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THE EFFECT OF SODIUM CHLORIDE ON STAPHYLOCOCCUS-PHAGE RELATIONSHIPS

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THE EFFECT OF SODIUM CHLORIDE

ON STAPHYLOCOCCUS-PHAGE

RELATIONSHIPS

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CHAPTER I

INTRODUCTION

The effect of the ionic environment on bacteriophages has been the subject of numerous investigations. These have been reviewed by Puck (1954), Tolmach (1957), Weidel (1958), Adams (1959), Elek (1959), and Groman (1961). Relatively few such studies, however, have been concerned with the effects of high concentrations of electrolytes on free phages or on host cell-phage relationships (Bronfenbrenner, 1925; Adams, 1949; Anderson, 1949, 1950; Herriott, 1951; Rountree, 1951). Moreover, as pointed out by Groman (1961), until recently most investigations have been restricted to studies of coli phages to the virtual exclusion of others. This concentration of effort led to rapid, cumulative advances in the understanding of the coli phages and, presumably, the nature of bacteriophages and their biological implications in general. Still, information on the non-enteric phages was relatively meager, unorganized, and, as a result, much of its significance has been ignored. With more information on the non-enteric phages, presently accepted generalizations may be verified or, if previously unrecognized cell-phage relationships are revealed, may have to be modified.

Bronfenbrenner (1925) observed that concentrations of sodium chloride greater than 1 per cent inactivated 99 per cent of a phage; the particular phage tested and time required for inactivation were not specified in his report.

Burnet and Lush (1935) found that coli and staphylococcal phages in the presence of 0.1 M solutions of sodium, potassium, and ammonium salts were much more susceptible to the inactivating effects of temperature (60°C) than when the same phages were suspended in broth. The addition of a small amount of calcium, magnesium, or barium salt partially or completely prevented this inactivation. Callow (1927) observed that high concentrations of calcium chloride, lanthanum chloride and sodium chloride inactivated staphylococcal bacteriophages.

Adams (1949) examined the kinetics of the inactivating effects of temperature on coli phages, particularly T5, in concentrations of sodium chloride ranging from 0.01 M to 2 M. He found that inactivation of phage T5 was almost complete after one hour at 37°C in solutions containing less than 0.2 M sodium chloride, whereas at concentrations of 0.4 M and 0.8 M sodium chloride there was no significant reduction in plaque count. The seven T phages, which lyse Escherichia coli, were much more rapidly inactivated by heat when suspended in a 0.1 M solution of sodium salts in distilled water than when suspended in broth containing equivalent amounts of these salts. In either condition the kinetics of inactivation followed those of a first order reaction. The rate of inactivation of phage T5 in 0.1 M sodium chloride at 37°C could be greatly decreased by the addition of 10^{-3} M concentrations of divalent cations such as calcium, magnesium, barium, strontium, manganese, cobalt, nickel, zinc, cadmium, and copper. It was proposed that the increase in stability of phage T5 in the presence of various cations was the result of complex formation between the phage and metal ion. Phage T5 was found to require calcium for replication but not for adsorption. Other T phages required calcium for adsorption as well as for replication. One per cent sodium citrate was found to inhibit phage multiplication but not multiplication of *E. coli* host cells. This inhibition was due, presumably, to the removal of the calcium ion by the citrate. Rountree (1951) noted that, in the free state, some staphylococcal phages were partially inactivated by 1 per cent sodium citrate. Lark and Adams (1953) found that the tail tip of *coli* phage T5 contained a calcium-activated enzyme which was necessary for the injection of phage nucleic acid. It was concluded by them that sodium citrate complexed with the calcium ion and thus prevented the activation of the injection enzyme. This conclusion was confirmed by Luria and Steiner (1954).

Rountree (1951; 1955) observed that not only was calcium involved in adsorption of the staphylococcal typing phages to host cells but also in a later step of the phage reproductive cycle. The amount of calcium giving maximal stimulation of phage development was usually 100 μg./ml. with the exception of phages 31 and 31B which required 200 μg./ml. Blair and Williams (1961) recommend the routine incorporation of 400 μg./ml. of calcium chloride to produce propagating phage to be used in typing procedures.

Puck et al. (1951) observed that the rate of attachment of coli phages to host cells could be varied by changing the concentration of salts. In the T1-E. coli B system attachment of the phage to the cell did not occur in distilled water, but if the proper concentration of salts was present the rate of attachment of phage to host cells could be adjusted to any desired value up to the maximum limit set by the diffusion rate of the virus. Salts of calcium, magnesium, barium, and manganese in a concentration of 5 x 10^{-4} M brought about a reaction rate representing 100 per cent collision efficiency. Both larger and smaller concentrations depressed the attachment velocity. Salts of sodium, potassium, ammonium, and lithium showed a similar pattern but required a ten-fold greater concentration than that of the divalent group in order to produce the same effect. The maximum velocity which could be obtained by using monovalent cations was only half that achieved with the divalent cations.

Some attempts have been made to correlate the effect of sodium chloride on bacterial growth as a factor in phage-host cell relationships. Fong and Krueger (1949) reported that 1 M sodium chloride interfered with adsorption of phage K to the propagating strain of staphylococcus. These investigators also noted that the total bacterial cell count was the same in ordinary sodium chloride broth and in the same broth containing 1 M sodium chloride, but the number of viable staphylococci by colony count in the high salt broth was much less than that in the control broth. Holm and Sherman (1921), Sherman and Holm (1922) and Sherman et al. (1922) reported that 0.2 M sodium chloride was optimal for accelerating the rate of growth of E. coli and shortening the lag period of bacterial growth. Greater or lesser amounts of sodium chloride delayed logarithmic growth.

Rountree (1951) studied the effects of electrolytes on free staphylococcal phage and on phage adsorption to host cells. It was found that the minimum amount of electrolyte giving 90 per cent adsorption after 20 minutes at 37°C varied from 5 to 400 μg./ml. in the case of both calcium chloride and magnesium chloride. The minimum concentration of sodium chloride necessary for 90 per cent adsorption varied from 25 to 1000 μg./ml. In these studies, 0.1 per cent was the greatest concentration of sodium chloride used. Salts of citrate and oxalate prevented multiplication of staphylococcal phages. Adsorption of staphylococcal phages 3A, 3B, 3C, 6, 7, 42B, 44, 47, 47A, 47B, and 47C was prevented by 0.5 per cent sodium citrate. Phages 31, 31B, 42C, 52, and 52A were too unstable in distilled water solutions of citrate and in distilled water alone to determine the effect of citrate on these phages. By using phage 3A in the presence of 400 μg. sodium chloride per ml., it was learned that, although the amount of free phage in the supernatant indicated 95 per cent adsorption, only 25 per cent of the adsorbed phage was recovered. This result was similar to that obtained by Puck (1949), who found that coli cells in the absence of calcium ions could not be recovered. In these reports neither Puck nor Rountree considered the possible effect of rapid decrease in osmotic concentrations in the inactivation of phage. Anderson (1949,

1950) and later Herriott (1951) demonstrated that certain *coli* phages when taken rapidly from solutions of high salinity to those of relatively low concentrations of 0.06 M formed viral "ghosts". Due to osmotic shock the infectious nucleic acid was released from the viral particles prior to adsorption. When the ratio of phage to cells was one-hundred to one respectively, these viral ghosts still possessed the ability to lyse host cells, but plaque forming ability was reduced by 99 per cent.

The present study was suggested by the observation that staphylococcal phage 81 lysed its propagating strain of staphylococcus on agar medium containing as high as 10 per cent sodium chloride, whereas a number of other staphylococcal phages were inactive at sodium chloride concentrations greater than 3 per cent. The specific objectives of the study were to examine the effect of high concentrations of salts, primarily sodium chloride, on (1) free staphylococcal phages, (2) host staphylococci in the absence of phage, (3) adsorption of phage to host cells, (4) the ability of host cells to replicate phage, and (5) the phage typing patterns of staphylococci. The possibility that, under the influence of electrolytes, nontypable strains of staphylococci might become susceptible to phage lysis was hopefully considered. The report of Ma and Mandle (1961) that, under the influence of heat shock, some nontypable strains of *Staphylococcus aureus* became phage typable encouraged this idea. Preliminary observations on alteration of phage susceptibility of staphylococcal strains treated with sodium chloride and certain other salts suggested that differences in the concentration of sodium chloride at different body surfaces, i.e., nasal mucosa and skin, might play a primary role in the selection of staphylococci susceptible to phage action, or to a physiological alteration of the cells that permits varying stages of the phage reproductive cycle to proceed. Bertani and Nice (1954) observed that reduction of temperature from 37°C to 20°C increased the rate of lysogenization with *Shigella dysenteriae* phage P1. Rountree (1959) observed that, following lysogenization with certain serological group A and F phages, staphylococcal strains in which the multiplication of B phages was partially blocked became fully sensitive to these B phages. Related observations were made by Asheshov and Rippon (1959); Blair and Carr (1961); Rountree and Asheshov (1961); and Sakurai *et al*. (1961). Conceivably sodium chloride might affect lysogenization, as did temperature, and thus alter phage patterns.

CHAPTER II

MATERIALS AND METHODS

Phages and Staphylococci

Phages were selected on the basis of preliminary observations and as examples of each of the staphylococcal typing groups. Arranged according to typing group they were:

Group I.	Phages 29, 52, 52A, 79, 80
Group II.	Phages 3A, 3B, 3C, 55, 71
Group III.	Phages 6, 7, 42B, 42E, 44A, 47, 47C, 77, 83
Group IV.	Phage 42D
Miscellaneous	Phages 81, 187

Staphylococci used were the phage propagating strains (PS) originally obtained, together with the phages, through the courtesy of Dr. J. E. Blair, as well as certain hospital and laboratory strains of *Staphylococcus aureus*. Among the latter, the Smith strain (Sm), originally studied by Smith and Dubos (1956), and its mouse virulent, phage nontypable mutant (Sv), first described by Hunt and Moses (1958), were kindly provided by Dr. G. A. Hunt. Hospital staphylococci and strains derived from them were represented by Hv1, a clumping factor-negative mutant of a typical coagulase-positive strain; E35, an antibiotic resistant strain belonging to phage type 52/80; and W, an antibiotic resistant strain belonging to phage type 52/80/81.

Phage Techniques

Preparation of Stock Phage

The method used to propagate the staphylococcal phages was an adaptation of the soft agar procedure introduced by Swanstrom and Adams (1951) for coli phages and later advocated for propagation of staphylococcal phages (Blair and Williams, 1961). Only phage filtrates which gave semi-confluent to confluent lysis of the corresponding propagating strains of staphylococci at dilutions of 10^{-4} or higher were used.

Phage Typing Technique

Except for the modification of the routinely used media (trypticase soy broth and trypticase soy agar) by the addition of increased concentrations of sodium chloride and, in certain experiments, of calcium chloride, the technique used was the same as that first described by Wilson and Atkinson (1945) and later modified by Blair and Carr (1953, 1960). Both the routine test dilution (RTD) and 1000 X RTD were used throughout the study. The degree of lysis was recorded as four plus (4), confluent lysis; three plus (3), semiconfluent lysis or confluent lysis with secondary colonies; two plus (2), fifty or more discrete phage plaques; one plus (1), twenty to fifty plaques; plus-minus ($\pm$), less than twenty plaques. Two plus lysis is considered to be a significant and specific reaction which contributes to the phage type of a staphylococcus. In routine phage typing, any degree of lysis from fifty plaques to confluent lysis is now recorded as two plus (Blair and Williams, 1961). Thinning of growth, generally referred to as inhibition and recorded as zero according to the International Subcommittee on Phage Typing of Staphylococcus is interpreted as a type of lytic reaction designated "shadow" (S) or S lysis in this study.

Phage Plaque Counts

Phage assay is based on the ability of a single phage particle to increase in number in the presence of a large excess of growing bacteria fixed in a thin, continuous film of agar medium (Gratia, 1936; Hershey et al., 1943). In this study the following plaque count method was employed. Within five to ten minutes after dilution of stock phage in trypticase soy broth (Baltimore Biological Laboratories), 0.3 ml. of the phage-broth was withdrawn and dispensed in 0.1 ml. amounts to each of three 13 x 100 mm. tubes which contained 0.1 ml. of a six hour (37°C) trypticase soy broth culture of the appropriate staphylococcus. Immediately thereafter, 3 ml. of melted, cooled soft agar (trypticase soy broth plus 0.6 per cent agar) was added to each tube. Contents of the tube were mixed and layered over the surface of regular trypticase soy agar (BBL) in a standard (100 mm. diameter) petri plate. When the soft agar layer was firm, plates were inverted and incubated at 30°C. All plaque counts except those of phages 71 and 79 were made after 24 hours incubation. Plaques of phages 71 and 79 were counted after 18 hours because further incubation resulted in overgrowth by the staphylococci.

Sodium Chloride Tolerance of Free Phages

Dilutions of each of 22 phages designed to contain from 100 to 300 plaque forming units per 0.1 ml., were made in 10 ml. amounts of (1) commercially prepared trypticase soy broth (BBL) of 0.5 per cent sodium chloride content and (2) the same broth to which sufficient salt was added to make a final concentration of 10 per cent. At 10 per cent sodium chloride concentration the pH of the broth was reduced from 7.2 to 6.8. No adjustment of pH was made since, in previous experiments, variation of hydrogen ion concentration within this range had no effect on the plaque forming ability of 10 representative phages tested in media containing 0.5, 3, 7.5, and 10 per cent sodium chloride.

The initial (0) sample was removed as soon after preparation as possible. The remainder of the suspension was incubated for 24 hours at 30°C. Additional samples were taken from all suspensions after 10 to 12 hours incubation and again at the end of 24 hours. With selected strains samples were taken at intervals of 2, 4, and 10-12 hours to determine phage inactivation at intermediate times. For ease of presentation of the results, since inactivation was relatively constant with time, only the initial (0) and twenty-four (1) hour findings are presented in Figures 1 and 2.

Sodium chloride inactivation of free phage was determined by the soft agar overlay method described for determining phage plaque counts.

Propagation of Phage 81 in Sodium Chloride Media

This procedure was patterned after that used by Rountree (1951), except that instead of there being 10 to 15 plaque forming units in the sample to be tested, the sample used here contained approximately 500 plaque forming units per 0.02 ml. Into 10 test tubes were pipetted 0.9 ml. amounts of trypticase soy broth containing 0.5, 2, 3, 4, 5, 6, 7, 8, 9, and 10 per cent sodium chloride, respectively. To each of these tubes was added 0.02 ml. of a six hour trypticase soy broth culture of PS 81. Immediately, 0.1 ml. of phage 81-broth, containing 25,000 plaque forming units per 0.02 ml., was added to each tube. Two-hundredth ml. samples were removed from each tube with 0.2 ml. pipettes as soon after mixing as possible and again after 2, 4, 6, 12, 24, and 30 hours. These samples were dispensed into tubes which contained 0.1 ml. samples of a six hour broth culture of PS 81. Three ml. of trypticase soy-0.6 per cent agar were added to each tube and thereafter the procedure followed that of the soft agar overlay technique. After 24 hours incubation at 30°C the degree of lysis was noted and recorded. Whenever possible individual plaques were counted. When the number of plaques increased to the point where individual plaques were not discernable, the relative degree of lysis was recorded as follows: ++, plaques too numerous to count; +++, semi-confluent lysis; ++++, confluent lysis.

Effect of Osmotic Shock on Inactivation of Phages 80 and 81

Phages 80 and 81 were compared in this experiment. A modification of the method used by Anderson (1949) and Herriott (1951) was followed. Two volumes of 20 per cent sodium chloride-trypticase soy broth were added to one volume of phage-broth which contained 10^9 plaque forming units per ml. After allowing five minutes for equilibration, 50 volumes of distilled water were added rapidly. Serial ten-fold dilutions in trypticase soy broth were made immediately and these were used in the soft agar overlay plates to determine the decrease, if any, in plaque forming units. One-tenth ml. phage samples and 0.1 ml. of bacterial cell suspension were mixed with 3 ml. trypticase soy-0.6 per cent agar and plated by the overlay technique. PS 81 was used as the host bacterium for each phage. Control suspensions of the phages, made in regular trypticase soy broth, were treated in a similar manner. Plaque counts were determined after 24 hours incubation at 30°C.

Effect of Sodium Chloride on Adsorption and Replication of Phage 81

Delbrück and Luria (1942) estimated adsorption by determining the total number of plaque forming units in the phage-host cell mixture and subtracting from this the number of plaque forming units remaining (free, unadsorbed virus) after removal of the infected cells by centrifugation. The Delbrück and Luria method was followed in the estimation of phage adsorption in the experiments described below.

Phage Adsorption in Trypticase Soy Broth with Replication in Salt Medium

Two ml. of phage 81 in trypticase soy broth, containing 200 to 600 plaque forming units per 0.1 ml., were mixed with an equal volume of a six hour broth culture of PS 81. Twenty minutes at room temperature were allowed for phage adsorption. Rountree (1951) found that about 95 per cent adsorption of staphylococcal phage to host cells occurred in 20 minutes. One-tenth ml. samples were placed in tubes to each of which was added 3 ml. of melted, cooled, trypticase soy-0.6 per cent agar containing sodium chloride to give a final concentration of 0.5, 3, 7.5, and 10 per cent respectively. After mixing the contents were poured to the surface of agar plates containing sodium chloride in the same proportions as the soft agar layer. Plaques were counted after 24 hours (30°C) incubation of the solidified plates. After the initial samples of the phage-staphylococcus mixture were taken, the remainder of the mixture was centrifuged at 2500 rpm for five minutes in an angle head centrifuge. One-tenth ml. of the phage-containing supernatant was added to tubes to which an equal volume of a six hour broth culture of PS 81 was dispensed. Following mixing, the contents of the five tubes were plated by the overlay technique. Plaques were counted after 24 hours incubation at 30°C.

Phage Adsorption in Salt Broth with

Replication in Salt Medium

The procedure described above was repeated except that phage 81 was suspended in 2 ml. of trypticase soy broth which contained double strength sodium chloride in order that the final salt concentration would be 0.5, 3, 7.5, and 10 per cent. Immediately thereafter, 2 ml. of phage-broth were mixed with 2 ml. of a six hour broth culture of PS 81 and 0.1 ml. samples, in replicates of five, were plated as before, initially and after centrifugation.

Phage Adsorption in Salt Broth with Replication in Trypticase Soy Agar

Phage suspensions were prepared in double strength sodium chloride broth as described above. However, in this experiment 0.1 ml. samples of the phage-cell mixture, taken before and after centrifugation, were plated to the surface of regular (0.5 per cent sodium chloride) trypticase soy agar plates by the soft agar overlay technique in replicates of five. The time and temperature of incubation were the same as in previous experiments.

Effect of Sodium Chloride on Staphylococci as Phage Host Cells

Effect of Salt Medium on Number of Viable Staphylococci

One-tenth ml. amounts of a suspension of PS 81, containing 100 to 300 viable cells per 0.1 ml., were placed in five tubes to each of which were added 3 ml. trypticase soy-0.6 per cent agar containing 0.5, 3, 7.5, and 10 per cent sodium chloride respectively. Contents of these tubes were mixed and poured to the surface of trypticase soy agar plates containing the same levels of sodium chloride. Colony counts were made after 48 hours incubation at 30°C.

Adaptation of Staphylococci to Growth in Salt Medium

Adaptation of PS 81 to growth in medium of high sodium chloride content was attempted in the following manner. PS 81 was subcultured daily, for eleven successive days, in the presence of increasing concentrations of sodium chloride, initially from regular 0.5 per cent sodium chloride trypticase soy agar to the same medium containing 1 per cent sodium chloride and, subsequently, by daily increments of 1 per cent of salt, to 10 per cent sodium chloride medium. Thereafter, four additional transfers on 10 per cent salt medium were made. One-tenth ml. samples of a trypticase soy broth suspension containing approximately 300 viable cells per 0.1 ml. were placed in each of eight tubes, in replicates of five, and 3 ml. of melted trypticase soy-0.6 per cent agar containing 0.5, 3, 5, 6, 7, 8, 9, and 10 per cent sodium chloride, respectively, was pipetted to each tube. After mixing, the contents of each tube was poured to the surface of plates of trypticase soy agar which contained the same concentration of sodium chloride as in the agar overlay. Plates were incubated at 30°C for 48 hours when colony counts were made.

Phage 81 Replication on Washed Cells after
Growth on Salt Medium

PS 81 was grown for approximately 15 hours at 37°C on regular (0.5 per cent sodium chloride) trypticase soy agar and on the same medium containing 10 per cent sodium chloride. Cells were washed from the slope cultures with trypticase broth and cell suspensions of each were made with optical density adjusted to that of the MacFarland #3 $BaSO_4$ standard. Another portion of each cell suspension was washed three times in trypticase soy broth and the density adjusted as before.

One-tenth ml. samples of suspensions of the washed and unwashed cells, grown on the low and high salt medium, were placed in tubes in replicates of five and 0.1 ml. of phage-broth, containing approximately 900 plaque forming units, was added to each. After allowing 20 minutes for adsorption, 3 ml. of melted trypticase soy-0.6 per cent agar were added to the tubes and the soft agar overlay method was used to enumerate phage plaques. Four ml. of each of the phage-cell suspensions were centrifuged at 8°C in an International refrigerated centrifuge at 3000 rpm for 20 minutes. Samples of the supernatant fluid were removed and counts determined by the soft agar overlay technique.

Effect of Sodium Chloride on Phage Susceptibility of Heat Shocked Cells

In order to test for the effect of sodium chloride on the phage susceptibility of heat shocked cells, the following procedure was employed. Staphylococci were subcultured daily to trypticase soy agar slopes and incubated at 45°C for three successive days. On the fourth day 2 ml. of trypticase soy broth was inoculated from the last slope culture and incubated at 45°C until the broth was quite turbid (about six hours). Control cultures were treated in a similar manner except that the incubation temperature in each instance was 37°C. The broth culture was streaked to the surface of trypticase soy agar plates, half of which contained increasing levels of sodium chloride and half contained no added salt over the amount (0.5 per cent) normally present. Phages 29, 52A, 79, 80, and 81 at 1000 X RTD were then dropped to the surface of the plates. As soon as the plates were dry, they were inverted and incubated at 30°C for 24 hours when they were read for lysis.

Experiments described in this chapter were performed in replicates of at least three. With the exception of the heat shock experiment, each experiment was repeated at least once.

CHAPTER III

RESULTS

Staphylococcal Phage Patterns on Sodium Chloride Agar

Figure 1 illustrates the enhancing effect of sodium chloride on the lytic activity of certain phages on PS 81. Of the phages tested (29, 52, 52A, 79, 80, 81, 71, 42B, 47C and 47), routine test dilutions (RTD) of phages 52, 80 and 81 gave semi-confluent to confluent lysis on 0.5 per cent sodium chloride trypticase soy agar. At RTD, in the presence of 3 per cent salt, only phage 81 completely lysed the propagating staphylococcus. This phage showed diminished lytic activity at 7.5 and 10 per cent concentrations of sodium chloride. At 1000 X RTD phage 52 gave confluent lysis on routine typing medium containing 0.5 per cent salt, but at 3 per cent sodium chloride only a shadow reaction appeared, and at higher salt levels no reaction occurred. Similar effects were noted with phages 52A and 80. At 1000 X RTD phage 42B, which showed numerous discrete plaques on routine test medium, was enhanced to semi-confluent lysis at 3 per cent salt concentration, while at 7.5 per cent salt its activity resulted in an extremely thin growth or what could be interpreted as either lysis or inhibition. At 1000 X RTD phage 81, which was derived originally from phage 42B (Bynoe _et al_., 1956) continued to give confluent lysis on 10 per cent sodium chloride

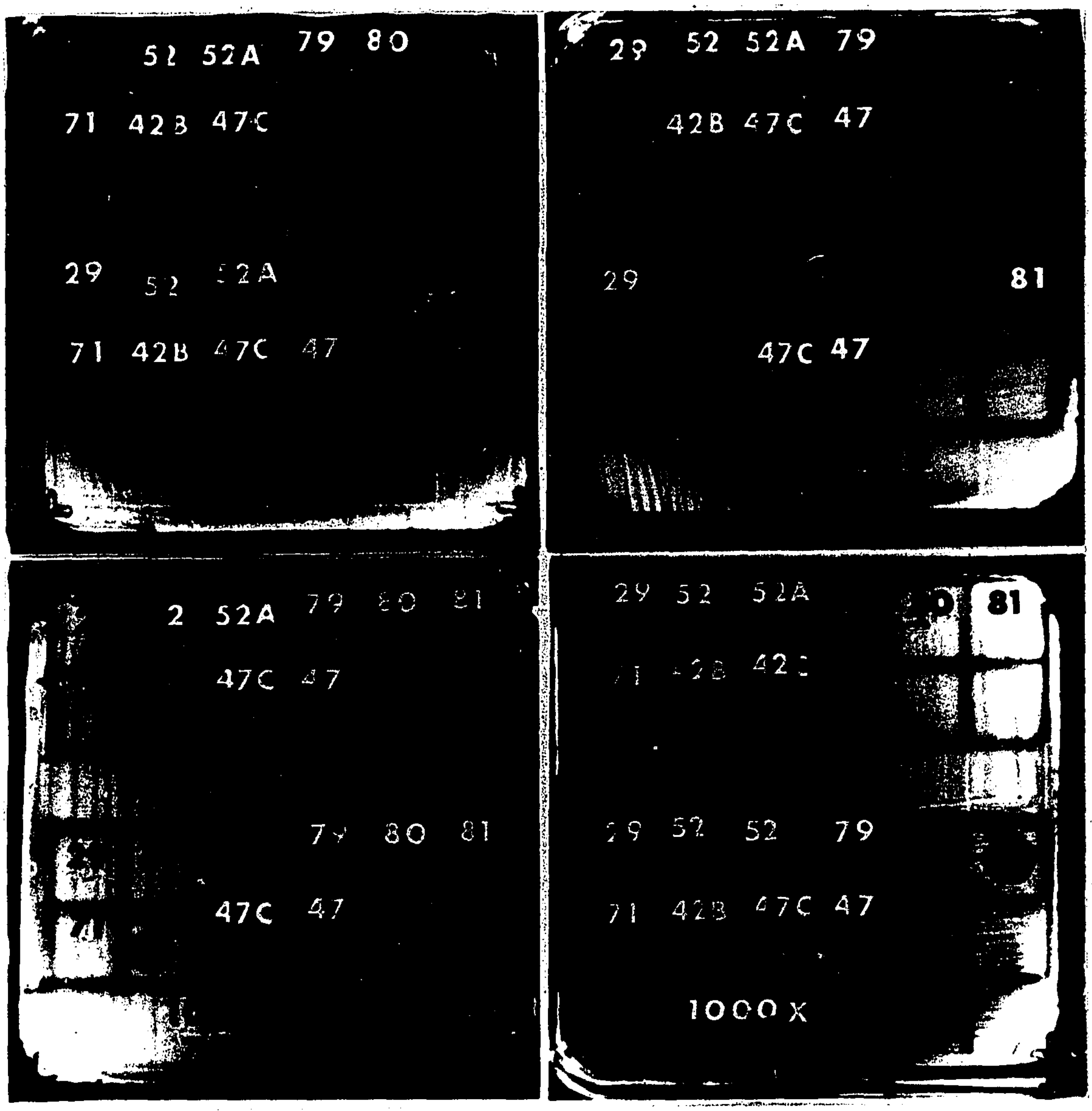

Figure 1. The effect of increasing sodium chloride concentration on lysis of PS 81 by staphylococcal phages.

medium.

An extension of this study using 21 phages, 20 propagating strains, and strains Hv1, E35, and W is presented in summary form in Figures 2, 3, and 4 and in greater detail in Tables 1 through 23. All phages at the routine test dilution (RTD) regularly showed diminished lytic activity as the salt concentration was increased. At 1000 X RTD, some strains of phage, which showed little or no lytic activity at low sodium chloride concentrations, gave almost complete lysis at increased levels. Examples of this phenomenon were observed in the following instances: Phage 42B on PS 80, PS 81, PS 54, Hv1, and W (Figures 1, 3, Tables 4, 5, 15, 20, and 22); phage 71 on PS 3B, PS 3C, PS 55 (Figure 3, Tables 7, 8, and 9); phage 47C on PS 6, PS 75, and Hv1 (Figure 4, Tables 11, 16, and 22). On routine typing medium, at 1000 X RTD, some phages gave "shadow lysis", usually referred to as inhibition or thinning of the bacterial growth. Some phages, unreactive on staphylococci in routine medium, gave the shadow reaction only in the presence of higher levels of sodium chloride. Examples of this type of reaction were noted with each staphylococcus studied except PS 71 (Table 10). In the case of those phages which gave lytic reactions on routine typing medium the shadow reaction generally replaced lysis as the concentration of sodium was increased. This would seem to indicate that the shadow reaction is a form of lysis. Phages belonging to group I (29, 52, 52A, 79, and 80) and group III (6, 42B, 42E, 47C, 47, 75, and 77) were usually the ones which showed increased shadow activity as the sodium chloride concentration in the typing medium increased. Phage 71, belonging to phage group II, frequently showed shadow activity with staphylococci which were normally lysed by phages of other groups at 3 and 7.5 per cent salt but not at 0.5 or 10 per cent concentrations. Phage 3B in group II, on occasion, showed enhanced activity at elevated salt levels. Examples of phages at 1000 X RTD which regularly exhibit lytic activity in 10 per cent sodium chloride typing medium are phage 81 on PS 80 and PS 81 (Figure 2, Tables 4 and 5), phages 3B and 3C on PS 3C and phage 71 on PS 3B (Figure 3 and Tables 7 and 8), phages 6, 47, and 75 on PS 6 (Figure 4 and Table 11), phages 6, 7, 47, and 75 on PS 7 (Figure 4 and Table 12), phages 7 and 42E on PS 42E (Figure 4 and Table 14), phages 47 and 75 on PS 54 (Figure 4 and Table 15), and phage 47 on PS 75 and PS 83 (Figure 4 and Tables 16 and 18).

As the salt concentration was increased in a stepwise fashion to 7.5 per cent, increased activity of phages 29, 52, 52A, 79, 80, 7, and 47 on the Smith strain of *Staphylococcus aureus* was indicated by thinning shadows (S) or enhanced degrees of lysis (Table 24). This was usually pronounced at the 3, 4, and 5 per cent sodium chloride levels. Conversely, phage 71 gave diminished activity on this strain as the concentration of salt was increased. The "normal" phage type of the Smith strain proved to be 29/71. However, phages 52, 52A, 79, and 80, which were inactive on routine test medium, gave confluent clearing at salt concentrations greater than 0.5 per cent. Phages 7 and 47, which exhibited only shadow activity on routine test medium, gave lysis at salt concentrations 4 through 7.5 per cent. The Smith non-typable

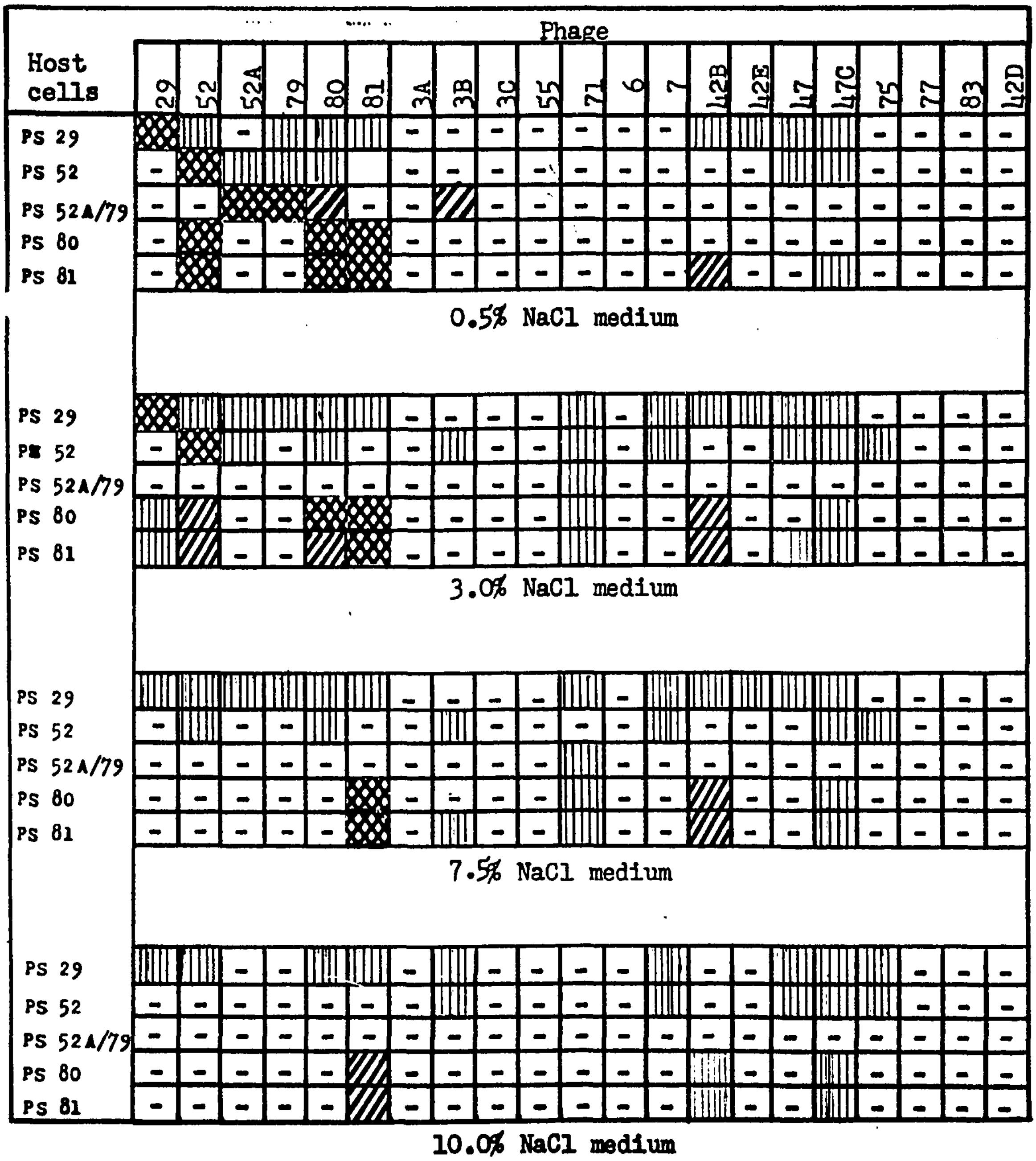

Figure 2. Summary of bacteriophage patterns of Group I propagating staphylococci (PS) and PS 81 on trypticase soy agar containing increasing concentrations of sodium chloride.

Shadow lysis at RTD

Shadow lysis at 1000 X RTD

Lysis at RTD

Lysis at 1000 X RTD

____ No reaction

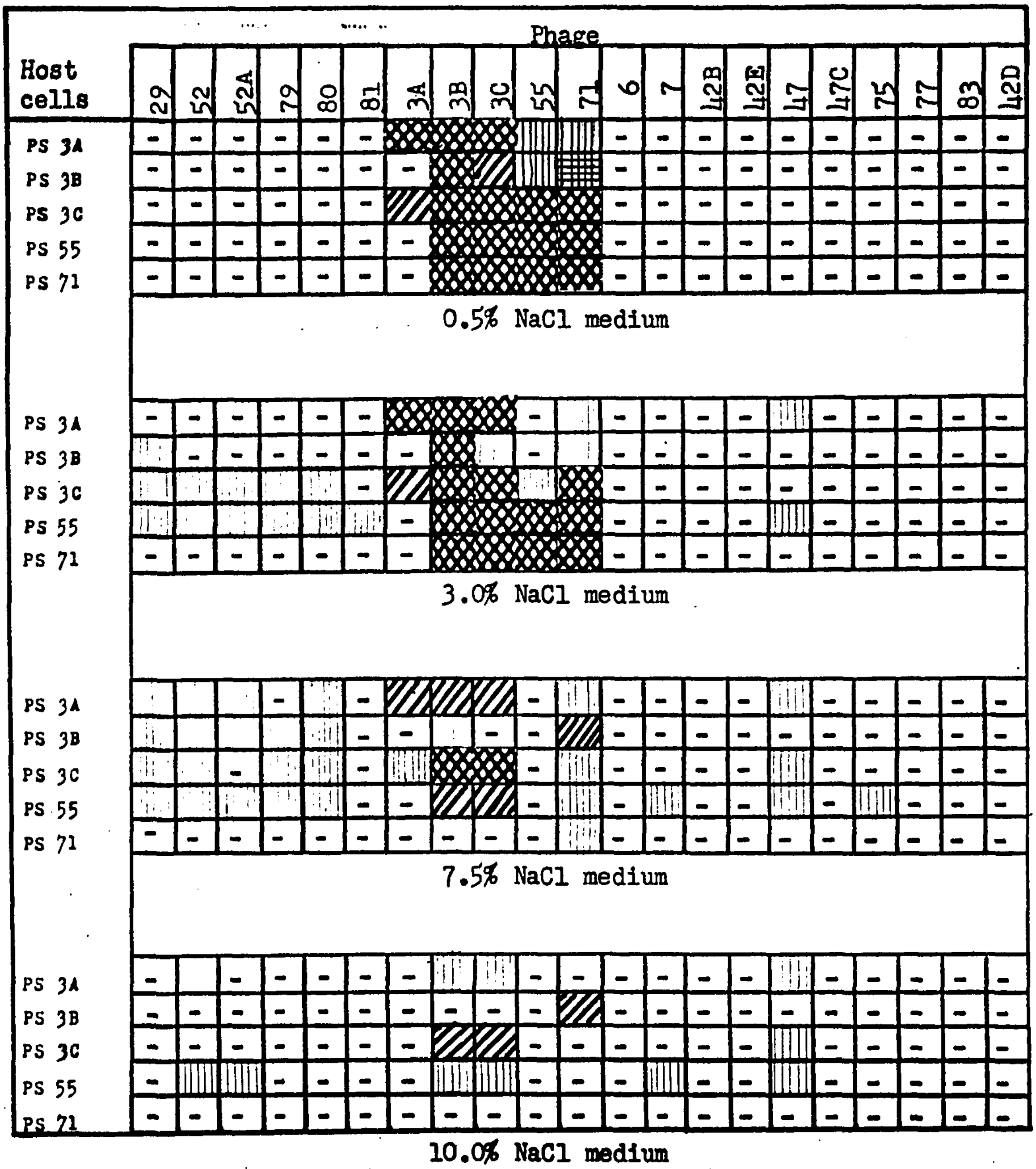

Figure 3. Summary of bacteriophage patterns of Group II propagating staphylococci (PS) on trypticase soy agar containing increasing concentrations of sodium chloride.

Shadow lysis at RTD

Shadow lysis at 1000 X RTD

Lysis at RTD

Lysis at 1000 X RTD

____ No reaction

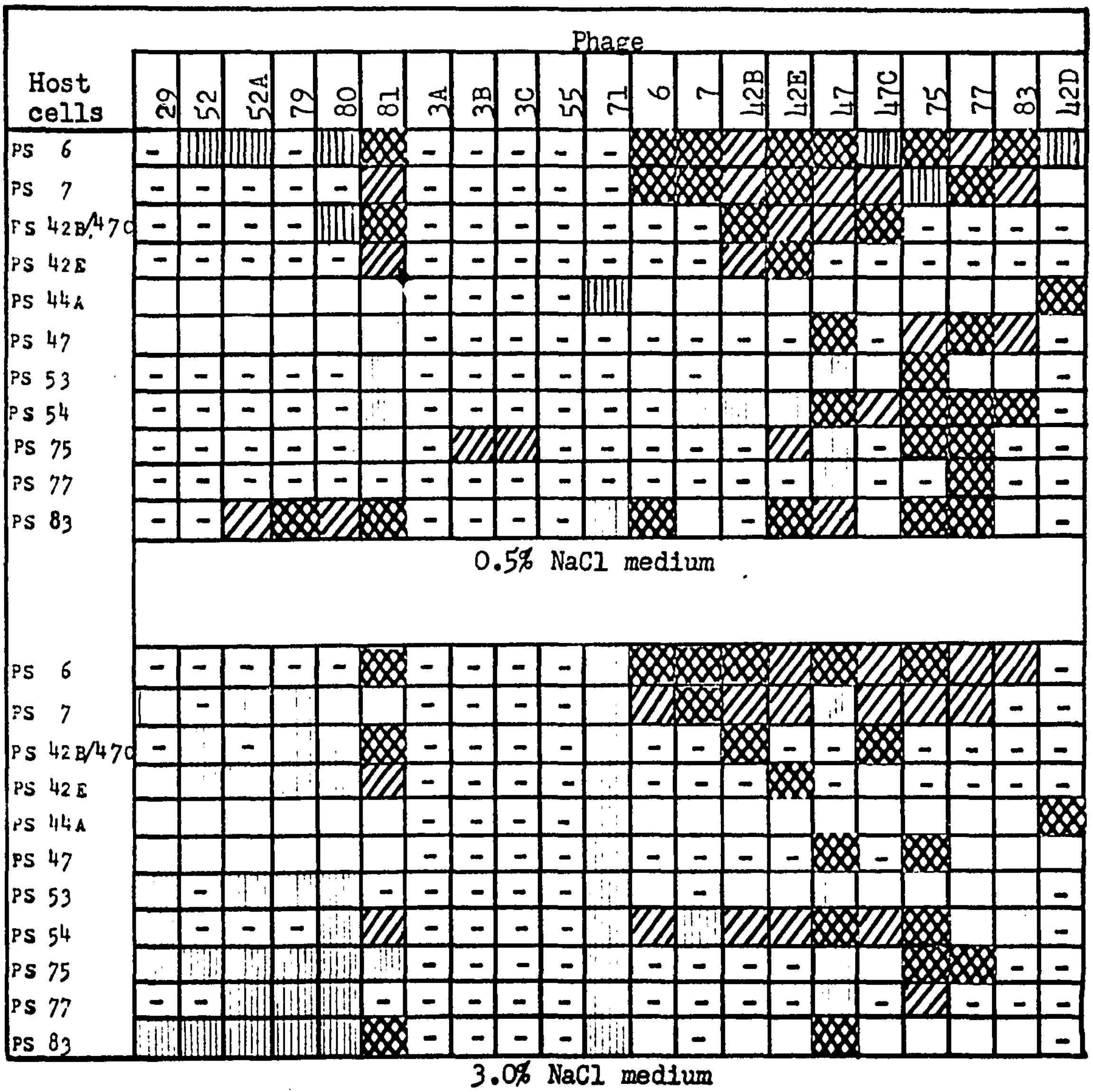

Figure 4. Summary of bacteriophage patterns of Group III and IV propagating staphylococci (PS) on trypticase soy agar containing increasing concentrations of sodium chloride.

Shadow lysis at RTD — Lysis at RTD

Shadow lysis at 1000 X RTD — Lysis at 1000 X RTD

____ No reaction

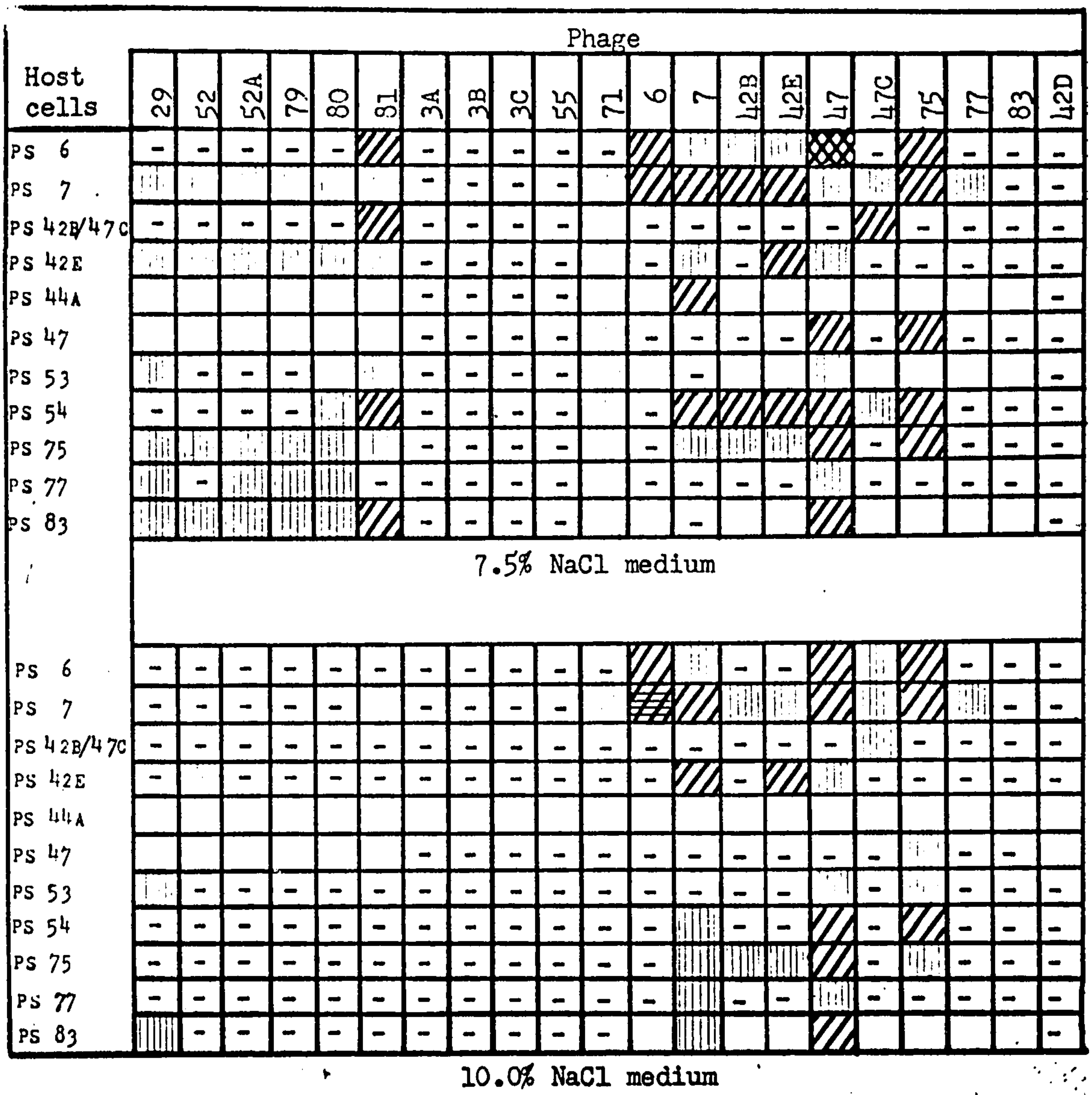

Figure 4, cont. Summary of bacteriophage patterns of Groups III and IV propagating staphylococci (PS) on trypticase soy agar containing increasing concentrations of sodium chloride.

Shadow lysis at RTD — Lysis at RTD

Shadow lysis at 1000 X RTD — Lysis at 1000 X RTD

____ No reaction

strain (Sv) was non-reactive on routine test medium, although at 6 per cent sodium chloride levels scattered plaques were observed with phage 47. On 7.5 per cent salt medium this same phage showed only shadow type lysis with the Smith non-typable strain.

Staphylococcal Phage Patterns on Calcium Chloride Agar

Although concentrations of calcium chloride in the typing medium were varied from 0.1 to 5 per cent, only the results with concentrations of 0.1 per cent through 0.5 per cent are presented in Tables 25 through 35. At concentrations of 1 per cent or more of calcium chloride the growth of staphylococci was reduced to such an extent that results were meaningless. In only a few instances was enhanced lytic effect noted. Phages which gave no lysis at the routine test dilution but gave lytic reactions with added calcium chloride were phages 52A on PS 52 (Table 26) and phage 80 on PS 52A/79 (Table 27). Phage 81 on PS 52A/79 (Table 27), which showed no lysis at 1000 X RTD on routine test medium, gave shadow reactions in the presence of 0.1 per cent through 0.5 per cent calcium chloride.

Shadow activity occurred at 1000 X RTD with phage 71 on PS 29 (Table 25) in the presence of 0.1 and 0.2 per cent added calcium chloride. Shadow activity was also noted with phages 52, 80, and 81 at 1000 X RTD when the Smith strain was tested on 0.1 through 0.4 per cent calcium chloride medium (Table 35). Phage 52 gave shadow lysis only with 0.1 per cent added calcium chloride, phage 80 gave shadow lysis at 0.1 and 0.2 per cent calcium chloride, and lastly, phage 81 gave shadow lysis with added calcium chloride from 0.1 through 0.4 per cent. No activity with these phages was observed in the absence of added calcium chloride. It will be recalled that increased concentrations of sodium chloride enhanced the effect of phage 80 on the Smith strain. At the levels of salts used phage activity was enhanced more by increased concentrations of sodium chloride than by increased levels of calcium chloride.

Sodium Chloride Tolerance of Free Phages

The sodium chloride tolerance of free phages in 0.5 per cent and 10 per cent salt broth was examined before and after 24 hours incubation at 30°C. Phages 3A, 3B, 3C, 7, 42B, 42E, 47, 47C, 75, 81, and 42D were not inactivated in 0.5 or 10 per cent salt broth (Figures 5 and 6). Conversely, the plaque forming ability of phages 29, 52, 52A, 79, 80, 55, 71, 44A, 77, 83, and 187 were reduced drastically after 24 hours incubation at 30°C in 10 per cent salt broth (Figure 5). Reduction in plaque forming ability of phages 80, 55, 77, and 187 was also pronounced after 24 hours incubation at 30°C in 0.5 per cent salt broth.

These results, plus knowledge of the serological group of each phage, showed that phages which were remarkably stable in 10 per cent sodium chloride broth belonged to serological group A or F, whereas the serological group B staphylococcal phages were regularly inactivated when exposed, in the free state, to 10 per cent sodium chloride. This finding correlates with Rountree's (1949) observation that serological group B phages were more unstable to heat and to storage at 4°C than phages belonging to serological groups A and F. The sodium chloride stability of group A and F phages was also decidedly greater than that of the single group L phage tested (phage 187).

Of the ten group A phages studied, only phage 47C showed as much as 33 per cent reduction in plaque-forming units, whereas the others of this group retained 72 per cent or more of their activity after exposure to the higher concentration of salt (Figure 5). The mean survival of the 10 group A phages, after 24 hours in 10 per cent salt broth, was 87 per cent. Conversely, eight of the nine group B phages tested showed 88 per cent or more inactivation under the same conditions (Figure 6). The one exception, phage 83, though less sensitive to sodium chloride than the rest of the group B phages, was inactivated to approximately the same degree in 0.5 per cent salt broth as in 10 per cent salt broth, i.e., 41 and 44 per cent reduction respectively. (Results with phage 83 do not appear in Figure 6 because its serological group became known, through personal communication from Miss Miriam Carr, after completion of the figure.) The salt sensitivity of the group B phages was also evidenced by a drop in the number of plaque-forming units within 5 to 10 minutes after initial contact of most of these phages with 10 per cent salt broth. Such an immediate effect was not produced by exposure of group A phages to the same salt broth. Results with group F phages (42D and 77) and group L phage 187 indicated that they were more salt tolerant than group B phages, but only phage 42D resembled a typical group A phage in this respect (Figure 6).

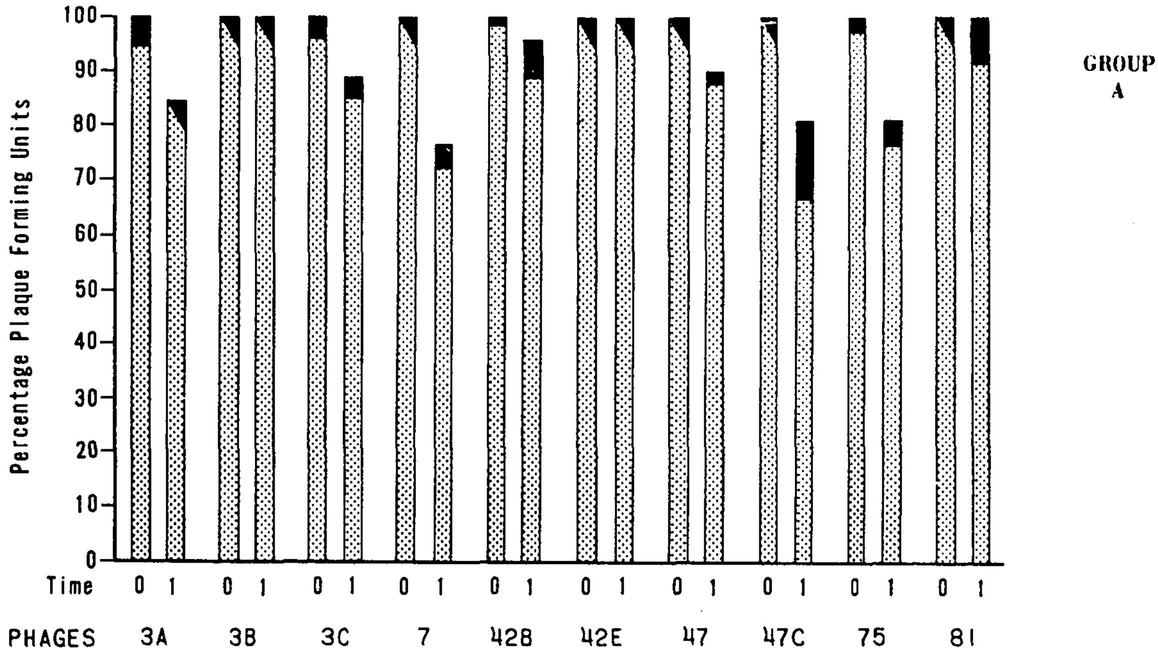

Figure 5. Percentage survival of active serological group A staphylococcal phages in 0.5 per cent and 10 per cent sodium chloride-trypticase soy broth at 30°C based on plaque counts at the time of initial inoculation (0) and after 24 hours (1).

--0.5% NaCl Broth

--10% NaCl Broth

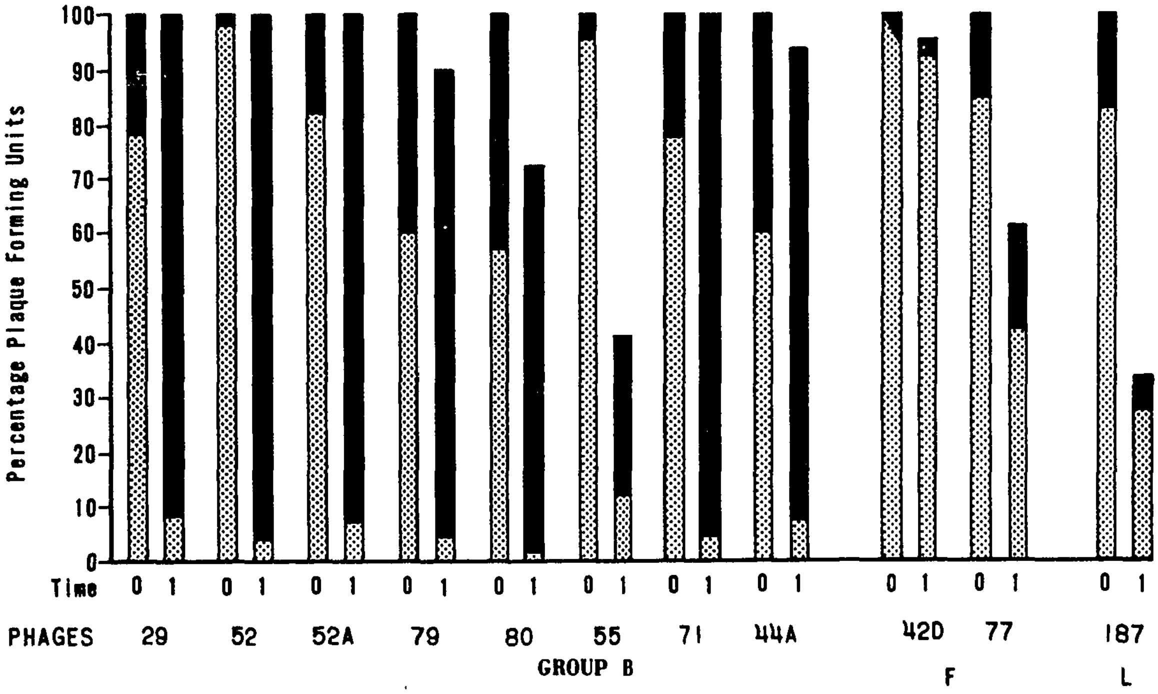

Figure 6. Percentage survival of active serological group B and certain other staphylococcal phages in 0.5 per cent and 10 per cent sodium chloride-trypticase soy broth at 30°C based on plaque counts at the time of initial inoculation (0) and after 24 hours (1).

Comparison of the mean per cent reduction in plaque counts after holding the group A and group B phages in 0.5 per cent salt broth and in 10 per cent salt broth for 24 hours is presented in Table 36. The extremes in variation of the stability of individual phages within each group are also shown in this table. It will be noted that survival of active group A phages in 0.5 per cent and 10 per cent salt broth are remarkably similar. Most of the group B phages as well as those of group A, appeared to be quite stable in 0.5 per cent salt broth, although plaque count reduction in the case of phage 55 after 24 hours incubation in 0.5 per cent salt broth was 59 per cent, as compared to 88 per cent in 10 per cent salt broth. The instability of phage 55 in ordinary trypticase soy broth was exceeded only by that of group L phage 187, which proved to be 67 per cent inactive after the same time in 10 per cent salt broth.

In other experiments, plaque counts of samples taken at intervals throughout the incubation period revealed that, when the activity of a phage was reduced after 24 hours incubation in the presence of either 0.5 or 10 per cent sodium chloride, a continuous decline in the quantity of active phage occurred throughout this period. Some typical results of plaque counts made during the first half of the period are given in Table 37. In the case of phage 29, for example, reduction in plaque-forming units after incubation in 10 per cent salt broth for four hours was 24 per cent; after 12 hours it was 70 per cent; this phage was over 90 per cent inactivated after 24 hours. Phage 80 showed similar inactivation resulting in over 90 per cent reduction of plaque-forming units after only 12 hours incubation in 10 per cent sodium chloride at 30°C. Both phages 42B and 81 were not inactivated, or showed only slight inactivation, after 12 hours incubation in 10 per cent salt broth at 30°C.

Propagation of Phage 81 in Sodium Chloride Media

Examination of Table 38 shows that phage 81 was replicated in media containing from 0.5 to 10 per cent sodium chloride. The delay in reaching maximum phage titer was proportional to the increase in sodium chloride content of the propagating medium. At 10 per cent sodium chloride, after 24 or 30 hours incubation, only a two-fold rise in the number of phage plaques occurred.

When the time of appearance of two plus lysis of PS 81 by phage 81 at 0.5, 2, 3, 4, 5, 6, 7, 9, and 10 per cent sodium chloride concentrations was plotted at 2, 4, 6, 12, 24, and 30 hour time intervals, delay in phage release was clearly shown (Figure 7). It became evident that there was roughly a delay of one hour to reach equivalent levels of lysis for each 1 per cent increase in sodium chloride. No additional phage release could be detected in 10 per cent salt broth after 15 to 16 hours incubation at 30°C.

Effect of Osmotic Shock on Propagation of Phages 80 and 81

Since, in the study on the effect of high sodium chloride concentrations on free phages, the phages had been taken from 10 per cent salt broth to trypticase soy-0.6 per cent agar (0.5 per cent sodium chloride), it was desirable to know the effect of precipitous drops in

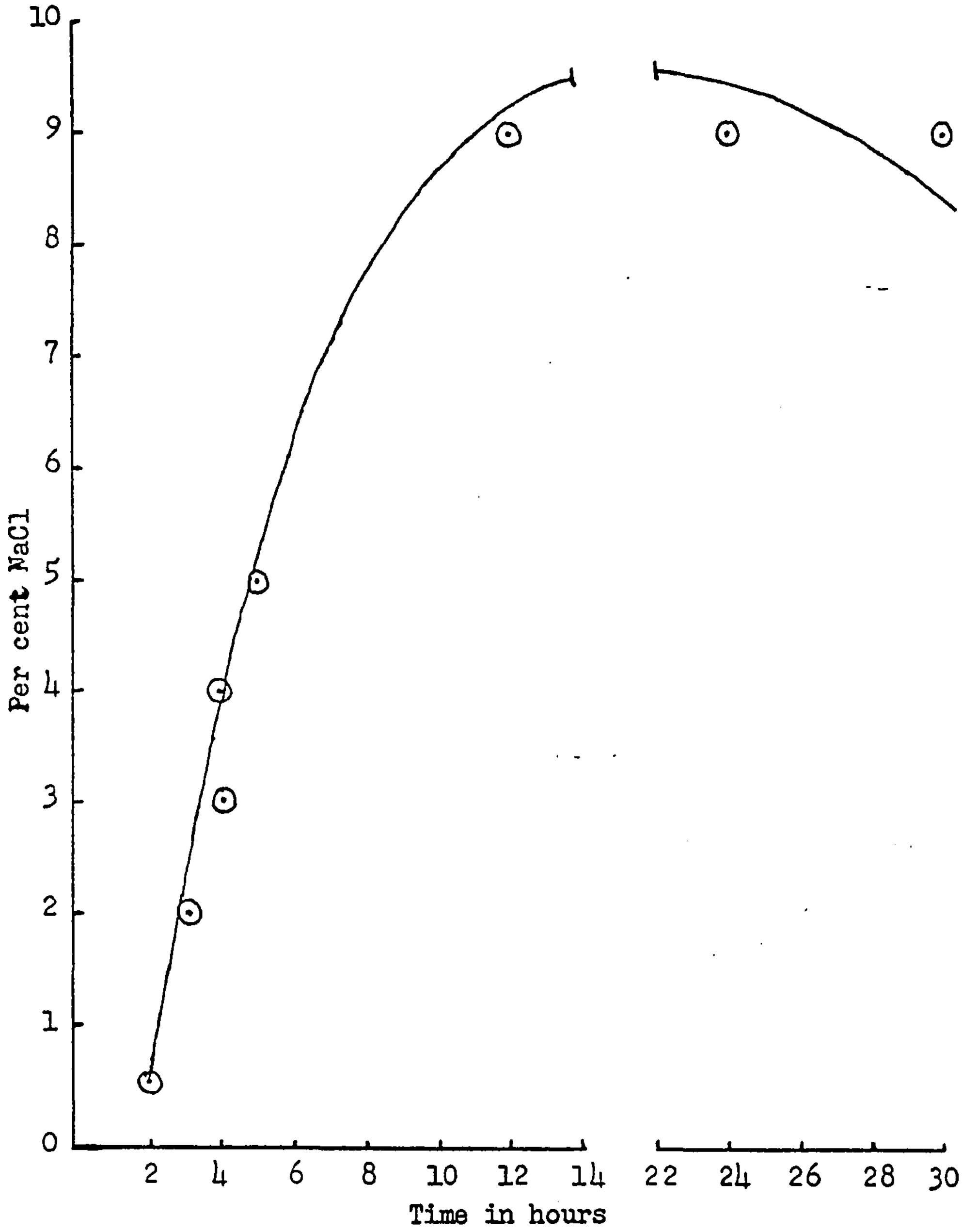

Figure 7. Time of appearance of two plus lysis (plaques too numerous to count, but not semi-confluent lysis) of PS 81 by phage 81 at increasing levels of sodium chloride.

osmotic pressure on staphylococcal phages. Anderson (1949) and Herriott (1951) observed that when certain coli phages were taken from solutions of high salinity to those of relatively low concentrations (0.6 M sodium chloride) viral "ghosts" were formed. These phage ghosts, when present at a phage-cell ratio of one-hundred to one, still possessed the ability to lyse host cells, but plaque forming ability was reduced by 99 per cent.

A precipitous drop in osmotic pressure appeared to have no effect on either phage 80, which previously was shown to be unstable at high levels of sodium chloride, or on the salt-stable phage 81 (Table 39).

Effect of Sodium Chloride on Adsorption and Replication of Phage 81

To help clarify the role of elevated levels of sodium chloride on adsorption and multiplication of phage 81, experiments were planned using the PS 81-phage 81 system where (1) adsorption would be in trypticase soy broth but replication would occur in semi-solid media containing increasing levels of salt, (2) adsorption would occur at elevated levels of sodium chloride and phage replication would occur at these same salt levels, and (3) phage would be adsorbed in the presence of increasing levels of sodium chloride but phage replication would occur on regular (0.5 per cent sodium chloride) medium. Table 40 illustrates that when 97 per cent adsorption had occurred in trypticase soy broth, phage replication (determined by plaque count) was 301 plaque-forming units in media containing 0.5 per cent salt; plaque count was diminished to 124 in 3 per cent salt medium; and, no plaques could be detected at the 7.5 and 10 per cent salt levels. When adsorption of phage to host cells took place in 0.5, 3, 7.5, and 10 per cent sodium chloride broth, and replication was in semi-solid media containing the same levels of salt, the number of phage plaques was 240 on 0.5 per cent salt medium, 103 on 3 per cent salt medium, and none were detected on medium containing 7.5 and 10 per cent sodium chloride. On the other hand, when adsorption of phage occurred in 0.5, 3, 7.5, and 10 per cent salt broth, but replication was in trypticase soy-0.6 per cent agar containing 0.5 per cent sodium chloride, the plaque count was 126, 129, 132, and 118 respectively. Estimates of the plaque forming units in the supernatant fluid after the 20 minute adsorption period indicated that phage adsorption varied inversely with sodium chloride content of the broth.

Effect of Sodium Chloride on Staphylococci as Phage Host Cells

Effect of Sodium Chloride Medium on Number of Viable Staphylococci

Growth of salt-adapted and nonadapted PS 81 were compared in ability to form colonies in media containing increasing levels of sodium chloride (Figure 8). It is apparent that a growth advantage of the salt adapted PS 81 occurred. Whereas a 50 per cent reduction of the colony count of the nonadapted strain occurred after 48 hours on the 5.5 per cent sodium chloride medium, there was a reduction of only 35 per cent of the salt adapted strain under the same growth conditions. This difference became more pronounced at 7 per cent salt levels where growth of the nonadapted strain approached zero. Growth of the salt adapted strain at 7 per cent salt was 50 per cent above that of the nonadapted strain. The effect of increasing salt levels on (1) free phage 81, (2) growth of PS 81, (3) growth of a "salt adapted" PS 81, (4) lysis of PS 81, after previous adsorption of phage 81, before placing on high salt agar, and lysis of PS 81 on regular 0.5 per cent salt agar after previous adsorption of phage in the presence of high concentrations of salt are compared in Figure 9. Free phage 81 showed little inactivation after 24 hours in the presence of 10 per cent sodium chloride. Growth of PS 81 decreased progressively to a point where a 50 per cent reduction in growth activity was obtained at about the 5.5 per cent sodium chloride level. The "salt adapted" PS 81 appeared to have a slight growth advantage in sodium chloride media when compared with cells not previously exposed to salt. If phage were adsorbed in 0.5 per cent salt broth but allowed to replicate at increased salt levels the plaque count dropped precipitously. Phage adsorption was progressively diminished with increasing salt concentration until, at about 7.5 to 10 per cent sodium chloride, adsorption remained constant (approximately 30 per cent).

Replication of Phage 81 on Washed Cells of Sodium Chloride Adapted and Nonadapted Staphylococci

No difference in plaque forming ability of phage 81 was observed whether the host cells were washed or not before exposing them to phage. It was evident, however, that the salt-grown cells did not

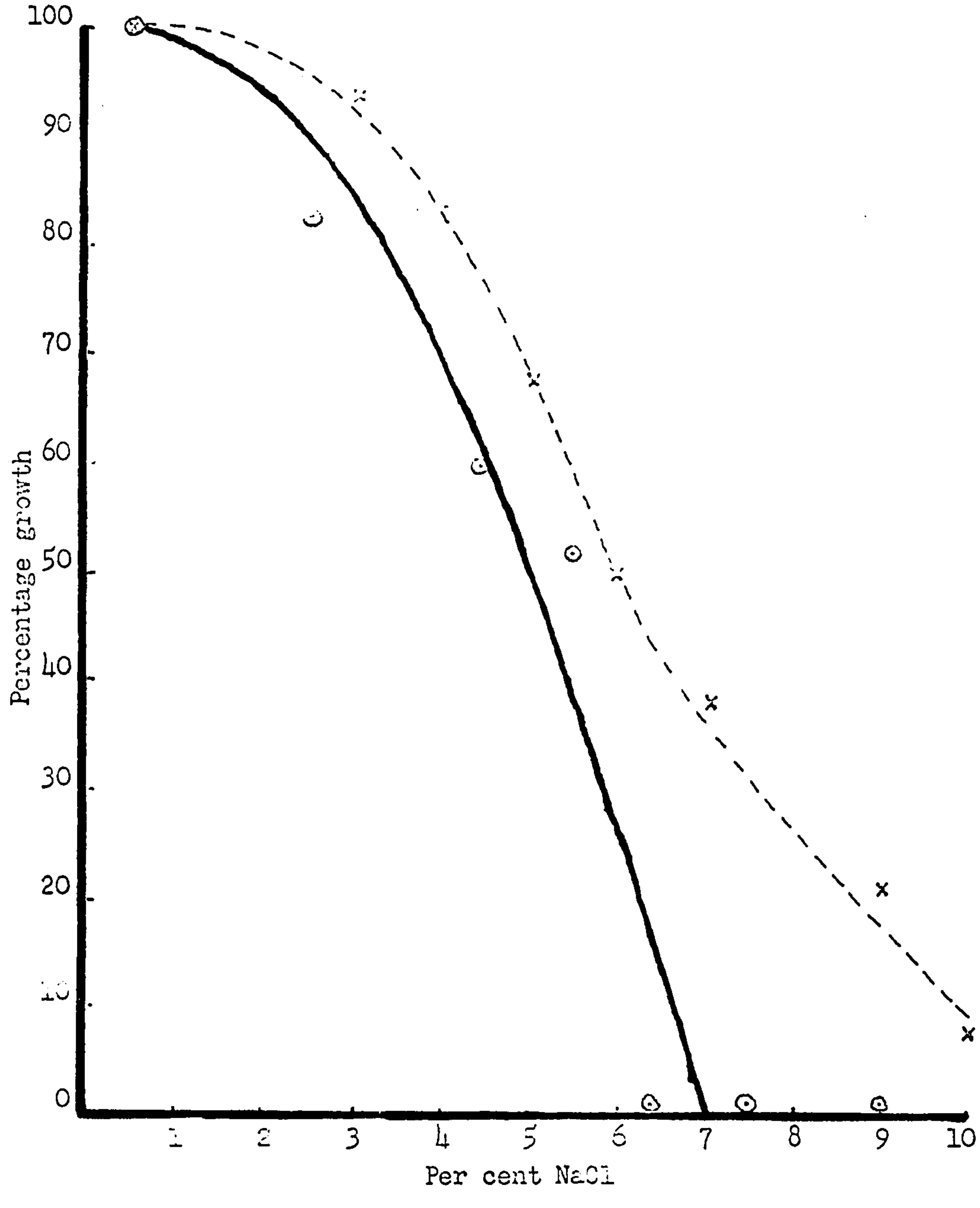

Figure 8. Comparison of growth of salt adapted and nonadapted PS 81 at increasing levels of sodium chloride.

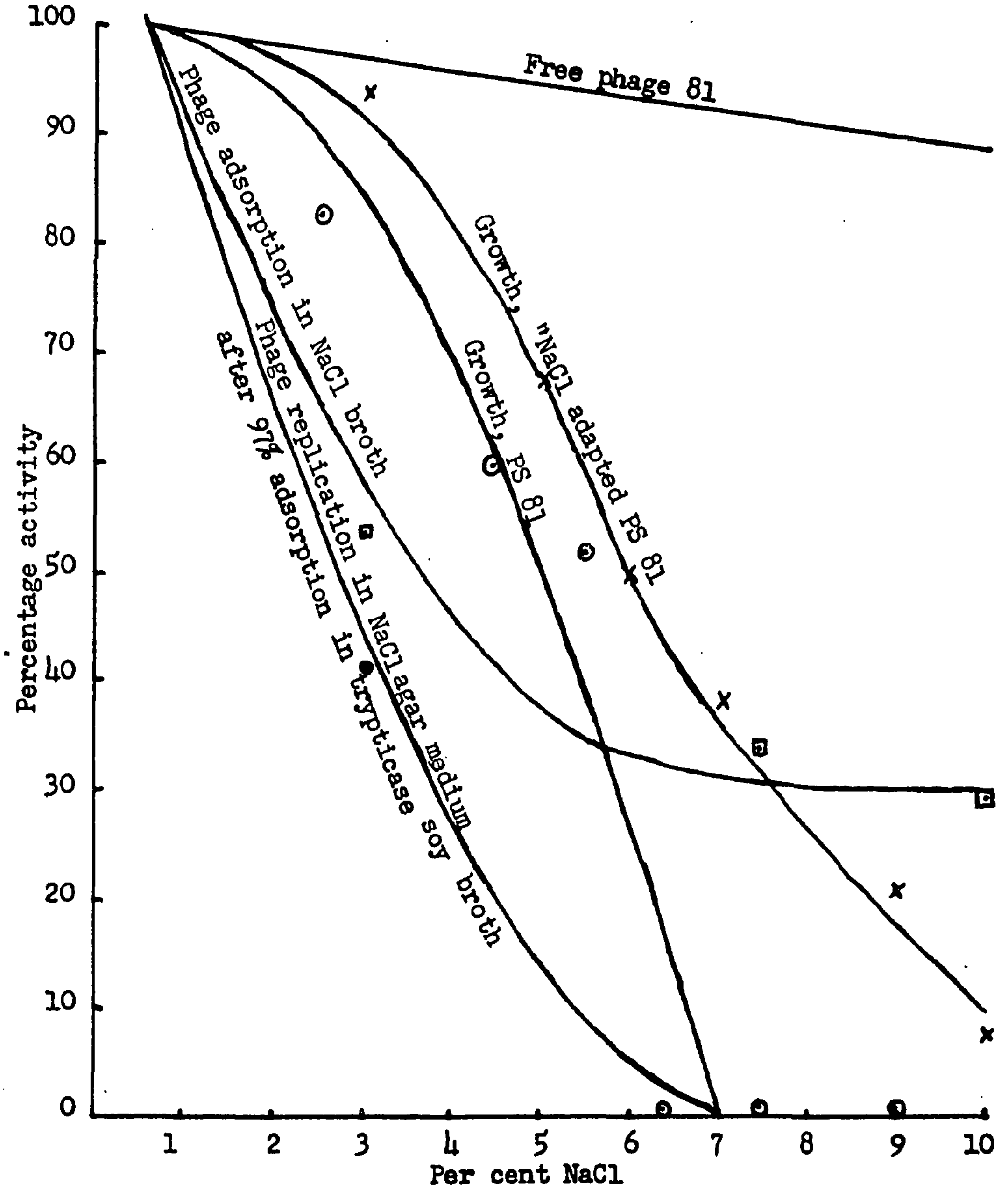

Figure 9. Comparison of percentage activity, in sodium chloride, of free and adsorbed phage 81 as well as growth of its propagating strain.

produce as many plaques as the cells which had not been grown on medium containing high concentrations of sodium chloride. The decrease in plaques in the case of the salt grown cells might be due to (1) decreased adsorption efficiency or (2) death or inactivation of many of the cells taken from the salt medium cultures. About 30 per cent of phage could not be accounted for when the cells which were grown on the medium containing high sodium chloride concentrations were examined (Table 41). This missing virus probably was adsorbed to dead or inactivated host cells which could not replicate the phage and thus produce plaques.

Effect of Sodium Chloride on Phage Susceptibility of Heat Shocked Cells

Propagating staphylococcus 29, 52A/79, 80, 81, 3A, 42B/47C, and 42D were cultivated at elevated temperatures and the lytic effect of selected phages (29, 52A, 79, 80, and 81) at increasing salt concentrations was observed (Table 42). Lytic or inhibitory reactions of phages were enhanced when the propagating staphylococcus had been grown at 47°C. After incubation at 45°C PS 29 was either lysed or its growth thinned (shadow reaction) to a greater extent on 0.5 per cent sodium chloride medium by phages 52A, 79, 80, and 81 than were the control cells which had been grown at 37°C. This was true also of phage 29 when the host organism was PS 52A/79. With PS 81 the effect was less pronounced but shadow activity by phage 52A was enhanced in the case of cells grown at elevated temperature. PS 3A, at 37°C, was not lysed or inhibited by phages 29, 52A, 79, 80, and 81 at low levels of sodium chloride, but was after growth of PS 3A cells at 45°C. No enhancement of lytic or inhibitory effects on salt media occurred with cells of PS 42B/47C when they were grown at 45°C. Lysis was enhanced by phage 81 when PS 42D had been grown at 45°C on 0.5 and 3 per cent sodium chloride medium. Conversely, the shadow reaction with phage 29 on the 3 and 7.5 per cent salt media disappeared after incubating PS 42D at 45°C.

TABLE 1

BACTERIOPHAGE PATTERNS OF PROPAGATING STAPHYLOCOCCUS 29 ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF SODIUM CHLORIDE

% NaCl	Phage Conc.	Phage 29	52	52A	79	80	81	3A	3B	3C	55	71	6	7	42B	42E	47	47C	75	77	83	42D
0.5	RTD	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3.0	RTD	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7.5	RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10.0	RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.5	1000 X RTD	4	S	-	S	S	S	-	-	-	-	-	-	-	S	S	S	S	-	-	-	-
3.0	1000 X RTD	4	S	S	S	S	S	-	-	-	-	S	-	S	S	S	S	S	-	-	-	-
7.5	1000 X RTD	S	S	S	S	S	S	-	-	-	-	S	-	S	S	S	S	S	S	-	-	-
10.0	1000 X RTD	S	S	-	-	S	S	-	S	-	-	-	-	S	S	-	S	S	S	-	-	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
± less than 20 plaques
S shadow (partial lysis or "inhibition")
- no reaction

TABLE 2

BACTERIOPHAGE PATTERNS OF PROPAGATING STAPHYLOCOCCUS 52 ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF SODIUM CHLORIDE

% NaCl	Phage Conc.	Phage 29	52	52A	79	80	81	3A	3B	3C	55	71	6	7	42B	42E	47	47C	75	77	83	42D
0.5	RTD	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3.0	RTD	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7.5	RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10.0	RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.5	1000 X RTD	-	4	S	S	S	S	-	-	-	-	-	-	-	S	S	S	S	-	-	-	-
3.0	1000 X RTD	-	4	S	-	S	-	-	S	-	-	S	-	S	-	-	S	S	S	-	-	-
7.5	1000 X RTD	-	S	-	-	S	-	-	S	-	-	-	-	S	-	-	S	S	S	-	-	-
10.0	1000 X RTD	-	-	-	-	-	-	-	S	-	-	-	-	S	-	-	S	S	S	-	-	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
± less than 20 plaques
S shadow (partial lysis or "inhibition")
\- no reaction

TABLE 3

BACTERIOPHAGE PATTERNS OF PROPAGATING STAPHYLOCOCCUS 52A/79 ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF SODIUM CHLORIDE

% NaCl	Phage Conc.	Phage 29	52	52A	79	80	81	3A	3B	3C	55	71	6	7	42B	42E	47	47C	75	77	83	42D
0.5		-	-	4	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3.0	RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7.5		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10.0		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.5		-	-	4	4	3	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
3.0	1000 x RTD	-	-	4	4	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-
7.5		-	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-
10.0		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
$\pm$ less than 20 plaques
S shadow (partial lysis or "inhibition")
\- no reaction

TABLE 4

BACTERIOPHAGE PATTERNS OF PROPAGATING STAPHYLOCOCCUS 80 ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF SODIUM CHLORIDE

% NaCl	Phage Conc.	Phage 29	52	52A	79	80	81	3A	3B	3C	55	71	6	7	42B	42E	47	47C	75	77	83	42D
0.5	RTD	-	3	-	-	4	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3.0		-	-	-	-	2	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7.5		-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10.0		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.5	1000 X RTD	-	4	-	-	4	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3.0		S	4	-	-	4	4	-	-	-	-	S	-	-	3	-	-	S	-	-	-	-
7.5		-	-	-	-	-	4	-	-	-	-	S	-	-	4	-	-	S	-	-	-	-
10.0		-	-	-	-	-	4	-	-	-	-	-	-	-	S	-	-	S	-	-	-	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
$\pm$ less than 20 plaques
S shadow (partial lysis or "inhibition")
\- no reaction

TABLE 5

BACTERIOPHAGE PATTERNS OF PROPAGATING STAPHYLOCOCCUS 81 ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF SODIUM CHLORIDE

% NaCl	Phage Conc.	Phage 29	52	52A	79	80	81	3A	3B	3C	55	71	6	7	42B	42E	47	47C	75	77	83	42D
0.5	RTD	-	3	-	-	4	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3.0	RTD	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7.5	RTD	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10.0	RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.5	1000 X RTD	-	4	-	-	4	4	-	-	-	-	-	-	-	2	-	-	S	-	-	-	-
3.0	1000 X RTD	S	4	S	S	4	4	-	-	-	-	S	-	-	3	-	S	S	-	-	-	-
7.5	1000 X RTD	S	S	-	-	S	4	-	S	-	-	S	-	-	S	-	-	S	-	-	-	-
10.0	1000 X RTD	-	-	-	-	-	4	-	-	-	-	-	-	-	S	-	-	S	-	-	-	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
± less than 20 plaques
S shadow (partial lysis or "inhibition")
\- no reaction

TABLE 6

BACTERIOPHAGE PATTERNS OF PROPAGATING STAPHYLOCOCCUS 3A ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF SODIUM CHLORIDE

% NaCl	Phage Conc.	Phage 29	52	52A	79	80	81	3A	3B	3C	55	71	6	7	42B	42E	47	47C	75	77	83	42D
0.5	RTD	-	-	-	-	-	-	4	3	3	-	-	-	-	-	-	-	-	-	-	-	-
3.0		-	-	-	-	-	-	3	3	3	-	-	-	-	-	-	-	-	-	-	-	-
7.5		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10.0		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.5	1000 X RTD	-	-	-	-	-	-	4	4	4	S	S	±	±	±	±	-	-	-	-	-	-
3.0		-	-	-	-	-	-	4	4	4	-	S	-	-	-	-	S	-	-	-	-	-
7.5		S	S	S	-	S	-	4	4	4	-	S	-	-	-	-	S	-	-	-	-	-
10.0		-	S	-	-	-	-	-	S	S	-	-	-	-	-	-	S	-	-	-	-	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
± less than 20 plaques
S shadow (partial lysis or "inhibition")
\- no reaction

TABLE 7

BACTERIOPHAGE PATTERNS OF PROPAGATING STAPHYLOCOCCUS 3B ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF SODIUM CHLORIDE

% NaCl	Phage Conc.	Phage 29	52	52A	79	80	81	3A	3B	3C	55	71	6	7	42B	42E	47	47C	75	77	83	42D
0.5	RTD	-	-	-	-	-	-	-	3	-	-	S	-	-	-	-	-	-	-	-	-	-
3.0		-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-
7.5		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10.0		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.5	1000 X RTD	-	-	-	-	-	-	-	4	4	S	S	-	-	-	-	-	-	-	-	-	-
3.0		S	-	-	-	-	-	-	4	S	-	S	-	-	-	-	-	-	-	-	-	-
7.5		S	S	S	S	S	-	-	S	-	-	3	-	-	-	-	-	-	-	-	-	-
10.0		-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
± less than 20 plaques
S shadow (partial lysis or "inhibition")
- no reaction

TABLE 8

BACTERIOPHAGE PATTERNS OF PROPAGATING STAPHYLOCOCCUS 3C ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF SODIUM CHLORIDE

% NaCl	Phage Conc.	Phage 29	52	52A	79	80	81	3A	3B	3C	55	71	6	7	42B	42E	47	47C	75	77	83	42D
0.5		-	-	-	-	-	-	-	4	4	2	3	-	-	-	-	-	-	-	-	-	-
3.0	RTD	-	-	-	-	-	-	-	4	4	-	2	-	-	-	-	-	-	-	-	-	-
7.5		-	-	-	-	-	-	-	3	3	-	-	-	-	-	-	-	-	-	-	-	-
10.0		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.5	1000 x RTD	-	-	-	-	-	-	3	4	4	4	3	-	-	±	±	-	-	-	-	-	-
3.0		S	S	S	S	S	-	3	4	4	S	4	-	-	-	-	-	-	-	-	-	-
7.5		S	S	-	S	S	-	S	4	4	-	S	-	-	-	-	S	-	-	-	-	-
10.0		-	-	-	-	-	-	-	4	4	-	-	-	-	-	-	S	-	-	-	-	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
± less than 20 plaques
S shadow (partial lysis or "inhibition")
- no reaction

TABLE 9

BACTERIOPHAGE PATTERNS OF PROPAGATING STAPHYLOCOCCUS 55 ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF SODIUM CHLORIDE

% NaCl	Phage Conc.	Phage 29	52	52A	79	80	81	3A	3B	3C	55	71	6	7	42B	42E	47	47C	75	77	83	42D
0.5	RTD	-	-	-	-	-	-	-	4	4	3	3	-	-	-	-	-	-	-	-	-	-
3.0	RTD	-	-	-	-	-	-	-	4	4	3	3	-	-	-	-	-	-	-	-	-	-
7.5	RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10.0	RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.5	1000 X RTD	-	-	-	-	-	-	-	4	4	4	3	-	-	-	-	-	-	-	-	-	-
3.0	1000 X RTD	S	S	S	S	S	S	-	4	4	S	4	-	-	-	-	S	-	-	-	-	-
7.5	1000 X RTD	S	S	S	S	S	-	-	4	4	-	S	-	S	-	-	S	-	S	-	-	-
10.0	1000 X RTD	-	S	S	-	-	-	-	S	S	-	-	-	S	-	-	S	-	-	-	-	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
$\pm$ less than 20 plaques
S shadow (partial lysis or "inhibition")
- no reaction

TABLE 10

BACTERIOPHAGE PATTERNS OF PROPAGATING STAPHYLOCOCCUS 71 ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF SODIUM CHLORIDE

% NaCl	Phage Conc.	Phage 29	52	52A	79	80	81	3A	3B	3C	55	71	6	7	42B	42E	47	47C	75	77	83	42D
0.5	RTD	-	-	-	-	-	-	-	3	3	2	4	-	-	-	-	-	-	-	-	-	-
3.0	RTD	-	-	-	-	-	-	-	3	1	2	4	-	-	-	-	-	-	-	-	-	-
7.5	RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10.0	RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.5	1000 X RTD	-	-	-	-	-	-	-	3	4	4	3	-	-	-	-	-	-	-	-	-	-
3.0	1000 X RTD	-	-	-	-	-	-	-	S	S	3	3	-	-	-	-	-	-	-	-	-	-
7.5	1000 X RTD	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-
10.0	1000 X RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
$\pm$ less than 20 plaques
S shadow (partial lysis or "inhibition")
\- no reaction

TABLE 11

BACTERIOPHAGE PATTERNS OF PROPAGATING STAPHYLOCOCCUS 6 ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF SODIUM CHLORIDE

% NaCl	Phage Conc.	29	52	52A	79	80	81	3A	3B	3C	55	71	6	7	42B	42E	47	47C	75	77	83	42D
		Phage																				
0.5	RTD	-	-	-	-	-	3	-	-	-	-	-	3	1	-	±	4	-	4	1	1	-
3.0	RTD	-	-	-	-	-	2	-	-	-	-	-	3	1	1	±	3	-	3	-	-	-
7.5	RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-
10.0	RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.5	1000 X RTD	-	S	S	-	S	4	-	-	-	-	-	4	3	4	4	4	S	4	S	4	S
3.0	1000 X RTD	-	-	-	-	-	4	-	-	-	-	S	4	3	4	4	4	2	4	1	3	-
7.5	1000 X RTD	-	-	-	-	-	3	-	-	-	-	-	3	S	S	S	4	S	4	-	-	-
10.0	1000 X RTD	-	-	-	-	-	-	-	-	-	-	-	3	S	-	-	4	S	4	-	-	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
± less than 20 plaques
S shadow (partial lysis or "inhibition")
- no reaction

TABLE 12

BACTERIOPHAGE PATTERNS OF PROPAGATING STAPHYLOCOCCUS 7 ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF SODIUM CHLORIDE

% NaCl	Phage Conc.	Phage 29	52	52A	79	80	81	3A	3B	3C	55	71	6	7	42B	42E	47	47C	75	77	83	42D
0.5	RTD	-	-	-	-	-	-	-	-	-	-	-	±	4	-	±	-	-	-	±	-	-
3.0		-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	±	-	-
7.5		-	-	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-
10.0		-	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	S	-	-	-
0.5	1000 X RTD	-	-	-	-	-	3	-	-	-	-	-	3	3	3	4	3	2	S	3	2	3
3.0		S	-	S	S	S	S	-	-	-	-	S	3	3	3	4	S	S	S	3	-	-
7.5		S	S	S	S	S	S	-	-	-	-	S	3	4	3	3	S	S	3	S	-	-
10.0		-	-	-	-	-	S	-	-	-	-	S	2	4	S	S	3	S	3	S	-	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
± less than 20 plaques
S shadow (partial lysis or "inhibition")
\- no reaction

TABLE 13

BACTERIOPHAGE PATTERNS OF PROPAGATING STAPHYLOCOCCUS 42B/47C ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF SODIUM CHLORIDE

		Phage																				
% NaCl	Phage Conc.	29	52	52A	79	80	81	3A	3B	3C	55	71	6	7	42B	42E	47	47C	75	77	83	42D
0.5	RTD	-	-	-	-	-	4	-	-	-	-	-	-	-	4	-	-	4	-	-	-	-
3.0		-	-	-	-	-	3	-	-	-	-	-	-	-	3	-	-	3	-	-	-	-
7.5		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10.0		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.5	1000 X RTD	-	-	-	-	S	4	-	-	-	-	-	-	-	4	1	1	4	-	-	-	-
3.0		-	S	-	S	S	4	-	-	-	-	S	-	-	4	-	-	4	-	-	-	-
7.5		-	-	-	-	-	3	-	-	-	-	S	-	-	-	-	-	3	-	-	-	-
10.0		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
$\pm$ less than 20 plaques
S shadow (partial lysis or "inhibition")
- no reaction

TABLE 14

BACTERIOPHAGE PATTERNS OF PROPAGATING STAPHYLOCOCCUS 42E ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF SODIUM CHLORIDE

% NaCl	Phage Conc.	Phage 29	52	52A	79	80	81	3A	3B	3C	55	71	6	7	42B	42E	47	47C	75	77	83	42D
0.5	RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-
3.0		-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-
7.5		-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-
10.0		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.5	1000 X RTD	-	-	-	-	-	4	-	-	-	-	-	-	-	1	4	-	-	-	-	-	-
3.0		S	S	S	S	S	3	-	-	-	-	S	-	-	-	4	S	-	-	-	-	-
7.5		S	S	S	S	S	S	-	-	-	-	S	-	S	-	4	S	-	-	-	-	-
10.0		-	S	-	-	-	-	-	-	-	-	-	-	2	-	4	S	-	-	-	-	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
± less than 20 plaques
S shadow (partial lysis or "inhibition")
\- no reaction

TABLE 15

BACTERIOPHAGE PATTERNS OF PROPAGATING STAPHYLOCOCCUS 54 ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF SODIUM CHLORIDE

% NaCl	Phage Conc.	Phage 29	52	52A	79	80	81	3A	3B	3C	55	71	6	7	42B	42E	47	47C	75	77	83	42D
0.5	RTD	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	3	±	3	2	2	-
3.0	RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	3	±	-	-
7.5	RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	±	-	-	-
10.0	RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.5	1000 X RTD	-	-	-	-	-	S	-	-	-	-	-	-	S	S	S	4	3	4	3	4	-
3.0	1000 X RTD	s	-	-	-	s	3	-	-	-	-	s	-	3	3	3	4	3	4	3	s	-
7.5	1000 X RTD	-	-	-	-	s	3	-	-	-	-	s	-	3	3	3	4	s	4	-	-	-
10.0	1000 X RTD	-	-	-	-	-	-	-	-	-	-	-	-	S	-	-	3	-	3	-	-	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
± less than 20 plaques
S shadow (partial lysis or "inhibition")
- no reaction

TABLE 16

BACTERIOPHAGE PATTERNS OF PROPAGATING STAPHYLOCOCCUS 75 ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF SODIUM CHLORIDE

% NaCl	Phage Conc.	Phage 29	52	52A	79	80	81	3A	3B	3C	55	71	6	7	42B	42E	47	47C	75	77	83	42D
0.5	RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	3	-	-
3.0	RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	2	-	-
7.5	RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10.0	RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.5	1000 X RTD	-	-	-	-	-	S	-	1	1	-	-	-	-	-	1	S	-	4	4	-	-
3.0	1000 X RTD	S	S	S	S	S	S	-	±	-	-	S	-	-	-	-	S	4	3	-	-	-
7.5	1000 X RTD	S	S	S	S	S	S	-	-	-	-	S	-	S	S	S	3	-	4	-	-	-
10.0	1000 X RTD	-	-	-	-	-	-	-	-	-	-	-	-	S	S	S	3	-	S	-	-	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
± less than 20 plaques
S shadow (partial lysis or "inhibition")
- no reaction

TABLE 17

BACTERIOPHAGE PATTERNS OF PROPAGATING STAPHYLOCOCCUS 77 ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF SODIUM CHLORIDE

% NaCl	Phage Conc.	Phage 29	52	52A	79	80	81	3A	3B	3C	55	71	6	7	42B	42E	47	47C	75	77	83	42D
0.5	RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-
3.0	RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	±	-	-
7.5	RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10.0	RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.5	1000 X RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	-	4	-	-	-
3.0	1000 X RTD	-	-	S	S	S	-	-	-	-	-	S	-	-	-	-	S	-	4	-	-	-
7.5	1000 X RTD	S	-	S	S	S	-	-	-	-	-	S	-	-	-	-	S	-	-	-	-	-
10.0	1000 X RTD	-	-	-	-	-	-	-	-	-	-	-	-	S	-	-	S	-	-	-	-	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
± less than 20 plaques
S shadow (partial lysis or "inhibition")
\- no reaction

TABLE 18

BACTERIOPHAGE PATTERNS OF PROPAGATING STAPHYLOCOCCUS 83 ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF SODIUM CHLORIDE

% NaCl	Phage Conc.	Phage 29	52	52A	79	80	81	3A	3B	3C	55	71	6	7	42B	42E	47	47C	75	77	83	42D
0.5	RTD	-	-	-	1	-	1	-	-	-	-	-	4	-	3	-	S	-	-	4	4	-
3.0		-	-	-	-	-	1	-	-	-	-	-		-			4					-
7.5		-	-	-	-	-	-	-	-	-	-	-		-			-					-
10.5		-	-	-	-	-	-	-	-	-	-	-		-			-					-
0.5	1000 X RTD	-	-	1	4	1	4	-	-	-	-	S		-			4					-
3.0		S	S	S	S	S	4	-	-	-	-	S		-			4					-
7.5		S	S	S	S	S	2	-	-	-	-	S		-			4					-
10.0		S	-	-	-	-	-	-	-	-	-	-		S			4					-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
± less than 20 plaques
S shadow (partial lysis or "inhibition")
\- no reaction

TABLE 19

BACTERIOPHAGE PATTERNS OF PROPAGATING STAPHYLOCOCCUS 42D ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF SODIUM CHLORIDE

% NaCl	Phage Conc.	Phage 29	52	52A	79	80	81	3A	3B	3C	55	71	6	7	42B	42E	47	47C	75	77	83	42D
0.5	RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4
3.0		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4
7.5		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10.0		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.5	1000 X RTD	-	-	-	S	-	S	-	-	-	-	-	-	-	-	-	2	-	-	-	-	4
3.0		S	S	S	S	S	S	-	-	-	-	S	-	S	-	-	S	-	-	-	-	S
7.5		S	S	S	S	S	S	-	S	-	-	S	-	S	-	-	S	S	S	-	-	-
10.0		-	S	-	-	S	-	-	-	-	-	-	-	S	-	-	S	S	S	S	-	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
± less than 20 plaques
S shadow (partial lysis or "inhibition")
\- no reaction

TABLE 20

BACTERIOPHAGE PATTERNS OF PROPAGATING STAPHYLOCOCCUS W ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF SODIUM CHLORIDE

% NaCl	Phage Conc.	Phage 29	52	52A	79	80	81	3A	3B	3C	55	71	6	7	42B	42E	47	47C	75	77	83	42D
0.5		-	3	-	-	4	4	-	-	-	-	-	-	-	±	-	-	-	-	-	-	-
3.0	RTD	-	1	-	-	2	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7.5		-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10.0		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.5	1000	-	4	-	-	4	4	-	-	-	-	-	-	-	3	-	-	S	-	-	-	-
3.0	X	-	4	-	-	4	4	-	-	-	-	S	-	-	3	-	S	S	S	-	-	-
7.5	RTD	-	S	-	-	S	4	-	-	-	-	S	-	-	4	-	S	S	-	-	-	-
10.0		-	-	-	-	-	3	-	-	-	-	-	-	-	3	-	S	S	-	-	-	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
± less than 20 plaques
S shadow (partial lysis or "inhibition")
- no reaction

TABLE 21

BACTERIOPHAGE PATTERNS OF PROPAGATING STAPHYLOCOCCUS E35 ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF SODIUM CHLORIDE

% NaCl	Phage Conc.	Phage 29	52	52A	79	80	81	3A	3B	3C	55	71	6	7	42B	42E	47	47C	75	77	83	42D
0.5	RTD	-	3	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3.0	RTD	-	3	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7.5	RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10.0	RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.5	1000 X RTD	-	4	-	-	4	4	-	-	-	-	-	-	-	±	-	-	S	-	-	-	-
3.0	1000 X RTD	S	4	S	S	4	S	-	-	-	-	S	-	-	1	-	S	S	-	-	-	-
7.5	1000 X RTD	S	S	S	S	S	-	-	-	-	-	S	-	-	-	-	S	S	-	-	-	-
10.0	1000 X RTD	S	S	S	S	S	-	-	-	-	-	S	-	S	-	-	S	S	-	-	-	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
± less than 20 plaques
S shadow (partial lysis or "inhibition")
\- no reaction

TABLE 22

BACTERIOPHAGE PATTERNS OF PROPAGATING STAPHYLOCOCCUS HV1 ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF SODIUM CHLORIDE

		Phage																				
% NaCl	Phage Conc.	29	52	52A	79	80	81	3A	3B	3C	55	71	6	7	42B	42E	47	47C	75	77	83	42D
0.5	RTD	-	2	-	-	4	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3.0		-	-	-	-	2	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7.5		-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10.0		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.5	1000 X RTD	-	4	-	-	4	4	-	-	-	-	-	-	-	2	-	-	S	-	-	-	-
3.0		-	4	-	-	4	4	-	-	-	-	S	-	-	3	-	-	S	-	-	-	-
7.5		-	S	-	-	S	4	-	-	-	-	S	-	-	4	-	-	3	S	-	-	-
10.0		-	-	-	-	-	4	-	-	-	-	-	-	-	3	-	-	S	-	-	-	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
± less than 20 plaques
S shadow (partial lysis or "inhibition")
\- no reaction

TABLE 23

BACTERIOPHAGE PATTERNS OF PROPAGATING STAPHYLOCOCCUS Sm ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF SODIUM CHLORIDE

% NaCl	Phage Conc.	Phage 29	52	52A	79	80	81	3A	3B	3C	55	71	6	7	42B	42E	47	47C	75	77	83	42D
0.5	RTD	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-
3.0	RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7.5	RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10.0	RTD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.5	1000 X RTD	S	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-
3.0	1000 X RTD	4	S	S	S	S	-	-	-	-	-	S	-	-	-	-	S	-	-	-	-	-
7.5	1000 X RTD	S	S	S	S	S	-	-	S	-	-	S	-	S	-	-	S	-	-	-	-	-
10.0	1000 X RTD	-	-	-	-	-	-	-	S	-	-	-	-	S	-	-	S	-	-	-	-	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
$\pm$ less than 20 plaques
S shadow (partial lysis or "inhibition")
\- no reaction

TABLE 24

BACTERIOPHAGE ACTIVITY OF SELECTED PHAGES ON STAPHYLOCOCCUS Sm ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF SODIUM CHLORIDE

% NaCl	Phage 29	52	52A	79	80	81	71	6	7	42B	42E	47	47C	75	42D
0.5	3	-	-	-	-	-	4	-	S	-	-	S	S	S	-
2.0	3	-	-	3	3	-	3								
3.0	4	3	3	3	3	-	3	-	S	-	-	S	-	S	-
4.0	3	3	3	3	3	-	3	-	S	-	-	3	-	S	-
5.0	3	3	3	3	3	-	3	-	1	-	-	3	-	S	-
6.0	3	3	3	3	3	-	3	-	2	-	-	3	-	S	-
7.5	3	3	3	3	3	-	3	-	1	-	-	3	-	S	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
$\pm$ less than 20 plaques
S shadow (partial lysis or "inhibition")
- no reaction

TABLE 25

BACTERIOPHAGE ACTIVITY OF SELECTED PHAGES ON PROPAGATING STAPHYLOCOCCUS 29 ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF CALCIUM CHLORIDE

	Phage															
%	RTD								1000 X RTD							
$CaCl_2$	29	52	52A	79	80	81	71	83	29	52	52A	79	80	81	71	83
TSA	4	-	-	-	-	-	-	-	4	3	S	3	3	3	-	-
0.1	4	-	-	-	-	-	-	-	4	3	S	3	3	3	S	-
0.2	4	-	-	-	-	-	-	-	4	3	S	3	3	3	S	-
0.3	4	-	-	-	-	-	-	-	4	3	S	3	3	3	-	-
0.4	4	-	-	-	-	-	-	-	4	3	S	3	3	3	-	-
0.5	4	-	-	-	-	-	-	-	4	3	S	3	3	3	-	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
± less than 20 plaques
S shadow (partial lysis or "inhibition")
- no reaction

TABLE 26

BACTERIOPHAGE ACTIVITY OF SELECTED PHAGES ON PROPAGATING STAPHYLOCOCCUS 52 ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF CALCIUM CHLORIDE

%	Phage															
	RTD								1000 X RTD							
$CaCl_2$	29	52	52A	79	80	81	71	83	29	52	52A	79	80	81	71	83
TSA	-	4	-	-	3	-	-	-	-	4	3	S	3	S	-	-
0.1	-	4	1	-	3	-	-	-	-	4	3	S	3	S	-	-
0.2	-	4	1	-	3	-	-	-	-	4	3	S	3	S	-	-
0.3	-	4	1	-	3	-	-	-	-	4	3	S	3	S	-	-
0.4	-	4	1	-	3	-	-	-	-	4	3	S	3	S	-	-
0.5	-	4	$\pm$	-	3	-	-	-	-	4	3	S	3	S	-	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
$\pm$ less than 20 plaques
S shadow (partial lysis or "inhibition")
\- no reaction

TABLE 27

BACTERIOPHAGE ACTIVITY OF SELECTED PHAGES ON PROPAGATING STAPHYLOCOCCUS 52A/79 ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF CALCIUM CHLORIDE

	Phage															
%	RTD								1000 X RTD							
$CaCl_2$	29	52	52A	79	80	81	71	83	29	52	52A	79	80	81	71	83
TSA	-	-	4	4	-	-	-	-	-	3	4	4	-	-	-	-
0.1	-	-	4	4	±	-	-	-	-	3	4	4	3	S	-	-
0.2	-	-	4	4	±	-	-	-	-	2	4	4	3	S	-	-
0.3	-	-	4	4	±	-	-	-	-	1	4	4	3	S	-	-
0.4	-	-	4	4	-	-	-	-	-	1	4	4	3	S	-	-
0.5	-	-	4	3	-	-	-	-	-	±	4	4	3	S	-	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
± less than 20 plaques
S shadow (partial lysis or "inhibition")
\- no reaction

TABLE 28

BACTERIOPHAGE ACTIVITY OF SELECTED PHAGES ON PROPAGATING STAPHYLOCOCCUS 80 ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF CALCIUM CHLORIDE

%	Phage															
	RTD								1000 X RTD							
$CaCl_2$	29	52	52A	79	80	81	71	83	29	52	52A	79	80	81	71	83
TSA	-	3	-	-	4	4	-	-	-	4	1	-	4	4	-	-
0.1	-	3	-	-	4	4	-	-	-	4	1	-	4	4	-	-
0.2	-	3	-	-	4	4	-	-	-	4	1	-	4	4	-	-
0.3	-	1	-	-	3	4	-	-	-	3	S	-	4	4	-	-
0.4	-	1	-	-	3	4	-	-	-	3	S	-	4	4	-	-
0.5	-	±	-	-	3	3	-	-	-	3	S	-	4	4	-	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
± less than 20 plaques
S shadow (partial lysis or "inhibition")
- no reaction

TABLE 29

BACTERIOPHAGE ACTIVITY OF SELECTED PHAGES ON PROPAGATING STAPHYLOCOCCUS 81 ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF CALCIUM CHLORIDE

	Phage															
%	RTD								1000 X RTD							
$CaCl_2$	29	52	52A	79	80	81	71	83	29	52	52A	79	80	81	71	83
TSA	-	4	-	-	4	4	-	-	-	4	S	-	4	4	-	-
0.1	-	3	-	-	4	4	-	-	-	4	S	-	4	4	-	-
0.2	-	3	-	-	4	4	-	-	-	3	S	-	4	4	-	-
0.3	-	2	-	-	3	4	-	-	-	3	S	-	4	4	-	-
0.4	-	1	-	-	3	3	-	-	-	3	S	-	4	4	-	-
0.5	-	-	-	-	3	3	-	-	-	3	S	-	4	4	-	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
± less than 20 plaques
S shadow (partial lysis or "inhibition")
\- no reaction

TABLE 30

BACTERIOPHAGE ACTIVITY OF SELECTED PHAGES ON PROPAGATING STAPHYLOCOCCUS 3A ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF CALCIUM CHLORIDE

	Phage															
%	RTD								1000 X RTD							
$CaCl_2$	29	52	52A	79	80	81	71	83	29	52	52A	79	80	81	71	83
TSA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	-
0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	-
0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	-
0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	-
0.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	-
0.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
$\pm$ less than 20 plaques
S shadow (partial lysis or "inhibition")
\- no reaction

TABLE 31

BACTERIOPHAGE ACTIVITY OF SELECTED PHAGES ON PROPAGATING STAPHYLOCOCCUS 3B ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF CALCIUM CHLORIDE

%	Phage															
	RTD								1000 X RTD							
$CaCl_2$	29	52	52A	79	80	81	71	83	29	52	52A	79	80	81	71	83
TSA	-	-	-	-	-	-	S	-	-	-	-	-	-	-	3	-
0.1	-	-	-	-	-	-	S	-	-	-	-	-	-	-	3	-
0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-
0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-
0.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-
0.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
$\pm$ less than 20 plaques
S shadow (partial lysis or "inhibition")
\- no reaction

TABLE 32

BACTERIOPHAGE ACTIVITY OF SELECTED PHAGES ON PROPAGATING STAPHYLOCOCCUS 3C ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF CALCIUM CHLORIDE

% $CaCl_2$	Phage RTD 29	52	52A	79	80	81	71	83	Phage 1000 X RTD 29	52	52A	79	80	81	71	83
TSA	-	-	-	-	-	-	3	-	-	-	-	-	-	±	3	-
0.1	-	-	-	-	-	-	3	-	-	-	-	-	-	-	3	-
0.2	-	-	-	-	-	-	3	-	-	-	-	-	-	-	3	-
0.3	-	-	-	-	-	-	3	-	-	-	-	-	-	-	3	-
0.4	-	-	-	-	-	-	3	-	-	-	-	-	-	-	3	-
0.5	-	-	-	-	-	-	3	-	-	-	-	-	-	-	3	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
± less than 20 plaques
S shadow (partial lysis or "inhibition")
- no reaction

TABLE 33

BACTERIOPHAGE ACTIVITY OF SELECTED PHAGES ON PROPAGATING STAPHYLOCOCCUS 55 ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF CALCIUM CHLORIDE

	Phage															
%	RTD								1000 X RTD							
$CaCl_2$	29	52	52A	79	80	81	71	83	29	52	52A	79	80	81	71	83
TSA	-	-	-	-	-	-	4	-	-	-	-	-	-	-	3	-
0.1	-	-	-	-	-	-	4	-	-	-	-	-	-	-	3	-
0.2	-	-	-	-	-	-	4	-	-	-	-	-	-	-	3	-
0.3	-	-	-	-	-	-	4	-	-	-	-	-	-	-	3	-
0.4	-	-	-	-	-	-	4	-	-	-	-	-	-	-	3	-
0.5	-	-	-	-	-	-	4	-	-	-	-	-	-	-	3	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
$\pm$ less than 20 plaques
S shadow (partial lysis or "inhibition")
\- no reaction

TABLE 34

BACTERIOPHAGE ACTIVITY OF SELECTED PHAGES ON PROPAGATING STAPHYLOCOCCUS 71 ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF CALCIUM CHLORIDE

	Phage															
%	RTD								1000 X RTD							
$CaCl_2$	29	52	52A	79	80	81	71	83	29	52	52A	79	80	81	71	83
TSA	-	-	-	-	-	-	4	-	-	-	-	-	-	-	3	-
0.1	-	-	-	-	-	-	3	-	-	-	-	-	-	-	3	-
0.2	-	-	-	-	-	-	3	-	-	-	-	-	-	-	3	-
0.3	-	-	-	-	-	-	3	-	-	-	-	-	-	-	3	-
0.4	-	-	-	-	-	-	3	-	-	-	-	-	-	-	3	-
0.5	-	-	-	-	-	-	S	-	-	-	-	-	-	-	3	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
$\pm$ less than 20 plaques
S shadow (partial lysis or "inhibition")
\- no reaction

TABLE 35

BACTERIOPHAGE ACTIVITY OF SELECTED PHAGES ON PROPAGATING STAPHYLOCOCCUS Sm ON TRYPTICASE SOY AGAR CONTAINING INCREASING CONCENTRATIONS OF CALCIUM CHLORIDE

	Phage															
%	RTD								1000 X RTD							
$CaCl_2$	29	52	52A	79	80	81	71	83	29	52	52A	79	80	81	71	83
TSA	±	-	-	-	-	-	1	-	3	-	-	-	-	-	4	-
0.1	±	-	-	-	-	-	1	-	3	S	-	-	S	S	3	-
0.2	±	-	-	-	-	-	1	-	3	-	-	-	S	S	3	-
0.3	-	-	-	-	-	-	±	-	2	-	-	-	-	S	3	-
0.4	-	-	-	-	-	-	-	-	S	-	-	-	-	S	3	-
0.5	-	-	-	-	-	-	-	-	S	-	-	-	-	-	3	-

TABLE 36

PERCENTAGE INACTIVATION OF STAPHYLOCOCCAL PHAGES AFTER 24 HOURS INCUBATION IN 0.5 PER CENT AND 10 PER CENT SODIUM CHLORIDE-TRYPTICASE SOY BROTH AT 30°C

		Percentage reduction in plaque count after 24 hr., 30°C		
Serological group	% NaCl in broth	Group mean	Most stable phage	Most stable phage
A (10 phages)	0.5	11	0	24
	10	13	0	33
B (9 phages)	0.5	17	0	59
	10	88	44	99
F (2 phages)	0.5	-	4	39
	10	-	8	58
L (1 phage)	0.5	-	67*	-
	10	-	72*	-

*Only one phage (187) studied.

TABLE 37

PLAQUE COUNTS OF TYPICAL SEROLOGICAL GROUP A PHAGES (42B AND 81) AND GROUP B PHAGES (29 AND 80) AFTER INITIAL DILUTION AND INCUBATION AT 30°C IN 0.5 PER CENT AND 10 PER CENT SODIUM CHLORIDE-TRYPTICASE SOY BROTH

	Number of Plaque Forming Units							
	Phage 42B		Phage 81		Phage 29		Phage 80	
Time (hr.)	0.5% NaCl broth	10% NaCl broth	0.5% NaCl broth	10% NaCl broth	0.5% NaCl broth	10% NaCl broth	0.5% NaCl broth	10% NaCl broth
0	250	247	137	137	188	178	203	179
4	247	260	128	141	171	135	142	43
10 - 12*	217	223	120	112	168	53	103	16

*All were 12 hour counts except those of Phage 81 which were made after 10 hours.

TABLE 38

REPLICATION OF PHAGE 81 IN INCREASING SODIUM CHLORIDE CONCENTRATIONS

	Plaque Forming Units						
% NaCl	0 hr.	2 hr.	4 hr.	6 hr.	12 hr.	24 hr.	30 hr.
0.5	256	++	+++	++++	++++	++++	++++
2.0	298	388	+++	++++	++++	++++	++++
3.0	235	253	++	+++	++++	++++	++++
4.0	298	372	++	+++	++++	++++	++++
5.0	223	268	313	++	+++	++++	++++
6.0	260	321	332	++	+++	++++	++++
7.0	222	247	216	265	+++	+++	+++
9.0	229	237	208	273	++	+++	+++
10.0	248	265	271	258	402	617	582

++++ confluent lysis
+++ semi-confluent lysis or confluent lysis with resistant colonies
++ more than 50 plaques

TABLE 39

THE EFFECT OF RAPID DILUTION, FROM 10 PER CENT SODIUM CHLORIDE TO DISTILLED WATER, ON PLAQUE FORMATION BY PHAGES 80 AND 81

	Phage 80		Phage 81	
Phage Dilution	Plaque Forming Units Control	Test	Plaque Forming Units Control	Test
10^{-2}	++++	++++	++++	++++
10^{-3}	++++	++++	++++	++++
10^{-4}	+++	+++	+++	+++
10^{-5}	++	++	++	++
10^{-6}	++	++	++	++
10^{-7}	156	152	150	146

++++ confluent lysis
+++ semi-confluent lysis or confluent lysis with resistant colonies
++ more than 50 plaques

TABLE 40

THE EFFECT OF VARYING CONCENTRATIONS OF SODIUM CHLORIDE UPON ADSORPTION AND REPLICATION BY BACTERIOPHAGE 81

Per Cent NaCl			Plaque Forming Units	
Adsorption in TSB	Replication in TSA	Percentage Adsorption after 20 min.	Suspension before Centrifugation	Supernatant after Centrifugation
0.5	0.5	97	301	20
	3.0	97	124	1
	7.5	97	0	0
	10.0	97	0	0
0.5	0.5	99	240	3
3.0	3.0	53	103	1
7.5	7.5	34	0	0
10.0	10.0	29	0	0
0.5	0.5	99	126	1
3.0		53	129	55
7.5		34	132	87
10.0		29	118	84

TSB trypticase soy broth
TSA trypticase soy agar

TABLE 41

THE EFFECT OF WASHING CELLS ON PLAQUE FORMATION

	Plaque Forming Units			
Organism	Unwashed Cells	Washed Cells	Supernatant Unwashed Cells	Supernatant Washed Cells
PS 81	876	809	3	3
PS 81 Adapted to 10% NaCl	583	571	33	9

TABLE 42

THE EFFECT OF ELEVATED TEMPERATURE ON HOST CELL SUSCEPTIBILITY TO BACTERIOPHAGE

Staph. Strain No.	% NaCl	Inoculum grown at 37°C					Inoculum grown at 45°C				
		Phage					Phage				
		29	52A	79	80	81	29	52A	79	80	81
PS 29	0.5	4	-	S	S	S	4	3	3	3	3
	3.0	4	S	S	S	S	3	S	S	S	S
	7.5	S	S	S	S	S	S	S	S	S	S
PS 52A/79	0.5	-	4	4	3	-	3	4	3	3	-
	3.0	-	4	4	-	-	-	3	3	S	-
	7.5	-	-	-	-	-	-	S	-	-	-
PS 80	0.5	-	-	-	4	4	-	S	-	4	4
	3.0	S	-	-	4	4	-	S	-	4	4
	7.5	-	-	-	-	4	S	S	-	S	4
PS 81	0.5	-	-	-	4	4	-	S	-	3	3
	3.0	S	S	S	4	4	-	S	-	3	3
	7.5	S	-	-	S	4	-	S	S	S	4

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
S shadow (partial lysis or "inhibition")
- no reaction

TABLE 42—Continued

Staph. Strain No.	% NaCl	Inoculum grown at 37°C					Inoculum grown at 45°C				
		Phage					Phage				
		29	52A	79	80	81	29	52A	79	80	81
PS 3A	0.5	-	-	-	-	-	1	S	S	-	2
	3.0	-	-	-	-	-	-	S	S	S	3
	7.5	S	S	-	S	-	S	S	S	S	1
PS 42B/47C	0.5	-	-	-	S	4	-	-	-	S	4
	3.0	-	-	S	S	4	-	-	-	S	4
	7.5	-	-	-	-	3	-	-	-	-	-
PS 42D	0.5	-	-	S	-	S	-	-	S	-	3
	3.0	S	S	S	S	S	-	S	S	S	3
	7.5	S	S	S	S	S	-	S	S	S	-

4 confluent lysis
3 semi-confluent lysis or confluent lysis with resistant colonies
2 more than 50 plaques
1 20 to 50 plaques
S shadow (partial lysis or "inhibition")
- no reaction

CHAPTER IV

DISCUSSION

Relationship of Sodium Chloride Tolerance to Serological Groups of Staphylococcal Phages

In addition to distinctive antigenicity, serological groups of staphylococcal phages are characterized by certain other properties, including host range, plaque size, ability to form lysogenic systems, and stability (Rippon, 1956). Seto *et al*. (1956) made electron micrographs of serological group A and B staphylophages and found that group A phages were large particles with elliptically contoured flattened heads and long tails; group B phages were much smaller particles with circular or polygonally shaped heads with long tails. The tails of both groups had a pronounced terminal bob. Rountree (1949) pointed out the relative instability of serological group B phages, particularly their marked sensitivity to heat and storage at 4°C, compared to that of the group A and F phages. In the first serological investigation of staphylococcal phages, Burnet and Lush (1935) observed that these phages could be divided into two categories, citrate-sensitive and -insensitive. Elek (1959), in his review of the bacteriophages, states that the resistance of free phage is unrelated to that of the host; gram-negative bacilli are killed by concentrations of chloroform, toluene, and other agents which had no effect on their bacteriophages. The staphylococcal phages were found to vary among themselves, however, in their resistance to chloroform (Liegeois-Muller and Fredericq, 1952). Rountree (1951) reported that most staphylococcal phages, when in the free state, were inactivated to some degree by 1 per cent sodium citrate and those most completely inactivated belonged to serological group B. In the same study, which was concerned chiefly with the effect of electrolytes on adsorption, evidence was presented that each phage has a characteristic calcium requirement. Sodium citrate inhibited adsorption of all but two of the phages examined and this effect was attributed to the action of the citrate anion which, it was suggested, bound the calcium on the surface of the phage and thus, blocked the adsorption site. In general, serological group A staphylococcal phages have only a partial calcium requirement; whereas in the case of serological group B phages, calcium is an absolute requirement for propagation (Blair and Williams, 1961).

In the present study it was found that the free staphylococcal phages which were least stable in 10 per cent sodium chloride-trypticase soy broth were also those of serological group B, whereas serological group A phages were stable at these same salt levels. The marked reduction in the activity of serological group B phages might be due to the effect of the sodium chloride on free phage prior to contact with the staphylococci or, possibly, due to failure of the phages to adsorb to cells in the presence of sodium chloride introduced with the phage-salt broth suspension to the phage-cell mixture. If inhibition of adsorption, as proposed by the latter explanation, were solely responsible, equal reduction in plaque count should occur in the initial sample and the 24 hour sample which were both taken from the 10 per cent salt broth. Mixing of 0.1 ml of this broth with an equal volume of the broth culture of the propagating strain of staphylococcus, followed by immediate addition of 3 ml. soft agar, quickly diluted the concentration of sodium chloride to about 0.8 per cent. The quantity of sodium chloride was further reduced on plating in the soft agar. Rountree (1951) found that in the case of the citrate-sensitive phages, adsorption in the presence of sodium chloride was variable. When it occurred, recovery of active phage from the cells infected in the presence of up to 1000 μg. per ml. of sodium chloride was less than from cells infected in the presence of up to 400 μg. per ml. of calcium chloride. These conclusions were based on experiments in which phages and cells were held 20 minutes in aqueous solutions of sodium chloride at lower concentrations than those in the phage cell mixtures tested here. The present study indicates that the most unstable phages of group B lyse susceptible staphylococci when 1 per cent sodium chloride-trypticase soy agar is employed in the routine typing procedure.

If inactivation of free phage occurs in the salt broth prior to mixing with the staphylococcal cells, one would expect a continuing decrease in the number of plaque-forming units with increase in time. This was observed to occur. In the case of group B phages there was an immediate reduction, followed by progressive decrease in plaque count until, at the end of 24 hours, 12 per cent or less of the original activity remained. Group A phages showed little, if any, decrease in activity on initial contact with 10 per cent sodium chloride-broth; when activity was reduced after 24 hours the plaque counts made at 4 and 10 to 12 hour intervals reflected a decrease in plaque forming ability. The inactivation of staphylococcal phages observed here appears to be an effect of sodium chloride on free phage.

The sodium chloride instability of serological group B staphylococcal phages observed in this study parallels their requirement for calcium. Two phages other than those of serological group B which have an absolute calcium requirement are 47C (group A) and 187 (group L) Blair and Williams,(1961). In the present study phage 47C proved to be the most sensitive to sodium chloride of all the group A phages tested, but it was much more salt stable than any of the other group B phages. The salt tolerance of phage 187 and of the exceptional group B phage 83 was intermediate between those of the average group A and group B phages, but they were almost as unstable in 0.5 per cent sodium chloride-broth as in 10 per cent sodium chloride-broth. In general, inactivation in the higher concentration of sodium chloride was independent of stability in regular broth. Most phages retained their activity in 0.5 per cent sodium chloride-broth, although there were individual differences within each group. No phage was more stable in 10 per cent sodium chloride-broth than in 0.5 per cent sodium chloride-broth.

Sodium chloride tolerance appears to be a characteristic of certain staphylococcal phages, particularly those of serological group A, and this property may be useful in presumptive identification of serological group or in other classification schemes. This property may also serve as an aid in isolating new group A phages from mixtures that occur following natural or artificial induction of staphylococci.

Inhibition, Adsorption, and Replication of Staphylococcal Phages and Inhibition of Host Cells by Phage at Elevated Sodium Chloride Levels

The unusual lytic or inhibitory reactions of 1000 X RTD phage on propagating staphylococci may be related to the presence of inhibitors in concentrated phage filtrates. Some early reports of the phage inhibiting effect of bacterial extracts were those of Freeman (1937) and Rountree (1947). Hood and Lowbury (1954) reported that an inhibitor of phage propagation which was found in undiluted staphylococcal filtrates was of the same order of size as the lytic phage but more heat labile than the phage. Williams and Rippon (1952) reported that high titer phage filtrates contain, in addition to phage, an agent which inhibits growth of staphylococci. This agent was not identical with autolysin, it could not be removed by Gradacol and Seitz filters, and it could not be detected in filtrates from which phage was removed by adsorption to heat killed cocci, nor when phage had been neutralized by antiserum.

The distinction between inhibition of bacterial growth and true lysis of staphylococci is not always a clear one. Blair and Williams (1961) differentiate the two by suggesting that titration of the inhibited strain will not produce discrete plaques at any dilution whereas true lysis will produce discrete plaques. In the same paper, in a footnote to a table, two examples were reported where true lysis and "inhibition" vary from time to time. They reported that discrete plaques may appear on one occasion and growth inhibition at another time.

In the present study some phages, at 1000 X RTD, gave no degree of lysis or any other observable reaction on routine typing medium, but showed degrees of lysis or of a reaction usually interpreted as inhibition of growth on typing medium supplimented with increasing amounts of sodium chloride. Burnet and McKie (1930) pointed out that a balance of sodium and calcium cations was required for phage activity, with the Na/Ca ratio being about 20/1 for optimal activity. Cherry and Watson (1949) found that lysis of Streptococcus lactis cells by homologous phage was stimulated by sodium chloride, but the levels used were 0.005 M to 0.05 M. Delbrück (1940) found that adsorption varied with the physiological state of the bacterium. However, others (Puck et al., 1951; Puck and Sagik, 1953) believed that electrolytes primarily affect the phage in its ability to adsorb to host cells. The adsorption rate of phage to ion exchangers or to glass was essentially the same as to host cells and could be varied by altering the electrolyte concentration (Puck and Sagik, 1953). It has been suggested that for a particular phage-host cell system there exists an optimal salt concentration at which the rate of attachment is maximal. A large proportion of the phages examined by various investigators have a specific calcium requirement for adsorption and, in some cases, calcium is apparently essential at some step after adsorption. Although Rountree (1951) showed that divalent cations were required for adsorption of staphylophages and that monovalent cations were not efficient in this respect, she did not use levels of monovalent salts, including sodium chloride, in a concentration greater than 1000 µg. per ml. Hershey et al. (1944) reported that the relative efficiency of plating (EOP) of phage T2 varied with the concentration of electrolyte. The EOP values ranged from 0.001 or less in the absence of added electrolyte to 1.0 in the presence of 0.2 M sodium ions. Similar results were obtained with other monovalent cations, although the EOP was independent of the nature of anions used. Puck and Tolmach (1954) found that attachment of phage to cell proceeded normally and specifically in the presence of sufficient complexing agent to bind any multivalent ions; thus the possibility that divalent salts were needed to act as bridges between virus and host cell was eliminated. It was found that phage T2 would attach readily to E. coli B even in the presence of 1.2 M sodium chloride. Rakieten and Rakieten (1937) and Rakieten and Tiffany (1938) pointed out that two polyvalent staphylococcal phages, designated by them as M-13 and M-16, will adsorb nonspecifically to some strains of Bacillus subtilis and enterococci. In the present study nonspecific adsorption of staphylococcal phages and simultaneous lytic enzyme induction with no phage release may account for some of the so-called "inhibition" reactions, but the specificity of the reactions, plus the appearance of plaques at concentrations of sodium chloride not normally encountered in routine typing, suggests that the shadow reactions and occasional phage plaques observed are due to specific lysis. This does not rule out the possibility that growth inhibition of the host cells is due to specific adsorption by phage or to an inhibitor present in concentrated phage filtrates.

A phage induced enzyme, designated virolysin, which is capable of lysing staphylococcal cells has been described (Ralston _et al_., 1955; Ralston _et al_., 1957a and b). Virolysin could be differentiated from staphylococcal autolysin on the basis of serological and operational criteria. Both virolysin and autolysin proved to be proteins, behaved as enzymes, and appeared to act on the bacterial cell wall. Both enzymes lysed heat-killed cells but could not attack viable log phase cells. If a sensitization step occurred, then log phase cells could also be lysed. Sensitization could be accomplished by the adsorption of five viable or ultraviolet-inactivated phage particles per cell. Sensitization was independent of temperature between 4°C and 37°C. Penicillin was also reported as capable of sensitizing cells. From the present study it seems reasonable that other means of sensitization of cells is possible, including perhaps high levels of sodium chloride.

Enzymes which are produced as a result of phage-host cell interactions and which are capable of acting on cell surfaces have been observed in other systems (Adams and Park, 1956; Park, 1956; Murphy, 1957; and Richmond, 1959). Groman (1961) interprets lysis in the following way. Virolysin is released each time the host cell releases phage. Virolysin will not attack other cells unless there is sufficient free phage present for sensitization. Thus, observations in which either phage-resistant or -sensitive cells are lysed secondarily become understandable on the basis of either the action of virolysin alone or in combination with phage. A cell may be sensitized by steps associated with adsorption or penetration or both. In some cases, sensitization may not be required or may occur by other means. Sensitization appears to involve an alteration of the cell wall, although the exact mechanism of action is unknown.

Delbrück (1940) showed that "lysis from without" occurred with phage-cell mixtures if the phage-host cell ratio was about 100/1. Andrewes and Elford (1932) observed that 95 per cent killing of E. coli occurred when 10^7 or more phage particles were in the presence of 10^3 to 10^7 cells. The greater the number of E. coli cells present, the more phage was required for the killing effect. These observations were confirmed by Cohen and Anderson (1946). Beumer-Jochmans (1953) reported that some phages were bacteriostatic for certain staphylococci after adsorption, although they were not lytic. A phage which was lytic for a certain strain of staphylococcus at 37°C was lethal when adsorbed at 44°C. Elek (1959) believes that death of host cells, and hence inhibition of growth, may result from the adsorption of a single phage particle to host cells although the phage cannot propagate in the host cell. Cell autolysis, which is independent of lysis by phage, might follow this killing effect. Inhibition of growth, due to the killing action of phage, is believed to render the use of undiluted phage impracticable in the phage typing of staphylococci. The present study indicates that the contrary may be true.

Puck (1954), using coli phage, regarded lysis from without as an unchecked and hence destructive operation of a lytic mechanism which, in limited form, is part of the normal sequence of virus penetration. When normal invasion occurred this lytic reaction was controlled somehow, but the leakage controlling mechanism might be inhibited by an overwhelming dose of virus particles to the same cell. Puck and Lee (1955) reported that no leakage occurred at low electrolyte concentrations where penetration did not occur following attachment of phage to host cells. If the sodium ion concentration were raised to 0.1 M, leakage increased progressively. The maximum concentration of sodium ion used in their study was 0.1 M (about 0.6 per cent sodium chloride). They also found that potassium was far less efficient than sodium in promoting leakage. In the presence of the divalent cation, magnesium, leakage was diminished. Leakage was greater in older stored cells than in cells in the log phase of growth. If cells in the present study were physiologically older due to the high salt concentrations then this could explain the enhanced activity of phage.

The earliest observation that viable staphylococci could be recovered from high levels of sodium chloride was made by Martens (1888)*. Hill and White (1929) and Koch (1942) used this information to devise selective media for the separation of the staphylococci from certain contaminating bacteria. Christian and Waltho (1961) found that sodium chloride tolerance of staphylococci was dependent upon the ability of the cells to concentrate potassium internally. Fong and Kreuger (1949) found that after three hours incubation at 42°C, in either plain broth or in broth containing 1 M sodium chloride, the total staphylococcal count was increased from 1 x 10^8 cells per ml. to 6.5 x 10^8 cells per ml.; on the other hand the viable count in plain broth at the end of three hours was 55 per cent of the total count. The viability of

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*<u>In</u> Hill, J. H. and White, E. C. (1929) J. Bacteriol., 18, 53-57.

the cells grown in 1 M sodium chloride was only 33 per cent of the total count. This finding, plus evidence in the present study on growth reduction in increasing levels of sodium chloride, lends support to the idea that concentrated phage acts on damaged staphylococcal cells by enhancing lysis or enhancing growth inhibition.

Fong and Krueger (1949) also observed that 1 M sodium chloride interfered with adsorption of phage K to the propagating strain of staphylococcus. More definitive kinetic studies of electrolytes on staphylophage adsorption were made by Rountree (1951, 1955). In the presence of sodium chloride concentrations greater than 1000 μg. per ml., phage was either inactivated or failed to adsorb to its host. Her 1955 studies showed that, depending upon the phage, latent periods varied from 35 to 60 minutes, with lysis completed at 55 to 75 minutes. The burst size for the 29 phages ranged from 10 to 140 active phage particles.

Similar observations were made in the present study in the case of the PS 81-phage 81 system. Phage adsorption diminished progressively as the sodium chloride level was increased to 10 per cent concentration. Although adsorption was diminished at high salt levels it obviously did not cease. The finding that PS 81 replicates phage 81 in the presence of 10 per cent sodium chloride has not been reported previously. Insofar as could be determined from a search of the literature, no other phage-cell systems have been reported in which phage replication occurs at such high salt levels, although several such systems were observed in the present study. The delay in time of lysis of salt grown cells might be explained on the basis of decreased phage adsorption at high levels of sodium chloride. This decreased adsorptive ability may be overcome with time. An alternative hypothesis is that reduced metabolic activity of cells growing in high sodium chloride concentrations will not permit phage replication to occur at a rate comparable to that of control cells which are growing in 0.5 per cent sodium chloride medium. The reduction in colony count and the size of the colonies formed in high salt media, when compared with similar colonies grown at lower salt concentrations, suggests that these uninfected bacteria metabolize at different rates. Decreased adsorptive ability and reduced metabolic activity probably contribute to the delay in time of lysis of cells in the presence of high concentrations of sodium chloride.

If a phage enzyme which affects lysis reacts better at higher salt concentrations than specificity of adsorption, this fact, coupled with high multiplicities of infection, would explain the unusual lytic reactions of host cells at high levels of sodium chloride. Weidel (1958) concludes that lytic enzyme comes from the phage and not from the cell as most other workers propose. He believes that lysis from without occurs when damage to the cell wall happens as a result of a comparatively large dose of phage enzyme activity which acts faster than the ability of the cell to repair that damage. He concludes further that repair, if it can be done at all, does not always reconstitute the original cell wall structure in every detail. In the case of multiply infected cells it is evident that holes caused by the phage enzyme can stay relatively small only as long as the areas attacked are still relatively far apart. When the zones of enzyme action approach one another too closely disproportionately large pieces of the cell wall suddenly come loose, and this results inevitably in complete breakdown of cell structure. The foregoing hypothesis was given as an alternate explanation of the observations of Puck and Lee (1955) relating to leakage. The latter authors had found the following sequence to occur when cells were multiply infected. First there was leakage of cytoplasmic constituents of low molecular weight, followed by constituents of high molecular weight, and eventually lysis. Weidel (1958) believes that normal lysis is caused by exces- phage enzyme produced during phage synthesis. He believes that induction of lysogenic strains with resultant enzyme supports this view in that if the cells are to be lysed then free lytic enzyme is produced which is presumably phage enzyme. He concludes that in spite of the vast literature on the subject of attachment and penetration the details are still largely speculative. The latter conclusion is appropriate for the present study relating to unusual lytic effects in the presence of high salt media.

CHAPTER V

SUMMARY

The stability of staphylococcal phages in the presence of high sodium chloride concentrations was found to be related to serological group. Twenty-two staphylococcal bacteriophages representing serological groups A, B, F, and L were examined for activity after incubation of free phage in 10 per cent sodium chloride-trypticase soy broth for 24 hours at 30°C. The mean reduction in plaque forming units for 10 phages of serological group A was 13 per cent; in no instance was inactivation of any group A phage greater than 33 per cent. All but one of nine group B phages were 88 per cent or more inactive after incubation in 10 per cent sodium chloride-broth; the single exception was phage 83 with slightly more than 40 per cent inactivation in both 0.5 per cent and 10 per cent sodium chloride-broth. Serological group F phage 42D resembled phages of group A, whereas phage 77 of group F and phage 187 of group L were intermediate between group A and B phages with respect to sodium chloride stability.

Some unusual lytic and inhibitory effects of staphylococcal phages in the presence of high concentrations of sodium chloride were examined. Twenty-one phages were tested by typing their propagating strains of staphylococcus in media containing increasing concentrations of sodium chloride. At RTD the only phage showing lytic activity above 0.5 per cent sodium chloride was phage 81. At 1000 X RTD certain additional effects were noted. Certain phages which gave confluent lysis on standard typing medium containing 0.5 per cent sodium chloride were reduced in activity to what was called a 3+ shadow lysis when the sodium chloride concentration was raised to 3 per cent. This type of activity on standard typing medium is usually referred to as inhibition of growth. As the salt concentration was increased the phage activity decreased. As an example of another kind of behavior, phage 42B gave numerous plaques on standard medium but, as the sodium chloride concentration was increased to 3 per cent the lytic activity increased to semi-confluent lysis. Above this salt concentration phage activity again diminished. In other instances phages which showed no activity against host cells on standard typing medium were enhanced in lytic or inhibitory activity as the sodium chloride level was increased. At 1000 X RTD phage 81 and certain other phages lysed their respective propagating strain of staphylococcus in media containing from 0.5 per cent through 10 per cent sodium chloride.

The activity of phage 81 on its propagating strain of staphylococcus, PS 81, was examined in greater detail. Free phage 81 had been shown to be unharmed by incubation in 10 per cent salt broth for 24 hours. Growth of PS 81, however, was steadily inhibited by increasing concentrations of sodium chloride. Sodium chloride-adapted PS 81, although better prepared for growth in high salt media than its nonadapted counterpart, was also steadily inactivated as it was exposed to increasingly higher sodium chloride concentrations. As the sodium chloride content of the medium was increased the ability of phage 81 to adsorb to its host cells decreased sharply until the level of adsorption reached roughly 30 per cent in 7 per cent sodium chloride medium where it remained constant to 10 per cent sodium chloride levels.

Samples taken at intervals from liquid media containing increasing amounts of sodium chloride indicated that phage 81 replication occurred in the presence of 10 per cent salt, although at this salt level the amount of phage produced was greatly diminished. When phages 80 and 81 were subjected to osmotic shock by rapid dilution of the high salt-cell suspension with distilled water their plaque forming ability was not affected.. The observation that plaque formation was less on washed and unwashed salt-adapted cells grown in 0.5 per cent sodium chloride medium permits the conclusion that replication of phage 81, although diminished, continues in the presence of 10 per cent sodium chloride. One explanation is that phage 81 is adsorbed to staphylococcal cells which are so altered physiologically that only a few plaque forming units per cell are produced.

Growing host cells at 45°C was found to increase the susceptibility of these cells to phage lytic activity.

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