

48. PERIPHERAL NERVE CONDUCTION AND ULTRASOUND: MAMMALS

Contributor: Lele, Padmakar P.

Reference: Lele, P. P. 1963. *Exptl. Neurol.* 8:47.

49. ULTRASONIC DESTRUCTION AND INJURY: CELLS AND MICROORGANISMS

The effects of ultrasound on cells and microorganisms in liquid suspensions have been studied largely in the presence of cavitation, although cavitation is by no means the only mechanism by which the effects of ultrasound are manifested [4]. The threshold of cavitation varies with the geometrical configuration of the sound field, frequency, chemical composition of the suspending fluid, temperature, viscosity, and pressure [4]. However, the relative rates at which different types of biological cells are destroyed are constant over a wide frequency range for specified values of other parameters [1,2]. So long as 1% of the population remains undamaged, the rate of destruction can be described by $dN/dt = RN$, where N is the cell concentration, t is time, and R (the rate constant) can be considered a measure of cell fragility, provided the physical conditions are maintained invariant. The production of heat during irradiation apparently plays a secondary role in the production of the observed effects. The survival of the microorganism, however, depends on the temperature of the irradiated medium [5], reflecting the decrease in cavitation threshold with increasing temperature [4]. At 4.5 watts per square centimeter and 800 kilocycles for 20 minutes, 50% of *Serratia marcescens* survived a temperature of 6°C, 15% survived 20°C, and 2% survived 40°C [5]. For a comprehensive review of the subject, consult references 3 and 6.

Contributor: Dunn, Floyd

References: [1] Ackerman, E. 1960. *Intern. Conf. Med. Electron., Proc.*, 3rd, p. 437. [2] Ackerman, E. 1962. *Biophysical science*. Prentice-Hall, Englewood Cliffs, N. J. [3] El'piner, I. E. 1964. *Ultrasound: physical, chemical, and biological effects*. Consultants' Bureau, New York. [4] Fry, W. J., and F. Dunn. 1962. In W. L. Nastuk, ed. *Physical techniques in biological research*. Academic Press, New York. v. 4, p. 261. [5] Fuchtbauer, H., and H. Theismann. 1949. *Naturwissenschaften* 36:346. [6] Grabar, P. 1953. *Advan. Biol. Med. Phys.* 3:191.

Part I. CELL SIZE AND RELATIVE FRAGILITY

Relative Fragility: Values for fragility are relative to human red blood cells taken as unity, and were determined at frequencies of 200 cycles/sec to 20 kc. No simple relationship exists between size and fragility; however, large cells tend to be more fragile.

| Cell Type | Average Diameter μ | Relative Fragility |
|----------------------------------|------------------------|--------------------|
| 1 <i>Amoeba proteus</i> | 200 | 0.4 |
| 2 <i>Paramecium caudatum</i> | 150 | 4 |
| 3 <i>P. aurelia</i> , G | 80 | 16 |
| 4 <i>Tritrichomonas foetus</i> | 12 | 2 |
| 5 Human RBC | 6 | 1 |
| 6 Rabbit sperm | 5 | 0.7 |
| 7 <i>Escherichia coli</i> , U.W. | 1 | 0.15 |
| 8 T-2 bacteriophage | 0.01 | 0.2 |

Contributor: Dunn, Floyd

General References: [1] Ackerman, E. 1952. *J. Cellular Comp. Physiol.* 39:167. [2] Ackerman, E. 1962. *Biophysical science*. Prentice-Hall, Englewood Cliffs, N. J. p. 228.

Part II. CELL SIZE AND OPTIMUM DESTRUCTIVE FREQUENCY

Although the relative rates of cell destruction are the same at most frequencies, some cells exhibit greatly increased sensitivities to destruction at particular frequencies, i. e., rupture occurs more readily at characteristic frequencies than at neighboring frequencies. These increased sensitivities at particular frequencies have been interpreted as a resonance phenomenon.

| Cell Type | Diameter, μ | | Optimum Frequency kc |
|------------------------------|-----------------|---------|----------------------|
| | Minimum | Maximum | |
| 1 <i>Paramecium caudatum</i> | 63 | 223 | 1.2 |
| 2 <i>P. bursaria</i> | 51 | 118 | 1.7 |
| 3 <i>P. aurelia</i> , G | 29 | 124 | 3.3 |
| 4 <i>P. trichium</i> | 38 | 80 | 4.1 |
| 5 <i>Amphiuma</i> RBC | 10 | 45 | 16.5 |

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General References: [1] Ackerman, E. 1952. *J. Cellular Comp. Physiol.* 39:167. [2] Ackerman, E. 1960. *Intern. Conf. Med. Electron., Proc.*, 3rd., p. 437. [3] Ackerman, E. 1962. *Biophysical science*. Prentice-Hall, Englewood Cliffs, N. J. p. 228.

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Part III. DESTRUCTION TIMES

A relationship exists between the composition of the suspending liquid and the destructive effect of ultrasound, so that proteins appear to inhibit destructive effects more than do lipids and carbohydrates [1]. This relationship also reflects, to some extent, the increase in cavitation threshold with increasing viscosity of the suspending medium [2]. For sufficiently high intensities, the effectiveness of the ultrasonic action depends upon the cell concentration [3].

| Cell Type | Composition of Medium or Cell Concentration | Time for Complete Destruction, sec | Reference |
|--------------------------------|---|------------------------------------|-----------|
| 1 Gonococci | Twice-distilled water | 240-300 | 1 |
| 2 | Physiological saline | 300-360 | |
| 3 | Blood serum | 600 | |
| 4 | Peptone bouillon | 2400-3000 | |
| 5 RBC | 1:5 dilution in isotonic saline | 4500 | 3 |
| 6 | 1:25 dilution in isotonic saline | 420 | |
| 7 <i>Trypanosoma gambiense</i> | 14,100 specimens/mm ³ | 20 | 3 |
| 8 | 48,000 specimens/mm ³ | 75 | |

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References: [1] El'piner, I. E. 1964. Ultrasound: physical, chemical, and biological effects. Consultants' Bureau, New York. [2] Fry, W. J., and F. Dunn. 1962. In W. L. Nastuk, ed. Physical techniques in biological research. Academic Press, New York. v. 4, p. 261. [3] Schoenaers, F. 1948. Compt. Rend. Soc. Biol. 142:182.

Part IV. DESTRUCTION (at 9 kc) RELATED TO pH AND TEMPERATURE

At pH 7, increasing temperature had little effect on the rate of destruction of *Escherichia coli*, *Micrococcus varians*, and *Serratia marcescens*. Destruction of *Pseudomonas aeruginosa* increased with increasing temperature, but the pH level appeared to have no effect.

| Species | pH | Exposure Time min | % of Bacteria Destroyed at Temperature of | | | | Reference | |
|----------------------------------|----|-------------------|---|------|------|------|-----------|----|
| | | | 15°C | 25°C | 35°C | 45°C | | |
| 1 <i>Escherichia coli</i> | 4 | 10 | 44 | 60 | 48 | 71 | 1 | |
| | | 20 | 55 | 43 | 65 | 82 | | |
| | | 30 | 35 | 39 | 46 | 47 | | |
| | 5 | 20 | 61 | 62 | 73 | 67 | | |
| | | 7 | 10 | 41 | 49 | 40 | | 49 |
| | | | 20 | 65 | 65 | 55 | | 63 |
| 7 <i>Micrococcus varians</i> | 4 | 80 | 59 | 56 | 71 | 99 | 1,2 | |
| | 5 | 80 | 28 | 36 | 48 | 80 | | |
| | 7 | 80 | 48 | 39 | 52 | 59 | | |
| 10 <i>Pseudomonas aeruginosa</i> | 4 | 10 | 44 | 51 | 79 | 55 | 1 | |
| | | 20 | 60 | 66 | 79 | 89 | | |
| | | 30 | 46 | 41 | 58 | 72 | | |
| | 5 | 10 | 71 | 71 | 79 | 91 | | |
| | | 7 | 10 | 46 | 51 | 63 | | 75 |
| | | | 20 | 67 | 72 | 77 | | 87 |
| 16 <i>Serratia marcescens</i> | 4 | 5 | 26 | 24 | 36 | 71 | 1 | |
| | | 7.5 | 38 | 22 | 38 | 77 | | |
| | | 10 | 41 | 42 | 51 | 83 | | |
| | | 5 | 5 | 31 | 30 | 33 | | 39 |
| | | | 7.5 | 39 | 37 | 48 | | 52 |
| | | | 10 | 44 | 43 | 55 | | 65 |
| | 7 | 5 | 27 | 29 | 24 | 33 | | |
| | | 7.5 | 42 | 45 | 37 | 46 | | |
| | | 10 | 47 | 52 | 49 | 59 | | |

Contributor: Dunn, Floyd

References: [1] Ackerman, E., et al. 1953. WADC Tech. Rept. 53-82. [2] Kinsloe, H., E. Ackerman, and J. J. Reid. 1954. J. Bacteriol. 68:373.

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49. ULTRASONIC DESTRUCTION AND INJURY: CELLS AND MICROORGANISMS

Part V. INJURY RELATED TO POSITION IN ULTRASONIC FIELD

The effect of ultrasound on living organisms is dependent on position in a standing wave field. Injury to *Spirogyra* filaments in agar was markedly more pronounced at acoustic pressure nodes than at antinodes.

| Plate Volts | In Water | | | | In Agar | | | | Agar Conc % |
|----------------|------------------------------|--------------|----------|------------------------------|--------------|----------|-------------------|---|-------------------|
| | Expo- sure Time sec | % Injured at | | Expo- sure Time sec | % Injured at | | Agar Conc % | | |
| | | Node | Antinode | | Node | Antinode | | | |
| 1 | 1000 | 10 | 74 | 80 | 5 | 49 | 0 | 1 | |
| 2 | 10 | 95 | 100 | 40 | 74 | 0 | 4 | 4 | |
| 3 | 25 | 56 | 75 | 50 | 40 | 0 | 2 | 2 | |

| Plate Volts | In Water | | | | In Agar | | | | Agar Conc % |
|----------------|------------------------------|--------------|----------|------------------------------|--------------|----------|-------------------|---|-------------------|
| | Expo- sure Time sec | % Injured at | | Expo- sure Time sec | % Injured at | | Agar Conc % | | |
| | | Node | Antinode | | Node | Antinode | | | |
| 4 | 1000 | 30 | 85 | 65 | 24 | 30 | 0 | 2 | |
| 5 | 1400 | 5 | 70 | 78 | 0.41 | 17 | 0 | 2 | |
| 6 | 15 | 83 | 100 | 0.25 | 47 | 7 | 1 | 1 | |

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Reference: Goldman, D. E., and W. W. Lepeschkin. 1952. J. Cellular Comp. Physiol. 40:255.

50. RESPONSES TO ULTRASOUND: PLANTS

Part I. QUANTITATIVE MEASUREMENTS

Exposure medium was water. An asterisk (*) after the species indicates that the data were subjected to statistical analysis by the author. **Effect:** *ns* = statistically not significant. Values in parentheses are ranges, estimate "c" (see Introduction).

| Species & Part Treated | Frequency kc/sec [Index of Sonic Amplitude] | No. of Plants/ Treat- ment | Exposure | | Effect | Ref- er- ence |
|--|---|---|------------|---|--|---------------------|
| | | | Temp °C | Time | | |
| 1 2 3 4 5 6 7 8 9 10 <i>Allium cepa*</i> Seeds, un- soaked | 1000 [2 w/cm ²] | 100 x 5 repli- cate | 15-18 | 1 min | 100% germinated [germination rate, 105%] ¹ | 7 |
| | | | | 2 min | 98% germinated, <i>ns</i> ² [germination rate, 105%] ¹ | |
| | | | | 4 min | 98% germinated, <i>ns</i> ² [germination rate, 97%, <i>ns</i> ³] ¹ | |
| | | | | 8 min | 99% germinated, <i>ns</i> ² [germination rate, 109%] ¹ | |
| | 1000 [4 w/cm ²] | 100 x 5 repli- cate | 15-18 | 0.5 min | 96% germinated, <i>ns</i> ² [germination rate, 107%] ¹ | |
| | | | | 1 min | 99% germinated, <i>ns</i> ² [germination rate, 106%] ¹ | |
| | | | | 2 min | 99% germinated, <i>ns</i> ² [germination rate, 105%] ¹ | |
| | | | | 4 min | 95% germinated [germination rate, 107%] ¹ | |
| | | | | 12 min | 88% germinated [germination rate, 120%] ¹ | |
| | | | | 16 min | 84% germinated [germination rate, 118%] ¹ | |
| 11 12 13 14 15 16 17 <i>Allium cepa*</i> Seeds, soaked 24 hr | 1000 [0.5 w/cm ²] | 100 x 5 repli- cate | 15-18 | 0.5 min? | 99% germinated, <i>ns</i> ² [germination rate, 94%] ¹ | |
| | | | | 1 min | 100% germinated, <i>ns</i> ² [germination rate, 100%, <i>ns</i> ³] ¹ | |
| | 1000 [1 w/cm ²] | 100 x 5 repli- cate | 15-18 | 0.5 min | 98% germinated, <i>ns</i> ² [germination rate, 99%, <i>ns</i> ³] ¹ | |
| | | | | 1 min | 99% germinated, <i>ns</i> ² [germination rate, 98%, <i>ns</i> ³] ¹ | |
| | | | | 2 min | 98% germinated, <i>ns</i> ² [germination rate, 99%, <i>ns</i> ³] ¹ | |
| 1000 [2 w/cm ²] | 100 x 5 repli- cate | 15-18 | 0.5 min | 101% germinated, <i>ns</i> ² [germination rate, 109%] ¹ | | |
| 18 19 | 1000 [4 w/cm ²] | 100 x 5 repli- cate | 15-18 | 0.5 min 2 min | 89% germinated [germination rate, 109%] ¹ 83% germinated [germination rate, 109%] ¹ | |
| 20 21 <i>Beta vulgaris</i> Seeds ⁴ , soaked 24 hr | 425 [1 w/cm ²] | 25 g of seeds x 5 repli- cate | | 0 | Plant wt, 394 g; root wt, 150 g; sugar yield, 21.0 g; total N, 0.409% | 1 |
| | | | | 2 min | Plant wt, 479 g; root wt, 196 g; sugar yield, 28.4 g; total N, 0.377% | |

¹ As % of controls. ² Not significantly different from "unexposed % germinated" figure. ³ Not significantly different from "% germinated" figure. ⁴ Fruit clusters.

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