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The Effects of Various Physical and Chemical Agents on a Staphylococcus Bacteriophage

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The *Butler University Botanical Studies* journal was published by the Botany Department of Butler University, Indianapolis, Indiana, from 1929 to 1964. The scientific journal featured original papers primarily on plant ecology, taxonomy, and microbiology. The papers contain valuable historical studies, especially floristic surveys that document Indiana's vegetation in past decades. Authors were Butler faculty, current and former master's degree students and undergraduates, and other Indiana botanists. The journal was started by Stanley Cain, noted conservation biologist, and edited through most of its years of production by Ray C. Friesner, Butler's first botanist and founder of the department in 1919. The journal was distributed to learned societies and libraries through exchange.

During the years of the journal's publication, the Butler University Botany Department had an active program of research and student training. 201 bachelor's degrees and 75 master's degrees in Botany were conferred during this period. Thirty-five of these graduates went on to earn doctorates at other institutions.

The Botany Department attracted many notable faculty members and students. Distinguished faculty, in addition to Cain and Friesner , included John E. Potzger, a forest ecologist and palynologist, Willard Nelson Clute, co-founder of the American Fern Society, Marion T. Hall, former director of the Morton Arboretum, C. Mervin Palmer, Rex Webster, and John Pelton. Some of the former undergraduate and master's students who made active contributions to the fields of botany and ecology include Dwight. W. Billings, Fay Kenoyer Daily, William A. Daily, Rexford Daudenmire, Francis Hueber, Frank McCormick, Scott McCoy, Robert Petty, Potzger, Helene Starcs, and Theodore Sperry. Cain, Daubenmire, Potzger, and Billings served as Presidents of the Ecological Society of America.

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THE EFFECTS OF VARIOUS PHYSICAL AND CHEMICAL AGENTS ON A STAPHYLOCOCCUS BACTERIOPHAGE

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In recent years considerable attention has been focused on a group of organisms known as bacterial viruses or bacteriophages. These minute virus particles are parasitic upon bacterial cells and probably most bacteria are susceptible to one or more of them. There are several excellent reviews and symposia covering the various steps of bacteriophage multiplication and the effects of inhibiting agents (1, 29, 30). The reader is referred to them for an explanation of the processes involved in attachment to and multiplication within the host cell.

The experimental work in this paper is divided into two sections: (1) the effect of physical agents and (2) the effect of chemical agents on a staphylococcus bacteriophage. The physical treatments include thermal inactivation, ultrasonic vibration, lyophilization, long term storage, effect of temperature on adsotption rate and photoreactivation after exposure to ultraviolet light. Chemical treatments included suspension in various salt solutions and the effect of several pH values on phage stability. It is apparent that some of these procedures could be classified as either physical or chemical in action or more properly as physico-chemical. However, for the purpose of this paper the more simple distinction will be followed beginning with the effects of physical agents. Preliminary experiments had shown that the phage used was inactivated in 30 minutes when suspended in broth at 60° and that it gradually loses activity when stored in broth at 4°. High speed centrifugation procedures also cause rapid inactivation.

GENERAL MATERIALS AND METHODS

Media: Tryptose phosphate broth (TPB) was used to grow the host organism and as a diluent. One percent base layer agar plates, 1.5% agar slants and 0.7% top layer agar tubes were prepared by adding sufficient agar to the TPB. All media were sterilized by autoclaving 15 minutes at 15 pounds. The final pH was 7.4.

Phage and Host Bacterium: The host bacterium SK9 and the phage P1 are isolates from cultures used routinely in antiphage assays at Eli Lilly and Company. Lysates of SK9 by P1 titer from 5 to 7 x 10^9/ml. after Selas filtration.

Phage Titering: The P1 samples were titered using the method described by Adams (1). The plates were inverted, then incubated overnight at 37° and counted on a Quebec colony counter.

ADSORPTION RATE AT VARIOUS TEMPERATURES

The first step in virus multiplication is adsorption, or attachment to the surface of the host cell prior to penetration of the cell membrane. Information regarding the adsorption phenomenon is of value since this action or a similar series of events is assumed to be universal among all plant, mammalian or bacterial viruses as the initial step in infection of the host cell.

Material and Methods: SK9 was grown in TPB to 2 x 10^7 organisms/ml., centrifuged and resuspended in an equal amount of physiological saline. The phage was diluted to 1 x 10^8/ml. in saline and both phage and host organism were brought to the desired temperature before mixing. At t=O, one ml. of P1 was added to nine ml. of SK9 and the tube was shaken. One half ml. samples were removed at intervals then diluted 1/2000 in saline at 2° to stop further adsorption. A phage titer was taken to determine the original titer and the samples were centrifuged 10 minutes at 5900g. The supernate was titered. Velocity constants were computed from the formula:

$$k = \frac{2.3}{t \times \text{cell count}} \times \log \frac{\text{initial phage titer}}{\text{final phage titer}}$$

Results and Discussion: Velocity constants for the attachment of P1 to SK9 in saline range from 489 x 10^{-12} cm^3 min^{-1} at 1° to 729 x 10^{-11} at 45° (fig. 1). At a given temperature the maximum rate of virus adsorption is attained and additional increases in temperature do not result in a correspondingly larger velocity constant. The maximum rate of adsorption for this system lies between 28° and 37° since no increase occurs above the higher temperature. This same effect was shown by Puck, et al. (2) with coliphage T1 in broth or buffer with Mg++ where adsorption reaches a maximum around 37° and falls off with decreasing or increasing temperatures. In addition to the temperature effect adsorption is influenced by the presence of certain co-factors and salts and the physiological condition of the host cell.

LONG TERM STORAGE

Information regarding the ability of microorganisms to remain viable when stored under various conditions is of prime importance. Long term storage of cultures can be best accomplished by first freeze-drying the material. The results found using this technique with P1 are described elsewhere in this paper. However, bacteriophage suspended in liquid remains viable for some time. It is with this type of storage that the following experiments were concerned.

Material and Methods: A freshly prepared phage stock was diluted to approximately 4 x 10^5/ml. in broth (pH 7.4), saline (pH 6.8) and in distilled water (pH 6.8). A sample of each was stored at 4°, room temperature (23°) and 37° in stoppered test tubes. Titers were made at intervals.

Results and Discussion: P1 was completely inactivated in three days when suspended in distilled water at room temperature or 37°. Rapid inactivation also occurs at 4° in water as only 10% was active after one week. In four weeks over 99.9% was inactive. P1 was found unstable at 37° or room temperature in saline and is 60% inactivated after one week at 4°. The least inactivation occurs when P1 is held in TPB. In this medium 90% of the phage is lost in one week at 37°. Only 1% remains active after two months at room temperature. When P1 is suspended in TPB and stored at 4° it loses very little titer in three weeks. In two months the titer drops to 20% of the original value. This phage can be stored in broth for short periods but a considerable titer drop is to be expected. Freeze-drying of stock preparations is preferable in order to retain viable phage partieles.

LYOPHILIZATION

Several bacteriophages have been lyophilized with varying success. Six dysentery phages were prepared using this procedure by Schade and Caroline (3, 4, 5). Five of these six lost no activity after one year over a dessicant at 37°. Coliphage T6, grown in synthetic media and lyophilized by Putnam, et al. (6) was inaetivated. Many mammalian viruses resist freeze-drying and were found viable after years of storage. Hofstadt, et al. (7) and Scherp, et al. (8). Bacteriophage P1 was lyophilized using standard procedures.

Material and Methods: Freshly prepared lysates were filtered through an 02 Selas candle and titered. Twenty-five ml. were placed in a round bottom flask and rapidly shell frozen in an alcohol/CO_2 bath. The freezing required less than one minute. The flask was atached to a lyophilization apparatus, evacuated and dried for 21 hours. At the conclusion of the drying process the material, in the form of brown flakes, was scraped off the sides of the flask, weighed, and placed in a tightly stoppered tube. Other phage lysates were first dialized at 4° in saline or water then lyophilized.

Results and Discussion: Approximately 85% of the phage is lost in the process of lyophilization. An additional 6% loss occurred in storage after 30 days but no further decrease was seen during six additional months at 4°. In spite of such a large drop in phage activity no difficulty was experienced in preparing fresh P1 lysates from the lyophilized material. Lysates which had been dialyzed in water or saline before freeze-drying lost no titer. When these preparations were lyophilized and then reconstituted in either saline or TPB over 99% of

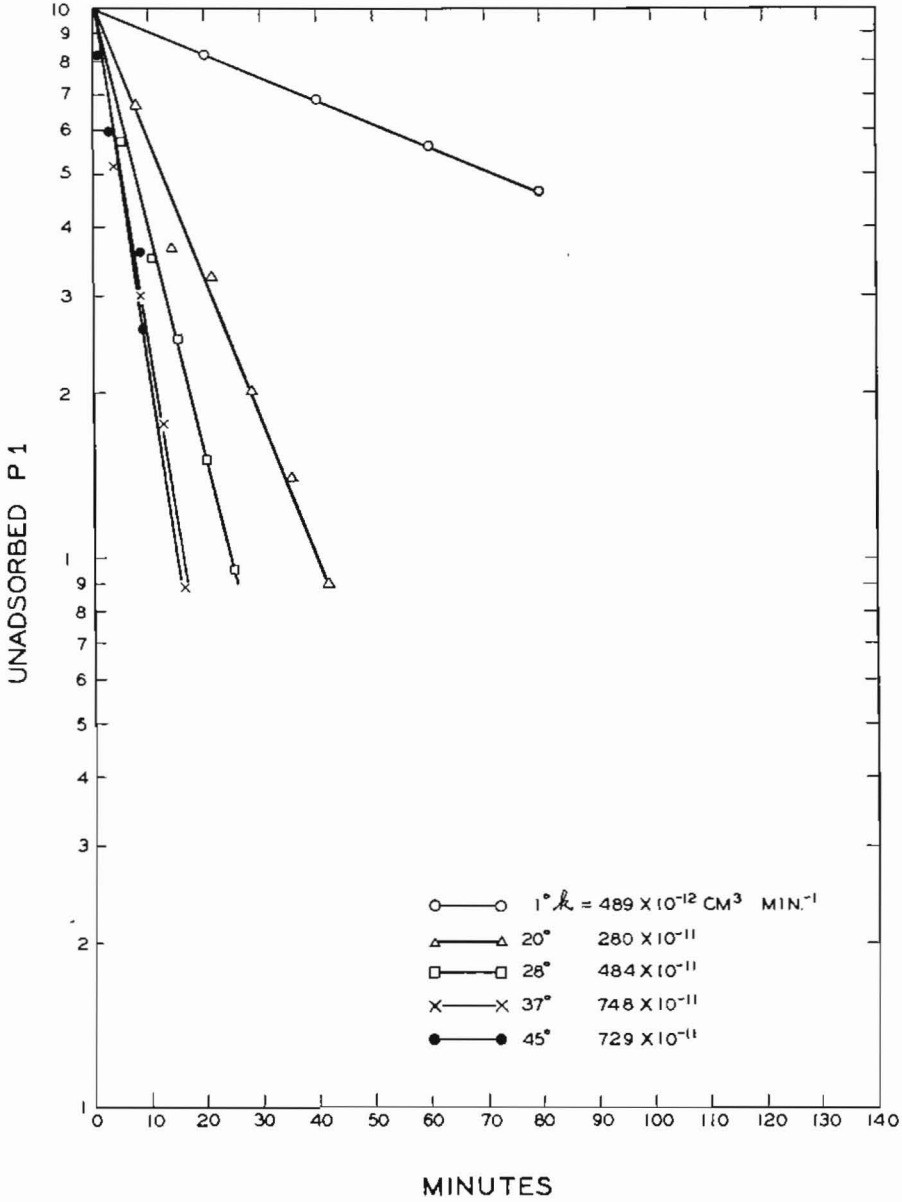

FIGURE 1

The effect of heat on the adsorption rate of PI bacteriophage to its host cell *S. aureus* SK9 in saline.

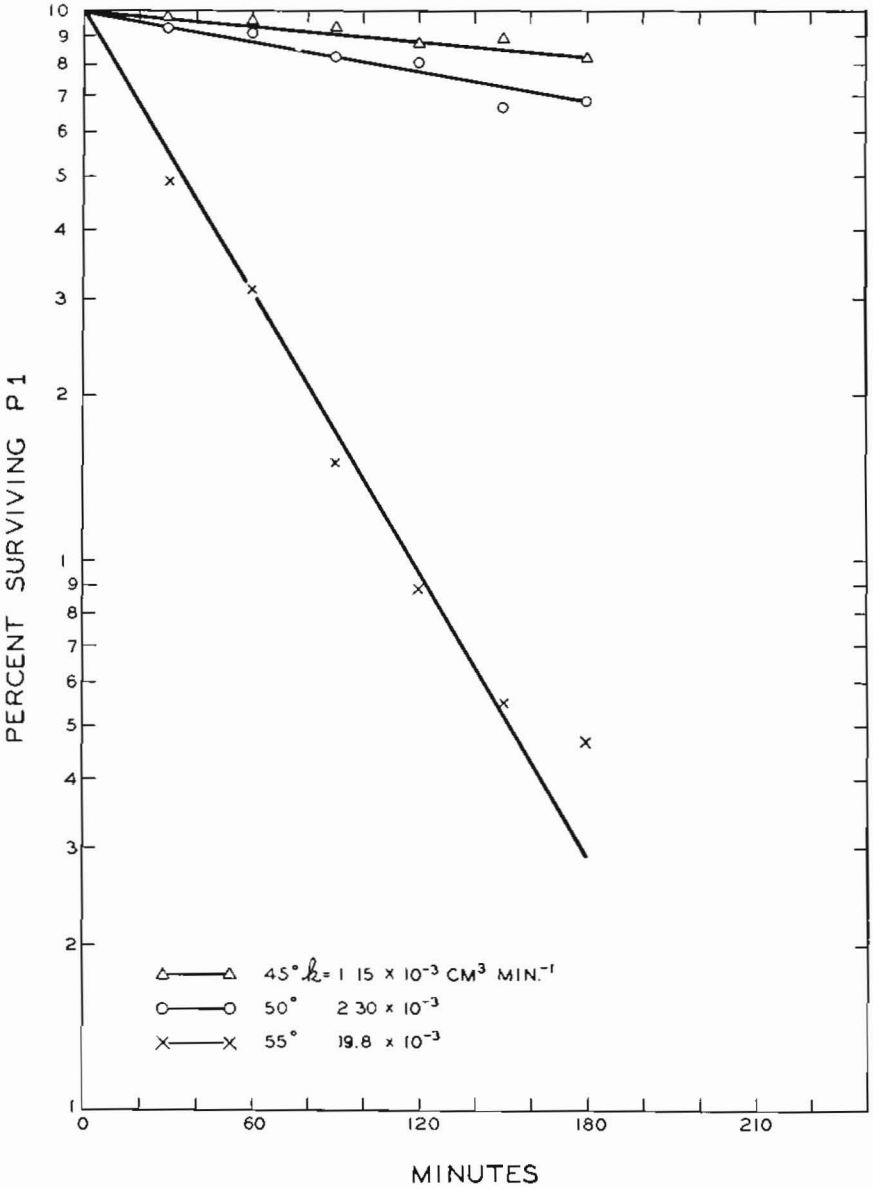

FIGURE 2

Log of uninactivated bacteriophage PI in broth at various temperatures as a function of time. 45°-55°.

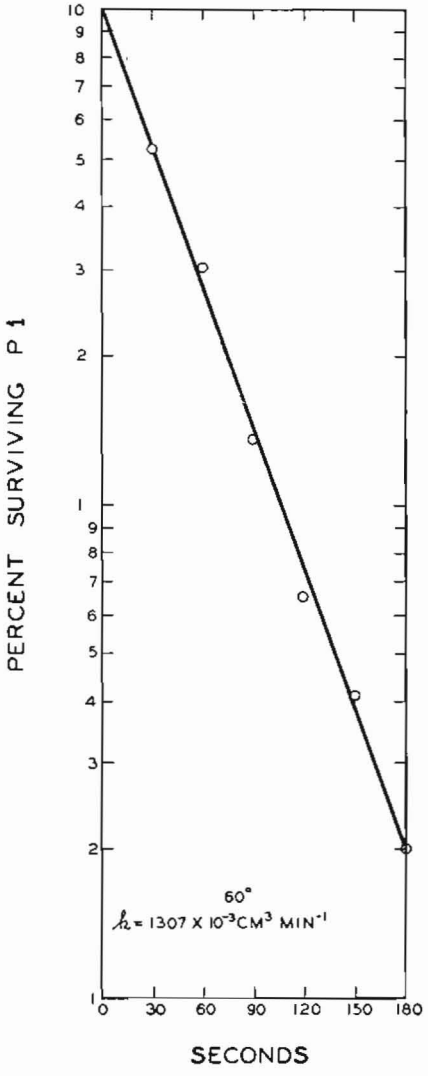

FIGURE 3
Log of uninactivated bacteriophage PI in broth at 50° as a function of time.

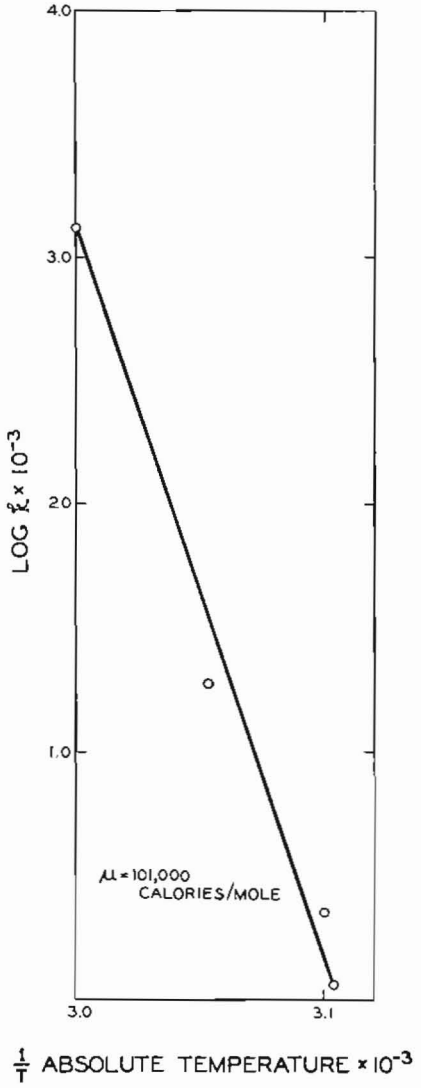

FIGURE 4
Log of the first order specific reaction rates for inactivation of PI bacteriophage as a function of temperature.

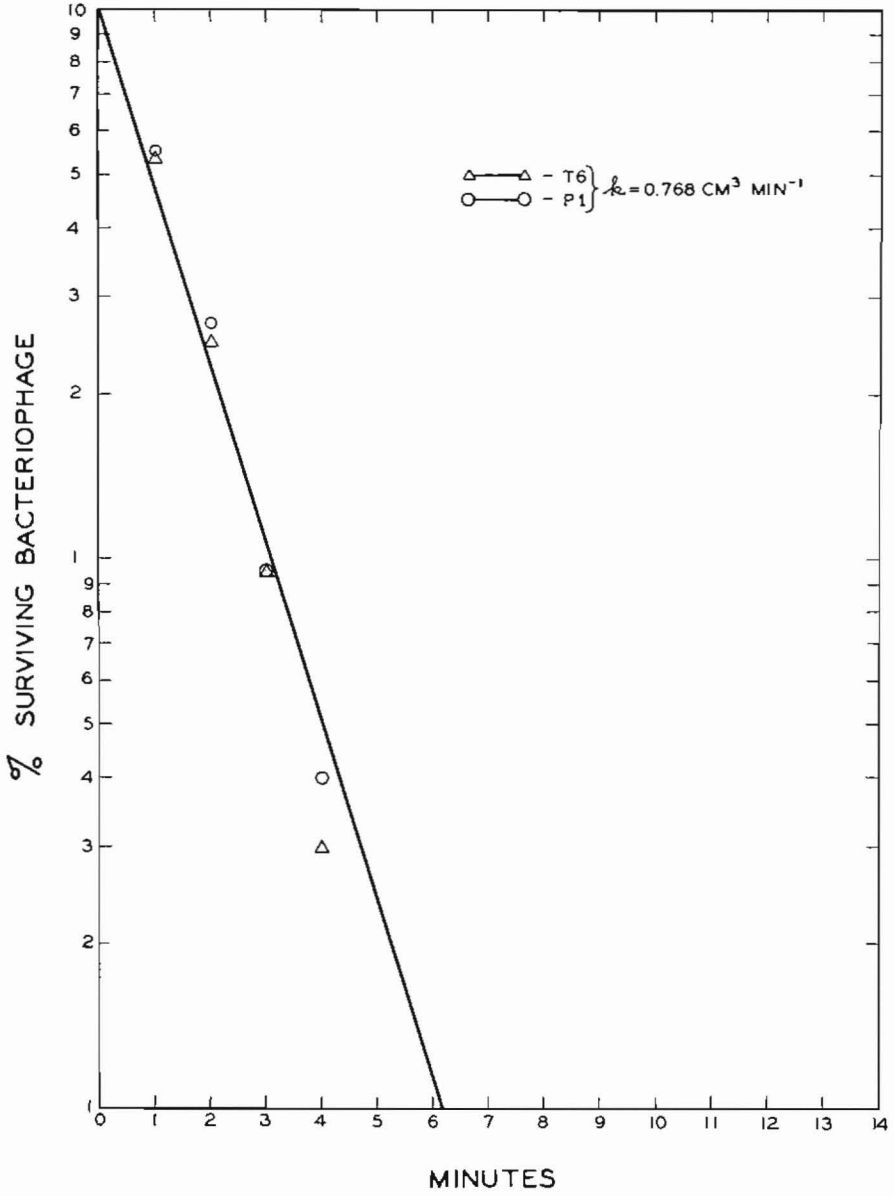

FIGURE 5

Log of uninactivated bacteriophages PI and T6 in broth treated with radio frequency oscillation as a function of time.

the phage was found to be inactivated. If P1 were lyophilized in ampoules under vacuum or dry nitrogen assuring the complete absence of moisture no doubt its stability would be greatly increased.

ULTRA-SONIC VIBRATION

Bacteriophages and other viruses have been shown to be very vulnerable to the effects of high frequency vibration. Such inactivation usually proceeds as a first order reaction.

Materials and Methods: The phage was diluted in TPB and 50 ml. placed in the water cooled Raytheon Magnetostriction sonic oscillator (Model DF-101). The frequency produced by this instrument is 10,000kcs. One tenth ml. samples were removed at intervals, diluted and plated. A sample of T6 coliphage was also prepared and treated in the same manner in order to compare the sonic effects on both phages. Velocity constants were determined using the formula,

$$k = \frac{2.3}{t} \times \log \frac{\text{initial phage titer}}{\text{final phage titer}}$$

cited in Pollard and Reaume (12).

Results and Discussion: P1 was found to be inactivated at approximately the same rate as the coliphages T2, T4, T5 and T6 and megatherium phages M2 and M3. In one minute one half of the phage was destroyed and only 2% remained active after five minutes exposure (fig. 5). All of the T series of coliphages have been subjected to this form of inactivation by Anderson, et al. (9). He found T2, T4, T5 and T6 to be more rapidly inactivated than the smaller phages T1, T3 and T7, and suggested that tbe larger, more complex phages were more susceptible to sonic action. Friedman and Cowles (10), working with a group of five *B. megatherium* phages could find no relationship berween size and relative sensitivity to sonoration. A staphylococcus phage was reported by Krueger (11) to be over 99% inactivated after 10 minutes exposure ro high frequency vibration. Velocity constants for both P1 and T6 were $0.77 cm^3 min^{-1}$.

THERMAL INACTIVATION

Velocity constants for the heat inactivation of various phages have been determined. Cherry (13) investigated the effect of heat on a *Streptococcus lactis* phage at temperatures from 30 to 65°. Other workers, Chang, et al., (14), Pollard and Reaume (15) and Adams (16) observed the effects of heat on the coliphage series. Friedman and Cowles (17) ran heat inactivation curves on their group of *B. megatherium* phages. In this paper a series of experiments were performed with P1 at temperatures of from 45 to 60°.

Materials and Methods: P1 was diluted in broth to 5 x 10^6/ml. One tenth ml. was added to a tube containing 9.9 ml. of TPB which had been previously brought to the desired temperature in a thermostatically controlled water bath. The tube was shaken, samples removed at intervals, diluted immediately in chilled TPB, then plated. The rate of inactivation (k) was calculated from the inactivation curves (figs. 2 and 3) for each temperature using the velocity constant equation. The logarithms of the k values for each temperatures were plotted against the reciprocals of the corresponding absolute temperatures in the manner developed by Arrhenius in order to illustrate the relationship between reaction rate and temperature.

Results and Discussion: Seventeen percent of the phage P1 was inactivated at 45° for 180 minutes and approximately twice that amount was dead at 50° for the same length of time. When P1 was treated at 55° for 180 minutes 97% of the phage was destroyed (fig. 2 and 3). Experiments performed at 65° indicate that over 99% was lost in less than 30 seconds. Velocity constants were not prepared for this temperature due to the difficulty in performing accurate sampling. Velocity constants (k) range from 1.15 x 10^{-3}cm^{3}min^{-1} at 45° to 1307 x 10^{-3} at 60°. All thermal inactivation studies for this phage compare well with those found by Krueger (17) in 1932 for a staphylococcus phage. The temperature characteristic of thermal inactivation (μ) has not been determined for all phages subjected to heat inactivation. In some cases the curve was not linear over its entire length. Cherry (13) found his S. *lactis* phage to have a μ value of 11,000 calories between 30 and 55° with an increase to 76,000 calories from 55 to 65°. The coliphage studies by Chang et al. (14) also had a non-linear curve with two μ values. The value of μ found for P1 was 100,000 calories and the curve was linear. This figure was also found by Krueger (17) with a staphylococcus phage (fig. 4).

PHOTOREACTIVATION

A description of the phenomonon known as photoreactivation (PHTR) of bacteriophages was first published in 1949 by Dulbecco (18). He found that all of the T coliphages, after exposure to ultraviolet light and plating on susceptible host cells, would, if incubated under strong visible light, result in higher titers than similarly treated coliphages incubated in the dark. Pre-illumination of the host cells or of the irradiated phage with visible light resulted in no PHTR and irradiated phage could only be reactivated after adsorption to the host cell. Hill and Rossi (19) working with a dried preparation of phage T1, showed that no PHTR took place with the dry material. They concluded that PHTR was dependent upon the state of the phage at the time of exposure to ultraviolet light. Dulbecco (18) and Watson (20) found no PHTR to occur in phage which had been treated with x-rays. PHTR in P1 staphylococcus phage was shown to occur in the following experiments.

Materials and Methods: P1 was irradiated using a 15 watt GE germicidal lamp with a filter giving maximum emission at 2570 A. Samples of phage diluted in TPB were exposed for periods of from three to three and one half minutes so as to cause approximately 80% inactivation. The irradiated phage was titered on 12 plates. Four were immediately placed in total darkness, four were placed beneath one 40 watt fluorescent lamp at a distance of 26 cm. and four beneath two 40 watt lamps at the same distance. All plates were incubated at 26° for 18 hours and then counted.

Results and Discussion: In these experiments reactivation titers amounted to twice the titer of the irradiated phage not treated with visible light. Titers of irradiated phage not reactivated averaged 15% of the original untreated phage but exposure to two fluorescent lamps for 18 hours resulted in titers equal to 29% of the untreated original phage. This effect has not previously been demonstrated using a staphylococcus phage. It is interesting to note that exposure of the irradiated phage to the visible light produced by one lamp did not cause the reactivation that two lamps produced in the same length of time. Also, if phage plates previously exposed to one lamp received additional visible light after the initial 18 hour period no additional reactivation occurred (table III). The maximum PHTR occurs in 18 hours or less but the total PHTR for a given period is dependent upon the intensity of the illumination for that period. Perhaps, and this possibility was not investigated, the only time when PHTR actually takes place is during the adsorption of the irradiated phage particle immediately after plating and all subsequent visible light treatment has no effect on phage titer.

HYDROGEN ION CONCENTRATION

Friedman and Cowles (10) found two of five *B. megatherium* phages to be stable for one hour at 37° in broth at pH 6 to 9 and one each stable from pH 5 to 9, 5 to 10 and 6 to 10. Purified coliphage T1 is most stable at pH 6 but little loss of titer occurs at pH values of from 4.3 to 7 after 24 hours in broth (Pollard and Reaume, 15). T7 is most stable from pH 6 to 8 (Kerby, et al. 21) and T6 is stable from pH 4.9 to 8.6 (Putnam, 22). No information regarding the stability of staphylococcus phages over a range of pH was found in the literature.

Materials and Methods: Bacto tryptose and NaCl were dissolved in water, brought to the desired pH, and sterilized in the autoclave. Glucose and Na_2HPO_4 were dissolved separately in water and sterilized, cooled and added to the tryptose-NaCl solution. Hydrochloric acid (0.1M) and NaOH (0.1M) were employed where necessary in adjusting to the exact pH. The phage was diluted in the appropriate pH broth, then held in a 37° water bath or stored at 4° for 24 hours. Samples were removed at one and 24 hours for titering.

TABLE I

The effect of various pH values on PI bacteriophage suspended in broth.

		pH Value									
	Percent PI active after:	3	4	5	6	7	8	9	10	11	12
Samples held at 37°	1 hour	0	0	86	95	98	51	35	0	0	0
	24 hours	0	0	16	60	72	44	5	0	0	0
Samples held at 4°	1 hour	0	96	100	100	95	67	63	0	0	0
	24 hours	0	79	96	96	98	49	51	0	0	0

TABLE II

The effect of various concentrations of NaCl on PI bacteriopbage at 37°.

Percent PI active after	Molar Concentration of NaCl								
	4	3	2	1	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
1 hr.	94	95	94	88	88	82	76	74	36
24 hrs.	22	24	27	24	28	24	6	4	5

TABLE III

Photoreactivation of irradiated bacteriophage PI.

	Exp. 1	Percent of Untreated Titer	Exp. 2	Percent of Untreated Titer	Exp. 3	Percent of Untreated Titer
Phage titer before irradiation	2.76×10^4		4.20×10^4		5.89×10^3	
Irradiated 3½ minutes, plates incubated in darkness	4.15×10^3	15	5.34×10^3	13	9.20×10^2	16
Irradiated, plates incubated under one 40 watt lamp	4.88×10^3	18	9.03×10^3	22	1.30×10^3	22
Irradiated, plates incubated under two 40 watt lamps	7.02×10^3	26	1.24×10^4	30	1.85×10^3	30

Results and Discussion: P1 was found to be relatively stable from pH 5 to 7 for one hour at 37° dropping off shatply at pH 4 and down to 35% at pH 9. All the phage was inactivated at pH values below 5 and above 9. A 25% drop in titer was seen when P1 was held in pH 7 TPB for 24 hours at 37°.

P1 in TPB at 4° was inactivated at an expected slower rate. Little titer loss occurred at pH's from 4 to 7 after one hour but complete inactivation occurred at pH 3 and pH 10. The phage remained stable after 24 hours at 4° in pH 5 to 7 in TPB. Fifty percent was destroyed at pH 8 (table I). In general, both mammalian viruses and bacteriophages are most stable when held in suspensions at pH values near neutrality. The zone of pH stability, however, varies over a range of pH 4 to 10 with very few viruses surviving except for short periods at pH's above 10 or below 4.

CITRATE ION

Several phage systems have been examined regarding the effect of citrate ions on adsorption and multiplication. It was suggested at one time that the classification of these organisms could be partially based on their behavior in the presence of citrate (Burnet, 23). Multiplication of coliphages T1 and T5 is inhibited in the presence of citrate but adsorption is uneffected (Adams, 24). None of the other T series phages are effected. Burnet and McKie (25) studied a large group of dysentery-Salmonella phages and found considerable variation in regard to citrate sensitivity. Rountree (26) divided a number of staphylococcal phages into sensitive and insensitive groups. All five of the megatherium phages examined by Friedman and Cowles (10) were sensitive to varying degrees.

Material and Methods: Top layer agar tubes and bottom layer agar plates were prepared containing from 10^{-1} to 10^{-4}M sodium citrate. P1 was diluted in TPB to 5 x 10^3/ml. then titered using the citrate agar. Controls were titered on standard TP agar.

Results and Discussion: The multiplication of P1 on the host cell was not inhibited by any concentration of sodium citrate tested.

DIVALENT SALTS

Burnet and McKie (25) investigated the effect of heat and salt concentration on a group of *Salmonella* and dysentery phages and found dilution in sodium, potassium or ammonium salts resulted in rapid loss when the phage was heated at 60°. The addition of small quantities of divalent salts such as calcium, magnesium or barium partially or completely prevented this inactiva-

tion. The addition of any one of four divalent salts at 10^{-3} M prevents phage loss at 37° for one hour on threc megatherium phages (Friedman, 27). The other two megatherium phages were only partially stabilized by the same salts. All except Hg++ and Pb++ had a definite protective effect on coliphage T5 in saline (Adams, 24).

Material and Methods: All divalent salts were prepared in 0.15 M NaCl and brought to pH 7. The phage was diluted and added to the salt concentrations then incubated at 37° in a water bath. Titers were made at one and 24 hours.

Results and Discussion: P1 was completely inactivated in one hour at 37° in the presence of 10^{-2} or 10^{-3} M Cu++, Pb++, or Fe++ ions and lost 80% in 10^{-2} M Cd++. None of the other salts used at these concentrations prevented inactivation any more than the 0.15 M NaCl control. The stability of P1 is not increased when diluted in divalent salt solutions.

SODIUM CHLORIDE

Only one group of phages has been examined for the effect of different NaCl concentrations on phage stability. This group consisted of the five *B. megatherium* phages studied by Friedman (18). Four of these phages were inactivated completely in NaCl concentrations of from 10^{-1} to 5 x 10^{-2} M after one hour at 37°. All were stable for the same length of time in 1 M solutions.

Material and Methods: Salt solutions were prepared using reagent grade NaCl dissolved in deionized water. The phage was diluted in deionized water to the desired concentration then 0.1 ml. was added to the tubes containing 9.9 ml. of each salt solution at 37°. Incubation was continued at 37° and titers were made at one and 24 hours.

Results and Discussion: In one hour at 37° from 5% to 26% of the P1 had been inactivated in the solutions containing from 4 M to 10^{-4} M NaCl. The least inactivation occurred at NaCl concentrations of 2 M to 4 M. Approximately 75% of the phage was lost in 24 hours at salt concentrations of 4 M to 10^{-2} M and ovet 90% at 10^{-3} M to 10^{-5} M (table II).

SUMMARY

A staphylococcus bacteriophage lysate was treated with various physical and chemical agents and the following results were obtained:

Bacteriophage P1 is inactivated by heat at a logarithmic rate and possesses a temperature characteristic (μ) of 100,000 calories/mole. Inactivation by

high frequency oscillation is a first order reaction with a velocity constant of 0.77 cm^3min^{-1}. Photo-reactivation occurs when ultraviolet treated P1 is exposed to strong light in the visible spectrum. Adsorption constants of P1 to SK9 range from 489 x 10^{-12} at 1° to 748 x $10^{-11}cm^3min^{-1}$ at 37°. P1 is most stable at pH values from 5 to 7. Of the mono- or divalent ions tested, none contributed significantly to the stability of the phage; the presence of citrate ions does not prevent phage multiplication. If P1 is held at 4° in TPB little loss of titer occurs in one month. P1 can be successfully lyophilized but there is considerable loss in the process.

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LITERATURE CITED

1. Adams, Mark. 1950. Methods of study of bacterial viruses. Methods in Medical Research, Vol. 2. The Yearbook Publishers, Chicago, Illinois.

2. Puck, Theodore T., Alan Garen, and Jewell Cline. 1951. The mechanism of virus attachment to host cells, I. The role of ions in the primary reaction. J. Exper. Med., 93:65-88.

3. Schade, Arthur L., and Leona Caroline. 1943. The preparation of a polyvalent dysentery bacteriophage in a dry and stable form. I. Preliminary investigations and general procedure. J. Bacr. 46:463-473.

4. ————. 1944. The preparation of a polyvalent dysentery bacteriophage in a dry and stable form. II. Factors affecting the stabilization of dysentery bacteriophage during lyophilization. J. Bacr. 48:179-190.

5. ————. 1944. The preparation of a polyvalent dysentery bacteriophage in a dry and srable form. III. Stability of rhe dried bacteriophage toward hear, humidity, age, and acidiry. J. Bact. 48:243-251.

6. Putnam, Frank W., Lloyd M. Kozloff, and Janice C. Neal. 1949. Biochemical studies of virus reproduction. I. Purification and properties of *Escherichia coli* bacteriophage T6. J. Biol. Chem. 179:303-323.

7. Hoffstadt, Rachel E., and Helen B. Tripi. 1946. A srudy of the survival of certain strains of viruses after lyophilizarion and prolonged storage. J. Infect. Dis. 78:183-189.

8. Scherp, H. W., E. W. Flosdorf, and D. R. Shaw. 1938. Survival of influenza virus under various conditions. J. Immunol. 34:447-454.

9. Anderson, Thomas F., Shiela Boggs, and Betty C. Winters. 1948. The relative sensitivities of bacterial viruses to intense sonic vibration. Science 108:18.

10. Friedman, Murray, and P. B. Cowles. 1953. The bacteriophages of *Bacillus megatherium*. I. Serological, physical and biochemical properties. J. Bact. 66:379-385.

11. Krueger, Albert P., B. B. Brown, and E. J. Scribner. 1941. The effect of sonic vibrations on phage, precursor and the bacterial substrate. J. Gen. Physiol. 24:691-698.

12. Pollard, Ernest. 1953. The physics of viruses. Academic Press, Inc. New York, N. Y.

13. Cherry, William B., and D. Watson. 1949. The *Streptococcus lactis* host-virus system. I. Factors influencing quantitative measurement of the virus. J. Bact. 58:601-610.

14. Chang, Shih L., Mary Willner, and Lois Tegarden. 1950. Studies on the destruction of bacterial virus. II. Kinetics in the thermodestruction of bacterial virus (bacteriophage against *E. coli*) in water. Am. J. Hyg. 52:194-201.

15. Pollard, Ernest, and Marjorie Reaume. 1951. Thermal inactivation of bacterial viruses. Arch. Biochem. and Biophys. 32:278-287.

16. Adams, Mark. 1951. Classification of bacrerial viruses: characteristics of the T5 species and of the T2, C16 species. J. Bact. 64:387-396.

17 Krueger, Albert P. 1932. The heat inactivation of antistaphylococcus bacteriophage. J. Gen. Physiol. 15:363-368.

18. Dulbecco, Renato. 1949. Reactivation of ultra-violet-inactivated bacteriophage by visible light. Nature 163:949-950.

19. Hill, Ruth F., and Harold Rossi. 1952. Absence of photo-reactivation in T1 bacteriophage irradiated with ultraviolet in the dried state. Science 116:425.

20. Watson, James D. 1950. The properties of x-ray inactivated bacteriophage. I. Inactivation by direct effect. J. Bact. 60:697-718.

21. Kerby, G. P., R. A. Gowdy, M. L. Dillon, D. G. Sharp, E. S. Dillon, T. Z. Csaky, and J. W. Beard. 1949. Purification, pH stability, and sedimentation properties of the T7 bacteriophage of *E. coli*. J. Immunol. 63:93-107.

22. Putnam, Frank W. 1951. Physiological properties of bacteriophages. II. Sedimentation of bacteriophage T6. J. Biol. Chem. 190:61-74.

23. Burnett, F. M. 1933. The classification of dysentery-coli bacteriophages. III. A correlation of the serological classification with cerrain biochemical tests. J. Path. and Bacr. 37:179-184.

24. ADAMS, MARK. 1949. The stability of bacterial viruses in solutions of salts. J. Gen. Physiol. 32:579-594.

25. BURNETT, F. M., AND MARGET MCKIE. 1930. Balanced salt action as manifested in bacteriophage phenomena. Australian J. Exper. Biol. and M. Sc. 7:183-200.

26. ROUNTREE, PHYLLIS M. 1951. The role of certain electrolytes in the adsorption of staphylococcal bacteriophages. J. Gen. Microbiol. 5:673-680.

27. FRIEDMAN, MURRAY. 1953. The bacteriophages of *Bacillus megatherium*. II. Stability in mono- and divalent ions. J. Bact. 66:501-502.

28. SYMPOSIA ON QUANTITATIVE BIOLOGY. 1953. Vol. XVIII Monumental Publishing Company, Baltimore, Maryland.

29. SECOND SYMPOSIUM OF THE SOCIETY FOR GENERAL MICROBIOLOGY. 1953. The nature of virus multiplication. University Press, Cambridge, England.