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Agglutination of *Staphylococcus aureus* by Rabbit Sera

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Of 137 *Staphylococcus aureus* strains, 87 agglutinated in normal rabbit serum. The agglutination was shown to be caused by the Fc-part of immunoglobulin G (IgG). $F(ab^1)_2$-fragments of IgG and immunoglobulin M (IgM) in corresponding concentrations were unreactive. The agglutinating strains had a high or moderate content of protein A. Strains with a low content of protein A and protein A-negative mutants did not agglutinate. The importance of the reaction between the Fc part of IgG and protein A for serotyping of *S. aureus* is demonstrated. Two alternative methods for serotyping *S. aureus* are suggested, using either $F(ab^1)_2$ fragments of IgG or intact IgM.

Agglutination of several *Staphylococcus aureus* strains by normal sera (man, rabbit, mouse) has been observed by many investigators (3, 4, 9, 16). Protein A, a cell wall component of *S. aureus* (6, 13, 15, 23, 34) which reacts with the Fc fragment of immunoglobulin G (IgG) from different species (8, 9, 18, 20), has been suggested to be identical with the agglutinogen (16, 17, 34). Other investigators have shown that protein A and the agglutinogen are not identical substances (3, 4, 11). It has been reported that the agglutinogen responsible for the agglutination of *S. aureus* in normal rabbit sera is not identical with protein A but with some other substance present in *S. aureus* and preparations of crude protein A (11, 12).

Because of the conflicting reports, based mainly on work with rabbit sera, the present investigation was undertaken to reexamine the role played by protein A in agglutination of *S. aureus*. As serum from rabbits is generally used for serotyping of *S. aureus*, a reaction between normal rabbit immunoglobulin and *S. aureus* could interfere with the serotyping reactions. If *S. aureus* agglutinates in normal rabbit sera due to a reaction between protein A and the Fc part of IgG, intact antibodies of the IgG type would seem unsuitable for serotyping of *S. aureus*.

The work described in this report demonstrates that *S. aureus*, but not protein A-deficient mutants of *S. aureus*, is agglutinated by normal rabbit IgG and by Fc fragments, but not by $F(ab^1)_2$ fragments derived from normal IgG or by immunoglobulin M (IgM). The importance of the agglutination of *S. aureus* in normal rabbit sera for serological typing has also been investigated, and two alternative methods for serotyping are suggested, using the $F(ab^1)_2$ fragments of IgG or Igm.

MATERIALS AND METHODS

Strains and cultivation technique. *S. aureus* type Cowan I (originally obtained from P. Oeding, the Oeding type collection of 15 *S. aureus* strains), 141 *S. aureus* strains (isolated in the routine laboratory), and *Escherichia coli* 0111 were used. All *S. aureus* strains were cultivated on nutrient agar and *E. coli* 0111 on dextrose agar.

Mutants and revertants. The mutants and revertants used in this paper were described in a previous report (7). *S. aureus* Cowan I was exposed to nitrosoguanidine or ethylmethanesulfonate, and survivors were screened on nutrient agar plates containing rabbit anti-protein A serum for loss of protein A production. More than half of all protein A-deficient mutants also lacked nuclease, coagulase, α-hemolysin, fibrinolysin, mannitol utilization, and the phage type pattern. Mutants with various combinations of these deficiencies were also isolated. Induced or spontaneous revertants of the mutants were obtained.

Sera. Pooled serum from 25 nonimmunized rabbits was used as normal rabbit serum. Anti-staphylococcus serum was obtained by weekly serial intravenous injections of Formalin-killed bacteria in rabbits by the method of Oeding (28). Antiserum to *E. coli* was obtained by weekly injections of living bacteria until a slide agglutination titer of 1:64 was recorded. Antiserum to protein A was produced by immunizing rabbits with highly purified protein A. Two subcutaneous injections of 0.25 mg of protein A in Freund's adjuvant were given 3 weeks apart. Blood was collected 2 weeks after the last injection (9).

Preparation of rabbit IgG. Ammonium sulfate precipitation followed by chromatography on a diethylaminoethyl (DEAE) cellulose column in

0.0175 M Na_2HPO_4 (*p*H 6.3) was used for purification of rabbit IgG (9). By applying a gradient from 0.0175 M sodium phosphate to 0.0175 M sodium phosphate plus 0.5 M sodium chloride (*p*H 6.3), two further major peaks could be eluted from the DEAE cellulose columns. By immunoelectrophoresis, the first peak was shown to contain IgG. IgG was purified from that fraction by chromatography on Sephadex G 200. A single line corresponding to IgG was found in immunoelectrophoresis when the material was tested against donkey anti-rabbit serum. Each IgG sample was concentrated in an ultrafiltration cell (Diaflo m 50, Amicon Corp.). The samples were divided into two portions. One was dialyzed against 0.15 M NaCl and adjusted to a protein concentration of 20 mg/ml. The protein concentration was determined by measuring the optical density at 280 nm by using a molar extinction coefficient of 13.4. The other portion was dialyzed against 0.1 M sodium acetate before pepsin digestion.

Preparation of rabbit IgM. IgM was prepared from normal rabbit serum and rabbit immune serum harvested 1 month after the start of immunization with *S. aureus* strain 17 A. The serum was centrifuged for 3 hr at 50,000 × *g* and 4 C to remove lipoproteins. Seven to eight milliliters of the serum was then chromatographed on a column of Sephadex G-200 (85 by 3.5 cm) which was equilibrated and eluted with 0.1 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, 1.0 M NaCl, *p*H 8.0 (5). The first peak, containing the IgM globulin, was dialyzed against 0.02 M Na_2HPO_4 (*p*H 8.0). It was then chromatographed on a DEAE cellulose column equilibrated with the dialysis buffer (5). After elution of a small amount of protein with the dialysis buffer, IgM-containing material was eluted with a gradient from 0.02 M Na_2HPO_4 (*p*H 8.0) to 0.3 M Na_2HPO_4 (pH 8.0). Fractions emerging near the end of the gradient and showing a single IgM band in immunoelectrophoresis were pooled and concentrated by ultrafiltration (Diaflo m 50, Amicon Corp.) to a concentration of 5 mg/ml. Analytical ultracentrifugation of this preparation showed that it consisted of more than 95% of 19*S* material (Fig. 1). In immunoelectrophoresis, it gave a single line against a donkey anti-rabbit plasma protein serum (Fig. 2).

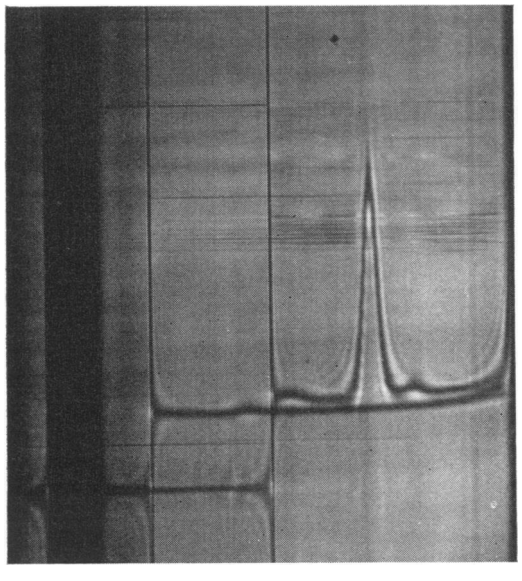

FIG. 1. *Schlieren pattern obtained in sedimentation velocity experiment on purified normal rabbit IgM in a synthetic boundary cell at a speed of 59,780 rev/min for 20 min after reaching full speed;* $s_{.01,w} = 18.6$.

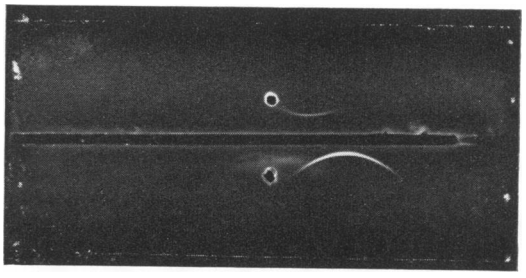

FIG. 2. *Immunoelectrophoresis at pH 8.6 of (a) top line, purified rabbit IgM; (b) lower line, facto, serum* h_1 *according to Oeding against donkey anti-rabbit serum. Cathode is to the left, anode to the right.*

Pepsin digestion. Pepsin digestion of IgG was performed as described by Nisonoff (27). Pepsin (Worthington Biochemical Corp.) was used in the following proportion: 1 mg of enzyme per 50 mg of IgG in 0.1 M sodium acetate (*p*H 4.5). As a control, anti-*E. coli* IgG was added before digestion with pepsin in a concentration corresponding to 2% of the IgG concentration of the sample.

When pepsin-digested IgG preparations were tested for their ability to agglutinate *E. coli* 0111, no significant decrease in titer was observed as compared with the nondigested material, indicating that the specific antibody-reactive sites were intact (1).

Papain digestion and separation of papain fragments. IgG globulin was digested by the method of Porter (30). One milligram of papain (Worthington Biochemical Corp.) per 100 mg of protein was added, and digestion was allowed to occur in 0.1 M sodium phosphate buffer (*p*H 7.0), 0.01 M cysteine, and 0.002 M ethylenediaminetetraacetic acid (EDTA). The digestion mixture was incubated at 37 C for 16 hr and then dialyzed against distilled water for 48 hr. After dialysis against 0.01 M sodium acetate (*p*H 5.5), the column was first eluted with 200 ml of starting buffer, after which a gradient from 0.1 M sodium acetate (*p*H 5.5) to 0.9 sodium acetate (*p*H 5.5) was applied. Three peaks were eluted, and peak III was shown to move more slowly than peaks I and II in immunoelectrophoresis.

Immunoelectrophoresis. To characterize IgG and IgM globulin and the $F(ab^1)_2$, Fab, and Fc-fragments of IgG globulin, immunoelectrophoresis (31) was

performed with anti-rabbit plasma protein from donkey (Behring Werke AG).

Heat treatment. Heat treatment of serum was performed at 60 C for 10 to 30 min to inactivate degradation products of fibrinogen (14, 22).

Agglutination. Agglutination was performed on slides by using 40 μliters of serum or immunoglobulin solution in appropriate dilutions. Smears were made with a platinum loop and the reaction was recorded as positive if definite agglutination occurred within 5 min. All experiments included controls for spontaneous agglutination performed in 0.15 M NaCl.

Adsorption. Adsorption experiments were performed as described by Oeding (28).

Determination of protein A. Protein A was extracted from *S. aureus* by the method of Jensen (16). The extract was diluted approximately 1:1 in 0.1 M Tris-hydrochloride (*p*H 8.0), so that 1 ml of the final solution of crude protein A corresponded to 5 ml of broth culture of *S. aureus* containing 10^9 colony-forming units/ml. The hemagglutination technique for quantitation of protein A (32) was applied to doubling dilutions of the material to be tested. Precipitation in gel was performed against anti-protein A serum in 1% agar (Special Agar Noble; Difco) in 0.15 M Tris-hydrochloride (*p*H 7.2) with protein A extracts concentrated 60 times by freeze drying. All strains producing protein A as determined by the hemagglutination technique were also shown to produce protein A as shown by immunodiffusion in gel.

RESULTS

Agglutination reactions with rabbit serum. *S. aureus* strains consecutively isolated from the routine laboratory were tested by slide agglutination. Four strains agglutinated spontaneously in 0.15 M NaCl. The 137 strains not agglutinating were tested against pooled serum from 25 non-immunized rabbits; 87 (63%) of the strains agglutinated in undiluted serum, and 76 (55%) were agglutinated by serum diluted 1 to 10. None of the strains agglutinated in serum diluted more than 1 to 40.

Isolation of the agglutinating factor in serum. By analyzing fractions obtained by gel filtration of pooled normal rabbit serum on Sephadex G-200 (10), all agglutinating activity could be shown to be located in the second peak, corresponding to IgG (Fig. 3). When pooled serum was precipitated with 22% ammonium sulfate, all agglutinating activity was found in the precipitate. By chromatography of the precipitated globulin fraction on a DEAE cellulose column, almost all agglutinating material could be eluted with the IgG fraction in 0.0175 M sodium phosphate buffer (*p*H 6.3). By applying a gradient from 0.0175 M sodium phosphate to 0.0175 M sodium phosphate plus 0.5 M sodium chloride, more agglutinating material could be eluted. Immunoelectrophoresis

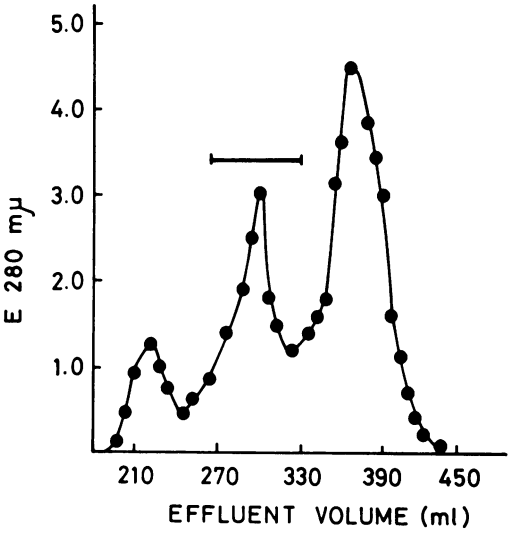

FIG. 3. *Chromatography on Sephadex G-200 of 6 ml of pooled normal rabbit serum. The column (95 × 2.5 cm) was equilibrated with 0.1 M Tris-hydrochloride, 1.0 M NaCl (pH 8.0). Fractions (6 ml) were collected at a flow rate of 18 ml/hr. Bar indicates fractions agglutinating S. aureus.*

after gel filtration of the agglutinating fractions on Sephadex G-200 showed that all agglutinating activity was confined to IgG. No agglutination was obtained with purified IgM. The agglutinin titer was not reduced by heat treatment at 60 C, which further excludes the possibility that degradation products of fibrinogen are responsible for agglutination of *S. aureus* in normal rabbit sera (14, 22).

Reactions with IgG from normal rabbits. All strains (87 of 137) which agglutinated in normal rabbit serum also gave a positive reaction with isolated IgG in a concentration of 10 mg/ml. Table 1 shows that IgG in a concentration of 1 mg/ml agglutinated 76 of 137 strains tested. The same strains were agglutinated by serum diluted 1 to 10. In Table 1, the strains are arranged in order according to their content of protein A as determined by hemagglutination. The strains were also agglutinated by Fc fragments of IgG. High percentages of the strains having high contents of protein A were agglutinated by IgG and Fc fragments, whereas lower percentages of the strains containing less protein A were agglutinated. Strains without detectable protein A or with cell-associated protein A giving a hemagglutination titer of 1 to 1 were not agglutinated. No reactions were obtained with IgM. When $F(ab^1)_2$ fragments of IgG were tested in a concentration of 6.6 mg/ml, a positive reaction was obtained with many of the strains. This was interpreted as indicating

the presence of some specific antibody activity. However, $F(ab^1)_2$ fragments in a concentration of 0.66 mg/ml did not agglutinate any of the *S. aureus* strains, indicating that the concentration of normally occurring specific antibodies was low. As a control, *E. coli* was shown to be agglutinated by $F(ab^1)_2$ fragments derived from anti-*E. coli* IgG in the same titer as for intact anti-*E. coli* IgG.

Agglutination of protein A mutants of S. aureus. Table 2 shows mutants of *S. aureus* obtained by exposing *S. aureus* Cowan I, to nitrosoguanidine or ethylmethanesulfonate and screening survivors for loss of protein A production.

A spectrum of mutants with different properties was obtained. For mutants lacking protein A, no agglutination reaction was recorded. However, mutants with a leaky production of protein A were agglutinated, as were protein A-producing revertants of protein A-negative mutants.

Agglutination with IgG from immunized rabbits. Figure 4 shows agglutination reactions obtained with IgG from rabbits immunized with *S. aureus* strain 17 A (Oeding). The *S. aureus* strains tested are type strains used for serotyping of *S. aureus*. As shown in the figure, $F(ab^1)_2$ fragments of IgG gave a lower agglutination titer than did intact IgG (two to four titration steps) with strain 17 A, used for immunization, and strains 670, Cowan I, and 1503. All of these strains are stated to have antigenic similarities, with antigens a5 and h1 in common, according to Oeding (29). Strain 5687 is also known to be antigenically related to these strains. However, with this strain, which has a low content of protein A, the same agglutination titer was recorded for $F(ab^1)_2$ and intact IgG. With strains 2235 and 1015, which lack antigenic similarities to strain 17 A, intact IgG gave agglutination reactions at titers 1 to 40 and 1 to 20, but $F(ab^1)_2$ gave no detectable reaction. For anti-*E. coli* IgG, which was mixed with anti-*S. aureus* 17 A IgG as a control, the same titer was obtained for IgG and $F(ab^1)_2$ when tested in slide agglutination against *E. coli*.

Agglutination obtained with IgM from immunized rabbits. IgM was prepared from anti-*S.*

TABLE 1. *Agglutination of 137 Staphylococcus aureus strains in normal rabbit immunoglobulin G (1 mg/ml) and $F(ab^1)_2$ fragments (0.7 mg/ml) of the globulin*

Protein A content	No. of strains tested	Strains agglutinating in 1 mg of IgG per ml[a]	Strains agglutinating in 0.3 mg of Fc fragment per ml	Strains agglutinating in 0.7 mg of $F(ab^1)_2$ fragment per ml	Strains agglutinating in 1.0 mg of IgM per ml
0–1[b]	30	0 (0)	0	0	0
2	12	5 (46)	3	0	0
4	16	9 (56)	8	0	0
8	25	18 (72)	16	0	0
16	28	23 (81)	16	0	0
32	22	17 (77)	15	0	0
64	4	4 (100)	4	0	0

[a] Values in parentheses express per cent of strains agglutinating.

[b] Reciprocal hemagglutination titer of extracted protein A in a broth culture with 10^9 colony-forming units per ml.

TABLE 2. *Agglutination of Staphylococcus aureus Cowan I and various derived mutants and revertants in normal rabbit IgG and $F(ab^1)_2$ fragments of the globulin in concentrations of 1 mg/ml and 0.7 mg/ml, respectively*

Determination	Agglutination with		Production of					Mannitol utilized	Phage type
	IgG	$F(ab^1)_2$	Protein A[a]	Nuclease	Coagulase	α-Hemolysin	Fibrinolysin		
Strains									
1 (Cowan I)	+	−	64	+	+	+	+	+	52/52A/80/81
Mutants									
12	+	−	1–4	+	+	+	+	+	52/52A/80/81
1	+	−	2	+	−	−	+	+	52/52A/80/81
5	−	−	−	+	+	+	+	+	NT[b]
11[c]	−	−	−	±	±	±	−	±	NT
26[c]	−	−	−	−	−	−	−	−	NT
Revertants									
3	+	−	4–32	+	+	+	+	+	52/52A/80/81

[a] Reciprocal hemagglutination titer of extracted protein A in a broth culture with 10^9 colony-forming units per ml.

[b] NT, not typable.

[c] Two spontaneously agglutinable mutants excluded.

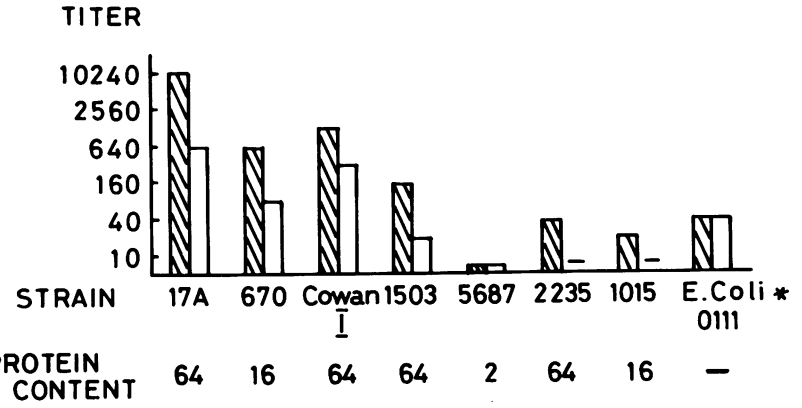

FIG. 4. *Agglutination of S. aureus strains in 10 mg of rabbit anti-S. aureus strain 17 A IgG per ml (shadowed columns) and in 6.6 mg of F(ab')$_2$-fragments of the globulin per ml. Protein A content of the strains is indicated in the figure. Bar (—) indicates no agglutination recorded; * indicates immunoglobulin preparations including 2% anti-Escherichia coli 0111 IgG.*

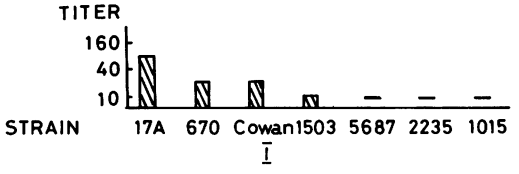

FIG. 5. *Agglutination of S. aureus strains in rabbit anti-S. aureus strain 17 A IgM (1 mg/ml). Bar (—) indicates no reaction recorded.*

aureus 17 A serum, the same serum used for isolation of specific IgG. Figure 5 shows that IgM agglutinated bacteria of strain 17 A used for immunization, and also strains 670, Cowan I, and 1503, which have antigens in common with 17 A. Strains 2235 and 1503, which are antigenically dissimilar, were not agglutinated. Strain 5687, which gave a weak reaction with F(ab')$_2$ from IgG was not agglutinated by IgM in a concentration of 1 mg/ml. At higher concentrations of IgM, a weak reaction was recorded.

Adsorption of factor sera by Oeding method. Rabbit anti-*S. aureus* 17 A serum was adsorbed by the Oeding scheme for production of specific factor sera a5 and h1. Figure 2 shows the results obtained when the anti-*S. aureus* 17 A serum had been adsorbed with strains 670 and 1503 to produce factor serum h1. No IgG line was any longer detectable in immunoelectrophoresis. The adsorbed serum contained factor h1 antibodies, probably of IgM class. The same result was obtained when the anti-*S. aureus* 17 A serum was absorbed with strains 1503 and 640 to produce specific factor serum a5. The results indicate that the current methods for serotyping *S. aureus* are based on reactions with IgM.

DISCUSSION

Protein A (antigen A) was originally described by Jensen (16) as a common antigen in *S. aureus* strains. He found antibodies to this antigen to be widespread and concluded that these antibodies to protein A are responsible for the agglutination of most *S. aureus* strains by normal sera. The role of protein A as an "agglutinogen" was demonstrated by adsorbing normal sera with different *S. aureus* strains. The adsorption reduced the agglutinin titers of normal sera to very low values. Soluble protein A added to normal human sera was also shown to inhibit the agglutination of *S. aureus*. Similar results were obtained by Yoshida et al. (34) who demonstrated that their purified protein A removed 94% of the agglutinins in normal human sera, Löfqvist (23), using specific rabbit antiserum to protein A, was able to remove the agglutinins of *S. aureus* in serum by adding protein A. Similar results were obtained by Hofstad (15).

From the adsorption experiments with protein A described above (15, 16, 23, 34), it is impossible to conclude that protein A is the substance responsible for agglutination by normal rabbit sera. It is now well known that protein A reacts with the Fc part of mammalian IgG (8.9, 18). It has been shown that adsorption with *S. aureus* can remove more than 90% of the IgG in normal rabbit serum (21). In the experiments performed in this study, no IgG could be detected by immunoelectrophoresis of rabbit sera after adsorption with *S. aureus*. From these experiments, it is obvious that specific agglutinins to *S. aureus*, if present, can be removed by protein A.

Contrary to the findings of Jensen, Yoshida et

al., Hofstad, and Löfqvist, Cohen et al. (3, 4) using sera from nonimmunized rabbits and mice concluded from adsorption experiments that the agglutinogen is not protein A. In further studies, the agglutinogen was considered to be a complex (2). Cohen et al. based their objection on the finding of agglutinins against *S. aureus* in normal rabbit serum, which in agar-gel experiments did not precipitate protein A. Neither could Grov et al. (11, 12) show that the agglutinogen is identical to protein A. They conclude that the agglutinogen is identical to some other substance in a preparation of crude protein A. Grov et al. (12) further report that agglutination of staphylococci by normal sera from man, hog, horse, and sheep is confined to the Fab part of IgG. Agglutination occurred in normal rabbit serum but not in IgG derived from it.

The report of Grov et al. (12) that normal rabbit serum agglutinates *S. aureus* Cowan I in a titer of 1:20 is in accordance with our results. However, their finding that normal rabbit IgG does not agglutinate *S. aureus* Cowan I is in sharp contrast with our experience. Our IgG preparations give a clear-cut and strong agglutination with 87 of 137 *S. aureus* strains, among them Cowan I. The difference is difficult to explain.

In this study it was not possible to show any agglutination of *S. aureus* by any component in serum other than IgG. Agglutination could be demonstrated with low concentrations of Fc fragments derived from normal rabbit IgG but not with $F(ab^1)_2$ fragments at similar concentrations. High concentrations of $F(ab^1)_2$ fragments caused weak agglutination of many of the *S. aureus* strains tested. The presence in low concentration in normal sera of antibodies to teichoic acid has been reported (24, 25). The present results show that the Fc part of the IgG molecule is mainly responsible for the agglutination of *S. aureus* by normal rabbit sera and that specific antibody activity in the $F(ab^1)_2$ part of the IgG has a weak agglutinating effect in high concentrations. Strong evidence that agglutination of *S. aureus* is due to a reaction with protein A is provided by the demonstration in this study that protein A-deficient mutants of *S. aureus* are not agglutinated by normal rabbit IgG, whereas protein A-producing revertants of such mutants are agglutinated.

The results of our experiments performed with normal rabbit serum are in accordance with those of Kronvall (17), who was able to demonstrate agglutination of *S. aureus* by human myeloma IgG. As rabbit antisera are used in current methods for typing *S. aureus* by means of agglutination reactions, interference by protein A with the specific antigen-antibody reaction must be of importance for serotyping. Difficulties with the reproducibility of serotyping of *S. aureus* are well known (33). A reaction between protein A and the Fc part of antibodies used for serotyping can obviously contribute to these difficulties.

Our study shows that IgG from rabbits immunized with *S. aureus* strains reacts with the strains used for immunization and with antigenically similar strains and, in addition, with strains containing a significant amount of protein A. The $F(ab^1)_2$ fragments obtained by pepsin digestion of the IgG molecules do not react with protein A but give a specific agglutination reaction with *S. aureus* strains containing antigens in common with the strain used for immunization. We have further shown that careful adsorption with *S. aureus* to produce specific factor sera removes IgG but that the adsorbed serum still agglutinates the *S. aureus* strain used for immunization and strains with common antigens, IgM was shown to be responsible for this agglutination. It is therefore suggested that $F(ab^1)_2$ fragments of IgG or intact IgM be used for specific serotyping of *S. aureus*.

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LITERATURE CITED

1. Cerottini, J.-C., and F. Fitch. 1968. Complement-fixing ability and antibody activity of rabbit $F(ab^1)_2$ antibody. Int. Arch. Allergy **34**:188–200.
2. Cohen, J. O. 1965. Studies of group agglutinogens of *Staphylococcus aureus*. Nature (London) **205**:917–918.
3. Cohen, J. O., G. S. Cowart, and W. B. Cherry. 1961. Antibodies against *Staphylococcus aureus* in nonimmunized rabbits. J. Bacteriol. **82**:110–114.
4. Cohen, J. O., W. L. Newton, W. B. Cherry, and E. L. Updyke. 1963. Normally occurring staphylococcal antibodies in germfree mice. J. Immunol. **90**:358–367.
5. Forsgren, A. 1968. Protein A from *Staphylococcus aureus*. V. Reaction with guinea pig γ-globulins. J. Immunol. **100**:921–926.
6. Forsgren, A., and U. Forsum. 1970. Role of protein A in nonspecific immunofluorescence of *Staphylococcus aureus*. Infect. Immunity **2**:387–391.
7. Forsgren, A., K. Nordström, L. Philipson, and J. Sjöquist. 1971. Protein A mutants of *Staphylococcus aureus*. J. Bacteriol. **107**:245–250.
8. Forsgren, A., and J. Sjöquist. 1966. "Protein A" from *Staphylococcus aureus*. I. Pseudo-immune reaction with human γ-globulin. J. Immunol. **97**:822–827.
9. Forsgren, A., and J. Sjöquist. 1967. "Protein A" from *Staphylococcus aureus*. III. Reaction with rabbit γ-globulin. J. Immunol. **99**:19–24.
10. Flodin, P., and J. Killander. 1962. Fractionation of human serum proteins by gel filtration. Biochim. Biophys. Acta **63**:403–410.
11. Grov, A. 1968. Studies on antigen preparations from Staphylococcus aureus. 5. Inhibition of bacterial agglutination by protein A preparations. Acta Pathol. Microbiol. Scand. **73**: 407–412.

12. Grov, A., P. Oeding, B. Myklestad, and J. Aasen. 1970. Reactions of staphylococcal antigens with normal sera, γG-globulins, and γG-globulin fragments of various species origin. Acta Pathol. Microbiol. Scand. Sec. B 78:106–111.
13. Grov, A., and S. Rude. 1967. Immunochemical examination of phenylhydrazine treated Staphylococcus aureus cell walls. Acta Pathol. Microbiol. Scand. 71:417–421.
14. Hawiger, I., S. Niewiarowski, V. Gurewich, and D. P. Thomas. 1970. Measurement of fibrinogen and fibrin degradation products in serum by staphylococcal clumping test. J. Lab. Clin. Med. 75:93–108.
15. Hofstad, T. 1965. Studies on the antigenic structure of the 80/81 complex of Staphylococcus aureus. 7. Preparation and properties of cell walls from strain 263. Acta Pathol. Microbiol. Scand. 65:151–160.
16. Jensen, K. 1959. Undersøgelser over staphylococcernes antigenstruktur. Munksgaard, Copenhagen.
17. Kronvall, G. 1970. Protein A—an "incomplete" staphylococcal agglutinogen. Acta Pathol. Microbiol. Scand. Sec. B 78:127–128.
18. Kronvall, G., U. S. Seal, J. Finstad, and R. C. Williams, Jr. 1970. Phylogenetic insight into evolution of mammalian Fc fragment of γG-globulin using staphylococcal protein A. J. Immunol. 104:140–147.
19. Lenhart, N. A., S. Mudd, A. Yoshida, and I. W. Li. 1963. The common protein agglutinogen of *Staphylococcus aureus*. I. Distribution in international serotypes and corresponding antibody in human populations. J. Immunol. 91:771–776.
20. Lind, I., I. Live, and B. Mansa. 1970. Variation in staphylococcal protein A reactivity with γG-globulins of different species. Acta Pathol. Microbiol. Scand. Sec. B 78:673–682.
21. Lind, I., and B. Mansa. 1968. Further investigation of specific and nonspecific adsorbtion of serum globulin to Staphylococcus aureus. Acta Pathol. Microbiol. Scand. 73:637–645.
22. Lipinski, B., I. Hawiger, and I. Jeljaszewicz. 1967. Staphylococcal clumping with soluble fibrin monomer complexes. J. Exp. Med. 126:979–988.
23. Löfkvist, T. 1966. Further studies of a preparation of protein A (Jensen's antigen A) from *Staphylococcus aureus*. Immunization of rabbits—serological experiments including bacterial agglutination, quantitative precipitation and indirect haemagglutination, Int. Arch. Allergy 29:240–253.
24. Löfqvist, T. 1969. Staphylococcal precipitins in normal and postinfectious human sera. Acta Pathol. Microbiol. Scand. 75:323–330.
25. Martin, R. R., H. Daugharty, and A. White. 1966. Staphylococcal antibodies and hypersensitivity to techoic acids in man. Antimicrob. Ag. Chemother. 1965, p. 91–96.
26. Mudd, S. 1970. Infectious agents and host reactions. W. B. Saunders Co., Philadelphia.
27. Nisonoff, A. 1964. X. Enzymatic digestion of rabbit gamma globulin and antibody and chromatography of digestion products. Meth. Med. Res. 10:134–141.
28. Oeding, P. 1957. Agglutiniability of pyogenic staphylococci at various conditions. Acta Pathol. Microbiol. Scand. 41:310–324.
29. Oeding, P. 1966. Antigenic structure of staphylococci. Postepy Mikrobiol. 5:221–228.
30. Porter, R. R. 1959. The hydrolysis of rabbit γ-globulin and antibodies with crystalline papain. Biochem. J. 73:119–126.
31. Scheidegger, I. 1955. Une microméthode de l'immunoelectrophorèsis. Int. Arch. Allergy 7:103–110.
32. Sjöquist, J., and G. Stålenheim. 1969. Protein A from *Staphylcoccus aureus*. IX. Complement-fixing activity of protein A-IgG complexes. J. Immunol. 103:467–473.
33. Cohen, J. O. 1969. Serology of the Micrococcaceae. *In* J. B. G. Kwapinsk (ed.) Analytical serology of microorganisms. Interscience Publishers, Inc., New York.
34. Yoshida, A., S. Mudd, and N. A. Lenhart. 1963. The common protein agglutinogen of *Staphylococcus aureus*. II. Purification, chemical characterization, and serologic comparison with Jensen's antigen. J. Immunol. 91:777–782.