SYMPOSIUM ON RELATIONSHIP OF STRUCTURE OF MICROORGANISMS TO THEIR IMMUNOLOGICAL PROPERTIES[1]

IV. Antigenic and Biochemical Composition of Hemolytic Streptococcal Cell Walls

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Introduction

Evidence for a close biological relationship among groups A, C, and G hemolytic streptococci is derived from a study of various bacteriological properties. For example, the human strains for each of these groups produce similar extracellular enzymes, such as streptokinase and deoxyribonuclease. Additional evidence to underscore the relationship among these groups of hemolytic streptococci is presented in this review of the antigenic and biochemical composition of the cell walls.

Hemolytic streptococci were initially classified by Lancefield into various groups on the basis of a specific carbohydrate extracted from the organism by boiling at pH 2 (12). The major constituents of this antigen were identified as rhamnose and glucosamine (22). Subsequent work has revealed that this group-specific carbohydrate is a component of the streptococcal cell wall (15, 15*a*). The type-specific M protein of group A streptococci has also been identified as a cell-wall component (2, 20). Thus, two major streptococcal antigens, the group carbohydrate and the type-specific M protein, initially characterized as somatic substances, are clearly cell-wall constituents. This review is limited to special features of the structure of the streptococcal cell wall and emphasizes the immunochemical properties of the group-specific carbohydrates. In particular, evidence delineating the character of the antigenic determinants is reviewed. The work to be reported here is primarily that of M. McCarty and the author. It should be emphasized that a comprehensive treatment of the research on the streptococcal cell wall would include the work of other investigators.

Streptococcal Structure

Several of the structural elements of the streptococcus which have been delineated are illustrated in Fig. 1. The hyaluronic acid of the outer capsule is composed of equimolar quantities of *N*-acetylglucosamine and glucuronic acid. Streptococcal hyaluronate appears to be indistinguishable from mammalian hyaluronate. Recently, Markovitz and Dorfman (14) demonstrated the production of hyaluronic acid by streptococcal protoplast membranes.

The cell wall of the streptococcus has a complex protein component and a polysaccharide fraction. Several antigens, designated M, T, and R, have been identified in the protein component of the wall. The M antigen has received particular attention, because it is responsible for the production of type-specific antibodies which are the basis of type-specific immunity in man. M protein

[1] This symposium was held at the Annual Meeting of the American Society for Microbiology, Cleveland, Ohio, 7 May 1963, under the sponsorship of the Division of Medical Microbiology and Immunology, with Edgar Ribi as convener.

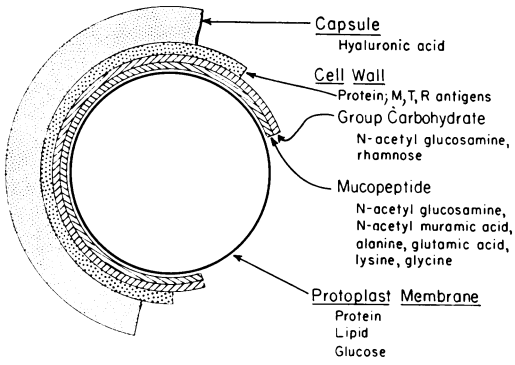

FIG. 1. *Diagrammatic representation of the subcellular components of hemolytic streptococci. The monosaccharides listed here for the carbohydrate are those of group A streptococci.*

has been identified as a virulence factor, and, although the mechanism of virulence enhancement is somewhat obscure, this characteristic may be dependent upon the capacity of M protein to impede phagocytosis. These features of the M protein are discussed in a recent review by Lancefield (13).

Cell walls, treated with trypsin and chymotrypsin, are essentially devoid of protein. The polysaccharide moiety of the residual walls after such treatment is separable into two components: the group-specific carbohydrate, and the mucopeptide residue. Rhamnose has been identified in the carbohydrate of several groups of streptococci, including A, C, and G (15, 15*a*, 22). The amino sugar content is not identical for the carbohydrates of the various groups. For instance, *N*-acetylglucosamine has been identified in the carbohydrate of group A streptococci, and *N*-acetylgalactosamine has been identified in that of group C. The cell-wall mucopeptide contains *N*-acetylglucosamine, *N*-acetylmuramic acid, and four major amino acids (9). Although Barkulis has shown that the amino sugars of the wall are *N*-acetylated (2), in the work reported here the percentage composition will be expressed as the amino sugar on the basis of the Elson-Morgan determination (9, 19).

Cell walls, collected by differential centrifugation of streptococci disrupted in a Mickle disintegrator, are depicted in the top electron micrograph of Fig. 2. The rigid envelope nature of the cell walls is evident in this figure. Protoplast membranes are depicted in the lower electron micrograph of Fig. 2. It is to be noted that these structures appear to be more delicate than the cell walls. The isolation of membranes requires the preparation of protoplasts from whole streptococci. This has been achieved by removing the cell wall from the bacteria in a hypertonic environment with the cell wall-dissolving enzyme, phage-associated lysin (5, 7, 8). The protoplasts are then ruptured by dilution into a hypotonic environment, and the membranes are collected by centrifugation. Freimer studied the chemical and immunological nature of the protoplast membrane in some detail (4).

ISOLATION AND IMMUNOCHEMICAL CHARACTERISTICS OF GROUP CARBOHYDRATES

Before beginning the discussion of the extraction of group A, A-variant, and C carbohydrates, and of the analysis of the distinguishing immunochemical features of these antigens, it is necessary to describe the relationship of A-variant strains of streptococci to group A streptococci. A group A-variant strain was first isolated by Wilson after repeated mouse passage of group A streptococci (23). It was noted that antigen extracts of this strain no longer reacted with group A antisera; however, the persistence of the type-specific M antigen in the variant strain ensured that it was derived from the group A organism originally injected into the mice. Subsequent work established the fact that the A-variant strain had not lost the carbohydrate antigen; rather, it possessed a carbohydrate which differed immunologically and chemically from the group A antigen. The group A-variant strains injected into rabbits stimulated the production of antibody which was specific for the A-variant carbohydrate and did not cross-react appreciably with group A carbohydrate.

A survey of the Rockefeller Institute collection of 1815 group A strains in search of A-variant strains revealed 10 which possessed the group A-variant antigen (16). Each of the A-variant strains had been subjected to animal passage, and in no instance was an A-variant strain encountered which had been isolated directly from human sources. It thus appears unlikely that the occurrence of the A-variant strain is a common event in the natural human host. Accidental infection of the nasopharynx of laboratory personnel indicates, however, that at least one A-variant strain is virulent for man and does not

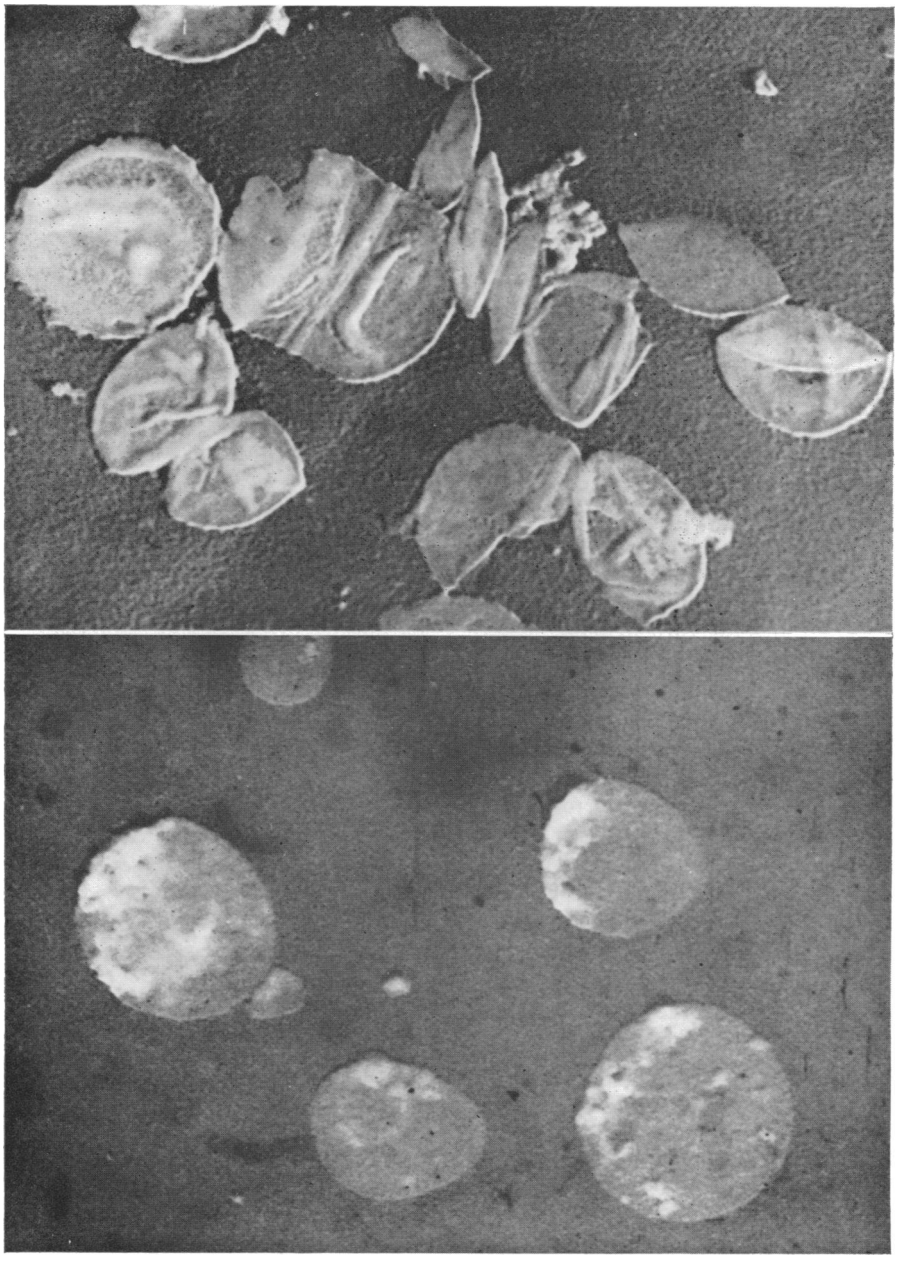

FIG. 2. (*top*) *Electron micrograph of trypsin-treated cell walls of group A streptococci.* (*bottom*) *Electron micrograph of trypsin-treated group A streptococcal protoplast membranes. Magnification: approximately* × *10,000. From Freimer, Krause, and McCarty* (*5*).

revert to the group A form upon subsequent human passage. In the work to be presented, it is clear that the study of the A-variant carbohydrate contributed important information on the nature of group A carbohydrate.

Methods for Extraction of the Carbohydrates from Cell Walls

Several procedures have proved valuable for the extraction of the group carbohydrate antigen, in addition to the initial procedure of boiling at

pH 2. In his initial studies, McCarty employed a cell wall-dissolving enzyme isolated from culture filtrates of *Streptomyces albus* (15, 15*a*). Group C streptococci undergoing lysis from a virulent phage infection also produce a cell wall-dissolving enzyme which releases the carbohydrate into solution (8). The polysaccharide isolated from cell-wall digests obtained by either enzyme, although composed primarily of group-specific carbohydrate, contains some mucopeptide material (9). It has not proved feasible to free these preparations of antigens from the mucopeptide by various fractionation procedures. Extensive studies indicate that the substrate of the cell wall-dissolving enzymes is the mucopeptide moiety of the cell wall (9). The lytic action of these enzymes thus resembles that of lysozyme (21).

Attention has been directed recently to the earlier method of Fuller for the extraction of carbohydrate from the cell walls (6). In this procedure, the cell walls are treated with formamide at 180 C, and the carbohydrate, readily isolated from the extract, is devoid of elements of the mucopeptide. The residue, the other product obtained by this treatment of the cell walls, is composed primarily of mucopeptide, and contains only a small percentage of the initial carbohydrate antigen. Electron microscopy of the insoluble residue reveals particles with a discrete disc structure similar to, but of less density than, the cell walls (Fig. 3). It seems likely that the mucopeptide is the rigid structural element of the intact cell walls. The evidence suggests that the mucopeptide structure isolated by formamide extraction is, in fact, the substrate of the intact cell wall for the phage-associated lysin and the *S. albus* enzyme (9). Both enzymes release a soluble glucosamine-muramic acid-peptide complex from the mucopeptide residue. Although groups A and C streptococcal cell walls are not lysed by lysozyme, the formamide residue is readily digested by this enzyme. This suggests that the removal of the group polysaccharide from the cell wall by formamide has exposed the linkages of the mucopeptide, which are hydrolyzed by lysozyme (9).

It seems clear that different linkages in the whole cell wall have been hydrolyzed by the cell wall-dissolving enzymes, on the one hand, and by hot formamide, on the other. The enzymes release soluble carbohydrate-mucopeptide complexes and soluble mucopeptide fragments and, therefore, probably act on a glycosidic linkage in the mucopeptide polymer. The hot formamide releases the serologically reactive carbohydrate moiety devoid of mucopeptide, which suggests that hydrolysis has occurred between the carbohydrate and the mucopeptide (9).

Group-Specific Carbohydrates Extracted with Hot Formamide

The results of hot formamide extraction of the cell walls of group A, A-variant, and C streptococci are illustrated in Tables 1, 2, and 3, respectively. Each table gives the chemical compositions of the cell walls, the extracted soluble carbohydrate, and the insoluble residue.

TABLE 1. *Composition of group A cell walls and of the soluble carbohydrate (CHO) and the insoluble residue after hot formamide extraction*

Constituent	Cell walls	Formamide treatment		
		Extracted A CHO	Formamide residue	
			Amount	Mole ratio
	%	%	%	
Rhamnose	34.0	60.0	1.2	—
Glucosamine	18.8	30.0	11.0	1.6
Muramic acid	6.4	—*	9.4	(1)
Alanine	17.4	—*	31.0	9.3
Glutamic acid	7.9	—*	17.0	3.1
Lysine	8.0	—*	14.0	2.6
Glycine	1.0	—*	0.9	0.3

* Less than 1%.

TABLE 2. *Composition of group A-variant cell walls and of the soluble carbohydrate (CHO) and the insoluble residue after hot formamide extraction*

Constituent	Cell walls	Formamide treatment		
		Extracted A-variant CHO	Formamide residue	
			Amount	Mole ratio
	%	%	%	
Rhamnose	36.0	85.0	<1.0	—
Glucosamine	7.2	3.0	6.9	1.4
Muramic acid	5.0	—*	6.8	(1)
Alanine	16.0	—*	22.7	9.4
Glutamic acid	8.4	—*	12.6	3.2
Lysine	8.8	—*	11.2	2.9
Glycine	1.9	—*	0.7	0.3

* Less than 1%.

TABLE 3. *Composition of group C cell walls and of the soluble carbohydrate (CHO) and the insoluble residue after hot formamide extraction*

Constituent	Cell walls	Formamide treatment		
		Extracted C CHO	Formamide residue	
			Amount	Mole ratio
	%	%	%	
Rhamnose	20.3	43.0	1.0	—
Galactosamine	10.5	35.1	<1.0	—
Glucosamine	5.8	3.9	8.2	1.2
Muramic acid	4.6	—*	9.3	(1)
Alanine	16.6	—*	25.1	7.6
Glutamic acid	7.5	—*	12.1	2.2
Lysine	7.6	—*	12.2	2.2
Glycine	2.1	—*	2.8	1.0

* Less than 1%.

It is to be noted that cell walls of each of these three groups have similar amino acid and muramic acid concentrations. Rhamnose is a significant component in each case, but there is considerable variation in the amino sugar content. Group A cell walls have a higher concentration of glucosamine than do group A-variant, and group C cell walls have galactosamine in addition to glucosamine. The cell-wall residue for each group after hot formamide extraction has a similar composition. The low rhamnose content indicates that the bulk of the carbohydrate antigen has been removed by the extraction process. The amino sugars and the amino acids of the cell wall, which are components of the mucopeptide, are recovered in the residue.

The extracted carbohydrates are essentially devoid of muramic acid and the four amino acids. Considerable variation is noted in the composition of the carbohydrates. Group A carbohydrate contains 60% rhamnose and 30% glucosamine, whereas that of group A-variant contains 85% rhamnose and only 3% glucosamine. Group C contains 43% rhamnose, but, unlike group A, it has galactosamine as the major amino sugar.

The compositions of the three carbohydrates are tabulated on the right in Fig. 4; on the left are depicted schematic representations of the chemical determinants of specificity for the three carbohydrate antigens. The present view is that in the group A carbohydrate the antigenic determinants are terminal *N*-acetylglucosaminide residues attached through a beta linkage to rhamnose-rhamnose linkages of the molecule. Group A-variant carbohydrate possesses a rhamnose moiety similar to that of group A and is devoid of terminal *N*-acetylglucosaminide residues, so that the antigenic determinants are the rhamnose-rhamnose linkages. In the case of group C, the rhamnose-rhamnose linkages of the carbohydrate are also similar to those of the other two groups, but the terminal determinant sugar is *N*-acetylgalactosamine instead of *N*-acetylglucosamine. The evidence to support such a general view of the relationship among these carbohydrates has been obtained from several avenues of investigation, all of which cannot be detailed here. Those studies selected for consideration include: antigen-antibody inhibition with specific constituent sugars of the carbohydrates, degradation of the carbohydrates by enzymes which destroy their serological reactivity, and an analysis of variant forms of groups A and C carbohydrates.

Inhibition of Antigen-Antibody Precipitation by Specific Constituent Sugars

Quantitative precipitin tests between group A carbohydrate and group A antiserum are depicted in Fig. 5 (16). The view that *N*-acetylglucosaminide residues are of primary importance in determining specificity of group A carbohydrate is demonstrated by the fact that free *N*-acetylglucosamine clearly inhibits the reaction between group A carbohydrate and its antibody. Rhamnose has no inhibitory effect. The existence of beta linkages between *N*-acetylglucosaminide residues and the rhamnose portion of the carbohydrate is supported by the fact that the beta anomer of phenyl-*N*-acetylglucosaminide is more effective as an inhibitor of the precipitin reaction than the alpha anomer (17). Additional evidence in support of this view will be detailed later.

The inhibitory effect of the three monosaccharide constituents of group C carbohydrate on its reactivity with antibody was tested by the quantitative precipitin technique. The results (Fig. 6) indicate that *N*-acetylgalactosamine, at a final concentration of 1%, markedly inhibits the precipitin reaction, whereas there is essentially no inhibition with *N*-acetylglucosamine or rhamnose at the same concentration (10). This result suggests a dominant role for *N*-acetylgalactosamine in the antigenic determinant of group C carbohydrate.

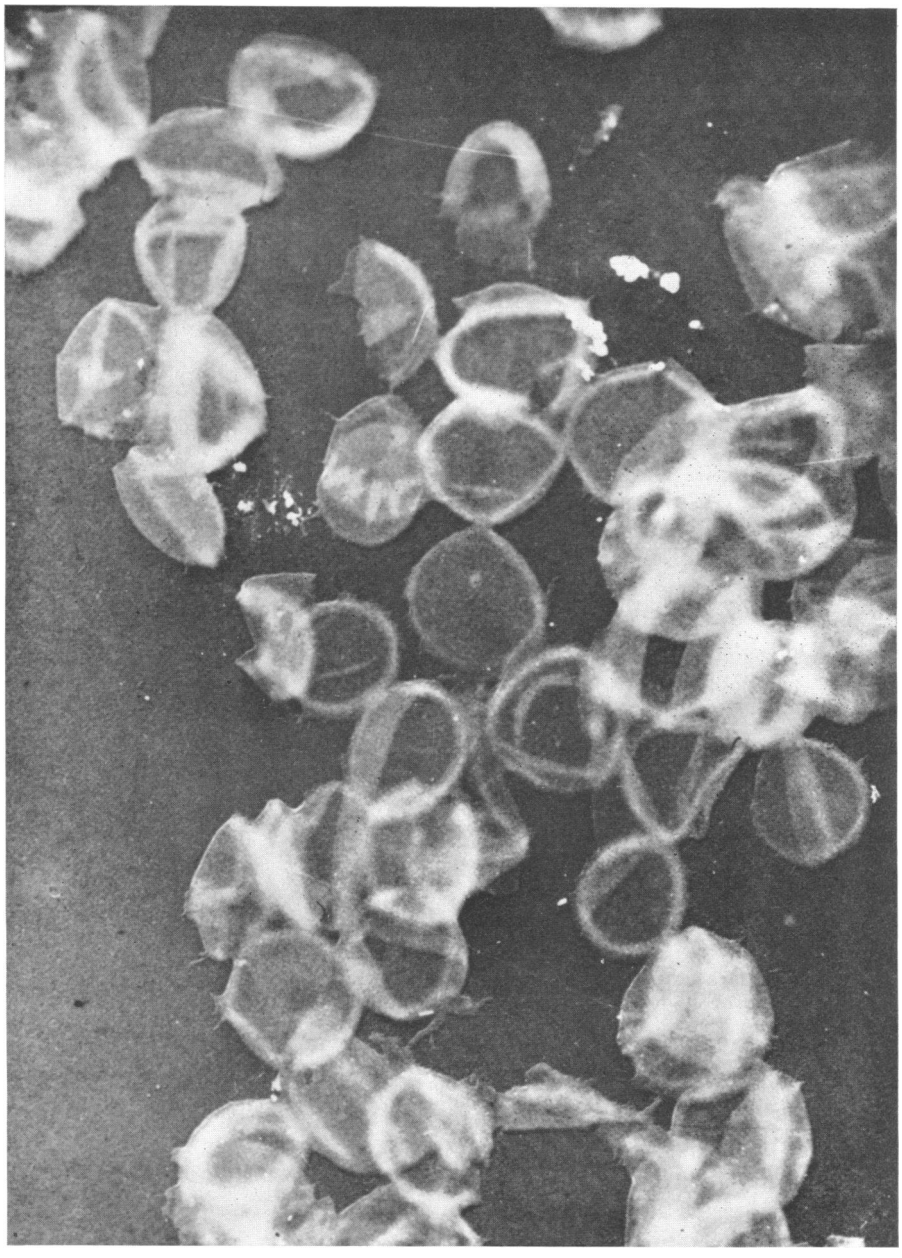

FIG. 3. *Electron micrograph of hot formamide extraction residue of T12 cell walls. These discrete disc structures are composed of mucopeptide. Magnification: approximately × 15,000. From Krause and McCarty (9).*

Effect of Induced Enzymes on the Carbohydrate

The relationship between groups A and A-variant carbohydrates and the chemical basis for antigenic specificity were clarified to a considerable extent by studies with induced enzymes (of soil organisms) capable of attacking the carbohydrates. McCarty isolated an induced enzyme, termed A enzyme, which has the capacity to destroy the serological reactivity of group A carbohydrate, and another enzyme,

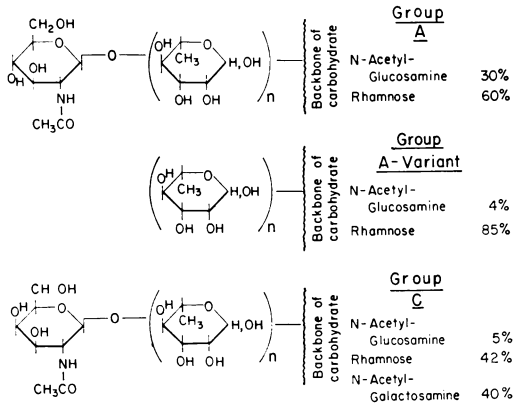

FIG. 4. *Chemical composition of the antigenic carbohydrates and a diagrammatic representation of the antigenic determinants of groups A, A-variant, and C streptococci.*

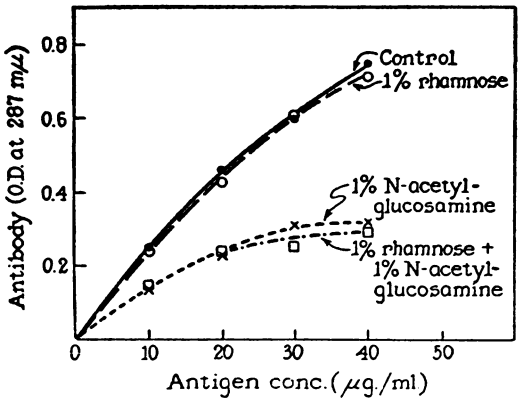

FIG. 5. *Inhibition of the precipitin reaction between A carbohydrate and group A antiserum by N-acetylglucosamine. From McCarty (16).*

termed V enzyme, which destroys the A-variant carbohydrate (16). There is, however, no similarity in the manner in which the two enzymes produce their effects.

The action of the A and V enzymes on groups A and A-variant carbohydrates is illustrated diagrammatically in Fig. 7 and 8. In Fig. 7, the A enzyme is represented as having no effect on the A-variant carbohydrate. The V enzyme, however, hydrolyzes the carbohydrate extensively to dialyzable split products of rhamnose and nondialyzable material, and both fractions have completely lost serological reactivity with homologous antiserum. The dialyzable products were mono-, di-, and oligosaccharides of rhamnose. It

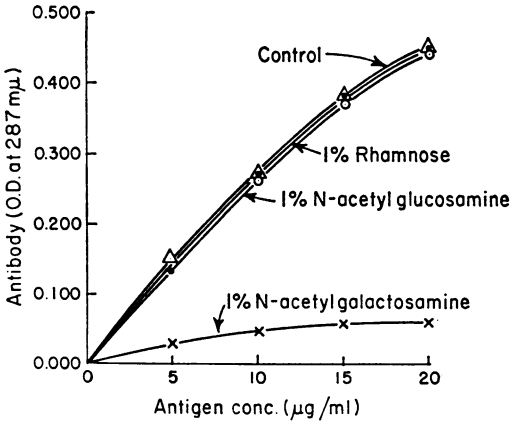

FIG. 6. *Inhibition of precipitin reaction between group C carbohydrate and group C antiserum with N-acetylgalactosamine. From Krause and McCarty (10).*

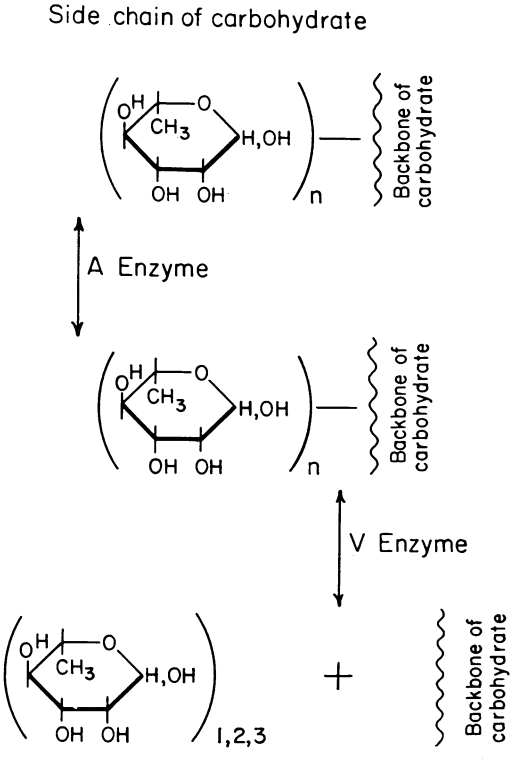

FIG. 7. *Diagrammatic representation of the action of A enzyme and V enzyme on group A-variant carbohydrate.*

is of special significance that the monosaccharide of rhamnose did not inhibit the precipitin reaction between V carbohydrate and homologous antiserum, but the rhamnose disaccharide exerted a

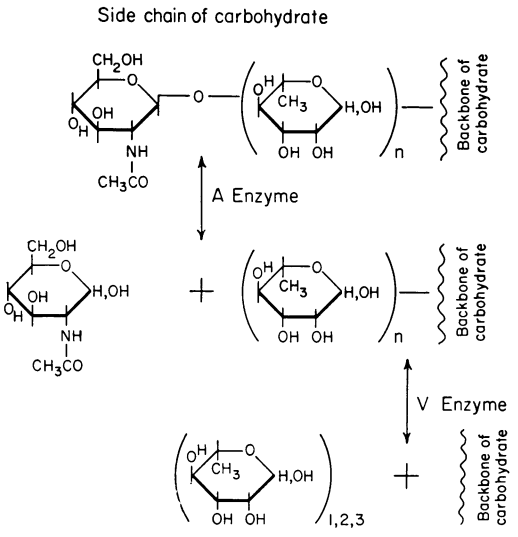

FIG. 8. *Diagrammatic representation of the action of A enzyme and V enzyme on group A carbohydrate.*

marked inhibitory effect. An experiment which shows this inhibitory activity on the precipitin reaction between A-variant carbohydrate and V antiserum is depicted in Fig. 9 (16). In the quantitative precipitin test (left of Fig. 9), it is to be noted that 1 mg per ml of the rhamnose disaccharide had a marked inhibitory effect on the precipitin reaction. In the quantitative precipitin test with A carbohydrate and A antiserum (right of Fig. 9), however, the disaccharide had no appreciable effect on the precipitin reaction. This evidence supports the view that rhamnose-rhamnose linkages are determinants of A-variant carbohydrate specificity but have no significant role in the antigenic determinant of A carbohydrate.

The effect of A enzyme on group A carbohydrate is depicted in the first reaction of Fig. 8. Chemical analysis reveals that the bulk of the amino sugar is released as free *N*-acetylglucosamine, and the residual carbohydrate is composed primarily of rhamnose. As would be expected, the antigen at this juncture exhibits a concomitant loss of serological reactivity with homologous antiserum but at the same time develops a marked cross-reactivity with A-variant antiserum. It is not surprising, therefore, that A carbohydrate, devoid of *N*-acetylglucosamine after A enzyme treatment, is attacked by the V enzyme with release of mono-, di-, and oligosaccharides of rhamnose and with concomitant

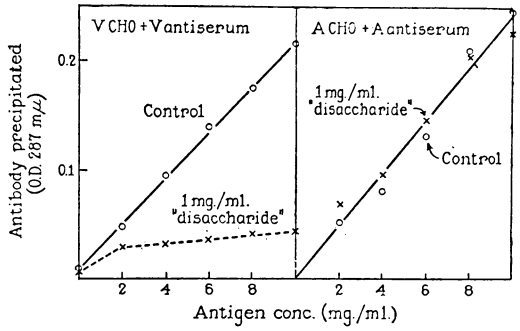

FIG. 9. *Inhibitory effect of rhamnose disaccharide on the precipitin reaction between V carbohydrate and V antiserum. From McCarty (16).*

loss of reactivity when tested against A-variant antiserum. These studies suggest that the rhamnose moieties of groups A and A-variant carbohydrates have similar rhamnose-rhamnose linkages, but, in the case of group A carbohydrate, the rhamnose-rhamnose linkages are masked by terminal *N*-acetylglucosaminide residues and, therefore, do not react with A-variant antiserum. In other studies, the A enzyme was shown to be specific for β-glucosaminides, thus supporting the view that the terminal determinants of A carbohydrate are not α- but β-*N*-acetylglucosaminide residues.

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. The induced A enzyme, a *N*-acetyl-β-glucosaminidase, although less effective, also has *N*-acetyl-β-galactosaminidase activity when tested with a substrate such as *p*-nitrophenol-N-acetyl-β-D-glucosaminide. It would be expected, therefore, that the A enzyme would split terminal *N*-acetylgalactosaminide residues from the group C carbohydrate. However, after prolonged exposure of the C carbohydrate to high concentrations of the A enzyme, there was no detectable loss of serological activity and no release of *N*-acetylgalactosamine. In view of these findings, although the *N*-acetylglucosaminide residues are attached through beta linkages in the case of group A carbohydrate, the nature of the linkage of the *N*-acetylgalactosamine residues to the remainder of the carbohydrate in the case of group C remains obscure (10).

Variation in the Specific Carbohydrates of Groups A and C Streptococci

Variant strains of streptococci, in addition to the group A-variant described earlier, include group A-intermediate, group C-variant, and group C-intermediate. Immunochemical data suggest that each of these strains possesses identical rhamnose-rhamnose linkages which are reactive with group A-variant antiserum. A special strain termed group A-intermediate was detected during the course of the survey for group A-variant streptoccocci. An antigenic extract of this strain, C121/39, reacted with both group A and group A-variant antisera. Bacteriological studies indicated that this serological activity was not due to a mixture of groups A and A-variant streptococci, because antigen extracts of single-colony isolates of the culture reacted with both groups A and A-variant antisera. Furthermore, immunochemical data indicated that the A-intermediate strain did not possess two types of carbohydrate but rather contained a single antigen which was reactive with both of the antisera (18).

During the course of routine serological identification of hemolytic streptococci, the extract of an occasional group C strain gives a cross-reaction with group A-variant antiserum. A survey of the group C streptococci of the Rockefeller Institute collection revealed that extracts of 14 of 152 strains tested gave an appreciable cross-reaction with group A-variant antiserum (11). Immunochemical evidence indicates that these special group C strains which cross-react with group A-variant antiserum are analogous to the A-intermediate strains. Thus, the C-intermediate strains possess a carbohydrate antigen which is reactive with both groups C and A-variant antisera. As would be expected, the C-intermediate strains give rise to antibodies which precipitate both C and A-variant carbohydrates.

It is of particular interest that certain variant group C strains have recently been shown to possess a carbohydrate which does not react with group C antiserum but does cross-react with group A-variant antiserum. These strains, termed group C-variant, were isolated from group C streptococci which survived exposure to virulent group C bacteriophage (1). Detailed immunochemical studies suggest that group C-variant strains possess a carbohydrate antigen similar to that of group A-variant streptococci. As would be expected, the group C-variant strains stimulate the production of antibody in rabbits which cross-reacts with A-variant carbohydrate.

The relationships between the carbohydrates of groups A, A-intermediate, and A-variant and of groups C, C-intermediate, and C-variant are depicted in Fig. 10 and 11. To the right in Fig. 10 are tabulated the percentage compositions of the A carbohydrates. Group A-intermediate has 17% *N*-acetylglucosamine, a value intermediate between the 30% of group A and the 4% of group A-variant. As would be expected, the rhamnose content of group A-intermediate is higher than that of group A and lower than that of group A-variant. A stylized representation of the antigenic determinants of the carbohydrates is depicted to the left in Fig. 10. Although the cross-reaction of A-intermediate carbohydrate with A-variant antiserum supports the view that it possesses some rhamnose-rhamnose linkages devoid of terminal *N*-acetylglucosaminide residues, additional evidence is derived from studies with the V enzyme. Group A-intermediate carbohydrate treated with V enzyme is no longer reactive with A-variant antisera. Concomitant with this loss of cross-reactivity, a portion of the rhamnose content of the antigen is rendered dialyzable.

In Fig. 11, the composition of C carbohydrates

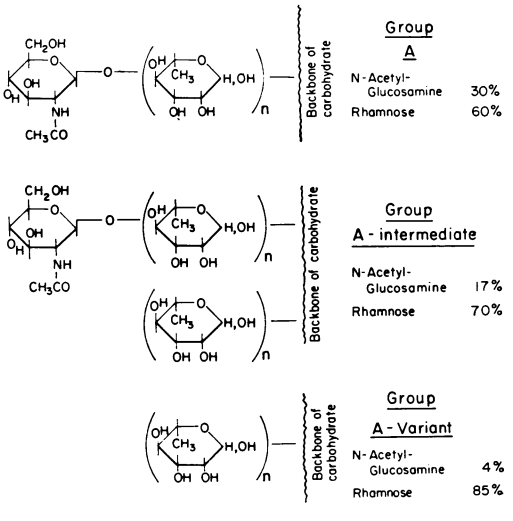

FIG. 10. *Chemical compositions and diagrammatic representations of the antigenic determinants of groups A, A-intermediate, and A-variant carbohydrates.*

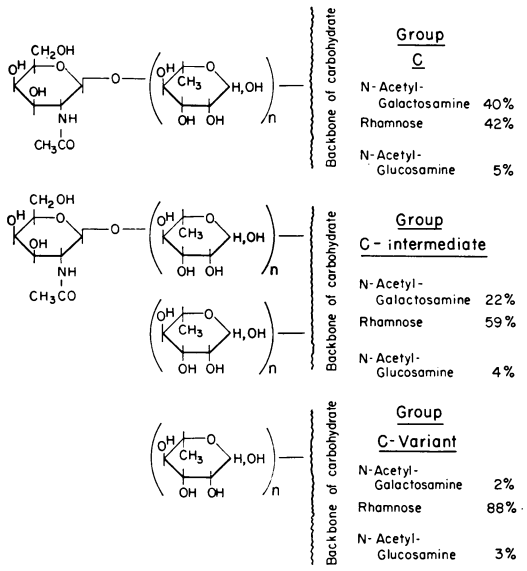

FIG. 11. *Chemical compositions and diagrammatic representations of the antigenic determinants of groups C, C-intermediate, and C-variant carbohydrates.*

is tabulated to the right. C-intermediate carbohydrate has 22% *N*-acetylgalactosamine, less than the 40% of group C and greater than the 2% of group C-variant. Correspondingly, the rhamnose content of 59% is intermediate between that of group C and that of group C-variant. Each of the carbohydrates contains a small percentage of *N*-acetylglucosamine. A stylized representation of the antigenic determinants is illustrated to the left in Fig. 11. Group C-intermediate is depicted as possessing rhamnose-rhamnose linkages with a terminal *N*-acetylgalactosaminide residue and linkages which are devoid of amino sugar. In addition to the serological data, this view is supported by the fact that C-intermediate carbohydrate, after treatment with V enzyme, is no longer reactive with A-variant or C-variant antisera. Concomitant with this loss of reactivity, a portion of the rhamnose content is released as dialyzable fragments. These chemical and immunological considerations of the carbohydrates of variant strains of groups A and C streptococci suggest that the rhamnose moiety of the A and C carbohydrates possesses similar rhamnose-rhamnose linkages. These linkages are exposed in the case of groups A-variant and C-variant carbohydrates, and are thus the determinants of variant specificity. In the case of group A, the rhamnose-rhamnose linkages possess terminal β-*N*-acetylglucosaminide residues, thus confirming group A specificity and at the same time masking the variant activity of the rhamnose moiety. The rhamnose-rhamnose linkages of group C carbohydrate possess terminal *N*-acetylgalactosaminide residues, a feature responsible for the group C reactivity of the carbohydrate and, at the same time, masking the variant reactivity of the rhamnose moiety.

Group G Streptococci

As was indicated in the Introduction, there are certain basic similarities between the cell-wall composition of group G streptococci and that of groups A and C. The monosaccharide and the amino acid content of group G cell walls is presented in Table 4 (3). The cell walls, like those of other groups discussed here, contain rhamnose, hexosamine, including muramic acid, and alanine, glutamic acid, lysine and glycine. Unlike the cell walls of the other groups, galactose is also present. The carbohydrate extracted with hot formamide contains rhamnose, galactosamine, and only traces of muramic acid and the amino acids. The residue contains the amino sugars and the amino acids of the mucopeptide and is similar to that of the groups of streptococci discussed previously.

If it were not for the presence of galactose, the

TABLE 4. *Composition of group G cell walls and of the soluble carbohydrate (CHO) and the insoluble residue after hot formamide extraction*

Constituent	Cell walls	Formamide treatment		
		Extracted G CHO	Formamide residue	
			Amount	Mole ratio
	%	%	%	
Rhamnose	19.6	40.7	5.5	—
Galactose	8.9	23.0	<1.0	—
Hexosamine	8.2	—	—	—
Galactosamine	—	20.6	0.0	—
Glucosamine	—	0.0	10.4	2.2
Muramic acid	3.3	—*	6.6	(1)
Alanine	16.9	—*	24.9	10.6
Glutamic acid	7.3	—*	11.1	2.9
Lysine	8.0	—*	11.5	3.0
Glycine	0.9	—*	0.9	0.4

* Less than 1%.

composition of group G carbohydrate would resemble that of group C. Both group C and group G carbohydrates contain rhamnose and galactosamine, although galactosamine is present in somewhat greater abundance in group C carbohydrate than in group G carbohydrate. Nevertheless, the similarity of chemical composition between groups C and G carbohydrates, except for the presence of galactose in the latter, suggested the possibility that galactose was associated with the determinant of antigenic specificity of group G carbohydrate. This hypothesis was tested in quantitative precipitin inhibition studies with galactose and other oligosaccharides which contain this sugar. No appreciable inhibition of the precipitin reaction was noted when galactose, lactose, or stachyose, at a final concentration of 1%, was added to the antigen-antibody system. Quantitative precipitin inhibition studies were performed, therefore, with the other monosaccharides of the group G carbohydrate. Although *N*-acetylgalactosamine had no appreciable inhibitory effect on the quantitative precipitin reaction, rhamnose, at a final concentration of 1%, markedly inhibited the precipitin reaction between group G carbohydrate and its antiserum. When the inhibition of the precipitin reaction was tested at antigen-antibody equivalence with various concentrations of rhamnose, as little as 10 μmoles per ml (or 0.15%) were sufficient to effect significant inhibition. Although these results are preliminary, they suggest that rhamnose may be a major determinant of the serological specificity of group G carbohydrate.

It will be recalled from earlier data in this paper than an oligosaccharide of rhamnose is the determinant grouping of group A-variant carbohydrate. The view that the determinant of group A-variant is a rhamnose oligosaccharide is supported by the fact that the precipitin reaction between A-variant carbohydrate and its antiserum is not inhibited by the monosaccharide of rhamnose but is inhibited by di- or oligosaccharides of rhamnose. A fundamental difference between the role of rhamnose in the determinant grouping of group A-variant on the one hand and group G on the other is suggested by the fact that group G and A-variant carbohydrates do not cross-react with the opposite antisera. Furthermore, group A-variant carbohydrate does not inhibit the group G precipitin reaction, and group G carbohydrate does not inhibit the group A-variant precipitin reaction.

It is interesting to speculate on the possible structural arrangement of the sugars in the group G carbohydrate. One can envision a structure similar to group C but with an additional rhamnose terminal to the *N*-acetylgalactosamine. It is conceivable that a single rhamnose molecule in such a position would confer specificity distinct from the rhamnose oligosaccharide determinant of group A-variant carbohydrate. In addition, the rhamnose terminal to the *N*-acetylgalactosamine would mask the potentially reactive group C determinant. Experiments now in progress are designed to answer these questions.

SUMMARY

In summary, the details of these experiments are in agreement with the hypothesis that the rhamnose moieties of the carbohydrates of groups A and C streptococci are composed of similar rhamnose-rhamnose linkages but that the antigenic determinants in the case of group A are terminal β-*N*-acetylglucosaminidase residues, whereas the determinants of group C are terminal *N*-acetylgalactosaminide residues. The serological reactivity of the rhamnose-rhamnose linkages is masked by the terminal *N*-acetyl amino sugars, and is only evident if the terminal groups are absent, either as in the natural circumstance of the variant carbohydrates or as a result of removal from group A carbohydrate by enzymatic action. Group G carbohydrate contains rhamnose, *N*-acetylgalactosamine, and galactose as the major constituent sugars, and quantitative precipitin inhibition studies suggest that rhamnose may be the major determinant of antigenic specificity.

The findings presented here underscore the close biological relationship of hemolytic streptococci, a relationship long accepted on a taxonomic basis. Despite this close relationship from a bacteriological point of view, it should be reemphasized that infections with groups C and G streptococci, unlike those with group A, are seldom if ever followed by the nonsuppurative complications, acute rheumatic fever and acute glomerulonephritis. For this reason, investigation of biological differences between group A streptococci on the one hand and groups C and G streptococci on the other warrants continued attention.

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