In Vitro Effect of Weak Direct Current on Staphylococcus Aureus

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Stone[8] in 1909, described a marked increase in the bacterial growth rate of naturally occurring milk and water flora subjected to galvanic current from immersed rods of 2 different metals. However, Beattle and Lewis[1] in 1925 and Prescott[5] in 1927 found that electrical current significantly reduced the bacterial count of milk and other fluids.

In 1965, Rosenberg *et al.*[6] and in 1969, Parielleux and Sicard[4] noted the lethal effect of low voltage alternating current on *Escherichia coli*. However, in 1927, Rowley[7] reported essentially no effect of alternating current on *E. coli* generation times, but an appreciable increase in generation times with direct current.

Cieszynski[2] has determined that bacterial growth is inhibited at the negative pole and stimulated at the positive pole with stainless steel electrodes. He has transferred this finding to his clinical experience in treating osteomyelitis with favorable results.[3]

Likewise, Wolcott *et al.*[9] describe the use of a negative electrode in treating infected decubiti.

The apparent contradictions, the paucity of published experimental data and the possibility of further clinical application of electrical current to bacteria, prompted this study.

MATERIALS AND METHODS

The organism, *Staphylococcus aureus*, coagulase positive, was chosen because it is the most common pathogen encountered and was readily available to our laboratory.

Sixty mm petri dishes were fashioned so that 2 identical wire electrodes, either silver (99.99%), gold (99.99%), platinum (99.9%) or stainless steel (surgical #316-L), could be fastened inside each bottom plate (Fig. 1) The bare portions were 20 mm in length, 25 mm apart, and of known gauge so that current densities at the electrode surface could be estimated for each experimental run. The

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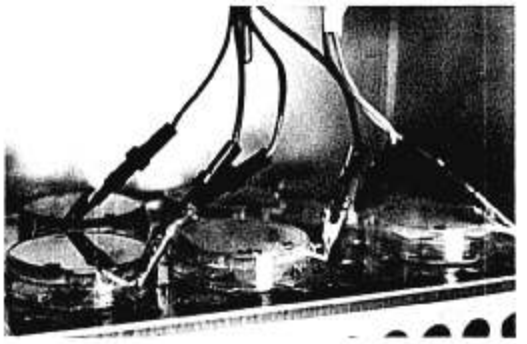

FIG. 1. Incubator set-up with petri dishes and lead wires in place. Constant current DC generators were located outside of the incubator.

rest of the wires were teflon insulated and their entry points into the petri dishes were coated with epoxy cement. Thin pour plates were prepared by inoculating sterile dishes with 0.5 ml of a broth suspension of the organisms harvested in mid-log phase. The suspension was mixed thoroughly with 3 ml of brain-heart infusion agar at 48 C and then cooled.

The plates were incubated at 37 C with the implanted electrodes attached to positive and negative poles, respectively, of constant current generators set to deliver predetermined currents of 0.4, 4.0, 40 or 400 microamperes (Fig. 1). Controls for each experimental run consisted of similarly fashioned plates not attached to the current generator. Each run consisted of an individual metal tested at each current and

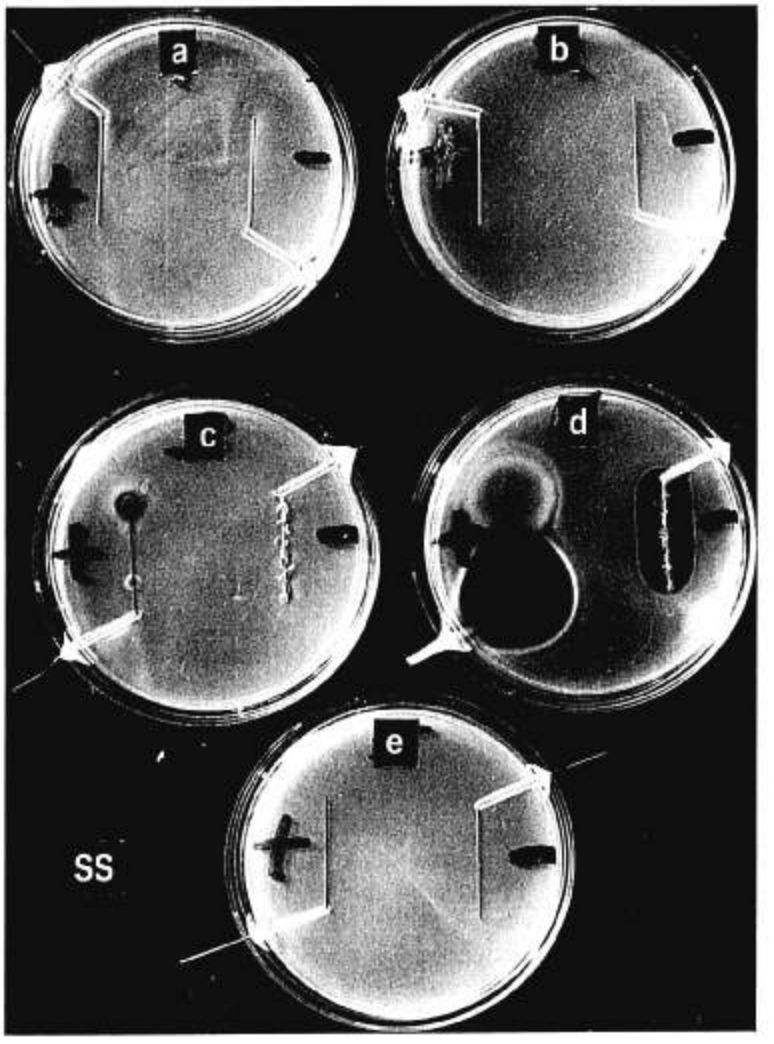

FIG. 2. Pour plates with stainless steel electrodes incubated for 24 hours with 0.4, 4.0, 40, 400 and 0.0 (control) microampere currents, respectively. Note the lack of inhibition in the lower current ranges and the noxious changes around the positive electrode especially at 400 microamperes. In this and following figures the petri dish diameter is 58 mm and the positive electrode is on the left.

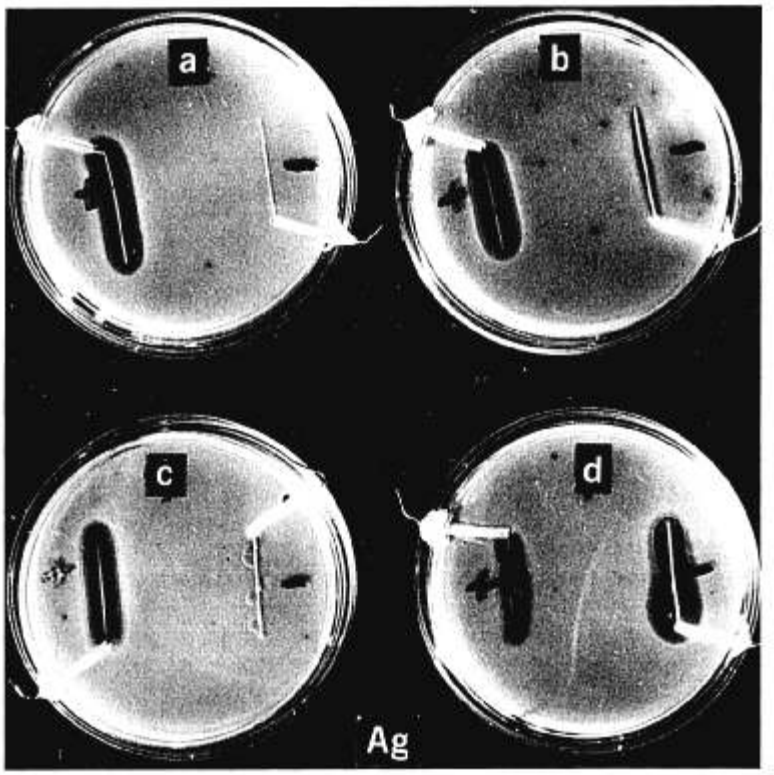

FIG. 3. Pour plates with silver electrodes incubated for 24 hours with 0.4, 4.0, 40, and 400 microamperes, respectively. Note the uniformity of inhibition at the positive electrodes, despite the current levels, and also the general lack of toxic reactions and severe discoloration of the medium. The clear zones were proved sterile with follow-up cultures.

2 controls. All runs were repeated at least twice with each metal tested.

Data were obtained at 24 hours and 48 hours after initiation of current flow. The inhibitory zones were measured and photographed. Corrosion and gas evolution were noted and recorded. Stab cultures were taken from the electrode sites and at 3 mm intervals between each electrode pair. Growth on these culture plates were smeared and stained for identification of the bacterial type. pH determinations were made at each electrode site and also at 3 mm intervals between the electrodes.

RESULTS

Stainless Steel. At 0.4–4.0 microamperes, no inhibition occurred at either the positive or the negative pole (Fig. 2). Slight inhibition was noted at the positive pole at 40 microamperes and marked inhibition occurred at both poles when the applied current was 400 microamperes. However, at 400 microamperes, gas formation and medium discoloration occurred (Fig. 2). Also a significant shift in pH was noted at both electrodes.

Platinum. Again, at 0.4 and 4.0 microamperes no inhibition occurred, while at 40 microamperes, slight inhibition was noted at the positive electrode. At 400 microamperes, marked inhibition occurred at the positive and negative poles, but again, gas formation and medium discoloration occurred, though to a lesser degree than with stainless steel. The pH shifts at 400 microamperes, however, were the more marked with platinum. The positive electrode region shifted from 7.2 to 1.5 and the negative from 7.2 to 10.0 pH units.

Gold. The inhibitory capacity of gold, in the lower ranges tested, exceeded that of stainless steel and platinum and the untoward effects were less. However, while inhibition occurred at the positive gold electrode at 40 microamperes and at both electrodes at 400 microamperes, pH shifts, gas

TABLE 1. Results of Electrochemical Treatment of *Staphylococcus Aureus In Vitro* with Microampere Direct Currents

Electrode (*area*)	*Current* (*micor-A*)	*Potential at 24 hrs* (*volts*)	*Clear Zone* (*inhibition*)		*pH Shift*		*Corrosion*		*Discoloration of Medium*		*Gas Formation*		*Viability at Electrode*	
			Pos	*Neg*	*Pos*	*Neg*	*Pos*	*Neg*	*Pos*	*Neg*	*Pos*	*Neg*	*Pos*	*Neg*
Silver	0.4	0.4	++	0	0	0	0	0	0	0	0	0	0	+++
(0.24 cm²)	4.0	0.7	+++	+	0	0	+	0	0	0	0	0	0	+++
	40	1.2	+++	0	0	+	++	0	0	0	0	+	0	++
	400	9.3	+++	+++	0	0	+++	0	+	0	0	+	0	0
Platinum	0.4	1.3	0	0	0	0	0	0	0	0	0	0	+++	+++
(0.13 cm²)	4.0	1.7	0	0	0	0	+	0	0	0	0	0	+++	+++
	40	2.6	+	0	0	+	++	0	+	0	+	++	++	+++
	400	3.4	+++	+++	—	+++	0	0	+	0	+	++	0	0
Stainless steel	0.4	1.0	0	0	0	0	0	0	0	0	0	0	+++	+++
(0.26 cm²)	4.0	2.3	0	0	0	0	0	0	0	0	0	0	+++	+++
	40	1.5	+*	+	0	+	+	0	++	0	0	+	++	+++
	400	9.1	+++*	+++	—	+++	0	0	+++	0	0	++	0	0
Gold	0.4	1.2	0	0	0	0	0	0	0	0	0	0	+++	+++
(0.16 cm²)	4.0	1.6	+	++	0	0	0	0	0	0	0	0	+++	0
	40	2.8	++	0	—	++	++	0	+	0	++	+++	0	+++
	400	3.8	+	+++	—	+++	0	0	+++	0	+	++	0	0

* Zones of dark discoloration.

Note: 0 = no effect, + = slight effect, ++ = moderate effect, +++ = strong effect. Details of the effects observed are described in the text and the figures. For pH shifts from control values, the scale is as follows: 0 = 0–0.5 units, ± = 0.5–1. units, ±± = 1.–2. units, and ±±± = > 2. units. The potentials expressed are typical of those at 24 hrs, but not constant since controlled currents were applied. The viability was determined by stab subcultures, where 0 = sterile in several trials, + = growth in ⅓ of the trials, ++ = growth in ⅔ of the trials, and +++ = growth in all trials (control level).

formation and medium discoloration were noted in these ranges.

Silver. Inhibition over the 0.4–40 microampere range with the positive silver electrode was far superior to the other electrodes tested (Fig. 3). Also, no increase in inhibitory capacity of the positive electrode was found by increasing the current to 40 or 400 microamperes (Fig. 3). Furthermore, in the lower ranges tested, the side effects of gas formation, corrosion pH shifts and medium discoloration were negligible. At 400 microamperes, inhibition at the positive and negative were marked and essentially equal, but AgCl anodization, gas formation and medium discoloration were evident.

In all instances, stab cultures confirmed that the areas of inhibition (clear zones) noted were sterile, while the controls and other sites cultured grew *Staphylococcus.*

DISCUSSION

The occurrence of wire corrosion, gas formation, medium discoloration and pH changes do not, by themselves, explain the inhibitory effects of electrical current since inhibition occurs without these effects at lower ranges in several cases. Rosenberg *et al.*[6] have shown the inhibition of *E. coli* by alternating electrical current (2–3 amperes) to be due to an indirect effect of the electrolysis products from Pt electrodes and they have shown this also occurs with other type VIII B transition elements. However, at these higher levels (> 400 microamperes in our system) any specific advantages of electrode type and polarity may be overcome by the formation of a toxic milieu. Certainly, the 4 metals tested in this study, at the highest level, were capable of bacterial inhibition at either positive or negative pole. Cieszinski[2] found excellent bacterial inhibition at the negative pole of a stainless steel wire driven at a current level of 150 microamperes (4.5V) and noted pitting and corrosion of the wire when the experiment was terminated. Our study corroborates the effectiveness of negative stainless steel, or any other negative electrode tested, at this high level of current. However, the demonstration of excellent inhibition and negligible toxicity of the positive silver electrode at very low levels of direct current (0.4 microamperes) makes this electrode most practical in considering future *in vivo* experimentation and possible chemical application.

Results of this study show that bacterial growth can be inhibited *in vitro* in the vicinity of metal electrodes by the application of direct current at levels of 0.4–400 microamperes (2–2,000 microamperes/cm^2 at the electrode surface). Either the positive or negative wire electrode of stainless steel, gold, platinum or silver can inhibit bacterial growth at 400 microamperes of current, but at this level *toxic* effects also occur. At lower levels of 0.4–4.0 microamperes, the positive silver electrode has excellent inhibitory capacity and negligible toxic effects. *In vivo* experiments should be devised utilizing electrochemical reactions at the positive silver electrode at 0.4–4.0 microamperes (2–20 microamperes/cm^2) to inhibit localized bacterial infections.

SUMMARY

Direct current at levels of 0.4–400 microamperes inhibits growth of *Staphylococcus aureus* with negligible toxicity. Tests on 4 different electrodes, *e.g.*, surgical stainless steel, pure platinum, gold and silver, showed that a positive silver electrode is superior at levels of 0.4–4.0 microamperes, (2–20 microamperes/cm^2) because it produced the highest level of inhibition and lowest levels of toxicity.

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