

# Lethal Effects of Electric Current on *Escherichia coli*

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An attempt has been made to use low-voltage alternating current to kill microorganisms such as *Escherichia coli*. The bactericidal effect depends on the energy passing through the suspension and on the time during which the cells are left standing in the medium after the treatment. Most of the toxicity is due to an indirect effect developed with unalterable electrodes in the presence of chlorides in the medium. This method might be applied to eliminate pollution of natural waters.

The bactericidal or bacteriostatic action of certain chemicals has already been tested to reduce pollution by microorganisms. Several attempts have also been made to use electric current as a killing agent. It has been found that milk or drinking water can be sterilized when low-value alternating current passes through the liquid (2, 3, 10). High frequency discharges or high voltage sparks (1, 4, 5) also have a bactericidal effect. The loss of viability depends upon the amount of energy used. Brandt et al. (4) suggested that the bactericidal effect occurring after electrical discharge may be due to oxidizing substances, probably in the form of free radicals. Under similar conditions, Gilliland and Speck have shown that the use of electrodes made of different metals may cause a high residual toxicity (6). However, it appears that it is much easier to use low-voltage electric current rather than high voltage discharges, especially for industrial purposes.

We have then studied the effects of low-frequency alternating current (50 cycles/sec) ranging from 10 to 200 ma on the viability of *Escherichia coli*.

## MATERIALS AND METHODS

**Growth of the cells.** *E. coli* K-12 was grown in a liquid medium at 37°C; this medium contained peptone (Difco, 8 g/liter), nutrient broth (Difco, 3 g/liter), NaCl (5 g/liter). A log-phase culture was centrifuged and washed. The pellet was resuspended in the same volume of minimal medium without glucose (7) (medium I in Table I). This suspension was then treated with electric current. After treatment, samples were taken to estimate the surviving fraction. Dilutions were made in minimal medium without glucose, and samples were plated on complete medium and incubated overnight at 37°C.

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**Electric equipment.** The bacterial suspensions were exposed to electric current in a 0.75-ml plastic cell. The two flat electrodes were connected to a potential transformer related to an alternating current power supply (50 cycles/sec; Fig. 1).

## RESULTS

**Effect of current value on viable count.** Since several authors have mentioned that the bactericidal effect observed in experiments with alternating current is mainly due to heat, our experiments were performed in such a way that the temperature of the bacterial suspension did not reach 40°C at the end of the treatment, as measured with a mercurial microthermometer in standard experiments (2, 3, 10). The total time of exposure never exceeded 10 sec.

The effect of electric current on viability of *E. coli* is shown in Fig. 2. The lethal activity depends upon the strength of the electric current passing through the liquid. The minimum energy required corresponds to 25 ma. Beyond this lower limit, the viability decreases with the current values.

**Indirect effect.** Since in all these experiments the number of viable cells decreases with time (Fig. 3a), the bacteria are kept in the treated medium for 30 min before the surviving fraction is determined. Brandt et al. (4) have already noticed that the killing effect of submerged electrical discharges increases when the bacterial suspensions are left standing for some time before the viable count is taken. When the plating is done immediately after exposure, there is no decrease in the number of viable cells. Therefore it seems likely that there is no direct lethal effect, but an indirect effect.

When untreated cells are added to a medium through which the current has just passed, the loss of viability is not as high as the loss observed after treatment of the cells in suspension (Fig.

TABLE 1. *Composition of media used for bacterial suspension<sup>a</sup>*

Medium I (minimal medium)	Medium II	Medium III	Medium IV
K <sub>2</sub> HPO <sub>4</sub> 3 KH <sub>2</sub> PO <sub>4</sub> 1	K <sub>2</sub> HPO <sub>4</sub> 3 KH <sub>2</sub> PO <sub>4</sub> 1		K <sub>2</sub> HPO <sub>4</sub> 3 KH <sub>2</sub> PO <sub>4</sub> 1
NH <sub>4</sub> Cl 5 NH <sub>4</sub> NO <sub>3</sub> 1 Na <sub>2</sub> SO <sub>4</sub> 2	NH <sub>4</sub> Cl 5 NH <sub>4</sub> NO <sub>3</sub> 1 Na <sub>2</sub> SO <sub>4</sub> 2	NH <sub>4</sub> Cl 5 NH <sub>4</sub> NO <sub>3</sub> 1 Na <sub>2</sub> SO <sub>4</sub> 2	
CaCl <sub>2</sub> 0.1 <sup>b</sup> MgSO <sub>4</sub> 0.1			

<sup>a</sup> Values are in grams per liter unless otherwise indicated.

<sup>b</sup> Milligrams per liter.

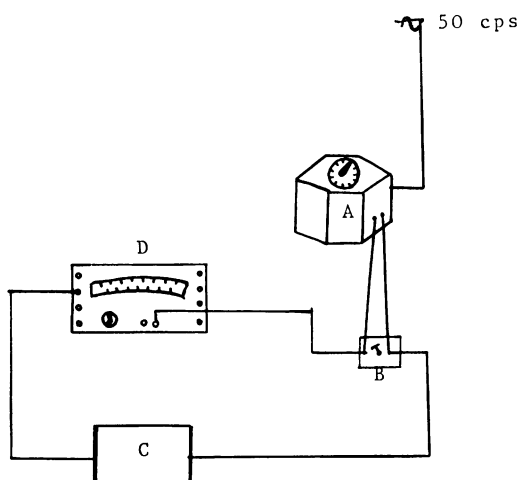


FIG. 1. *Electric equipment.* (A) Potential transformer; (B) switch; (C) cell; (D) milliammeter.

3b). After 30 min, the treated medium loses its toxic activity completely. The toxicity might be due to labile compounds whose effect can be inhibited by the addition of cysteine or protein in the medium. (Table 2). The indirect effect produced by the treated medium cannot explain all the toxicity of the treatment as suggested by experiments on inactivation by electrohydraulic shock (5). We then tried to measure the direct effect on the treated cells which were immediately separated from the suspension medium. One half of such treated cells were suspended in an untreated medium, and the other half suspended in a medium that had just been treated. The first batch gave rise to the initial number of bacteria, the second gave rise to the same number of cells as was found after the treatment of the whole suspension (Table 3).

According to the results, direct damage of the cells is not evident but we must assume that

there is nevertheless a direct effect, since the toxicity of the treated medium alone cannot account for the low survival observed when the whole suspension is treated.

**Response to chloride-containing medium.** The medium used for treatment of the bacterial suspension consists of different salts (*see* Table 1). We prepared different media ranging from the complete medium to a phosphate solution (media I to IV), in which cells were resuspended.

The killing effect is still observed in media II and III but not in medium IV. We then prepared the solutions of each of the three salts of medium III at a final concentration of 0.1 M in phosphate buffer. The loss of viability occurred only in the ammonium chloride and sodium chloride solutions (Table 4). It can be concluded that the chlorides must be involved in the lethal effect of an electric current.

**Metallic nature of electrodes.** All our experiments were performed with stainless steel electrodes. The lethal effect of alternating current is identical when we use platinum electrodes, but lethal effect could not be detected with electrodes made of ordinary steel.

## DISCUSSION

In addition to the increase in temperature, it is obvious that electrical current has a strong lethal activity. We have shown that the presence of chlorides is essential for the development of the killing effect in the treated medium. This activity is mainly due to an indirect effect, since the bacteria lose their viability when added to treated medium. However the bacteria can be completely repaired after treatment if they are

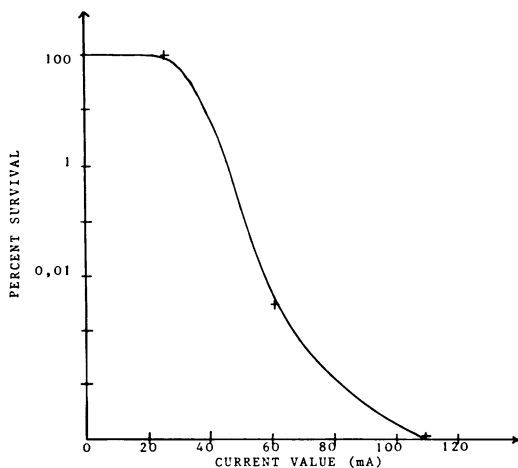


FIG. 2. *Effect of current values on viable count of Escherichia coli.* The bacterial suspensions are held for 30 min after treatment before plating.

plated immediately on complete medium. The toxicity lasts about 20 min and disappears with the addition of cysteine. The toxic products seem to be oxidizing substances similar to free radicals, as observed after irradiation (8, 9) or electrical discharges (1, 4, 5). However,

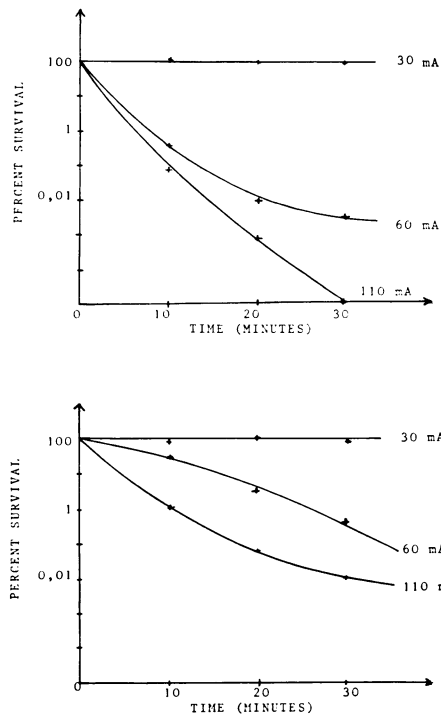


FIG. 3. Effect of holding time on viable count of *Escherichia coli*. (a) Bacterial suspensions treated with electric current; (b) untreated bacteria added to the treated medium.

TABLE 2. Protection by cysteine or albumin against toxicity of electric current<sup>a</sup>

Concn	Bacteria <sup>b</sup>	
	Untreated cells	Treated cells
Cysteine		
0.5 × 10 <sup>-5</sup> M	2.9 × 10 <sup>7</sup>	1.8 × 10 <sup>7</sup>
0.5 × 10 <sup>-6</sup> M	2.9 × 10 <sup>7</sup>	1.2 × 10 <sup>7</sup>
0	2.3 × 10 <sup>7</sup>	0
Albumin		
4 × 10 <sup>-2</sup> mg/ml	2.5 × 10 <sup>7</sup>	4 × 10 <sup>5</sup>
4 × 10 <sup>-4</sup> mg/ml	2.5 × 10 <sup>7</sup>	0.9 × 10 <sup>3</sup>
0	2.5 × 10 <sup>7</sup>	0

<sup>a</sup> Treatment: 10 sec, 110 ma, 30-min delay before plating.

<sup>b</sup> Values are number of bacteria per milliliter.

TABLE 3. Survival of treated bacteria in untreated or newly treated medium as a measure of the indirect effect<sup>a</sup>

Bacteria per ml	After immediate plating	After 30-min delay
Treated cells in untreated medium.....	3 × 10 <sup>7</sup>	3.1 × 10 <sup>7</sup>
Treated cells in newly treated medium.....	3 × 10 <sup>7</sup>	0

<sup>a</sup> Treatment: 10 sec, 60 ma.

(a)

TABLE 4. Survival of bacteria in salt-containing phosphate buffer after treatment with electric current<sup>a</sup>

Salt	After immediate plating	After 30-min delay
NH <sub>4</sub> NO <sub>3</sub>	2 × 10 <sup>7</sup>	1.8 × 10 <sup>7</sup>
Na <sub>2</sub> SO <sub>4</sub>	1.5 × 10 <sup>7</sup>	1.6 × 10 <sup>7</sup>
NH <sub>4</sub> Cl	1.9 × 10 <sup>7</sup>	0
NaCl	1.7 × 10 <sup>7</sup>	0

<sup>a</sup> Treatment: 10 sec, 160 ma.

electrohydraulic shock is effective even in de-ionized water, whereas alternating current requires the presence of chlorides in the medium.

Gilliland and Speck suggest that the "residual toxicity" obtained after discharges using copper-core electrodes is caused by cupric ions shed in the buffer (5). In our experiments, the toxicity cannot be of this nature, since we get a bactericidal effect by using electrodes made of platinum or stainless steel, and not with ordinary steel. It is possible that some metal ions are formed as electric current is passed through the medium, and they would inhibit cell division (11).

By using direct current, we tried to show the formation on one electrode of chlorine containing compounds such as hypochlorite. But these experiments did not give reproducible results

In any case, the existence of an indirect effect is not sufficient to explain the loss of viability of the cell suspensions treated with electric current. The cells are probably sensitized to the treated medium when they are themselves treated. However, since we get the initial number of bacteria after plating, we have to assume that repair mechanisms completely restore the ability of the cells to divide. Although the exact nature of the bactericidal effect is not yet elucidated, the results indicate the possibility of using low-voltage alternating current to kill the microorganisms in chloride-containing suspensions such as polluted natural waters.

This method might be applied to the destruction of sulphur reducing bacteria in oil field waters to avoid biological corrosion.

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