THE TYPE CLASSIFICATION OF *STAPHYLOCOCCUS AUREUS*: A COMPARISON OF PHAGE-TYPING WITH SEROLOGICAL TYPING

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INTRODUCTION

The species *Staphylococcus aureus* has been divided into a series of 'types' by two different methods—serological and bacteriophage. The foundations of the serological typing methods now in use were laid by Cowan's (1939) recognition, by slide agglutination, of three main serotypes. Cowan's work was extended by Christie & Keogh (1940) and later by Hobbs (1948), who finally recognized a total of 13 serotypes. The principal difficulty in Hobbs's method lay in the absorption of the sera to reduce cross-reactions, and even with the method as published the distinction between the separate types was not always clear-cut.

Oeding approached the serological classification from observations of a particular strain of *Staph. aureus* that was widely spread in Norwegian maternity hospitals and responsible for many cases of mastitis; this strain appeared to be distinguishable from others by its possession of a particular antigenic component, recognizable by slide agglutination. Oeding then sought other similar antigens and by a process of specific absorption obtained what appeared to be a series of single-factor sera (Oeding, 1952). Investigations of the character of the antigens led to various modifications of the original typing technique, but in its latest form (Oeding, 1957), eight 'factor' sera are prepared and used for slide agglutination of living 5 hr. agar cultures of the staphylococci, which are emulsified in drops of the sera on a slide by means of a platinum loop.

Phage typing of *Staph. aureus* was developed from Fisk's (1942*a*, *b*) early work by Wilson & Atkinson (1945), and their methods have been followed without substantial modification by present-day workers (see, for example, Anderson & Williams, 1956). A set of 21 phages is now employed and the 'types' are recognized as the 'patterns' of strong lysis by one or more of these phages.

Phage-typing has been widely employed in epidemiological studies in the past 15 years and, although difficulties in interpretation certainly occur, especially with strains that are susceptible only to low dilutions of phage, the method has proved of undoubted value. Serological typing has hitherto been far less widely used for comparable epidemiological studies, but it too has proved satisfactory in the hands of certain investigators (e.g. Sompolinsky, Hermann, Oeding & Rippon, 1957).

In seeking to compare typing methods one can ask two questions. (1) Do the types recognized by one method correspond in any regular way to the types recognized by the other? (2) If a number of separate cultures isolated from one naturally existing clone are tested independently by the two methods, do both agree in classifying all the cultures as one and the same type?

There are certainly other characteristics to study in judging between two typing methods, such as the number of types recognized (and the distribution of staphylococci across them), the proportion of untypable strains, technical convenience, and so forth. But the second of the two questions mentioned above seems to us by far the most important, because unless a typing method classifies as identical a set of subcultures from a single clone it is useless for epidemiological work, in which the pertinent question is practically always 'Could strain A have been derived from strain B?'

Five comparisons of serological and phage-typing have been published (Hobbs, 1948; Oeding & Vogelsang, 1954; Pillet, Calmels, Orta & Chabanier, 1954; Sompolinsky *et al.* 1957; Oeding & Sompolinsky, 1958). The first three confined themselves to seeking a correlation between particular types; both Hobbs and Pillet *et al.* considered that there was a tendency for strains of Cowan's types I, II and III to fall into what are now known in phage typing as Lytic Groups I, II and III, but the correlation was not exact, and Oeding & Vogelsang found many discrepancies. In recent work Oeding & Sompolinsky (1958) have provided an epidemiological comparison of the two methods. They concluded that the two methods generally gave identical results in picking out epidemiologically related strains, but that their respective advantages and drawbacks varied in relation to the source of the strains. These authors considered it an advantage to use both methods simultaneously in epidemiological work.

The present paper records a comparative study, in which we attempt to answer both the questions mentioned above. Frequency distributions of the types recognized by the two methods are also presented, because these are needed if one is to judge the significance of an observation that two strains isolated from different sources belong to one type.

MATERIALS AND METHODS

Most of the staphylococci, all of which were coagulase-positive, were derived from the material sent by hospitals and public health laboratories to the Staphylococcus Reference Laboratory, Colindale, for typing; some had been isolated in Norway, Sweden or Israel and sent to the Gade Institute for typing.

The phage-typing of the strains carried out at the Staphylococcus Reference Laboratory followed the methods already described (Williams & Rippon, 1952; Anderson & Williams, 1956); the Norwegian strains were phage-typed by the same method in the Gade Institute. The 'types' reported are lists of the phages from the 'basic set' (and phage 187 where indicated) that produced strong lysis. Type designations given in italics were obtained with phages used at 1000 times the concentration of the ordinary routine test dilution (R.T.D.); the suffix 'w' indicates a weak reaction.

Serological typing was carried out in the Gade Institute using the latest modification of the method (Oeding, 1957). The types are lists of the antibody factors in the sera causing agglutination, weak reactions being given in parentheses.

For all the analyses an attempt was made to employ only 'independent' strains: no more than one strain of any one type was taken from any patient or any single-type epidemic. For this purpose strains were only regarded as of 'one type' if they were identical, except for trivial variations, in both phage-type and serological type. Where a single source yielded, for example, two strains of identical phage type but differing serotype, both were included in the analysis.

Although independent, the strains were not randomly selected, and two phage types—3A and 187—are somewhat over-represented because they seemed to have an especially well-marked correlation with particular serotypes. Otherwise the strains were mostly selected as being typical of those encountered in epidemics of hospital infection and of staphylococcus food-poisoning.

RESULTS

Direct comparison of types

The typing results obtained by the two methods on 223 strains are illustrated in Table 1. There was a tendency for association of some serotypes with particular phage-types. Thus strains lysed by phage 187, which practically never enters into patterns with other typing phages, were all agglutinated by the *k* factor serum (Serological group *4*), usually in association with other antibody factors. Although some strains of phage-types, other than 187, were agglutinated by the *k* factor serum, the reaction in these cases was usually either weak or agglutination also occurred with other sera. Strains of phage-type 3A were very often spontaneously agglutinable, though after autoclaving were usually agglutinable by the *i* factor serum and most spontaneously agglutinable strains proved to be phage-type 3A. Nine of 13 strains of phage-type 80 had the serotype *abceh*.

There was, however, no example of complete correlation between the two methods and in some cases there was a broad spread in the reactions. Strains lysed by phage 52, for example, fell into serotypes *abceh*, *aeh*, *abeh* and *abch*; and strains of serotype *abch* had phage patterns 52, 3C/55/71, 75/77, 6/7/47/53/54/75, 6/7/47/54/75 and 73. Several other similar examples could be found.

Phage-types have been previously classified into three major groups (see, for example, Rippon, 1956). It has now been found that these correspond broadly to four groups that can be defined among the serotypes on the basis of three 'determinant' antigens, as follows:

Serological group *1*: reaction with *e* factor serum, and others.
Serological group *2*: no reaction with *e*, *i* or *k* factor sera.
Serological group 3: reaction with *i* factor serum, usually with others.
Serological group *4*: reaction with *k* factor serum, often with others.

Strains with both *e* and *i* reactions are classed as serological group *1*.

Table 2 summarizes the association between the groups. Some 80% of phage-group I strains fell into serological group *1*; half those in phage-group II fell into

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serological group *2* (and the association would have been stronger if it had not been for the number of type 3A, spontaneously agglutinable, strains included), while 80 % of the strains of phage-group III fell into serological groups *2* or *3*. The strains of serological group *2* that belonged to phage-group II were mostly types *ah* or *h*,

Table 1. *Comparison of serological types with phage sensitivity patterns*

			Serological type													
			Group * *1*				Group *2*				Group *3*			Group *4*		
Lytic group	Phage pattern	No. of strains	*abceh*	*aeh*	*abeh*	Others	*abch*	*ah*	*h*	Others	*abchi*	*i*	Others	*k* alone or with others	Spont. agglutinable	Untypable
I	80	13	9	2	1	.	.	.	.	.	.	.	.	1	.	.
	52	7	2	1	1	.	1	.	.	1	.	.	.	.	.	1
	52/52A	5	1	3	1	.	.	.	.	.	.	.	.	.	.	.
	52/52A/80	3	2	.	.	.	.	.	.	.	.	.	.	1	.	.
	52A/79	3	1	1	.	1	.	.	.	.	.	.	.	.	.	.
	29/52/80	3	1	1	1	.	.	.	.	.	.	.	.	.	.	.
	80 v.w.	3	.	.	.	.	.	.	.	.	.	1	.	2	.	.
	Others	16	7	4	1	1	.	.	1	1	.	.	.	.	.	1
II	3A	10	.	.	.	.	.	1	.	.	.	.	3	.	6	.
	3C/55/71	6	1	.	.	.	1	2	1	.	.	.	.	.	1	.
	Others	7	.	.	.	.	.	5	2	.	.	.	.	.	.	.
III	75/77 and 77+	20	.	1	.	.	5	.	.	1	8	2	1	.	1	1
	75	3	1	.	.	.	.	.	.	.	1	.	.	1	.	.
	6/7/47/53/54/75	6	.	.	.	.	3	.	.	1	1	.	1	.	.	.
	6/7/47/54/75	3	.	.	.	.	2	.	.	1	.	.	.	.	.	.
	7/47/54/77	3	.	.	.	.	.	.	.	.	3	.	.	.	.	.
	73	6	.	.	.	1	1	.	.	1	.	2	1	.	.	.
	73+	4	.	.	.	1	.	.	.	1	.	.	.	2	.	.
	53w	3	.	.	.	.	.	.	.	3	.	.	.	.	.	.
	Others	45	6	1	.	2	11	.	.	2	12	7	3	.	1	.
Miscellaneous																
	187	19	.	.	.	.	.	.	.	.	.	.	.	19	.	.
	Others	17	4	5	.	.	.	.	1	1	.	.	3	1	.	2
Untypable		18	2	2	1	2	.	.	1	.	1	1	2	4	.	2
Total		223	37	21	6	8	24	8	6	13	26	13	14	31	9	7

* Serological groups are defined on p. 447.

Table 2. *Comparison of serological groups and phage sensitivity groups*

	Serological group						
Lytic group	1	2	3	4	Spontaneously agglutinable	Untypable	Total
I	42	4	1	4	0	2	53
II	1	12	3	0	7	0	23
III	13	32	42	3	2	1	93
Miscellaneous	9	2	3	20	0	2	36
Untypable	7	1	4	4	0	2	18
Total	72	51	53	31	9	7	223

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while those in phage-group III were mostly *abch*. The strains of 'miscellaneous' phage-types were mostly lysed by phages of more than one group; those lysed by group I phages were usually in serological group *1*.

Comparison of groups of strains with common origin

We can only be certain that a set of staphylococcal cultures all have a common origin if they are a set of laboratory subcultures. But for a practical interpretation of the significance of typing results we need to know what variability may be expected when the subcultures are carried out in a set of human beings, not test-tubes. We have therefore examined a number of sets of strains that were isolated from different sites on one person, from different persons, or from persons and infected fomites in circumstances that made it very probable that all the strains had a common parent. All were shown to be of one phage-type at Colindale. When these strains were tested for serotype in Bergen the results were quite consistent (Table 3), in that all the strains thought, from their origin and phage-type, to have a common source, had identical or very similar serotypes.

Table 3. *Comparative typing of groups of strains thought to have a common source*

Source of strains	No. of strains	Phage-type	No. showing variations in phage-type	Serotype	No. showing variations in serotype
Food-poisoning outbreak	8	6/47/75+	0	*abch*	0
Nose and septic lesion 1 person	2	3C/55/71+	1 lacked the minor reactions indicated by the +	*h*	0
	2	71+	0	(*ah*)	1=(*h*)
	3	3A+	0	Spont. agglutinable (auto: *i*)	0
	2	29/52/80+	0	(*ab*)*eh*	1=(*a*)*eh*
	3	6/7/47/53/54+	1 had 75 as well	*abci*	1=(*abci*), 1=*abc*(*h*)*i*
	3	80	0	(*ab*)*eh*	1=(*a*)*eh*
Hospital epidemic	3	80	0	*abceh*	0
Food-poisoning outbreak	3	42E	0	*i*	0
Hospital epidemic	3	75/77	0	(*abh*)*i*	1=(*ab*)*i*
Clothes of 1 individual	4	3C/55/71	0	*h*	0
Food-poisoning outbreak	5	47/53/73+w	3 had the 47 and/or 53 reactions weaker	*i*	1=(*b*)*i*

This comparison was extended by examining the typing results from pairs of strains isolated from successive cultures from the nose or throat of 28 individuals (Table 4). In 21 cases both methods would have led to the same conclusion on the similarity or dissimilarity of the two strains. The limits of variation with the serological typing method are less well established than with phage-typing (see Williams & Rippon, 1952) so that it is difficult to know how much weight should be put on the differences in the serotypes of the remaining 7 strains; for this reason the detailed typing results are given in the table.

Since the strains were selected on the basis of origin and phage-type it is natural that the variation in the phage reactions was less than in the serological reactions. It was unfortunately not practicable to test a comparable group selected first on the basis of serotype.

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Frequency distributions of types

Tables 5 and 6 give frequency distributions of types observed by the two methods. Some condensation has been necessary because of the great number of distinct patterns that are observed. In an actual epidemiological investigation where

Table 4. *Comparative typing of pairs of strains from nose or throat of* 28 *individuals*

Conclusion from typing by: Phage	Conclusion from typing by: Serology	No. of pairs	Serotypes where discrepant
Identical	Identical	17	—
	Moderate differences	2	*abch, abc*; *a(b)k, ahk*
	Different groups	1	*abeh*, S.A. (auto: *abch*)
Insignificant differences*	Identical	1	—
	Moderate differences	2	*ab, a*; *a(b)eh, aeh*
Different	Moderate differences	2	*ab, abc*; *abceh, aeh*
	Different	3	—

* Strains were considered significantly different only if one was lysed strongly by 2 phages that did not lyse the other.

Table 5. *Frequency distribution of serological types among* 239 *independent strains*

Serological group		No. of strains. Specified types	Total for group
1	*abceh, (abc)eh, abc(e)h, (a)bceh, a(bc)eh*	40	
	aeh, (a)eh	19	
	abeh, (ab)eh, (abe)h, a(b)eh	6	74
	(ac)eh, a(b)e, ae, e, (e)	7	
	(abc)ehi, abcei	2	
2	*abch, a(bc)h*	27	
	abc	5	
	abh, (ab)h	3	59
	ah, (ah), h, (h)	17	
	a, (a), (b), ab	7	
3	*abchi, (a)bchi, abch(i)*	29	
	i	13	
	(ab)hi, ab(h)i, (abh)i	3	56
	ai, (a)i, (ab)i, abci	5	
	bi, (b)i, b(h)i, h(i)	6	
4	*k, (k)*	3	
	ak, (ak), (a)k	6	
	abk, (a)bk	4	
	ahk, hk	3	31
	abhk, (abhk), ab(h)k	4	
	aehk, (abe)k, (aeh)k, aek, a(b)eh(k), (abc)ehik, (ei)k	8	
	(abc)k, aik, (abi)k	3	
Spontaneously agglutinable			
	After autoclaving: *i*	9	
	After autoclaving: *abch*	2	11
	Untypable	8	
Total		239	

Note. This table is based on strains isolated from patients and carriers in hospitals in England, Bergen, Tel-Aviv and Stockholm. It is probable that group *4* is over-represented.

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a sufficient number of strains of an 'epidemic type' are tested to establish its range of variability, one can often make finer distinctions than those entered in the table, but the different types given represent those that could generally be regarded as distinct even on the basis of single strains.

Table 6. *Frequency distribution of phage-types among 750 independent typable strains*

Lytic group	Phage patterns	No. of strains: Specified types	No. of strains: Related patterns	Total for lytic group
I	29	20	—	
	29 + other phages of group I	—	18	
	52/52A, 52, 52A	41	—	
	79	27	—	212
	52A/79, 52/52A/79	63	—	
	80	29	—	
	80 + other phages of group I	—	14	
II	3A	8	—	
	3C, 3C/55, 3C/55/71, 55/71, 55	36	—	84
	71	21	—	
	Other group II patterns	—	19	
III	6/7/47/53/54/75 and similar patterns all with 6 and 7	28	—	
	6/47/54, 6/47/53, 6/47/53/75, 6/53/54/75, etc.	35	—	
	Other patterns including phage 6	—	7	
	7/47/53/54/75/77 and similar, all with 7 and 47	36	—	
	Other patterns with 7 but not 6	—	16	
	42E	15	—	
	Others with 42E, not 6 or 7	—	6	348
	47/53/75/77, 47/53/77, 47/75/77 and similar	36	—	
	Others with 47, not 6, 7, 42E	—	4	
	75/77, 75, 77	78	—	
	53	35	—	
	53/75, 53/75/77, 53/77	19	—	
	Others with 53, 54, etc., not 6...47	—	17	
	70	5	—	
	73	10	—	
	Others with 70...77	—	1	
IV	42D or 42F	5	—	5
Miscellaneous	47A, 52B, 69, 81, 187, 44, 31	—	8	8
Mixed	Phages of groups I and II	—	78	93
	Other mixed-group patterns	—	15	
	Total typable strains	547	—	750

The table is based on the 750 apparently independent strains in a total of 2394 strains isolated from the lesions (wound, pemphigus, mastitis, enteritis, etc.) of patients in investigations of hospital infection. 99 strains (4·1 %) were untypable. 134 of the 750 independent types were recognized by use of phage 1000 times stronger than R.T.D. only; these are not distinguished in this table.

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DISCUSSION

Two conclusions seem to emerge clearly from this study. First, the receptors concerned with the serological typing must differ from those for phage-typing, since there was very little correlation between the individual types recognized by two methods, although some interesting associations were observed. Strains agglutinated by the *k* factor serum were almost all lysed by phage 187 and spontaneously agglutinable strains by phage 3A. Secondly, when strains thought to be derived from one source were found to be of one type by the phage method, they were also all of one serological type. Thus we have the situation that three strains isolated from different sites of one patient all proved to be phage-type 80 and serotype (*ab*)*eh* or (*a*)*eh*, which may be regarded as the same. But strains of phage-type 80 from various situations were of types *aeh*, *abceh*, or *abk*, and serotype *aeh* strains from different sources were, by phage-typing, 77 + w, 29/52/79 +, 7/54/70/73 +, 52A, 52A/79 or *80/42E* w. The relative stability of the reactions with both methods, with strains from a common source, indicates that this variation cannot arise from technical faults; there must, it seems, be an almost random distribution of the two sorts of receptor in different clones of staphylococci.

The results of Tables 3 and 4, if confirmed on a larger series, would indicate that either method could be used for tracing the source of an epidemic and either should recognize the strains of the epidemic type with equal reliability. In practice, however, the reliability of the two methods may not be equal. Serological typing recognizes many fewer types than the phage-typing method and consequently the former may identify strains as belonging to the epidemic type, when they are in fact unrelated. Four types, *abceh*, *aeh*, *abch* and *abchi*, account for 50% of the strains distinguishable by the serotyping method. To reach the same proportion some 10 phage-types have to be included, namely 75/77, 52A/79, 52/52A, 47/53/75/77, 7/47/53/54/75/77, 3C/55/71, 6/47/54, 53, 80, 6/7/47/53/54/75.

With the serological method very few untypable strains are encountered. With phage-typing 20–40% may be untypable at the routine test dilution; most of these are lysed when the phages are employed 100 or 1000 times more concentrated, but difficulties in interpretation are increased when the strong phage filtrates have to be used.

The epidemiological advantage of the greater number of types recognized by the phage method has to be set against several disadvantages, of which the first may indeed be that the number of types that one is tempted to recognize is too great, and the distinction between the types is not always clear. By the study of replicate cultures from individuals, and sets of strains from epidemics it has been possible to assess the variability of phage-types (Williams & Rippon, 1952; Williams, 1957; and unpublished analyses in progress) and to provide some rules for deciding when two strains with overlapping phage patterns ought to be regarded as belonging to different types. It is not yet possible to offer similar analyses for the serological typing method, but there is undoubtedly variation in, at least, the weak reactions. Thus one strain typed as *abc*(*e*)*h* on one day might be *abceh* on another and a second strain typed as *abc*(*e*)*h* might be *abch* on another day. It is a general

impression that variation leading to alteration of serological group is unlikely to occur.

There is no doubt that the reagents used for phage-typing are more complex and probably more difficult to prepare than typing sera. Detailed testing of the phages is essential if they are to be preserved through many successive propagations. It has, nevertheless, proved possible to maintain the phages successfully, and comparison of the reactions obtained with successive batches propagated at intervals over the last 5 years shows remarkable stability.

Although typing sera seem easier to prepare, and store better, than phage filtrates, we have not yet sufficient experience to judge how similar successive batches may be, and a testing routine such as is followed in phage work has yet to be defined. Hitherto sera have been absorbed immediately before use, but this is probably unnecessary.

At the bench both methods have their advantages: serological typing gives the answer more rapidly, but is less well adapted to testing large numbers of strains since the ephemeral agglutination must be inspected by an experienced worker; phage-typing takes at least 18 hr., but more of the work can be done by junior technicians and the plates can be inspected at convenience. Phage-typing is much more extravagant of media than serological typing.

It seems therefore that for most purposes the decision as to which method to use will have to rest on the number of different types which we need to distinguish. Where few will suffice, serological typing has advantages; where many are needed, phage-typing must be employed. In some circumstances the use of both methods together may give the best results.

SUMMARY

A comparison has been made between the bacteriophage and serological methods for identifying types among coagulase-positive staphylococci. In only a few cases was one particular serotype clearly related to a particular phage-type, and in several cases single 'types' recognized by one method contained several different 'types' when tested by the second method. Nevertheless, when sets of strains isolated in the investigation of epidemics or of the nose and skin carrier state of particular individuals were tested by the two methods, consistent results were obtained: an individual combination of phage-type and serotype appeared to be stable.

The principal advantages of phage-typing are the facts that it is able to recognize more types than the serological method, and that the distinctions between the types are based on a greater number of different reactions. The advantages of the serological method are the smaller number of untypable strains and its greater technical simplicity.

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