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Direct-Current Bactericidal Effect on Intact Skin

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Positive carbon-containing electrodes conveying 5 or more μA of constant direct current per cm^2 showed bactericidal activity on intact back skin of 13 human subjects. This effect increased with the duration of stimulation up to a total surface bacterial kill at 20 h. When total current and current density were varied independently on 16 sites on the backs of eight subjects, the effect was dependent on current density, not on total current. Electrodes driven by similar voltages but which removed the electrochemical reaction from inoculated sites on the backs of three subjects failed to reduce the numbers of colony-forming units as compared with those sampled from control sites. This showed the bactericidal effect to be electrochemical in origin, probably mediated by local acidity generated at the surface of the positive carbon-containing electrodes. With an adhesive tape stripping technique on three sites on each of six subjects, it was determined that the effect extended into the epidermis of the human back. No effect was observed beneath negative or control electrodes under the same conditions.

Direct-current stimulation of a biological subject may produce effects resulting from ion flow within the subject or from electrochemical events at the tissue-electrode interface or both. Metallic ions released electrochemically at positive electrodes have been reported to exhibit local antimicrobial activity (1, 2, 4–7). However, little is known about the effects of direct current delivered through nonmetallic carbon electrodes.

The present study investigated the effects of constant direct-current stimulation through carbon-filled electrodes on microorganisms in intact human skin.

MATERIALS AND METHODS

Electrical apparatus. Two types of stimulating electrodes were used. In the first type of electrode, Condulon film (Bemis Co., Inc., Minneapolis, Minn.), a polyvinyl chloride film containing carbon, served as the electrode surface on a 6-V flat-pack dry cell. This battery-electrode complex (4.5 cm by 5 cm by 2 mm) was made by modifying a Leclanche P-70 battery (Ray-O-Vac Division, The International Nickel Co. of Canada, Ltd., Madison, Wis.). When manufactured without its stainless steel back plate, the P-70 battery is sufficiently flexible for this application. The second type of electrode consisted of 4-ml (0.1-mm thick) Velostat film (3-M Co., Minneapolis, Minn.) laminated to a 2-ml (0.05-mm thick) layer of aluminum foil. Velostat is a polyolefin plastic containing carbon. The voltage source was a remote 9-V transistor battery. Both circuits were completed through current-limiting devices designed to deliver no more than the specified total number of microamperes of direct current (±1%).

All electrodes were masked with nonconductive vinyl tape (Permacel, Division of Johnson & Johnson, New Brunswick, N.J.) so that only a 10-cm^2 circle of the carbon film was exposed as the stimulating surface. This surface was separated from the skin by a 10-cm^2 circle of rayon fabric weighing 101 g/m^2 which served as a reservoir for the inoculum while providing a moist interface with the skin and promoting uniform conduction across the stimulated area.

Test organisms. Each test site was inoculated with a pure culture of a bacterium known to be a resident of the test subject. The microorganisms had previously been isolated from a high dilution of a wash from the subject's skin after the skin had been occluded with Saran Wrap (Dow Chemical Co., Indianapolis, Ind.) for 24 h. All isolates had been grown on Trypticase Soy Agar (BBL Microbiology Systems, Cockeysville, Md). for 48 h at 35°C. Cell suspensions harvested from these cultures were stored in small-volume aliquots at −75°C until used.

All isolates had been identified as strains of *Staphylococcus epidermidis*. The inoculum level on any given test day was determined by inoculating and sampling a control site adjacent to the test sites.

Sampling and observations. Bacteria in the intact skin were enumerated by a scrubbing technique (8). This technique is reliable to ±1 $\log_{10}$ colony-forming unit (CFU) per cm^2 of skin surface. For 30 s, 1.5 ml of sterile saline was stirred vigorously in a 28-mm-diameter cylinder centered over the test site. A 1-ml amount of this solution was transferred to a test tube containing 9 ml of sterile peptone water. The saturated rayon fabric was placed in a separate test tube containing 9.6 ml of diluent. Aliquots from these tubes and serial dilutions made from them were plated in duplicate on Trypticase Soy Agar plus 1% Tween 80 (Sigma Chemical Co., St. Louis, Mo.). All plates were incubated at 35°C for 48 h before CFUs were counted. Because there were no consistent differences between skin and fabric samples, these were combined in the tables summarizing results. An inoculated control site

was sampled at the start of each test. Other sampling times are specified in each experiment.

At each sampling time, all sites were examined for erythema, edema, and pH of the intact skin, and rayon fabric was measured with sterile multi-range pHydrion brand pH paper (Micro Essential Laboratory, Brooklyn, N.Y.).

Subjects. A total of 18 male and 10 female volunteers participated in the experiment after giving informed consent. No attempt was made to control diet or clothing, although bathing and vigorous physical activity, such as tennis playing or running, were discouraged to reduce variability in conductivity and permit adhesion of the tapes holding the apparatus in place.

Treatment of test sites. Test sites on each subject's back were covered with rayon fabric inoculated with 0.4 ml of an 0.85% saline suspension of the test organism. Positive, negative, or control electrodes or similar Saran-Wrap patches, a laboratory standard, were then applied, counterbalancing position effects on the backs of the various subjects. Each treatment was held in place with Dermicel taffeta tape (Johnson & Johnson). Positive and negative electrodes were 10 to 15 cm apart from center to center. Current flow was monitored before, during, and at the end of stimulation by completing the stimulating circuit through a Weston model 4442 digital multimeter (Weston, Inc., Newark, N.J.).

Sterilization and testing of materials. Before each experiment, all materials contacting the skin were sterilized with 2.5 Mrads of cobalt irradiation. All were tested for bacteriostatic activity on Nutrient Agar (BBL Microbiology Systems) streaked with test organisms. No bacteriostatic activity was observed in any of the test materials.

RESULTS

Determination of bactericidal activity. A total of 29 sites on the backs of human volunteers were covered with fabric inoculated with 10^7 microorganisms and sampled after 4 or 18 h of stimulation with 0, 10, 25, 50, 75, or 100 μA of total direct current.

The numbers of CFUs/cm^2 recovered from the intact skin and the rayon fabric beneath the positive electrode decreased with increasing stimulation time and increasing current density over 50 μA of total direct current or 5 μA per cm^2 of stimulated intact skin (Fig. 1). All current data are presented in terms of total current. For calculating current density, total current was divided by 10 cm^2, the effective electrode area.

In contrast, no significant effect of direct current stimulation was seen after any duration of stimulation or at any current density beneath negative electrodes in the same circuits. More than 10^5 CFUs/cm^2 were recovered from the skin surface and fabric on all negatively stimulated and control sites.

Menstruum effects. The effects of various menstrua were investigated both in vivo and in vitro. Six sites on the backs of each of three human volunteers were inoculated with 10^6 microorganisms, with either 0.85% saline (three sites) or 0.1% peptone in distilled water (three sites) as the menstruum. One site inoculated with each menstruum was then covered with either a control Condulon electrode or a positive or negative electrode conveying 14 to 90 μA of direct current to the skin.

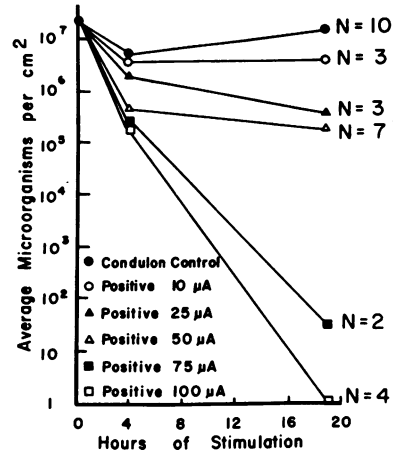

FIG. 1. *Effect of stimulation with positive direct current on inocula of normal microorganisms on human intact skin. N, Number of samples averaged per point.*

In vitro, 10^6 *S. epidermidis* isolated from human skin were suspended in 0.85% saline, 0.1% peptone, double distilled water, or citrated sheep's blood. Two layers of rayon fabric were inoculated with 0.4 ml each of the suspension and then placed between electrodes conveying 400 μA of direct current for 4 or 24 h. In this model, it was not possible to separate the effects of positive and negative electrodes because electrochemical by-products from both electrodes comingled in the fabric. However, the combined effects of positive and negative electrodes were investigated with the various menstrua.

In vivo, fewer microorganisms were recovered from both the intact skin and the fabric beneath positive electrodes conveying 2 or more μA/cm^2 for 18 h than from beneath negative or control electrodes (Table 1). This effect was evident with either saline or peptone suspension medium.

In vitro, at 40 μA/cm^2, the bactericidal effect was seen at 4 and 24 h with saline, double distilled water, and peptone water menstrua (Table 2). However, in the sheep's blood menstruum, a bactericidal effect was seen only after 24 h of stimulation.

Current density dependence of the bactericidal effect. Total direct current (microam-

TABLE 1. *Comparison of saline and peptone water as the moisturizing interfaces between electrodes and skin in vivo*

Subject	Test time (h)	CFUs/cm² in skin and fabric					
		Saline		Control	Peptone		Control
		+[a]	−[b]		+[a]	−[b]	
1	4	7.5×10^6	3.2×10^6	1.4×10^7	1.8×10^6	2.7×10^6	6.7×10^6
2	4	8.8×10^5	5.4×10^6	1.9×10^6	4.7×10^3[c]	$>1.5 \times 10^6$	3.6×10^6
3	4	1.6×10^6	3.6×10^6	3.2×10^6	3.2×10^6	8.6×10^6	7.5×10^6
1	18	8.5×10^4	8.4×10^6	2.6×10^6	4.7×10^5	5.2×10^7	4.0×10^6
2	18	2.4×10^2[c]	6.2×10^6	6.8×10^6	5[c]	10^6	2.4×10^6
3	18	1.1×10^4[c]	9.9×10^6	5.8×10^6	2.4×10^4[c]	1.1×10^7	1.2×10^7

[a] +, Positive electrode.
[b] −, Negative electrode.
[c] Significant reduction from control.

TABLE 2. *In vitro effect of 40 μA of direct current per cm² on bacteria at 4 and 24 h with different menstrua*

No. of tests	Menstruum	Stimulation time (h)	Avg no. of CFUs recovered from rayon fabric saturated with indicated menstruum placed between electrodes conveying	
			0 μA/cm²	40 μA/cm²
3	Saline	4	2.2×10^7	5
1	Saline	24	8.8×10^6	<5
2	Water	4	1.9×10^7	3.8×10^5
1	Water	24	9.7×10^6	<5
2	Peptone	4	2.0×10^7	5.0×10^4
1	Peptone	24	2.1×10^7	<5
2	Sheep's blood	4	1.7×10^7	10^7
1	Sheep's blood	24	2.4×10^6	<5

peres) and current density (microamperes per square centimeter) were varied independently to determine whether the effect of direct-current stimulation on microflora was dependent on total current or current density. Two sets of electrodes were applied simultaneously to the backs of each of four human subjects. One set conveyed a total of 50 μA through 10-cm² Condulon electrodes; the second set conveyed 10 μA through 2-cm² electrodes. Thus, both circuits delivered a current density of 5 μA/cm², although total current differed. Condulon control electrodes were either 10 or 2 cm² in area. To moisten the fabric and skin uniformly and avoid a bias in conductivity, inocula of 10^4 microorganisms were contained in 0.4 ml for a 10-cm² electrode area, 0.08 ml for a 2-cm² electrode area, and 0.04 ml for a 1-cm² electrode area.

Two more subjects were stimulated simultaneously with either 100 μA delivered through 10-cm² electrodes or 10 μA delivered through 1-cm² electrodes, yielding a current density of 10 μA/cm² through both sets of electrodes.

Two more subjects were stimulated simultaneously with each of two circuits conveying 10 μA through either 10- or 1-cm² electrodes, yielding current densities of 1 or 10 μA/cm², respectively. Test sites were sampled after 4 and 20 h of stimulation which followed the application of inocula. All patches of rayon fabric matched the electrodes in size and shape.

Current density and not total current was most closely associated with bacterial kill on human intact skin after both 4 and 20 h of stimulation (Table 3). The log viable microorganisms recovered from stimulated sites after 20 h of stimulation correlated −0.87 with current density but only −0.40 with total current in this set of experiments, in which the two variables were investigated independently. A correlation of ±0.78 is statistically significant at $\alpha \leq 0.05$.

Electrochemical dependence of the effect. This study determined whether the effects observed were electrochemical in origin. Electrodes were constructed from silver screen in such a way that the silver conductor was separated from the intact skin by filter paper and a dialysis membrane. These electrodes permitted current to flow in the same voltage range as that observed in the carbon film electrodes, less than 2.0 V per electrode, but prevented electrochemical by-products from reaching the skin or inoculated fabric.

Two sets of 10-cm² electrodes conveyed 10 μA/cm² to inocula of 10^4 bacteria on sites on the backs of each of three human volunteers for 4

and 18 h. Positive, negative and control silver screen and Velostat electrodes were contrasted on each subject.

The bactericidal effect of positive direct current was shown to occur only beneath the carbon-containing positive electrodes, which produced local acidity in the range of pH 3.0 or less (Table 4). Interestingly, the pH of 9.0 or more observed at the corresponding negative electrodes was not associated with a bactericidal effect.

Depth of the effect. The depth of the effect of direct current on human intact skin microflora was determined.

Three sites (2.5 × 4 cm) on the backs of each of six volunteers were inoculated with 10^4 bacteria, which were then allowed to proliferate under a Saran-Wrap film dressing for 24 h. The sites were then stimulated for 24 h with ± 10.0 or 7.5 $\mu A/cm^2$ or covered with a control Velostat electrode. Immediately after treatment, the microorganisms present in the stimulated skin were sampled with a tape stripping technique. A rectangle (2 × 3.5 cm) of sterile Dermicel taffeta tape was removed from its backing, applied to the center of the stimulated area, and pressed down firmly with sterile forceps. The Dermicel tape, termed strip no. 1, was then stripped off with an adhering layer of stratum corneum cells and resident bacteria and shaken for 15 min in a flask containing 10 ml of 10% Shell Tolusol (Shell Chem, Houston, Tex.), 5% aqueous HLB8, and glass beads to dissolve the adhesive mass. Serial dilutions were then plated on Trypticase Soy Agar. This procedure was repeated for the sampling of strip no. 2. Rectangles (2.5 × 4 cm) of highly tacky clear plastic tape were then used to remove successive strips of stratum corneum 3 to 10, which were not sampled. Dermicel tape samples were again taken on strips 11, 12, 21, 22, 31, and 32, with unsampled clear plastic tape being used to remove successive strips of the skin intervening between the sampled tapes.

Colonies on the plates made from the sampled strips were incubated for 48 h and then counted to determine the depth of the effect of direct current on the microorganisms in human skin.

The bactericidal effect extended at least 32 tape strippings beneath the skin surface to the Malpighian layer beneath the stratum corneum

TABLE 3. *Experimental conditions and results of current density experiments*

No. of subjects	Total current (μA)	Electrode area (cm^2)	Current density ($\mu A/cm^2$)	Time (h)	Mean viable microorganisms recovered from indicated site		
					Control	Positive DC[a]	Negative DC
2	10	10	1	4	4.3×10^4	1.1×10^4	3.1×10^4
				20	7.2×10^6	6.1×10^2	5.9×10^4
4	10	2	5	4	10^4	2.1×10^2	2.2×10^3
				20	1.7×10^7	4.5×10	2.3×10^6
4	50	10	5	4	1.9×10^2	2.1×10	7.4×10
				20	1.5×10^6	<1[b]	1.6×10^6
4	10	1	10	4	2.4×10^4	1.8×10^2	1.5×10^4
				20	2.5×10^5	<5[b]	3.1×10^4
2	100	10	10	4	7.4×10^2	2.7×10	1.9×10^3
				20	7.9×10^2	<1[b]	1.1×10^3

[a] DC, Direct current. A 2-log microbial reduction was considered significant (applies to all values in this column but 1.1×10^4).

TABLE 4. *Total CFUs recovered from skin surface and fabric after stimulation with 10 $\mu A/cm^2$ through Velostat or silver screen electrodes*

Treatment[a]	4 h of stimulation		18 h of stimulation	
	Skin pH	Avg CFUs recovered[b]	Skin pH	Avg CFUs recovered[b]
Inoculum		7.0×10^5		3.3×10^4
Untreated skin		1.9×10^3		1.6×10^4
Silver control	5.0	3.2×10^4	6.0	1.1×10^6
Silver +	5.0	1.1×10^5	6.7	1.5×10^6
Silver −	5.0	2.8×10^4	6.7	9.3×10^4
Velostat control	5.0	6.3×10^4	5.0	1.8×10^5
Velostat +	2.5	1.9×10^3	1.0	2.0×10^c
Velostat −			9.1	1.4×10^4

[a] +, Positive electrode; −, negative electrode.
[b] Average of three subjects.
[c] Significant reduction from control.

TABLE 5. *Microflora sampled from tape strippings of human back skin after 24 h of stimulation with direct current*

No. of observations	Current density ($\mu A/cm^2$)	Tape strippings (from surface)	CFUs recovered beneath[a]		
			Velostat +[b]	Velostat −	Velostat control
1	10.0	1, 2	8.5×10^2	1.6×10^5	1.3×10^6
		11, 12	5	2.0×10^3	1.2×10^4
		21, 22	<5	7.4×10^2	6.3×10^3
		31, 32	4.0×10	3.0×10^2	3.6×10^3
5	7.5	1, 2	3.2×10^2	1.2×10^5	6.0×10^5
		11, 12	4.2×10^2	2.0×10^4	3.0×10^4
		21, 22	2.4×10^2	1.2×10^4	2.7×10^4
		31, 32	3	1.4×10^4	8.2×10^3

[a] +, Positive electrode; −, negative electrode.
[b] Significant reduction from control for all values in this column.

(Table 5). In one subject in which the effect was observed, bleeding was noticeable after 21 tape strippings, indicating that the bactericidal effect extended to the peripheral dermis.

DISCUSSION

The bactericidal effect seen beneath positive carbon-containing electrodes conveying 5 or more μA of direct current per cm^2 was clearly independent of voltage and total current. The variables most closely associated with bacterial kill were current density and acidity, which was electrochemically generated at the positive electrode. The pH values observed at the effective stimulation sites are sufficient alone to produce bactericidal effects in the presence of various salt solutions (3), suggesting that the reduction of pH at positive carbon-containing electrodes may be the source of the effect.

Interestingly, a strongly alkaline environment generated electrochemically at negative electrodes conveying the same current density had no noticeable effect.

The electrochemical events leading to these local changes in pH are outlined in reactions 1 and 2. At the positive electrode, ions are attracted to the electrode surface from the medium, giving up electrons (e^-) to the electrode, as follows:

$$2\,H_2O - 2e^- = O_2\uparrow + 4\,H^+ \quad \text{at } E \geq +1.23\ V \quad (1)$$

At the negative electrode, electrons are repelled, as follows:

$$2\,H_2O + 2\,e^- = H_2\uparrow + 2\,OH^- \quad \text{at } E \leq -0.83\ V \quad (2)$$

It should be noted that these are only the initial events occurring in electrodes contacting pure water at the indicated voltages. Reactions differ and become considerably more complex when various suspension media are used. For example, in the presence of chloride ions, H_3O^+ may form complexes of hypochlorite or weak hydrochloric acid at the positive electrode. In the presence of the numerous ions and proteins in blood, the complex chemicals formed and the lengths of their existence(s) become difficult to predict. It may be the presence of some component of blood or its high buffering capacity which retards this bactericidal effect in the presence of citrated sheep's blood.

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