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Composition and Properties of a Group A Streptococcal Teichoic Acid

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Teichoic acid-like material extracted by cold trichloroacetic acid from lyophilized whole cells of streptococci from groups A, D, E, O, and T was shown to give a positive precipitin reaction with group antisera. Similar material from cells of groups B, C, F, G, H, K, L, M, N, P, Q, R, and S did not give a positive reaction with group antisera. The group A material also reacted with anti-E serum; however, the opposite did not occur. A similar result was also obtained on the group T material and anti-O serum. The group A teichoic acid was purified by Sephadex column chromatography, and was shown to be free of cell wall peptidoglycan and polysaccharide, and ribitol teichoic acid. It was composed of glycerol, phosphate, alanine, and glucosamine. Alkaline hydrolysis showed the presence of ester-linked alanine and glucosaminylglycerol. Phosphorus was released from ester linkage by alkaline phosphatase. *N*-acetylglucosamine produced a 72% inhibition of the precipitin test at a level of 10 μmoles, and D-alanine methyl ester was significantly stronger than the L-alanine ester. A single precipitin band was seen with group A serum. The data indicate that teichoic acid of group A streptococci is a polymer composed of glycerol phosphate and containing *N*-acetylglucosamine and alanine. Antisera to these streptococci contain antibodies specific for the alanine and the glucosamine linkages. The use of serum containing antibodies to alanine-polyglycerophosphate shows that the occurrence of this type of teichoic acid is widespread among the streptococci.

Information on the teichoic acids present in the streptococcal cell is limited to those occurring in serological groups A, D, and N. In group D the teichoic acid serves as the group antigen (22, 23). It is a polymer of glycerol phosphate containing glucose and alanine, and is associated with the protoplast membrane (14, 16). A teichoic acid from two strains of group N streptococci is known to react with group N antiserum, and is reported to be a galactosyl glycerophosphate polymer (7). In group A, a teichoic acid occurs in all serological types (9), but does not function as the group antigen. It contains glycerol, phosphate, and alanine (10). The location in the cell of the latter two components is not known.

The antigens of streptococci are significant for an understanding of the pathogenicity and antigenicity of the microorganisms and their relationship to its structure. The present study was designed to investigate the distribution of teichoic acids among a few strains of each of the serological groups. A reaction between the material isolated from a group A strain and both group A and E antisera led to a detailed study of the composition of the substance and its immunological properties. A preliminary report has been presented (T. Matsuno and H. D. Slade, Bacteriol. Proc., 1969, p. 106.

MATERIALS AND METHODS

Streptococcal strains. The cells of serological group A (strain Richards, type 3) were obtained, through the courtesy of Aaron Lane, from Difco Laboratories, Detroit, Mich. Group B, C, D, E, F, G, H, K, L, M, N, O, P, Q, R, S, and T cells were grown in Todd-Hewitt broth plus a glucose-salts mixture (8); they were washed three times with water and lyophilized.

Preparation of crude teichoic acid. Lyophilized whole cells (1 to 3 g) from each serological group were extracted with 30 to 90 ml of 10% trichloroacetic acid at 4 C for 17 hr with continuous stirring. The suspension was centrifuged at 4 C for 15 min at 3,000 × *g*, and the extraction of the cells was repeated twice. Two volumes of absolute ethanol were added to the supernatant and held for 17 hr at 4 C. The precipitate was removed by centrifugation, washed with acetone, ethanol, and ether, and dried in a vacuum. A 1-mg amount was dissolved in 1 ml of 0.85% saline and tested for the presence of antigen by capillary precipitin tests (20).

Purification of group A teichoic acid. A 50-g amount of lyophilized whole cells was extracted with 700 ml of 10% trichloroacetic acid as above. The residue was then extracted three times with 300 ml of 10% trichloroacetic acid, and the extracts were combined; 2.9 liters of absolute ethanol was added, and the mixture was centrifuged. The supernatant fluid was concentrated by flash evaporation at 40 C to 1.6 liters. Three liters of acetone was added and the solution was held at 4 C for 24 hr. The precipitate was removed by centrifugation and dissolved in 200 ml of 10% trichloroacetic acid at 4 C; 200 ml of acetone was added. After centrifugation, the precipitate was washed once with 30 ml each of acetone, ethanol, and ether, and was then dried in a vacuum. The yield was 2.4 g. The material was then suspended in 50 ml of water, and the insoluble fraction was removed by centrifugation. The supernatant fluid was lyophilized, dissolved in 15 ml of water, adjusted to *p*H 7.0 with 1 N NH_4OH, and passed through a Sephadex G50 column (40 by 1 cm). The quantity of nucleic acid in the teichoic acid material was measured at 260 nm after dilution, and a qualitative determination was made of the antigen present by the capillary precipitin test against specific group A rabbit antiserum. The positive fractions were lyophilized to yield 72.6 mg. The ethanol residue from the above extraction was separately purified by a similar procedure. The yield was 8.5 mg.

Thin-layer chromatography. A 2-mg amount of teichoic acid was hydrolyzed in 4 N HCl (1 ml) in a sealed tube for 12 hr at 100 C. The acid was removed by evaporation in vacuo over NaOH and P_2O_5, and the residue was dissolved in water (1 ml). A 5-μliter amount (10 μg) was spotted on precoated thin-layer chromatographic plates (Cellulose F, E. Merck AG). For the separation and identification of sugars and amino acids, a solvent composed of *n*-butyl alcohol-pyridine-water (4:6:3 v/v) was used. For glycerol, the solvent used was *n*-propanol-NH_3-water (6:3:1 v/v). Reducing sugars and α-glycol were detected by $AgNO_3$ (21), and amino acids by ninhydrin (5).

For the identification of glucosaminylglycerol, 2 mg of teichoic acid was hydrolyzed in 1 N NaOH (1 ml) for 3 hr at 100 C. The hydrolysate was passed through a column (2.5 by 0.6 cm) of Dowex 50 (NH_4^+ form) and evaporated to dryness. A 5-μliter amount (10 μg) was applied to a cellulose F plate and run in the same solvent as used for glycerol. To increase the intensity of the color of both glycerol and the glucosaminyl derivative, the plates were heated at 90 to 100 C for 5 min after dipping in the $AgNO_3$ reagent.

To detect ester-linked alanine, 1 mg of teichoic acid was shaken at 37 C for 17 hr in 1 ml of N NH_4OH. The alanine was identified on a thin-layer chromatographic plate, or quantitated on a Spinco amino acid analyzer.

Analytical methods. (i) *Enzymatic.* Monoester phosphate was determined by treatment with bacterial alkaline phosphatase (Worthington Biochemical Corp., Freehold, N.J.). A 100-μg amount of teichoic acid in 100 μliters of water was mixed with 50 μliters of tris(hydroxymethyl)aminomethane buffer (0.01 M, *p*H 10.0), and 10 μg of enzyme in 10 μliters of water was added. The mixture was incubated for 4 hr at 37 C, and the free inorganic phosphate was determined by the addition of 1 ml of reagent. The latter was a mixture of 9 ml of reagent A (1 ml of M sodium-acetate, 1 ml of 2.5% ammonium molybdate, 7 ml of water) and 1 ml of reagent B (10% ascorbic acid). Color development was maximal at 90 min at 37 C and was read at 820 nm.

D-Glucose was determined by the oxidase method as previously described (17).

(ii) *Chemical.* Total phosphorus was determined after 10 to 50 μg of dry teichoic acid was oxidized with 100 μliters of a mixture of 30.6 ml of H_2SO_4 and 6.7 ml of 70% $HClO_4$, plus water to 100 ml. The oxidation was carried out for 1 hr at 95 C and for 2 hr at 165 C. A 1-ml amount of molybdate reagent (A and B) was added after cooling, and measurement was made as above.

Rhamnose and total hexosamine were measured on the HCl hydrolysate as previously described (17). Nucleoprotein was measured chemically (13) and by extinction at 260 nm.

(iii) *Chromatographic.* Glucosamine was identified and determined in the HCl hydrolysate on a Beckman-Spinco amino acid model 116 analyzer (19). Glycerol was released from the teichoic acid (1 mg) by hydrolysis in 1 ml of 2 N HCl for 3 hr at 100 C. Water (2 ml) was added and the *p*H was adjusted to 9.0 with NH_4OH. Intestinal phosphatase (0.5 mg; Worthington Biochemical Corp.) was added, and the solution was held at 37 C for 17 hr. Enzyme protein was removed by the addition of trichloroacetic acid to a final concentration of 3%. The acid was removed by six extractions with 2 ml of ether and dried. A solution (100 μliters) containing 1 ml of pyridine, 0.2 ml of 1,1,1,3,3,3-hexamethyl disilizane, and 0.1 ml of chlorotrimethylsilane was added (18). After 5 min at room temperature, 1 to 2 μliters was injected into a Varian Aerograph model 1200 gas chromatograph; 100 μg of glycerol was used as a standard.

Serological procedures. The quantitative precipitin determination was described previously (17). The procedure for the inhibition of this determination was as follows. To 25 μliters of group A antiserum was added 1 to 10 μliters of M alanine (or its derivative) or glucosamine, and 0.85% NaCl to a total volume of 45 μliters. The tubes were incubated at 37 C for 30 min. Teichoic acid (12.5 μg in 5 μliters of saline) was added, and the solution was incubated at 37 C for 90 min, followed by 17 hr at 4 C. The quantity of antigen-antibody precipitate was determined as previously described (17).

Agar-diffusion analysis of the antigen-antibody complex was carried out as described by Ouchterlony (11).

Sources of sera and chemicals. Alanine methyl ester and glycosamine-6-phosphate were obtained from Sigma Chemical Co., St. Louis; D-alanine methyl ester from Cyclo Chemical Co., Los Angeles, Calif,; D-glucosamine HCl from Phanstiehl Chemical Corp., Waukegan, Ill.; and *N*-acetylglucosamine HCl from Mann Research Laboratories, New York, N.Y. The L-alanyl-L-alanine and the D-alanyl-D-alanine peptides were kindly provided by Francis Neuhaus. Grove Wiley kindly supplied a serum active against alanine-

polyglycerophosphate (ala-PGP; *see* Wilson and Wiley, 25). This serum was obtained by the injection of whole group A type 39 streptococcal cells into rabbits. In some cases we adsorbed this serum with lyophilized group A or H streptococcal cells (40 mg of cells/ml of serum). Streptococcal group antiserum was obtained from the National Communicable Disease Center, Atlanta, Ga., or prepared as previously described (15).

RESULTS

Lyophilized cells from all the known streptococcal groups were extracted with cold trichloroacetic acid (6). Extracts of A, D, E, O, and T cells reacted with specific rabbit antiserum (Table 1). The intensity of the reaction varied, however; the time required for the antigen-antibody precipitate to form was considerably less with group O than the other four sera. The yield of material in each case from 1 g of whole cells was as follows: group A, 5 mg; group D, 19 mg; group E, 4 mg; group O, 21 mg; and group T, 5 mg. As shown by further studies on the A and E material, quantities of inactive material were present in each case. Table 1 shows that the material extracted from A cells reacted with E as well as A antiserum, and that T material also reacted with O serum. In the case of E and O extracts, however, no cross-reaction was seen. Consequently, our attention was directed toward a study of the immunologically active material present in the A cells.

When quantities of lyophilized cells (50 to 100 g) were extracted, ethanol precipitated only small quantities of material from the acid-soluble fraction, whereas much larger quantities were precipitated with acetone. Figure 1A shows the elution pattern of the serologically active acetone precipitate and the nucleoprotein after passage through Sephadex G50. The active material began to appear soon after the void volume of the column had been collected. It is evident that a large part of the active material was present with nucleoprotein. The first 30 ml was concentrated and passed again through the same column after the column was washed with NaCl and water. The first 20 ml was concentrated and again passed through the column. The elution pattern after the third passage is shown in Fig. 1B. This first 18 ml of eluate was lyophilized and used for chemical analysis and immunological activity. It is evident that most of the nucleoprotein was removed, and analysis showed a content of only 1.1%. The glucose content was 1.1%.

Table 2 shows the compounds which were detected on thin-layer chromatographic plates in the acid and alkaline hydrolysates. The absence of rhamnose and muramic acid in the acid fraction indicates the absence of group A-specific polysaccharide and cell wall peptidoglycan in the teichoic acid preparations. Chemical determinations of rhamnose were also negative, and the Spinco amino acid chromatogram was negative for muramic acid. The presence, however, of glycerol and glycerol phosphate indicates a glycerol-type teichoic acid, and the negative ribitol value indicates that the former is the only teichoic acid present.

The alkaline hydrolysate (Table 2) contained glucosaminylglycerol, indicating that glucosamine is a part of the teichoic acid and directly attached to the glycerol unit. The resistance of 2-O-glucosaminyl-glycerol to alkaline hydrolysis has been shown (12).

Chemical and enzymatic analysis of the teichoic acid material is shown in Table 3. The large quantities of glycerol, phosphorus, alanine, and

TABLE 1. *Precipitin reactions between teichoic acid-like materials and streptococcal group antisera*[a]

Trichloroacetic acid extract		Group antiserum				
Group	Strain	A	D	E	O	T
A	Richards	+	−	+	−	−
D	8177	−	+	−	−	−
E	K129	−	−	2+	−	−
O	B357	−	−	−	2+	−
T	6496/62	−	−*	−	3+	+

[a] Material extracted from lyophilized cells with cold trichloroacetic acid was precipitated with ethanol and washed with acetone, ethanol, and ether. Extracts of cells from groups B, C, F, G, H, K, L, M, N, P, Q, R, and S were also tested against specific antisera and were found to be negative.

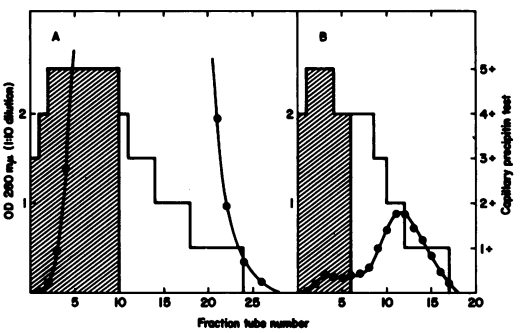

FIG. 1. *Pattern of acetone-precipitated teichoic acid on elution with water from G50 Sephadex column. Pyramid portion represents intensity of precipitin test against group A antiserum.* ●, *Nucleo-protein content of fractions; 3 ml in each fraction. Cross-hatched areas were subjected to further purification. A, First passage through column; B, third passage.*

TABLE 2. *Compounds present in acid and alkaline hydrolysates as shown by thin-layer chromatography*

Compound	Acid hydrolysate	Alkaline hydrolysate
Glycerol	+	+
Glycerol phosphate	+	+
Ribitol	−	−
Alanine	+	+
Glucose	+	−
Galactose	−	−
Rhamnose	−	−
Glucosamine	+	−
Galactosamine	−	−
Glucosaminylglycerol	−	+
Muramic acid	−	−

TABLE 3. *Chemical analysis of group A streptococcal teichoic acid*

Compound	Per cent (dry wt)	μMoles/mg (avg)
Glycerol	15.6 -17.0[a]	1.77
Total phosphorus	15.9 -17.8	5.42
Monoester phosphorus	0.42- 0.66[b]	0.17
Ester alanine	4.8	0.54
Glucosamine	6.6 - 6.7	0.37

[a] Range of several assays on two separate preparations.

[b] These values represent 2.6 to 3.7% of the total phosphorus.

glucosamine provide further evidence of the presence of a teichoic acid.

Figure 2 illustrates the antigen-antibody precipitin curves obtained when the teichoic acid reacted with group A-specific antiserum and an antiserum containing antibodies against ala-PGP (25). In both cases peaks were obtained with only 5 to 8 μg of the teichoic acid material.

Agar-gel diffusion (Fig. 3) showed that the group A teichoic acid produces a single band of precipitate upon reaction with A serum, whereas it showed no reaction with antisera to streptococci known to possess the group-specific teichoic acids, D and N. Antisera against groups E, O, and T were also negative.

To obtain an indication of the relationship of the constituents of the teichoic acid to its ability to react with antibody, various compounds were tested for their ability to inhibit the reaction. The results are given in Table 4. It is apparent that alanine methyl ester, glucosamine, and *N*-acetylglucosamine possess significant activity, with the latter most active. The absence of activity by alanine as compared to the alanine ester indicates that the ester linkage in the antigen is important for its immunological specificity. Of even greater significance, however, is the role of *N*-acetylglucosamine in the specificity and antigenicity of the teichoic acid. It is a major determinant.

DISCUSSION

Table 1 shows that crude teichoic acid from group A cells, prepared by ethanol precipitation of cold trichloroacetic acid extracts, cross-reacts with group E antiserum. In contrast, the acetone-precipitated and purified teichoic acid did not react with the same serum. The ethanol material was found to contain 8.7% nucleoprotein in addition to glycerol teichoic acid. This preparation was not as readily soluble in water as that prepared by acetone precipitation. The ethanol material may be combined with nucleoprotein as reported for teichoic acids from group D streptococci and lactobacilli (6, 22). After treatment of the ethanol teichoic acid preparation with ribonuclease, the cross-reaction with group E antiserum disappeared. The nucleoprotein free of teichoic acid did not react; consequently, it is possible that antibodies are present which are directed against a teichoic acid-nucleoprotein combination.

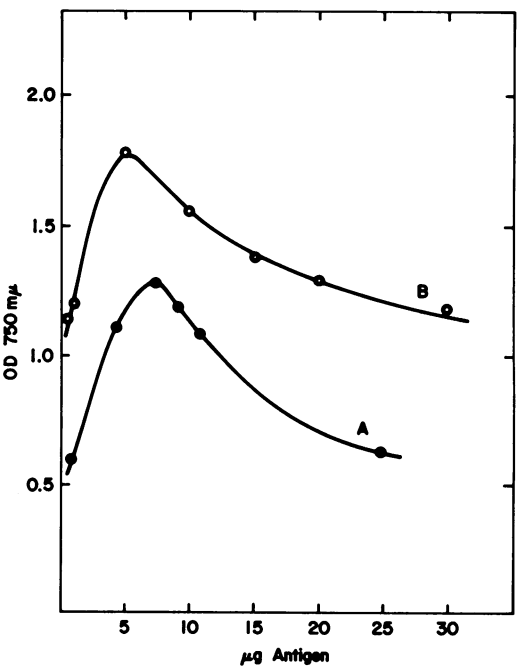

FIG. 2. *Precipitin curves of teichoic acid against; A, group A antiserum; B, alanine-polyglycerophosphate antiserum. For curve A, 25 μliters of antigen solution and 25 μliters of serum were used; for curve B, 50 μliters of antigen solution and 50 μliters of serum were used.*

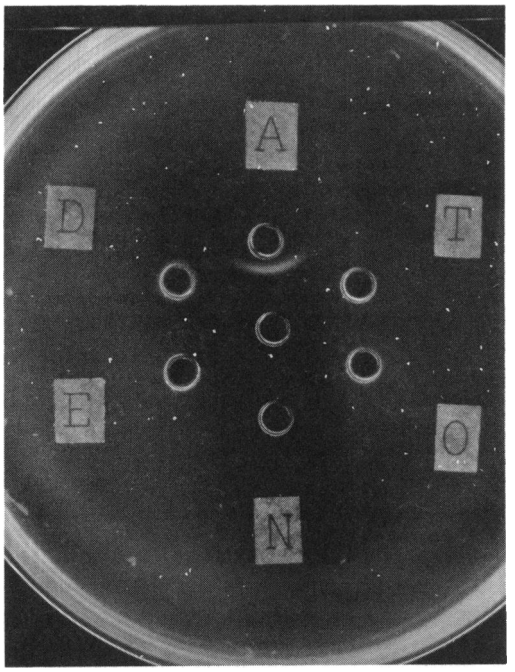

FIG. 3. *Reaction in agar of group A teichoic acid (acetone precipitate) and anti-A serum. Center well contained 5 μliters of saline containing 1 μg of antigen/μliter. The outer wells contained antisera specific to the streptococcal groups designated by the letters.*

An attempt has been made to determine the location of the group A teichoic acid antigen in the streptococcal cell. The material employed for chemical and serological studies was extracted from whole cells; however, cell walls extracted in a similar manner yielded only a trace of serologically active material based on the precipitin test with specific antiserum. Consequently, it is likely that this teichoic acid is associated with the protoplast membrane, as has been demonstrated with a group-specific teichoic acid of a group D streptococcal strain (14, 16).

A membrane teichoic acid from *Staphylococcus aureus* H is reported to contain as many as 35 glycerol units (12) based upon the ratio of monoester phosphate to total phosphate. In the present case, the average ratio is 32; however, it is not certain that the non-ester phosphate is also a part of the polymer chain. In all cases, values on the length of the repeating unit are subject to the method of extraction employed. The use of extracting agents other than trichloroacetic acid has been shown to release polymers with a variable number of repeating units (4). The quantity of phosphate in the teichoic acid (Table 3) is of interest. A 1:2 ratio of glycerol to phosphate has been shown in *S. lactis* I3 (2). In that case two glycerol-phosphate ester linkages were present, the second involving *N*-acetylglucosamine. Additional studies are required to establish the nature of the phosphate in the present case.

Table 3 shows that 4.8% alanine is released by mild alkaline hydrolysis and, as such, is present in ester linkage. We have found that, when ethanol is used as a precipitant after cold trichloroacetic acid extraction, the teichoic acid obtained contained 8.6% ester-linked alanine. The lability of this linkage in a group A streptococcal prepation has been reported (10). In addition to the lability of the ester alanine linkage, the present results also illustrate that the presence of glucosamine is affected by the extraction procedure employed. Studies in another laboratory on group A teichoic acid showed the presence of a polyglycerophosphate polymer (9) which contained alanine (10). No glucosamine was present. The extraction procedure employed 0.2 M acetate (*p*H 6.0) at 37 C for a total of 60 min. Glucosamine is a major constituent of the teichoic acid isolated by cold trichloroacetic acid (Table 2). When the streptococcal cells used in the present study were extracted with *p*H 6 acetate buffer, the teichoic acid obtained did not contain glucosamine; however, the same buffer had no effect on the glucosamine content of our purified teichoic acid. It is likely that the action of a glucosaminidase was responsible for the removal of the amino sugar in the work reported (10). The possibility of the presence of a second teichoic acid, similar in all respects except for the absence of glucosamine, must also be kept in mind.

The occurrence of a polymer of glucosamine-6-phosphate as found in *S. lactis* I3 and *Micrococcus hyicus* (3) is not indicated in the present case because of the lack of any inhibition (Table 4). The inhibition obtained with alanine methyl ester

TABLE 4. *Specific inhibition of teichoic acid precipitin reaction*

Compound	Inhibition observed with	
	10 μmoles	5 μmoles
	%	%
L-Alanine	0	0
D-Alanine	0	0
L-Alanyl-L alanine		0
D-Alanyl-D alanine		0
L-Alanine methyl ester	20.0	7.5
D-Alanine methyl ester	31.5	19.4
D-Glucosamine	40.0	17.0
N-acetylglucosamine[a]	72.0	50.0
D-Glucosamine-6-phosphate		0

[a] Inhibition with 1 μmole was 18.7%.

indicates the importance of this linkage in the immunological specificity, and as was to be expected, the D isomer was more effective than the L isomer. The absence of inhibition with either alanine or alanyl-alanine peptide further substantiates these results. Consequently, the group A material as isolated in the present case contains specificities against the glycerophosphate polymer, ester-linked alanine, and *N*-acetylglucosamine.

The chemical analysis as shown in Table 3 indicates that the group A teichoic acid is a polymer composed of glycerol, phosphate, ester-linked alanine, and glucosamine. The alanine could exist in a glycerol-2-O linkage or in a glucosamine-6-O linkage. The 2-O linkage appears more likely because it has been isolated free of glucosamine but containing alanine (10). If such is the case, of each 10 glycerol units, 3 would possess a 2-O ester linkage with alanine, 2 would possess a 2-O glycerol linkage with glucosamine, and 5 would be unsubstituted.

It is apparent from the present results and those of others (9, 10) that the glycerophosphate polymers of the streptococci from different serological groups do not display the same ease of extraction from the cell. Association with the protoplast membrane would not necessarily require a common behavior on extraction. Although all group A strains contain a glycerophosphate polymer only a limited number are capable of serving as antigens, or adsorbing antibodies from sera (10). Similarly, immunologically specific cell wall polysaccharide antigens of the streptococci show differences in location (24) and in ease of extraction under the same conditions (17).

Serum containing antibodies against ala-PGP has been obtained by the injection of whole cells of a type 39 strain of a group A streptococcus (25). This serum has been found to give a positive precipitin reaction with trichloroacetic acid extracts of A, E, F, H, M, Q, and T cells. After adsorption of the serum with whole cells of group A or H, these extracts no longer reacted with the adsorbed serum. These results indicate that an ala-PGP-type teichoic acid occurs in many streptococcal groups.

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LITERATURE CITED

1. Archibald, A. R., and J. Baddiley. 1966. The teichoic acids, p. 323–375. *In* M. L. Wolfrom and R. S. Tipson (ed.), Advances in carbohydrate chemistry, vol. 21. Academic Press Inc., New York.
2. Archibald, A. R., J. Baddiley, and D. Button. 1968. The glycerol teichoic acid of walls of *Staphylococcus lactis* I3. Biochem. J. **110**:543–557.
3. Archibald, A. R., J. Baddiley, D. Button, S. Heptinstall, and G. H. Stafford. 1968. Occurrence of polymers containing *N*-acetyl glucosamine-1-phosphate in bacterial walls. Nature (London) **219**:855–856.
4. Burger, M. M. 1966. Teichoic acids: antigenic determinants, chain separation, and their location in the cell wall. Proc. Nat. Acad. Sci. U.S.A. **56**:910–917.
5. Consden, R., and A. H. Gordon. 1948. Effect of salt on partition chromatograms. Nature (London) **162**:180–181.
6. Critchley, P., A. R. Archibald, and J. Baddiley. 1962. The intracellular teichoic acids from *Lactobacillus arabinosus* 17-5. Biochem. J. **85**:420–431.
7. Elliott, S. D. 1963. Teichoic acid and the group antigen of lactic streptococci (group N). Nature (London) **200**:1184–1185.
8. Hess, E. L., and H. D. Slade. 1955. An electrophoretic examination of cell free extracts from various serological types of group A hemoltyic streptococci. Biochim. Biophys. Acta **16**:346–353.
9. McCarty, M. 1959. The occurrence of polyglycero-phosphate as an antigenic component of various gram-positive bacterial species. J. Exp. Med. **109**:361–378.
10. McCarty, M. 1964. The role of D-alanine in the serological specificity of group A streptococcal glycerol teichoic acid. Proc. Nat. Acad. Sci. U.S.A. **52**:259–265.
11. Ouchterlony, O. 1958. Diffusion-in-gel methods for immunological analysis. Prog. Allergy **5**:1–9.
12. Rajbhandary, U. L., and J. Baddiley. 1963. The intracellular teichoic acid from *Staphylococcus aureus* H. Biochem. J. **87**:429–435.
13. Schneider, W. C. 1957. Determination of nucleic acids in tissues by pentose analysis, p. 680–684. *In* S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, vol. 3. Academic Press Inc., New York.
14. Shockman, G. D., and H. D. Slade. 1964. The cellular location of the streptococcal group D antigen. J. Gen. Microbiol. **37**:297–305.
15. Slade, H. D., and W. C. Slamp. 1962. Cell-wall composition and the grouping antigens of streptococci. J. Bacteriol. **84**:345–351.
16. Slade, H. D., and Shockman, G. D. 1963. The protoplast membrane and the group D antigen of *S. faecalis*. Iowa State J. Sci. **38**:83–96.
17. Slade, H. D. 1965. Extraction of cell-wall polysaccharide antigen from streptococci. J. Bacteriol. **90**:667–672.
18. Sloneker, J. H. 1968. Gas chromatography of carbohydrates, p. 87–135. *In* H. A. Szymanski (ed.), Biochemical applications of gas chromatography, vol. 2. Plenum Press, New York.
19. Spackman, D. H., W. H. Stein, and S. Moore. 1958. Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem. **30**:1190–1206.
20. Swift, H. F., A. T. Wilson, and R. Lancefield. 1943. Typing group A hemolytic streptococci by M precipitation reactions in capillary pipettes. J. Exp. Med. **78**:127–133.
21. Trevelyan, W. E., D. P. Procter, and J. S. Harrison. 1950. Detection of sugars on paper chromatograms. Nature (London) **166**:444–445.
22. Wicken, A. J., and J. Baddiley. 1963. Structure of intracellular teichoic acids from group D streptococci. Biochem. J. **87**:54–62.
23. Wicken, A. J., S. D. Elliott, and J. Baddiley. 1963. The identity of the streptococcal group D antigen with teichoic acid. J. Gen. Microbiol. **31**:231–239.
24. Willers, J. M., P. A. Deddish, and H. D. Slade. 1968. Transformation of type polysaccharide antigen synthesis and hemolysin synthesis in streptococci. J. Bacteriol. **96**:1225–1230.
25. Wilson, A. T., and G. G. Wiley. 1963. The cellular antigens of group A streptococci. J. Exp. Med. **118**:527–556.