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LABORATORY AND EPIDEMIOLOGICAL RESEARCH ON HEMOLYTIC STREPTOCOCCI

For the Period: 1 March 1965 to 28 Feb 1966

Responsible Investigator: Richard M. Krause, M.D.
Washington University
School of Medicine
660 South Euclid
St. Louis, Missouri 63110

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Annual report to the Commission on Streptococcal and Staphylococcal Diseases of the Armed Forces Epidemiological Board

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Richard M. Krause, M.D.
Washington Univ. School of Medicine
St. Louis, Missouri 63110

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LABORATORY AND EPIDEMIOLOGICAL RESEARCH ON HEMOLYTIC STREPTOCOCCI

ABSTRACT

Streptococcal epidemiology.-Epidemiologic research on Group A hemolytic streptococci at Loring Air Force Base, Maine, has directed attention to the role of intrafamilial transmission of the organism in the persistence and spread of streptococcal disease in the community. Throat cultures were performed on 3,590 family contacts of 1,065 patients with upper respiratory disease who harbored Group A hemolytic streptococci; and throat cultures were performed on 2,231 family contacts of 709 acute respiratory disease cases who had negative throat cultures for hemolytic streptococci. The prevalence percentage of A-positive contacts was at least five times higher for the streptococcal index cases than for the nonstreptococcal index cases.

Streptococcal group-specific antibodies in man.-Two serologically distinct antibodies directed against different antigenic sites on the Group A carbohydrate were detected in human sera by agglutination techniques. Agglutinins with A-variant specificity were demonstrable by the direct agglutination of purified A-variant cell walls , whereas indirect Coombs type agglutinins with Group A specificity were demonstrable with Group A cell walls. There was an obvious correlation between the magnitude of the antistreptolysin O titer and the direct Group A-variant and indirect Group A titers.

Indexing terms

Hemolytic streptococci -- Streptococcal epidemiology
Antigens, streptococcal - Antibodies, streptococcal

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Streptococcal group-specific antibodies in rabbits.

The development of the immune response to group carbohydrate.-Immunization of rabbits with a vaccine of either whole Group A streptococci or cell walls is followed by the production of Groups A and A-variant antibodies. The cell wall agglutination procedure was employed for their detection. The Groups A and A-variant agglutinins were separated out of an immune rabbit serum by an absorption-elution procedure which employs Group C-variant cell walls. In a modification of this work a fluorometric method was adopted to measure the relative concentration of Groups A and A-variant antibodies in streptococcal Group A rabbit antisera. In this method FITC-labeled antibodies are absorbed onto streptococcal cell walls and, after subsequent elution, measured in a fluorometer.

Characteristics of purified rabbit group-specific antibody.-In certain instances rabbits immunized with hemolytic streptococcal vaccine yield sera which possess markedly elevated γ-globulin. In one case the γ-globulin was greater than 50 mgm/ml. The bulk of this γ-globulin, identified as γG, was homogeneous by zone electrophoresis and possessed specificity directed against the carbohydrate antigen. The L chains isolated from the specific antibody exhibited electrophoretic homogeneity on acid urea starch gel electrophoresis, whereas L chains of normal γ-globulin were distributed as a diffuse band.

Immunochemistry of streptococcal antigens.

Group D Type 1 antigen.-Although the Group D Type 1 antigen extracted by the hot formamide procedure contains no cell wall mucopeptide, the serologic reactivity is destroyed. The Type 1 antigen can be successfully extracted by a new technique involving autolysis of the cell walls at pH 6.2, as well as by the S. albus enzyme procedure and by lysozyme. Quantitative precipitin inhibition studies with Type 1 antigen and antibody indicate the D-glucose and/or N-acetylglucosamine may be components of the antigenic determinant.

Streptococcal mucopeptide.-The antigenic basis for the serological specificity of streptococcal mucopeptide has been investigated by immunochemical techniques. The results suggest that the antigenic reactivity of the mucopeptide employed here is dependent upon the peptide moiety rather than the hexosamine polymer.

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A. Streptococcal epidemiology.-Epidemiological research on the transmission and spread of hemolytic streptococci has continued at Loring Air Force Base, Maine. The unique feature of this study is that almost the entire population of a community of 14,000 was under essentially complete surveillance: one, for the occurrence of streptococcal pharyngitis and streptococcal-associated respiratory disease; and two, for the incidence of positive throat cultures of Group A streptococci among the family contacts of such patients. From the data obtained it is apparent that during endemic periods, Group A streptococci are not randomly distributed throughout the populace but are largely clustered within discrete family units. This would suggest and support the idea that the transmission of the organism requires a fairly direct and close degree of contact between carriers and potential susceptibles and that at a base such as Loring A.F.B. the major locus of this combination of persons and environment is the home.

Patients with pharyngitis or upper-respiratory-tract infection were evaluated clinically by history and physical examination. A throat culture was taken in all cases. Criteria for the diagnosis of cases of "strict criteria" Group A streptococcal pharyngitis are listed in Table 1. Those patients who did not meet these criteria were classified as streptococcal-associated respiratory disease. These are patients with either a streptococcal infection which is too mild to diagnose clinically with any degree of certainty, or a viral infection with incidental carriage of Group A streptococci in the throat.

The number of patients with "strict criteria" streptococcal pharyngitis reporting to the hospital dispensary for 1963-64 and 1964-65 is tabulated in Table 2. In general there were fewer cases during the winter and early spring of 1964-65 than during a similar period of 1963-64.

The total number of patients with respiratory disease from whom Group A streptococci were isolated from the pharynx ("strict criteria" streptococcal pharyngitis and streptococcal-associated upper respiratory disease) for 1963-64 and 1964-65 is tabulated in Table 3.

The intrafamilial aggregation of β-hemolytic streptococci was detected by culturing the throats of the family contacts of both A-positive and A-negative index cases. Data collected from these studies was reported in detail last year. In general 45% of all contacts of A-positive index cases harbored Group A streptococci, whereas less than 10% of all contacts of A-negative index cases harbored this organism. All positive contacts received penicillin or some other form of antibiotic therapy.

There is no consensus of opinion on the usefulness of treating all positive family contacts for the prevention of streptococcal disease in the contacts and for the prevention of spread of streptococcal disease in the community. Clearly, what is needed is a therapeutic trial in which one group of asymptomatic contacts is treated with antibiotics and another similar group is not treated. A comparison of the subsequent illness rates and serological responses of the two groups should in part settle the question of the degree of personal danger involved in not treating such contacts. In this connection a small pilot study was conducted in the present population this past winter. No difference was found in subsequent illness rates between treated and untreated contacts although treatment was over 95% effective in eliminating the carrier state. However, the number of patients involved in this study was small and further work on this problem is required to determine the value of routine treatment of asymptomatic A-positive contacts. Additional studies are particularly needed to identify the positive contact carrier as an important element in the spread of the disease to other susceptible individuals in the population.

B. Streptococcal group-specific antibodies in man.- Last year's report described methods to detect antibodies to the carbohydrate of Group A streptococci in human sera which employ direct and indirect agglutination of streptococcal cell walls. The data suggested that human sera contain indirect-agglutinins with specificity directed against the Group A determinant of the carbohydrate and direct agglutinins with specificity directed against the A-variant moiety of the carbohydrate. These studies have been extended and, in general the magnitude of the ASO titer bears a direct correlation to that of the two anti-carbohydrate antibody titers. The anti-Group A agglutinins were detected by a modified Coombs technique which employs Group A cell walls. The anti-Group A-variant agglutinins were detected by a direct agglutination technique which employs Group A-variant cell walls. The Group A and the Group A-variant agglutinins and the antistreptolysin O titers were determined on a battery of human sera. Although it is probable that the group-specific antibodies in human sera represent an antibody response to a streptococcal infection, it is not feasible to describe the nature of this antibody response because none of the sera studied here were collected in series on a single patient after an untreated illness. It is to be anticipated, however, that if the anti-group carbohydrate agglutinins are indicative of a post-streptococcal antibody response, the magnitude of the titer should bear a direct relationship to that of the antistreptolysin O titer. Direct A-variant agglutinins and antistreptolysin O determinations performed on 84 sera and indirect Group A agglutinins and antistreptolysin O determinations performed on 47 sera are presented in Tables 4 and 5 respectively. There is an obvious correlation between the magnitude of the antistreptolysin O titers and that of the direct A-variant and the indirect Group A agglutinin titers. These findings are consistent with the view that the anti-carbohydrate antibodies are a feature of the immune response which develops after streptococcal infections. Studies on serial sera collected from a single patient are planned for the future.

C. Streptococcal Group-specific antibodies in rabbits

1. The development of the immune response to group carbohydrate.- The development of the immune response in rabbits to streptococcal group-specific carbohydrates following intravenous immunization with streptococcal vaccine has been studied in some detail. Immunization with Group A cell walls is followed by two serologically distinct agglutinins with specificity for different antigenic sites in the Group A carbohydrate. The principal agglutinins are directed against the terminal N-acetyl glucosaminide residues upon which the Group A specificity is dependent, whereas the secondary agglutinins are directed against the rhamnose disaccharide determinant of the rhamnose moiety of the carbohydrate. These agglutinins can also be detected by labeling them with fluorescein isothiocyanate and staining both Group A and Group A-variant cell walls by the fluorescent antibody technique. However, major disadvantages of either this procedure or the agglutination method are that the assays involve two-fold dilution of the test serum or conjugate and that the antibody titer is determined by a subjective visual estimation of the end point.

Other means were sought to determine more precisely the relative concentrations of antibodies with Group A and Group A-variant specificity in Group A antisera. A fluorometric method was developed which employs the absorption of fluorescein isothiocyanate labeled antibodies onto purified cell walls. The absorbed labeled antibodies are subsequently eluted from the cell walls and the degree of fluorescence measured in a fluorometer. The fluorometric reading is a direct measure of the amount of antibody which had been adsorbed and eluted from the cell walls. Inhibition of the reaction between Group A conjugate and homologous cell walls by Group A-variant carbohydrate confirms the immunological specificity of the two antibodies. In the experiment depicted in Fig. 1, 10 mgm/ml of Group A carbohydrate had no effect. In a similar fashion C-variant

carbohydrate markedly inhibited the reaction between the A-variant conjugate, whereas Group A carbohydrate failed to do so.

It should be emphasized that this fluorometric method affords an estimate of the relative concentrations of Groups A and A-variant streptococcal antibodies, but it is not a test of antibody concentration in the usual sense. However, certain refinements of the method, such as the determination of the FITC units conjugated to the antibody protein, would permit quantitative measurements of antigen-antibody reactions.

2. Characteristics of purified group-specific antibody.- During the course of the studies on the development of the immune response in rabbits to streptococcal group-specific carbohydrate, certain rabbits were noted to develop an unusually high level of streptococcal group-specific antibodies. Examination of the zone electrophoresis patterns of these immune sera revealed a remarkably sharp and narrow band within the γ-globulin region. The immunological, chemical and physical properties of the specific γG globulin isolated from these sera was studied in some detail. The findings suggest a selective manufacture in certain rabbits of specific immune γG globulin which exhibits a restricted range of electrophoretic properties compared to that of the normal complement of γ-globulin.

The protocol for the immunization of rabbits 80 and 81 with Group A-variant streptococcal vaccine is presented in Table 6. Also recorded are the values for the serum total protein, γ-globulin, and Group A-variant agglutinin titers before, during, and at the end of immunization.

It will be noted that serum 5 of rabbit 80 had an A-variant cell wall agglutination titer of 40,000, whereas serum 5 of rabbit 81 had a titer of 640. Rabbit 80 developed 55 mgm/ml of γ-globulin. The patterns obtained by

zone electrophoresis of sera 1 and 5 of rabbit 80 are depicted in Fig. 2. A strikingly distinct, narrow band is present in the γ-globulin region of serum 5. Specific absorption studies identified streptococcal group-specific antibodies as the major component of the γ-globulin peak. The antibody isolated from precipitating antigen-antibody complexes was identified as a 7S protein and a γG globulin. Further analysis suggested that the normal complement of γ-globulin of pre-immunized rabbits had greater electrophoretic diversity than the specific immune globulin.

Evidence for specific antibody with a restricted range of electrophoretic properties compared to that of normal γ-globulin was derived from several sources. The specific immune globulin exhibited only one band on alkaline starch gel electrophoresis, whereas the γ-globulin from pre-immunized serum was distributed in two bands. The mobility patterns in acid urea starch gel electrophoresis of L chains isolated from both specific immune γ-globulin and normal γ-globulin also supported the view that the immune γ-globulin represented a restricted population of antibodies. The L chains isolated from the specific immune γ-globulin migrated as a single narrow band rather than the usual diffuse smear exhibited by L chains of normal γ-globulin.

Although the biological process which gives rise to exceptionally high levels of electrophoretically homogeneous antibodies is obscure, it may be dependent upon several factors such as the chemical nature of the antigen, the physical state of the antigen, the route of immunization, and the genetic background of the rabbits. The studies conducted thus far indicate that one in five to ten New Zealand red rabbits after immunization exhibit an electrophoretically homogeneous antibody peak which possesses Group A-variant antibodies. Although the findings at this point are preliminary, similar results have not been obtained with New Zealand white rabbits. It is thus conceivable that the breed of rabbits may be an important factor

in the occurrence of this hyperimmune response. Studies are underway to select New Zealand red rabbits which exhibit hyperimmune responses and to follow the immune response in their offspring after similar immunization.

In view of the current interest in highly purified specific antibodies, examination of sera from animals immunized with various bacteria may yield additional antibodies with physical-chemical properties and electrophoretic characteristics which are distinctive from those observed for the normal complement of γ-globulin.

D. Immunochemistry of streptococcal antigens.- Work is continuing on the chemical nature of streptococcal antigens which give rise to antibodies after streptococcal infections. Particular attention has been devoted to the immunochemical nature of the cell wall carbohydrate antigens of Group D streptococci and to the cell wall mucopeptide.

1. Group D Type 1 antigen.- The type-specific carbohydrates of Group D streptococci are components of the cell wall and are the structural and chemical counterparts of the specific carbohydrates of Groups A, B, C, and G streptococci. Although a number of serologic types of Group D streptococci have been identified, the immunochemical basis of this antigenic classification has not been elucidated. Work this past year has been concerned primarily with the immunochemical properties of the type antigen of Group D Type 1.

The data in Table 7 affords a comparison between the chemical composition of the serologically inactive formamide extracted carbohydrate and that obtained by: an autolytic process, the Streptomyces albus enzymes, and the lysozyme procedures. It is to be noted that the formamide carbohydrate is devoid of mucopeptide, while the carbohydrates obtained with enzymes contain an appreciable amount of this material. The low content of the

amino acids in the S. albus carbohydrate is probably due to the fact that the S. albus enzyme solution contains an amidase or peptidase. Purified lysozyme does not have such activity, and this is reflected in the high content of amino acids in the lysozyme carbohydrate. The autolytic carbohydrate has a higher content of amino acids than that of the S. albus carbohydrate but a lower content than that of the cell walls. This diminution of the amino acid content may be dependent upon an amidase or proteolytic activity during the autolysis process. All of the carbohydrate preparations except the formamide carbohydrate are reactive with homologous type-specific antisera but are unreactive with Group D antisera. The high content of rhamnose, hexosamine, and glucose and the lower content of the mucopeptide components in the case of the S. albus carbohydrate suggests that a greater purification of antigen is achieved with the S. albus enzyme than with lysozyme or the autolysis procedure.

Quantitative precipitin inhibition studies were undertaken to ascertain whether glucose or one of the hexosamines was a significant component of the antigenic determinant of the Type 1 carbohydrate. In these quantitative precipitin inhibition tests, carried out at antigen-antibody equivalence, S. albus carbohydrate was employed as the antigen. Final concentration of antigen was 30 micrograms per ml, and the final concentration of sugar inhibitor was 55 micromoles per ml. In addition to the known constituent sugars of the carbohydrate, a number of other sugars were also tested. From an inspection of the data in Table 8, it is clear that D-glucose and N-acetylglucosamine are effective inhibitors. In addition, D-fucose, D-galactose, and L-arabinose, which have the same symmetry about carbons 2 and 3 as that of D-glucose, also inhibit the reaction. If glucose is altered at the third carbon by methylation, the product is not inhibitory, however, 2 deoxy-D-glucose and N-acetylglucosamine, which represent alterations at the second carbon, have inhibitory capacity similar to D-glucose.

Inhibition of the Group D Type 1 precipitin reaction with disaccharides is presented in Table 9. Sucrose and maltose, both alpha glucosides, are particularly effective inhibitors of the precipitin reaction, whereas the beta glucoside, D-cellobiose, identical to maltose except for the beta linkage between the glucosyl residues, is an ineffective inhibitor. Results of other precipitin inhibition studies with maltose indicate that 45% inhibition could be achieved with as little as 14 micromoles per ml. In addition, alpha methyl D-glucose was inhibitory, whereas beta methyl substituted glucose was not inhibitory. These studies suggest that both glucose and N-acetylglucosamine may be major components of the determinant and that the terminal residue has an alpha linkage to the remainder of the carbohydrate. Current studies are in progress to identify the terminal sugar of the determinant.

2. Mucopeptide antigen.- The antigenic basis for the serological specificity of streptococcal mucopeptide has been investigated by immunochemical techniques. Mucopeptide, solubilized by sonication or lysozyme, and rabbit antisera, developed against purified mucopeptide, gave a precipitin reaction. A haptenic inhibitor of this reaction was isolated from an S. albus enzymes (muralytic enzyme and amidase) digest of mucopeptide. Fractions of the dialyzable portion of the digest were eluted from a DEAE cellulose column with an increasing concentration of $(NH_4)_2CO_3$ (pH 8.6). The third fraction, eluted with 0.02 molar $(NH_4)_2CO_3$, was an active inhibitor, whereas the other fractions were ineffective in this respect. Greater than 90% inhibition of the mucopeptide quantitative precipitin reaction was achieved with as littled as 1 mg/ml of active inhibitor. The final inhibitory fraction was retarded on a G-25 Sephadex column. It consisted of alanine, lysine, and glutamic acid in a mole ratio of 3:1:1 and was devoid of glucosamine and muramic acid. These results suggest that the antigenic reactivity of mucopeptide employed here is dependent upon the peptide moiety rather than the hexosamine polymer.

E. Summary and conclusions

Streptococcal epidemiology.- Epidemiologic research on Group A hemolytic streptococci at Loring Air Force Base, Maine, has directed attention to the role of intrafamilial transmission of the organism in the persistence and spread of streptococcal disease in the community. The personnel at Loring Air Force Base, unlike that at a large recruit training command, consists predominantly of married military personnel and their dependents. Group A hemolytic streptococcal prevalence was determined for the family contacts of index cases of streptococcal-associated respiratory disease and index cases of non-streptococcal respiratory disease. The prevalence percentage of A-positive contacts was at least five times higher for the streptococcal index cases than that for the non-streptococcal index cases. This suggests that the home is the major locus and the school the secondary locus for the spread of streptococci in the community. These studies emphasize that patients with streptococcal disease are a potential hazard to the other members of the family. Under certain circumstances the identification of streptococcal pharyngitis in one member of a family may warrant bacteriologic procedures to detect possible spread to other family members.

Thus the spread of streptococcal disease in a population such as that at Loring Air Force Base is more typical of a civilian community than a military population such as a recruit training command. From the point of view of military medicine, control of streptococcal pharyngitis among the dependents can be an important feature in the control of disease in the military.

Streptococcal group-specific antibodies in man.- Two serologically distinct antibodies directed against different antigenic sites on the Group A carbohydrate were detected in human sera by agglutination techniques. Agglutinins with A-variant specificity were demonstrable by the direct agglutination of purified A-variant cell walls,

whereas indirect Coombs type agglutinins with Group A specificity were demonstrable with Group A cell walls. There was an obvious correlation between the magnitude of the antistreptolysin O titer and the direct Group A-variant and indirect Group A titers. The ASO test is the most popular and reliable current method which detects streptococcal antibodies in the sera of patients. Because this test shows a significant antibody rise in only about 80% of the patients with streptococcal disease, there is a real need for an auxiliary method to detect antistreptococcal antibodies. It is conceivable that the method described here for the detection of anti-Group A carbohydrate agglutinins may prove useful for more widespread use in the diagnostic laboratory, and it would appear that a field trial of the test is warranted.

Streptococcal group-specific antibodies in rabbits

The development of the immune response to group carbohydrate.- A growing body of evidence suggests that the pathogenesis of rheumatic fever is dependent upon an altered or aberrant immune mechanism, and there is thus a need for a continued description of the immune response in man and animals to the streptococcal antigens.

Immunization of rabbits with a vaccine of either whole Group A streptococci or cell walls is followed by the production of Groups A and A-variant antibodies. The cell wall agglutination procedure was employed for their detection. The Groups A and A-variant agglutinins were separated out of an immune rabbit serum by an absorption elution procedure which employs Group C-variant cell walls. In a modification of this work a fluorometric method was adopted to measure the relative concentration of Groups A and A-variant antibodies in streptococcal Group A rabbit antisera. In this method FITC-labeled antibodies are absorbed onto streptococcal cell walls and after subsequent elution measured in a fluorometer.

Characteristics of purified rabbit group-specific antibody.- In certain instances rabbits immunized with hemolytic streptococcal vaccine yield sera which possess

markedly elevated γ-globulin. In one case the γ-globulin was greater than 50 mgm/ml. The bulk of this γ-globulin, identified as γG, was homogeneous by zone electrophoresis and possessed specificity directed against the carbohydrate antigen. The L chains isolated from the specific antibody exhibited electrophoretic homogeneity on acid urea starch gel electrophoresis, whereas L chains of normal γ-globulin were distributed as a diffuse band.

The study of antibody structure is dependent, in part, upon the isolation of highly purified antibody, developed against relatively simple haptenic groups. Examination of sera from other rabbits, as well as other animals immunized with various bacteria, may yield additional antibodies with physical-chemical properties and electrophoretic characteristics which are distinctive from that observed for the normal complement of γ-globulin.

Immunochemistry of streptococcal antigens.- These studies are part of a long-range program to identify the chemical and immunological properties of the hemolytic streptococcal antigens.

Group D Type 1 antigen.- Although the Group D Type 1 antigen extracted by the hot formamide procedure contains no cell wall mucopeptide, the serologic reactivity is destroyed. The Type 1 antigen can be successfully extracted by a new technique involving autolysis of the cell walls at pH 6.2, as well as by the S. albus enzyme procedure and by lysozyme. Quantitative precipitin inhibition studies with Type 1 antigen and antibody indicate the D-glucose and N-acetylglucosamine may be components of the antigenic determinant.

Streptococcal mucopeptide.- The antigenic basis for the serological specificity of streptococcal mucopeptide has been investigated by immunochemical techniques. The results suggest that the antigenic reactivity of the mucopeptide employed here is dependent upon the peptide moiety rather than the hexosamine polymer.

There is yet much to be learned about the chemical features of bacterial mucopeptides, and immunochemical analysis should prove helpful in this respect.

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TABLE 1

Criteria for the Diagnosis of Streptococcal Pharyngitis in Patients with Upper-Respiratory-Tract Diseases*

Criterion	No. of Points
History of sore throat	1
Pharyngeal injection	1
Tonsillar or pharyngeal exudate	1
Temperature 101°F or higher	1
White-cell count ≥ 10,000	1
+ or ++ throat culture, Group A	1
+++ or ++++ throat culture, Group A	2

*Cases in patients 0-5 yr. of age who had at least 4 of 7 possible points diagnosed as "strict-criteria" streptococcal pharyngitis; cases in those ≥ 5 yr. of age who had at least 5 of 7 possible points diagnosed as "strict-criteria" streptococcal pharyngitis.

TABLE 2

Patients with "Strict Criteria" Group A Streptococcal Pharyngitis at Loring Air Force Base, Maine

1963-64 Season

Patient Age Group	2 Aug. 29 Aug.	30 Aug. 27 Sept.	28 Sept 24 Oct	25 Oct 21 Nov	22 Nov 19 Dec	20 Dec 16 Jan	17 Jan 13 Feb	14 Feb 12 Mar	13 Mar 9 Apr	10 Apr 7 May	8 May 4 June	Total
Pre-school	16	9	14	30	18	31	50	53	34	26	30	311
Elem.school	3	9	11	7	14	9	21	24	14	14	23	149
Jr.H.school	0	0	0	0	0	4	3	3	0	1	3	14
High school	1	1	0	1	0	3	0	3	2	0	1	12
Mothers	0	3	5	4	1	3	10	6	5	2	5	44
Fathers	0	2	2	2	1	3	3	5	7	0	2	27
Bachelors	4	0	1	1	2	7	9	7	4	0	1	36
Total	24	24	33	45	36	60	96	101	66	43	65	593

1964-65 Season

Patient Age Group	31 July 27 Aug.	28 Aug 24 Sept	25 Sept 22 Oct	23 Oct 19 Nov	20 Nov 17 Dec	18 Dec 14 Jan	15 Jan 11 Feb	12 Feb 11 Mar	12 Mar 8 Apr	9 Apr 6 May	7 May 3 June	Total
Pre-school	9	25	25	27	15	43	21	26	18	20	10	239
Elem.school	9	14	9	26	43	22	21	24	20	16	16	220
Jr.H.school	2	2	3	0	0	2	2	3	0	0	2	16
High school	0	0	1	0	2	2	1	0	0	0	4	10
Mothers	1	1	2	8	5	8	4	3	3	1	2	38
Fathers	3	4	0	2	2	3	5	3	5	0	2	29
Bachelors	3	2	8	5	2	8	4	13	5	1	4	55
Total	27	48	48	68	69	88	58	72	51	38	40	607

*Patients were listed as streptococcal pharyngitis if they met the diagnostic criteria listed in Table 1.

TABLE 3

Total number of Patients with U.R.I. from whom Group A Streptococci were isolated*

1963-64 Season

Patient Age Group	2 Aug 29 Aug	30 Aug 27 Sept	28 Sept 24 Oct	25 Oct 21 Nov	22 Nov 19 Dec	20 Dec 16 Jan	17 Jan 13 Feb	14 Feb 12 Mar	13 Mar 9 Apr	10 Apr 7 May	8 May 4 June	Total
Pre-school	29	28	34	60	37	81	121	121	89	66	72	738
Elem.school	15	24	23	16	32	50	96	56	63	31	39	445
Jr.H.school	1	2	1	2	3	9	10	14	8	1	5	56
High school	1	2	0	2	1	9	1	10	10	2	3	41
Mothers	2	11	5	11	8	22	40	36	34	17	16	202
Fathers	5	11	5	5	1	19	30	40	27	4	3	150
Bachelors	5	9	6	7	9	22	35	34	26	11	9	173
Total	58	87	74	103	91	212	333	311	257	132	147	1805

1964-65 Season

Patient Age Group	31 July 27 Aug	28 Aug 24 Sept	25 Sept 22 Oct	23 Oct 19 Nov	20 Nov 17 Dec	18 Dec 14 Jan	15 Jan 11 Feb	12 Feb 11 Mar	12 Mar 8 Apr	9 Apr 6 May	7 May 3 June	Total
Pre-school	26	47	38	48	36	61	55	77	72	61	31	552
Elem.school	16	36	21	47	73	54	64	63	58	43	41	516
Jr.H.school	4	4	5	1	1	4	4	6	3	1	3	36
High school	0	0	1	4	3	4	5	3	8	3	6	37
Mothers	5	4	12	17	7	13	18	12	17	10	7	122
Fathers	6	12	2	8	7	10	12	17	14	5	3	96
Bachelors	7	12	16	6	8	20	16	33	22	16	7	163
Total	64	115	95	131	135	166	174	211	194	139	98	1522

*These totals include the patients with streptococcal pharyngitis listed in Table 2.

TABLE 4

The direct anti-Group A-variant agglutination titers and the antistreptolysin O titers on 84 human sera

ASO Titer	Anti-A-Variant Agglutination Titer						
	< 20	20	40	80	160	320	640
25	17	3					
50	6						
100	10	5	2				
125	2	1					
166	3	2					
200	1	1					
250	5	1	1	1	4	1	1
333		1		3	1		1
400		1			1		
500		7	2	1			
625				2	2		
1280			1		1		

$r = 0.440$, $P < 0.01$

TABLE 5

The indirect anti-Group A agglutination titers and the antistreptolysin O titers on human sera

ASO Titer	<20	20	40	80	160	320	640	1280
25	4	1	3	1	1			
50	2							
100	1	4		1	1			
125								
166	1				1			
200			1			1		
250			2	1	1	1		
333				1				
400			1					
500				1	3		1	1
625				3	1	1		1
1280				1		2		2

$r = 0.440$, P 0.01

TABLE 6

Immunization Schedule with Group A-variant Streptococcal Vaccine and Immune Globulin Response

		Rabbit No. 80				Rabbit No. 81			
Day	ml of Vaccine	Serum Number	gms % Total Serum Protein	gms % Gamma Globulin	A-var. Agg. Titer	Serum Number	gms % Total Serum Protein	gms % Gamma Globulin	A-var. Agg. Titer
1	0.25	1	6.48	0.68	20	1	6.66	0.50	20
2	0.25								
3	0.25								
8	0.50	2	4.85	0.71	160	2	-	-	-
9	0.50								
10	0.50								
15	0.75	3	6.59	1.34	320	3	6.63	0.81	1280
16	0.75								
17	0.75								
22	1.00	4	-	-	-	4	-	-	-
23	1.00								
24	1.00								
29		5	10.79	5.57	40,000	5	8.14	2.33	640
39		6	8.89	2.94	20,000	6	-	-	-
44		7	8.23	1.91	640	7	-	-	-
71		8	7.06	1.15	80	8	-	-	-

TABLE 7

Composition of Type 1 (D76) Carbohydrates
Extracted by Four Different Methods

	Extraction procedure			
	Hot formamide	Autolysis	S.albus enzyme	Lysozyme
	per cent	per cent	per cent	per cent
Rhamnose	37.6	24.6	30.5	22.5
Hexosamine	11.5	15.7	14.9	11.9
Glucose	19.8	15.6	16.3	14.4
Muramic acid	<1.0	3.4	2.8	4.2
Alanine	<1.0	5.3	0.9	11.7
Glutamic acid	<1.0	1.6	0.3	5.5
Lysine	<1.0	2.4	0.6	5.8

TABLE 8

Inhibition of Type 1 (D76) Precipitin Reaction by Monosaccharides

Inhibitor	Inhibition
55 micromole/ml	per cent
N-Acetylglucosamine	59.4
N-Acetylgalactosamine	16.1
D-Glucose	59.0
L-Glucose	19.0
D-Galactose	45.1
D-Fucose	51.2
L-Fucose	22.9
L-Arabinose	42.0
D-Arabinose	12.3
L-Rhamnose	0.0
D-Mannose	24.7
L-Mannose	3.1
2-Deoxy-D-Glucose	43.1
3-O-Methyl-D-Glucose	9.8

All precipitin reactions were carried out on antigen-antibody equivalence.

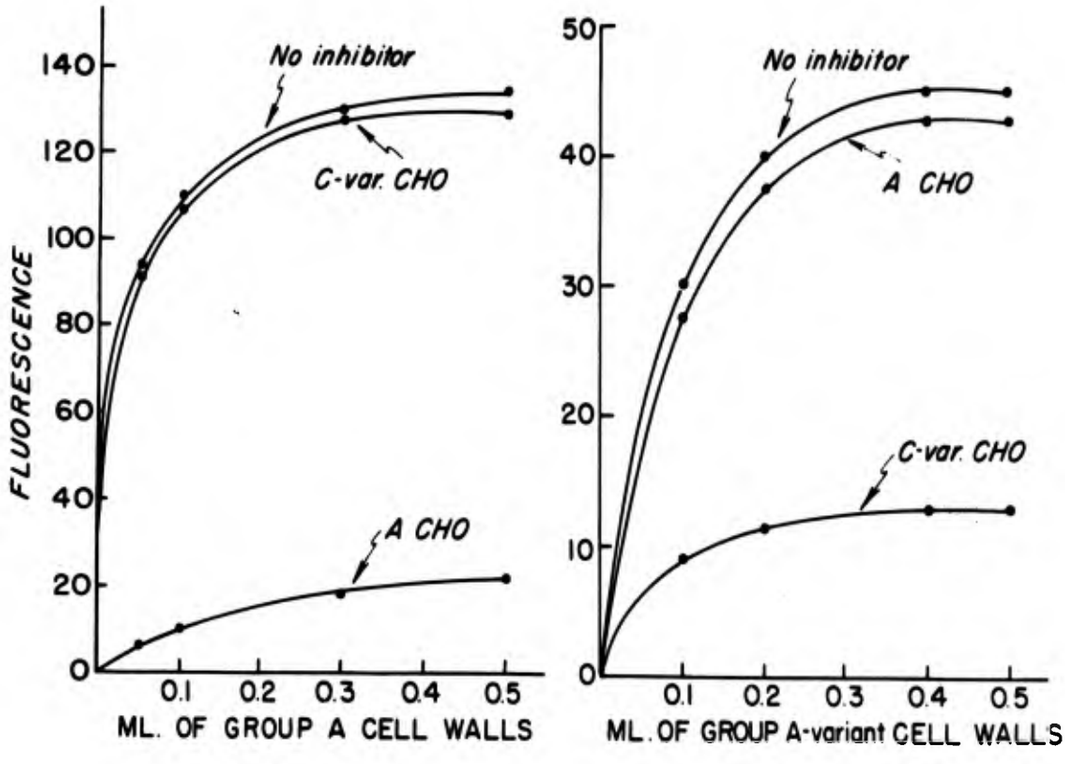
TABLE 9

Inhibition of Type 1 (D76) Precipitin Reaction by Disaccharides and Methylglucosides

Inhibitor	Inhibition
55 micromole/ml	per cent
Sucrose	100.0
Maltose	96.7
D-Cellobiose	18.2
Beta-Lactose	13.6
Alpha-Methyl-D-Glucose	100.0
Beta-Methyl-D-Glucose	12.2

All precipitin reactions were carried out at antigen-antibody equivalence.

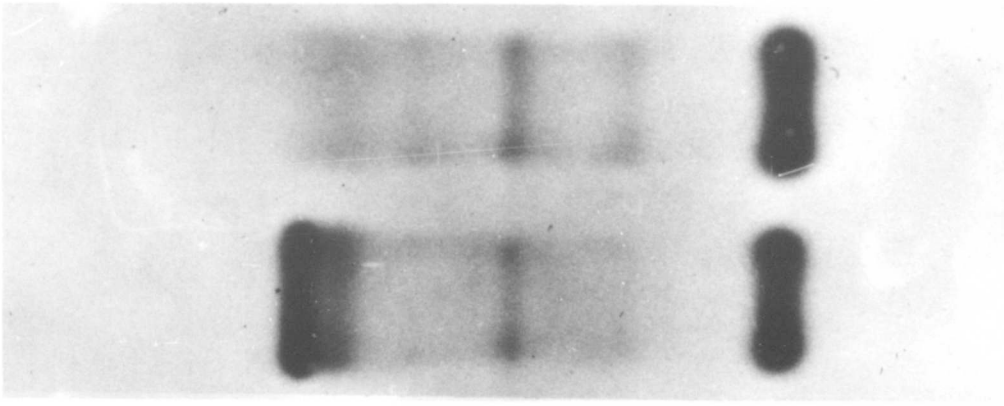
Figure 1



On the left: inhibition with Group A carbohydrate of the reaction between Group A conjugate and Group A cell walls.

On the right: inhibition with Group C-variant carbohydrate of the reaction between Group A-variant conjugate and Group A-variant cell walls.

Figure 2



Zone electrophoresis of sera collected from rabbit 80 which had been immunized with Group A-variant vaccine. The pattern of serum 1 obtained prior to immunization is at the top and the pattern for serum 5 obtained at the end of immunization is at the bottom.