EXPOSURE OF MICROORGANISMS TO FOCUSED AND UNFOCUSED SOUND FIELDS

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Previous investigations (Kinsloe et al., 1954) of the effect of time, pH, temperature and acoustic field on the destruction of microorganisms by vibratory energy showed the destruction to be due largely to mechanical breakdown and that the rate of destruction was dependent on the acoustic field. The objectives of the present study include a comparison of the effect on microorganisms of a focused and unfocused sound field and a comparison of the relative resistance to a focused sound field of strains of a species known to differ in their resistance to ultraviolet light and various oxidizing agents.

MATERIALS AND METHODS

The energy sources employed in this investigation were a 10 kc "raytheon" magnetostriction oscillator, Model DF-101 Serial No. 1-081 and an 800 kc vibrator employing a Brush piezo-electric barium titanate ceramic spherical section transducer.

Operated at maximum power control setting, the alternating electric power to the transducer of the magnetostriction oscillator is approximately 200 w with a voltage of approximately 180. The nominal sonic power obtained in the treatment cup is 50 w, according to the manufacturer.

The 800 kc vibrator was constructed by the Physics Department of The Pennsylvania State University. The transducer is driven by a specially constructed electronic generator the output of which is monitored with an oscilloscope to check wave form and voltage output, and with a radio frequency ammeter to measure the current drawn by the transducer. This generator can supply a relatively clean sine wave from 50 kc up to 1 mc, with a voltage of the order of 100 v r.f. and r.f. currents as high as 10 amp. The sound field in the exposure tube is measured with probe hydrophones and the output read on a Hewitt Packard vacuum tube voltmeter. Measurements made in this manner indicate that this equipment can produce an output greater than 1 atm acoustic pressure in the exposure tube from 70 kc to 1 mc. The output frequency is completely independent of the load characteristics, although the output size and form of the wave is altered by the load. The output wave form is monitored with a "heathkit" type 0-10 oscilloscope, and the current is monitored with a 5 or 10 amp r.f. ammeter.

The 800 kc bowl and the housing used for its support were manufactured by the Brush Electronics Co., Cleveland, Ohio. The housing contains electrical terminals and water cooling coils. The exposure tube consists of a glass test tube of the type used in the Klett-Summerson photoelectric colorimeter and a brass ring to hold the test tube in the cover plate. The brass ring is cemented to the glass tube; a small hole in the ring fits a pin in the cover plate and in this manner it is assured that the relative positions of the tube and transducer do not vary during a series of exposures.

An acoustical field is an elastic disturbance consisting of stresses and strains which propagate through the medium. In a homogeneous isotropic medium such as a fluid, the excess stress is called the acoustic pressure, the time derivative of the displacement is termed the local or particle velocity and the rate at which the disturbance moves is called the wave velocity.

At some pressure the liquid will start to pull apart. Under these conditions both the density and the bulk modulus will be altered. If more acoustic energy is applied to the liquid it will cavitate more vigorously. This being the case, the acoustic pressure will rise very slowly but the particle velocity will not be affected to this extent and will serve to give an indication of the cavitation intensity.

In the magnetostriction oscillator cavitation occurs primarily at the vibrator surface, whereas
in a focused system such as the 800 kc transducer, it occurs first in the body of the liquid. For a focused system, pressure readings outside the focal region indicate applied acoustic energy values far above the cavitation threshold in the focal region. The cavitation thresholds obtained at 800 kc in the focused transducer are much sharper than the cavitation thresholds at 10 kc in the magnetstriction oscillator.

Relative particle velocities were determined within and without the cavitating regions and below the cavitation threshold under varying r.f. current. As acoustic pressure cannot be measured adequately in a region where cavitation is occurring, the acoustic pressures were measured within and without the focal region before cavitation started and outside the focal region during cavitation. Calculations from these measurements were made to determine the acoustic pressure which would be present in that region were cavitation not occurring. On this basis it was determined that the acoustical pressure in the focal region of the 800 kc transducer with r.f. current of 2.5 amp would have approximated a value of 16 atm in the absence of cavitation. In a similar manner it was determined that the pressure with r.f. current of 5.0 amp would have approximated a value of 32 atm in the absence of cavitation. It was also determined that the particle velocity at focus with r.f. current of 5.0 amp was double that at 2.5 amp.

The test microorganisms included the following strains of *Escherichia coli*: strain B obtained from the Johnson Foundation of the University of Pennsylvania, strain B/r obtained from the Biological Laboratory, Long Island Biological Association, Cold Spring Harbor, N. Y., strain C-30 from the culture collection of The Pennsylvania State University. *Micrococcus pyogenes* var. *aureus* strain 52A from the culture collection of The Pennsylvania State University was used also.

The cell suspensions were prepared as previously described by Kinsloe et al., (1954). Thirty ml of the buffered suspensions were used in the exposures conducted in the 10 kc magnetstriction oscillator and 5 ml in the 800 kc vibrator. Exposures were made at 15 C with an exposure time of 20 min.

Relative resistance of the strains of *E. coli* to ultraviolet irradiation was determined as follows: 24 hr broth cultures were centrifuged for 15 min, the supernatant decanted and the cells resuspended in 10 ml of 0.85% saline, dilutions of 1:10 were prepared and placed in sterile petri dishes. A General Electric 15 w germicidal lamp was calibrated to deliver approximately 300 ergs/mm² per min. Exposures were conducted in open petri dishes with agitation accomplished by an eccentric rotor adjusted to set up a series of standing waves in the suspension through its vibratory impulses. *E. coli* strain B was exposed to intensities of 0 to 900 ergs/mm², *E. coli* strains B/r and C-30 to intensities of 0 to 1200 ergs/mm².

Photoreactivation tests were conducted with the use of a 300 w tungsten projection lamp in a projector. Thirty ml aliquots of suspensions exposed to the magnetstriction oscillator and 5 ml aliquots of suspensions exposed to the 800 kc vibrator were illuminated at 30 C, at the focal point of the light beam in a glass water bath.

Determinations of viable cells, other than in the case of ultraviolet exposures, were made in accordance with plating techniques as described in Standard Methods for the Examination of Dairy Products, 10th Edition, with the exception of the medium employed and temperature of incubation. Nutrient agar was used as the plating medium and all plates were incubated at 37 C. Counts of viable cells following exposure to ultraviolet radiation were made by placing 0.1 ml of the dilution on nutrient agar plates, spread by the use of a glass rod spreader and counted following incubation at 37 C for 48 hr.

Cells of *M. pyogenes* var. *aureus* exposed to the sound field of the 800 kc vibrator and unexposed cells of the organisms were plated on Chapman Stone agar to detect changes in pigment production and on blood agar to determine hemolytic activity. The Chapman Stone agar plates were examined at the end of 24, 48, and 72 hr and the blood agar plates at the end of 24 hr incubation. All plates were incubated at 37 C.

**RESULTS AND DISCUSSION**

Studies conducted with the 10 kc magnetstriction oscillator using three strains of *Escherichia coli* and *Micrococcus pyogenes* var. *aureus*. Bryson and Davidson (1951) have reported that mutants of *E. coli* strain B such as B/r possess increased resistance to numerous oxidizing agents. Braun (1953) states that radiation resistant *E. coli* strains can be obtained by isolating crystal violet or proflavine resistant strains employing the techniques used in obtaining strains resistant to
antibiotics. These findings have led investigators in this field to the conclusion that resistance to ultraviolet irradiation implies resistance to oxidizing influences. It would follow, therefore, that if the lethal action of vibratory energy is associated with the production of hydrogen peroxide, the radiation resistant strains B/r and C-30 should exhibit greater resistance to vibratory energy than the radiation susceptible strain B.

For comparison with these rod shaped bacteria, a beta hemolytic strain of M. pyogenes var. aureus was included. Typical data obtained in this comparison are presented in table 1.

The relative resistance of the strains of E. coli to ultraviolet irradiation was determined in a series of trials employing dosages ranging from 50 to 1200 ergs/mm². These data are presented in figure 1.

The data presented in table 1 and figure 1 may be summarized as follows: first, the strain of M. pyogenes var. aureus proved more resistant to vibratory energy under the conditions of this study than did the three strains of E. coli. Second, E. coli strains B/r and C-30 although more resistant to ultraviolet light than E. coli strain B proved equally susceptible to vibratory energy. The percentage of organisms killed under the conditions of this study was dependent upon output energy in addition to species, confirming previous plate voltage studies (Kinsloe et al., 1954).

Photoreactivation of cells of E. coli strains B and B/r previously exposed to the 10 kc magnetostriiction oscillator for a period of 20 min at 15 C with dosages ranging from r.f. current of 0.5 to 0.85 amp was attempted without success. Treated cells exposed to white light for a period of 1 hr failed to show survival greater than that of aliquots plated without exposure to light.

The similarity in susceptibility to the sound field of E. coli strains B, B/r and C-30 and the failure to demonstrate photoreactivation of cells exposed to the sound field suggest that the mechanism of killing by vibratory energy and by ultraviolet radiation have little in common. These observations and those previously reported (Kinsloe et al., 1954) on the comparison of exposure temperatures from 15 to 45 C and the effect of pH lend support to the theory that the principal mechanism of death in the sound field is mechanical rather than chemical in nature.

Studies conducted with the 800 kc ceramic transducer using Escherichia coli strain B and Micrococcus pyogenes var. aureus. Buffered suspensions of these strains were exposed to the sound field at 15 C and dosages ranging from r.f. current of 2.5 to 5.0 amp. Typical data obtained are presented in table 2.

The data presented in table 2 show a significant change in death rate of E. coli between r.f. current of 3.5 and 4.0 amp. A similar change in death rate of M. pyogenes var. aureus was observed between r.f. current of 4.0 and 4.5 amp. This suggests that under the conditions obtaining in this study "crit-

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**Table 1**

<table>
<thead>
<tr>
<th>Amperes r.f.</th>
<th><strong>Escherichia coli</strong></th>
<th><strong>Micrococcus pyogenes</strong></th>
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<tbody>
<tr>
<td></td>
<td>B</td>
<td>B/r</td>
</tr>
<tr>
<td>0.50</td>
<td>53.2</td>
<td>50.5</td>
</tr>
<tr>
<td>0.55</td>
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<tr>
<td>0.75</td>
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<td>5.3</td>
</tr>
<tr>
<td>0.85</td>
<td>—</td>
<td>2.4</td>
</tr>
</tbody>
</table>

**Figure 1.** Per cent survival of Escherichia coli strain B, B/r and C-30 irradiated with ultraviolet.
organisms were examined for evidence of change in colony characteristics that might be associated with exposure to vibratory energy. Only in the case of *M. pyogenes* var. *aureus* exposed at r.f. current 4.5 and 5.0 amp in the focussed field of the 800 kc transducer were such changes noted.

Colonies of this organism produced by unexposed cells were at all times deep golden orange in color following 24 hr incubation at 37°C on the plating medium. Similar results were obtained with all exposures at output levels from 2.5 to 4.0 amp. Results obtained with exposures of 10, 15 and 20 min at r.f. current of 4.5 and 5.0 amp are shown in table 3.

The findings do not appear to be selective killing inasmuch as some effect was noted on colonies with a survival of 85 per cent, although complete loss of pigment was not found under these conditions. These findings were confirmed by plating on Chapman Stone agar. Isolations were made from the grayish white colonies to Chapman Stone agar and were carried on this medium through a minimum of 10 serial transfers. The ability to produce the golden orange pigment was not regained. Blood agar was employed to determine possible losses in hemolytic ability that might be associated with loss in pigment formation. All isolates, however, proved to be Beta hemolytic, a characteristic of the parent strain.

Photoreactivation was also attempted without success on cells of *E. coli* B previously exposed to the sound field of the 800 kc vibrator for a period of 20 min at 15°C with dosages ranging from r.f. current 2.5 to 5.0 amp. Treated cells exposed to white light for a period of 1 hr failed to show survival greater than aliquots plated without exposure to the light.

Of all the observations made in the course of this investigation three may be considered to possess special significance. These are first, the similarity in susceptibility of *E. coli* strains B, B/r and C-30 to the sound field of the 10 kc oscillator; second, the apparent “critical” intensity effect obtained with both species in the sound field of the 800 kc transducer and third, the effect on pigment formation by *M. pyogenes* var. *aureus* obtained at r.f. current of 4.5 to 5.0 amp in the 800 kc transducer.

**SUMMARY**

Buffered suspensions of strains of *Micrococcus pyogenes* var. *aureus* and *Escherichia coli* were exposed to the sound fields of a 10 kc raytheon
magnetostriction oscillator and an 800 kc barium titanate ceramic bowl transducer. Twenty min exposures were made at 15 C and at various levels of power output.

The strain of *Micrococcus pyogenes* var. *aureus* employed was found to be more resistant to the lethal effects of the sound field than were the strains of *Escherichia coli* used. Increase in power output resulted in an increase in lethal effect. Attempts to demonstrate photoreactivation following exposure to a sound field were not successful.

Ultraviolet resistant strains proved to be as susceptible to the sound field as did the ultraviolet susceptible strain. This suggests that oxidative effects are of little significance in the lethal mechanism of vibratory energy.

Breaks in the death curves obtained with the more powerful transducer suggest that "critical" intensities were reached.

REFERENCES


