The Effect of the Pressure Shock Wave and Some Electrical Quantities in the Microbicidal Effect of Transient Electric Arcs in Aqueous Systems

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SUMMARY

Micro-organisms in aqueous suspensions were killed when voltages above a certain threshold value were discharged through electrodes immersed in the suspensions. The relation of the peak pressure, peak current and the arcing time to the killing effect were studied, but no clear-cut relationship was ascertained. At the discharges shock waves with durations of 20–105 μsec. and pressure amplitudes of 40–250 bar were generated. Although bigger and more solid objects were considerably damaged, the micro-organisms were inconspicuously affected by the pressure shock wave alone.

INTRODUCTION

When micro-organisms suspended in water are subjected to submerged high-voltage electrical discharges, a large proportion of them are killed (Brandt et al. 1962; Allen & Soike, 1966). The spectrum of sensitive organisms is wide and the efficiency high, which might make this method useful for practical disinfection purposes. Several physical and chemical activities are initiated by the electrical discharge. From the plasma channel emanate a pressure shock wave, photon radiation and more or less unstable chemical compounds. Information about the relation of the microbicidal effect to the different non-biological effects of the discharge might elucidate the mechanism of action and lead to a high microbicidal efficiency of the process being obtained.

METHODS

The electric circuit used and its physical and electrical quantities

The electric circuit used is shown in Fig. 1. The step-up transformer (3) is supplied with a variable voltage from a variac (2) connected to the a.c. mains (1). The high voltage capacitor (6) is charged through a resistor (4) and a rectifier (5). The capacitor voltage will reach a ceiling value, \( U \), determined by the spark gap (8) operating in air. The breakdown of gap (8) causes a current to flow between the submerged electrodes (9). The capacitor will discharge through the circuit elements (7–8–9) of Fig. 1. The capacitance, \( C \), is measured in microfarad (\( \mu \text{F.} \)) and the inductance, \( L \), in microhenry (\( \mu \text{H.} \)). The electrodes (9) were submerged in water.
To initiate a breakdown of the water between the submerged electrodes and to bridge the electrodes with an arc the capacitor must be initially charged with a voltage \( U \geq U_b \). The breakdown voltage \( U_b \) is a function of the electrode separation \( s \), the water conductivity and the capacitance \( C \). The current through the submerged gap is a damped sinusoidal current (e.g. Fig. 5 of Brandt et al. 1962). The arc is extinguished after one or more half-cycles of current.

![Electric circuit diagram](image)

**Fig. 1.** The electric circuit. 1, Mains; 2, variac; 3, step-up transformer; 4, resistor; 5, rectifier; 6, capacitor; 7, choke; 8, spark gap; 9, submerged electrodes. \( U \), initial capacitor voltage; \( u \), voltage between submerged electrodes; \( i \), discharge current; \( C \), capacitance; \( L \), inductance.

The pressure shock wave originates from the plasma arc between the submerged electrodes. It travels through the liquid with the speed of sound. The peak pressure develops during the rise of current during the first current cycle. The variation of the peak pressure with voltage \( U \), choke inductance \( L \) and electrode separation \( s \), was studied with the electrodes submerged in tap water in a steel tank of about 500 l. capacity (fig. 2 of Brandt et al. 1962). The pressure was recorded by a piezoelectric tourmalin crystal connected with a cathode-ray oscilloscope. The crystal diameter (0.25 in.) limited the measuring accuracy of the pressure rise time. The pressure was also recorded behind membranes of variable thickness.

Standard deviation, \( \sigma = \sqrt{\frac{(x - \bar{x})^2}{n - 1}} \), where \( n \) is the number of observations, \( x \) the measured value and \( \bar{x} \) the arithmetic mean.

**Microbiological methods**

*Escherichia coli* B was cultivated in 200 ml. nutrient broth (Difco) on a shaker table at 37°. After incubation for 18 hr the culture was cooled to 0°, centrifuged, the deposited organisms washed once in 0.01M-sodium phosphate (pH 7.0) and then suspended in distilled water. The total count was estimated after dilution and counting in a Buerker Chamber with 0.01 mm. depth. Colony counts were made by spreading 0.1 ml. of tenfold dilutions on the surface of nutrient agar plates. A standard diluent containing Difco nutrient broth, 1 g.; NaCl, 5 g.; distilled water, 1000 ml., was used throughout. The plates were incubated at 37° for 1 day, and those with 20-400 colonies were counted. Each value given is the arithmetic mean of three individual determinations. Phage suspensions were prepared according to Frick (1961) and titrated by the plaque technique (Adams, 1959).
The experiments on the microbicidal effect of the discharges were made in a vertical stainless-steel tube vessel (Fig. 2). Immediately after the discharge 0.5 ml. of treated microbial suspension was transferred to 4.5 ml. diluent and kept in an ice-water bath until further dilution and plating. This storage did not appreciably affect the colony count. When no dilution was to be done, 0.1 ml. was directly pipetted on to nutrient agar plates.

Fig. 2. Vertical stainless steel tube discharge vessel. 1, Water; 2, high-voltage electrode; 3, plastic insulation; 4, earthed electrode.

In some experiments the suspension of micro-organisms was isolated from all other recognized effects of the submerged discharges except the pressure by putting the suspension in a metal box placed on the bottom of the discharge vessel (Fig. 2). The box-wall facing the electric arc consisted of a steel membrane of different thicknesses. In other experiments the suspension was contained in a cellophan bag (tube diameter 6.35 mm.) and placed on the bottom of the discharge vessel. Samples of these suspensions were taken with syringe and needle.

RESULTS

Separate discharges performed in the same kind of liquid with the same electrical set-up produced different results with respect to the peak value and duration of the pressure and the current, and to the killing effect (Table 1). Also the sound of the bang accompanying the discharge varied markedly. A loud bang was generally accompanied by a higher bactericidal effect than a weak one. This variation is supposed to be inherent in the nature of the discharge, which is rapid and far from equilibrium. Several experiments were therefore made to determine the results of a certain type of discharge.
At voltages \( U < U_b \) no plasma channel was formed in the water. At such voltages the noise accompanying the discharge was much weaker or inconspicuous, and no killing effect or pressure shock wave were recorded. At higher voltages \((U > U_b)\) the peak pressure and the peak current increased with voltage. At these voltages an oscillating current was produced (e.g. fig. 5 of Brandt et al. 1962). As seen from Fig. 3, the threshold voltage for killing effect was higher at greater electrode separation \((s)\) and the killing effect increased with voltage until a plateau was reached. This maximum killing effect was smaller and was attained at lower voltages at shorter distances between the electrodes.

When the circuit inductance was increased, the peak pressure and the peak current decreased and the arcing time increased (L. Edebo & I. Selin, unpublished). The bactericidal effect was affected by changes in the circuit inductance as shown in Table 1. For several different micro-organisms, voltages and electrode separations, the maximum bactericidal effect was achieved at an intermediate inductance value in the range of 40–400 \( \mu H \). (Fig. 4).

The pressure behind steel membranes of different thicknesses was oscillographically

![Graph](image)

**Table 1. Escherichia coli B17 suspended in tap water and subjected to one discharge**

<table>
<thead>
<tr>
<th>Inductance ((\mu H))</th>
<th>Maximum (\times 10^4)</th>
<th>Minimum (\times 10^4)</th>
<th>Mean (\bar{x}) (\times 10^4)</th>
<th>Standard deviation, (\sigma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>7.0 (\times 10^4)</td>
<td>2.6 (\times 10^4)</td>
<td>4.9 (\times 10^4)</td>
<td>23</td>
</tr>
<tr>
<td>126</td>
<td>1.7 (\times 10^4)</td>
<td>7.8 (\times 10^2)</td>
<td>1.2 (\times 10^4)</td>
<td>20</td>
</tr>
<tr>
<td>767</td>
<td>3.8 (\times 10^4)</td>
<td>1.2 (\times 10^4)</td>
<td>2.2 (\times 10^4)</td>
<td>38</td>
</tr>
</tbody>
</table>
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recorded and compared with recordings without membrane (L. Edebo & I. Selin, unpublished). Within each group there was considerable difference between individual discharges. The average peak pressure behind a membrane, however, deviated less than ±11 % from the pressure without a membrane. This deviation is smaller than the standard deviation of individual discharges from the same experimental set-up. Consequently, the insertion of a thin metallic membrane between the arc and a suspension of micro-organisms should not cause any appreciable change in the pressure effect on the micro-organisms.

Table 2. Effect on Escherichia coli B in a steel box with different membrane thicknesses and in a cellophan bag

<table>
<thead>
<tr>
<th>Container</th>
<th>No. of discharges</th>
<th>Colony count (bacteria/ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>0</td>
<td>7.8 × 10⁶</td>
</tr>
<tr>
<td>Box, membrane 0.06 mm.</td>
<td>40</td>
<td>6.5 × 10⁶</td>
</tr>
<tr>
<td>Box, membrane 0.093 mm.</td>
<td>40</td>
<td>6.6 × 10⁶</td>
</tr>
<tr>
<td>Box, membrane 0.291 mm.</td>
<td>40</td>
<td>6.2 × 10⁶</td>
</tr>
<tr>
<td>Box, lid 3.83 mm.</td>
<td>40</td>
<td>6.7 × 10⁶</td>
</tr>
<tr>
<td>Cellophan bag</td>
<td>4</td>
<td>2.4 × 10⁵</td>
</tr>
</tbody>
</table>

Table 3. Effect on coliphage T5 in different containers

<table>
<thead>
<tr>
<th>Container</th>
<th>No. of discharges</th>
<th>Plaques/ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>0</td>
<td>5.7 × 10⁴</td>
</tr>
<tr>
<td>Box, membrane 0.06 mm.</td>
<td>20</td>
<td>7.1 × 10⁴</td>
</tr>
<tr>
<td>Cellophan bag kept in the box above</td>
<td>20</td>
<td>5.3 × 10⁵</td>
</tr>
<tr>
<td>Cellophan bag on the bottom of the discharge</td>
<td>3</td>
<td>5</td>
</tr>
</tbody>
</table>

A steel box with a steel membrane cover and containing a suspension of Escherichia coli was submerged in water in which electrical discharges were initiated. No significant change in the number of colonies was observed (Table 2). When very dilute suspensions of micro-organisms were used, a minor killing effect was noticed, but such an effect was produced even without discharges. When, instead of enclosing the bacteria in a metal box, the bacteria were kept in a cellophan bag, a considerable killing effect was achieved with discharges. Whereas 40 discharges did not produce any significant decrease in colony count of a suspension kept in the metal box, 4 discharges decreased the colony count 20-40-fold in the cellophan bag. No killing effect by discharges was observed in experiments when the bag was placed in the metal box, or placed below a piece of U-shaped iron plate standing on its legs, or when it was wrapped into a piece of black plastic sheet.

Experiments similar to those described for Escherichia coli B were also made with bacteriophages. Suspensions of coliphage T5 enclosed in the steel box and subjected to discharges showed little or no inactivation of the phages. When, however, phage enclosed in a cellophan bag was subjected to a much smaller number of discharges, there was almost complete inactivation (Table 3).
DISCUSSION

The experiments described in this paper were planned to investigate whether the killing effect might be correlated to any one of the main physical or electrical activities of the discharge. By increasing the circuit inductance, the peak pressure, the pressure gradient and the peak current were decreased without affecting the energy content of the discharge. Since a moderate increase of the circuit inductance increased the microbialicidal effect, none of the above-mentioned activities can be held solely responsible for the killing effect.

It was possible to separate the pressure shock wave from other activities by screening off the discharge gap with a thin steel membrane. Although pressure measurements showed no appreciable change of the shock wave when passing through the membrane, the killing effect was completely extinguished. We conclude that the pressure alone had no microbialicidal effect.

It might seem surprising that pressure shock waves which were capable of buckling 0.5 mm. thick iron plate did not kill the micro-organisms. However, when the physical nature of the shock wave in relation to the micro-organism was considered, the results seemed less strange. Assume a pressure wave with a peak pressure of 50 bar and a rise time of 10 μsec. The velocity of a pressure shock wave in water is about 1.5 mm./μsec. Hence: the corresponding pressure front is about 15 mm. and the pressure gradient 50/15 bar/mm. An Escherichia coli bacterium is generally shorter than 3 μ along its long axis. This means that the pressure difference along the length of the bacterium is about 0.003 x (50/15) bar, i.e. 0.01 bar. This is quite small compared with the intracellular pressure of E. coli which has been estimated as 4 bar (Mitchell & Moyle, 1956). Consequently, the mechanical shearing stress on a bacterium by the pressure shock wave is negligible. Electron photomicrographs of bacteria subjected to 40 discharges, which left a surviving fraction less than 10⁻⁶, did not show any morphological changes in the bacteria. Staphylococci and streptococci, which are smaller and have considerably higher intracellular pressure than E. coli, were affected to approximately the same extent by the electrical discharges (Brandt et al. 1962). All these data support the view that the killing effect of the discharges depended little on the pressure gradient.

Besides the shearing stress, the pressure gradient can also be considered as a sudden squeezing of the micro-organisms. Hydrostatic pressures of the magnitude measured in our experiments have no known killing action on micro-organisms (Larson, Hartzell & Diehl, 1918; Johnson & Lewin, 1946; Rutberg, 1964; Hedén, 1964). For Escherichia coli a pressure of 1058 atmospheres for 5 min. is required to cause an almost 90% decrease of the colony count (Rutberg, 1964). The sudden application and removal of the moderate pressures formed by discharges did not seem to affect their innocuousness for micro-organisms.

Bacteria kept in a cellophan bag were killed by the discharges, in contrast to bacteria within a steel box. This might indicate that, instead of pressure, the photon radiation was important for the killing effect. This supposition was further supported by the fact that bacteria suspended in water but not in broth or milk were effectively killed by discharges.

Below a certain threshold voltage no killing effect could be demonstrated. At higher voltages an increase in voltage enhanced the killing effect until a plateau was reached.
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Since the energy consumption of the discharge increases with the square of the voltage, a voltage increase after the plateau has been reached would lead to waste of energy under these conditions. Studies on the maximum efficiency will be reported later.

Recently a method for sterilization by electrohydraulic treatment has been described (Allen & Soike, 1966). In those experiments the microbicidal effect of multiple discharges of 14 kV. and 5–95 μF. has been investigated. Although the voltage was lower and the capacitance higher than those used by us, their results indicate that the activities responsible for the killing effect are similar.

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REFERENCES


