

The Gratia Polyvalent Staphylococcal Bacteriophage (34764)

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Gratia (1) in 1921 isolated from untreated fresh green vaccine pulp a bacteriophage (2) for *Staphylococcus aureus*. "Staphylococcus is therefore, the first gram-positive bacterium to possess a marked inhibiting and dissolving action on the growth of staphylococci, and this lysis could be carried on indefinitely from one culture to another" (1). This bacteriophage played a lively role in the controversy of that time regarding the nature of the principle of transmissible lysis (3).

The Gratia phage has been maintained in the laboratory for 48 years and for at least three decades has been used in therapy (4, 5). The present communication examines the present host range of the Gratia phage, and the degree of inhibition of lysis in the presence of dilutions of human serum.

Staphylococcal bacteriophage lysate. History of bacteriophage. The polyvalent bacteriophage presently distributed in staphylococcal bacteriophage lysate³ under USPHS license was isolated in 1921 by Gratia (1). It was transferred to Hooker and Follansby at Boston University School of Medicine by Larkum (5). The propagating strains of *S. aureus* on which the phage has been maintained were isolated in 1946 and 1947 from staphylococcal infections. They were typed, respectively, as Serologic Types I and III (6). The propagating strains have been main-

tained at the Delmont Laboratories in lyophilic form. Lysates are prepared according to NIH regulations and dispensed as Serologic Types I or III and as Types I and III combined. Tests against the "Blair" strains of *S. aureus* and against a large series of wild strains made at Delmont Laboratories by Roos with bacteriophages diluted 1:10 and observed after 48 hr at 37° were reported as showing lysis against the majority of staphylococcal strains tested.

Materials. Bacteriophage preparations were "Staphage Lysate Type I" and "Staphage Lysate Type III," obtained from Delmont Laboratories. Bacterial cultures were: (i) the standard propagating strains used for producing and testing staphylococcal typing phages (7), (ii) the homologous propagating strains for the two bacteriophages being tested, and (iii) "wild" strains of staphylococci of various phage groups which had been submitted to the NCDC for phage typing. Pooled normal human serum was obtained from a local blood bank. Trypticase-soy broth and agar were used for growing and testing all cultures. Trypticase-soy broth was also used as diluent for the serum and bacteriophage.

Methods. Each bacteriophage preparation was incubated in the presence of pooled normal human serum, in varying concentrations, for 2 hr at 37°. To do this, the serum was supplemented with Trypticase-soy broth and phage suspension to yield final serum concentrations of 10, 20, 40, 60, and 80%, and final phage dilutions in each case of 1:10. Following incubation, each phage-serum mixture was serially diluted in tenfold steps to 10⁻⁶. In each case, the same percentage of serum was used for diluent as had been used for 37° incubation. One drop of each serial dilution was then placed on a Trypticase-soy agar

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plate which had been seeded with a 4-6-hr broth culture of the test strain, and the plates were incubated at 30° overnight. Controls consisted of: (i) phage dilutions made without serum but incubated in the manner indicated above, and (ii) serum diluted and incubated as above, but without added phage. After overnight incubation each plate was then observed for lysis and results were translated into "efficiency of plating" ratings as recommended for determining lytic spectra of phages (7). According to these recommendations, lysis of a given propagating strain by its homologous phage is rated "5." Any other lytic reaction of the same intensity by the same phage and at the same dilution, but on another strain, is rated 5 also. If the same intensity reaction occurs at phage dilutions

10-fold or 100-fold stronger, the rating is 4. Similarly, if the same intensity reaction requires a dilution 10³ or 10⁴ times stronger, the rating is 3; and if the required dilution is 10⁵ or 10⁶ times stronger, the rating is 2. A rating of 1 is usually reserved for those lytic reactions which are very weak (+ or ±) with undilute phage.

Results. Table I shows that each of the two phages has a broad range of activity against the standard staphylococcus propagating strains. In the absence of serum, both phage lysates lysed 18 of the 27 strains tested. Both phages are active on the same strains; the only significant difference is that phage I is somewhat more lytic and strains 54 and 75 than is phage III. Both phages are highly active against strains of phage group

TABLE I. Lytic Spectra of Lysates I and III in the Presence of Normal Human Serum (pooled).

PS	Lysate: % Serum:	Efficiency of plating											
		I						III					
		0	10	20	40	60	80	0	10	20	40	60	80
29		—	—	—	—	—	—	—	—	—	—	—	—
52		—	—	—	—	—	—	—	—	—	—	—	—
52A/79		4	4	4	4	4	3	3	3	3	3	3	2
80		5	4	4	4	4	4	4	4	3	3	3	3
3A		4	4	4	4	4	4	5	5	4	4	4	4
3C		5	5	5	4	4	4	5	5	5	5	5	5
55		5	4	4	4	4	4	5	5	4	4	4	4
71		5	5	4	4	4	4	5	5	5	4	4	4
6		4	3	3	—	—	—	3	2	—	—	—	—
Int 7		4	3	3	2	—	—	3	—	—	—	—	—
42E		5	4	4	4	4	4	4	3	3	3	2	2
47		—	—	—	—	—	—	—	—	—	—	—	—
53		—	—	—	—	—	—	—	—	—	—	—	—
54		4	4	4	4	3	3	2	2	1	1	—	—
75		4	4	4	4	3	3	2	2	1	1	—	—
77		—	—	—	—	—	—	—	—	—	—	—	—
42D		—	—	—	—	—	—	—	—	—	—	—	—
187		5	4	4	4	4	3	4	3	3	3	2	—
84		—	—	—	—	—	—	—	—	—	—	—	—
85		—	—	—	—	—	—	—	—	—	—	—	—
86		—	—	—	—	—	—	—	—	—	—	—	—
81		5	5	4	4	4	4	4	4	3	3	3	3
2009		2	—	—	—	—	—	2	—	—	—	—	—
8719		4	4	3	3	1	1	4	3	3	3	2	2
18Z		5	5	4	4	4	4	4	4	4	3	3	3
I		5	4	4	3	3	2	4	3	3	2	—	—
III		5	4	3	3	2	2	5	4	4	3	2	2

II (3A, 3C, 55, 71), but phage III is slightly less active against other strains tested than is phage I. This is shown by the slightly lower efficiency of plating on these various strains. The inhibitory action of serum was greater on phage III than on phage I (Table I).

The Efficiency of Plating (Table I) gives a slightly distorted picture of the effect of serum, since there is a two-dilution range for ratings 1-4. Therefore, comparisons of the highest dilutions which produced lysis have been made (Table II). The significant changes in these dilutions are shown in Table II under the heading "Log drop in titer." It is immediately apparent that exposure to serum had a greater effect on the lytic activity of each phage on its homologous host than it did against the phage group II strains. This may

have been because of different ionic requirements for adsorption and lysis, differences in receptor sites on the cells, differences in "normal" antibodies in the serum, or for other reasons.

Table II also shows relatively little difference in the effect of the serum on the two phages. The drop in titer of both phages was quite similar on a given propagating strain, with the exception of strain 6. It is also apparent that phage which has a high lytic titer against a strain retains much of that titer even in the presence of 80% serum.

The effect of serum on the lytic capability of lysates I and III against "wild" strains of staphylococci is shown in Table III. For this experiment, lysates I and III were suspended in 10, 40, and 80% serum at a final

TABLE II. Negative Logarithm of Highest Dilution of Phages I and III Yielding + Lysis in Normal Human Serum.

PS	% Serum:	Phage I						Log drop in titer	Phage III						Log drop in titer	
		0	10	20	40	60	80		0	10	20	40	60	80		
29		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
52		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
52A/79		3	3	2	2	2	1	2	1	1	1	1	0	0	1	1
80		4	3	3	3	3	3	1	2	2	1	1	1	1	1	1
3A		3	3	2	2	2	2	1	4	4	3	3	3	3	3	1
3C		4	4	4	3	3	3	1	4	4	4	4	4	4	4	0
55		4	3	3	3	3	3	1	4	4	3	3	3	3	3	1
71		4	4	3	3	3	3	1	4	4	4	3	3	3	3	1
6		3	2	1	—	—	—	4 ^a	1	0	—	—	—	—	—	2
Int 7		2	1	1	0	—	—	3	1	—	—	—	—	—	—	2
42E		4	3	3	3	2	2	2	2	1	1	1	0	0	2	2
47		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
53		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
54		2	2	2	2	1	1	1	0	0	0	0	—	—	—	1
75		2	2	2	2	1	1	1	0	0	0	0	—	—	—	1
77		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
42D		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
187		4	3	3	3	2	1	3	2	1	1	1	0	—	3	3
84		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
85		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
86		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
81		4	4	3	3	3	3	1	2	2	1	1	1	1	1	1
2009		0	—	—	—	—	—	1	0	—	—	—	—	—	—	1
8719		2	1	0	0	0	0	2	2	1	1	1	0	0	2	2
18Z		4	4	3	3	3	3	1	2	2	2	1	1	1	1	1
I		4	2	2	1	0	0	4	2	0	0	0	—	—	—	3
III		4	2	1	0	0	0	4	4	2	2	1	0	0	4	4

^a A drop of 3 to — is measured as a 4-log drop, since there was no lysis with undilute phage.

TABLE III. Effect of Normal Serum on Lytic Activity of Lysates I and III on "Wild" Strains of Staphylococci of Various Phage Typing Groups.
Values indicate numbers of cultures lysed strongly.

Phage group and no.	% Serum:	Lysate I, in				Lysate III, in			
		0	10	40	80	0	10	40	80
I	(42)	15	15	15	9	2	1	1	1
II	(47)	33	30	29	21	36	30	29	24
III	(78)	16	14	7	5	4	3	1	1
Misc.	(9)	2	2	2	0	1	1	1	1
Mixed	(41)	14	13	10	8	0	0	0	0
"80/81"	(10)	7	7	7	7	0	0	0	0
Nontypable	(52)	10	9	7	2	2	2	0	0
Totals	(279)	97	90	77	52	45	37	32	27
Titration of phages									
Host PS I		+++	++	++	++	±	±	—	—
Host PS III		+++	++	++	—	+++	++	+	±

dilution equal to the routine test dilution (RTD) of each phage. These mixtures were incubated as in other tests, but no dilutions were made after the incubation period. Each serum-phage mixture was then used to type 279 cultures of staphylococci of various phage groups (7) and from various sources. The "80/81 complex" phage group was composed of strains which reacted with the standard phages 52, 52A, 80, and 81 either alone or in any combination.

Lysate I showed a broad range of lytic activity against these "wild" strains by lysing 97 of the 279 strains in the absence of serum. In the presence of 40% serum, 77 strains were lysed, and 80% serum still permitted lysis of 52 strains (54% of the 97 susceptibles). Lysate I seemed to retain its activity against strains of the "80/81 complex" quite well, since all 7 strains which were susceptible in the absence of serum were still lysed in the presence of 80% serum. The high degree of activity of both lysates against phage group II strains is notable, because such strains are well-documented agents of impetigo and other skin infections, but are less frequently encountered in systemic and other staphylococcal infections. The only significant activity exhibited by lysate III was against strains of phage group II, and this activity was retained well even in the presence of 80% serum.

The data in Table III illustrate that the lytic spectra of lysates I and III on the standard propagating strains (Table I) do not correspond to their value in typing "wild" strains of staphylococci. Although both lytic spectra were similar and both lysates were used at about the same concentration, lysate I was much more effective against "wild" strains than was lysate III. On the basis of the effect of serum on the titration of the two lysates, lysate III was more susceptible to the action of normal serum, but this difference could not be detected when lysates were used against "wild" strains. Table II did support this difference, since in 40% serum, lysate III was still active against only 20 (71.5%) of 28 strains; whereas, 47 (87.5%) of 55 strains were still lysed by lysate I.

Discussion. A number of interesting considerations are apparent from studying Tables I, II, and III. The broad range of capability to lyse staphylococci, both of the international typing strains and of "wild" types, is indeed remarkable. This broad polyvalence is present after 48 years of propagation in the laboratory. The two subtypes of phage, carried for more than 20 years on propagating strains, respectively, of serotypes I and III, are qualitatively similar in lytic spectra against the standard propagating strains. At least in the samples examined, however,

subtype I is slightly more active as a lytic agent against the standard propagating strains than is subtype III. Against "wild" strains subtype I is more effective than subtype III and less inhibited by serum.

The practical objectives of the present study were, of course, (i) to test the lytic spectra of Gratia phage against both laboratory and "wild" strains of staphylococci, and (ii) to ascertain the degree of inhibition of lysis by human serum, under conditions approximating, as closely as practicable, conditions of clinical use. Concerning serum inhibition, the results were frankly more favorable than anticipated. Although it is impossible to make a quantitative judgment on this point, it seems reasonable to conclude that phage lysis of susceptible staphylococci need not be effectively prevented by the amount of serum proteins present in nasal sinuses, on respiratory membranes, or indeed in other sites of topical application.

It may be pertinent to recall that Bartell *et al.* (8) found significant action of a specific phage in mice even after administration of the phage intravenously; the mice were challenged in these experiments by intraperitoneal injection of the propagating strain of staphylococci.

The question of the possible efficacy of bacteriophage therapy under appropriate conditions has had a long and interesting history, which we do not propose to recount in the present communication. We do, however, wish to record our personal judgment that this question has not been definitely decided one way or the other, and that it still deserves thorough and critical investigation.

A recent aspect of staphylococcal phage lysate therapy, namely its efficacy as a hypsensitizing agent, is discussed elsewhere (9).

Summary. Lytic spectra of the Gratia staphylococcal bacteriophage have been determined both against the standard international typing strains of *Staphylococcus aureus* and against "wild" strains. The broad range of lytic capability of the Gratia phage is remarkable. The substrain of Gratia phage propagated on Serotype I lysed more than half of the standard typing strains and 31% of 279 "wild" strains. Inhibition of lytic activity by pooled human serum was not complete in the majority of instances even in 80% serum. These data we believe to be compatible with the supposition that direct lysis of infecting staphylococci may be a factor in the therapeutic efficacy of the staphylococcal bacteriophage lysate under appropriate conditions.

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