Antigens of *Streptococcus mutans*

II. Characterization of an Antigen Resembling a Glycerol Teichoic Acid in Walls of Strain BHT

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Received for publication 11 September 1973

Cold 10% trichloroacetic acid was used to extract antigens from purified cell walls of *Streptococcus mutans* BHT. Column chromatography on Biogel P-100 resolved two serologically reactive fractions (B and C). These fractions were ascertained to be relatively pure by recycling on Biogel P-100, Ouchterlony double-diffusion analysis, and immunoelectrophoresis. Fractions B and C demonstrated bands of absolute homology by double-diffusion but different mobilities by immunoelectrophoresis. Chemical analysis indicated that fraction B is a polysaccharide composed principally of rhamnose and galactose, with smaller amounts of glucose and glucosamine. Small quantities of glycerol and phosphorus also were found. Fraction C was composed mainly of galactose, glycerol, and phosphorus. Alkaline hydrolysis of this fraction yielded products typically released by the degradation of a glycerol teichoic acid, such as glycerol monophosphate, glycerol diphosphate, inorganic phosphorus, and several glycosyl glycerol phosphates. Diglycerol triphosphate was not detected. Side-group analysis revealed that glycerol was substituted by mono- and trigalactosyl moieties. Fraction C was deduced to contain 25 glycerol phosphate units per polymer length. Hapten inhibition studies revealed a \( \beta \)-galactosyl side-group as the probable hapten on this antigen. The BHT teichoic acid reacted strongly with FA-1 antiserum. It showed bands of homology with both BHT and FA-1 crude acid extracts upon double-diffusion, using antisera to either strain. The BHT teichoic acid also displayed immunoelectrophoretic behavior identical to one of the mobile FA-1 cell wall antigens, again using either serum to develop precipitin bands. It is concluded this antigen may possess a serotype-specific determinant for *S. mutans* serotype b.

Dental caries in humans and rodents is the result of a bacterial infection of teeth and the subsequent degradation of enamel and dentinal layers by lactic acid and possibly proteolytic enzymes. One organism usually isolated from carious lesions is *Streptococcus mutans* (10, 12, 13, 20). Bratthall (8) defined five serotypes (a through e) within this cariogenic species by using comparative immunoelectrophoresis of whole-cell acid extracts. Extracts of *S. mutans* serotype e cross-reacted with antisera to Lancefield group E organisms, but extracts of serotypes a through d did not cross-react with any other known serological group.

The immunochemical natures of serotype-specific determinants of *S. mutans* have been the subject of recent investigations. Van de Rijn and Bleiweis (19) proposed that a membrane-associated glycerol teichoic acid antigen is common to all strains recognized as members of *S. mutans* serotype a. This antigen possesses a haptenic side-group identified as a disaccharide composed of D-galactose and D-glucose. Mukasa and Slade (16) demonstrated two antigens localized in the walls of *S. mutans* FA-1, a serotype b strain. One was a polysaccharide composed mainly of rhamnose and galactose; the other was a mucoprotein composed of galactose and a unique protein estimated to be 40% of the total dry weight. The haptenic determinants of each antigen were D-galactose and D-galactosamine.

In previous publications (5, 6), Bleiweis et al. reported the presence of glycerol and phosphorus in cell walls of *S. mutans* BHT and FA-1, both serotype b strains. Preliminary evidence suggested that a glycerol teichoic acid could be an important cell-wall antigen in *S.
mutans BHT and possibly in FA-1 as well (5). Mukasa and Slade (16), however, did not isolate such a polymer from FA-1. The present report describes the isolation and characterization of a teichoic acid-like antigen found in cell walls of S. mutans BHT. Because this purified antigen also reacts strongly with antisera prepared against FA-1, we believe it to possess a serotype-specific determinant for S. mutans, serotype b.

**MATERIALS AND METHODS**

**Bacterial strains and growth conditions.** Two strains of S. mutans were used in this study. BHT, a human isolate, was obtained from J. M. Jablon (University of Miami, Fla.), and FA-1, a rat isolate, was obtained from R. J. Fitzgerald (Veterans Administration Hospital, Miami, Fla.). Each strain was characterized as to species by colonial morphology and fermentative activity (i.e., utilization of mannitol and sorbitol) and as to serotype by precipitin reactions using antisera to BHT obtained from Dr. Jablon and FA-1 obtained from Dr. Bratthall (University of Göteborg, Göteborg, Sweden). At the conclusion of the present study, a subculture of BHT was returned to Dr. Jablon, who reconfirmed its identity.

Conditions for large-batch growth and subsequent harvesting were identical to those described previously (19).

**Extraction of cell-wall antigens by trichloroacetic acid.** Purified cell walls were obtained by breaking whole cells with glass beads in a Braun tissue homogenizer and treating the crude walls with trypsin, ribonuclease, and deoxyribonuclease according to known methods (7). Cell walls, at a concentration of 25 mg/ml, were suspended in cold 10% trichloroacetic acid and stirred for 20 h at 4°C. The partially extracted walls were collected by centrifugation at 33,000 × g for 10 min and reextracted. The pooled supernatants from three extractions were shaken with ether (5 volumes) to remove the acid from the aqueous fraction until a final pH of 5.0 was attained in the aqueous layer; the ether was discarded. Next, the extract was dialyzed for 24 h against 200 volumes of distilled water at 4°C, after which it was lyophilized. This material served as the crude extract for gel chromatography. A typical yield of extract was 1.5 g obtained from 11.5 g of pure cell walls, or 13% recovery.

**Purification by column chromatography.** Columns (2.7 by 90 cm) of Biogel P-100 (Biorad Laboratories, Richmond, Calif.) were prepared by using distilled water. Samples (50 to 75 mg) were loaded onto the columns and eluted with distilled water at room temperature. Flow rates of 10 ml/h were maintained, and fractions (2.0 ml) were collected in a Gilson Mini-Ecargot fraction collector (Gilson Medical Electronics Inc., Middleton, Wis.). Fractions were analyzed for rhombose and phosphorus as described below and for antigenic reactivity by capillary precipitin tests using homologous antiserum.

**Alkaline hydrolysis and diethylaminoethyl (DEAE)-cellulose chromatography.** Samples of purified antigen were alkaline-hydrolyzed to determine the nature of phosphorylated end products and the types of sugar side-groups on glycerol moieties. Base hydrolysates were subjected to chromatographic separation on DEAE-cellulose columns. All procedures used were identical to those described by van de Rijn and Bleiweis (19).

**Quantitative assays.** Phosphorus was determined by the method of Lowry et al. (15), with absorbancies measured at 820 nm. Glucose and galactose were determined by using the Glucostat and Galactostat reagents (Worthington Biochemical Corp., Freehold, N.J.), respectively. Glycerol was measured by use of the Glycerol Stat-Pack (Calbiochem, Atlanta, Ga.). Rhinomose was assayed by the method of Dische and Shettles (11). Amino acids and amino sugars were measured on a JEOL model JLC-6AH automated amino acid analyzer (JEOL, Inc., Cranford, N.J.). Sample hydrolysates were prepared for the above quantitative assays as described previously (19).

**Paper chromatography.** The following solvent systems were used: (i), n-propanol-aqueous ammonia (specific gravity 0.88)-water (6:3:1 by volume); and (ii), ethyl acetate-pyridine-water (10:4:3 by volume). Both solvent systems were used with Whatman 3MM paper (descending) for the separation of products of alkaline hydrolysis. Solvent system (i) separated phosphorylated glycerides initially resolved by chromatography on DEAE-cellulose columns, and system (ii) separated nonphosphorylated glycerides found in the water fractions obtained by using the above columns. Chromatograms to be eluted were run as bands on paper previously washed with 2 M acetic acid followed by water. Products were detected on papers as follows: alkaline silver nitrate for free sugars (18) and modified for glycosides (9); and molybdate for phosphoric esters (14).

**Immunological procedures.** The initial paper in this series (19) detailed the methods used for preparation of rabbit antiserum, preparation of Lancefield extracts, quantitative precipitin assays, quantitative precipitin inhibition assays, gel diffusion, and immunoelectrophoresis. These techniques were used with no changes except where noted below.

**Chemicals.** Organic solvents and acids and most common salts were obtained from Mallinckrodt (Scientific Products, Chamblee, Ga.). Amino acids, amino sugars, l-rhamnose, glycerol monophosphate, glycerol diphosphate, and a-methyl galactopyranoside were obtained from Sigma Chemical Co., St. Louis, Mo.; D-glucose and D-galactose were from Calbiochem; 3-methyl galactopyranoside was from Schwarz/Mann, Orangeburg, N.Y.; and ninhydrin and amino acid standard solutions were from Pierce Chemical Co., Rockland, Ill.

**RESULTS**

**Recovery of antigenic components by gel chromatography of trichloroacetic acid extracts.** Bleiweis et al. (5) reported the extraction of polysaccharide antigens from S. mutans BHT cell walls by using 0.5 N NaOH, as first described by Archibald et al. (3). One important antigen was partially purified by a combi-
nation of ion-exchange and gel chromatography, and was preliminarily characterized as a glycerol teichoic acid (5). Since that publication, however, Archibald et al. (2) have reported extensive degradation of certain teichoic acids by their procedure and concluded "it is clear that, although alkali extraction may be generally useful for obtaining fragmented teichoic acid for structural analysis of subunits, it cannot serve as a general method for the isolation of polymeric material." We decided to change extraction procedures for this reason and because of the potential loss of alkali-labile alanine esters. The procedure we used (see Materials and Methods) was the widely used cold trichloroacetic acid extraction technique of Armstrong et al. (4).

Crude trichloroacetic acid-extract (50 to 75 mg) was loaded onto a Biogel P-100 column, and four products (A, B, C, and D) were resolved upon elution with distilled water at room temperature (Fig. 1). Tubes 80 to 125 contained large amounts of rhamnose, little phosphorus, and no antigenic reactivity. These were pooled as fraction A. Tubes 126 to 162 also contained much rhamnose and little phosphorus, but reacted moderately (1+ or 2+) with homologous antiserum. These were pooled as fraction B. Tubes 163 to 215 contained essentially no rhamnose but much phosphorus, and reacted strongly (4+) with antiserum. These were pooled as fraction C. Finally, tubes 216 to 238 possessed no rhamnose and much phosphorus, and did not react serologically. These were pooled as fraction D.

**Examination of antigenic fractions for purity.** Restricting our studies to the antigenic fractions B and C, we examined them for indications of immunological and physical purity. Upon recycling on Biogel P-100, fraction C yielded a single peak with chemical and serological characteristics identical to those depicted in Fig. 1. Fraction B, however, sometimes showed slight contamination with fraction C material. For this reason, only small amounts of pure fraction B material were collected, and the studies emphasized in the present report are limited to fraction C.

Precipitation of the antigens by specific antibody by using the Ouchterlony double-diffusion technique is shown in Fig. 2. Fractions B and C reacted with the serotype b (BHT) antiserum to yield single homologous bands. Apparently, a common determinant is present in these antigenic fractions. Furthermore, the appearance of single bands indicated a lack of gross contamination by other antigenic moieties. The latter finding was verified by immunoelectrophoresis of the purified fractions and mild Lancefield extracts of cell walls (Fig. 3). Each fraction produced a single precipitin band, migrating toward the anode, upon reaction with homolo-

![Fig. 2. Immunodiffusion in gel demonstrating the precipitin pattern of fractions B and C derived from S. mutans BHT. The lower well contains homologous antiserum (A-BHT).](http://iai.asm.org/)

![Fig. 3. Immunoelectrophoresis of fractions B and C and a mild Lancefield extract of S. mutans BHT cell walls. Precipitin bands were developed by using homologous antiserum placed in the troughs. Mobility was toward the anode.](http://iai.asm.org/)
gous antiserum. But the electrophoretic mobility of each purified antigen was different and matched one of the two bands obtained with the crude cell-wall extract. Immunoelctrophoresis, therefore, was routinely used to check the purities of the two antigenic fractions after separation by gel chromatography.

Comparative chemical compositions of antigenic and nonantigenic fractions. Table 1 presents the chemical compositions of whole cell walls of S. mutans BHT, crude trichloroacetic acid extracts, residue from acid extractions, and column fractions B and C (see Fig. 1). Purified cell walls possess large amounts of sugars, including rhamnose, galactose, and glucose, significant amounts of the teichoic acid components glycerol and phosphorus, as well as the characteristic peptidoglycan components glucosamine, muramic acid, lysine, glutamic acid, and alanine in the molar ratios of 1.0:1.0:1.0:1.0:3.9. Only trace amounts of other amino acids were detected.

Upon acid extraction, much of the teichoic acid and polysaccharide was released (see Table 1), whereas the residue contained virtually all of the peptidoglycan material. The residue also contained significant amounts of the other compounds, indicating that the procedure was not totally effective in extracting non-peptidoglycan material.

Although fraction B contained some glycerol and phosphorus, it was mainly composed of sugars; amino acids were absent. The chemical composition of fraction B, as well as its immunoelectrophoretic characteristics (Fig. 3), are similar to the S. mutans FA-1 antigen 1 of Mukasa and Slade (16). Fraction C, however, had only three major components: glycerol, phosphorus, and galactose. Only small amounts of rhamnose and glucose were measured. This chemical composition suggests that we isolated a teichoic acid, although the molar ratio of glycerol to phosphorus (1.0:1.4) indicates either contamination by other sources of phosphorus or a novel structure not previously reported. Furthermore, the amino acid alanine is present in only trace amounts. d-Alanine is common to most teichoic acids, although Slabiyj and Panos (17) recently reported the absence of this amino acid in teichoic acids obtained from S. pyogenes L-forms. Fraction C has an immunoelectrophoretic mobility (Fig. 3) similar to the S. mutans FA-1 antigen 2 of Mukasa and Slade (16), but its chemical composition is far different from that mucoprotein. The nonantigenic fraction D is also a teichoic acid-like compound, but with significantly less galactose (not shown in Table 1).

Alkaline hydrolysis and identification of resolved products. Studies were undertaken to characterize fraction C as a glycerol teichoic acid. Fraction C was base hydrolyzed, and products were separated on a DEAE-cellulose column with a buffer gradient of ammonium carbonate (Fig. 4). After initial loading of the hydrolyzed sample onto the column, 200 ml of water was used to elute noncharged components. Four phosphorus-containing peaks were detected.

![Table 1. Chemical compositions of purified cell walls of S. mutans BHT and of various fractions derived by trichloroacetic acid extraction]

<table>
<thead>
<tr>
<th>Major component*</th>
<th>Cell walls</th>
<th>Crude extract†</th>
<th>Residue‡</th>
<th>Column fractions§</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μg/mg</td>
<td>μmol/ mg</td>
<td>μg/mg</td>
<td>μmol/ mg</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>14.9</td>
<td>0.48</td>
<td>39.0</td>
<td>1.26</td>
</tr>
<tr>
<td>Glycerol</td>
<td>22.8</td>
<td>0.25</td>
<td>72.0</td>
<td>0.78</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>224.0</td>
<td>1.36</td>
<td>194.0</td>
<td>1.18</td>
</tr>
<tr>
<td>Galactose</td>
<td>133.1</td>
<td>0.74</td>
<td>292.0</td>
<td>1.62</td>
</tr>
<tr>
<td>Glucose</td>
<td>11.0</td>
<td>0.06</td>
<td>26.7</td>
<td>0.15</td>
</tr>
<tr>
<td>Glucosamine</td>
<td>86.9</td>
<td>0.39</td>
<td>15.5</td>
<td>0.07</td>
</tr>
<tr>
<td>Muramic acid</td>
<td>93.1</td>
<td>0.31</td>
<td>3.8</td>
<td>0.01</td>
</tr>
<tr>
<td>Lysine</td>
<td>54.8</td>
<td>0.37</td>
<td>3.3</td>
<td>0.02</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>55.9</td>
<td>0.38</td>
<td>1.6</td>
<td>0.01</td>
</tr>
<tr>
<td>Alanine</td>
<td>127.5</td>
<td>1.43</td>
<td>3.0</td>
<td>0.03</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>4.1</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* All values are uncorrected for water of hydration or ash content.
† Amino sugars are reported as acetylated derivatives.
‡ See Materials and Methods for extraction procedure using trichloroacetic acid.
§ See Fig. 1 for separation of listed fractions.
*—*, Absence of component. Amino acids not listed were absent.
then resolved by the buffer gradient (200 ml). The water fraction and several phosphorus-containing fractions were pooled separately, lyophilized, and brought to constant volume (0.5 ml) with water for further analysis.

Table 2 summarizes the several phosphorylated compounds found in peaks I through IV, after further resolution by paper chromatography using solvent system (i). Glycerol monophosphate, glycerol diphosphate, and phosphorus were clearly evident and are normally the main end products of the alkaline-hydrolysis of glycerol teichoic acids. There were also present, however, at least seven unknown glycerol phosphate products. Each was eluted from paper chromatograms, acid-hydrolyzed, and chemically analyzed. The unknown products contained galactose, glycerol, and phosphorus in varying molar proportions; no other compounds were found. It must be surmised that this teichoic acid polymer is highly substituted by galactose and that the unknown products represent different types of fragments yielded by alkaline hydrolysis.

None of the products listed in Table 2 has the characteristic Rf or molar chemical constituency of diglycerol triphosphate, a hydrolysis product indicative of a 1-3 phosphodiester linkage in the original polymer. Archibald and Baddiley (1) have shown, however, that this compound is obtained only when two adjacent, unsubstituted glycerol moieties are present in the teichoic acid. Little or no diglycerol triphosphate is released by base hydrolysis of highly substituted polymers.

The water fraction was subjected to paper chromatography in solvent system (ii). Three distinct spots were detected with the modified silver nitrate spray reagent. The fastest-moving product co-chromatographed with authentic glycerol. Two slower-moving compounds were also detected (R gly 0.704 and 0.247). Each compound was eluted with water from resolved chromatograms, concentrated by lyophilization, brought up to 1.0 ml with 2 M HCl, and hydrolyzed in sealed ampoules for 3 h at 100 C. After washing to remove acid, the final dried samples were brought to constant volume with water and analyzed for glycerol, galactose, glucose, and phosphorus. Glucose and phosphorus were absent from each hydrolysate. The slower-moving compound (R gly 0.247) contained galactose and glycerol in a molar ratio of 2.9:1.0, whereas the faster-moving product (R gly 0.704) had a molar ratio of 1.0:1.0. The compound with the R gly 0.247 was clearly the major glycosyl glyceride in the water fraction. It would appear, therefore, that a trigalactosyl side-group is present on the native polymer, with smaller amounts of monogalactosyl groups.

**Polymer chain length.** Fraction C (1 mg) was treated with alkaline phosphatase (0.5 mg) in 0.5 ml of 0.02 M ammonium carbonate buffer (pH 9.5) for 18 h at 37 C to release terminal phosphoric acid groups. The total phosphorus content was measured, and the ratio of total phosphorus to labile phosphorus established a chain length of 25 glycerol phosphate units. This length is common to many teichoic acids studied previously (1). Interestingly, the nonantigenic fraction D revealed an average chain length of only 3 units by the above procedure.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Compound</th>
<th>R glycerol monophosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Glycerol</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Unknown glycosyl glycerol</td>
<td>0.36, 0.43, 0.51</td>
</tr>
<tr>
<td>II</td>
<td>Phosphorus</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Unknown glycosyl glycerol</td>
<td>0.61, 0.71, 0.83</td>
</tr>
<tr>
<td>IV</td>
<td>Glycerol diphospathate</td>
<td>0.24</td>
</tr>
</tbody>
</table>

* See text for further separation.
* All compounds listed as "unknown glycosyl glycerol phosphates" contained galactose, glycerol, and phosphorus in varying molar proportions. No further studies were made due to lack of samples.
Quantitative precipitin reaction and hapten inhibition studies. A quantitative precipitin experiment was run to establish the serological equivalence point for subsequent hapten inhibition studies. Homologous antisera were incubated with varying amounts of teichoic acid (fraction C) for 4 days, at which time the precipitates were collected and analyzed for antibody protein contents. All samples were run in triplicate. Controls for nonspecific precipitating antibody were negative. Equivalence was obtained at approximately 90 µg of teichoic acid.

After diluting the antiserum 1:5, a quantitative precipitin inhibition assay was set up in duplicate using 75 µmol of inhibitor and 20 µg of antigen (Table 3). The greatest amount of inhibition was obtained by β-methyl galactopyranoside and free galactose. Since α-methyl galactopyranoside demonstrated less inhibition, the native hapten appears to be in the β-configuration. The main component of the teichoic acid backbone, glycerol monophosphate, allowed only slight inhibition.

Comparative Ouchterlony and immunoelectrophoretic analyses. Purified fraction C, as well as crude acid extracts of S. mutans BHT, failed to react in capillary precipitin tests with antiserum to S. mutans serotypes a, c, and d organisms. Strong cross-reactivity with antiserum to S. mutans FA-1 was obtained, as expected. Ouchterlony analysis illustrated the cross-relatedness of the two strains. Fig. 5 demonstrates the strong reactivity of FA-1 antiserum with both crude acid extracts of BHT and its purified teichoic acid (fraction C). The band of absolute homology shown suggests that the teichoic acid may be a key antigen in this serotype. The reverse experiment (Fig. 6), using BHT antiserum reacting with both crude acid extracts of FA-1 and the BHT teichoic acid, revealed a similar pattern. That is, a band of absolute homology was obtained between the BHT teichoic acid antigen and the FA-1 extract.

Immunoelectrophoresis of crude acid extracts of whole cells of S. mutans BHT and the purified fractions B and C is shown in Fig. 7. Homologous antiserum was used and revealed three bands in the crude extract, all migrating toward the anode. Fractions B and C migrated the same distance as the two slower-moving antigens, but the fast-moving third component was unexpected and has not been previously shown. Importantly, it is absent from crude acid-extracts of BHT cell walls (Fig. 3). Immunoelectrophoresis of S. mutans FA-1 whole-cell extracts likewise revealed three bands, whereas FA-1 cell-wall extracts showed only the usual two bands. In these studies (Fig. 8), the mobilities of the two antigens in S. mutans FA-1 cell-wall extracts were identical to those of fractions B and C of S. mutans BHT. This was true when antisera to either organism were used and is a further indication of the close serological relationship between the two strains.

### DISCUSSION

It is necessary to consider the data presented above for S. mutans BHT in light of the findings by Mukasa and Slade (16) for S. mutans FA-1.

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**Table 3. Hapten inhibition of the quantitative precipitin reaction between teichoic acid (fraction C) and antiserum against S. mutans BHT**

<table>
<thead>
<tr>
<th>Hapten</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>d-Galactose</td>
<td>45.7</td>
</tr>
<tr>
<td>α-Methyl galactopyranoside</td>
<td>34.3</td>
</tr>
<tr>
<td>β-Methyl galactopyranoside</td>
<td>46.9</td>
</tr>
<tr>
<td>Glycerol monophosphate</td>
<td>9.2</td>
</tr>
</tbody>
</table>

* A 75-µmol amount was added in each case.
The polysaccharide antigen 1 of FA-1 appears to be identical to fraction B extracted from BHT cell walls by trichloroacetic acid. Both chemical composition and immunoelectrophoretic mobility closely relate the two fractions to each other. The mucoprotein antigen 2 of FA-1, however, appears to be absent from BHT cell walls. In fact, purified BHT cell walls were found to possess only trace amounts of the non-peptido-glycan amino acids (Table 1). The FA-1 mucoprotein antigen, therefore, may be unique to that organism.

The fraction C isolated by acid extraction of BHT cell walls is very likely a glycerol teichoic acid. Probably because of extensive substitution of glycerol at C-2 by galactose, base hydrolysis failed to yield diglycerol triphosphate, a moiety indicative of the characteristic 1-3 phosphodiester linkage of glycerol teichoic acids. For this reason alone, it is inappropriate to be dogmatic about the chemical nature of this antigen. But, the chemical constituency of fraction C and the nature of other products of base hydrolysis strongly suggest that we are dealing with a glycerol teichoic acid. This polymer is apparently substituted by trigalactosyl and monogalactosyl side-groups. Results of hapten inhibition studies (Table 3) suggest that the antigenic determinant is a β-galactoside. This sugar has been found to be the probable hapten in the two FA-1 antigens (16). Further studies using other antisera are in progress to define the haptenic configuration more precisely. The absence of D-alanine is surprising, since most teichoic acids possess this compound in an alkali-labile ester linkage. All extractions and washings were done at acid or neutral pH levels, so it is unlikely that D-alanine present in the native polymer was lost.

The present studies indicate strong cross-reactivity between the BHT teichoic acid and FA-1 antiserum (Fig. 5). Also, Ouchterlony analyses revealed absolute homology between precipitin bands formed by the BHT teichoic acid and FA-1 acid extracts (Fig. 6). The immunoelectrophoretic mobility of the BHT teichoic acid is identical to the more mobile antigen in FA-1 cell-wall acid extracts, using either antiserum to develop precipitin bands (Fig. 8). These findings suggest the occurrence of a similar antigen in FA-1 cell walls; however, Mukasa and Slade (16) did not report the presence of such a polymer. Studies of FA-1 are in progress to determine the presence of a cell-wall teichoic acid. It is apparent, however, that the BHT teichoic acid possesses the serotype b determinant.

I. Van de Rijn, J. Zabriskie, and A. S. Bleiweis (manuscript in preparation) recently found that rabbit antisera to BHT react with human myocardial tissue, as detected by immunofluorescent assays. There was no reactivity with tissues from several other organs. It was possible to remove heart-reactive antibody from the rabbit antiserum and from human rheumatic sera by absorption with the purified cell-wall teichoic acid described in this report. The immunochemical relationship of the BHT teichoic acid to S. pyogenes membrane antigens is being investigated.

ACKNOWLEDGMENTS

This investigation was supported by a grant from the Florida Agricultural Experiment Station (BC-01440) and by Public Health Service grant DE-02901-06 from the National Institute of Dental Research.

We thank E. M. Hoffmann for helpful comments during the preparation of this manuscript.
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