

EFFECT OF SODIUM SULFATE ON THE PHAGE-BACTERIUM REACTION

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(Accepted for publication, June 10, 1938)

Two definite types of electrolyte effect on the phage-bacterium reaction have been reported. Krueger and West (1) showed that minute concentrations of Mn^{++} accelerate lysis by depressing the lytic threshold; *i.e.*, by lowering the ratio of phage to bacteria requisite for lysis. Furthermore, the Mn ion shifts phage distribution, increasing the extracellular fraction to approximately four times the normal value. Due to the fact that less phage is required to lyse a given number of bacteria, the cellular phage-producing mechanism is destroyed earlier in the reaction and there is a corresponding decrease in the total quantity of phage produced.

Scribner and Krueger (2) investigated a phenomenon first noted by Northrop; namely, that the addition of fairly large amounts of sodium chloride to the medium during the period of reaction between phage and susceptible organisms leads to an augmented end titre of phage. They concluded that 0.25 M NaCl raises the lytic threshold 5- to 10-fold. Therefore, the increased total yield is merely an expression of the fact that the bacteria are less susceptible to phage action and more phage must accumulate in the medium before lysis is initiated. Of particular significance with regard to the basic mechanism of phage formation is the further observation that 0.7 hour prior to the onset of lysis bacterial growth ceases whereas phage production continues at an undiminished rate. It would appear then that under some conditions bacterial growth is not an essential prerequisite for phage formation.

It is evident that variations in the concentration of certain electro-

*The authors wish to express their appreciation for support by grants-in-aid from the American Medical Association, The Board of Research of the University of California, and from various interested friends.

lytes can profoundly influence the kinetic relationships between the two reactants, phage and bacteria. We wish to present here the results of experiments in which bacteriophagy took place in the presence of sodium sulfate.

Methods¹

The symbols used are as follows:

[*P*] = concentration of phage activity units/ml.

[*B*] = concentration of bacteria/ml.

[*P*]₀ = initial concentration of phage/ml.

[*B*]₀ = initial concentration of bacteria/ml.

P.U. = phage activity units

1. The bacterial suspensions consisted of freshly harvested 16-hour cultures of *Staphylococcus aureus* (strain *S₃K*) grown on nutrient agar in Roux flasks and washed once in Locke's solution. Cell concentrations were determined by the centrifuged sediment method of Krueger (3). Broth was standard beef infusion containing 1 per cent Difco neopeptone, 0.5 per cent sodium chloride, and was adjusted to pH 7.4.

2. Phage titres were determined by the activity method of Krueger (4). The activity unit is the smallest amount of phage which will cause lysis when added to a certain number of susceptible cells under standard conditions. Our standard phage contains 1×10^{10} activity units. Because Na_2SO_4 tends to slow down the phage-bacterium reaction its presence must be taken into account in titrating experimental solutions. We have made it a practice to titrate 10^{-2} , 10^{-3} , and 10^{-4} dilutions of all phage unknowns; these dilutions obviate any effect of Na_2SO_4 on the titration system itself.

3. Determination of [bacteria]. As noted above the centrifuged sediment method was employed in preparing the living bacterial suspensions for each day's experiments. In the experimental series variations in [bacteria] were followed by three methods.

A. Turbidity readings were made directly on the sample 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.).

B. Direct counts were used to check the turbidity measurements. Samples were diluted in gentian violet containing formalin and the numbers of cells were counted in the Hauser chamber.

C. Plate counts were made on broth cultures of *S₃K* containing no phage. Despite multiple sampling the plate count method was found to be inadequate for quantitative work.

¹ The experimental methods employed were essentially those reported in previous publications appearing for the most part in the *Journal of General Physiology*, 1929-38.

4. Na_2SO_4 effect. Sterile $\frac{M}{1}$ Na_2SO_4 was added to mixtures of phage and bacteria in broth in sufficient quantity to produce the desired final molarity. The mixtures were shaken in the 36°C . water bath.

5. Rate of phage production. To observe the effect of Na_2SO_4 on the rate of phage production special concentrations of bacteria, phage, and Na_2SO_4 were used. $[B]_0$ was 3×10^7 bacteria per ml., $[P]_0$ was 1×10^8 P.U. per ml., and $[\text{Na}_2\text{SO}_4]$ was $\frac{M}{8}$. These concentrations were selected to assure the reaction being completed within a reasonable length of time and to provide for adequate dilutions of phage unknowns so that there would be no possibility of Na_2SO_4 or bacteria carried over from the reacting mixture affecting the titration system. The mixtures were made in broth at pH 7.4 and were maintained at 36°C . in the water bath shaker. For total phage determinations samples were removed at intervals and were promptly diluted in broth kept at 5°C . The diluted samples from one experiment were accumulated and titrated together. For extracellular phage determinations samples of the reacting suspension were cooled in salt ice mixture and the bacteria were thrown down in the angle centrifuge. Dilutions of the supernatants were made in cold broth and were kept at 5°C . until time for titration.

6. Lytic threshold experiments. To detect the effect of Na_2SO_4 on the lytic threshold of resting bacteria washed cells were grown in broth in the presence of Na_2SO_4 and in its absence, using an initial concentration of 1×10^7 cells per ml. When [bacteria] reached 6×10^8 cells per ml. growth was stopped by icing the suspensions and mixtures containing a final concentration of 6×10^7 bacteria per ml. and varying amounts of phage were prepared. The mixtures were placed in the water bath shaker at 36°C . and bacterial concentrations were determined at 0.2 hour intervals. The lowest ratio of phage to bacteria giving lysis without bacterial growth was selected as the critical value.

RESULTS

When the phage-bacterium reaction takes place in the presence of increasing concentrations of sodium sulfate two results are outstanding. First, the time of lysis is prolonged and second, the end titre of the lysate is increased. As shown in Table I the increase in time of lysis is roughly proportional to the salt content. The end titre, however, reaches a maximum in $\frac{M}{8}$ Na_2SO_4 and further increase in salt concentration, while delaying the time of lysis, does not affect the titre of the lysate.

A comparison of the phage production curves in Figs. 1 and 2 shows that there is quite a lag phase in the presence of sodium sulfate.

In one hour the salt-containing suspension has formed 4×10^8 P. U./ml. while the control mixture has produced 1.0×10^{10} P. U./ml. From 1 hour on the rate of phage production in the presence of Na_2SO_4 becomes logarithmic with time but the rate of increase is only 40-fold per hour as contrasted with the control rate of a 125-fold increase per hour.

Phage distribution between cells and medium likewise is altered by Na_2SO_4 . By 0.5 hour in the control 80 per cent of the phage is in or on the cells while the corresponding figure for the intracellular fraction in the salt mixture is 40 per cent. At 1.0 hour 50 per cent of

TABLE I
Time of Lysis and Total Phage Produced in Broth Containing Various Concentrations of Na_2SO_4

The mixtures were each prepared in a total volume of 10 ml. of broth and the tubes were shaken at 36°C .

Molarity of Na_2SO_4	$[P]_0$	$[B]_0$	t lysis <i>hrs.</i>	Total phage formed
0.05	1×10^7	3×10^7	2.50	7×10^{10}
0.075	"	"	2.70	1×10^{11}
0.10	"	"	3.20	1.5×10^{11}
0.125	"	"	3.60	3.0×10^{11}
0.150	"	"	3.97	3.0×10^{11}
0.175	"	"	4.74	3.0×10^{11}
0.200	"	"	6.00	3.0×10^{11}
0.250	"	"	No lysis	
Control	"	"	2.10	2×10^{10}

the total phage still remains extracellular in the $\frac{M}{8}$ Na_2SO_4 suspension.

Casual inspection of Figs. 1 and 2 shows that the lytic threshold for actively growing bacteria is raised by Na_2SO_4 . In the control set (Fig. 1) the critical value is 80 P. U. per bacterium whereas in the salt mixture the threshold rises to 310 P. U. per bacterium (Fig. 2). Tests carried out with resting cells likewise showed the threshold to be four times greater under the influence of $\frac{M}{8}$ Na_2SO_4 (Table II).

Another point of interest in the phage-bacterium reaction occurring in $\frac{M}{8}$ Na_2SO_4 broth is the long period just before lysis during which bacterial growth ceases while phage production continues. The

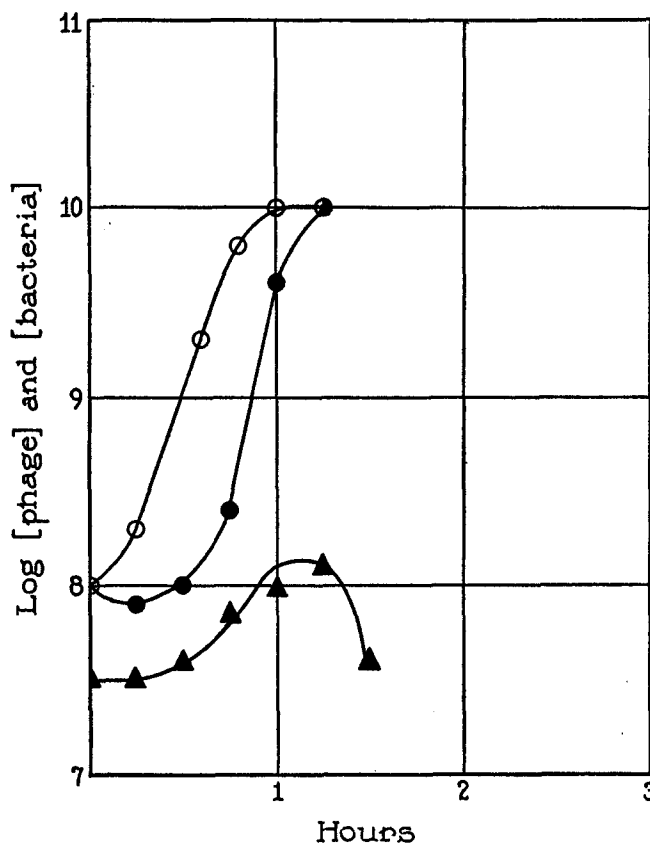


FIG. 1. The growth of bacteria and phage production in plain broth. $[B]_0 = 3 \times 10^7$ bacteria/ml.; $[P]_0 = 1 \times 10^8$ P.U./ml.; \circ = total phage in activity units/ml.; \bullet = extracellular phage in activity units/ml.; \blacktriangle = bacteria/ml.; 36°C .; pH 7.4.

bacterial growth curves were followed by means of turbidity measurements, direct microscopic counts, and in the case of suspensions containing no phage by means of plate counts. Evidently $\frac{M}{8}$ Na_2SO_4 has

no effect on bacterial growth either in the presence or in the absence of phage except for the production of the prelytic plateau noted above.

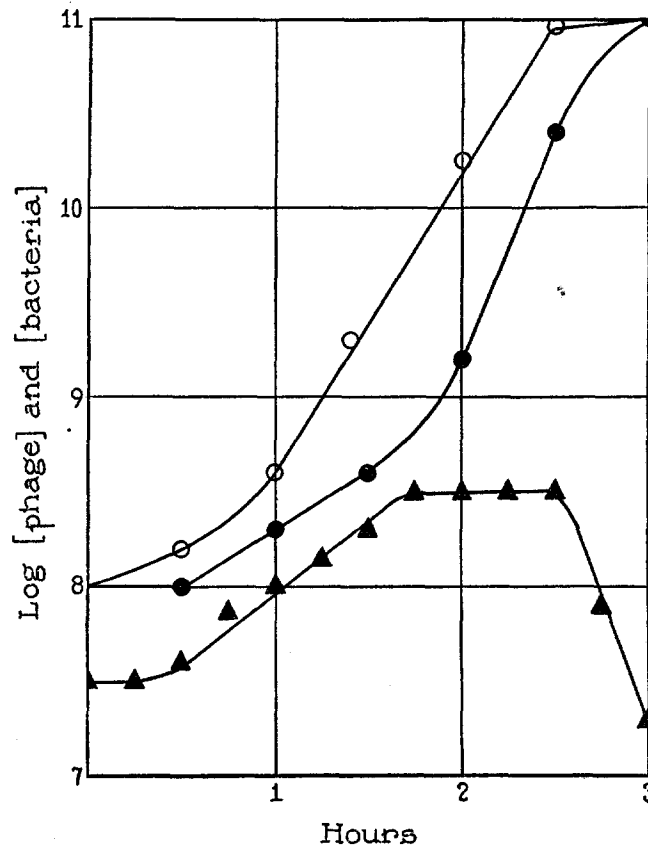


FIG. 2. The growth of bacteria and phage production in $\frac{M}{8}$ Na_2SO_4 broth. $[B]_0 = 3 \times 10^7$ bacteria/ml.; $[P]_0 = 1 \times 10^8$ P.U./ml.; ○ = total phage in activity units/ml.; ● = extracellular phage in activity units/ml.; ▲ = bacteria/ml.; $[\text{Na}_2\text{SO}_4] = \frac{M}{8}$; 36°C .; pH 7.4.

The influence of Na_2SO_4 on distribution can be demonstrated in another way. Mixtures containing 5×10^8 bacteria/ml. and 1×10^8 P. U./ ml. in broth with and without $\frac{M}{8}$ Na_2SO_4 are held at 5°C . At intervals samples are removed, the resting cells centrifuged down,

and the supernatants titrated. The suspension containing Na_2SO_4 comes to equilibrium with 40 per cent of the phage outside the cells; in the control mixture 10 per cent of the phage is extracellular.

TABLE II

Effect of Na_2SO_4 on the Lytic Threshold of Resting Cells. Determination of Critical Phage/Bacteria Ratio for Lysis in Absence of Bacterial Growth. Staphylococcus-Phage Mixtures with [Bacteria] Constant ($[B]_0 = 6 \times 10^7$ Bacteria/Ml.) and [Phage] Varying in Plain Broth and in Broth

Containing $\frac{M}{8} \text{Na}_2\text{SO}_4$

The bacterial suspensions consisted of young cells. Growth was inhibited by preliminary icing. After the mixtures were made they were placed in the water bath shaker at 36°C . and turbidity readings were made every 0.2 hr. Multiply figures in columns by 10^7 .

The critical phage/bacteria ratio in plain broth is 83 P.U./bacterium; in $\frac{M}{8} \text{Na}_2\text{SO}_4$ it is 330 P.U./bacterium.

Standard phage 1×10^{10} P.U./ml. or Na_2SO_4 phage 1×10^{11} P.U./ml.	8		7		6		5		4		3		2		1	
	1		2		3		4		5		6		7		8	
Broth	1		1		1		1		1		1		1		1	
Bacteria 6×10^9 /ml.	1		1		1		1		1		1		1		1	
Time	Control		Na_2SO_4		Control		Na_2SO_4		Control		Na_2SO_4		Control		Na_2SO_4	
	Control	Na_2SO_4	Control	Na_2SO_4	Control	Na_2SO_4	Control	Na_2SO_4	Control	Na_2SO_4	Control	Na_2SO_4	Control	Na_2SO_4	Control	Na_2SO_4
hrs.																
0.2	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
0.4	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
0.6	3	6	3	6	3	6	3	6	8	6	8	6	8	6	8	8
0.8	0	3	0	3	0	3	0	3	6	3	8	3	10	3	10	8
1.0		0		0		0		0	0	0	4	0	8	0	8	4
1.2											0		4		4	0
1.4													0		0	

DISCUSSION

The reaction between *Staphylococcus aureus* and the homologous bacteriophage in the presence of Na_2SO_4 has several interesting aspects. In the first place bacterial growth shows no departure from the normal. Phage production, however, has a rather long lag phase after which it proceeds logarithmically with time but at only one-

third the normal rate. Apparently it is the action of $\frac{M}{8}$ Na_2SO_4 in altering phage distribution which is responsible for both the long lag phase and the lower rate of phage production. Somehow the salt changes the bacterium's surface so that much less than the usual amount of phage is taken up. The phage initially present in the mixture is thus kept from optimal contact with the phage precursor in the actively growing cells and the phage-forming mechanism consequently functions at a slower pace.

$\frac{M}{8}$ Na_2SO_4 has a marked influence on the susceptibility to phage action of both resting and growing bacterial cells. It takes four times the normal amount of phage to lyse a bacterium in the presence of this concentration of sodium sulfate. This phage resistance is attained without observable destruction of the phage-forming system and it is because of the increased resistance that the bacteria are enabled to continue manufacturing phage with the eventual result that from ten to twenty times the customary phage yield is attained.

SUMMARY AND CONCLUSIONS

Bacteriophagy taking place in the presence of $\frac{M}{8}$ Na_2SO_4 has the following pronounced characteristics:

- A. Time of lysis is considerably prolonged.
- B. The bacteria take up less than the normal amount of phage.
- C. Phage production occurs at one-third the customary rate.
- D. It takes four times as much phage to lyse a Na_2SO_4 -treated bacterium than a normal one.

E. Bacterial growth is not affected by Na_2SO_4 .

The lag phase and the lowered rate of phage production can be attributed to the Na_2SO_4 effect on the cell surface. Less phage is taken up by the cells and contact of phage with the bacterium's precursor-producing mechanism is impeded.

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