THE STABILITY OF BACTERIAL VIRUSES IN SOLUTIONS OF SALTS*

By MARK H. ADAMS

(From the Department of Microbiology, New York University College of Medicine, New York)

(Received for publication February 19, 1949)

Burnet and McKie (1) in 1930 published a report on balanced salt action as manifested in bacteriophage phenomena. They found that *coli* and staphylococcal bacteriophages when diluted in 0.1 N solutions of sodium, potassium, and ammonium salts were much more susceptible to the inactivating effects of temperature (60°C.) than were the same phages diluted in broth. The addition of a small amount of calcium, magnesium, or barium salt partially or completely prevented this inactivation. They interpreted this phenomenon as another example of the physiological ion antagonism studied by Ringer, Jacques Loeb, and many other physiologists. Gratia (2) has drawn similar conclusions from experiments on the stability of a *megatherium* phage in salt solutions.

In a study of the properties of the *coli*-dysentery phages in chemically defined media we have found that these phages exhibit a similar phenomenon, which, however, cannot be explained on the basis of ion antagonism. The present paper is a report on the kinetics of inactivation of phages in the presence of various ions and on the effect of the environment on the rate of inactivation.

Materials and Methods

The group of seven *coli*-dysentery phages studied by Demerec and Fano (3) was used. Certain properties of this group of bacterial viruses have been summarized by Delbrück (4). These phages were grown on *Escherichia coli*, strain B, in a chemically defined medium containing per liter 1 gm. NH_4Cl, 0.1 gm. $MgSO_4$, 1.5 gm. KH_2PO_4, 3.5 gm. Na_2HPO_4, and 9 gm. lactic acid. The medium was adjusted to pH 6.5 by the addition of NaOH. Since phage T5 is not produced in the absence of calcium ion, calcium chloride to a final concentration of 0.001 M was added when preparing stocks of this phage. All phage stocks used contained between 10^9 and 10^{10} plaque-forming particles per ml. All phage assays were made on strain B of *E. coli* using the agar layer technique of Gratia as modified by Hershey (5). The plating medium was Difco nutrient agar to which 0.5 per cent of sodium chloride was added. The broth used in certain experiments was Difco nutrient broth with 0.5 per cent of sodium chloride. The pH of these broth-containing media was 6.8.

All glassware used in this study was cleaned with acid dichromate and repeatedly rinsed with hot distilled water since, as will be shown later, very small amounts of

* Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

salts markedly affect the results. Solutions used in the kinetic studies were made up in distilled water redistilled from an all glass apparatus.

EXPERIMENTAL

Preliminary Experiments.—In connection with certain experiments with phage T5 in a chemically defined medium, sodium citrate to a concentration of 0.001 M was added to form a weakly ionized complex with small amounts of calcium ion present in the medium. It was found that under these conditions the infectivity of the phage was rapidly lost. The stability of phage T5 was then tested in various media. The phage was diluted in 0.01 M phosphate buffer at pH 7.00 to a concentration of 5×10^4 infectious particles per ml. It was then further diluted 1/10 in 0.01 M phosphate buffer, in buffer plus 0.002 M

TABLE I

The Stability of Phage T5 at 37° C. and pH 7.0 When diluted in 0.01 M Phosphate Buffer, and in Buffer Plus Citrate or Calcium

Diluent	Phage assay at time		
	0	30 min.	2 hrs.
0.01 M PO_4 buffer	380	353	158
Same + 0.001 M $CaCl_2$	333	438	376
" + 0.002 M citrate	44	2	0

The phage assays are the number of plaques formed when 0.1 ml. samples are plated on nutrient agar.

sodium citrate, and in buffer plus 0.001 M calcium chloride. These solutions were incubated at 37°C. and assayed at intervals for phage activity with the results shown in Table I.

From the results in Table I it is evident that the phage is stable in the presence of calcium ion, loses activity slowly in 0.01 M phosphate buffer, and is very rapidly inactivated in the presence of citrate ion. In the latter case almost 90 per cent of the phage is inactivated before the zero time sample could be taken, that is in less than a minute.

Prevention of Inactivation by Divalent Cations.—From other experiments it became evident that the inactivation of phage T5 was not attributable to the anions present, that the effect of citrate was due to removal of calcium and magnesium ions through complex formation, and that the inactivation of T5 could be prevented by any one of a number of divalent cations. To demonstrate the latter point a diluent was used which contained 0.001 M phosphate buffer at pH 7.0, 0.15 M sodium chloride, and 0.1 mg. of Eastman salt-free gelatin per ml. to prevent surface inactivation of the virus in long term experiments (6). To this diluent various salts were added and the pH was checked. The

phage was diluted to a concentration of 10^6 infectious particles per ml. in the diluent, then further diluted 1/100 in the diluent plus various additions. The phage solutions were incubated at 37°C. and sampled at intervals for assay with the results shown in Table II.

From the data in Table II it is evident that all the divalent metals tested at a concentration of 10^{-3} M have a definite protective effect against the inactivation with the exception of lead and mercuric salts. Calcium ion at a concentration of 10^{-4} M still exerts a considerable protective effect, but protection is not evident under these conditions with calcium at 10^{-5} M concentration.

TABLE II

The Effect of Various Divalent Cations on the Inactivation of Phage T5 in 0.15 N Sodium Ion at pH 7.0 and 37°C.

Diluent	Phage assay at time		
	0	2 hrs.	24 hrs.
Diluent alone	736	2	0
" + 10^{-3} M Ca^{++}	778	436	384
" + 10^{-4} M Ca^{++}	932	300	25
" + 10^{-5} M Ca^{++}	960	2	0
" + 10^{-3} M Ba^{++}	760	441	359
" + 10^{-3} M Sr^{++}	697	321	288
" + 10^{-3} M Mg^{++}	632	393	369
" + 10^{-3} M Mn^{++}	468	335	364
" + 10^{-3} M Co^{++}	706	310	297
" + 10^{-3} M Ni^{++}	699	321	298
" + 10^{-3} M Zn^{++}	594	279	265
" + 10^{-3} M Cd^{++}	376	268	171
" + 10^{-3} M Cu^{++}	622	282	55
" + 10^{-3} M Pb^{++}	550	1	0
" + 10^{-3} M Hg^{++}	430	5	0

Kinetics of Inactivation in the Presence of Low Concentrations of Sodium Ion.— Since the inactivation of phage T5 in solutions of sodium ion seemed to be rather sensitive to the presence of small amounts of other ions, it was deemed desirable to purify specially some salt of sodium. A concentrated aqueous solution of reagent grade trisodium phosphate was boiled for some time, yielding a considerable amount of amorphous precipitate which was removed by filtration. The solution was allowed to crystallize, and the crystals subjected a second time to the same treatment. The twice recrystallized salt remained clear and colorless on boiling in concentrated solution. A weighed sample of the twice recrystallized salt was dissolved in redistilled water, adjusted to a pH of 7.0 by the addition of redistilled reagent grade HCl, and diluted with water to a sodium concentration of 1 N. This solution was further diluted with

water to give a series of solutions of known sodium ion concentration which were used as diluents for phage T5. The phage was diluted in these diluents to a concentration of 10^4 infectious particles per ml., incubated at 37°C., and sampled at intervals for assay with the results shown in Table III.

From the data of Table III it would seem that phage T5 is relatively stable at concentrations of sodium ion of 0.4 N or higher. However, at sodium ion concentrations of 0.2 N and 0.1 N the phage is rapidly inactivated. The kinetics of inactivation are of the first order with a velocity constant of 0.08 $min.^{-1}$ for 0.2 N sodium ion and 0.9 $min.^{-1}$ for 0.1 N sodium ion at 37°C. and pH 7.0.

TABLE III

The Inactivation of Phage T5 in Various Concentrations of Sodium Phosphate at 37°C. and pH 7.0

Time	The No. of plaque-forming particles surviving at the indicated times in diluent containing				
	0.8 N Na^+	0.6 N Na^+	0.4 N Na^+	0.2 N Na^+	0.1 N Na^+
min.					
1	1541	1465	1781	1304	461
3	—	—	1413	971	81
5	—	—	—	856	10
10	—	—	—	730	1
20	—	—	—	226	3
30	—	—	—	191	2
60	1340	1586	1354	30	2

The dashes indicate that the plates concerned were not counted but appeared to have about the same number of plaques as those in the same series that were counted. The rate of inactivation is significant only at sodium ion concentrations of 0.2 N and 0.1 N.

To insure that the inactivation of T5 in the presence of low concentrations of the neutralized Na_3PO_4 was not merely a peculiarity of the particular lot of salt chosen these experiments were repeated with NaCl. Reagent grade sodium chloride was twice recrystallized from a saturated solution in distilled water by the addition of alcohol. A normal solution of this salt was prepared in redistilled water and appropriately diluted in redistilled water and 0.01 N phosphate buffer, prepared as described above, so that the final solution contained a known concentration of sodium chloride and sodium phosphate buffer at 0.001 N and pH of 6.0. The T5 phage was diluted in these solutions at room temperature to a concentration of 3×10^5 infectious particles per ml. and then further diluted 1/100 in the same solution at 37°C. and sampled at intervals for assay. At concentrations of sodium chloride of 0.8 N and 0.4 N the inactivation of T5 was inappreciable in 1 hour. The first order velocity constants for inactivation of T5 were 0.11 $min.^{-1}$ at 0.2 N NaCl and 1.0 $min.^{-1}$ at 0.1 N NaCl. Experiments were carried out also at sodium ion concentrations of 0.075 N, 0.05 N, 0.026 N,

0.011 N, 0.006 N, and 0.001 N, the first order velocity constants over this entire range varying randomly between 1 min.$^{-1}$ and 2 min.$^{-1}$.

The Effect of Temperature on the Inactivation of Phage T5 in Dilute Sodium Chloride.—The diluent used in these experiments contained 0.1 N sodium chloride and 0.001 N sodium phosphate buffer at pH 6.0, both salts being twice recrystallized as described above and dissolved in redistilled water. Phage T5 was diluted to about 2×10^6 particles per ml. in the diluent at 0°C. This was

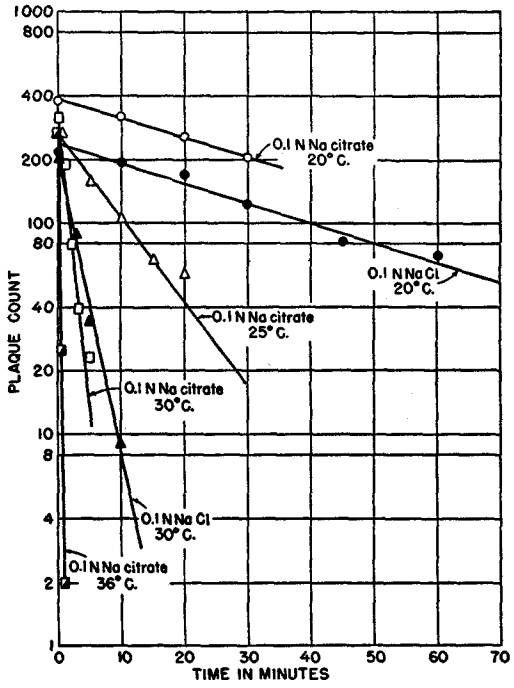

FIG. 1. Plaque counts per sample plotted on a logarithmic scale against the time of sampling for phage T5 in 0.1 N NaCl or 0.1 N Na citrate at various temperatures.

then diluted 1/100 in the diluent which had been already equilibrated at the temperature chosen for the experiment. Zero time for the experiment was taken as the moment at which this 1/100 dilution was made. At appropriate intervals samples were removed and diluted 1/10 in broth to stop the inactivation, since phage T5 is not inactivated at an appreciable rate in broth below 50°C. At a convenient time 0.1 ml. samples of these broth dilutions were plated by the agar layer method for assay. The zero time counts were taken from a direct assay of the phage in the diluent at 0°C.

The data of these experiments are given graphically in Fig. 1, in which the plaque counts of the samples are plotted on a logarithmic scale against the

time of sampling. Each point represents the count of a single plate. Also included in Fig. 1 are data for the inactivation of phage T5 in 0.1 N sodium citrate adjusted to pH 6.0 with HCl. The rates of inactivation are probably not significantly different whether the anion involved is citrate, phosphate, or chloride.

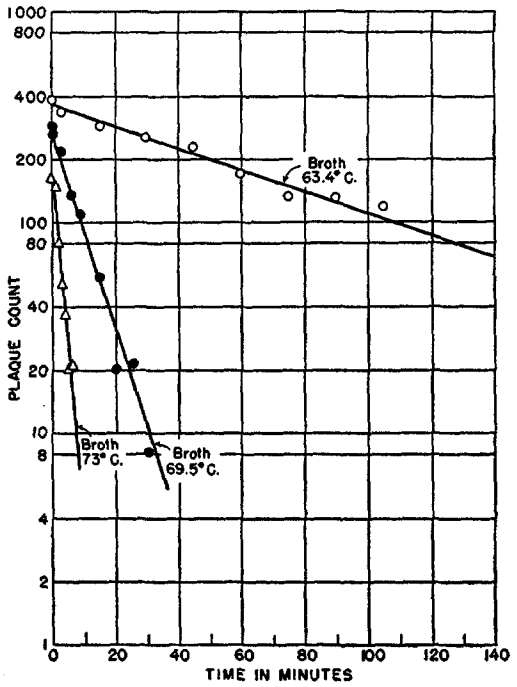

FIG. 2. Plaque counts for the inactivation of phage T5 in broth at various temperatures as a function of time.

In all cases the plaque count decreases as an exponential function of time with no initial lag. The inactivation of phage T5 under these conditions follows the kinetics of a first order reaction, as is usual in the inactivation of viruses whether by heat, by irradiation, or by chemical action. The first order velocity constants were calculated from the slope of the inactivation curve for each experiment and are presented in the form of an Arrhenius plot in Fig. 6.

As noted above, phage T5 is markedly more stable in broth than in 0.1 N sodium salts. However, at temperatures above 60°C. this virus in broth is inactivated at readily measurable velocities. The plaque counts as a function of time of exposure to three different temperatures are given in Fig. 2. The rate of inactivation is an exponential function of time, and the first order velocity constants calculated from the slopes of the curves are presented in the form of an Arrhenius plot in Fig. 6.

The Effect of Magnesium Ion on the Rate of Inactivation of Phage T5 in Dilute Solutions of Sodium Salts.—Since the marked protective effect of various divalent cations against the inactivation of phage T5 in 0.1 N sodium salts had been noted (Table II), it was decided to determine the effect of various concentrations of magnesium ion in the presence of 0.1 N NaCl and 0.001 N phosphate buffer on the velocity of inactivation of phage T5 at various temperatures. The results of these experiments are given in graphical form in Figs. 3, 4, and 5. In all cases the kinetics of inactivation are of the first order. The addition of small

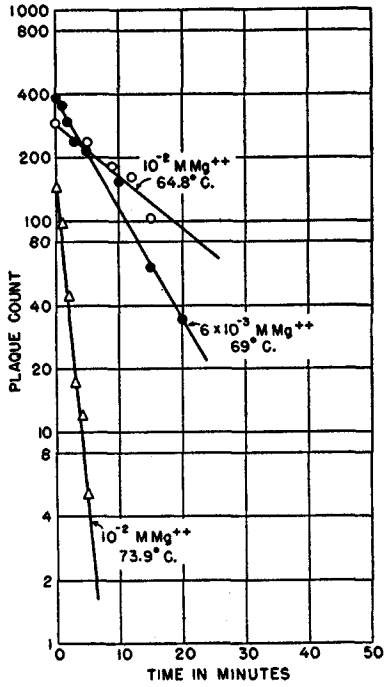

FIG. 3. Plaque counts for the inactivation of phage T5 in 0.1 N NaCl plus added magnesium at various temperatures as a function of time.

amounts of magnesium ion to the saline diluent increases the stability of phage T5 so that the temperature must be raised to bring about inactivation at a measurable rate. The first order velocity constants for each experiment have been calculated and are given in Fig. 6 in the form of Arrhenius plots.

In Fig. 6 the logarithms of the first order velocity constants for inactivation of phage T5 are plotted against the reciprocal of the absolute temperature at which the inactivation was carried out (Arrhenius' method of demonstrating the relationship between reaction rate and temperature). The lines have been drawn through the points, with the result that the slopes of the curves vary in a

random manner. This is misleading because there is reason to believe that all the curves should be parallel to the broth curve with the exception of the curves which contain no added magnesium. The rate of inactivation of phages in salt diluents is markedly affected by traces of impurities such as detergents and oxidizing agents and by ions such as magnesium or citrate. For this reason any single rate determination in salt diluents is subject to some uncertainty. This

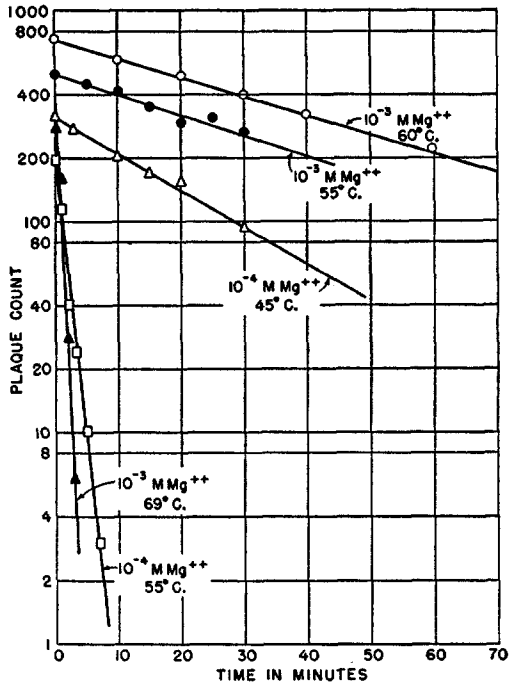

FIG. 4. Plaque counts for the inactivation of phage T5 in 0.1 N NaCl plus added magnesium at various temperatures as a function of time. Note that the time scale for the experiment with 10^{-3} M magnesium ion at 55°C. is to be multiplied by six, that is the ten minute sample was taken at 1 hour, etc.

is not true of the experiments in broth since the properties of this diluent are not affected by traces of impurities. For these reasons the Arrhenius plots in Fig. 6 unduly exaggerate the experimental errors inherent in work of this kind. Nonetheless the relative positions of the curves along the temperature axis adequately demonstrate the marked effect of small amounts of magnesium salts on the stability of phage T5.

In order to compare the effects of varying magnesium ion concentrations on the velocity constants for inactivation of phage T5, the Arrhenius plots of Fig. 6 have been extrapolated to a common temperature ordinate. A temperature of

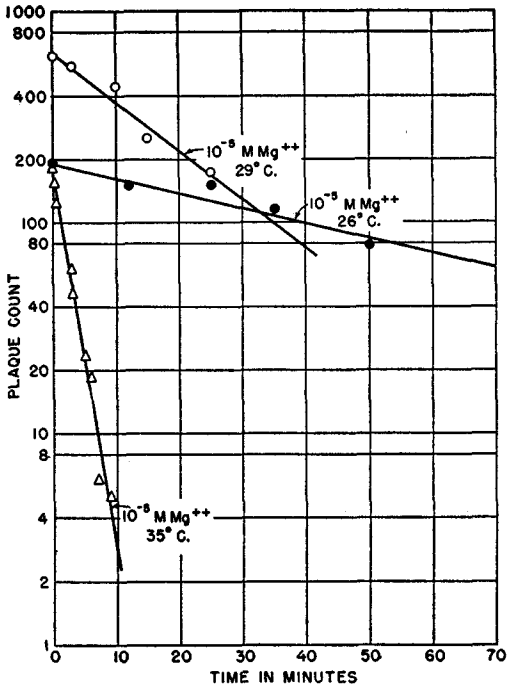

FIG. 5. Plaque counts for the inactivation of phage T5 in 0.1 N NaCl plus added magnesium at various temperatures as a function of time.

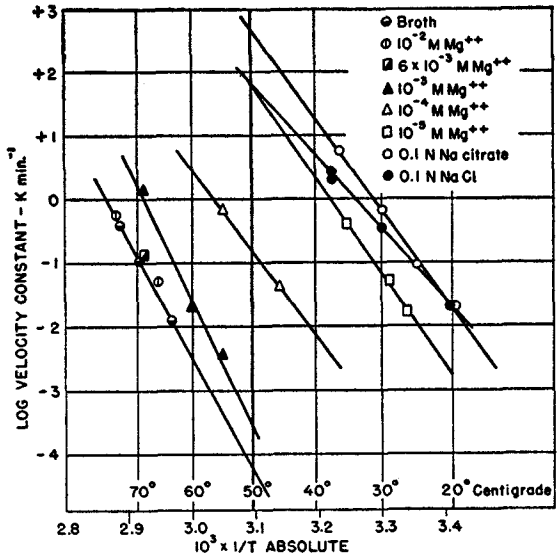

FIG. 6. The log of the first order velocity constants for inactivation of phage T5 as a function of temperature and of the magnesium concentration.

50°C. was chosen since it is near the midpoint of the experimentally available temperature range and hence would require less extrapolation of the Arrhenius plots. From the values of the intercepts it is evident that the rate of inactivation of phage T5 in 0.1 N saline at 50°C. is at least one million times that in broth at the same temperature, and that any velocity between these two limits can be achieved by adding a suitable concentration of magnesium salt. The relationship between velocity constant and magnesium concentration is expressed graphically in Fig. 8 in which the log of the first order velocity constant at 50°C. is plotted against the log of the magnesium concentration. The slope of this line is -2.7 which means that the velocity of inactivation is inversely proportional to the second or third power of the magnesium concentration. Since the protective effect of magnesium is neutralized by an excess of citrate ion, it would appear that the effective agent is magnesium ion rather than the total amount of magnesium salt present. Therefore, a more precise quantitative determination of the effect of magnesium ion on phage stability would require an independent assay of the magnesium ion activity in these highly diluted solutions.

The Effect of Increased Concentrations of Sodium Ion on the Inactivation of Phage T5.—As was noted in Table III, higher concentrations of sodium ion seemed to protect phage T5 from temperature inactivation in the same way as did the addition of divalent cations. The results of the experiments involving various concentrations of sodium ion are included in Fig. 7 in which the log of the first order velocity constant for inactivation of phage T5 is plotted against the reciprocal of the absolute temperature at which the experiment was done. It is evident from these Arrhenius plots that an increase in the concentration of sodium ion results in an increase in the stability of the phage to temperature, the phage being as stable in 2 N NaCl as it is in broth. In Fig. 8 is included a plot of the first order velocity constants extrapolated to 50°C. as a function of the concentration of sodium ion in the medium. From this plot it would appear that the rate of inactivation of phage T5 at 50°C. is inversely proportional to the fifth or sixth power of the sodium ion concentration. The precise relationship between the velocity constants and the sodium ion concentration is uncertain because of the experimental errors involved. The relationship may not be a simple one because changing the sodium concentration from 0.1 N to 2 N involves a large change in ionic strength, the effect of which on the inactivation of phage is not known. It is, however, certain that the effect of sodium ion on the velocity constant is quantitatively different from the effect of magnesium ion which renders it highly unlikely that the protective effect of higher concentrations of sodium ion is due to contamination with small amounts of magnesium ion.

The Relative Stability of Other Phages in Broth and Dilute Saline.—Data for the inactivation of phages T1, T4, and T7 in broth and in 0.1 N sodium chloride

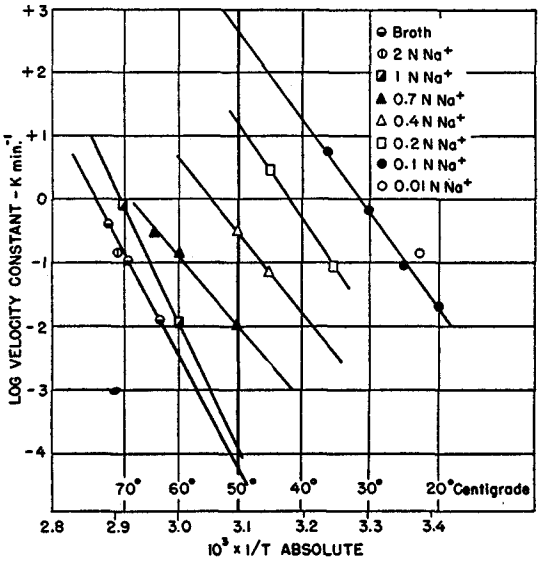

FIG. 7. The log of the first order velocity constants for inactivation of phage T5 as a function of temperature and of the sodium concentration.

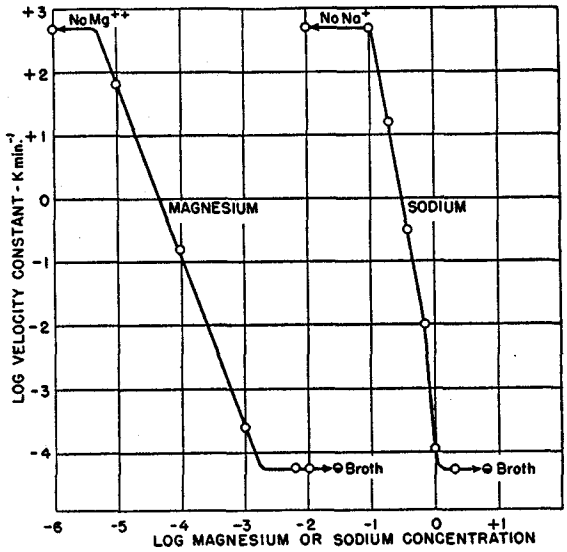

FIG. 8. The log of the first order velocity constants for inactivation of phage T5 at 50°C. as a function of the log of the magnesium or sodium concentrations in moles per liter.

are presented in Fig. 9. In all cases it may be seen that the phage is more stable in broth than in saline although with phage T4 the difference is much

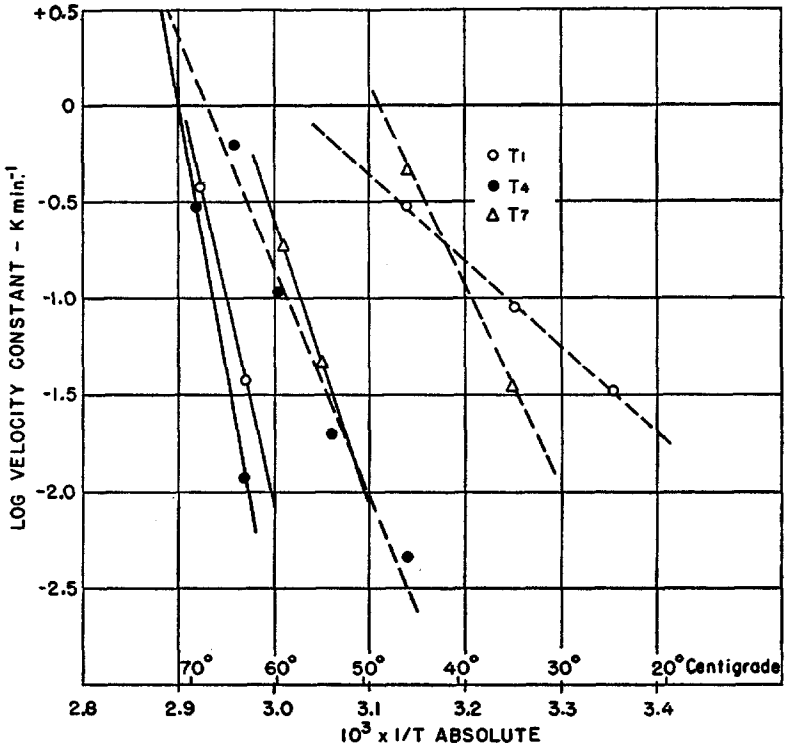

FIG. 9. The log of the first order velocity constants for the inactivation of phages T1, T4, and T7 as a function of temperature and of the medium. The solid lines represent inactivation in broth, while the dashed lines represent inactivation in 0.1 N sodium chloride.

less marked than in the other cases. Phage T3 resembles T7 and phage T6 is similar to phage T4 when subjected to heat in the two media.[1]

[1] It is probable that phage T2 is similar to T4 and T6 but it was not possible to study this phage because the inactivation did not follow the kinetics of a first order reaction. The inactivation proceeded very slowly at first, then accelerated, eventually becoming approximately first order when 90 per cent of the infectivity was gone. The velocity of inactivation of the residual 10 per cent of the phage was very close to that of T4 and T6 at the same temperature. This type of behavior might be expected if the virus consisted largely of pairs or small clumps of virus particles in which the inactivation of one member of the pair or clump would not result in a decrease in the number of the infective centers. This supposition is born out by the fact that phage

The Arrhenius constants for the inactivation of the phages may be calculated by multiplying the slopes of the curves in Figs. 6, 7, and 9 by 4.58. These constants for experiments in broth are given in Table IV.

It may be seen from Table IV that the Arrhenius constants for heat inactivation of phages in broth are of the order of magnitude of those determined for the heat denaturation of proteins and for the heat inactivation of enzymes and of bacterial toxins.

TABLE IV

The Arrhenius Constants for the Heat Inactivation of Phages T1, T4, T5, and T7 in Broth

Phage	Arrhenius constant in broth
T1	106,000
T4	131,000
T5	86,000
T7	77,000

The effect of hydrogen ion concentration on the rate of inactivation of phage T5 in 0.1 N NaCl at 37°C. was also studied. Over the range from pH 5.5 to 7.5 at least, the rate of inactivation seemed to be independent of hydrogen ion concentration, but below 5 or above 9, the rate of inactivation was greatly accelerated. The effect of pH on the rate of inactivation of the other phages has not been measured.

DISCUSSION

The *coli*-dysentery phages of the T group with the possible exception of T2 are inactivated by heat at an exponential rate. With T5 the kinetics are of the first order whether the inactivation is at 20°C. in 0.1 N sodium salts, or at 70°C. in broth. The inactivation of bacteriophages by other agencies such as x-radiation (7), ultraviolet irradiation (8), heat (12), and surface denaturation (6) has also been found to follow the kinetics of a first order reaction. The destruction of infectivity of influenza virus by heat was found to be a first order reaction (9). It would seem from these examples that in general the destruction of the infectivity of a virus follows the kinetics of a first order reaction regardless of the agent responsible for the destruction. Exceptions to this generalization as in the case of phage T2 may probably be accounted for by

---

T2 diluted in broth at 37°C. actually shows an increase in the number of infective centers often doubling in titer in the course of an hour. An investigation of the conditions required for the dissociation of phage T2 in various salt solutions would be necessary before the kinetics of inactivation of T2 could be studied.

the clumping of two or more virus particles to form a single infective unit with increased resistance to inactivation (10).

From the data presented in this paper it is evident that the bacteriophages studied are markedly less stable in the presence of 0.1 N sodium ion than they are in broth. The lowered stability in dilute sodium ion is manifested by a lower temperature at which a given first order velocity constant for inactivation is reached. Since the difference in stability seemed to be most marked in the case of the phage T5, most of the work has been done with this phage. The addition of a variety of divalent cations at a concentration of 10^{-3} M to a salt medium containing 0.15 N sodium ion resulted in a marked increase in the stability of phage T5 at 37°C. In a more quantitative study in which various concentrations of magnesium ion were added to a salt solution containing 0.1 N sodium ion it was found that the stability of phage T5 increased with increasing concentrations of magnesium ion reaching an optimal stability indistinguishable from that in broth at a magnesium concentration of 6×10^{-3} M. Since the Arrhenius plots for the inactivation of phage T5 in 0.1 N sodium ion and in the presence of 10^{-2} M magnesium ion converge, it would appear that the stabilizing effect of magnesium ion decreases with increasing temperature becoming zero at the point of intersection. In the case of phage T5 the velocity of inactivation at the point of intersection is too high to be measured. With T4, however, the point of intersection of the two curves is about 74°C. and at a velocity constant of about 5 $min.^{-1}$ which corresponds to a rate of inactivation just a little faster than can be measured by our techniques. It is conceivable that with other viruses the point of intersection would occur at velocities of inactivation too low to be conveniently measured, in which case there would be no detectable difference in stability of the virus whether tested in broth or in salt solutions.

When the log of the velocity constant for inactivation of phage T5 at 50°C. is plotted against the log of the magnesium ion concentration an S-shaped curve is obtained, the linear portion of which has a slope between -2 and -3 (Fig. 8). This means that over the range of the linear portion of the curve, the first order velocity constant for inactivation is inversely proportional to the second or third power of the magnesium ion concentration.

This relationship might be most simply explained by assuming that the phage reacts with Mg^{++} ion to form a weakly dissociated complex in accordance with the equation: phage $+ n\ Mg^{++} \rightarrow$ phage-Mg_n. The free phage undergoes irreversible inactivation at 50°C. at a rate which is about 10^6-fold greater than the rate of inactivation of the phage-magnesium complex. The actual rate of inactivation of phage in any given concentration of magnesium ion is largely determined by the concentration of free phage which in turn is determined by the magnesium ion concentration in accordance with the equation. The phage-magnesium complex must dissociate to yield free phage and mag-

nesium ion at a rate which is at least as great as the rate of inactivation of phage under the experimental conditions.

It would appear from the data summarized in Fig. 8 that the phage will also form a complex with sodium ion, and that the dissociation constant for such a complex must be much higher than that for the magnesium complex. Since the velocity constant of inactivation is inversely proportional to the fifth or sixth power of the sodium ion concentration, it would seem that the phage is able to associate more sodium ions than magnesium ions. The situation in the case of the sodium ion, however, is so complex, involving marked changes in ionic strength as well as temperature when the sodium ion concentration is increased, that mathematical analysis of the available data would have little value.

Phage T5 is one of the phages (11) which requires relatively high concentrations of calcium ion in the medium for reproduction to take place. The remaining phages of the T group require much less or no calcium ion for growth, and the host cell, *E. coli*, also appears to require little or no calcium ion for multiplication. For instance plaque formation with phage T5 on nutrient agar plates is completely inhibited by 1 per cent sodium citrate whereas growth of the host cell is not appreciably affected. The calcium ion is not needed for adsorption of phage T5 to the host cell, but is required for some later step in the life cycle of the virus. The calcium requirement cannot be met by magnesium ion or other common cations. Since the calcium requirement is specific for the virus rather than for the host cell, it is likely that the calcium ion takes part in some metabolic reaction peculiar to phage multiplication, perhaps activating some enzyme which is part of the virus. The ability of the virus T5 to form complexes with various cations leading to increased heat stability may possibly be related to the requirement of calcium for some metabolic purpose.

SUMMARY

1. The seven bacterial viruses of the T group, active against *E. coli*, are much more rapidly inactivated by heat when suspended in 0.1 N solutions of sodium salts than when suspended in broth.

2. The kinetics of this inactivation whether in salt solutions or in broth are those of a first order reaction.

3. The rate of inactivation of phage T5 in 0.1 N NaCl at 37°C. can be greatly decreased by the addition of 10^{-3} M concentrations of such divalent cations as Ca, Mg, Ba, Sr, Mn, Co, Ni, Zn, Cd, and Cu.

4. An increase in the cation concentration in the suspending medium results in an increase in the stability of phage T5 to the inactivating effects of temperature.

5. The hypothesis is proposed that the increase in stability of phage T5 in

the presence of various cations is the result of complex formation between the phage and the metal ion.

The author wishes to acknowledge the assistance of Miss Nancy J. Collins in the experimental work.

REFERENCES

1. Burnet, F. M., and McKie, M., *Australian J. Exp. Biol. and Med. Sc.*, 1930, **7,** 183.
2. Gratia, A., *Compt. Rend. Soc. biol.*, 1940, **133,** 443, 445, 702.
3. Demerec, M., and Fano, U., *Genetics*, 1945, **30,** 119.
4. Delbrück, M., *Biol. Rev.*, 1946, **21,** 30.
5. Hershey, A. D., Kalmanson, G., and Bronfenbrenner, J., *J. Immunol.*, 1943, **46,** 267.
6. Adams, M. H., *J. Gen. Physiol.*, 1948, **31,** 417.
7. Luria, S. E., and Exner, F. M., *Proc. Nat. Acad. Sc.*, 1941, **27,** 370.
8. Luria, S. E., and Latarjet, R., *J. Bact.* 1947, **53,** 149.
9. Lauffer, M. H., Carnelly, H. L., and MacDonald, E., *Arch. Biochem.*, 1948, **16,** 321.
10. Rahn, O., *J. Gen. Physiol.*, 1929, **13,** 179, 395.
11. Burnet, F. M., *J. Path. and Bact.*, 1933, **37,** 179.
12. Krueger, A. P., *J. Gen. Physiol.*, 1932, **15,** 363.