B. pertussis does not interfere with localization of antibodies in tissues and that it does not make the tissues hypersensitive to an antigen-antibody reaction. This has been previously suspected since it has been observed that, although there is a marked difference in amount of antibody needed to produce fatal anaphylaxis, there is hardly any observable difference in amount of antibody required to produce anaphylactic symptoms such as ruffling of the hair, forced respiration, slight cyanosis, lethargy, etc.(3).

Summary. Mouse antibody was found to be more effective than rabbit antibody in sensitizing the mouse uterus to the specific antigen. It was also found that *B. pertussis* cells do not affect the sensitization of the mouse uterus when the mouse is sensitized by passive means. When the active method of sensitization of mice was employed, however, *B. pertussis* accelerated the appearance of sensitivity of the uterus as demonstrated by the Schultz-Dale reaction.

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New Phages Active Against Staphylococci that Are Resistant to the Conventional Phage Set.* (26243)

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During studies on staphylococcal infections at Boston City Hospital, it was found that a large and probably increasing number of infections were caused by staphylococci that were insensitive to the phages generally used for typing, even at concentrations of 1000 x the routine test dilution (RTD)(1). A large proportion of these strains had been cultured from serious infections such as septicemias and pneumonias and they were usually resistant to several of the antibiotics in general use. The isolation and some of the properties of 5 new phages that were found to be useful in typing these "nontypable" (NT) strains is described in this paper. Some findings with 2 other new phages related to 80/81 are also included.

Isolation of New Phages. For this purpose 108 nontypable strains cultured from different sources and with a varying antibiotic sensitivity were selected. They were all cross-cultured by the method of Fisk(2). An overnight culture of each strain was flooded on plates of trypticase soy agar and the inoculum was allowed to dry. A drop of a broth culture of each of the other strains was then applied on the surface in a similar manner as in the bacteriophage typing. The plates were read after incubation at 30°C for 18 hours. Some (24 of 5040 combinations) of these cross-cultures showed evidence of lysis. The areas of good lysis (7 in all) were

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scraped off together with some of the underlying agar, suspended in 2 ml broth and shaken vigorously. Each supernate, after centrifugation at 3000 rpm for 20 minutes was spotted on the 2 members of the combination, on a new plate, to determine the lysogenic (resistant) and the indicator (sensitive) strain. After filtration to remove the lysogenic strain, the phage was purified by picking a single plaque which was further propagated.

Propagation of Phages. After a few passages on plates to increase the yield of phage, propagation in broth was used and proved satisfactory. To 20 ml broth in flasks was added 1 ml sterile solution of 1% $CaCl_2$, 0.2 ml 4-hour broth culture of the propagating strain and 0.5 ml of undiluted phage. The cultures were incubated at 37°C for 6 hours. At that time a moderate growth was generally observed. After storage at 4°C overnight the cultures often turned clear. Serial 10-fold dilutions of the supernates after centrifugation were then tested on the propagating strain. A satisfactory titer was thus obtained.

Each of the 7 phages thus isolated was tested on all the new propagating strains, all of which were resistant to the conventional phages; 5 of the new phages proved to be distinct, each lysing only its own propagating strain. These 5 phages were selected for further study; their origins were:

Provisional designation of phage	Lysogenic strain		Indicator strain	
	No.	Source	No.	Source
A	206/60	Sputum	38/60	Sputum
B	258/60	Abscess	57/60	Stool
C	187/60	Sputum	113/60	Sputum
D	286/59	Urine	157/59	"
E	199/60	"	222/60	Abscess

Two other phages, provisionally called F and G, were isolated from colonies of the original culture plates of clinical specimens; both had caused a motheaten appearance of a few among the many colonies of *Staph. aureus* on these plates.§ Phage F was obtained from a motheaten colony grown from an ear discharge by suspending it in a small amount of broth. After vigorous shaking, the suspension was centrifuged; colonies of normal appearance on the same plate proved to be sensitive to the phage in the supernate, in contrast to the subculture from the nibbled colony which was resistant. A normal looking colony was used as the propagating strain (PS) and the phage propagated as described above. With the standard phages, both the normal colony (the PS) and the nibbled colony (the carrier) were lysed by phage 80 and weakly by phage 52.

Phage G was isolated in the same manner from a sputum culture. A few colonies on a plate had a motheaten appearance while the rest looked normal; the latter were sensitive to the phage whereas the nibbled colony was not. A normal colony was chosen for further propagation of the phage; it typed with the conventional set of phages as 80/81/KS6/42B, but the carrier colony was lysed only by phage 80. PS-F was lysed by phage F but not by G; PS-G was lysed by F and G, both of which proved to be closely related to 80 and 81 and they nearly always gave identical lysis of a large number of strains tested. Phage G thus appears to be of little significance and was eventually discarded.

Results. Lytic spectrum of new phages, as compared to standard phages. The lysis produced at RTD by the new phages and the standard set of phages used at this laboratory (3-5) on the propagating strains of all these phages is shown in Table I. Besides full lysis of their own PS, the new phages showed the following spectrum: Phage A lysed PS-6 and 83; phages B and E lysed no others; phage C produced weak lysis in PS-6 and 7; phage D lysed PS-83; phage F lysed PS-80, 81, 73, 42B and G; and phage G lysed PS 80, 81, 73 and (weakly) 42B.

PS-A, B, C, D, and E were each lysed only by its corresponding phage; PS-F was lysed by phages 80 and F, and PS-G by 80, 81, KS6 and G. The phages A to E thus have a narrow host range which would make them suitable for typing purposes. A, C, and D, seem to be related to the group III phages, whereas F and G are related to phages 80

§ We are indebted to Miss A. Kathleen Daly, Bacteriology Lab., Boston City Hospital, for detection of these phages.

TABLE I. Lytic Spectrum of 7 New Phages on Their Propagating Strains: Comparison with Conventional Phages.

Propagating strains	Conventional phages																							New phages						
	29	52	52A	79	80	81	3A	3B	3C	55	71	6	7	42E	47	53	54	73	75	77	83	42D	187	42B	KS6	A	B	C	D	E	F	G
29	+																															
52		+																														
52A/79			+	+	—																											
80					+	+																			+						+	+
81					+	+																			+						+	+
3A							+																									
3B								+	—	+																						
3C								+	+		+																					
55								+	—	+	+																					
71								+	—	+	+																					
6												+	—		+	—	+	—	+							+		—				
7													+															—				
42E														+																		
47															+	—			+		—											
53																+	—		+	—	+											
54															+	+	+		+	—	+											
73								+	+			+	+	+	+		+	+	+					+							+	+
75																			+													
77																				+												
83												+			+	+				+	+					+			+			
42D																						+										
187																							+									
42B						+																		+	+						+	—
KS6		—	—		—																				+							
A																										+						
B																											+					
C																												+				
D																													+			
E																														+		
F					+																										+	
G					+	+																			+						+	+

+ = good lysis; — = weak or poor lysis.

and 81 and phages B and E did not lyse any other PS.

Typing with new phages of strains nontypable with standard phages. 158 strains isolated in 1959-1960 and nontypable with the standard phages even at 1000 x RTD were tested with the new phages A through G. Since phages F and G gave almost identical results, the latter is not included in the following analysis. Most of these untypable strains were identified during another study (1); 123 were from clinical sources (22 from outpatients and 101 from hospitalized patients) and 35 were obtained at autopsy. The results are listed in Table II. Of the 158 strains, 70 were lysed by phage A alone; 35 of these by the RTD and 35 only by 1000 x RTD; 4 to 11 strains were lysed by each of the other new phages. Only 7 strains were lysed by more than one of these 6 phages; 42 (27%) of the conventionally nontypable strains were negative with the new phages as well. Type A was particularly common among the strains isolated from autopsy material, representing 72% of all nontypable autopsy strains; the negatives, on the other hand, predominated among strains from outpatients. The hospitalized cases of type A infections were distributed among 19 wards, 8 of them in a single medical ward, and strains from the latter were resistant to all the common antibiotics except novobiocin.

Antibiotic sensitivity of strains of types A to F was tested with 2-fold dilutions in agar, using an inoculum of about 10^6 organisms in a replica-inoculating apparatus(6). The results are summarized in Table III. Strains of each type generally had a characteristic antibiotic spectrum. Type A strains were nearly all resistant to penicillin, streptomycin, tetracycline and erythromycin; 43% of them were also resistant to chloramphenicol and 30% to novobiocin.‖ Strains of type B were generally resistant to penicillin, but sensitive to the others; those of type C were generally resistant to all antibiotics, whereas most of those of type F and all of type E were sensitive. These results suggest the possible usefulness of the new phages for typing.

TABLE II. Susceptibility to 6 New Phages of Pathogenic Staphylococci Nontypable with Conventional Phages (1000 × RTD).

New phages lysed	Source of strains: Clinical* OP	Clinical* IP	Autopsy	Total: No.	Total: %
A†	1	44	25	70	44.3
B	2	9		11	7.0
C		7	2	9	5.7
D		5		5	3.2
E	1	3		4	2.5
F	2	7	1	10	6.3
Misc.‡	2	5		7	4.4
Neg.	14	21	7	42	26.6
Total	22	101	35	158	100
% neg.	64	21	20	27	

* OP = outpatient case; IP = inpatient (hospital case).

† Includes those lysed weakly (or only by 1000 × RTD).

‡ Various combinations of these phages.

TABLE III. Antibiotic Resistance of Strains Nontypable with Conventional Phages but Lysed by 6 New Phages.

New phages lysed	No. of strains tested	No. resistant to:* P	S	T	CM	E	N
A†	70	68	68	69	30	66	21
B	11	8	0	0	0	0	0
C	9	9	8	8	7	8	6
D	5	5	2	3	1	2	1
E	4	0	0	0	0	0	0
F	10	6	3	3	2	2	1
NT	42	21	6	7	2	6	0

* Not inhibited in a 2-fold dilution test by (μg/ml): Penicillin 0.8 (P); streptomycin 12.5 (S); tetracycline 12.5 (T); chloramphenicol 25 (CM); erythromycin 1.6 (E); novobiocin 1.6 (N).

† Includes those lysed weakly or by 1000 × RTD.

Activity of the new phages on strains typable with the standard phages. 1. *Relation to "Group 80/81."* 116 strains that previously had typed as 80/81/KS6, 80/KS6, 80, 80/81, 81 or KS6 (here referred to as "group 80/81" for convenience) were tested with the new phages. All except 4 strains were lysed by phage F; 108 were lysed only by F, and 4 by another of these phages in addition. Two strains were lysed only by phage B and 2 were not lysed by any of them. These results demonstrate the close relationship of phage F to phages 80/81; the identity is not complete, however. Ten strains that did not

‖ For definition of "resistance" see footnote in Table III. Values chosen represent the point where the cumulative percent distribution curve of sensitivity has flattened out, as shown previously(3).

TABLE IV. Activity of 6 New Phages on 100 Strains of Conventional Phage Patterns Other Than "Group 80/81."

Pattern with conventional phages	No. of strains tested	Pattern with new phages‡
Group I		
29*	8	B(1), B/F(3), F(1), NT(3)
52	2	B(2)
52A/79	11	B(10), D(1)
79	3	B(3)
Group II	10	NT(10)
Group III		
42E	6	B/D(2), NT(4)
6/47	2	NT(2)
7	11	C(9), C/D(2)
47/53/83	2	A(2)
53	8	A(8)
54	9	F(5), NT(4)
77	9	C(9)
6/47/53/54/75/77/83	4	C/D(4)
Others†	12	A/B/C/D(1), B(1), B/D(1), C(1), D(1), F(1), NT(6)
Miscellaneous		
187	3	NT(3)

* Also includes those lysed by other phages in addition.
† 10 different patterns of Group III phages.
‡ No. of strains in parentheses.

respond to any standard phages, including 80, 81 and KS6, were lysed by phage F (Table II).

2. *Relation to other phage groups.* One hundred randomly chosen strains of a variety of other phage types were similarly tested with phages A to F; a variety of responses emerged as shown in Table IV. Most strains in phage Group I were lysed by phage B. All 10 Group II strains tested were negative. Among 63 strains of Group III, 26 were lysed by phage C, 11 by A, 11 by D, 6 by F and 4 by phage B. Phage E which was active against only a small number of nontypable strains (Table II) had no activity against any strains of these 2 series.

Discussion. Phage typing is an important tool in study of staphylococci, particularly in epidemiological surveys. In the last few years the marked predominance of 80/81, often considered as a single type, has, however, somewhat decreased the value of this method. Another limitation is the nontypable strains; an appreciable proportion of strains are nontypable with this method and thus do not permit conclusions regarding epidemiological significance. The frequency of the nontypable strains generally varies between 10 and 20%. Since nontypable strains were most often recovered from healthy nasal carriers and less frequently from clinical lesions, they have been considered less virulent, and not much attention has been given to them.

A study of staphylococci recovered from patients admitted to Boston City Hospital from Sept. 1959 to Feb. 1960 disclosed a large, and probably increasing number of strains resistant to the standard set of phages used (20-30% of all strains). The infections caused by these organisms were often severe and included septicemias, pneumonias, etc. and this was also reflected by the high frequency of nontypable strains in postmortem cultures made at autopsy. Moreover, these staphylococci often turned out to be resistant to most or all of the antibiotics in general use.

The need for an improved phage typing technic was thus considered to be great. Of the two simplest methods available to find new phages, namely, adaptation of a known phage to a strain with low sensitivity to it or isolation of new "wild" phages, the latter method was chosen. For the crosscultures only nontypable strains were selected for the purpose of finding phages that attacked this group of strains. The attack rate in the crosscultures of such strains was low, only about 0.5%.

The 5 new phages that were selected proved to be useful for differentiation of strains within the group of hitherto nontypable strains. Together with another new phage, the effect of which was visible on primary culture from an ear discharge, the 6 phages were able to type 73% of 158 nontypable strains tested. One phage proved to be related to phages 80 and 81, but the other 5 seem to be quite specific. They attacked only a few of those strains that characterize the different standard phages, and, generally only one of them attacked the nontypable strains tested, thus giving 5 characteristic

"types." Provisionally the phages have been called A, B, C, D, E and F. A, C, and D, seem to be related to the Group III phages, F to 80/81, B to other Group I phages and E to none of the others.

Summary and conclusions. The isolation of 7 new staphylococcal phages is described: 5 of them appear to be quite distinct and specific and the other 2 are related to the 80/81 phages. With the 5 new and specific phages and one of the others it was possible to type 73% of 158 strains which were recently isolated from infected lesions, nontypable with the conventional set of phages and most of them resistant to the most frequently used antibiotics. These new phages may, therefore, prove useful for phage typing of pathogenic staphylococci.

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Comparative Activity of Various Penicillins Against Penicillinase-Producing and Nonpenicillinase-Producing Staphylococci.* (26244)

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The isolation of 6-aminopenicillanic acid (APA) from penicillin fermentations was heralded as an important discovery making possible the synthesis of many new penicillin derivatives which could not be prepared by direct biosynthesis.(1). The introduction, within less than a year, of two new, therapeutically useful semisynthetic penicillins(2, 3) with enhanced activity against penicillin-resistant staphylococci and the likelihood of the development fairly soon of other such penicillins with substantially or radically different antibacterial and other properties, has already resulted from this discovery. Comparisons of the true therapeutic value against human infections of the new semisynthetic penicillins with those already available, or which can be prepared by direct fermentation methods, is extremely arduous and some penicillins which have similar *in vitro* and pharmacologic properties, may be therapeutically indistinguishable. Nevertheless, any valid *in vitro* or *in vivo* comparisons may be useful in the long run in correlating chemical structure with therapeutic activity for the ultimate purpose of synthesizing "tailor-made" drugs. The results of comparisons of the absorption and excretion of the first of the semisynthetic penicillins with others prepared by fermentation(4,5), and of their *in vitro* activity against common bacterial pathogens (6), have already been reported from this laboratory. This paper deals with more detailed comparisons of the antistaphylococcal activity of a number of penicillins, including the two semisynthetic ones that were recently introduced.

Materials and methods. The strains of staphylococci used in this study were chosen from among recent isolates from infected le-

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