INTERGROUP PHAGE REACTIONS AND TRANSDUCTION BETWEEN GROUP C AND GROUP A STREPTOCOCCI*

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The possibility that bacteriophages may influence important biological properties of Group A streptococci and may serve as vectors of genetic information in these organisms has bolstered interest in this area despite technical difficulties in working with this phage-host system (1, 2). Much valuable background information has been contributed by the studies of Evans and Sockrider (3), Kjems (4), Maxted (5), Krause (6), Fischetti and Zabriskie (7), and Friend and Slade (8).

Zabriskie (9) has documented that streptococcal phage is concerned in the production of scarlatinal toxin, by a mechanism of phage multiplication or lysogenic conversion. Transduction of streptomycin-resistance between strains of Group A streptococci has been reported by Leonard et al. (10) and by Malke (11). Colón and co-workers (12) have described transduction of resistance to this antibiotic from Group A streptococci to Group G streptococci, with the Group A strain serving as the donor. Intergroup transduction experiments, in which Group A streptococci have successfully served as recipients, have not been previously described.

Earlier studies from this laboratory have dealt with the characterization of phages from nephritogenic and nonnephritogenic strains of Group A streptococci (13). The present communication records our studies of intergroup reactions and transduction of streptomycin and bacitracin resistance between Groups C and A streptococci, in which Group A streptococci are shown to be capable of receiving genetic information from a heterologous group of streptococci.

Materials and Methods

Phages.—Virulent Group A phages designated as A6, A12, A25, and A27 were kindly provided by Dr. W. R. Maxted (5). The virulent Group C phage designated as C1 was received from Dr. John Zabriskie (7).

The virulent Group A phage designated as A25 was used for all transduction experiments. The phage has been characterized by Malke (14) and by Read and Reed (15).

Temperate phages were obtained by spontaneous release and propagation or by induction with mitomycin C as previously described (13). The preparation of mitomycin C used here

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was from a different commercial source (Sigma Chemical Co., St. Louis, Mo.) than that previously used.

Streptococci.—

Donor strains: Transduction experiments included the following: strain GT-9440-str^R is a spontaneous mutant resistant to 2 mg/ml of streptomycin, which was selected from a Group A, M Type 6 strain. The original streptomycin-sensitive strain (GT-9440) was received from Dr. R. W. Reed (designated T-17 by him) and was isolated from a nonnephritic control subject (16). The streptomycin-sensitive strain was used as a donor control in some transduction experiments.

Strain 70-380-str^R is a spontaneous mutant resistant to 2 mg/ml streptomycin selected from a Group C strain. The original streptomycin-sensitive strain (70-380), isolated from a sputum culture of a patient at the University of Minnesota Hospitals, was used as a donor control in some experiments.

Strain 70-380-str^R-bac^R is a spontaneous mutant resistant to 80 μg/ml bacitracin and 2 mg/ml streptomycin which was obtained by selection from the streptomycin-resistant Group C strain (70-380-str^R).

Recipient strains: Transduction experiments included the following: a Group, A M Type 12 strain, designated K56, received from Dr. E. Kjems (4). A strain designated as GT-8747, which is the K56 strain lysogenized with a temperate phage isolated from strain GT-6527. This latter strain was a Group A, M Type 14/51 strain isolated at Warren Air Force Base, Cheyenne, Wyo. (17). A Group C strain, designated 70–152 obtained from Lowry Air Force Base, Denver, Colo.

Propagating strains.: Included are K56, GT-9440-str^R, and two additional strains. PF-4603-str^R is a spontaneous mutant resistant to 2 mg/ml streptomycin obtained by selection from a Group C strain, which was originally isolated from a throat culture on the Red Lake Indian Reservation, Red Lake, Minn. (18). A Group A, M Type 12 strain designated as GT-9372 which was obtained from Dr. W. R. Maxted in Colindale, England and was the original propagating strain for the virulent A12 phage (5).

Indicator strains: The host-range experiments included the standard indicator strains K56 (Group A) and PF-4603-str^R (Group C). Additional Group C strains used as indicators included 71 strains from various locations (Red Lake, 8 strains; Univ. of Minn. Hospitals, 32 strains; Lowry Air Force Base, 28 strains; and 1 strain each from Colindale, England, Bucharest, Rumania, and the Univ. of Minn. St. Paul campus) and isolated from various sources (throat, 59 strains, skin lesions, 3 strains, sputum, 2 strains; and 1 strain each from nose, urine, rectum, perineal drainage, throat from epidemic of acute nephritis, bovine mastitis, and unidentified site).

Group A indicator strains included 30 strains, approximately half of which were M Type 12 and 49 strains used in an earlier study (13), and the remainder were non-M-typable strains with T-patterns of 5/27/44, 14/19, and 8/25/Impetigo 19 received from U. S. Navy laboratories at Great Lakes, Ill. and at Orlando, Fla.

Media.—The two basic broth media used in these experiments were modifications of those described by Malke (11) and are here designated as no. 1 broth and no. 3 broth: no. 1 broth consists of proteose peptone no. 3 (Difco Laboratories, Detroit, Mich.) 6%, NaCl 0.3%, $CaCl_2$ 0.02%, Na_2HPO_4 0.07%, glucose 0.05%, and horse serum 5%. The broth was prepared and autoclaved without the $CaCl_2$, glucose, and horse serum. Concentrated solutions of $CaCl_2$ and glucose were sterilized by autoclaving and were added along with the sterile horse serum to the autoclaved broth. The final pH was approximately 7.1. No. 3 broth is a modification of no. 1 broth in which 1% proteose peptone no. 2 (Difco) is substituted for the proteose peptone no. 3, the glucose is increased to 0.1% and the horse serum to 10%, and 2.5% Heart Infusion Broth (Difco) is added.

No. 1 broth was used in phage propagation for all Group A to Group A transduction ex-

periments and in phage propagation for transduction of streptomycin resistance from Group C to Group A streptococci in the presence of phage antibody. No. 3 broth was used in propagation of phage for Group C to Group C transduction experiments and in phage propagation for Group C to Group A system without phage antibody. No. 3 broth was also used for growing the recipient strains for transduction experiments. Neopeptone (Difco) 1% was added to the no. 3 broth for growing the recipient strain in some Group A to A experiments.

Plates for selection of transductants consisted of three layers containing no. 3 broth with the following modifications and additions: Bottom layer (total volume approx. 30 ml): agar (Difco) 2%, Heart Infusion Broth 0.5%, and horse serum 2%. For the Group C to C system, horse serum was omitted from this layer. Middle layer (total volume 5 ml): 3 ml consisting of agar 0.7%, Heart Infusion Broth 1%, and horse serum 20% mixed with 2 ml of the transduction mixture. For the Group C to C system, horse serum was omitted from this layer. Top layer (total volume 8 ml): agar 0.7%, Heart Infusion Broth 1%, horse serum 5%, and streptomycin, 1,000 μg/ml for Group A to A system and 1,500 μg/ml for other systems, or bacitracin 40 μg/ml.

For the host range experiments, agar plates were prepared containing proteose peptone no. 2 2%, NaCl 0.3%, Na_2HPO_4 0.07%, $CaCl_2$ 0.02%, glucose 0.05%, horse serum 5%, agar 1%, and hyaluronidase (Sigma Chemical Co.) at final concentration of 68 μg/ml. Sterile horse serum and separately autoclaved concentrated solutions of $CaCl_2$ and of glucose were added after autoclaving as previously indicated, along with the appropriate amount of a concentrated solution of hyaluronidase sterilized by filtration. No. 1 broth was used for growing propagating strains and for growing strains to be inoculated onto plates for streptococcal lawns. No. 3 broth was used for propagating phage on Group C streptococci. No. 1 broth containing hyaluronidase (Sigma), 40 μg/ml, was used for propagation of virulent phages on Group A streptococci.

Propagation of Phages.—One drop of a broth culture of the propagating strain (which had been quick frozen in log phase and thawed just before use) was added to 5 ml of broth (see above) and incubated at 30°C for 18 h. Propagations were made in broth at 37°C or 26°C and for 5–18 h as indicated under results. A typical preparation contained 1 ml of an 18 h streptococcal culture and 5–10 ml of high titer lysate in 100 ml of broth. Hyaluronidase (Sigma) at a final concentration of 40 μg/ml was added in some instances as indicated in the results section. Phage lysates were sterilized by filtration through a Millipore 0.45 μm HA filter (Millipore Corp., Bedford, Mass.) Phage lysates to be used in transduction experiments were quick frozen and stored up to a year at −70°C.

Titration of Phage Lysates and Determination of Host Range.—Indicator lawns were prepared as previously described (13) except that plates were dried at 34°C. 10-fold dilutions of phage, ranging from undiluted to 10^{-4}, were applied with a Lidwell phage-typing machine (Biddulph and Co., Manchester, England) as previously described (19). Plates were incubated at 34°C overnight and were examined the following morning with the aid of a hand lens.

Transduction.—For transduction, the A-25 phage was propagated to high titer (10^9), filtered, and stored as indicated above. Phage lysates were thawed and irradiated before use in transduction experiments. Two General Electric G25T8 25 W ultraviolet germicidal lamps (General Electric Co., Lamp Glass Dept., Richmond Heights, Ohio) were used as a source of irradiation. The intensity of irradiation at the plate level was 7,000 ERG per s per cm^2 measured with a Blak-ray ultraviolet meter (Ultra-violet Products Inc., San Gabriel, Calif.). A 5 ml sample of thawed phage lysate was placed in a 15 × 100 mm Petri dish to a depth of 1–2 mm. The plates were swirled under the ultraviolet light for 1–16 min as indicated under results.

The transduction procedure used was similar to that described by Malke (11). Cultures of strains to be used as recipients were quick frozen in log phase. One drop of the thawed culture was inoculated into 10 ml of broth and grown for 18 h at 37°C.

1 ml of the 18 h culture was mixed with 1 ml of the irradiated phage and incubated at 37°C

for 20 min to allow for adsorption of phage to bacteria. 3 ml of soft serum agar with ingredients as described above were added to this mixture and the mixture (middle layer) poured onto the bottom layer. In some experiments, as indicated, up to 1 ml of antiserum for A-25 phage was incorporated in the middle layer. After drying on the bench the plate was incubated at 34°C for 2 h to allow for phenotypic expression of antibiotic resistance. The top layer containing selective antibiotic was then added to the plate. Streptomycin was used at a concentration of 1,000 μg/ml for the A to A transductions and at a concentration of 1,500 μg/ml for the C to C and C to A transductions. Bacitracin was used at a concentration of 40 μg/ml.

Plates were incubated at 34°C and were examined daily for 4 days for evidence of colony formation by transductants. The antibiotic resistance of the transductants was confirmed by serial transfer of selected colonies to antibiotic-containing media. Controls consisting of transducing lysates alone and recipient streptococci alone or in which antibiotic sensitive donors were used showed no colonies, with the exception of an occasional spontaneous mutant with the recipient strain (70–152) in the Group C to C system.

Phage Antiserum Production.—The A25 phage was propagated on strain 70-380-str^R to high titer (10^{10}). The lysate was filtered, then centrifuged in a Beckman Spinco ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) at 96,000 *g* for 4 h and washed once with 0.9% NaCl. The pellet was resuspended in saline at 10% of the original volume, filtered, and stored at +5°C. 1-ml samples of the suspension were injected into rabbits daily for 5 days with a second series of daily injections after a 1–2 wk interval. Rabbits were bled when a test bleeding gave serum which at a 1:10 dilution would reduce the plaque titer of a homologous lysate containing 10^7 plaque-forming units by 2 logs in 30 min.

RESULTS

Host Range of Virulent Phages on Strains of a Heterologous Group.—Earlier observations in this laboratory in which virulent and temperate phages from one serological group of streptococci were spotted onto lawns of another group of streptococci suggested some variability in the group specificity of streptococcal phages.

The ability of virulent Group A phages to plaque on lawns of Group C strains was examined systematically (Table I). Four virulent Group A phages (A6, A12, A25, and A27) and one virulent Group C phage (C1) were propagated on a Group C streptococcal strain (strain PF-4603-str^R) and applied to lawns prepared from a battery of 71 Group C strains obtained from a variety of sources. With the four virulent Group A phages, the percent of Group C strains whose lawns showed plaques varied from 34 to 48%, as compared to 70% with the virulent Group C phage. Among those Group C strains which did support plaque formation, a rather wide range in plating efficiency (plaque count per milliliter) was observed, with the virulent Group C phage as well as the virulent Group A phages. Plaque counts of 10^6 or greater were obtained by specific Group A phages on 15–35% of the 71 Group C indicator lawns. Comparable plaque counts were obtained with the virulent Group C phage control on 56% of the Group C lawns.

Since by adaptation or selection, the propagating strain might have an effect on the results obtained in such an experiment, further studies were done comparing lysates of virulent Group A phages prepared on one serological group

with respect to their plaquing efficiency on strains of the same group and on strains of the heterologous group (Table II). In examining preparations of the A6, A12, A25, and A27 phages propagated on both Group A and Group C strains, little or no difference was found in the ability of an individual lysate to form plaques on a Group A or a Group C lawn. The greatest difference observed was with an A25 lysate prepared on a Group C strain which showed a decreased plaque-forming efficiency on a Group A as compared with a Group C lawn (1+ log difference).

Further studies were done to examine the range in ability of these Group A phages to form plaques on a number of Group C lawns after propagation on

TABLE I

Ability of Virulent Group A Phages Propagated on Group C Strain to Plaque on Lawns of 71 Group C Strains

Virulent phage	Plaque counts* on control lawns		No. of Group C indicator strains showing			No. of Group C strains showing plaque counts* of						
	K56‡	PF4603-str^R§	No plaques or lysis	Lysis only	Plaques	10^3	10^4	10^5	10^6	10^7	10^8	10^9
A6	10^7	10^8	35	3	33 (47%)	6	3	3	2	14	5	0
A12	10^7	10^7	46	1	24 (34%)	5	6	2	8	3	0	0
A25	10^8	10^9	36	1	34 (48%)	4	3	2	6	11	3	5
A27	10^7	10^8	36	3	32 (45%)	4	3	2	7	14	2	0
C1 (Control)	10^4	10^9	21	0	50 (70%)	1	4	5	9	20	4	7

* Plaque count per milliliter: $10^3 = 1\text{–}9 \times 10^3$, $10^4 = 1\text{–}9 \times 10^4$, etc.

‡ Standard Group A indicator strain.

§ Group C strain used for preparing lysate.

Group A and Group C strains. From the previous experiment (Table I), 24 Group C strains which had shown plaque counts of 10^6 or higher with at least one of the Group A phage lysates were selected for use as lawns in these additional studies (Table III). For each of the four virulent Group A phages, the percent of Group C indicator lawns which showed plaques was unrelated to the serological group of the propagating strain. With the A6 and A25 phages, plaque counts of 10^6 or greater were seen with equal frequency on the Group C lawns regardless of the group of the propagating strain. With the A12 phage, lysates produced by propagation on a Group A strain yielded plaque counts of 10^6 or greater more frequently than those produced by propagation on a Group C strain. The reverse was found with the A27 phage.

Lysis of Streptococcal Lawns by Temperate Phage Lysates of a Heterologous

Group.—In view of the finding that virulent Group A phages form plaques on lawns of a number of Group C streptococcal strains, the ability of undiluted temperate phage lysates to lyse lawns of a heterologous streptococcal group was

TABLE II

Group A Virulent Phages Propagated on Group A and C Strains: Comparison of Ability to Form Plaques on Group A and Group C Indicator Lawns

Virulent phage	Propagating strain*	Indicator strain‡	Dilutions of phage lysate				
			Undil.	10^{-1}	10^{-2}	10^{-3}	10^{-4}
A6	Group A	Group A	Cl§‖	Cl	Cl	+++	+
		Group C	Cl	Cl	Pl	+++	++
	Group C	Group A	Cl	Cl	Pl	+++	+
		Group C	Cl	Cl	Pl	+++	+
A12	Group A	Group A	Cl	Cl	Pl	++++	++
		Group C	Cl	Cl	++++	++++	0
	Group C	Group A	Pl	++++	++	+	0
		Group C	Pl	++++	+++	+	0
A25	Group A	Group A	Cl	Cl	Cl	Cl	+++
		Group C	Cl	Cl	Cl	Pl	++
	Group C	Group A	Cl	Cl	+++	0	0
		Group C	Cl	Cl	Pl	+++	+
A27	Group A	Group A	Cl	Pl	++	+	0
		Group C	Pl	++++	++	+	0
	Group C	Group A	Cl	Cl	Pl	+++	+
		Group C	Cl	Cl	Pl	+++	+

* Propagating strains were GT-9440-str^R and GT-9372 (Group A) and PF4603-str^R (Group C). See Table III footnote for details.

‡ Indicator strains were K56 (Group A) and PF-4603-str^R (Group C).

§ Cl, Complete lysis; Pl, Partial lysis; ++++, >100 plaques; +++, 51–100 plaques; ++, 11–50 plaques; +, 1–10 plaques; 0, no lysis or plaques.

‖ A ++ recording at a dilution of 10^{-4} corresponds to a plaque count of 7.5×10^7 per milliliter.

examined (Table IV). In testing the ability of temperate phages from Group A strains to lyse Group C strains, considerable variability was found. Thus, a single Group C strain was sensitive to 67% and 83% of phage lysates freshly induced from Type 12 and Type 49 strains. However, when two phages obtained by spontaneous release from a Type 12 and a Type 49 strain and propa-

gated on a Group A strain were spotted on 64 Group C indicator strains, only 5% and 19% of combinations showed lysis. Moreover, when temperate phage lysates prepared in this manner from 20 different Group A strains of a variety of serological types were applied to lawns of 11 Group C indicator strains, lysis was observed in less than 1% of combinations.

In a more limited experience with temperate Group C phages, lysates obtained by mitomycin induction of 10 Group C strains rarely lysed Group A lawns (approx. 1% of combinations). However, an individual lysate obtained

TABLE III

*Comparison of Group A Virulent Phages Propagated on a Group A Strain with Same Phages Propagated on a Group C Strain: Ability to Form Plaques on Lawns of 24 Group C Strains**

Virulent phage	Propagating strain‡	Plaque counts on control lawns		No. of Group C indicator strains showing			No. of Group C strains showing plaque counts of					
		K56	PF-4603-str^R	No plaques or lysis	Lysis	Plaques	10^3	10^4	10^5	10^6	10^7	10^8
A6	Group A	10^7	10^7	4	0	20 (83%)	1	0	2	5	9	3
	Group C	10^7	10^7	3	1	20 (83%)	0	1	2	5	11	1
A12	Group A	10^7	10^7	5	1	18 (75%)	1	2	2	5	8	0
	Group C	10^6	10^6	4	0	20 (83%)	2	2	11	4	1	0
A25	Group A	10^8	10^7	1	0	23 (96%)	0	2	2	2	12	5
	Group C	10^6	10^7	2	1	21 (88%)	0	0	2	8	11	0
A27	Group A	10^6	10^6	5	0	19 (79%)	1	6	7	4	1	0
	Group C	10^7	10^7	2	0	22 (92%)	2	1	3	9	7	0

* See footnotes for Table I.

‡ The Group A propagating strain was GT-9440-str^R for the A6, A25, and A27 phages and GT9372 for the A12 phage. The Group C propagating strain was PF-4603-str^R.

from one Group C strain was unusual in that it lysed 53% of 30 Group A indicator strains. A temperate phage lysate obtained by spontaneous release from a Group C streptococcal strain and propagation on a Group A strain lysed 6 indicator lawns from 15 different Group A strains (40% of combinations). When propagated on a Group C strain, this phage lysed only 1 of the 15 Group A indicator strains (7%).

In the experiments dealing with temperate phages, it is difficult to assess the significance of the lysis seen since, without titration or attempts to propagate, it is impossible to distinguish lysis from without or the effect of a phage-associated lysin from phage replication.

Intragroup and Intergroup Transduction Systems.—The finding that virulent Group A phages would often form plaques on Group C strains as well as on

Group A strains suggested that they may serve as agents in transduction between streptococcal strains of both homologous and heterologous serological groups. As will be detailed below, successful transduction of streptomycin-resistance was achieved between Group A strains, between Group C strains, and also from Group C to Group A strains. In addition, bacitracin-resistance was transduced from a Group C to a Group A strain.

TABLE IV

Lysis of Streptococcal Lawns by Undiluted Temperate Phage Lysates Obtained from Streptococci of a Heterologous Group

Temperate phages				Indicator strains		Phage-indicator combinations		
Strain source		Lysate prepared by	No. tested	Group	No. tested	No. tested	With lysis or plaques	
Group	Type						no.	
								%
A	12	Mitomycin induction	21	C	1*	21	14	67
A	49	Mitomycin induction	6	C	1*	6	5	83
A	12	Propagation on Group A strain§‖	1‡	C	64	64	3	5
A	49	Propagation on Group A strain§‖	1¶	C	64	64	12	19
A	Mixed	Propagation on Group A strain§‖	20	C	11	220	2	1
C	—	Mitomycin induction	10	A	7	70	1	1
C	—	Mitomycin induction	1**	A	30	30	16	53
C	—	Propagation on Group A strain§‖	1**	A	15	15	6	40
C	—	Propagation on Group C strain‖‡‡	1**	A	15	15	1	7

* Strain PF-4603.

‡ From strain GT-2172, isolated from patient with acute nephritis in Cleveland.

§ Strain K56.

‖ Spontaneously released phage propagated by infection.

¶ From strain GT-8760, isolated from epidemic of pyoderma at Red Lake in 1966.

** From strain GT-9934, isolated from guinea pig and obtained from Bucharest.

‡‡ Strain GT-9930, isolated from epidemic of nephritis in Bucharest.

Initial experiments were done to examine the effect of ultraviolet irradiation of phage lysates to be used in transduction and to determine the optimal irradiation time for the various transducing systems. Experiments were also done to examine the effect of variation in the temperature of lysate production on the frequency of transduction.

As shown in Table V, ultraviolet irradiation of the transducing phage (A25) increased the number of transductants obtained in all of the transduction sys-

tems which were examined. The optimal irradiation time varied from one system to another. A longer irradiation time was required for best yields in the Group A to A system, whereas shorter irradiation times appear to be optimal in the Group C to C and the Group C to A systems.

As shown in Table VI, in all of the transducing systems examined, a higher frequency of transduction was obtained when the transducing lysate (A25) was prepared at 26°C rather than at 37°C. In our experience, the effect of lowering the propagation temperature of the transducing lysate was less impressive in the Group A to A than in the other two systems. With the A to A system the number of transductants obtained was sometimes less at the lower temperature even

TABLE V

Number of Transductants per Plate as Related to Irradiation of Transducing Lysate*

Transducing system‡		Irradiation time					
Donor strain	Recipient strain	0	1	2 *min*	4	8	16
Group A§	Group A‖	792	ND¶	ND	864	2,112	103
Group C**	Group C‡‡	6	288	440	200	29	ND
Group C**	Group A‖	3	21	24	39	20	1

* Each plate contained 2 ml of transducing mixture. (See Methods.)
‡ Streptomycin resistance was marker transduced in all instances.
§ Strain GT-9440-str^R.
‖ Strain K56.
¶ ND, Not done.
** Strain 70-380-str^R.
‡‡ Strain 70-152.

though the transduction frequency was higher. This apparent discrepancy can be explained by the difference in phage yield at the two temperatures, which appeared to be greater with the Group A to A than with the other systems.

Details of the Group C to C and Group C to A transduction experiments in which the most nearly optimal conditions were used and the highest yields of transductants were obtained are presented in Table VII. Larger numbers of transductants and higher transduction frequencies were seen with the intragroup than with the intergroup transduction systems (Tables VI and VII). In comparing transduction systems in which the donor and the recipient strain were of the same serological group, the Group A system was found to be more efficient than the Group C system. Fewer transductants and appreciably lower transduction frequencies were obtained with the intergroup (C to A) system. With the intergroup transduction system, the streptomycin resistance marker was transduced more efficiently than the bacitracin marker. Indeed, it was

necessary to add phage antibody to the transducing system to obtain transductants in the latter system.

In many instances a higher transduction frequency was observed with phage lysates which had been aged for several months in comparison with those which were used within 1 wk or 1 mo of preparation. These aged preparations usually had a somewhat lower phage titer and consequently the multiplicity of infection

TABLE VI

Number of Transductants and Transduction Frequency As Related to Temperature of Propagation of Transducing Lysate

Transducing system*						
Donor strain	Recipient strain	Propagation temperature	Phage titer‡	MOI§	No. of transductants‖	Transduction frequency¶
Group A**	Group A‡‡	26°C	6×10^7	0.03	1,013	1.7×10^{-5}
		37°C	1.5×10^8	0.08	1,558	1.0×10^{-5}
Group C§§	Group C‖‖	26°C	1.8×10^9	6.0	440	2.4×10^{-7}
		37°C	2.1×10^9	7.0	26	1.2×10^{-8}
Group C§§	Group A¶¶	26°C	1.8×10^9	0.3	29	1.6×10^{-8}
		37°C	2.1×10^9	0.4	2	9.5×10^{-10}

* Streptomycin resistance was marker transduced in all instances.

‡ Phage titer: plaque-forming units (PFU) per mililiter before irradiation.

§ Multiplicity of infection (MOI) = plaque-forming units (PFU)/colony-forming units (CFU). To obtain MOI per cell, it is necessary to adjust for the chain length. The mean chain length for the Group A recipient strain K56 grown in no. 3 broth was 3.87.

‖ Per plate. (Each plate contains 2 ml of transducing mixture. See Methods.)

¶ Transductants per plaque-forming unit.

** Strain GT-9440-str^R.

‡‡ Strain K56.

§§ Strain 70–380-str^R.

‖‖ Strain 70-152.

¶¶ Strain GT-8747.

was often lower in the experiments in which they were used. It is therefore impossible to be certain whether this was an effect of age per se or of the lower multiplicity of infection which Malke (11) has reported to be an important factor in the frequency of transduction in the Group A to A system.

DISCUSSION

The original view that streptococcal phages are rather strictly group specific (6, 20) was based on examination of a limited number of strains. Krause (6)

found no lysis of 10 Group C strains by four virulent Group A phages (including 3 of the 4 reported here) whereas 9 of the 10 Group C strains were lysed by the virulent Group C phage C1. In his study, inactivation of the C1 phage by Group C carbohydrate prepared from a *Streptomyces albus* enzyme extract strengthened the concept of group specificity of this system and suggested that the group-specific carbohydrate may serve as the phage receptor site. However, it has not been possible in his or subsequent studies (7) to inactivate Group A phages with Group A carbohydrate.

Several findings in this early report by Krause (6) support a lack of strict

TABLE VII

*Details of Group C to C and Group C to A Transducing Systems in Experiments Yielding Highest Number of Transductants**

Transducing system								No of transductants	Transduction frequency
Marker	Donor strain	Recipient strain	Propagation temperature	Irradiation time	Strep. count‡	Phage titer	MOI		
Streptomycin resistance	Group C§	Group C‖	26°C	2 min	2.1×10^9	4.5×10^9	2.0	839	1.9×10^{-7}
Streptomycin resistance	Group C§	Group A¶	26°C	4 min	3×10^9	9×10^8	0.3	86	9.6×10^{-8}
Bacitracin resistance**	Group C§§	Group A‖‖	26°C	4 min	1.8×10^9	9×10^9	5	38	4.2×10^{-9}

* See footnotes for Table VI.
‡ Colony-forming units (CFU) per milliliter added as recipient strain.
§ Strain 70-380-str^R.
‖ Strain 70-152.
¶ Strain GT-8747.
**Phage-antibody used in this system only.
§§ Strain 70-380-str^R-bac^R.
‖‖ Strain K56.

group specificity: (*a*) the observation that the C1 phage did form small indistinct plaques on a Group A strain (T25/41) when plated in high concentration and (*b*) lysis of a Group A strain and its Group A variant by a virulent Group A phage, A1.

The observation by Fischetti and Zabriskie (7) that the virulent Group A phage, A25, absorbs to a strain of Group C and a strain of Group G streptococci almost as well as to a strain of Group A streptococci also suggested a certain amount of nongroup specificity, at least with respect to this initial phase of phage-host interaction. These authors found that only one of three virulent Group C phages was inactivated by enzymatically prepared group-specific carbohydrate and that none was inactivated by chemically extracted group-specific carbohydrate. These findings indicated that virulent Group C phages

differ with respect to their inactivating site on the streptococcal cell wall. The additional observation that Group A virulent phages failed to adsorb to heat-killed Group A streptococcal cells suggested that other factors, apparently factors produced by the living cell, may be needed for inactivation of these phages.

Subsequent studies by Colón et al. (21) showed that a temperate phage isolated from a Group G streptococcus formed plaques on a few strains of Group A and Group C streptococci. In the same study it was recorded that several virulent and one temperate Group A phage would lyse certain Group C and Group G strains. A further report by the same group of authors (12) indicated a high frequency (74%) of lysis of Group C streptococcal strains by the virulent Group A phage, A25, but the frequency of demonstration of plaques was not indicated. Moreover, propagation on the lysed strains was achieved only in certain instances although the authors presented some indirect evidence that an infectious cycle rather than lysis from without or production of a cell wall-lysing enzyme was responsible for the lysis observed on the indicator lawns.

The findings presented here indicate that intergroup reactions can be rather frequently demonstrated between virulent Group A phages and Group C streptococci. Distinct plaque formation, often in rather high titer, was found on 34–48% of lawns prepared from Group C strains. Propagation of these virulent Group A phages on a heterologous strain (Group C) did not consistently alter the plaque titers as demonstrated on a Group A and a Group C indicator strain. The percent of Group C strains showing plaques with these virulent Group A phages was essentially the same regardless of whether they were propagated on a Group A or a Group C strain.

The situation with respect to intergroup reactions of temperate Group A and Group C phages is less clear. The studies of Colón et al. (12) indicate lysis of a moderate percentage of Group C strains by one Group A temperate phage and infrequent lysis of Group C strains by two other Group A temperate phages. Our studies showed a high frequency in some circumstances and a low frequency in others. A high frequency was found with a collection of temperate Group A phages when a specific Group C indicator strain was used and with a number of Group A indicator strains when certain lysates of a temperate Group C phage were applied. Some variations were noted between temperate Group A phage lysates prepared by induction and those prepared by propagation (i.e. infection) and some differences in host range were observed when a temperate Group C phage was propagated on a strain of homologous or heterologous serologic group. These findings suggest the possibility of host-controlled modification and restriction, a phenomenon well known with other bacterial viruses and recently described in Group A streptococci (22). Limited studies with Group C temperate phages also indicated an ability of at least one induced and one propagated phage to lyse a significant number of Group A indicator strains. Other observa-

tions[1] suggest that a Group A indicator strain sensitive to a temperate Group C phage will develop lysogenic immunity to this phage when lysogenized with a number of Group A temperate phages, again indicating some lack of group specificity. Further studies are needed to define the nature and the frequency of intergroup reactions with temperate phages. The recent demonstration that certain temperate phages, in contrast to earlier reports (4, 20), require hyaluronidase[1] may extend the host range of these phages and thereby facilitate such studies.

Genetic exchange involving Group A streptococci has been rather infrequently demonstrated and the mechanisms available may indeed be limited in comparison with other organisms. In transformation experiments, Group A streptococci have been employed as DNA donors but Group A streptococci have never successfully served as recipients (23). The reasons for this failure are not clear. Production by Group A streptococci of large amounts of several different nucleases which attack DNA has been proposed as an explanation (24), but attempts to transform Group A streptococci by neutralizing the activity of these nucleases have not been successful (25).[2]

Bacteriophages as vectors for transmission of genetic information in Group A streptococci have been more rewarding. A role for temperate phage in directing scarlatinal toxin production or in release of toxin has been suggested by the studies of Zabriskie (9). Lysogenization of a non-toxin-producing Group A strain resulted in the appearance of erythrogenic toxin, perhaps by a mechanism of phage conversion (26). Transduction of streptomycin resistance between strains of Group A streptococci has been described by Leonard et al. (10). These workers (10) and Malke (11) have provided evidence for the protective role of the bacterial hyaluronic acid capsule in transduction by Group A virulent phages. In the same study, Malke has shown that it is possible to improve the efficiency of the Group A to A transduction system by using a double temperature-sensitive phage mutant at the restrictive temperature and also by decreasing the propagation temperature of the transducing lysate. In addition, ultraviolet irradiation has been shown to increase the frequency of transduction (27, 28).

Our studies confirm the transduction of streptomycin resistance between Group A streptococci (10, 11) and demonstrate that transduction of this marker can also be accomplished between strains of Group C streptococci, using the same transducing phage (A25) which has proved most efficient in the Group A to A system (10). The increase in transduction frequency which can be achieved in the Group A to A system by lowering the propagation temperature (11) and by ultraviolet irradiation of the transducing lysate (27, 28) has been shown to apply in the new systems reported here, Group C to C and Group C to A transduction.

[1] Skjold, S., and L. W. Wannamaker. Unpublished observation.

[2] Wannamaker, L. W. Unpublished observation.

Intergroup transduction of streptomycin resistance, with a strain of Group A streptococcus as the donor and a strain of Group G streptococcus as the recipient, has been recently reported by Colón et al. (12). In the independent studies described here we have also demonstrated intergroup transduction, using a transducing lysate prepared from the same phage (A25) but with a strain of Group C streptococcus as the donor and a strain of Group A streptococcus as the recipient. Both streptomycin resistance and bacitracin resistance were used as markers in these Group C to A transduction reactions. The significance of the observations reported here lie not so much in the description of additional transformation systems but in the first demonstration of transduction between different groups of streptococci in which the Group A strain has been the recipient of genetic information.

SUMMARY

In a study of intergroup reactions, four virulent Group A streptococcal phages were found to form plaques in high titer on lawns prepared from a number of Group C streptococcal strains. Whether the phages were propagated on the homologous (Group A) strain or a heterologous (Group C) strain did not appear to influence consistently the plaque-forming efficiency on lawns prepared from a homologous (Group A) or a heterologous (Group C) strain or to alter significantly the percent of Group C strains which showed plaque formation.

Considerable variability was found in the ability of temperate phages to lyse strains of a heterologous group. A single Group C indicator strain was lysed by a high percentage of freshly induced temperate Group A phages. A single temperate Group C phage lysed a significant proportion of Group A strains when freshly induced or when propagated on a Group A strain.

Intragroup transduction of streptomycin resistance was demonstrated between Group C strains. Intergroup transduction of streptomycin resistance and also bacitracin resistance was achieved between Group C and Group A streptococci. These observations provide evidence that Group A streptococci can serve as recipients in intergroup transmission of genetic information.

Ultraviolet irradiation of the transducing lysate and lowering the propagation temperature of the transducing lysate increased the frequency of transduction in both the intragroup and intergroup transduction systems.

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