

TEICHOIC ACIDS OF GROUP D STREPTOCOCCI WITH
SPECIAL REFERENCE TO STRAINS FROM PIG MENINGITIS
(*Streptococcus suis*)*

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The group antigen of Group D streptococci is a glucose-substituted polymer of α -glycerophosphate (1, 2). In streptococcal extracts it is found in two forms, one, lipid-bound (lipoteichoic acid), the other, lipid-free (3). The two forms are often found together but in differing relative proportions depending on the source of the material and method of extraction. In phenol extracts from washed cocci, the lipoteichoic acid predominates. It is probably released by phenol from association with the streptococcal cell membrane and carries with it a small fragment of the membrane lipid (4). In hydrochloric acid or trichloroacetic acid extracts of streptococci and in culture supernatant fluids, the lipid-free form predominates.

Both forms of teichoic acid react with Group D antiserum. In our experience, phenol extracts from all Group D strains tested, including representatives of the sub-groups *Streptococcus faecalis*, *Streptococcus faecium*, *Streptococcus suis*, and *Streptococcus bovis*, give group-specific precipitation reactions. HCl is less effective than phenol in extracting the lipoteichoic acid. The conventional method for extracting streptococcal group antigens by heating in HCl at 100°C and pH2 yields, from *S. faecalis*, sufficient free teichoic acid to precipitate with potent Group D antisera, but from *S. suis* such extracts may give weak or equivocal reactions (5). In an attempt to account for this difference between *S. faecalis* and *S. suis*, we describe here our examination of teichoic acid preparations from a variety of Group D streptococci, including representatives of these two subgroups.

Materials and Methods

Streptococcal Strains. Of the Group D streptococci used in this investigation strains D76 and C1 are classified as *S. faecalis*, strain C3 as *S. faecium*, (6), strains A227, A228, D930, and D958 as *S. suis* (7, 8), and strains A481 and A482, formerly designated C101 and C33, as *S. bovis* (9). Strain D958 is a noncapsulated variant of strain D930; *S. suis* type 1 (A228), type 2, (D930), and provisional type 3 (A227) correspond to deMoor's groups S, R, and T, respectively (5). Strains A227 and A228 (formerly designated PM23) have been described (7) Strain D930 was isolated from a human case of pig meningitis (8)

Streptococcal Extracts. HCl (pH2) extracts were prepared as previously described (10)

Phenol extracts were prepared from cultures grown in Todd-Hewitt broth for 18 h at 37°C. The cocci harvested by centrifugation from 5 liters of culture were washed twice in saline and resuspended in 25 ml H₂O. To this suspension was added 25 ml 90% phenol. The phenol suspension

* Supported by U S Public Health Service grant HL-03919.

was thoroughly mixed and then incubated for 60 min at 60°C followed by 18 h at 4°C. After centrifugation the aqueous layer was collected and the phenol layer re-extracted once with 25 ml H₂O. The combined aqueous phases were treated with ethanol (2 vol) containing 1% ammonium acetate. The resulting precipitate was washed twice in ethanol, once in anhydrous ether, and then dried under vacuum.

Preparation of Precipitating Antiserum in Rabbits. Serum R2045 was raised against Group D streptococcus strain D76 (*S. faecalis*). In addition to Group D antibody this serum contained low-titred type-specific antibody directed against cell-wall carbohydrate. Serum R1998 was raised against strain H69D5 (*S. faecalis*). Serum R1875 was raised against Group A streptococci (type 13) and contained antibody specific for alanine-substituted Group A polyglycerophosphate (11). Serum W3750 was raised against Group A streptococci type 38 (strain C94) and contained antibody directed against Group A polyglycerophosphate (i.e., "teichoic acid backbone" antiserum).

These rabbits were immunized by a procedure previously described using formolized vaccines for Group D strains and heat-killed vaccines for the Group A strains (12).

Immunoelectrophoretic Analysis This was performed in Veronal buffer 0.04 M pH 8.6. The supporting medium was 1% Noble agar (Difco Laboratories, Inc., Detroit, Mich.) in 0.04 M Veronal buffer on microscope slides (3 × 1 inch) employing 5 MA per slide. In all experiments the electrophoretic patterns were developed with Group D antiserum R2045.

Precipitin Tests. These were carried out using the capillary technique (13). In precipitin inhibition tests equal volumes of antiserum, inhibitor (or saline), and antigen solution were introduced into the capillary tube in that order. The tests were read immediately and after about 30 min at room temperature.

Results

Yield of Teichoic Acid in Phenol Extracts from Various Group D Streptococci. Phenol extracts were prepared from seven strains of Group D streptococci classified as *S. faecalis* (strains D76 and C1), *S. faecium* (strain C3), *S. suis* type 1 (strain A228), type 2 (strains D930 and D958), and provisional type 3 (strain A227). Each strain was grown for 18 h at 37°C in 5 liters of Todd-Hewitt broth. The cocci were harvested by centrifugation and extracted in 45% aqueous phenol as described under Materials and Methods.

Table I shows the weight of material recovered in this way from each strain together with the percentage content of RNA in each extract. The weight of bacterial cells from which each extract was prepared was not determined, but a comparative estimate was provided by the packed-cell volume of a 500-ml sample from each 5-liter culture. It can be seen that *S. faecium* and *S. suis* produced less growth in Todd-Hewitt broth than did *S. faecalis*. This may account in part for the smaller yield of material, exclusive of nucleic acid, in the phenol extracts from *S. faecium* and *S. suis* as compared with the yield from strain D76 (*S. faecalis*).

Serological Reactivity of Phenol Extracts with Group D and Group A Teichoic Acid Antisera. Table II shows the results of precipitation reactions between phenol extracts from the Group D strains listed in Table I and two different teichoic acid antisera, R2045 and W3750. Serum R2045 was raised against strain D76; it contained mostly antibody directed against Group D antigen and a negligible amount of type-specific antibody directed against D76 cell-wall carbohydrate. Serum W3750 was raised against a Group A streptococcus, strain C94; it contained high-titred antibody to unsubstituted Group A polyglycerophosphate ("backbone" antiserum).

From Table II it can be seen that, when tested against Group D antiserum (R2045), the phenol extracts from *S. faecalis* and *S. faecium* had about four to

TABLE I
Phenol Extracts from Various Group D Streptococci Yield of Aqueous Phase Components

Streptococcus extracted	Packed-cell volume	Total material extracted in aqueous phase	RNA* in aqueous phase
	ml	mg	%
Strain D76 (<i>S. faecalis</i>)	1.4	61	44
C1 (<i>S. faecalis</i>)	1.3	31.5	66
C3 (<i>S. faecium</i>)	0.7	10	2
A228 (<i>S. suis</i>)	1.1‡	66	80
D930 (<i>S. suis</i>)	1.1‡	64	89
D958 (<i>S. suis</i>)	0.8	83	97
A227 (<i>S. suis</i>)	0.8	50	84

* The ribose nucleic acid content of extracts was estimated on the basis of OD at 260 nm on a Beckman DU spectrophotometer assuming that 50 mg per ml RNA gives an OD of 1.0.

‡ Capsulated cocci packed with difficulty.

TABLE II
Reactivity of Phenol Extracts with Group D Antiserum and Teichoic Acid "Backbone Antiserum"

Phenol extract used in precipitin tests	Precipitin reactions Antiserum prepared with													
	Strain D76 Group D (R2045)							Strain C94 Group A (W3750 "backbone antiserum")						
	Extract (1 mg per ml) diluted													
	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:1	1:2	1:4	1:8	1:16	1:32	1:64
D76 (<i>S. faecalis</i>)	++	++	++	+	+	±	-	++	++	++	+	+	±	-
C1 (<i>S. faecalis</i>)	++	++	++	+	+	±	-	++	++	++	+	+	±	-
C3 (<i>S. faecium</i>)	++	++	+	+	±	-	-	++	++	++	+	+	±	-
A227 (<i>S. suis</i>)	++	++	+	±	-	-	-	+	±	-	-	-	-	-
A228 (<i>S. suis</i>)	+	+	±	-	-	-	-	±	-	-	-	-	-	-
D958 (<i>S. suis</i>)	++	+	±	-	-	-	-	+	±	-	-	-	-	-

In this and subsequent table ± to ++ indicates various strengths of precipitin reactions

eight times the reactivity of the extracts from *S. suis*. The comparatively low reactivity of the *S. suis* extracts was partly, though, as will be shown later, not solely due to their higher content of nucleic acid. With the backbone antiserum, W3750, the *S. faecalis* and *S. faecium* extracts showed the same amount of reactivity as with serum R2045. By contrast, the *S. suis* extracts showed significantly less reactivity with the backbone than with the Group D antiserum. This suggested a possible difference in the structure of lipoteichoic acid from *S. suis* as compared with lipoteichoic acid from *S. faecalis* and *S. faecium*, e.g., a different arrangement of glucosyl residues on the glycerophosphate chain. We decided to explore this possibility by comparing the serological reactivity of *S. faecalis* and *S. suis* phenol extracts after they had been subjected to heating in HCl at pH 1.8. This treatment was chosen because, in the past, it has been used as a means of extracting Group D antigen without loss of specific serological reactivity.

Effect of 100°C at pH 1.8 on Serological Activity of Phenol Extracts. It has been shown that treatment with 10% trichloroacetic acid at 4°C causes slow deacylation of Group D lipoteichoic acid (4). It therefore seemed possible that

TABLE III
Effect of 100°C at pH 1.8 on Reactivity of Phenol Extracts with Group D Antiserum and Backbone Antiserum

Phenol extract used in precipitin tests	Precipitin reactions Antiserum prepared with													
	Group D strain D76 serum R2045						Group A strain C94 serum W3750 (backbone antiserum)							
	Phenol extract (1 mg per ml) diluted													
	11	12	14	18	116	132	164	11	12	14	18	116	132	164
D76 (<i>S. faecalis</i>)	++	++	++	+	+	±	-	++	++	++	+	+	±	-
D76 heated at pH 1.8	+	+	±	±	-	-	-	+	+	±	±	-	-	-
D968 (<i>S. suis</i>)	+	+	+	±	-	-	-	+	±	-	-	-	-	-
D968 heated at pH 1.8	+	+	+	±	-	-	-	+	±	-	-	-	-	-

more drastic treatment, such as heating in HCl to 100°C at pH 1.8, might lead not only to its deacylation but also to disruption of its backbone structure without destroying the specific serological reactivity of the glucosyl residues. Accordingly, solutions (1 mg per ml) in HCl (0.02 M pH 1.8) were prepared with phenol extracts from all the Group D strains listed in Table I. Part of each preparation was heated to 100°C for 5 min. Both heated and unheated portions were then neutralized and tested against serum R2045 and serum W3750.

The results of precipitin reactions with the heated and unheated phenol extracts from strains D76 and D958 are shown in Table III. Results similar to those with strain D76 were obtained with extracts from strains C1 (*S. faecalis*) and C3 (*S. faecium*), and the results with strain D958 were the same as those obtained with the other *S. suis* extracts. It can be seen that heating at pH 1.8 caused a significant reduction in the reactivity of the D76 extract with both the Group D and the backbone antiserum. With the *S. suis* extracts, on the other hand, heating at pH 1.8 caused no significant change in serological reactivity with either serum.

Because the loss of reactivity suffered by the heated D76 extract was the same in both antisera, it seemed possible that a single component, the teichoic acid backbone, had been damaged. If this were so, all the serological reactivity of the D958 extract and the heated D76 extract with Group D serum R2045 was probably attributable to the group-specific part of the teichoic acid, i.e., the glucosyl determinant. Previous attempts to specifically inhibit with glucose Group D precipitation reactions involving group antigen from *S. faecalis* have been unsuccessful. This may have been due to the group determinant in *S. faecalis* taking the form of a di- or trisaccharide (2). It seemed worthwhile to re-examine the possible role of glucose as a specific inhibitor of Group D reactions involving lipoteichoic acid from *S. suis*.

Specific Inhibition of Group D Serological Reactions by Dextrose. Table IV presents the results of experiments in which four hexoses (dextrose, galactose, mannose, and *N*-acetyl glucosamine) were tested for their ability to inhibit precipitation reactions between phenol extracts of Group D streptococci and Group D antiserum R2045. It can be seen that dextrose, in a concentration of 50

TABLE IV
Effect of Monosaccharides on Precipitation of Phenol Extracts by Group D Antiserum R2045

Phenol extracts* from streptococcus strain	Precipitation reactions with serum R2045 in presence of stated hexose‡			
	Dextrose	Galactose	Mannose	<i>N</i> -acetyl glucosamine
D958 (<i>S. suis</i>)	±	++	++	++
A227 (<i>S. suis</i>)	±	++	++	++
A228 (<i>S. suis</i>)	±	++	++	++
D76 (<i>S. faecalis</i>)	++	++	++	++
C3 (<i>S. faecium</i>)	++	++	++	++
A481 (<i>S. bovis</i>)	±	++	++	++
A482 (<i>S. bovis</i>)	±	++	++	++

* *S. suis* and *S. faecium* extracts used in concentration of 1 mg per ml *S. faecalis* extracts 0.1 mg per ml. *S. bovis* extracts A481, 0.3 mg per ml; A482, 1.7 mg per ml.

‡ Hexoses tested in concentration of 50 mg per ml

mg per ml, inhibited almost completely the reaction of *S. suis* extracts with serum R2045; it also inhibited the reaction of these extracts with another antiserum, R1998, raised against a *S. faecalis* of serological type different from that used in the preparation of serum R2045. Dextrose also inhibited the reaction between phenol extracts from two strains of *S. bovis* (A481 and A482) and serum R2045, but it did not inhibit the reaction of these extracts with serum R1998. Dextrose did not inhibit the reaction of phenol extracts from *S. faecalis* or *S. faecium* with either of the Group D antisera tested.

We concluded from these results that the glucosyl determinants in *S. suis* teichoic acid are probably monosaccharides instead of the di- or trisaccharides which have been postulated as the glucosyl determinants in *S. faecalis* teichoic acid (2). It is hard to understand why dextrose should inhibit the reactivity of *S. bovis* extracts with antiserum R2045 but not with antiserum R1998. It is possible that inhibition of precipitation referable to the glucosyl determinant occurred in both antisera but that in serum R1998 this inhibition was obscured by precipitation referable to the complete glucosylglycerophosphate antigen. This explanation assumes that serum R2045 had more antibody to the glucosyl determinant of glucosylglycerophosphate than to the complete glucosylglycerophosphate antigen itself, whereas serum R1998 had more to the complete antigen than to the glucosyl determinant.

Examination of Phenol Extracts by Immunoelectrophoresis. To see whether mixtures of lipid-bound and free teichoic acid were present in the phenol extracts listed in Table I, samples of each extract were subjected to immunoelectrophoresis as described in Materials and Methods. The electrophoretic patterns were developed with Group D antiserum R2045.

The electrophoretic patterns given by phenol extracts from strain D76 (*S. faecalis*) and strain D958 (*S. suis*) are shown in Figs. 1 and 2. It can be seen that the extract from strain D76 showed two major components: one, fast-moving and with mobility only slightly less than that of free teichoic acid found in HCl extracts from strain D76; the other, slower and with a characteristically sharply

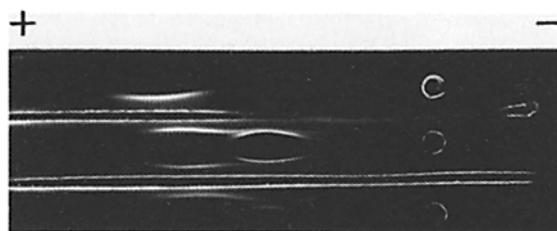


FIG. 1. (*S. faecalis*) Upper well contained D76 pH 2 extract; middle, D76 phenol extract 5 mg per ml; lower, D76 phenol extract heated to 100°C at pH 1.8 for 5 min. In all figures the electrophoretic patterns were developed with Group D antiserum R2045.

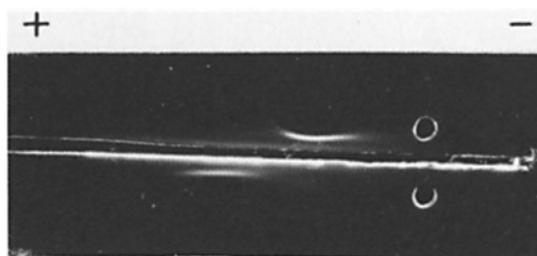


FIG. 2. (*S. suis*) Upper well contained D958 phenol extract; lower, D958 phenol extract heated to 100°C at pH 1.8 for 5 min.

defined precipitin pattern possibly due to lipoteichoic acid. This slow component showed two parallel lines of precipitation: one, barely visible in Fig. 1, was a faint line close to the serum trough and continuous with the line of the "fast" component; the other, a sharply defined line further from the serum trough, appeared to represent a serologically distinct lipid-bound component. Mixtures similar to this were also found in phenol extracts from strain C1 (*S. faecalis*) and strain C3 (*S. faecium*). By contrast, it can be seen that the phenol extract from strain D958 (*S. suis*) showed only one component. This was slightly less mobile than the slow component in *S. faecalis* extracts, but its precipitin pattern had the same sharply defined character. Patterns similar to that shown by strain D958 were shown by phenol extracts from all the *S. suis* strains.

These results suggested that whereas phenol extracts from *S. faecalis* contain a mixture of free and lipid-bound teichoic acid, phenol extracts from *S. suis* contain only lipid-bound material. Because such a possibility, if verified, might help explain the difficulty in extracting Group D antigen from *S. suis*, further experiments were designed to characterize with more certainty the group-reactive components in phenol extracts.

Effect of Acid on Phenol Extracts as Shown by Changes in Electrophoretic Pattern. It has been reported that in Group D lipoteichoic acid the linkage between lipid and glycerophosphate is destroyed by 10% trichloroacetic acid at 4°C (4). It therefore seemed possible that the nature of the components seen in the phenol extracts might be further elucidated by changes in the electrophoretic pattern brought about by treatment with acid.

EFFECT OF HEATING PHENOL EXTRACTS TO 100°C AT pH 1.8. Phenol extracts from strains D76 (*S. faecalis*) and D958 (*S. suis*) were dissolved in HCl 0.02 N to

give final concentrations of 5 and 2 mg per ml, respectively, at pH 1.8. Of each preparation, part was heated to 100°C for 5 min. This and the unheated portion were then subjected to immunoelectrophoresis at pH 8.6 with Group D antiserum R2075 as the developing serum.

From Figs. 1 and 2 it can be seen that heating to 100°C at pH 1.8 eliminated the slow-moving component from the D958 extract and much reduced it in the D76 extract. With the D958 extract a new fast-moving component, absent in the unheated preparation, appeared in the heated sample. In the heated D76 extract there appeared to be an increased concentration of the fast-moving component as shown by intensification of its electrophoretic pattern.

These changes in electrophoretic pattern brought about by acid treatment of phenol extracts confirmed our suspicion that the slow-moving component was indeed lipid bound, and the fast component, free teichoic acid.

EFFECT OF INCUBATING PHENOL EXTRACTS AT 37°C AND pH 4.5. Grown in nutrient broth containing 1% dextrose, cultures of *S. faecalis* achieve a pH level between 4 and 4.5 (14). It was obviously of interest to know whether this degree of acidity would detach the lipid from the lipoteichoic acid in phenol extracts of *S. suis* and possibly account for the mixture of free and lipid-bound teichoic acids found in *S. faecalis*. Accordingly, phenol extracts from three different strains of *S. suis* were dissolved in 0.4 ml amounts of acetate buffer (0.1 M, pH 4.5) to give a concentration of 10 mg per ml and incubated at 37°C for 18 h. Control solutions in saline (20 mg per ml) were held at 4°C. After 1 and 18 h, samples were taken from each preparation and subjected to immunoelectrophoresis.

No change was seen in any of the preparations after 1-h incubation, but after 18 h all three preparations incubated at 37°C and pH 4.5 showed two components assumed to be free and lipoteichoic acid. The control preparations remained unchanged with only one component, probably lipoteichoic acid. Figs. 3 *a* and *b* show the change in electrophoretic pattern of two of the preparations incubated at pH 4.5 after 18 h.

Effect of pH Level of Suspending Medium on Intracellular Teichoic Acid. The mixture of free and lipid-bound teichoic acids found in phenol extracts of *S. faecalis* could not be attributed solely to acid conditions generated in the culture medium during growth of these streptococci. We drew this conclusion from the result of an experiment in which strain D76 (*S. faecalis*) was grown in broth maintained at a pH level between 7 and 7.8 by the addition of sodium bicarbonate during the phase of active bacterial proliferation. A phenol extract from the resulting cocci contained a mixture of free and lipoteichoic acids similar to that found in a culture in which the pH level had dropped to pH 6.3 during growth. The only difference demonstrable by immunoelectrophoresis of the two extracts was that in the extract from the cocci grown under alkaline conditions both components showed higher mobility than did the components of the extract made from cocci grown under slightly acid conditions. The difference in mobility was possibly due to loss of alanine from the teichoic acid of the alkaline-grown cocci (11). This explanation was supported by the result of precipitin reactions with a teichoic acid antiserum possessing alanine specificity, R1875 (see Materials and Methods). In this antiserum the phenol extract from the acid-grown cocci had twice the reactivity of the extract from the

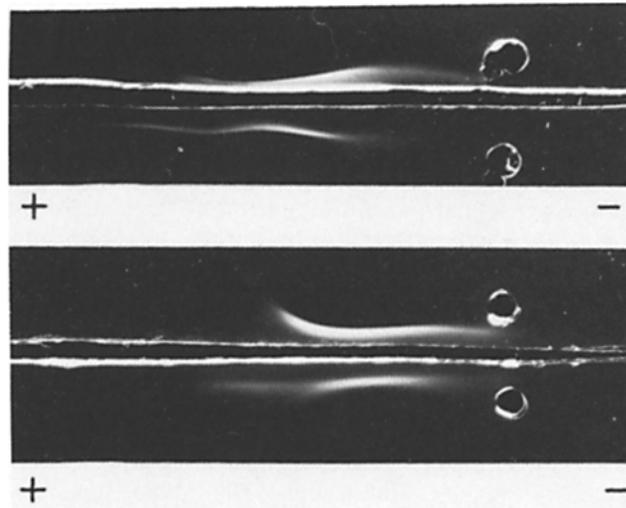


FIG. 3. (a) (*S. suis*) Upper well contained D958 phenol extract; lower, D958 phenol extract incubated at 37°C in acetate buffer pH 4.5 for 24 h. (b) (*S. suis*) Upper well contained A227 phenol extract, lower, A227 phenol extract incubated in acetate buffer pH 4.5 for 24 h

alkaline culture, whereas the reverse was found with a teichoic acid backbone antiserum lacking alanine specificity, W3750. From this experiment it was concluded that in *S. faecalis* there is an intracellular mixture of teichoic acids regardless of the pH level at which the cocci are cultivated. The teichoic acids of the actively growing cocci were accessible to the reaction of the culture medium. A low pH level might therefore contribute to deacylation of the lipoteichoic acid but some other factors, possible enzymatic, probably come into play. In this connection it should be said that we have been unable to detect any change in the electrophoretic pattern of *S. suis* lipoteichoic acid as a result of treatment with either phospholipase C or phospholipase D (Worthington Biochemical Corp., Freehold, N. J.).

Because of their inability to grow in broth at a pH level below 6, it was not possible with *S. suis* to study the effect of a lower pH level on actively growing cocci. Instead, the cocci harvested from a broth culture of strain D958 (*S. suis*) were incubated at 37°C in acetate buffer 0.1 M pH 4.5. Electrophoresis of a phenol extract made after 18 h revealed a single component of lipoteichoic acid indistinguishable from that found in a control extract from D958 cocci suspended in saline. From this experiment we concluded that with *S. suis* the intracellular lipoteichoic acid of resting or senescent cocci is not accessible to acid conditions in the suspending medium. It seems possible that the inability of *S. suis* to grow under acid conditions may protect the intracellular teichoic acid from deacylation.

Discussion

Our investigation had the limited objective of trying to understand why the group antigen is more difficult to recognize in some kinds of Group D streptococci than in others. Our results must be interpreted within the limits imposed

by the methods used: these were immunological techniques employed in the examination of unpurified streptococcal extracts. For example, we have assumed that the changes in the electrophoretic pattern of phenol extracts brought about by acid treatment were due to removal of lipid from the teichoic acid. Alternatively, they could be attributed to an altered dispersion of teichoic acid micelles (4) or to some change in the structure of the glycerophosphate polymer.

From the evidence presented here it appears that the difficulty in extracting teichoic acid from *S. suis* by heating in acid may result from combination of the intracellular teichoic acid with lipid. We have confirmed the finding of Joseph and Shockman (3) that phenol extracts of *S. faecalis* and *S. faecium* contain mixtures of free and lipid-bound teichoic acid. In phenol extracts from *S. suis* we have been able to demonstrate by electrophoresis only the lipid-bound form. We suggest that lipoteichoic acid, though readily extractable by phenol, is less accessible to extraction by heating at pH 2 than is free teichoic acid. It is possible that the low pH level achieved during the growth of some varieties of Group D streptococci in glucose broth deacylates part of the intracellular lipoteichoic acid and thus makes free teichoic acid available for extraction by heating at pH 2. This may account for the heretofore unexplained finding of Medrek and Barnes (15) that improved yields of Group D antigen were obtained from *S. bovis* grown in broth containing 1% dextrose.

Because of difficulty in the recognition of Group D antigen in streptococci from pig meningitis (*S. suis*) three new serological groups (groups R, S, and T) have been established for their accommodation (5). Two factors, at least, may have contributed to this difficulty: first, the difficulty in preparing good diagnostic Group D antiserum; second, the unsuitability of methods designed for the extraction of cell-wall group carbohydrates (e.g., heating at pH 2 or in formamide) when used for the extraction of lipoteichoic acid. For the identification of streptococci whose group antigen is suspected of being intracellular teichoic acid, a different method of extraction is called for, e.g., either physical disruption of the cocci or extraction in aqueous phenol.

It has become clear that the Group D antigen, glucosyl glycerophosphate, is distributed among a wide range of streptococci with widely differing characteristics. It may be said that the assembly of such a heterogeneous collection in a single group has little practical value. Alternatively, it can be argued that, in conjunction with serological typing and the establishment of subgroups, group identification is a useful screening device that reveals the relationship of previously unidentified strains to established serological types of known characteristics and potential.

Summary

Immunoelectrophoresis revealed in phenol extracts from *S. faecalis* and *S. faecium* a mixture of free and lipid-bound teichoic acids, both reactive with Group D antisera. In phenol extracts from *S. suis* only lipid-bound teichoic acid, also reactive with Group D antiserum, was seen. This difference probably accounts for the low yield of Group D antigen from *S. suis* as compared with *S. faecalis* and *S. faecium* when heating at pH 2 is used for extraction. When phenol is used good yields are obtained from *S. suis* as well as from *S. faecalis* and *S. faecium*.

Lipoteichoic acids from *S. faecalis* and *S. faecium* have a backbone structure the same as or similar to that of Group A streptococcal teichoic acid. Lipoteichoic acid from *S. suis* has a structure differing from that of *S. faecalis* and *S. faecium*, e.g., possibly in the attachment of its glucosyl substituents.

Precipitation reactions between *S. suis* lipoteichoic acid and Group D antisera were specifically inhibited by glucose. Reactions between *S. bovis* phenol extracts and some Group D antisera were also specifically inhibited by glucose, but extracts from *S. faecalis* and *S. faecium* were not. This may indicate a monosaccharide glucosyl substituent in teichoic acid from *S. suis* and *S. bovis* instead of the di- or trisaccharide previously postulated as the glucosyl substituent in the teichoic acid of *S. faecalis*.

Received for publication 18 October 1976.

References

1. Elliott, S. D. 1962. Teichoic acid and the group antigen of group D streptococci. *Nature (Lond.)*. **193**:1105.
2. Wicken, A. J., S. D. Elliott, and J. Baddiley. 1963. The identity of streptococcal group D antigen with teichoic acid. *J. Gen. Microbiol.* **31**:231.
3. Joseph, R., and G. D. Shockman. 1975. Synthesis and excretion of glycerol teichoic acid during growth of two streptococcal species. *Infect. Immunol.* **12**:333.
4. Toon, P., P. E. Brown, and J. Baddiley. 1972. The lipid-teichoic acid complex in the cytoplasmic membrane of *Streptococcus faecalis* N.C.I.B.8191. *Biochem. J.* **127**:399.
5. Moor, C. E. de. 1963. Septicaemic infection in pigs caused by hemolytic streptococci of new Lancefield groups designated R, S and T. *Antonie van Leeuwenhoek J. Microbiol. Serol.* **29**:272.
6. Elliott, S. D. 1960. Type and group polysaccharides of group D streptococci. *J. Exp. Med.* **111**:621.
7. Elliott, S. D. 1966. Streptococci infection in young pigs. 1. An immunochemical study of the causative agent (PM streptococcus). *J. Hyg.* **64**:205.
8. Windsor, R. S., and S. D. Elliott. 1975. Streptococcal infection in young pigs. IV. An outbreak of streptococcal meningitis in weaned pigs. *J. Hyg.* **75**:69.
9. Medrek, T. F., and E. M. Barnes. 1962. The physiological and serological properties of *Streptococcus bovis* and related organisms isolated from cattle and sheep. *J. Appl. Bacteriol.* **26**:169.
10. Lancefield, R. C. 1933. A serological differentiation of human and other groups of hemolytic streptococci. *J. Exp. Med.* **57**:571.
11. McCarty, M. 1964. The role of d-alanine in the serological specificity of Group A streptococcal glycerol teichoic acid. *Proc. Natl. Acad. Sci. U.S.A.* **52**:259.
12. Lancefield, R. C. 1938. A microprecipitin technique for classifying hemolytic streptococci and improved methods for producing antisera. *Proc. Soc. Exp. Biol. Med.* **38**:473.
13. Swift, H. F., A. T. Wilson, and R. C. Lancefield. 1943. Typing Group A haemolytic streptococci by M precipitin reactions in capillary pipettes. *J. Exp. Med.* **78**:127.
14. Sherman, J. M. 1937. The streptococci. *Bacteriol. Rev.* **1**:1.
15. Medrek, T. F., and E. M. Barnes. 1962. The influence of the growth medium on the demonstration of a Group D antigen in faecal streptococci. *J. Gen. Microbiol.* **28**:701.