THE EFFECT OF NaCl ON THE PHAGE-BACTERIUM REACTION*

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Investigations dealing with the effects of salts on bacteriophage have been rather limited in scope and predominantly qualitative or roughly quantitative in character. For the most part efforts have been restricted to pointing out analogies between the balanced salt solutions required for the growth of common living organisms and those requisite for phage production. While the former data are essentially accurate and subject to adequate control the corresponding experiments with phage suffer from a lack of controlled experimental conditions and also from the use of inaccurate methods for determining phage quantitatively.

Consequently it is not surprising that there is no general agreement in the literature about salt effects. For example Brutsaert (1–2) concludes that phage may develop and bacterial lysis take place in peptone water without salt. Ciuca (3), Lisbonne and Carrere (4), and da Costa Cruz (5–7) on the contrary hold that electrolytes are essential for both phage production and bacterial lysis. Arloing and Chavanne (8) and Bordet and Renaux (9) stress not only the necessity for electrolytes in bacteriophagy but also the importance of the particular electrolyte used. The observations of Bordet (10), Stassano and de Beaufort (11), Planteureux (12), and Anciaux (13) indicate that the calcium ion is necessary for the lytic action of phage on bacteria.

Burnet and McKie (14) report experimental evidence for the protective action of divalent ions against inactivation of phage by heat and dyes. In Bronfenbrenner's work (15–16) it appears that the greater the concentration of electrolytes, the greater the percentage inactivation of phage by alcohol and acetone.

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Krueger and West (17) in an analysis of the kinetics of the phage-bacterium reaction in the presence of the manganous ion find that extremely small concentrations of Mn^{++} cause an acceleration of lysis due to a lowering of the lytic threshold; *i.e.*, the ratio of phage to bacteria requisite for lysis. There is a concomitant increase in the extracellular phage fraction and a decrease in the total quantity of phage produced.

Northrop (private communication) has noted that the presence of increased concentrations of sodium chloride during the period of reaction between phage and susceptible organisms leads to an augmented end titre of phage. We felt that a study of this reaction might furnish a better understanding of the way electrolytes effect bacteriophagy and might also be of aid in analyzing the mechanisms of phage production and bacterial lysis.

Methods

1. Quantitative determinations of phage were routinely made by the activity method of Krueger (18).

a. Unit of Phage Defined.—The minimum quantity which will cause complete lysis when added to a total of 12.5×10^7 cells of *S. aureus* (a 16 hour culture grown on agar and counted by the centrifuged sediment method of Krueger (19)), in a total volume of 5 ml. of beef infusion broth held at 36°C. at pH 7.4, in infinite time. 1×10^{-10} ml. of the standard phage prepared by adding 1 ml. of phage to 2×10^9 staphylococci contained in a total volume of 100 ml. of broth and allowing the mixture to lyse, suffices to produce lysis under these conditions and therefore contained 1×10^{10} units of phage per ml.

b. Since there is a linear relation between the time of lysis and the log (initial concentration of phage), *i.e.* log $[P]_o$, an unknown may be run according to the following procedure:

$[P]$, concentration of phage in units per ml.

$[P]_o$, initial concentration of phage in units per ml.

$[B]$, concentration of susceptible staphylococci per ml.

$[B]_o$, initial concentration of susceptible staphylococci per ml.

4 ml. aliquots of appropriate dilutions in broth of phage-containing solutions are pipetted into standard tubes. To each is added 1 ml. of a broth suspension of staphylococci prepared as described above and containing 12.5×10^7 organisms per ml. The tubes are placed in the water bath shaker and after 1.5 hours readings are made first at 0.2 hour and later, as lysis is initiated, at 0.1 hour intervals until the arbitrary end-point of 8×10^7 cells per ml. is reached. With this series

dilutions of the original standard phage are run in the same manner. Usually four dilutions 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, are used. Log $[P]_o$ is plotted against the time of lysis of these standard dilutions. Knowing the time of lysis for a given dilution of the unknown sample, the original concentration of phage in the mixture may be determined by reference to the standard curve.

2. The bacterial suspensions used in all the experiments described consisted of freshly harvested 16 hour cultures of *Staphylococcus aureus* (strain S_3K) grown on agar and washed once in Locke's solution. Broth was standard beef infusion containing 1 per cent Difco Neopeptone, 0.5 per cent sodium chloride, and adjusted to pH 7.4.

3. Except where otherwise indicated, a volume of 1 M NaCl sufficient to give a final concentration of 0.25 M NaCl was used in the entire test series. These aliquots of one molal sodium chloride were added to beef infusion broth. In all cases control mixtures were included, consisting of the same constituents, similarly treated except that the volume of the stock solution of 1 M NaCl used was replaced by an equal volume of physiological saline 0.85 per cent (0.145 M).

4. *Growth Curves.*—To 38.5 ml. of broth and 17.5 ml. of 1 M NaCl, 14 ml. of a broth suspension of staphylococci containing 12.5×10^7 bacteria per ml. were added. This mixture, and a control mixture were placed in the water bath shaker at 37°C. Turbidity readings were made directly or on an aliquot sufficiently diluted to come within the range of a formalinized standard series (range 5×10^7 to 20×10^7 bacteria per ml.). Readings were made at intervals of 0.5 hour over a period of 4 hours, diluting the preparations when necessary to keep $[B]$ within the range of the turbidity standards.

5. *Rate of Phage Production and Phage Distribution As Related to Bacterial Growth.*—To 31.5 ml. of broth were added 7 ml. of standard staphylococcus phage diluted 1/1000 (final concentration of phage = 1×10^6 units per ml.), 14 ml. of staphylococcus culture containing 12.5×10^7 bacteria per ml., and 17.5 ml. of 1 M NaCl. This was paralleled by a control mixture. These suspensions were placed in the water bath shaker at 37°C. At intervals of 0.5 hour turbidity measurements for bacterial growth were made on 5.0 ml. aliquots. At the same time 1 ml. was removed from the mixture, diluted 1/10, and kept in ice and salt for subsequent titration with the remainder of the series; a 5 ml. aliquot was transferred to a 15 ml. centrifuge tube, iced, and centrifuged for 20 minutes at 2100 R.P.M. 1 ml. of the supernatant fluid from the centrifuged sample was diluted with 9.0 ml. broth to be titrated for the amount of extracellular phage. The quantity of intracellular phage was then determined by difference: total phage − extracellular phage = intracellular phage.

6. *Control to Determine the Effect of NaCl on Phage Alone.*—Phage left in infusion broth with 0.25 M NaCl at 37°C. for 4 hours and at 0° for 24 hours was subsequently titrated.

7. *Control to Determine the Effect of NaCl on Staphylococci Alone.*—A suspension of staphylococci, 12.5×10^7/ml., was left in the NaCl 0.25 M infusion broth for 3

hours at 0°C. and the suspension used in a titration of standard phage along with a control series. A similar suspension, initially containing 2.5×10^7 B/ml. was grown in 0.25 M NaCl for 3.5 hours, diluted to 12.5×10^7 B/ml., and used in a titration of standard phage along with a control suspension.

8. For determining the salt effect at the lytic threshold in static mixtures, aliquots of 5 ml. total volume containing 1×10^8 B/ml. and varying concentrations of phage, were left at 0°C. for 0.75 hour and then were placed in the water bath shaker at 37°C. The ratios of log $[P]_o/[B]_o$ represented in these mixtures ranged from 1.0 to 2.0. Turbidity measurements were made on these mixtures at 0.10 hour intervals.

9. In order to determine the relative effect of salt on bacteria and on phage in mixtures at the lytic threshold, 9 ml. of broth, 2 ml. of a 10^{-4} dilution of standard phage, 5 ml. of 1 M NaCl solution, and 4 ml. of a suspension of staphylococci containing 12.5×10^7 organisms per ml. were mixed and placed in the water bath shaker at 37°C. A similar mixture without the salt was set up as control. At 0.4 hour before the end-point of lysis (time determined previously in an identical mixture) two 5 ml. aliquots of the control mixture and two 5 ml. aliquots of the test mixture were removed from the bath, placed in 15 ml. centrifuge tubes, the tubes packed in ice and salt in centrifuge cups, and centrifuged in the cold room for 12 minutes. The supernatant fluids of one test portion and one control were interchanged and the organisms uniformly resuspended. Two portions as controls, one with 0.25 M NaCl and one without, were resuspended without interchanging supernatants, and were returned in standard size tubes to the bath at 37°C. The end-point of lysis for these four suspensions was observed, along with that of the two control tubes which were left in the bath at 37°C. during the treatment of the other four portions. The lysates of these test portions were subsequently diluted and titrated in the usual manner for total phage.

10. To determine the possible relative osmotic effect of the salt of a divalent anion, mixtures of phage and bacteria were set up in broth containing various concentrations of NaCl and Na_2SO_4, and the times of lysis noted. The lysates were also titrated for $[P]$.

11. The rate of oxygen consumption of the *Staphylococcus aureus* strain was measured during normal growth, during growth with phage present, and during growth with 0.25 M NaCl and phage present. The constant volume type of manometric apparatus was used with flasks of approximately 20 ml. capacity. 1 ml. of a staphylococcus suspension containing 2×10^8 organisms per ml. was added to 1 ml. of beef infusion broth. Similar mixtures containing 5×10^6 phage units in the 1 ml. of broth or with 0.50 M NaCl in the 1 ml. of broth-phage mixture were prepared. 0.4 ml. of 20 per cent NaOH was added to the filter papers placed in the absorption chambers of the flasks. The mixtures were allowed to come to equilibrium in the 37°C. water bath shaker (90–100 oscillations per minute) for a period of 0.5 hour before the stop-cocks were closed. Readings were made over a period of 2 hours at intervals of 10 minutes.

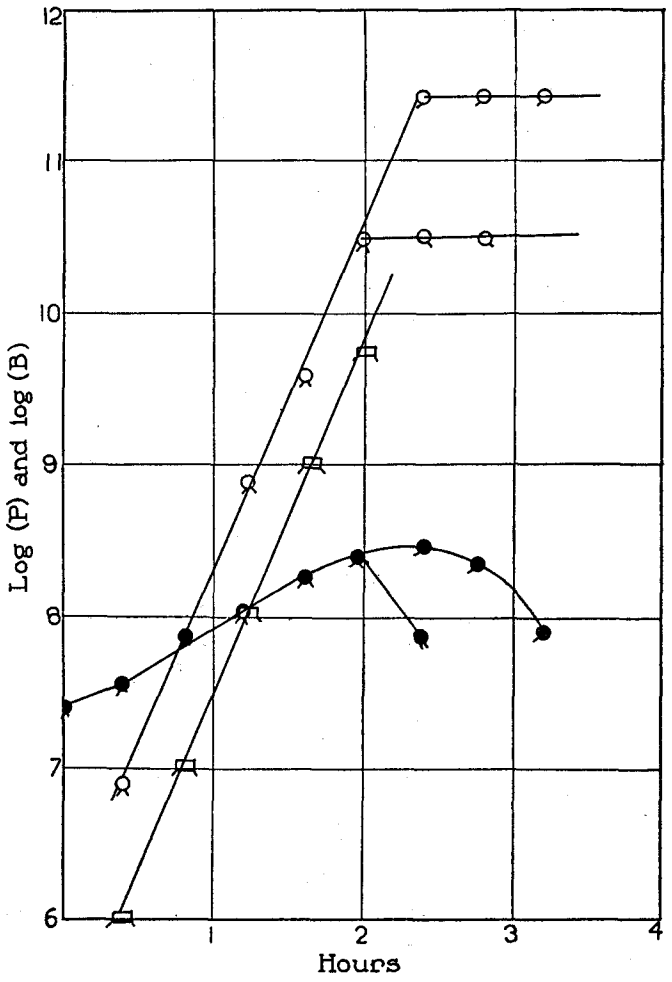

FIG. 1. Composite plot of several experiments in which staphylococci were grown at 36°C. in the presence of 0.25 M NaCl and without NaCl. $[B]_o = 2.5 \times 10^7$; $[P]_o = 1.0 \times 10^6$. ○ = total [P] in the salt mixture; ○ = total [P] without salt; □ = extracellular [P] in the salt mixture; □ = extracellular [P] without salt; ● = [B] in salt mixture; ● = [B] in mixture without salt.

RESULTS

Kinetic analysis of the reaction between a susceptible staphylococcus and its homologous phage in the presence of 0.25 M NaCl gave the following results:

1. The growth curve of the staphylococcus strain remains normal under the influence of 0.25 M NaCl (Fig. 1).

2. The activity of phage left in contact with 0.25 M NaCl at 0° (18 hours) and at 37°C. (4 hours) is not affected (Table I).

There is no effect on organisms exposed to salt action and then used for titrations of standard phage (Table II).

TABLE I

Effect of Exposing Phage to 0.25 M NaCl at 0°C. and 37°C.

7.5×10^9 phage units/ml. = initial phage concentration.
NaCl in test solution 0.25 molal.
Mixtures kept 18 hours at 0°C. and 4 hours at 37°C.

0°C.		37°C.	
Control	Salt	Control	Salt
Final [phage] 7.3×10^9	7.5×10^9	7.5×10^9	7.4×10^9

TABLE II

Effect of Exposing Organisms to 0.25 M NaCl and Subsequently Using Them for Titration of Standard Phage

12.5×10^7 *B*/ml. left 4 hrs. at 0° in 0.25 M NaCl and in normal saline. Both preparations subsequently used in titration of standard phage.

Initial log $[P]_o$	T lysis (hrs.)		End titre, log $[P]$	
	Salt	Control	Salt	Control
7.9	1.75	1.75	10.5	10.5
6.9	2.25	2.25	10.7	10.7
5.9	2.65	2.65	10.9	10.9

2.5×10^7 *B*/ml. grown at 37° in broth containing 0.25 M NaCl and in broth containing 0.145° M NaCl. Both preparations subsequently used in titration of standard phage.

Initial log $[P]_o$	T lysis (hrs.)		End titre, log $[P]$	
7.9	1.2	1.2	10.4	10.4
6.9	1.6	1.6	10.5	10.5
5.9	2.1	2.1	10.75	10.75

3. Sodium chloride, (0.25 M), has no effect on bacterial growth, rate of phage production, or phage distribution in growing mixtures of phage and bacteria up to the point of lysis. At this point, however, in the mixtures containing salt, lysis is delayed for approximately 0.6 to 0.75 hour as compared with the control mixtures. When the

end-point of lysis is reached in the control mixture, the phage concentration rapidly increases in the salt mixture but no further bacterial growth takes place; an extraordinary phenomenon in view of all the experimental evidence establishing bacterial growth as an essential conditioning factor for phage production (15). The end titre of the lysate is five to ten times greater than that of the control mixture. Hence, at the point of lysis in the salt mixture the concentration of

TABLE III

Determination of the Phage/Bacterium Ratios Requisite for Lysis without Bacterial Growth in the Presence and Absence of Salt

NaCl 0.25 M (*S*)
Control (*C*)
$[B]_o = 1 \times 10^8$/ml.
Readings by turbidity, $[B] \times 10^7$/ml.

Value of $[P]_o/[B]_o$ initially.....	0.9		1.1		1.3		1.5		1.7		1.9	
Time	*C*	*S*	*C*	*S*	*C*	*S*	*C*	*S*	*C*	*S*	*C*	*S*
hrs.												
0.2	10	10	10	10	10	10	10	10	10	10	10	10
0.4	12	12	12	12	12	12	12	12	10	10	10	10
0.6	16	16	16	16	16	16	12	12	6	9	6	9
0.7	18	18	18	16	12	16	7	12		9		9
0.8	22	22	16	16	8	12		12		6		6
0.9	12	20	5	12		10		6				
1.0	8	16		10		8						
1.1		14		8								
1.2		12										
1.3		10										
1.4		6										
Increase in end titre.....	3.6*x*		3.6*x*		3.0*x* (3 experiments)		2.5*x*		3.0*x*		3.0*x*	

phage per bacterium, that is, the lytic threshold, is increased approximately five to tenfold, although the concentration of bacteria per milliliter is approximately the same as that of the control (Fig. 1).

4. Determinations of the lytic threshold in static mixtures with 0.25 M NaCl indicate that the lytic threshold, *i.e.* that mixture in which the ratio of phage to bacteria is such that lysis without growth takes place, is not changed. Although the time of lysis is delayed

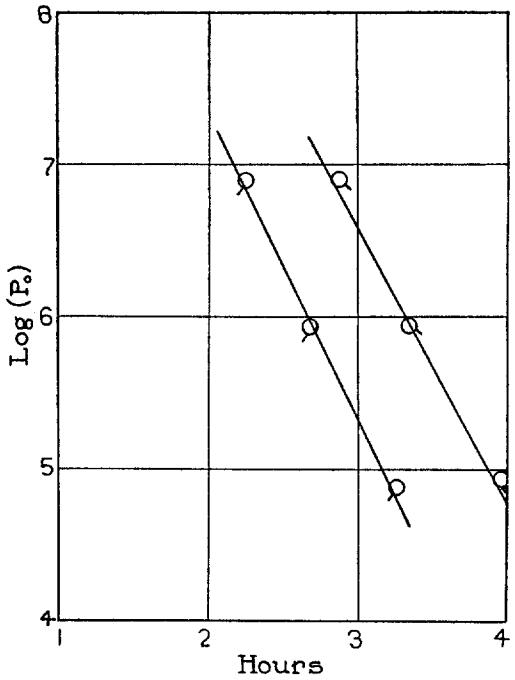

FIG. 2. The delay in $t_{(lysis)}$ caused by 0.25 M NaCl is independent of P_o. Q = phage and bacteria mixtures in broth containing 0.25 M NaCl; $[B]_o = 2.5 \times 10^7$; $[P]_o = 8.0 \times 10^6$, 8.0×10^5, and 8.0×10^4 activity units/ml. respectively. O = identical mixtures without salt. Temperature—36°C.

TABLE IV

Effect of Varying Initial Concentration of Bacteria on Time of Lysis and Final Titre of Lysate, in the Presence and Absence of Salt

[NaCl] = 0.25 M in test series.
$[P]_o$ = 1×10^6 units/ml.

$[B]_o$/ml.		2.5×10^7	5×10^7	7×10^7	1.25×10^8
T_{lysis} *hrs.*	Control	2.6	2.68	2.78	3.15
	Salt	3.35	3.45	3.5	3.90
ΔT_{lysis}		0.75	0.77	0.72	0.65
End titre Log	Control	11.25	11.35	11.45	11.35
	Salt	12.0	12.0	12.1	12.0

considerably in the presence of salt, there is no increase in bacterial growth but there is a slight increase in phage titre of the lysates, which is constant over the range of phage and bacterial concentrations used (Table III).

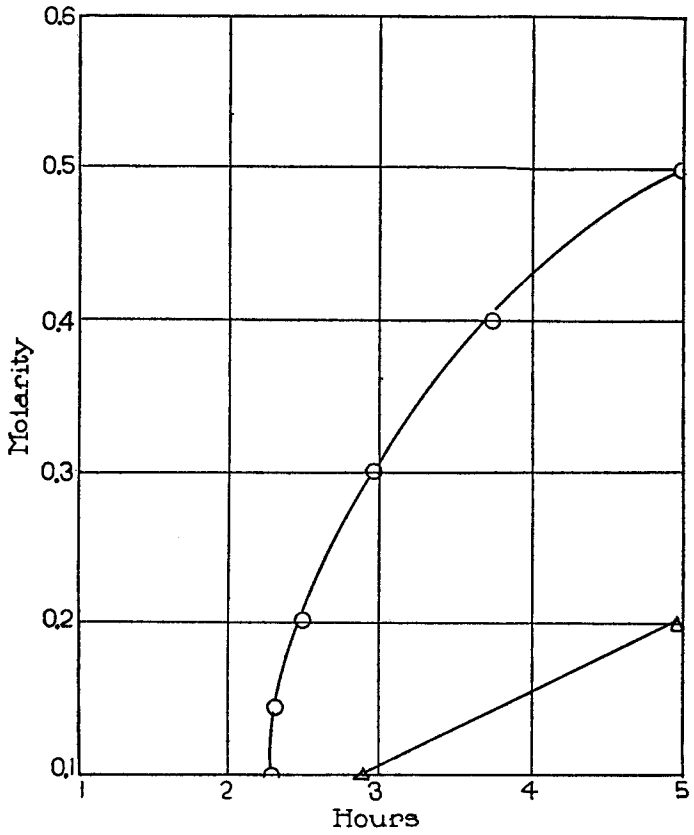

FIG. 3. The effect of increasing [NaCl] on $t_{(lysis)}$. $[P]_o = 1 \times 10^6$ activity units/ml. $[B]_o = 2.5 \times 10^7$ B/ml. with varying concentrations of NaCl (○), and Na_2SO_4 (△). Increasing concentrations of both salts produce a considerable delay in the time of lysis.

5. The delay of lysis by salt and the increase in end titre of the lysate are independent of the initial phage and bacterial concentrations used (Fig. 2 and Table IV).

6. Within limits an increase in NaCl beyond 0.20 M causes a corre-

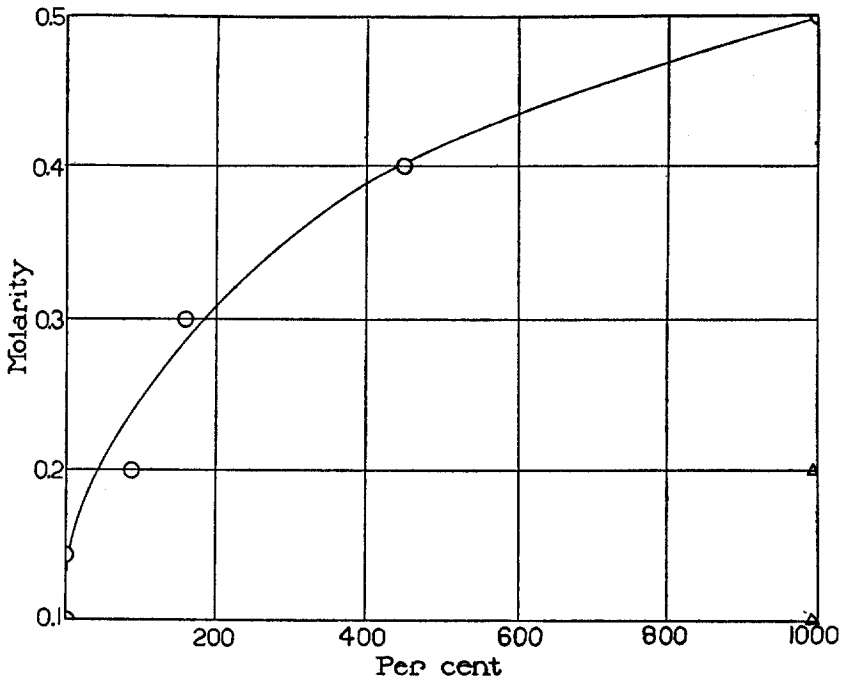

FIG. 4. The effect of increasing [NaCl] on the end titre of a phage-bacteria mixture. $[P]_o = 1 \times 10^6$ activity units/ml.; $[B]_o = 2.5 \times 10^7$ B/ml. Mixtures maintained at 36°C.

TABLE V

Effect of 0.25 M NaCl on Phage Distribution between Bacteria and Broth

Mixture of resting bacteria and phage left 0.75 hr. at 0°C.

$[B]_o = 1 \times 10^8$ B/ml.

$[P]_o = 1 \times 10^9$ phage units/ml.

Mixtures titrated for total phage and extracellular phage/ml.

	Extracellular phage, *per cent*	
	Control	0.25 M salt
1	5.6	7.9
2	7.9	17.0
3	4.0	8.0
4	6.0	14.0
5	13.0	16.0
6	4.0	4.0

sponding delay in lysis and an increase in the titre of the lysate. However, neither of these effects is strictly proportional to salt concentration. Corresponding increases in Na_2SO_4 produce greater delays in time of lysis (Figs. 3 and 4).

7. The effect of NaCl on phage distribution between resting cells and the medium was tested by keeping a mixture containing 1×10^9 phage units per ml. and 1×10^8 bacteria per ml. for 0.75 hour at 0°C. The suspension was then centrifuged and determinations were made

TABLE VI

Effect of Adding Salt to a Phage-Bacterial Mixture Just Prior to Lysis

$[B]_o = 2.5 \times 10^7$/ml.

$[P]_o = 1 \times 10^6$ units/ml.

1.5 ml. 1 M NaCl added to test series at varying times with reference to the control end-point and replaced at 37° to lyse. 1.5 ml. normal saline added to control mixtures instead of 1 M NaCl.

Controls:

1. Salt, 0.25 M NaCl present initially.
2. No salt present initially.

Time before control end-point at which salt solution was added	T_{lysis} (hrs.)		Increase in titre over control lysate
No salt added	Control	2.2	
0.25 M NaCl present initially	Salt	2.7	5x
0.9	Control	2.23	
	Salt	2.68	3.2x
0.6	Control	2.23	
	Salt	2.53	3.2x
0.3	Control	2.23	
	Salt	2.38	1.6x

of total phage and extracellular phage per ml. As shown in Table V and Fig. 1 no change in phage distribution was detected.

8. When 5 ml. portions of phage-bacterial mixtures initially containing 2.5×10 B/ml. and 1×10 phage units/ml. are allowed to grow in the water bath shaker and 1.5 ml. of 1 M NaCl are added at varying times before the end-point of lysis of a control mixture, lysis is delayed and the titre of the lysate is increased. The earlier the salt is added the greater is the effect (Table VI).

9. Attempts were made to extract from bacteria a catalytic agent

hypothetically responsible for the observed increase in phage titre. No such substance could be extracted by the procedures outlined below:

a. 2.5 × 10 *B*/ml. are grown for 2.6 hours in the presence of 0.25 M NaCl and without increased salt concentration. Both bacterial suspensions are then added to 5 ml. phage, iced to prevent growth of

TABLE VII

Test for a Hypothetical Activator of Phage Extracted from Bacteria by NaCl

5 ml. of 2.5 × 10^7 *B*/ml. grown 2.6 hrs. in presence of 0.25 M NaCl and without NaCl, iced 1 hr., added to 5 ml. of phage, 1 × 10^{10} phage units per ml. Iced 1 hr. Centrifuged. Titrated for total and extracellular phage.

	Total phage log [*P*]	Extracellular phage log [*P*]	Extracellular phage
			per cent
Control	9.7	9.1	26
0.25 M NaCl	9.7	9.1	26
Control	9.6	8.6	10
0.25 M NaCl	9.6	8.6	10

TABLE VIII

Test for a Hypothetical Activator of Phage Extracted from Bacteria by NaCl

Two 5 ml. aliquots of 5 × 10^9 *B*/ml. each left 2 hrs. at 0°C. in 0.25 M NaCl and in physiological saline. Added to 5 ml. phage. Iced 1 hr. Titrated for total and extracellular phage.

	Total phage log [*P*]	Extracellular phage log [*P*]	Extracellular phage
			per cent
Control	9.7	8.7	10
0.25 M NaCl	9.7	8.7	10
Control	9.4	8.6	16
0.25 M NaCl	9.5	8.6	11

organisms, and after 1 hour of contact the mixtures are titrated for total phage/ml. and extracellular phage/ml. (Table VII).

b. 5 × 10 *B*/ml. are left in 0.25 M NaCl without growth occurring and are then treated as in *a* (Table VIII).

c. 1 × 10 *B*/ml. are left 24 hours in salt and in salt and glycerine mixtures, centrifuged, and the supernatant added to an equal volume of phage and titrated (Table IX).

10. In order to determine whether the effect at the lytic threshold was primarily on the phage or on the organisms, phage-bacterial mixtures were grown with and without salt, centrifuged, the supernatants interchanged 0.45 and 0.9 hour before lysis in the control mixture, and they were then replaced to lyse at 37°C. No consistent results were obtained. Since the mixtures were either at the lytic

TABLE IX

Test for a Hypothetical Activator of Phage Extracted from Bacteria by NaCl

5 ml. of 1 × 10^9 *B*/ml. left at 0°C. 24 hrs. in the following mixtures:

A. 0.8 M NaCl.

B. 0.6 M NaCl, 50 per cent glycerine.

Centrifuged 1/2 hr. 1 ml. of the supernatant added to 1 ml. of phage 1 × 10^{10} units/ml. Left 1 hr. at 0°C. Titrated for total phage.

	log [*P*]	
	Exp. 1	Exp. 2
0.8 M NaCl	9.5	9.6
A. Control (normal saline)	9.6	9.7
0.6 M NaCl	9.9	9.7
B. Control (normal saline)	9.9	9.7

TABLE X

Effect of Low Temperature on Time of Lysis and End Titre of Lysate

	37°C.	0°C.
Time of lysis		
0.25 M NaCl	2.6	4.1
Control	2.05	2.6
Titre of lysate log [*P*]		
0.25 M NaCl	10.75	8.5
Control	10.15	9.6

threshold or just starting to lyse, it was difficult to obtain samples in exactly the same condition each time. However, there were some indications that for the maximum effect the salt must act on phage and bacteria together at the threshold. Organisms grown in salt and added to control supernatants, for the most part did not show any changes. If the interchange was made nearer the end-point of lysis

of the control, the results were very erratic, sometimes showing a slight increase in titre of the lysate and other times none at all.

11. Gram stains of organisms during the period of lysis in phage-salt mixtures show no deviations from similar preparations containing no salt.

12. When mixtures of phage and bacteria made with 0.25 M NaCl and without salt are grown at 36°C. to within 0.4 hour of lysis and are

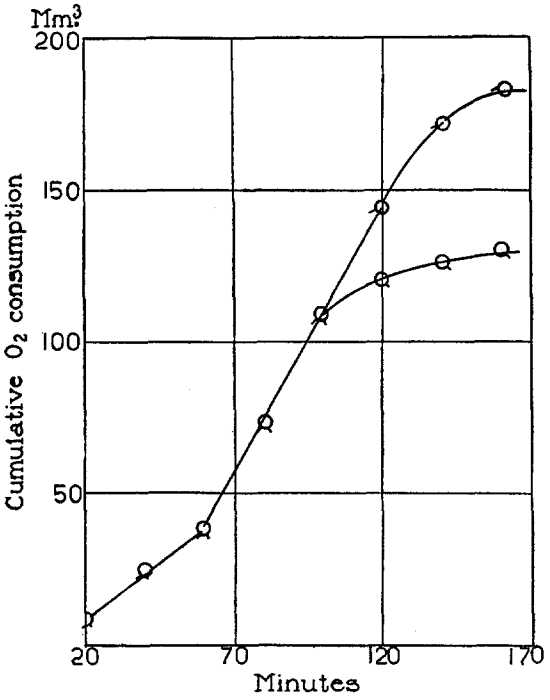

FIG. 5. Cumulative oxygen consumption of organisms grown in the presence of phage with and without salt. $[B]_o = 1 \times 10^8$ B/ml.; $[P]_o = 5 \times 10^6$ activity units/ml. Temperature—36°C. Q = mixture without salt; ○ = mixture with 0.25 M NaCl.

then removed to a water bath at 4°C., lysis occurs in the salt mixture as much as 5 hours later than in the control. Both mixtures exhibit a tenfold diminution in end titre (Table X).

13. Organisms grown in the presence of 5×10^6 phage units per ml. showed the same rate of oxygen consumption as the controls grown without phage. The rates differ only during the period of lysis as would be anticipated. The rate of oxygen consumption during

growth in the presence of 0.25 M NaCl does not differ from the normal rate. The same holds true of organisms exposed to both 0.25 M NaCl and 5×10^6 phage units per ml. during growth.

DISCUSSION

The foregoing analysis of the influence of 0.25 M NaCl on the kinetics of the phage-bacterium reaction leads to the conclusion that the lytic threshold is increased five to tenfold during a period just preceding the end-point of lysis in the salt mixture and that the titre of the resulting lysate is five to tenfold greater than that of the control.

Lysis occurs about 0.7 hour later than in the mixture containing no salt and it is during the prolonged maximal growth stationary phase preceding lysis that the increase in the total phage concentration develops. The extent of delay in lysis caused by salt and the increase in the titre of the lysate rise with increasing salt concentration over a small range and then attain a maximum. Turbidity measurements and the rates of oxygen consumption of the bacteria during growth and phage production in the presence of 0.25 M NaCl indicate that there is no growth of organisms taking place during the period of phage production at the lytic threshold. This finding is of considerable significance since previous work has emphasized bacterial growth as an essential conditioning factor for phage production (20–24). If bacterial growth is really requisite for phage production, the only way in which phage could be produced while the bacterial growth curve remains flat would necessitate growth of young cells and an exactly equivalent lysis of older cells. The data on oxygen consumption would rule out this possibility so apparently there exist conditions under which phage production can occur in the absence of bacterial growth.

We have attempted to use other compounds in investigating specific ion and osmotic effects on bacterial growth and phage production. Unfortunately there are inherent limitations in this approach; for example the salts of trivalent anions and cations and other salts of univalent and bivalent ions either inhibit growth of the staphylococcus or form insoluble compounds with the constituents of the medium at the pH necessary for growth. Hexoses which are sufficiently soluble are fermented by the staphylococcus and other carbohydrates which are not fermented are not sufficiently soluble.

CONCLUSIONS

1. The presence of 0.25 M NaCl during the reaction between a staphylococcus phage and susceptible organisms results in a five to tenfold increase in the amount of phage produced.

2. Analysis of the reaction indicates that normal kinetic relationships exist until just before lysis occurs. At this time the organisms enter the stationary phase, lysis is delayed approximately 0.7 hour as compared with control mixtures and phage continues to be produced at the usual rapid rate.

3. Apparently there are conditions under which phage can be produced in the absence of bacterial growth although previous work has uniformly emphasized growth of the bacterial substrate as the prime conditioning factor for formation of phage.

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