INVESTIGATIONS ON THE TYPING OF STAPHYLOCOCCI BY MEANS OF BACTERIOPHAGE

I. THE ORIGIN AND NATURE OF LYSOGENIC STRAINS

By H. WILLIAMS SMITH, Ph.D., M.Sc., M.R.C.V.S.,
London School of Hygiene and Tropical Medicine

This work was begun with the intention of applying the phage-typing method devised by Wilson & Atkinson (1945) to the study of the epidemiology of staphylococcal infections in animals. It was soon found that a high proportion of coagulase-positive strains obtained from cattle were susceptible to one phage, phage 42D, as has also been found by Macdonald (1946). It was, therefore, considered advisable to search for further proof that these 42D type strains were really identical. The resulting investigations are reported in this paper.

TECHNIQUE

The technique of bacteriophage typing was that described by Wilson & Atkinson (1945) with a few slight modifications.

Preparation of 'crude phages' from lysogenic strains. Lysogenic strains were detected by the cross-culture method (Fisk, 1942). Strains to be examined were grown in nutrient broth for 18 hr. at 37° C. Each strain was then used in turn as a 'basal' strain. Four drops (0·1 ml.) of the culture were spread over the whole surface of a nutrient agar plate, and, after the plate had dried, the remaining cultures were 'spotted' upon it. The plates were then incubated at 37° C. for 6–8 hr. and left at room temperature overnight. Phage action was revealed next morning by the presence of either discrete plaques or a narrow zone of inhibition of growth, less than 0·5 mm. in width around the edge of the spotted culture. The growth of any pair of strains showing evidence of phage action was scraped off the agar and suspended in a small volume of broth. This was centrifuged, and the supernatant fluid, or 'crude phage', was tested against both strains to find out which was the lysogenic and which the susceptible strain.

Purification of phages. The 'crude phage' was plated with the susceptible strain and a single plaque was picked off, and spread over the surface of an agar plate. This plate was incubated at 37° C. for 6–8 hr., and then left at room temperature overnight. A fresh plaque was picked off next morning, and the process repeated once. The growth from the second plating was scraped off, suspended in 5 ml. of broth, and centrifuged. The phage in the supernatant fluid was then propagated.

Propagation of phages. This was carried out by a plate method, a broth method, or a combination of both these methods.

(*a*) *Plate method.* This method, which was suggested by Prof. G. S. Wilson, has been used by other workers. A few drops of an 18 hr. broth culture of the susceptible strain were mixed with a small quantity of the purified phage preparation and spread over the surface of an agar plate. This plate was then incubated at 37° C. for 5–6 hr. for lysis to take place, and then kept in the ice-chest overnight. The next morning, 5 ml. of broth were poured over the surface of the agar plate, and left for 30 min. This was then collected and centrifuged. Some of the supernatant fluid was then used for another passage. The phage was washed off the second agar plate on the following morning with the remainder of the supernatant fluid obtained on the previous day, with or without additional broth. Several agar plates were used in each of the final passages, and the amount of propagating culture per plate was increased.

(*b*) *Broth method.* The purified phage preparation and six drops of an 18 hr. broth culture of the propagating strain were added to 10 ml. of nutrient broth. This was incubated at 37° C. until complete lysis had taken place. More culture was then added, and incubation continued until lysis again occurred. This process was repeated several times. If secondary growth appeared, the material was filtered, after which propagation was continued. Lysis would sometimes take place three times in 8 hr. and a potent filtrate could be obtained in a short time. Phages were propagated, whenever possible, by the broth method. Some had to be propagated by the plate method owing to the rapidity with which secondary growth occurred. Others were propagated by the plate method in the earlier stages, and then by the broth method. The final phage cultures were filtered through a Seitz E.K. pad, and kept in the ice-chest without preservative.

Titration of lytic filtrates. The phage filtrates were

https://doi.org/10.1017/S002217240003610X Published online by Cambridge University Press

diluted 1/10 to 1/100,000 with quarter-strength Ringer solution. Four drops (0·1 ml.) of an 18 hr. broth culture of the susceptible strain were spread evenly over the surface of an agar plate, allowed to dry, and then 'spotted' with a standard loopful of each dilution of the filtrate. Plates were incubated for 6 hr., kept at room temperature overnight, and read next morning. The highest dilution producing confluent lysis was regarded as the test dilution, i.e. that suitable for routine tests.

Preparation of lytic filtrates ('adapted phages') for untypeable strains. Strains that showed no phage action with the test dilutions of the available lytic filtrates were tested with the undiluted filtrates. If evidence of phage action was observed with any filtrate, a single plaque was picked off with some of the surrounding growth, and spread over an agar plate. Propagation of the phage was then carried on by one of the methods already described.

Method of setting up tests. Nutrient agar plates containing 2·0 % agar were dried at 37° C. for 1 hr. with the lid partly removed. Four drops (0·1 ml.) of an 18 hr. broth culture of the strain to be typed were spread evenly over the surface of a plate. After the plates had dried, a standard loopful of each of the phage filtrates, in test dilution, was spotted on to the culture. When several strains were to be tested, the filtrates were spotted on to the culture by means of a dropping pipette (150 drops/ml.). For the sake of convenience the bottom of the plate was divided by a glass-writing diamond into twenty five ½-in. squares, so that twenty-five phages could be tested, if desired, on any one strain. The plates were then incubated at 37° C. for 6 hr., left at room temperature overnight, and read next morning. The scheme of notation used is as follows:

Confluent lysis with no secondary growth	CL
Confluent lysis with secondary growth	+ + +
Numerous semi-confluent plaques	+ +
Discrete plaques	+
Less than twenty plaques	±

Production of phage-resistant strains of staphylococci. One drop of an 18 hr. broth culture of a susceptible strain was spread evenly over a small area (about 2 in. by ½ in.) of the surface of an agar plate. When this was dry, one drop of an undiluted phage filtrate was spotted on to the surface of the culture which was then incubated at 37° C. A zone of complete lysis was observed after about 4–5 hr. incubation. Incubation was then continued until secondary growth appeared within this zone. A little of this secondary growth was picked off into broth, and incubated. This broth culture was plated on agar, a single colony picked off and replated; finally a single colony from this plating was picked off into broth, and incubated. The final culture was then tested on an agar plate to find whether it was completely resistant to the undiluted phage filtrate. 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 had been made resistant. The words 'resistant strains' are used throughout this paper to mean phage-resistant strains.

Cross-resistance tests. These were carried out by a modification of the original method devised by Bail (1923). Resistant strains were prepared by the action of several phage filtrates on one strain of staphylococcus. These phage filtrates were then titrated, in the manner previously described, against each of the resistant strains.

Since publication of this paper, Wilson & Atkinson have renamed their phages. The new names are included, in parentheses, in their diagnostic schema which is reproduced in Table 1. Three new phages, 29A, 42D and 42E, have also been added to the eighteen that they previously used in routine typing. Most of the phages prepared during the course of this work were designated by the number of the lysogenic strain from which the phage was derived followed by the number of the strain upon which it was propagated. New phages prepared by adaptation of one of the original phages were designated by the number of the original phage, preceded by the letter 'p' and followed by the number of the strain to which it was adapted.

RESULTS

The presence of lysogenic 42D *type strains*

It was first considered probable that if the 42D type strains were identical, none of them would be carrying a phage that would lyse any of the others. If one of them was carrying such a phage, some of the strains would be susceptible to it while at least one, the phage-carrying or lysogenic strain, would be insusceptible to it. This would result in a division of the group. Twenty-three 42D type strains were examined for the carriage of phage by the cross-culture method; seven of the strains were shown to be carrying phage to the other sixteen strains. When any two strains that were carrying phage to the non-phage-carrying group were superimposed upon each other, the results varied. In some instances no phage action was noted, suggesting that both strains were carrying a similar, or closely related, phage; in others, both strains were susceptible to the 'crude phage' used for test, suggesting that these two strains carried dissimilar phages; in others, only one strain was lysed, suggesting that one strain carried a phage not present in the other. The results indicated that 42D group could probably be subdivided by the use of the phages carried by the lysogenic strains; the non-lysogenic strains would be lysed by all the phages and the lysogenic

https://doi.org/10.1017/S002217240003610X Published online by Cambridge University Press

strains only by some of them. Further division of the lysogenic strains might also be possible.

The twenty-three 42D type strains (seven lysogenic and sixteen non-lysogenic) were tested against twenty-five strains belonging to other phage types to find whether lytic action occurred between any of them. No lytic action was observed. It was, therefore, provisionally concluded that when lytic action is noted between two strains, these two strains are closely related to each other.

On the other hand, only three of the lysogenic strains were lysed completely by this dilution. The rest, including strain 1363, the propagating strain of phage 42D, were not lysed completely by phage filtrate 42D in dilutions greater than 1 in 200. As the dilution of a phage filtrate used in routine tests is that which produces confluent lysis of its propagating strain, it can be seen from Table 2 why some of these lysogenic strains were originally classified as 42D type, and why some were not. If one of the non-lysogenic strains had been used as the propagating strain of phage 42D, instead of strain 1363, the test dilution would be such that only three of the lysogenic strains would have been typed in routine tests. The remainder, including strain 1363, would have been regarded as untypeable.

Table 1. *Bacteriophage type designations of staphylococci* (Wilson & Atkinson, 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.

Phage 42D, which is mentioned frequently in this paper, was prepared by Wilson & Atkinson after publication of the table shown above. It was prepared by adapting phage 42C to lyse an hitherto untypeable strain, strain 1363. None of the phage types 1–14 were susceptible to this phage.

Confluent lysis = +; semi-confluent lysis = ±; lesser degrees of lysis or no lysis = −.

The titration of phage filtrate 42D *against* 42D *type strains and other strains*

Phage filtrate 42D was now titrated against a number of 42D type strains, nine of which were non-lysogenic, and twelve of which were lysogenic. Seven untypeable strains were included in these titrations because they were lysogenic to phage 42D type strains. The results are illustrated in Table 2. The nine non-lysogenic strains were lysed completely by phage filtrate 42D diluted 1 in 400.

The titration of phages lytic for 42D *type strains*

Seven phages were now propagated; five were obtained from lysogenic strains and two were adapted phages. The five phages were 14/94,

https://doi.org/10.1017/S002217240003610X Published online by Cambridge University Press

E174/16, 1363/14, 30/16 and 129/16. The two adapted phages were p7/E168 and p42D/E193. The seven phage filtrates were titrated against a representative group of 42D type strains and against untypeable strains that had been shown to be lysogenic to 42D type strains. Four non-lysogenic strains, strains 8, 16, 86 and 118, were fully susceptible to all seven phages, but the remainder of the strains, which were lysogenic, gave varying results. The lysogenic untypeable strains were fully susceptible to one or more, but not to all of these phages. Table 3 has been compiled to illustrate whether positive reactions (complete lysis) would be given by these phage filtrates upon the strains tested if the filtrates were used in test dilution. The result of the action of phage 42D on these strains is also shown. This table shows that the sixteen strains, previously regarded as of phage 42D type or untypeable, could now be classified into nine phage types according to the pattern of reactions that they gave with the test phages. The non-lysogenic strains, being fully susceptible to all the phages, could not be distinguished from each other.

The seven phage filtrates referred to above did not lyse any of the strains against which they were titrated in a higher dilution than they did their propagating strains. This was in contrast to phage filtrate 42D as this filtrate (Table 2) completely lysed its propagating strain, the lysogenic strain 1363, at a dilution of 1 in 40, and the non-lysogenic strains at a dilution of 1 in 400. As phage 42D had been prepared by adaptation of phage 42C to lyse strain 1363, it was thought possible that the reason for this might be that adaptation had not been complete. Phage 42D was therefore subjected to further propagation on strain 1363 by the agar-plate method for fifteen passages. Titrations then showed the non-lysogenic strains and strain 1363 to be

Table 2. *Titration of phage filtrate* 42D *against various strains of staphylococci*

Strain no.	Dilutions of phage filtrate 42D 1 in										End-point 1 in
	10	20	40	80	100	200	320	400	800	1600	
	Non-lysogenic 42D type strains										
8	CL	CL	CL	CL	CL	CL	CL	CL	++	+	400
86	CL	CL	CL	CL	CL	CL	CL	CL	+++	+	400
16	CL	CL	CL	CL	CL	CL	CL	CL	++	±	400
118	CL	CL	CL	CL	CL	CL	CL	CL	++	±	400
20	CL	CL	CL	CL	CL	CL	CL	CL	++	±	400
91	CL	CL	CL	CL	CL	CL	CL	CL	++	±	400
27	CL	CL	CL	CL	CL	CL	CL	CL	++	+	400
46	CL	CL	CL	CL	CL	CL	CL	CL	++	+	400
87	CL	CL	CL	CL	CL	CL	CL	CL	++	+	400
	Lysogenic 42D type strains										
E75	CL	CL	CL	CL	CL	CL	CL	CL	++	+	400
100	CL	CL	CL	CL	CL	CL	CL	CL	++	+	400
104	CL	CL	CL	CL	CL	CL	CL	CL	++	±	400
14	CL	CL	CL	CL	CL	CL	++	++	+	−	200
E170	CL	CL	CL	CL	CL	CL	++	++	+	−	200
E174	CL	CL	CL	CL	CL	CL	++	+	−	−	200
30	CL	CL	CL	CL	CL	+++	++	+	+	−	100
150	CL	CL	CL	CL	CL	++	++	+	±	−	100
161	CL	CL	CL	CL	++	++	+	+	±	−	80
95	CL	CL	CL	CL	++	++	+	±	−	−	80
99	CL	CL	CL	+++	++	+	−	−	−	−	40
1363*	CL	CL	CL	+++	++	++	+	+	−	−	40
	Untypeable strains lysogenic to 42D type strains										
13	CL	CL	++	++	++	+	+	±	±	−	20
94	CL	CL	++	++	+	+	+	+	−	−	20
E168	+++	+++	++	++	+	+	±	−	−	−	−
129	+++	++	++	++	+	±	−	−	−	−	−
E210	+++	+	±	−	−	−	−	−	−	−	−
E77	±	−	−	−	−	−	−	−	−	−	−
E193	±	−	−	−	−	−	−	−	−	−	−

The degree of lytic action is designated as indicated in the text.
The end-point is the highest dilution of filtrate producing complete lysis of the strain upon which it was tested.
* Strain 1363 is the propagating strain of phage 42D.

https://doi.org/10.1017/S002217240003610X Published online by Cambridge University Press

equally susceptible to this new phage 42D, but the other resistant strains were still partly or completely resistant.

Cross-resistance tests were now carried out to study the relationship to each other of the phages shown in Table 3. The resistant variants were prepared from the non-lysogenic strain 16. The results showed that all the phages, except E 174/16 and p 7/E 168 were different from each other.

Although the classification of the strains shown in Table 3 appeared to be based upon the resistance of the lysogenic strains to some of the test phages, it was considered advisable to search for further proof that this was so. Resistant variants of the non-lysogenic strain 16 were prepared to the phages carried by the twelve lysogenic strains shown in Table 3. These phages were prepared in the usual way except that they were not purified by growing from single plaques. The 'crude phages' were propagated instead (on strain 16) as it was thought possible that some of the strains might be carrying more than one phage. The test phages shown in Table 3 were then titrated against the twelve resistant variants of strain 16 and the twelve lysogenic strains referred to above. The resistant variants, in all cases, were acted upon by the test phages in exactly the same manner as were their corresponding lysogenic strains. For example, the resistant variant of strain 16 to the phage carried by strain 129 was acted upon in exactly the same manner as was strain 129 itself. Thus the acquired phage resistance of the lysogenic strains was responsible for the classifications of the strains shown in Table 3 and for several of them being previously regarded as untypeable.

Table 3. *Results of titrations of phages lytic for* 42D *type strains*

	Phage filtrates					
	E 174/16 p 7/E 168 14/94	1363/14	30/16	p 42D/E 193	129/16	42D
Non-lysogenic strains:						
8, 16, 86, 118	+	+	+	+	+	+
Lysogenic strains:						
E 75	+	+	−	+	+	+
14	−	+	−	−	+	+
30	+	−	−	+	−	+
E 170, E 174	−	−	−	+	+	+
1363	−	−	−	−	−	+
129, E 168	+	+	−	+	−	−
13, 94	+	−	−	+	−	−
E 193, E 77	−	−	−	+	−	−

+ = positive reaction (complete lysis).

While this work was proceeding, 200 strains of coagulase-positive staphylococci isolated from cattle had been subjected to routine phage-typing using the phages of Wilson & Atkinson. These strains were also tested for carriage of phage to 42D type strains. (It was found convenient for this purpose to 'spot' a non-lysogenic 42D type strain, strain 16, on each culture during typing.) Of these 200 strains, ninety-three strains, forty-seven of which were lysogenic to strain 16, were of phage 42D type. Forty-five of the strains belonged to types other than 42D and were non-lysogenic to strain 16. The remaining sixty-two strains were untypeable although a number of them were slightly susceptible to phage 42D. Of these sixty-two strains, forty-seven were lysogenic to strain 16, and fifteen were non-lysogenic. These sixty-two untypeable strains were now tested with the phages shown in Table 3; the forty-seven lysogenic strains were fully susceptible to one or more, but not all of these phages, indicating that acquired phage resistance was probably the reason for their being previously regarded as untypeable.

The resistance of lysogenic strains of phage X 2 *type*

It now became necessary to determine whether lysogenic strains belonging to phage types other than 42D exhibited any resistance to the test phages which was related to their lysogenic state. A considerable number of strains from sheep had been examined by this time and phage filtrates had been prepared to aid in their identification. One of these new phages was designated X 2. Of 158 sheep strains, sixty-two were of phage X 2 type, sixty were of other phage types, and thirty-six were untypeable. Twenty of the phage X 2 type strains and twenty-eight of the untypeable strains were lysogenic to strain 56, the propagating strain of phage X 2. (Strain 56 had been shown consistently to be non-lysogenic.) The other strains were non-lysogenic to strain 56. The lytic effect of phage X 2, in its test

https://doi.org/10.1017/S002217240003610X Published online by Cambridge University Press

dilution, on the strains that were lysogenic to strain 56, but untypeable, was as follows:

++ (6 strains), +(12 strains), −(10 strains).

Phage filtrate X 2 was titrated against a selection of the lysogenic strains, resistant variants of strain 56 to the phages carried by these strains, and non-lysogenic strains. The conclusions reached were exactly the same as in the case of the phage 42D type strains.

The ability of phages adapted to resistant strains to lyse similar but non-resistant strains

Twenty-two of the propagating strains of the phages used in routine typing were examined by the cross-culture method to see whether they were lysogenic. Seventeen (77·3 %) of the strains were lysogenic. As a considerable number of the routine-test phages had been prepared by adapting existing phages to untypeable strains which were now shown to be lysogenic, it was necessary to determine whether a phage, to which a certain non-resistant strain was susceptible might, after adaptation to a resistant variant of the same strain, fail to produce complete lysis of the original strain. The evidence so far did not support this. However, further proof was sought.

Eight new phage filtrates were prepared by adapting six of the phages that were lytic to strain 16 to resistant variants of the same strain. These filtrates were then titrated against the resistant variants of strain 16, strain 16 itself and three non-lysogenic strains of the same phage type as strain 16, i.e. phage 42D type. Strain 16 and the other three non-lysogenic strains were lysed completely by the eight phage filtrates in the same dilutions as each of these phages lysed the resistant variants of strain 16 upon which they had been propagated.

An interesting example of the adaptation of a phage to strains that appeared to differ only by virtue of acquired resistance to different phages is shown in Table 4. Phage 42D was adapted to a lysogenic strain, strain E 193, and this new phage, p 42D/E 193, was then adapted to another lysogenic strain, strain 1363, the propagating strain of phage 42D. Each of the adapted phages was fully active on its propagating strain and on the non-lysogenic strain 16 but it had only a slight lytic effect on the strain that had previously been its propagating strain. Thus a phage filtrate in its test dilution may fail to lyse its original propagating strain (only if lysogenic) after it has been adapted to another lysogenic strain apparently identical with the first except for acquired resistance to a different phage.

The occurrence of non-lysogenic resistant strains

The investigations so far had been facilitated by the fact that the resistant strains that had been studied were lysogenic. It has been noted, however, from a study of the relevant literature that resistant variants of a bacterium may be either lysogenic or non-lysogenic. It was decided to investigate the behaviour of different phage types in this respect.

Resistant strains were prepared by the action upon their propagating strains of eighteen of the phages used by Wilson & Atkinson in routine typing. These resistant strains were then tested to find whether they were lysogenic for the strains from which they were prepared. The usual method for detecting lysogenic strains was used except that each resistant strain was used only as a basal strain and the strain from which it was prepared was spotted upon it. Twelve tests were made on different occasions. Six of the resistant strains were not shown at any of the tests to be lysogenic to the strains from which they were prepared, while seven were lysogenic at all the tests. The remaining five resistant strains gave varying results; they were shown to be lysogenic on some occasions but not on others. For example, one resistant strain was detected to be lysogenic in only two of the twelve tests, while another was lysogenic in all but two tests.

Table 4. *Examples of phage adaptation (see Text)*

	Phage filtrate 42D				Phage filtrate p 42D/E 193				Phage filtrate p 42D/E 193/1363			
Strains	N	10^{-1}	10^{-2}	10^{-3}	N	10^{-1}	10^{-2}	10^{-3}	N	10^{-1}	10^{-2}	10^{-3}
16	CL	CL	CL	++	CL	CL	CL	++	CL	CL	CL	++
E 193	+++	+	−	−	CL	CL	CL	++	+++	++	±	−
16 (E 193/16)	+++	+	−	−	CL	CL	CL	+	+++	++	±	−
1363*	CL	CL	CL	++	+++	+	−	−	CL	CL	CL	+
16 (1363/16)	CL	CL	CL	++	+++	+	−	−	CL	CL	CL	+

N = undiluted phage filtrate.

* 1363 is the propagating strain of phage 42D.

The degree of lytic action is designated as indicated in the text.

https://doi.org/10.1017/S002217240003610X Published online by Cambridge University Press

Further efforts were now made to demonstrate lysogenicity in the six resistant strains that had not been shown to be lysogenic to the strains from which they were prepared. The cross-culture method was again tried, but this time using broth cultures of the strains in various dilutions. It was found that the best method was to use the 'susceptible' strain as a basal strain and the resistant strain, diluted 100 times, as the spotting strain. Two of the six resistant strains were shown to be lysogenic by this method. Growth of the resistant and 'susceptible' strains together in nutrient broth, centrifugation, and the use of the supernatant fluid for spotting on to the 'susceptible' culture did not serve to demonstrate lysogenicity in the other four resistant strains.

It seemed from these results that some strains of staphylococci might carry the phage to which they had been made resistant and that others might not, although there was evidence to suggest that all resistant strains may carry phage but that the methods of demonstrating lysogenic strains were not wholly satisfactory. The results, however, have to be interpreted in the light of the fact that most of the strains from which the resistant strains were prepared were lysogenic. (This was inevitable as 77·3 % of the propagating strains had been shown previously to be lysogenic.)

Observations on strains of staphylococci rendered resistant to more than one phage

The results of the previous experiment indicated that lysogenic strains could be made resistant to yet another phage; some of the 'double' resistant strains were shown to be carrying the second phage to which they had been made resistant, and others were not. It was decided to make strains resistant to more than one phage, and then to determine whether the resistant strains could be shown to be carrying all the phages to which they had been made resistant.

Resistant variants of strain 16 to phages 14/94 and 129/16 were prepared. Both the resistant variants were lysogenic to strain 16. Strain 16 (14/94) was susceptible to phage 129/16 and strain 16 (129/16) to phage 14/94. Strain 16 (14/94) was then made resistant to phage 129/16 and strain 16 (129/16) to phage 14/94. Strains 16 (14/94 + 129/16) and 16 (129/16 + 14/94) were lysogenic to strains 16 (129/16) and 16 but not to strain 16 (14/94). The 'crude-phages' obtained by the action of each of these two 'double' resistant strains on strain 16 were plated with strain 16. A number of isolated plaques picked from each plate and propagated on strain 16 yielded phages that were all lytic for strain 16 (129/16) but not for strain 16 (14/94).

This experiment was then repeated, but using phages E 174/16 and E 168/16 instead of 14/94 and E 168/16 (phages 129/16 and E 168/16 were later shown to be identical). Strain 16 (E 174/16) was susceptible to phage E 168/16, and strain 16 (E 168/16) to phage E 174/16. The two 'double' resistant strains, 16 (E 174/16 + E 168/16) and 16 (E 168/16 + E 174/16) were lysogenic to strains 16 (E 174/16), 16 (E 168/16) and 16. The phages prepared in this case from the two 'double' resistant strains were of two kinds; some were lytic only for strain 16 (E 168/16) and others only for strain 16 (E 174/16). The two 'double resistant' strains were now plated out on nutrient agar a number of times, single colonies being carefully picked into broth each time, incubated, and replated. Ten colonies were picked from the final plating into broth and incubated. The resulting cultures were lysogenic to strains 16 (E 174/16), 16 (E 168/16) and 16. This was done to minimize the risk that the cultures used for test might have been derived from more than one coccus, some of which were carrying phage E 174/16 and some E 168/16.

Thus the resistant variant of strain 16 to phages E 174/16 and E 168/16 was carrying both phages, but the resistant variant to phages 129/16 and 14/94 could only be demonstrated to be carrying one phage, phage 14/94. Strains 16 (14/94 + 129/16) and 16 (129/16 + 14/94) were made resistant to another phage, phage 1363/14, but only phage 14/94 could be demonstrated in the resistant strains that resulted.

The same results were also obtained with 'double' resistant strains prepared simply by growing the 'single' resistant strains together in broth. This work will be reported more fully in a later paper. Field strains that were apparently identical with the 'double' resistant strain of strain 16 to phages E 174/16 and E 168/16 were also encountered. These field strains were shown to be carrying two phages, one similar to phage E 174/16 and the other to phage E 168/16.

DISCUSSION

The results indicate that acquired phage resistance is probably a factor of considerable importance in the typing of staphylococci by the bacteriophage method. At first sight, it would appear to enhance the value of phage-typing in epidemiological studies as strains could be classified into a larger number of types than would be otherwise possible. For example, by the use of the seven phages of the 42D group, the sixteen strains, previously regarded as belonging to phage 42D type or as untypeable, were classified into nine types. For other purposes, it is obviously a complicating factor as strains that are identical apart from the acquired phage resistance of one or both of them might be classified as different types.

The phage-resistant strains of the 42D group were all lysogenic to strain 16, the non-lysogenic 42D type strain, but not to strains of other phage types. The studies on resistant variants of strains of other phage types revealed that a high proportion of them could be shown to be lysogenic to the strains from which they were prepared. It is probable, therefore, that the demonstration of lysogenicity will be a useful method of identifying phage-resistant strains and of identifying strains that differ only by virtue of acquired phage resistance. The non-lysogenic 42D type strains were also fully susceptible to all the phages of the 42D group, but the lysogenic ones were only susceptible to some of them. The testing of a large number of strains with a given set of phages may consequently indicate which of these phages can be grouped together as serving to distinguish between strains that differ from each other only by virtue of acquired phage resistance. Bearing this in mind, a study of the diagnostic schema of Wilson & Atkinson (Table 1) suggests that acquired phage resistance may be responsible for the differentiation of the sub-types of types 1, 2 and 3. It is hoped to report the examination of strains belonging to these sub-types and other types in a later paper.

The results of making a strain resistant to more than one phage are difficult to explain; the doubly resistant strains, 16 (E 174/16 + E 168/16) and 16 (E 168/16 + E 174/16) were carrying both phages to which they had been made resistant, whereas strains 16 (129/16 + 14/94) and 16 (14/94 + 129/16) were shown to be carrying only phage 14/94. It is, of course, possible that the two latter strains might have been carrying both phages, but, when the strains were tested for lysogenicity, the activity of phage 129/16 might have been inhibited by phage 14/94. The 'key-enzyme' theory of Delbrück & Luria (1942) can account for these two strains only carrying phage 14/94; in the case of the resistant strains 16 (129/16 + 14/94) prepared by the action of phage 14/94 on strain 16 (129/16) it is possible that phage 14/94 replaced phage 129/16 in the 'key-enzyme' system. Neither the 'key-enzyme' theory nor the 'penetration hypothesis' of Delbrück (1945) can explain why strains 16 (E 174/16 + E 168/16) and 16 (E 168/16 + E 174/16) were latently infected with two phages. Both these theories postulate that each bacterial cell can contain only one phage.

SUMMARY

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

2. Strains previously considered as belonging to one phage type, type 42D, or as untypeable, were classified into a number of types by the use of additional phages. This further classification was rendered possible by the acquired phage resistance of some of the strains. All the resistant strains were lysogenic.

3. It was possible to produce a resistant variant of a strain to two phages. Some of these resistant variants carried both phages. Others could be demonstrated to be carrying only one phage.

I wish to thank Prof. G. S. Wilson for his valuable advice and encouragement.

I have to acknowledge the assistance given at various stages of the work by Dr V. D. Allison, Dr B. C. Hobbs and Mr J. D. Atkinson of the Central Public Health Laboratory, Colindale.

The expenses of the work were defrayed by a grant from the Wellcome Research Foundation administered by the Veterinary Educational Trust. I am grateful to Dr W. R. Wooldridge, Scientific Director of the Trust, for his continued interest.

REFERENCES

BAIL, O. (1923). *Z. ImmunForsch.* **38**, 57.
DELBRÜCK, M. (1945). *J. Bact.* **50**, 137, 151.
DELBRÜCK, M. & LURIA, S. E. (1942). *Arch. Biochem.* **1**, 111.
FISK, R. T. (1942). *J. infect. Dis.* **71**, 153.
MACDONALD, A. (1946). *Mon. Bull. Min. Hlth, Lond.*, **5**, 230.
WILSON, G. S. & ATKINSON, J. D. (1945). *Lancet*, **1**, 647.

(*MS. received for publication* 2. XII. 47.—Ed.)