ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1974, p. 637-642

Vol. 6, No. 5
Printed in U.S.A.

Antibacterial Effects of Silver Electrodes with Weak Direct Current

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Received for publication 19 June 1974

Silver, platinum, gold, stainless-steel, and copper electrodes were used with low currents (0.02 to 20 $\mu A/mm^2$) to explore their electrochemical effects on the growth of four bacterial species. In the higher current ranges, all electrodes inhibited growth at both poles, usually in conjunction with electrolytic breakdown of the medium and severe corrosion of the metal. Silver, however, was extremely bacteriostatic, even at the lowest current, when used as the anode. Quantitative studies showed that most of this inhibition takes place in a few hours and is not accompanied by changes in pH. Electrochemically injected silver from the anode is probably the instrumental agent, being effective in concentrations of about 5 $\mu g/ml$. This is the equivalent concentration of silver sulfadiazine that has been shown to give complete inhibition of bacteria, but without the sulfonamide moiety.

The inhibition of bacterial multiplication by use of electric currents and metal electrodes has been reported (3, 5, 6, 8). Whether alternating or direct currents were used, most authors agree that electrochemical products at the electrodes and/or metal ions are instrumental in this effect. In most cases, however, the currents were high enough to produce severe changes in the medium, especially near the electrodes.

The present study was undertaken to explore the effects of relatively weak direct currents (and their electrochemical products) on agar plate bacterial cultures of *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, and *Pseudomonas aeruginosa*. Attention is paid to: (i) the electrode metal, (ii) the surface current density at the electrode, and (iii) the spatial character and polarity of the effects. We also report the results of more detailed measurements of viable organisms in the region of silver electrodes in liquid broth cultures as a function of time, current, and polarity.

In a preliminary study (1) on *S. aureus*, it was found that currents of the order of 10^{-8} A/mm^2 with silver electrodes can give rise to significant local inhibition. Much higher currents were required for platinum, gold, and stainless steel, usually in association with the breakdown of the electrolyte, to achieve similar effects.

MATERIALS AND METHODS

Qualitative studies. Culture plates (45 mm) were prepared by mixing 0.5 ml of broth medium (GIBCO 1-t-1859) containing exponential-phase bacteria (optical density of 0.17 to 0.23 at 500 nm) with 3 ml of nutrient agar (GIBCO 1-t-1904) at 48 C. Identical electrode pairs (20 by 0.2 to 0.4 mm) were included in the plates and emerged through the sides. Pure silver (99.99%), platinum (99.9%), gold (99.99%), surgical stainless-steel (no. 316L), or copper (99.99%) wires were used. Battery-operated, constant-current generators (modifications of Vitron LIDC generators kindly supplied by Jack TerBeek of the Ritter Co., Rochester, N.Y.) were applied to the electrodes (except controls) after solidification of the agar and throughout incubation (37 C, 24 h). Current levels were 0.4, 4.0, 40, and 400 μA. In some cases the plates were incubated without current for 24 h, and then current was applied for the next 24 h to determine the effect on already established bacterial colonies. Currents and applied potentials were monitored at the beginning and the end of the culture periods in all cases. After incubation the plates were examined and photographed; subcultures and pH were taken at 3-mm intervals between the electrodes and at control points in all plates. The pH test paper used (Hydrion) had a least-count of 0.4 pH unit. The experimental organisms selected, *S. aureus*, *E. coli*, *P. vulgaris*, and *P. aeruginosa*, were all patient derived at this hospital.

Quantitative studies. Pairs of special Plexiglas chambers, 3 ml in volume and connected by thin agar-salt bridges, were used to study bacterial growth around silver electrodes with *S. aureus* and *E. coli* (Fig. 1). Each chamber containing 2.5 ml of broth medium (GIBCO 1-t-1859) was inoculated with 10^8 organisms and treated with constant currents as above for 4 h. The electrode surface was 24 mm^2. At hourly intervals 0.1 ml was removed from anode and cathode sides, and dilutions and standard plate counts were made. Silver concentrations in the me-

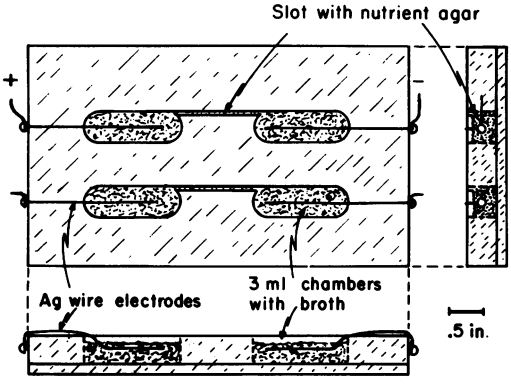

FIG. 1. *An example of the Plexiglas chambers used in the quantitative studies of the effects of silver electrodes on S. aureus and E. coli. Each chamber pair is connected to a separate, battery-operated, constant-current generator. A loose-fitting cover was placed over the chambers during incubation.*

dium after 4 h of current flow and incubation were determined by emission spectrographic analysis for each current level. (Arc emission analysis on a grating spectrograph was performed after a portion of the culture mixture was dried in graphite cup electrodes. Li_2CO_3 was used as the buffer and Cu^{2+} was added to the mixture as an internal standard. Spectral lines Ag 3280/Cu 3274 were used as the analysis pair after the recording of the spectra on film.)

RESULTS

Lower current ranges: 0.4 to 4.0 μA. At 0.4- and 4.0-μA current levels (equivalent to 20 and 200 nA/mm² at the electrode), platinum and stainless steel had no effect on the bacteria (Table 1). Significantly, the positive silver electrode inhibited all organisms tested in a 5- to 7-mm clear zone around the electrode, even with 0.4 μA. The silver cathode showed little if any inhibition (Fig. 2). Also, silver and copper were the only metals to maintain low potentials (0.1 to 0.7 mV) at these currents, all others being in excess of 1 V. The gold cathode had a measurable inhibition at 4 μA for *S. aureus* and *E. coli*. The copper anode was effective against *E. coli* at 4.0 μA. No noteworthy discoloration, gas formation, pH shifts, or corrosion were seen for any metal tested at these levels.

Higher current ranges: 40 to 400 μA. For all

TABLE 1. *Bacterial inhibition near metal electrodes with weak direct currents*

Current (μA)	Electrode (area)	Potential[a] (V)	Inhibition near electrode[b]							
			S. aureus		*E. coli*		*P. vulgaris*		*P. aeruginosa*	
			Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
0.4	Silver (24 mm²)	0.38	+++	0	+++	0	++	0	+++	+
	Platinum (13 mm²)	1.1	0	0	0	0	0	0	0	0
	Gold (16 mm²)	1.2	0	0	0	0	0	0	0	0
	Stainless (24 mm²)	1.3	0	0	0	0	0	0	0	0
	Copper (16 mm²)	0.086	0	0	+	0	0	0	0	0
4.0	Silver	0.70	+++	0	+++	0	+++	+++	+++	0
	Platinum	1.7	0	0	0	0	0	+	0	0
	Gold	1.9	0	+++	0	+++	0	++	0	+
	Stainless	1.7	0	0	0	0	+	0	0	0
	Copper	0.69	0	+	+++	+	0	+	0	0
40.0	Silver	1.5	+++	+	+++	+++	+++	+++	+++	++
	Platinum	2.5	+	0	0	+++	+	+++	0	0
	Gold	3.0	+++	0	+++	+++	+++	+	+++	0
	Stainless	1.7	+	0	++	0	0	++	0	0
	Copper	1.3	++	+	+++	+++	+++	0	0	+
400.0	Silver	≥9.5	+++	+++	+++	+++	+++	+++	+++	+++
	Platinum	3.5	+++	+++	+++	+++	+++	+++	+++	+++
	Gold	3.4	+++	+++	+++	+++	+++	+++	+++	+++
	Stainless	≥9.5	+++	+++	++	0	++	+++	+++	+++
	Copper	≥9.5	+++	+++	+++	+++	+++	+++	+++	+++

[a] At 24 h.

[b] Inhibition was judged by both the extent of the clear zone and the sterility of the region by subculture tests. 0, No inhibition; +, slight inhibition (with about ⅓ of stab tests sterile); ++, medium inhibition (with about ⅔ of stab tests sterile); +++, strong inhibition (with 5- to 10-mm clear zone and all stab tests sterile). Pos, Positive; Neg, negative.

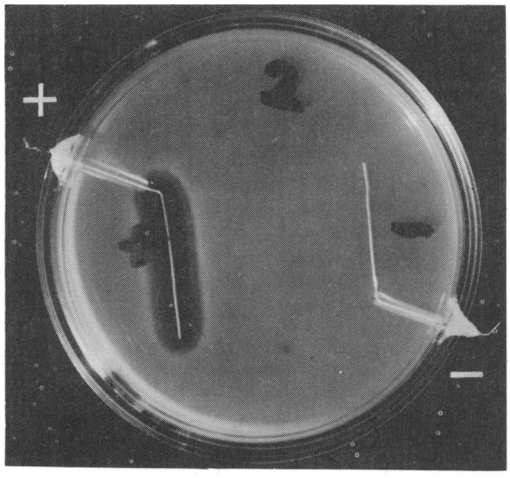

FIG. 2. *Culture plate of S. aureus after 24 h of incubation with a 0.4-μA current and silver electrodes. Note the inhibition (clear zone) at the anode. No untoward effects were observed at this current. The outer diameter is 58 mm.*

metals at the highest current range (equivalent to 20 μA/mm² at the electrode), bacterial inhibition occurred for all organisms tested in a 5- to 10-mm zone around both positive and negative poles (Fig. 3; Table 1). Interelectrode potentials, however, were 3 V or more, and significant gas formation occurred at the cathodes. Medium discoloration and corrosion were extensive at the anodes. For all but silver, pH shift of several units were measured at both electrodes and could explain the inhibition. Similar but less marked effects were found in all cases at 40 μA (2 μA/mm²). Inhibition at 40 and 400 μA was also produced when spectrographic graphite electrodes were used instead of the metals; corrosion was absent in this case. The bacteriostatic action at these higher current densities, therefore, is probably a result of deleterious toxic changes in the medium via electrolysis.

Other qualitative observations. Subcultures confirmed the sterile nature of the clear zones at the electrodes. It was noted that incubation beyond 24 h, with or without current, did not tend to enlarge the zones. We also found that introducing the bacteria by stabs into the gelled agar (rather than by mixing them into the ungelled agar at 48 C) did not affect the outcome of these experiments. This suggests that the presence of a large amount of bacterial by-product does not influence the inhibitory activity near the electrodes.

Trials with *S. aureus* in which current was applied to the plates after the bacteria were permitted to grow unimpeded for 24 h showed turbidity changes near the silver anodes at all current levels. These 3- to 8-mm zones, although not clear, proved to be sterile on subculture, indicating that the organisms within were either killed or irreversibly inactivated.

Quantitative studies with silver electrodes. The bacterial counts made for silver electrodes with *E. coli* and *S. aureus* grown in liquid nutrient medium generally confirmed the inhibitory effects of the silver anode seen in the agar plate experiments above. The quantitative studies indicated further that the anodic inhibition occurs within the first few hours of such treatment and is faster, the higher the current. The detailed behavior as a function of time, current, and polarity is shown in Fig. 4 and 5. In only 4 h the 4.0- and 40-μA anodes (approximately 0.2 and 2 μA/mm² at the electrode surface, respectively) were especially effective, reducing bacteria by at least 3 orders of magnitude from initial levels and by 5 orders of magnitude from peak levels. The cathodes were not effective in this regard even at the 40-μA level and behaved much like the control electrodes (i.e., with no current) during the incubation period.

A few similar trials were performed in which bacteria (*E. coli*) were inoculated into mouse bone marrow cultures to test the effect of the current and silver electrodes on the organism in the presence of a normal animal cell population. Preliminary results showed that leukocyte and erythrocyte populations were unchanged from controls after treatment with 4.0- or 40-μA

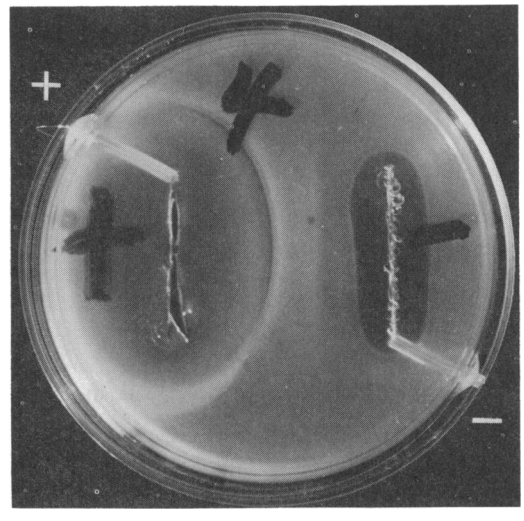

FIG. 3. *Culture plate of S. aureus after 24 h of incubation with a 400-μA current and platinum electrodes. The bacteria were inhibited at both electrodes, but large pH shifts and gas formation were also encountered.*

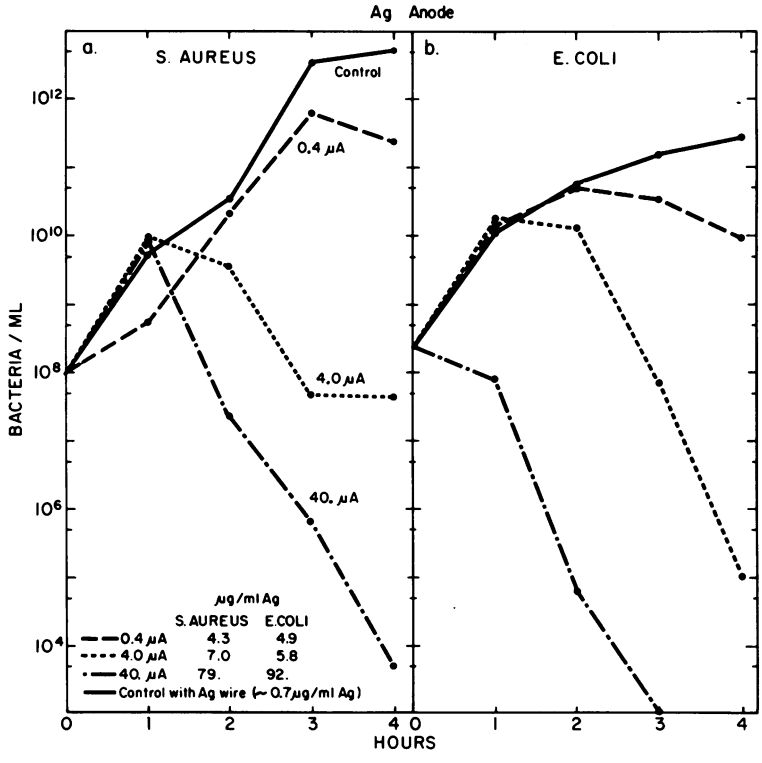

FIG. 4. *(a) Results of standard plate counts of S. aureus in the silver anode chamber of Fig. 1, as a function of time and current level. Note the inhibitory effects of the 4- and 40-μA electrodes. The concentration of Ag (in micrograms per milliliter) measured at 4 h in the broth is shown in the legend (in this and in the following figure). (b) Results of standard plate counts of E. coli in the silver anode chamber. E. coli are more sensitive to the electrochemical products than S. aureus.*

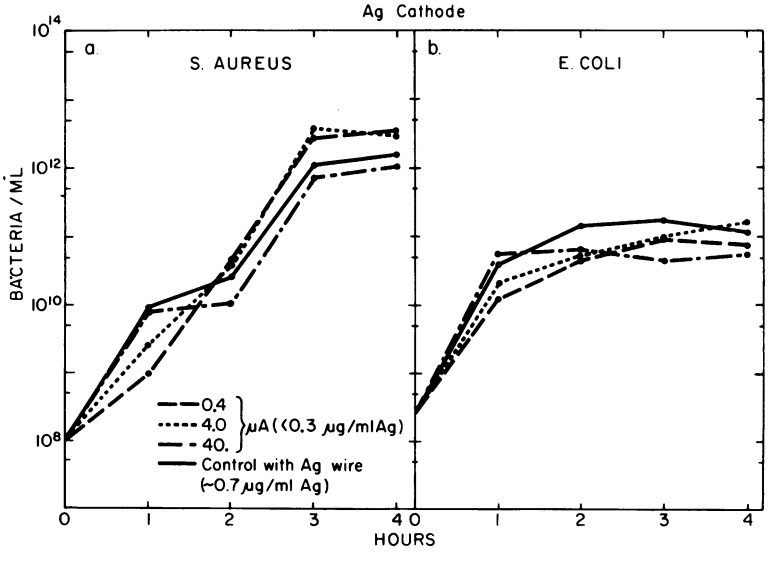

FIG. 5. *(a) Results of standard plate counts of S. aureus in the silver cathode chamber. Note lack of any significant inhibition, even at 40 μA. (b) Results of standard plate counts of E. coli in the silver cathode chamber.*

currents. The cells' morphology at 4.0 μA remained normal after 4 h of treatment, as viewed by light microscopy, although the bacteria in the anode chamber had been almost completely eliminated. (Cell viability was confirmed by trypan blue exclusion tests.) At 40 μA some morphological changes in the nuclei of the neutrophils, as well as some cell lysis, were observed.

Silver evolution from the anode. The concentration of silver determined spectrographically in the broth, after 4 h of incubation, at each electrode and current is shown on the graphs of Fig. 4 and 5. This represents the total amount of silver leaving each electrode since no attempt was made to separate bound, dissolved, or precipitated fractions. Around the cathodes, very little silver was detected (<0.3 μg/ml) as would be expected. Around 0.4- and 4-μA anodes, concentrations of 4 to 5 and 6 to 7 μg/ml, respectively, were found. These levels are only twice the amount expected from the solubility of AgCl in water at 38 C. In the 40-μA anode chambers, concentrations of 80 to 90 μg/ml were present. Thus, the final concentration of Ag was only partly related to the measured bacterial inhibition. It did not correlate well, for example, with the large differences in inhibition observed for the 0.4- and 4-μA cases.

DISCUSSION

The results with low currents confirm that metal-specific electrochemical products from the electrode interface are bacteriostatic and, as stated by Rosenberg et al. (6), that pH and temperature changes per se are not necessary. The remarkable efficacy of the silver anode, and secondarily of the gold cathode, in bacterial inhibition may be in some way related to their location in the same column of the periodic table and near to the group VIII-B elements implicated by Rosenberg et al. It is likely, although not completely certain from the present study, that the Ag ion or a Ag complex is responsible for the inhibition when this metal is used as a low-current anode. This view is supported by the following observations. (i) When bacteria were introduced into agar in which current had previously been injected via silver, the organisms were also inhibited despite the fact that the current had been discontinued. (ii) Silver (bound plus unbound) concentrations in the bacteriocidal medium at the anode were far from zero, although only twice the amount expected from the solubility of AgCl in water. (iii) Since silver complexes strongly with many proteins, large concentrations may not be required to effect bacterial inhibition.

Recent research on the use of silver sulfadiazine (AgSD) as a topical antibiotic has also provided strong evidence that the silver ion is primarily responsible for its activity (4, 7) either by acting on the cell surface (2) or the nuclear deoxyribonucleic acid (4, 9). In fact, the concentration of AgSD (0.05 μmol/ml) for complete inhibition in 4 h of *P. aeruginosa* (4) corresponds closely to the 6 to 7 μg of Ag per ml measured in the 4-μA anode chamber in the present study. Thus, it appears as if the electrically injected Ag ion is at least as effective as that carried by AgSD.

The somewhat anomalous inhibition seen around the gold cathode at 4 μA was reproducible and may warrant further investigation. It is probably due to the formation of electrochemical reaction products, particular to this metal and the prevailing electrode potential, that are bacteriocidal.

The bacteriostatic effects of the other metal electrodes used in these experiments were far less marked. The effectiveness of these metal ions per se, however, cannot be determined from our results since electrolytic changes in the medium would mask any effect of the ions evolved from the electrodes. Moreover, the higher currents required for any consistent bacteriostatic action with Pt, Au, stainless steel, or Cu gave rise to significant corrosion of the anodes. This was especially strong for stainless steel and copper and would contra-indicate the use of such electrodes for in vivo applications when current densities in excess of 1 μA/mm^2 at the surface are contemplated.

Finally, this study suggests that electrochemically injected silver ions in nanomolar concentrations be considered for further testing and for a possible use as a "topically" applied bacteriostatic treatment for infections of poorly vascularized areas such as burns, chronic skin ulcerations, and osteomyelitis. Advantages may include a greater depth of tissue penetration compared with the simple diffusion resulting from the topical applications of silver sulfadiazine, as well as obviating the need for the accompanying sulfonamide with its possible toxic reactions.

ACKNOWLEDGMENTS

We are grateful to Jack TerBeek and his staff at the Ritter Company for support of this work and for supplying the constant current generators. We also thank Jack Duffy, Supervisor, Microbiology Section, V.A. Hospital, Syracuse, N.Y., for providing the bacterial cultures.

This work was supported by Public Health Service re-

search training grant 05603 and project grant 07626, by Veterans Administration research grant 0865, and by a grant from the Ritter Company, Division of Sybron Corporation.

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