STUDIES ON THE CHEMICAL STRUCTURE OF THE 
STREPTOCOCCAL CELL WALL* 

II. THE COMPOSITION OF GROUP C CELL WALLS AND CHEMICAL BASIS FOR 
SEROLOGIC SPECIFICITY OF THE CARBOHYDRATE MOIETY 

BY RICHARD M. KRAUSE, M.D., AND MACLYN McCARTY, M.D. 

From The Rockefeller Institute 

Received for publication, September 28, 1961 

In the preceding paper of this series, the trypsinized cell walls of Groups A 
and A-variant streptococci were shown to be composed of their respective 
group-specific polysaccharide antigen and a mucopeptide polymer (1). The data 
presented here indicate that Group C streptococcal cell walls contain the same 
two components. The mucopeptide is similar in composition to that of Group A 
streptococci with N-acetylglucosamine, N-acetylmuramic acid, and alanine, 
lysine, glutamic acid, and glycine as the principal constituents. The group-
specific polysaccharide also resembles that of Group A in that it is composed 
of rhamnose and hexosamine. However, in Group C polysaccharide the pre-
dominant hexosamine is N-acetylgalactosamine rather than N-acetylglucos-
amine. Previous studies have shown that the specificity of Group A carbohydrate 
is largely determined by terminal β-N-acetylglucosaminide residues (2, 3) and 
in the present paper evidence is presented which indicates that N-acetyl-
galactosamine plays a comparable role in determining the serological specificity 
of group C carbohydrate. 

Materials and Methods 

The following organisms were used for the preparation of cell walls and isolation of group-
specific carbohydrate: Group A, strain T12; Group A-variant, strain K43; Group C, 
strains H46A and 28RP95. A large quantity of strain H46A was kindly supplied by Lederle 
Laboratories. The methods for preparing cell walls and muralytic enzymes, and for extrac-
tions of carbohydrate both by enzymatic and chemical procedures have been previously de-
scribed (1). 

Analytical Methods.—Analytical methods for rhamnose, glucosamine, muramic acid, and 
aminos acids were the same as previously described (1). 

Because galactosamine as well as glucosamine is present in the cell wall of Group C strep-
tococci, methods were required to estimate the relative amounts of these two sugars. For the 
preliminary work which required only an approximate determination of the relative amounts 
of glucosamine and galactosamine in a preparation the method of Tracey (4) was satisfactory. 
With this method a borate buffer is employed in the Elson-Morgan procedure which results 

* This investigation was supported in part by research grant H3919 from the National 
Heart Institute, Division of Research Grants, United States Public Health Service.
in a greater depression of the color intensity for glucosamine than for galactosamine. The
difference in the color intensity between samples tested with and without borate buffer pro-
vides an estimate of the glucosamine:galactosamine ratio in the mixture.

An estimate of galactosamine content can also be obtained by a modification of the Elson-
Morgan procedure. As suggested by Crumpton (5) and as discussed in the previous report (1),
the ratio of the optical density of the color reaction at 505 and 530 m\textmu; after 18 hours varies for
the different hexosamines. In the present study, this ratio ranged from 0.7 to 0.75 for galactosa-
mine, 0.8 to 1.0 for glucosamine, and 2.5 to 3.1 for muramic acid. These relationships are
illustrated in Fig. 1, which gives the absorption spectrum for the Elson-Morgan color reaction
for 50 \mu;g of glucosamine, galactosamine, and muramic acid at 30 minutes and 18 hours. At 18

![Spectrophotometric analysis of the Elson-Morgan reaction of glucosamine,
galactosamine, and muramic acid. The ratios of readings at 505 and 530 m\textmu;,
characteristic of each amino sugar is given under each curve.](image-url)

hours the peak of the absorption curve for glucosamine shifts slightly from 530 to 520 m\textmu;,
while that of galactosamine remains at 530 m\textmu;. As a result the 18 hour 505 m\textmu;:530 m\textmu; ratio
for glucosamine approaches 1.0 while the ratio for galactosamine remains low. Mixtures of
glucosamine and galactosamine give 505 m\textmu;:530 m\textmu; ratio values intermediate between those
for glucosamine and galactosamine. Galactosamine cannot be estimated by this method in a
sample which contains muramic acid because of the high 18 hour 505 m\textmu;:530 m\textmu; ratio of the
latter. For this reason the procedure is performed after glucosamine and galactosamine have
been separated from the hydrolysate containing muramic acid by the method of J. T. Park as
described by Perkins and Rogers (6).

For a more reliable estimation of galactosamine the column chromatography method of
Gardell (7) was employed. The sample was hydrolyzed with 2 N HCl for 4 hours, evaporated to
dryness, taken up in a small volume of 0.3 N HCl, and loaded onto a 8 X 39 cm column of
Dowex 50 W 200 to 400 mesh. The column had been previously standardized with a known
mixture of glucosamine and galactosamine. The fractions were used directly for the Elson-
Morgan procedure as modified by Gardell.
Quantitative Precipitin Determinations.—Quantitative precipitin determinations were carried out as previously described (2). The washed precipitates are dissolved in 0.1 N NaOH and assayed spectrophotometrically.

Preparation of N-Acetylgalactosamine.—N-acetylgalactosamine was prepared from commercial galactosamine hydrochloride (Mann Research Laboratories, New York) by the acetylation method of Roseman and Ludowieg (8). The product was assayed for purity by paper chromatography and gave the expected color value (30 to 35 per cent of N-acetylglucosamine) in the N-acetylhexosamine method of Reissig et al. (9).

EXPERIMENTAL

The cell walls of Groups A, A-variant, and C hemolytic streptococci contain rhamnose, glucosamine, muramic acid, and alanine, glutamic acid, lysine, and glycine. In addition, as reported by Cummins and Harris (10), the cell walls of Group C streptococci contain galactosamine. The group-specific haptens isolated from these cell walls by the muralytic enzymes contain all of these cell wall constituents. A comparison of the percentage composition of the cell walls and the carbohydrates prepared from them by the enzyme method in the case of these three groups of streptococci is shown in Table I. In each instance the feature which distinguishes the composition of the carbohydrate from the cell walls is the higher percentage of rhamnose and the lower percentage of muramic acid and amino acids in the carbohydrate. In addition, it is to be noted that the Group C carbohydrate as well as the cell walls contains galactosamine.

Lysis of Group C streptococci by phage-associated lyasin.—Group C streptococci and their isolated cell walls are lysed by Streptomyces albus enzyme and phage-associated lyasin. In previous studies (1) it was reported that group-specific carbohydrate and mucoprotein fractions are released during lysis of Group A cell walls by phage-associated lyasin. A similar experiment was designed, there-
fore, to identify the substances of Group C cell walls which had been solubilized by phage-associated lysin.

Trypsinized Group C cell walls were prepared from streptococcal strain 28RP95, and dissolved with phage-associated lysin. At the completion of the lytic process the digestion mixture was dialyzed in the cold against distilled water, and the dialysate was concentrated in vacuo to a small volume. A small amount of insoluble material, removed from the digestion mixture by centrifugation, contained essentially none of the cell wall sugars, and this fraction was therefore discarded. Soluble protein material was removed from the digestion mixture after precipitation by the addition of two volumes of absolute alcohol. This precipitate did not contain significant quantities of cell wall sugars and it was also discarded. The group-specific carbohydrate was precipitated from the alcohol-water mixture by the addition of 4 volumes of acetone, collected by centrifugation, and redissolved in distilled water. The residual acetone-alcohol-water mixture was concentrated in vacuo to a small volume. Chemical and serological analyses were performed in three fractions; the dialyzable fraction, the non-dialyzable acetone-precipitable fraction and the non-dialyzable acetone-alcohol-soluble fraction.

Chemical analysis of the fractions released from the cell wall by phage-associated lysin is shown in Table II. The acetone-precipitated fraction contained almost all of the rhamnose and, as would be expected, serological testing indicated that this fraction contained the Group C specific carbohydrate. Galactosamine was detected in the group-specific carbohydrate but not in the other two fractions. The dialyzable fraction and the non-dialyzable acetone-soluble fraction contained muramic acid, glucosamine, and the amino acids of the mucopolysaccharide. The acetone-soluble fraction contained significant quantities of amino acids and sugars, as indicated by the data presented in Table II.

### TABLE II

<table>
<thead>
<tr>
<th></th>
<th>Dialyzable Fraction</th>
<th>Non-Dialyzable Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmols</td>
<td>µmols</td>
</tr>
<tr>
<td>Acetone-precipitated</td>
<td>342.0</td>
<td>1.8</td>
</tr>
<tr>
<td>Acetone-soluble fraction</td>
<td>30.0</td>
<td>11.7</td>
</tr>
<tr>
<td>Dialyzable fraction</td>
<td>18.0</td>
<td></td>
</tr>
<tr>
<td>Acetone-precipitated</td>
<td>9.5</td>
<td>8.5</td>
</tr>
<tr>
<td>Acetone-soluble fraction</td>
<td>120.0</td>
<td>22.6</td>
</tr>
<tr>
<td>Dialyzable fraction</td>
<td>44.5</td>
<td>31.7</td>
</tr>
<tr>
<td>Acetone-precipitated</td>
<td>41.3</td>
<td>‡</td>
</tr>
<tr>
<td>Acetone-soluble fraction</td>
<td>42.2</td>
<td>‡</td>
</tr>
<tr>
<td>Dialyzable fraction</td>
<td>14.2</td>
<td>‡</td>
</tr>
</tbody>
</table>

* This strain possesses a higher concentration of rhamnose and a lower concentration of galactosamine in the cell walls than that found in typical Group C streptococci.

‡ Because the dialyzable fraction contains the electrolytes of the digestion mixture, quantitative determination of the amino acids was performed after dialyzable material had been eluted from a darco G60 column as recorded in Table III.
peptide. The bulk of the total muramic acid was in the non-dialyzable acetone-soluble fraction and the dialyzable fraction, and less than one-fourth was associated with the group-specific carbohydrate fraction. Preliminary data indicated that the dialyzable amino sugars existed as an oligosaccharide, and the amino acids as a peptide. The following experiment revealed that the peptide was combined with the oligosaccharide.

An aliquot of the dialyzable fraction of the previous experiment was loaded onto a darco G60 column as described previously (1) and eluted with 300 ml of water, and then successively with two 300 ml volumes of 15 per cent alcohol, 25 per cent alcohol, and 50 per cent alcohol. The eluates were concentrated in vacuo to approximately 10 ml and then analyzed after hydrolysis for amino sugars and amino acids.

### TABLE III

<table>
<thead>
<tr>
<th></th>
<th>Group C cell wall</th>
<th>Non-dialyzable mucopeptide</th>
<th>Dialyzable mucopeptide eluted from a darco G60 column</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muramic acid</td>
<td>1</td>
<td>1.4</td>
<td>(1)</td>
</tr>
<tr>
<td>Glucosamine</td>
<td>10.1</td>
<td>14.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Alanine</td>
<td>2.8</td>
<td>7.9</td>
<td>2.2</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>2.8</td>
<td>5.0</td>
<td>1.9</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.5</td>
<td>3.7</td>
<td>1.4</td>
</tr>
<tr>
<td>Glycine</td>
<td></td>
<td></td>
<td>1.0</td>
</tr>
</tbody>
</table>

Chemical analyses of the eluates from the column are included in Table III. No material was eluted from the column by water and 15 per cent alcohol. The 50 per cent fraction contained approximately twice as much material as the 25 per cent fraction. Elution of material with both the 25 and 50 per cent alcohol indicates that the dialyzable material was not homogeneous. The view that the peptide is associated with the oligosaccharide is supported by the fact that no detectable material was eluted from the column by a concentration of alcohol below 25 per cent.

Table III also affords a comparison of the compositions of the eluates from the darco G60 column and the non-dialyzable mucopeptide (the acetone-soluble material of Table II) to that of Group C cell walls. The relative amounts of glucosamine, muramic acid, and the amino acids are similar for the intact cell walls and the enzymic split products. Because galactosamine is associated with the group-specific carbohydrate it is not included in the table. These results indicate that for Group C streptococci as is the case for Group A, cell wall mucopeptide is the substrate for phage-associated lysin.
Preparation of Group-Specific Carbohydrate.—A disadvantage of the group-specific carbohydrates prepared by muralytic enzymes is that they contain a portion of the mucoprotein component of the cell wall. The previously described methods for isolating Groups A and A-variant carbohydrate without a mucoprotein component and the mucoprotein without appreciable group-specific carbohydrate also proved useful for Group C streptococci. As with Group A streptococci, hydrolysis of Group C cell walls by boiling at pH 2 releases only a portion of the group-specific carbohydrate. The extracted carbohydrate is free of mucoprotein material, but the cell wall residue still contains large amounts of carbohydrate. Hot formamide extraction, on the other hand, separates the cell wall into two fractions: the soluble group-specific carbohydrate and the insoluble mucoprotein residue.

An example of hot formamide extraction of Group C cell walls is given in Table IV. Included in the table is the composition of the initial cell wall preparation, and the extracted group-specific carbohydrate and the mucoprotein residue. The formamide residue has several features which are similar in most respects to the residues obtained from Groups A and A-variant cell walls (1). It is composed of the elements of the mucoprotein; it occurs in the form of particles with a discrete disc appearance when examined by phase microscopy; and it appears to be the substrate in the intact cell wall for the muralytic enzymes. Like that of Group A, the formamide residue is lysed by those enzymes which

<table>
<thead>
<tr>
<th></th>
<th>Cell walls</th>
<th>Hot formamide</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Extracted C carbohydrate</td>
<td>Residue</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>26.9</td>
<td>53.7*</td>
<td>1.0</td>
</tr>
<tr>
<td>Galactosamine</td>
<td>8.1</td>
<td>17.4</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>Muramic acid</td>
<td>4.5</td>
<td>—</td>
<td>9.3</td>
</tr>
<tr>
<td>Glucosamine</td>
<td>5.4</td>
<td>2.0</td>
<td>8.3</td>
</tr>
<tr>
<td>Alanine</td>
<td>14.9</td>
<td>—</td>
<td>25.1</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>7.4</td>
<td>—</td>
<td>12.1</td>
</tr>
<tr>
<td>Lysine</td>
<td>7.4</td>
<td>—</td>
<td>12.2</td>
</tr>
<tr>
<td>Glycine</td>
<td>1.5</td>
<td>—</td>
<td>2.8</td>
</tr>
</tbody>
</table>

* The carbohydrates extracted from typical Group C streptococci contain approximately the same percentage of rhamnose and galactosamine. The carbohydrate of this strain contains a considerably higher proportion of rhamnose than galactosamine, and serologically cross-reacts with Group A-variant antiserum.
Fig. 2. Analysis of soluble carbohydrate and residue of Group C cell walls extracted with hot formamide. The glucosamine, galactosamine, and muramic acid in hydrolysates of these materials were separated by a Dowex 50 W 200 to 400 mesh column. The Elson-Morgan reaction was performed on each fraction off the column, and the ratios of the spectrophotometric readings at 505 and 530 m\(\mu\) of the color reactions after 18 hours, characteristic for each amino sugar, are listed under each curve.

lyse the streptococcal cell wall and also by lysozyme which does not attack the whole cell wall.

Of particular interest is the finding that the group-specific carbohydrate extracted with hot formamide is composed primarily of galactosamine and rhamnose and contains only a small percentage of glucosamine. Data for this clear
association of the galactosamine with the carbohydrate were obtained by analyzing the hot formamide extracted carbohydrate and the mucopeptide residue for galactosamine by chromatographic column procedures described by Gardell (7). The results of analysis, given in Fig. 2, indicate that approximately 90 per cent of the hexosamine in the carbohydrate is galactosamine and 10 per cent is glucosamine. The analyses obtained for the mucopeptide residues are complicated by the fact that galactosamine and muramic acid are eluted from the column in the same fractions. However, the other procedures described under methods which can be employed to distinguish glucosamine, galactosamine, and muramic acid indicate that the two amino sugars eluted from the column loaded with mucopeptide were glucosamine and muramic acid. The 18 hour spectropho-

<table>
<thead>
<tr>
<th>Carbohydrates</th>
<th>Acid extracted</th>
<th>Formamide extracted</th>
<th>Formamide residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhamnose</td>
<td>46.5</td>
<td>43.0</td>
<td>(1.0)</td>
</tr>
<tr>
<td>Galactosamine</td>
<td>36.5</td>
<td>35.1</td>
<td>—</td>
</tr>
<tr>
<td>Glucosamine</td>
<td>4.1</td>
<td>3.9</td>
<td>7.8</td>
</tr>
<tr>
<td>Muramic acid</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>7.5</td>
</tr>
<tr>
<td>Alanine</td>
<td>—</td>
<td>—</td>
<td>22.5</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>—</td>
<td>—</td>
<td>10.5</td>
</tr>
<tr>
<td>Lysine</td>
<td>—</td>
<td>—</td>
<td>10.5</td>
</tr>
<tr>
<td>Glycine</td>
<td>—</td>
<td>—</td>
<td>1.6</td>
</tr>
</tbody>
</table>

tometric values at 505 and 530 \( \text{m} \mu \) of the Elson-Morgan determinations on the column fractions gave characteristic ratios as noted in Fig. 2, which are similar to those obtained for the respective amino sugar standards.

As was indicated previously, hydrolysis at \( \text{pH} \) 2 does not remove all of the group-specific carbohydrate from the cell wall, but the material which is removed has a composition similar to that obtained by formamide extraction. Analyses of the carbohydrates obtained from Group C, strain H46A, by acid and formamide extraction are given in Table V in comparison with that of the residue remaining after formamide extraction. The two carbohydrates are similar chemically in that they are composed primarily of rhamnose and galactosamine. There is no significant mucopeptide component. Quantitative precipitin analysis as shown in Fig. 3, indicates both preparations of carbohydrate have similar serologic activity.

It is clear from these experiments that the principle amino sugar in Group C carbohydrate is \( \text{N}-\text{acetyl} \)galactosamine. The amino sugar in Group A carbohy-
drate, on the other hand, is N-acetylglucosamine. It has been shown previously that the N-acetylglucosamine has a terminal position on side chains of the Group A carbohydrate molecule and is the primary determinant of immunologic specificity (2). The demonstration that N-acetylgalactosamine is the major hexosamine in Group C carbohydrate suggested that it might play a comparable role in the antigenic specificity of this hapten. The results of serologic studies presented in the following section support this view.

**Precipitin Inhibition with N-Acetylamino Sugars.**—The inhibitory effect of the three monosaccharide constituents of Group C carbohydrate on its reactivity with rabbit antibody was tested by the quantitative precipitin technique. As indicated by the quantitative precipitin curves presented in Fig. 4, N-acetylgalactosamine in a final concentration of 1 per cent strongly inhibits the precipitin reaction, while N-acetylglucosamine and rhamnose have no discernible effect at this concentration. Thus, these data suggest a dominant role for N-acetylgalactosamine in the serological specificity of the carbohydrate.

Further data on the inhibitory effect of N-acetylgalactosamine and a comparison with the effect of N-acetylglucosamine on the Group A reaction are illustrated in Fig. 5. These tests were carried out with constant antigen and antibody and varying concentration of inhibitor, and the data are expressed as per cent inhibition of the precipitin reaction in comparison with controls. It will be noted that effective inhibition of the Group C reaction is again obtained with N-acetylgalactosamine, although N-acetylglucosamine is totally without effect. The reverse is true in the case of the Group A reaction in which only N-acetyl-
glucosamine exerts a detectable inhibitory effect. In each case, a measurable inhibition was obtained with a concentration as low as 0.1 per cent of the effective N-acetylhexosamine.

 Attempts at Enzymatic Degradation of Group C Carbohydrate.—In establishing the importance of terminal N-acetylglucosaminide residues in the serological specificity of Group A carbohydrate, the initial evidence came from experiments with an induced bacterial enzyme capable of destroying serological activity of the carbohydrate (2). It was shown that the enzyme removed two-thirds of the total hexosamine as free N-acetylgalactosamine leaving the remainder of the carbohydrate as a non-dialyzable residue. Specificity studies demonstrated that the enzyme is an N-acetyl-β-glucosaminidase (3). Repeated attempts to isolate a soil organism which produces an enzyme active against the Group C carbohydrate have met with failure. Thus, this type of additional evidence for the role of N-acetylgalactosamine as the primary determinant of specificity is not available.

Recent studies by Woollen, Walker, and Heyworth (11) have demonstrated that N-acetyl-β-glucosaminidase preparations from a wide variety of sources all contain N-acetyl-β-galactosaminidase activity. On the basis of this, and other work from their laboratory, the possibility is raised that a single active site on the same enzyme protein is responsible for both activities. A sample of the bacterial N-acetylglucosaminidase active against the Group A carbohydrate (induced with phenyl-N-acetyl-β-glucosaminide) was submitted to these workers. It was found, as in the case of the other preparations examined, that the induced enzyme also has N-acetyl-β-galactosaminidase activity (personal com-
The substrates employed were \( p \)-nitrophenyl \( N \)-acetyl-\( \beta \)-D-glucosaminide and the corresponding galactosaminide, and the

![Diagram](https://via.placeholder.com/150)

**Fig. 5.** Inhibition of the precipitin reaction between A and C carbohydrates and their antisera with \( N \)-acetylglucosamine and \( N \)-acetylglactosamine respectively. The results are expressed as per cent inhibition. The Group A reaction was performed with 20 \( \mu \)g of carbohydrate and 0.2 ml of antiserum in a final volume of 1 ml, and the Group C reaction was performed with 10 \( \mu \)g of carbohydrate and 0.2 ml of antiserum in a final volume of 1 ml.

Tests were carried out at pH 5.9. The \( N \)-acetyl-\( \beta \)-galactosaminidase activity was approximately 8 per cent of the \( N \)-acetyl-\( \beta \)-glucosaminidase activity. This result is comparable to that which they had obtained with other bacterial preparations, three out of four of which also had large glucosaminidase to galactosaminidase ratios. It is of interest that the induced enzyme is not entirely specific for the \( N \)-acetyl-\( \beta \)-glucosaminide substrate.
The foregoing findings suggest that the Group A enzyme should be able to split terminal N-acetyl-galactosaminide residues from the Group C carbohydrate. However, when this possibility was reexamined using long incubation periods and much larger amounts of enzyme than are necessary to inactivate Group A carbohydrate completely, there was no change in the serological activity of Group C carbohydrate and no detectable release of N-acetylgalactosamine.

One possible explanation for the lack of effect of the bacterial N-acetyl-β-glucosaminidase on the Group C carbohydrate is that the terminal N-acetyl-galactosaminide residues are attached by α- rather than β-linkages. In this event, one might expect some degree of cross-reactivity between the Group C carbohydrate and the blood-Group A substance, since α-linked N-acetylgalactosaminide end groups are associated with specificity in this case (12, 13). However, preliminary tests have failed to show any suggestion of cross-reactivity in either direction. While this finding could possibly be dependent on the large differences in the remainder of the side chain, it reduces the likelihood that the N-acetylgalactosamine is linked through α-linkages in the streptococcal Group C carbohydrate. The nature of the linkage remains to be determined by future work.

DISCUSSION

These studies, in conjunction with those reported previously (1), indicate certain essential similarities in the cell walls of Groups A, A-variant, and C streptococci. A rigid mucoprotein structure, composed of N-acetylglucosamine, N-acetylmuramic acid, and alanine, lysine, glutamic acid, and glycine, has been identified in the cell wall of each of these three groups of streptococci. The evidence suggests that lysis of intact bacteria or isolated cell walls occurs with enzymatic hydrolysis of this component by the muralytic enzymes.

The other component in Group C cell walls is the group-specific carbohydrate antigen. Hot formamide extraction has proved particularly useful to obtain preparations of this antigen which contain none of the mucoprotein material. In contrast to the Group A carbohydrate which is composed of rhamnose and N-acetylglucosamine and the Group A-variant carbohydrate which contains primarily rhamnose, the Group C carbohydrate contains N-acetylgalactosamine, rhamnose, and only a small percentage of N-acetylgalactosamine. These facts can be related to the determinants of antigenic specificity. The antigenic specificity of Group A carbohydrate is determined by terminal N-acetylgalactosamine on side chains of rhamnose. The Group A-variant carbohydrate lacks the terminal N-acetylgalactosamine so that the rhamnose component determines antigenic specificity (2). This view is confirmed by enzyme studies. When the terminal N-acetylgalactosamine residues are removed by the induced N-acetylgalactosaminidase, the residual carbohydrate loses Group A serologic activity but acquires Group A-variant activity.
The finding that N-acetylgalactosamine is present in Group C carbohydrate, and that the Group C precipitin reaction can be specifically inhibited by N-acetylgalactosamine, supports the view that the primary difference between Group C and Group A carbohydrate is in the specific amino sugar, and that N-acetylgalactosamine is the determinant of Group C serologic specificity. It has not yet been possible to isolate a soil organism which will produce an enzyme capable of removing N-acetylgalactosamine from Group C carbohydrate. It seems likely that if this were accomplished the residual carbohydrate would have Group A-variant serologic activity. Some support for this view is derived from work now in progress with certain strains of Group C streptococci. The carbohydrate of these strains possesses a higher concentration of rhamnose but less than the usual amount of N-acetylgalactosamine. (Compare Tables IV and V.) In serological tests the carbohydrate reacts almost equally well with both Group C and Group A-variant antisera. These Group C strains appear to be analogous to the Group A intermediate strains reported by McCarty and Lancefield (14) which react with both Groups A and A-variant antisera.

SUMMARY

The trypsinized cell walls of Group C streptococci contain two components, the group-specific carbohydrate and a mucopeptide polymer. Hot formamide extraction of Group C cell walls results in a soluble group-specific carbohydrate fraction and an insoluble mucopeptide residue. This mucopeptide, similar in composition to that of Groups A and A-variant streptococci, contains N-acetylgalactosamine, N-acetylmuramic acid, alanine, glutamic acid, lysine, and glycine. It is dissolved by the muralytic enzymes, including lysozyme, which does not attack the whole cell wall. Lysis of the cell wall by phage-associated lysin results in the release of soluble fragments composed of the elements of mucopeptide.

Group C carbohydrate extracted with formamide is composed primarily of N-acetylgalactosamine and rhamnose. Serological studies suggest that the specificity of Group C carbohydrate is determined by the N-acetylgalactosamine.

BIBLIOGRAPHY

4. Tracey, M. V., A rapid colorimetric distinction between glucosamine and galactosamine, Biochim. et Biophysica Acta, 1955, 17, 159.