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Evidence for a Multiplicity of Capsular Types Among *Staphylococcus aureus* Strains

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The Smith diffuse variant and the wound mucoid strain of *Staphylococcus aureus* were shown to exhibit serologically distinct capsules. The Welwood and K-6 strains of *S. aureus* were tested to determine their capsular types. Both Welwood and K-6 were found to be representative of the Smith capsular type. An additional 13 isolates of *S. aureus* from mice were tested. Gel double-diffusion tests and immunoelectrophoresis of staphylococcal antigens disclosed the possible existence of at least two additional capsular types. Passive hemagglutination tests carried out with cells sensitized with 1 mg of antigen per ml showed a multiplicity of cross-reacting antigens. However, cells sensitized either with 0.1 or 0.05 mg of antigen per ml and reacted with antisera absorbed with 10 or 1 μg/ml showed the presence of a specific antigen in each strain of *S. aureus*. Corroborative evidence for a multiplicity of capsular types was obtained by the specific capsular reaction. At least four capsular types of *S. aureus* were found. The prototypic strains for these antigens are the RLM or wound strain, the Smith diffuse strain, and mouse strains designated 36T and 43R. We propose to designate these types 1, 2, 3, and 4, respectively.

The paradoxical behavior of two encapsulated variants of *Staphylococcus aureus* in mice led to experiments designed to test the hypothesis that laboratory mice were susceptible to one strain and resistant to the other owing to carriage by these mice of one of the strains (19). It was proposed that the observed resistance was the result of elaboration of anticapsular antibodies reactive with the strain to which the mice were resistant. Subsequent experiments (19) revealed the frequent occurrence of anticapsular antibodies in mice reactive with only one of the types of *S. aureus*. Efforts to detect anticapsular antibodies against the Smith diffuse strain of *S. aureus* were unsuccessful, whereas 55% of the animals possessed anticapsular antibodies reactive with the wound mucoid strain of *S. aureus*. These same experiments resulted in isolation of a total of 13 mucoid strains from the throat and rectum of laboratory mice. All strains were coagulase-positive, fermented mannitol anaerobically, and gave some indication of being encapsulated in wet mounts in India ink. Specific capsular reactions carried out with young cultures of these strains disclosed the possible existence of additional capsular types. The present report presents evidence for this conclusion.

MATERIALS AND METHODS

Strains. *S. aureus* strain W (the wound mucoid strain) and strain Sd (the Smith diffuse strain) were described in a previous paper (19). Welwood and K-6 strains were received from R. Tompsett, Baylor University Medical Center, Dallas, Texas. The mouse strains, 36T, 43R, 47R, 50R, and 51R, were isolated on Mannitol Salt Agar plates (Difco) inoculated from throat and rectal swabs taken on DAL Swiss Webster mice. Single colonies were tested for mucosity after 48 hr of incubation at 37 C.

Media and cultivation. Mannitol Salt Agar plates were used for primary isolation of *S. aureus*. For cloning and transferring cultures, buffered nutrient glycerol (BNG) or buffered nutrient dextrose (BND) was used (18). When fermentation tests revealed that a given strain of *S. aureus* did not vigorously ferment glycerol, BND was used. BNG or BND was used in the coagulase test, for specific capsular reactions, and for cultivation of organisms used in the extraction procedures for preparation of capsular antigens. The semisynthetic Casamino Acids glycerol or dextrose broth was described in detail previously (18). Incubation was carried out at 37 C.

Coagulase tests. Tube coagulase tests were done as described in an earlier paper (19).

Specific capsular reactions. The specific capsular reaction was carried out as previously described (19).

Acid extraction of antigens. The procedure described by Morse (14) was slightly modified. Extracts

were centrifuged prior to neutralization at 13,000 × *g* for 1 hr in a Sorvall centrifuge (model SS-4) with a GSA rotor and again at 13,000 × *g* for 1 hr for clarification after neutralization. Five volumes of acetone were then added to each supernatant fluid to precipitate the antigens, and the mixtures were stirred and refrigerated overnight at 4 C. The following day, the supernatant fluids were decanted and the precipitate was dissolved in a minimal volume of distilled water. The resulting solutions were dialyzed against distilled water in the cold at 4 C for 3 days. The water was changed twice daily. The dialyzed material was lyophilized and stored over Drierite in a dessicator until needed.

Preparation of crude capsular material. The method for isolation of capsular antigens from culture supernatant fluids was previously described (20); it was based on the technique of Heidelberger et al. (7). However, the deproteinization step with chloroform and *n*-butyl alcohol was omitted.

Vaccines and immunization of animals. The vaccines were prepared as previously described (19) and immunization of animals was carried out as previously described (19).

Phosphate-buffered saline (PBS). The composition of the PBS used throughout the work was as follows: anhydrous Na_2HPO_4, 1.58 g, anhydrous KH_2PO_4, 0.346 g; NaCl, 8.0 g; and water to make 1 liter. The *p*H was adjusted to *p*H 7.2 to 7.3.

Absorption of antisera. Antisera and antigen solutions were mixed in equal volumes, incubated at 37 C for 2 hr, and refrigerated overnight. The mixture was centrifuged for 1 hr at 1,085 × *g* by use of a Sorvall centrifuge with a type A rotor. The antigen solutions were prepared in PBS in concentrations of 10 μg/ml and 1 μg/ml.

Gel diffusion tests. The technique of Ouchterlony described in Kabat and Mayer (10) was followed. Unwashed agar (Difco) was used in a concentration of 1% and made up according to the directions in Kabat and Mayer (10). Merthiolate in a final concentration of 1:10,000 was added as a preservative. Plastic petri dishes (90 mm in diameter) were filled with 15 ml of molten agar per dish. After the agar had hardened, the wells were cut with a Feinberg agar-gel cutter (Colab Laboratories, Inc., Chicago Heights, Ill.) having a central well (10 mm in diameter) and six peripheral wells (7 mm in diameter). To the peripheral wells, a 1% antigen solution in PBS (*p*H 7.3) was added; to the center well, 0.2 ml of antiserum was added. The wells were filled only once.

Immunoelectrophoresis. Colab gel contact troughs (no. 2576) were connected to a Vokam 2541 power supply (Colab Laboratories). A barbital buffer, ionicity 0.1, *p*H 8.5, was used (LKB, Stockholm 12, Sweden). A slide frame (LKB, no. 6801A) containing six glass slides (LKB, no. 6890-03) was filled with Noble agar (Difco). The proportions of agar-buffer-water in the solution were 1:25:75. The slide frame was placed on the gel contact troughs at an angle. A constant current of 1.2 ma per slide was applied across the terminals. Electrophoresis was carried out for 1 hr, after which troughs were cut in the agar and rabbit antiserum was added. The slides were then placed in a moist chamber for up to 72 hr at room temperature to allow development of the precipitin arcs.

Indirect hemagglutination test. Rabbit red blood cells were obtained from the central ear artery of the rabbit and mixed with Alsever solution (2). Samples of the rabbit blood were washed three times in cold PBS (*p*H 7.3). The cells were packed by centrifugation at 755 × *g* for 30 min in a type A Sorvall centrifuge with a type A rotor and diluted to make a 5% suspension. The partially purified antigen extracts prepared by acid extraction of whole cells or by concentration from the culture supernatant fluids were diluted 1:1,000, 1:5,000, 1:10,000, and 1:20,000 in PBS (*p*H 7.3). Equal volumes of 5% cells and dilutions of extracts or crude capsular antigen were mixed. The mixture was incubated in a water bath for 2 hr at 37 C. The test tubes were gently inverted every 15 min to resuspend the settled erythrocytes. At the end of 2 hr, the cells were centrifuged and washed three times with cold PBS (*p*H 7.3). The antiserum was diluted 1:4 through 1:512 in twofold dilutions; 0.12 ml of each dilution of antiserum was then added to each test tube, followed by 0.5 ml of saline and 0.1 ml of sensitized cells. The final dilutions were considered to be sixfold greater than the initial dilutions, owing to the additional dilution by saline and sensitized cells. The tubes were incubated at 37 C for 2 hr, and the tubes were shaken every 30 min during incubation. The following controls were used: (i) 0.62 ml of PBS (*p*H 7.3) plus 0.1 ml of sensitized cells; (ii) 0.62 ml of PBS (*p*H 7.3) and 0.1 ml of unsensitized cells; (iii) 0.12 ml of antiserum 1:4, 0.5 ml of PBS (*p*H 7.3), and 0.1 ml of unsensitized cells. After incubation at 37 C, the tests were refrigerated overnight at 4 to 6 C before being read.

Determination of mouse mortality ratios. Estimations of virulence of staphylococcal isolates were made by the method previously described (19).

RESULTS

It was decided to first examine two strains of *S. aureus* well known to workers in the staphylococcal field to ascertain whether they are encapsulated and to which capsular type they belong. These strains, designated Welwood and K-6, were received from R. Tompsett, Baylor University Medical Center, Dallas, Texas, and were known to be mouse-virulent. A high-titered rooster antiserum against *S. aureus* Sd and a rabbit antiserum against *S. aureus* W were used in the specific capsular reactions carried out with these strains. Table 1 shows the results of the specific capsular reactions. Both K-6 and Welwood proved to be representative of *S. aureus* Sd and exhibited mortality ratios in white mice similar to that of the diffuse variant of the Smith strain.

It was postulated that mice might owe their

susceptibility to Sd staphylococci to lack of contact with the organism or absence of protective antibodies; a survey of mice for carriage of *S. aureus* was undertaken. One hundred mice were surveyed to assess their carriage of *S. aureus*. Particular attention was focused on possibly encapsulated strains occurring naturally in mice. Both throat and rectal swabs were taken. All swabs were streaked directly on Mannitol Salt Agar. Mannitol fermenting colonies were picked off and examined directly in wet mounts of India ink. Mucosity of the colonies was also noted at the time of picking. All isolates were tested for free coagulase and clumping factor. None of the isolates reacted with the antiserum against the Sd variant strain of *S. aureus* in the specific capsular reaction. Since we were interested in discovering possibly different capsular types, we selected strains of greatest mucosity showing evidence of a halo in India ink mounts and negative-specific capsular reactions with anti-W and anti-Sd sera to be used for immunization of rabbits. Formalinized vaccines were used to immunize the animals. Antigens for immunoelectrophoresis were prepared by the technique described in Materials and Methods.

TABLE 1. *Specific capsular reactions of four strains of S. aureus*

Strain	Antiserum prepared against:[a]	
	Sd	W
Sd	+	−
K-6	+	−
Welwood	+	−
W	−	+

[a] Positive reaction (+), negative reaction (−).

The extracts of supernatant fluids were made up to a concentration of 1% in PBS for use in immunoelectrophoresis. Two antisera were quite specific and did not react with extracts or crude capsular antigen prepared from heterologous strains. These were the antisera prepared against the Sd and the 43R strains. The latter strain was isolated from the rectum of a mouse. The immunoelectrophoretic patterns of the extracts prepared from the Sd and 43R strains, when reacted with their homologous antisera, are shown in Fig. 1. Similar patterns were exhibited by the crude capsular antigens. The extract of Sd gave a single precipitin arc with homologous antiserum, whereas the extracts from the W, 36T, or 43R strains did not react with Sd antiserum (Fig. 1, a and b). The extract from the 43R strain gave two precipitin arcs with homologous antiserum, whereas the extracts from Sd, W, and 36T did not react with 43R antiserum (Fig. 1, c and d). Immunoelectrophoresis indicated that extracts or crude capsular antigen from the Sd and 43R strains were serologically distinct from each other and any of the other strains.

The situation with the other strains was more complicated. The original strain with which so much of our work has been done is designated the W strain. Another strain included here and designated the 36T strain originated in the throat of a mouse. Figure 2 shows the results of im-

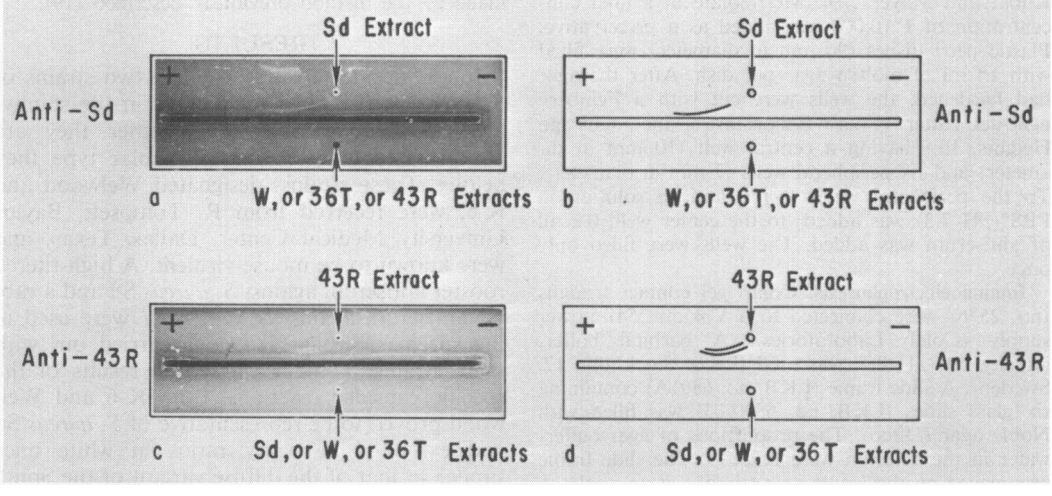

FIG. 1. *Immunoelectrophoresis of acid extracts of encapsulated staphylococcal strains. Development of precipitin arcs was allowed to proceed for 72 hr at room temperature, after which the photographs (a and c) were made; (b and d) diagrammatic representations of the reactions, shown for purposes of clarity.*

munoelectrophoresis of extracts of these strains when reacted with their homologous antisera. Results with the crude capsular material were similar; hence, only the results with the extracts will be presented here. The extract of the W strain produced three precipitin arcs when reacted with homologous antiserum. Neither 36T nor 43R extracts exhibited any reactivity with W antiserum (Fig. 2, a and b). The extract prepared from the 36T strain yielded two precipitin arcs when reacted with homologous antiserum. The extracts of the Sd and W strains did not react with 36T antiserum (Fig. 2, c and d). Figure 3 (a and b) depicts the three precipitin arcs exhibited by W extract when reacted with homologous antiserum. The extract of the Sd strain cross-reacted with W antiserum to produce a weak precipitin arc. The extract of 43R yielded a weak precipitin arc when reacted with 36T antiserum (Fig. 3, c and d). It was considered possible that the cross-reaction between the Sd extract and W antiserum and that between 43R extract and 36T antiserum could have resulted from the presence prior to immunization of

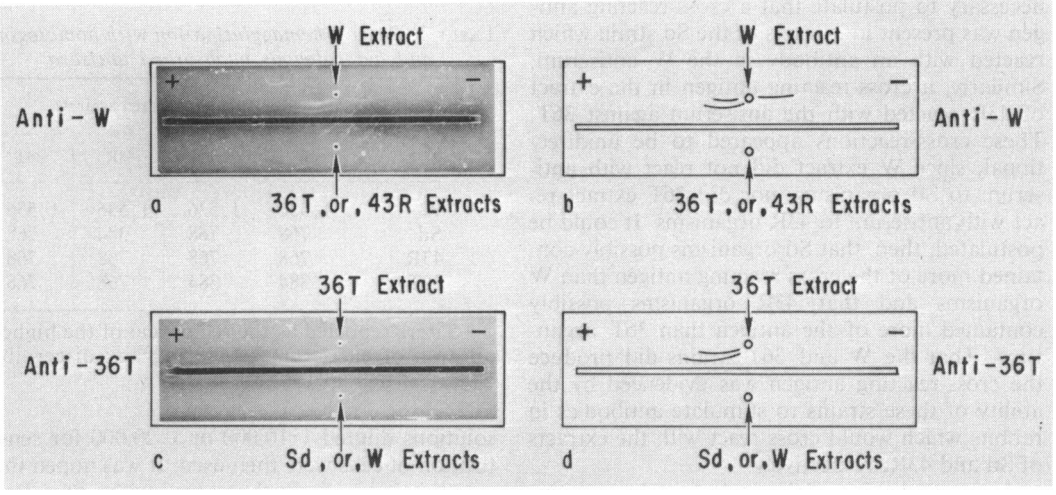

FIG. 2. *Immunoelectrophoresis of acid extracts of encapsulated staphylococcal strains. Development of precipitin arcs was allowed to proceed for 72 hr at room temperature, after which the photographs (a and c) were made; (b and d) diagrammatic representations of the reactions.*

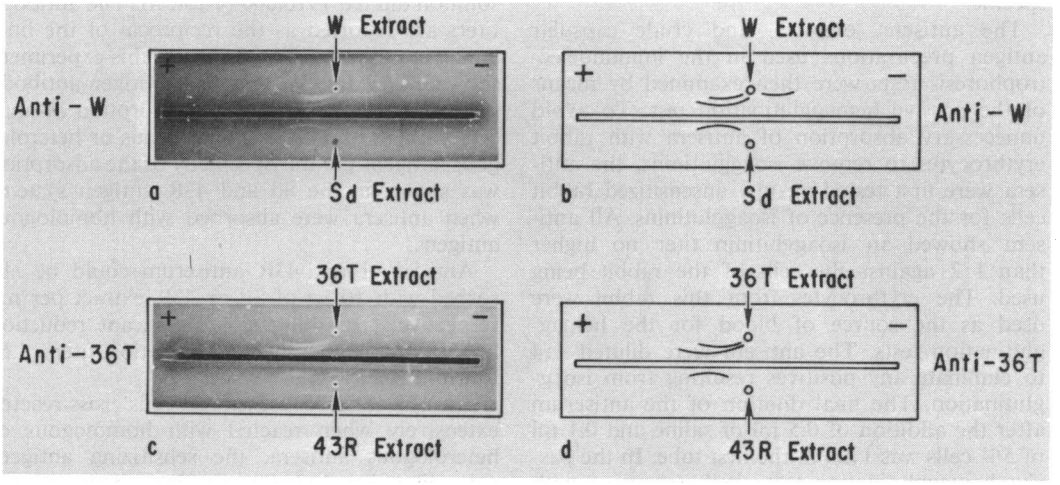

FIG. 3. *Immunoelectrophoresis of acid extracts of encapsulated staphylococcal strains. Development of precipitin arcs was allowed to proceed for 72 hr at room temperature, after which the photographs (a and c) were made; (b and d) diagrammatic representations of the reactions.*

natural antibodies against antigens in the extracts. Such a possibility was not remote, since previous work in our laboratory had disclosed the frequent presence of natural antibodies to staphylococci in normal rabbits (18). The results of immunoelectrophoretic tests carried out with the preimmunization serum from the rabbits used to prepare the W antiserum and 36T antiserum, respectively, were negative; i.e., 43R extract and Sd extract failed to react with the preimmunization serum, thus invalidating any hypothesis explaining cross-reactivity as being due to natural cross-reacting antibodies. It was necessary to postulate that a cross-reacting antigen was present in extracts of the Sd strain which reacted with an antibody in the W antiserum. Similarly, a cross-reacting antigen in the extract of 43R reacted with the antiserum against 36T. These cross-reactions appeared to be unidirectional, since W extract did not react with antiserum to Sd organisms, nor did 36T extract react with antiserum to 43R organisms. It could be postulated, then, that Sd organisms possibly contained more of the cross-reacting antigen than W organisms and that 43R organisms possibly contained more of the antigen than 36T organisms. That the W and 36T strains did produce the cross-reacting antigen was evidenced by the ability of these strains to stimulate antibodies in rabbits which would cross-react with the extracts of Sd and 43R, respectively.

A summarization of the various cross-reactions just described is shown in Table 2. It is apparent that, except for the two cross reactions described, the extracts and antiserum for each strain were specific.

The antisera, extracts, and crude capsular antigen preparations used in the immunoelectrophoresis tests were then examined by means of the passive hemagglutination test. To avoid unnecessary absorption of antisera with rabbit erythrocytes to remove isoagglutinins, the antisera were first tested against unsensitized rabbit cells for the presence of isoagglutinins. All antisera showed an isoagglutinin titer no higher than 1:2 against the cells of the rabbit being used. The erythrocytes from this rabbit were used as the source of blood for the hemagglutination tests. The antisera were diluted 1:4 to eliminate any positives resulting from isoagglutination. The final dilution of the antiserum after the addition of 0.5 ml of saline and 0.1 ml of 5% cells was 1:24 in the first tube. In the passive hemagglutination tests, cells sensitized with antigen 1:1,000 or 1:5,000 showed marked cross-reactivity when reacted with unabsorbed or absorbed antisera. All sera agglutinated these sensitized erythrocytes equally (Table 3). Antigen solutions diluted 1:10,000 or 1:20,000 for sensitization of cells were then used. It was hoped that sensitization of erythrocytes with the least amount of antigen required would eliminate binding to the cells of cross-reacting antigens. Rabbit erythrocytes were sensitized with antigen solution diluted 1:10,000 (Table 4). The antibody titers are reported as the reciprocal of the final antiserum dilution. The results of this experiment showed that the W and 36T antigen-antibody systems were not affected by adsorption of antisera with 10 or 1 μg of homologous or heterologous antigens per ml. Specificity of the adsorption was shown in the Sd and 43R antigen systems when antisera were absorbed with homologous antigens.

Anti-Sd or anti-43R antiserum could be absorbed with 10 μg of Sd or 43R extract per ml, respectively, resulting in a significant reduction in the homologous hemagglutination titers, i.e., fourfold reduction.

Since W- and 36T-sensitized cells cross-reacted extensively when reacted with homologous or heterologous antisera, the sensitizing antigens were diluted to 1:20,000. Table 5 shows hemagglutination titers before and after absorption when cells were sensitized with antigen diluted to 1:20,000. Both W and 36T antigen-antibody systems showed at least fourfold reductions in

TABLE 2. *Summarization of immunoelectrophoresis reactions*

Antigen extracted from	Antiserum prepared against:			
	W	Sd	36T	43R
W	3[a]	—[b]	—	—
Sd	1	1	—	—
36T	—	—	2	—
43R	—	—	1	2

[a] Indicates the numbers of precipitin arcs.
[b] Indicates the absence of precipitin arcs.

TABLE 3. *Passive hemagglutination with homologous and heterologous unabsorbed antisera*

Cells sensitized with antigen 1:1000 prepared from:	Antiserum prepared against:			
	W	Sd	43R	36T
W	1,536[a]	1,536	1,536	1,536
Sd	768	768	384	768
43R	768	768	768	768
36T	384	384	768	768

[a] Titers reported as the reciprocal of the highest dilution of antiserum producing any discernible degree of hemagglutination.

TABLE 4. *Absorption of capsular antibodies from homologous antisera with homologous or heterologous antigens*

Cell sensitized with homologous antigen 1:10,000 prepared from:	Unabsorbed antiserum titer[a]	Antiserum titer after absorption with:[a]							
		W antigen		Sd antigen		43R antigen		36T antigen	
		10 μg/ml	1 μg/ml	10 μg/ml	1 μg/ml	10 μg/ml	1 μg/ml	10 μg/ml	1 μg/ml
W	192	192	192	192	192	384	384	384	192
Sd	384	192	384	0[b]	24[b]	384	384	768	384
43R	384	192	384	192	192	96[b]	192	192	384
36T	384	192	384	192	192	192	192	192	192

[a] Reciprocal of final antiserum dilution.
[b] Significant reduction in titer.
[c] Uninterpretable.

TABLE 5. *Absorption of capsular antibodies from homologous antisera with homologous or heterologous antigens*

Cells sensitized with homologous antigen 1:20,000 prepared from:	Unabsorbed antiserum titer[a]	Antiserum titer after absorption with:[a]							
		W antigen		Sd antigen		43R antigen		36T antigen	
		10 μg/ml	1 μg/ml	10 μg/ml	1 μg/ml	10 μg/ml	1 μg/ml	10 μg/ml	1 μg/ml
W	384	24[b]	96[b]	192	384	384	384	384	384
Sd	U[c]	U	U	U	U	U	U	U	U
43R	384	192	192	192	192	48[b]	96[b]	192	192
36T	384	192	384	192	384	192	192	96[b]	96[b]

[a] Reciprocal of final antiserum dilution; U, uninterpretable.
[b] Significant reduction in titer.
[c] Uninterpretable.

titers when antisera were absorbed with 10 or 1 μg of homologous antigens per ml. The 43R antigen-antibody system still showed a significant reduction in titer, but the results with the Smith diffuse antigen-antibody system were uninterpretable, owing to the very weak reactions. The final and most definitive test used to disclose the serological specificity of the four strains of *S. aureus* described here was the specific capsular reaction carried out with each strain and its homologous antiserum (Table 6). The serological specificity of the Sd, W, and 36T strains is clearly shown. A group of cross-reacting strains comprised of 43R, 47R, 50R, and 51R represented a separate group distinguishable from all the others, but not from each other, by the specific capsular reaction. The specific capsular reaction titers of anti-43R and anti-36T were less than 1:4, but high-titered antiserum is now being prepared in roosters against 36T and 43R.

TABLE 6. *Specific capsular reactions of staphylococci*

Strains	Antisera						
	W	Sd	36T	43R	47R	50R	51R
W	+	−	−	−	−	−	−
Sd	−	+	−	−	−	−	−
36T	−	−	+	−	−	−	−
43R	−	−	−	+	+	+	+
47R	−	−	−	+	+	+	+
50R	−	−	−	+	+	+	+
51R	−	−	−	+	+	+	+

DISCUSSION

Various investigators have isolated mouse-virulent strains of *S. aureus* that grow in a diffuse manner in serum-soft agar (1, 12). Gel diffusion tests have shown the presence of an antigen in the Sd strain which was absent from the Smith compact strain of *S. aureus* (12). Experimental data from our laboratory had shown definitively that the Sd strain was encapsulated and that the capsule was serologically different from that of the wound strain (19). Smith surface antigen (SSA) was chemically analyzed and found to be a polymer of 2-amino-2-deoxy-D-glucuronic acid, a heretofore undescribed component of

bacterial capsules (4, 5, 13). Gel diffusion tests with SSA and extracts from Welwood and K-6 have shown that SSA was extractable from these strains (14). The present report showed that the Sd, Welwood, and K-6 strains were of the same capsular type as shown by the specific capsular reaction tests. Immunoelectrophoresis of extracts prepared by the method of Morse (14) and Wiley and Wonnacott (20), from the W, Sd, 43R, and 36T strains, showed the presence of antigens specific for each strain. The observation that anti-W serum cross-reacted with Sd extract and anti-Sd serum did not cross-react with W extract was explained on the basis that these reactions were one-way cross-reactions similar to those observed by Pease and Laughton (15) with mycoplasma antigens.

Our results do not correspond to those reported by Morse (14). In every instance in this work, except where an extract of Sd was employed, we obtained multiple precipitin arcs with the extracts. Usually there were two arcs, but in the case of the W extract, we obtained three precipitin arcs. One of the anti-Sd sera employed consistently gave a single arc when reacted with the Sd extract, but a second anti-Sd serum gave two precipitin arcs with Sd extract. The precipitin arc detected in extracts of the Sd strain when reacted against anti-W serum could not have represented the capsular antigen of the Sd strain because we showed previously (19), and quite definitively, that capsules of the two strains are serologically distinct. The possibility that the rabbit used in the preparation of the anti-W serum possessed naturally occurring antibodies reactive with the Sd extract was ruled out by our failure to detect a precipitin arc when reacting Sd extract and a preimmunization serum. The best explanation seems to be that acid extraction of staphylococci by the method of Morse (14) extracts multiple antigens in many instances. In all deference to Morse, it is worth pointing out that we employed antigen concentrations 10 times stronger than his, and we used a procedure potentially more sensitive and capable of better resolution of multiple antigens when we used immunoelectrophoresis. It is wise to sound a word of caution here, that interpretation of results in immunoelectrophoresis or double diffusion should be made cautiously. When multiple precipitin arcs are present, it may be quite difficult to ascribe one of them as being due to capsular antigen. Our results would have been difficult to interpret without the specific capsular reaction which we use as a definitive test to detect capsular antigens.

Among the most sensitive methods for detecting antibodies is the passive hemagglutination test. It is capable of detecting from 0.003 to 0.006 μg of antibody nitrogen per ml, whereas the qualitative precipitin test in gels is capable of detecting 3 to 5 μg of antibody nitrogen per ml (8). The passive hemagglutination test was used to detect antibodies to antigen in extracts of staphylococci (6, 11, 17). The passive hemagglutination tests reported here disclosed that an acid extract of staphylococci as well as crude capsular material were capable of sensitizing erythrocytes and rendering them agglutinable by antiserum against *S. aureus*. When large amounts of the extracts or crude capsular material were used, there was little evidence of strain-specific antigens in hemagglutination tests. When the sensitizing antigens were diluted to 1:10,000 or 1:20,000, however, and the antisera were absorbed with homologous and heterologous antigen diluted to 1:100,000 or 1:1,000,000, strain specificity was evident, for homologous antigen reduced the titer of the antiserum fourfold or more. Since it was evident in most instances that multiple antigens were present in the antigen extracts, the cross-reactivity evident in hemagglutination tests carried out with antigen diluted 1:1,000 was not surprising.

Rantz et al. (16) isolated a non-species specific antigen from gram-positive bacteria that was capable of sensitizing erythrocytes to subsequent agglutination by antiserum. Grov et al. (3) isolated a substance they termed protein A from *S. aureus* strains and showed that it was capable of sensitizing tanned sheep cells to subsequent agglutination by antisera against *S. aureus*. Most normal human sera also were capable of agglutinating tanned sheep erythrocytes sensitized with protein A. The point we make here is that extracts of staphylcocci may indeed contain a variety of antigenic moieties such as teichoic acid, protein A, Rantz antigen, or capsular antigen. When multiple precipitin arcs are detected, it is difficult to identify each of the antigens producing the precipitin reactions, unless purified antigens are available for purposes of identification. It seems likely that Rantz antigen could be present in our antigen extracts and this could explain the marked cross-reactivity of the antisera in the hemagglutination test when antigen diluted 1:1,000 or 1:5,000 was used. When the hemagglutination tests were carried out with antigen diluted 1:10,000 or 1:20,000 and absorption tests were set up, specificity of the hemagglutination reaction was shown by failure of heterologous antigen to diminish the homologous hemagglutination titers. In all instances, absorption of homologous antiserum by homologous antigen at a concentration of 1 μg/ml or 10 μg/ml resulted in at least a fourfold reduc-

tion in the homologous hemagglutination titer. The final conclusion that at least four capsular types of *S. aureus* exist was based on the results of specific capsular reactions carried out with rabbit antiserum. Two of the capsular types represented by strains 36T and 43R have never been described before to our knowledge.

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