INVESTIGATIONS ON THE TYPING OF STAPHYLOCOCCI BY MEANS OF BACTERIOPHAGE

II. THE SIGNIFICANCE OF LYSOGENIC STRAINS IN STAPHYLOCOCCAL TYPE DESIGNATION

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Earlier work (Williams Smith, 1948) on the bacteriophage method of typing staphylococci of Wilson & Atkinson (1945) revealed that strains previously regarded as belonging to one phage type, phage type 42D, could be further classified into a number of types by the use of additional phage filtrates. Some strains hitherto regarded as untypeable strains were also susceptible to these filtrates. The strains were distinguished by the pattern of reactions that they gave with these filtrates; the non-lysogenic strains were susceptible to all of the filtrates and the lysogenic strains only to some of them. The acquired phage resistance of the lysogenic strains was shown to be responsible for making possible this further classification. As the 42D pattern was similar in appearance to the patterns of the sub-types of types 1, 2 and 3 of Wilson & Atkinson (Table 1), it was decided to investigate whether acquired phage resistance was responsible to any extent for the present classification of staphylococci by the bacteriophage method.

Table 1. *Bacteriophage type of designations of staphylococci* (Wilson & Atkinson, *Lancet*, 26 May 1945)

Phage type of coccus	Bacteriophage filtrates * (3A) 3/284	(3B) 3/211	(51) 51/145	(6) 6/3	(7) 7/4	(42B) 42/1163	(47) 47/36	(47C) 47/1163	(29) 29/33	(31) 31/18	(52) 52/144	(3A) 3/284	(3C) 3B/1339	(42C) 42A/1307	(44) 44/18	(44A) 44/373	(47) 47/761	(47B) 47/987	(47C) 47/1163	(51) 51/145	(52) 52/144	(52A) 52/925
1A	+	−	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1B	−	+	−	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1C	±	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2A	.	.	.	+	−	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2B	.	.	.	±	+	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2C	.	.	.	−	−	+	−	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2D	.	.	.	−	−	−	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3A	.	.	.	.	.	.	.	.	+	+	−	.	.	.	.	.	.	.	.	.	.	.
3B	.	.	.	.	.	.	.	.	−	+	−	.	.	.	.	.	.	.	.	.	.	.
3C	.	.	.	.	.	.	.	.	−	+	+	.	.	.	.	.	.	.	.	.	.	.
4	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.
5	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.
6	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.
7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.
8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.
9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.
10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.
11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.
12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.
13	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.
14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+

* The new names given to the bacteriophage filtrates by Wilson & Atkinson are shown in parentheses.
Confluent lysis = +; semi-confluent lysis = ±; lesser degrees of lysis or no lysis = −.

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TECHNIQUE

The technical procedures and nomenclature used in this work have been fully described in a previous paper (Williams Smith, 1948). Phages prepared during the course of these studies were designated by the number of the lysogenic strain from which the phage was originally derived, followed by the number of the strain upon which it was propagated. Resistant strains were designated by the number of the susceptible strain of staphylococcus followed by the number (in parentheses) of the phage to which it was made resistant.

Investigations were carried out by the method that had been successful in elucidating the relationship to each other of the strains that were susceptible to one or more of the 42D group of phages. Strains that were susceptible to one or more of a group of phages used in routine typing and that were distinguished from each other by the pattern of reactions that they gave with these phages were examined for lysogenicity to each other by the cross-culture method (Fisk, 1942). The 'crude phage' obtained from any pair of strains was propagated on the susceptible strain. A resistant variant of this strain to the 'crude phage' was prepared. A comparison of the lytic action of the group of test phages on the original lysogenic strain, the susceptible strain, and the resistant variant would then decide whether the difference in susceptibility of the lysogenic strain and the susceptible strain to the test phages was dependent only on the acquired phage resistance of the lysogenic strain. To quote a simple, but hypothetical example. Strain 1 was susceptible to phages A and B. Strain 2 was susceptible only to phage A. The resistant variant of strain 1 to the phage carried by strain 2, strain 1 (2/1), was also found to be susceptible only to phage A. Therefore, the difference in phage susceptibility of strains 1 and 2 to phages A and B was due solely to the acquired resistance of strain 2 to the phage it carried.

RESULTS

Phage 3A, 3B, 3C *and* 51 *group.* Six strains that were lysed by one or more of these phages were studied. Strains that were lysed completely by all four phages in their test dilutions were not encountered. All the strains except one, strain 284, were shown to be lysogenic. A study of the lytic action of these four phages in test dilution on the six strains and on some of the resistant variants that were prepared from some of these strains (Table 2) showed that acquired phage resistance was responsible for strains 1394, 1335, 145, 211 and 284 being considered as separate phage types. There was no direct evidence for including strain 1339 in this relationship; the resistant variant of strain 1335 to the phage carried by strain 1339, i.e. strain 1335 (1339/1335) was insusceptible, however, to phages 3A and 51.

Table 2. *The lytic action of phages of the* 3A *group*

	Phage filtrates in test dilution			
Strain no.	3A	3B	3C	51
1394	CL	CL	CL	++
1335	++	CL	CL	++
1339	–	+	CL	–
145	–	+	–	CL
211	–	CL	++	–
284	CL	++	–	+
Resistant strains				
284 (145/284)	–	+	–	+
145 (1394/145)	–	+	–	+
1335 (211/1335)	–	CL	++	–
1394 (145/1394)	–	+	–	++
1394 (211/1394)	–	CL	++	–
1335 (145/1335)	–	+	–	+
1335 (1339/1335)	–	CL	CL	–

CL = Confluent lysis with no secondary growth.
++ = Numerous semi-confluent plaques.
+ = Discrete plaques.
± = Less than twenty plaques.
The resistant strains are designated as indicated in the text.

Table 3. *The lytic action of phages of the* 6 *group*

	Phage filtrates in test dilution				
Strain no.	6	7	42B	47	47C
4	+	CL	CL	++	+
3	CL	±	CL	CL	CL
1417	–	+	CL	–	±
36	–	–	–	CL	CL
1163	–	–	CL	–	CL
Resistant strains					
3 (36/3)	–	–	–	CL	CL
4 (36/4)	–	–	–	++	+
4 (1163/4)	–	–	CL	±	+
1163 (1417/1163)	–	–	CL	–	+
3 (4/3)	CL	–	CL	CL	+
4 (1417/4)	–	CL	CL	–	–

Phage 6, 7, 42*B*, 47 *and* 47*C group.* Five strains that were lysed by one or more of these five phages were studied. Strains that were lysed completely by the five phages in test dilution were not encountered. The five strains were lysogenic. The results (Table 3) showed that acquired phage resistance accounted for these five strains being considered as different phage types. The phage carried by strain 4 had some effect on strain 3 as strain 3 (4/3) only gave a + reaction with phage 47C.

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However, as the other results suggested that strains 3 and 36, and 4 and 36, apart from acquired phage resistance, were identical, it followed that strains 3 and 4 must also be identical. The phage carried by strain 1417 had the expected result on strain 1163, as the resistant strain 1163 (1417/1163) gave only a + reaction with phage 47C and a CL reaction with phage 42B. Strain 4 (1417/4) was insusceptible to phages 47 and 47C but it still, however, gave a CL reaction with phage 7. In view of previous work (Williams Smith, 1948), these reactions of strains 1163 (1417/1163), 4 (1417/4), and 3 (4/3) strongly suggested that strains 1417 and 4 had been infected with more than one phage, had become resistant to both of them, but were now different phage types. No direct evidence could be obtained to prove that this phenomenon was responsible for the other six strains being considered as different phage types; five of them were not shown to be lysogenic and the phage obtained from strain 1312 was not responsible for its resistance to some of the phages of this group (see strain E88 (1312/E88)). However, to quote one example, the fact that the phage carried by strain 144 was responsible for the resistance of strain 144 to phages 29, 29A, 31, 42C and 52A (see strain E88 (144/E88)), and the fact that phages 31 and 44 were propagated on the same strain, strain 18, suggested that acquired phage resistance was responsible for strains being differentiated into types

Table 4. *The lytic action of phages of the* 29 *group*

	Phage filtrates in test dilution						
Strain no.	29	29A	31	42C	44	52	52A
E88	CL	CL	++	CL	–	CL	CL
1430	CL	+	CL	–	–	CL	++
E30	++	CL	CL	±	–	CL	++
1312	–	–	CL	++	–	CL	–
E66	CL	+	+	–	–	CL	±
144	–	–	–	–	–	CL	+
1307	–	–	–	CL	–	–	–
148	CL	–	CL	–	–	+	–
202	CL	–	–	CL	–	CL	–
18	–	+	CL	+	CL	–	–
1351	–	CL	–	–	–	–	–
925	–	–	–	–	–	–	CL
Resistant strains							
E88 (144/E88)	–	–	–	–	–	CL	+
1430 (144/1430)	–	–	–	–	–	CL	+
E30 (1430/E30)	++	+	CL	–	–	CL	+
E88 (E66/E88)	CL	+	+	–	–	CL	+
E88 (1430/E88)	CL	+	++	–	–	CL	++
E88 (148/E88)	CL	+	++	–	–	–	–
1430 (148/1430)	CL	±	CL	–	–	–	–
E88 (1312/E88)	CL	CL	++	CL	–	CL	CL

carrying only one demonstrable phage. It is probable that a similar position existed in the case of strain 1339 (see phage 3A, 3B, 3C, 51 group).

Phage 29, 29A, 31, 42C, 44, 52, 52A *group*. This was a more complex group of phages than the two which had been investigated previously, but routine typing had revealed strains of staphylococci that were lysed by these phages in a manner suggesting that they should be considered as one group. Strains that were fully susceptible to all seven phages had not been encountered. Despite repeated efforts, only six of the twelve strains that were studied were shown to be lysogenic. These six were strains 148, 1312, E30, 1430, E66 and 144. The results shown in Table 4 indicated that acquired phage resistance was responsible for strains E88, 1430, 144, E30, E66 and 148 being considered as according to their susceptibility to the seven phages of this group. A few more strains belonging to the same phage types as the non-lysogenic strains were also examined. They, also, were not shown to be lysogenic.

Phage 88, 88A *group*. A number of strains that were susceptible either to phage 88, 88A or to both of these phages, were studied. Two strains, E108 and E109, were included as they were unique among the untypeable strains in being lysogenic to the propagating strain of phage 88. The results (Table 5) indicated that acquired phage resistance was responsible for the phage 88 type strains, the phage 88–88A type strains, and the two untypeable strains belonging to different phage types. No direct evidence was obtained in the case of the phage 88A type strains as they were not shown to be lysogenic.

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Thus, in the majority of cases, it was possible to show that the reason why some staphylococcal strains had been classified into different types or sub-types according to their susceptibility to either the 3A group of phages, the 6 group, the 29 group, the 42D group, or the 88 group was because some of them had been infected previously with one or more different phages. The phage resistance they acquired, as a result, accounted for their resistance to some of the test phages forming the group.

Table 5. *The lytic action of phages* 88 *and* 88A

Strain no.	Phage filtrate in test dilution: 88	88A
E118	CL	CL
E129	CL	CL
E139	CL	–
E126	CL	–
E127	–	CL
E152	–	CL
E108	–	–
E109	–	–
Resistant strains		
E118 (E139/E118)	CL	–
E118 (E126/E118)	CL	–
E118 (E108/E118)	–	–
E118 (E109/E118)	–	–
E129 (E139/E129)	CL	–
E129 (E126/E129)	CL	–
E129 (E109/E129)	–	–
E129 (E108/E129)	–	–

Table 6 represents an attempt to classify staphylococci, by phage methods, into types that differ other than by virtue of acquired phage resistance. These types, *for convenience*, are called genetic types so as to distinguish them from phage types. Twelve types are shown in the table; strains that are susceptible to any of the phages forming one of the groups referred to above are considered as one genetic type. As more strains are examined and new phages are prepared to identify untypeable strains, it is realized that this table will constantly be subject to alteration. It is also realized that though the existence of a high proportion of resistant strains makes this form of classification into genetic types a difficult one, it does not appear to affect the usefulness of phage-typing in epidemiological studies provided acquired phage resistance is a permanent and constant character, and strains do not acquire resistance subsequent to phage infection during the course of a short-term epidemiological study.

The stability of resistant strains of staphylococci. Twenty lysogenic strains of the 42D type and twenty resistant variants of the non-lysogenic strain 16 prepared to the phages carried by these strains were titrated with the phage filtrates of the 42D group. These titrations were repeated three times in a year, the strains being sub-cultured several times between each titration. The results for each strain were the same on all four occasions. Many strains, lysogenic and non-lysogenic, were phage-typed several times during the course of this

Table 6. *The classification of staphylococci into genetic types*

Genetic type	Phage filtrates in test dilution: 3A, 3B, 3C, 51	6, 7, 42B, 47, 47C	29, 29A, 31, 42C, 44, 52, 52A	p42D/E193, 30/16, 129/16, 1363/14, 14/94, E174/16, 42D	42E	44A	47A	47B	×2	S7	88A, 88	G12
1	CL	.	.	.	.	.	.	.	.	.	.	.
2	.	CL	.	.	.	.	.	.	.	.	.	.
3	.	.	CL	.	.	.	.	.	.	.	.	.
4	.	.	.	CL	.	.	.	.	.	.	.	.
5	.	.	.	.	CL	.	.	.	.	.	.	.
6	.	.	.	.	.	CL	.	.	.	.	.	.
7	.	.	.	.	.	.	CL	.	.	.	.	.
8	.	.	.	.	.	.	.	CL	.	.	.	.
9	.	.	.	.	.	.	.	.	CL	.	.	.
10	.	.	.	.	.	.	.	.	.	CL	.	.
11	.	.	.	.	.	.	.	.	.	.	CL	.
12	.	.	.	.	.	.	.	.	.	.	.	CL

The genetic types have been numbered for the purpose of this table only and are not recognized designations.
CL = confluent lysis.

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work; their susceptibility to the test phages did not vary. The strains that were lysogenic were never shown to lose this property.

The effect of growing two strains of staphylococci together. In view of the ease with which the phage carried by a lysogenic strain can be propagated by growing the lysogenic strain with a strain known to be susceptible to the phage carried by the lysogenic strain, it was decided to investigate this process more fully to find out whether it might be of any significance in epidemiological studies.

Eighteen-hour broth cultures of two strains of staphylococci were diluted 100 times with broth, and 0·02 ml. of each diluted culture was inoculated into the same test-tube containing 5 ml. of broth. This was incubated at 37° C. and one loopful of the contents was plated on nutrient agar at 6, 24, 48 hr., 4 days and 14 days after the commencement of incubation. Twenty colonies from each plate were picked off into broth and phage-typed in the usual manner. The two strains with which the broth had been inoculated were phage-typed at the same time. The strains used for each mixed culture were chosen because one or both of them was known to be carrying a phage to which the other was susceptible. Eight such mixed cultures were studied in all, five inoculated with strains susceptible to the 42D group of phages, two with strains susceptible to the 3A group, and one with strains susceptible to the 6 group. The following three examples are typical of the results that were obtained. The phage types of staphylococci found in the mixed cultures are shown first, and then the proportion of these present in the twenty cultures examined at each isolation is shown in tabular form. The phage filtrates were used in test dilution.

Strains E 168 *and* E 174. Each of these strains was carrying a phage to which the other was susceptible.

Phage types present in the mixed culture	Test phages						
	42D	E 174/16	14/94	1363/14	129/16	30/16	p42D/E 193
A (i.e. strain E 168)	±	CL	CL	CL	–	–	CL
B (i.e. strain E 174)	CL	–	+	–	CL	–	CL
C*	±	–	+	–	–	–	CL

Phage type	No. of each phage type present in the twenty cultures examined at each isolation				
	6 hr.	24 hr.	48 hr.	4 days	14 days
A	8	6	8	0	0
B	12	12	9	2	0
C*	0	2	3	18	20

* This phage type can be prepared either by making strain E 168 resistant to the phage carried by strain E 174, or strain E174 resistant to the phage carried by strain E168.

Strains E 193 *and* 16. Strain E 193 was lysogenic to the non-lysogenic strain 16.

Phage types present in the mixed culture	Test phages						
	42D	E 174/16	14/94	1363/14	129/16	30/16	p42D/E 193
A (i.e. strain E 193)	±	–	+	–	–	–	CL
B (i.e. strain 16)	CL	CL	CL	CL	CL	CL	CL

Phage type	No. of each phage type present in the twenty cultures examined at each isolation				
	6 hr.	24 hr.	48 hr.	4 days	14 days
A	4	19	20	20	20
B	16	1	0	0	0

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Strains 145 *and* 1394. Each of these strains was carrying a phage to which the other was susceptible.

Phage types present in the mixed culture	Test phages			
	3A	3B	3C	51
A (i.e. strain 145)	–	+	–	CL
B (i.e. strain 1394)	CL	CL	CL	++
C*	–	+	–	++

No. of each phage type present in the twenty cultures examined at each isolation

Phage type	6 hr.	24 hr.	48 hr.	4 days	14 days
A	10	4	6	8	12
B	10	16	14	9	4
C*	0	0	0	3	4

* This phage type can be prepared either by making strain 145 resistant to the phage carried by strain 1394, or strain 1394 resistant to the phage carried by strain 145.

The growing together of two strains, one of which was non-lysogenic and susceptible to the phage carried by the other (e.g. strains E 193 and 16), resulted eventually in the isolation only of cultures of the same type as the lysogenic strain. These cultures probably represented the original lysogenic strain and, possibly, some resistant variants of the non-lysogenic strain to the phage carried by the lysogenic strain. When both strains were lysogenic to each other (e.g. strains E 174 and E 168, and strains 145 and 1394) a phage type different from the two phage types that were grown together was found in the mixed culture. This new phage type was obviously a resistant variant of one of the two strains to the phage carried by the other. In the case of strains E 174 and E 168, the twenty strains tested after 14 days incubation were all of the 'variant' type—and it is probable that these variants had been produced from both strains E 174 and E 168.

Several of the new phage types that had been produced were plated out and ten colonies from each plate were phage-typed; the ten colonies from each plate were found to be of the same phage type. This was done to ensure that the new types were not merely mixtures of the strains that had been grown together.

When the nutrient agar plates streaked with the mixed cultures were examined after incubation, some of the colonies were found to be of the transparent degenerate kind originally referred to by Twort (1915). These colonies were not used for phage-typing as they were actively infected with phage and when spread on agar plates produced only a very thin transparent growth.

Five other mixed cultures were studied in exactly the same way as the ones described above. Each tube of broth was inoculated with two strains, both lysogenic, but neither susceptible to the phage carried by the other. Two different strains were used for each mixed culture. In these five mixed cultures, both strains with which the tubes were inoculated were isolated after every period of incubation from 6 hr. to 14 days.

The results obtained by growing strains E 168 and E 174 together were especially interesting because strains of the same phage types as these strains and their resistant variant had been isolated from individual milk samples taken from twenty-two cows in the same herd. Of the twenty-two strains that were examined, six were of the same phage type as strain E 168, nine of the same phage type as strain E 174 and seven (e.g. strain E 193) of the same phage type as the resistant variants that had been obtained by growing strains E 168 and E 174 together. As a result of previous work (Williams Smith, 1948) it was thought that if strain E 193 was a resistant variant of either strain E 168 or strain E 174 to the phage carried by the other, it might be carrying two phages—the phage carried by strain E 174 and the phage carried by strain E 168. The 'crude phage' obtained by the action of strain E 193 on the non-lysogenic strain 16 was plated out with strain 16 so as to yield isolated plaques. The phages prepared from some of these plaques were of two kinds; one which lysed strain E 174 and not strain E 168 and one which lysed strain E 168 and not strain E 174. One of the resistant variants obtained by growing strains E 174 and E 168 together was studied in the same way as strain E 193, and was also shown to be carrying two different phages. Cross-resistance tests were now carried out to show the relationship to each other of these four phages and the phages carried by strains E 174 and E 168. For this purpose, resistant variants of strain 16 to these six phages were prepared. The six phages were then titrated against the six resistant variants. The results showed quite conclusively that strain E 193 and the resistant variant obtained by growing strains E 174 and E 168 together were both carrying two phages, one identical with the phage carried by strain E 174 and the other identical with the phage carried by strain E 168.

It is highly probable that, in the herd referred to above, the strains of the same phage type as strain E 193 were produced from strains of the same phage types as strains E 174 and E 168 in a manner similar to that in which the resistant variants of strains E 174 and E 168 had been produced in the laboratory. When strains E 193 and 16 were grown together in the laboratory, only strains of the same phage type as strain E 193 were isolated (see above). Two strains isolated from milk samples taken from cows in this herd were of the same phage type as

strain 16 and it is problematic whether the strains once present in this herd were all of this phage type. It was hoped to make further studies on this herd, but unfortunately the cows had been subjected to udder infusion with a penicillin preparation in the meantime.

DISCUSSION

The results, together with those reported in a previous paper (Williams Smith, 1948), confirm the observations of Wilson & Atkinson (1945) that staphylococcal strains can be classified into a number of different types by their sensitivity to bacteriophage, but it has been shown that apparent differences between certain strains may, in fact, be due to acquired phage resistance. Since a considerable proportion of strains are lysogenic and hence resistant, it is clear that the classification of staphylococci by phage methods is dependent to a considerable extent on the past experience of the organisms with regard to phage infection. For example, two strains that are identical apart from the fact that one of them has acquired a resistance to a phage may be classified as two different phage types.

The phage types were quite stable in character provided they did not become infected with phage. It has been shown that a staphylococcal strain may undergo a change of type if it is grown together with another strain that is carrying a phage to which it is susceptible. Evidence has been produced to suggest that this change can also take place in the field. It is obvious also that all strains shown to be lysogenic must have undergone, at some time, some change in phage susceptibility. While change of type is likely to be a rare occurrence in an epidemiological investigation of short duration, such as that of an outbreak of staphylococcal food poisoning, it may well limit the usefulness of the method in inquiries conducted over a long period. Whatever significance change of type may have in epidemiological studies, it is certainly sound practice to plate infected material directly on solid media rather than to give the organisms an opportunity to grow together by previous incubation in broth, and so run the risk of development of a resistant variant.

Although an attempt was made to classify strains into types that differed other than by virtue of acquired phage resistance (i.e. into so-called genetic types), it is realized that such a classification is open to objections of its own. The large number of strains that are phage resistant is one reason for this. Some strains may acquire a resistance to more than one phage yet may be shown to be carrying only one of these phages (Williams Smith, 1948). Also it is difficult to demonstrate lysogenicity in some resistant strains, and the possibility exists that all resistant strains of staphylococci may not be lysogenic. The evidence suggesting that the strains susceptible to one or more of the 29 group of phages differed only by virtue of acquired phage resistance was also not so conclusive as was the case in the other groups. The lack of evidence may have been due to the fact that some of these strains were resistant but not lysogenic, or that their lysogenicity could not be demonstrated owing to the lack of suitable 'susceptible' strains. On the other hand, factors other than acquired phage resistance may have been responsible for the reactions of some of these strains with this group of phages.

The appearance of transparent glassy degenerate colonies as well as normal colonies on agar that had been streaked with a broth culture of two strains suggested that the calf-lymph used by Twort (1915) in his original observations on transmissible bacterial lysis might have contained two strains of staphylococci, one of which was carrying a phage to which the other was susceptible. Such an explanation might also account for the origin of the staphylococcal phages obtained by Callow (1922), Burnet & Lush (1935), and others by the filtration of pus; and for the fact that faeces and sewage, which contain strains of many different bacteria, are a prolific source of various phages. Lysogenic strains have also been identified in many species of bacteria by the filtration of strains that were normal in appearance. Callow (1927) was apparently able to obtain concentrated phage filtrates from such strains of staphylococci. This observation was not confirmed by Fisk (1942), or during this present work in which the filtrates of twenty strains of staphylococci known to be lysogenic were shown not to contain any phage. While such factors as adsorption and filtration technique undoubtedly play an important part in the demonstration by this method that a bacterial culture is lysogenic, the state of the phage-bacterium relationship at the time of filtration may also be concerned; any disturbance in this relationship, though not sufficient to produce visible changes in the bacterial culture, might result in a filtrate that contained phage.

SUMMARY

1. Investigations have been carried out on the bacteriophage method of typing staphylococci developed by Wilson & Atkinson (1945).

2. Acquired phage resistance has been shown to be responsible for the classification of many strains as different phage types.

3. Repeated testing of the same strains showed that the phage types were stable in character when the strains were maintained under laboratory conditions.

4. When two strains of staphylococci, one of

which was lysogenic to the other, were grown together in broth, the susceptible strain underwent a change in phage type. As a result of this, it is considered that, in epidemiological studies, infective material should be plated directly on to a solid medium and not incubated first in broth, as is sometimes done. Evidence has been obtained suggesting that this change of type may also take place in the field. This phenomenon may be of some significance in epidemiological studies extending over a long period of time.

5. An attempt has been made to classify staphylococcal strains by phage methods into types that differ other than by virtue of acquired phage resistance. These are called, *for convenience*, genetic types. Reasons are given for the view that this may not be a satisfactory method of classification in practice.

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REFERENCES

BURNET, F. M. & LUSH, D. (1935). *J. Path. Bact.* **40**, 455.
CALLOW, B. R. (1922). *J. infect. Dis.* **30**, 643.
CALLOW, B. R. (1927). *J. infect. Dis.* **41**, 124.
FISK, R. T. (1942). *J. infect. Dis.* **71**, 153.
TWORT, F. W. (1915). *Lancet*, **2**, 1241.
WILLIAMS SMITH, H. (1948). *J. Hyg., Camb.*, **46**, 74.
WILSON, G. S. & ATKINSON, J. D. (1945). *Lancet*, **1**, 647.

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