, and therefore, yields a less powerful silver ion solution. Because of this reduction in ion strength, there is a decrease in the anti-septic function after time.

Mild Silver Proteins

The mild silver proteins consist of 19 to 23% silver. This demonstrates how silver oxide particles are protected by a suitable protein colloid. There is only a small fraction of the silver in the mild silver protein solution that is ionized. Even though the amount of silver that is ionized is minimal, it is remark-ably consistent. Various reports indicate that dilution's as weak as 0.01% of a standard 23% solution will still experience an ion concentration very close to that of a more concentrated solution. This is an important factor because much of the solution will be diluted by body fluids. Nevertheless, a weak dilution still remains effective.

Electrolytic Colloidal Silver

The literature review further identified various concepts suggesting colloidal silver can be produced by arching an electrical current across two silver electrodes sub-merged in distilled

water, or water with a mild electrolyte. The electric arc creates a nitrogen-free ozone that, as a dissolved gas, may help destroy harmful anaerobic bacteria. However, regardless of the potential ozone benefits, the process and silver benefit may be of little value given the extremely small amount of silver that remains suspended in solution.

CANCER AND SILVER

It is widely believed that except for bone marrow cells, humans are unable to pro-duce primitive cells. However, while researching the effects of silver compounds on bone healing, Dr. Robert Becker discovered that fibroblast cells in the body, upon exposure to silver ions, dedifferentiated (became generic, not one specific type). The cells multiplied rapidly and produced many primitive cells which differentiated into whatever variety of tissue-producing cells were needed at that place. The body regenerated itself!

Dr. Becker details this amazing phenomenon in his book *Cross Currents.* And he asks, **"If the electrically generated silver ion dedifferentiated normal human fibroblast cells, would it also dedifferentiate human cancer cells?"**

His answer is excerpted from his book:

"I also had a patient with a severe, chronic bone infection who had an associated cancer in the wound. He refused amputation, which would have been the treatment of choice, and insisted that I treat his infection with the silver technique. After three months, the infection was under control, and the cancer cells in the wound appeared to have changed back to normal. When I last heard from him, eight years after the treatment, he was still fine."

WHAT HAPPENED TO THE SILVER SHEEN?

Although various fields of science have lagged behind in the conventional warfare involving mutant strains of "superbugs," they are beginning to reexamine the merits of silver. For example, Dr. Charles Fox of Columbia University developed a product, approved by the FDA, known as sulfadiazine (also as silvadene). This product is now being used in many burn centers around the world to control Pseudomonas Aeruginosa burn infections.

A few years ago, with the introduction of many so-called "miracle drugs," silver compounds apparently fell out of favor. Despite the initial benefits of the "miracle drugs," some of the literature indicated these drugs can pro-duce patient sensitization and may become ineffective with prolonged use. On the other hand, sensitization with silver compounds is low if it occurs at all.

Data in available literature substantiates that silver compounds offer broad, consistent anti-microbial protection against common pathogens, specifically against mycotic and viral infections.

Although the word "oligodynamic" was first used more than one-hundred years ago, potential uses of silver's oligodynamic effect are still being discovered. As reported in *Science Digest,* March 1978, Dr. Richard L. Davies, Executive Director of the Silver Institute which monitors silver technology in 37 countries, states:
"In the last four years, we've described 87 important new medical uses for silver. We're just beginning to see to what extent silver can relieve suffering and save lives."

SAMPLE CASE STUDIES

Included in the article, "Silver—New Magic in Medicine," *Science Digest* March 1978, were several case histories describing the benefits of silver treatments. In burn injuries, those suffering from burns covering more than 80% of their bodies usually die from infections rather than from the actual burns.

The first case study reported an 18-year-old victim of an auto accident with burns on 80% of his body. Remarkably, the victim was able to leave the hospital four months after the accident. Doctors credited the use of a silver compound (sulfadiazine) to his recovery. At the time, the silver compound was being used in 70% of the burn centers in the United States.

The second case study reported on an individual who was a diabetic. People who are afflicted with this health problem often are susceptible to infections that do not heal.

The subject was a 65-year-old who acquired an infection from a cut on his leg. It was reported that after a year the infection had digressed to a "stasis ulcer." Amputation was considered. A number of antibiotics were tried without success, and the patient was referred to a clinic which pre-scribed a silver compound. The ulcer was healed within two months.

Eleven patients suffering from chronic osteomyelitis and non-union bone fractures were treated. Each was given treatments of direct, electrically generated silver ions into the areas of the fracture and infection. Three other patients suffering from chronic osteomyelitis without the fractures were also given the same treatment.

The treatments resulted in control of the infection in eleven of the 14 cases and partial control in two of the cases. Only one of the 14 cases resulted in failure to control the infection and to heal the fracture. Two of the cases resulted in partial healing of the fractures. All other cases resulted in total healing. There were no undesirable side effects resulting from the silver treatment in any of these cases.

In five of the cases, the silver treatment produced an additional benefit. It was reported that substantial amounts of new bone tissue were deposited during the treatments. Common to all successful treatments was major growth stimulation to the bone, soft tissue and skin areas of the wounds.

CHELATED SILVER & ELECTROLYTES

Because of the potential toxic side effects from colloids derived from silver nitrate and other harsh chemicals, some researchers are hesitant to use current silver technology with-out some modifications. For example, the most pure forms of silver colloids are electrically generated and will stay in sus-pension only in trace amounts. Even if kept in suspension, however, their particle size may be a deterrent. When taken orally, very little silver gets to the vascular system to fight viral and microbial diseases, and any silver finding its way into the bloodstream most likely would never contact viral infection lodged inside the cell.

When injected intravenously much greater amounts of silver colloids can be introduced into the blood stream. However, the dilution caused by body fluids reduces electrolytically generated silver colloids to ineffective levels. Chemically derived colloids also could be used intravenously,

but their attendant adverse effects restrict use to small amounts for limited duration.

In all, it seems the most desirable form of silver for internal use is a non-toxic solution taken orally and in effective amounts. If the silver is attached to an electrolyte and cell nutrient, it will be absorbed into the blood and can interact with contagions found there. In this form it can also gain entrance to cells to interact and destroy intercellular, invasive viral infection. This can be accomplished by using fulvic acids with silver.

Fulvic acids are water-based electrolytes. Their chelating ability bonds essential minerals and enhances them for use by living organisms. Fulvic acids have proven to be a powerful organic electrolyte, avail-able to balance cell life. They are an ideal chelator of silver compounds.

Electrolytes and Human Health

Medical researchers have long known the vital role electrolytes play in human health. Researchers now report a breakthrough in the ability to dissolve and attach electrically generated silver (in substantial quantities) to a naturally occur-ring "chelate."

Not only has chelation been achieved, but the sub-stance used for chelation is of immense value as a natural electrolyte and cell nutrient. Of this naturally occurring electrolyte, Dr. William R. Jackson, in his book, *Organic Soil Conditioning* says:

"The physical well being of the organism, whether plant or animal, can be expressed in terms of electrical potential. Fulvic acid has proven to be a powerful organic electrolyte, serving to balance cell life. It is available at times as a donor and at other times as an acceptor, based on the cell's requirements for balance. If the individual cell is restored to its normal chemical balance and thereby, in turn, its electrical potential, we have given life where death and disintegration would normally occur within plant and animal cells."

SUMMARY: QUESTIONS AND FINDINGS

What is the ideal concentration for silver supplements taken internally? It has been suggested that the number of pathogens killed relates directly to the number of silver ions present, but there is no ideal concentration for all cases. It appears from the review of literature, that severe cases would require increased dosages.

Can small amounts of silver be beneficial? Small amounts of Vitamin C are beneficial, but they do not prevent scurvy. Likewise, those involved in the various case studies used silver compounds in amounts relative to need.

Are all colloidal silver supplements the same? The research data indicated multiple vendors are marketing silver compounds under a variety of trade names, all containing greater or lessor amounts of silver or combinations of silver compounds.

What is the relationship between total silver content and effective results? The research data confirmed that it is not the total amount of silver in suspension, but the total amount of ionized silver in solution, that determines effectiveness.

Will ionized silver destroy friendly microorganisms? Schweizer (1929), using a silver sponge combined with electropotential, verified that silver kills pathogens, including E. coli, without harming helpful airborne and waterborne microorganisms.

How do commercial silver products differ? It appears that most commercially available products are a preparation of silver protein powders derived from the precipitation of silver nitrate solutions. While the silver is beneficial, the nitrate radical may do harm to cells and tissues.

What is argyria? Argyria is a darkening of the skin or tissues resulting from injections of excessive amounts of silver (or its compounds) or from topical applications of these same compounds to broken skin for a prolonged period of time. Studies published in 1957 *(Antiseptics, Disinfectants, Fungicides and Chemical and Physical Sterilization)* indicated that no cases of argyria had been reported where silver was taken in amounts consistent with its oligodynamic benefits. Moreover, the amounts of silver required to cause argyria are much greater than amounts necessary for oligodynamic action. Fortunately, apart from discoloration, there are no negative physiological symptoms associated with the condition of argyria.

Can silver colloids in small amounts purify water? Research tests performed by Supfle and Werner (1951) and Schioppa (1936) provide some answers. Their data confirms that silver ion solutions work best and appear superior to any other form of silver. They also demonstrated that factors such as temperature and the hardness of the water affected results. They were successful it identifying the specific amount; of silver ions in the solutions necessary to kill all pathogens. These amounts were measured in micrograms (gamas) per liter of water treated.

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Silver for Human Health is produced by the
Environmental Health Foundation,
an organization dedicated to research and to the
production of natural ingredients for good health.

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 2008, p. 2171–2178 Vol. 74, No. 7
0099-2240/08/$08.00+0 doi:10.1128/AEM.02001-07

Antibacterial Activity and Mechanism of Action of the Silver Ion in *Staphylococcus aureus* and *Escherichia coli*[▽]

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Received 31 August 2007/Accepted 21 January 2008

The antibacterial effect and mechanism of action of a silver ion solution that was electrically generated were investigated for *Staphylococcus aureus* and *Escherichia coli* by analyzing the growth, morphology, and ultrastructure of the bacterial cells following treatment with the silver ion solution. Bacteria were exposed to the silver ion solution for various lengths of time, and the antibacterial effect of the solution was tested using the conventional plate count method and flow cytometric (FC) analysis. Reductions of more than 5 $\log_{10}$ CFU/ml of both *S. aureus* and *E. coli* bacteria were confirmed after 90 min of treatment with the silver ion solution. Significant reduction of *S. aureus* and *E. coli* cells was also observed by FC analysis; however, the reduction rate determined by FC analysis was less than that determined by the conventional plate count method. These differences may be attributed to the presence of bacteria in an active but nonculturable (ABNC) state after treatment with the silver ion solution. Transmission electron microscopy showed considerable changes in the bacterial cell membranes upon silver ion treatment, which might be the cause or consequence of cell death. In conclusion, the results of the present study suggest that silver ions may cause *S. aureus* and *E. coli* bacteria to reach an ABNC state and eventually die.

Since ancient times, the silver ion has been known to be effective against a broad range of microorganisms. Today, silver ions are used to control bacterial growth in a variety of medical applications, including dental work, catheters, and the healing of burn wounds (17, 30, 31). Silver ions are also used for a number of nonmedical purposes, such as in electrical appliances (14, 36). The slow-release "nanosilver" linings of laundry machines, dishwashers, refrigerators, and toilet seats are also marketed and advertised. It is clear that we are exposed to a wide range of mostly unfamiliar uses of silver-containing products intended to function as antimicrobial biocides. Therefore, it is necessary to elucidate the antimicrobial activity of the silver ion, which is widely used in these products.

The mechanism of the antimicrobial action of silver ions is closely related to their interaction with thiol (sulfhydryl) groups (1, 5, 9, 10), although other target sites remain a possibility (27, 34). Amino acids, such as cysteine, and other compounds containing thiol groups, such as sodium thioglycolate, neutralized the activity of silver against bacteria (18). By contrast, disulfide bond-containing amino acids, non-sulfur-containing amino acids, and sulfur-containing compounds, such as cystathione, cysteic acid, L-methionine, taurine, sodium bisulfate, and sodium thiosulfate, were all unable to neutralize the activity of silver ions. These and other findings imply that the interaction of silver ions with thiol groups in enzymes and proteins plays an essential role in its antimicrobial action, although other cellular components, like hydrogen bonding, may also be involved (10). Silver was also proposed to act by binding to key functional groups of enzymes. Silver ions cause the release of K^+ ions from bacteria; thus, the bacterial plasma or cytoplasmic membrane, which is associated with many important enzymes, is an important target site for silver ions (9, 22, 25, 29).

In addition to their effects on bacterial enzymes, silver ions caused marked inhibition of bacterial growth and were deposited in the vacuole and cell wall as granules (6). They inhibited cell division and damaged the cell envelope and contents of bacteria (27). Bacterial cells increased in size, and the cytoplasmic membrane, cytoplasmic contents, and outer cell layers all exhibited structural abnormalities. Finally, silver ions interact with nucleic acids (35); they interact preferentially with the bases in DNA rather than with the phosphate groups, although the significance of this in terms of their lethal action is unclear (12, 24, 34, 37).

The following silver compounds and silver are listed in *Martindale: the Extra Pharmacopoeia*: silver metal, silver acetate, silver nitrate, silver protein, and silver sulfadiazine (26a). The silver ion can be generated by electrolyzing the silver metal or dissolving the silver compounds. It is known that the electrically generated silver ion appeared to be superior to the silver compounds in antimicrobial activity (3, 4). However, most of the aforementioned studies which determined a mechanism of action of silver used silver ions produced from silver compounds like silver nitrate or silver sulfadiazine, and thus there has been limited research on the electrically generated silver ion. Recently, a laundry machine that emits electrically gener-

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▽ Published ahead of print on 1 February 2008.

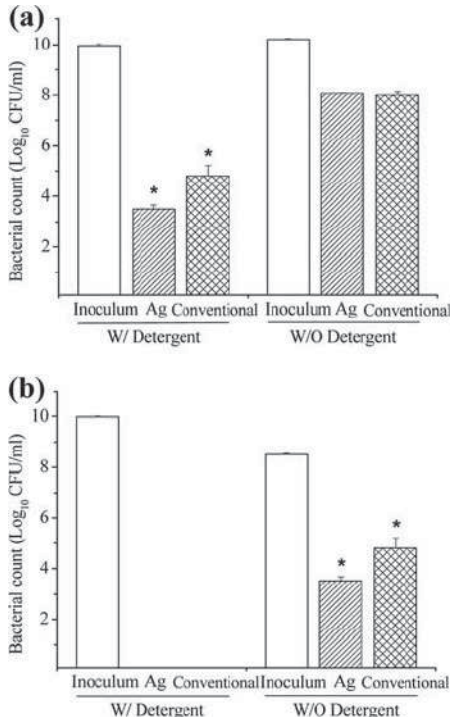

FIG. 1. Viable counts (mean ± standard error) of *Staphylococcus aureus* (a) and *Escherichia coli* (b) bacteria after washing bacteria-contaminated textile pieces using silver and conventional laundry machines with (W/) or without (W/O) detergent. Each group contained three pieces of test textiles. Inoculum, preinoculation bacterial count; Ag, result for silver laundry machine (Samsung); Conventional, result for conventional laundry machine (Samsung). Significant differences ($P < 0.05$) in viable counts of each bacteria between the silver and conventional laundry machines are denoted with asterisks.

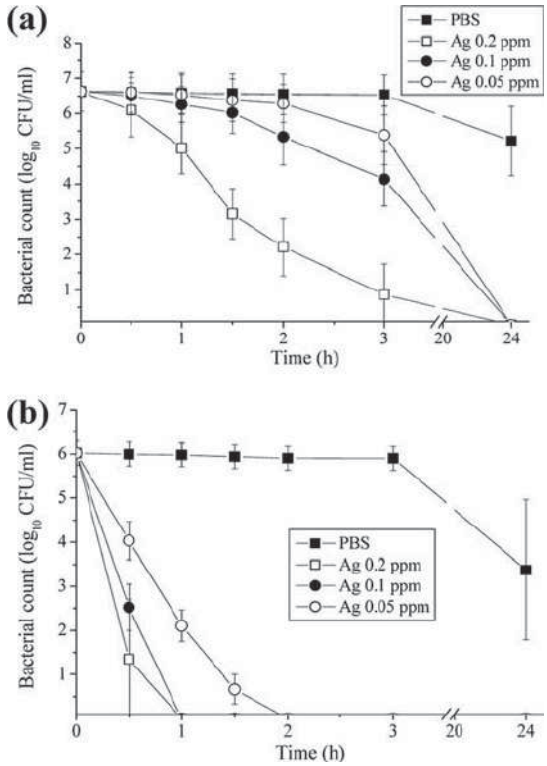

FIG. 2. The effect of the silver ion solution on *Staphylococcus aureus* (a) and *Escherichia coli* (b) was investigated by conventional plate counting. The tested silver ion concentrations were 0.2 ppm, 0.1 ppm, and 0.05 ppm, and PBS was used as a control.

ated silver ions was developed for hygiene, namely, in order to prevent easily transmissible bacterial and fungal skin infections from being transmitted by contaminated laundry. In particular, it can be beneficial to hospitals and homes in which immunocompromised people (the elderly, children, and medical patients) or pets may dwell. Our previous study demonstrated the antifungal activity of a laundry machine that electrically generates silver ions (14). In the present study, we used conventional plate counting, flow cytometry (FC), and transmission electron microscopy (TEM) to investigate the antibacterial activity and mechanism of action against *Staphylococcus aureus* and *Escherichia coli* bacteria of a silver ion solution generated from the laundry machine.

MATERIALS AND METHODS

Bacterial strains. *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 were used in this study. The strains were grown in 5% sheep blood agar (Promed, Gyeonggi, Korea).

Antibacterial efficacy test of household laundry machines. A silver laundry machine (Samsung, Gyeonggi, Korea) and a conventional laundry machine (Samsung) which was the same as the silver laundry machine except for the fact that it did not emit silver ions were used as the experimental and control machines, respectively. The silver laundry machine is designed to release silver ions twice during the laundry process: once during the main washing step (for 30 min) and once during the final rinsing step (for 20 min). Powerclean Max (Oxy, Seoul, Korea) was used as the detergent.

The method for testing the antibacterial properties of household laundry machines was performed as previously described (17a) with minor revision. The bacteria were enumerated by the conventional plate count method. The test textile (100% cotton) was 5 cm × 5 cm. Three pieces of test textile were attached to the edge of a 1-m × 1-m laundry textile (100% cotton). Each test and laundry textile was autoclaved and dried, and then the test textiles were inoculated with *S. aureus* or *E. coli*. The bacteria were diluted to 10^9 to 10^{10} CFU/ml using 0.85% sterile saline. One milliliter of each adjusted bacterial culture was inoculated to the test textiles, and then textiles were washed in each laundry machine.

Two pieces of laundry textile with three pieces of test textile and 28 pieces of laundry textile without test textile, which were used to adjust the weight of the laundry to be 3 kg, were processed at the same time with or without detergent using the silver and the conventional laundry machine. The laundry textile with the test textile attached to it was taken out at the end of the laundry process. The test textile was then removed from the laundry textile and pummeled with 10 ml of sterile buffered peptone water (Becton Dickinson, Sparks, MD). The buffered peptone water rinse solution was then serially diluted with saline, and bacteria were counted using the conventional plate count method.

Silver ion preparation. A silver ion solution in phosphate-buffered saline (PBS; pH 7.4) was prepared from the silver laundry machine (Samsung), and this solution was used in all subsequent experiments (conventional plate counting, FC analysis, and TEM). The silver ions were produced from two silver plates while PBS was passed through the silver kit, which was made with polypropylene housing. The water from the tap passed through the silver kit housing and went down to the drum. Both the anode and cathode were 99.9% silver metal plates with surface areas of 12.5 cm^2, and two electrodes were installed parallel to each other with 5 mm of distance between them. The volume of the silver kit housing was 30 ml. The flow rate through the silver kit housing was regulated to be 10 liter/min, and the electric current was controlled at 80 mA by changing the input voltages from 2 to 24 V. The electric current was applied only during water supply. The concentration of the silver was determined by inductively coupled plasma mass spectrometry (ELAN 6100; Perkin-Elmer SCIEX, Norwalk, CT) at the National Center for Inter-University Research Facilities, Seoul National University, and it was approximately 0.2 ppm.

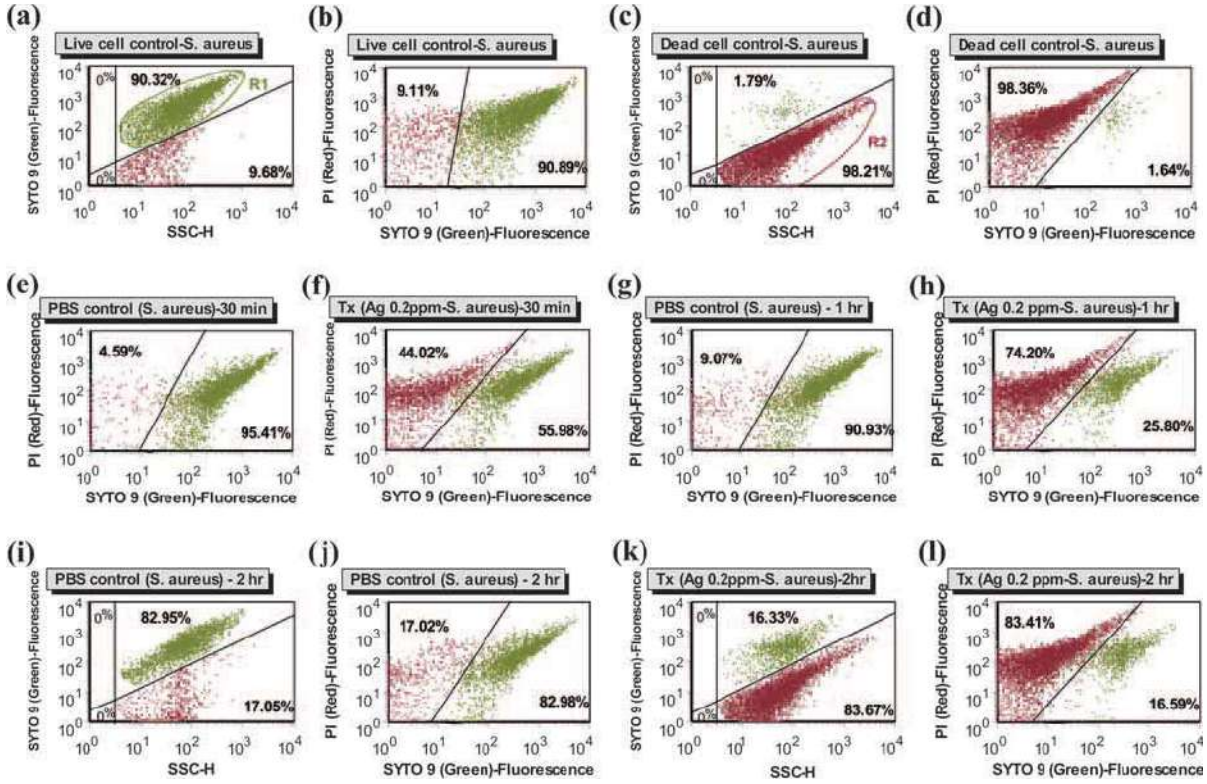

FIG. 3. Representative dot plot profiles of *Staphylococcus aureus* cells treated with PBS (e, g, i, and j) or silver ion solution (0.2 ppm) (Tx; f, h, k, and l) for 30 min, 1 h, and 2 h analyzed by FC after staining with SYTO 9 and PI. For controls, suspensions of fresh live (untreated) (a and b) and dead (70% isopropyl alcohol treated) (c and d) cells were also analyzed. The quadrants show the division between live cells in gate 1 (a; R1-green) and damaged or dead cells in gate 2 (c; R2-red) with the relative frequencies of cells in each gate before treatment with PBS or silver ion solution. All of the profiles were analyzed with gates placed on 1 and 2. SSC-H, side-scatter height.

Determination of antibacterial effect of silver ions by conventional plate counting. The silver ion solution made with PBS was autoclaved at 121°C for 15 min and tested for its antibacterial efficacy. The concentrations of silver ions tested were 0.2, 0.1, and 0.05 ppm. Ninety-nine milliliters of the test solution and 1 ml of the bacterial suspension in PBS were mixed to a final bacterial concentration of 10^5 to 10^6 CFU/ml. The mixture of solution and bacteria was incubated at 37°C with shaking and counted at 30-min intervals from 30 to 180 min and then again at 24 h using the conventional plate count method, with serial 10-fold dilutions with saline plated on plate count agar (Becton Dickinson).

FC analysis of antibacterial effect of silver ions. After the bacterial suspensions (10^5 to 10^6 CFU/ml) were treated with silver ion solution (0.2 ppm) or PBS for 30 min, 1 h, 1.5 h, 2 h, and 3 h, the bacterial cells (*S. aureus* or *E. coli*) were washed two times with PBS and resuspended in SYTO 9 and propidium iodide (PI) from a Live/Dead *Bac*Light bacterial viability kit (Molecular Probes, Inc., Eugene, OR) (2, 28). The suspension was incubated for 15 min in the dark at room temperature. In the control group, suspensions of fresh live (untreated) and dead (70% isopropyl alcohol treated) cells were stained as described above, and the green and red fluorescence generated by SYTO 9 and PI staining, respectively, as well as the size (side scatter height) were also read by FC analysis. After reading the parameters of the live and dead cell controls, with the resulting live cells in gate 1 (R1-green) and damaged or dead cells in gate 2 (R2-red) as discriminated by FC analysis, the relative frequencies of cells in each gate before treatment with silver ion solution or PBS were determined, with all of the experimental profiles being analyzed with gates 1 and 2 by FC analysis. The green fluorescence of the SYTO 9 dyes (FL1) was collected using a 530-nm ± 30-nm band-pass filter. The red fluorescence emitted from PI (FL3) was collected using a 650-nm ± 13-nm band-pass filter. The proportions of live and dead cells were determined and analyzed by using a FACSCalibur with the CellQuest program (Becton Dickinson Immunocytometry Systems, San Jose, CA) and FCS Express software (De Novo Software, Ontario, CA), respectively.

For the enumeration of esterase-active bacteria, 900 μl of bacterial cells, which were treated with silver ion solution or PBS and washed as described above, were supplemented with 90 μl of sterile 1.0 M phosphate buffer (pH 8.0) and 10 μl of 50 mM EDTA. Then, carboxyfluorescein diacetate (CFDA; Molecular Probes, Inc.) stock solution in dimethyl sulfoxide was added to the sample at a final concentration of 10 μM, and the sample incubated at 35°C in the dark for 10 min (11). Following incubation, the cells were washed and resuspended in sterile 1.0 M phosphate buffer (pH 8.0), and esterase-active bacteria were enumerated by the enhanced-green-fluorescence intensity as determined by FC analysis. Positive-control live cells and negative-control dead cells were prepared and stained as described above.

TEM. Unstained cells of *S. aureus* and *E. coli* were observed for the presence of electron-dense precipitates by TEM. The two bacterial strains were diluted to a final concentration of 10^5 to 10^6 CFU/ml with silver ion solution (0.2 ppm) or PBS. The mixture of solution and bacteria was incubated at 37°C for 2 h with shaking, centrifuged at 1,320 × *g* for 30 min to obtain cell pellets, and then diluted with 1 ml of PBS. A drop of the mixture was placed on a glow-discharged Formvar-coated copper grid for 1 min. The excess liquid was drained off with a filter paper, and the preparation was air dried for 5 min. The specimens were examined with an energy-filtering TEM (LIBRA 120; Carl Zeiss, Oberkochen, Germany) operated at an accelerating voltage of 120 kV. Zero-loss energy-filtered images were recorded with a 4 K slow-scan charge-coupled-device camera (4000 SP; Gatan, Pleasanton, CA).

In addition, the detailed ultrastructural changes induced by the silver ion treatment in embedded bacterial cells were examined. The cell pellets of the two bacterial strains were fixed with modified Karnovsky's fixative consisting of 2% (vol/vol) glutaraldehyde and 2% (vol/vol) paraformaldehyde in 0.05 M sodium cacodylate buffer (pH 7.2) at 4°C for 2 h (15). They were then washed three times with the same buffer for a period of 10 min. The specimens were postfixed with 1% (wt/vol) osmium tetroxide in the same buffer at 4°C for 2 h and washed briefly with distilled water twice. The postfixed specimens were dehydrated in a graded ethanol series (once in 30, 50, 70, 80, and 95% and three times in 100% for 10 min each). The specimens were further treated with propylene oxide twice each for 10 min as a transitional fluid and then embedded in Spurr's resin (33).

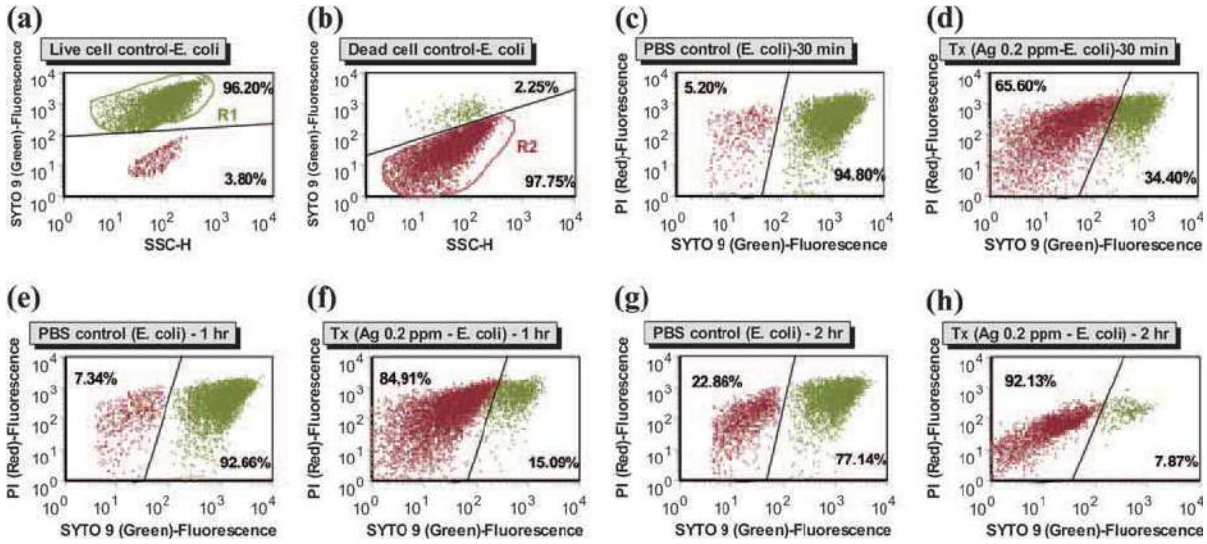

FIG. 4. Representative dot plot profiles of *Escherichia coli* cells treated with PBS (c, e, and g) or silver ion solution (0.2 ppm) (Tx; d, f, and h) for 30 min, 1 h, and 2 h analyzed by FC after staining with SYTO 9 and PI. For controls, suspensions of fresh live (untreated) (a) and dead (70% isopropyl alcohol-treated) (b) cells were analyzed. The quadrants show the division between live cells in gate 1 (a; R1-green) and damaged or dead cells in gate 2 (b; R2-red) with the relative frequencies of cells in each gate before treatment with PBS or silver ion solution. All the profiles were analyzed with gates placed on 1 and 2. SSC-H, side-scatter height.

Ultrathin sections (approximately 60-nm thickness) were cut with a diamond knife using an ultramicrotome (MT-X; RMC Inc., Tucson, AZ) and then mounted on bare copper grids. They were stained with 2% uranyl acetate and Reynolds' lead citrate (26) for 7 min each, followed by examination with the electron microscope.

Statistical analysis. The data from triplicate experiments are presented as the mean ± standard error of the mean. An unpaired *t* test analysis was performed using Origin 6.1 (OriginLab, Northampton, MA) to compare the viable bacterial counts within different samples that underwent different washing treatments (detergent and laundry machines) and to compare the viable bacterial counts between the silver ion treatment and nontreatment groups. The proportions of live or dead *E. coli* or *S. aureus* determined by FC analysis in the silver ion treatment groups treated for different periods of time (30 min, 1 h, 1.5 h, 2 h, and 3 h) were compared with those in the control (PBS) group using the Kruskal-Wallis one-way analysis of variance by rank. Significant differences in the data that originated from the same group but were determined at different times were analyzed by the Wilcoxon signed-rank test using Analyze-it software (Analyze-it Software Ltd., Leeds, United Kingdom). The level of significance was set at a P value of <0.05.

RESULTS

Antibacterial efficacy of household laundry machines. The efficacy test results of the two laundry machines against *S. aureus* and *E. coli* conducted with or without detergent are shown in Fig. 1. The *S. aureus* bacterial count was significantly reduced by the silver laundry machines with detergent in comparison to the results with the conventional laundry machine ($P < 0.05$). All of the inoculated *E. coli* bacteria were eliminated when detergent was used in both the silver and conventional laundry machines. In the absence of detergent, *E. coli* was significantly reduced by the silver laundry machine in comparison to the results with the conventional laundry machine ($P < 0.05$).

Effect of the silver ions on the bacterial reduction rate. The antibacterial effects of the silver ion solution at different concentrations of silver ions against *S. aureus* and *E. coli* bacteria as determined by the conventional plate count technique are shown in Fig. 2. The total number of *S. aureus* bacteria was reduced by over 5 $\log_{10}$ CFU/ml after treatment with the original silver ion solution (0.2 ppm) for 90 min, demonstrating that the antibacterial activity of the silver ion solution was significantly greater than that of PBS treatment ($P < 0.05$). The *E. coli* bacterial count was reduced from the inoculum size (10^5 CFU/ml) to the limit of detection (<20 CFU/ml) within 30 min at a silver ion concentration of 0.2 ppm. All of the tested silver ion solutions (0.2, 0.1, and 0.05 ppm) significantly eliminated *E. coli* cells in comparison to PBS treatment ($P < 0.05$).

FC analysis in conjunction with a *Bac*Light kit was also performed to examine the antibacterial effect of the original silver ion solution (0.2 ppm) against *S. aureus* and *E. coli* bacteria in terms of damage to the cell membrane, shown in different colors (green in live cells and red in damaged or dead cells). In addition, CFDA staining was used for the enumeration of esterase-active bacteria because CFDA is cell permeant and undergoes hydrolysis of the diacetate groups into fluorescent carboxyfluorescein by intracellular nonspecific esterases. Based on the side light scatter and green (FL1) fluorescence, the R1 and R2 gates were used to identify live and damaged or dead cells, respectively. The proportions of damaged or dead cells (both *S. aureus* and *E. coli*) in the silver ion solution-treated groups were significantly greater ($P < 0.05$) at 30 min, 1 h, 1.5 h, and 2 h of treatment than with the control (PBS) groups (Fig. 3 and 4). Longer treatment times (from 30 min to 2 h) had a positive effect on the antibacterial effect of the silver ion solution ($P < 0.05$); however, there were no significant differences in the proportions of live or dead cells when both *S. aureus* and *E. coli* cells were treated with the silver ion solution for 2 or 3 h ($P > 0.05$).

The antibacterial-efficacy results determined by conventional plate count and FC analyses are compared in Fig. 5. For

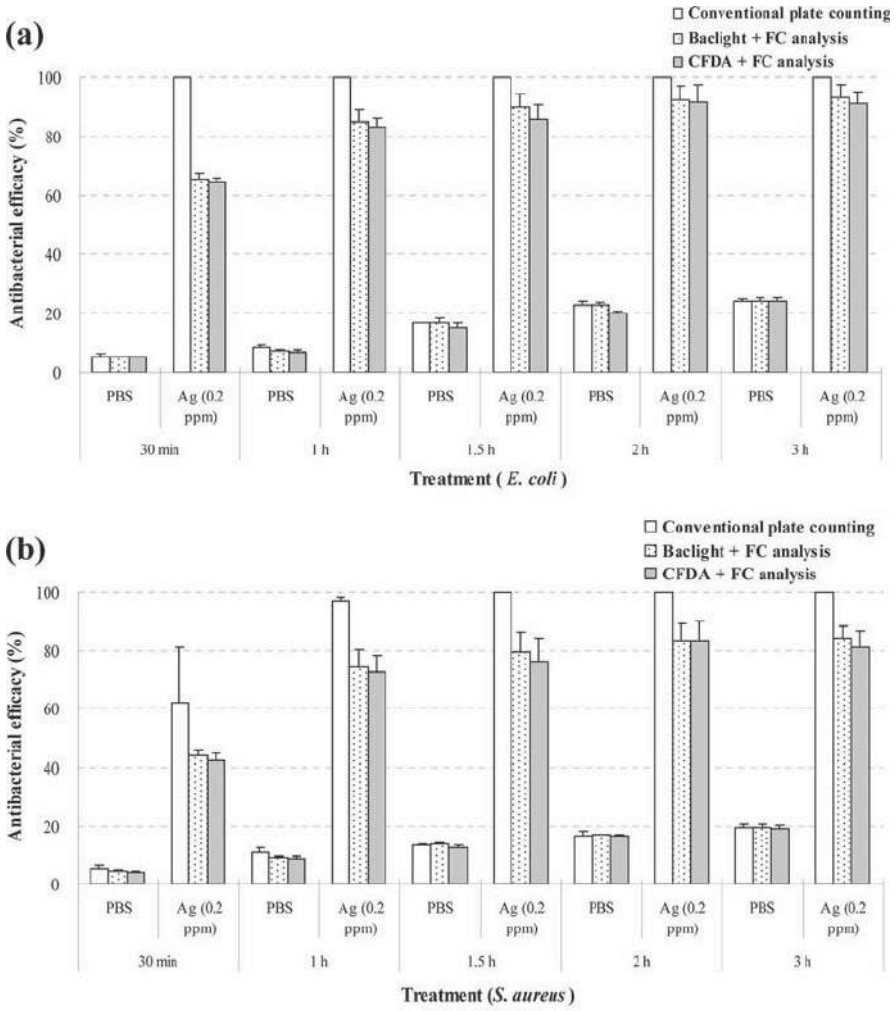

FIG. 5. Comparative analysis of the antibacterial efficacy of the silver ion solution (0.2 ppm) against *Staphylococcus aureus* (a) and *Escherichia coli* (b) bacteria as determined using the conventional plate count method and FC analysis. PBS was used as a control. Antibacterial efficacy was calculated using the following formula: antibacterial efficacy = $[(A - B)/A] \times 100$, where A is the preinoculation bacterial count (CFU/ml) and B is the bacterial count after treatment with silver ion solution or PBS (CFU/ml).

the PBS-treated control group and silver ion-treated experimental groups tested, both the *Bac*Light kit and the CFDA assay gave similar antibacterial efficacies ($P > 0.05$). The number of physiologically active bacteria enumerated by FC analysis in conjunction with the *Bac*Light kit or the CFDA assay was relatively higher than the bacterial count determined by conventional plate counting ($P < 0.05$), except for *E. coli* bacteria after 2 and 3 h of treatment. This difference appeared to be nonlinear across different treatment times, suggesting that the difference in antibacterial efficacy determined by the two analyses decreased as the silver ion treatment times approached 2 and 3 h.

Morphological changes in *S. aureus* and *E. coli* cells after silver ion treatment. TEM analysis of unstained bacteria showed the external morphological features of the two bacterial strains. The untreated *S. aureus* cells retained their coccal morphology (ca. 600 nm in diameter) and seemed to be normal (Fig. 6a). In contrast, *S. aureus* cells treated with the silver ion solution for 2 h appeared to undergo lysis, resulting in the release of their cellular contents into the surrounding environment, and finally became disrupted (Fig. 6b to d). It was common to find electron-dense particles or precipitates around damaged bacterial cells that were electron translucent in comparison to undamaged cells. In cross section, the untreated cells of *S. aureus* showed normal cell characteristics and homogeneous electron density in the cytoplasm. Their cell walls and membranes were intact, showing a well-preserved peptidoglycan layer and cytoplasmic membrane (Fig. 7a and b). However, significant morphological changes were observed in *S. aureus* cells treated with the silver ion solution. They showed lysed cells with broken walls and membranes and decreases and heterogeneity in electron density in the cytoplasm (Fig. 7c and d). The localized separation of the cell membrane from the cell wall could be discerned.

E. coli cells diluted in PBS showed normal morphology having many filaments, such as flagella and fimbriae (Fig. 8a). The fimbriae were peritrichous, approximately 7 nm wide, and up to 900 nm long. Meanwhile, the bacterial cells after silver ion

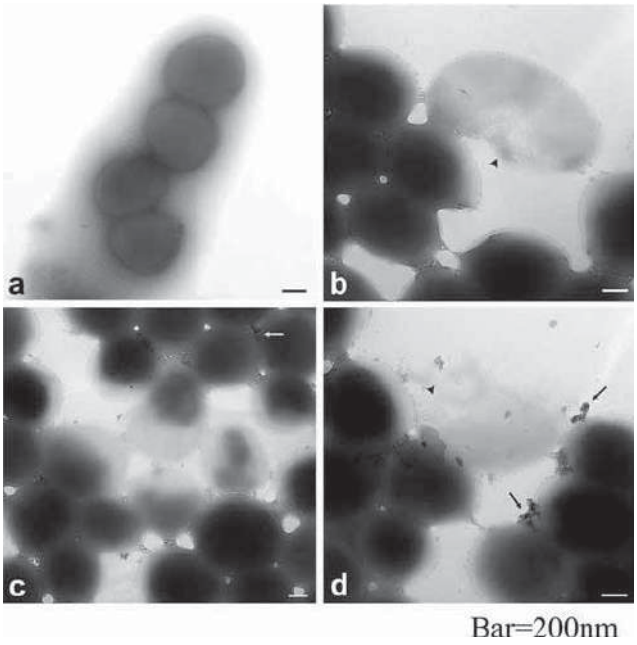

FIG. 6. External morphology of unstained *Staphylococcus aureus* cells observed by TEM. (a) Untreated bacteria. (b, c, and d) Bacteria treated with silver ion solution (0.2 ppm). Electron-dense particles were found around damaged cells (arrows). Note the release of cellular contents (arrowheads).

treatment for 2 h appeared to be seriously damaged (Fig. 8b to d). The cells showed aberrant morphology; they were cracked and ruptured. Electron-dense particles or precipitates were also observed around damaged bacterial cells. The internal

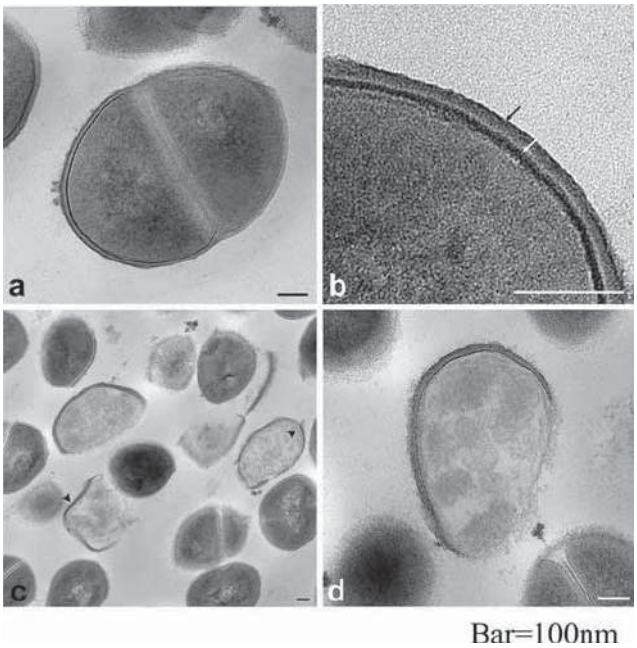

FIG. 7. Internal structure of *Staphylococcus aureus* observed by TEM. (a and b) Untreated bacteria. (c and d) Bacteria treated with silver ion solution (0.2 ppm). Black and white arrows indicate peptidoglycan layer and cytoplasmic membrane, respectively. Note the separation of cell membrane from the cell wall (arrowheads).

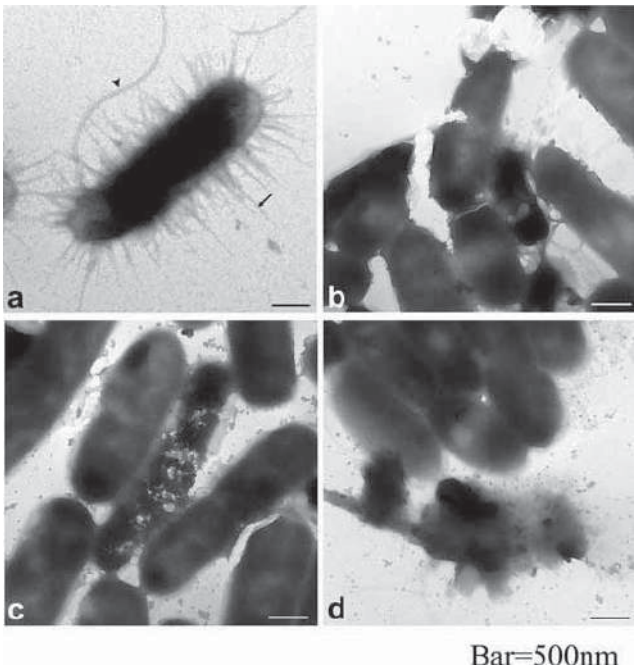

FIG. 8. External morphology of unstained *Escherichia coli* observed by TEM. (a) Untreated bacteria. An arrow and an arrowhead indicate fimbriae and a flagellum, respectively. (b, c, and d) Bacteria treated with silver ion solution (0.2 ppm).

structure of the untreated *E. coli* cells appeared to be normal, showing a multilayered cell surface consisting of an outer membrane, a peptidoglycan layer in the periplasmic space, and a cytoplasmic membrane (Fig. 9a and b). Damaged cells showed either localized or complete separation of the cell membrane from the cell wall (Fig. 9c). The cellular degradation was also accompanied by electron-translucent cytoplasm and cellular disruption in the damaged cells (Fig. 9d).

DISCUSSION

The electrically generated silver ion solution exhibited good bactericidal efficacy against *S. aureus* and *E. coli* both in experiments using the silver laundry machine with contaminated fabric and in those using the silver ion suspension generated from the silver laundry machine. The efficacy of the silver ion solution showed better activity against the gram-negative *E. coli* than against the gram-positive *S. aureus*. This was possibly due to the thickness of the peptidoglycan layer, which may prevent the action of the silver ions through the bacterial cell wall, and this result was consonant with the results of other studies (8, 23). Although the *S. aureus* and *E. coli* bacteria were effectively eliminated from the contaminated fabric by the silver washing course, it was not confirmed that the silver ions killed the bacteria. It is possible that the bacteria were removed from the fabric by the washing course. Therefore, the antibacterial effect of the silver ions was confirmed by the conventional plate count, FC, and TEM analyses in this study.

The number of bacteria determined by conventional plate counting, which counts only culturable colonies in media, was significantly lower than the number determined by FC analysis,

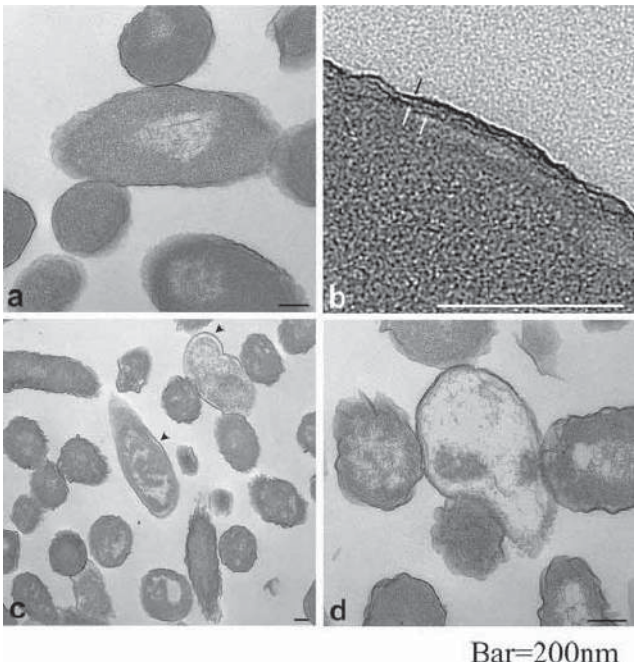

FIG. 9. Internal structure of *Escherichia coli* observed by TEM. (a and b) Untreated bacteria. (c and d) Bacteria treated with silver ion solution (0.2 ppm). Arrows indicate outer membrane, peptidoglycan layer, and cytoplasmic membrane from the outside of the cell. Arrowheads indicate separation of the cell membrane from the cell wall.

suggesting that the cell membrane and intracellular esterase activity of the bacteria treated with the silver ion solution might be damaged. Bacteria in the environment are exposed to various conditions that lead to survival stress. To counter this condition, some bacteria are capable of maintaining metabolic activity while developing recalcitrance to culture. Such a state in bacteria is often defined as an "active but nonculturable (ABNC)" state, a state in which the bacteria exhibit measurable traits of physiological activity but fail to grow to a detectable level (16). A state of ABNC or sublethal injury of bacteria seems to be induced by exposure to silver ions, thus rendering bacteria nonculturable in media (7, 21). This may serve as a possible explanation for the discrepancy in the results determined by the two methods used in this study, and this observation is consistent with the findings of other studies (11, 13). This finding may be expected because bacteria previously exposed to environmental stresses may only be able to divide a limited number of times, which would give a positive result in the FC analysis, but they would be unable to produce visible colonies on solid media.

The differences between the results of the conventional plate count and FC analyses were nonlinear, and the difference rate between the results of the two methods was reduced as time progressed. The reason for this aspect might be that the bacteria in the ABNC state started to die after 2 h of treatment with the silver ions.

Similar phenomena were also observed in the silver ion-treated cells of *S. aureus* and *E. coli* by the TEM studies. Following the silver ion treatment, the cytoplasm membrane shrank and became separated from the cell wall. Cellular contents were then released from the cell wall, and the cell wall was degraded. These phenomena suggest possible antibacterial mechanisms by which silver ions inhibit bacterial growth, as well as cellular responses of both the gram-positive and gram-negative bacteria to the silver ion treatment. Although the mechanisms underlying the antibacterial actions of silver are still not fully understood, several previous reports (20, 23, 32) showed that the interaction between silver and the constituents of the bacterial membrane caused structural changes and damage to the membranes and intracellular metabolic activity which might be the cause or consequence of cell death, as demonstrated in this study. Analytical electron microscopy remains to be done to identify the elemental composition of the electron-dense particles or precipitates around damaged bacterial cells. In conclusion, the results of the present study clearly show that the electrically generated silver ion solution exerts its antibacterial effect by inducing bacteria into a state of ABNC, in which the mechanisms required for the uptake and utilization of substrates leading to cell division were disrupted at the initial stage and caused the cells to undergo morphological changes and die at the later stage. These findings suggest that the use of the silver ion solution may have valuable applications in various fields, such as the manufacture of household appliances and medical devices.

ACKNOWLEDGMENTS

We thank Young Hwan Paik for his technical assistance.

This study was supported by Korea Research Foundation grants (KRF-2006-005-J02903 and KRF-2007-331-E00254), a grant from the Technology Development Program for Agriculture and Forestry provided by the Ministry of Agriculture and Forestry (grant no. 305003–3), and the Korea Bio-Hub Program of the Korea Ministry of Commerce, Industry Energy (2005-B0000002). Additional support was provided by the Research Institute of Veterinary Science, Department of Veterinary Microbiology, College of Veterinary Medicine, and the BK21 Program for Veterinary Science, Seoul National University.

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Silver Toxicity and Resistance In Wound Care

May 2010

Recent articles in the lay press have suggested that medical and non-medical uses of silver pose a threat to human health or the environment. Such articles, which do not include data, at best can represent only the opinion of the author(s) and should not be considered as conclusive scientific studies.

As a manufacturer of silver-containing medical products, Argentum Medical LLC encourages open discussion of the indications and benefits of silver therapies. Proper forums for such discussion include the scientific meetings of reputable medical organizations and manuscripts that are published in peer-reviewed medical journals. To facilitate discussion and exchange of research ideas, Argentum Medical LLC maintains a Medical Advisory Board of physicians and scientists with significant expertise in the fields of microbiology, burn surgery, wound care and reconstructive surgery. Members of the Advisory Board are available to discuss specific medical concerns or proposals for appropriate research studies.

The environmental impact of silver-containing medical devices is vastly overstated. Silver is not a super-toxic disinfectant developed in a chemical laboratory: rather it is a naturally-occurring element that is ubiquitous in nature. Humankind has had significant interaction and exposure to silver since antiquity. Silver has been widely utilized for currency, jewelry, water purification, cooking or serving vessels, plates, utensils, and as an electrical conductor. If silver represented a threat to human health, the use of silverware, silver plates and silver earrings would have ceased long ago.

In terms of global ecology, the medical use of silver for wound dressings represents a tiny fraction of annual total global silver production. The Silver Institute maintains statistics on annual silver supply and demand. Since 1999, annual worldwide silver demand averages approximately 900 million ounces. By comparison, as one of the top five manufacturers of silver based bandages, Argentum used approximately ***7,000 ounces*** of silver in 2009. This represents 0.0008 % (0.000008) of global annual silver consumption.

As a precious metal, industrial silver is often recycled or reclaimed. The one valid environmental question regarding silver-containing medical dressings is whether or not the silver in such products can be recycled. Research into this question is clearly indicated. Finally, it could be argued that the use of a naturally occurring (and potentially recyclable) element as a wound dressing can replace the use of toxic chemicals or late-generation antibiotics presently utilized for these indications: for this reason, the use of silver medical dressings may actually benefit the environment.

The second concern is the potential for silver-containing medical devices to cause microbial resistance. The answer here is both simple and complex: clinically relevant microbial resistance to silver might occur, but we presently lack clinical evidence and appropriate laboratory methodology to measure or quantify such resistance (1). It is reassuring to note that over 100 years of clinical experience with silver-containing products strongly suggests that microbial resistance to silver has little or no clinical impact.

Traditionally, microbial resistance to antibiotics has been measured either in terms of zones of growth inhibition on agar plates or by quantitative antimicrobial assays using serial dilutions to determine a minimal inhibitory concentration (MIC) necessary to achieve appropriate 'kill levels'. Neither assay is useful to assess the efficacy of silver, which exerts an antimicrobial effect largely through release of silver ion (Ag+). The problem is that the microbial growth media utilized for both methodologies contain multiple substances such as chloride ions, sulfate ions, phosphate ions and organic anions that react and bind with ionic silver. When agar diffusion assays are utilized, it is difficult to determine what we are measuring by microbial growth endpoint. Does the zone of inhibition represent dilution of the silver ion beyond that required for a bactericidal effect, the limit of silver inactivation of a component necessary for growth of the test organism, a critical level of silver ion binding to substrates or a combination of all three? When performing serial dilution assays to determine MIC, a similar problem occurs. We are diluting the concentration of silver ion, but not the concentrations of bindable chloride, sulfate and organic ions in the growth medium, leading to a nonlinear and misleading relationship.

To be clinically useful, the minimal inhibitory concentration levels that correlate with categorical breakpoints (susceptible, intermediate or resistant) must be established (1). This is usually done by a professional organization such as the European Committee on Antimicrobial Susceptibility Testing (1). Unfortunately, there is no consensus on what MIC constitutes silver resistance. Tenfold variations in MIC (8-80 mg/L for *Staphylococcus aureus* and 8 – 70 mg/L for *Pseudomonas aeruginosa*) have been reported(1). Because the multiple silver-containing products on the market all differ in rate and level of silver delivery, MIC values determined for one product would not correlate with efficacy in other products (1). Finally, zones of inhibition or MIC values (developed for use of systemic antibiotic therapy) may have little correlation with clinical practice, where a topical wound dressing is typically delivering bactericidal silver ion at very high concentrations directly to the wound bed.

Reports of clinical failures due to silver resistance have not been documented. Silver-resistance genes have rarely been found, however unlike many of the past and present parenteral agents, a silver "resistance" gene linkage to multiply antibiotic resistance transfer mechanisms has *not* been reported as a clinical reality. At this time clinical observation of wound condition, as unscientific as this may be, is the only practical evaluation for the effectiveness of specific silver dressings. It is unreasonable to conclude that clinically significant silver resistance cannot occur; *the fact is that it simply has not been demonstrated.*

Discussion points concerning the clinical utilization of silver:

- In 1881, it was discovered that application of silver nitrate solutions to the eyes of newborns would prevent ophthalmia neonatorium, an infection that can lead to serious eye damage or blindness. This practice quickly became a standard of care and was mandated by state law in most US jurisdictions by the early 1900's. As recently as 1978, the US Centers for Disease Control and the American Academy of Pediatrics advocated silver nitrate eye drops as one of three antibiotic choices for newborn eye prophylaxis (2,3), and as recently as 1981, 11 states allowed only silver nitrate drops to be utilized for this purpose (2,3). One hundred and twenty nine years after the initial discovery, silver nitrate eye drops are still used by some clinicians in the United States.

- Burn patients are an immunosuppressed population with large open wounds. With loss of the protective skin barrier, it would be expected that any topical agent applied to burn patients would also have systemic effects. Silver-containing solutions have been utilized as topical burn therapy for over 75 years with little adverse effect:

- Ten percent solutions of silver nitrate were applied as escharotics over burn wounds as early as 1935 (2,4)

- In 1965, Moyer et al described the use of 0.5 % silver nitrate solution as a topical therapy for patients with large burns (5). The choice of silver nitrate was influenced by the experiences of one of his coauthors, who had been utilizing silver nitrate solution in the management of necrotizing fasciitis and other contaminated wounds since 1941 (5). Sixty-five years after the first case reports, silver nitrate continues to be utilized as the primary topical antimicrobial in some burn centers (4).

- In 1968, Fox introduced silver sulfadiazine cream for the management of burn wounds (6). For forty-two years, this combination of sulfa drug and silver ion has been utilized as the primary topical antimicrobial agent in burn centers around the world.

- Since 2003, Silverlon® dressings have been extensively utilized by the US Military. US Service members and Coalition Partners burned in the war zone are frequently treated in Silverlon® dressings. Silverlon® dressings are also considered a standard of care for long-range aeromedical transportation of burn patients (8), including transcontinental flights.

- 1% silver sulphadiazene cream ($C_{10}H_9AgN_4O_2S$) that is 30% silver by weight, would provide 950 µg/cm² *per application* assuming a coating thickness of .125". If applied twice per day over 7 days, the total silver load would be 13,300 µg.

- By comparison, the silver bio-burden to the patient is much lower when using Silverlon®. The *total* amount of silver coating contained in Silverlon® wound dressings is approximately 5,799 µg / cm² . As measured by Atomic Absorption Spectrometry, *only 6.5% (50-60 ug/mL) of silver is actually measured in the test medium (tryptic soy broth) after seven days of immersion.*

- Reported microbial resistance to silver is exceptionally uncommon. A recent literature search covering the medical literature from 1950 to April 2010 combining the search terms 'silver compounds/ or silver' and drug resistance, microbial' yielded only 56 references. All were either in-vitro (bench) studies, literature reviews or letters to the editor***. There were no studies demonstrating any clinical significance of silver microbial resistance.***

- Genes that confer silver resistance to bacteria have been documented but are probably of little clinical significance. At least one study suggests that silver-containing wound dressings are effective in killing methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria that possess silver-resistance genes (7).

- Dressings that deliver low (or even sub lethal) levels of silver ion may play a role in increasing bacterial resistance to silver (1). Products such as Silverlon®, which deliver consistent and high levels of silver ion, may have an advantage in this regard.

- One article in the popular (nonmedical) press states that “silver threatens the use of antibiotics”. In reality, the opposite is more likely to be true in that *silver can reduce the need for systemic antibiotics*. Topical silver dressings are frequently utilized for patients with chronic wounds colonized with multiple drug-resistant (MDR) organisms as a result of long-term antibiotic therapy. Dressings that release ionic silver are ideal in this application, because the high silver levels achieved are usually lethal to MDR organisms. Chronic non-healing wounds frequently are associated with poor blood circulation, limiting the availability of systemic antibiotics to the wound itself. Topical silver therapy does not have this limitation.

- Topical silver dressings are beneficial to the patient. The ability to leave a dressing intact for several days decreases the number of painful dressings that a patient must undergo and is cost-effective in terms of saving time for the nursing staff. Topical silver dressings frequently allow management of chronic wounds in the outpatient rather than inpatient setting. This limits exposure of the hospital environment to the MDR organisms frequently found in chronic wounds and limits patient exposure to multiple drug-resistant hospital flora.

In summary, extensive medical use of silver ion for over one hundred years has shown that this mode of therapy is both highly effective and well tolerated. While microbial resistance to silver is a theoretic possibility, to date, the clinical significance of silver resistance is minimal to absent. In an era where antibiotic over-use has resulted in the development of multiple drug-resistant flora, it makes sense to utilize topical silver dressings instead of systemic antibiotics whenever possible.

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