INTRODUCTION

Ever since Twort (118) observed the moth-eaten appearance of colonies from glycerinated calf vaccine, it has been known that staphylococci may be infected with an agent which has a lytic activity upon the cells. Although Twort's bacterial strain was described as "white" and his "lysin" as much less active on strains of Staphylococcus aureus from the boils of man, d'Herelle (49) soon reported the isolation of this lytic principle, "bacteriophage," from the golden staphylococci of an infected finger. In the 40 years since these early observations, the accumulation of knowledge concerning the staphylococcal bacteriophages and their lytic action has led to a technique for the differentiation of staphylococcal strains on the basis of phage susceptibility. Understanding of the natural history of staphylococcal disease has always been hampered by the lack of any means for real differentiation within the genus. Attempts to identify the staphylococci by means of serological techniques have only divided them into a few broad groups (23, 29, 78) and have not provided the definitive typing necessary for tracing the spread of infection. Bacteriophage typing, which also provides useful information about other genera, has been particularly significant for the staphylococci which are so difficult to distinguish on any other basis.

Early phage isolates were not of a type suitable for differentiation of host strains, for they had broad lytic power, lysing most staphylococcal cultures. Callow (22) was the first to obtain a series of phages which differed in lytic activity.

1 On leave from the Ohio Department of Health.

From 14 cases of acute purulent infection, she obtained six phages with varying ability to lyse stock strains isolated from similar sources. Although Burnet and Lush (19) found little strain specificity among a group of phages which they studied, Williams and Timmins (136) used four of these "Au" (aureus) phages in an epidemiological study. With these, they were able to determine six "types" among strains from 19 cases of acute osteomyelitis, and found that in 12 cases the strain from the patient's lesion appeared to be the same as that from his nose, throat, or blood. These phages were termed "strong" because they produced complete lysis of susceptible strains with clearing of broth cultures, in contrast to "weak" phages which did not propagate well in broth, but produced lysis on solid media (19). Therefore, clearing in broth was used as the criterion of phage action.

Fisk (38), who utilized lysis on solid media, laid the foundation for a practical typing technique. Since the majority of the phages which contribute to the specificity of strain differentiation do not produce the clearing in broth of the "strong" phages, the evolution of a typing technique has come from modifications of Fisk's methods. Fisk isolated his phages by streaking broth cultures of staphylococci across agar plates and spotting broth cultures of other strains along the streaks, in what has become known as the "Fisk cross-culture" technique. He found that 44% of the strains he examined were "carriers of bacteriophage." The phages he isolated and propagated were totally inactive against his coagulase-negative strains and, moreover, showed a specificity in host range such that 24
phages could be identified by difference in lytic activity. The investigation of 95 cultures with these phages (39) showed that strains from related sources were susceptible to the same phages, and strains maintained on laboratory media, or passed through mice, retained the phage susceptibility of the freshly isolated cultures. Since Fisk’s phages were identified merely by letters and are no longer available, nothing is really known of their specificity and they are not involved in the present-day technique of phage typing. That they were of practical use is shown by the fact that Fisk and Mordvin (40) used them in an epidemiological investigation, as did McClure and Millar (72) in the study of staphylococcal food poisoning.

The work of Wilson and Atkinson (137) contributed many of the phages used in typing. These British workers made two very important contributions to the technique of staphylococcal phage typing. Whereas Fisk (38) had applied his phage as undiluted lysate, they adopted the use of the “routine test dilution,” i.e., the highest dilution giving confluent lysis of the propagating strain, as described by Craigie and Yen (30) for typing typhoid bacilli.

Many difficulties beset the use of undiluted lysates. Rountree (92) showed that coagulase-positive cocci adsorb all the typing phages, irrespective of their lytic activity, and this adsorption may be lethal to the cocci whether followed by phage replication or not. Ralston et al. (85) described lysis-from-without of S. aureus strain K with phage-cell ratios greater than 17, this destruction being independent of phage infectivity. Williams and Rippon (134) found that certain lysates caused inhibition of growth in bacterial lawns when used undiluted, but when these lysates were progressively diluted the effect was lost without passing through any dilution which produced isolated plaques. In addition, it is now well known that at least 75% of the propagating strains are themselves lysogenic (67, 90, 93), and concentrated lysates can be expected to contain not only the phage propagated but also one or more temperate phages carried by the host culture. Preparations of phage may also contain host-range mutants which produce plaques on a strain which the parent phage does not infect. It has been shown that plating concentrated lysates with lysogenic bacteria may cause prophage induction with resultant plaque formation (97).

Thus, it is impossible to know, when using undiluted lysates, whether the lysis produced on a test strain is truly an indication of sensitivity to the typing phage. The use of test dilutions of phage eliminated most of these complications by reducing cross reactions and greatly increasing specificity.

Wilson and Atkinson (137) also supplied future workers with a set of phages which still forms the nucleus of that now used for typing. From 26 lysogenic cultures, they isolated seven different phages. They then found that they could type additional cultures by “adapting” these to new propagating strains to obtain new phage stocks. These “adaptations” were either host-range mutations, host-induced modifications (97), or a combination of these two effects (1). They were able to recognize 21 “types” among 460 staphylococcal cultures with their 18 phage stocks. Of these types, 14 were lysed by only one phage and the remaining by several phages.

The successful use of this technique in the investigation of outbreaks of food poisoning, pneumococcus neonatorum, and wound infection (34, 50, 77) showed its usefulness as an epidemiological tool, and in 1946 a routine phage-typing service was established in the Central Public Health Laboratory at Colindale, London, under the direction of V. D. Allinson. This laboratory, under his direction until 1948, and then under that of R. E. O. Williams until 1960, has been a recognized center for the distribution of phages and a focal point for research on staphylococcal disease. The present Staphylococcal Reference Laboratory (Director, M. T. Parker) has been designated as the International Reference Center by the Subcommittee on Phage Typing of Staphylococcus of the Nomenclature Committee of the International Association of Microbiological Societies.

Since the establishment of this laboratory, centers using the Colindale phages for typing have arisen in other countries. Among the first to use this procedure were Wahl and LaPeyre-Mensignac (128) in France, Saint-Martin, Charest, and Desanelleau (106) in Canada, and Blair and Carr (14) in the United States. In 1952, Williams and Rippon (134) published a detailed description of the Colindale techniques, and many laboratories have based their methodology upon these suggestions or those of Blair and Carr. Recently, Blair and Williams (18) prepared a report on recommended procedures on behalf of the Subcommittee on Phage Typing (18). The
committee feels that standardization of techniques is essential for comparison of one laboratory's results with those of another.

**Typing Phages**

In 1952, the Colindale laboratory employed routinely a set of 24 phages, 21 contributed by Wilson and Atkinson and 3 added by Dr. Allison (134). Workers in other laboratories often supplemented this group with their own phages, and the lack of a standard nomenclature makes it difficult to identify the phages employed by various investigators. The typing phages from published reports and some information about their sources are listed in Table 1.

Wilson and Atkinson (137) originally identified their phages by "phage number/propagating strain number" (as noted under Former Designation in Table 1). They later renamed them (112), adopting the numbers now in use. The propagating strains were assigned the same numbers as their phages, prefixed by "PS" (87). At first, phages derived from primary isolates were identified by addition of a letter to the number of the parent phage, e.g., 52A from 52. This system did not develop logically, indeed it could not, since more phages were "adapted" from those already assigned letters. Thus, phages 47A, 47B, and 47C were all isolated by plating phage 47 on different bacterial hosts; on the other hand, 42A, 42B, and 42E were likewise derived from 42, but 42C from 42A and then 42D from 42C. Serological investigation (94) disclosed that 42C and 42D differed from the others. From a study of the lysogenic state of the propagating strain PS 42A, it appeared that 42C is not a mutant of 42A but is more likely the temperate phase of the lysogenic propagating strain, which had been present in the 47A lysate in low concentration. This example illustrates the difficulty of determining the origin of any "adapted" phage. The present system avoids this dilemma by assigning new numbers to "adapted" phages. Phages accepted by the International Subcommittee are assigned official numbers; other phages go by the designation of the laboratory of origin.

Very early it was observed that a given bacterial culture was lysed by more than one of the typing phages, resulting in "phage patterns." It was also seen that phage lysis was not randomly distributed in these patterns. Some reactions were frequently associated with one another, but other combinations rarely, if ever, occurred. Williams and Rippon (134) therefore grouped the phages by similarity of host range into three broad groups, which they referred to as the 3A, 6/47, and 52 groups. Blair and Carr (14) came to the same general conclusions from their own observations. Their "types" 1, 2, and 3 correspond to the above-mentioned groups. There is an overall, although not strict, correlation between these lytic groups and the antigenic groups established by Cowan (29, 51, 125). That this was a logical classification has been confirmed by subsequent typing of many cultures. Although overlapping of groups does occur, it is much less frequent than association of phage reactions within a group.

Rippon (86, 87) established a classification for the known staphylococcal phages based on their serological relationships and the character of their lytic spectra. The typing phages all belong to the general class, "phages with restricted host range." They fall within serological divisions A, B, F, and L, and are subdivided into lytic groups according to host range. Table 2 shows the relationship between the lytic and serological classification of the principal typing phages.

This classification has been adopted by most workers, but Wahl and Fouace (125) classify 44 and 44A as group III phages and all of the 42 series, along with 47B and 47C, as group IV. Since they found their group IV phages as frequently associated with group I as with group III they felt these phages could not be classified with either of those groups.

As the number of possible typing phages increased, there arose the problem of choosing a practical number for routine work. Since the inception of the International Subcommittee in 1953, its members have attempted to solve this problem for investigators by recommending a basic set which could be supplemented, if necessary, by phages of special value in a certain locale or situation. Subsequent conferences have made minor changes in the basic set, but it remains essentially the same. Experience (21, 76, 101) suggests that the list may require further minor revision if strains which cause widespread disease but are not lysed by phages of the basic set continue to appear. The currently recommended set includes 29, 52, 52A, 79, and 80 in group I; 3A, 3B, 3C, 55, and 71 in group II; 6, 7, 42E, 47, 53, 54, 75, 77, and 83A in group III; 42D in group IV; and 81 and 187 as miscellaneous.

The basis for selection of such a set has been admirably discussed by Williams (5). Because of
<table>
<thead>
<tr>
<th>Phage</th>
<th>From</th>
<th>Former designation</th>
<th>Investigator</th>
</tr>
</thead>
<tbody>
<tr>
<td>3A</td>
<td>Phage 3</td>
<td>3/284</td>
<td>Wilson and Atkinson</td>
</tr>
<tr>
<td>3B</td>
<td>Phage 3</td>
<td>3/211</td>
<td>Wilson and Atkinson</td>
</tr>
<tr>
<td>3C</td>
<td>Phage 3B</td>
<td>3B/1339</td>
<td>Wilson and Atkinson</td>
</tr>
<tr>
<td>6</td>
<td>Strain 42†</td>
<td>6/3</td>
<td>Wilson and Atkinson</td>
</tr>
<tr>
<td>7</td>
<td>Strain 5</td>
<td>7/4</td>
<td>Wilson and Atkinson</td>
</tr>
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<td>Strain 21</td>
<td>29/33</td>
<td>Wilson and Atkinson</td>
</tr>
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</tr>
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<td>Strain 24</td>
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</tr>
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<td>Phage 31</td>
<td></td>
<td>Rountree and Thompson</td>
</tr>
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<td>Phage 42</td>
<td>42/30</td>
<td>Wilson and Atkinson</td>
</tr>
<tr>
<td>42B</td>
<td>Phage 42</td>
<td>42/1163</td>
<td>Wilson and Atkinson</td>
</tr>
<tr>
<td>42C</td>
<td>Strain 36</td>
<td>42A/1307</td>
<td>Wilson and Atkinson</td>
</tr>
<tr>
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<td>Phage 42C</td>
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<td>Phage 42D</td>
<td>42D/E193</td>
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<td>Goeling, Hien, and Bots</td>
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<td>Strain 17</td>
<td>47/36</td>
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<td>P-2311</td>
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</tr>
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<td>68</td>
<td>Phage 44</td>
<td></td>
<td>Wahl and LaPeyre-Mensignac</td>
</tr>
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<td>69</td>
<td>Strain</td>
<td></td>
<td>Hood</td>
</tr>
<tr>
<td>70</td>
<td>Strain</td>
<td></td>
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<td>71</td>
<td>Strain</td>
<td></td>
<td>Hood</td>
</tr>
<tr>
<td>73</td>
<td>Strain 10</td>
<td>10/56-A</td>
<td>Fisk</td>
</tr>
<tr>
<td>75</td>
<td>Strain</td>
<td></td>
<td>Hood</td>
</tr>
<tr>
<td>75A</td>
<td>Phage 75</td>
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<td>Hood</td>
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<td>75B</td>
<td>Phage 75 or 75A</td>
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<td>Hood</td>
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<td>52AV</td>
<td>Rountree and Freeman</td>
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<td>Phage 42B</td>
<td>81/LH66459</td>
<td>Bynoe, Elder, and Comtois</td>
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<td>82</td>
<td>Phage 52A</td>
<td>'52AV'/LH552280</td>
<td>Comtois and Bynoe</td>
</tr>
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<td>Va4</td>
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</tr>
<tr>
<td>142</td>
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<td>187</td>
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<td>523</td>
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<td>Strain</td>
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<td>Wallmark</td>
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</table>

* References: 14; 24; 27; 45; 86; 87; 101; 103; 112; 126; 129; 130; 134; 135; 137; Jevons, personal communication.
† Lysogenic S. aureus strain, number given where known.

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TABLE 2. Lytic and serological groups of the typing phages

<table>
<thead>
<tr>
<th>Lytic groups</th>
<th>A</th>
<th>B</th>
<th>F</th>
<th>L</th>
<th>G</th>
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<tbody>
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<td>I</td>
<td>29, 29A, 31A, 52, 52A, 79, 80</td>
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<td></td>
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<tr>
<td>II</td>
<td>3A, 3B, 3C, 51</td>
<td>55, 71</td>
<td>61, 62</td>
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<tr>
<td>III</td>
<td>6, 7, 42A, 42B, 42E, 47, 47A, 47B, 47C, 54, 70, 73, 75</td>
<td>42C, 53, 76</td>
<td>83A 77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td></td>
<td></td>
<td></td>
<td>42D*</td>
<td></td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>57, 78, 81, 82, KS6</td>
<td>31, 31B, 44A, 44B, 52B, 69</td>
<td>187 65, 66, 68†</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Variant of the original 42D now used routinely in typing.
† Polyvalent phages no longer used in typing.

the numerous distinct patterns which could be obtained if every known phage were employed, it is necessary to make a reasonable compromise between the relative nonspecificity needed to give some lysis on the majority of strains and the need for enough specificity and independence of phage action to adequately differentiate strains for epidemiological purposes.

TECHNIQUES OF PHAGE PROPAGATION

Methods have been described for the propagation of phage in liquid media, on the surface of agar plates, and by agar-layer techniques. Early reports described modifications of Fisk's (38) methods for propagation on agar plates, but this method has been largely supplanted by propagation in broth or soft agar. Although broth propagation is simpler, it does not give as high titers as the soft-agar methods, particularly with some of the phages (18). Williams and Rippon (134) were the first to employ a soft agar, using a freeze and thaw technique. Jackson, Dowling, and Lepper (56) modified the agar-layer method which Swanstrom and Adams (114) used for propagation of the T phages. Jackson et al. added phage-culture inocula to 0.9% agar which was then poured over a base layer of standard agar medium. After incubation, the plate was flooded with 5 ml of broth, and the soft agar was scraped free from the base layer. Centrifugation yielded a supernatant fluid free from agar and of good phage titer. Present soft-agar methods employ less agar, 0.5% being recommended for overlays (18).

When White, Foster, and Knight (132) compared soft-agar with broth propagation, they found that broth with added calcium gave titers equal to those from agar propagation. Of their typing phages, 13, including all of the B phages, would not propagate in Heart Infusion Broth without additional calcium. Rountree (96) had found that divalent cations were required for phage adsorption and that neither magnesium nor strontium could be substituted for the calcium requirement of the B phages. She also showed that calcium is required for some step late in the latent period. Although White et al. used Heart Infusion Broth for phage propagation, their soft-agar propagation was done with Trypticase Soy Agar (BBL). Dowell and Rosenblum (32) have shown that lysis by group B phages is inhibited on Brain Heart Infusion Agar, though not that of the A phages. Phosphates were the major inhibitory component of the Brain Heart medium, and phosphates added to Trypticase Soy Agar made it inhibitory. Difficulties in phage propagation are often associated with media, and even with a suitable medium occasional lots may give poor results, for reasons not yet understood (16, 134).

An efficient means for phage propagation, by virtue of simplicity and excellence of product, is the cellophane technique, originally described by Burnet and Lush (19) and recently advocated by Liu (66). Liu believes that retention of phage by soft agar can be avoided by overlaying the base agar layer with a cellophane disc rather than with soft agar. His method employs only 3 hr of incubation, is technically simple, and yields high-titer lysates.

Different methods for phage propagation have been compared at the Staphylococcus Reference Laboratory, Colindale, and the technique recommended for each typing phage may be found in the report of Blair and Williams (18). The optimal phage inoculum, and time and temperature of incubation, which vary with the phage, are given for each.
Since the titer of a phage preparation is a function both of the phage being propagated and of the technique employed, under standard conditions the test dilution of a phage is characteristic of that phage and remains fairly constant (14, 16, 184). Phage stocks with titers of $10^8$ to $10^9$ plaque-forming units per ml and routine test dilutions (RTD) of $10^4$ to $10^5$ can be regularly maintained (16, 18). Published titers for RTD values have varied from $10^3$ to $10^4$ without any correlation with the techniques employed for propagation. It is difficult to compare the titers reported by one investigator with those of another, since the details of titration are seldom given. Some workers have used a single pipette to prepare serial dilutions of phage, a technique known to result in significantly higher titers than when a fresh pipette is used at each step (16). Titers stated in terms of RTD should be directly proportional to plaque count, but White et al. (132) have shown that the relationship is not always consistent, since it is influenced by plaque size. They found a 100-fold difference in the concentrations of phages 42E and 52A required for confluent lysis.

The real problem associated with phage propagation is not one of obtaining high-titer lysates, but of maintaining phage with the same specificity of host range. The "lytic patterns" of the typing phages may be determined by typing a standard set of strains (the propagating strains), with the RTD, but this method does not always reveal significant changes in host range. It is currently recommended that any new phage stock be titered in tenfold dilutions on each propagating strain which it lyses when undiluted. The titer on each propagating strain should remain a constant fraction of the titer on the homologous strain. Detailed directions for this more complicated technique and standards for acceptable phage stocks have been published by Blair and Williams (18). Minor variations in lytic spectra are acceptable, but any major variation indicates that the stock should be rejected. The appearance of a high-titered reaction where none should exist, or the lack of a reaction where one should occur, is a major variation. No phage stock should be used for typing or further propagation unless it conforms to the requirements specified. When testing lytic spectra in this manner, precautions must also be taken to guard against changes in phage susceptibility of the bacterial cultures. When major variations in lytic spectra are observed, the bacterial strain involved should be retested with phages whose lytic patterns have been previously established. If any change is now found in the phage type of the bacterial strain, it should be discarded and a new propagating strain obtained. In no case should an apparently altered culture be used for the propagation of typing phage, since the lytic range of the phage may be affected by host-induced modification. Strict adherence to the controls recommended (18) should ensure the standard bacterial and phage strains essential for reproducible results.

Even in the hands of the most experienced workers, significant variation in the host range of certain phages has occurred in the past. The best known example is the change in the lytic range of phages 52, 42B, and 44A (13). The HJD strains of these phages being used by workers in the United States resulted in the identification of 80/81 strains as 42B/44A/52/80/81 (109) or as 52/42B/81 (37). The LH phage strains from the London Reference Laboratory did not lyse these staphylococci, which are properly typed as 80/81. From the epidemiological standpoint, this may have been a fortunate happenstance, since it permitted the identification of strains which were otherwise untypable prior to the use of phages 80 and 81. However, such a change leads to much confusion, and it is essential that each laboratory maintain the lytic spectra of the typing phages unchanged, if the results from one laboratory are to be compared with those of another.

Because of the difficulty of maintaining host specificity, the International Reference Laboratory plans to send phages and propagating strains to the National Reference Laboratories every 3 years. The United States has two reference laboratories: one which supplies phages and consultation to hospitals, universities, and research institutions (J. E. Blair, Hospital for Joint Diseases, New York, N.Y.); and another which advises and supplies phage to public health laboratories, including the present 24 regional typing centers (E. L. Updyke, Communicable Disease Center, Atlanta, Ga.).

Sterilization and Preservation of Lysates

Phage lysates can be freed from bacterial contamination either by filtration or with chemicals. Heat cannot be used to sterilize lysates (except phage 73), since the staphylococcal phages, unlike some others, are destroyed by temperatures below those which kill the host bacteria (94).
Zephiran (38), thymol (128), and glycerol (31) have been used to preserve lysates, but the residual bactericidal effect of chemical preservation is undesirable and precludes the use of undiluted lysates. Any type of filter which removes bacteria without removing too much phage is satisfactory, and Gradocel (134), Zigmundy (21), and Millipore membrane (43) as well as sintered-glass (56, 70) and Seitz (16) filters have been used. Hsie (54) found that the recovery rate for staphylococcal phages after Seitz filtration varied greatly with particle size, so that Seitz filtration may not be advantageous for routine use with all the phages, especially if small amounts are filtered.

It is fortunate that the staphylococcal phages are relatively stable, since the preparation of large amounts of phage and the continued use of the same phage stock is best for typing. Undiluted phage stocks have remained viable at 4°C for more than 1 year (16). Even test dilutions keep their titers for weeks or months under refrigeration, although Rountree (94) found that phages of serological groups A and F were considerably more stable than those of group B.

Lyophilization is preferred for long-term storage. The phages and their propagating strains have been maintained and distributed in this form from the National Collection of Type Cultures, London (74). Stocks may be adequately maintained at −10°C for 1 to 2 years where facilities for lyophilization are not readily available. Ghitter and Wolfson (43) have suggested the routine use of lyophilized test dilutions which can be reconstituted when needed, but it does not appear certain that all routine test dilutions will maintain full phage titer upon lyophilization.

Technique of Phage Typing

Determining the bacteriophage type of a staphylococcal culture is a relatively simple procedure, in which an agar plate is inoculated with sufficient bacterial culture to give a lawn of confluent growth, and drops of suitably diluted phage lysates are placed at designated positions on the agar surface. After incubation, the degree of lysis or the number of plaques produced by each phage is recorded, and the stronger reactions are reported as the "phage pattern" or "phage type." Techniques have developed on a more or less empirical basis, since the variables which affect the degree of lysis are not yet fully understood.

Although glucose agar was first used for typing (128, 130, 137), Jackson et al. (56) found that glucose-free media gave less resistant overgrowth, and more plaque contrast. Nutrient agar (5, 31, 74) or meat infusion agars were then employed (88). Blair and Carr (14) found that Trypticase Soy Agar was more satisfactory than Tryptone or Brain Heart Infusion, and it is now widely used in the United States. Menolasco (75) has suggested the addition of 2,3,5-triphenyltetrazolium chloride to this agar to enhance the contrast between bacterial lawn and lytic areas.

Agar plates to be used for typing should be incubated before use to insure sterility and dry surfaces (16). Some investigators have used porcelain tops for drying at room temperature (43, 79).

Whatever the agar used for typing, the corresponding broth has usually been employed for growing the bacterial inocula. The staphylococci to be typed must be coagulase-positive strains in pure culture. This requirement is sometimes overlooked by laboratories submitting cultures to typing centers. Cultures from noses and throats may yield mixtures of staphylococcal strains (9, 70); unless these strains are separated on primary isolation, one strain may overgrow the lysis produced on the other and a result of "nontypable" can be recorded (70). The practice of transferring confluent growth from what appears to be a pure culture of staphylococci on an agar plate, rather than an isolated colony, should be discouraged.

The bacterial strains to be typed are usually grown in broth at 37°C for 4 to 6 hr (16, 132, 134), although longer incubation does not affect the typing (56, 134) and 18-hr incubation has been commonly employed (14, 21, 79, 106, 130). Plates for typing may be marked on the bottom with a diamond writing pencil (134, 137) or with an inked rubber stamp (56, 79, 130) to designate the areas for phage spotting. Hoeprich and Whitesides (52) described a heated metal mold for grid branding polystyrene plates. Permanently etched plates (Lifetime Red, Corning Glass Works, Corning, N.Y.) are commercially available and, though expensive, are very convenient (16).

The most uniform lawns of bacterial growth are obtained by flooding the plates with 1 to 2 ml of broth culture (56, 134), but the inocula can be spread with a bent glass rod (128, 134) or with a sterile cotton swab (14, 16, 132). Swabbed plates should be dried 5 to 10 min and flooded plates 30 to 45 min with the lids removed before the phage dilutions are spotted. Drying time should
not exceed 1 hr, since intervals greater than 1 to 1.5 hr between bacterial and phage inoculation have resulted in a decrease in phage action (130, 134).

Some workers have considered the advisability of using diluted broth cultures for bacterial inoculation. Wallmark (130) compared the results obtained with 18-hr broth cultures undiluted and diluted 1:10 and 1:100. The undiluted inocula gave weaker reactions than the 1:10 dilution, whereas growth from the 1:100 dilution was too thin for good reading. Since these were 18-hr cultures, the results cannot be assumed to apply to 4- to 6-hr cultures. However, Matějovská and Jellinek (71) titrated cultures of propagating strains incubated for 5 hr against phage dilutions in a block-type titration. In 63% of their tests, the phage titers rose tenfold as the cultures were diluted tenfold; after further bacterial dilution, the titers fell. In 14% of the trials, there was no change in phage titer with moderate bacterial dilution, but in 23% of the titrations the phage titers fell progressively as the cultures were diluted. Therefore, it is difficult to tell whether the additional work required to prepare diluted bacterial inocula would be worthwhile.

Platinum loops (31, 137), fine capillary tubes (79), finely tipped capillary pipettes (5, 16), or tuberculin syringes and needles (56, 132) have been used for applying phage to the bacterial lawn. Spotting with a platinum loop is an arduous task and has been replaced by either capillaries or syringes. Since capillary pipettes must be drawn to a fine tip and do not always deliver drops of the same size, syringes and needles probably give a more uniform inocula. In general, 27-gauge 0.5-in. needles have been recommended (18), but 30-gauge needles are commercially available (Beckman, Dickinson Co., Rutherford, N. J.) and deliver a small drop with greater ease.

Mechanization has entered this field as all others, and efforts have been made to reduce the time consumed in applying phages individually. Tarr (115) designed a semiautomatic apparatus using nichrome loops; Lidwell (65) introduced further mechanization. Goldberg devised a simple means for applying phage with rods to an uninoculated plate (16); Torheim (117) described a similar rod apparatus.

Zierdt, Fox, and Norris (140) constructed a multiple-syringe applicator said to apply 26 phages at the rate of 300 plates per hr. The syringes are held in a stainless-steel framework so that the apparatus can be cleaned or sterilized as a unit. Simulated typings using sterile broth for phage and alternating uninoculated plates with plates spread with staphylococci or Proteus did not transfer bacterial inocula from one plate to succeeding ones.

An apparatus which dispenses phage from cartridges (138) is now available (Accu-Drop Dispenser, Scientific Products, Evanston, Ill.), and cartridges containing lyophilized phage may be obtained commercially with this model. However, any laboratory wishing to employ commercially prepared phages should take note of the recommendations of the Committee on Staphylococcal Phage Typing of the American Public Health Association (4). In an earlier communication (3), this committee discouraged the use of commercially prepared phages which did not meet the specifications of the National Reference Laboratories. When lyophilized phages are used, they should conform to the same standard lytic spectra and should never be used without propagating strains for the usual control tests. So far, none of the mechanical devices for applying phage has been officially recommended by committees on typing.

At each typing, every test dilution of phage should be spotted on its propagating strain to check its potency. Although it has been suggested that this control procedure can be done at weekly or semiweekly intervals, it might best accompany each set of typings to insure dependability of results. It is only necessary to spread a small sector of a plate with a broth culture of the propagating strain and add a drop of the homologous phage.

When the phage inocula are dry, the plates are incubated overnight at 30° C (5, 16, 56). If 30° C incubation is not available, plates can be incubated at 37° C for 4 to 6 hr and then left at room temperature overnight, this procedure having been used by several investigators (21, 128, 130, 137). "Room temperature" is, however, an uncontrollable variable and is certain to differ from climate to climate and with the seasons. Jackson et al. (56) found that overnight incubation at 30° C gave better results than did the 37° C, room-temperature method.

The establishment of a routine test dilution (RTD) determines the concentration of phage stock to be used. Though many investigators
have followed the procedure of Wilson and Atkinson (137), using as RTD the highest dilution giving confluent lysis of the propagating strain, variations of this procedure have been tried. Blair and Williams (18) now define the RTD as the highest dilution which just fails to give confluent lysis. If no tenfold dilution conform to this definition, intermediate dilutions are tested. Near-confluent lysis is now recommended because it falls within a narrow dilution range and can be more precisely determined than confluent lysis. Two to ten times more phage is required for confluent than for near-confluent lysis.

Other investigators have preferred to strengthen rather than reduce the original RTD. Jackson et al. (56) termed the highest dilution giving confluent lysis as their “critical test dilution” and used for typing a concentration ten times the critical one. This modification has been used in epidemiological investigations (57, 120, 139) and does not seem to have given patterns of undue complexity. Since strains which show no lysis with the RTD are usually retested with more concentrated phage preparations, Zierdt and Marsh (141) have suggested that a single typing at 100 times the RTD would eliminate this second step and yield a more reproducible phage pattern. However, Williams (5) contends that this concentration yields patterns much too complex.

The use of test dilutions ten times the critical dilution does tend to minimize the minor variations, often seen in control spot testing, that constantly raise the question of changing the test dilution (Wentworth et al., unpublished data). But RTD values of 10 or 100 times the critical dilution can only be employed with high-titered lysates, where contaminating temperate phages, mutant particles, and “inhibition” are still diluted to extinction. The test dilution should be the one which yields the greatest number of typable strains, while providing maximal reproducibility without the disadvantage of very complex patterns. The original RTD was an arbitrarily chosen dilution (5), and some studies comparing various modifications might prove helpful.

It has been generally observed that 10 to 30% of the staphylococcal cultures examined do not show any lysis with the RTD, the percentage varying with the number of phages used and the source of the cultures. Williams and Rippon (134) began the practice of retesting such cultures with undiluted phage preparations, but by 1956 (87) this had been changed to testing with 1,000 times the RTD to avoid the inhibitory action of some undiluted lysates. Bynoe et al. (21) proposed a more complicated procedure involving titration on the test culture of all phages lysing at a 1:10 dilution. Since only those phages lysing at greatest dilution were reported, this method seems to approximate testing at 10 or 100 times the RTD. Because concentrated phage preparations tend to give patterns much more complex and nonspecific than do test dilutions, their interpretation is more difficult. It has been recommended that this procedure be reserved for those cultures showing no lysis at the RTD and that the results be interpreted with caution (5, 18).

Inhibition is sometimes seen even with testing at 1,000 times the RTD, and occasionally seems to be a constant characteristic of epidemiologically related strains which are otherwise untypable. Although usually not reported, in these unusual cases it may provide a clue to the probable identity of such strains (16).

Ma and Mandle (69) reported that 29% of 242 untypable cultures would type if grown in broth at 45 C instead of 37 C, and that a further 26% became typable after heat shock (55 C for 5 min). The epidemiological significance of phage susceptibility demonstrated in this way has not yet been determined. Harrison, Beavon, and Griffin (47) reported that growth of typable organisms in sublethal concentrations of neomycin resulted in loss of phage susceptibility, although sensitivity was regained after three serial passages in drug-free media. It may be that a certain proportion of nontypable cultures are merely in a “refractory” state, but Wallmark (130) concluded from repeated testing of typable and nontypable strains that temporary loss of phage susceptibility only occurs under rare and exceptional circumstances.

Reading and Reporting

After incubation, plates are examined for lysis with the aid of bright light, a dark background, and the moderate magnification of a hand lens, Quebec colony counter, stereoscopic microscope, etc. (5, 16, 18, 21). Degrees of activity are indicated by “+” values, the system varying from one published report to another.
Originally, only confluent or semiconfluent lysis was regarded as a significant result (14, 130, 137), but there has been an increasing tendency to emphasize lesser reactions. Early workers (125, 130, 137) attempted to classify their cultures into a restricted number of types and subtypes, each characterized by the attack of certain phages or combination of phages, but it soon became apparent that these systems were inadequate and that the only practical approach was to report a phage pattern for each staphylococcal strain (14).

For most reporting, it has been arbitrarily decided that reactions showing less than 50 plaques are not to be included in the reported pattern, and the current system indicates all reactions ranging from confluent lysis down to 50 plaques by + +, these being reported as the phage pattern; counts of 20 to 50 plaques are recorded as +, and less than 20 plaques as ± in the laboratory records. These degrees of activity correspond, respectively, to the "strong, moderate, and weak" terminology employed by some workers (88, 129, 134). The phage type is reported by listing the numbers of those phages which give a + + reaction and separating them by slant lines, e.g., 6/53/54/75. In some publications, this list is followed by a +, ±, or w to indicate additional weaker reactions.

Ruys and Borst (105) suggested a more complicated, but indeed more informative, method for reporting. They report all visible reactions in order of conspicuity. The phage numbers are separated by commas rather than slant lines, the lines being reserved to indicate degrees of reactivity. The phages giving complete lysis are listed first, followed by a slant line; then those which give at least a + + reaction, followed by a colon; next reactions which are still easily read, followed by a line; and, finally, the weak reactions. This system may be illustrated by: 6, 7/47, 53, 54:75,77. The phage type ordinarily reported for such a series of reactions would be 6/7/47/53/54, but Ruys and Borst feel that the "tail" of weaker reactions is extremely important for true differentiation of strains and is of epidemiological significance. However, the International Committee has decided that this method is too complicated for routine use (55). As yet, there is not sufficient epidemiological evaluation of these lesser reactions to indicate the value of such detailed reporting.

Reproducibility of Phage Types

Since so little is known of the genetic basis for phage susceptibility of the staphylococci, the technique of phage typing has had to stand or fall upon the basis of epidemiological evaluation. That bacteriophage typing has contributed to the knowledge of staphylococcal disease is now undeniable and widely appreciated. The many published reports of the investigation of outbreaks prove that typing does serve as an epidemiological tool. Considering the many laboratories using phage typing as such a tool, there is a disturbing lack of published data about its reproducibility. Both epidemiologist and laboratory are constantly plagued by the recurring question—are these phage patterns the same?

Williams and Rippon (134) undertook to analyze the extent of reproducibility by measuring the variation in multiple typings of a single stock culture and the variation in multiple isolates from a single source. When they typed, on the same day, multiple plates from a single broth culture, multiple broth subcultures from a single stock culture, and multiple colonies from a single plate culture, only about 35% showed no variation at all. About 50% varied no more than the loss or gain of one weak reaction, while 1 to 3% showed a significant (+ +) variation. Multiple isolates from the same individual had about the same amount of variation as multiple tests of a stock culture, if typed on the same day. As might be expected, cultures from related sources showed more discrepancy. When the same comparisons were made, but the typings done on different days, a significantly greater variation was observed. Even broth cultures from the same stock culture, when typed on different days, showed a loss or gain of one + + reaction in 12% of the comparisons. They concluded that exact reproduction of phage patterns is infrequent and some degree of variation must be expected. From the typing of epidemiologically related cultures they concluded also that this variation increases with the remoteness of two isolates from a presumed common source.

When variation was analyzed by lytic group, phages of group III showed more variability than those of group I or II, and this variation was greater than could be explained merely by the presence of more phages in group III. Williams and Rippon (134) decided that two typing pat-
terns of group III phages may be considered the same (and therefore presumably the cultures) if the patterns differ by only one strong reaction, but that patterns of group I and group II phages are not the same if they differ by as much as one strong reaction. It has been generally accepted that a difference of two significant reactions is a yardstick for judging similarity of types, but not a hard and fast rule.

Vogelsang and Östervold (124) found this criterion valid for deciding the identity of pairs of nose and throat isolates typed on the same day. They concluded that preparation of media and uniformity of phage titers are important to stability of phage types: that when doubtful results are obtained, cultures should be retested with the same lot of medium on the same day.

Fortunately, the problems of interpretation are ordinarily limited to a relatively small number of cultures, the majority of phage types being either sufficiently different or enough alike to cause no difficulty. Neither is the problem so great when the laboratory can type a collection of cultures from one outbreak at the same time. But interpretation becomes infinitely more complicated for the typing center which constantly receives cultures from many sources. Unless there is some set standard for judging similarity of reported types, the laboratory must interpret its results. Blair and Williams (18) think the laboratory can indicate on its report which strains are reasonably considered to have a common source. Their guide for interpretation is that two cultures are different when one is lysed strongly by two phages which produce no lysis of any degree upon the other. The validity of this interpretation depends upon the knowledge of weak reactions which are not reported, so only the laboratory can apply this criterion. In other words, two cultures are considered to have the same phage type when the phage pattern of one has weak reactions with two phages that produce strong lysis of the other. But a culture lysed strongly by two phages which do not give even weak reactions on a second strain is considered to be a different phage type from the second. This is probably the best criterion presently available, since it allows for variations in the strength of reactions due to technique as well as other factors. But it seems unfortunate that a procedure should require the extensive interpretation presented in this report.

The typing laboratory should be able to evaluate reproducibility by retesting cultures at intervals. It has become more and more evident, as illustrated by the remarks of Ruys and Borst (105) among others, that the typing laboratory must have a very careful analysis of results in order to decide the identity or nonidentity of strains. As pointed out in excellent discussions of this problem (16, 18), adequate analysis requires a knowledge of the source and history of the cultures typed. Only by constant correlation of phage typing with other epidemiological data can one recognize proper performance and interpretation. A typing laboratory can, however, with careful attention to standardization of techniques and its own system for testing reproducibility, maintain a high degree of consistency as judged by epidemiological evidence (18; Wentworth et al., unpublished data).

ASSOCIATION OF PHAGE SUSCEPTIBILITY WITH OTHER PROPERTIES OF THE STAPHYLOCOCCI

Although assigning an individual culture to one of the lytic groups has no epidemiological significance, investigators have noted the frequency with which the various groups are encountered and have attempted to correlate lytic group with other strain characteristics. Table 3, showing group distribution, summarizes the findings from 40 investigations covering a variety of cultural sources. In summarizing the material, epidemic strains were compiled separately to

<table>
<thead>
<tr>
<th>Table 3. Per cent distribution of phage lytic groups*</th>
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<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>I</td>
</tr>
<tr>
<td>II</td>
</tr>
<tr>
<td>III</td>
</tr>
<tr>
<td>Misc*</td>
</tr>
<tr>
<td>Special*</td>
</tr>
<tr>
<td>NT*</td>
</tr>
</tbody>
</table>

* References: 2, 7, 11, 12, 17, 21, 31, 33, 37, 41, 45, 46, 48, 53, 56, 58, 60, 61, 62, 63, 70, 74, 76, 81, 84, 88, 89, 95, 100, 101, 102, 104, 108, 110, 119, 121, 122, 123, 124, 135.

* Includes group IV and all strains typing with phages of more than one group and with unclassified phages.

* Includes types 80/81, 71, 52A/79, and 44B.

* Nontypable.
avoid a bias in group distribution. There was no evidence in these data of any tendency toward a shift in group distribution among strains collected between 1948 and 1960. It was apparent that the prevalence of any group depends upon the clinical source of the cultures under study. Reports of hospital-associated strains (2, 21, 42, 57, 62, 123) showed a predominance of group III strains due to the prominence of "antibiotic-resistant hospital strains," but group III strains were not more frequent among cultures from healthy carriers (31, 41, 102) or from disease conditions in patients not hospitalized (11, 41, 45, 60, 81, 102, 104). Cultures collected in the preantibiotic era contained fewer group III and more group I strains.

Numerous studies have attempted to relate the production of toxins and enzymes by the staphylococci to their ability to produce disease. Coagulase production is the attribute most commonly associated with pathogenicity, and the term "pathogenic staphylococci" is often equated with "coagulase-positive staphylococci." This is really a misnomer. Although it has been definitely established that almost every staphylococcal strain from a pathological source produces coagulase, there is no evidence to show that all those strains which produce coagulase can also cause disease in man (24). Methods for typing, or further differentiating, this heterogeneous group of coagulase-positive organisms have been hopefully aimed at pointing out the disease producers. The typing phages lyse only coagulase-positive strains; thus, susceptibility to the typing phages may be said to be associated with coagulase production even though a significant fraction of the strains producing coagulase are not lysed by these phages.

Although some other associations have been recorded for certain phage types or groups, it appears that the ability to produce toxins and enzymes correlates much better with the clinical source of cultures than with their phage susceptibility. Association of pigment or hemolysin production with phage type varies with the source of the culture, and no association with any particular phage type has been demonstrated (36, 58, 64, 70). English workers (84, 116) found serum opacity production and inhibition of growth of Corynebacterium diphtheriae associated with type 71 cultures from impetigo. According to Pan and Blumenthal (80), strains of group I generally produce more acid phosphatase than do those of other groups. Faber and Rosendal (35) found a correlation between phage type and hyaluronidase production.

There does appear to be a real correlation between production of enterotoxin and susceptibility to the phages of group III. Phage typing of agents from food poisonings have shown 64 to 92% to be group III strains, the rest being either 42D or nontypable (83, 135).

There is also an association between lytic group and antibiotic resistance. Although any particular phage type may show any degree of antibiotic resistance, multiply resistant strains, when typable, are most commonly 80/81 or group III types (2, 7, 21, 37, 53, 60, 62). Barbour and Edwards (10) suggested that the staphylococci of group III are less stable than those of other groups, since they found higher mutation rates to streptomycin resistance and dependence among group III strains than among those of group I or II. Penicillin resistance seems to be correlated with susceptibility to group III phages irrespective of the clinical source of the cultures (11, 53, 70, 101, 102, 123). Table 4 shows a summary of investigations relating penicillin, streptomycin,

<table>
<thead>
<tr>
<th>Group</th>
<th>Penicillin</th>
<th>Streptomycin</th>
<th>Tetracycline</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Total</td>
<td>% Resistant</td>
<td>Total</td>
</tr>
<tr>
<td>I</td>
<td>4,218</td>
<td>42</td>
<td>2,052</td>
</tr>
<tr>
<td>II</td>
<td>2,549</td>
<td>19</td>
<td>1,169</td>
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<tr>
<td>III</td>
<td>7,917</td>
<td>69</td>
<td>4,206</td>
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<tr>
<td>Misc</td>
<td>2,009</td>
<td>46</td>
<td>1,402</td>
</tr>
<tr>
<td>Special</td>
<td>1,240</td>
<td>91</td>
<td>1,453</td>
</tr>
<tr>
<td>NT</td>
<td>2,886</td>
<td>35</td>
<td>1,012</td>
</tr>
<tr>
<td>Total</td>
<td>20,819</td>
<td>10,534</td>
<td>8,480</td>
</tr>
</tbody>
</table>

* References. Penicillin: 2, 7, 8, 10, 11, 21, 31, 33, 45, 46, 48, 57, 58, 62, 63, 70, 81, 84, 89, 95, 101, 102, 110, 121, 122, 123, 135. Streptomycin: 10, 21, 42, 48, 58, 62, 63, 70, 123. Tetracycline: 21, 48, 57, 58, 62, 63, 68, 70, 123.

* Includes group IV and all strains typing with phages of more than one group and with unclassified phages.

* Includes types 80/81, 71, and 44B.

* Nontypable.
and tetracycline resistance to phage group. Chronological arrangement of the data did not indicate any progressive increase in resistance within any group.

Phage typing has cast a great deal of light upon the epidemiology of staphylococcal disease, and we now speak of "epidemic types." Though neither epidemic nor "epidemic type" has been strictly defined, it is clear that there are outbreaks of disease where the majority of the cases are caused by a single phage type. The pandemic of 80/81 infections which has occurred since 1954 needs little elaboration here. Rountree and Freeman (101) first identified a type "80" as the cause of outbreaks in Australia, then Bynoe et al. (21) a type "81" in Canada. Shaffer et al. (109) called attention to 80/81 as the cause of epidemics both in the United States and in other countries. Reports identifying 80/81 as the cause of furunculosis and postoperative and postnatal infection are much too numerous to review here. They leave no doubt of the role of the 80/81 type in disease production, but furnish no explanation for its world-wide prevalence. Williams (133), in discussing the concept of the epidemic type, makes it clear that 80/81 is not the only epidemic strain known, in spite of its present prominence. He defined "epidemic type" as one isolated from three or more associated cases of infection, and found six phage types responsible for more than 50% of the epidemics whose cultures were typed by the Reference Laboratory between 1954 and 1957. These six were 80/81, 52A/79, 71, 7/47/53/54/75, 47/53/75/77, and 75/77.

It would seem that there are two kinds of epidemic strains, those which cause hospital cross infection and those which cause infection in the community-at-large. Numerous reports incriminate the group III strains as the chief cause of postoperative infection, although no single phage type has been implicated to the extent of the 80/81 type (2, 12, 15, 42, 62, 111). But even among the group III strains so common in hospitals, some are disease producers and some apparently not. Although susceptibility to the group III phages is correlated with antibiotic resistance and both properties are characteristic of the strains causing hospital infections, this does not mean that all strains with these two properties are serious pathogens. Shooter et al. (111) have demonstrated that there is no correlation between the prevalence of a phage type in the hospital environment and its presence in infections, since some organisms widely disseminated in a hospital ward caused no disease among the patients.

Although many group III strains appear to act as opportunists, causing secondary infection in the susceptible hospital patient, there are those epidemic strains which, when carried into the community by the newborn infant, cause primary disease in healthy family members. The epidemiology of such outbreaks is best exemplified by studies of 80/81 epidemics (109, 131), but similar situations have been reported for other types, as shown in Table 5. Though there appears to be a correlation between phage type and the ability to cause this kind of an epidemic, it is difficult to decide whether this may be considered a correlation with virulence (133). No laboratory test is available for demonstrating virulence for man, so this quality must be evaluated upon clinical and epidemiological grounds. Rountree and Freeman (101) and Cooper and Keller (28) described 80/81 cases as unusually severe, but Jessen et al. (59) could ascribe no difference in mortality in bacteremia cases to the phage type of the infecting strain. Hospital-acquired infections and those caused by antibiotic-resistant strains had more serious prognoses than did others, regardless of the phage type of the infecting strain. But, as noted by Williams (133), modern antibiotic therapy leaves little opportunity for evaluating the virulence of an antibi-

<table>
<thead>
<tr>
<th>Phage type</th>
<th>Investigator</th>
<th>Date</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>44, 47B</td>
<td>Wilson and Atkinson</td>
<td>1945</td>
<td>137</td>
</tr>
<tr>
<td>57</td>
<td>Colbeck</td>
<td>1949</td>
<td>24</td>
</tr>
<tr>
<td>8A</td>
<td>Parker and Kennedy</td>
<td>1949</td>
<td>82</td>
</tr>
<tr>
<td>71</td>
<td>Barrow</td>
<td>1955</td>
<td>11</td>
</tr>
<tr>
<td>62A</td>
<td>Barber and Burton</td>
<td>1955</td>
<td>7</td>
</tr>
<tr>
<td>71</td>
<td>Tomlinson and Parker</td>
<td>1956</td>
<td>116</td>
</tr>
<tr>
<td>52A/79</td>
<td>Sherman et al.</td>
<td>1956</td>
<td>110</td>
</tr>
<tr>
<td>52A/79</td>
<td>Wentworth et al.</td>
<td>1958</td>
<td>131</td>
</tr>
<tr>
<td>NT* (756/950)</td>
<td>Milch</td>
<td>1960</td>
<td>76</td>
</tr>
</tbody>
</table>

* Not typable with basic set of phages.
otic-sensitive organism, and one can only conclude that the antibiotic-resistant types causing epidemic disease have something that other, equally resistant, phage types do not. Whether this property is increased virulence or something more properly termed "epidemogenicity" remains to be seen. It is certain that this property is not dependent upon phage susceptibility, since extensive nasal colonization of newborn infants with so-called epidemic types without subsequent illness has been reported (9, 73). Nevertheless, it must be assumed from the evidence correlating certain phage types with epidemic disease that these types should be considered particularly dangerous until proved otherwise.

LYSOCIN AND PHAGE SUSCEPTIBILITY

Almost all attempts to demonstrate lysogeny in staphylococci have met with success. Indeed, the extent to which the staphylococci are lysogenic makes the interpretation of their phage susceptibility difficult. Interference effects in the staphylococci appear limited; strains exhibit multiple lysogeny, and prophage immunity may not extend even to closely related phages (93, 97). Such extensive lysogeny raises the question of the extent to which natural changes in the lysogenic state affect the reliability of identification by phage typing.

Lysogeny appears to be a rather stable and permanent characteristic of a staphylococcal strain (93, 113), although Wahl and Fouace (126) reported a change in phage susceptibility in two strains which they attributed to spontaneous loss of prophage. Early work demonstrated that artificial lysogenization could be accomplished readily (20) and that such lysogenization could alter the phage type (67, 93, 113). This alteration sometimes consisted only of immunity to the lysogenizing phage, but in many cases multiple loss of phage susceptibility occurred. One prophage thus controlled resistance to several phages (127). The immunity conferred by lysogenization was serospecific; that is, lysogenization with an A phage resulted in immunity to other A phages but not to B, and vice versa (97). Although no exceptions to the specificity of A versus B phages have been reported, there have been reports of cross immunity between sero-group F and A phages (67, 127). Artificial lysogenization may involve either the addition of a new prophage to pre-existing ones or may result in prophage substitution (44, 127).

Smith (113) and Rountree (93) both concluded that lysogeny is only partly responsible for variations in susceptibility to the typing phages. The fact that the temperate phages carried by lysogenic strains have a host range which places them in the same lytic group as the phages to which the lysogenic strains are susceptible supports this conclusion (90, 127), since, if lysogeny were the principal determinant of phage susceptibility, one would expect to find the reverse situation wherein prophage would provide immunity to phages of similar serology and host range. It is difficult also to relate phage susceptibility, even on a group basis, to antigenic structure, since the correlation between antigenic grouping and phage grouping is so limited. While Hobbs (61) and Wahl and Fouace (125) found a broad general correspondence between Cowan’s serological groups I, II, and III and the lytic groups, more extensive investigation with factor sera have shown that there is no strict correlation between phage type and serological type, and that the correlation is only good when the strains are epidemiologically related (78). Lack of phage susceptibility is usually associated not with lack of cell receptors for adsorption but with some block in the steps which follow adsorption. It has also been shown that artificial lysogenization of two strains having dissimilar phage types, each with a different phage, can result in lysogenized strains showing the same phage type. Therefore, serological typing cannot be expected to correlate absolutely with phage type.

The isolation of strains typing 52/52A/80/81 strain 80/81 in conjunction with 80/81 strains in epidemic situations suggested the possibility that one of these types might arise from the other by lysogenization. Of the phage types tested for lysogeny, the strains typing 80/81 have yielded the fewest frankly lysogenic strains (6, 26, 91). Rountree (98) found an F phage in three of her strains, but it was a phage very difficult to propagate and with a limited host range. Lysogenization of these apparently nonlysogenic 80/81 strains with sero-group A phages resulted in a change in phage type which involved not only acquired immunity to the A phage, but also the loss of immunity to B phages 52 and 52A (6, 27, 101, 109). Phages capable of producing this result were termed “converting” and the results “conversion” by Rountree (98), without any implications as to the exact mechanism involved.
Subsequent work (99) has shown that this "conversion" is due to prophage substitution, the A prophage substituting for a defective prophage carried by 80/81 strains which is capable of recombination with "converting" phages, but not of phage production.

Lysogenization of 80/81 types also gave rise to strains which were completely untypable (25, 91), a finding which may have serious epidemiological implication if these nontypable strains retain the same ability to produce disease as the 80/81 strains. Milch (76) has reported the isolation of an untypable epidemic strain from a nursery outbreak. A number of 81 types were isolated in conjunction with these untypables, and both strains appeared to be causing disease. But more severe disease, with a fatality rate of 25%, was produced by the nontypable strain. Milch was able to type these routinely untypable strains with two newly isolated group B phages.

It has been suggested that typing by means of lysogeny could be a useful procedure, further differentiating strains of the same phage type (61, 90, 112). Sakurai et al. (107) have shown that phage types 52/52A/80, 52/52A/80/81, and 80/81 can further be divided into subtypes on the basis of their susceptibility to and change in phage type after in vitro lysogenization. Differentiation of 80/81 subtypes was helpful in clarifying some epidemiological problems. Undoubtedly, strains with the same phage type do exhibit different lysogenic states, but the epidemiological significance of these differences has not been sufficiently investigated. Rosenblum and Dowell (90) showed that each of the phages they isolated from a lysogenic strain had such a unique host range, not duplicating those of the typing phages, that the phage typing scheme could be extended almost indefinitely if so desired. The practical reasons for employing a restricted number of typing phages has already been discussed, and the technical difficulties of "lysogenic typing" would seem to preclude its routine use.

Rosenblum and Dowell (90) suggested that the classification of typing phages could be improved by taking into account the lysogenicity of the propagating strains. They would place phages 42B, 47C, and 81 in group I, and phage 187 in group II, since their propagating strains either carry or are lysed by phages of these groups. Though this suggestion would provide a means for assigning group designations to phages now unclassified, it might be better to leave the classification as it stands, since all the present group I phages are serologically B, whereas 42B, 47C, and 81 are A phases.

Investigation of the lysogenic state of the staphylococci has shown that lysogeny is widespread and undoubtedly does determine phage susceptibility to some degree, but there are no data which can be applied to the natural situation to estimate the probability of change in phage type resulting from changes in the lysogenic state. Epidemiological evidence still appears to support Smith’s conclusion (113) that change of phage type is apt to be rare in an investigation of short duration, but may significantly affect long-term studies.

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