

THE TYPE-SPECIFIC POLYSACCHARIDES  
OF *STREPTOCOCCUS SUIS*\*

BY S. D. ELLIOTT‡ AND JOSEPH Y. TAI

*From The Rockefeller University, New York 10021*

The association of *Streptococcus suis* types 1 and 2 with meningitis in pigs has been the subject of previous communications (1, 2). Alternatively designated group S (type 1) and group R (type 2) (3), these streptococci have attracted renewed attention because type 2 has been identified as a cause of meningitis in man, particularly in men whose employment brings them into association with pigs (4).

*S. suis* constitutes a subgroup in Lancefield's serological group D (1). In this subgroup, two serological types, types 1 and 2, are distinguishable by the immunological specificity of a component thought to be a capsular polysaccharide (2). In bactericidal tests, well capsulated strains grow readily in heparinized porcine and human blood. Such growth is inhibited by phagocytosis in the presence of type-specific antibody directed against the capsule (5). It was, therefore, of interest to determine whether vaccination with capsular polysaccharide would elicit the production of type-specific antibodies in pigs. If this were so, such vaccination might protect against infection with homologous streptococci.

In this report we describe the extraction and purification of capsular polysaccharides from *S. suis* types 1 and 2. We also present the results of a chemical and serological examination of these substances. A later paper will describe an investigation into the immunogenicity of type 2 polysaccharide in pigs.

Materials and Methods

*Streptococcal Strains.* Strains A228 and D928 belong to *S. suis* type 1 and were isolated from piglets with meningitis. Strain D930 belongs to *S. suis* type 2 and has been previously described (6).

*Streptococcal Antisera.* Hyperimmune rabbit antisera were prepared with formalized vaccines by a method previously described (7). Serum R800 was raised against strain A228 (type 1), serum R5037 against strain D930 (type 2), and serum R1647 against strain D76 (*Streptococcus faecalis*).

*Precipitin Tests.* These were carried out using the capillary technique (8).

*Analytical Methods.* The protein content of the purified types 1 and 2 polysaccharides was determined by amino acid analyses. Polysaccharide (1 mg) was hydrolyzed in 4 N methane sulfonic acid (9) and the analysis was performed on a Durrum D-500 automatic amino acid analyzer using a one-column system (Durrum Instrument Corp., Sunnyvale, Calif.). Sugar analyses were performed by gas-liquid chromatography as described by Clamp et al. (10). Polysaccharide (0.1 mg) was hydrolyzed in 1.5 N methanolic HCl at 80°C overnight, and the monosaccharides were analyzed as the trimethylsilyl ethers on a Varian model 3700 gas-liquid

\* Supported by U.S. Public Health Service grant HL-03919 and by a bequest from the Hochschild Foundation.

‡ Present address: Department of Pathology, Cambridge, England.

chromatography unit equipped with a CDS-111 integrator (Varian Associates, Instrument Div., Palo Alto, Calif.). Appropriate sugar standards were hydrolyzed and analyzed using identical conditions. Free amino groups of the polysaccharides were determined by trinitrophenyl sulfonic acid (TNBS) as described by Fields (11).

## Results

*Isolation of Capsular Polysaccharides from S. Suis Types 1 and 2.* *S. suis* was found to be susceptible to the lytic action of lysozyme which resulted in the release of capsular polysaccharide from both types 1 and 2. A 75–80% lysis of the cells was observed after 6–8-h incubation with lysozyme at 37°C. We describe in this paper the procedure followed in a typical experiment for the extraction of capsular polysaccharide from type 2. The same procedure was used for the isolation of type 1 capsular polysaccharide.

*S. suis* type 2 (strain D930) was grown for 18 h at 37°C in 5 liters of Todd-Hewitt broth. The cocci were harvested by centrifugation, washed once in saline, and resuspended in 100 ml glycine buffer (0.1 M, pH 9.2) containing 10 mg crystalline, salt-free egg-white lysozyme (Worthington Biochemical Corp., Freehold, N. J.). Sodium azide in a final concentration of 0.02% was added to inhibit the growth of contaminants. The streptococcal suspension was then incubated for 6–8 h at 37°C with continuous mixing. During this time most of the capsular material, together with nucleic acid and other streptococcal components, was released into the supernatant fluid. After incubation of the lysozyme digest, the residual streptococcal debris was separated by centrifugation. From the supernatant fluid, nucleic acid was then removed by precipitation with ethanol (25% vol/vol) and CaCl<sub>2</sub> (0.1 M). Finally the capsular polysaccharide was precipitated from solution by increasing the ethanol concentration to 80% vol/vol. The precipitate was dissolved in 20–30 ml H<sub>2</sub>O and lyophilized after dialysis against H<sub>2</sub>O.

The dry polysaccharide was dissolved in 1/30 M phosphate buffer pH 8.0 and purified further by column chromatography on Sepharose 6B (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.) and eluted with the same buffer. Phenol sulfuric acid assay (12) and capillary precipitin tests with appropriate rabbit antisera were used to identify the capsule polysaccharides and the group D teichoic acid (Fig. 1 and 2). It can be seen that the polysaccharide passed through the column unimpeded and was thereby separated from other streptococcal components including the Group D glycerol teichoic acid. The eluted capsular polysaccharide was dialyzed exhaustively against distilled water and then lyophilized. The final yield was 180 mg of type 2 polysaccharide from 5 liters of culture. For type 1 (strain D928), the yield was 60 mg polysaccharide from 5 liters of culture.

*Characterization of Types 1 and 2 Capsular Polysaccharides.* The purity of the isolated types 1 and 2 capsular polysaccharides was indicated by chemical and serological analyses. Type 1 polysaccharide was found to contain <0.1% of nucleic acid by 260 nm absorption and 0.3% of amino acids; type 2 also had <0.1% of nucleic acid and 0.49% of amino acids. Type 1 polysaccharide is composed of five sugars: galactose, glucose, *N*-acetyl glucosamine, *N*-acetyl galactosamine, and sialic acid in a molar ratio of 2.42:1.00:1.00:1.13:1.39 (Table I). Type 2 polysaccharide was found to differ only in one sugar: rhamnose substitutes for *N*-acetyl galactosamine which is absent in type 2. The type 2 polysaccharide contains rhamnose, galactose, glucose, *N*-acetyl glucosamine, and sialic acid in a molar ratio of 1.07:3.17:1.00:0.94:1.00 (Table I). Quan-

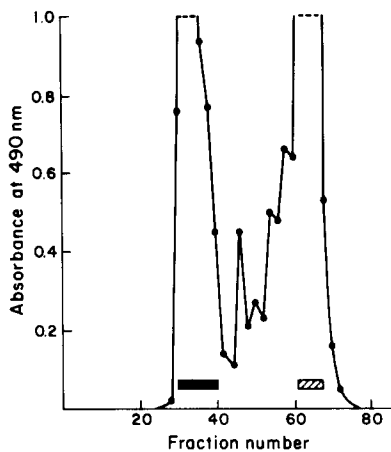


FIG. 1. Purification of *S. suis* type 1 polysaccharide on Sepharose 6B column (2.5 × 180 cm) in M/30 PO<sub>4</sub> buffer pH 8.0. Polysaccharides were analyzed by phenol-sulfuric acid assay. Solid and hatched bars indicate the capillary precipitin test against type 1 and teichoic acid antisera, respectively.

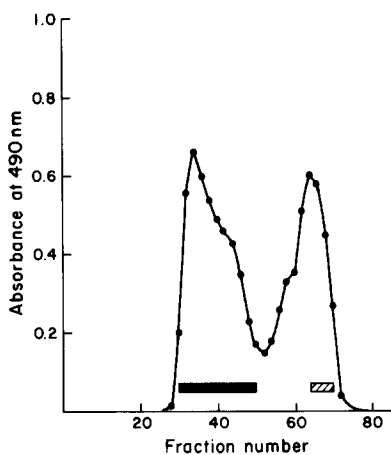


FIG. 2. Purification of *S. suis* type 2 polysaccharide on Sepharose 6B column (2.5 × 180 cm) in M/30 PO<sub>4</sub> buffer pH 8.0. Polysaccharides were analyzed by phenol-sulfuric acid assay. Solid and hatched bars indicate the capillary precipitin test against type 2 and teichoic acid antisera, respectively.

TABLE I  
Chemical Compositions of *Streptococcus Suis* Type 1 and Type 2 Capsular Polysaccharides

Composition	Type 1			Type 2		
	mg/100 mg	μmol/100 mg	molar ratio	mg/100 mg	μmol/100 mg	molar ratio
Rhamnose	—	—	—	11.14	76	1.07
Galactose	26.27	162	2.42	36.46	225	3.17
Glucose	10.89	67	1.00	11.53	71	1.00
<i>N</i> -acetyl gluco- samine	13.69	67	1.00	13.58	67	0.94
<i>N</i> -acetyl galac- tosamine	15.39	76	1.13	—	—	—
Sialic acid	26.94	93	1.39	20.61	71	1.00

TABLE II  
*Serological Reactivity of Purified Capsular Polysaccharide*

Polysaccharide		Precipitin reactions with rabbit antisera against		
Source	Concentration	Type 1 (R800)	Type 2 (W5037)	Group D (R1647)
	<i>mg/ml</i>			
Type 1, strain D928	1.0	++	-	-
	0.01	+	-	-
Type 2, strain D930	1.0	-	++	-
	0.01	-	+	-

titative free amino group determinations with TNBS indicated that  $\cong 23\%$  of the amino sugars in type 1 polysaccharide is present as glucosamine and galactosamine and 25% of the amino sugar in type 2 polysaccharide is nonacetylated glucosamine. Type 1 polysaccharide appears to be larger than type 2 polysaccharide as indicated by chromatography on Sepharose 4B columns (13). Type 1 polysaccharide gave a  $K_d$  value of 0.074, whereas type 2 polysaccharide had a  $K_d$  value of 0.185.

*Serological Reactivity of the Purified Capsular Polysaccharide.* Table II shows the reactivity of the purified capsular polysaccharides with streptococcal antisera of homologous and heterologous type. Tested with hyperimmune rabbit antisera raised against *S. suis* type 1 (serum R800) and type 2 (serum W5037), the polysaccharides in a concentration of 0.01 mg/ml showed immediate precipitation when mixed with antisera of homologous type. In concentrations up to 1.0 mg/ml, neither polysaccharide showed any reaction with serum of heterologous type or with group D antiserum (R1647) raised against strain D76 (*S. faecalis*).

### Discussion

Our examination of the supernatant fluid from lysozyme digests of *S. suis* suggests that the two main components released by lysis of the streptococci are nucleic acid and capsular polysaccharide. We have also detected in digest fluids glycerol teichoic acid, the group D antigen, although much of this component remained with the insoluble streptococcal debris from which it could be extracted by aqueous phenol. So far, we have found in the digest supernates of capsulated strains no counterpart for the cell-wall carbohydrates that contribute to the structure of most hemolytic streptococci. The capsular polysaccharides of *S. suis* are readily distinguishable from typical streptococcal cell-wall carbohydrates by their high molecular weight and lability in hot acid. The behavior of these capsular polysaccharides on Sepharose columns suggests molecular weights of at least 100,000. The loss of serological activity that occurs when the type 2 polysaccharide is heated in HCl to 100°C at pH 2 is in striking contrast to the resistance shown by typical wall carbohydrates to such treatment (2, 3). Our failure to detect wall carbohydrate by serological tests may result from lack of a suitable antiserum. We have detected in type 1 digests a small amount of rhamnose which, because it is not part of the type 1 capsule, may have originated in a cell-wall carbohydrate. This possibility is under investigation.

It is of interest to compare the capsular type-specific polysaccharides of *S. suis*, the causal agent in porcine neonatal meningitis, with the type polysaccharides of group B streptococci responsible for human neonatal meningitis. In *S. suis*, the polysaccharides are readily detached from the cocci by digestion with lysozyme. Group B

streptococci are resistant to lysozyme, but they are lysed with the releases of type-specific polysaccharide by digestion with muralytic enzymes from *Streptomyces albus* (14). In group B streptococci and in *S. suis*, individual-type polysaccharides are each composed of four or five different sugars (14-16) of which glucose, galactose, *N*-acetyl glucosamine, and sialic acid are common to all. *S. suis* type 1 streptococci elicit in pigs antibodies that protect against infection type specifically (5), but this has yet to be confirmed with type 2. Group B streptococci induce in rabbits antibodies that protect mice type specifically (17); some of the group B type-specific polysaccharides appear capable of eliciting antibodies with more than one homologous specificity directed against different parts of the same molecule (14, 15).

### Summary

*Streptococcus suis* types 1 and 2 were subjected to digestion with lysozyme. Serologically type-specific capsular polysaccharides were isolated from the lysates by ethanol precipitation followed by Sepharose 6B chromatography. The purified type 1 polysaccharide has a  $K_d$  value of 0.074 on a Sepharose 4B column and contains galactose, glucose, *N*-acetyl glucosamine, *N*-acetyl galactosamine, and sialic acid in a molar ratio of 2.42:1.00:1.00:1.13:1.39. The type 2 polysaccharide has a  $K_d$  value of 0.185 and is composed of rhamnose, galactose, glucose, *N*-acetyl glucosamine, and sialic acid in a molar ratio of 1.07:3.17:1.00:0.94:1.00. A comparison is drawn between the type polysaccharides of *S. suis* and those of group B streptococci.

Dr. Elliott wishes to thank Dr. Maclyn McCarty for the hospitality of his department.

Received for publication 8 September 1978.

### References

1. Elliott, S. D. 1966. Streptococcal infection in young pigs. I. An immunochemical study of the causative agent (PM streptococcus). *J. Hyg.* **64**:205.
2. Windsor, R. S., and S. D. Elliott. 1975. Streptococcal infection in young pigs. IV. An outbreak of streptococcal meningitis in weaned pigs. *J. Hyg.* **75**:69.
3. Moor, C. E. de. 1963. Septicaemic infection in pigs caused by haemolytic streptococci of new Lancefield groups designated R, S, and T. *Antonie van Leeuwenhoek J. Microbiol. Serol.* **29**:272.
4. Agass, M. J. B., C. P. Willoughby, A. J. Bron, C. J. Mitchell, and R. T. Mayon-White. 1977. Meningitis and endophthalmitis caused by *Streptococcus suis* type II (group R). *Br. Med. J.* **2**:167.
5. Agarwal, K. K., S. D. Elliott, and P. J. Lachman. 1969. Streptococcal infection in young pigs. III. The immunity of adult pigs investigated by the bactericidal test. *J. Hyg.* **67**:507.
6. Elliott, S. D., M. McCarty, and R. C. Lancefield. 1977. Teichoic acids of group D streptococci with special reference to strains from pig meningitis. *J. Exp. Med.* **145**:490.
7. Elliott, S. D. 1960. Type and group polysaccharides of group D streptococci. *J. Exp. Med.* **111**:621.
8. Swift, H. F., A. T. Wilson, and R. C. Lancefield. 1943. Typing group A haemolytic streptococci by M precipitin reactions in capillary pipettes. *J. Exp. Med.* **78**:127.
9. Simpson, R. J., Neuberger, M. R., and T.-Y. Liu. 1975. Complete amino acid analysis of proteins from a single hydrolysate. *J. Biol. Chem.* **251**:1936.
10. Clamp, J. R., T. Bhatti, and R. E. Chambers. 1972. The examination of carbohydrate in glycoproteins by gas-liquid chromatography. *Glycoproteins.* **5**:300.

11. Fields, R. 1972. The rapid determination of amino group with TNBS. *Methods Enzymol.* **25**(part B):464.
12. Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28**:350.
13. Ackers, G. K. 1964. Molecular exclusion and restricted diffusion processes in molecular-sieve chromatography. *Biochemistry.* **3**:723.
14. Tai, J. Y., E. C. Gotschlich, and R. C. Lancefield. 1979. Isolation of type-specific polysaccharide antigen from group B type 1b streptococci. *J. Exp. Med.* In press.
15. Kane, J. A., and W. W. Karakawa. 1978. Existence of multiple immunodeterminants in the type-specific capsule substance of group B type Ia streptococci. *Infect. Immun.* **19**:983.
16. Baker, C. J., D. L. Kasper, and C. E. Davis. 1976. Immunochemical characterization of the "native" type III polysaccharides of group B streptococcus. *J. Exp. Med.* **143**:258.
17. Lancefield, R. C., M. McCarty, and W. N. Everly. 1975. Multiple mouse-protective antibodies directed against group B streptococci. *J. Exp. Med.* **142**:165.