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Some Specific Cellular Effects of Electrically Injected Silver and Gold Ions *

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Summary

We have shown that silver anodes are bacteriostatic *in vitro* at nominal current densities as low as 10 nA/mm². Even antibiotic resistant strains are quite susceptible to the injected silver. *Staphylococcus aureus*, treated with a silver anode, showed abnormal mesosomes; and in *E. Coli*, induced protein production (β-galactosidase) is blocked by the silver. These observations suggest that the action occurs on the cell membrane. *In vivo* experiments, completed thus far, show that the silver anode effect can be clinically useful in the treatment of localized infections in animals and man.

In experiments on rabbits with an induced form of rheumatoid arthritis, gold ions were injected directly into the inflammed joint by means of a gold wire anode and μA direct current. Results to date suggest that the proliferation of synovial cells and/or their subsequent invasion into the joint surfaces can be significantly reduced with this technique. The mechanism of action is not known.

Introduction

The antibacterial effects of the silver compounds have been known for a long time. At present, a few silver complexes and silver nitrate are used against local infections. [1] Others have shown that the responsible agent in these compounds is the silver ion, [2] but several possible mechanisms of action have been shown, including; interference with the respiratory chain, [3] the binding of Ag ions to cellular DNA, [4,5] and binding to the cell membrane. [6] In the course of experiments to test the effects of metal electrodes on bacteria, we found that the silver anode was also

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extremely bactericidal. [7-9] This effect occurred at low current densities (20 nA/mm^2) and was not associated with any electrolytic breakdown of the medium (see Fig. 1). Here we present some further evidence that the electrochemical injection of silver ions is an effective method for inhibiting bacteria *in vitro* and *in vivo*, and also that interference with membrane–related activity is responsible.

Since systemic gold therapy has been used in the treatment of rheumatoid arthritis, it is also possible that the electrochemical injection of gold ions, directly into the affected joints, would have therapeutic value, without toxic side–effects. Evidence that such a technique could be useful is also presented.

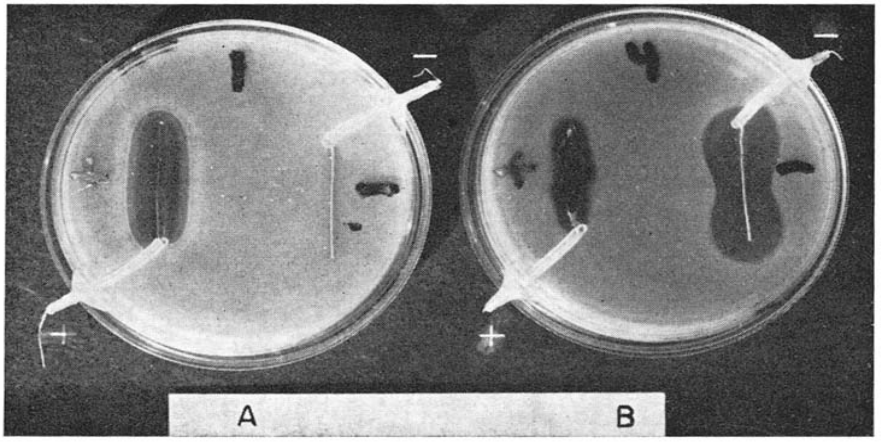

Fig. 1.
Bacterial culture plates (*S. aureus*) with silver electrodes after 24 hrs. incubation. (A) 0.4 μA; (B) 400 μA. Note inhibition (clear zone) around anode in (A). Inhibitory zones in (B) were associated with gas evolution, corrosion and large pH shifts and not specific to silver.

Experimental

Bacterial cultures [1] used in these experiments were obtained either from clinical isolates or standard test organisms. They were grown in nutrient broth or agar (GIBCO) as indicated. Electrodes were 99.99 % pure wires, 0.4 mm in diameter, 2 cm long. Current was supplied by constant–current generators operating from 0.04 to 400 μA. Silver concentrations were measured by arc emission spectroscopy. [8] Other experimental details are given with the results, or are published elsewhere. [7-9]

Results

Bacterial susceptibility to the silver anode

Several species of bacteria were tested for susceptibility to electrically generated silver by culturing the organism in small wells with silver electrodes as previously described.[8] Fig. 2 shows the results of standard plate counts of viable bacteria in the anode wells as a function of time and current-level for *S. aureus* and *E. coli*. The bacteria were reduced to below innoculum levels within 3 hours for the 4.0 and 40 μA levels. Also shown in Fig. 2 are typical values for the silver concentrations measured in the anode well after 4 h. The bacteria in the cathode wells grew as well as the controls.

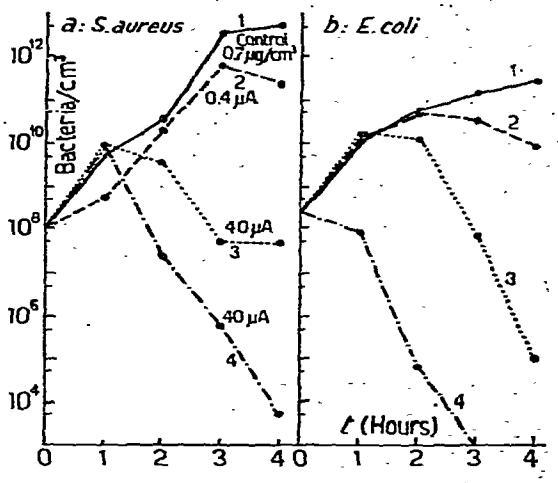

Fig. 2.
Inhibition of *S. aureus* and *E. coli* in the Ag anode wells for different current levels[8]. The ordinate is number of viable organisms per cm³ as measured by standard plate counts. The silver cathode (not shown) was completely ineffective. Reprinted with permission. *a* 1: control with Ag wire (~0.7 μg Ag/cm³); 2: 4.3 μg Ag/cm³, 0.4 μA; 3: 7.0 μg Ag/cm³, 4 μA; 4: +9 μg Ag/cm³, 40 μA; *b* 1 control (see *a*); 2: 4.9 μg Ag/cm³, 0.4 μA; 3: 5.8 μg Ag/cm³, 4 μA; 4: 92 μg Ag/cm³, 40 μA.

The minimum inhibitory concentrations (MIC) of electrically generated silver ions have been determined for a number of bacterial species *in vitro* and are generally found to be 1–100 times lower than those for other common antibiotics, including silver sulfadiazine.[9]

A measurement was made of the silver concentration remaining in the anode broth (*a*) after removal of any spontaneous precipitates and (*b*) after precipitation of proteins with trichloracetic acid (TCA). While no appreciable silver is apparently lost by spontaneous precipitation, approximately 5 % remained after TCA precipitation. This fraction is probably the most effective against the bacterial cell since it is free to combine rapidly with cell organelles. From Fig. 2, it can be shown that to inhibit *S. aureus* and *E. coli* an upper limit of 4×10^6 and 5×10^5 Ag^+ ions per cell, respectively, are required. Reducing these concentrations by the fraction of *unbound* silver found above, yields 2×10^5 and 2.5×10^4 ions per cell, or less, required for inhibition in this nutrient broth medium.

To test for any adverse effects of anodically generated silver on normal mammalian cells, a medium with 4 μg/cm³ of Ag was used to culture mouse bone marrow cells. After 18 h in culture differential counts were made of this mixed cell population. No abnormal cell lysis, adhesion or distortion was observed. Relative cell populations were found only slightly different from control cultures (without silver). [9] Thus it appears that electrochemically generated silver ions are quite effective against many bacteria, while leaving mammalian cells intact.

EM study – Staphylococcus aureus

S. aureus (penicillin resistant strain), was cultured in 2.5 cm³ wells in the presence of silver wire electrodes as previously described. [8] After exposure for 4 hours (15 μA) the bacteria were removed and prepared for electron microscopy. Bacteria from the anode chamber showed many incomplete, erratic septa, dense, enlarged mesosomes, and plasma membrane separation (see Fig. 3). Bacteria from the cathode side and from normal controls did not show these effects. This observation would suggest that the mechanism of action of the released silver ions is (for this species) at the cell plasma membrane. Mesosomes in gram positive bacteria are known to be elaborations of the plasma membrane and are involved in the formation of septa during the division process. Confirmation of these observations and experiments with other species of bacteria will be required before further conclusions can be made.

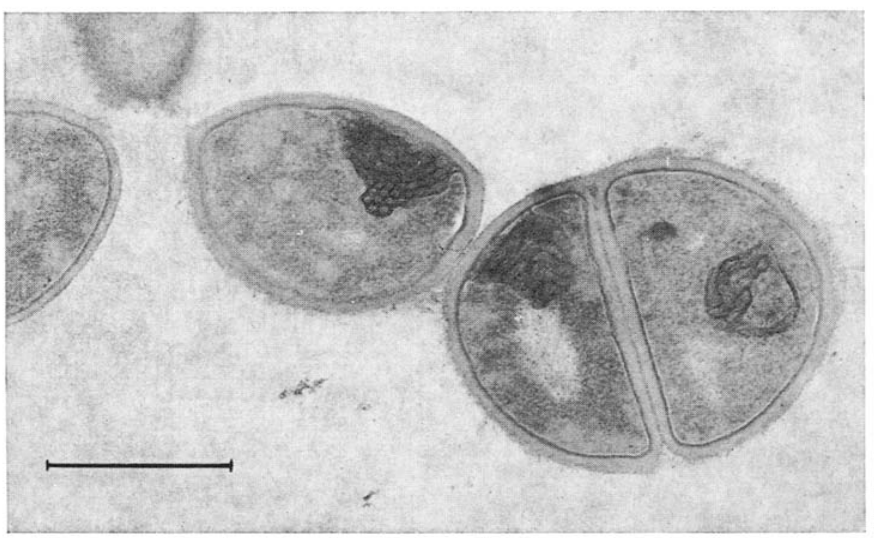

Fig. 3.
Electron micrography of cells of *S. aureus* after 4 h exposure to the silver anode region. Note large, dense mesosomes. Cells fixed with glutaraldehyde and osmium tetroxide and stained with uranyl acetate. Bar indicates 0.5 microns.

Inhibition of protein synthesis – E. coli

A study was made of the effect of anodically generated silver ions on the induction of β–galactosidase (β–gal) in *E. coli* grown in a glycerol–salts minimal medium.[10] Depression of the β–gal gene occurs by the addition of *iso*–propyl–thio–galactoside (IPTG) to the culture. The induction was measured colorimetrically following the cleavage of *ortho*–nitro–phenyl–β–*D*–galactoside (ONPG) and release of the chromophore by β–gal. The cultures were grown in small wells containing silver electrodes, as above, with current of 0.04, 0.4, 4.0 and 40 μA. Aliquots were taken at intervals from 0–130 minutes after induction with IPTG and β–gal measured. The results (Fig. 4) show clearly that the production

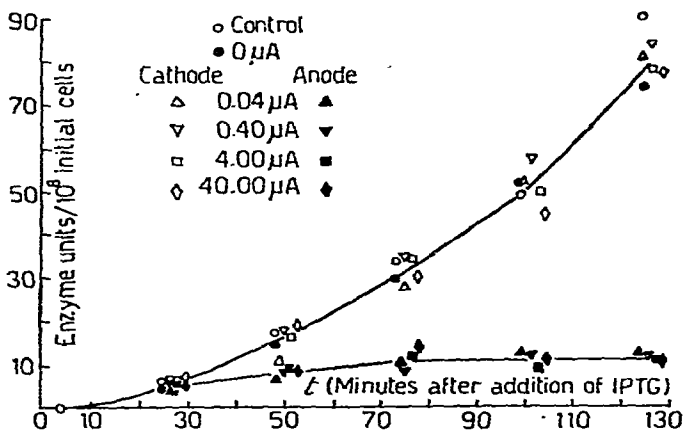

Fig. 4.
Induction of beta–galactosidase by IPTG in *E. coli* as a function of time and current level in cultures exposed to silver electrodes. Note almost complete inhibition on the anode side.

of the enzyme was inhibited almost completely in the anode well, as compared to cathode and control cultures. Further tests showed that much higher silver concentrations are required to inhibit β–gal itself and therefore inhibition is most likely in the production of the protein. If one delays the start of current flow to the anode until after de–repression of the β–gal gene has occurred, the enzyme production is still inhibited. This indicates that the inhibition is either at the level of transcription or translation. Since the former process is membrane–related in *E. coli* this is further evidence that the inhibition by Ag may be primarily on the cell membrane, although other mechanisms cannot be eliminated.

In vivo study of bone infections in rabbits

In order to be clinically useful, it is necessary to demonstrate the effectiveness of electrochemically injected silver ions against infections *in vivo*. A portion of this study is now complete in which infections produced in the tibia of 22 rabbits were treated with silver anodes. [11] The rabbit model of a bone infection (with metallic rod fixation) proved to be workable after much initial experimentation with regard to dose, site, and method of administration. Such localized bone infections (osteomyelitis) are often difficult to treat in man.

The rabbits were infected, through a hold drilled into each tibia, with 0.2 cm^3 agar suspension of *S. aureus* (10^5–10^6 bacteria/cm^3). A 2 mm diameter silver rod was inserted on one side and a stainless steel rod on the control side. The Ag side was either :

a. run anodically at 40 μA for 1 h after insertion ;
b. connected to the positive lead of an implanted battery pack ($\leqslant$20 μA);
c. previously anodized in NaCl solution before insertion. Return electrodes were subcutaneous wires several cm from the surgical site.

The control rods were not powered.

After 5 weeks the rabbits were sacrificed, X-rayed and the tibias examined and cultured for bacteria. The results are summarized in Table 1. In 7 out of 22 cases the Ag anode site was completely normal in appearance radiographically (see Fig. 5) while the control side suffered obvious bone destruction typical of osteomyelitis. In 4 cases, hardly any change had occurred on either side ; in 3 cases both sides were severly

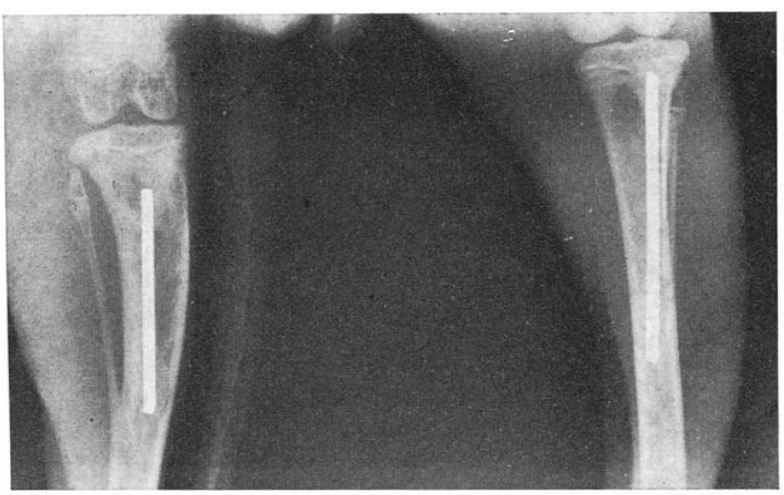

Fig. 5.
Radiograph of the tibia of a rabbit 5 weeks after infection with *S. aureus* and insertion of silver and stainless steel rods. The silver rod received 40 μA anodic current for 1 hour after implantation. Note bone destruction on the stainless steel (control) side.

infected. In the rest, the Ag anode side was noticeably less damaged. These results indicate that for the infection model chosen, the Ag anode has a therapeutic effect, especially for the two actively stimulated modes of application. Further study of the implanted Ag electrode, the fate of the electrochemical products *in vivo* and the optimum parameters of stimulation are required.

Table 1. Evaluation of Ag anode treated infections in rabbit tibia at 5 weeks.

Experimental Mode	No. Animals	Mean infection score *		% Cases improved
		Ag Side	Control side	
Current at surgery only: 40 μA, 1 h	11	0.73	1.92	73
Battery implant ⩽ 20 μA, continuously	6	0.17	2.00	83
Anodized Ag Rod	5	1.20	1.80	40
Total	22	0.68	1.96	68

* Severity of infection and destruction of bone: 0 = normal, 1 = slight change, 2 = moderate destruction, 3 = severe destruction. Control side = stainless steel rod (Steinman pin).

Electrically injected gold in arthritic joints in rabbits

Systemically administered gold compounds are effective in treating rheumatoid arthritis, but their use is often accompanied by toxic reactions. As an alternative, the direct injection of gold from a metal electrode into the synovial fluid was evaluated. [12] Arthritic inflammation, characteristic of the disease in man, was induced bilaterally in 40 rabbit knees using FREUNDS' complete adjuvant. [13] One week later, one knee in each animal was treated by inserting a 99.99 % pure gold wire into the joint space

(insulated except for 1 cm) and applying a constant positive potential of 0.9 volts to the wire with respect to a large skin electrode. It was estimated that about 10^{-7} moles of the Au would be administered during the 2 h treatment. Groups of 5 animals were sacrificed at intervals from 1–60 days thereafter, and the knees dissected, examined, and histological specimens taken.

Results to date are based only on visual inspection of the dissected joints, performed by an orthopedic surgeon (R.O.B.) without prior knowledge of the treated side. Signs of improvement included reduced invasion of cartilage, reduced synovial hypertrophy, and clarity of synovial fluid. During the first two weeks after treatment with the gold anode, 60–80 % of the treated knees showed marked improvement compared to the untreated side. In the 30–60 day groups, only 40 % improved. If confirmed by histological analysis and electron microscopy, the direct injection of gold may have important therapeutic potential.

As in the case of silver, further exploration of the electrochemical events and mechanisms *in vivo* associated with such treatment are required. Extension of principles derived from such studies on the treatment of viruses and tumors (which also involve rapidly dividing cell populations) may also be quite fruitful.

Aknowledgements

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