THE IN VIVO INTERACTION BETWEEN STAPHYLOCOCCUS BACTERIOPHAGE AND STAPHYLOCOCCUS AUREUS*

By P. F. BARTELL, PH.D., I. S. THIND, M.D., T. ORR, AND W. S. BLAKEMORE, M.D.

(From the Harrison Department of Surgical Research, School of Medicine, University of Pennsylvania, Philadelphia)

(Received for publication, March 14, 1963)

The use of bacteriophage lysates in the treatment of staphylococcal infection has remained a controversial subject for many years. One major difficulty in the acceptance of such rationale has been the failure to demonstrate *in vivo* interaction between bacteriophage and infecting bacterial cell.

The studies to be described in this communication were undertaken to ascertain whether or not *Staphylococcus aureus* 80/81 and bacteriophage 81 were capable of *in vivo* interaction and to observe the effects of, and to define certain factors which influence this interaction.

Methods

Mice.—Swiss mice of the Huntingdon Farm strain were employed and at time of use weighed 14 to 16 gm. They were housed 10 to a cage and fed Purina mouse chow and water ad libitum.

Staphylococci.—The strain of *Staphylococcus aureus*, phage Type 80/81, employed in these studies was originally isolated from a wound infection. It was coagulase-positive, produced alpha and beta hemolysins, fermented mannitol, and was resistant to penicillin, streptomycin, erythromycin, and tetracycline. Stock suspensions of this bacterium were prepared from 18-hour growth on trypticase soy agar (BBL) in Roux bottles. The growth from each bottle was harvested in 10 ml broth, placed into ampules, glass sealed, and stored at −20°C. Sufficient quantities were prepared to provide material for several experiments, and over a period of 8 to 12 weeks the viable count showed little change. Infectivity titrations were correspondingly reliable. Infection of 14 to 16 gm mice was accomplished by the intraperitoneal injection of 0.2 ml containing 1.5×10^9 viable cells suspended in 5 per cent gastric mucin (Wilson granular mucin, type 1701-W). This inoculum was invariably lethal to 100 per cent of the untreated mice within a 24 hour period; therefore, the 7 to 10 day observation proved to be more than adequate. Certain experiments, however, were observed as long as 14 days for comparative purposes.

Bacteriophage.—The staphylococcus bacteriophage 81 employed in these studies was received from the Communicable Disease Center, Chamblee, Georgia. Lysates were prepared employing the above described *S. aureus* 80/81 as the propagating strain, in 1 liter Erlenmeyer flasks containing 200 ml 4 per cent peptone (Difco Laboratories, Inc., Detroit). Each flask was seeded with a phage to bacteria ratio of approximately 1:5. The suspensions were

* This investigation was supported by research grant E-2385, from the National Institute of Allergy and Infectious Diseases, Public Health Service.

placed on a reciprocating shaker at low speed and maintained at room temperature. The turbid suspensions gradually cleared within 4 to 6 hours and held overnight at 4°C before centrifugation at 1230 RCF for 30 minutes followed by filtration through Seitz EK filters. Such lysates, containing approximately 2 to 4 × 10^{10} PFU/ml (plaque-forming units per ml), were placed into ampules, glass sealed, and stored at −20°C.

Bacteriophage titrations were performed by the agar layer technique as described by Adams (1). All titrations were performed, at least, in duplicate. Circulating phage levels were established by bleeding groups of mice from the tail at certain intervals of time. Blood was collected in cold heparinized saline (50 u/ml), and centrifuged at 1230 RCF for 10 minutes. Supernates were titrated as described. Peritoneal washings were obtained by rinsing the peritoneal cavity

TABLE I

The Specific Protective Activity of Staphylococcus Bacteriophage 81 on Staphylococcus aureus 80/81 Infection in Mice

Inocula*	No. mice	Survivals
		per cent
S. aureus 80/81 in 5 per cent mucin + bacteriophage 81	248	81
S. aureus 80/81 in 5 per cent mucin + bacteriophage 3b	40	0
S. aureus 80/81 in 5 per cent mucin + bacteriophage 81 (inactivated)‡	40	0
S. aureus 80/81 in 5 per cent mucin + 4 per cent peptone broth	160	0
S. aureus 80/81 in 5 per cent mucin + saline	40	0
5 per cent mucin + bacteriophage 81	40	100
5 per cent mucin + bacteriophage 3b	40	100
S. aureus 44A in 5 per cent mucin + bacteriophage 81	40	0
S. aureus 44A in 5 per cent mucin + 4 per cent peptone broth	40	0
S. aureus 80/81 in TSB§ + bacteriophage 81	30	93
S. aureus 80/81 in TSB + 4 per cent peptone broth	30	20

* Viable bacteria (1.5 × 10^9) injected immediately before bacteriophage (1.5 × 10^9 PFU). All inoculations 0.2 ml, intraperitoneally.

‡ 70°C, 1 hour.

§ Trypticase soy broth.

with cold sterile saline containing 50 u/ml heparin. The suspension was centrifuged at 1230 RCF for 10 minutes. Supernates were titrated as described.

RESULTS

Protective Activity of Staphylococcus Bacteriophage Lysates.—The ability of staphylococcus bacteriophage 81 to protect mice was demonstrated in a series of 15 experiments involving 248 mice.

The infecting inoculum contained in 0.2 ml, was injected into one side of the peritoneum and the same number of bacteriophage particles or other substances in 0.2 ml was immediately injected into the opposite side of the peritoneum.

The results of these experiments are summarized in Table I. Of the total number injected, 201 mice or 81 per cent survived an otherwise lethal infection.

The various controls,—which received heat-inactivated (70°C, 1 hour) staphylococcus bacteriophage 81, active but heterologous staphylococcus bacteriophage 3b, saline, or broth,—all failed to demonstrate any protective activity against *S. aureus*, Type 80/81. When infection was initiated with *S. aureus*, Type 44A active preparations of staphylococcus bacteriophage 81 failed to encourage any survivors.

Thus, it was concluded that preparations of staphylococcus bacteriophage 81 were capable of exerting a protective effect in mice infected with *Staphylococcus aureus*, Type 80/81. Moreover, this protective activity appeared to be dependent upon the use of active, type-specific bacteriophage.

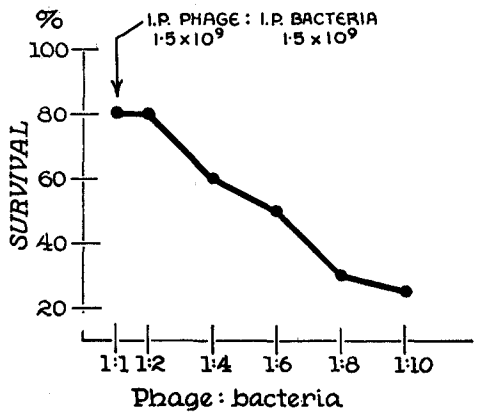

FIG. 1. Influence of bacteriophage to bacteria ratio on mouse survival.

Influence of Bacteriophage: Bacteria Ratio at the Site of Infection.—When intraperitoneal infection was initiated with 1.5×10^9 viable cells it was shown that administration of an equal number of bacteriophage particles protected 81 per cent of the mice. It appeared desirable to determine the effectiveness of various quantities of bacteriophage delivered to the site of infection.

Infection of all mice was accomplished as described. Various dilutions of bacteriophage 81 were injected so that the following ratios of phage-bacteria were tested: 1:1, 1:2, 1:4, 1:6, 1:8, 1:10. At 1:1 both phage and bacteria were 1.5×10^9. Injections of 0.2 ml were made one immediately following the other into opposite sides of the peritoneum.

The results shown in Fig. 1 indicate that maximal protection was obtained at a ratio of 1:2 with 80 per cent mouse survival. An increased quantity of bacteriophage did not appear to influence this value. However, decreased quantities of bacteriophage, at the site of infection, resulted in a corresponding decrease in the number of survivors. Thus, within a certain range there appeared to be a linear relationship between the bacteriophage-bacteria ratio and the ability of mice to survive infection.

The Effect of Time and Sequence of Administration.—Despite the fact that the staphylococcal mouse infection employed in these experiments was extremely rapid and left much to be desired, it was considered desirable to obtain some information relating to the prophylactic and therapeutic potentials of the bacteriophage lysate material.

An inoculum of 1.5×10^9 phage was administered to mice at 3, 6, and 12 hours before; and 5, 15, and 30 minutes after infection. At zero time both phage and the infecting inoculum were injected intraperitoneally, one immediately after the other. The phage to bacteria ratio employed was 1:1.

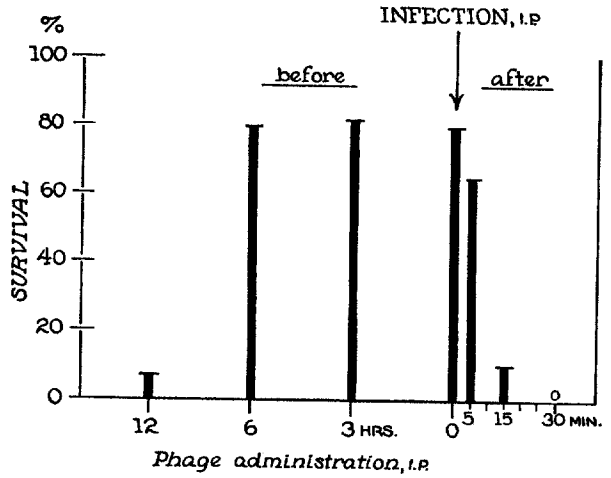

FIG. 2. The effect of time and sequence of administration on mouse survival.

It can be seen in Fig. 2, when both bacteriophage and bacterium were injected at zero time, 80 per cent survival was observed. When bacteriophage was administered 3 or 6 hours before infection the percentage survival remained unchanged. However, a sharp decline in the number of survivals was noted by the 12th hour when only 7 per cent of the mice survived. On the other hand, when infection was established first and administration of bacteriophage delayed, protective activity was severely diminished. At 5 minutes, 65 per cent survival was noted and this fell off to 10 per cent survival if 15 minutes elapsed before phage was injected. After 30 minutes none of the mice survived. Under these circumstances it appeared that the outcome of this infection was determined very early in the infectious process, and bacteriophage administration after this time interval could not reverse the process.

In Vivo Interaction of Bacteriophage and Infecting Bacterial Cell.—While the experiments which have been described suggested a certain relationship between the bacteriophage lysate material and mouse survival, it appeared obvious

that a more direct approach was necessary to demonstrate a positive *in vivo* interaction between bacteriophage and its host cell, *S. aureus*.

Groups of mice were infected with *S. aureus* 80/81. All mice were immediately injected with 1.5×10^9 PFU bacteriophage 81 into the opposite side of the peritoneum. The bacteriophage to bacteria ratio employed was 1:1. Groups of 5 mice each were bled from the tail at the time intervals indicated, postinjection.

In Fig. 3 the circulating bacteriophage levels are presented. It can be seen that in normal non-infected mice bacteriophage levels were evident as early as

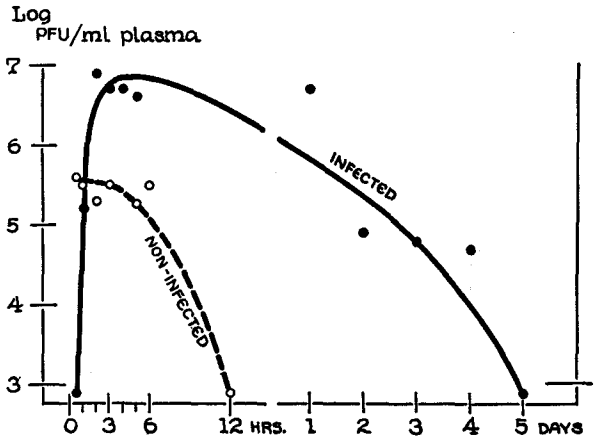

FIG. 3. Plasma levels of bacteriophage 81 after administration to mice infected with *S. aureus* 80/81 and to non-infected mice.

5 minutes after inoculation and persisted at approximately the same level for 6 hours. However, by 12 hours circulating levels of bacteriophage were no longer detectable, that is, less than 10^3 PFU/ml plasma. On the other hand, infected mice which were treated with bacteriophage responded quite differently. The 30 minute bleeding indicated a circulating bacteriophage level of less than 10^3 PFU/ml, and at 1 hour the levels attained were still below those observed in the non-infected groups. However, by 1½ to 2 hours circulating bacteriophage levels of infected mice were significantly higher than those observed in non-infected mice, and these levels persisted as long as 4 days.

It should be pointed out that the infected curve does not represent peak values of circulating bacteriophage which may be obtained but serves simply to illustrate the point being made here.

Thus, in the system described, we have observed: (*a*) a delayed appearance of bacteriophage, (*b*) increased circulating titers, and (*c*) extended duration of detectable levels. These observations suggest that staphylophage 81 in the

presence of a sensitive host cell is capable of *in vivo* interaction and subsequent propagation.

It was also observed that the phage-bacterium infectious cycle did not continue to final eradication of the infecting bacterial cell. Repeated isolations revealed cells which appeared to be unchanged in regard to the properties tested, which included phage and antibiotic sensitivity, alpha and beta hemolysins, coagulase, and mannitol.

Specificity of the Interaction as Demonstrated by Circulating Levels.—Protection experiments (Table I) indicated that survival of mice infected with *S. aureus* 80/81 depended upon the use of active, type-specific bacteriophage

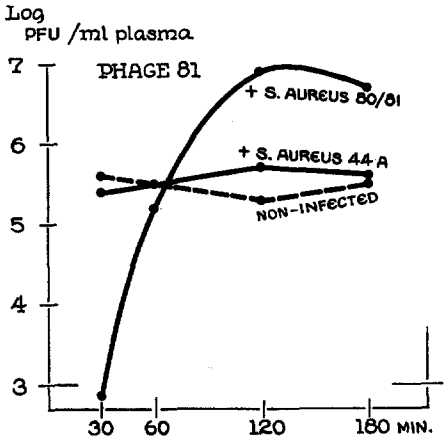

FIG. 4. Plasma levels of staphylococcus bacteriophage 81 after administration to mice infected with *S. aureus* 80/81 and *S. aureus* 44A, and to non-infected mice.

81 preparations. It was of interest to determine if and how this might be expressed by following circulating bacteriophage levels.

Groups of mice were infected with *S. aureus* 80/81 or *S. aureus* 44A in 5 per cent mucin. Control mice received 5 per cent mucin alone. All mice were immediately injected with 1.5×10^9 PFU bacteriophage 81 into the opposite side of the peritoneum. The bacteriophage to bacteria ratio employed was 1:1. Groups of 5 mice each were bled from the tail at 30, 60, 120, and 180 minutes postinjection.

In Fig. 4 it can be seen that bacteriophage levels in non-infected mice and those bearing the heterologous infection with *S. aureus* 44A roughly paralleled each other. In each case, levels of approximately $10^{5.5}$ PFU/ml were observed at 30 minutes which remained comparatively unchanged through the 180 minute observation period. However, mice infected with the sensitive *S. aureus* 80/81 responded quite differently with a delayed appearance of circulating bacteriophage. The 30 minute bleeding revealed levels of less than 10^3 PFU/

ml, after which the circulating levels rose sharply to exceed those observed in the other groups.

Thus, it was quite clear that only in those infected mice which received type-specific bacteriophage lysate material was there *in vivo* interaction as evidenced by production of bacteriophage.

Relationship between Circulating Bacteriophage Level and Mouse Survival.—The foregoing experiments indicated that bacteriophage 81 lysates were effective in mouse protection and that a definite *in vivo* interaction occurred between bacteriophage 81 and its sensitive host cell *S. aureus* 80/81.

The following experiments were executed to determine the relationship between circulating bacteriophage levels and mouse survival.

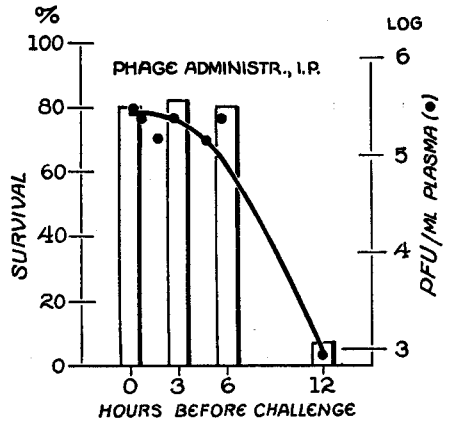

FIG. 5. Relationship between circulating bacteriophage levels and survival of mice.

Groups of mice were inoculated intraperitoneally with 1.5×10^9 PFU bacteriophage 81 and then challenged with 1.5×10^9 *S. aureus* 80/81 in 5 per cent mucin, intraperitoneally, at 0, 3, 6, and 12 hours. Tail bleedings were made prior to inoculation of the challenge dose at the time indicated.

Fig. 5 depicts the relationships which were observed. It may be seen that when circulating bacteriophage levels were approximately $10^{5.5}$ PFU/ml a correspondingly high degree of protective activity was observed. On the other hand, when circulating bacteriophage levels fell to less than 10^3 PFU/ml protective activity was poor.

Activity of Intravenously Administered Bacteriophage.—The experiments discussed so far have dealt exclusively with bacteriophage preparations inoculated directly into the site of infection. It was of interest to determine if bacteriophage was capable, after administration by the intravenous route, of reaching and acting at the site of intraperitoneal infection.

Groups of mice were injected either intraperitoneally or intravenously (tail vein) with 1.5×10^9 PFU bacteriophage 81. Control mice received sterile broth. Challenge with *S. aureus* 80/81 in 5 per cent mucin was performed at various time intervals.

Table II presents the results of this experiment. It is evident that when bacteriophage was administered intravenously the number of survivals was sharply reduced (20 per cent) as compared to groups which received bacteriophage intraperitoneally (80 per cent). However, it should be noted that while a decreased activity was observed it was quite definite, protecting against an

TABLE II

Influence of intravenous or intraperitoneal administration of bacteriophage on survival of mice infected intraperitoneally

Before challenge‡	Bacteriophage administration*				Broth controls			
	Intravenous		Intraperitoneal		Intravenous		Intraperitoneal	
	Survivals/Group	Survival	Survivals/Group	Survival	Survivals/Group	Survival	Survivals/Group	Survival
hrs.		*per cent*		*per cent*		*per cent*		*per cent*
0	6/30	20	24/30	80	0/20	0	0/20	0
3 to 6	4/20	20	16/20	80	0/20	0	0/20	0
12 to 24	0/20	0	1/20	5	0/20	0	0/20	0

* Bacteriophage 81; 1.5×10^9 in 0.2 ml.

‡ *S. aureus* 80/81; 1.5×10^9 in 5 per cent mucin, 0.2 ml intraperitoneally.

otherwise 100 per cent lethal infection, and further, that termination of protective activity occurred within the same time interval for both groups.

It was felt that this point would be better demonstrated if the interaction could be detected in peritoneal washings of treated mice.

Groups of mice were inoculated intravenously (tail vein) with 1.5×10^9 PFU bacteriophage 81 and immediately challenged with *S. aureus* 80/81 in 5 per cent mucin, intraperitoneally. Peritoneal washings were obtained at 30, 60, 90, 120, and 180 minutes postchallenge.

Fig. 6 illustrates the interaction as observed in the peritoneal washings of infected mice. Bacteriophage levels found in the peritoneal washings of uninfected controls are shown for comparison. Thus, it appears that bacteriophage was capable of leaving the circulatory system and interacting with its sensitive host cell at the site of infection.

Extended Protection of Treated Mice.—Approximately 81 per cent of the infected mice which received bacteriophage were immediately protected against *S. aureus* (Table I). In addition to promoting survival of mice this treatment resulted in increased levels of bacteriophage which persisted in the blood stream 4 to 5 days, and for somewhat longer periods in peritoneal washings and homog-

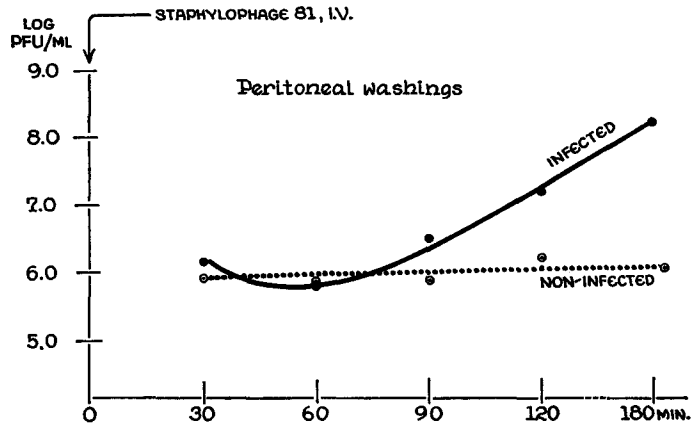

FIG. 6. Peritoneal interaction between intravenously administered staphylophage 81 and *S. aureus* 80/81, intraperitoneal infection.

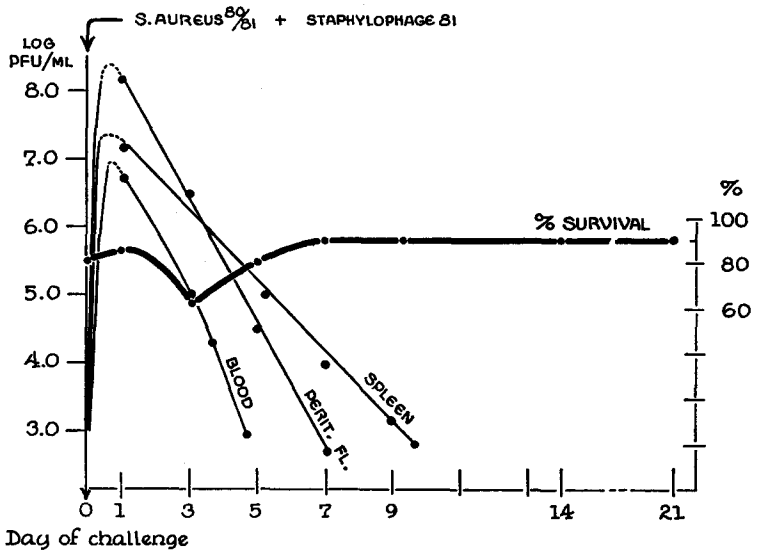

FIG. 7. Relationship of bacteriophage levels in blood, peritoneal fluids, spleen of treated mice, and survivals on various days of challenge subsequent to treatment.

enates of spleen. It became of interest to determine what effect this treatment had on mice, especially after bacteriophage declined to non-detectable levels.

Groups of mice were infected with *S. aureus*, 80/81 intraperitoneally and treated with 1.5×10^9 PFU bacteriophage 81. Mice were bled from the tail and challenged on days 1, 3, 5, 7, 9, 14, and 21 days with 1.5×10^9 *S. aureus* 80/81 in 5 per cent mucin.

The results are illustrated in Fig. 7. Bacteriophage levels present in peritoneal washings and splenic homogenates of treated mice were obtained in separate experiments. Mice challenged 24 hours after such treatment responded with a somewhat increased resistance to infection (83 per cent). However, on the 3rd day only 63 per cent survival was observed, and it was striking that the decreased ability to withstand challenge coincided with a decreasing availability of active bacteriophage. Beginning with the 5th day increased resistance to challenge was noted, and at 7 through 21 days 90 per cent of the mice survived the challenge inoculum. (This curve has been reproduced through 4 experiments and results have been consistent with particular attention to the decreased per cent survival seen on the 3rd day.)

DISCUSSION

Recent hospital experiences with antibiotic resistant strains of *Staphylococcus aureus* have stimulated a resurgence of interest in the control of this microorganism. Ironically, those very antibiotics which had performed so reliably also tended to reduce interest in the search for additional means of combating this disease. However, the dramatic experience apparently left its mark and revitalized effort has already seen the development of newer and more effective therapeutic agents. To some, the immunoprophylactic approach, despite its many difficulties, holds the greatest promise for reliable control. Interest has also focused on the potential therapeutic and immunoprophylactic usefulness of bacteriophage lysate preparations. This approach lost its appeal due to the many conflicting clinical reports, the lack of laboratory evidence to strengthen such rationale, and the advent of successful antibiotic therapy. The literature concerning itself with bacteriophage therapy is voluminous and for obvious reasons we have restricted our comments to staphylococcal bacteriophage.

Experimentally, Krueger (2), Robinson (3), Sulkin (4), and others failed to observe any beneficial effect when bacteriophage was administered to staphylococcus-infected animals. Sulkin concluded that conditions conducive to bacterial lysis could not be established *in vivo*. In support of this were the results of Colvin (5), Gratia and Mutsaars (6), and Applebaum and MacNeal (7) who reported on the *in vitro* inhibition of bacteriophage activity by pus, blood, serum, and other body fluids. However, Colvin's observations indicated that such inhibition appeared to be of a variable nature, being dependent upon the particular specimen, as well as the strain of staphylococcus or bacteriophage employed. MacNeal, McRae, and Colmers (8) demonstrated an increased opsonic effect when optimal quantities of phage and staphylococcus were mixed and added to human blood. *In vivo*, Walker (9) observed a reduced severity of staphylococcal lesions through the use of bacteriophage lysates, and Muir and Blakemore (10) first successfully protected staphylococcal infected mice. More recently, a definite *in vivo* interaction has been reported (11).

Clinical use of bacteriophage lysates received encouraging comment from MacNeal and Frisbee (12, 13) and Larkum (14). Such reports dealing with the use of staphylococcus bacteriophage, of their very nature, have been difficult to assess or evaluate

(15, 16). Many attempts, made out of sheer desperation, were doomed to failure. Without benefit of *in vivo* experimental evidence the acceptance of any rationale advocating the use of this material was indeed difficult. While experimentation with the laboratory animal does not guarantee extrapolation to the human being, it does provide an accessible and easily managed *in vivo* system with which one may study mechanism of action. On the basis of existing meager knowledge, it appeared premature to cast aside a weapon of such potential in our continuous struggle to control pathogenic staphylococci.

In the present study *Staphylococcus aureus* 80/81 and bacteriophage 81 were shown to be capable of *in vivo* interaction. This was demonstrated by the following series of events which occurred immediately after inoculation of infected mice with bacteriophage: (*a*) non-detectable bacteriophage levels (period of adsorption) followed by (*b*) rapidly increased circulating bacteriophage levels (period of release from infected bacterial cells) and (*c*) extended duration of detectable bacteriophage. It was also observed that the interaction was dependent upon the use of active, type-specific bacteriophage which resulted in 81 per cent survival of mice against an otherwise 100 per cent lethal challenge. The bacteriophage to bacteria ratio as well as time and sequence of administration were found to be influencing factors. It was also shown that mice capable of withstanding lethal challenge carried high levels of circulating bacteriophage, whereas those succumbing to intraperitoneal infection had only low levels of circulating bacteriophage. These circulating levels apparently reflected active bacteriophage available at the site of peritoneal infection. Of great interest was the demonstration that bacteriophage administered intravenously was capable of reaching and interacting with the infecting bacteria at the site of infection, as evidenced by bacteriophage production and mouse survival. A consideration of the rapidly fatal infecting system employed and the availability of adequate levels of bacteriophage at the site of infection appear to be likely explanations for the limited survival rates observed in this circumstance.

The exact mechanism by which bacteriophage may protect mice from the lethal effects of injected staphylococci is unknown since, in the first place, the basic cause of death in untreated mice is still vague. However, it is evident that bacteriophage does interfere with some further process required for expression of the lethal effect. Infection of cocci by bacteriophage might accomplish this by (*a*) altering bacterial metabolism so that cellular processes are diverted from their usual metabolic functions and (*b*) destruction of bacterial cells so as to reduce the critical number of cocci below the lethal level. Recently, Smith (17) has suggested that elaboration of the lethal effect is closely associated with continued bacterial multiplication after inoculation, and the basic cause of death appears to be chemical but associated with the coccus rather than its products.

It was evident that a continued reinfection of bacterial host cells by bacterio-

phage did not occur. This interruption of the phage-bacterium infectious cycle has not, as yet, been closely studied, however, it did not appear to be due to emergence of phage resistant mutants, since repeated isolations yielded bacterial cells which appeared to be unchanged from the original infecting inoculum in those characteristics tested.

Infected mice which were treated with bacteriophage lysates were shown to respond with an altered resistance to infection. The results of several experiments suggested that protective activity at zero time and possibly up to 24 to 48 hours was due to the availability of sufficient active, type-specific bacteriophage. Treated mice responded with a decreased resistance to challenge on the 3rd day, a time which corresponded with the period of decreasing bacteriophage levels. However, beginning with the 5th day, a protective mechanism was apparently operative in the absence of significant bacteriophage levels which continued to be effective for at least 21 days. The system at this point is evidently very complex and several factors appear to be simultaneously operative. Those factor(s) which may be responsible for such high levels of protective activity are unknown. However, one might consider (*a*) stimulated phagocytosis and/or (*b*) altered cellular capacity for destruction of bacteria brought about through the production of certain specific or non-specific substances. In addition, it would appear reasonable to expect antigenic substances present in lysates to be effective in the stimulation of antibody. The possibility exists that other substances may be induced or formed under *in vivo* circumstances which may participate in the elaboration of protective activity. It is known, for example, that antigenic changes may be initiated in bacterial cells through the activity of bacteriophage, and further, that these changes do not require lysogenization of the host cell, but may be brought about by bacteriophage in the vegetative state. Certain salmonella bacteriophages (18–20) have been shown to induce the synthesis of polysaccharide moieties of the 0 antigen, and diphtherial bacteriophages (21) in the synthesis of toxic protein. However, it must be stated that we have no evidence that any of these conversions may occur under *in vivo* conditions.

Future studies have been oriented to further elucidate the *in vivo* characteristics of this interaction. Similar consideration will be extended to include other *S. aureus* and bacteriophage strains.

SUMMARY

Staphylococcus bacteriophage 81 is capable of *in vivo* interaction with *Staphylococcus aureus*, Type 80/81. This is immediately made evident by increased levels of bacteriophage and concomitant survival of 81 per cent infected mice. The reaction is dependent upon the use of active, type-specific bacteriophage. The maximal protective effect is observed at a bacteriophage to bacteria ratio of 1:2 and decreased quantities of bacteriophage result in de-

creased protection. Time and sequence of administration are also determining factors. It is evident that bacteriophage administered intravenously is capable of interaction with the infecting bacterial cell at the site of infection. *In vivo* produced bacteriophage is apparently eliminated or otherwise rendered non-detectable fairly rapidly, occurring within a period of 5 to 10 days. However, it appears that host defense mechanisms are stimulated in the process and actively play a protective role against subsequent challenge inocula administered up to 3 weeks later.

BIBLIOGRAPHY

1. Adams, M. H., Bacteriophages, New York, Interscience Publishers, Inc., 1960.
2. Krueger, A. P., Lich, R., and Schulze, K. R., Bacteriophage in experimental staphylococcal septicemia, *Proc. Soc. Exp. Biol. and Med.*, 1932, **30,** 73.
3. Robinson, G. H., The interference of antistaphylococcus bacteriophage, *J. Bact.*, 1940, **39,** 72.
4. Sulkin, S. E., Douglass, D. D., and Bronfenbrenner, J., Bacteriophage therapy. IV. Effect of bacteriophage in experimental staphylococcal septicemia in rabbits, *J. Inf. Dis.*, 1942, **70,** 92.
5. Colvin, M. G., Behavior of bacteriophages in body fluids and in exudates, *J. Inf. Dis.*, 1932, **51,** 527.
6. Gratia, A., and Mutsaars, W., L'action inhibitrice du serum normal sur la lyse du staphylocoque dore par les bacteriophages staphylococciques polyvalents, *Compt. rend. Soc. biol.*, 1931, **106,** 943.
7. Applebaum, M., and MacNeal, W. J., The influence of pus and blood on the action of bacteriophage, *J. Inf. Dis.*, 1931, **49,** 225.
8. MacNeal, W. J., McRae, M. A., and Colmers, R. A., Further observations on bacteriophage action in the presence of blood, *J. Inf. Dis.*, 1938, **63,** 25.
9. Walker, J. E., The action of bacteriophage and chemical disinfectants on staphylococcus in subcutaneous injections, *J. Inf. Dis.*, 1930, **46,** 324.
10. Muir, W. R., and Blakemore, W. S., Staphylococcal bacteriophage in experimental infection in mice, *Surg. Forum*, 1960, **10,** 339.
11. Bartell, P., Thind, I. S., Spencer, L., and Blakemore, W. S., Staphylococcus bacteriophage in experimental infection, *Fed. Proc.*, 1962, **21,** 457.
12. MacNeal, W. J., and Frisbee, F. C., Bacteriophage as a therapeutic agent in staphylococcus bacteremia, *J. Am. Med. Assn.*, 1932, **99,** 1150.
13. MacNeal, W. J., and Frisbee, F. C., One hundred patients with staphylococcus septicemia receiving bacteriophage service, *Am. J. Med. Sc.*, 1936, **191,** 179.
14. Larkum, N. W., Bacteriophage treatment of staphylococcus infections, *J. Inf. Dis.*, 1929, **45,** 34.
15. Eaton, M. D., and Bayne-Jones, S., Bacteriophage therapy: Review of the principles and results of the use of bacteriophage in the treatment of infections, *J. Am. Med. Assn.*, 1934, **103,** 1769.
16. Elek, S. D., Staphylococcus pyogenes and its relation to disease, London, E. & S. Livingstone Ltd., 1959, 146–148.

17. Smith, I. M., Wilson, A. P., Hazard, E. C. H., Hummer, W. K., and Dewey, M. E., Death from staphylococci in mice, *J. Inf. Dis.*, 1960, **107,** 369.
18. Zinder, N. D., Lysogenic conversion in *Salmonella typhimurium*, *Science*, 1957, **125,** 1237.
19. Stocker, B. A. D., Lysogenic conversion by the A phages of *Salmonella typhimurium*, *J. Gen. Microbiol.*, 1958, **18,** ix.
20. Uetake, H., Luria, S. E., and Burrous, J. W., Conversion of somatic antigens in *Salmonella* by phage infection leading to lysis or lysogeny, *Virology*, 1958, **5,** 68.
21. Barksdale, L., Phage multiplication and toxin production in *Corynebacterium diphtheriae*, *J. Gen. Microbiol.*, 1958, **18,** x.