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Mechanisms of the bactericidal activity of low amperage electric current (DC)

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The mechanisms whereby low amperage (10–100 μA) electric current (DC) is bactericidal were investigated with *Staphylococcus epidermidis* and *Staphylococcus aureus*. A zone of inhibition test involving the insertion of an anode and cathode into an agar plate inoculated with a lawn of bacteria was used to study the antimicrobial activity of electric current. A zone of inhibition was produced around the cathode when 10 μA (DC) was applied for 16 h. The diameter of the zone was greatly reduced in the presence of catalase. There was no zone around the cathode when the test was carried out under anaerobic conditions. H_2O_2 was produced at the cathode surface under aerobic conditions but not in the absence of oxygen. A salt-bridge apparatus was used to confirm that H_2O_2 was produced at the cathode and chlorine at the anode. The antimicrobial activity of low amperage electric current under anaerobic conditions and in the absence of chloride ions against bacteria attached to the surface of a current carrying electrode was also investigated. Antibacterial activity was reduced under anaerobic conditions, which is compatible with the role of H_2O_2 as a primary bactericidal agent of electricity associated with the cathode. A reduction in chloride ions did not significantly reduce the antibacterial activity suggesting that chlorine plays only a minor role in the bactericidal activity towards organisms attached to anodal electrode surfaces. The localized production of H_2O_2 and chlorine and the intrinsic activity due to electric current may offer a useful method for eradicating bacteria from catheter surfaces.

Introduction

Infection is a major clinical complication associated with the use of central venous catheters (CVCs).[1] Treatment of these infections by antibiotics is relatively expensive, not always successful and frequently necessitates catheter removal. Bacteria attached to devices as biofilms are typically highly resistant both to antimicrobial agents and to host defences.[2,3] Prevention is therefore an important strategy to reduce CVC infections.[4] Previous approaches to the prevention of these infections have included modifying the catheter material and incorporating antimicrobial agents into the catheters to reduce colonization by microorganisms.[5] One novel approach is the use of low amperage electric current (DC) which repels micro-organisms from an electroconducting, carbon-impregnated catheter,[6] prevents intraluminal microbial migration[7] and is bactericidal against organisms attached to catheters.[8]

Bactericidal activity of electric current, both DC and AC, has been demonstrated in synthetic urine,[9] water[10,11] and salt solutions[12] and on human skin.[13] However, it has been suggested that electric current alone is not able to eradicate bacteria in a biofilm, but rather that it can act synergically with biocides.[14] Most of these investigations have used electric current of hundreds of milliamperes, either AC or DC.[11,12,15] At the milliampere level, toxic substances produced as a result of electrolysis, including H_2O_2, oxidizing radicals and chlorine molecules, have been suggested as the main bactericidal agents.[11,12] Other direct effects described include the oxidation of enzymes and

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coenzymes such as NADH, membrane damage leading to leakage of essential cytoplasmic constituents[16] and decreasing respiratory rate.[17]

In contrast, there are very few reports of the antimicrobial effects of electric current with amperage sufficiently low (<20 μA) as to be useful *in vivo*. Matsunaga *et al.* reported bactericidal activity of 10–20 μA DC when the surface to which bacteria attached became +0.74 V.[18] They demonstrated reduction of cellular functional enzyme CoA and concluded that electric current oxidizes this enzyme which leads to inhibition of cell respiration and eventually to cell death.[19] Similarly, we reported that 10 μA was bactericidal to organisms attached to electroconducting catheter surfaces.[8] Furthermore, previous studies have not differentiated antimicrobial activity of the cathode and anode when AC electrical current was used, nor when the electrodes were contained within an enclosed system with DC applied. In this present study, these variables were investigated further so that the mechanisms of the bactericidal activity of low amperage (up to 100 μA) DC on bacteria would be elucidated further.

Materials and methods

Catheters

Intravascular catheters made of 15% carbon-impregnated polyurethane, with an external diameter of 2.3 mm, and which conducted electricity were kindly supplied by Ohmeda (Swindon, UK). Catheters were steam-sterilized at 100°C for 30 min; this sterilization method does not disrupt the plastic polymers.

Electrical device

The electrical device consisted of a 9 V alkaline battery (Ever Ready, PP9), in series with a variable high-stability carbon film resistor (RS Components, Corby, UK) to generate a constant current of 10–100 μA (DC). In all experiments, flow of electric current was confirmed by placing an ammeter in series in the circuit.

Organisms

Experiments were carried out with two clinical isolates, *Staphylococcus epidermidis* 983 and 811, and two type cultures, *Staphylococcus aureus* NCIMB 6571 and *S. epidermidis* NCIMB 12721. The majority of the experiments were carried out with the type cultures.

Culture media and reagents

All culture media and reagents were obtained from Unipath Ltd, Basingstoke, UK, and chemicals were obtained from Sigma Chemical Co., St Louis, MO, USA. Nutrient agar was prepared by adding Agar No. 1 (L1), final concentration 1.5% (w/v), to nutrient broth (CM1). Blood agar was prepared by supplementing Columbia agar (CM331) with 7% defibrinated horse blood. For the investigation into the effect of electric current on bacteria attached to a catheter surface, a modification of the continuous culture broth for biofilm (BFB) described by Duguid *et al.* was used.[20] It consisted of glycerol, 10 mM; $(NH_4)_2SO_4$, 6 mM; $MgSO_4$, 0.5 mM; KCl, 13.5 mM; KH_2PO_4, 28 mM; Na_2HPO_4, 72 mM; thiamine, 1 mg/L; biotin, 0.5 mg/L; peptone, 3 mg/L. The medium was buffered to pH 7.4. All media were sterilized by autoclaving at 121°C for 15 min.

Mechanisms of the bactericidal activity of electricity as investigated by zone of inhibition tests

Three colonies obtained from cultures on nutrient agar slopes were inoculated into 5 mL of nutrient broth. The resulting bacterial suspension was then incubated at 37°C for 2 h. Four microlitres of the staphylococcal suspension (approximately 10^5 cells) was inoculated on to the surface of a nutrient agar plate. Three nutrient agar plates were prepared for each bacterium studied. Two carbon catheters (2.5 cm long) were placed perpendicularly in one nutrient agar plate. The catheters were then connected to an electrical device which generated 10 μA DC via external leads with one catheter acting as a cathode and the other as an anode (Figure 1). The agar plate was then incubated at 37°C, in air for 16 h. The second agar plate was flooded with 0.5 mL of a freshly prepared catalase solution (EC 1.11.1.6 of bovine liver origin) which had 1500 units of activity (one unit decomposes 1.0 μmol of H_2O_2 per min at pH 7.0 at 25°C at 10.3 mM H_2O_2). This agar plate was then left at room temperature for up to 5 min to allow the catalase to be absorbed by the agar. Two catheters were then placed perpendicularly in the agar, and electric current of 10 μA

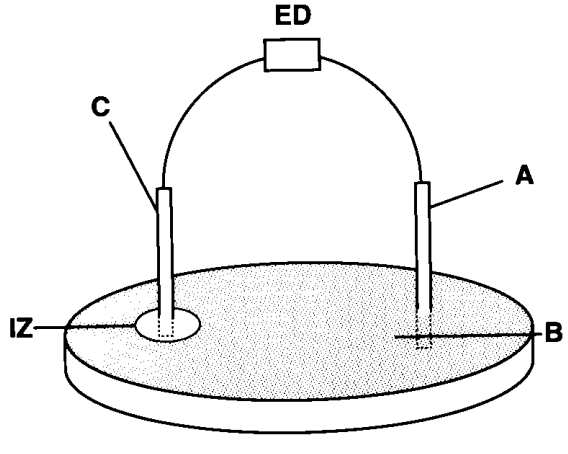

Figure 1. Mechanisms of the bactericidal activity of electricity as investigated by the zone inhibition test. A, catheter as anode; B, lawn growth of bacteria; C, catheter as cathode; ED, electrical device; IZ, inhibition zone.

was applied as described above. This plate was then incubated at 37°C, in air, for 16 h. The final agar plate of the set was placed in an anaerobic cabinet (Model Anaerobic 22290, Microflow Anaerobic System, Andover, UK) with an atmosphere of 10% CO_2, 10% H_2 and 80% N_2 at 37°C. After 10 min, two catheters were placed in the nutrient agar plate and connected to one electrical device as described above. The plate was then incubated under these conditions for 16 h. Following incubation, catheters were removed from the plates and the diameters of any zones of inhibition around the catheter insertion sites into the agar were measured with a vernier calliper. The diameter was taken as the total distance between the outer edges of the zone with the centre of the catheter acting as the midpoint. The staphylococcal strains were tested on six separate occasions. Oxygen concentration in the anaerobic cabinet was measured with an oxygen meter (OX 20, Walden Precision Apparatus Ltd, Cambridge, UK).

Effect of various concentrations of catalase on the zones of inhibition

To quantify the effect of catalase on the zones of inhibition, various amounts of the enzyme were dissolved in phosphate-buffered saline (PBS) and added to 20 mL of molten nutrient agar that had been cooled to 44°C (final concentration of catalase ranged from 50 to 2500 units/mL agar). After thorough mixing, the plates were allowed to set. Four microlitres of staphylococcal suspension prepared as above were then spread on to each plate. Two carbon catheters were placed perpendicularly in the agar and connected to the electrical device which generated 10 μA DC. All plates were incubated in air, at 37°C for 16 h. After the catheters had been removed, the diameters of the zones of inhibition were measured. *S. aureus* NCIMB 6571 and *S. epidermidis* NCIMB 12721 were tested six times at each concentration of catalase.

Effect of microaerophilic and anaerobic atmospheres on the zones of inhibition

Four microlitres of staphylococcal suspension were spread on to each of five nutrient agar plates. Two catheters were then placed in each nutrient agar plate as described above. An electrical device that generated 10 μA was connected to the catheters of one of the five plates and was then incubated in air, at 37°C. Another plate with the two catheters connected to an electrical device that generated 10 μA was placed in an anaerobic container. A palladium catalyst pack and a Gas Generating Kit, Campylobacter System (Oxoid BR 60), prepared according to the manufacturer's instructions, were immediately placed in the container which was then sealed and incubated at 37°C. Microaerophilic conditions, with approximately 5% oxygen, were achieved within 30 min. The remaining three nutrient agar plates with the catheters were placed in an anaerobic cabinet. An electrical device that generated either 10, 75 or 100 μA DC was then connected to the catheters of each plate via external leads. All five nutrient agar plates were incubated for 16 h and then the diameters of the zones of inhibition measured as described above. *S. aureus* NCIMB 6571 and *S. epidermidis* NCIMB 12721 were each tested six times.

Detection of hydrogen peroxide

After measuring the diameter of the zones of inhibition, a Merckoquant strip (E. Merck, Darmstadt, Germany) for detecting H_2O_2 was placed on the nutrient agar within the zone. The strip contained a peroxide that transfers oxygen from a peroxide or a hydroperoxide group to an organic redox indicator. The indicator is then converted to a blue-coloured oxidation product which can be detected visually. Each strip measures 0.5–25.0 mg/L H_2O_2 semiquantitatively. The presence of $<$0.5 mg/L of H_2O_2 was indicated by the presence of blue colour in the reaction area.

Detection of H_2O_2 and chlorine by salt-bridge apparatus

The salt-bridge is a standard apparatus for investigating electrolysis (Figure 2). The system used here consisted of two glass Universal containers of 30 mL volume. Fifteen millilitres of BFB were placed in each container, and the two containers were connected via a salt-bridge, which consisted of a polyurethane tube (internal diameter 1.5 mm, length 150 mm) filled with the same solution as in the containers. One carbon catheter (2.5 cm long), which acted as an electrode, was then placed in the broth of each container. The catheters were then connected to an electrical device which completed the circuit. The ions in the solutions acted as conductors of electric current. A current of either 10 or 100 μA was applied for 14 h and H_2O_2 was detected by a Merckoquant strip. Free chlorine was

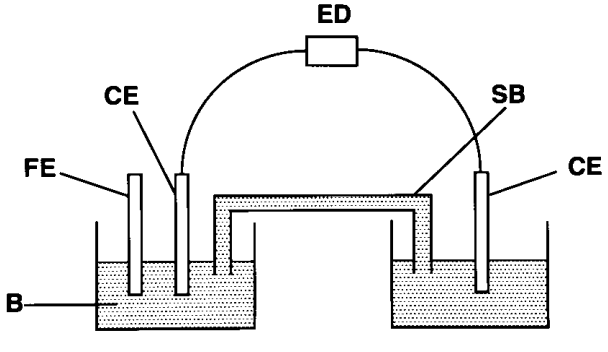

Figure 2. Salt-bridge apparatus. B, bacterial culture broth; CE, catheter as electrode, ED, electrical device; FE, field catheter; SB, polyurethane tube as salt-bridge.

detected by the *N*,*N*-diethyl-*p*-phenylene diamine colorimetric method using a Lovibond chlorine kit (Tintometer Ltd, Salisbury, UK) with a minimum detection limit of 0.1 mg/L free chlorine.

Colonization of catheters

Two or three colonies of *S. epidermidis* NCIMB 12721 from a nutrient agar slope were inoculated into 5 mL of nutrient broth which was then incubated at 37°C in air for approximately 2.5 h. The resultant bacterial suspension was then diluted into 100 mL nutrient broth to attain a viable count of 1×10^6 cfu/mL. Segments of carbon catheters (2.5 cm) were placed in the broth for 2 h at 37°C with continuous stirring to allow the staphylococci to attach to the catheter surface. After colonization, the catheters were removed from the broth and a metal wire with a loop at the end was threaded through their lumen. The catheters were then suspended in 30 mL PBS in a Universal container. The containers with the catheters were then centrifuged at 660**g** for 5 min so that any loosely attached organisms were removed.

Influence of anaerobic conditions on the antibacterial activity of electrical current

A salt-bridge apparatus placed in an anaerobic cabinet was used to study the antibacterial activity of electric current on organisms attached to a catheter surface. The containers and the linking bridge were filled with BFB without magnesium ions. Two colonized catheters, one of which acted as the cathode, were placed vertically in a 300 mL glass container containing 100 mL of broth. All the broth had been previously stored in the anaerobic cabinet for at least 24 h. A sterile, non-colonized catheter which acted as the anode was placed in another container, which also contained 100 mL broth. The broth of the two containers were connected with a salt-bridge as described above. An electrical device was connected to one of the two catheters in both containers to generate a constant current of 10 μA. The other catheter in the first container, which was the field catheter, was not connected to any electrical source. After 14 h all catheters were removed and examined by the roll-plate technique as described by Maki *et al.*[21] Catheters were rolled on Columbia agar supplemented with 7% defibrinated horse blood which was then incubated at 37°C for 24 h in air. The number of cfu on the plate was then counted with a colony counter (Gallenkamp, UK). When the number of cfu exceeded 1000, this was recorded as >1000 as an accurate count over this number was not possible. The dissolved oxygen concentration in the broth was measured with an oxygen meter.

Influence of chloride ions in the test medium on the antibacterial activity of electric current

The salt-bridge apparatus was prepared as described above, but used under aerobic conditions. One hundred microlitres of BFB, with or without potassium chloride, was added to each container and the linking salt-bridge was filled with the same broth. Two colonized catheters were placed in the first container, with one catheter acting as the anode and the other as the field catheter. One sterile non-colonized catheter was placed in the second container as the cathode. An electrical device that generated 100 μA DC was applied to the catheters via external leads and the apparatus was incubated at 37°C, in air, for 14 h. All the catheters were then removed and roll-plated to determine the viable count. Chloride concentration in the sterile biofilm broth without potassium chloride was measured by ion chromatography using a Dionex Chromatograph (Sunnyville, CA, USA) with a detection limit of 0.01 mg/L.

Table I. Zones of inhibition produced around the cathode under aerobic and anaerobic conditions with or without catalase. The catalase (1500 units in total) was spread over the nutrient agar plate.

Organism	Zone diameter (mm)[a] aerobic	aerobic, with catalase	anaerobic, without catalase
S. aureus NCIMB 6571	11.5 ± 1.2	–[b]	–
S. epidermidis NCIMB 12721	10.2 ± 1.0	5.4 ± 2.1[c]	–
S. epidermidis 983	11.3 ± 1.0	7.2 ± 1.1[c]	–
S. epidermidis 811	11.1 ± 3.2	<4.0[d]	–

[a] Mean ± S.D.; $n = 6$. The zone of inhibition was measured from edge to edge across the centre of the catheter and the external diameter of the catheter was 2.3 mm.
[b] No zone of inhibition observed, as the bacteria grew up to the catheter–agar interface.
[c] Significantly different from the zone sizes under aerobic conditions, $P < 0.01$, Wilcoxon Signed Rank Test.
[d] No zone of inhibition observed ($n = 3$) and mean = 4.0 mm ($n = 3$).

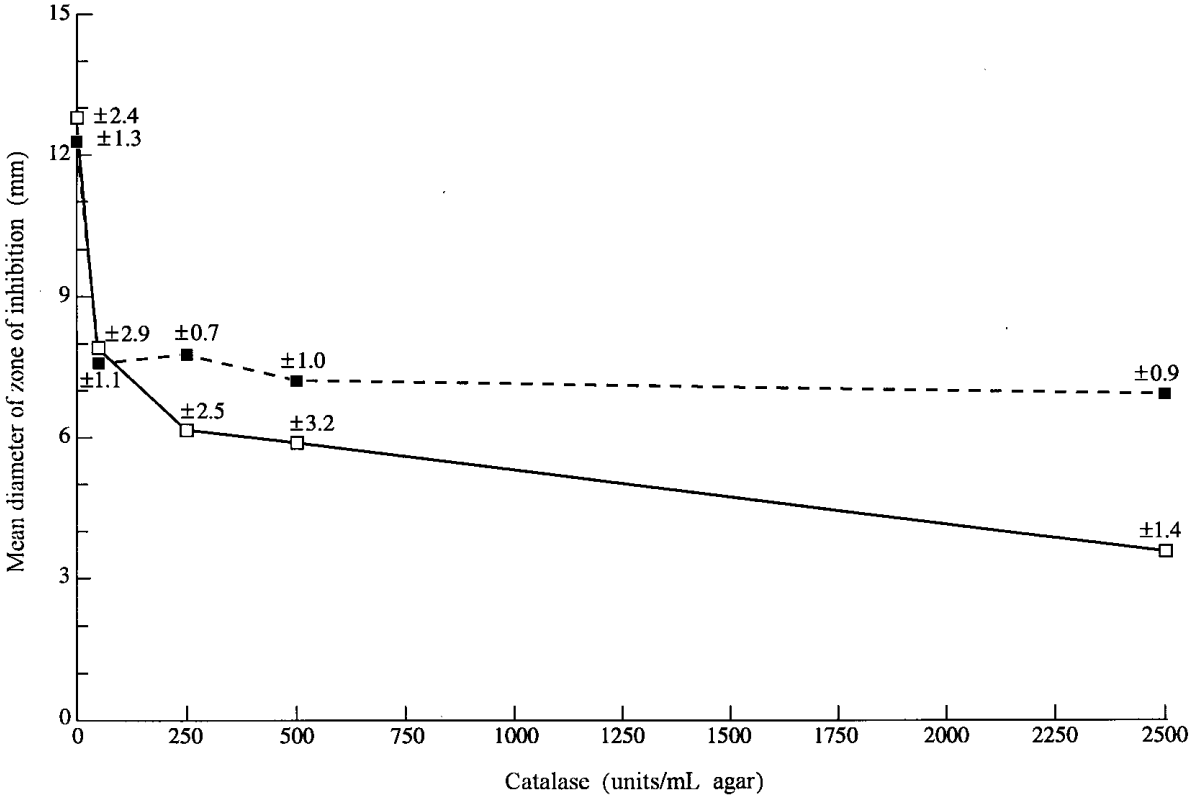

Figure 3. Effect of various concentrations of catalase added to medium on the size of the zone of inhibition around the cathode (mean ± 1 S.D., $n = 6$). – – ■ – –, *S. epidermidis* NCIMB 12721; ——□——, *S. aureus* NCIMB 6571.

Results

Mechanisms of bactericidal activity of electricity as investigated by the zone of inhibition test

In the majority of experiments, there were no zones of inhibition around the anode with all the staphylococcal strains tested. On a few occasions, a rim (<1 mm width) of clear area around the catheter–agar interface was observed. The diameters of the zone of inhibition produced around the cathode in air, in air with catalase and in anaerobic conditions without catalase are shown in Table I. The diameters of the zones of inhibition in air for all staphylococcal strains were similar (mean 10.2–11.5 mm). Addition of catalase to the agar reduced the zone size for the three strains of *S. epidermidis* and there was no zone with *S. aureus* NCIMB 6571. When the zone of inhibition tests were carried out in anaerobic conditions (gaseous oxygen concentration < 0.1%), there were no zones around the cathode for any of the organisms tested.

Effect of various concentrations of catalase on the diameter of the zone of inhibition

Addition of catalase as low as 50 units/mL agar significantly reduced the zone size ($P < 0.01$, Wilcoxon signed rank test) as compared with control (Figure 3). When *S. epidermidis* NCIMB 12721 was tested, increasing the amount of catalase from 50 to 2500 units/mL agar did not reduce the diameter of the zones of inhibition any further or eliminate them completely. With *S. aureus* NCIMB 6571, increasing the amount of catalase reduced the zones of inhibition gradually, but still did not eliminate them completely even at the highest concentration (2500 units/mL agar) of the enzyme.

Effect of microaerophilic and anaerobic atmospheres on the zones of inhibition

The diameter of the zone of inhibition around the cathode was reduced as the oxygen concentration decreased under microaerophilic conditions (Table II). When the zone of inhibition tests were carried out in anaerobic conditions, no zone was formed around the cathode, and the organisms grew up to the catheter–agar interface. Increasing the electric current to 100 μA did not alter this effect. However, the absence of oxygen had no influence on the activity of the anode. In addition, as the amperage increased under anaerobic conditions, the zone size increased similarly.

Detection of H_2O_2

H_2O_2 was detected in the agar within the zone of inhibition around the cathode only. The concentration was up to 20 mg/L (0.59 mmol/L) at the catheter–agar interface and

Table II. Zones of inhibition produced under different atmospheric conditions

Atmospheric conditions[b]	Current (μA)	Zone diameter (mm)[a] *S. aureus* NCIMB 6571 cathode	*S. aureus* NCIMB 6571 anode	*S. epidermidis* NCIMB 12721 cathode	*S. epidermidis* NCIMB 12721 anode
Aerobic	10	12.3 ± 1.0	4.3 ± 0.5	8.0 ± 0.9	2.6 ± 1.0
Microaerophilic	10	6.8 ± 0.8[c]	3.2 ± 1.3	6.0 ± 1.4[c]	0.0
Anaerobic	10	–[d]	3.8 ± 2.2	–	3.8 ± 1.0
Anaerobic	75	–	12.8 ± 3.5	–	13.6 ± 1.0
Anaerobic	100	–	16.2 ± 1.2	–	15.7 ± 1.4

[a] Mean ± S.D.; $n = 6$. The zone of inhibition was measured from edge to edge through the centre of the catheter and the external diameter of the catheter was 2.3 mm.
[b] Oxygen concentrations under different conditions were: aerobic, 20.9%; microaerophilic, approximately 5%; anaerobic, 0.1%.
[c] Significantly different from the zone sizes under aerobic conditions ($P < 0.01$, Wilcoxon signed rank test).
[d] No zone of inhibition observed, as the bacteria grew up to the catheter–agar interface.

gradually diminished over a 2–3 mm distance from the catheter surface. Within the zone of inhibition in the agar with added catalase, H_2O_2 was not detected.

Detection of H_2O_2 and chlorine in the salt-bridge apparatus

After the application of 10 μA DC, H_2O_2 was detected only in the broth associated with the cathode. It was detected semi-quantitatively by the Merckoquant strip after 4 h of application of electric current and increased to 5–10 mg/L (0.15–0.30 mmol/L) after 16 h. However, chlorine was not detected in the broth associated with the anode throughout the 16 h. When 100 μA was applied for 16 h, 10–20 mg/L H_2O_2 was detected in the broth associated with the cathode, and 0.1 mg/L chlorine in the broth associated with the anode.

Influence of anaerobic conditions on the antibacterial activity of electric current

Ten microamperes DC has been shown to be bactericidal to organisms attached to the surface of an electroconducting catheter when the test was carried out in aerobic conditions.[8] Therefore, if the activity of electric current applied to the organisms attached on the catheter surface was examined under anaerobic conditions, any bactericidal activity present at the cathode would indicate mechanisms other than electrolysis are involved. When the activity of electric current (10 μA DC) on organisms attached to catheter surfaces was carried out in anaerobic conditions, the mean viable count (241 cfu/cm length of catheter) on the cathodal catheter surface was reduced. This was significantly lower ($n = 4$, $P < 0.01$) than the control catheter and the field catheter since both viable counts were >1000 cfu/cm length catheter. The dissolved oxygen concentration in the broth was 0.1 mg/L and H_2O_2 was not detected by the Merckoquant strip.

Influence of chloride ions in the test medium on the antibacterial activity of electric current

As the removal of oxygen from the test medium did not eliminate the bactericidal activity of the cathode, the effect of omitting chloride from the BFB on the antibacterial activity of the anode was also examined. At 10 μA, the antibacterial activity at the anode was not consistently observed in the zone inhibition test, therefore 100 μA was applied. When chloride was omitted from the BFB, the viable count at the anode surface was still significantly lower than that in the control (Table III). The reduction in viable count for the catheter suspended in broth without chloride was significantly different from the broth with added chloride. The broth without potassium chloride contained 1.25 mg/L chloride ions.

Discussion

In a previous report we described the formation of a zone of inhibition around catheters that acted as cathodes when DC electrical charge was applied to bacteria grown on nutrient agar.[7] By using a low amperage (10 μA) DC, antibacterial activity was observed around the cathode but not the anode.[8] Formation of a zone of inhibition around a current carrying catheter placed in a lawn of bacteria suggested the effect of a concentration gradient and indicated the presence of a diffusible substance. The effect on the diameter of the zone of inhibition of spreading catalase on the agar shows that this substance is H_2O_2. The presence of catalase reduced the diameter of the zones of inhibition, but the four staphylococcal strains showed various degrees of reduction in zone diameter. Catalase is one of the most

Table III. Viable counts of *S. epidermidis* NCIMB 12721 on the catheter surfaces in test media with/without chloride ions added, after 14 h of application of 100 μA DC under aerobic conditions ($n = 6$) (cfu/cm length of catheter)

	1.35 mM KCl added			No KCl added		
	control[a]	field[b]	anode	control	field	anode
Mean	975	645	7	794[c]	469[d]	36
Median	1000	704	4	818	409	3
Range	800–>1000	88–1000	0–19	489–>1000	74–1000	0–207

[a] Control catheters were placed in their respective broths under the same conditions as tests, but without an electric current.
[b] Field catheter was placed in the anodal bath, but not connected to the electrical device.
[c] Significantly lower than the control with 1.35 mM KCl added ($P < 0.01$, Wilcoxon signed rank test).
[d] Significantly lower than the viable count of the field catheter with 1.35 mM KCl ($P < 0.01$, Wilcoxon signed rank test).

efficient enzymes, with a turnover of 4×10^7 (mole substrate)/(mole enzyme)/s.[22] With 1500 units of catalase spread on the agar surface, no zone of inhibition was recorded with *S. aureus*. This was probably a result of the higher intracellular content of catalase in some strains of *S. aureus*.[23] This intrinsic content of catalase in combination with the added enzyme may have been sufficient to nullify completely the effect of any H_2O_2 produced.

The high efficiency of catalase was demonstrated further by the effect that adding various amounts of catalase in the agar had on the diameter of the zones of inhibition. Concentrations as low as 50 units of catalase per mL agar reduced the zone size significantly, whereas increasing the catalase from 50 to 2500 units/mL agar had no drastic effect. H_2O_2 production must be very rapid and its concentration near the catheter–agar interface must be so high that low concentrations of catalase cannot decompose it fast enough to eliminate a zone of inhibition completely. The presence of H_2O_2 in the zone of inhibition was confirmed by the detection with the Merckoquant strip, which detects peroxide and hydroperoxide groups. The highest concentration (up to 0.6 mmol/L) of H_2O_2 produced was at the catheter–agar interface and diminished outwardly over a distance of 2–3 mm. Therefore, it appeared that the zone size around the cathode in the presence of catalase in the medium was determined by the rate of diffusion of, and the rate of catalytic decomposition of H_2O_2.

The production of H_2O_2 at the cathode required oxygen. This is consistent with the half-cell reaction describing the electrolysis of hydrogen ions in the presence of oxygen:

$$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2 \quad (+0.68\ V)^{24}$$

The amount of H_2O_2 produced is in a stoichiometric relation with the concentration of oxygen. If H_2O_2 is the main vehicle for inhibition, the zone should become smaller in the absence of oxygen. Under microaerophilic conditions less H_2O_2 is produced and the zone size decreased as predicted by the half-cell equation. This was confirmed in experiments carried out under anaerobic conditions, where no zones of inhibition were observed even when the amperage was increased to 100 μA. Previous studies demonstrated the production of H_2O_2 peroxide when relatively high (400 mA) AC was applied to phosphate buffers without chloride ions.[15] However, the production of H_2O_2 was not related to either the cathode or anode because AC was used. In the present study, using the zone of inhibition test, H_2O_2 was shown for the first time to be associated with the cathode when lower levels of DC (10 μA) were applied.

Chlorine and chlorine-related compounds have been shown previously to be the bactericidal agents when 400 μA DC was applied in synthetic urine, but chlorine production was not related to either anode or cathode.[25] As there was no strip method to detect chlorine in agar, the role of chlorine when low current was applied was investigated in a salt-bridge apparatus in this study. By linking the broth of the two containers, each with an electrode, via a salt-bridge, their contents remained separate but a complete electrical circuit was achieved. Any antibacterial substances produced in either container would therefore be independent of the corresponding electrode. When 10 μA was applied, no chlorine was detected in either containers. However, 0.1 mg/L chlorine was produced in the broth containing the anode when the electric current was increased to 100 μA DC. It is therefore likely that the zone of inhibition observed around the anode at ⩾75 μA is caused partly by chlorine. At the anode, chloride ions lose their electrons to produce chlorine molecules:

$$2Cl^- \rightarrow Cl_2 + 2e^- \quad (+1.40\ V)^{24}$$

In a multi-ion situation such as a bacterial culture broth and with a potential difference of 9.0 V, all ions, including chloride and hydronium ions, may be electrolysed. However, which electrolysis reaction takes place depends on factors such as the half-cell potential, the concentrations of the ions and the physical state of the electrolysis products.[24] The lower the half-cell potential, the easier for the reaction

to take place. The potential for the formation of H_2O_2 (+0.68 V) is lower than the formation of oxygen from water at the anode (+1.23 V) or chlorine from chloride ions (+1.40 V).[24] Furthermore, oxygen is available in large quantity in aerobic conditions, and so facilitates the electrolysis of oxygen to H_2O_2. As the half-cell potential for the production of chlorine from chloride by electric current is higher than that of H_2O_2, chlorine was probably present only when an electric current of $\geqslant$75 μA was applied as observed in the experiment. The amount of electrolysis product is also proportional to the amount of electric charge available as is evident by the increase in zone size around the anode as the current increased.

Our finding is the first report to demonstrate that an electric current as low as 10 μA when applied to a multi-ion solution, electrolysed ions in the solution, and consequently produced the antibacterial substances, H_2O_2 and chlorine, at the cathode and anode respectively. Furthermore, as current was increased, generation of H_2O_2 (+0.68 V) appeared to precede the production of chlorine (+1.4 V). This may be due to the relative instability and minute quantity of free chlorine produced, which did not result in a zone of inhibition at 10 μA. As the current increased (at 100 μA), both reactions took place concurrently. It is possible that other bactericidal substances such as HO_2, ozone and OCl^- are also produced. Their presence is difficult to demonstrate since they are unstable and no practicable specific detection methods are available.

Other studies have previously used metallic electrodes to demonstrate the inhibitory effects of electric current on microorganisms.[14,26] However, electrolysis takes place even at low amperage levels. If metallic electrodes were used in studies on the effect of current on bacteria, they would interfere with the process. In particular, they can corrode and affect the bactericidal activity of electric current.[9]

While these results established the products of electrolysis firmly as the bactericidal agents, electricity *per se* may have antibacterial activity. Bacteria attached to the surface of an electrode are exposed to the effects of electric current including electric field and potential gradient. In comparison, bacteria suspended in the associated broth would not be exposed to the same degree. To investigate this intrinsic activity of electricity we applied electric current to bacteria attached to a catheter surface and suspended it in broth after the removal of chloride ions and oxygen. When these ions and molecules were excluded, the viable counts of bacteria on the catheter surfaces were still reduced significantly as compared with the control. This bactericidal activity associated with the cathode cannot be attributed to H_2O_2 despite the presence of residual of oxygen in the test medium. The dissolved oxygen concentration in the test medium was 0.1 mg/L (6 μmol/L) and if all the oxygen were to be converted to H_2O_2, only 3 μmol/L would be produced. This concentration of H_2O_2 is below the MIC of *S. aureus* (0.15 mmol/L).[27] The sensitivity of *S. epidermidis* NCIMB 12721 to H_2O_2 is similar to that of *S. aureus* NCIMB 6571 in the zone inhibition test and so the bactericidal effect cannot be due to H_2O_2. The concentration of residual chlorine in the BFB was 1.25 mg/L, and if all these chloride ions were to be electrolysed to chlorine there would be 0.6 mg/L chlorine molecule produced. This concentration of chlorine would only be bactericidal if it was all concentrated on the catheter surface. This is unlikely to have occurred here as the broth in the experiment was continuously mixed. It therefore appears that electricity *per se* at the anode also has antibacterial activity. This antibacterial activity is effective only on the electrode surface as the bacteria attached to the field catheter were still viable even when 100 μA DC was applied. The mechanism of this activity may include the disruption of the integrity of the bacterial membrane or electrolysis of molecules on the cell surface.

The initial stage of colonization of a catheter is the attachment of bacteria to the surface. Once they are attached, they then develop into microcolonies. Disrupting this stage of colonization of the catheter is thus an important step in preventing catheter-related infections. The antibacterial activity of an electroconducting catheter acting as cathode fulfils this requirement. The intrinsic activity is effective against organisms attached on the catheter surface. The production of H_2O_2, again at the surface of the electrode, enhances the activity of electric current further. The concentration of dissolved oxygen in plasma is 0.14 mmol/L.[28] There is therefore likely to be sufficient oxygen in the blood to facilitate continuous production of H_2O_2. Any organisms adhered to a current-carrying catheter should therefore be exposed continually to the H_2O_2 produced. Furthermore, free H_2O_2 eventually decomposes to harmless oxygen and water. This novel method of preventing catheter-related infections delivers the antibacterial H_2O_2 to specific sites where it is most needed. Although chlorine production requires a higher amperage, it could be useful in other body sites, such as the urinary tract where a larger amount of electric current might be tolerated.

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