

VARIATION IN THE GROUP-SPECIFIC CARBOHYDRATE OF GROUP C HEMOLYTIC STREPTOCOCCI*

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In the course of serologic identification of hemolytic streptococci occasional strains of Group C have been encountered which give a precipitin cross-reaction with Group A-variant antiserum. In a previous communication (1) the carbohydrate antigen of one of these strains was shown to have a rhamnose-hexosamine ratio of 2.8, whereas the ratio for the carbohydrate of typical Group C strains varies between 1.1 and 1.7. The major hexosamine of both forms of Group C carbohydrate is *N*-acetylgalactosamine and previous results suggest that this is the primary determinant of antigenic specificity.

Group A-variant carbohydrate, the antibody of which cross-reacts with these special Group C strains, is composed primarily of rhamnose with glucosamine as a minor component. Immunochemical studies have shown that side chains of rhamnose oligosaccharides are the major determinants of antigenic specificity of Group A-variant carbohydrate (2). In the carbohydrate of typical Group A strains, this rhamnose specificity is masked by the presence of terminal *N*-acetylglucosaminide units on the side chains. McCarty and Lancefield (3) have described the occurrence of a Group A streptococcal strain which consistently yields carbohydrate preparations that are almost equally reactive with Group A and Group A-variant antisera. This strain was designated Group A-intermediate, and the available evidence indicates that its serological behavior results from the presence on the same molecule of side chains both with and without terminal *N*-acetylglucosaminide units (2, 3).

The data presented in the present report suggest that the Group C strains which cross-react with Group A-variant antisera are analogous to the Group A-intermediate strain. Thus, some of the rhamnose side chains of the carbohydrate lack the terminal *N*-acetylgalactosaminide residues which determine Group C specificity so that they are now available to react with A-variant antibodies. The findings underscore the close relationship between Group A and Group C streptococci.

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Materials and Methods

The following organisms were used for the preparation of cell walls and the isolation of group-specific carbohydrate: Group C strains H46A and K64, Group C-intermediate strains 28RP95 and B636, Group A-variant strain T27A.

The methods for preparing cell walls and the isolation of the group-specific carbohydrates have been previously described (4). In these studies all of the carbohydrate preparations were extracted by Fuller's formamide procedure (5).

An induced enzyme from soil organisms which attacks Group A-variant carbohydrate was prepared as previously described (2).

Analytical Methods.—Analyses for rhamnose, glucosamine, and galactosamine were performed by previously described methods (4).

Quantitative Precipitin Tests.—Rabbit antisera reactive against a group-specific carbohydrate were prepared as previously described (3). Quantitative precipitin analyses were performed by dissolving the washed antigen-antibody precipitate in 0.1 N NaOH and assaying the antibody spectrophotometrically at 287 m μ (3).

EXPERIMENTAL

Survey of Group C Streptococci for Cross-Reactions with Group A-Variant Antiserum.—In the course of routine serological identification of hemolytic streptococci, the extract of an occasional strain of Group C will react not only with homologous antiserum but also with Group A-variant antiserum. A survey was made, therefore, of the Group C streptococcal collection to identify those strains which cross-react with Group A-variant antiserum. A total of 152 strains were rechecked by the usual capillary precipitin technique for Group C and Group A-variant reactivity. While all strains reacted with Group C serum, 14 strains gave an appreciable precipitin reaction with Group A-variant serum. The source of these strains was from animal and human material. Of those strains isolated from human beings several were from the nasopharynx, one was from the blood of a patient with septicemia,¹ and one was from the lesion of impetigo.² Two of these strains, 28RP95 and B636, were selected for chemical and serological studies on the carbohydrate antigen. In the following experiments comparison is made between these strains and typical Group C strains, H46A and K64.

Chemical Analysis of Carbohydrates.—The carbohydrates employed in these experiments were extracted from the cell walls by the hot formamide method of Fuller (5). Rhamnose, *N*-acetylgalactosamine, and *N*-acetylglucosamine are the principle components of these carbohydrates. In all of the results reported here, however, the amino sugar analyses are for the non-acetylated form. Previous work indicates that approximately 90 per cent of the hexosamine in Group C carbohydrate is galactosamine and 10 per cent is glucosamine. For this reason the chemical analysis for hexosamine by the Elson-Morgan reaction

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² Kindly furnished by Dr. Elaine L. Updyke, Communicable Diseases Center, Atlanta 22, Georgia.

has been determined by comparison with a galactosamine standard. The results of analysis of the carbohydrates are presented in Table I. The rhamnose:hexosamine ratio for Group C carbohydrate of strains H46A and K64 is 1.1 to 1.7, respectively, a finding consistent with previous values for Group C strains (6). In contrast to this, strains 28RP95 and B636 which cross-react with Group A-variant antiserum have ratios of 2.4 and 2.6, respectively. These strains have been given the designation of Group C-intermediate.

Qualitative Serological Studies.—Qualitative precipitin studies of the carbo-

TABLE I
Chemical Composition of Group-Specific Carbohydrate Extracted with Formamide

Group	Strain	Galactosamine	Rhamnose	R/G ratio
		<i>per cent</i>	<i>per cent</i>	
C	H46A	39.0	43.0	1.1
C	K64	30.4	51.3	1.7
C-intermediate	B636	21.0	55.3	2.6
C-intermediate	28RP95	24.4	58.8	2.4

TABLE II
Comparison of the Qualitative Precipitin Tests with the Three Varieties of Carbohydrate

Antigen	Tests in C antiserum							Tests in A-variant antiserum						
	Concentration of antigen solution, mg/ml													
	1.0	0.1	0.05	0.025	0.012	0.006	0.003	1.0	0.1	0.05	0.025	0.012	0.006	0.003
Typical Group C	±	+++	++++	+++	+++	++	+	±	-	-	-	-	-	-
Group C-intermediate.....	+	+++	++++	+++	+++	+	+	+	++	+	±	±	tr	-
Group A-variant.	-	-	tr	±	tr	-	-	±	+++	+++	++	++	++	+

hydrates with Groups C and A-variant antisera were carried out in capillary precipitin tubes. The results, shown in Table II, demonstrate the appreciable cross-reaction in the usual capillary precipitin test between the Group C-intermediate carbohydrate and the Group A-variant antiserum. The Group C-intermediate carbohydrate reacts with both antisera and although the reaction with Group C antiserum is predominantly stronger, as little as 6 μ g of antigen reacts with Group A-variant antiserum. The data in Tables I and II illustrate the fact that those strains with a high rhamnose:hexosamine ratio cross-react with the Group A-variant antiserum.

The cross-reaction of the Group C-intermediate carbohydrate with Group A-variant antiserum depicted in Table II illustrates a finding which was noted with several different Group A-variant antisera. However, it should be pointed

out that there was considerable variation in the strength of the cross-reaction among the antisera. Furthermore, the strongest cross-reaction was not necessarily obtained with the most potent antiserum. Characteristically, the cross-reactions developed more slowly than the homologous reactions.

Quantitative Precipitin Analysis.—Additional information on the immunologic behavior of the carbohydrates was obtained from quantitative precipitin analysis. Fig. 1 illustrates quantitative precipitin tests on Groups C and C-intermediate carbohydrates with Groups C and A-variant antisera. The Group

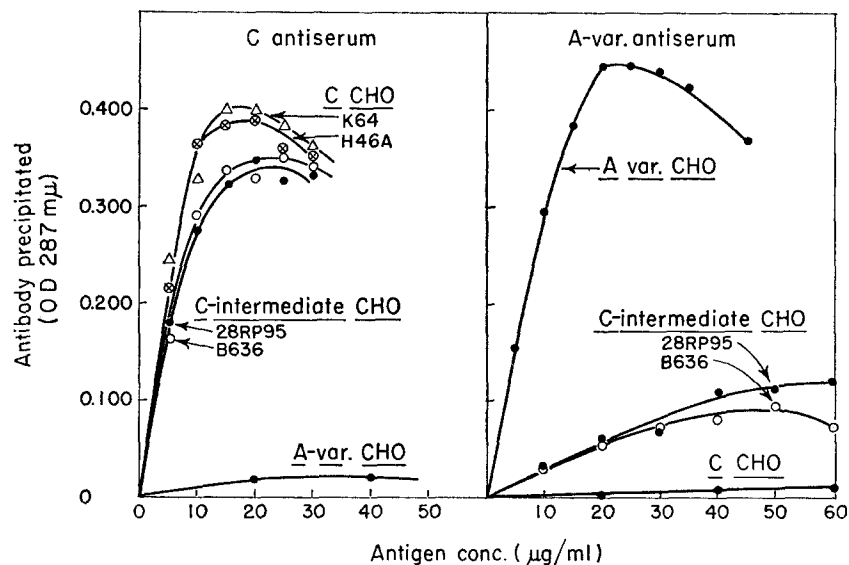


FIG. 1. Comparison of the quantitative precipitin analyses of Groups C, C-intermediate, and A-variant carbohydrates with C and A-variant antisera.

C carbohydrates 28RP95 and B636 gives somewhat less precipitin reaction with Group C antiserum than the Group C carbohydrates H46A and K64, a finding which has been consistent with several different Group C antisera. The A-variant carbohydrate gives no appreciable reaction with these antisera. On the other hand, the carbohydrates of both Group C-intermediate strains cross-react with Group A-variant antisera to give broad, flat precipitin curves. Typical Group C carbohydrate lacks this cross-reactivity.

Quantitative Precipitin Studies with Group C-Intermediate Antiserum.—Group C-intermediate strains 28RP95 and B636 were used to prepare a pepsin-digested, heat-killed vaccine as previously described (3). Although vaccines of these strains appear to be less effective in the induction of precipitating anti-carbohydrate antibodies than those of typical Group C strains, satisfactory

antisera for analysis were obtained. Quantitative precipitin reactions with Group C-intermediate serum and Groups C, C-intermediate, and A-variant carbohydrates are illustrated in Fig. 2. It is clear that the Group C-intermediate serum reacts with the Group A-variant carbohydrate as well as the Groups C and C-intermediate carbohydrates. This is in contrast to the Group C serum, as depicted in the left half of Fig. 1, which gives no appreciable cross-reaction with A-variant carbohydrate.

These studies support the view that Group C-intermediate carbohydrate is

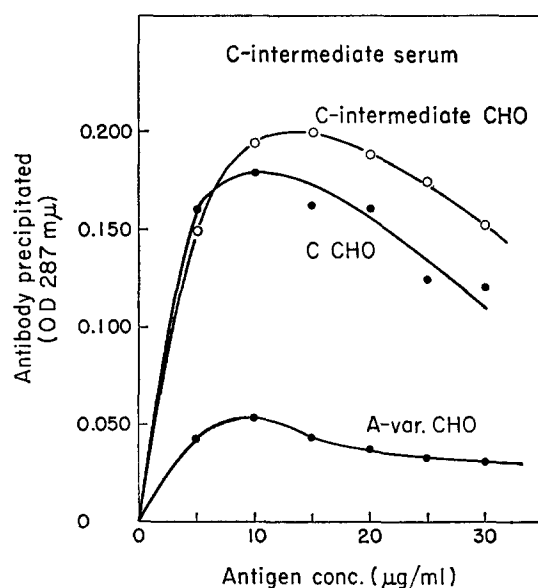


FIG. 2. Quantitative precipitin analysis with C-intermediate antiserum.

related antigenically to Group A-variant carbohydrate as well as to that of Group C. The following experiments indicate that the Group C-intermediate carbohydrate contains a rhamnose oligosaccharide determinant similar to that found in Group A-variant carbohydrate.

Effect of Induced Enzymes on the Serologic Activity of Group C-Intermediate Carbohydrate.—McCarty (2) described an induced enzyme isolated from the culture filtrate of a soil organism which destroys the serologic activity of Group A-variant carbohydrate. Dialyzable oligosaccharides of rhamnose are released during enzymatic hydrolysis which inhibit the Group A-variant precipitin reaction. By fractionation of the split products, one component with the properties of a rhamnose disaccharide was isolated and shown to act as a specific inhibitor. It was concluded from these studies that rhamnose oligo-

saccharide side chains of undetermined length are the primary determinants of specificity of the A-variant carbohydrate. The cross-reaction reported here between Group C-intermediate carbohydrate and Group A-variant serum suggests that this carbohydrate also contains rhamnose oligosaccharide determinants. If this is the case the variant enzyme would be expected to destroy the Group A-variant activity of Group C-intermediate carbohydrate with concomitant release of dialyzable rhamnose fragments. Such a result was obtained in the following experiment.

10 mg of the Group C-intermediate carbohydrate in a small volume of saline was treated with variant enzyme during simultaneous dialysis against saline at 37°C for 12 hours. Subsequent dialysis of the digestion mixture at 4°C for the next 48 hours was against changes of distilled water. The non-dialyzable digestion mixture and the combined dialysates after con-

TABLE III
Effect of Induced Variant Enzyme on Group C-Intermediate Carbohydrate

		Non-dialyzable	Dialyzable	Recovery
		<i>mg</i>	<i>mg</i>	<i>per cent</i>
C-intermediate CHO + variant enzyme	Rhamnose	4.25	1.50	99
	Galactosamine	2.24	0.23	100
C-intermediate CHO control	Rhamnose	5.92	0.23	106
	Galactosamine	2.35	0.07	99

centration were saved for chemical and serologic analysis. Another 10 mg sample of the carbohydrate, which served as a control was treated in a similar fashion except that variant enzyme was not employed. Chloroform was used throughout as a preservative.

Chemical analysis of the non-dialyzable and the dialyzable fractions of the Group C carbohydrate treated with variant enzyme, and the corresponding control fractions are represented in Table III. Recorded in the table are the values for rhamnose and galactosamine content of the fractions after acid hydrolysis. In the case of the control carbohydrate which was not treated with variant enzyme only a small percentage of the initial material was recovered in the dialysate. As a result of the enzymic treatment of the carbohydrate, on the other hand, 25 per cent of the total rhamnose but only 10 per cent of the galactosamine was recovered in the dialysate. This finding suggests that the Group A-variant component of the Group C-intermediate carbohydrate has been removed by the enzyme. The results of serologic analysis on the fractions support this view.

Quantitative precipitin analysis of the untreated Group C-intermediate carbohydrate and the sample treated with variant enzyme are illustrated in Fig. 3. Results are given for Groups C, C-intermediate, and A-variant antisera.

With Group C antiserum, the precipitin curves of enzyme-treated and control carbohydrate are essentially similar, indicating that no change in Group C reactivity had occurred with enzyme treatment. Differences between the precipitin curves of the enzyme-treated and control carbohydrates are seen when Groups C-intermediate and A-variant antisera are employed. The enzyme-treated carbohydrate precipitates almost no antibody from Group

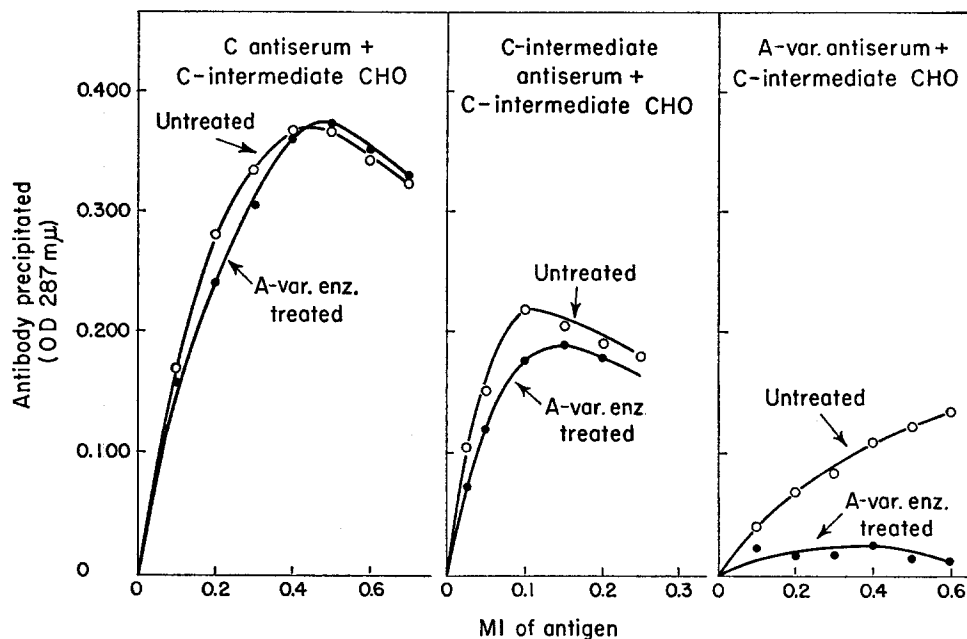


FIG. 3. Effect of induced A-variant enzyme on the serological reactivity of C-intermediate carbohydrate with C, C-intermediate, and A-variant antisera. The antigen solutions are the non-dialyzable portion of the C-intermediate carbohydrate treated with the A-variant enzyme and the control carbohydrate sample. The samples were diluted 1:20 for use in the precipitin tests.

A-variant serum. The treated carbohydrate also precipitates somewhat less antibody than control material when tested with Group C-intermediate serum. This latter finding indicates that the treated carbohydrate is unable to precipitate the Group A-variant antibody present in the Group C-intermediate serum. These results demonstrate that Group C-intermediate carbohydrate, after removal of the rhamnose determinants of Group A-variant activity, is unable to react with Group A-variant antibody.

Collaborative evidence that the variant enzyme removes the rhamnose portion of the C-intermediate carbohydrate, which determines the A-variant cross-reactivity, was obtained with precipitin inhibition studies illustrated in

Fig. 4. The dialyzable fraction obtained from the C-intermediate carbohydrate after treatment with variant enzyme, and employed in the previous experiment, was found to inhibit markedly the precipitin reactions between Groups A-variant and C-intermediate carbohydrates and Group A-variant antiserum. Extensive chemical studies have not been made on this dialyzable material but preliminary evidence suggests that it is a mixture of rhamnose oligosaccharides.

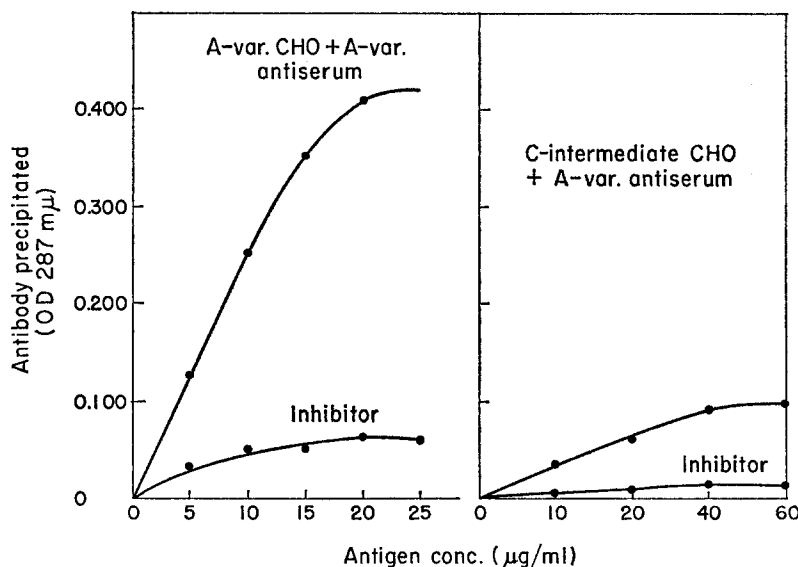


FIG. 4. Inhibitory effect of dialyzable rhamnose oligosaccharides on the reactivity of A-variant and C-intermediate carbohydrates with A-variant antiserum. The rhamnose oligosaccharides in the inhibitor solution was obtained by dialysis of C-intermediate carbohydrate treated with A-variant enzyme. The oligosaccharide solution added to the inhibition tests contained 60 μ g of rhamnose. The precipitin tests were done in a final volume of 1 ml.

DISCUSSION

The specific carbohydrates of Groups A and C streptococci differ primarily in the determinant amino sugar. Both carbohydrates contain rhamnose but that of Group A contains *N*-acetylglucosamine while that of Group C contains *N*-acetylgalactosamine (1). The specific carbohydrate of an antigenic variant of Group A streptococci, designated A-variant, is composed primarily of rhamnose (4). The work of McCarty (2) with induced enzymes from soil organisms has identified the principle determinants of specificity in the Groups A and A-variant carbohydrates. The induced enzyme which attacks Group A is a β -glucosaminidase, while that which attacks Group A-variant removes dialyzable rhamnose oligosaccharides. The antigenic determinant of A-variant

carbohydrate is a rhamnose oligosaccharide as indicated by the fact that the products of enzymic hydrolysis inhibit the homologous precipitin reaction. In the case of Group A, *N*-acetylglucosamine is removed from the carbohydrate by the induced β -glucosaminidase with an associated loss of Group A serologic activity. Concomitant with this alteration, the enzymic residue composed primarily of rhamnose reacts with the Group A-variant antiserum (2). The residue, unlike the initial Group A carbohydrate is hydrolyzed by the variant enzyme with the release of rhamnose oligosaccharides. These and other studies (7) have led McCarty to suggest that an essential feature of Group A carbohydrate structure is terminal *N*-acetylglucosamine on side chains of rhamnose oligosaccharide, and the Group A-variant carbohydrate differs from this primarily in the absence of the terminal *N*-acetylglucosamine.

An interesting streptococcal variant of Group A has been termed A-intermediate (3). The carbohydrate from this organism reacts with both A and A-variant antisera. The analytical values for rhamnose and glucosamine in the A-intermediate carbohydrate fall midway between those of Groups A and A-variant. The evidence suggests that only a portion of the rhamnose oligosaccharide side chains possess terminal *N*-acetylglucosamine. The rhamnose oligosaccharide which does not possess *N*-acetylglucosamine is susceptible to the action of the variant enzyme with the result that the residual carbohydrate reacts with Group A antisera but not with A-variant antisera.

The present work provides some support for the view that C carbohydrate is structurally similar to A carbohydrate. This evidence has developed from the finding that certain strains of Group C streptococci possess a carbohydrate antigen which also reacts with Group A-variant antiserum. The carbohydrate of these strains, termed Group C-intermediate, differs from that of typical Group C strains in the proportion of rhamnose to hexosamine. The Group C-intermediate carbohydrate has a higher concentration of rhamnose and a lower concentration of hexosamine than typical Group C antigens. The reactivity of this antigen with A-variant antiserum is due to the fact that only a portion of the oligosaccharide side chains possess terminal *N*-acetylgalactosamine. The side chains from which the terminal *N*-acetylgalactosamine is absent determine the reactivity with A-variant antiserum. Support for this view is derived from the fact that treatment of C-intermediate carbohydrate with variant enzyme removes 25 per cent of the rhamnose with a concomitant loss of A-variant reactivity. Thus, these particular rhamnose oligosaccharide side chains of both C-intermediate and A-intermediate carbohydrates are similar in that their reactivity with A-variant antiserum is destroyed with variant enzyme.

From these studies it is clear that the C and C-intermediate carbohydrates have an antigenic relationship which is analogous to that of A and A-intermediate carbohydrates. The results are in agreement with the hypothesis that

the carbohydrates of Groups A and C streptococci are composed of similar rhamnose oligosaccharide side chains but that the terminal amino sugar in the case of Group A is *N*-acetylglucosamine whereas that of Group C is *N*-acetylgalactosamine.

SUMMARY

Certain strains of Group C hemolytic streptococci, termed Group C-intermediate, contain a group-specific carbohydrate antigen which gives a precipitin cross-reaction with A-variant antiserum. The carbohydrate antigens of these strains have a rhamnose:hexosamine ratio ranging from 2.4 to 2.6 whereas the ratio of typical Group C strains varies between 1.1 and 1.7. *N*-acetylgalactosamine, the major hexosamine in all of these strains is the principle determinant of Group C specificity. The high concentration of rhamnose in the C-intermediate carbohydrate suggests that a portion of the rhamnose oligosaccharide side chains are devoid of terminal *N*-acetylgalactosamine and thus react with Group A-variant antiserum. This view is supported by the fact that the induced variant enzyme, which destroys A-variant carbohydrate reactivity with the liberation of rhamnose oligosaccharides, has a similar action upon the Group C-intermediate carbohydrate. C-intermediate carbohydrate, after treatment with variant enzyme which removed approximately 25 per cent of the rhamnose, does not react with A-variant antisera.

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