# BACTERIOPHAGE TYPING OF *STAPHYLOCOCCUS AUREUS*

By R. E. O. WILLIAMS, B.Sc., M.D. AND JOAN E. RIPPON, A.R.C.S.,
B.Sc., DIP.BACT.

*Staphylococcal Reference Laboratory, Public Health Laboratory Service, Colindale*

(With Plates 13 and 14, and 1 Figure in the Text)

CONTENTS

## A. INTRODUCTION

Two methods have been widely used in an attempt to recognize stable types within the species *Staphylococcus aureus* (or *pyogenes*) defined by the coagulase test: the serological method by slide agglutination first described by Cowan (1939) and subsequently studied by Christie & Keogh (1940) and Hobbs (1948); and the bacteriophage method suggested by Fisk (1942*a, b*). In both cases the approach

was largely the practical one of devising a method of type identification, and no satisfactory antigenic analysis of the species has yet been reported.

A suggestion that specific phages might be used for typing was made by Williams & Timmins (1938), but the starting-point for the development of the present method was Fisk's isolation from strains of *Staph. aureus* of a series of bacteriophages that were apparently type- rather than species-specific (Fisk, 1942*a, b*). These phages were obtained by picking the plaques that developed when spot-inocula of several strains were grown on the surface of an agar culture of a single strain. Later Fisk & Mordvin (1944) utilized such phages to investigate the epidemiological relationships of a number of staphylococci. Wilson & Atkinson (1945) isolated phages by Fisk's cross-culture method and, with purification and propagation of the phages to relatively high titre, devised a method suitable for routine use. Since 1945, bacteriophage typing has been used by several workers in Britain and elsewhere for epidemiological work (e.g. Barber, Hayhoe & Whitehead, 1949; Rountree & Thomson, 1949; Wallmark, 1949; Denton, Kalz & Foley, 1950).

Smith (1948*a, b*) studied phage-carriage by staphylococci, and considered that in many cases the resistance of staphylococci to the typing phages could be accounted for on the supposition that they had become carriers of phages from other strains. Rountree (1949*a*) showed that some staphylococci carried several distinct phages, but in her experience these were usually distinct from the typing phages, and she did not think that phage-carriage was wholly responsible for resistance to the typing phages. Rountree (1949*b*) also showed that the typing phages fell into two distinct serological groups, which she named A and B, and that a number of their other characteristics were correlated with this serological grouping.

The only investigation of the technique of typing since Wilson & Atkinson's was that of Wahl & Lapeyre-Mensignac (1950*a, b*), who proposed a new method of classification of the phage types of staphylococci.

In 1946 a routine service of bacteriophage type-identification of strains of *Staph. aureus* isolated in epidemiological investigations was set up for the Public Health Laboratory Service in its Central Laboratories at Colindale, under the direction of Dr V. D. Allison. The method and phages of Wilson & Atkinson were employed. From 1946 until Dr Allison left the laboratory at the end of 1948, a great body of experience of the typing technique and of the interpretation of the results was accumulated, and this was a necessary basis for the analysis that we have made on the material examined during the last 3 years, and which we report in this paper.

Many workers have adopted the technique used at Colindale, and phage-typing results are frequently being quoted in the literature. It seems desirable, therefore, before analysing our results, to describe the routine methods of typing in some detail, even though they are in the main only developments of those already published.

## B. DESCRIPTION OF ROUTINE METHODS OF TYPING

### 1. *Material*

#### 1·1. *Source and nomenclature of typing phages*

Of the twenty-four bacteriophages used as a routine during this work, eighteen were those described by Wilson & Atkinson (1945), and three, known as 42 D, 42 E and 29 A, were isolated by them later. Phages 31 A, 53 and 54 were added to the set by Dr Allison.

Ten of the phages were isolated by the cross-culture technique of Fisk (1942 *a*), and the remaining fourteen were adapted from these or other phages similarly isolated.

Table 1. *List of standard set of staphylococcal bacteriophages; their source, serological group, propagating strains and N.C.T.C. reference numbers*

| | | Source | | | N.C.T.C. numbers | |
| Laboratory numbers | | Reference no. of lysogenic staphylococcus | Phage from which adapted | Serological group | | |
| Phage | Propagating strain | | | | Phage | Propagating strain |
|---|---|---|---|---|---|---|
| 3 A | 284 | . | 3 | A | 8408 | 8319 |
| 3 B | 211 | . | 3 | A | 8410 | 8321 |
| 3 C | 1339 | . | 3 B | A | 8411 | 8327 |
| 6 | 3 | ? 42 | . | A | 8403 | 8509 |
| 7 | 4 | 5 | . | A | 8404 | 8510 |
| 29 | 33 | 21 | . | B | 8413 | 8331 |
| 29 A | 1351 | . | 29 | B | 8423 | 8371 |
| 31 | 18 | 24 | . | B | 8402 | 8508 |
| 31 A | R 48/2329 | . | 31 | B | 8424 | 8373 |
| 42 B | 1163 | . | 42 | A | 8419 | 8355 |
| 42 C | 1307 | . | 42 A | B | 8417 | 8353 |
| 42 D | 1363 | . | 42 C | B‡ | 8414 | 8341 |
| 42 E | 1670 | . | 42 | A | 8418 | 8357 |
| 44 | 18 | 35 | . | B | 8405 | 8508 |
| 44 A | 373 | . | 44 | B | 8422 | 8369 |
| 47 | 36 | 17 | . | A | 8409 | 8325 |
| 47 A | 761 | . | 47 | A | 8407 | 8317 |
| 47 B | 987 | . | 47 | A | 8415 | 8345 |
| 47 C | 1163 | . | 47 | A | 8421 | 8355 |
| 51 | 145 | 40 | . | A | 8416 | 8349 |
| 52 | 144 | Rad. 2 | . | B | 8401 | 8507 |
| 52 A | 925 | . | 52 | B | 8420 | 8363 |
| 53* | R 48/3292 | R 48/2311 | . | B | 8406 | 8511 |
| 54† | R 48/R 3303 | R 48/3298 | . | A | 8412 | 8329 |

\* 53 was previously known as P 2311.
† 54 was previously known as P 3298.
‡ Or group F (unpublished observations).

The propagating strains may also be referred to by the number of the phage, prefixed by 'PS', e.g. PS. 3 A for strain 284 and PS. 31/44 for strain 18.

Table 1 shows the twenty-four phages used, together with their source and their propagating strains. Two propagating strains (18 and 1163) are listed twice, because each serves for the propagation of two different phages.

Phages isolated by cross-culture were given a number, and adapted phages were given the number of the parent phage followed by a letter, e.g. 52 A was a phage adapted from the primary isolate, 52. However, this system of numbering the phages became somewhat cumbersome if followed logically when numerous adaptations were carried out from one phage; and in practice the designation of adapted phages has not always been logical. For instance, 3C was adapted from 3B and not from the original '3' phage, now lost. In any case the process of adaptation is not as simple as it appears (see, for example, Rountree, 1949b). Adapted phages are generally obtained by propagation from an isolated plaque produced when the parent phage is applied undiluted to a strain that was not lysed by any phage used at the normal working dilution. It is assumed that the isolated plaque was produced by a variant of the parent phage. However, this is not necessarily true. Nearly all staphylococci carry phages which may be released during growth; and this may occur during the propagation of the typing phage. The final filtrate may therefore be contaminated with one or more phages, and an isolated plaque may be due to one of the contaminants. Rountree has shown that this was probably the source of phage 42C, but it is not known how often mixtures of this sort were the source of the 'adapted' phages.

### 1·2. *Propagating strains of staphylococci*

Agar slope cultures of the strains used for propagating the phages are kept in the refrigerator; subcultures are made from these to nutrient broth once or twice a week, and subcultures from the broths are used for day-to-day tests during the week.

A stock collection of agar stab cultures is kept in the refrigerator, and these are used when any strain grows poorly from an agar slope; dried cultures are also available.

The propagating strains are typed periodically; their lytic patterns are given in Table 2.

### 1·3. *Media*

One medium is used both for propagation and for typing, namely digest broth (Hartley, 1922) with sufficient agar to give a rather soft plate, the actual percentage of agar being varied according to the batch in use. For fluid cultures, nutrient broth is used. For routine typing, but not for propagation, the Hartley agar is underlayered with peptone-water agar made with 1 % Evans peptone, 0·5 % NaCl, and agar.

### 2. *Routine methods*

### 2·1. *Propagation of phages*

In the past, propagation of the phages was carried out serially from batch to batch. However, filtrates of all the routine phages have now been freeze-dried by the National Collection of Type Cultures, and each propagation is carried out from a fresh ampoule of this stock.

The phages are propagated on the surface of 15 cm. agar plates, which are poured to a depth of about 5 mm. with Hartley agar, and dried at 37° C. for about

Table 2. *Phage patterns of the propagating strains of staphylococci*

Phage no.

| Propagating strain | | 3A | 3B | 3C | 6 | 7 | 29 | 29A | 31 | 31A | 42B | 42C | 42D | 42E | 44 | 44A | 47 | 47A | 47B | 47C | 51 | 52 | 52A | 53 | 54 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. | Used for phage | | | | | | | | | | | | | | | | | | | | | | | | |
| 284 | 3A | ++ | + | + | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | ± | . | . | . | . |
| 211 | 3B | . | ++ | ++ | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | ± | . | . | . | . |
| 1339 | 3C | . | . | ++ | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 3 | 6 | . | . | . | ++ | + | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | + | + |
| 4 | 7 | . | . | . | + | ++ | . | . | . | . | ± | . | . | ± | . | . | + | . | . | ± | . | . | . | + | ++ |
| 33 | 29 | . | . | . | . | . | ++ | . | ± | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1351 | 29A | . | . | . | . | . | . | ++ | ± | . | . | . | . | . | . | . | . | . | . | . | . | . | ± | . | . |
| 18 | 31, 44 | . | . | . | . | ± | + | ± | ++ | . | ± | . | . | + | . | ± | + | ± | ± | . | . | ± | ± | . | ± |
| R.2329 | 31A | . | . | . | . | . | . | . | . | ++ | ± | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1163 | 42B, 47C | . | . | . | . | . | . | . | . | . | ++ | . | . | . | + | . | . | . | . | ++ | . | . | . | . | . |
| 1307 | 42C | . | . | . | . | . | . | . | . | . | ++ | ++ | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1363 | 42D | . | . | . | . | . | . | . | . | . | . | . | ++ | . | . | . | . | . | . | . | . | . | . | . | . |
| 1670 | 42E | . | . | . | . | . | . | . | . | . | . | . | . | ++ | . | . | . | . | . | . | . | . | . | . | . |
| 373 | 44A | . | . | . | . | . | . | . | . | . | . | . | . | . | . | ++ | + | . | . | . | . | . | ± | . | . |
| 36 | 47 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | ++ | + | + | + | . | + | . | + | + |
| 761 | 47A | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | ++ | . | . | . | . | . | . | . |
| 987 | 47B | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | ++ | ++ | . | . | . | . | . | . |
| 145 | 51 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | ++ | . | + | . | . |
| 144 | 52 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | ++ | + | . | . |
| 925 | 52A | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | ± | ++ | . | . |
| R.3292 | 53 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | ++ | ± |
| R.3303 | 54 | . | . | . | . | + | . | . | . | . | ± | . | . | . | . | . | + | + | + | ± | . | . | . | ++ | ++ |

++ = more than 50 plaques, including confluent lysis.   + = 20–50 plaques.   ± = less than 20 plaques.

1 hr. The dried plates are spread thinly with a 4–6 hr. broth culture of the propagating strain, using a glass spreader to distribute the minimum number of drops that will give a confluent growth. When the culture has been absorbed into the agar, the phage, suitably diluted, is spread over all but a small segment of the plate. The plates are then incubated at 30° C. overnight.

The phage dilution used is somewhat stronger than the routine test dilution (R.T.D.), which is the highest dilution at which the phage gives confluent lysis on the propagating strain, and is the dilution used for routine phage typing (§ 2·3).

After incubation, the unlysed control area should show good growth with no evidence of spontaneous lysis; any plates showing spontaneous lysis are discarded, since the filtrate would necessarily be contaminated with the 'spontaneous' phage. As a rule the highest titres are obtained from plates showing slight secondary growth amid confluent lysis; plates showing complete lysis with a glassy surface often give low titres, presumably because there was an excess of phage, and almost all the cocci were lysed by the phage soon after the inoculation of the plate, and the substrate available for further phage multiplication was therefore restricted.

The agar with the unlysed growth is removed from the satisfactory plates with a sterile scalpel, and the remaining agar is frozen by holding the plates at − 20° C. for 24 hr., or − 60° C. for 1–2 hr. After freezing, the plate is allowed to thaw at room temperature, when the agar disintegrates and the liquid is expressed (see also § 3·2). The fluid is pipetted off, and centrifuged; a preliminary titration is then carried out and the bulk is stored at 4° C. Titrations are made by applying standard 0·02 ml. drops of tenfold dilutions of phage in broth to a plate previously spread with the appropriate indicator strain.

With phages of serological group A an R.T.D. greater than 1/1000, corresponding to $10^8$ to $10^9$ phage particles per ml., is usually obtained; but with the group B phages the average R.T.D. is 1/100 to 1/1000.

If the preliminary titration is satisfactory, the bulk is filtered through a Gradocol membrane (A.P.D. 0·7–0·9 $\mu$) for small volumes, or through a Seitz filter (Ford 'Sterimat' grade S.B.) for larger volumes. Tests have shown that, with filtrates of reasonably high titre, neither method of filtration consistently leads to a serious drop in titre (Table 3). If the initial titre is below 1/100, filtration results in considerable loss of phage, and such lysates are generally discarded.

Filtrates are tested for sterility by inoculating about 0·5 ml. into 5 ml. broth and incubating for 5 days.

(See § 3·3 for a discussion of propagation in broth and § 3·4 for notes on the stability of the phages.)

## 2·2. *Testing of new batches of phages*

The typing phages have a characteristic lytic spectrum on the set of propagating strains. Each new batch of phage prepared is therefore first spotted, undiluted, on these strains to see that it retains the usual spectrum (Table 4); it is then titrated on those strains that it lyses. The phage titre on any one strain should remain in practically constant ratio to that on the propagating strain. When phages are propagated to high titres, the range of strains lysed by the undiluted filtrate is often

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Table 3. *Effect of filtration on phage titre*

| | Original titre | | Titre after filtration through | | | | |
| | | | Seitz pad | | Gradocol membrane | | |
| Phage | R.T.D. | Count ($\times 10^7$) | R.T.D. | Count ($\times 10^7$) | R.T.D. | Count ($\times 10^7$) |
|---|---|---|---|---|---|---|
| 3 B | 1/1,000 | 27 | 1/10,000 − * | 75 | 1/1,000 + | 55 |
| 42 D | 1/100 | 3·5 | 1/100 | 0·5 | 1/100 | 1·5 |
| 70 | 1/10,000 | 250 | 1/10,000 | 230 | 1/10,000 | 50 |
| 71 | 1/1,000 | 18 | 1/1,000 | 4 | 1/1,000 | 14 |
| 61 | 1/10,000 | 40 | 1/10,000 | 65 | 1/10,000 | 80 |
| 54 | 1/1,000 + | 30 | 1/1,000 | 85 | 1/1,000 | 45 |
| 29 | 1/1,000 | . . + | 1/100 + | . . | 1/100 + | . . |
| 53 | 1/1,000 | 10 | 1/1,000 | . . | 1/1,000 | 5 |
| 55 | 1/100,000 | . . | 1/100,000 − | 180 | 1/100,000 − | 130 |
| 68 | 1/5,000 | . . | 1/5,000 | 45 | 1/1,000 | 10 |
| 47 | 1/10,000 | . . | 1/10,000 + | . . | 1/10,000 + | 500 |

\* − or + against R.T.D. = slightly less than, or slightly more than the tabulated figure.
† . . = not tested.
Apparent increases in titre are considered to be within the limits of experimental error.

increased, but even so, on dilution to the R.T.D., the pattern should remain constant.

It is most important that full tests should be made of each new batch of phage, as contamination and slight variations in the phage may occur and be otherwise undetected.

Certain phages, when propagated to high titre, have produced what we term the 'inhibitory effect', that is, the undiluted filtrate inhibits the growth of many, if not all, of the propagating strains. The inhibition may be complete, giving a picture resembling complete lysis, or it may be partial. On dilution to 1/10 or more, true phage plaques are seen on only some of the strains inhibited by the undiluted filtrate (see also § 3·5). Phage filtrates showing this effect cannot be used undiluted for typing (see § 3·9).

### 2·3. *Dilution and routine testing of typing phages*

The dilution of phage filtrate used for routine typing (R.T.D.) is the highest that gives confluent lysis on the propagating strain. We use tenfold dilutions for determining the R.T.D. and if the dilution just giving confluent lysis appears to lie between two of the tenfold levels, the half-way dilution is taken.

For routine use the phages are diluted in broth. The titres of the diluted phages are checked twice a week by spotting one drop of the working dilution of each phage on to its propagating strain. Any filtrates showing less than a strong reaction are renewed.

### 2·4. *Routine typing technique*

Petri dishes (9 cm.), etched on the bottom with a grid of twenty-five 12 mm. squares, are poured with the Hartley agar on a peptone-water agar base to give a total volume of about 25 ml. and dried at 37° C. for 60–90 min. before use.

Table 4. *Typical lytic spectra of undiluted filtrates of the typing bacteriophages on the propagating strains of staphylococci*

| Propagating strain No. | Used for phage | 3A | 3B | 3C | 6 | 7 | 29 | 29A | 31 | 31A | 42B | 42C | 42D | 42E | 44 | 44A | 47 | 47A | 47B | 47C | 51 | 52 | 52A | 53 | 54 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 284 | 3A | 5 | 4 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 211 | 3B | 3 | 5 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | 3 | . | . | . | . |
| 1339 | 3C | . | 2 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | 3 | . | . | . | . |
| 3 | 6 | . | . | . | 5 | 4 | 1 | 3 | . | . | 4 | 3 | 3 | 4 | . | . | 4 | . | 3 | 4 | . | . | . | 4 | 5 |
| 4 | 7 | . | . | . | 4 | 5 | . | 3 | 2 | . | 4 | 4 | 3 | 4 | 1 | . | 3 | . | 3 | 4 | . | . | . | 4 | 4 |
| 33 | 29 | . | . | . | . | . | 5 | . | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1351 | 29A | . | . | . | 3 | . | 4 | 5 | 3 | . | 4 | 3 | 3 | 4 | 3 | . | 3 | . | 3 | 3 | . | 2 | 3 | . | 3 |
| 18 | 31, 44 | . | . | . | . | 3 | 4 | 4 | 5 | 2 | 4 | 3 | 3 | 4 | 5 | . | 4 | 2 | 3 | 3 | . | 4 | 3 | 3 | 3 |
| R 48/2329 | 31A | . | . | . | . | . | . | 1 | 1 | 5 | 5 | . | 1 | 1 | 2 | . | . | . | . | . | . | . | . | . | 1 |
| 1163 | 42B, 47C | . | . | . | . | . | . | . | . | . | 3 | 5 | 3 | 2 | 2 | 5 | 1 | . | . | 5 | . | . | . | . | 1 |
| 1307 | 42C | . | . | . | . | . | . | . | . | . | 1 | 5 | . | 1 | 2 | . | . | . | . | . | . | . | . | 3 | 1 |
| 1363 | 42D | . | . | . | . | . | . | . | . | . | 1 | . | 5 | 5 | . | 5 | . | . | . | . | . | . | . | . | . |
| 1670 | 42E | . | . | . | . | . | . | . | . | . | 1 | 4 | 2 | 5 | 1 | 5 | 5 | . | . | . | . | . | . | 3 | . |
| 373 | 44A | . | . | . | 1 | 4 | . | 3 | 1 | 1 | 1 | . | 3 | 1 | 1 | 5 | 5 | 2 | 3 | 2 | . | 4 | 3 | 3 | 3 |
| 36 | 47 | . | . | . | . | 2 | . | 3 | . | . | 1 | 4 | 3 | 1 | 1 | . | 5 | . | 4 | 4 | . | . | . | 3 | . |
| 761 | 47A | . | . | . | . | 1 | . | . | 1 | . | . | . | . | . | . | . | . | 5 | . | . | . | . | . | . | . |
| 987 | 47B | . | . | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | 5 | . | . | . | . | . | . |
| 145 | 51 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | 5 | . | . | . | . |
| 144 | 52 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | 5 | 3 | . | . |
| 925 | 52A | . | . | . | . | . | . | . | . | . | . | . | . | . | 1 | . | . | . | . | . | . | 3 | 5 | . | . |
| R 48/3292 | 53 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | 5 | 3 |
| R 48/3303 | 54 | . | . | . | . | 4 | . | . | . | . | 3 | . | . | 3 | 5 | . | 5 | . | 4 | 3 | . | . | . | 4 | 5 |

5 = maximum titre; 4 = 1/10–1/10²; 3 = 1/10²–1/10⁴; 2 = 1/10⁵ or more of maximum titre; 1 = 1/10⁵ or more of maximum titre with certain batches only.

The strains for typing are subcultured to nutrient broth in the morning and incubated at 37° C. until well grown (4–6 hr.). The plates are then flooded with these cultures, excess liquid being pipetted off, and left to dry with the lids tilted up for about 30 min. Drops of the typing phages are then applied in a constant order, one in the area over each etched square, with fine pipettes (giving approximately 0·01 ml. drops). A drop of saline is also applied to each plate to assist in detecting non-specific lysis (see below). The drops are allowed to dry and the plates are then incubated at 30° C. overnight.

In applying the phage drops it is important that the tip of the pipette should not touch the surface of the culture, since if it does some cocci may be carried over on to subsequent strains. If the first staphylococcus happens to carry a phage to which other staphylococci in the same batch are susceptible, this may result in the appearance of 'non-specific lysis'. At the same time, if the saline pipette is allowed to touch the plates, the development of plaques in the area of the saline drop will show whether inadvertent touching of the plates with the phage pipettes was likely, in this particular case, to lead to non-specific lysis, and indicate the size of the plaques so produced.

## 2·5. *Examination of plates and methods of reporting*

During 1949 we followed Wilson & Atkinson's (1945) notation for recording lysis, employing the range 'trace' to $+ + + +$, but this was later modified to the following:

$+ + =$ more than 50 plaques $=$ 'strong lysis',
$+ =$ 20–50 plaques $\quad=$ 'moderate lysis',
$\pm =$ less than 20 plaques $=$ 'weak lysis'.

The 'pattern' of the strains, which is reported, is confined to the list of phages giving strong lysis. Additional symbols used in reporting are:

$( + ) =$ additional weak reactions present,
$w =$ weak reaction (less than $+ +$),
$- =$ non-typable, i.e. no lysis seen.

The appearance of the plates varies considerably. The $+ +$ or 'strong' level, which corresponds to the $+ + + +$, $+ + +$, and $+ +$ levels of Wilson & Atkinson, includes reactions ranging from complete lysis to fifty plaques, and it also includes the reaction where the area of lysis is filled with resistant growth (Pl. 13). This resistant growth may be thick or thin, and in the latter case may also contain plaques, presumably caused by a variant of the phage capable of attacking the cells that are resistant to the predominant phage. The plaques vary considerably in size, and when small may be difficult to see, especially if the staphylococcus has grown luxuriantly. With some strains the plaques may be filled with resistant growth, and occasionally a phage gives both clear plaques and such opaque plaques on one strain.

Non-specific lysis due to the transfer of lysogenic cocci from previous staphylococci of the same batch can usually be distinguished from normal weak reactions by the irregular distribution of the plaques in the drop area (Pl. 14).

## 3. *Investigations into methods*

### 3·1. *Media*

We have made certain experiments to find a simple, reproducible medium that would support the growth of the staphylococci and the phages, but have not been successful. The activity of phages of serological group B on some strains appears to be particularly susceptible to changes in the medium, of an as yet unspecified nature, to which the other phages are more resistant.

### 3·2. *Methods of harvesting phage*

In a few experiments, little difference in phage yield was found when the method of harvesting used in this laboratory was compared with the method described by Rountree (1947), namely emulsification of the agar in broth and extraction overnight at 4° C. before spinning. Emulsification of the agar in broth is a time-consuming operation, and the spinning of the agar takes longer than the spinning of the fluid expressed in the freezing and thawing method, but the method may have to be used if a $-20°$ C. freezing chamber is not available.

### 3·3. *Propagation in broth*

Propagation in fluid culture has been used in the past for staphylococcal phages (e.g. Wilson & Atkinson, 1945; Wahl & Lapeyre-Mensignac, 1950*a*). Rountree (1949*b*) investigated propagation in buffered glucose broth (modified from Todd & Hewitt, 1932) and found that, though some of the phages of serological group A (3A, 3B, 3C and 51) multiplied in this medium, others required the addition of tryptophan; the group B phages could only be propagated in broth enriched with certain factors in the vitamin B complex. No reasons were given why these additional substances should be required for propagation in broth but not on agar.

Preliminary experiments on propagation in fluid media with some phages of serological group A showed that there was an optimum concentration of staphylococci, above and below which phage multiplication decreased. In one experiment with phage 3A in nutrient broth it was found that 2 and 4 hr. cultures gave better phage multiplication than a 6 hr. culture.

On the basis of these preliminary experiments, a 1/100 dilution of a 2–3 hr. broth culture of the appropriate staphylococcus, with a phage inoculum of the order of $10^3$–$10^4$ particles per ml., was used for subsequent work. The cultures were incubated at 37° C. for 6–9 hr. The media used were nutrient broth (N.B.), modified Todd-Hewitt broth (T.H.), 0·2 % glucose peptone water (G.P.W.) made with 1 % Evans peptone, and glucose phosphate peptone water (G.P.P.W.) (0·2 % glucose, 0·5 % $K_2HPO_4$); to these were added when indicated DL-tryptophan (usually at 0·008 mg./ml.) and 1 % Yeastrel sterilized by Seitz filtration.

All the group A phages multiplied in one or more of these media, giving final titres generally at least as high as with agar propagation (Table 5), and little difference was noted between the media. The group B phages multiplied well in glucose peptone water, irregularly in nutrient broth, and only phage 42D multiplied

Table 5. Results of phage propagation in fluid media

| Phage | Typical titres from agar* | Maximum titre from fluid culture* | Results with unenriched media | | | | Effect of tryptophane in | | | Effect of Yeastrel | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | T.H.† | N.B. | G.P.W. | G.P.P.W. | T.H. | N.B. | G.P.W. | T.H. | N.B. | G.P.W. |
| | | | Serological group A | | | | | | | | | |
| 3 A | $10^8$–$10^9$ | $10^9$ | 1‡ | 1 | .. | .. | 0§ | .. | .. | .. | .. | .. |
| 3 B | $10^8$–$10^9$ | $10^8$ | 1 | 1 | 1 | .. | 0 | 0 | 0 | .. | .. | .. |
| 6 | $10^8$–$10^9$ | $10^9$ | 1 | 1 | 2 | .. | 0 | 0 | 0 | .. | .. | .. |
| 7 ‖ | $10^8$–$10^9$ | $10^5$ | 1 | 1 | 1 | .. | 0 | 0 | ± | .. | .. | .. |
| 42 B | $10^7$–$10^9$ | $10^9$–$10^{10}$ | .. | 1 | 1 | .. | .. | 0 | 0 | .. | .. | .. |
| 42 E | $10^8$–$10^9$ | $10^8$–$10^9$ | 2 | 2 | 1 | .. | .. | .. | .. | .. | .. | .. |
| 47 | $10^8$–$10^9$ | $10^8$–$10^9$ | 1 | 1 | .. | .. | 0 | .. | .. | .. | .. | .. |
| 47 A | $10^8$–$10^9$ | $10^{10}$ | .. | 1 | .. | .. | .. | .. | .. | .. | .. | .. |
| 47 B | $10^8$–$10^9$ | $10^9$ | .. | 1 | .. | .. | .. | .. | .. | .. | .. | .. |
| 47 C | $10^7$–$10^8$ | $10^8$ | 2 | 1 | 2 | .. | 0 | 0 | + | .. | .. | .. |
| 51 | $10^7$–$10^8$ | $10^8$ | .. | 1 | .. | .. | .. | .. | + + | .. | .. | .. |
| 54 | $10^6$–$10^8$ | $10^9$ | 1 | 1 | 1 | 3 | 0 | 0 | 0 | .. | .. | .. |
| | | | Serological group B | | | | | | | | | |
| 29 | $10^7$ | Not more than 100-fold increase | .. | 1 | 1 | .. | .. | .. | .. | .. | 0 | .. |
| 29 A | $10^6$–$10^7$ | $10^9$ | .. | 1 | 1 | .. | .. | .. | .. | .. | .. | .. |
| 31 | $10^8$–$10^8$ | $10^8$ | 3 | 3 | 1 | .. | 0 | 0 | 0 | + | 0 | 0 |
| 42 C | $10^7$–$10^8$ | $10^9$ | 3 | 1 | 1 | .. | 0 | 0 | 0 | + | 0 | 0 |
| 42 D | $10^7$ | $10^8$ | 1 | 1 | 1 | 1 | 0 | 0 | 0 | .. | .. | .. |
| 44 | $10^7$–$10^8$ | $10^9$ | 3 | 1 | 1 | .. | 0 | 0 | 0 | 0 | 0 | 0 |
| 44 A | $10^7$–$10^8$ | $10^9$ | 3 | 1 | 1 | .. | 0 | 0 | 0 | 0 | 0 | 0 |
| 52 | $10^8$ | $10^6$ | .. | 1–2 | 1 | .. | .. | 0 | 0 | .. | .. | .. |
| 52 A | $10^7$–$10^9$ | $10^9$ | 3 | 3 | 1 | .. | 0 | 0 | 0 | + | 0 | 0 |
| 53 | $10^8$ | $10^9$ | 3 | 3 | 1 | .. | 0 | 0 | 0 | 0 | 0 | 0 |

\* Titres expressed as phage particles/ml.

† T.H. = modified Todd-Hewitt broth; N.B. = nutrient broth; G.P.W. = 0·2% glucose peptone water; G.P.P.W. = 0·2% glucose peptone water with 0·5% K₂HPO₄.

‡ Results: 1 = maximum titre obtained on any medium; 2 = 1/10–1/100 of maximum titre; 3 = less than 1/100 of maximum titre; .. = not tested.

§ 0 = no difference; ± = 10-fold increase; + = 100-fold increase; + + = 1000-fold increase.

‖ Result of one experiment, using only one phage/culture ratio.

well in T.H. Addition of tryptophan had little effect in any of the three media, although in one experiment the multiplication of phages 7 and 47 C in G.P.W. was enhanced by it. The addition of Yeastrel was tested only on the group B phages; it improved the multiplication of phages 31, 42 C and 52 A in T.H. but had no effect in N.B. or G.P.W.

Propagation in fluid is therefore satisfactory for most staphylococcal phages, if the right media and inocula are chosen. The inhibitory agent (§ 3·5) was observed in five of the ten propagations of group B phages in glucose peptone water.

### 3·4. *Phage titres and stability*

The initial titres of the filtrates after preparation vary between 1/100 and 1/100,000 being, as a rule, higher for phages of serological group A than for those of group B; the lower titres generally seem to be due to bad batches of medium.

Rountree (1949 b) claimed that phages of serological group B are less stable than those of group A, and in our routine bi-weekly checking of the working phage dilutions, most of the group A phages have proved more stable than the group B. Wahl & Lapeyre-Mensignac (1950 a) found that the phages were less stable when prepared in glucose peptone water than when prepared in nutrient broth. We examined ten pairs of phage filtrates, each prepared on the same day by propagation in nutrient broth and in glucose peptone water, over a period of 2–5 months, but failed to demonstrate any difference in the stability of the phage grown in different media.

### 3·5. *The inhibitory effect*

As already noted, high titre filtrates of certain phages, from either agar or broth, sometimes contain in addition to the phage an agent that inhibits the growth of the staphylococci (see § 2·2). The phenomenon has been encountered particularly with phages of the B group, although 47 A has shown the effect, and to a lesser extent 42 B and 3 A.

This agent appears to differ from the autolysin described by Welsch & Salmon (e.g. 1950), which, in our hands, does not inhibit cocci growing on solid media. At the same time, many of our phage filtrates have an effect similar to that of the autolysin in that they lyse heat-killed staphylococci to a slight extent, but this action is not related to the 'inhibitory agent'. The inhibitory agent, which passes readily through Seitz pads and Gradocol membranes, can no longer be detected in filtrates from which the phage has been removed by absorption on to heat-killed cocci, or in which the phage has been neutralized by antiserum. It therefore seems that this inhibitory agent can only act in the presence of phage. Wahl & Josse-Goichot (1950 a, b) have described a 'bacteriostatic factor' produced by a certain *Streptococcus lactis* phage which appears to be similar to the inhibitory agent, although it apparently has a more limited range of action.

### 3·6. *Methods of isolation of cultures for typing*

The work of Smith (1948 b, d) suggested that it would be undesirable to use fluid-culture methods for the isolation of staphylococci that were to be typed by

'the phage method, because the original material might contain a mixture of staphylococci carrying different phages, and incubation in the broth culture might result in interchange of their phages and consequently a change in their susceptibility to the typing phages. It is clearly impossible to show that this never happens, but it seems to us that the risk has been over-emphasized and, in fact, mixtures of types seem to be uncommon in most material (§ 5·1). Results were available from twenty-six sets of four strains from single sources, two of the strains being picked from a direct culture plate, and two from the plate subculture of a primary broth culture. The differences between the phage reactions of strains isolated by the two methods were no greater than the differences between those of pairs of strains isolated by each separate method.

### 3·7. *Minor modifications of typing routine*

It has often been suggested that minor alterations in such points as the age of the cultures used for typing may have a large effect on the results obtained. A small number of staphylococci were therefore typed in parallel using different methods, but no detectable effect resulted from any of the following alterations: (*a*) The use of 18 hr. broth cultures in place of 4 hr. cultures. (*b*) Drying the agar plates for 2 or 3 hr. open, or for 24 or 48 hr. closed, instead of 1 hr. open. (*c*) Inoculation of the plates with four drops of culture and spreading with a bent glass rod instead of flooding and removing the excess culture; or even spreading single loopfuls of culture over an area sufficient for a single phage drop. (*d*) Increase in the time between inoculation of the plate with staphylococci and the application of the phage drops, up to about $1\frac{1}{2}$ hr. at room temperature or $\frac{1}{2}$ hr. at 37° C. Longer drying reduced the phage reactions. (*e*) Incubation of the plates at 37° C. for 5–6 hr. followed by holding at room temperature overnight, instead of overnight incubation at 30° C. Overnight incubation at 37° C. gave heavy growth of staphylococci which obscured the phage plaques. (*f*) Holding plates at 4, 30, or 37° C. after incubation at 30° C. overnight.

### 3·8. *Methods for the isolation of new phages*

New phages for typing have been isolated from staphylococci by the following two methods: (*a*) By the cross-culture technique of Fisk (1942*a*). As an alternative to this method drops of young broth culture supernatants may be spotted on suitable indicator strains. (*b*) From spontaneously lytic strains. Method (*b*) gives phages which may be propagated on the parent strain.

Phages isolated by either of these methods are first purified by picking a single plaque to 0·5–1·0 ml. of broth, which is then plated on the sensitive strain. Three such pickings and platings are made before propagation.

Phages obtained in any of these ways can sometimes be 'adapted' to give strong lysis on staphylococci on which the original preparation gives only a few plaques by repeated picking and plating as for purification of new phages; a total of ten such passages has been used.

3·9. *Investigation of untypable strains*

Approximately 60 % of strains of *Staph. aureus* are lysed by one or more of the twenty-four phages described in this paper, when used at their R.T.D. There appear to be at least two possible reasons why some strains are untypable. First, the right phage may not be available; Rountree & Thomson (1949) reported an epidemic of cross-infection due to a staphylococcus lysed only by a newly adapted phage, 31 B. Secondly, a strain may be, perhaps temporarily, in a state in which it is partially or completely resistant to the phages that, at other times, could lyse it. If partially resistant it is lysed by undiluted phages, if completely resistant it is not lysed by any phage at any concentration.

An indication that strains may sometimes be partially resistant was given by the results of testing 154 untypable routine strains with undiluted filtrates of the routine typing phages; seventy-six (49·4 %) were lysed by one or more of the filtrates, and such reactions were observed with all groups of phages.

Some phages have been tested in an attempt to discover any that would be useful against the untypable staphylococci encountered in our routine work, which are a much more varied collection than those studied by Rountree & Thomson. One hundred and forty-one untypable strains were tested with the undiluted routine phages, and with twenty phages isolated by ourselves and others from the following sources: (1) cross-culture, 13; (2) adaptation, 5; (3) a spontaneously occurring phage, and an adaptation from it, 2.

Fifty-six of the 141 strains were not lysed by the undiluted routine phages and only one of these fifty-six was lysed by any of the new phages. The remaining eighty-five strains were lysed by the undiluted routine phages and nineteen of them were also lysed moderately or strongly by one or more of the new phages. Only fifteen of the twenty new phages acted on any strain; eight of these lysed one, two lysed two, two (31 B and 57) lysed three, and one (52 B) lysed twelve strains. This last phage was obtained by adapting phage 52 to an untypable strain isolated from a nasal culture. Clearly further search for new phages is needed, but the fact that all but one of the strains lysed by this set of new phages were also lysed by one or other of the undiluted routine phages is perhaps further evidence that some strains are untypable because they are resistant to phage action rather than because the right phage is not available.

It is noteworthy that untypable staphylococci are more commonly encountered in cultures from the normal nose or skin than from lesions (39·8 % of 265 and 24·8 % of 302 strains respectively; the difference is highly significant), and there is some evidence that skin and nose strains are less virulent than those from lesions (Schwabacher, Cunliffe, Williams & Harper, 1945; Elek & Levy, 1950). It might be that this difference reflects some metabolic change that is also associated with a change in phage susceptibility.

In practice, testing with the undiluted filtrates is often a useful method of examining untypable strains, but the interpretation of the results is difficult, partly because the filtrates vary greatly in titre, and partly because the plaques are often obscured by secondary growth. Although the reaction-patterns observed

with the undiluted phages are broader than those with the diluted phages, multiple minor reactions do not often occur. Undiluted filtrates used for such tests should not contain the inhibitory agent (§ 3·5).

3·10. *The use of phage pools*

It seems probable that more than twenty-four phage filtrates will be needed if we are to type the greatest possible number of staphylococci. The use of pools of those phages that lyse only a few strains was envisaged by Dr Allison, and the method has recently been tested extensively by Mr A. M. Hood (personal communication), who has been able to show that there is no interference between phages. Since the work reported in this paper was completed, we have relegated three phages, 29A, 31A and 47A to pools, mixed with a number of other new phages and by this means we have been able to type a few additional strains without recourse to undiluted phage filtrates.

In investigations of the staphylococci from a single community such as a hospital, the use of particular additional phages may be necessary. For example, Rountree & Thomson (1949) found a great number of their strains lysed only by phage 31B, a phage for which we have found little use in our routine typing of staphylococci from many sources.

## C. ANALYSIS OF RESULTS OF TYPING

There are two ways in which staphylococci might be distinguished by the use of the phages. In the first, the phages might be found to be 'type-specific', in that staphylococci were commonly lysed by only one phage, or perhaps by one or other of a small group of phages, the individual phages or groups being mutually exclusive. In the second, more complex patterns of phage action might be observed, with considerable overlapping between patterns, and in this case the staphylococci could only be distinguished by comparison of their general patterns of phage susceptibility. Most of the phages that we have used appear to give pattern reactions rather than truly type-specific lysis. The nature and number of these patterns seem to us to make impossible the separation of 'types' in the sense that the word has been used in connexion with, for example, the *Salmonella typhi* phages. Analysis of the reactions of sets of strains thought to have a common origin shows, however, that there is a considerable degree of stability in the pattern of strains of any one population of staphylococci, a 'population' being a group of cocci and their progeny growing in one natural site, as distinguished from a 'strain' which is the succession of laboratory cultures grown from a single colony. Tentative rules can be given for distinguishing between strains of different populations.

## 4. *The patterns observed in phage typing*

4·1. *Frequency of lysis by particular phages*

To study the relative frequency with which staphylococci were lysed by the different phages, we examined the typing results of one strain from each of the 567 distinct sources represented among the 5331 strains typed as a routine in the period January 1949 to March 1950 (Table 6). Thus only one strain was included

Table 6. *Source of strains analysed for frequency of lysis by the typing phages*

| | No. of strains | | | |
|---|---|---|---|---|
| Source of strains | Total | 'Typable' | No reactions at R.T.D. | Non-specific lysis |
| Normal persons, or patients not suffering from staphylococcal infection: | | | | |
| Nose | 202 | 104 | 82 | 16 |
| Faeces, perianal swab, rectal swab | 56 | 33 | 18 | 5 |
| Skin | 7 | 1 | 5 | 1 |
| Septic lesions: | | | | |
| Adults | 109 | 67 | 29 | 13 |
| Pemphigus, etc., in infants | 55 | 39 | 10 | 6 |
| Lung, sputum and nose from patients with pneumonia | 98 | 64 | 29 | 5 |
| Vomit or food in outbreaks of food-poisoning | 37 | 24 | 7 | 6 |
| Miscellaneous | 3 | 3 | 0 | 0 |
| Totals | 567 | 335 | 180 | 52 |

from any one outbreak of food-poisoning or of hospital infection where an 'epidemic type' was recognized, and only one from any particular individual. When the results for more than one strain from a source were available we took the first to appear in our day-book.

One limitation of this material should be made clear directly. The results were in all cases from routine work, and although the titres of the phages were checked frequently there is no doubt that an unrecognized drop in titre, or defect in the medium, must sometimes have led to loss of action by phages to which a strain was in fact susceptible.

We first tabulated the number of strains giving weak, moderate, or strong reactions (as defined on p. 328) with each of the twenty-four typing phages in use, regardless of the other phages that attacked the strain (Table 7). It is noteworthy that the phages attacking most strains produced strong lysis more often than those attacking few strains.

### 4·2. *Combinations of phage reactions*

A simple tabulation of the relative frequency of attack by various phages is, however, of limited interest since staphylococci are commonly attacked by several different phages. Thus, of the 229 strains lysed strongly by one or more phages, only 118 were lysed strongly by a single phage; the numbers lysed strongly by 2, 3, 4, 5, 8 and 10 phages were respectively 60, 27, 17, 4, 2 and 1. We therefore attempted to define the combinations of reactions observed in the set of 567 strains. For these analyses we excluded the 180 strains attacked by none of the typing phages at their test dilution, and the fifty-two strains giving non-specific lysis, the cause of which was not at the time appreciated.

It was soon clear that analysis of these results would be extremely difficult. For example, when the strong reactions alone were considered, no fewer than

Table 7. *Frequency of lysis by the twenty-four typing phages*

| Phage no. | No. of strains lysed | | | | Total, as percentage of the 335 strains giving specific lysis | + + reactions as percentage of total for the phage |
|---|---|---|---|---|---|---|
| | ± | + | + + | Total | | |
| 54 | 39 | 33 | 55 | 127 | 37·9 | 43·3 |
| 47 | 17 | 28 | 72 | 117 | 34·9 | 61·6 |
| 53 | 46 | 27 | 30 | 103 | 30·8 | 29·1 |
| 47 B | 32 | 39 | 30 | 101 | 30·2 | 29·7 |
| 47 C | 46 | 34 | 17 | 97 | 29·0 | 17·5 |
| 52 A | 28 | 17 | 44 | 89 | 26·6 | 49·4 |
| 42 B | 29 | 27 | 18 | 74 | 22·1 | 24·4 |
| 6 | 15 | 19 | 35 | 69 | 20·6 | 50·7 |
| 7 | 24 | 14 | 21 | 69 | 20·6 | 30·4 |
| 52 | 33 | 7 | 27 | 67 | 20·0 | 40·3 |
| 3 C | 23 | 21 | 19 | 63 | 18·8 | 30·2 |
| 42 E | 31 | 15 | 13 | 59 | 17·6 | 22·0 |
| 3 B | 19 | 9 | 12 | 40 | 11·9 | 30·0 |
| 31 | 25 | 9 | 5 | 39 | 11·6 | 12·8 |
| 51 | 14 | 14 | 9 | 37 | 11·0 | 24·3 |
| 29 | 21 | 8 | 4 | 33 | 9·9 | 12·1 |
| 44 A | 22 | 3 | 4 | 29 | 8·7 | 13·8 |
| 44 | 20 | 4 | 4 | 28 | 8·4 | 14·3 |
| 42 C | 19 | 5 | 3 | 27 | 8·1 | 11·1 |
| 3 A | 14 | 5 | 6 | 25 | 7·5 | 24·0 |
| 31 A | 15 | 4 | 1 | 20 | 6·0 | 5·0 |
| 42 D | 10 | 4 | 3 | 17 | 5·1 | 17·6 |
| 47 A | 13 | 3 | 1 | 17 | 5·1 | 5·9 |
| 29 A | 10 | 3 | 0 | 13 | 3·9 | 0 (<7·7) |

eighty-two distinct patterns could be recognized among the 229 strains. The number of distinct patterns was even greater if the lesser degrees of lysis were included: with the moderate and strong levels of lysis taken together, 295 strains fell into 132 different patterns. The greatest number of strains regarded as identical was thirty-three when strong reactions alone were considered, or taking both strong and moderate reactions, thirty-nine; the numbers of patterns represented by single strains were fifty-three and ninety-two, respectively (Table 8).

However, both Wilson & Atkinson (1945) and Dr Allison had observed that phages forming the patterns were not randomly assorted and that, for each phage, there were some other phages with which it was commonly, and some with which it was rarely associated. To give a quantitative illustration of this association of the phages we have used the cultures in our sample that showed strong or moderate lysis with one or more phages. For any one phage we first listed all the strains in the sample that were lysed by it. We then counted the number of these strains that were also lysed by each of the other phages. When a strain was lysed by, for example, the four phages 3A, 3B, 3C and 51, we had twelve entries for our table since, starting with 3A, we had the associations with 3B, with 3C, and with 51; starting with 3B, there were the associations with 3A, 3C, and 51; and so forth. In Text-fig. 1 the results of this enumeration are set out in the form of a block diagram. Each block indicates the frequency with which two phages were observed together lysing one staphylococcus. For example, the third horizontal row of four

Table 8. *Frequency of occurrence of phage reaction patterns*

| | Patterns comprising | | | | |
|---|---|---|---|---|---|
| | Moderate (+) and/or strong (++) reactions | | | Strong (++) reactions only | |
| No. of strains per pattern | No. | Actual patterns | No. | Actual patterns | |
| 1 | 92 | * | 53 | * | |
| 2 | 19 | * | 7 | * | |
| 3 | 4 | 3A, 6/47/47B/53/54, 47B, 51 | 4 | 3A, 3C/51, 47/47B, 47/47B/54 | |
| 4 | 3 | 29, 6/47/53/54, 47/47B/47C | 2 | 3B, 6 | |
| 5 | 3 | 3B/3C/51, 6/47, 42B | 3 | 42B, 51, 52/52A | |
| 6 | 1 | 47/47B/47C/54 | 3 | 3B/3C, 6/47, 7 | |
| 7 | 3 | 3B/3C, 47/54, 54 | 2 | 47/53, 53 | |
| 8 | 2 | 52, 52/52A | 3 | 6/47/54, 47, 54 | |
| 9 | 2 | 3C/51, 42E | 3 | 3C, 42E, 47/54 | |
| 11 | . | .. | 1 | 52 | |
| 12 | 1 | 53 | . | .. | |
| 14 | 1 | 3C | . | .. | |
| 33 | . | . | 1 | 52A | |
| 39 | 1 | 52A | . | . | |
| Totals | 132 | | 82 | | |

\* There were too many patterns in these categories for tabulation.

blocks gives the pairings observed with phage 3A. In the first column, under '3B', is indicated the percentage (45, see scale at foot) of the strains lysed by phage 3A that were also lysed by 3B. The second column gives the percentage (35) of the strains lysed by 3A that were also lysed by 51, and the fourth column the similar percentage (35) lysed by 3C. The third column, under 3A, gives the percentage (25) lysed by 3A alone. The lightly shaded blocks are those based on fewer than ten reactions by the index phage. Since the phages are set out in the same order in the top and left margins, the block representing lysis by one phage alone always lies on the diagonal of the table.

The taller blocks in the figure represent more common pairings of phages; the grouping of blocks represents wider groups of 'related' phages.

The phages are set out in the figure to give the most clear-cut segregation of groups. One group stands out distinctly, namely that comprising phages 3B, 51, 3A and 3C, only the last of which was at all commonly found in combination with phages outside this group. That there are two other broad groups is also apparent, one comprising phages 47, 53, 7, 54, 6, 42B, 47B, 47C, 42E and 47A, and one comprising phages 44, 44A, 52, 52A, 31, 29, 31A and 29A; but there was considerable overlap between these two groups and in particular it is not easy to determine the position of 42C and 42D. The phages of each of the three main groups show some other common characteristics and we shall refer to the groups respectively as the 3A, 6/47, and 52 groups; by extension staphylococci may also be said to belong to these groups.

It is possible to extend this kind of analysis to more complex patterns. Instead of entering each phage separately in a table and setting out the relative frequency

of pairing with each of the other phages, one can enter in the table each pair that showed significant association in Text-fig. 1, and set out the frequency with which trios, quartets, and so on are found with other phages. For example, starting with
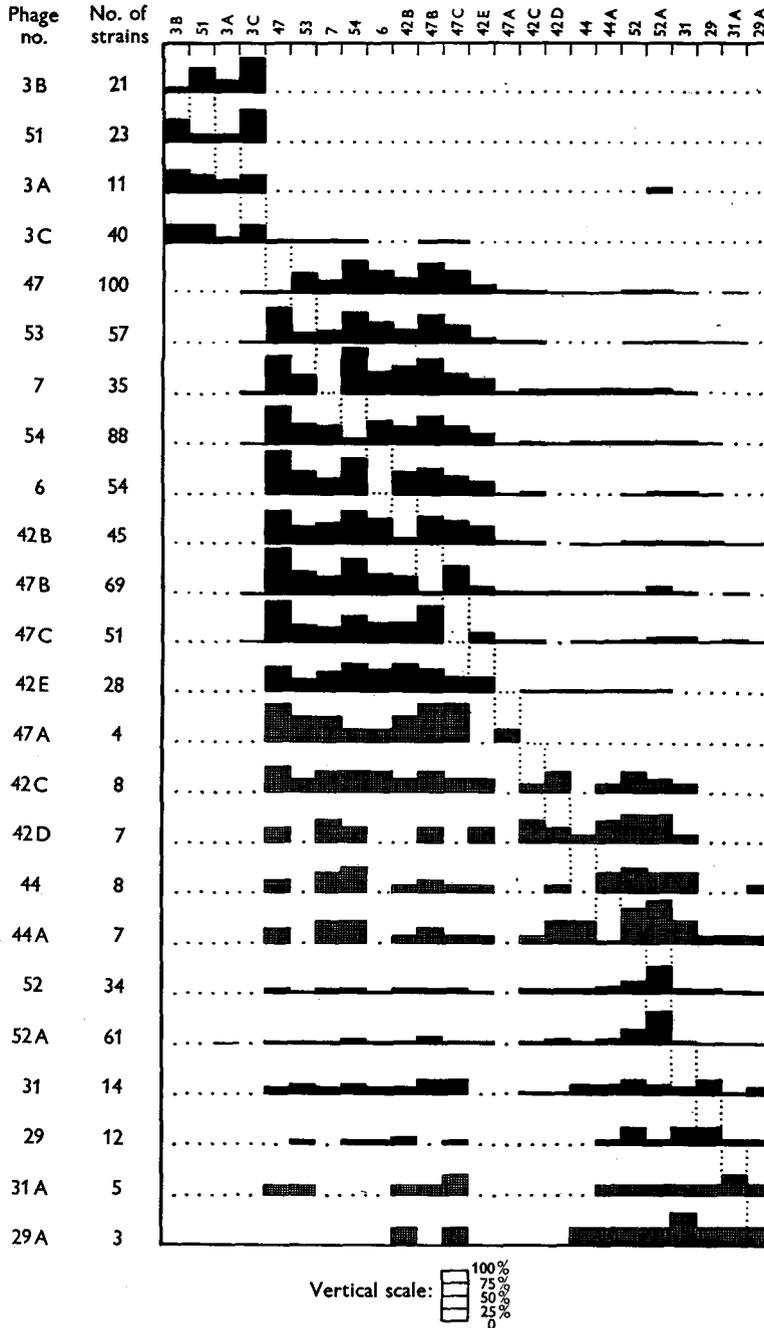


Text-fig. 1. Frequency of occurrence of pairs of reactions in the phage patterns of staphylococci (see text).

phage 7 and arbitrarily assuming that the association is significant if another phage is found lysing 70 % or more of the strains that phage 7 lyses, we can define more complex patterns as follows. Of all the attacks by phage 7, 86 % were in combination with phage 54; of the 7/54 attacks, 83 % were in combination with 47; and again of the 7/54/47 attacks, 84 % were in combination with 47 B. There was no one other phage found in 70 % or more of the 7/47/47 B/54 attacks. Therefore 7/47/47 B/54 may be called a stable group. The groups recognized by this method are, however, very numerous and often cumbersome; and there is no justification for regarding them as epidemiologically significant. For example, one strain may be lysed by phages 7/47/47 B/54 and another by phages 6/7/47/47 B/54. The important question then is, whether these two might be derived from a common source. To answer this question it is necessary to study the variation in patterns actually observed in the field.

Text-fig. 1 also gives some further information on the usefulness of the various phages. For example, we can see which of the phages in the set were commonly found acting alone, from the percentage frequencies on the diagonal. We can also see whether any phages were of relatively little importance because of close association with another phage. At first glance one might think that it was unnecessary to use both 47 and 47 B, since, of the 47 B attacks, 88 % were accompanied by attacks by 47; but in this case, as in most other similar cases, the reciprocal combination frequency was not so high, for only 61 % of the 47 reactions were accompanied by 47 B.

## 5. *The stability of phage patterns*

### 5·1. *Variability in sets of strains with a common origin*

The results so far presented illustrate the characteristic feature of this typing method, namely that staphylococci show patterns of phage susceptibility, and that the number of patterns observed is very great. The staphylococci from a single outbreak of, for instance, food-poisoning, where many persons are infected from a single source, usually have the same, or almost the same, reaction patterns. A typical set of strains, isolated in the investigation of an outbreak of food-poisoning, will serve as an example (Table 9). From the general appearance of the patterns there can be no real doubt that strains 1–8 are all of one type, and are certainly different from strain 9, and almost certainly from strain 10. But there are a number of differences both in the range of phages that lyse the strains and in the degree of lysis. We need, therefore, some measure of the variation commonly observed in staphylococcal populations to indicate when two patterns should be regarded as different, and when one should be considered a variant of the other.

No purely laboratory investigations of the frequency of various patterns can indicate the epidemiological validity of distinction between patterns; this can only be obtained by a study of the variation actually observed in material collected in the field. We have therefore attempted to measure the range of variation in the phage-reaction patterns in various sets of cultures. This analysis is of necessity rather complex, but it nevertheless seems desirable that it should be presented in full. The general conclusions derived from it are summarized in § 5·2.

Table 9. *Typing results for staphylococci from an outbreak of food-poisoning*

| No. | | Degree of lysis with phages | | | | | | | | | | | | | | | | Reported as |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3C | 6 | 7 | 29 | 31 | 42B | 42C | 42D | 42E | 47 | 47B | 47C | 52 | 52A | 53 | 54 | |
| 1 | Foodstuff 1 | ± | ± | ++ | . | . | + | . | . | + | + | ++ | + | . | . | + | + | 7/47B+ |
| 2 | Foodstuff 2 | . | ± | ++ | . | . | ± | . | . | ± | ± | + | ± | . | . | + | + | 7+ |
| 3 | Foodstuff 3 | . | + | ++ | . | . | + | . | ± | + | + | + | ± | . | . | + | + | 7+ |
| 4 | Patient 1, vomit | . | ± | ++ | . | . | ± | . | . | ± | ++ | + | ± | . | . | + | ± | 7/47+ |
| 5 | Patient 2, faeces | . | ± | ++ | . | . | ± | . | . | . | . | ± | . | . | . | ± | ± | 7+ |
| 6 | Patient 3, faeces | + | + | ++ | . | ± | ± | . | . | + | ± | ++ | + | . | . | + | + | 7/47B+ |
| 7 | Food-handler 1 | + | + | ++ | . | . | + | . | . | + | + | ++ | + | . | . | + | ++ | 7/47B/54+ |
| 8 | Food-handler 2 | ± | ± | ++ | ± | . | ± | . | ± | ± | ± | + | ± | . | . | + | + | 7+ |
| 9 | Food-handler 3 | ++ | . | . | . | . | . | + | ++ | ++ | . | . | . | ++ | + | . | . | 3C |
| 10 | Food-handler 4 | . | . | ++ | . | . | . | . | ++ | ++ | . | . | . | ++ | . | . | ++ | 7/42D/42E/52/54+ |

The sets of cultures used for this analysis were:

(1) Duplicate plates spread from one broth culture.

(2) Duplicate broth cultures seeded on one day from one stock culture.

(3) Duplicate broth cultures seeded on different days from one stock culture, and thus typed on different days.

(4) Broth cultures grown from different colonies picked from one primary culture plate, e.g. of a nose swab.

(5) Cultures from different sites on a single subject.

(6) Sequential cultures from a single source, e.g. the nose of a persistent carrier.

(7) Multiple cultures from a number of foods or of victims in outbreaks of food-poisoning, all apparently infected simultaneously, as judged by the epidemiological evidence.

(8) Multiple cultures from patients all involved in one outbreak of hospital infection, but not necessarily all infected together or even from an immediate common source.

The first three of these groups—involving repeated typing of a single culture—may be regarded as tests of the method; the remaining five tests are presented as measures of the variability observed in natural staphylococcal populations. In examining the material of groups (4)–(6) we found that mixtures of staphylococci having quite unrelated phage patterns were rare. We therefore assume that in the great majority of cases the several strains tested were drawn from a single population. Had mixtures of staphylococci been common in these sites it seems improbable that the strains in any one set, though varying somewhat from one another, would practically always have shown closely related phage patterns.

There is an additional practical reason for studying the variation in sets of strains from various sites in single individuals. We often wish to know whether staphylococci isolated on one day from the nose of a carrier could be of the same type as those isolated from patients thought to have been infected from the carrier at some time previously.

For assessing the degree of variation between a pair of reaction patterns we have used the classification given in Table 10, rows 11–19. When the variation included the gain or loss of reactions, we ignored any degree of variation in the reactions of phages lysing both strains.

For assessing variation in sets of strains we ordinarily regarded the first strain to be entered in our day-book as the 'index' strain, and compared the other strains with it; in sets from epidemics, when more strains were studied, it seemed better to take as the index the modal strain, that is the one that varied least from the greatest number of the remaining strains. In analysing results from outbreaks of food-poisoning, the strains from patients and food handlers have been compared with the index strain from the food, or from the vomit when no food strain was available.

Much of our material was derived from routine work when it was not always possible to obtain equal numbers of strains for each set, and the sets on which our analyses are based therefore contained varying numbers of strains. For presentation we wished to obtain a composite picture from all the sets in any one group, and

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we have therefore used the following rules. Each strain in a set was classified according to its difference from the index strain, as shown in Table 10 (rows 11–20). To assess the average variation in sets in one group we gave each set the same weighting, whether it consisted of two or more strains. Thus in a set of two strains, 1 point was entered under the appropriate difference; in a set of six strains where, for example, 2 were classed as 'none' and 3 as 'in strength of reaction only' the amounts entered for these two categories were 2/5 and 3/5 respectively.

The values set out in the table may therefore be taken as an average of the amount by which two-culture sets from the same sources would be expected to vary.

Sets of strains from six different sources were analysed. Tests of duplicate and repeat typings were made on the staphylococci used for propagating the phages, and on material derived from our routine typing. For examination of variation between different colonies picked from a plate, and cultures from different sites on one person, we have relied largely on routine material and on two investigations of the carriage of staphylococci carried out respectively by Dr Lynette M. Dowsett at Norwich and Dr N. S. Mair at Manchester. In the former, cultures were made from the nose and faeces of patients admitted to an isolation hospital; in the latter a number of healthy men working in an engineering factory had their noses, hands and any minor skin wounds swabbed weekly over 2 months. For some of this material, and more particularly as a source of sequential cultures, we are indebted to Dr J. S. Porterfield of the Common Cold Research Unit, Salisbury (C.C.R.U.), who supplied us with cultures from the noses of volunteers at the time of arrival at and departure from that Unit. A number of the sequential culture sets were also collected in a study with Mr Neave, of the National Institute for Research in Dairying, on staphylococcal infection of cows' udders. Sequential cultures were usually tested at intervals of 1–4 weeks.

*Results.* From the results of the analysis (Table 10) it can be seen that the sets rarely contained typable strains that differed completely from the index in sharing no reactions with it and were therefore considered distinct (row 17); in the large groups of multiple colonies from single cultures and of multiple cultures from single subjects (cols. 4 and 5) the percentages were only 1·2 and 1·8 respectively.

Interpretation of the results is, however, made difficult by the fact that in most of the groups there was a proportion of sets containing a mixture of typable and untypable strains (row 16). A small proportion (1·7 and 0·95 %) of such sets were observed in duplicate typings of one strain carried out on one day (cols. 1 and 2), but the reactions of the typable strains in these sets were weak so that this result is not unexpected. However, with repeat typings of one strain on different days about 12 % were untypable at one of the two tests. In this instance the untypable strain cannot be regarded as belonging to a different population from the typable, and we must assume that the difference in the results of typing was due either to difference in technique—and we know that variations in media may have this effect—or to a real change occurring on storage. In the remaining groups there are two possible explanations for the existence of untypable strains in an otherwise typable set. They may represent a mixture of populations—one typable and the

## Table 10. *Variation in typing results*

| | Multiple plates from 1 broth (1) | Multiple broths from 1 culture on one day (2) | Repeat typings on different days (3) | Multiple colonies from 1 primary culture (4) | Multiple cultures from 1 person (5) | Sequential cultures from 1 site (6) | Outbreaks: Food-poisoning (17 outbreaks) — Foods (7) | Vomit and/or faeces (8) | Food-handlers (9) | Hospital cross-infection lesions (10) |
|---|---|---|---|---|---|---|---|---|---|---|
| **Source of strains (see text):** | | | | | | | | | | |
| 1. Propagating strains | 22 | 22 | | | | | | | | |
| 2. Routine investigations | 98 | 81 | 123 | 87 | 69 | 33 | 13 | 23 | 12 | 19 |
| 3. Volunteers at C.C.R.U. | ·· | ·· | ·· | 17 | ·· | 50 | ·· | ·· | ·· | ·· |
| 4. Volunteers at Manchester | ·· | 2 | 15 | 18 | 18 | 13 | ·· | ·· | ·· | ·· |
| 5. Nose and faeces survey | ·· | ·· | ·· | 112 | 38 | ·· | ·· | ·· | ·· | ·· |
| 6. Cows' udders | ·· | ·· | ·· | ·· | ·· | 13 | ·· | ·· | ·· | 19 |
| 7. Total number of sets of strains or cultures | 120 | 105 | 138 | 234 | 125 | 109 | 13 | 23 | 12 | 19 |
| 8. No. of cultures tested | 240 | 216 | 276 | 768 | 306 | 218 | 79 | 83 | 40 | 153 |
| 9. Mean no. cultures tested per set | 2·0 | 2·06 | 2·0 | 3·3 | 2·4 | 2·0 | 6·1 | 3·6 | 3·3 | 12·4 |
| 10. Range of no. of cultures tested | ·· | 2–5 | ·· | 2–10 | 2–7 | ·· | 2–18 | 1–22 | 1–5 | 2–25 |
| **No. of sets with variation:** | | | | | | | | | | |
| 11. None | 37·0 *30·8† / 39·8 | 30·0 28·6 / 35·5 | 16·0 11·6 / 14·7 | 47·9 20·5 / 32·8 | 22·6 18·1 / 30·4 | 7·0 6·4 / 11·7 | 1·2 9·6 / 9·7 | 1·9 8·3 / 10·1 | 0·7 5·9 / 6·8 | 1·0 5·3 / 6·3 |
| 12. In strength of reaction only | 6·0 5·0 / 6·5 | 8·5 8·1 / 10·1 | 7·0 5·1 / 6·4 | 20·4 8·7 / 13·9 | 11·4 9·1 / 15·3 | 3·0 2·8 / 5·0 | 3·0 23·5 / 24·2 | 4·5 19·6 / 23·8 | 0·45 3·8 / 4·4 | 1·3 6·6 / 8·2 |
| 13. Loss and/or gain of ± or + reactions only | 47·0 39·2 / 50·5 | 44·0 41·9 / 52·0 | 56·0 40·6 / 51·4 | 53·4 22·8 / 36·5 | 25·0 20·0 / 33·6 | 22·0 20·2 / 36·7 | 6·2 47·7 / 50·0 | 9·7 42·2 / 51·3 | 5·9 49·2 / 57·0 | 6·4 33·7 / 40·5 |
| 14. Loss or gain of one ++ with or without gain and/or loss of + and ± reactions | 1·0 0·8 / 1·1 | 1·0 0·95 / 1·2 | 13·0 9·4 / 11·9 | 4·9 2·1 / 3·3 | 1·0 0·8 / 1·3 | 11·0 10·1 / 18·3 | 0·1 0·8 / 0·8 | 0·1 0·4 / 1·5 | 0·8 6·7 / 7·7 | 1·9 10·0 / 12·1 |
| 15. Loss and/or gain of more than one ++ reaction, etc. | 0 0 | 0 0 | 0 0 | 1·3 0·6 / 0·9 | 1·2 1·0 / 1·6 | 6·0 5·5 / 10·0 | 1·2 9·2 / 9·7 | 0·6 2·6 / 3·2 | 1·3 10·8 / 12·5 | 1·5 7·9 / 9·5 |
| 16. One strain untypable | 2·0 1·7 / 2·1 | 1·0 0·95 / 1·2 | 17·0 12·3 / 15·6 | 18·5 7·9 / 12·6 | 13·3 10·6 / 17·8 | 11·0 10·1 / 18·3 | 0·7 5·4 / 5·6 | 2·1 9·1 / 11·1 | 1·2 10·0 / 11·6 | 3·7 19·5 / 23·4 |
| 17. No common reactions | 0 0 | 0 0 | 0 0 | 2·9 1·2 | 2·5 1·8 | 0 0 | 0 0 | 2·1 9·1 | 1·25 10·4 | 1·3 6·8 |
| 18. Non-specific lysis in one or both strains | 16·0 13·3 | 11·5 10·9 | 12·0 8·7 | 9·3 4·0 | 2·2 1·8 | 11·0 10·1 | 0·6 4·6 | 2·0 8·7 | 0·4 3·3 | 1·4 7·4 |
| 19. Both untypable | 11·0 9·2 | 9·0 8·6 | 17·0 12·3 | 75·3 32·2 | 46·2 37·0 | 38·0 34·8 | 0 0 | 0 0 | 0 0 | 0·5 2·6 |
| 20. Total, less rows 17, 18 and 19 | 93·0 84·5 | 84·5 | 109·0 | 146·4 | 74·5 | 60·0 | 12·4 | 18·9 | 10·35 | 15·8 |

\* For explanation of fractional values in rows 11–20, see p. 342.

† The upper black figure of each pair is the percentage of the total number of sets examined (row 7) and the lower is the percentage of the total, less rows 17, 18 and 19 (row 20).

other untypable; or it may be that a proportion of the cells in one population have a reduced sensitivity to the phages. It seems likely that both causes operate. If the first were the whole explanation one might have expected to find a large proportion of sets in which there was a clear mixture of typable populations, but this is not generally the case: in almost all groups the proportion in row 17 is much smaller than that in row 16. If diminished phage sensitivity were the explanation one might have expected to find that the typable strains of the set were below the average sensitivity of phages generally and showed only weak or moderate reactions, but this was also not the case. However, some further indications may be obtained by testing the strains with undiluted phage filtrates (see p. 333), and this was done in twenty-six pairs of strains from the material in cols. 4–6 (row 16) of Table 10. In these tests, nine (34·6 %) of the untypable strains could be shown to be of a different type from the typable members of their set and eight (30·8 %) could be shown to be broadly the same; the remaining nine (34·6 %) were untypable with the undiluted phages. It appears, therefore, that the mixed typable and untypable sets represent to some extent mixtures of populations, and probably also to some extent variations in sensitivity.

The point that is of particular interest in this analysis is the degree of variation observed in strains thought to be from one population. For such an analysis it is reasonable to exclude sets in which all the strains were untypable (row 19, Table 10), and also sets that were clearly mixtures of two typable populations (row 17). The results from strains showing non-specific lysis (row 18) cannot be analysed, and since we have no reason to think that their omission will bias the results we have ignored them.

As already discussed, some of the sets containing an untypable strain (row 16) represent mixtures of populations and some represent mixtures of strains more and less resistant to the phages. These sets are included in the analysis, so that the 'total' number of sets on which the percentages are based (row 20) really includes a number of mixtures, and therefore the tabulated proportions of sets showing a particular degree of variation (the lower of each pair of black figures in Table 10) are underestimates of the proportion to be expected in sets that contain no mixtures.

The first three columns of Table 10 summarize our tests of the variability of the typing method. It will be seen that identity of patterns was rare, even in duplicate plates seeded from one broth. Lysis by the same set of phages, regardless of the degree of lysis, was observed in only 46·3 % of duplicate platings (rows 11 and 12) and identity in both range and degree of lysis in only 39·8 % (row 11, col. 1). It seems that, with the technique that we have used, we must accept some variation in the range of phages that are active as well as in the strength of their reactions. Sets entered in row 13 differed only by the gain or loss, or both, of one or more weak or moderate reactions, and this degree of variation was observed in some 50 % of sets. Only one of the sets of duplicate platings from one broth (col. 1) and one of the duplicate broths from one culture (col. 2) showed gain or loss of a strong reaction. As was to be expected, the degree of variation within the sets was greater when one culture was tested on different days (col. 3), since the media used for typing would

often be from a different batch: 11·9 % of such sets differed by the gain or loss of one strong reaction (row 14).

The variability in staphylococcal populations (cols. 4–8) was naturally greater than that in single cultures (cols. 1–3), but even so gain or loss of one strong reaction was only observed in 4·2 and 2·9 % respectively of the sets of multiple colonies and multiple cultures tested on one day (cols. 4 and 5, rows 14 and 15). Sequential cultures from single sites (col. 6) showed considerable variation: no fewer than 28·3 % differed by one or more strong reactions. This large number of strains differing strongly is presumably due partly to differences of the sort mentioned above, which occur when a single culture is tested on different days, and partly to real variation in the population in its natural habitat. On several occasions, retesting of such sequential cultures in one batch, to avoid differences in media, gave improved agreement between patterns. We therefore carried out a formal experiment in an attempt to distinguish between these two sources of variation by isolating strains from nasal carriers at intervals of about a month, and typing them, first, at their respective times of isolation, and, secondly, together at the end of the experiment. Unfortunately only seven of the twenty-two carriers yielded typable strains, and among these there was no detectable difference in the degree of variation at the two tests; further work is needed on this question.

It should be noted that with the duplicate typings of cols. 1–3 there was no set differing by more than one strong reaction (row 15); in the remainder about one-third of the sets showing 'strong' differences differed by two or more strong reactions.

The variation among sets of strains from toxic foodstuffs, vomit, and the lesions of victims of hospital infection appeared to be intermediate between that of the multiple cultures and that of the sequential cultures, but in view of the small numbers no great confidence can be attached to the exact proportions.

We have not so far distinguished the phage groups (3A, 6/47 and 52, see p. 337) in discussing variations in phage-patterns, and the values tabulated are shown as average values over all typable staphylococci. However, this does not represent the whole truth, since strains lysed by phages in the three groups show different degrees of variation (Table 11). Such differences are to some extent to be expected, since the number of phages forming the patterns in each of the three groups is different, but the differences seem to be too big to be explained by this alone. In the 3A group variation in phage pattern was absent in 40–47 %, and in degree only in 12–21 %. In the 6/47 group variation including the gain or loss of moderate and weak reactions was common (48 and 57 %). In the 52 group variations were little more frequent than in the 3A group. It was notable, however, that mixtures of typable and untypable strains were commoner in the 52 group than in either of the others; and there is some evidence that phages of this group are the most exacting in their nutritional requirements.

Lastly we may note that of eighty sets (from the material of cols. 4–6, Table 10) in which all the strains were untypable with the diluted phages and were retested with the undiluted filtrates, forty-seven (59 %) were apparently all of the same type, and four (5 %) probably the same; twenty-four (30 %) remained untypable,

and only five (6 %) contained strains that were clearly different. This last proportion is similar to the corresponding proportion observed with the typable strains.

Table 11. *Variation in typing results in the different phage groups*

| | Phage group | | | | | |
| | 3 A | | 6/47 | | 52 | |
| Variation | 1* | 2 | 1 | 2 | 1 | 2 |
| --- | --- | --- | --- | --- | --- | --- |
| None | 47·2 | 40·2 | 11·8 | 12·1 | 35·2 | 37·2 |
| In strength of reaction only | 11·6 | 21·3 | 14·5 | 11·2 | 12·7 | 15·3 |
| Loss and/or gain of + and ± reactions only | 22·4 | 15·2 | 47·6 | 56·9 | 28·6 | 14·3 |
| Loss or gain of one + + with or without gain and/or loss of + and ± reactions | 1·2 | 0 | 6·3 | 0 | 2·4 | 0 |
| Loss and/or gain of more than one + +, etc. | 4·0 | 0 | 0 | 0 | 0·7 | 4·7 |
| One strain untypable | 4·0 | 13·0 | 14·5 | 14·7 | 17·8 | 28·5 |
| No common reactions | 1·2 | 4·4 | 1·7 | 4·3 | 0 | 0 |
| Non-specific lysis in one or both strains | 8·4 | 5·9 | 3·7 | 0·9 | 2·7 | 0 |
| No. of sets | 25 | 23 | 56 | 29 | 45 | 21 |
| Mean no. of reactions per strain: † | | | | | | |
|    All reactions | 2·3 | | 5·8 | | 3·1 | |
|    + + reactions only | 1·4 | | 2·3 | | 1·4 | |

\* 1 = multiple colonies; 2 = multiple cultures (cols. 4 and 5 of Table 10).
† Based on the 299 classifiable strains from the sample defined on p. 335.

### 5·2. *Suggested conventions for interpretation of typing results*

Summarizing the conclusions of § 5·1, it is clear that complete identity of phage pattern in two cultures thought to be the same was relatively rare, and, as was to be expected, the degree of variation increased with the remoteness of the cultures from their presumed common source. Duplicate tests of one culture showed less variation than duplicate cultures from one source, and these in turn showed less variation than successive cultures from one source. Nevertheless, in sets of cultures all tested on 1 day probably fewer than 5 % differed by the complete loss of more than one strong reaction.

These investigations offer guidance for the interpretation of typing results obtained in routine work. In general, in sets tested all on the same day, we consider that two strong differences, that is, the complete absence in one strain of lysis by two phages giving strong lysis in the other, may be regarded as indicating a distinct population; but that a single strong difference usually indicates a different population among staphylococci of the 3 A and 52 groups. When one or more strains of a set are not lysed by any phage at the test dilution, they should be retested with undiluted phage filtrates before being regarded as different; if they then give patterns similar to those observed with the diluted filtrates in other members of the set, they are generally regarded as the same as those other members despite the quantitative difference in phage sensitivity. Such quantitative differences have been most frequently observed with the 52 group of phages. When none of the strains is lysed by any phage at the test dilution, all should be retested with undiluted filtrates.

When the typing is carried out on different days it is more difficult to be certain

as to the amount of variation permissible; the rules given above would be followed, but the results reported with less confidence. It may help if one or two strains of the first batch to be typed are included as a standard with each batch typed subsequently.

One of the greatest practical advantages of a scheme for classifying phage patterns into 'Types' would be the simplification of reporting, but in view of the observations already discussed we have thought it best to continue to report the actual phage pattern observed. Our general rule is to present a list of the numbers of the phages that give strong lysis. Thus a staphylococcus reported as 6/7/47/47C+

Table 12. *Examples of method of reporting phage patterns*

| Strain no. | Phage reactions | | | | | | Phage pattern reported | Strain considered the same as numbers |
|---|---|---|---|---|---|---|---|---|
| | 6 | 7 | 42D | 47 | 53 | 54 | | |
| 1 | ++ | ± | − | ++ | + | ++ | 6/47/54+ | 2, 3 |
| 2 | ++ | ++ | − | ++ | ++ | ++ | 6/7/47/53/54 | 1, 3 |
| 3 | ++ | − | − | ++ | ± | ± | 6/47+ | 1, 2 |
| 4 | − | ++ | ± | ++ | − | − | 7/47+ | 5 |
| 5 | − | ++ | + | + | + | − | 7+ | 4, ? 6 |
| 6 | − | − | ++ | − | − | − | 42D | ? 5, none |

showed strong lysis with the four phages, 6, 7, 47 and 47C, and weak lysis with other phages. In the presence of a strong reaction we do not ordinarily report the weaker reactions in detail, although if these are present we indicate the fact by adding '+' to the list of phages (see also § 2·5, p. 328). Such descriptions are better referred to as 'patterns' than as 'types'. A written comment should be added to the report.

As an example we may consider the set of reactions in Table 12. Clearly strains 1, 2 and 3 must all be regarded as indistinguishable, since none shows two or more strong differences from any other, and all three are different from each of strains 4, 5 and 6. But the patterns reported for 1, 2 and 3 make the strains appear different. When all the typings are reported on one day it would clearly be possible to derive a 'composite' type for reporting, in this case perhaps 6/7/47/53/54; but if strain 3 were the first to be reported it would be impossible to foresee the direction in which the strains to be typed subsequently would vary. Strain 6 cannot strictly be regarded as certainly different from 5, since the two do not differ by two strong reactions, although it would be held to be different from 4. Nevertheless, we know that 42D is a phage commonly found acting alone on a strain, and for this reason, and because of the several moderate reactions in strain 5, we should regard 5 and 6 as different. Cases such as this, where experience of the common phage patterns necessarily plays a part in deciding on the identity of two strains, are not uncommon, and we are not at present in a position to formulate rules which could never be broken.

We have chosen extreme examples for discussion, and most sets of strains isolated in routine work do not offer such great difficulties (e.g. Table 10). Some difficulties of interpretation are unavoidable but they are reduced if all the strains from one inquiry are typed at the same time.

## D. DISCUSSION

*A method for type-identification of staphylococci needs to be considered in relation* to the situations for which it is required. Widespread epidemics of staphylococcal infection are unknown, and interest usually centres on spread within a small community or on possible self-infection from nose or skin. It is therefore more important to be able to make fine distinctions between a number of strains from one investigation than to recognize as identical, strains recovered from widely separated places or at different times.

The distinction of types in, for example, *Strep. pyogenes, Salm. typhi* or *Salm. paratyphi* B (Felix, 1951) depends on the recognition of antigens or receptors, or patterns of receptors, which are not shared amongst the types. With the phages now used, typing of staphylococci depends on the recognition of receptors that are or may be shared by different types, and strains from various sources are distinguished by their patterns of sensitivity to numerous phages. Each phage may take part in numerous patterns, which may differ from one another little or much. For example, among the 567 strains analysed, 132 distinct patterns were defined by consideration of moderate and strong lytic reactions; and some of the phages concerned took part in as many as thirty different patterns.

To summarize and interpret the phage patterns as defining 'types', Wilson & Atkinson (1945) took note only of confluent lysis and regarded most of their phages as type-specific because strains were lysed confluently by only one of them; a small number of their 'types' were determined by patterns of confluent lysis with groups of two, three, or four phages. Wahl & Lapeyre-Mensignac (1950b) suggested a different approach: they disregarded different degrees of lysis, and treated five of the phages as 'major' phages which determined the group into which a staphylococcus fell. For example, a strain lysed by the 'major' phage 3C, and by some of the minor but none of the other major phages, falls into group D2, no matter what the degree of lysis by 3C.

There seem to us to be difficulties with both of these schemes. We do not think that it is practicable at present to rely on confluent lysis for the determination of type because, in sets of strains presumed to come from one source, we have commonly noted variation from confluent lysis to semi-confluent lysis or even discrete plaques. For example, in fifty sets of strains each of which was thought to be of one type and all of which had one strain showing confluent lysis with one or more phages, twenty-one sets included at least one strain in which the confluent reaction was reduced to the level of discrete plaques. Moreover, if only confluent reactions are accepted, the proportion of strains regarded as typable is low. Thus in a random sample of 100 strains lysed to some degree by one or more phages, only thirty-four showed a confluent reaction, and would therefore have been classifiable by Wilson & Atkinson; seventy-three gave a strong reaction, i.e. fifty or more plaques. If degrees of lysis less than confluent are used, it is no longer true that a great proportion of strains are lysed by only a single phage, and, as we have already shown, the number of distinct patterns observed is so large that we see no way of extending the few groupings of the Wilson & Atkinson scheme. The difficulties of this scheme

are, therefore, that too many strains are unclassifiable, either because they show confluent lysis with no phage or because they show strong but not necessarily confluent lysis with too many; and that variation from confluent lysis to a lower degree of lysis may occur within one population.

Wahl & Lapeyre-Mensignac (1950*b*) adopted an approach akin to ours described in § 4, but they have defined more subgroups than we consider practicable. Broadly, however, their three principal groups correspond to ours. Wahl & Lapeyre-Mensignac have not discussed the epidemiological validity of differences between the patterns within one of their groups or subgroups, although we understand from personal discussion with Dr Wahl that he would certainly regard strains falling into different groups or subgroups as having a different origin. In practice this convention would probably give substantially the same results as our own method, although there might be some differences, as for example with two strains one of which was lysed by phage 52 strongly and 52A weakly, and the other by phage 52 alone. These would fall into different subgroups in Wahl's classification, but would be regarded as indistinguishable by us.

Another distinct scheme proposed by Wallmark (1949), and similar to that of Wilson & Atkinson, is simply a compilation of the patterns observed, however infrequently. While this may be useful for the description of a restricted investigation, it does not, for the reasons already stated, seem to us satisfactory as a general method.

Our own investigations show first that, while there is commonly some variation in the degree of lysis produced by one phage on separate cultures of one strain, and gain or loss of weak reactions is common, it is rare for one culture of a set of related strains to show strong lysis when another shows none at all. At the same time we have been unable to recognize any method of simplifying the phage patterns to allow the definition of types. We prefer therefore to regard the present set of phages as not competent to recognize 'types' and to consider that the most that we can achieve, after the broad classification into the 3A, 6/47 and 52 groups, is to decide whether or not any pair of strains is or is not identical. Our analysis offers a guide towards making this decision.

It has been suggested that staphylococcal phage typing would be more reliable if the typing phages were all adapted from one common parent, as are those used in the typing of *Salm. typhi* (Craigie & Felix, 1947). This could have two advantages. The adapted phages might be, as in the *Salm. typhi* set, truly type-specific with few or no overlapping reactions; and theoretically the staphylococci would have had little or no opportunity to encounter such adapted phages in nature, and so to become resistant to them. The latter advantage is, in the present state of our knowledge, largely speculative since we have no idea of the real risk of alteration in phage susceptibility as a result of chance meetings in nature. Moreover, it seems doubtful whether adaptation of staphylococcal phages as commonly practised is often more than selection of one phage from a mixture, so that the theoretical basis of the argument is in some doubt.

As yet little work has been reported on attempted adaptation of staphylococcal phages; but none of our adapted phages appears to be specific in the sense that the

*Salm. typhi* Vi phages are specific: they have all at one time or another lysed a strain that was also lysed by one or more of the other adapted phages. It can of course be argued that even if one phage was specific, its action would be masked in our tests by all the other non-specific phages. It may well be that the correct parent phage has yet to be discovered and recognized, but it is worth noting, in the absence of clear proof, that it is very unlikely that the species of *Staph. aureus* is nearly as homogeneous as the Vi-antigen-containing members of the species *Salm. typhi*. Cowan's three serological groups among staphylococci correlate fairly well with the three major phage groups—I with the 52 group, II with the 3A group, and III with the 6/47 group—and perhaps specific phages exist for each of these groups.

One of the phages used extensively by Wahl & Lapeyre-Mensignac in their typing scheme, namely 68, is a phage with an exceedingly wide range of action. Through the kindness of Dr Wahl we have been able to examine this phage, which appeared as a mutant of phage 44A, and we have found it to differ serologically from all the other typing phages. Indeed, the only other phages that are serologically similar are two isolated by Dr. G. Wallmark of Stockholm, and referred to by him as phages 155 and 166, which we have also had the opportunity to examine. We propose to refer to these phages as belonging to serological group G. All of them have a very wide range of activity, and two of the three have been found to lyse strains of all the three major groups. We regard them therefore as unsuitable for use in typing; Wahl (personal communication) has subsequently put less stress than he did initially on lysis by phage 68.

A search for a suitable phage from which adaptations might be made to yield type-specific phages must clearly be pursued, and in addition to the typing phages themselves, we now have as possible parent phages the group G phages described in the last paragraph, the group F phages isolated by Rountree (1949a), as well as those appearing spontaneously in some strains. Nevertheless, such work as we have carried out, and similar attempts made earlier by Wilson & Atkinson, and by Allison (personal communications), suggested that the search may need to be a very long one. Indeed it might even prove that a method of type identification by recognition of the phages carried by the staphylococci, similar to that used by Boyd (1950) for *Salm. typhi-murium*, is better than the recognition of phage susceptibility.

Apart from the difficulties that arise from the use of phages that are not truly type-specific, there is one great difficulty with the phage-typing method in its present form, namely that of obtaining completely reproducible results from day to day. We have made numerous investigations of various points in the technique in the hope of finding one that was critical, and perhaps responsible for the bulk of the variation. We have not been successful; and, as described in § 3, even quite large variations in the time and temperature relations of the various steps in the technique made little difference to the results observed. We are forced to conclude that some undetected differences in the media are responsible for much of the variation, and we have commenced a number of investigations of simpler or better-defined media than the Hartley digest agar hitherto used as a routine.

We have been at some pains to stress the defects and difficulties of the bacterio-phage method of typing staphylococci because we think it important that its limitations, in comparison with the methods of typing other bacteria, should be fully recognized. But if these limitations are admitted, and if the use of the method is confined to answering the sort of question that it is competent to answer—namely the identity or non-identity of sets of cultures—there can be little doubt of its value. This has been shown clearly in various investigations of the spread of epidemics of neonatal sepsis and cross-infection in hospitals, and of the source of outbreaks of food-poisoning, as well as in more general studies of the ecology of staphylococci. Detailed accounts of some of these investigations have already been published (e.g. Barber *et al.* 1949; Rountree & Thomson, 1949; Allison, Hobbs & Martin, 1949; Oddy & Clegg, 1947; Millar & Pownall, 1950) and others will appear shortly. We hope to present a separate report giving the relative frequencies of different phage patterns in cultures from different sources, in both healthy and sick people.

## E. SUMMARY

The routine methods for propagation of staphylococcal typing bacteriophages, and for their use in identifying strains of staphylococci, are described.

Most of the phages can be propagated in fluid media as well as on agar, and for some glucose-peptone-water is a better medium than nutrient or Todd-Hewitt broth.

Many phage filtrates derived from broth or agar propagation contain, in addition to the phage, an agent that inhibits the growth of staphylococci.

Investigations of variations in the routine typing technique showed that considerable latitude is permissible in the age of cultures used for typing and in the inoculation procedures. It is, however, important to test all phage filtrates after propagation for purity and freedom from the inhibitory agent; and to repeat the tests frequently during use to detect alterations in titre.

About 40% of staphylococci are not lysed by the phages used at their test dilution; about half of these untypable strains are lysed by undiluted phage filtrates.

An analysis was made of the results of typing 567 independent strains of staphylococci; 229 of these showed strong lysis by one or more phages, but there were no fewer than 82 distinct phage patterns represented, and only 118 strains were lysed strongly by a single phage. Certain phages tend to appear together in patterns and on the basis of such associations three main phage groups can be distinguished; they are known respectively as the '3A', '6/47' and '52' groups.

No method was discovered for segregating patterns into 'types', but conventions have been devised on the basis of the variation observed in sets of strains from various sources, for distinguishing between different patterns.

Dr G. Wallmark, Stockholm; Dr H. Williams Smith, Poultry Research Station, and Mr A. M. Hood, Birmingham.

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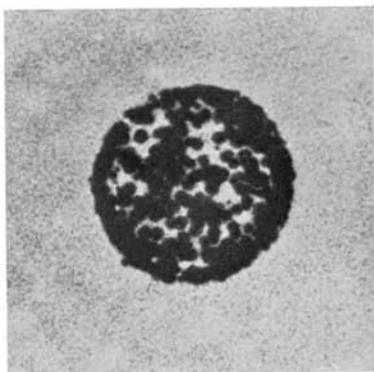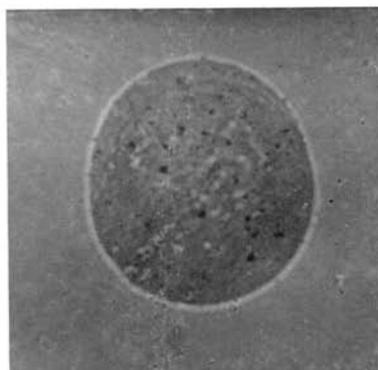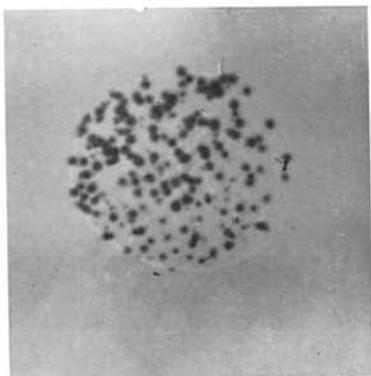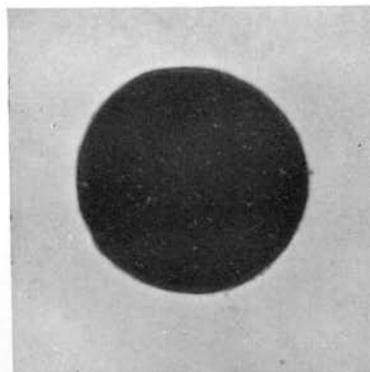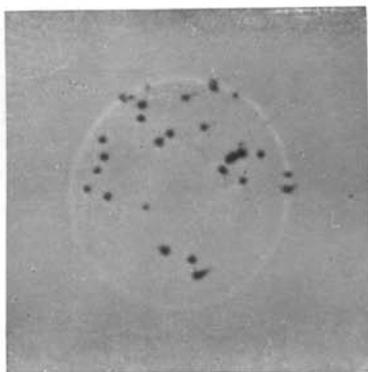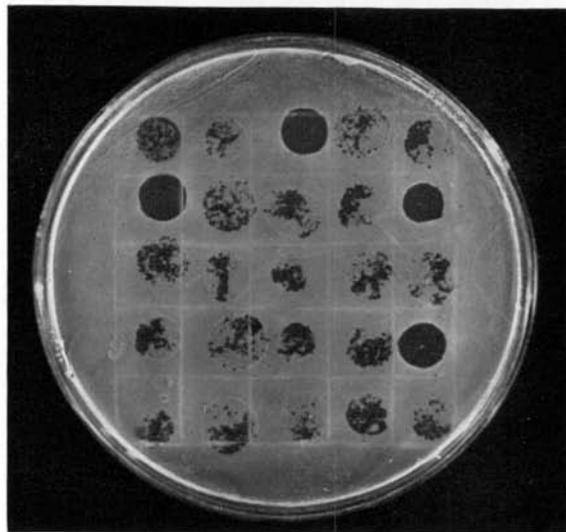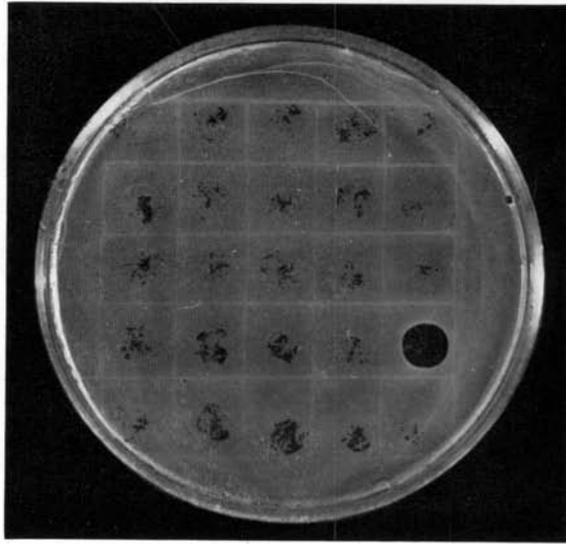
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### EXPLANATION OF PLATES 13 AND 14

#### PLATE 13

The range of variation of phage reactions: (a) discrete plaques (less than 50); (b) discrete plaques (more than 50); (c) semi-confluent lysis; (d) confluent lysis; (e) secondary growth in area of confluent lysis, with subsequent secondary plaque formation. (× 3.)

#### PLATE 14

Cultures showing non-specific lysis in the areas of the phage drops.

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