Disinfection by Electrohydraulic Treatment

Abstract. *Electrohydraulic treatment was applied to suspensions of* Escherichia coli, *spores of* Bacillus subtilis *var.* niger, Saccharomyces cerevisiae, *and bacteriophage T2 at an input energy that, in most cases, was below the energy required to sterilize. The input energy was held relatively constant for each of these microorganisms, but the capacitance and voltage were varied. Data are presented which show the degree of disinfection as a function of capacitance and voltage. In all cases, the degree of disinfection for a given input energy increases as both capacitance and voltage are lowered.*

Electrohydraulics, the discharge of a high-voltage arc under the surface of a liquid medium, has been shown to be an effective way to sterilize suspensions of microorganisms in water. Data reported previously (*1*) demonstrated complete kill of a variety of microorganisms and showed that logarithmic decrease in the concentration of viable microorganisms (disinfection) is essentially a straight-line function of input energy. This report shows the relation of the decrease in concentration of viable miroorganisms to the voltage and capacitance levels of the electrohydraulic discharge at an input energy approximately constant for each organism tested.

Cultures of the following organisms were tested: *Escherichia coli* ATCC 11229, *Bacillus subtilis* var. *niger* ATCC 9372, *Saccharomyces cerevisiae* ATCC 2338, and *E. coli* T2 bacteriophage (*2*).

Escherichia coli was cultured on nutrient agar slants in milk dilution bottles at 37°C for 18 to 20 hours. Cells were suspended by gentle washing in 0.01*M* phosphate buffer and were passed through Whatman No. 1 filter paper. *Bacillus subtilis* var. *niger* was cultured in similar manner at 37°C for 48 hours. After 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. *Saccharomyces cerevisiae* was grown on Sabouraud's glucose agar at 25°C for 20 hours. Cells were removed by

Table 1. Experimental conditions and results. Volume of the organism suspension was 1.2 liters; total treatment time was less than 1 minute.

Organism	Initial conc. per ml	Capacitance (μfarads)	Voltage (kv)	Joules per discharge	Total discharges	Watt-hours per liter	Final conc. per ml
Escherichia coli	1–1.6 × 10^8	18	15	2025	6	2.80	9.8 × 10^3
		18	12	1296	9	2.69	8.8 × 10^3
		18	10	900	13	2.70	9.8 × 10^2
		18	8	576	19	2.53	8.2 × 10^2
		12	15	1350	8	2.50	9.7 × 10^3
		12	12	864	13	2.61	2.9 × 10^2
		12	10	600	19	2.64	95
		12	8	384	30	2.66	3
		12	6	216	53	2.63	0
		6	12	432	27	2.69	7 × 10^2
		6	10	300	38	2.64	8
		6	8	192	60	2.67	0
Spores of *Bacillus subtilis* var. *niger*	9 × 10^5	36	14	3528	3	2.45	1.45 × 10^4
		36	11	2178	5	2.52	7.1 × 10^3
		36	9	1458	8	2.70	1.23 × 10^3
		36	7	882	13	2.65	128
		36	6	648	18	2.71	20
		36	5	450	25	2.60	0
		27	14	2646	4	2.46	9.2 × 10^3
		27	11	1624	7	2.63	1.35 × 10^3
		27	9	1094	10	2.54	910
		27	7	662	17	2.60	35
		18	14	1764	7	2.86	180
		18	11	1089	11	2.77	177
		18	9	729	16	2.70	122
		18	7	441	26	2.66	0
Saccharomyces cerevisiae	1.6 × 10^7	18	12	1296	22	6.60	6.6 × 10^5
		18	9	729	40	6.75	2.9 × 10^5
		18	5	225	128	6.67	1.5 × 10^4
	8.3 × 10^6	9	9.6	415	70	6.72	3 × 10^4
		9	5	113	255	6.67	9
Bacteriophage T2	10^9	45	9	1823	6	2.54	1.9 × 10^4
		45	7	1103	10	2.56	0
		36	11	2178	5	2.53	9.1 × 10^3
		36	9	1458	8	2.70	2.75 × 10^3
		36	7	882	13	2.66	0
		27	14	2646	4	2.48	1.35 × 10^5
		27	11	1624	7	2.63	1.03 × 10^4
		27	9	1094	10	2.53	8.5 × 10^2
		27	7	662	17	2.60	0
		18	14	1764	7	2.86	4.7 × 10^3
		18	9	729	16	2.70	9 × 10^2
		18	7	441	26	2.65	0
		9	14	882	14	2.86	6.4 × 10^2
		9	9	365	32	2.70	2 × 10^2
		9	7	220	52	2.65	0

gentle washing in 0.01*M* phosphate buffer. Immediately prior to treatment, these three organisms were diluted in a 10- to 100-fold volume of sterile distilled water. The number of viable cells (or spores) was determined by duplicate plate counts on growth medium.

Bacteriophage T2 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.

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.

After treatment, samples of bacteria or yeast 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 T2 was assayed in the same manner by plating serial tenfold dilutions on nutrient agar seeded with the host organism *E. coli* B.

In order to have a basis for comparison of different voltage levels and capacitances, the total electrohydraulic input energy was held as close as possible to 2.6 watt-hours per liter for *E. coli*, *B. subtilis* spores, and the phage. Because of the greater resistance of *S. cerevisiae*, tests were performed at an electrohydraulic input energy as close as possible to 6.6 watt-hours per liter. The total energy for each single electrohydraulic discharge is given by $J = \frac{1}{2} CE^2$, where J is the energy in joules or watt seconds; C, the capacitance in farads; and E, the voltage. Therefore, as C and E are varied, the energy per discharge J varies. Constant input energy was maintained by varying the total number of discharges fired into the 1.2-liter volume of organisms suspended in sterile distilled water in the sterilizing tank. In all cases, total treatment time was less than 1 minute.

The experimental conditions and results are given in Table 1. Variation in total input energy for any one organism is caused by the necessity of firing whole numbers of discharges at any given joule input level. In all cases, increased disinfection at constant input energy occurs at lower capacitance and lower voltage.

The equipment utilized in this study was limited to a low capacitance of 6 μfarad, and data below that level could not be taken. The equipment was capable of operation at voltage levels below 5 kv, but oscilloscope traces of the electrical characteristic (voltage and current versus time) of the electrohydraulic discharge showed a rapid deterioration as voltage was lowered below about 4.5 kv. This deterioration of electrical characteristic, a function of electrode design, would have introduced other variables into the experiment, so that testing was limited to a low voltage level of 5 kv.

The fact that disinfection by electrohydraulic treatment is maximized at low voltages and low capacitances means that equipment is less expensive to frabricate and the costs of power are minimized. As a result, this disinfection technique continues to look attractive.

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References and Notes

1. M. Allen and K. Soike, *Science* **154**, 155 (1966).
2. ATCC is the American Type Culture Collection, Rockville, Md.

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Turnover of Rat Liver Tyrosine Transaminase: Stabilization after Inhibition of Protein Synthesis

Abstract. *Turnover of the rat liver tyrosine transaminase in vivo was measured by a label and chase procedure under conditions where the amount of enzyme undergoes no change. Half-life of the ^{14}C-labeled enzyme in this basal condition was found to be 1.5 ± 0.3 hours. Inhibitors of protein synthesis (cycloheximide or puromycin) do not appreciably influence the basal enzyme level over a 5-hour period, although these drugs will block hormonal induction of this enzyme. In pulse-labeling experiments, cycloheximide blocked transaminase synthesis almost completely. The conclusion that enzyme degradation, as well as synthesis, must be blocked when protein synthesis is stopped was confirmed in experiments showing that labeled enzyme is stable in the liver of rats treated with cycloheximide. The participation of a continuously synthesized polypeptide in the degradative phase of transaminase turnover is suggested.*

The tyrosine α-ketoglutarate transaminase of rat liver is generally considered to undergo unusually rapid turnover in vivo. This was first shown by measurements of the rate of decay of the elevated enzyme level in hydrocortisone-treated animals (*1*) and later confirmed by a crude isotopic analysis of turnover in the steady-state or basal condition (*2*), in which the enzyme level undergoes no change. These and subsequent measurements based on the rate of hormonal-induced change (*3*) have yielded half-life estimates ranging from 1.7 to 3.5 hours. The widely used inhibitors of synthesis of RNA and of protein (actinomycin D, puromycin, and cycloheximide) do not appreciably affect the basal level of this enzyme (*4*) except in some special circumstances (*5*), but these inhibitors are all effective in blocking changes in the rate of transaminase synthesis brought about by various hormones (*4*). The failure of actinomycin to lower the basal enzyme level has been interpreted as indicating that synthesis of this enzyme occurs on stable templates (*6*), but no explanation for the similar results with inhibitors of protein synthesis has been presented. The present experiments were done (i) to determine the rate of transaminase turnover in the basal condition with the increased precision that is now possible due to improved immunological techniques and (ii) to resolve the apparent discrepancy which arises in the failure of inhibitors of protein synthesis to lower the level of an enzyme which normally must be maintained by continual enzyme synthesis.

The data of Fig. 1 are from two experiments in which the normal rate of transaminase turnover was determined. Livers of adrenalectomized, fasted (18 hours) rats, killed at hourly intervals after a single injection of