ISOLATION AND PROPERTIES OF A SURFACE ANTIGEN OF STAPHYLOCOCCUS AUREUS*

By STEPHEN I. MORSE, M.D.

(From The Rockefeller Institute)

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Knowledge of the nature and function of the antigenic components of *Staphylococcus aureus* is limited, particularly with respect to those factors which endow the organism with pathogenicity and virulence. A substantial body of information is available concerning the extracellular products of the organism which have the potential of altering host tissues and body fluids; *e.g.*, coagulase, the hemolysins, fibrinolysin, and leucocidin. However, none of these substances has thus far been shown to be a major determinant of the initiation and persistence of staphylococcal disease.

Phagocytosis and intracellular killing of bacteria by host leucocytes constitute the primary defense against microbial invasion. It is well known that efficient phagocytosis of many virulent bacteria requires the presence of specific immune opsonins. Thus, in the absence of antibody against the capsular polysaccharides of pneumococci, these organisms are not readily phagocyted and overwhelming microbial invasion may ensue. In the presence of specific antisera the anti-phagocytic properties of the surface polysaccharides are neutralized and the organisms are readily ingested.

The role of immune opsonins in experimental and clinical staphylococcal disease has been a point of controversy. Until recently it was not appreciated that opsonizing antibody was required for the efficient *in vitro* phagocytosis and killing of strains of *S. aureus* by rabbit polymorphonuclear leucocytes (1). Such antibody was not found in the serum of normal rabbits or rabbits immunized with *S. albus*, but was produced in high titer in animals injected with heat-killed cells of *S. aureus*. In order to pursue these studies further and extend them to *in vivo* systems, it was necessary to attempt the isolation of the antigenic substances reponsible for the insusceptibility to phagocytosis.

The present study concerns the isolation, properties, and biologic activities of a surface antigen of the Smith strain of *S. aureus* responsible for the resistance of this organism to *in vitro* phagocytosis by rabbit leucocytes. Injection of this apparently homogeneous antigen into experimental animals resulted in protection against lethal infection with the homologous organism.

A preliminary account of these investigations has appeared elsewhere (2).

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Materials and Methods

Staphylococcus aureus Smith.—This organism was originally isolated from a patient with osteomyelitis, and has been maintained on artificial media in the laboratory of R. J. Dubos. The organism produces mucoid colonies with golden pigmentation, coagulase, and slight hemolysis on rabbit blood agar. As recently as 1959, the Smith strain was readily lysed by phages 44A and 42E at the usual typing dilutions (1). However, the strain is now non-typable in this and other laboratories (3).

Medium and Method of Producing Mass Cultures.—*Staphylococcus aureus* Smith was cultured in the medium and by the techniques described by Goebel *et al.* (4). The basic medium consisted of 1 per cent technical casamino acids (Difco) in tap water buffered to pH 7.4 with 0.03 M phosphate. To 15 liters of this medium was added 12 ml of 50 per cent glucose and a concentrate of the dialysate of 75 gm of yeast extract.

The medium was then inoculated with the slant washings of an overnight growth of strain Smith on penassay agar. After stationary incubation at 37°C for 18 hours, 450 ml of glucose was added and aeration initiated. The pH was maintained at 7.4 by automatic titration with 2 M sodium carbonate. After further incubation for 10 hours, the number of viable bacterial units was 1×10^{10}/ml. 300 ml of 50 per cent ethanol and 200 ml of 88 per cent phenol were then added and the culture held at 4°C for 48 hours to ensure sterilization.

Physical Methods.—Electrophoretic analyses were carried out at 4°C in the Tiselius apparatus using the schlieren scanning method of Longsworth (5). Mobilities were calculated from descending patterns by the method of Tiselius and Kabat (6). Ultricentrifuge analyses were performed in the model E Spinco apparatus. Ultraviolet absorption spectra were determined in a Beckmann ratio recording spectrophotometer.

Chemical Analyses.—Nitrogen was determined by a modification of the procedure of Koch and McMeekin (7) and amino acid content by the method of Moore *et al.* (8). Reducing sugar was determined by the Nelson modification of the Somogyi technique (9); hexosamine by a modification of the Elson-Morgan method (10); phosphorus by the Fiske and Subbarow procedure (11); total acetyl was estimated after hydrolysis in *p*-toluene sulfonic acid (12); and sulfur was determined by the Elek and Hill technique (13). Lipid was measured by the method of Folch *et al.* (14).

Serologic Methods.—Strains of *S. aureus* and *S. albus* used for immunization, and the method of preparation of rabbit antisera have been previously described (1). Agglutination tests were performed using standard techniques (15). Qualitative precipitin reactions were carried out in capillary tubes and quantitative precipitin reactions were estimated by the method of Libby (16). Double diffusion precipitin studies in agar gel were performed by the method of Ouchterlony (17), and immunoelectrophoretic studies by the microtechnique of Scheidegger (18).

Bacteria-Leucocyte Interactions.—The method utilized for the quantitative estimation of *in vitro* phagocytosis and killing by rabbit leucocytes has been described in detail elsewhere (1).

RESULTS

Isolation of the Smith Surface Antigen (SSA).—Preliminary experiments indicated that the supernatant fluid from cultures of strain Smith contained serologically reactive material. Since many of the known bacterial surface antigens are released into the growth medium, effort was directed towards the separation of the Smith surface antigen from cultures rendered bacteria-free.

Fifteen liters of a sterile culture of strain Smith was passed through a Sharples centrifuge (Type T—41-24 1HY) and the turbid effluent was recentrifuged. The resultant clear super-

natant fluid was concentrated to 900 ml. by distillation *in vacuo* at 72°C. and dialyzed against distilled water for 36 hours. The solution containing the non-dialyzable substances was then concentrated to 200 ml. and passed through a celite (Johns Mansville) pad on a coarse sintered glass filter. The filtrate was again dialyzed against distilled water for 72 hours and dried from the frozen state. The yield from 15 liters of culture was 3.2 gm.

The crude material gave a faintly positive Molisch test and contained 9.1 per cent nitrogen; 4 distinct bands were produced in gel-diffusion reactions with homologous antiserum.

The crude mixture of antigens was dissolved in 300 ml. of 2 per cent aqueous sodium acetate. Two volumes of absolute ethanol were added and the resultant precipitate was discarded. Six more volumes of ethanol were added to the supernatant fluid and the turbid solution was stored at 4°C for 18 hours. A tenacious gummy precipitate which had formed was isolated by centrifugation and redissolved in 260 ml of 2 per cent aqueous sodium acetate. Upon the addition of 3 volumes of ethanol a voluminous precipitate formed which was isolated, redissolved in water, dialyzed for 48 hours and lyophilyzed.

The resulting material amounted to 1.2 gm with a nitrogen content of 10.5 per cent and less than 0.2 per cent of phosphorus. This material was deproteinized by a modification of the Sevag procedure (19). The product was dissolved in 250 ml of 0.02 M acetate buffer at pH 5.0 and 70 ml of chloroform–caprylic alcohol (3:1) was added. The mixture was emulsified at high speed (Lourdes, multi-mix) at 4°C for 15 minutes and then centrifuged at 6000 RPM ×60 minutes in the Lourdes centrifuge. The resultant filmy emulsion cake was discarded and the process was repeated twice more.

The fluid was then dialyzed against distilled water for 72 hours and lyophilyzed. The yield was 790 mg of Smith surface antigen.

General Properties of SSA.—The Smith surface antigen was white, amorphous, and readily soluble in water, physiologic saline, and 50 per cent ethanol. Aqueous solutions were clear and viscid. Precipitation did not occur on the addition of copper or barium salts, trichloroacetic acid, or saturated picric acid. Marked humin production was noted upon heating SSA in dilute mineral acids. At a concentration of 0.1 per cent, the material did not coagulate either rabbit or human citrated plasma.

Physical Properties of SSA.—The ultraviolet absorption spectra of aqueous solutions of SSA are depicted in Fig. 1. At a concentration of 0.1 per cent there was only a slight absorption between 250 and 280 mμ, and none when the concentration was 0.01 per cent. The absence of absorption in this range suggested the presence of no more than minimal amounts of nucleic acids and aromatic amino acids.

The relative viscosity of a 0.1 per cent aqueous solution, determined in an Oswald viscometer at 25°C, was 3.5. The sedimentation properties of SSA in the ultracentrifuge are presented in Fig. 2. The material sedimented as a single symmetrical peak; $S_{20} = 1.33$ Svedberg units. The electrophoretic mobility of SSA in the Tiselius apparatus is depicted in Fig. 3. A 1 per cent solution of the antigen was prepared in barbital buffer (pH 8.6, ionic strength 0.1) and dialyzed against this buffer at 4°C for 48 hours before electrophoresis. Representative patterns are depicted and it is apparent that the ascending boundaries were symmetrical. The electrophoretic mobility was -11.4×10^{-5} cm^2/volt sec.

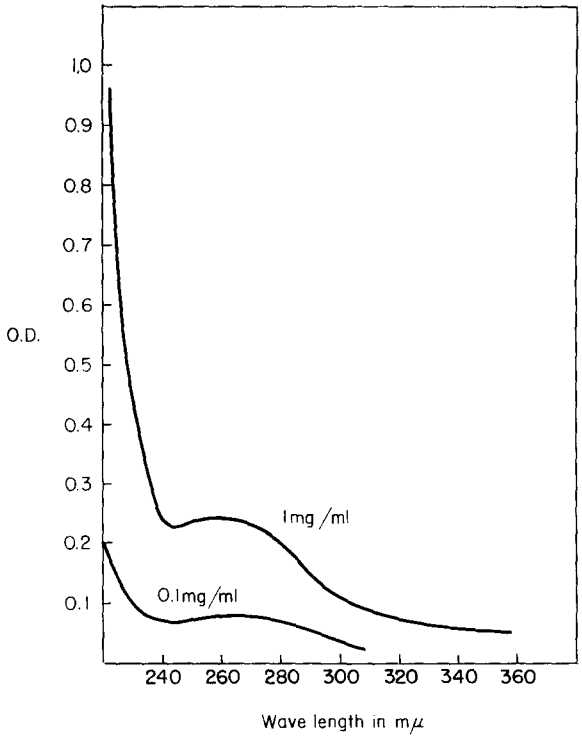

FIG. 1. The absorption in the ultraviolet of aqueous solutions of SSA

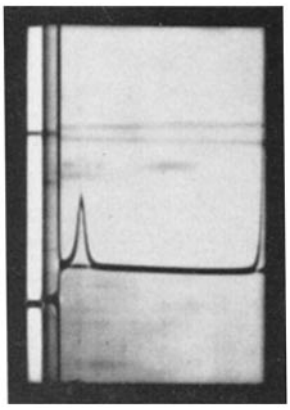

FIG. 2. The sedimentation pattern of a 0.5 per cent solution of SSA in 0.15 M NaCl recorded after 110 minutes at 54,000 RPM in the model E Spinco.

Chemical Properties of SSA.—As indicated in Table I, approximately 30 per cent of SSA was accounted for by the presence of amino acids. The amino acids found were those usually associated with the cell walls of staphylococci, and were present in the same proportions as in the cell walls. Muramic acid, also a constituent of staphylococcal cell walls (20), was not detectable in SSA by the Perkins and Rogers procedure (21). No lipid could be demonstrated in

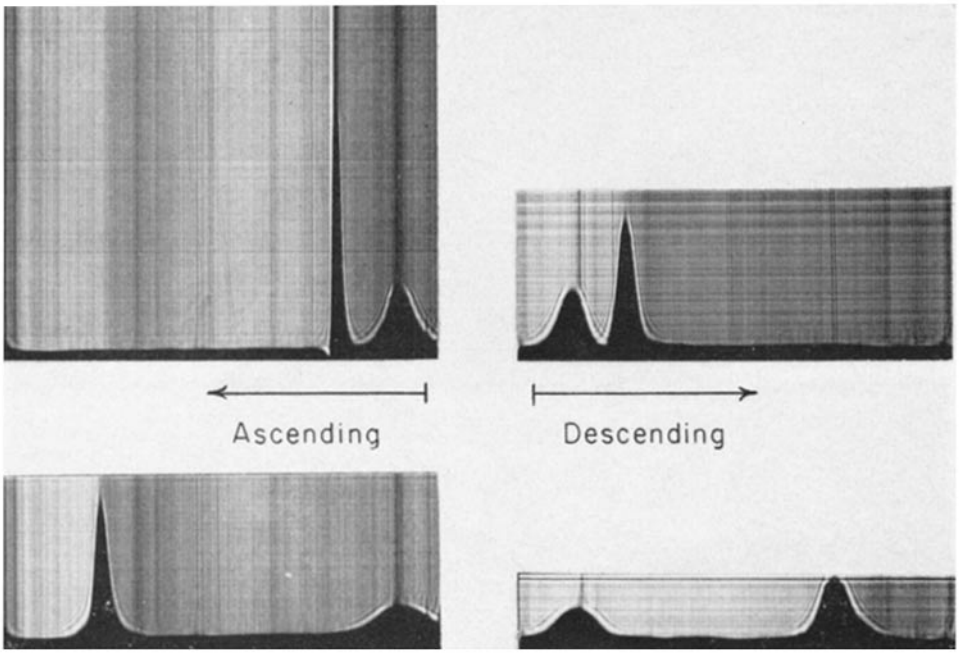

FIG. 3. The electrophoretic pattern of a 1.0 per cent solution of SSA in barbital buffer of pH 8.6 and 0.1 ionic strength. The upper pattern was recorded at 1,200 seconds and the lower at 6,062 seconds at a potential gradient of 6.4 volts per cm.

SSA even after hydrolysis in 0.5 N hydrochloric acid for 30 minutes. Thus, 70 per cent of the antigen was presumed to be carbohydrate.

Determinations of reducing capacity and release of Elson-Morgan reacting material were performed after hydrolysis in 2 N hydrochloric acid at 100°C for varying time periods. Glucose was utilized as a standard for reducing properties and glucosamine hydrochloride was the standard in the Elson-Morgan test. The latter values were then calculated as the free base.

As indicated in Fig. 4, the reducing properties as calculated from glucose amounted to 14.2 per cent of SSA. This maximum value was obtained after 6 hours of hydrolysis. In contrast, the maximum of Elson-Morgan reactive material, expressed as free glucosamine, appeared after 1 hour of hydrolysis

and amounted to 25 per cent of SSA. Since glucose and glucosamine possess virtually identical molecular weights and reducing capacity, it is possible that the non-conformity of the experimental values may be due to material other than hexosamine reacting in the Elson-Morgan test. This is further indicated by the instability of the reactive material over the time period of hydrolysis, whereas hexosamines are usually stable.

TABLE I

The Chemical Properties of SSA

	per cent		*per cent*
Nitrogen	8.49	Reducing sugar	14.2
Phosphorus	<0.2	"Hexosamine"	25.0
Sulfur	<0.3		
Total acetyl	21.6	Amino acids	28.9
Lipid	0	(Ala. 9.1; Glu. 6.7; Lys. 6.6; Asp. 4.9; Gly. 1.1; Ser. 0.25; Thr. 0.25)	

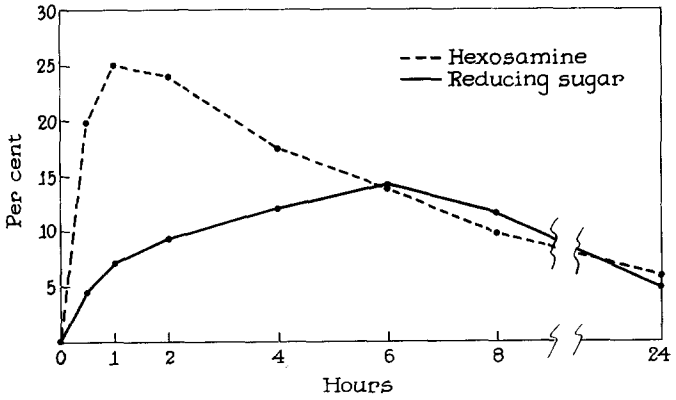

FIG. 4. The release of reducing sugar and hexosamine during hydrolysis of SSA in 2 N HCl at 100°C.

Conventional methods for the detection of hexose, pentose, methyl pentose, heptose, hexuronic acid, and nonulosaminic acids did not reveal the presence of any of these substances.

Numerous attempts at separation and characterization of monosaccharide units by paper chromatography were unsuccessful, despite the use of a variety of hydrolytic conditions, solvents, and staining reagents. Only one moiety appeared consistently and this material migrated with glucosamine in *n*-butanol:acetic acid:water (4:1:5), and stained with ninhydrin, silver nitrate, and the Elson-Morgan reagent. Presumably this compound was hexosamine,

but on the basis of quantitative analysis, true hexosamine constituted not more than 50 per cent of the carbohydrate present in the SSA and hence less than 35 per cent of SSA.

Serologic Activity of SSA.—Tests were performed in capillary tubes. Undiluted serum was layered over an equal volume of the appropriate dilution of antigen in 0.15 M NaCl. The tubes were kept at 4°C and examined for the presence of precipitate at 30 minutes and at 24 hours. The final concentrations of antigen ranged from 1 mg per ml to 0.25 μg per ml.

The results of qualitative precipitin tests are summarized in Table II. Within 30 minutes immune serum prepared against *S. aureus* Smith or *S. aureus* Stern had reacted with SSA. The lowest concentration of antigen reacting with the sera was 2 μg per ml. In contrast, at the end of 30 minutes, no reaction had occurred with antiserum against *S. aureus* strains Stovall or O'Hara, nor with antiserum against *S. albus* strains Greaves or Prengel. In each case antiserum from at least 3 different rabbits was used.

TABLE II

Qualitative Precipitin Reactions of SSA with Normal and Immune Sera

Serum	Immunizing agent	Lowest concentrationof SSA giving a precipitin reaction in	
		30 min.	24 hrs.
		μg/ml	μg/ml
Rabbit	*S. aureus* Smith	1.9	0.5
"	" " Stern	1.9	0.5
"	" " O'Hara	*	62.5
"	" " Stovall	*	250.0
Rabbit	*S. albus* Greaves	*	*
"	" " Prengel	*	*
Rabbit	None	*	*
Human	"	*	*
Guinea pig	"	*	*

* No visible precipitate.

At the end of 24 hours immune serum prepared against *S. aureus* Smith and Stern had reacted with concentrations of SSA as low as 0.5 μg per ml. In contrast, the lowest concentration of antigen reacting with antisera against strains Stovall and O'Hara were 250 μg per ml and 62.5 μg per ml respectively. Normal serum from several species and rabbit antiserum against *S. albus* strains did not react. The turbidimetric method of Libby was utilized to evaluate the quantitative precipitin test.

Various quantities of SSA were added to a standard amount of Smith antiserum previously diluted 1:5. After standing for 15 minutes, the turbidity which developed was determined by means of a photoelectric turbidimeter. The turbidity, which is proportional to the amount of antibody nitrogen precipitated, was recorded in arbitrary galvanometric units, as shown in Fig. 5.

The shape of the curve was similar to many other polysaccharide antigen-antibody systems. There was approximately 60 per cent inhibition of precipita-

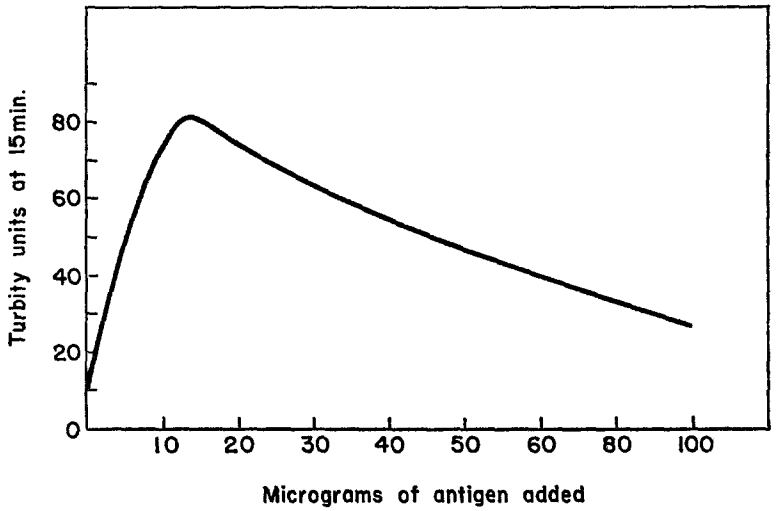

FIG. 5. Turbidimetric precipitin reactions of SSA with Smith antiserum

tion in the region of antigen excess. The results of immunoelectrophoretic studies are presented in Fig. 6.

Electrophoresis of a 0.1 per cent solution of SSA was carried out for 1 and also for 2 hours at a potential of 40 volts. The developing antisera were added and the slides incubated for 18 hours at 25°C.

The antigen moved rapidly to the anode as expected and only one clearly demarcated precipitin band was apparent in the reaction against Smith immune serum. No reaction occurred with normal rabbit serum.

In double diffusion reaction in gel, the antigen (500 μg per ml in 0.15 M sodium chloride) precipitated with undiluted Smith and Stern antiserum, forming a single broad band; these bands formed reactions of identity. At higher antigen concentrations (1 mg per ml) double parallel lines were encountered. The significance of double band formation was unclear. It has been reported that minor structural alterations of carbohydrate antigens such as deacetylation or acetylation will alter the number of bands produced in gel diffusion precipitin tests (22).

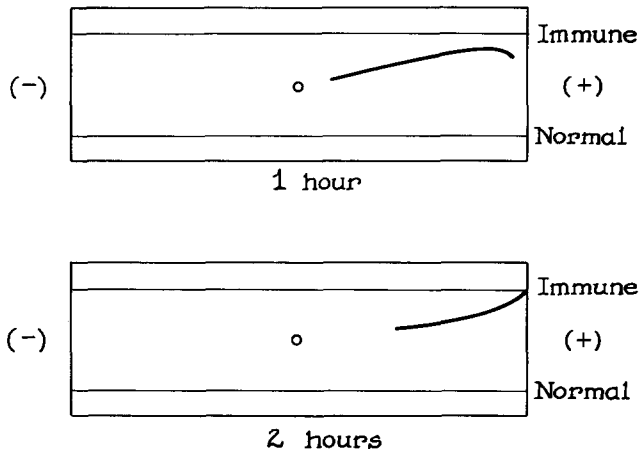

FIG. 6. Immunoelectrophoretic studies of SSA. The antigen was subjected to electrophoresis for 1 hour (top) and 2 hours (bottom). Smith antiserum was then added to both upper troughs and normal rabbit serum to the lower troughs.

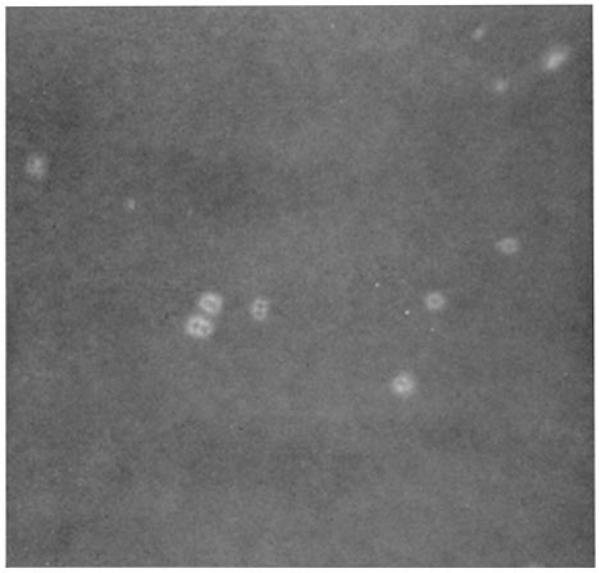

FIG. 7. Cocci from a 4 hour broth culture of *S. aureus* Smith suspended in dilute India ink. × 930.

The Role of SSA as an Agglutinogen.—*S. aureus* Smith grown in broth has a clearly demarcated capsule when examined with dilute india ink under phase microscopy (Fig. 7). However, in contrast to the reports of Wiley (23), no Quellung reaction could be demonstrated with immune serum. Therefore,

inhibition of the Quellung reaction could not be used to demonstrate convincingly the location of SSA on the surface of strain Smith, or its identity with the capsule. In order to show that SSA was indeed on the surface of the organism, agglutination reactions were studied after absorption of Smith antisera by SSA at the equivalence point.

Smith antiserum was diluted 1:5 in 0.15 M saline and 2.5 ml of this dilution was dispensed to each of two lusteroid tubes. 0.15 ml of saline containing 18.75 μg of SSA was added to one and the tube was mixed and kept at 4°C for 18 hours. The precipitate was removed by centrifugation and the supernatant fluid held at 4°C until use (absorbed immune serum). To the other tube 0.15 ml of saline was added and the serum was processed in the same fashion (unabsorbed immune serum). Qualitative precipitin tests performed after absorption revealed that the absorbed immune serum no longer reacted with SSA while the activity of

TABLE III

The Agglutination of S. aureus Smith by Antiserum Absorbed with SSA

Serum	Reciprocal of the final serum dilution					
	50	100	200	400	800	1600
Normal rabbit	0	0	0	0	0	0
Absorbed Smith antiserum	0	0	0	0	0	0
Unabsorbed " "	+++	++++	++++	++++	++	+

* All sera were diluted in 0.15 M sodium chloride containing 10 per cent normal rabbit serum.

the unabsorbed serum was unchanged. The sera were then serially diluted in 10 percent normal rabbit serum to provide complement and agglutination tests were carried out using heat-killed Smith cells as antigen.

Table III indicates that absorption with SSA completely removed the agglutinins in Smith antiserum and supported the contention that SSA was a surface component of the organism.

The Effect of SSA on the Phagocytosis-Promoting Properties of S. aureus Antiserum.—It has previously been suggested that phagocytosis of *S. aureus* Smith was inhibited by antigenic substances surrounding the bacterial cell. Numerous attempts to produce antibodies by the injection of purified SSA into rabbits by a variety of procedures and techniques were unsuccessful. In view of the inability to produce antibodies to the isolated material, an indirect test was utilized to demonstrate the decisive role played by SSA in resistance to *in vitro* phagocytosis.

Rabbit polymorphonuclear leucocytes obtained from glycogen-induced peritoneal exudates were suspended in balanced salt solution containing 10 per cent normal rabbit serum to provide adequate complement. Aliquots of the suspensions were dispensed to roller tubes and various quantities of Smith antiserum absorbed at equivalence with SSA or unabsorbed

immune serum were added. An inoculum of *S. aureus* Smith was then introduced and the number of viable organisms assayed immediately and after 120 minutes of incubation with constant agitation at 37°C. The concentration of leucocytes was 30 million per ml and the leucocyte-bacteria ratio was 1:2–3. The results of one such experiment are presented in Fig. 8.

Significant killing of *S. aureus* Smith did not occur in the presence of immune serum absorbed with SSA. In leucocyte suspensions containing a 1:125 dilution of absorbed serum, only 40 per cent of the organisms were killed. In the pres-

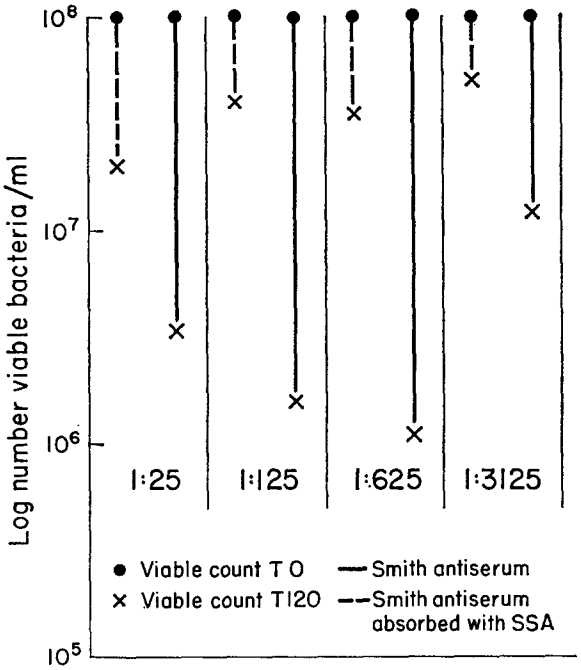

FIG. 8. The fate of *S. aureus* Smith in suspensions of rabbit granulocytes containing various dilutions of unabsorbed and absorbed Smith antiserum.

ence of normal rabbit serum alone, no significant killing of the inoculum occurred.

The survival of *S. aureus* Smith in suspensions of leucocytes containing absorbed serum could be attributable to two mechanisms: (*a*) minute traces of unbound antigen might have been leucotoxic and produced dysfunction of either the phagocytic or bactericidal properties of the leucocytes; or (*b*) opsonizing antibodies may have been removed by absorption. In order to establish which mechanism was operative the following experiments were performed.

The first experiment was designed to determine the localization of surviving organisms after incubation of *S. aureus* Smith with leucocytes in the presence of immune serum.

Rabbit granulocytes were suspended in 10 per cent normal rabbit serum and aliquots were dispensed to three roller tubes. Absorbed immune serum was added to one tube and unabsorbed immune serum to another; the final dilution of each was 1:150. No antiserum was present in the third tube. An inoculum of *S. aureus* Smith was added to each tube, and the

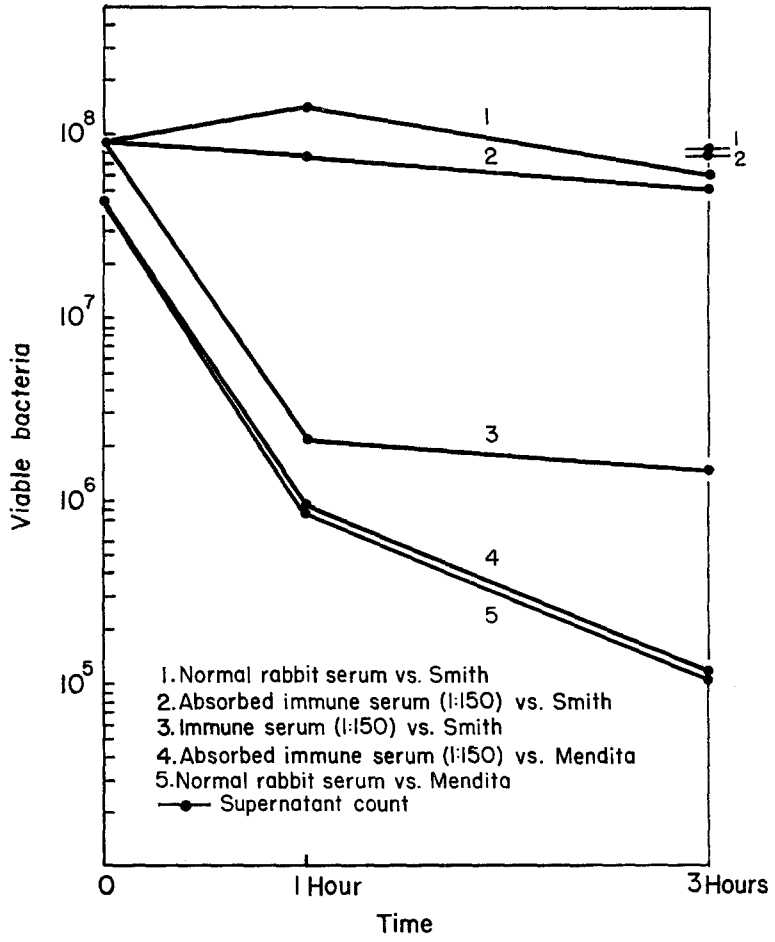

FIG. 9. The fate and localization of *S. aureus* Smith and *S. albus* Mendita in leucocyte suspensions containing Smith antiserum absorbed with SSA.

total number of viable bacteria was assayed during 3 hours of incubation. At the end of the incubation period, the leucocytes were deposited by low speed centrifugation and the viable organisms in the supernatant fluid were enumerated.

As indicated in Fig. 9, virtually no killing of *S. aureus* Smith was noted in the absence of immune serum, or in the presence of absorbed immune serum

(Curves 1 and 2). Furthermore, all of the viable organisms could be accounted for in the extracellular fluid. In contrast, *S. aureus* Smith was efficiently ingested and killed in the presence of unabsorbed immune serum. These results indicated that survival of *S. aureus* Smith in the presence of antiserum absorbed with SSA was not attributable to intracellular viability, but to a lack of phagocytosis.

The question of leucotoxicity was next examined by determining the effect of absorbed antiserum on the phagocytosis and killing of a strain of *S. albus* (Strain Mendita). Curve 4 of Fig. 9 represents the reduction of the number of viable Mendita in the presence of absorbed immune serum and normal rabbit serum alone. It is evident that the rates of bacterial destruction were the same over the course of 3 hours. Similar results were obtained when *Escherichia coli* was used as the test organism. Therefore, absorbed immune serum was not leucotoxic.

Cutaneous Reactions Produced by SSA.—As has previously been indicated, the injection of SSA into rabbits was not followed by the production of precipitating antibody. However, rabbits which had been immunized with heat-killed cells of strain Smith exhibited cutaneous hypersensitivity when small quantities of SSA were inoculated intradermally. Whereas intracutaneous inoculation of 100 μg of SSA into normal rabbits produced no local reaction, the injection of as little as 1 μg into immunized animals was followed by the development of an area of erythema and swelling 1 to 2 cm in diameter (Fig. 10). These reactions were maximal at 8 to 12 hours after injection and gradually receded over the next 48 hours.

The Protective Effect of SSA against Lethal Infection with S. aureus Smith.—Recently, Fisher has described a protective action of the supernatant fluid obtained from a culture of an organism similar to, if not identical with, *S. aureus* Smith (3). Injections of small amounts of the culture supernatant fluid 2 weeks before challenge protected mice against lethal infection with this strain of *S. aureus* suspended in mucin. It was therefore of interest to determine whether the isolated and purified SSA would have the same kind of protective activity.

NCS mice of both sexes weighing between 20 and 22 gm were utilized (24). 12 to 14 days before challenge, they were injected subcutaneously with various quantities of SSA in a volume of 0.2 ml of 0.15 M NaCl. Control mice were injected subcutaneously with physiological saline alone.

The challenge dose was derived from an overnight culture of *S. aureus* Smith which had been diluted in penassay broth and then diluted in 5 per cent mucin (hog gastric mucin Type 1701W, Wilson Laboratories, Chicago) prepared according to the manufacturer's directions. 1 ml of the suspension was injected intraperitoneally. Mice were observed for at least 7 days after injection.

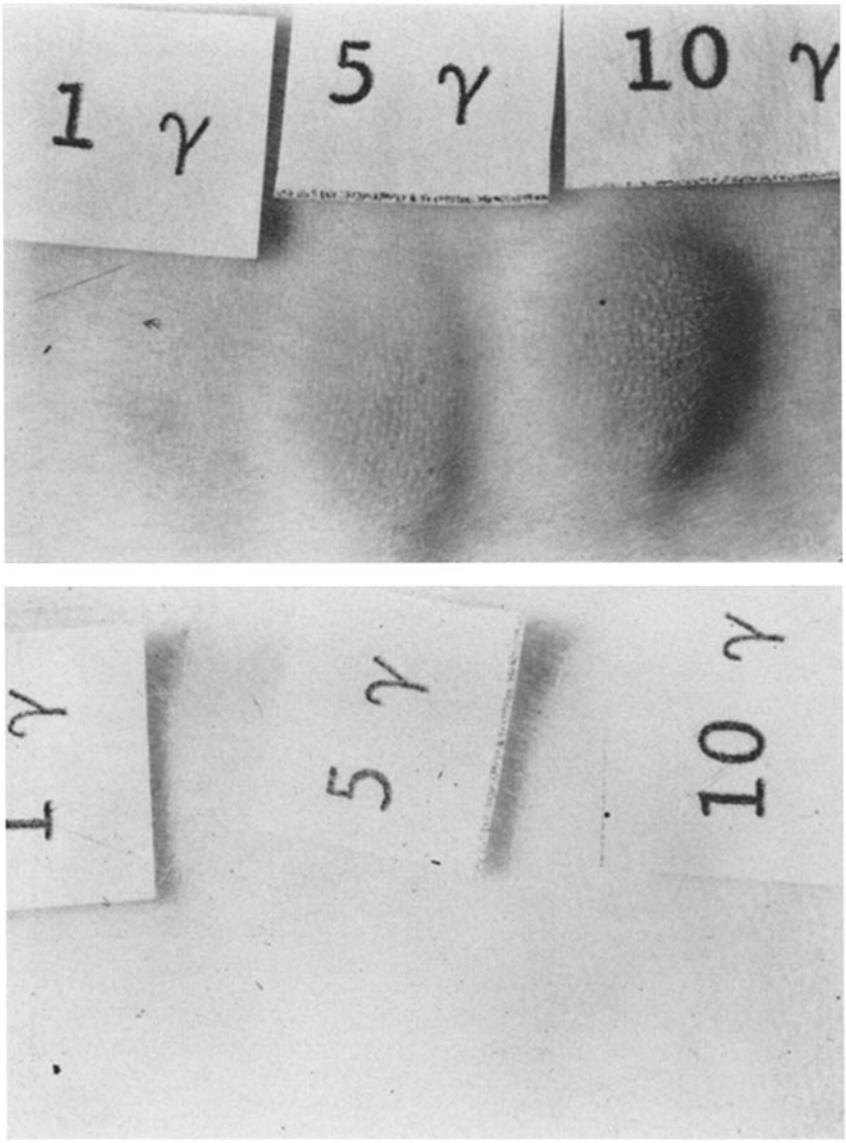

FIG. 10. The cutaneous reactions occurring 3 hours after the intradermal injection of SSA into rabbits immunized with heat-killed strain Smith (top). There was no reaction in normal animals (bottom).

As noted in Table IV, the injection of as little as 0.01 μg of SSA 14 days before challenge resulted in significant protection against an intraperitoneal inoculum of 10^5 organisms in mucin (50 to 100 LD_{50}'s). Increasing the immunizing dose to 10 or 100 μg per mouse, led to a diminished effect when compared with doses between 0.01 and 1.0 μg. This finding of a maximum as well as a minimum protective dose is in accord with mouse protection studies utilizing other purified polysacchride antigens.

An immunizing dose of 0.1 μg afforded maximum protection against the mucin challenge but did not alter the survival rate when mice were challenged

TABLE IV

The Protective Effect of Immunization of Mice with SSA

Immunizing dose of SSA	No. of mice	Survivors*	
		1 day	7 days
μg.			
100	20	11	3
10	20	9	4
1	20	15	12
0.1	20	19	18
0.01	20	14	10
0.001	20	6	0
0‡	40	10	0

* All mice received an intraperitoneal injection of 10^5 viable cells of *S. aureus* Smith suspended in mucin 14 days after immunization.

‡ Injected with saline 2 weeks before challenge.

with 0.2 ml of an overnight broth culture of *S. aureus* Smith (10^8 organisms = *ca.* 5 to 10 LD_{50}'s). It is possible that the large numbers of organisms necessary to produce lethal infection in the absence of mucin results in the rapid production of large amounts of lethal toxin before sufficient numbers of phagocytic cells can enter and kill the bacteria within the peritoneal cavity.

Further studies are in progress in order to determine the specificity of the protection afforded by SSA.

SUMMARY

A technique is described for the isolation and purification of an antigen released into the culture medium by *Staphylococcus aureus* strain Smith.

The antigen was found to be homogeneous when examined by free electrophoresis and analytic ultracentrifugation. Immunologic homogeneity was established by immunoelectrophoresis and quantitative precipitin tests using high titer antiserum prepared against the homologous organism.

Chemical analysis showed that the antigen contained 70 per cent carbohydrate, of which approximately 30 to 35 per cent was believed to be glucosamine. The analytic data suggested that another amino sugar, probably carboxylated, was also present, but extreme lability of this compound to mild hydrolytic procedures has thus far precluded further identification. The remainder of the antigen was composed of alanine, glutamic acid, aspartic acid, lysine, glycine, serine, and threonine. No muramic acid was found.

The chemical and physical data indicate that the antigen described herein is a previously unrecognized component of *Staphylococcus aureus*.

The purified compound was capable of absorbing agglutinating antibody from antiserum prepared against *S. aureus* Smith, indicating that it was a surface component of this encapsulated staphylococcus. It is proposed that the antigen be known as the Smith surface antigen (SSA).

The injection of SSA into rabbits did not produce precipitating antibodies. However, SSA did precipitate at low concentrations (0.5 μg/ml) with antiserum prepared against *S. aureus* Smith and one other strain of *S. aureus* tested. Antiserum against two other *aureus* strains reacted only with high concentrations of SSA. SSA did not react with *S. albus* antiserum or with normal sera from several animal species. Experiments are in progress to define further the distribution of SSA.

Intradermal injection of small quantities of SSA into rabbits immunized with *S. aureus* Smith evoked a reaction of cutaneous hypersensitivity, which was maximal in 8 to 12 hours.

SSA appeared to be the substance responsible for the ability of *S. aureus* Smith to resist engulfment by phagocytes, since absorption of Smith antiserum with SSA effectively removed opsonizing antibodies.

SSA induced protection in mice against experimental staphylococcal disease. The subcutaneous injection of 0.1 μg resulted in protection against a subsequent intraperitoneal challenge with 50 to 100 LD_{50}'s of *S. aureus* Smith suspended in mucin. Increasing as well as decreasing the immunizing dose resulted in significantly less protection.

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