The Effect of the Photon Radiation in the Microbicidal Effect of Transient Electric Arcs in Aqueous Systems

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SUMMARY

The importance of photon radiation for the microbicidal effect of electrical discharges in water has appeared from a series of experiments. (a) An ultra-violet (u.v.)-sensitive strain of *Escherichia coli* was more susceptible to discharges than a u.v.-resistant mutant derived from it. (b) The kinetics of the inactivation by discharges were under certain conditions similar to those of continuous u.v. irradiation. (c) Addition of u.v.-absorbing substances to the discharge liquid decreased the bactericidal efficiency of the discharges to an extent which paralleled the u.v. absorbance. (d) The bactericidal effect decreased as the distance from the spark increased. (e) Bacteria enclosed in a cellophan bag were killed by discharges outside the bag, even when the bag was kept in the air above the discharge liquid. (f) Also discharges in air were active. (g) Bacteria inactivated by discharges were accessible to photoreactivation, but the magnitude of the reactivation was generally less than that obtained with bacteria inactivated by continuous u.v.-irradiation. In addition to direct radiation effects other kinds of microbicidal activities were produced by the electric discharges.

INTRODUCTION

Submerged electrical discharges in water have a potent microbicidal effect for a wide range of micro-organisms (Brandt *et al.* 1962; Allen & Sioke, 1966). The physico-chemical effect of the discharges consists of, amongst others, ionization, heat, a pressure shock wave, radiation, and chemical effects. Previous experiments (Edebo & Selin, 1968) showed that the microbicidal effect of the pressure shock wave was insignificant, nor could any of the main electrical quantities be correlated with the killing effect. However, a microbial suspension enclosed in a dialysis bag was inactivated by discharges, unless it was shielded from the electric arc (Edebo & Selin, 1968). These results indicated that radiation from the arc might be essential for the microbicidal effect.

METHODS

The *Escherichia coli* B strains B15 (u.v.-sensitive) and B17 (u.v.-resistant) were obtained from Dr G. Bertani, Department of Microbial Genetics, Karolinska Institutet, Stockholm.

The electrical equipment, cultivation of micro-organisms and colony counts were made as described previously (Edebo & Selin, 1968). When the killing effect in free suspensions in the discharge vessel (fig. 2 of Edebo & Selin, 1968) was investigated, each value was calculated as the arithmetic mean of three individual determinations.

The difference between each one did not usually exceed 30%. In some experiments suspensions of bacteria were enclosed in four separate cellophan bags, fastened to a metal frame and placed in the discharge vessel. Discharges were made and then samples taken from the cellophan bags by means of a syringe and needle. The spread of colony count values was greater in these cases, sometimes, particularly at high killing effects, the largest value being nearly 9 times the smallest. Therefore each such experiment was calculated as the geometric mean of the four individual determinations.

After treatment with discharges or continuous u.v. irradiation 3 ml. samples of the bacterial suspensions in test-tubes were subjected to visible light (Philips Attralux, 24 V., 150 W, > 3800 Å.) for photoreactivation. The samples were immersed in a water bath (24–28°) to prevent heating, and light was admitted through 45 × 62 mm. windows in the wall of the container. The distance from the wall of the container to the centre of the test-tube was 90 mm., the illumination time 30 min.

Standard electrical arrangement (SEA): capacitance (C) = 0.6 μ F., inductance (L) = 43 μ H., discharge voltage = 45 kV.; electrode separation = 11 mm.; electrode tips consisted of copper + tungsten alloy; liquid volume in the discharge vessel = 1200 ml.

Symbols in the Figures unless otherwise stated: solid circles and continuous lines represent samples diluted 1/10 in the diluent (nutrient broth 1 g., NaCl 5 g./l.) immediately after the discharges; open circles and broken lines those after overnight incubation in the refrigerator. Each circle is the result of one experiment from which three different samples were taken.

The characteristics of the tap water were: colour < 5 mg. Pt/l., permanganate 4–6 mg. KMnO_4 /l., conductivity 354–413 μ mho./cm., pH 7.1–7.7, total hardness calculated as Ca 101–108 mg./l.; ammonium < 0.1, manganese < 0.05, bicarbonate 262–286, chloride 18–21, sulphate 26–34, nitrate 9–36, nitrite < 0.01, phosphate < 0.01 mg./l. Bacteria: nutrient gelatine at 20° for 48 h < 1 colony/ml.; lactose broth 37° < 1/100 ml.

RESULTS

Microbicidal effect of discharges with relatively high energy content (620 J.)

The microbicidal effect on various concentrations of bacteria was investigated for *Escherichia coli* strains B15 and B17 in 0.001 M-KCl and in tap water (Figs. 1–4). Potassium chloride was added to distilled water, because a certain conductivity was needed to produce this type of discharge (Edebo, Holme & Selin, unpublished results). When suspensions of *E. coli* B15 in 0.001 M-KCl were subjected to discharges of SEA (see methods), a large proportion of the bacteria were killed. When the logarithm of the colony count was plotted against the number of discharges, it approached a straight line (Fig. 1). The killing effect might therefore be approximately described by $S = e^{-NK}$, where S is the fraction of surviving colony-forming units, N is the number of discharges, and K is a constant characteristic for the electrical arrangement the suspending fluid and the micro-organism investigated. For practical reasons the base 10 was chosen instead of e , $S = 10^{-Nx}$, and the microbicidal effect of the individual discharges ($-x$) expressed as log per discharge (l.p.d.). For suspensions of *E. coli* B15 whose initial colony counts were between 2×10^6 and 2.5×10^7 bacteria/ml. the microbicidal effect was 3.0–2.8 l.p.d. For more concentrated suspensions it became less, successively diminishing with higher bacterial concentrations. When the bacterial

suspensions which had been subjected to discharges were left overnight in the refrigerator before dilution and plating, the number of colonies recovered was further decreased, while the count of untreated bacteria remained almost constant. Suspensions with initial colony counts of 10^7 bacteria/ml. and less did not show any growth (i.e. < 10 bacteria/ml.), when subjected to one discharge and kept overnight at 4° . For *E. coli* B15 in tap water the microbicidal effects at the initial concentrations 2.2×10^6 , 2.2×10^7 and 2.2×10^8 bacteria/ml. were 2.4, 1.9 and 0.4 l.p.d. respectively (Fig. 2). No protracted killing effect was observed when these suspensions were kept overnight in the refrigerator.

Fig. 1

Fig. 2

Fig. 1. *Escherichia coli* B15 suspended in 0.001 M-KCl and subjected to discharges (SEA and symbols, see Methods).

Fig. 2. *Escherichia coli* B15 suspended in tap water and subjected to discharges (SEA).

The microbicidal effect on *Escherichia coli* B17 suspended in 0.001 M-KCl at concentrations between 9.6×10^5 and 8.3×10^6 bacteria/ml. was 2.0 l.p.d. (Fig. 3). At moderately higher concentrations the inactivation was less but fairly constant in consecutive discharges. At an initial concentration higher than 10^8 bacteria/ml. the killing curve deviated considerably from a straight line. The fourth and the fifth discharge had the greatest killing effect. The protracted killing effect achieved by keeping overnight in the refrigerator was, however, considerable also at high concentrations; only for initial concentrations above 10^7 bacteria/ml. were the colony counts sufficiently high to allow an approximate estimation. In tap water at concentrations of 2.7×10^6 and 2.9×10^7 bacteria/ml. the bactericidal effect varied between 1.6 and 1.2 l.p.d. (Fig. 4). At a concentration of 2.5×10^8 bacteria/ml. it was decreased to about 0.2 l.p.d. The protracted effect was generally negligible.

In these experiments the u.v.-sensitive B15 strain of *Escherichia coli* was more strongly inactivated by discharges than the u.v.-resistant strain B17. However, the

Fig. 3

Fig. 4

Fig. 3. *Escherichia coli* B17 suspended in 0.001 M-KCl and subjected to discharges (SEA).

Fig. 4. *Escherichia coli* B17 suspended in tap water and subjected to discharges (SEA).

kinetics of the inactivation of strain B17 deviated considerably from that of continuous u.v. radiation.

The influence of albumin on the microbicidal effect

The presence of albumin at 100 mg./l. ($A_{260m\mu} = 0.125$) in a liquid containing *Escherichia coli* B15 decreased the immediate killing effect of discharges slightly

Fig. 5

Fig. 6

Fig. 5. *Escherichia coli* B15 suspended in different concentrations of albumin and subjected to one discharge (SEA). Upper horizontal lines represent viable counts of samples not subjected to discharges. —, Conductivity; — · —, absorbance.

Fig. 6. The relation of the u.v.-absorbing capacity of the discharge liquid to the bactericidal effect on *Escherichia coli* B17 enclosed in cellophan bags and immersed in the discharge liquids. SEA, two discharges. ●, DNA; ◆, RNA; ×, albumin; □, dextran; ■, blue dextran; ■, green dextran; ■, yellow dextran; ■, red dextran; ○, yeast suspension.

(Fig. 5); at higher concentrations the effect was gradually decreased. In suspensions containing albumin, 1 g./l., no bactericidal effect was observed. The protracted bactericidal effect was still more sensitive to albumin. No protracted effect could be demonstrated at concentrations ≥ 200 mg./l.; at 50–100 mg./l., 98–99 % of the bacteria surviving immediately after the discharge were killed later, and at 20 mg./l. and below, < 10 to 40 bacteria/ml. were recovered after keeping overnight in the refrigerator. These experiments showed that albumin decreased the immediate and the protracted killing effect. They did not show, however, whether this was due to protective action on the bacteria themselves or to a general neutralization of some physical or chemical activities of the discharges.

Fig. 7

Fig. 7. Absorbance of 1 g./l. solution of DNA (—), RNA (---), albumin (-.-.), and dextran (.....).

Fig. 8

Fig. 8. Absorbance of g./l. solution of variously coloured dextrans: (—), blue; (---), green; (-.-.), yellow; (.....), red.

The microbicidal effect on Escherichia coli suspensions enclosed in cellophan tubing immersed in discharge liquids containing light-absorbing substances

When cellophan tubing containing *Escherichia coli* B17 was placed on the bottom of the discharge vessel and two discharges made, the microbicidal effect was strongly influenced by the nature of the surrounding liquid (Fig. 6). Substances with high u.v. absorbing capacity, e.g. DNA, RNA, albumin (Fig. 7), decreased the microbicidal effect even at low concentrations; the nucleic acids were particularly active. Dextran at 1 g./l. influenced the bactericidal effect little (this solution had low u.v. absorbance); however, with a dextran concentration at 10 g./l. the bactericidal effect was almost completely extinguished. This dextran solution (10 g./l) was viscous and the sound accompanying a discharge was weak, which might imply that the discharge itself was affected. Before the discharges all the dextran was not dissolved; after the discharges, however, no undissolved material remained. When solutions with chromophores chemically bound to the dextran molecules were used (substances provided by AB Pharmacia, Uppsala), the decrease of the killing effect was considerably greater. These substances showed a fairly strong absorption in the u.v. region (Fig. 8) which paralleled their decrease of the killing effect. No correlation with other parts of the spectrum was observed.

The microbicidal effect on suspensions enclosed in cellophan tubing and placed at different distances from the spark

When a suspension of *Escherichia coli* strain B was enclosed in a cellophan tube and placed in a large discharge vessel (fig. 2 of Brandt *et al.* 1962) at different distances from the spark gap, the bactericidal effect became less at greater distances from the spark gap. At a distance of 8 cm. one 45 kV. discharge decreased the colony count more than 300-fold; at distances more than 20 cm. the killing effect was hardly measurable.

The microbicidal effect of discharges in liquid and in air

When different volumes of an *Escherichia coli* strain B 15 suspension were subjected to one discharge, the bactericidal effect was dependent on the volume (Fig. 9). The surviving fraction had a maximum at 600 ml., i.e. when the surface of the liquid was just below the electrodes so that the discharge took place in the air. In these experiments it should be observed that at the same spark gap (fig. 1:8, Edebo & Selin, 1968) the discharge voltage with electrodes in the air was higher than that with submerged electrodes.

Fig. 9. The effect on different volumes of a suspension of *Escherichia coli* B 15 in tap water of discharges in the suspension (> 700 ml). and in the air above the suspension (< 700 ml.). One discharge. SEA except spark gap = 12 mm. (i.e. 38 kV. with electrodes in water) and $s = 12.9$ mm. No discharge = 1.6×10^5 bacteria/ml.

Cellophan tubing containing *Escherichia coli* B 15 was placed on the bottom of the discharge vessel and at the same distance above the electrodes, and discharges were made with different volumes of tap water in the vessel, similar to the experiments described in Fig. 9. With no liquid and 600 ml. in the discharge vessel the discharge took place in the air, with 800 and 1200 ml. it occurred in the water. The cellophan tubes placed above the electrodes were surrounded by air. Except when no tap water was added the tubes below the electrodes were immersed in water. The colony counts of the samples kept in either medium were greatly decreased by discharges in water and in air. The greatest killing effect on the average was obtained with 600 ml. water in the discharge vessel. With regard to the original colony count the differences between the

results with the different volumes were small. Therefore the efficiency of discharges in water and in air could not be adequately compared, since also reflexion, refraction and absorption of the radiation varied between different experiments, and at the same spark-gap a higher voltage was needed for discharges in air. Experiments where air was injected through the lower of two vertical electrodes while discharges were performed gave low bactericidal effects.

The microbicidal effect of submerged discharges with relatively low energy content (91 J.) compared with discharges in air and continuous u.v. radiation

The bactericidal effect of 620 J. discharges did not show the typical u.v. inactivation curves on *Escherichia coli* B17 (Figs. 3, 4). Therefore discharges with less energy

Fig. 10

Fig. 11

Figs. 10, 11. *Escherichia coli* after treatment with submerged (1200 ml., ●) and air (200 ml., ×) discharges, and with continuous u.v. irradiation (■). The units along the abscissa signify no. of discharges or min. u.v. treatment. Solid lines and NPR = without photoreactivation (PR), broken lines and PR = with PR. ○, bacteria enclosed in cellophan tubing. Fig. 10 = *E. coli* strain B15 (u.v.-sensitive), Fig. 11 = *E. coli* strain B17 (u.v.-resistant).

($C = 0.6 \mu\text{F}$, $L = 43 \mu\text{H}$., spark gap 5 mm., i.e. 17.4 kV with electrodes in water, $s = 4.5$ mm., iron electrodes, 91 J. per discharge) were used to compare the bactericidal effect of discharges in water and in air with that of continuous irradiation from an ordinary u.v. lamp (Philips TUV, 6 W., 2537 Å., distance to sample 15 cm.). The effect of submerged discharges was tested with 1200 ml. water and that of air discharges with 200 ml. water in the discharge vessel. In Figs. 10 and 11 the units along the abscissa signify number of discharges or minutes of u.v. treatment. Due to few colonies viable counts < 100 bacteria/ml. were not reliable. All the inactivation curves of *E. coli* strain B15 ran fairly close (Fig. 10); the general tendency was that they were slightly concave upwards. With the units chosen the inactivation by air discharges was somewhat greater; the energy content of the air discharges was greater and the volume treated smaller than that of submerged discharges. The curves for *E. coli* strain B17

(Fig. 11) were more convex upwards and did not follow each other so closely. The B 17 organisms were particularly sensitive to high doses of continuous u.v. radiation. The upward bend of the air-spark curve at low colony counts was presumably an artifact due to contamination from the part of the wall of the discharge vessel which had been located in the shadow.

Photoreactivation

Escherichia coli strains B 15 and B 17 inactivated by submerged discharges were reactivated by illumination with visible light (Figs. 10, 11). At comparable inactivation rates the magnitude of the reactivation of bacteria inactivated by discharges in the suspension was smaller than that of continuous u.v. irradiation and discharges in air. In contrast, bacteria inactivated by submerged discharges while enclosed in cellophan tubing were very liable to photoreactivation.

DISCUSSION

As a consequence of earlier results (Edebo & Selin, 1968) experiments were designed to test the hypothesis that the electric arc used produced bactericidal quantities of u.v. radiation. Two different strains of *Escherichia coli* B were used: strain B 15 was u.v.-sensitive and strain B 17 was u.v.-resistant. When the bacteria were suspended in 0.001 M-KCl (Figs. 1, 3) or tap water (Figs. 2, 4) at concentrations less than 2.5×10^7 bacteria/ml. the fraction of bacteria killed by one discharge was fairly constant. Strain B 15 was more susceptible than was strain B 17. The bactericidal effect per discharge was decreased at higher concentrations of bacteria and when albumin was dissolved in the discharge medium (Fig. 5). It was also decreased for bacterial suspensions enclosed in cellophan tubing and immersed in the discharge liquids, when u.v.-absorbing material was present in the discharge medium (Fig. 6). Consequently the protective effect of these materials was not a result of intimate contact between bacteria and protective agent but a neutralization of something passing through the discharge medium. Most likely the neutralization was caused by absorption of u.v. radiation, as the extent of the neutralization paralleled u.v. absorbance, and u.v. radiation has a strong microbicidal effect. Since the distance between the electrodes and the cellophan tubes was 65–75 mm., the radiation transmitted to the tubes with bacteria in a liquid with an absorbance/cm. (A/cm.) of 0.1 was 20 % and in one of 0.2 it was 4 % of that in 0.001 M-KCl. In agreement with this were the results (Fig. 6) that the bactericidal effect was moderately decreased at A/cm = 0.1 and almost extinguished at A/cm = 0.2. At the same absorbance coloured dextrans neutralized the bactericidal effect slightly more than the nucleic acids did; this might be due to their stronger absorption at wavelengths longer than 300 m μ . Also *Saccharomyces cerevisiae* suspended in the discharge medium decreased the bactericidal effect. At the same absorbance the decrease by yeast suspensions was less than that of u.v.-absorbing solutions. The absorbance values of the yeast suspensions were not quite comparable to the values of the solutions, however, since light scattering by the yeast particles contributed to the extinction measured in the spectrophotometer. In addition, suspensions of carbon and of calcium carbonate decreased the bactericidal effect. Experiments showing that the bactericidal effect was smaller at greater distances from the spark, and that the killing effect was transmitted through and generated in air (Fig. 9), also supported the initial hypothesis.

However, the kinetics of the inactivation by discharges with an energy content of 620 J. (Figs. 3, 4) did not show the characteristics of u.v.-killing for *Escherichia coli* strain B 17. Assume that more than one u.v. quantum hit was necessary to kill a B 17 bacterium, and that some bacteria were always protected from direct u.v. radiation by the shadow cast by the nylon insulation of the electrodes. When the intensity of the radiation was very strong, almost all bacteria outside the shadow should have been killed. At the next discharge the process was repeated and as the discharge also brought about good mixing of the suspension a nearly straight inactivation curve should result. Since the u.v. radiation probably was very strong with 620 J. per discharge, the generally straight inactivation curves at this energy content (Figs. 3, 4) agreed with u.v. killing, although they did not show the usual u.v. inactivation kinetics. However, since the inactivation of u.v.-sensitive strain B 15 (Figs. 1, 2) exceeded that of u.v.-resistant strain B 17 (Figs. 3, 4) there could not be a well-defined shadow. The size of the arc, reflexion against the walls of the discharge vessel, and the presence of high concentrations of bacteria contribute to make the shadow less absolute. At lower energies (91 J.; Fig. 11), where the light intensity was less, the inactivation kinetics of discharges were more like those of continuous u.v. radiation.

Rentschler, Nagy & Mouromseff (1941) found that the same dose of u.v. radiation (2537 Å) given in periods ranging from a few microseconds to several hours yielded the same bactericidal effect, provided that the time of treatment did not involve an appreciable part of the life-cycle of the organism under exposure. Marcovich (1956), who studied the induction of *Escherichia coli* K 12 (λ) by u.v. radiation showed that the same dose given in 0.1 or 10,000 sec. had the same effect. In most biological systems the surviving fraction of a given dose is independent of the intensity of the incident radiation (Zelle & Hollaender, 1955). Consequently, the yield of active photons from the arc should be decisive for the microbicidal effect. One may, however, expect different kinds of cell damage by the different wavelengths (Zelle & Hollaender, 1955) which seem to be produced by discharges.

The biological effect of u.v. radiation is dependent on several mechanisms. A large proportion of the effect is caused by thymine dimerization in DNA. Cross-linking between DNA and messenger RNA or DNA and protein may also occur (Wacker, 1963; McLaren & Shugar, 1964). Another mechanism has been postulated (Witkin 1964). Radiation-resistant *Escherichia coli* B strains are probably capable of repairing or getting around the dimer blocks, whereas sensitive bacteria are not (Setlow, Swenson & Carrier, 1963). The repair processes are enhanced by 3000–5000 Å radiation which is called photoreactivation (PR). Two kinds of PR have been described, one of which is almost independent of the dose rate during the PR treatment (Jagger & Stafford, 1965). Assume that the smaller PR of bacteria inactivated by submerged discharges (Figs. 10, 11) was due to the fact that some PR did already take place during the discharge. If this were the case, this mechanism would be still more pronounced with bacteria in cellophan tubing, since, due to preferential absorption of light of shorter wavelengths by the water and the wall of the tube, the proportion of light of longer wavelengths was greater. Since the PR of bacteria inactivated in cellophan tubes often was even greater than that after continuous far ultraviolet (2537 Å) inactivation (Figs. 10, 11), the PR by discharges was probably of little importance.

Consider also the possibility that the smaller PR after submerged discharges was a consequence of uneven distribution of microbicidal photons in the suspension. Under

such conditions bacteria close to the spark might be hit so heavily that they could not be photoreactivated, and some bacteria would not be hit at all. Due to stirring of the suspension by each discharge the number of hits on each bacterium should be more nearly equal after several discharges. The relation between the u.v. radiation dose required for killing a certain fraction of the bacteria without and with PR may be expressed as the dose-reduction factor which in some systems is constant (Rupert, 1964). When *Escherichia coli* strain B 17 was inactivated by u.v. irradiation this factor was not constant but moderately decreasing at higher inactivation rates, being 0.5 at 10 min of u.v. treatment. At inactivation by discharges the factor was moderately increasing, being 0.8 at 10 discharges (Fig. 11). Since the number of photon hits on individual bacteria was more nearly equal after several discharges, these observations suggest that further microbicidal mechanisms, not subject to photoreactivation, exist. The observation that the bactericidal effect on bacteria suspended in 0.001 M-KCl and subjected to one discharge was increased on standing in the refrigerator overnight (Figs. 1, 3), showed that at least under such conditions photon radiation was not the only killing mechanism.

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