A Study of the Antigenic Specificity of Staphylococcal Coagulase in Relation to Bacteriophage Group

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SUMMARY: Rabbit antisera were prepared separately against coagulase from staphylococci of each of the three main bacteriophage groups. Neutralization tests showed that each of the three antisera caused considerable delay in the clotting of plasma by coagulase from the autogenous staphylococcus and other strains of the same phage group, but little or no delay in the action of coagulase from strains of other groups. Staphylococci of certain phage types yielded coagulase which was not neutralized by any of the three antisera. Amongst these were types 3A and 42E which are usually included in groups II and III, respectively, as well as unclassified types (e.g. 187). Antisera prepared against coagulase from types 3A and 42E caused significant neutralization of coagulase of the same type only.

Just over fifty years ago Loeb (1903-04) demonstrated the specific action of *Staphylococcus aureus* in causing clotting of goose plasma. This finding was confirmed by Much (1908), who emphasized the correlation between the clotting ability of staphylococci and pathogenicity. Subsequent workers have studied this correlation (Chapman, Berens, Peters & Curcio, 1934; Cruickshank, 1937) and the properties and mode of action of the clotting agent (Smith & Hale, 1944; Tager & Hales, 1948*a, b*; Duthie & Lorenz, 1952; Duthie, 1954). Contrary to the observations of earlier workers (Gross, 1931; Smith & Hale, 1944) it has recently been shown that staphylocoagulase is antigenic (Tager & Hales, 1948*b*; Rammelkamp, Hezebicks & Dingle, 1950; Duthie & Lorenz, 1952; Duthie, 1952). Rammelkamp *et al.* (1950) and Duthie (1952) found that coagulase derived from different staphylococci of human origin could be divided into three distinct antigenic types. Duthie also had a fourth antigenic type of coagulase from a strain of bovine origin. In later work Duthie (1954) showed that bound and free staphylocoagulase were distinct entities and immunologically unrelated.

The present study was concerned solely with free staphylocoagulase. It was begun in order to see whether there was any correlation between quantitative production of coagulase and virulence. In preliminary experiments to devise a simple quantitative test, however, significant differences in the optimum conditions of coagulase production and release were found with strains belonging to different bacteriophage groups. Artificial immunization of rabbits with coagulase preparations from staphylococcal strains of bacteriophage groups I and III were then prepared, and it was found that the two coagulases were of quite distinct antigenic types. A study of the antigenic specificity of coagulase in relation to bacteriophage type was, therefore, undertaken.

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METHODS

Organisms. All strains of staphylococci used in this study yielded free coagulase (Duthie, 1954) during growth in broth. Some of them had been recently isolated from patients and staff in St Thomas's Hospital, and some had been cultivated in the laboratory for many years. A number of other strains of representative bacteriophage types were kindly supplied by Dr R. E. O. Williams of the Central Public Health Laboratories at Colindale.

Selection of variants yielding large quantities of coagulase. For some purposes, especially for the production of neutralizing antisera, it was necessary to use staphylococcal strains which yielded large quantities of free coagulase. Duthie & Lorenz (1952) selected one such strain (Newman) by using fibrinogen plates; but strains of this sort seem to occur rarely in nature. Smith, Morrison & Lominski (1952) have, however, described 'fast clotting' variants which occurred in old cultures of staphylococci and which give rise to rough colonies on heated blood agar medium. Variants yielding large amounts of coagulase were therefore sought in cultures of a number of strains grown in different media. Six such variants isolated from three strains were sent for bacteriophage typing, together with their parent stock. In every case the phage patterns were similar.

Media. Three different media were used during this work. They were: peptone yeast-extract broth consisting of 1% (w/v) peptone (Evans), 0.1% (w/v) yeast extract (Difco), 0.5% (w/v) NaCl, pH 7.2; bullock-heart infusion broth; fibrinogen agar which contained bovine fibrinogen (Armour fraction V), human plasma, trypsin inhibitor, nutrient broth and 1.5% (w/v) agar (Difco; see Klemperer & Haughton, 1957). On the latter medium colonies of coagulase-positive staphylococci were surrounded by a halo of opacity whose diameter was usually found to be related to the concentration of coagulase formed when the organisms were grown in fluid culture.

Phosphate + citrate buffer. All dilutions of coagulase, plasma or fibrinogen were made in buffer which comprised 1% (w/v) $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ + 1% trisodium citrate in distilled water. The pH value was adjusted to 7.0 with N-NaOH and the solution sterilized by autoclaving.

Production of free coagulase

Two methods were used for the production of high concentrations of coagulase.

Method 1. Selected variants were inoculated into 200 ml. amounts of peptone yeast-extract medium which were distributed in shallow layers in Roux bottles. These were incubated at 37° and sampled at intervals. When the samples were found to contain sufficient coagulase to give a clot in the standard test within 5 min. (after 1-3 days of incubation, depending on the strain), the organisms were removed by centrifugation and the coagulase in the supernatant fluid was concentrated by the cadmium sulphate method (see below).

Method 2. Another method for producing large yields of free coagulase has been developed by Dr E. S. Duthie (personal communication). The method

depends upon the fact that coagulase is released by nearly all strains of *Staphylococcus aureus* in the presence of albumin (Davies, 1951; Duthie, 1954). For large-scale preparations, strains were inoculated into a number of 8 oz. medical flat bottles, each of which contained 5.0 ml. heart infusion broth. These bottles were mounted in a Kahn shaker and were continuously shaken for 18 hr. at 37°. After this, 45 ml. peptone yeast-extract medium to which had been added 0.15 % (w/v) bovine serum albumin (Armour fraction V), and which had been warmed to 37°, was added and the cultures again shaken for 2 hr. The cocci were then removed by centrifugation and the coagulase present in the supernatant fluid concentrated by the ammonium sulphate method (see below).

This method was also used on a smaller scale with 0.5 ml. heart infusion broth in test tubes 150 × 16 mm. After shaking for 18 hr., 4.5 ml. peptone yeast-extract medium with albumin were added.

Concentration of coagulase

Cadmium sulphate method. Coagulase produced by method 1 was concentrated by precipitation with 0.5 % (w/v) CdSO₄ at pH 5.8 in the cold (Duthie & Lorenz, 1952). After holding in the refrigerator overnight, the precipitate was collected, dissolved by the gentle addition of N-HCl to a final pH value of about 2.0, and then dialysed against running tap water in the refrigerator. When free from cadmium, the dialysed material was clarified by centrifugation and stored in the cool until required. This method of concentration was unsuitable when method 2 had been used for coagulase production, since under this condition the coagulase was not precipitated by cadmium sulphate. This was probably due to the presence of albumin.

Ammonium sulphate method. Coagulase produced by method 2 was concentrated by adding 2 vol. of saturated ammonium sulphate (Dr E. S. Duthie, personal communication). After storage overnight in the refrigerator the opalescent precipitate was collected, washed once in 60 % saturated (NH₄)₂SO₄ and taken up in distilled water.

Preparations of coagulase obtained by either of the above methods showed a 10- to 20-fold concentration.

Detection and estimation of free coagulase

The supernatant fluids from liquid cultures or concentrated preparations obtained as described above were tested for their ability to clot fresh citrated human plasma. With the supernatant fluids, further elaboration of coagulase by remaining bacteria was prevented by adding sodium thiomersalate to give a final concentration of 1/10,000 (Davies, 1951). For the test, 0.2 ml. volumes of suitable dilutions were mixed with an equal volume of clotting system in a 75 × 8 mm. test tube and incubated at 37° in a water bath. The formation of a clot indicated free coagulase. The test was made quantitative by measuring the time taken for a clot to develop. Dilutions which gave clots in from 5 to 60 min. gave the most accurate results. No attempt was made to

convert the readings into 'units' of activity; they have been recorded directly as clotting times in minutes.

Clotting system. Fresh citrated human plasma, always taken from the same individual, was made up to 20 % (v/v) in buffer, and bovine serum albumin (Armour fraction V) was added to give a final concentration of 3 % (w/v).

Preparation of antisera

Strains with high coagulase activity and representative of each of the three main bacteriophage groups were first selected as follows: strain M, phage-type 52/80+ (Group I); strain W120, phage-type 3B/3C/55+ (group II); and strain Newman, phage type 7/47/53/54/73/75/77 (group III). Later one strain each of phage types 3A and 42E were also used. Antigens for injection were prepared by adding 0.4 g. potassium aluminium sulphate to 15 ml. of coagulase preparation and adjusting the pH value to 6.8 with N-NaOH. Preparations of coagulase concentrated by either method were used, but those prepared by ammonium sulphate precipitation were found to have a high toxicity which could not be attributed to α -toxin. The coagulase activity of different preparations was very variable and was not standardized.

Antisera were prepared by injecting young adult rabbits with 0.5–1.0 ml. doses of alum-precipitated coagulase antigen once weekly for 4 or more weeks. Ten days after the last injection, the rabbits were bled and their sera separated. Sera were inactivated at 56° for 30 min. before use.

Coagulase neutralization test

Coagulases, either in the form of concentrated preparations or supernatant fluids of cultures after centrifugation, were used. After testing their activity the coagulase preparations were diluted to give a clot after incubation for 10 min. but not after 5 min. These dilutions were mixed with equal volumes (0.1 ml.) of serum, usually diluted 1/2, and left at room temperature for 1 hr. The residual activity was then measured by adding 0.2 ml. clotting system incubating at 37° and noting the clotting time.

Haemagglutination

Coagulase was adsorbed on to tanned erythrocytes (Boyden, 1951). Both human (group O) and sheep cells were used. After washing three times in the phosphate citrate buffer, 1 ml. amounts of packed cells were suspended in 9 ml. buffer containing 1/20,000 tannic acid (British Drug Houses Ltd.) and left at room temperature for 10 min. The tanned cells were then separated by centrifugation and washed once in buffer. Nine ml. of coagulase preparation at different concentrations was left in contact with 1 ml. of packed tanned cells for 10 min. The cells were then washed once and resuspended in buffer containing 0.4 % (v/v) normal rabbit serum. For haemagglutination tests 1 % (v/v) suspensions of cells were added to equal volumes of antiserum dilutions, and the results read after sedimentation.

RESULTS

Preliminary observations

Many preliminary experiments were made to determine the conditions under which different strains of *Staphylococcus aureus* gave high yields of free coagulase in liquid cultures. In peptone yeast-extract medium a significant difference was found between the behaviour of strains of bacteriophage groups I and III. The difference was particularly marked in relation to the effect of

Table 1. *Effect of sera of various animals on the production of free coagulase by staphylococci*

22 hr. shaken cultures in peptone yeast-extract medium						
Medium	Bacteriophage group of staphylococci					
	I			III		
	Strain of staphylococcus					
	M	SB25	SB26	SB21	N	Woods
	Relative amounts of coagulase					
Broth alone (v/v)	—	—	—	+++	++++	++
Broth + 1 % (v/v) bovine albumin	—	—	—	++++	++++	++
Broth + 10 % (v/v) human serum	+++	+++	+++	++++	+++	+++
Broth + 10 % (v/v) horse serum	+	+	+	+++	++++	+
Broth + 10 % (v/v) rabbit serum	—	—	—	+++	++++	+++
Broth + 10 % (v/v) fowl serum	—	—	—	+	++++	—
Broth + 10 % (v/v) guinea-pig serum	—	—	—	—	++	—
Broth + 10 % (v/v) cat serum	—	—	—	—	—	—

Clotting times of supernatant fluids + thiomersalate recorded as follows: ++++ = < 10 min.; +++ = 10–60 min.; ++ = 360 min.; + = clot overnight; — = not clot.

adding sera or albumin from various animals. A typical experiment is shown in Table 1, from which it will be seen that strains of group I produced little or no free coagulase except when grown in the presence of human serum, whereas strains of group III yielded moderate or large amounts of free coagulase under most conditions. It is interesting, however, that guinea-pig and cat sera appeared to inhibit either the production or activity of coagulase.

Production and release of free coagulase

The above results are recorded because they first called to our notice a difference between staphylococcal strains of different phage groups. In all further work the two methods described above for production of free coagulase were used. Consistently high yields were obtained by method 2 with nearly all strains of *Staphylococcus aureus* tested, and this method was, therefore,

used in all coagulase neutralization tests. Method 1 had the advantage that no albumin was added, and was therefore preferred for preparation of antisera, but good yields of free coagulase were only obtained by this method with the freak strain, Newman, or with 'fast-clotting' variants selected from other strains.

Selection of 'fast-clotting' variants

Twenty-four strains of *Staphylococcus aureus* were examined for the occurrence of variants giving high and low yields of free coagulase. Broth cultures were plated out on fibrinogen plates and the area of opacity round individual colonies noted. It was frequently found that colonies from a single strain showed gross variation in this respect. Colonies surrounded by large and small haloes were selected from each strain and tested for production of free coagulase in peptone-yeast broth, without the addition of serum or albumin. By this technique cultures showing gross variations in coagulase activity were isolated from 18 of the 24 strains. Typical results with 6 strains are shown in Table 2. The colonies of the 'fast-clotting' variants isolated in this way were

Table 2. *Production of free coagulase in peptone-yeast broth by selected colonies from 6 strains of Staphylococcus aureus*

Strain	Colony a	Colony b
	Clotting time (min.)	
M	< 5	45
26	< 5	45
W 120	2	360
PR	2	60
LKS	10	360
LAR	< 1	360

often small and rough in appearance, as described by Smith *et al.* (1952). The surrounding area of opacity was always large, although a direct quantitative relationship between this and production of free coagulase in broth has not been demonstrated. Typical appearances are seen in Pl. 1.

Evaluation of coagulase test

Relationship of coagulase concentration to time of clot. Tests were carried out in replicate with different dilutions of a preparation of coagulase, and the tubes were examined for clot formation every 2.5 min. The following results were obtained.

Dilutions	...	1/1	1/2	1/4	1/8
No. of replicates		10	10	20	20
Mean clotting time (min.)		6.75	11.25	29.5	55.5
Standard deviation		1.2	0.26	2.04	5.5

In a further experiment closer dilutions were used and the results are shown in Fig. 1. It will be seen that the relationship is linear over a period from 5 min. to 1 hr.

Factors which affect the tests. 20 % (v/v) fresh citrated human plasma from one individual was used for all tests. In early work it was found that the rate of clot formation was affected by the presence of albumin. Addition of bovine serum albumin to give a final concentration of 3 % (w/v) caused a two- to sixfold decrease in clotting time with all coagulase preparations tested. This was, therefore, incorporated in the clotting system (see Methods). Later, when

Fig. 1. Relationship between clotting time and dilution of coagulase. Both axes are plotted on logarithmic scales. O, last negative reading; x, first positive reading.

testing the effect of rabbit antisera, it was found that the presence of normal rabbit serum accelerated clot formation to a variable extent. This effect was most marked when the coagulase preparation and rabbit serum were left in contact with one another for a period before plasma was added, and was not altered by inactivating the serum at 56° for 30 min. For this reason, in coagulase neutralization tests, controls were left in contact with a volume of normal rabbit serum equivalent to that of the antiserum used in the tests.

Coagulase neutralization with rabbit antisera prepared against coagulase from staphylococcal strains of bacteriophage groups I, II and III

Activity of antisera. At first antisera were prepared against coagulase from one strain of staphylococcus from each of the three major bacteriophage groups. The neutralizing activity of each antiserum was tested after four or five injections of antigen. In most cases it was found that the antiserum possessed moderate or good neutralizing power against the homologous coagulase, but in some cases it was necessary to give a further course of injections. The factors which affected antibody response were not investigated, but in all

cases the antiserum finally used possessed good neutralizing ability against the homologous coagulase. Attention was, however, paid to the effect on neutralization of altering the proportions of coagulase and antibody present. A chessboard type of experiment was set up in which falling dilutions of antiserum (1/1–1/100) were each tested against falling dilutions of coagulase; the results are given in Table 3. Two practical points emerge. First, the antiserum was of low titre in the sense that however dilute the coagulase only low

Table 3. *Coagulase neutralization; effect of varying proportions of coagulase and antiserum*

The figures in the main part of the table represent the factors by which clotting times were delayed; — = delayed beyond the limits of experiment.

Dilution of antiserum ($\log_{10}$)	Dilution of coagulase ($\log_{10}$)				
	0	0.3	0.6	0.9	1.2
	Relative delays in clotting time				
0	3000	—	—	—	—
0.2	56	—	—	—	—
0.4	10	—	—	—	—
0.6	2.8	500	—	—	—
0.8	1	14	144	—	—
1.0	1	2.8	8	78	—
1.2	1	1	2.5	7.5	62
1.4	1	1	1.25	2	2.4
1.6	1	1	1.25	2	1.4
1.8	1	1	1	1.5	1.4
2.0	1	1	1	1.5	1

dilutions of serum had an effect; yet the antiserum was of high activity in the sense that these dilutions enormously prolonged the clotting time of all dilutions of coagulase. Secondly, small alterations in the dilution of coagulase had a dramatic effect on the degree of neutralization. In neutralization tests, therefore, antisera were always used at low dilution (1/2 or 1/4) and the coagulase preparations were carefully standardized to give a clot in 5–10 min., which corresponds to the dilution 0.6 $\log_{10}$ in Table 3.

Specificity of antisera. The neutralizing effect of each of these sera was then tested against each of the three preparations of coagulase; the results are shown in Table 4. It is clear that each antiserum has a dramatic effect on the

Table 4. *Coagulase neutralization by rabbit antisera*

Clotting times were determined after contact for 1 hr. at room temperature with rabbit sera diluted 1/2.

Coagulase preparation	Normal rabbit serum	Rabbit antisera		
		I (M)	II (W120)	III (N)
		Clotting time (min.)		
I (M)	10	270	10	10
II (W120)	10	10	180	10
III (N)	15	10	10	> 1440

homologous coagulase and none whatever on the others. We therefore decided to test coagulase from a number of strains of *Staphylococcus aureus* of known bacteriophage type.

Through the kindness of Dr R. E. O. Williams, of the Central Public Health Laboratory, we obtained a number of strains of commonly occurring phage types. Coagulase from each of these was tested against each antiserum. Clear evidence of neutralization by one only of the three antisera was noted with 42 of 49 staphylococcal strains tested. When the results were analysed in relation to bacteriophage group the correlation was good. It will be seen from Table 5 that there are few discrepancies and all of them are interesting.

Table 5. *Neutralization by rabbit antiserum of coagulase from 49 strains of Staphylococcus aureus of different bacteriophage groups*

Phage group	No. of strains tested	Average factor by which clotting time is increased			Remarks
		Antiserum I	Antiserum II	Antiserum III	
Group I	14	9.3 (>3-18)*	1.1 (0.75-2.0)	1.0 (0.6-1.5)	—
Group I	1	1.0	1.5	1.0	Bovine strain
Group II	8	1.4 (1.0-1.5)	5.0 (>2.5-8.0)	1.1 (1.0-2.0)	—
Group II	2	1.3 (1.3 and 1.3)	1.0 (1.0 and 1.0)	1.0 (1.0 and 1.0)	Both type 3A
Group III	19	1.1 (0.1-1.5)	1.0 (0.5-2.0)	14.0 (>6-36)	—
Group III	2	1.8 (1.5 and 2.0)	1.4 (1.3 and 1.5)	1.2 (1.0 and 1.3)	Both type 42E
Miscellaneous	2	1.4 (1.3 and 1.5)	1.3 (1.0 and 1.5)	1.0 (1.0 and 1.0)	Both type 187
Non-typable	1	>4.0	0.6	0.6	—

* Range is given in parentheses.

The one anomalous strain of phage group I was atypical in that it was of bovine origin. In group II both outstanding strains were of phage type 3A and they were the only strains of this phage type. A similar anomaly was noted in group III where the two exceptional strains were alone in being of phage type 42E. Strains of type 187 do not fall into any of the three major phage groups and our results confirm this. The non-typable strain would appear to have originated from a strain of phage group I. Two strains sensitive to phages of both groups I and III were also tested. The coagulase from one of these was specifically neutralized by antiserum I whilst that of the other was not affected by any of the antisera.

A further 30 strains of *Staphylococcus aureus* isolated in an investigation by Hare & Ridley (1957, unpublished) were typed by bacteriophage at the Central Public Health Laboratory, and tested by the coagulase neutralization method. Twenty-one of the strains fell clearly into one or other of the three main phage groups and with 20 of these the coagulase neutralization test gave good agreement. The remaining strain was classified as phage type 54/77+ but only reacted to concentrated phage preparations; by the coagulase neutralization test it appeared unequivocally to be group I. Seven of the 30 strains reacted to

phages of both groups I and III. Of the coagulases from these, 3 were neutralized by group I antiserum, 2 by group III antiserum, 1 was inhibited slightly by all three antisera and 1 was not affected by any of the antisera. Two strains of miscellaneous phage type (69 and 42D) also failed to react with any of the antisera.

Coagulase neutralization with further rabbit antisera

In the light of these findings antisera were prepared against coagulases from one strain of each of the types 3A and 42E. Neutralization tests of coagulases from 26 representative strains were done with these and the 3 original antisera. The results, shown in Table 6, confirm the finding that coagulase from

Table 6. *Neutralization by rabbit antiserum of coagulase from 26 strains of Staphylococcus aureus of different bacteriophage patterns*

Phage pattern	No. of strains tested	Average factor by which clotting time is increased				
		Antiserum I	Antiserum II	Antiserum III	Antiserum 3A	Antiserum 42E
Group I	6	10.7 (6.0-18.0)*	1.2 (1.0-2.0)	1.0 (0.5-1.5)	3.3 (1.5-5.5)	1.6 (1.0-2.5)
Group II	8	1.3 (1.0-2.5)	> 5.3 (> 2.5-> 8.0)	1.2 (1.0-2.3)	1.0 (1.0-1.0)	1.1 (1.0-2.5)
Group III	8	1.2 (1.0-1.5)	1.1 (1.0-2.0)	> 15.5 (> 6.0-36.0)	1.5 (1.0-2.5)	1.6 (1.0-2.5)
Type 3A	2	1.0 (1.0 and 1.0)	1.0 (1.0 and 1.0)	1.3 (1.3 and 1.3)	> 12 (> 12 and > 24)	1.0 (1.0 and 1.0)
Type 42E	2	1.4 (1.3 and 1.5)	1.4 (1.3 and 1.5)	1.5 (1.0 and 2.0)	2.5 (2.0 and 3.0)	6.5 (5.0 and 8.0)

* Range is given in parentheses.

strains of type 3A differs significantly from that of other group II strains, and similarly that coagulase from type 42E is distinct from that of other group II strains. It will be seen that the 5 antisera differed considerably in the degree of activity against coagulase of the homologous group. No attempt has so far been made to standardize the antisera.

Anomalous strains

Two strains of type 42D gave unexpected results. The coagulase of one (W) showed significant inhibition with group III antiserum, whereas that from the other (T) was unaffected. An antiserum was prepared against W coagulase. This caused a < 12-fold delay in the action of the autogenous coagulase and a 4 to 16-fold delay in that of all 8 strains of staphylococci of bacteriophage group III, but failed to inhibit coagulase from the other 42D strain. When tested with additional phages the two strains differed in that one (W) was strongly lysed by phage 52B, whereas the other (T) was not.

Coagulase from one strain of type 53 was only weakly inhibited by group III antiserum. An autogenous antiserum delayed its action by a factor of 10 and that of 15 strains of group III by a factor of 1.6-5.3 (average 3.7). In fact this antiserum affected the coagulase of some strains from all phage groups to a similar extent.

Coagulase neutralization by human sera

The coagulase neutralizing activity of sera from 29 human adults was tested against coagulase from 17 strains of *Staphylococcus aureus* representative of the three main phage groups and types 3A, 42E and 187; the results are shown

in Table 7. It will be seen that nearly all sera contained antibodies to group I coagulase, more than half to groups III and II, and about a third to each of the remainder. Only 2 sera had no antibodies to any coagulase. It is significant, in view of our findings with rabbit antisera, that antibodies to coagulase

Table 7. *Pattern of staphylococcal coagulase-neutralizing antibodies in 29 human sera*

No. of sera	I	II	3A	III	42E	187
4	+	+	—	—	—	—
3	+	—	—	—	—	—
2	+	+	—	+	—	+
2	+	+	—	+	—	—
2	+	+	+	+	+	+
2	+	+	+	+	+	—
2	+	—	—	+	+	—
2	—	—	—	—	—	—
2	+	+	+	+	—	+
1	+	—	—	—	—	+
1	+	—	—	+	+	+
1	+	—	+	+	+	+
1	+	—	+	+	+	—
1	—	—	—	+	—	—
1	+	—	—	—	+	+
1	+	+	—	+	+	+
1	+	—	+	—	—	—
Total						
29	26	15	9	17	11	11

of type 3A did not necessarily go with those to group II; similarly, with type 42E and group III. One serum inhibited the coagulase from only 5 out of 6 group I strains and another only 1 of 2 strains of type 187. In all other instances consistent results were obtained.

Haemagglutination

Because of the specificity of our results with the coagulase neutralization test, it was thought desirable to see whether similar specificity could be demonstrated by another technique. Coagulase was found to be readily adsorbed on to tanned, but not untanned, human and sheep red blood cells. Thus when preparations of coagulase which gave clotting in 10 min. were left in contact with one-tenth volume of tanned erythrocytes for 10 min., 25–50 % of the coagulase was adsorbed. Cells coated in this way by coagulase preparations of each of the three main groups of staphylococci were agglutinated by all three antisera to a similar degree, but not by normal rabbit serum. Since this demonstrated the impurity of the coagulase preparations, serial adsorptions with varying proportions of red cells were carried out. This procedure increased the specificity of haemagglutination in some experiments, but so far we have failed to standardize the method,

DISCUSSION

The results presented here show that there is a close relationship between the bacteriophage group of staphylococcal strains and the antigenic specificity of the free coagulase produced by the corresponding strains. Thus antiserum prepared against coagulase from staphylococci of one bacteriophage group neutralizes coagulase from most strains of the same group, but not coagulase from strains of other groups. The coagulase from staphylococci of phage type 3A appears distinct from that of other group II strains. Similarly, that from type 42E differs antigenically from that of other group III strains. Of 80 strains tested, apart from 7 of unclassified or mixed phage patterns, only 2 did not fall into one or other of these 5 coagulase groups.

In 1922 Julianelle in America and Hine in Great Britain divided pathogenic staphylococci into three serological types by means of agglutinating antisera. Subsequent workers have confirmed by more refined techniques the three main groups, and demonstrated other serological types or sub-types (Cowan, 1938; 1939; Christie & Keogh, 1940). Hobbs (1948) made an extensive study of the serological classification of staphylococci. By means of agglutination tests with absorbed sera, she recognized 7 specific serological types and a further 8 types with less specific reactions.

Staphylococci have also been classified by their susceptibility to bacteriophages. Fisk (1942*a, b*) showed that phages carried by many strains of *Staphylococcus aureus* lysed some but not all other strains. Wilson & Atkinson (1945), who used bacteria-free phage filtrates, defined 21 different phage types among pathogenic staphylococci. Williams, Rippon & Dowsett (1953), with 24 different phage filtrates, found that 1394 strains of *Staphylococcus aureus* fell into three broad groups. These corresponded to the three serological groups recognized by Cowan (1939). Hobbs (1948) showed a relationship between serological type and bacteriophage pattern and reading this paper in the light of the phage groups defined by Williams *et al.* (1953) and of our own results, it seems of interest to note that Hobbs found that strains of phage type 3A and 42E differed serologically from most strains lysed by other phages of group II or group III respectively.

The capacity of coagulase to produce antibodies has only recently been appreciated. Lominski & Roberts (1946) demonstrated a specific coagulase inhibitor in human sera, which was associated with the globulin fraction. Tager & Hales (1948*b*) were the first to record the experimental production of antibodies to coagulase. Rammelkamp *et al.* (1950) distinguished three antigenic types of coagulase by neutralization tests with sera from 148 medical students and 3 immunized monkeys. Duthie & Lorenz (1952) and Duthie (1952) by using antisera prepared in rabbits and 25 human sera were able to define 4 antigenic types of coagulase, one of which was derived from a bovine strain. None of these workers related coagulase type to bacteriophage type.

The close relationship which we have found between antigenic type of coagulase and bacteriophage type suggests either some direct association between coagulase and phage, or a difference in the genetic constitution of

staphylococci of different phage groups. Possible direct relationships are that coagulase acts as a receptor for phage or is concerned in events after absorption. Preliminary experiments to demonstrate such a relationship have been inconclusive.

A number of factors point to a difference in the genetic constitution of staphylococci of each of the three main phage groups. Besides the serological distinction we found differences in production and release of coagulase. Other workers have drawn attention to the association of certain phage groups of staphylococci with particular types of infection (cf. Anderson & Williams, 1956). Moreover, there is evidence to suggest that staphylococci of phage group III yield antibiotic-resistant variants more readily than do staphylococci of other groups (Barber & Whitehead 1949; Rountree & Thomson, 1949; Barber & Burston, 1955).

All investigators who have used agglutination or precipitation techniques have found cross-reactions so common that unabsorbed sera had little or no distinguishing value. It is remarkable that with coagulase neutralization, probably because of the crudity of the test and the fact that a single antigen is concerned, cross-reactions are relatively unimportant. This means that it has advantages over other methods of serological classification, and although it distinguishes fewer types than the bacteriophage method, it might be a useful procedure in small laboratories.

The presence of coagulase-neutralizing antibodies in human sera have been shown to be of common occurrence (Lominski & Roberts, 1946; Rammelkamp *et al.* 1950; Duthie 1952). We confirm the findings of Rammelkamp *et al.* (1950) and Duthie (1952) that an individual may have antibodies to one type of coagulase but not to another. Thus the incidence of neutralizing antibodies in human sera varies with the coagulase used for the test. Of the 29 individuals studied by us 26 had antibodies to coagulase of group I, 17 to that of group III and 15 to that of group II. Antibodies to coagulases from staphylococci of types 3A, 42E and 187 were less frequent. These results reflect the ubiquity of staphylococcal infection; and the high incidence of antibodies to group I coagulase parallels the frequency of small circumscribed infections, e.g. boils, due to staphylococci of this group.

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EXPLANATION OF PLATE

Fig. 1. Fibrinogen plate inoculated with cultures from 2 strains of *Staphylococcus aureus*, A and B. Growths on the left come from parents cultures and those on the right from 'fast-clotting' variants.

Fig. 2. A single strain (W120) of *Staphylococcus aureus* plated out on fibrinogen agar. Three types of colony will be seen: (a) large colonies with no halos, (b) large colonies with moderate halos, (c) small colonies with large halos (fast-clotting variants).

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