TEMPERATURE-DEPENDENT VARIATION IN THE SYNTHESIS OF GROUP-SPECIFIC CARBOHYDRATE BY STREPTOCOCCAL VARIANT STRAINS

I. Immunochemical Studies*

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The determinant of Group A serological reactivity for the β-hemolytic streptococcus is a polysaccharide with side chains of rhamnose and end-terminal *N*-acetylglucosamine, the latter amino sugar being responsible for the antigenic specificity of the Group A polysaccharide (1, 2). Removal of the terminal *N*-acetylglucosamine results in an alteration of the serological reactivity of the residual carbohydrate so that the antibody specificity becomes directed to the rhamnose moiety (1, 3). While such an alteration can be produced by either chemical or enzymatic hydrolysis of the Group A carbohydrate, absence of the terminal amino sugar has been described in naturally occurring variant strains of Group A streptococci (1, 3, 4).

Isolation of a variant strain of this type was initially reported by Wilson (4). Subsequently, McCarty and Lancefield (3) described a number of additional variant strains and also reported the isolation of another strain with a partial modification of its antigenic determinant. This latter strain, designated as intermediate, synthesizes a cell wall carbohydrate that reacts with antisera to both the Group A and variant antigens. Antibody prepared with the intermediate organism reacts with both Group A and variant carbohydrates.

The present study reports a temperature-dependent alteration in the synthesis of the cell wall carbohydrate by certain variant strains. Carbohydrate extracted from a previously identified variant strain was found to react with Group A antiserum when the organism was grown at room temperature, while the same strain grown at 37°C had a cell wall polysaccharide that reacted only with variant antiserum. A similar temperature-dependent variation in the serological reactivity of the carbohydrate was observed to occur with an intermediate strain. Immunochemical data are presented detailing the change associated with this temperature-related alteration, which appears to be a property of strains with intermediate characteristics.

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Materials and Methods

Streptococcal Strains.—The variant (V)[1] and intermediate (I) streptococcal strains were obtained from Doctors R. C. Lancefield and Maclyn McCarty. The A-486-Var strain was the V strain on which our initial observation of antigenic variation with temperature was made. Another strain, C 121/46/4, also labeled as a V strain and isolated after further mouse passage of strain C 121/39, showed similar variation when tested. Subsequently, the C 121/39/8, a subculture of the I strain C 121/39 (3), was obtained from Dr. McCarty and used as a prototype of the I group. Two V strains (T/27A-32-18 and B/403-1022) obtained from Doctors Lancefield and Zabriskie had been tested and found to maintain their V antigenic characteristic at 22° and 37°C. These strains were used as prototypes of the V group. The C-203-S strain (Group A, M-Type 3) and F-60 (a Group A, M-Type 49 strain obtained from a patient with impetigo and nephritis) were used as prototypes for this serological group.

Cultural Techniques.—Unless specified otherwise, bacterial growth was carried out in Todd-Hewitt broth (THB) (Difco Laboratories, Detroit, Mich.). After the initial observation of the temperature-related variation in serological reactivity, organisms grown for studies at various temperatures were subcultured from the lyophilized form into blood broth or onto 5% sheep blood agar. These subcultures were grown and maintained for the period of study at the same temperature as that of the final broth culture, i.e., either 37° or 22°C (room temperature). Incubation at 37° and at 22°C of cultures for bacterial growth was usually carried out for 18–24 h and for 48–72 h, respectively.

Preparation of Carbohydrates—The cells were harvested and cell walls prepared by disintegration with glass beads in a Mickle apparatus followed by differential centrifugation (5). The carbohydrate was solubilized from either whole cells or cell walls by extraction in hot acid (6) or hot formamide (7). Preparation of ^{14}C-labeled carbohydrates was performed using ^{14}C-uniformly labeled glucose, as described previously (8).

Serological Techniques.—Hyperimmune rabbit Group A and V antisera were initially provided by Dr. Rebecca Lancefield and subsequently prepared in our laboratory. The Group A antisera used showed maximal reactivity with a solution of formamide-extracted A carbohydrate (0.1 mg/ml), giving a maximal (++++) precipitation reaction by the qualitative capillary precipitin technique (9) and minimal reactivity (±) with formamide-extracted V carbohydrate (0.1 mg/ml). This latter reaction was not immediate as with the A antigen, but occurred slowly and increased in intensity while standing at room temperature over 15–20 min. The V antiserum utilized reacted maximally (++++) with the variant antigen and showed minimal (±) reactivity with Group A carbohydrate. In certain experiments the sera were adsorbed with the heterologous cross-reactive A or V cells before use.

Qualitative capillary precipitin techniques were performed according to the method of Swift, Wilson, and Lancefield (9). Testing with formamide-extracted carbohydrates was carried out at concentrations of 0.1 mg/ml in phosphate-buffered saline (PBS). Quantitative immune precipitin studies were performed as described by McCarty and Lancefield (3). Immunodiffusion studies were carried out in 1% agarose (10) and the plates incubated at room temperature (22°C). In these studies the antigen solution consisted of formamide-extracted carbohydrate at 0.1 mg per ml of PBS. Radioimmune precipitin technique studies were performed as previously reported (8).

Analytical Methods.—Rhamnose was determined by the method of Dische and Shettles (11). Quantitative glucosamine was determined by a modification of the Elson and Morgan procedure (12).

EXPERIMENTAL

Variation of Serological Reactivity with Culture Incubation Temperature.—Our initial observation on the alteration of the serological reactivity with culture

[1] *Abbreviations used in this paper:* I, intermediate; PBS, phosphate-buffered saline; THB, Todd-Hewitt broth; V, variant.

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incubation temperature was made when a THB culture of a "V" organism, the A-486-Var strain, was allowed to grow at room temperature (22°C) for 65 h. Formamide-extracted carbohydrate from cell walls of this culture showed no appreciable reactivity with V antiserum but reacted strongly with Group A antiserum. When the procedure was repeated with the modification of incubating the final THB culture at 37°C, the carbohydrate reacted with V antiserum and showed minimal reactivity with Group A antiserum. Repeat of the experiment using the same subculture with parallel incubation of the cultures at 22°C for 18 and 72 h and at 37°C for 18 h, yielded the results shown in Table I. Incubation at 22°C for 72 h enhanced the reactivity with Group A antiserum without altering the V reactivity.

Further evidence for the temperature-related effect of culture incubation on the serological reactivity of the A-486-Var carbohydrate was obtained when the cultures were incubated at 22°, 30°, and 37°C. Subcultures grown at these temperatures were used to inoculate 500 ml of THB, which were further incubated for 24 h at the same temperatures. The carbohydrate extracted from the cells by the hot formamide technique was reacted with Group A antiserum in the quantitative immunoprecipitin technique. As shown in Fig. 1, the degree of reactivity of the A-486-Var carbohydrate increased with decreasing culture incubation temperatures. Chemical analyses of the carbohydrates (Table II) revealed a direct correlation between the glucosamine content and the amount of antibody precipitated by the carbohydrate.

TABLE I

Variation of the Group Serological Reactivity of the Streptococcal A-486-Var Strains with Temperature of Culture Incubation

Strain	Incubation		Serological reactivity Qualitative capillary precipitin	
	Temperature	Time	A antiserum	V antiserum
		h		
A-486-Var	37°C	18	–	++++
	22°C	18	++	+
	22°C	72	+++	+

Effect of Repeated Subculture and Storage at Various Temperatures on Serological Reactivity.—Because the above "V" strain had been passed through several subcultures and stored for varying periods at 4°C, the following studies were performed with the same strain, A-486-Var, newly obtained in the lyophilized form.

(*a*) The effect of repeated subculture was tested by inoculating the lyophilized preparation into two blood broth tubes, one of which was incubated at 22°C and the other at 37°C. Subcultures were then made from each tube on blood agar plates that were incubated at 22° or 37°C. Thereafter, 12 colony picks were

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made from each plate and inoculated into 12 blood broth tubes, respectively. Six tubes from each were then incubated at either 22° or 37°C and subsequently used to inoculate corresponding THB cultures that were incubated at the same temperatures. The cells were harvested and extracted in hot acid. The results of

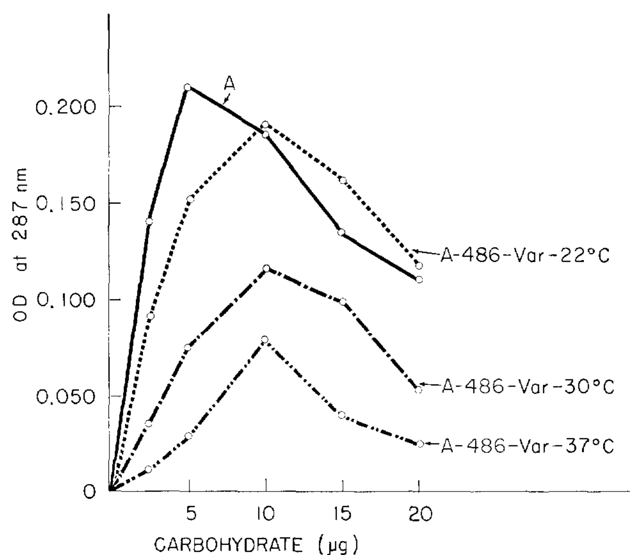

FIG. 1. Quantitative immune precipitin studies of Group A antiserum with Group A carbohydrate and carbohydrate synthesized by the A-486-Var organism at various temperatures. The chemical composition of these carbohydrates is presented in Table IV.

TABLE II

Composition of Formamide-Extracted Carbohydrates Synthesized by Group A and A-486-Var at Various Temperatures

Strain	Culture temperature	Rhamnose	Glucosamine	Molar ratio (rhamnose:glucosamine)
		%	%	
C-203-S (Group A)	37°C	54	27	2 4
A-486-Var	37°C	82	8	11.6
	30°C	71	12	6.5
	22°C	55	21	2.9

the serological reactivity of these extracts are shown in Table III. Regardless of the primary subculture incubation temperature, the majority of the organisms grown at 22°C reacted with Group A as well as with V antisera, while none of the organisms grown at 37°C showed significant reactivity with the Group A antiserum.

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(*b*) To test the effect of storage of the subculture at 4°C, an aliquot from the lyophilized preparation was incubated in blood broth and grown at 37°C overnight. A loopful was then streaked on a blood agar plate, which was incubated overnight at 37°C. After overnight growth, 20 colony picks were transferred to 20 blood broth tubes, 10 of which were incubated at 22°C while the other 10 tubes were incubated at 37°C. After 24 h incubation, an inoculum was transferred to THB tubes, which were incubated at the same temperatures for 48 h. Acid extracts of the harvested cells showed predominant reactivity (+++/++++) with V antiserum for all 20 cultures; five of the extracts from cultures grown at 22°C also showed minimal reaction (+/++) with the Group A antiserum.

In the interim, the 10 blood broth tubes that had been incubated at 22°C were

TABLE III

*Effect of Culture Incubation Temperature on the Serological Reactivity of Acid Extracts from Multiple Colony Picks of A-486-Var Strain**

First subculture		Second subculture		Serological reactivity			
No. of strains	Incubation temperature	No. of strains	Incubation temperature	No. of strains	A antiserum	No. of strains	V antiserum
12	37°C ↗	6	37°C	6 :	neg	1 : 5 :	± +++/++++
	37°C ↘	6	22°C	1 : 5 :	++ ++++	6 :	+++/++++
12	22°C ↗	6	37°C	6 :	neg	6 :	+++
	22°C ↘	6	22°C	1 : 5 :	+ +++	6 :	+++/++++

* Twelve colonies from blood agar plates were subcultured at 22° or 37°C (first subculture), then six of each grown at either 22° or 37°C (second subculture).

allowed to stand at 4°C for 5 days; aliquots were then transferred from these tubes to THB cultures that were grown at 22°C for 48 h. Acid extracts of the cells from these cultures now showed almost equal reactivity (+++) with the Group A antiserum as well as reactivity (++++) with the V antiserum.

Effect of Incubation Temperature on Serological Reactivity of Group A, Variant, and Intermediate Streptococci.—Although the dual reactivity with both A and V antisera was less marked in our initial experiments, the high degree of dual reactivity obtained in the above experiments suggested that the temperature-dependent variation in immunological reactivity was due to an enhanced formation of a polysaccharide with intermediate immunochemical composition at lower temperatures. To confirm this observation, similar studies were performed with other V strains: one of the strains was the C 121/46/4, which had been labeled

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as a "V" organism, and two V strains, T/27A-32-18 and B/403-1022. The C-203-S Group A streptococcus was also included for comparison. Table IV summarizes the range of serological reactivities of the extracts obtained in several comparable experiments with the various strains. The Group A organism maintained its serological specificity in cultures at both temperatures. However, the A-486-Var and C 121/46/4 strains behaved in similar fashion, both showed enhancement of reactivity with Group A antiserum when cultured at 22°C. In contrast, the V strains, T/27A-32-18 and B/403-1022, continued to maintain their antigenic identity with no demonstrable enhancement

TABLE IV

Serological Reactivity of Group A and Variant Streptococcal Strains after Culture at 22° and 37°C

Strain	Original group designation	Culture temperature	Growth	Serological reactivity*	
				A antiserum	V antiserum
			OD 650 nm		
C-203-S	A	37°C	—	++++	—
		22°C	—	+++	—
A-486-Var	"V"	37°C	0.300	−/+	++++
		22°C	0.220	++/+++	+++/++++
C 121/46/4	"V"	37°C	—	−/+	++++
		22°C	—	++	+
T/27A-32-18	V	37°C	0.270	—	++++
		22°C	0.330	—	+++/++++
B/403-1022	V	37°C	0.275	—	++++
		22°C	0.220	—	++++

* Range of reactivity obtained in four experiments.

of reactivity with Group A antiserum when the cultures were incubated at 22°C. Various attempts at inducing increased Group A serological reactivity, either by varying the subculture and culture temperatures or storing subcultures at 4°C and randomly picking several colonies from blood agar plate subcultures, failed to enhance the serological reactivity of the cell wall carbohydrate of the T/27A-32-18 and B/403-1022 strains with Group A antiserum.

Specificity of the Serological Reactivity of the Polysaccharides.—To characterize further the nature of the alteration of antigenic structure that occurs in the A-486-Var strain, immunodiffusion studies were carried out with formamide-extracted carbohydrates of this strain as well as Group A, V, and I organisms. As shown in Fig. 2 *a*, a single precipitin line occurs between Group A antiserum and carbohydrates of the Group A, I (grown at 22° or 37°C), and the A-486-Var

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grown at 22°C (A-486-Var-22°C) but not with extracts of the A-486-Var-37°C or the V organisms. Reaction with V antiserum (Fig. 2 *b*) occurred with all carbohydrates tested except for the Group A polysaccharide. These findings proved that the reactivity of the various polysaccharides with the antisera was specific and not due to cross-reactivity between the antisera used and the Group A or V antigens. As seen in these illustrations, the reactions of the various antigens with either the Group A or V antisera show a reaction of identity for all antigens reacting with the same antiserum. The specificity and relatedness of these reactions is further demonstrated in Fig. 2 *c*. Double lines of precipitation with reactions of partial identity were obtained in the reaction of the I and the A-486-Var-22°C antigens with the "AV" antiserum, while a single line of precipitation with a reaction of identity was obtained in the reaction of the A-486-Var-37°C and the V antigens with the AV antiserum.

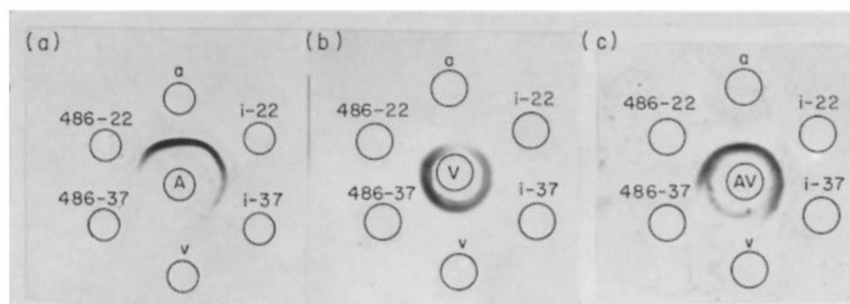

FIG. 2. Immunodiffusion precipitin reactions of carbohydrates from Group A (*a*), variant (*v*), intermediate (*i*), and A-486-Var (*486*) streptococci with Group A and V antisera as well as a combination of both antisera (*A V*). Carbohydrates A and V were obtained from organisms cultured at 37°C. Similar reactions were obtained with A and V carbohydrates from organisms cultured at 22°C.

Correlation of the Serological Reactivity with Chemical Composition.—Solutions of the formamide-extracted carbohydrates used in the above study were analyzed for their rhamnose and glucosamine content. The results of these analyses, shown in Table V, reveal that the compositions of the carbohydrates correlate with the anticipated serological reactivity as previously described (3). The molar ratios of the rhamnose to glucosamine are lowest for the Group A organism and highest for the V strain. As expected, the I strain shows a rhamnose to glucosamine ratio intermediate in value between the above. These ratios are similar for carbohydrates from A, V, and I organisms grown at 22° or 37°C, with a somewhat lower ratio for the I organism grown at 22°C than that obtained from the organism grown at 37°C. As can be seen, the rhamnose to glucosamine ratio obtained from the A-486-Var-22°C is similar to that of the I carbohydrate,

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while the ratio obtained for the A-486-Var-37°C approximates that of the V carbohydrate.

Comparison of Structures of the A-486-Var and Intermediate Carbohydrates.—Prior studies by McCarty and Lancefield showed that the dual reactivity of the I polysaccharide was due to the presence of a major component capable of reacting with both A and V antisera (3). To determine the basis for the dual reactivity of the A-486-Var-22°C carbohydrate, the following studies were carried out:

Immunodiffusion studies: Various carbohydrate antigens were reacted in agar gel diffusion with a preparation containing equal amounts of the A and V antisera (Fig. 3). Single lines of precipitation showing reactions of identity were obtained when either the A or the V antigens were reacted separately with the antisera (Fig. 3 *a* and *b*). An examination of the precipitation patterns obtained with the A-486-Var and I carbohydrates revealed certain differences: while the reaction of the A-486-Var-37°C carbohydrate (Fig. 3 *c*) shows a single line of precipitation similar to that obtained with the V carbohydrate (Fig. 3 *b*), the pattern obtained with the A-486-Var-22°C (Fig. 3 *d*) shows two lines of precipitation. No double lines of precipitation are evident in the reaction of the I carbohydrates with the antisera (Fig. 3 *e* and *f*), although a diffuse reaction may be observed in the reaction of the I-37°C with the antisera (Fig. 3 *e*).

TABLE V

Analysis of Rhamnose and Glucosamine Contents of Various Solutions of Formamide-Extracted Carbohydrates Used in Immunodiffusion Studies

Strain	Group	Culture temperature	Rhamnose	Glucosamine	Molar ratio
			μg	μg	
F-60	A	37°C	151	73	2.4
		22°C	195	99	2.3
T/27A-32-18	V	37°C	201	14	17.0
		22°C	296	19	18.0
C 121/39/8	I	37°C	278	58	5.5
		22°C	167	44	4.4
A-486-Var	"V"	37°C	226	21	12.0
		22°C	210	42	5.8

Radioimmune precipitin reactions: The precipitin patterns obtained above suggested certain differences in the nature of the antigenic composition of the A-486-Var-22°C and I polysaccharides. While the presence of single lines of precipitation for the I carbohydrate reactions was in line with the evidence presented by McCarty and Lancefield (3) regarding the presence of a major com-

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ponent capable of dual reactivity, the presence of double lines of precipitation in the reaction of the A-486-Var-22°C pointed to the presence of two carbohydrates with separate A and V reactivity. To confirm this possibility, the following studies utilizing the radioimmune precipitin technique were performed:

Samples containing approximately 1 μg of the various ^{14}C-labeled carbohydrate antigens were incubated with adsorbed A and V antisera or a combination of both antisera (AV antiserum). The amount of labeled antigen precipitated by each antiserum was assessed using the radioimmune precipitin technique described previously (8). As shown in Table VI, 97–100% of the A or V antigens were precipitated by their homologous antisera or by the AV antiserum. When a solution containing 0.5 μg of each antigen (A + V) was incubated with the same antisera, about half of the total carbohydrate was precipitated with either the A or V antisera, while all of the carbohydrate was precipitated by the AV antiserum.

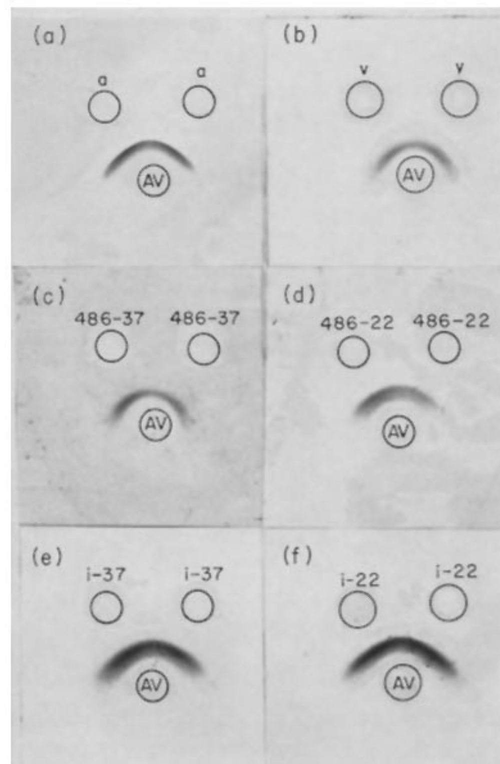

FIG. 3. Immunodiffusion precipitin reactions of various streptococcal Group A (*a*), variant (*v*), intermediate (*i*), and A-486-Var (*486*) carbohydrates with a combination of Group A and V antisera (*AV*). Aliquots of the same solution of the antigen were placed in the top two wells.

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The results obtained for the A-486-Var-22°C carbohydrate were similar to those obtained with the solution containing equal amounts of the A and V carbohydrates (A + V), indicating that the A-486-Var-22°C polysaccharide consists predominantly of almost equal proportions of separate molecules of A and V antigens. This finding contrasted with results obtained for the I carbohydrates. The fact that more than 90% of the I polysaccharide was precipitated by either the A or V antisera supported the conclusion that this carbohydrate is composed primarily of one molecule possessing the capacity to react with both A and V antisera.

Enhanced Production by I Organism of a Polysaccharide with Group A Serological Reactivity at Lower Incubation Temperatures.—Evidence suggesting an

TABLE VI

Precipitation of ^{14}C-Labeled A, V, and A-486-Var-22°C and I Carbohydrates by A and V Antisera

Carbohydrate antigen	Rhamnose content	Total radioactivity	Fraction of total carbohydrate precipitated*					
			A antiserum		V antiserum		AV antiserum‡	
	%	*cpm*	*cpm*	*%*	*cpm*	*%*	*cpm*	*%*
A	46	582	578	99	132	23	597	100
V	85	368	20	5	357	97	365	99
A + V§	—	465	298	64	250	54	463	100
A-486-Var-22°C	53	494	338	68	365	74	498	100
I-22°C	50	245	231	94	222	91	230	94
I-37°C	50	203	181	89	195	96	184	91

* Approximately 1 μg of carbohydrate was used in each test. Normal rabbit serum precipitated 5% of the A, 6% of the V, and 8% of the I antigens.

‡ Equal amounts of a twofold concentration of the A and V antisera.

§ Solution of A and V carbohydrates for a final concentration in the test of 0.5 μg of each antigen.

enhanced production of a carbohydrate with Group A serological reactivity was obtained in both the immunodiffusion and radioimmune precipitation studies performed above. A stronger and less diffuse precipitin reaction was obtained on immunodiffusion with the I-22°C than with the I-37°C carbohydrate (Fig. 3 *e* and *f*). In addition, an examination of the proportions of the carbohydrates precipitated by the antisera in the radioimmune precipitin studies showed that more of the I-22°C carbohydrate precipitated with the A antiserum, while more of the I-37°C carbohydrate precipitated with the V antiserum (Table VI). Further evidence supporting this observation was obtained by doubling the concentration of the ^{14}C-labeled carbohydrates used in the radioimmune precipitin technique. While no significant alteration in the proportions of (A + V) or A-486-Var-22°C carbohydrates precipitated by the various antisera was obtained, an exaggeration of the differences noted for the I-22°C and I-37°C occurred. Thus, when 2 μg of the carbohydrates were incubated with the same

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amount of antisera, 93% of the I-22°C carbohydrate was precipitated with A antiserum, while 86% precipitated with the V antiserum. Opposite results were obtained when the I-37°C carbohydrate was used: 77% of the carbohydrate precipitated with the A antiserum, while 93% precipitated with the V antiserum.

Chemical analysis of the ^{14}C-labeled I carbohydrate yielded a rhamnose to glucosamine ratio of 4.3 for the I-22°C carbohydrate and a ratio of 8.3 for the I-37°C carbohydrate. These results, together with the results obtained above, provide evidence for an enhanced production of carbohydrate with Group A composition and reactivity by the I organism cultured at 22°C.

DISCUSSION

The temperature-dependent variation in the synthesis of the cell wall carbohydrate appears to be a property of certain streptococcal strains. While the associated alteration of serological reactivity was very striking during the earlier stages of our studies, subsequent studies after repeated subculture of the same strains resulted in a partial waning of this phenomenon. Initially, a preponderant Group A reactivity was found when the A-486-Var strain was cultured at 22°C. This changed with further studies so that the reactivity with Group A antiserum became less intense and strong intermediate reactivity emerged.

An attempt to provide an explanation for the "sudden" appearance of this phenomenon would be highly speculative. The supposition that this phenomenon might be ascribed to a contamination of the variant culture by Group A organisms was not supported by the study on multiple colony picks. None of the 12 isolates cultured at 37°C (Table III) showed reactivity with the Group A antiserum. The finding of equal reactions with both Group A and V antisera by 11 of the 12 colony isolates grown at 22°C would suggest an alteration in the individual colony-forming organism related to incubation temperature.

The findings on the dual reactivity of the A-486-Var polysaccharide produced at 22°C parallel those reported by McCarty and Lancefield (3) on the I polysaccharide. However, our studies indicated the presence of a quantitative difference in the proportion and nature of the polysaccharide produced by these strains. In their studies on the intermediate carbohydrate, McCarty and Lancefield (3) concluded that the I polysaccharide is composed of a major component with dual reactivity with Group A and V antisera. This conclusion is strongly supported by our studies using the radioimmune precipitin technique, which allows direct quantitation of the amounts of antigen precipitated by the specific antiserum. The difference between the carbohydrate produced by the A-486-Var at 22°C and the I organism is elucidated by both the immunodiffusion and the radioimmune precipitin studies. Despite the persistent cross-reactivity of the adsorbed V antiserum with Group A carbohydrate, valid conclusions can be made from the results obtained with the A antiserum and the combination of the A and V antiserum (Table VI). The A antiserum showed no appreciable cross-reactivity with the V carbohydrate, inasmuch as the radioactivity precipitated

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with this antigen did not exceed that obtained with normal rabbit serum. Utilizing these data alone, one can conclude that about 50% of the A-486-Var-22°C polysaccharide possesses only Group A specific reactivity, with approximately 5–10% bearing dual antigenic reactivity, while about 90% of the I carbohydrate possesses dual reactivity with 5–10% having separate A or V antigenic specificity.

It is of importance to point out that this temperature-related variation occurred in the A-486 Var strain as well as in a strain that originally had a propensity for producing an intermediate carbohydrate, the C 121/46/4. Other strains that were tested showed only V reactivity when grown at 22° or 37°C. While the A-486-Var had been designated as a V strain, apparently it had been noted to give mixed reactions on occasion (R. C. Lancefield, personal communication).

One of the possibilities raised by these findings is that the group-specific carbohydrate synthesized by the Group A streptococcus and its variant strains is usually heterogeneous. While Group A or variant carbohydrates are predominantly synthesized under certain conditions, the synthesis of small proportions of the heterologous or intermediate carbohydrates may occur at all times. The present finding of an enhanced production of the A carbohydrate at lower temperatures by some variant and intermediate strains probably represents one condition that brings about a shift in the proportions of the carbohydrates synthesized for the particular strains investigated. Although similar changes could not be brought about by varying the culture incubation temperature of other variant strains, the possibility that other changes in the cultural conditions could also affect the proportions of carbohydrates synthesized by other A or variant strains remains to be explored.

SUMMARY

A temperature-dependent alteration in the synthesis of the group-specific polysaccharide was found to occur in two "variant" streptococcal strains, A-486-Var and C 121/46/4. These strains synthesize a polysaccharide with variant immunochemical characteristics when grown at 37°C. However, when these organisms are grown at lower temperatures, 22°C, an enhanced synthesis of Group A carbohydrate occurs. Other variant strains show no appreciable alteration of the cell wall carbohydrate composition when grown at lower temperatures. Studies on an intermediate strain show that this organism has a propensity for the synthesis of a polysaccharide with higher glucosamine content and enhanced Group A serological reactivity when grown at 22°C.

Immunochemical studies performed on the carbohydrates produced by the A-486-Var at various temperatures revealed that the appearance of Group A serological reactivity at lower temperatures is due to the additional synthesis of a polysaccharide with Group A specificity along with the continued synthesis of a variant carbohydrate. This finding contrasts with data obtained on the car-

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bohydrate produced by the intermediate organisms that appears to consist predominently of one molecule bearing dual A and variant antigenic determinants.

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