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Characterization of Neuraminidases Produced by Various Serotypes of Group B Streptococci

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Neuraminidase produced by 11 strains of group B streptococci (GBS), from serotypes Ia, Ib, Ic, II, and III, were characterized according to molecular weight, antigenic identity, and substrate specificity. Following growth in a chemically defined medium, ammonium sulfate-concentrated culture supernatants were assayed for activity with bovine submaxillary mucin as substrate. Neuraminidase produced by GBS strain 122 (serotype III) was purified by a combination of salt fractionation, affinity chromatography with Affi-Gel Blue, ion-exchange chromatography with DEAE-cellulose, and gel filtration on Sephadex G-200. Purified neuraminidase was used to immunize rabbits, and the resultant antiserum reduced the activity of purified neuraminidase from strain 122 by 87.7%. The antiserum also reduced the activity of neuraminidases produced by the other four serotypes by between 78.3 and 90%. Molecular weight estimates of the neuraminidases produced by the various serotypes were obtained by gel filtration chromatography on Sephadex G-200. The molecular weights obtained for the neuraminidases from the representative strains of each serotype ranged from 110,000 to 180,000. In addition, all of the GBS neuraminidases examined (regardless of the producing serotype) were active only on bovine submaxillary mucin. On the basis of these results, it appears that the neuraminidases produced by different GBS serotypes are quite similar.

During the past two decades, the group B streptococci (GBS) have emerged as important pathogens in the human host and are most noted for their capacity to produce infection, including septicemia and meningitis, in neonates and young infants (1, 3, 11). Many investigators have studied the production of extracellular products by these organisms in the hope of gaining insight into the mechanism by which strains of GBS are virulent for the human host. Consequently, a variety of extracellular products of GBS have been described, including an oxygen-stable, nonimmunogenic hemolysin (29), hyaluronidase (21), DNase (12), CAMP factor (6), proteases (28), extracellular type-specific antigen (9), and a carbohydrate toxin (17). The production of extracellular neuraminidase (sialidase) was first demonstrated in 1969 (16) with a sialomucoid preparation from bovine submaxillary mucin (BSM).

The enzyme neuraminidase has been implicated as a virulence factor in bacteria such as *Corynebacterium diphtheriae*, *Vibrio cholerae*, and *Streptococcus pyogenes* which are able to survive on mucosal surfaces (26). Gottschalk (15) demonstrated that the removal of sialic acid (SA) residues from salivary glycoproteins hindered the protective role of these secretions against invading microorganisms. Therefore, neuraminidase appeared to enhance the survival of these microorganisms. Similarly, it was found that all of 77 clinical pneumococcal isolates produced neuraminidase, whereas only 7 of 15 laboratory-adapted strains were able to do so (19), again suggesting that neuraminidase activity might be necessary for the survival of pneumococci in the host. In an examination (22) of a large number of strains of GBS for the production of neuraminidase, serotype III strains isolated from infants with invasive disease were found to be more capable of producing elevated levels of neuraminidase than were non-type-III strains from diseased infants or serotype III strains from asymptomatically colonized infants. In a subsequent study (23), the fundamental characteristics of the extracellular neuraminidase of type III GBS were examined. The results indicated that the type III (strain 110) enzyme differed from other bacterial neuraminidases in that it possessed a limited substrate specificity, since it was found to be active only against BSM. In addition, purified neuraminidase (strain 110) was found to possess a higher molecular weight (125,000), following chromatography on Sephacryl S-200, than those of neuraminidases produced by other bacteria, including *V. cholerae* (68,000) (10), *Clostridium perfringens* (56,000) (8), and *S. pneumoniae* (88,000) (10).

The majority of GBS neonatal infections in the United States have been shown to be due to type III strains (1, 3, 11). Since type III strains are capable of elaborating very high levels of neuraminidase (22), we thought that it would be interesting to further examine the characteristics of this enzyme. Specifically, we were interested in determining whether the type III neuraminidase differs in some way from enzymes produced by non-type-III strains. We therefore examined the substrate specificity, molecular weight, and antigenic identity of neuraminidase produced by several different GBS serotypes.

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. Eleven strains of GBS, from serotypes Ia (strains 158 and 161), Ib (strains 132 and 136), Ic (strains 165 and 172), II (strains 155 and 170), and III (strains 122, 126, and 178), were used. Strain 170 was classified as a nonproducer of the enzyme neuraminidase (<10 nmol of SA released per min per mg [dry weight] of cell); strains 158, 155, 161, and 172 were classified as low producers (>10 to ≤140 nmol of SA released per min per mg [dry weight] of cell); and strains 122, 126, 132, 136, 165, and 178 were classified as high producers (≥140 nmol of SA released per min per mg [dry weight] of cell) (22). These strains were kindly supplied by Carol J. Baker, Baylor College of Medicine. Their source of isolation, total neuraminidase production, and disease-producing

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TABLE 1. Release of SA from various substrates by neuraminidases produced by either *C. perfringens* or GBS strain 122

Substrate	α-Ketosidic linkage present	Enzyme activity[a]		
		C. perfringens neuraminidase[b]	GBS type III neuraminidase[b,c]	Acid hydrolysis[d]
BSM	2,6 SA to *N*-acetylgalactose	0.12	7.20	1.00
Colominic acid	2,8 *N*-acetylneuraminosyl residues	0.80	ND[e]	0.33
α_1-Acid glycoprotein	2,3 and 2,6 SA to galactose	0.31	ND	11.70
Fetuin	2,6 SA to galactose	0.14	ND	0.75
N-Acetylneuramin-lactose	2,6 and 2,3 (85%) SA to lactose	0.10	ND	1.00

[a] Each assay mixture contained substrate (100 μg of colominic acid, fetuin, α_1-acid glycoprotein, or *N*-acetylneuramin-lactose or 1 mg of BSM), 1 M sodium acetate (pH 6.5), and various amounts of neuraminidase (2 to 50 μl).

[b] Activity is expressed as micromoles of SA released per minute per milliliter.

[c] Stage I preparation of GBS strain 122 was used.

[d] Hydrolysis conditions were 0.025 M H_2SO_4 for 1 h at 80°C. BSM was used at 200 μg, while all other substrates were used at 20 μg. Activity is expressed as micromoles of SA released divided by micromoles of SA equivalents. The number of SA equivalents present was determined by using the formula weight of *N*-acetylneuraminic acid (309.1) and the percentage of SA found in each substrate.

[e] Activity was less than 0.005 μmol of SA per min per ml.

capacity were described in an earlier publication (22). The preparation of the chemically defined medium (FMC) containing human serum albumin and the procedures used for the growth of cultures and quantitation of bacterial mass were described previously (22, 24).

Purification of the extracellular neuraminidase produced by strain 122 and molecular weight estimates of the neuraminidases produced by different GBS serotypes. The extracellular neuraminidase of GBS strain 122 was purified by the procedure of Milligan et al. (23), including ammonium sulfate fractionation to 75% (stage I) and 40 to 60% (stage II), affinity chromatography with Affi-Gel Blue (Bio-Rad Laboratories) (stage III), and ion-exchange chromatography on DEAE-cellulose (stage IV). Gel chromatography on Sephadex G-200 (2.5 cm by 90 cm) (Pharmacia Fine Chemicals) was performed to obtain a molecular weight estimate for neuraminidase (stage V) produced by strain 122 as well as molecular weight estimates for neuraminidases produced by representative strains of serotype Ia (strain 158), serotype Ib (strain 132), serotype Ic (strain 165), serotype II (strain 155), and serotype III (strain 178). Elution volumes (V_e) were determined for aldolase (158,000), chymotrypsinogen (25,000), and ribonuclease (13,700) by recording the volume at which these proteins displayed maximal A_{280}. Partition coefficients (K_{av}) were then determined for the eluted peaks of each standard protein in addition to the peak of neuraminidase activity. The molecular weight of neuraminidase was estimated by interpolation from a graph of K_{av} versus $\log_{10}$ molecular weight.

Neuraminidase assays and analytical PAGE. The amount of neuraminidase activity present in concentrated culture supernatant fluids or purified preparations was quantitated by measuring the amount of SA released from BSM as previously described (24). In addition to BSM, we used various other substances with known α-ketosidic linkages, including *N*-acetylneuramin-lactose, colominic acid, fetuin, and α_1-acid glycoprotein (Sigma Chemical Co.). The various substrates were tested for the release of SA either in the presence of GBS neuraminidase, by acid hydrolysis, or by neuraminidase produced by *C. perfringens* (Sigma). Protein determinations were performed in duplicate by the method of Lowry et al. (20), in which bovine serum albumin (Sigma) was used as the standard. Samples corresponding to the various stages of the GBS strain 122 neuraminidase purification scheme were analyzed on 7% analytical polyacrylamide gels as previously described (23). For localization of the neuraminidase activity after electrophoresis, 0.5-cm sections of the gel were placed in dialysis tubing. After the addition of 0.5 ml of 100 mM sodium citrate (pH 6.5) and gentle mashing of the gel matrix, these sections were dialyzed against 100 volumes of the same buffer for 24 h. Samples of the dialysis bag contents were then assayed for neuraminidase activity (24), and purified neuraminidase preparations and reference proteins were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (PAGE) as previously described (23).

Preparation of antiserum to stage IV neuraminidase produced by GBS strain 122 and neuraminidase neutralization studies. New Zealand White rabbits were injected subcutaneously with 1 ml (180 μg) of the stage IV enzyme preparation mixed with an equal volume of Freund complete adjuvant (Difco Laboratories). In addition, 500 μl of the enzyme preparation was administered intravenously via the marginal ear vein three times a week during the first 2 weeks of the immunization regimen. Two weeks following the first subcutaneous immunization, rabbits were injected subcutaneously with 1 ml of the stage IV preparation in an equal volume of Freund incomplete adjuvant. Rabbit serum was separated from clotted blood by a method previously described (7).

The presence of neutralizing antibody to stage IV neuraminidase produced by strain 122 as well as to stage I neuraminidases produced by the various other GBS strains was determined by using the neuraminidase assay (24). The various enzyme preparations were incubated with either 0.5 ml of nonimmune serum or 0.5 ml of immune serum in the presence of sodium citrate (100 mM, pH 6.1). Neuraminidase activity was assayed immediately. The mixtures were incubated at room temperature for 30 min and then at 4°C overnight. Following incubation, the percent reduction in neuraminidase activity was determined by performing the neuraminidase assay (24) and comparing the difference in activities between the pre- and postincubation assays.

RESULTS

Substrate specificity of neuraminidases produced by strains of different GBS serotypes. The substrate specificity of neuraminidase produced by strain 122 (serotype III), utilizing compounds of known SA linkages (Table 1), was found to be quite limited. Only BSM was found to be susceptible to digestion (7.20 μmol of SA released per min per ml). Activities against colominic acid, fetuin, α_1-acid glycoprotein, and *N*-acetylneuramin-lactose were found to be less than 0.005 μmol of SA released per min per ml. Acid hydrolysis of these substrates (Table 1) indicated that each contained SA. In addition, each of these compounds was

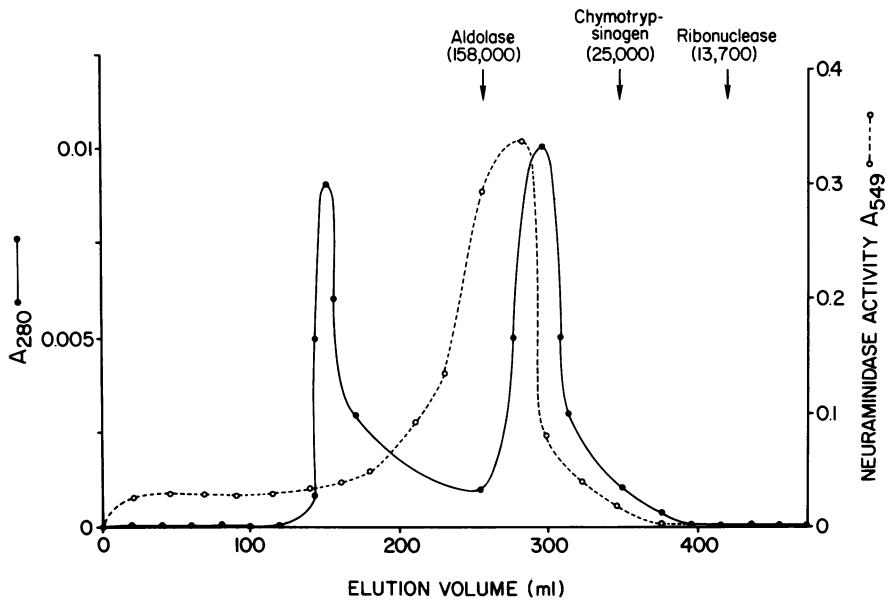

FIG. 1. Sephadex G-200 gel filtration of stage IV neuraminidase from GBS strain 122. Stage IV enzyme was applied to the column and eluted with 100 mM sodium citrate (pH 6.1)–100 mM NaCl. Every fifth fraction was collected, lyophilized, dialyzed against 100 mM sodium citrate (pH 6.1), and assayed for enzyme activity as previously described (24).

found to be susceptible to digestion by neuraminidase produced by *C. perfringens*. In an examination of the substrate specificities of neuraminidase produced by other type III strains (178 and 126) and strains of serotypes Ia, Ib, Ic, and II, once again only BSM was found to be susceptible to digestion. Activities against this substrate differed considerably among strains of different serotypes (Ic and II) and between strains of similar serotypes, ranging from less than 5 to 392 nmol of SA released per min per mg [dry weight] of cell. The activities for strains 161, 158, 132, 136, 172, 165, 170, 155, 178, and 126 were 0.11, 0.15, 0.86, 0.56, 0.26, 0.73, 0 (none detected), 0.19, 1.20, and 1.09 nmol of SA released per min per ml, respectively. Activities against colominic acid, α_1-acid glycoprotein, fetuin (type III), and *N*-acetylneuramin-lactose for all of these strains were found to be less than 0.005 μmol of SA released per min per ml.

Purification and molecular weight estimate of the neuraminidase produced by GBS strain 122. The banding pattern obtained following PAGE of the various stages of the GBS strain 122 neuraminidase preparations were essentially the same as those obtained previously for GBS strain 110 (23). The stage IV GBS neuraminidase preparation possessed a specific activity of 68.3 μmol of SA released per min per mg of protein. PAGE of this material indicated that it contained traces of human serum albumin and a slow-running diffuse band. All regions of this broad protein band contained active enzyme upon elution of a corresponding region from an unstained gel (23). Analysis of the stage IV neuraminidase preparation from GBS strain 122 on sodium dodecyl sulfate-PAGE demonstrated the presence of a large protein with a molecular weight of 110,000 (data not shown).

Molecular weight estimation on Sephadex G-200 of neuraminidase produced by GBS strain 122. The stage IV enzyme (strain 122) preparation was chromatographed on a previously calibrated column of Sephadex G-200 to obtain a molecular weight estimate. Figure 1 shows the elution of both protein (A_{280}) and neuraminidase activity (A_{549}). The elution profile shown in this figure demonstrates that neither of the two major protein peaks coeluted with the neuraminidase activity. This suggests that the enzyme protein is not responsible for the major protein peaks detected by measuring A_{280}. A plot of the $\log_{10}$ molecular weight of proteins with known molecular weight against their respective partition coefficients (K_{av}s) was made, and by using the K_{av} of the maximal peak of neuraminidase activity, the molecular weight of neuraminidase produced by strain 122 was estimated to be 120,000.

Molecular weight estimation of neuraminidases produced by other GBS serotypes. Stage III neuraminidase preparations from serotypes Ia (strain 158), Ib (strain 132), Ic (strain 165), II (strain 155), and III (strain 178) were chromatographed on Sephadex G-200 to obtain molecular weight estimates for each. The molecular weights of neuraminidases produced by strains 155 (serotype II), 178 (serotype III), 158 (serotype Ia), 165 (serotype Ic), and 132 (serotype Ib) were 120,000, 125,000, 145,000, 145,000, and 180,000, respectively.

Stage III neuraminidase from strain 122 was chromatographed on Sephadex G-200 to determine whether the molecular weight was altered by the purification procedure which produced the stage IV preparation. The elution of enzyme activity from Sephadex G-200 in the stage III preparation was quite similar to that obtained for stage IV. The molecular weight estimate obtained for the stage IV enzyme preparation (strain 122) was 120,000, and the estimate obtained for neuraminidase in the stage III preparation was 110,000.

Neutralization of neuraminidases produced by different GBS serotypes. The stage IV (strain 122) preparation was used to immunize New Zealand White rabbits. Following immunization, serum samples were assayed for the presence of neutralizing antibody to the stage IV enzyme preparation. In the presence of immune serum, an 87.7% reduction in enzyme activity of the stage IV preparation was observed (0.61 ± 0.09 μmol of SA released per min per ml preincubation versus 0.08 ± 0.02 μmol of SA released per min per ml postincubation; Table 2). A similar reduction (91.5%) in

TABLE 2. Antibody neutralization of neuraminidase produced by GBS strain 122 or *C. perfringens*

Enzyme preparation[c]	Nonimmune fraction[a]			Immune fraction[b]		
	Preincubation activity[d]	Postincubation activity[d]	% Reduction[e]	Preincubation activity[d]	Postincubation activity[d]	% Reduction[e]
Stage I	1.17 ± 0.10	0.95 ± 0.06	18	1.26 ± 0.24	0.12 ± 0.02	91.5
Stage IV	0.70 ± 0.13	1.00 ± 0.15		0.61 ± 0.09	0.08 ± 0.02	87.7
C. perfringens neuraminidase	0.12 ± 0.01	0.16 ± 0.01		0.19 ± 0.01	0.17 ± 0.01	8.1

[a] Nonimmune fractions were added to the incubation mixture and contained 0.5 mg of protein.
[b] Immune fraction were obtained following immunization of New Zealand White rabbits with stage IV neuraminidase from GBS strain 122; 0.5 mg of protein was added to the incubation mixture.
[c] Each preparation contained 0.066 μmol of SA released per min.
[d] Neuraminidase activity was expressed as micromoles of SA released per minute per milliliter. Immune or nonimmune serum was incubated with the enzyme preparation, and activity was assayed for immediately with BSM as substrate and following overnight incubation. Activities represent an average of two separate experiments.
[e] The percent reduction in activity was obtained by comparing neuraminidase activities before and after overnight incubation in the presence of either nonimmune or immune serum.

neuraminidase activity was observed when the stage I enzyme preparation from GBS strain 122 was incubated in the presence of immune serum. A similar experiment was performed with neuraminidase produced by *C. perfringens*. However, compared with the reduction in GBS neuraminidase activity observed as a consequence of incubation with immune serum (either stage I or stage IV produced by strain 122), only minimal reduction in activity occurred (8.11%).

The stage I neuraminidase preparations obtained from representative strains of serotypes Ia (strains 161 and 158), Ib (strain 132 and 136), Ic (strains 165 and 172), II (strain 155), and III (strains 178 and 126) were also incubated with either nonimmune or immune serum to stage IV (strain 122) neuraminidase and then assayed for activity. In the presence of immune fractions, the percent reduction in neuraminidase activity of stage I preparations obtained from each of these strains (Table 3) ranged from 78.3% (serotype Ib, strain 136) to 90.0% (serotype III, strain 126). The percent reduction in neuraminidase activity between the pre- and postincubation assays was quite similar, not only among strains of similar serotypes, but also between strains of different serotypes.

DISCUSSION

The purpose of this study was to determine whether the neuraminidases produced by GBS strains were similar with regard to molecular weight, substrate specificity, and antigenic identity. We had previously suggested that the ability to produce elevated levels of neuraminidase might be related to disease among neonates (22). However, in that study, we used a method for producing the enzyme that had been developed with type III strains. Our concern was that we may have been influencing our results by using an assay that was optimal for serotype III enzyme but not for the enzymes produced by other serotypes. Therefore, we attempted to examine the relationship between the GBS serotype III neuraminidase and the neuraminidases produced by the other serotypes.

The first parameter examined was that of substrate specificity. With compounds of known α-ketosidic linkages, neuraminidase produced by GBS strain 122 was found to possess a limited substrate specificity, since the release of SA by this enzyme was demonstrated only when BSM was used (Table 1). These results are similar to and an extension of those obtained in a previous study (24), in which neuraminidase produced by a serotype III strain (strain 110) was active only on BSM but not on human α_1-acid glycoprotein.

Similar substrate specificities were observed for all the neuraminidases produced by the various serotypes. The limited substrate specificity demonstrated by the GBS neuraminidase is interesting, since it differs from that observed for neuraminidase produced by a variety of other bacteria including *V. cholerae* (10), *S. pneumoniae* (18), *Arthrobacter* species (14), and *C. perfringens* (10), which have been shown to cleave SA from a variety of different substrates including the human α_1-acid glycoprotein. These

TABLE 3. Antibody neutralization of neuraminidases produced by strains of various GBS serotypes

Serotype/ strain[a]	Nonimmune fraction			Immune fraction		
	Preincubation activity[b]	Postincubation activity[b]	% Reduction[c]	Preincubation activity[b]	Postincubation activity[b]	% Reduction[c]
Ia/161	0.14 ± 0.01	0.12 ± 0.01	14.3	0.15 ± 0.01	0.02 ± 0.00	86.7
Ia/158	0.20 ± 0.02	0.14 ± 0.02	28.2	0.19 ± 0.01	0.02 ± 0.01	89.5
Ib/132	0.19 ± 0.01	0.15 ± 0.02	21.1	0.14 ± 0.03	0.02 ± 0.01	85.7
Ib/136	0.26 ± 0.06	0.20 ± 0.05	23.1	0.23 ± 0.05	0.05 ± 0.03	78.3
Ic/172	0.15 ± 0.02	0.11 ± 0.02	26.6	0.14 ± 0.01	0.02 ± 0.01	85.7
Ic/165	1.50 ± 0.31	1.50 ± 0.60	6.7	1.30 ± 0.38	0.19 ± 0.04	85.4
II/155	0.18 ± 0.01	0.13 ± 0.01	27.7	0.16 ± 0.03	0.02 ± 0.01	87.5
III/178	0.43 ± 0.07	0.35 ± 0.03	18.6	0.39 ± 0.04	0.04 ± 0.01	89.7
III/126	1.30 ± 0.17	1.10 ± 0.15	15.4	1.10 ± 0.16	0.11 ± 0.03	90.0

[a] Stage I neuraminidase preparations obtained from representative strains of each GBS serotype were used.
[b] Either nonimmune serum or immune serum (0.5 mg of protein) was incubated with stage I enzyme (0.066 μmol of SA released per min), and activity was assayed against BSM. The activities (μmol of SA released per min per ml) represent an average of two separate determinations.
[c] The percent reduction in neuraminidase activity was obtained by comparing neuraminidase activities before and after overnight incubation in the presence of either nonimmune or immune serum.

results also suggest not only that GBS neuraminidase exhibits a limited substrate specificity but that the site of cleavage may not necessarily be the α-2,6 linkage, since BSM, α_1-acid glycoprotein, and fetuin all contain α-2,6 linkages of SA to galactose, but only BSM was found to be susceptible to digestion.

The next parameter examined was the molecular weights of the neuraminidases produced by the various GBS serotypes. The only other reported molecular weight for a GBS neuraminidase was 125,000 for GBS strain 110, a serotype III strain, determined by gel filtration on Sephacryl S-200 (23). The molecular weights obtained for the neuraminidases of the various serotypes examined (see Results) ranged from 120,000 for GBS strain 122 (serotype III) to 180,000 for GBS strain 132 (serotype Ib). These molecular weights are somewhat higher than those previously determined for the neuraminidases of a variety of other bacteria. These include the neuraminidase of *Arthrobacter* species (14), *V. cholerae* (10), *Corynebacterium diphtheriae* (25), and *S. pneumoniae* (10), for which the molecular weights were 87,000, 68,000, 65,000, and 88,000, respectively.

Purified neuraminidase (stage IV from GBS strain 122) was used in the immunization of New Zealand White rabbits to produce antibody specific for the enzyme produced by this strain. The presence of antibody to this enzyme was detected by the use of the standard neuraminidase assay and was based on the reduction of thiobarbituric acid-reactive material following overnight incubation of the antibody-enzyme mixtures. The results of this study (Tables 2 and 3) indicate that neuraminidases produced by strains of different GBS serotypes are quite similar antigenically, at least on the basis of the presence of similar epitopes making up the enzymatically active site of these molecules. Any differences observed (e.g., slight differences in molecular weights or amount of neuraminidase produced) were not likely to be a function of whether the strain was isolated from an infected individual. This is because 10 of the 11 strains used in this study were isolated from cases of either early- or late-onset sepsis. Only strain 178 (a type III GBS) was isolated from the umbilicus of an asymptomatically colonized infant (22).

Serotype III strains have been shown to be particularly prominent among isolates from patients with late-onset disease or meningitis at any age (2, 30, 31). The cause of this phenomenon is not known, but it has been suggested that the phenomenon is related to the presence of SA (4) in the capsular material of these bacteria, as has been shown for the *Escherichia coli* K1 strains (27). This is unlikely, because all GBS serotypes have SA in their capsule (5), while only type III strains are consistently found in patients with meningitis (30). We recently showed that the production of high levels of neuraminidase is associated primarily with type III GBS strains (22). In this report, we show that the neuraminidases produced by the various serotypes of GBS are similar in substrate specificity, molecular weight, and serological identity. Therefore, the results of the previous study (22) are valid and are not a consequence of using an assay that was developed for the type III GBS neuraminidase, and that was less sensitive for the neuraminidases produced by the other serotypes.

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LITERATURE CITED

1. **Anthony, B. F., and D. M. Okada.** 1977. The emergence of group B streptococci in infections of the newborn infant. Annu. Rev. Med. **28**:355–369.
2. **Baker, C. J., and F. F. Barrett.** 1974. Group B streptococcal infections in infants. J. Am. Med. Assoc. **230**:1158–1160.
3. **Baker, C. J., F. F. Barrett, R. C. Gordon, and M. D. Yow.** 1973. Suppurative meningitis due to streptococci of Lancefield group B: a study of 33 infants. J. Pediatr. **82**:724–729.
4. **Baker, C. J., and D. L. Kasper.** 1976. Microcapsule of type III strains of group B streptococcus: production and morphology. Infect. Immun. **13**:189–194.
5. **Baker, C. J., and D. L. Kasper.** 1976. Identification of sialic acid in polysaccharide antigens of group B streptococcus. Infect. Immun. **13**:284–288.
6. **Brown, J., R. Farnsworth, L. W. Wannamaker, and D. W. Johnson.** 1974. CAMP factor of group B streptococci: production, assay, and neutralization by serum from immunized rabbits and experimentally infected cows. Infect. Immun. **9**:377–383.
7. **Campbell, D. H., J. S. Garvey, N. E. Cremer, and D. H. Sussdorf.** 1970. Methods in immunology, p. 218. W. A. Benjamin, New York.
8. **Cassidy, J. T., G. W. Jourdian, and S. Roseman.** 1965. The sialic acids. VI. Purification and properties of sialidase from *Clostridium perfringens*. J. Biol. Chem. **240**:3501–3506.
9. **Doran, T. I., D. C. Straus, and S. J. Mattingly.** 1980. Extracellular antigens of serotype III group B streptococci. Infect. Immun. **30**:890–893.
10. **Drzeniek, R.** 1972. Viral and bacterial neuraminidases. Curr. Top. Microbiol. Immunol. **59**:35–75.
11. **Eickhoff, T. C., J. O. Klein, A. K. Daly, D. Ingall, and M. Finland.** 1964. Neonatal sepsis and other infections due to group B *beta*-hemolytic streptococci. N. Engl. J. Med. **271**:1221–1228.
12. **Ferrieri, P., E. D. Gray, and L. W. Wannamaker.** 1980. Biochemical and immunological characterization of the extracellular nucleases of group B streptococci. J. Exp. Med. **151**:56–68.
13. **Fisher, L.** 1971. An introduction to gel chromatography, p. 396. North-Holland Publishing Co., Amsterdam.
14. **Flashner, M. P., P. Wang, J. Hurley, and W. S. Tannenbaum.** 1977. Properties of an inducible extracellular neuraminidase from an *Arthrobacter* isolate. J. Bacteriol. **129**:1457–1465.
15. **Gottschalk, A.** 1960. Correlation between composition, structure, shape and function of a salivary mucoprotein. Nature (London) **186**:945–951.
16. **Hayano, S., and A. Tanaka.** 1969. Sialidase-like enzymes produced by group A, B, C, G, and L streptococci and by *Streptococcus sanguis*. J. Bacteriol. **97**:1328–1333.
17. **Hellerquist, C. G., J. Rojas, R. S. Green, S. Sell, H. Sundell, and M. T. Stahlman.** 1981. Studies on group B *beta*-hemolytic streptococcus. I. Isolation and partial characterization of an extracellular toxin. Pediatr. Res. **15**:892–898.
18. **Hughes, R. C., and R. W. Jeanloz.** 1964. The extracellular glycosidases of *Diplococcus pneumoniae*. I. Purification and properties of a neuraminidase and a β-galactosidase: action on the α-1 acid glycoprotein of human plasma. Biochemistry **3**:1535–1543.
19. **Kelly, R. T., D. Greiff, and S. Farmer.** 1966. Neuraminidase activity in *Diplococcus pneumoniae*. J. Bacteriol. **91**:601–603.
20. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
21. **McClean, D.** 1941. The capsulation of streptococci and its relation to diffusion factor (hyaluronidase). J. Pathol. Bacteriol. **53**:13–27.
22. **Milligan, T. W., C. J. Baker, D. C. Straus, and S. J. Mattingly.** 1978. Association of elevated levels of extracellular neuraminidase with clinical isolates of type III group B streptococci. Infect. Immun. **21**:738–746.
23. **Milligan, T. W., S. J. Mattingly, and D. C. Straus.** 1980. Purification and partial characterization of neuraminidase from type III group B streptococcus. J. Bacteriol. **144**:164–172.

24. **Milligan, T. W., D. C. Straus, and S. J. Mattingly.** 1977. Extracellular neuraminidase produced by group B streptococci. Infect. Immun. **18**:189–195.
25. **Moriyama, T., and L. Barksdale.** 1967. Neuraminidase of *Corynebacterium diphtheriae*. J. Bacteriol. **94**:1565–1581.
26. **Pardoe, G. I.** 1974. The inducible neuraminidases of pathogenic microorganisms. Behring Inst. Mitt. **55**:103–122.
27. **Robbins, J. B., G. H. McCracken, Jr., E. C. Gotschlich, F. Ørskov, I. Ørskov, and L. A. Hanson.** 1974. *Escherichia coli* K1 capsular polysaccharide associated with neonatal meningitis. N. Engl. J. Med. **290**:1216–1220.
28. **Straus, D. C., S. J. Mattingly, T. W. Milligan, T. I. Doran, and T. J. Nealon.** 1980. Protease production by clinical isolates of type III group B streptococci. J. Clin. Microbiol. **12**:421–425.
29. **Todd, E. W.** 1934. A comparative serological study of streptolysins derived from human and from animal infections with notes on pneumococcal hemolysin, tetanolysin and staphylococcus toxin. J. Pathol. Bacteriol. **39**:299–321.
30. **Wilkinson, H. W.** 1978. Analysis of group B streptococcal types associated with disease in human infants and adults. J. Clin. Microbiol. **7**:176–179.
31. **Wilkinson, H. W., R. R. Facklam, and E. C. Wortham.** 1973. Distribution by serological type of group B streptococci isolated from a variety of clinical material over a five-year period (with special reference to neonatal sepsis and meningitis). Infect. Immun. **8**:228–235.