References and Notes

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2. R. Webster, *Gems, Their Sources, Descriptions and Identification* (Butterworths, London, 1962), vol. 1.
3. K. Wada, *Bull. Nat. Pearl Res. Lab.* **7**, 767 (1961).
4. Transverse, concavo-convex, commonly smooth, pseudopunctate, commonly nacreous, articulate brachiopods belonging to the family Strophheodontidae of the superfamily Strophomenacea. The Paleozoic family Strophheodontidae is characterized by a denticulate hinge instead of teeth and sockets, a bilobed cardinal process, and lack of a functional pedicle opening except in early stages of growth.
5. A. Williams, "North American and European strophheodontids: their morphology and systematics," *Geol. Soc. Amer. Mem. 56* (1953).
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Sterilization by Electrohydraulic Treatment

Abstract. *Electrohydraulic treatment was applied to suspensions of* Escherichia coli, *spores of* Bacillus subtilis *var.* niger, Saccharomyces cerevisiae, *and bacteriophage T-2, as well as to raw municipal sewage. These suspensions were all sterilized. Data are presented to show the different degrees of treatment required for each microorganism.*

Electrohydraulics is a new process for converting electrical energy directly to other useful forms of energy. It depends upon the discharge of a high-voltage arc under the surface of a liquid medium. Arc discharge results in the generation of extremely rapid-rise, high-pressure shock waves and highly active chemical species. The process results in no perceptible rise in temperature; it is virtually instantaneous and completely electrical in nature. Except for a trace of electrode-erosion products formed by the arc, no foreign materials or chemicals are added.

Suspensions of various microorganisms were subjected to multiple electro hydraulic discharges which resulted in complete sterilization of the medium, but no morphologic alteration in the microorganisms studied was observed after this treatment. The total energy necessary for complete sterilization varied with the species.

The electrical equipment consists of a power source fed to a high-voltage d-c power supply, a capacitor bank, charging resistor, and the necessary triggering switch circuit. Energy is stored in the capacitor and discharged to an underwater arc gap. In operation the capacitor is charged to the desired voltage by the power supply, thus storing energy: $J = \frac{1}{2}CE^2$, where J is the energy in joules or watt seconds, C is the capacitance in farads, and E is the voltage. The electrohydraulic effect is obtained by dumping (discharging) this stored energy through a cable into the gap located in the sterilizing tank containing the liquid to be treated.

The equipment used was capable of operating at variable voltage, to 14 kv, at a capacitance from 5 to 95 μfarad, and had an inductance in the range of 2.5 μhenry. It was designed to operate automatically at an arc discharge rate of 0.8 to 30 times per second. The repetition rate, however, had to be chosen commensurate with the energy discharged, so that the power-supply charging rate was not exceeded. The sterilizing tank consisted of a 5-inch (12.7-cm) length of schedule 160 stainless steel pipe, 5 inches in diameter, with flanges welded at both ends to accommodate bolted lids sealed with O-rings (Fig. 1). Electrodes covering a range of gap sizes were fabricated.

Cultures of the following organisms were tested: *Escherichia coli* ATCC 11229, *Bacillus subtilis* var. *niger* ATCC 9372, *Saccharomyces cerevisiae* ATCC 2338, and *E. coli* T-2 bacteriophage. Raw sewage was also tested in an effort to demonstrate efficiency in treating material composed of a naturally occurring variety of microorganisms.

Table 1. Electrohydraulic energy required to kill bacterial and viral species suspended in distilled water. The total time of treatment was less than 1 minute.

Organism	Initial conc. (cells or particles per milliliter)	Total energy (watt hr/gal)
Escherichia coli	2.2×10^7	6.9
Spores of *Bacillus subtilis* var. *niger*	9×10^5	10
Bacteriophage T2	10^8	10
Saccharomyces cerevisiae	9.3×10^6	31
Raw sewage	1.7×10^4	5

Escherichia coli was cultured on nutrient agar slants in milk dilution bottles, at 37°C for 18 to 20 hours. The cells were suspended by gentle washing in 0.01*M* phosphate buffer and were passed through Whatman No. 1 filter paper. Immediately prior to treatment they were diluted in a 10- to 100-fold volume of sterile distilled water. The number of viable cells was determined by duplicate plate counts in nutrient agar, the average number being 10^8 per milliliter. *Bacillus subtilis* var. *niger* was cultured in similar manner, at 37°C for 48 hours. After the cells had been washed from the agar surface with buffer solution and filtered through filter paper, the suspension was treated at 56°C for 30 minutes to destroy any vegetative cells, leaving only spores.

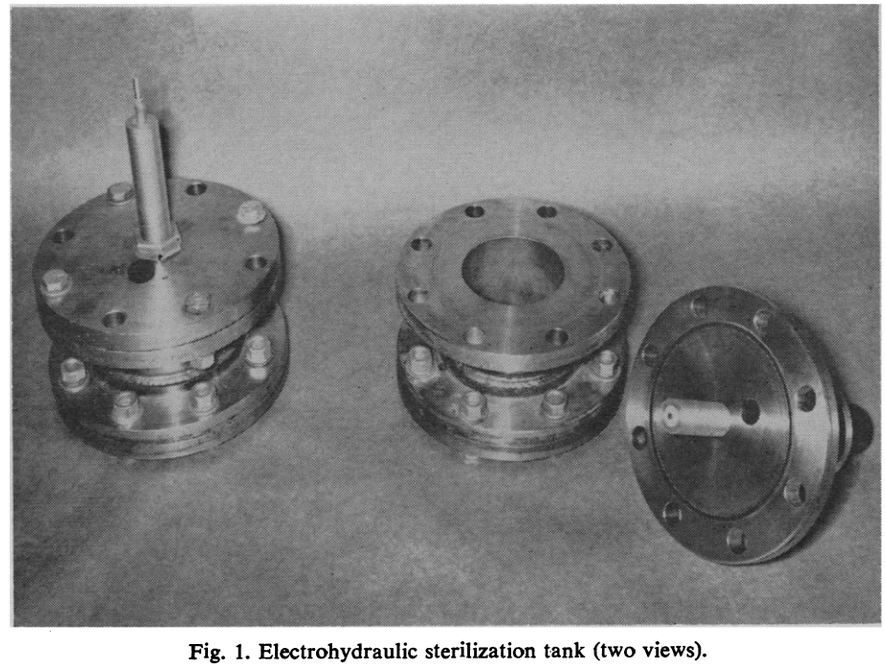

Fig. 1. Electrohydraulic sterilization tank (two views).

Duplicate plate counts were made after dilution of the spore suspension in sterile distilled water prior to electrohydraulic exposure. There were approximately 5×10^5 spores per milliliter of sample. *Saccharomyces cerevisiae* was grown on Sabouraud's glucose agar, at 25°C for 20 hours. Cells were removed by gentle washing in 0.01*M* phosphate buffer and diluted 1 : 10 to 1 : 20 in sterile distilled water. Prior to treatment, the number of viable cells was determined by duplicate plate counts on the growth medium; they averaged 10^7 cells per milliliter. Bacteriophage T-2 was cultured in nutrient broth on its host organism, *E. coli* B, and harvested at 18 hours by filtration through a Seitz filter. Following dilution in sterile distilled water, the number of phage particles was determined by plaque counts on nutrient agar seeded with the host organism. Raw municipal sewage without dilution was treated in the reaction chamber. Plate counts were performed on the sewage before treatment and yielded counts in the range of 10^4 organisms per milliliter.

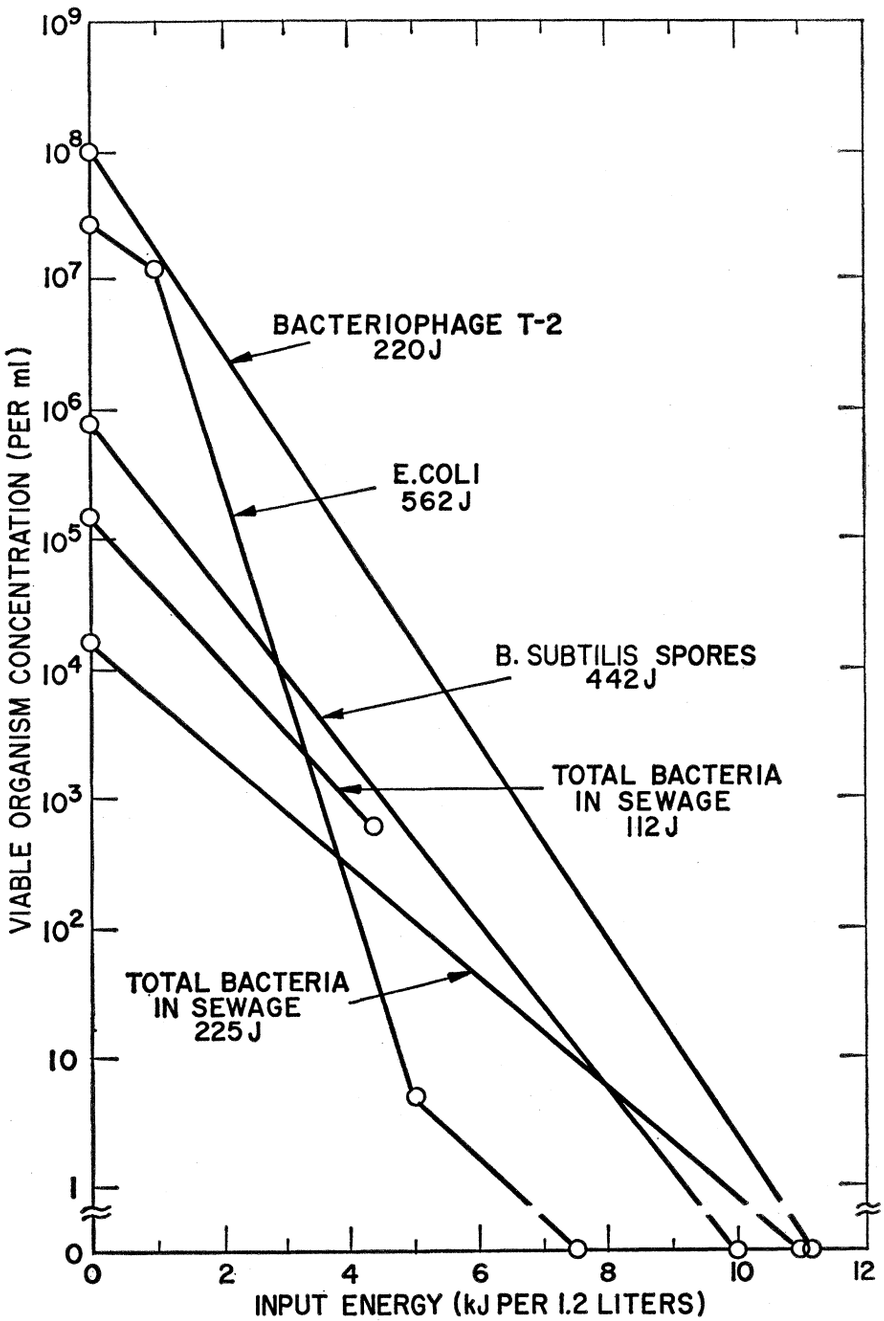

Fig. 2. Typical electrohydraulic sterilization data: viable organisms versus input energy. Numbers indicate joules per discharge.

For testing, 1200 ml of a suspension of the microorganism in sterile distilled water was put in the sterilizing tank. An initial sample was withdrawn and placed in an ice bath for determination of the number of organisms present prior to treatment. The tank was then sealed and the contents were subjected to a multiplicity of electrohydraulic discharges, with varying voltage and capacitance. Samples were removed at selected intervals that corresponded to increased doses of electrohydraulic energy. Tests were made in which the voltage was varied and the capacitance kept constant, and vice versa.

After treatment bacterial or yeast samples were plated on nutrient agar or Sabouraud's glucose agar, respectively, in duplicate, and the count of viable organisms was determined after 48 hours of incubation. The effect on bacteriophage T-2 was assayed in the same manner by plating serial 10-fold dilutions on nutrient agar seeded with the host organism, *E. coli* B.

Complete kill of a variety of microbial species in distilled water was obtained by electrohydraulic treatment (Table 1). In all cases total time of treatment was less than 1 minute. Suspensions of all organisms were completely sterilized by the use of sufficient energy. The degree and rate of kill at any given set of input parameters were dependent upon total energy input. The energy required to effect complete sterilization varied with the initial number of organisms in the suspension. Complete sterilization of the suspension of spores of *B. subtilis* var. *niger* required about one-and-a-half times the energy required for the suspension of *E. coli*. The most resistant organism tested, *S. cerevisiae*, absorbed more than four times this amount of energy before the count of viable organisms was reduced to zero. The rate of kill, essentially a straight-line function of input energy, is illustrated in Fig. 2 for certain selected data, and results are reproducible at established levels of voltage and capacitance. Rate of kill varies with changes in voltage and capacitance, the greatest effect being at lower voltages.

When treated suspensions of *E. coli* and *S. cerevisiae* were stained, no disruption of the cell wall was seen. Nor was the antigenic structure of *E. coli* altered by electrohydraulic treatment, since the treated organisms could be used in the preparation of a vaccine. Rabbits that received six intravenous in-

jections of the treated bacterial suspensions over a 2-week period showed no evidence of toxicity and responded with the same increase in antibody titer that rabbits treated with formalinized vaccine did.

The presence of protein in the reaction solution reduced the effectiveness of treatment, and increased energy was required for complete sterilization.

The mechanism by which death of the various species occurs is not known but is under investigation. The following facts are pertinent to this investigation. Death does not occur by disruption of the cell wall. Electrohydraulic discharge does result in the formation from the water of very active chemical species, such as free radicals and ions that are short-lived but may be responsible for the effect. The high shock-wave pressure resulting from the discharge may cause these active chemical species to be forced through the cell wall, thus enhancing their effect. The fact that increased energy is required when protein is present suggests that protein in solution may absorb these active radicals and ions and in this manner diminish the effect. Shock-wave pressures do not cause any perceptible damage to the cell wall, but they may cause internal damage, and this may result in death of the bacterial cell.

Electrohydraulic treatment is an extremely effective, essentially instantaneous, and inexpensive way to sterilize water and sewage without a rise in temperature or the addition of chemicals or foreign materials.

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Disinhibition of Visually Masked Stimuli

Abstract. *Backward-masking conditions were established for a pair of circular-patch stimuli. A third stimulus was then selected so as to mask the second when the second and the third were presented in the absence of the first. When all three stimuli were presented in serial order, the first and third were reliably detected but the second was not. Apparently, by masking the second flash, the third "disinhibited" the first.*

Crawford (*1*), in a now classic experiment, demonstrated that subsequent stimulation reduced sensitivity to a preceding flash. He found that when a second "conditioning flash" (CF) of 12° area and 524-msec duration followed a 10-msec, 0.5° "test flash" (TF) by as much as 100 msec, threshold sensitivity to TF was elevated. This phenomenon of backward masking has been reported subsequently by a number of investigators (*2*, *3*) and has been reviewed recently by Raab (*4*). It appears, moreover, to be a general property of sensory information processing, demonstrable in the skin sense (*5*), in audition (*6*), and in color vision (*7*).

Crawford's explanation of backward masking was rooted in "transmission-time" interactions; that is, CF, being greater in area and duration, overtakes TF in the processing through the visual radiations and arrives earlier at the cortical level. More recently observed conditions (*3*) in which the preceding TF must be *more* intense than the following CF, however, require a more dynamic neuroinhibitory mechanism than is implied in transmission effects. The experiment here reported describes a preliminary attempt to suggest such a process. Specifically, it extends classical backward-masking research by incorporating a *third* stimulus, one that follows TF and CF at an interval short enough to mask CF. Under these conditions, it was of interest to determine the resulting effects upon the detection of TF, the first in the train of three flashes.

Two male laboratory assistants, naive with respect to the specific experiment, were used as subjects. Stimuli were presented by means of a Scientific Prototype Three-Channel Tachistoscope. Each channel of this device holds a pair of argon-mercury vapor-discharge lamps. The associated timing circuits permit continuous control of the flash duration of each field and of the interval between successive flashes, from 110 μsec to 110 seconds. Stimulus area is varied by inserting fixed-diameter apertures in the frame-holders of each field, which are positioned anterior to the respective lamp compartments. Although coarse intensity controls are available, nonlinearities require the use of neutral density filters for accurate variation of luminance.

In the present experiment, three aperture diameters were used; field 1 contained a circular aperture of 2 mm; field 2, 4 mm; and field 3, 8 mm. At the viewing end, 18 inches (46 cm) from these apertures, these circular patches subtended 0.23°, 0.46°, and 0.92° of visual angle, respectively. With opal diffusing screens positioned between source and view, the overall configuration provided circular flashes of different areas and homogeneous luminance, the latter maintained at 5.0 mlam, for each field.

After 10 minutes of dark adaptation, subjects were given practice in identifying each area with an appropriate number; 0.23° = 1, 0.46° = 2, and 0.92° = 3. Subjects were told that, on any given trial, any one or combination of areas could be presented simultaneously or successively. Their task, at the end of a presentation, was to report whether 1, 2, or 3 or any combination had been presented. The experiment consisted of 400 trials per subject: 50 at each of four TF-CF intervals (25 msec, 50 msec, 75 msec, and 100 msec) in the absence of a third flash, and 50 trials at each of the same four intervals in the presence

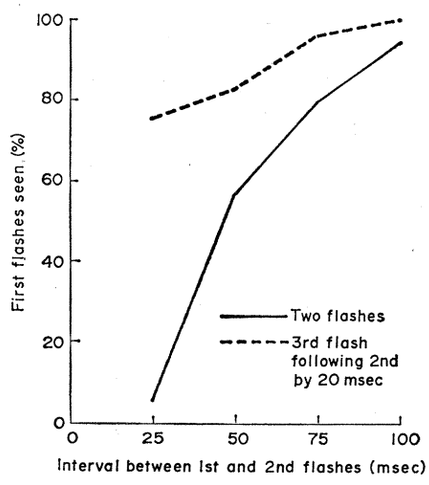

Fig. 1. Pooled data from two subjects showing percentage of first flashes detected as a function of the interval between first and second flashes for two conditions: one in which no third flash followed (solid curve), and one in which a third flash followed the second by 20 msec (dashed curve). Each point is based upon 100 trials.