The cell walls of streptococci

BY G. COLMAN AND R. E. O. WILLIAMS

*Department of Bacteriology, Wright-Fleming Institute,
St Mary's Hospital Medical School, Paddington, London, W. 2*

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SUMMARY

The cell-wall compositions of 197 strains of streptococci were determined by paper chromatography. The residues formed 26 patterns, but 117 strains had one or other of only 3 patterns. The results suggest possibilities for future investigations of some poorly defined taxa.

INTRODUCTION

The methods developed by Salton (1958) made possible the study of the cell-wall composition as a relatively simple procedure and recent work by others has suggested that cell-wall composition may be of use in classifying streptococci (Roberts & Stewart, 1961; Slade & Slamp, 1962). As part of a general study of the taxonomy of the streptococci we have collected over 300 strains representing the recognized Lancefield groups, other named species and a variety of presently unclassifiable strains. Each of the strains has been examined by a series of some 50 physiological and biochemical tests, the results of which will be presented separately; 197 of the strains have been subjected to cell-wall analysis and the results are presented here. The results obtained confirm previous work to the extent that the cell-wall structure of a few clearly defined species is constant, but the many different patterns of cell-wall residues in the less well-defined clusters of strains indicate that the study of cell-wall composition, as a method of classification, is subject to the same limitations as have long been recognized with other methods.

However, strains usually classified as, or with, *Streptococcus viridans* fell into two major divisions on the basis of cell-wall composition. The significance of this finding is not yet clear, but it does suggest possibilities for the investigation of the 'species' *S. viridans*, which is now defined in terms of the absence of distinguishing characters. The results also suggest that some, at least, of the non-haemolytic streptococci of Lancefield groups A, C and G could be classified, not with their respective groups, but with the non-haemolytic strains of group F and the proposed species *S. milleri* (Guthof, 1956).

METHODS

Strains. 103 of the strains were obtained from culture collections in this or in other countries, and 94 strains were freshly isolated. All cultures were preserved in the freeze-dried state.

Preparation of cell walls. The cocci were grown for 2 days in 0.5 l. amounts of Todd Hewitt broth, except for the strains of *Streptococcus pneumoniae* which were grown in a broth made up of pancreatic digest of casein (1%, w/v), yeast extract, Difco

(0.5 %), glucose (0.5 %) and K_2HPO_4 (0.5 %) at pH 7.0. Cultures of *S. cremoris* and *S. pneumoniae* were incubated at 32°; all other cultures were incubated at 37°.

The cocci were deposited by centrifugation and washed twice by resuspending in cold physiological saline after centrifugation. The first cell-wall preparations of six strains, the pneumococci and one strain in each of the Lancefield groups D, R and S were lost during handling. This was thought to be due to autolytic enzymes and in repeat preparations the washed cocci were heated in a boiling water bath for 10 min. before further treatment.

The washed cocci were resuspended in cold physiological saline, one drop of iso-octanol added, and the suspension shaken in a Mickle tissue disintegrator with an equal volume of Ballotini grade twelve glass beads for 10 min. The cell walls were separated from the glass beads by passing the mixture through a small sintered glass filter, and then the walls and the few remaining intact bacteria were deposited by centrifugation for 10 min at 12,000g. The deposit was washed twice with molar saline and resuspended in M/15 phosphate buffer (pH 8.0) containing trypsin 1 mg./ml. The suspension was incubated overnight at 37° with a few drops of toluene.

The cell walls were then washed twice with distilled water and twice with M-NaCl. The material was resuspended in molar saline and a differential centrifugation done by spinning at 1000g for 15 min. to deposit intact cocci and debris, and subsequently spinning at 12,000g for 10 min. to deposit the cell walls from the supernatant fluid of the previous operation. Loss of cell-wall material at the stage of differential centrifugation was great. The cell walls were then washed thrice with distilled water, or until the supernatant fluid was free from chloride and the preparation was then freeze-dried. The yield of material from 0.5 l. of broth varied between 1 and 32 mg. depending on the strain.

The method of preparation of cell walls is a modification of that described by Salton & Horne (1951) and was suggested by Salton (personal communication).

Paper chromatography. Three chromatographic systems were used routinely for all cell walls, but on occasions further tests were made and these are described below. For each chromatogram the products of acid hydrolysis of 2 mg. of cell walls were repeatedly dried in a vacuum desiccator containing concentrated sulphuric acid and potassium hydroxide pellets and finally applied to Whatman grade 1 paper.

To detect hexoses and deoxyhexoses, the dried walls were hydrolysed with 2 N-HCl for 2 hr at 100°. The residues were separated on a descending chromatogram on which the solvent was allowed to run for 16–20 hr. The solvent was butanol + pyridine + water (6 + 4 + 3, v/v). The paper was then air-dried and dipped through *p*-anisidine hydrochloride in butanol (1 %, w/v), and heated in an oven at 70–75° for 15 min. The detecting agent, used in this way, did not react with amino sugars, amino acids or polyols.

For the detection of polyols, the cell walls were hydrolysed in 2 N-HCl for 2 hr at 100°. These conditions largely converted ribitol to anhydrosorbitol. A periodate and benzidine reagent (Smith, 1960) was used to detect glycerol and anhydrosorbitol; ribitol was rarely present on the chromatograms. Separation of glycerol and anhydrosorbitol was achieved with a descending chromatogram on which butanol + acetic acid + water (12 + 3 + 5, v/v) was allowed to run for 40 hr.

Amino acids and amino sugars were detected in samples hydrolysed for 16 hr in 4 N-HCl at 100°. An ascending two-dimensional technique with 10 in. square papers was used. Butanol + acetic acid + water was the first solvent, and lutidine + water (13 + 7, v/v) was used as the solvent in the second direction. Residues were detected by dipping the air-dried papers through a solution of ninhydrin in acetone (0.2 %, w/v) and heating the paper in an oven (70–75° for 15 min.) These chromatograms were preserved by dipping them through an aqueous half-molar solution of nickel sulphate and then drying the papers in the oven.

Reproducibility of results. To test the reproducibility of the results reported below, fresh preparations of cell walls were made from 26 strains. The strains were not chosen at random, for with 7 of the strains the residue glycerol had previously been reported and anhydrosorbitol had been recorded as present in the residues of 12 strains. The same sugars, amino sugars and amino acids were recorded on the first and second occasions, but glycerol and anhydrosorbitol were each recorded on one less occasion in the second series of tests. This suggests that glycerol and anhydrosorbitol are indicative of polymers either less constantly formed or less firmly attached to the cell wall.

Grouping reactions. For the purposes of this paper we have taken the reaction of a strain with any Burroughs Wellcome Lancefield grouping antiserum of the groups A to S as indicating the presence of a group hapten. The cell wall composition is tabulated, where possible, by the presence of these haptens.

RESULTS

Table 1 lists the patterns of cell wall residues found and shows that, while twenty-six patterns of the occurrences of sugars and sugar-like substances were detected, more than half of the strains fell into only three of these patterns. Table 2 is a listing of these patterns against accepted or possible taxonomic groupings of streptococci and the final column of this table contains those strains in which the finding of further characters for differentiation would be useful. Table 3 gives the results obtained with individual strains from the National Collection of Type Cultures and the National Collection of Dairy Organisms.

The substances detected

Amino acids and amino sugars. The pair of solvents used to separate these compounds gave good separation of amino sugars, but had limitations for the separation of amino acids, which tended to lie diagonally across the paper. Aspartic acid and glutamic acid were only partly separated and thus, although aspartic acid was recorded as being present in sixty wall preparations, this may represent only a portion of the occurrences. Muramic acid, glucosamine, lysine, glutamic acid and alanine were detected in all cell walls. Valine and leucine (or isoleucine) were also present but in smaller amounts, and were absent from one strain (NCTC 6681). Glycine was found in small amounts in all but fifteen preparations, and serine was detected, as well as glycine, in the pneumococci and two other strains.

A residue with mobility between that of glucosamine and muramic acid in the solvent butanol + acetic acid + water was detected with ninhydrin in 11 strains. A positive Elson-Morgan reaction (Smith, 1960) indicated it was an amino sugar.

Table 2. *Strains examined for cell wall composition divided by taxonomic group and cell-wall constituents. The letters of the alphabet refer to Lancefield group reactions, and the numbers in the body of the table are numbers of strains*

Cell wall pattern											CO ₂ depend-	Ottens	<i>S.</i> <i>milleri</i>	Indif- erent A, C and G			<i>S.</i> <i>uberis</i>			<i>S.</i> <i>sanguis</i> (gp. H)	<i>S.</i> <i>sanguis</i>	K	<i>S.</i> <i>salivarius</i> (gp. K)	<i>S.</i> <i>salivarius</i>			Pneu- mococci	Strains lacking disting- uishing charac- ters	
	A	C	G	B	D	Q	R	S	T	L	F	types		N	E	P		H	M			O							
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26	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	1
No. of strains	3	5	5	7	15	1	2	2	2	4	2	20	7	8	7	7	6	1	3	6	7	11	6	5	4	4	7	3	37

Table 3. *The results of cell wall analysis of strains of streptococci obtained from the National Collection of Type Cultures and the National Collection of Dairy Organisms*

Strain no.	Species and serological group	Cell wall pattern no.
NCTC 8194	<i>S. pyogenes</i> gp. A	1
NCTC 8181	<i>S. agalactiae</i> gp. B	6
NCTC 4669	<i>S. dysgalactiae</i> gp. C	2
NCTC 7186	<i>S. equisimilis</i> gp. C	2
NCTC 6176	<i>S. zooepidemicus</i> gp. C	2
NCTC 7879	<i>S. faecium</i> gp. D	9
NCTC 8175	<i>S. faecalis</i> var. <i>liquefaciens</i> gp. D	10
NCTC 8176	<i>S. faecalis</i> var. <i>zymogenes</i> gp. D	11
NCTC 8177	<i>S. bovis</i> gp. D	11
NCTC 8213	<i>S. faecalis</i> var. <i>faecalis</i> gp. D	10
NCTC 8307	<i>S. durans</i> gp. D	11
NCTC 5385	<i>S. sp.</i> gp. E	13
NCTC 3858	<i>S. uberis</i> gp. E	6
NCTC 5389	<i>S. sp.</i> gp. F	11
NCTC 8037	<i>S. sp.</i> (MG) gp. F	11
NCTC 8547	<i>S. sp.</i> gp. G	3
NCTC 7868, NCTC 10231	<i>S. sp.</i> gp. H	6
NCTC 7863	<i>S. sanguis</i> gp. H	5
NCTC 7865	<i>S. sanguis</i> gp. H	6
NCTC 10232	<i>S. sp.</i> gp. K	24
NCTC 8606	<i>S. salivarius</i> gp. K	6
NCTC 8618	<i>S. salivarius</i> gp. K	11
NCTC 6404	<i>S. sp.</i> gp. L	11
NCTC 7760	<i>S. sp.</i> gp. M	9
NCTC 6681	<i>S. lactis</i> gp. N	4
NCDO 712	<i>S. lactis</i> gp. N	6
NCDO 176	<i>S. lactis</i> var. <i>diacetilactis</i> gp. N	4
NCDO 607	<i>S. cremoris</i> gp. N	11
NCDO 924	<i>S. cremoris</i> gp. N	6
NCTC 8081	<i>S. sp.</i> gp. O	24
NCTC 9824	<i>S. sp.</i> gp. P	15
NCTC 9938	<i>S. sp.</i> gp. Q	4
NCTC 10234	<i>S. sp.</i> gp. R	6
NCTC 7366	<i>S. salivarius</i>	6
NCTC 7864	<i>S. sanguis</i> type II	20
NCTC 7466	<i>S. pneumoniae</i>	21
NCTC 8165	<i>S. sp.</i> 'viridans' type	11

In the solvent butanol+pyridine+water the order of separation was the same as glucose and in the solvent phenol+aqueous ammonia (phenol 160 g., water 40 ml. in an ammoniacal atmosphere) the mobility of the unknown sugar was 2.0 relative to glucose (Crumpton, 1959). This unknown amino sugar was thus thought most likely to be fucosamine, and it showed identical mobilities with fucosamine obtained by extraction of *Chromobacterium lividum* (*C. violaceum*) NCTC 7917 (Crumpton & Davies, 1958) in these three solvent systems.

6-Deoxyhexoses. Rhamnose was present in considerable amounts in 155 strains and was recorded as being present in trace amounts in 10 further strains. Of these 10 strains, 7 would in everyday usage be classified as *Streptococcus viridans* and are included in the final column of Table 2, 2 were strains of *S. sanguis* and 1 belonged to Lancefield group 0. The possibility exists that if more sensitive methods had been

used for the detection of rhamnose, the proportion of strains recorded as showing smaller amounts of rhamnose would have been higher and the number of strains described as lacking rhamnose would have been correspondingly lower.

One strain (S320) received as *Streptococcus cremoris* but differing from typical strains of this species by its failure to yield an extract reacting with Lancefield group N serum gave a sugar travelling closer to the solvent front than rhamnose. This sugar was obtained in chromatographically pure form by elution from paper chromatograms. In the CyR10 reaction for 6-deoxyhexoses of Dische & Shettles (1948) samples of the unknown sugar gave spectra with the maximum value occurring between 396 and 400 m μ and a zero reading at 430 m μ ; indicating a 6-deoxyhexose. The R_{rhamnose} values were 1.09 in butanol+pyridine+water, and 1.20 in butanol+acetic acid+water. An authentic sample of 6-deoxy-L-talose showed the same mobility in the latter solvent, and similar colour reactions were given by both sugars with a vanillin+perchloric acid spray (MacLennan, Randall & Smith, 1959). Electrophoresis with constant current (0.1 mA./cm.) and a voltage range of 150 to 300 V. in borate buffer pH 9.5 showed identical mobilities of the unknown sugar and 6-deoxy-L-talose (MacLennan, Randall & Smith, 1961). The unknown sugar was therefore thought most likely to be 6-deoxytalose.

The strains examined

Lancefield group A (Streptococcus pyogenes). The cell-wall compositions of 26 strains of *S. pyogenes* (Lancefield group A) has been described by Michel & Gooder (1962). They found that rhamnose was the only neutral sugar present, a finding confirmed by our examination of 3 strains. *N*-acetylglucosamine is the determinant of the group A antigen (McCarty, 1958), but the conditions of hydrolysis used will de-acetylate such sugars and the detection of residues found in hydrolysates of cell walls would not distinguish between glucosamine as part of a cell wall polysaccharide and glucosamine as part of the mucopeptide. As mentioned previously glucosamine and muramic acid were detected in all cell walls.

Lancefield group C. Cell walls of 5 strains of group C were examined and galactosamine was the only residue additional to those found in strains of *Streptococcus pyogenes*. This sugar in the *N*-acetyl form is the determinant of the group C antigen (Krause & McCarty, 1962). All 5 strains were found to have the same cell wall composition but Slade & Slamp (1962) and Michel & Gooder (1962) have recorded glucose as an additional residue present in the cell walls of some group C strains. Three strains of group C were from the National Collection of Type Cultures and are listed in Table 3, and the two further strains were isolated from human clinical material.

Lancefield group B. Seven strains of *Streptococcus agalactiae* (Lancefield group B) were examined and, of the neutral sugars, all contained rhamnose, glucose and galactose and all lacked galactosamine. Five strains showed only trace amounts of glucose on the paper chromatograms. Strains with these three aldose sugars and lacking galactosamine form pattern 6 in Table 1. One strain (fw 57) gave a precipitate with Lancefield group R sera as well as a group B serum but has been included as a strain of *S. agalactiae* as it had the physiological and biochemical reactions of this species and was unlike strains of group R. Slade & Slamp (1962) and Wittner & Hayashi (1965) have both recorded galactosamine as an additional

constituent of the cell wall of group B streptococci. This cell wall composition in Table 1 is designated as pattern 11, which with pattern 6 comprises almost one half of the strains examined.

Lancefield group G. Curtis & Krause (1964) have presented evidence of an antigenic relationship between the cell wall polysaccharides of groups B and G streptococci. However, our studies showed differences in gross cell wall composition in that all 5 haemolytic strains of group G contained galactosamine and lacked glucose while none of the strains of group B contained galactosamine and all contained glucose.

Lancefield group D. Of the species forming Lancefield group D some wall hydrolysates contained glycerol, some anhydrosorbitol and some neither. This is the basis of the division of these strains into patterns 9, 10 and 11 in Table 1. Two strains (BS 347 and BS 361) have been included by us in this group, for, although grouping reactions failed, in all other respects they showed the reactions typical of *Streptococcus faecalis*. Six cultures were common to our study and that of Slade & Slamp (1962); they were the cultures from the National Collection of Type Cultures listed as group D in Table 3. The same sugars were detected in both studies.

Groups Q, R, S and T. A total of only 7 strains, from culture collections, were examined in the groups Q, R, S and T. The strains in groups R, S and T showed the residues of the two most frequently found patterns, those numbered 6 and 11 in the Tables 1 and 2. One strain in group S, s 1230, was unusual in that a large amount of glycine was found in the cell wall and this strain was also autolytic.

Group L. Analysis of cell wall preparations from four different cultures of group L streptococci showed a different composition for each strain. Two strains were probably isolated from animals, the other two were isolated from hospital patients.

CO₂ dependent streptococci. These two strains were isolated by Fitzgerald & Keyes (1960) and were capable of causing dental caries in the hamster. They differed from the 'minute' streptococci found in groups F and G (Deibel & Niven, 1955) in that they required carbon dioxide for growth on normal laboratory media, that they were non-haemolytic (indifferent) when grown on horse blood agar plates, and in their absence of a recognized group antigen. Both strains had the cell wall composition numbered 6 while the 'minute' streptococci of group F examined by us had the cell wall pattern numbered 11.

Group F and physiologically related streptococci. Streptococci of Lancefield group F, particularly the non-haemolytic strains, have many physiological characters in common with strains that lack a recognized group antigen but contain an Ottens antigen (Ottens & Winkler, 1962); with streptococci of the proposed species *Streptococcus milleri* (Guthof, 1956); and with the few non-haemolytic streptococci of groups A, C, and G that we have examined. The 42 strains thus grouped together show considerable similarity in cell wall composition. The compositions of the cell walls of the indifferent strains of groups A, C and G are closer to each other and to the strains of group F than to the haemolytic strains of groups A, C and G. The non-haemolytic streptococcus of group A mentioned in the paper of Michel & Gooder (1962) may be similar to the organisms included here.

One strain (FW 113, pattern 5), extracts of which gave a delayed precipitate with group F antisera was of interest, for its cell walls lacked detectable amounts of both galactose and galactosamine. Michel & Willers (1964) partially hydrolysed the

polysaccharide of a group F strain and obtained a disaccharide of glucose and galactosamine which was a most potent inhibitor of F/anti-F reactions. From the results of inhibition tests these authors thought glucose to be the major determinant of antigenicity and the finding of this strain provides further evidence for that postulate.

Group N. Six cultures, extracts of which gave a precipitate with group N serum, were studied. They belonged to the species *Streptococcus lactis*, *S. lactis* var. *diacetylactis*, and *S. cremoris*, but their species designation was not correlated with their wall composition. One strain sent to us as a typical representative of *S. cremoris* (s320, pattern 12) could not be identified by serological methods but it gave the physiological reactions expected of a strain of this species. It was this strain that contained the unusual sugar 6-deoxytalose, a sugar identified by MacLennan (1961) in *Actinomyces bovis*.

Group P. The three strains belonging to group P were notable in that they all contained fucosamine but lacked galactosamine. Fucosamine was reported by Wheat, Rollins & Leatherwood (1964) as a cell wall constituent of an organism identified as *Bacillus cereus*.

Group E. Six strains belonging to Lancefield group E were examined. One was a strain of *Streptococcus uberis*, two were haemolytic, and three strains showed greening on horse blood agar. One of the haemolytic strains (NCTC 5385, pattern 13) has been examined by Cummins & Harris (1956*a*), Michel & Gooder (1962), and Slade & Slamp (1962). All of these workers described mannose as present in the cell wall of this strain. Within the limits of our techniques we did not confirm this finding but did detect fucosamine. One of the greening strains (s322, pattern 14) contained an amino sugar we were unable to identify. The chromatographic mobility differed slightly from that of fucosamine.

Streptococcus uberis. One strain of *Streptococcus uberis* that did not belong to group E was examined, and it differed in composition from the strain of *S. uberis* of group E by the absence of galactose.

Group H and Streptococcus sanguis. A working definition of serological groups was given in the section on methods and this definition needs clarification here, for de Moor (1964) has pointed out that both group H and group K are serologically heterogeneous. With the sera we used to examine our collection, strain Challis (NCTC 7868) belonged to group H while the strain Perryer (F 90 A) examined by Slade & Slamp (1962) did not, although both strains were isolated and characterized by Professor R. Hare as group H.

Seven strains of *Streptococcus sanguis* serological group H were examined and the only unusual feature of the cell walls noted was that of the 4 strains with the cell wall pattern numbered 6 in Table 2, 3 strains contained glucose and galactose at the lowest limit of detection. Six strains were examined that belonged to group H and that failed to produce a polysaccharide on sucrose-containing media, and 4 of these strains contained small amounts of fucosamine (pattern 15).

Strains that are classified as *Streptococcus sanguis* but lack the group H hapten are of interest, for two general patterns of cell wall composition can be seen in Table 2. Some strains have the commonest patterns found among the streptococci, containing rhamnose, glucose and galactose and either containing galactosamine (pattern 11) or lacking it (pattern 6). With the exception of one strain, the other

strains of *S. sanguis* not of group H differ markedly. The residue anhydrosorbitol is to be found and the walls lack or contain only trace amounts of rhamnose. One of Washburn, White & Niven's (1946) strains of *S. sanguis* type II (NCTC 7864) shows the latter general pattern.

Group K and Streptococcus salivarius. With one exception, the strains of group K and the strains of *Streptococcus salivarius*, whether or not they contained the K antigen, showed one or other of three cell wall patterns. Eleven of the 15 strains were found to belong to the patterns 6 and 11 (Table 2). Three of the strains of group K that did not produce levan differed markedly in cell wall composition from the other 12 strains in that rhamnose was not present while anhydrosorbitol was detected.

Roberts & Stewart (1961) found rhamnose to be absent from a number of their strains of group K, but present in strains of *Streptococcus salivarius* that contained the K antigen. Our results thus confirm and extend their findings.

Groups M and O. Streptococci of group M are described as possessing rhamnose in the cell wall (Roberts & Stewart, 1961; Slade & Slamp, 1962). We found rhamnose in the only haemolytic strain we examined (NCTC 7760, pattern 9), while it was absent from the cell walls of the 3 greening strains isolated from man. In all strains, including the one with rhamnose, anhydrosorbitol was detected.

The group O serum used in the identification of our strains contained antibodies reacting with components of streptococci of more general occurrence than the 'O' antigen (Stewart, 1965). Thus a fuller serological analysis of the strains included in group O might lead to some being excluded. Of the total of 5 strains examined by Roberts & Stewart (1961) and Slade & Slamp (1962) none contained rhamnose. Six of our strains were found to lack rhamnose and to yield anhydrosorbitol (pattern 24) and the seventh strain was found to contain rhamnose in an amount at the lowest level of detection (pattern 25).

The pneumococci. Three pneumococcal strains were examined and our findings confirmed those of Roberts & Stewart (1961) in that we did not detect rhamnose but did detect both glucose and galactose (pattern 21). Of particular interest was the finding of large amounts of glycerol in the residues of hydrolysis. Glycerol was detected among the residues from a number of other cell walls but then always at the lowest level of detection.

Strains lacking positive distinguishing characters. The last column in Table 2 contains the results from 37 strains that lacked positive distinguishing characters. If these strains are divided on the basis of cell wall composition, one strain (pattern 26) is clearly unlike any other, for it had the residues usually found in the cell wall of a staphylococcus (Cummins & Harris, 1956*b*). The 16 strains included in the patterns 5 to 15 had the residues usually associated with streptococci, namely rhamnose, one or more additional neutral sugars and the amino acids common to nearly all strains. The 20 strains in patterns 16 to 25 lacked entirely, or contained only small amounts of, rhamnose. The 37 strains showed considerable diversity in physiological tests but the strains in patterns 23, 24 and 25 were physiologically similar to the strains included in Lancefield group O.

DISCUSSION

In this study the residues present in hydrolysates of entire cell walls have been investigated as part of a taxonomic survey of the genus *Streptococcus*. The results of previous investigations are confirmed to the extent that well-established species such as *Streptococcus pyogenes* and *S. agalactiae* differ from each other in their component cell wall sugars, but unfortunately these species are the exception and very few taxa are composed of strains showing one cell wall pattern.

The current classification of streptococci is based, first, on the possession of a Lancefield group antigen; second, in a few species on the basis of a single striking marker such as levan production (*Streptococcus salivarius*); and third, on a composite picture of physiological activities. The taxa defined by these different bases do not always coincide, as exemplified again with *S. salivarius* which may or may not be group K. Cell wall composition seems to constitute yet another dimension in the classification scheme. The production of a dextran-like polysaccharide from sucrose is the diagnostic feature of *S. sanguis*, the species is serologically heterogeneous, and in addition it shows 7 different patterns of cell wall composition.

At the same time there are some parts of the genus in which cell-wall composition may perhaps offer some help. Thus the rhamnose-deficient strains (patterns 23, 24 and 25) may have sufficient other characters in common to suggest that they form a significant cluster. Apart from the total pattern, cell-wall analyses may be of value when it can be shown that particular residues are associated with an antigen as, for example, glucose in the group F antigen.

It should also be possible to find methods for separating the various structural components of the cell wall and it is possible that this would provide a more comprehensible basis for a taxonomic classification, but much further work is needed before this is possible. Meanwhile the present results show that there is a sufficient variety in the cell wall composition of the different members of the genus to suggest it might be possible to find some more satisfactory grounds for defining *Streptococcus viridans*, hitherto defined on the lack of distinguishing characteristics of the strains concerned.

The residues found are presumably components of the various sorts of polymers found in cell walls and which have been discussed by Salton (1964). Glucosamine, muramic acid and the main amino acids are presumably components of the mucopeptide. The amino acids present in lesser amount could come from a trypsin resistant peptide that adheres to the cell wall, or the mucopeptide may be of more complex composition than is usually described. Cell wall polysaccharides whether they be 'group' or 'type' in nature could contain amino sugars and neutral sugars, and teichoic acids would be expected to contain a polyol, alanine and a sugar. Little is known about the lipids of bacterial cell walls, but perhaps the glycerol found in the cell walls of the pneumococci might be a component of the recently described glycolipids of Gram-positive bacteria (Brundish, Shaw & Baddiley, 1965).

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