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Isolation of an Acidic Surface Antigen from a Conventional Strain of *Staphylococcus aureus*

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Staphylococcus aureus strain 7007, a prototype isolated from a hospital burn unit, was shown to exhibit a significant degree of resistance to ingestion by mouse polymorphonuclear leukocytes. An acidic surface antigen was isolated from strain 7007 by a combination of 10% trichloroacetic acid extraction, ion-exchange chromatography, and gel filtration. Chemical analysis indicated that the surface antigen consists of an unknown aminouronic acid and an amino sugar. Immunochemical analysis suggested that the 7007 antigen is a common feature of all the strains collected from the burn unit. No cross-reactivity was observed between the carbohydrate preparations of various hospital staphylococcal strains and the 7007 heteropolymer. These results suggested the possibility that the surface antigen of strain 7007 represents a strain- or type-specific antigen.

It has been established that antiphagocytic cell-wall surface antigens play a major role in the invasive ability of most pyogenic bacteria (13). One exception to this is *Staphylococcus aureus*, for in only a single instance has a chemically defined antiphagocytic antigen been isolated from these organisms (13). Morse isolated an acidic polysaccharide from an encapsulated *S. aureus*, strain Smith, which was shown to be the factor responsible for the virulence of these organisms (2, 16). In an extension of these observations, numerous attempts to demonstrate the presence of antiphagocytic surface antigens among various strains of *S. aureus* resulted in a series of negative observations (17). Similar findings by other investigators have suggested that antiphagocytic surface antigens may be unique to only a few mucoid laboratory strains and absent in the nonmucoid conventional strains which are normally isolated from staphylococcal infections (11, 17).

Evidence is now accumulating which indicates that the conventional strains of *S. aureus* do possess a diversity of surface antigens (15, 25, 26). These observations underscore the possibility that *S. aureus* may possess a diversity of surface antigens which could be of potential immunological importance. It is conceivable, therefore, that strains of *S. aureus* can be divisible into a series of groups on the basis of common surface heteropolymers.

This report describes the isolation, partial purification, and immunological features of a surface antigen isolated from nonmucoid strains of *S. aureus* collected from a hospital burn unit. Evidence is also presented which indicates that this surface antigen is a strain- or type-specific antigen.

MATERIALS AND METHODS

Organisms. *S. aureus* strain K-93M was kindly supplied by F. Kelly, formerly of the University of Oklahoma, School of Medicine, Oklahoma City. *S. aureus* strain Smith was obtained from R. Ekstedt, Northwestern University, Chicago, Ill. Strain C-55 was obtained from L. Wanamaker, University of Minnesota, Minneapolis. Strains 1, 5, 7, 9, 14, 21, 22, 29, 37, 42, 55, 57, 4345, 7003, and 7007 were isolated from a military hospital in Japan by Melvin Smith.

Media and growth conditions. Lyophilized organisms were rehydrated with sterile saline and grown in beef heart infusion (BHI) broth supplemented with 3% sucrose and grown overnight under CO_2 tension at 37 C. Bulk liquid cultures were inoculated from starter cultures prepared from the lyophilized cultures according to the method described by McDonald and Karakawa (14).

Ion-exchange chromatography and gel filtration. The techniques used for diethylaminoethyl-cellulose chromatography and Bio-Gel filtration were previously described (10).

Analytical methods. Analysis for hexosamines and amino acids was performed by the method of Spackman et al. (23) as described by Karakawa and Krause (10). Analysis of total hexoses was previously described (4). Total hexosamine was determined by the method of Rondle and Morgan (22). Total phosphorus was determined by the method of Chen et al. (1).

Staphylococcal vaccines. The vaccine was prepared by the modified method described by McDonald and Karakawa (14). Cultures of staphylo-

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cocci were grown in BHI broth at 37 C for 4 h under 10% CO_2 tension. The cells were harvested by centrifugation and then treated with 3% formalinized saline for 24 h at 4 C. The killed cells were again centrifuged, resuspended in phosphate-buffered saline, and stored at 4 C until used.

Serological methods. Methods for the qualitative and quantitative precipitin tests have been previously described by McCarty and Lancefield (12). Double-diffusion studies in agar, as well as immunoelectrophoresis studies, have been described by Karakawa and Kane (9).

Phagocytosis studies. Strain 7007 and *S. aureus* strain K-93M, which possesses an aminouronic acid capsule, were grown in BHI broth supplemented with 3% sucrose for 4 h at 37 C under 10% CO_2 tension. The cultures were then inoculated into tissue culture tubes containing 2 ml of fresh heparinized rabbit blood and tumbled for 2 h at 37 C. The blood culture was then centrifuged for 5 min at 500 rpm. The turbid supernatant which contained unphagocytized organisms was transferred into BHI broth incubated for 8 h under 10% CO_2 tension, and were stored at −70 C. These cultures were used as starter cultures. Phagocytosis studies were performed by a modified method described by Roberts (20). Mid-log phase organisms (2 to 4 h) grown in BHI broth were centrifuged, and the packed cells were suspended in Gey balanced salt solution (Microbiological Associates, Inc., Bethesda, Md.). A one-tenth ml amount of a bacterial suspension (approximately 2×10^7 cells) was mixed with 0.1 ml of antiserum, 0.1 ml of fresh normal rabbit serum, and 0.3 ml of Gey balanced salts containing 0.1% gelatin (Difco Laboratories, Detroit, Mich.). To this mixture was added 0.5 ml of a leukocyte suspension obtained from the peritoneal cavity of mice inoculated with 0.1% glycogen (cp, Amend Drug and Chemical Co., Inc.) according to the method of Hirsch and Strauss (8) as described by Roberts (20). After tumbling for 30 min at 37 C, the mixture was centrifuged for 5 min at 500 rpm. The supernatant containing unphagocytized bacteria was dispensed into 1.0 ml of Gey solution and serially diluted. The diluted fractions were plated onto blood agar plates and incubated at 37 C for 12 h, and the colonies were counted as described by Roberts (20). The packed cells consisting of leukocytes and phagocytized bacteria were smeared on slides and stained with Diff-Quik stains (Harleco, Phialdelphia, Pa.). The degree of phagocytosis by polymorphonuclear leukocytes was evaluated by counting 200 polymorphonuclear leukocytes and determining the percentage of these leukocytes which contained more than five bacteria per leukocyte.

RESULTS

Results of agglutination studies on 15 selected strains of methicillin-resistant *S. aureus*, phage type 84/85, indicated that these organisms were incapable of agglutinating even in the presence of its homologous antiserum. The cells of strain 7007 failed to react with all the antisera (Table 1). In contrast, nonburn strain W, rich in teichoic acid, readily reacted with all of the antisera. The ability of strain W cells to react with anti-7007 clearly indicates that the anti-7007 serum contains teichoic acid agglutinins, and therefore it can be concluded that the teichoic acid moiety is an integral part of the strain 7007 cell surface. The results obtained from these agglutination studies suggest the possibility that the burn strains possess at least two cell wall-associated antigens, a common teichoic acid and a surface antigen, capable of masking the cell-agglutinin reaction.

Phagocytosis studies with strain 7007. Previous studies by a number of investigators have clearly demonstrated that slime material from selected strains of *S. aureus* can inhibit phagocytosis by polymorphonuclear leukocytes (6). In the present studies, efforts were made to detect antiphagocytic surface antigens in strain 7007 by using the in vitro phagocytosis test of Hirsch and Strauss (8) as described by Roberts (20). Illustrated in Table 2 are the results of the direct smear phagocytosis studies which used

TABLE 1. *Direct agglutination test using homologous and heterologous staphylococcal antisera and cells of staphylococcal strains 7007 and W*

Antisera	Agglutination titer[a] of staphylococcal strain:	
	7007	W
7007	<10	320
33	<10	320
W	<10	160
32	<10	320
B-2	<10	320
H	<10	80
Smith	<10	160

[a] Titer is the reciprocal of the highest dilution with a 3+ or 4+ agglutination reaction.

TABLE 2. *Phagocytic indexes with staphylococcal strains 7007 and K-93M and homologous and heterologous staphylococcal antisera*

Strain of organism	Antiserum	PMN containing 5 or more ingested bacteria (%)[a]
7007	Normal serum	2.3
7007	Anti-7007 serum	57.0
7007	Anti-K-93M serum	12.6
K-93M	Normal serum	1.5
K-93M	Anti-7007 serum	1.8
K-93M	Anti-K-93M serum	78.0

[a] Mouse polymorphonuclear leukocytes (PMN) were obtained from the peritoneal cavity of strain BALB/c mice. The smear method was used.

strains 7007 and K-93M, an encapsulated strain of *S. aureus*. Note that, after 30 min of incubation in the presence of normal serum or K-93M antiserum, the degree of ingestion of strain 7007 cells by mouse polymorphonuclear leukocytes was noticeably low. However, with the addition of specific anti-7007 serum devoid of teichoic acid antibodies, the degree of ingestion of the 7007 cells by leukocytes was significantly elevated from 2.3 to 57.0%. The control encapsulated organism, strain K-93M, in the presence of normal serum or 7007 antiserum was markedly resistant to phagocytosis by leukocytes. In the presence of homologous anti-K-93M serum, however, the K-93M cells were readily engulfed by the polymorphonuclear leukocytes. These observations clearly indicate that the cells of strain 7007 possess antiphagocytic surface antigens which can elicit the production of specific opsonins in rabbits. It should be emphasized that the phagocytic indexes obtained with strain 7007 cells and its homologous antiserum, although not as pronounced as that obtained with the fully encapsulated organism, were significantly constant. For example, similar phagocytic indexes were consistently obtained with strain 7007 and homologous antiserum by using the plate-count method which utilized the supernatant fraction of the leukocyte-bacteria mixture.

Isolation of surface antigens from strain 7007. To isolate the antiphagocytic surface antigens from the burn strains, two extraction procedures were used: the method described by McDonald et al. which used the culture filtrate, and the cold trichloroacetic acid method of Park and Hancock which used whole cells of *S. aureus*. Results of the extraction studies indicated that an antiphagocytic surface antigen consisting of 3.2% hexose, 0.8% uronic acid, 18.0% phosphorus, and 25% amino sugars can be isolated from both the whole cells and the culture filtrates of strain 7007, the burn strain prototype. In both instances, these antigen preparations were shown to be moderately effective in inhibiting the in vitro phagocytosis of strain 7007 cells by a combination of immune serum and leukocytes. Because the extraction of antigens from whole cells is a more convenient method than extraction from the culture filtrate, the former procedure was used throughout this report.

Chemical composition of the burn strain antigens. Depicted in Table 3 are the results of the chemical analysis of the trichloroacetic acid extracts of burn strains 7007, 1, 9, 37, and 7003. Note that the antigens contained appreciable levels of both amino sugars and phosphorus.

TABLE 3. *Chemical composition of the trichloroacetic acid preparations derived from the whole cells of burn strains 1, 9, 37, and 7003*

Component	Composition (μg/ml) of strains:				
	7007	1	9	37	7003
Total hexose[a]	30.9	14.0	25.4	83.6	30.9
Total amino sugars[b]	297.5	327.2	310.4	321.8	321.5
Carbazole (uronic acid)[c]	2.5	1.5	3.9	5.0	2.5
Phosphorus	114.3	113.8	164.9	124.9	114.3

[a] Determined by the Anthone method described by Scott and Melvin, 1953.

[b] Determined by the method of Rondle and Morgan (22).

[c] Determined by the method of Dische, 1947.

The high levels of phosphorus and amino sugars in the trichloroacetic acid extracts of these strains was suggested to be due to the presence of species-specific teichoic acids in the preparations. This was shown to be the case, because double-diffusion studies in agar using unadsorbed and adsorbed *S. aureus* sera clearly indicated that the antigen preparations of the selected burn strains contained of the teichoic acid polymer in addition to a possible type-specific antigen. Illustrated in Fig. 1 are the results of the double-diffusion studies in agar between the trichloroacetic acid extracts of strains 7007, 1, 9, 37, 7003, and 7 and anti-7007 serum. As depicted in this figure, the trichloroacetic acid extracts of strains 7007, 1, and 7003 formed more than one precipitin band with anti-7007 serum, whereas the extracts of strains 9, 37, and 7 formed only a single precipitin band with anti-7007 serum. These observations suggest that the outer precipitin band, common to all the extracts, may represent a type-specific antigen, and the inner precipitin band observed with strains 7007, 1, and 7003 may represent the teichoic acid antigen. Depicted in Fig. 2 are the results of the double-diffusion studies in agar between the antigenic preparations of the burn strains and adsorbed anti-7007 serum, devoid of teichoic acid antibodies. It should be noted that all the extracts formed precipitin bands with the adsorbed anti-7007 serum, and these bands merged to form lines of identity. These results indicate that the outer precipitin bands observed in Fig. 1 represent a possible type-specific antigen.

Characterization of the 7007 surface antigen. Illustrated in Fig. 3 are the results of the chromatographic analysis of a hydrolysate of the trichloroacetic acid-extracted antigen of

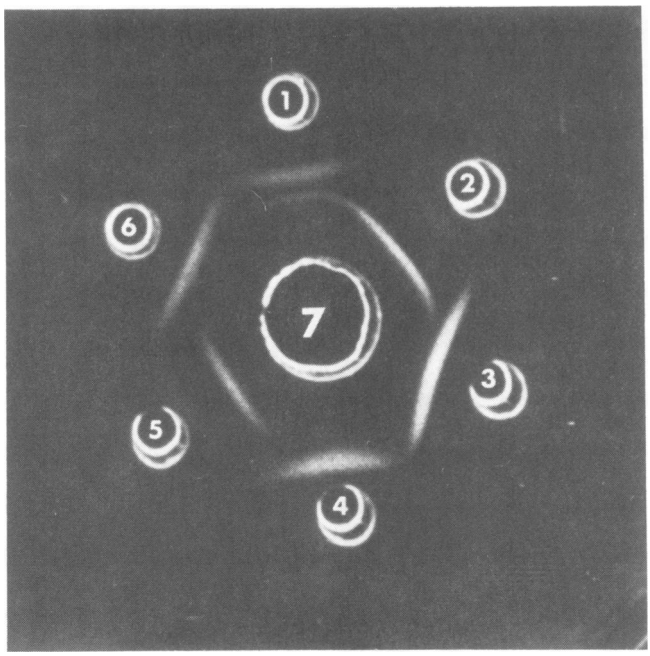

FIG. 1. *Immunodiffusion reactions in agar gel between crude trichloroacetic acid extracts of strains 7007, 1, 9, 37, 7003, and 7 and anti-7007 serum. Well 1, strain 7007; Well 2, strain 1; Well 3, strain 9; Well 4, strain 37; Well 5, strain 7003; Well 6, strain 7; and Well 7, anti-7007 serum.*

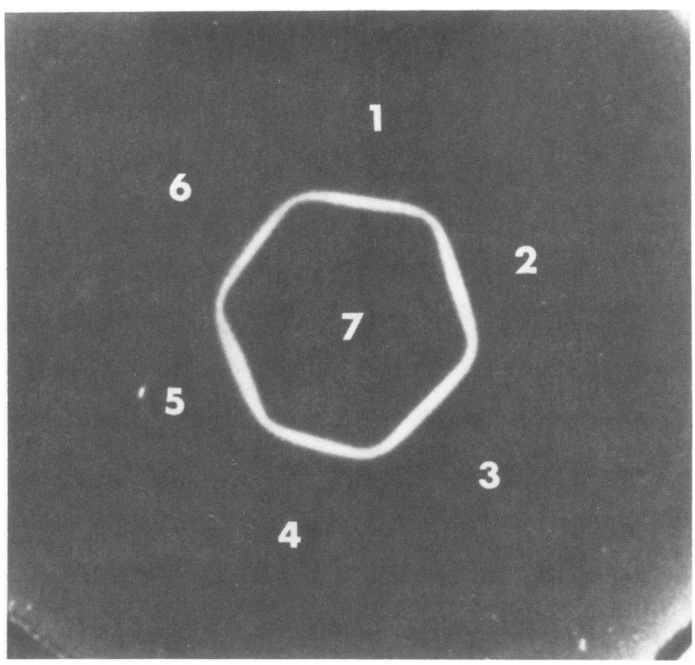

FIG. 2. *Immunodiffusion reaction in agar between antigens of strains 7007, 1, 9, 37, 7003, and 7 and adsorbed anti-7007 serum. Well 1, strain 7007; Well 2, strain 1; Well 3, strain 9; Well 4, strain 37; Well 5, strain 7003; Well 6, strain 7; and Well 7, adsorbed anti-7007 serum.*

strain 7007 before and after purification. The crude antigen (bottom frame) possessed many of the constituents of the teichoic acid-mucopeptide complex, namely muramic acid,

glutamic acid, glycine, alanine, and glucosamine. However, it should be noted that a distinct peak was eluted at approximately 250 min. The elution pattern of this compound was noticeably different from glucosamine or galactosamine, the common amino sugars of bacterial polysaccharides. Depicted in the top frame of Fig. 3 is the chromatographic analysis of the antigen of strain 7007 after purification by diethylaminoethyl-cellulose chromatography and gel filtration. It should be noted that the unknown peak initially observed in the hydrolysate of the crude 7007 antigen preparation is a major constituent of the purified 7007 antigen (Fig. 3, bottom frame). Subsequent analysis, using paper chromatography and quantitative chemical analysis, suggested that the 7007 antigen consists of two unknown amino sugars or a possible disaccharide (Fig. 4). Further analyses indicate that polymer is essentially devoid of amino acids, hexoses, and phosphorus.

Depicted in Fig. 5 are the results of the immunoelectrophoretic studies using the 7007 antigen. Various dilutions of purified 7007 antigen were placed in wells 1, 2, 3, and 4 and electrophoresed at 250 V for 30 min. After electrophoresis, anti-7007 serum and anti-1 serum were placed into the top and bottom troughs, respectively. A single precipitin band was formed between the purified antigens and antisera. The relative position of the antigen in reference to the origin suggests that the 7007 antigen is negatively charged and therefore acidic in nature. Preliminary evidence, resulting from a combination of borohydride reduction and paper chromatography, suggests the possibility that the acidic component of the 7007 antigen may be a mannosaminouronic acid.

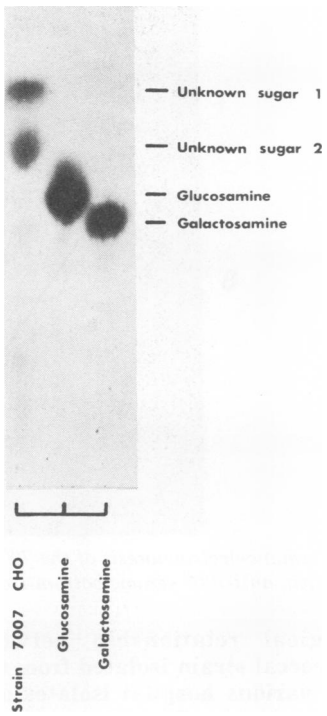

FIG. 4. *Paper chromatogram showing the hydrolytic products from the 7007 antigen after hydrolysis in 2.0 N HCl at 100 C for 18 h.*

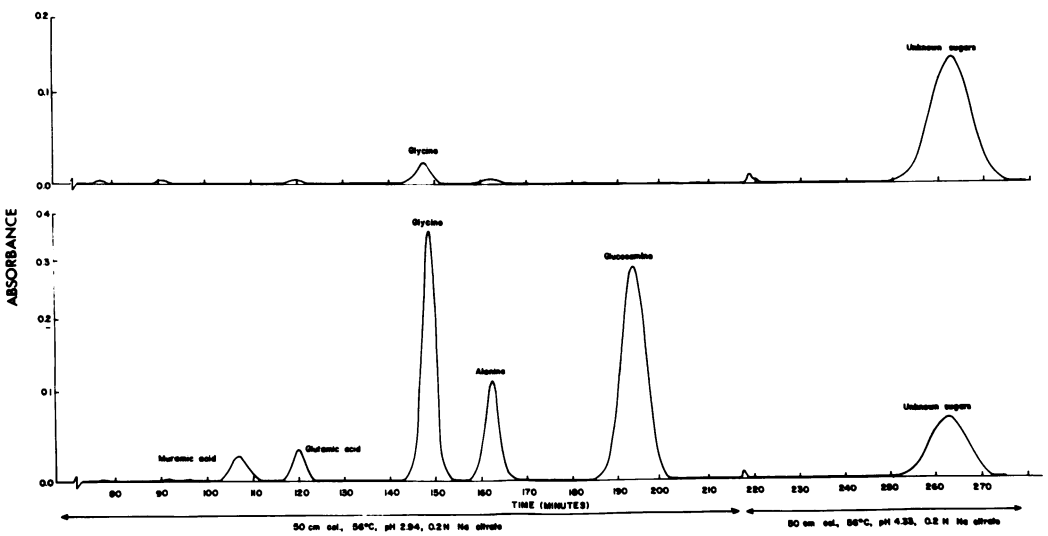

FIG. 3. *Bottom frame, Analysis of an acid hydrolysate of the crude trichloroacetic acid extract of strain 7007 whole cells; top frame, analysis of an acid hydrolysate of purified 7007 antigen. Hydrolysis was carried out under vacuum with 2 N HCl at 100 C for 1 h.*

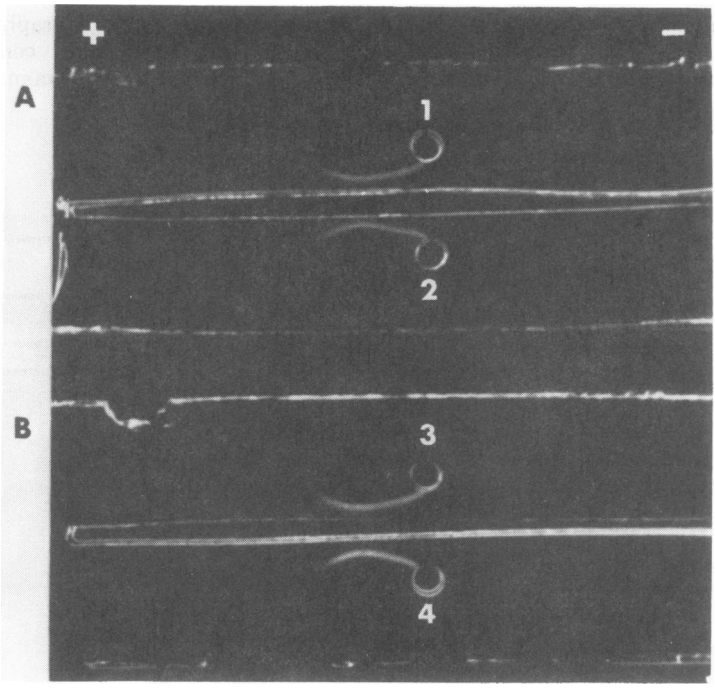

FIG. 5. *Immunoelectrophoresis of the 7007 antigen. Wells 1 and 3, 100 μg of 7007 antigen; Wells 2 and 4, 50 μg; top trough, anti-7007 serum; bottom trough, anti-1 serum.*

Serological relationship between the staphylococcal strain isolated from the burn unit and various hospital isolates of S. aureus. Illustrated in Fig. 6 are results of the quantitative precipitin test using the purified antigen of strain 7007 and trichloroacetic acid extracts of strains Smith, C-55, and 33 and anti-7007 serum, devoid of teichoic acid antibodies. The antigen isolated from strain 7007 gave a strong precipitin reaction with homologous antiserum. In contrast, the extracts of an impetigo strain C-55 (phage type 71) and strain 33 gave only minimal reactions with anti-7007 serum. The Smith capsular antigen also gave only a weak reaction with 7007 antiserum. Further serological analyses of the trichloroacetic acid extracts of an additional 25 hospital strains also gave similar negative reactions with anti-7007 serum.

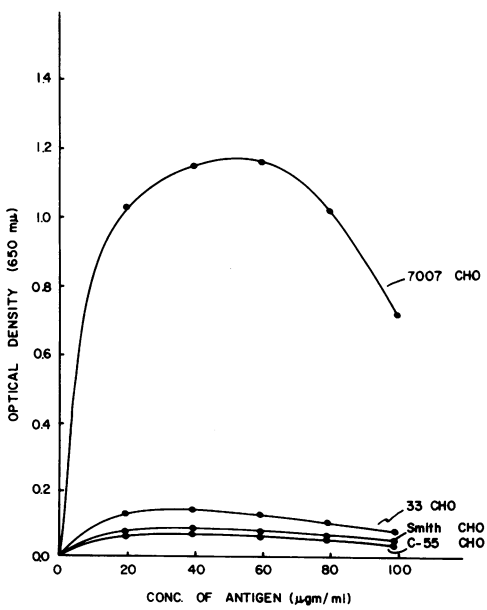

FIG. 6. *Quantitative precipitin reaction between the purified antigens of S. aureus strains 7007, Smith, 33, and C-55 and anti-7007 serum.*

DISCUSSION

It is evident that a systematic scheme for the classification of invasive bacteria is essential for the proper understanding and control of infectious diseases. Such studies are necessary not only for the elucidation of the biological and immunological relationship existing among various strains or types, but also for the development of effective means of immunological therapy. In the case of *S. aureus*, there is a noticeable absence of a simple, definitive scheme for the classification of these organisms into specific groups based on immunologically significant antigens. Presently, the agglutina-

tion procedure described by Cowan (3) and subsequently by Oeding (18) has proved to be moderately useful in the classification of *S. aureus*. However, the agglutinin factors present on the cell surface of *S. aureus* represent a multiplicity of ill-defined antigens which have not been shown to be critical in the virulence of these organisms. In this report, phagocytic studies suggest that a series of *S. aureus* strains collected from an epidemic involving patients in a hospital burn unit in Japan exhibited a significant degree of resistance to ingestion by polymorphonuclear leukocytes. Studies on the immune response of rabbits to the burn strain prototype, strain 7007, indicated that surface components of this strain can elicit the production of specific opsonins. An acidic polysaccharide, consisting of an aminouronic acid and an unknown amino sugar, was isolated from strain 7007. Paper chromatographic analyses of an acid hydrolysate of the 7007 antigen revealed the presence of two Elson Morgan-positive compounds with R_f values in the range of aminohexuronic acids and hexosamines, respectively (19). Preliminary evidence using a combination of borohydride reduction and paper chromatography suggests that the aminouronic acid of the 7007 antigen may be mannosaminouronic acid. The presence of a possible mannosaminouronic acid in the 7007 antigen suggests that this antigen may be immunologically related to the fucosamine-mannosaminouronic acid polymer recently isolated from *S. aureus* strain T by Wu and Park (24). Extracts from *S. aureus* strain T, obtained from J. T. Park, gave negative precipitin reaction with anti-7007 serum, and therefore it appears that these antigens may be distinct polymers.

Serological evidence was presented which indicated that the acidic polymer of strain 7007 was unique to the burn strains and probably represented a type-specific surface antigen. This observation is consistent with the results observed by other investigators (15, 25). Preliminary studies on a series of hospital strains indicate that the presence of type-specific surface antigens in fresh clinical isolates of *S. aureus* may be a more frequent occurrence than one suspected. Results presented here also indicate that the 7007 surface antigen can only be isolated in quantity from young BHI broth cultures inoculated with starter cultures which have been repeatedly revitalized by mouse passage.

Although the incidence of serious staphylococcal illness has been relatively infrequent in the past, this situation is slowly being altered due to the increased emergence of susceptible groups of individuals. The emergence of these groups can be attributed, in part, to the tremendous advance in medicine, nursing care, and surgery which has extended the life of man but has not concomitantly increased the proficiency of the patient's natural immunity barriers. Because staphylococci are normal inhabitants of man and have exhibited a high incidence of antibiotic resistance, infections due to these organisms can be a problem in hospitalized individuals. In light of these potential problems presented by the ubiquitous staphylococci, a successful means for active immunization would be a major advance in the control of staphylococcal infections in the small group of susceptible individuals.

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LITERATURE CITED

1. Chen, P. S., T. Y. Toribara, and H. Warner. 1956. Microdetermination of phosphorus. Anal. Chem. **28**:1756–1758.
2. Cohn, Z. A., and S. I. Morse. 1959. Interactions between rabbit polymorphonuclear leukocytes and staphylococci. J. Exp. Med. **110**:419–443.
3. Cowan, S. T. 1939. Classification of staphylococci by slide agglutination. J. Pathol. Bacteriol. **48**:169–173.
4. Curtis, S. N., and R. M. Krause. 1964. Antigen relationship between group B and G. streptococci. J. Exp. Med. **120**:629–637.
5. Davidson, E. A. 1968. Modified carbazole procedure, p. 55. *In* E. F. Neufeld and V. Ginsburg (ed.), Methods in enzymology, vol. 8. Academic Press, Inc., New York.
6. Ekstedt, R. D. 1965. Mechanisms of resistance to staphylococcal infection: natural and acquired. Ann. N.Y. Acad. Sci. **128**:301–333.
7. Ekstedt, R. D., and J. M. Bernhared. 1973. Preparation and characterization of a slime layer material produced by *Staphylococcus aureus*. Proc. Soc. Exp. Biol. Med. **142**:86–91.
8. Hirsch, J. G., and B. Strauss. 1964. Studies on heat-labile opsonin in rabbit serum. J. Immunol. **92**:145–154.
9. Karakawa, W. W., and J. A. Kane. 1972. Characterization of the surface antigens of *Staphylococcus aureus*, strain K-93M. J. Immunol. **108**:1199–1208.
10. Karakawa, W. W., and R. M. Krause. 1966. Studies on the immunochemistry of streptococcal mucopeptide. J. Exp. Med. **124**:155–171.
11. Koenig, M. G., and M. A. Melly. 1965. The importance of surface antigens in staphylococcal virulence. Ann. N.Y. Acad. Sci. **128**:231–250.
12. McCarty, M., and R. C. Lancefield. 1955. Variation in the group-specific carbohydrates of group A streptococci. I. Immunochemical studies on the carbohydrate of variant strains. J. Exp. Med. **102**:11–28.
13. McCarty, M., and S. I. Morse. 1964. Cell wall antigens of gram-positive bacteria. Advan. Immunol. **4**:249–286.
14. McDonald, H. C., and W. W. Karakawa. 1970. Immunochemical analysis of a uronic acid polymer of *Staphylococcus epidermidis*, strain 53. J. Immunol. **105**:389–395.
15. Maverakis, N. H., and B. B. Wiley. 1969. Evidence for a multiplicity of capsular types among *Staphylococcus aureus* strains. J. Bacteriol. **99**:472–479.

16. Morse, S. I. 1962. Isolation and properties of a surface antigen of *Staphylococcus aureus*. J. Exp. Med. **115**:295-311.
17. Morse, S. I. 1963. Distribution of a surface antigen of *Staphylococcus aureus*. Nature (London) **200**:1126-1127.
18. Oeding, P. 1965. Antigenic properties of staphylococci. Ann. N.Y. Acad. Sci. **128**:183-189.
19. Perkins, H. R. 1963. A polymer containing glucose and aminonexuronic acid isolated from the cell walls of *Micrococcus lysodeikticus*. Biochem. J. **86**:475-483.
20. Roberts, R. B. 1967. The interaction *in vitro* between group B meningococci and rabbit polymorphonuclear leukocytes. J. Exp. Med. **126**:795-818.
21. Rogers, D. E., and M. A. Melly. 1965. Speculation on the immunology of staphylococcal infections. Ann. N.Y. Acad. Sci. **128**:274-284.
22. Rondle, C. J. M., and W. T. Morgan. 1955. Determination of glucosamine and galactosamine. Biochem. J. **61**:1756-1958.
23. Spackman, D. H., W. H. Stein, and S. Moore. 1958. Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem. **30**:1190-1206.
24. Wu, T. C. M., and J. T. Park. 1971. Chemical characterization of a new surface antigenic polysaccharide from a mutant of *Staphylococcus aureus*. J. Bacteriol. **108**:874-884.
25. Yoshida, K. 1971. Demonstration of serologically different capsular types among strains of *Staphylococcus aureus* by the serum-soft agar technique. Infect. Immunity **3**:535-539.
26. Yoshida, K., and R. D. Ekstedt. 1968. Relation of mucoid growth of *Staphylococcus aureus* to clumping factor reaction, morphology in serum-soft agar, and virulence. J. Bacteriol. **96**:902-908.