THE EFFECT OF SONIC VIBRATIONS ON PHAGE, PHAGE PRECURSOR, AND THE BACTERIAL SUBSTRATE*

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During recent years mechanical vibrations produced by sonic and ultrasonic oscillators have been used extensively for two types of investigation in the field of bacteriology. Chambers, Mudd, Flosdorf, and coworkers at the University of Pennsylvania, have employed sonic vibrations to rupture bacterial cells and liberate antigenic substances. They have obtained detectable quantities of immunologically active components which normally are very labile, as for example, the soluble material of *H. pertussis* responsible for absorption of phase I agglutinins (1), the *E. typhi* Vi antigen (2), and the phagocytosis-promoting factor of the Lancefield C substance (3).

Another application has been the denaturation of various biologically active proteins such as enzymes and viruses. Flosdorf and Chambers (4) studied the inactivating effect of a frequency of 8900 cycles per second on egg albumin and found that the denaturation produced was comparable to that obtained with other denaturing agents. Rivers, Smadel, and Chambers (5) exposed vaccinia virus to the same frequency; the elementary bodies were inactivated but did not undergo disruption. Hapwood, Salaman, and MacFarlane (6) used ultrasonic waves generated by a quartz crystal vibrating at a natural frequency of 550 kc. per second for the treatment of vaccinia virus but did not observe any inactivation. In the case of tobacco mosaic virus, however, Stanley (7) found that the 550 kc. per second frequency produced fairly rapid inactivation. While conflicting results have been reported with reference to inactivation and denaturation, this is probably due in large measure to the wide variations in intensity, frequency, and mode of application of the vibrations.

We wish to report here the results of experiments undertaken to compare the rates at which phage, phage precursor, and staphylococci are destroyed by sonic vibrations.

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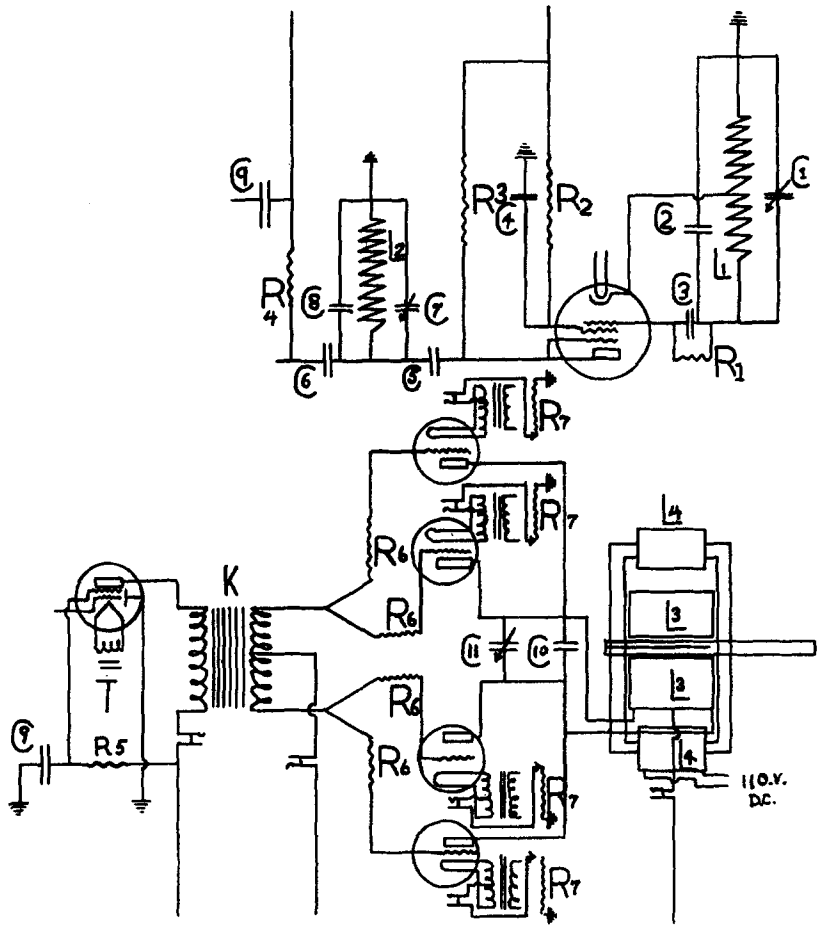

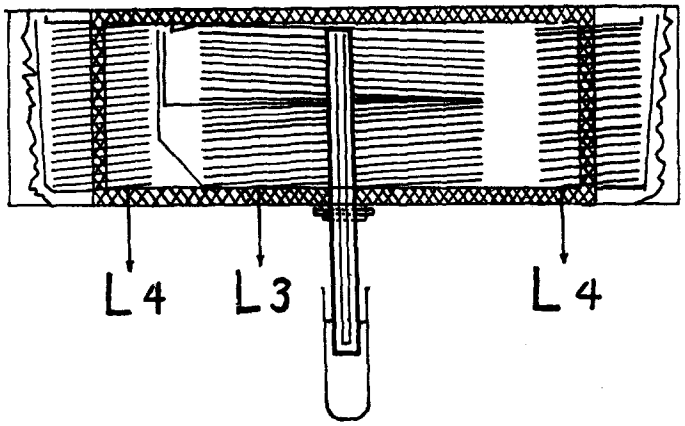

FIG. 1

Methods

A magneto-striction oscillator of approximately 320 watts output was constructed to produce vibrations of 9300 cycles per second frequency in a 20 gauge nickel tube having an outside diameter of $^{15}/_{32}$ inch. Fig. 1 gives the details of the circuit construction and the mounting of the nickel tube. In the oscillators previously reported the nickel tube has been pointed upward with the result that special provisions had to be made to maintain the material exposed in place over the tube. By constructing the tube so that it points downward it has been possible to circumvent many of the difficulties attending sonic treatment and we find that solutions to be studied can be placed in suitable open containers very conveniently. Two factors contributing to the operating efficiency of the oscillator are the improved tube suspension which diminishes damping to a great extent and the use of containers properly curved for focusing reflected sonic waves.

FIG. 1. Circuit diagram for sonic generator.

C-1	0.0005	μfd.	air condenser	500	volt
C-2	0.001	"	mica condenser	1,000	"
C-3	0.002	"	" "	1,000	"
C-4	1.000	"	" "	1,500	"
C-5	4.000	"	paper "	750	"
C-6	1.000	"	mica "	1,500	"
C-7	0.0015	"	air "	1,000	"
C-8	0.0025	"	mica "	2,500	"
C-9	0.01	"	" "	2,500	"
C-10	0.009	"	" "	7,500	"
C-11	0.00025	"	castor oil condenser	12,500	"

L_1 = 2000 turns No. 22 gauge enameled copper wire layer wound 1 × 1½ inches. L_2 = 2000 turns No. 22 gauge enameled copper wire layer wound 1 × 1½ inches. L_3 = 12 series connected, stagger wound pancake coils of No. 22 gauge enameled copper wire, 123 turns each (1476 turns total) center tapped. These coils wound on 1½ inch core and separated by 10,000 volt insulating spacers. Complete assembled unit is 4 inches thick × 8 inches diameter. L_4 = 2 series connected, layer wound coils of No. 22 gauge enameled wire 250 turns each, cored with ½ inch × 2 *C* shaped mild steel pieces, gaped on ends at 0.020 inch. These coils pick up high voltages due to eddy currents so are insulated with 5,000 volt varnished cambric. Magnet is activated by 110 volts D.C. at 13.6 amps. K = a choke coil approximately ¾ Henry. The 4 lb. core is 2 square inches laminated high grade silicon steel 0.008 inch air gap. Primary 2 sections of 500 turns total No. 25 guage enameled copper wire with secondary interposed. Four leads. Secondary, 400 turns No. 23 guage enameled copper wire center tapped.

R_1 = 250,000 ohms resistance. R_2 = 100,000 ohms resistance. R_3 = 20,000 ohms − 2 watts. R_4 = 20,000 ohms − 2 watts. R_5 = 20,000 ohms − 2 watts. R_6 = 100 ohms − 2 watts. R_7 = 3,000 ohms − 75 watts.

The lower diagram shows the oscillating coil and D.C. magnets, as centrally located in a copper tank, 12 × 12 × 18 inches. Coal oil, chosen because of its high flash point and high dielectric constant is circulated at 6°C. through this tank and delivered under pressure to the bottom of the nickel tube. This device insures that the maximum temperature attained in the sample is not greater than 16°C.

Experiments were performed to determine whether the withdrawal of aliquots at intervals with consequent reduction in total volume of the exposed material would sufficiently alter energy absorption conditions to affect the rates of change. It was found that aliquot sampling restricted to small volumes did not detectably influence the slope of the curves for cell destruction or phage inactivation. For the sake of uniformity and convenience this sampling procedure was employed throughout our work rather than the more cumbersome method of exposing successive identical volumes for various time periods.

To study the rate of phage inactivation standard staphylococcus phage containing 1×10^{10} activity units/ml. was diluted to a titer of 1×10^{9} units/ml. in Locke's solution of pH 7.2. The solution was exposed to the action of the oscillator and samples were removed at intervals for determination of residual [phage] by the activity method (8). The rate of phage inactivation was found to serve satisfactorily as a measure of the oscillator's operating efficiency and the energy output was checked daily by running a phage inactivation experiment. Besides measuring the effect of the vibrations on phage alone we have carried out experiments on the rate of inactivation of phage in the presence of various concentrations of homologous staphylococci.

To determine the killing effect of the vibrations on staphylococci, suspensions containing approximately 5×10^{10} cells/ml. in Locke's solution at pH 7.2 were exposed and sampled at intervals, the residual viable cells being determined by plate counts.

For the study of phage precursor inactivation the following procedure was employed:

18 hour Roux flask cultures of staphylococci grown on nutrient agar were washed twice in Locke's solution and a broth suspension was prepared containing 5×10^{9} organisms/ml. The culture was maintained at 37°C. for 1 hour while oxygen was bubbled through the broth. At the end of this time an equal volume of broth was added and the oxygenation at 37°C. was continued for an additional hour. The cells were then centrifuged down and re-suspended in Locke's solution at a final concentration of 5×10^{10} cells/ml. Such "activated" cells when added to phage produce a tenfold increase in activity titer after only 2 minutes contact at 0°C. This phage-augmenting capacity has been attributed to the presence of phage precursor in the bacteria (9). The rate at which supersonic vibrations destroy intracellular precursor was followed by interval sampling. To a 4.0 ml. aliquot of each sample diluted to a bacterial density of 5×10^{8} cells/ml. was added 1.0 ml. of phage containing 1×10^{9} activity units/ml. The mixtures were kept at 5°C. for 5 minutes to allow the conversion of residual precursor into phage and were then titrated for phage activity. Plate counts were also made on the same samples to eliminate the possibility that any apparent reduction in precursor content might be ascribable to the killing of bacteria with subsequent irreversible sorption of phage.

EXPERIMENTAL RESULTS

When phage solution is exposed to the action of sonic vibrations of 9300 cycles per second frequency, there is no demonstrable lag period in the phage destruction curve; within 10 minutes the phage is reduced to about 0.1 per cent of the original titer (Fig. 2). In the cases of the precursor inactivation processand the lethal effect on bacteria there are lag phases of about 3 minutes and 30 minutes respectively (Fig. 3). It should be pointed out

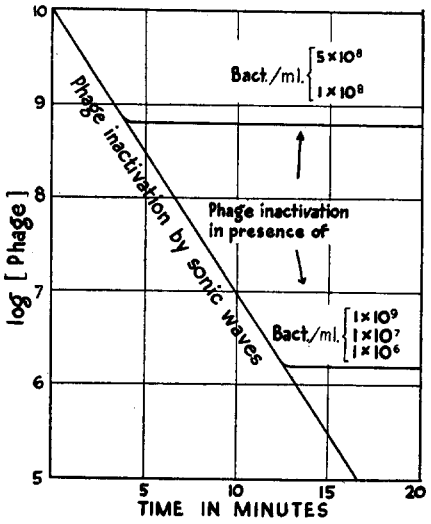

FIG. 2. Phage inactivation by sonic waves. The straight line represents the course of phage inactivation in the absence of bacteria. Phage inactivation in the presence of susceptible organisms follows the same curve for a time after which there is no further destruction of phage. The curve for phage alone is the average of twelve experiments and that for phage in the presence of bacteria is the average of ten experiments.

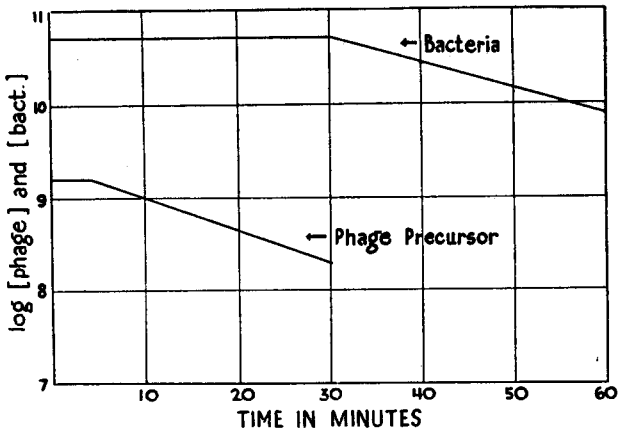

FIG. 3. The curves for inactivation of phage precursor and killing of bacteria by sonic waves. Residual intracellular precursor was measured by capacity of the cells to form phage when added to phage. Numbers of bacteria were determined by plate count. For both curves the data of eleven experiments were averaged.

that the curve for precursor inactivation is expressed as total phage produced when cells to be tested for precursor are added to a known amount of phage. The initial phage titer of the mixture is 2×10^8 activity units/ml. and any

increase above this level can be attributed to precursor reacting with phage to form more phage (10). The precursor inactivation experiments present a possible source of error in that the accumulation of any appreciable number of dead cells in the treated samples might bring about an apparent reduction in total phage formed without actually involving a direct loss of precursor. This depends upon the fact that dead cells can take up large amounts of phage; such sorption is irreversible and the attached phage does not participate in the titration (11). "Dead cells" as used here connote staphylococci which have lost their power to reproduce and if this criterion is employed there can be no error in the precursor inactivation curves for all the precursor is lost by the time the reproductive capacity of the staphylococci is measurably reduced. However, there remains the further possibility that cells treated with sonic vibrations may develop the property of taking up phage irreversibly before the reproductive mechanism is damaged. Control experiments were run on this point, using both normal and activated bacteria, and it was found that irreversible sorption does not take place until the cells have lost their ability to divide.

Once the lag phases are ended the destruction of precursor and the killing of staphylococci proceed logarithmically with time, the rate of the former process being somewhat faster than that of the latter. Theoretically one would expect the precursor to be destroyed much more rapidly than the cells, for it seems to be a protein (12) and its probable particle size may be assumed to be of the order of magnitude of phage itself; *i.e.*, very much smaller than the bacterium. Therefore the energy required for denaturation of the precursor molecule should be much less than the amount needed to destroy the cells. Actually, however, two facts modify this hypothesis. In the first place the precursor is intracellular and as a consequence is protected by the cell substance which no doubt absorbs a major share of the sonic vibrations. Secondly, in our experiments we have not measured the rate of cellular disruption but rather the rate at which the cells lose their ability to reproduce. Minor alterations of only a fraction of the total cell substance may be responsible for this loss. There is then no direct significance to be attached to the similarity between the two rates of inactivation. The fact that the precursor content of the cell can be abolished before the reproductive mechanism is damaged, is compatible with recently reported observations; namely that heat (12), iodoacetic acid (13), and methylene blue + light (14) can accomplish the same result.

The vast difference in particle size between the phage (about 50 $\mu\mu$) and the staphylococci (1,000 $\mu\mu$) can be invoked to account for the wide

variation in energy requirements for the destructive reactions. When phage is exposed to sonic vibrations in the presence of homologous organisms the rate of inactivation coincides initially with that for phage alone but plateaus are soon reached beyond which no further destruction of phage appears to occur. With [bacteria]'s of 5×10^8/ml. or 1×10^8/ml. the plateau begins after 4 to 5 minutes of exposure and the [phage] remaining is about 6 per cent of the original titer. Lower [bacteria]'s do not protect the phage as efficiently; with 1×10^7 and 1×10^6 bacteria/ml. the inactivation continues for 12 minutes and only 0.02 per cent of the original phage is left in active form. As in the case of precursor the inhibition of sonic inactivation probably depends upon absorption of energy by the staphylococcal cell substance. The organisms take up most of the phage and the intracellular phage fraction is protected by the relatively large volume of bacterial protoplasm around it. In general higher concentrations of bacteria confer a greater protective effect but for some unexplained reason the highest concentration of bacteria used, 1×10^9 cells/ml., gives no more protection than very low concentrations.

SUMMARY AND CONCLUSIONS

1. A nickel tube magneto-striction oscillator of 320 watts output producing sonic vibrations of 9,300 cycles per second frequency is described. Certain structural innovations contribute to operating efficiency and permit more convenient exposure of test materials than in earlier types.

2. The rate of phage inactivation by sonic waves proceeds logarithmically with time and serves as a satisfactory measure of energy output during operation of the generator. The curve for phage inactivation taking place in the presence of homologous staphylococci follows that for phage alone but soon reaches a plateau after which no further loss of activity is noted. In general higher concentrations of bacteria more effectively inhibit phage destruction than do lower concentrations.

3. Cells that have attained a resting state after a preliminary phase of rapid growth normally have the capacity of inducing a very rapid and marked increase in [phage] when added to phage. This effect has been attributed to the presence of intracellular phage precursor. The store of phage precursor in activated cells is destroyed by sonic waves in about 30 minutes. The number of cells (plate count) shows no reduction until after the precursor is entirely inactivated.

4. Attempts to extract phage precursor from activated staphylococci by exposing the cells to sonic vibrations were unsuccessful.

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