IMMUNOCHEMICAL STUDIES ON THE GROUP AND TYPE ANTIGENS OF GROUP F STREPTOCOCCI AND THE IDENTIFICATION OF A GROUPLIKE CARBOHYDRATE IN A TYPE II STRAIN WITH AN UNDESIGNATED GROUP ANTIGEN*

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It has been shown previously that many strains of Group F streptococci carry one out of five type antigens (1, 2). This classification of group and type antigens was primarily based on serologic analysis. Similar substances have been identified in a few streptococcal strains belonging to Groups A, C, G, and L (1, 2, 3, 4). The type antigens which have been examined here are of a carbohydrate nature and can be extracted from purified cell walls. They are probably on the surface of the cells and more superficial than the group antigen. It is consistent with this assumption that the type antigens, in contradistinction to the group antigens, are excreted in a soluble form into the medium (5).

Group F streptococci which contain a type antigen readily induce a type-specific immune response, but a group-specific response is much more difficult to achieve. The availability of antisera with only type antibodies and devoid of group antibodies led to the discovery of streptococcal strains which carry one of the type antigens, but apparently lack a serologically detectable group antigen (2). The question remained, however, whether such strains possessed the chemical equivalent of a group antigen.

In the present report the group and type antigens from an antigenic mixture derived from a Group F Type II streptococcus were separated and chemically purified. When the same procedure was applied to a strain carrying solely a

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Type II antigen, but no serologically detectable group antigen, two distinct carbohydrate polymers were again isolated. One was identified as pure Type II antigen. The other carbohydrate is analogous to the group antigens of hemolytic streptococci. These chemical studies confirm the serological classification described previously (1, 2).

Methods

Streptococcal Strains.—Antigens were prepared from: strain FII (La 60R) which was obtained from Dr. Rebecca C. Lancefield, The Rockefeller University, as strain H60R; and strains FO (I 124), OI (Is 8), and OII (H 189) described earlier (2). Antisera against these strains were prepared in rabbits by injection of heat-killed vaccines.

Methods for Extraction of Group- and Type-Specific Carbohydrate.—Group and type carbohydrates were extracted from whole streptococci with hot HCl at pH 2 (6), or with hot formamide (7). The initial step in the isolation of group and type antigens from these extracts was achieved by successive precipitations with alcohol and acetone.

For formamide extraction, lyophilized streptococci were suspended in formamide and heated at 160°C for 20 min. After cooling, 4 volumes of alcohol were added to the extract, and after 12 hr in the cold, the precipitate was collected by centrifugation. 5 volumes of acetone were added to the alcohol supernatant and the precipitate which was formed during 18 hr in the cold was collected by centrifugation. The bulk of the extracted type antigen, along with a portion of the group antigen, was precipitated by the addition of 4 volumes of alcohol. The precipitate obtained by the subsequent addition of 5 volumes of acetone contained the bulk of the group antigen and the remainder of the type antigen. The alcohol and acetone precipitates were treated with DNAse, RNAse, pepsin, and trypsin. After dialysis the enzymes were removed from the antigenic material by chromatography on DEAE-cellulose (5). In a typical formamide extraction of Group F Type II streptococcus strain FII, the final yield from 9.7 g of lyophilized streptococci was 650 mg of alcohol precipitate and 120 mg of acetone precipitate.

For acid extraction the streptococci were heated at 100°C for 10 min and reextracted twice. The extracts were combined, neutralized, and the volume brought down with a flash evaporator. The extracted material was treated with enzymes as described in the paragraph above.

Final separation and purification of the group and type antigens from the alcohol and acetone precipitates was achieved by the chromatographic procedures described below.

Chromatography on Cellulose.—After gross separation of the antigens from the extracts by alcohol and acetone precipitations, the materials were further purified on columns using standard grade Whatman cellulose powder (8), equilibrated with 90% alcohol containing 0.3% sodium acetate. Before use, a concentrated solution of sodium acetate was neutralized with dilute acetic acid. The column was eluted stepwise with decreasing concentrations of alcohol containing 0.3% sodium acetate. The carbohydrate content of the eluates was determined by the phenol sulfuric acid test (9).

Group and type antigens in the eluates were detected with specific antisera by the capillary precipitin test.

Purification of the Soluble Antigen from the Medium.—To isolate type-specific material from Todd-Hewitt medium, a 20 liter culture of the strain OII was freed of bacteria by centrifugation, and concentrated in dialysis tubing to one liter (20 times) against Carbowax 20,000. 80-ml portions of the dark syrup were loaded onto 90 × 5 cm column containing a P10 polyacrylamide gel (Bio-Gel P10, 50-150 mesh, Bio-Rad Laboratories, Richmond, Calif.). On elution with water, carbohydrate and large molecular impurities fell through but pigmented ma-

terial was completely retarded. The volume of the combined effluents was then reduced to a final volume of 100 ml by flash evaporation. The active material was completely precipitated by the addition of 2 volumes of alcohol to the concentrate and further treated with enzymes and DEAE-cellulose as described above for the antigen recovered by the formamide extraction procedure.

Chemical Analysis.—Glucosamine and galactosamine were estimated as described previously (10).

Analysis of the carbohydrate for rhamnose, mannose, glucose, and galactose was done by the chromatographic method of Walborg et al. (11). The carbohydrate was hydrolyzed for 3 hr in 3 N HCl at 100°C and neutralized with Dowex 2-X8 in the carbonate form. 1 mg of material was loaded onto a column prepared with Dowex 2-X8 200–400 mesh, which had been specially washed to remove coarse and fine particles (11). By using a resin with a total exchange capacity (dry weight) of 3.8 meq per g, a column size of 150 × 0.5 cm, and a jacket temperature of 56°C, complete separation of rhamnose, mannose, galactose, and glucose was obtained in 21 hr at a flow rate of 3.18 ml per hr. In subsequent studies resolution was maintained even though the flow rate was increased to 8 ml/hr. In this case an analysis was completed in 8.5 hr. Fractions were collected each 20 min. Using an automatic dilutor, the sugars were estimated in the column effluent fractions with the aniline orthophosphoric acid reagent (12). Rhamnose, glucose, and galactose were also determined by methods previously employed (5).

Precipitin Analysis.—Quantitative precipitin analyses and inhibition studies were performed according to the method of McCarty and Lancefield (13).

EXPERIMENTAL

Isolation of Group F and Type II Antigens.—Although both the group and type antigens of Group F streptococci are constituents of the cell wall, it is feasible to purify these antigens from hot formamide extracts of the whole organism. In the case of the streptococcal strain FII, the bulk of the type antigen and a portion of the group antigen was precipitated by the addition of 4 volumes of alcohol. This material is referred to as alcohol precipitate. Subsequent addition of 5 volumes of acetone precipitated the remainder of the group antigen and the residual type antigen. This material is referred to as acetone precipitate. The chromatographic method employed for the isolation of group and type carbohydrates from these antigen mixtures makes use of the fact that these substances exhibit different solubilities in alcohol.

9 mg of alcohol precipitate from strain FII was loaded onto a cellulose column (25 × 1.5 cm) and eluted with stepwise decreases in alcohol concentration. The acetone precipitate was handled in a similar fashion. The pattern of eluted carbohydrate material detected by the phenol–sulfuric acid reagent is depicted in Fig. 1. Both alcohol and acetone precipitates of a formamide extract gave a large peak on elution with 60% alcohol. The alcohol precipitate in addition yielded other substantial peaks at 40% and 30% alcohol. Preliminary serologic testing of the effluents indicated that only Group F antigen was found in carbohydrate material eluted with 60% alcohol and in some instances in the lesser portion eluted with 50% alcohol. The bulk of the Type II antigen was eluted with 40% alcohol, and the remainder with 30% alcohol. This pilot run

proved the feasibility of this method for the isolation of group and type antigens from a mixture.

Accordingly, 400 mg of the alcohol precipitate of a formamide extract of strain FII was dissolved in 8 ml of 0.3% sodium acetate and put on a cellulose column (45 × 5 cm), reequilibrated with 90% alcohol (1 liter) and eluted successively with 50% alcohol (2 liters) and 20% alcohol (2 liters). Elution with these concentrations of alcohol was selected for this batch procedure because the

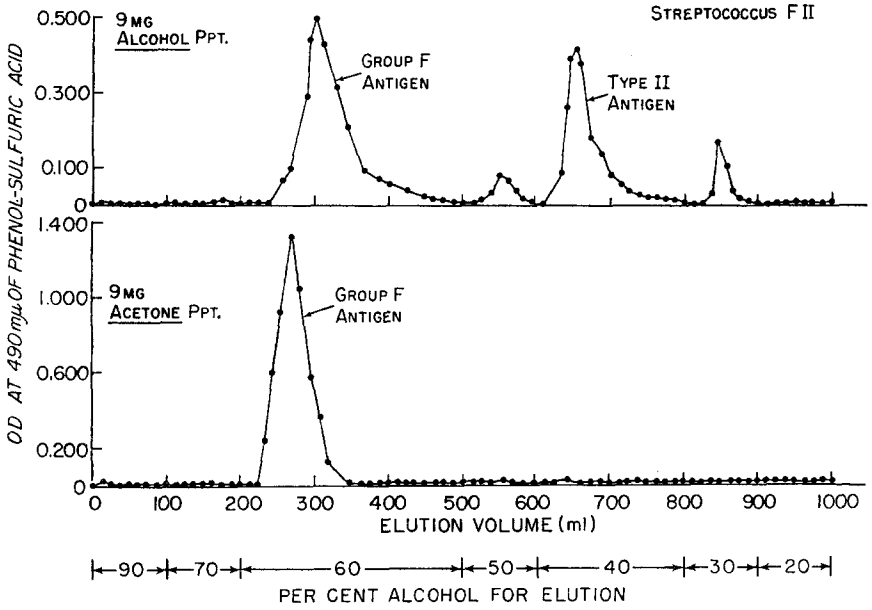


FIG. 1. Cellulose column chromatography of alcohol and acetone precipitates from a formamide extract of strain FII. 9-mg samples were employed. Column dimensions: 40 × 1 cm. The column was equilibrated with 90% alcohol, and was eluted with stepwise decreases of the alcohol concentration. The carbohydrate eluted at 60% alcohol reacted with Group F serum. The carbohydrate eluted with 40% alcohol or less reacted with Type II serum.

pilot run had indicated that all the material recovered from the column by elution between 90% and 50% alcohol was Group F carbohydrate and all the material recovered by elution between 40% and 20% alcohol was Type II antigen. The effluents were evaporated in vacuo, dialyzed, and lyophilized. The yield of group antigen collected between 90% and 50% alcohol was 92 mg and the yield of type antigen collected between 50% and 20% alcohol was 120 mg.

The results of quantitative precipitin tests which employed the Group F and Type II antigens isolated by the chromatographic procedure are shown in Fig. 2. Evidence that this method has resulted in an almost complete resolution of

the original antigen mixture into group and type components is provided by the fact that only minimal precipitin reactions were observed between Group F antigen and Type II serum and between Type II antigen and Group F serum. These minor reactions occurred only when high concentrations of antigens were employed. These findings indicate that a high degree of purification of the two antigens had been achieved.

70 mg of the acetone precipitate of a formamide extract of strain FII was chromatographed over the same column. In this case the yield of Group F antigen collected between 90% and 50% alcohol was 35 mg and the yield of Type II antigen collected between 50% and 20% alcohol was only 5 mg.

The chemical compositions of alcohol and acetone precipitates obtained from

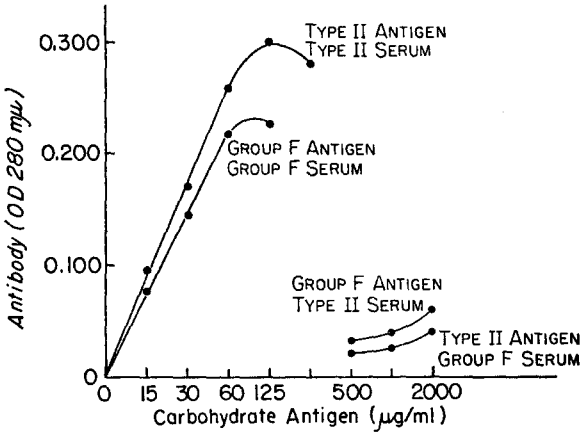


FIG. 2. Quantitative precipitin analysis of Group F and Type II antigens purified from the alcohol precipitate of a formamide extract.

a formamide extract of strain FII and the group and type antigens isolated by chromatography are given in Table I. As galactose is the only sugar which is present in the type antigen but not in the Group F carbohydrate (5, 14), this sugar is a good indicator for following the efficacy of the separation procedure. Although galactose was present in the initial alcohol precipitate, the Group F antigen recovered from chromatography was devoid of this sugar. The chromatographic patterns of the hydrolysates of the Group F and Type II antigens and of a standard sugar mixture are shown in Fig. 3.

Approximately the same concentration of galactosamine was present in both the Group F and Type II antigens. A small percentage of glucosamine was identified in the Group F carbohydrate but not in the Type II antigen.

Isolation of Antigens from Streptococcus Strain OII.—Previous reports described a streptococcal strain which possessed the Type II antigen but lacked any one of the known streptococcal group antigens (2). This strain was given the

designation OII. It was assumed that strain OII possessed a grouplike substance as a cell wall constituent, analogous to the Group F antigen, even through it had not been identified serologically. The purification of Group F and Type II antigens by cellulose chromatography from a formamide extract of strain FII led to the application of similar methods for the isolation of a grouplike carbohydrate and the Type II antigen from a formamide extract of strain OII.

Formamide extraction of 5.8 g of lyophilized streptococci, strain OII, gave a yield of 712 mg of alcohol precipitate and 213 mg of acetone precipitate. In an initial pilot study, 12 mg of each preparation was further resolved with cellulose column chromatography. As is shown in Fig. 4, the bulk of the carbohydrate

TABLE I

Chemical Compositions of Group F and Type II Antigens Isolated by Cellulose Chromatography

Constituent	Formamide extract of Group F streptococcus strain FII				
	Alcohol precipitate			Acetone precipitate	
	Before chromatography	After chromatography		Before chromatography	After chromatography
		Group F antigen	Type II antigen		Group F antigen
	%	%	%	%	%
Rhamnose	28.4	40.5	29.6	34.8	37.2
Glucose	17.7	12.4	30.0	14.5	15.2
Galactose	4.1	0.0	14.9	0.0	0.0
Mannose	0.0	0.0	0.0	0.8	0.5
Glucosamine	0.8	1.6	0.0	1.3	1.9
Galactosamine	18.2	22.3	17.2	14.6	19.2

contained in the acetone and alcohol precipitates is soluble in 60% and 40% alcohol and is eluted from the column. Presumably the carbohydrate eluted with 60% alcohol is the substance in the cell wall which is analogous to other group antigens such as Groups A, B, C, G, and F. The bulk of this material was eluted with 60% alcohol and the remainder with 50% alcohol. Type II antigen was identified serologically only in the fraction eluted with 40% alcohol and in some instances in a lesser portion eluted with 30% alcohol.

Both the alcohol and subsequent acetone precipitates of the formamide extract were then processed on a large cellulose column as described in the previous section. In the batch procedure the grouplike carbohydrate was eluted with 50% alcohol and the Type II antigen with 20% alcohol. Elution was limited to these two alcohol concentrations, because results of the pilot run indicated that only grouplike carbohydrate was eluted between 90% and 50% alcohol and Type II antigens between 40% and 20% alcohol. From 400 mg of

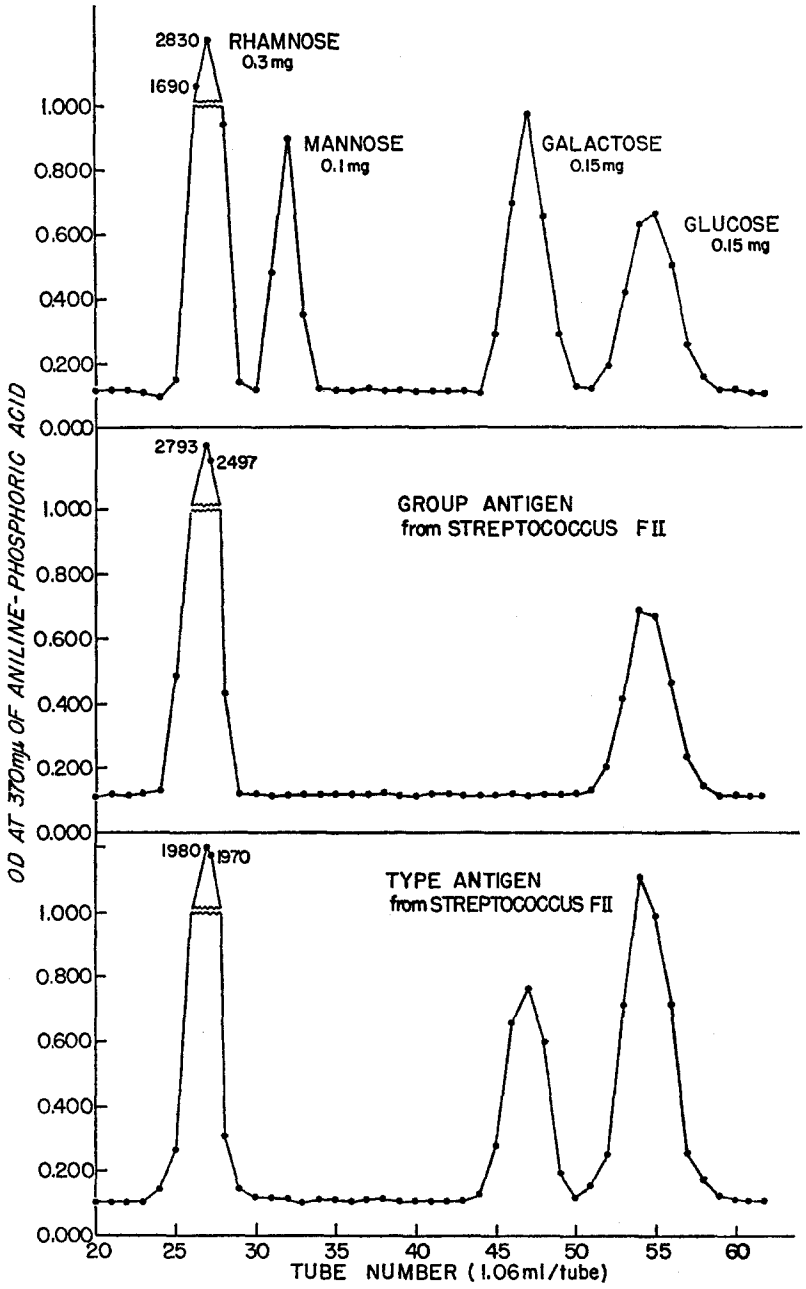


FIG. 3. Chromatographic procedure for the quantitative determination of rhamnose, mannose, galactose, and glucose in a mixture. Column dimensions with Dowex 2-X8 resin 150 × 0.5 cm. Depicted in the upper frame is the chromatographic pattern of a standard mixture of four sugars. Middle frame, sugars recovered from a hydrolysate of Group F antigen isolated from strain FII by cellulose chromatography. Lower frame, sugars recovered from a hydrolysate of Type II antigen also isolated from strain FII by cellulose chromatography. 1-mg samples were loaded on to the column. Standardization of the column with known sugars afforded calculation of the sugar content in the samples.

alcohol precipitate, 75 mg grouplike carbohydrate and 234 mg Type II antigen were obtained. Similarly, from 125 mg of acetone precipitate, 68 mg grouplike carbohydrate and 10 mg Type II antigen were recovered.

Table II presents the chemical compositions of the Type II antigen and the grouplike carbohydrate isolated by cellulose chromatography from alcohol and acetone precipitates of a formamide extract of strain OII. The data indicate

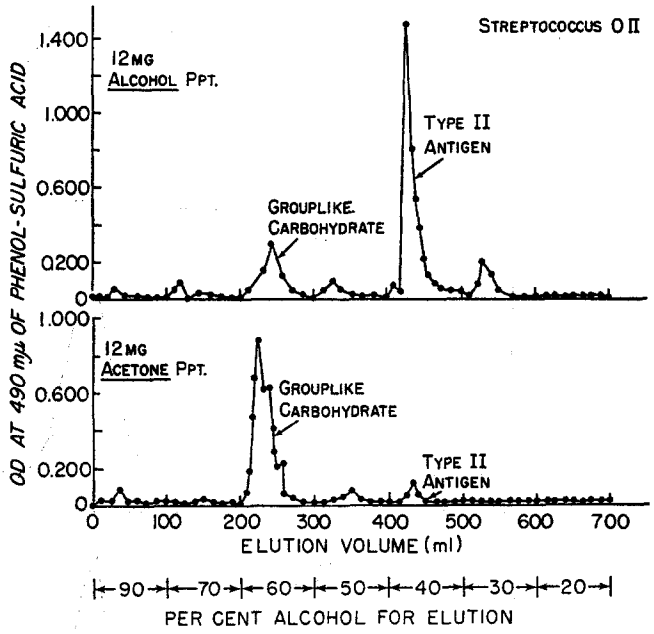


FIG. 4. Cellulose column chromatography of alcohol and acetone precipitates from a formamide extract of strain OII. 12-mg samples were employed. Column dimensions, 40 × 1 cm. The column, equilibrated with 90% alcohol, was eluted with stepwise decreases of the alcohol concentration. The carbohydrate eluted at 60% alcohol did not react with any of the streptococcal grouping sera. The carbohydrate eluted with 20% alcohol reacted with Type II serum.

that two polymers of distinct chemical composition were obtained. One polymer, the Type II antigen, derived from the alcohol precipitate and eluted between 40% and 20% alcohol, contained nearly all of the glucose and galactosamine of the initial material. The other polymer, designated grouplike carbohydrate, which was derived from the acetone precipitate following alcohol precipitation, was eluted between 90% and 50% alcohol and contained all the glucosamine. Galactose and rhamnose are about equally present in both polymers. The grouplike carbohydrate from strain OII is chemically unlike the Group F carbohydrate. Both carbohydrates contain rhamnose but the group-

like carbohydrate contains galactose and glucosamine, whereas the Group F carbohydrate contains glucose and galactosamine.

The quantitative precipitin tests in Fig. 5 afford a comparison of the immunologic activity between the Type II antigen prepared from strain FII to that from strain OII. Both preparations exhibit similar reactivity with Type II serum.

In the studies described thus far, formamide extraction has been employed to isolate the Type II antigen from either strains FII or OII. It is possible, however, to obtain the type antigen by other means. For example, a major portion

TABLE II

Chemical Compositions of Grouplike Carbohydrate and Type II Antigen Isolated by Cellulose Chromatography

Constituent	Formamide extract of streptococcus strain OII				
	Alcohol precipitate			Acetone precipitate	
	Before chromatography	After chromatography		Before chromatography	After chromatography
		Grouplike CHO	Type II antigen		Grouplike CHO
	%	%	%	%	%
Rhamnose	33.0	28.5	24.5	30.9	30.0
Glucose	15.8	3.2	25.9	8.4	0.0
Galactose	14.0	12.2	12.2	12.2	10.9
Mannose	0.0	0.0	0.0	1.8	0.0
Glucosamine	6.2	25.2	0.1	19.8	30.5
Galactosamine	11.3	1.8	15.8	2.4	0.0

CHO, Carbohydrate.

of the type antigen is solubilized by hot HCl extraction at pH 2, while much of the group carbohydrate remains in the bacterial residue. The extracted material was treated with DNAse, RNAse, pepsin, and trypsin. After dialysis the enzymes were removed from the antigenic material by chromatography on DEAE-cellulose (5). The final product was highly reactive with Type II antiserum. Further purification of Type II antigen obtained in this way was not attempted by the chromatographic methods which have been employed here.

Previous reports have indicated that the broth cultures of strain OII contain appreciable amounts of Type II antigen. Purification of this antigen was attempted because it has the obvious advantage over the extracted material in not having been exposed to drastic hydrolysis procedures. While some success was achieved in the purification of the Type II antigen from the broth, it is not clear whether the final product is free of impurities because chemical analysis

revealed only 4.6% rhamnose, 4.7% glucose, 15.2% galactose, 1.4% mannose, 15.8% glucosamine, and 11.5% galactosamine. Thus these constituents comprise only 55% of the substance. Treatment with trypsin, pepsin, RNAse, and DNAse did not alter this chemical composition. It is worth noting, however, that antigenic differences between the broth antigen and the HCl and formamide antigens were not detected by Ouchterlony double diffusion precipitin analysis. In the experiment depicted in Fig. 6a, these three antigen preparations form a precipitin line of identity when reacted with Type II antiserum.

It was previously shown that mannose was consistently present in substantial amounts in soluble Type II carbohydrate isolated from the medium

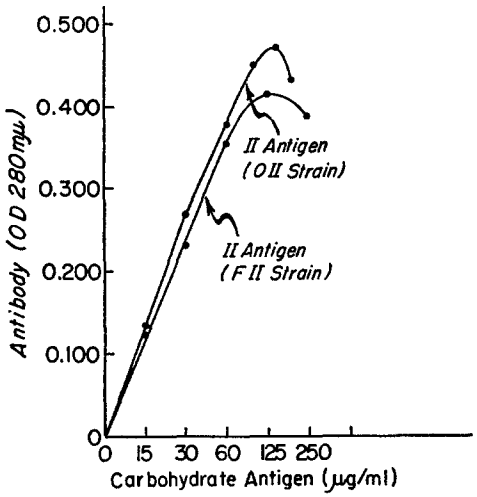
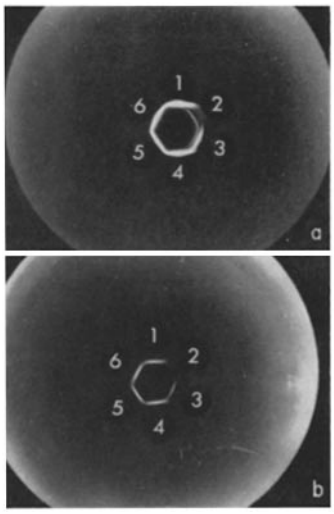


FIG. 5. Precipitin analysis of Type II antigens recovered from formamide extracts of streptococcal strains FII and OII.

(5). The amount of mannose found here was, therefore, much lower than expected. As the isolation of the active material in the previous work did not involve precipitation by alcohol, it is conceivable that mannose is not a component of the soluble antigen, but is part of another polymer which differs in alcohol solubility from both the soluble type antigen and the glucosamine-rich grouplike polymer.

Grouplike Carbohydrate.—The grouplike carbohydrate which was isolated from the acetone precipitable material of strain OII does not react with any of the known streptococcal grouping antisera. Immunological evidence obtained by using the double diffusion technique indicated that this glucosamine-rich carbohydrate reacts with an antibody in antiserum OII. In Fig. 6a, different antigenic preparations, all derived from strain OII, were placed in the peripheral wells and an anti-II serum, obtained from a rabbit immunized with strain OII,

was placed in the center well. The concentrations of each of the various Type II antigen preparations was that which gave, by prior testing, a point of equivalence in the Type II precipitin reaction. Two sets of lines of identity can be observed. The heavy outer line is formed against all antigen preparations which exhibit reactivity with anti-Type II sera in quantitative precipitin tests. Although 4 mg/ml of the grouplike carbohydrate was utilized, only a faint pre-



FIGS. 6 *a*–6 *b*. Antigenic analysis by double diffusion in agar of grouplike carbohydrate and Type II antigen. All antigen preparations are from streptococcal strain OII. Wells 1 and 4, Type II antigen recovered from an HCl extract of strain OII by precipitation with 2 volumes of alcohol. Well 2, grouplike carbohydrate recovered in the acetone precipitate from a formamide extract. Well 3, mixture of grouplike carbohydrate and Type II antigen recovered from a formamide extract. Well 5, the soluble Type II antigen isolated from the culture medium. Well 6, the Type II antigen in an alcohol precipitate of a formamide extract.

In Fig. 6 *a* the center well was filled with a serum prepared against streptococcal strain OII. In Fig. 6 *b* the center well was filled with a serum prepared against streptococcal strain OI.

cipitin line was formed and this was continuous with a faint line formed by the various Type II antigen preparations. This implies that the anti-OII serum contains two antibodies, antibodies in high concentration directed against the Type II antigen, and antibodies in low concentration directed against a carbohydrate with chemical properties analogous to the typical group antigen.

From previous studies (5) it is known that the Types I and II antigens obtained from strains OI and OII respectively, give weak cross-reactions with the opposite antisera. Although *N*-acetylgalactosamine (5) is the immunodominant determinant of both Types I and II antigens, the question arose whether the cross-reaction was due to partial identity of the type antigens or to common

antigenic determinants between the grouplike carbohydrates of strains OI and OII. Double diffusion tests were employed to answer this question. In Fig. 6b the outer wells were filled with the same antigens as those in Fig. 6a, but in this case the central well was filled with an anti-I serum obtained from a rabbit immunized with strain OI. This serum gave a weak cross-reaction with Type II antigen in standard precipitin tests. It is to be noted that the grouplike carbohydrate of strain OII does not react with the OI serum, but that all antigen preparations containing Type II antigen are reactive.

On inspection of Fig. 6b it is noted that three of four Type II antigen preparations exhibit closely adjacent double precipitin lines with Type I antiserum. While it is not clear why two lines are formed, additional evidence indicates that they both represent a cross-reaction between Type II antigen and Type I antiserum. For example, the addition of *N*-acetylgalactosamine as an inhibitor to the anti-I serum in the central well prevents the formation of both lines.

It should be noted that the precipitin reactions formed by the Type II antigen preparations in wells 3 and 4 merge with evidence of spur formation. While the reason for this is not entirely clear at this time, it may be a reflection of the fact that the antigen in well 3 was not recovered from an extract in the same manner as the Type II antigen in wells 1, 4, and 6.

It is not possible at this time to determine the antigenic relationship between the grouplike carbohydrates of strains OI and OII. When antisera are available which possess potent antibodies against these substances, it will be possible to determine whether the grouplike carbohydrates isolated from strains OI and OII are identical or are distinct.

DISCUSSION

Group F hemolytic streptococci were initially identified as a distinct serologic group by Lancefield and Hare (15). Subsequently, members of this Group (16) were found to contain a second carbohydrate representing the type antigen (1). Classification into four distinct types was based on precipitin and agglutination reactions with type antiserum. The fact that these strains were readily agglutinated by type antiserum, but not Group F antiserum, suggested that the type antigens were superficial to the cell wall group antigen.

More recently Ottens and Winkler observed that a number of Group F streptococci which were isolated from root canals of teeth also contained the type antigens (2). From a bacteriologic point of view, it was of interest that a number of streptococci isolated from the root canal cultures were not identifiable as members of any known group of streptococci. It was shown, however, that these strains did possess the type antigens associated with Group F streptococci (2). Occasional strains of Groups C and G streptococci possess these type antigens (1, 2), and the Type II antigen has been identified in strains of Group A streptococci (4).

Various means may be employed to obtain the Group F and Type II antigens

from Group F streptococci. An advantage of the hot formamide extraction procedure is that all of the cell wall carbohydrate antigenic material is solubilized while the residue consists predominantly of mucopeptide.

In the studies reported here both the Group F and Type II antigens have been isolated successfully from a hot formamide extract of a streptococcal strain which contains both Group F and Type II carbohydrate antigens as cell wall constituents. Purification was achieved by a fractionation procedure on a cellulose column initially equilibrated with 90% alcohol (8) which makes use of the fact that the Group F antigen is more soluble in alcohol than the Type II antigen. As a consequence, the two antigens are recovered separately when the column is eluted with decreasing concentrations of alcohol. The chemical composition of the Group F antigen is in agreement with that reported previously (5). Serologic evidence has indicated that a disaccharide with a proposed structure of 3-*O*-β-D-glucopyranosyl-*N*-acetylgalactosamine is the antigenic determinant of the Group F antigen (14). On the other hand, quantitative precipitin inhibition studies suggest *N*-acetylgalactosamine is a component of the Type II antigen (5). Additional studies on the structure of this antigen are in progress.

Group B streptococci also contain surface capsular type carbohydrates which are distinct from the Group B carbohydrate. Isolation of group and type antigens from a mixture was dependent on the fact that the type antigen is less soluble in alcohol than the group carbohydrate. By means of successive differential reprecipitations with alcohol, pure type antigen and pure Group B antigen have been isolated (17, 18). There is no serologic relationship between either the group or the type antigens of Groups B and F. It is known that the type-specific capsular antigens of Group B streptococci function as type-specific virulence factors and induce the formation of protective antibodies in rabbits immunized with encapsulated strains (19). It is unknown whether as in Group B, the superficial type substances of Group F streptococci enhance the virulence of these organisms or give rise to protective antibodies.

Those streptococcal strains which possess one of the Group F type antigens, but do not contain an antigen reactive with either Group F or the other streptococcal grouping antisera have been designated "zero" (2). The data presented here indicate that strain OII contains a grouplike carbohydrate in the cell wall which is analogous in every way to the carbohydrates of Groups A, B, C, G, and F streptococci. The fact that these unidentifiable strains and the Group F strains have been frequently isolated from root canal cultures suggests the possibility that they are in some way related. It will be recalled, for example, that Group A-variant streptococci were isolated from mice infected with Group A organisms (20, 13). In this case the antigenic alteration occurs because the group antigen of the A-variant organisms no longer possesses terminal *N*-acetylglucosaminide residues, the dominant determinant of Group A specificity (21). The Group F antigen contains glucose, galactosamine, and rhamnose whereas the grouplike carbohydrate of strain OII contains galactose, glucosa-

mine, and rhamnose. On the basis of this chemical analysis, it seems unlikely that the Group F antigen and the grouplike carbohydrate of strain OII have a similar antigenic relationship or that the source of strain OII was initially a Group F organism.

Although streptococcal strains OI, OIII, and OIV have not been identified as a member of a streptococcal group, each may also contain carbohydrates which are similar to the grouplike antigen of strain OII. That attempts to produce satisfactory antisera have been unsuccessful is a major handicap in the study of the grouplike antigens. It is not understood why the rabbits immunized with strain OII do not produce antibodies against the grouplike carbohydrate. One explanation might be that the presence of type antigens masks the antigenicity of the inner polymer. It is known, for example, that rabbits immunized with Group F streptococci which are devoid of type antigen exhibit antibody responses to the group substance (1, 2). Eventually the streptococci which contain the grouplike carbohydrates will require a taxonomic designation which is consistent with that employed for the hemolytic streptococci.

SUMMARY

Two antigens, the group-specific carbohydrate and the Type II carbohydrate, have been isolated by cellulose column chromatography from a formamide extract of a Group F streptococcus. Chemical and immunologic analyses indicate that both antigens are free of other cellular components. Both antigens are components of the cell wall although the Type II antigen is probably more superficial than the group antigen. The Type II antigen is composed of rhamnose, glucose, galactose, and galactosamine. The Group F antigen is composed of rhamnose, glucose, galactosamine and a small percentage of glucosamine.

A grouplike carbohydrate and the Type II carbohydrate have been isolated from a streptotoccal strain which lacks a serologically detectable streptococcal group antigen. This grouplike carbohydrate, which does not cross-react immunologically with Group F serum, is composed of rhamnose, galactose, and glucosamine.

No chemical or immunological differences were observed between the Type II antigen isolated from the Group F strain and the Type II antigen isolated from the nongroupable strain.

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