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Factors Influencing Release of Type III Antigens by Group B Streptococci

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The release of serotype III group B streptococcal polysaccharides into the supernatant fluid was examined under a variety of physiological conditions. Release of both high- and low-molecular-weight type III antigens was fairly constant throughout exponential growth, but increased markedly upon entering the stationary phase of growth. Increased glucose and decreased phosphate concentrations both caused a large increase in release of antigens. Inhibition of protein synthesis in exponentially growing cells by chloramphenicol (10 μg/ml) caused a condition of unbalanced growth in which antigen release was increased greatly over control values. Strain variability in antigen release was also observed. Strains which are known to be high neuraminidase producers released elevated levels of both low- and high-molecular-weight type III antigens. Non-neuraminidase-producing strains released considerably less high-molecular-weight antigen, but similar levels of the low-molecular-weight antigen compared with the high neuraminidase producers. Strain D136C, a type III non-neuraminidase producer, released negligible quantities of the high-molecular-weight antigen in the supernatant fluid. These results indicate that both the physiological environment and the type III strain are important in determining the quantity of type-specific antigen released into the culture fluid.

Immunity to invasive group B streptococcal disease appears to be associated primarily with antibody directed against the type-specific antigen (TSA) located on the surface of the organism (18). Five serotypes (Ia, Ib, Ic, II, III) of the group B streptococci (GBS) are recognized, although serotype III is the predominant organism isolated from infected infants (2, 38). Chemical analyses of TSAs have shown them to be carbohydrate in nature (19), with the exception of the Ibc protein, which is shared by both the Ib and Ic serotypes (37).

A variety of techniques have been used to extract TSAs from the cell surface of GBS, including hot HCl (18), cold trichloroacetic acid (20), saline (35), enzymes (33), and buffer extraction (5, 15). Several recent studies have demonstrated that both type III and group B antigens can be readily isolated and purified from the growth medium. By using a chemically defined medium (9) or a chemically defined broth supplemented with acid-hydrolyzed casein (8), large quantities of both group B and type III antigens were purified by ion-exchange or gel permeation chromatography or both. Doran et al. (9) demonstrated that two molecular species of type III antigen could be recovered from the supernatant fluid, a high-molecular-weight (HMW) antigen and a smaller form of the antigen (low molecular weight [LMW]). Although the smaller antigen did not give a positive capillary precipitin reaction, it gave a line of complete identity with the HMW antigen in immunodiffusion studies (9).

The present study was undertaken to further investigate the effect of various physiological factors such as culture age, optimum phosphate and glucose concentrations, requirement for protein synthesis, and strain variability of the release of TSA.

MATERIALS AND METHODS

Organisms and medium. Six clinical isolates of serotype III GBS were utilized in these studies, in addition to prototype strain, D136C (Table 1). These strains were classified by their ability to elaborate extracellular neuraminidase as determined by Milligan et al. (24). However, the majority of experiments were performed with GBS strain 110 (GBS 110), which has been previously described (26).

The organisms, one or two passages from the clinical situation, were stored in Todd-Hewitt broth at −70°C until needed. Thawed cultures were routinely streaked on sheep blood agar plates and incubated at 37°C for 18 to 24 h before use.

All experiments were performed with a chemically defined medium (FMC) which has been previously described (25) and which was modified for some of these experiments as described below.

Growth, harvest, and treatment of supernatant fluids. The growth conditions are described below for each individual experiment. Essentially, the organisms were inoculated from a fresh blood agar

TABLE 1. *Serotype III GBS strains*

Strains	Extracellular neuraminidase levels[a]	Disease/origin
110	High	Neonate, late onset meningitis, CSF[b]
120	High	Neonate, late onset sepsis, blood
130	High	Neonate, early onset meningitis, CSF
118	None	Neonate, early onset sepsis, blood
127	None	Neonate, late onset meningitis, CSF
142	None	Neonate, late onset sepsis, blood
D136C	None	Standard Lancefield immunizing type III strain

[a] Neuraminidase levels as determined by Milligan et al. (24).

[b] CSF, cerebrospinal fluid.

plate to starter cultures of FMC which were approximately 1/10th of the final culture volume. Optical densities were measured with a Coleman Junior II spectrophotometer at 675 nm, multiplied by 1,000, and converted to adjusted optical density units (AOD) so that the values would be in accordance with Beer's law and be proportional to bacterial mass (34). One AOD is equivalent to 0.43 μg of cellular dry weight per ml (26). Starter cultures were grown to mid-exponential phase (approximately 500 AOD units) and used to inoculate the remainder of the FMC (usually 1- to 8-liter total volume), giving a starting density of approximately 50 AOD units. All cultures were grown aerobically at 37°C in a circulating water bath. During growth, both pH and optical density were monitored, and the pH was maintained at 6.5 to 7.0 by slow titration with sterile 2.5 N NaOH. When cultures were ready for harvest, they were immediately chilled in an ice bath, a final AOD reading was made, and the culture volumes were determined. Cells were removed by centrifugation at 8,000 to 9,000 × *g* for 15 to 20 min with a Sorvall RC-5 refrigerated centrifuge at 4°C. The supernatant fluids were first dialyzed for 3 to 4 days at 4°C against 10 mM acetate buffer, pH 6.5 (1 liter of supernatant fluid per 8 liters of buffer) with daily buffer changes and then lyophilized to dryness.

Effect of growth phase. GBS 110 was grown in 4 liters of unmodified FMC (25). One-liter aliquots were withdrawn at several time points during the exponential phase of growth and 30 min after the onset of the stationary phase. Samples were immediately chilled and treated as described above.

Glucose and phosphate studies. The effects of varying the glucose and phosphate concentrations of the growth medium on release of type-specific antigen were examined with GBS 110. Unmodified FMC contained 0.065 M phosphate and 1% glucose. In the phosphate studies, FMC contained 1% glucose, and the phosphate levels were altered by varying the sodium phosphate concentration from 0 to 0.12 M. Potassium phosphate, a normal component of FMC (24), was present in all cultures at 0.005 M, yielding a final phosphate range of 0.005 to 0.125 M. The pH of the medium in each case was maintained between 6.5 to 7.0 by the addition of sterile 2.5 N NaOH.

The effect of varying levels of glucose on release of TSA was examined using a range of 0.2 to 5% glucose in FMC which contained 0.065 M phosphate in each case.

For all of the phosphate and glucose studies, 1-liter cultures of each type of medium were used. After growth, cultures were harvested immediately upon entering the stationary phase and treated as described above.

Effect of protein synthesis inhibition on release of TSA. Eight liters of standard FMC were used for growth of GBS 110. At each of several points during the exponential phase of growth and immediately upon entering the stationary phase of growth, two 1-liter aliquots were withdrawn. One aliquot of each pair was immediately chilled to serve as the control. The second culture was examined for effects of cessation of protein synthesis on release of TSA by immediately adding 10 μg of chloramphenicol per ml, which was thoroughly mixed with the culture. These cultures were incubated at 37°C for an additional 60 min with continued monitoring of optical density and pH, followed by chilling on ice. All cultures were then harvested and treated as described above.

Release of TSA by high- and non-neuraminidase-producing strains. Three high-neuraminidase-producing strains and three non-neuraminidase-producing strains of type III GBS (Table 1) were examined for release of TSA in standard FMC. In addition, strain D136C, a prototype strain widely used for production of type III-specific antiserum (21) and shown to be a non-neuraminidase-producing organism (24), was examined for extracellular antigen production. One liter cultures of each organism in standard FMC were grown as described above and harvested in early stationary phase. Cultures were treated as described above.

Chromatography and serological assays. The lyophilized supernatant fluids (above) were dissolved in a small quantity of 10 mM acetate buffer, pH 6.5 (0.02% azide), and chromatographed on a Sepharose 4B column (2.5 by 90 cm) as previously described (9). Fractions of 100 drops per tube (ca. 4.5 ml) were collected and assayed.

Fractions were tested for serotype III or group B antigen reactivity by the capillary precipitin test with type III and group B-specific rabbit sera prepared by the method of Lancefield et al. (21).

Purification of type III antigen for chemical analysis. Type III antigen was obtained from an early stationary-phase culture of GBS 110 grown in 10 liters of FMC (1% glucose, 0.065 M phosphate). The supernatant fluid was collected and treated as described above. The antigen was purified by Sepharose 4B chromatography (above), diethylaminoethyl-cellulose (9), followed by Sepharose 4B again.

Chemical assays and gas chromatography. Sialic acid was measured by the thiobarbituric acid assay of Aminoff (1). For column eluates, every third fraction was assayed for all samples. We have shown by rocket immunoelectrophoresis that sialic acid content is proportional to the quantity of antigen (9).

Other sugars were assayed after acid hydrolysis of purified antigen using 3 N HCl at 100°C for 4 h in vacuum-evacuated sealed vials. Amino sugars were assayed by the modified Elson-Morgan reaction (28). Neutral sugars and amino sugars were identified and quantitated by gas-liquid chromatography. A Shimadzu GC-4B gas chromatograph containing a 1.7 M column packed with 3% GE-SE-30 Gas-Chrom-Q with a 100/120 mesh was used. The flow of N_2 carrier gas was 25 ml/min.

Trimethylsilyl derivatives were prepared using 500 μg of hydrolyzed, neutralized antigen. Drying and neutralization were accomplished by addition of approximately an equal volume of ethanol to the hydrolysate and drying under a constant stream of N_2. Trimethylsilyl derivatives of the antigen were accomplished with 100 μl of a 10:2:1 mixture (vol/vol) of dry pyridine, hexamethyldisilizane reagent (Applied Science Laboratories, Inc.), and chlorotrimethylsilane (Applied Science Laboratories, Inc.) per 500 μg of antigen. The antigen was reacted with the trimethylsilyl reagent for 30 min at room temperature before applying the sample to the gas chromatograph. A temperature program was run from 128 to 170°C with an increase in temperature of 3°C/min. The detector oven was set at 280°C. Protein was determined by the Lowry procedure (22).

Calculations. All values for sialic acid are expressed as micrograms of sialic acid per milligram of cell dry weight. This allows comparison of samples from different time points in an experiment or comparison of samples from different experiments on the basis of equivalent cell mass. These results are calculated using the data determined from the thiobarituric acid assay by using the following formula: micrograms of sialic acid/milligrams of cell dry weight = (micrograms of sialic acid/milliliter × milliliter/fraction × 1,000 μg/mg)/0.43 μg of cell dry weight/AOD/milliliter × culture volume (milliliters) × AOD.

Comparative quantitation of weight data. Peak areas of sialic acid-containing material which eluted from the Sepharose 4B column were determined by a K and E planimeter (Keuffel and Esser Co., New York, N.Y.) and used to compare quantitatively the amount of antigen released.

RESULTS

Effect of growth phase on TSA release. A 4-liter culture of GBS 110 grown in a chemically defined medium (FMC) was used to study the release of TSA as a function of growth phase. One-liter aliquots were taken from two time points during exponential growth and 30 min after entering the stationary phase. The culture supernatant fluids were dialyzed, concentrated by lyophilization, and chromatographed on a Sepharose 4B column which previously has been demonstrated to separate the type III and group B antigens (9). The eluates were monitored for sialic acid (an integral component of the type antigens of group B streptococci (4) and for type III specificity by the capillary precipitin reaction. Sialic acid has been shown previously to correlate directly with the quantity of TSA by rocket immunoelectrophoresis (9). The data are expressed as micrograms of sialic acid per milligram of cell dry weight so that samples from the different phases of the growth cycle could be compared in terms of equivalent cell mass.

When the samples were chromatographed on Sepharose 4B (Fig. 1A to C), two major sialic acid-containing peaks were seen, as previously described (9). The HMW peak, centered near fraction 40, gave a strong capillary precipitin reaction with type III antiserum and no reaction with group B antiserum. The second peak, centered near fraction 78, is an LMW peak of TSA, which has been shown to give a line of complete identity with the HMW antigen in immunodiffusion analyses (9).

As evident in Fig. 1A and B, the cells released a fairly constant quantity of both forms of the antigen throughout the exponential phase of growth, although only the HMW antigen will be considered in detail in this study. However, within 30 min after onset of the stationary phase (Fig. 1C), there was a demonstrable increase in the quantity of both forms of TSA (3.1-fold for HMW TSA). There was no further change in the sialic acid profile 5 h after the onset of the stationary phase (data not shown). The release

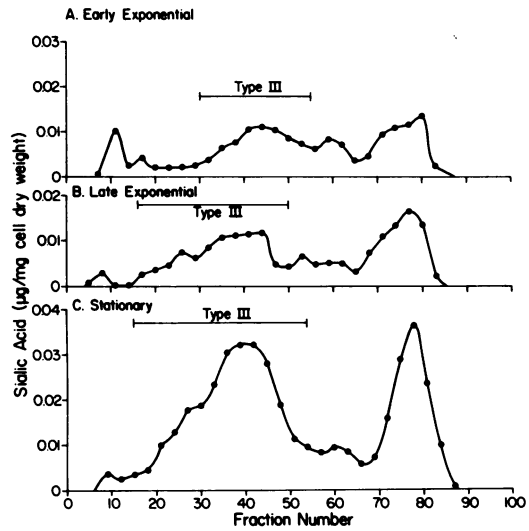

FIG. 1. *Sepharose 4B elution profiles of extracellular sialic acid-containing type III antigens of GBS 110. Samples were obtained during (A) early exponential, (B) late exponential, and (C) stationary phases of growth. The antigens were eluted with 10 mM sodium acetate buffer, pH 6.5. Every third sample was assayed for sialic acid by the thiobarbituric acid assay (1). Fractions were tested for type III specificity by the capillary precipitin test with rabbit antiserum against the type III antigen.*

of TSA was not attributable to cellular lysis since less than 1% of total [^{14}C]thymidine-labeled DNA was detectable in the supernatant fluid (data not shown).

Chemical composition of TSA. The chemical composition of the HMW form of TSA is shown in Table 2. The antigen contained galactose, sialic acid, glucose, and *N*-acetyl-glucosamine in approximately a 2:1:1:1 molar ratio. This value agrees well with the chemical composition determined by Greber et al. (T. F. Greber, R. B. Carey, G. D. Shockman, T. K. Eisenstein, D. Resavy, and K. W. Goldstein, R. M. Swenson, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, B1, p. 17) for the soluble GBS type III polysaccharide purified from supernatant fluid of strain M732 grown in casein hydrolysate-based medium, and of neutral buffer extracted type III antigen (14, 16). The chemical composition of the LMW antigen is currently being examined.

Effect of glucose concentration on release of TSA. GBS 110 was grown in 1-liter cultures of FMC containing 0.2 to 5.0% glucose and containing 0.065 M phosphate in each case. All cultures were harvested 30 min after onset of the stationary phase, and the supernatant fluids were fractionated by Sepharose 4B gel filtration. As expected, the final culture optical densities were generally lower for cultures containing lower glucose levels. The final AOD readings were: 530 AOD (0.2% glucose), 850 AOD (0.5%), 1,240 AOD (1.0%), and 1,650 AOD (2.0%). However, the culture containing 5.0% glucose appeared to be somewhat inhibited (1,215 AOD) compared with the 2.0% glucose culture.

The Sepharose 4B elution profiles for the various cultures (Fig. 2), corrected to equivalent cell masses, indicate that both forms of TSA (HMW and LMW) were released in progressively greater quantities with increasing concentrations of glucose up to 2.0% glucose. Compared with cultures grown in FMC containing 1.0% glucose and 0.065 M phosphate, the cultures released 0.2-fold (0.2% glucose), 0.4-fold (0.5% glucose), and 1.6-fold (2.0% glucose) the quantity of HMW antigen. The culture grown in 5.0% glucose, however, had reduced levels of the HMW form of TSA (0.6-fold), and the LMW antigen had slightly increased levels.

TABLE 2. *Chemical analysis of HMW type III antigen produced by GBS strain 110 grown in chemically defined medium containing 0.065 M phosphate and 1% glucose*

Component	%	Method
Sialic acid	25.1	TBA[a]
N-Acetylglucosamine	18.7	GLC[b]; Elson-Morgan (28)
Galactose	34.6	GLC
Glucose	17.3	GLC
Protein	<1	Lowry (22)
Rhamnose	ND[c]	GLC

[a] TBA, Thiobarituric acid assay (1).
[b] GLC, gas liquid chromatography; see text for complete details.
[c] ND, Not detected.

Effect of phosphate concentration on release of TSA. One-liter cultures of GBS 110 were grown in FMC containing 1.0% glucose, but modified to contain phosphate levels from 0.005 to 0.125 M. As before, cultures were harvested 30 min after onset of the stationary phase, and the supernatant fluids were fractionated by Sepharose 4B chromatography.

The quantity of the HMW form of TSA released into the culture supernatant fluid was found to be inversely related to the phosphate concentrations (Fig. 3). Cells grown in 0.125 M phosphate released 0.4-fold the quantity of HMW antigen compared to the cultures containing 1.0% glucose and 0.065 M phosphate. In addition, the lag-time before onset of exponential growth was greatly extended (data not shown), and the normal doubling time of 33 min (0.065 M phosphate) increased to 45 min (0.125 M phosphate) (25). In contrast, cells grown in 0.005 M phosphate had a doubling time of 27 min and released 2.6-fold greater levels of the HMW antigen and almost negligible levels of the LMW TSA, whereas 0.035 M phosphate-grown cells released 1.8-fold higher quantities of HMW antigen compared with cells grown in 0.065 M phosphate.

Effect of protein synthesis inhibition on release of TSA. The observation that most of the extracellular TSA was detected after cultures entered the stationary phase suggested the possibility that an inducible enzyme might mediate the release of the TSA. To examine this possibility, duplicate one-liter aliquots of GBS 110 grown in unmodified FMC (0.065 M phosphate, 1.0% glucose) were withdrawn at various times during the exponential and stationary phases of growth. Chloramphenicol (10 μg/ml) was added in quantities sufficient to completely inhibit protein synthesis in GBS 110 (unpublished data), following by an additional 60-min incubation period. Control cultures were taken from each time period, but were immediately chilled on ice to stop growth. The levels of extracellular TSA present in the control culture supernatant fluids represented the same level of antigen present in the chloramphenicol-treated culture before addition of the antibiotic.

The Sepharose 4B elution profiles indicated that inhibition of protein synthesis caused a 9.7-fold increase in the amount of TSA released during mid-exponential growth (Fig. 4a) com-

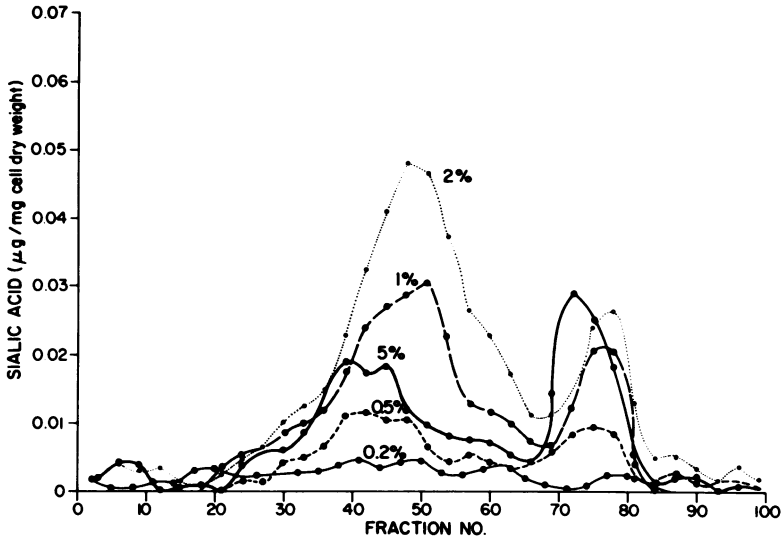

FIG. 2. *Sepharose 4B elution profiles of extracellular type III antigens of GBS 110 produced by cultures growing in chemically defined medium containing various concentrations of glucose as indicated. See legend for Fig. 1 for assay conditions.*

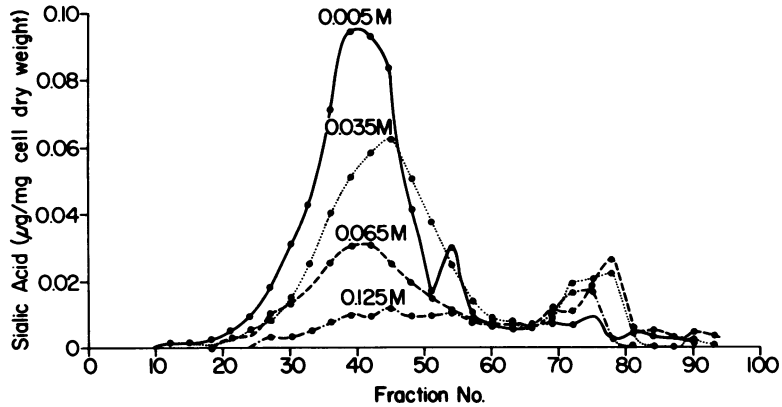

FIG. 3. *Sepharose 4B elution profiles of extracellular type III antigens of GBS 110 produced by cultures growing in chemically defined medium containing various concentrations of sodium phosphate as indicated. See legend for Fig. 1 for assay conditions.*

pared with the control. The effect was still present, but was reduced in late exponential growth (Fig. 4b) to 4.9-fold greater than control levels. Early stationary-phase cultures treated with chloramphenicol released about the same amount of TSA as the untreated cultures (Fig. 4c). These results, therefore, clearly indicate that protein synthesis is not required for TSA release.

Correlation of TSA release with extracellular neuraminidase production by GBS. Previous studies indicated that serotype III strains isolated from neonates with invasive disease were more often capable of elaborating elevated levels of extracellular neuraminidase than type III strains from asymptomatically colonized infants (24). However, non-neuraminidase-producing strains have been isolated from infected infants, suggesting that additional factors may contribute to the overall virulence of the type III organism. The classification of all type III organisms as either "high producers" or "nonproducers" suggested that there might be basic physiological differences between these two groups of organisms. This was further substantiated by comparison of the Sepharose 4B elution profiles of supernatant fluids from three high-producing and three non-neuraminidase-producing strains (Fig. 5).

The high-neuraminidase-producing serotype III strains (strains 110, 120, and 130) and the non-neuraminidase-producing strains (strains

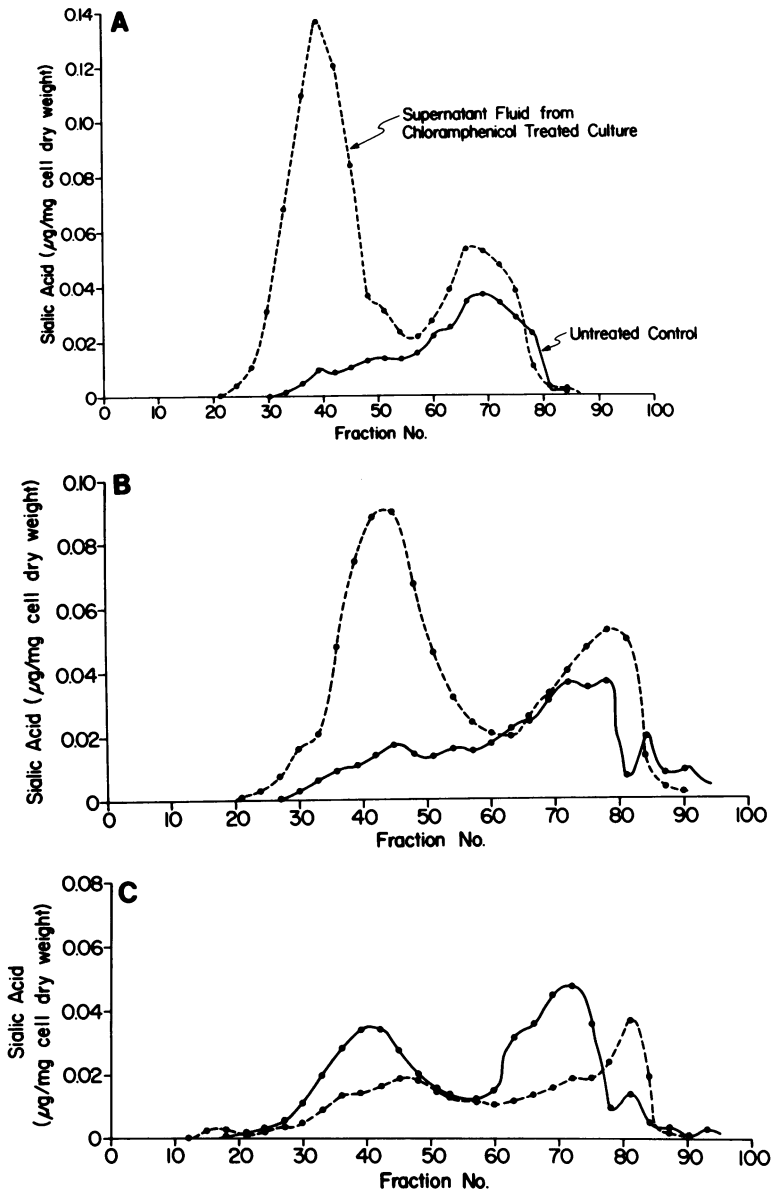

FIG. 4. *Sepharose 4B elution profiles of extracellular type III antigens of GBS 110 produced by untreated (—) and chloramphenicol (10 μg/ml)-treated cultures (- -) at (A) mid-exponential, (B) late exponential, and (C) stationary phases of growth. See legend for Fig. 1 for assay conditions.*

118, 127, and 142) were grown in unmodified FMC. The cultures were harvested in early stationary phase, and the supernatant fluids were fractionated by Sepharose 4B gel filtration.

The three high neuraminidase producers had very similar Sepharose 4B elution profiles (Fig. 5A). This pattern of two sialic acid-containing peaks of TSA had been previously described (9) and was also seen in Fig. 1 to 4.

The three neuraminidase nonproducers had patterns which differed considerably from the elution profiles of the high producers, although they were similar to each other (Fig. 5B). The HMW peak of TSA, previously described for strain 110 (a high neuraminidase producer), was also present in the supernatant fluids of the three nonproducers (fractions 20 to 50) at an average of 2.9-fold-lower levels. As before, this material gave a positive capillary precipitin test with serotype III antiserum. The LMW peak

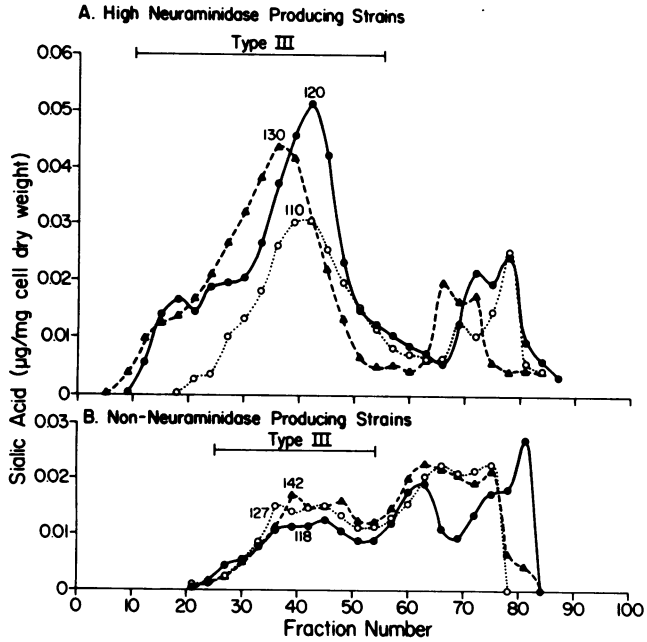

FIG. 5. *Sepharose 4B elution profiles of extracellular products of six strains of GBS classified as (A) high producers of extracellular neuraminidase (24) or (B) nonproducers of the enzyme. All cultures were grown to the stationary phase and analyzed as described for Fig. 1.*

(fraction 75 to 80), seen for the high neuraminidase producers, was also present in supernatant fluids of nonproducers.

Strain D136C, the prototype type III strain used extensively for preparation of type III specific antiserum, has been previously demonstrated to be a non-neuraminidase-producing organism (24). Examination of the Sepharose 4B elution profile (Fig. 6) of this strain indicated that it was similar to other non-neuraminidase-producing strains except that there was very little HMW TSA present, and it was of a lower molecular weight, as determined by its larger elution volume on the gel filtration column.

Although a definitive role for neuraminidase in TSA release is not readily apparent, these results suggest a possible correlation between high levels of extracellular neuraminidase and the release of increased levels of TSA. In addition, these studies demonstrate that physiological differences exist among serotype III organisms which may be related to degrees of virulence within this serotype.

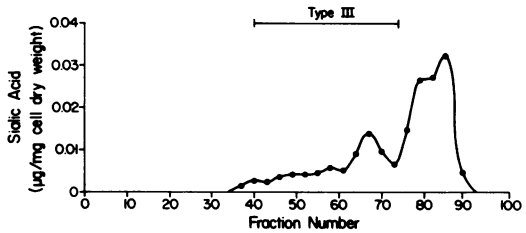

FIG. 6. *Sepharose 4B elution profile of extracellular type III antigens produced by GBS strain D136C, a neuraminidase nonproducer (24), and the prototype type III strain. The culture was grown to the stationary phase and analyzed as described for Fig. 1.*

DISCUSSION

The influence of physiological and nutritional factors on the synthesis or release of cell surface polymers or both in bacteria is well recognized. Duguid and Wilkinson observed in 1953 that *Aerobacter aerogenes* produced maximal levels of capsular polysaccharide with growth-limiting levels of phosphate (10). Other studies have described a phenotypic variation in *Bacillus subtilis* during phosphate-limited growth in which teichoic acids are replaced by teichuronic acids (D. C. Ellwood and D. W. Tempest, Biochem. J. **104**:69p, 1967). Cell wall thickening has been shown to occur upon deprivation of essential nonpeptidoglycan amino acids or inhibition of protein synthesis in streptococci (23, 30). Also, Baker and Kasper (3) used increased levels of glucose and phosphate in Todd-Hewitt broth to obtain greater yields of "native" cell-associated polysaccharide from type III GBS and provide greater buffering capacity for the medium.

Cell surface polymers, including lipids (13),

teichoic acids (17), and peptidoglycan (27), have also been shown to be released into the medium, particularly during unbalanced growth conditions. In the present study, type III GBS strain 110, a neonatal meningeal isolate, released low levels of the HMW type III antigen throughout exponential growth, but much higher quantities of the antigen upon entering the stationary phase of growth. Lysis of cells was eliminated as a possible explanation for presence of the antigen in the culture medium based on no observable decrease in culture turbidity and the release of <1% of the total [^{14}C]thymidine-labeled DNA into the medium. The amount of extracellular type III antigen generally increased (with the exception of medium containing 5% glucose) with increasing glucose levels. However, it is interesting that detectable levels of cell-free type III antigen were found even when the level of glucose (0.2%) was severely growth-limiting. The inverse relationship between the level of phosphate in the growth medium and the amount of type III antigen released suggests a possible role for phosphate in the attachment of the type III antigen to the cell surface, possibly to the peptidoglycan, as has been demonstrated for wall teichoic acids (7). Decreased levels of phosphate in the growth medium would leave fewer sites available for attachment of antigen to the cell surface, resulting in increased levels of antigen in the culture fluid. Other possible interpretations for antigen release attributable to decreased phosphate might be due to membrane alterations or effects on cell-free or cell-associated enzymes. However, phosphate effects were not due to pH changes since the pH was maintained between 6.5 and 7.0 in all experiments.

The observation that additional type III strains produced either high or low levels of extracellular type III antigen suggests that there may be significant physiological differences among type III strains of GBS. This observation assumes additional significance when one considers that high concentrations of extracellular type III antigen may remove protective antibody from the circulation, leaving the host more susceptible to disease. This concept was suggested previously in a study by Pollack (29) who showed that the presence of detectable *Klebsiella* capsular antigens in the serum of patients infected with *Klebsiella pneumoniae* appeared to correlate with the severity of the infection and with a poorer prognosis than in those patients in which no extracellular antigen was detected. Data which point to the in vivo production of extracellular TSA by the GBS can be found in several reports. These include studies by Hill (12), Wilkinson (36), Baker et al. (6) and Stechenberg et al., (31) who demonstrated the presence of TSA in the body fluids in infants by countercurrent immunoelectrophoresis, and those of Edwards et al., (11) who detected type III GBS antigens in the cerebrospinal fluids of 12 infants by latex particle agglutination.

Further support of the existence of physiological differences among the type III GBS strains was reported by Milligan et al. (24), who demonstrated that type III strains could be classified as either high-neuraminidase- or non-neuraminidase-producing organisms. Additionally, type III strains from infected infants were more often high neuraminidase producers than strains from asymptomatically colonized infants. Straus et al. (32) in a recent report indicated that type III strains producing high levels of extracellular neuraminidase and type III antigen also elaborated high levels of extracellular proteases compared with low type III antigen and non-neuraminidase-producing strains. Durham et al. (D. L. Durham, T. I. Doran, T. W. Milligan, S. J. Mattingly, and D. C. Straus, Intersci. Conf. Antimicrob. Agents Chemother. 20th, New Orleans, La., 1980, Abstr. No. 586) demonstrated that type III strains elaborating high levels of extracellular material (neuraminidase, type III antigen, and protease) were 100- to 1,000-fold more virulent in the mouse model than low producers of extracellular material. Which, if any, of these factors contribute to the increased virulence of high producing strains remains to be determined.

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