STUDIES ON A NON-HEMOLYTIC STREPTOCOCCUS ISOLATED FROM THE RESPIRATORY TRACT OF HUMAN BEINGS

III. Immunological Relationship of Streptococcus MG to Streptococcus salivarius Type I*‡

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The clearly defined cultural differences between streptococcus MG and *Str. salivarius* were described in a previous paper (1). Various serological cross-reactions between streptococcus MG and *Str. salivarius* type I have been mentioned briefly in the preceding paper (2). The possibility that an antigenic relationship between these two microorganisms might exist first became apparent when it was observed that *Str. salivarius* type I developed capsular swelling in the presence of antistreptococcus MG rabbit serum. This observation suggested that *Str. salivarius* type I might possess, like streptococcus MG, a biologically active specific capsular substance. Evidence will be presented below indicating that *Str. salivarius* type I does in fact possess such a substance. Moreover, it will be shown that streptococcus MG is serologically related to *Str. Salivarius* type I but is antigenically distinct from this microorganism.

Methods

Streptococci.—The strains of streptococcus MG and the strains of *Str. salivarius* used were identical with those described in the preceding paper (2).

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Serological Techniques.—All of the techniques used in this study are described in detail in the preceding paper (2).

Preparation and Chemical Properties of Soluble Substance Obtained from Str. salivarius Type I.—Cells of *Str. salivarius* type I were dried and extracted by the same procedures employed with streptococcus MG. The methods are described in the preceding paper (2). The yield of soluble substance obtained either by alkali or by water extraction was approximately 20 mg. per gram of cells. The results of biuret, Bial, and Dische tests were negative on preparations extracted by each method. The Molisch test, however, on each was positive at dilutions of 1:5000. Quantitative elementary analysis on one preparation obtained by method A and subjected to electrodialysis gave the following results: C 32.6 per cent; H 3.72 per cent; N 5.64 per cent; P 4.29 per cent. After acid hydrolysis this preparation yielded 100 per cent of reducing sugars and gave a positive Sörensen test for glucosamin. The high value for reducing sugars could be explained by the presence of fructose in the hydrolysate. It is of interest that Niven, Smiley, and Sherman (3) have reported previously that the levan synthesized by *Str. salivarius* from sucrose or raffinose yielded large amounts of fructose on acid hydrolysis.

The results of the chemical tests suggested that the soluble substance extracted from *Str. salivarius* type I, like that obtained from streptococcus MG, was a nitrogenous polysaccharide.

Soluble substance obtained from *Str. salivarius* type I by procedures identical to those used for the extraction of a similar substance from streptococcus MG was found to possess specific serological activity in high degree. In the presence of homologous immune rabbit serum, dilutions of 1:1,000,000 of this substance yielded definite precipitates. When mixed with anti-type II *Str. salivarius* rabbit serum, however, no precipitation occurred.

An immunological study of the antigenic relationships between streptococcus MG and *Str. salivarius* type I, as well as *Str. salivarius* type II, was carried out. Representative strains of each microorganism and the sera of rabbits immunized with each strain were employed. Tests were made by the agglutination, *Quellung*, precipitation, and complement-fixation techniques as described above.

Cross-Agglutination Tests.—Suspensions of streptococcus MG, *Str. salivarius* type I, and *Str. salivarius* type II, respectively, were tested by the agglutination technique against each of the homologous immune rabbit sera. The results of a typical experiment are recorded in Table I. It will be seen that anti-streptococcus MG serum in a dilution of 1:5120 agglutinated the homologous microorganism and, in a dilution of 1:640, also agglutinated *Str. salivarius* type I. On the other hand, this immune serum did not agglutinate *Str. salivarius* type II. The anti-type I *Str. salivarius* serum, in dilution of 1:2560, agglutinated the homologous microorganism and, in a dilution of 1:640, also agglutinated streptococcus MG. This immune serum agglutinated *Str. salivarius* type II, but only in serum dilutions of 1:80 or less. The anti-type II *Str. salivarius* serum, in dilutions of 1:5120 and 1:160, respectively, agglutinated the homologous microorganism and *Str. salivarius* type I. This immune serum

also agglutinated streptococcus MG but only in serum dilutions of 1:20 or less.

Many similar cross-agglutination tests were carried out with numerous suspensions of each of these three microorganisms and a number of homologous immune sera. The agglutination titer of each serum was determined repeatedly against each streptococcal suspension. The geometric means of the logarithms of these titers are presented graphically in Fig. 1. In the upper half of the figure the results indicate the extent to which each of the three varieties

TABLE I

Results of Cross-Agglutination Tests with Streptococcus MG, Str. salivarius Types I and II, and Immune Rabbit Sera

Rabbit serum against	Streptococcal suspension	Serum dilution									
		1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120
Str. MG	Str. MG	4	4	4	4	4	4	4	4	3	1
	Str. sal. I	4	4	4	4	4	3	1	0	0	0
	Str. sal. II	0	0	0	0	0	0	0	0	0	0
Str. salivarius type I	Str. MG	4	4	4	4	4	4	3	0	0	0
	Str. sal. I	4	4	4	4	4	4	4	4	3	0
	Str. sal. II	4	4	4	2	0	0	0	0	0	0
Str. salivarius type II	Str. MG	4	2	0	0	0	0	0	0	0	0
	Str. sal. I	4	4	4	4	3	0	0	0	0	0
	Str. sal. II	4	4	4	4	4	4	4	4	3	1
Normal serum	Str. MG	0	0	0	0	—					
	Str. sal. I	0	0	0	0	—					
	Str. sal. II	0	0	0	0	—					

Str. MG = streptococcus MG
Str. sal. = *Str. salivarius*

of streptococcal suspension was agglutinated by each of the three varieties of antiserum. In the lower half of the figure, the results indicate the extent to which each of the three varieties of antiserum was capable of agglutinating each of the three varieties of streptococcal suspension. It will be seen that these two methods of analysis yielded very similar results and that the two graphs shown in Fig. 1 are almost mirror images of each other as would be anticipated from theoretical considerations. The results of these tests show clearly the extent of the cross-agglutination reactions obtained with streptococcus MG and *Str. salivarius* type I. They show also that similar cross-agglutination reactions were not obtained with streptococcus MG and *Str. salivarius* type II.

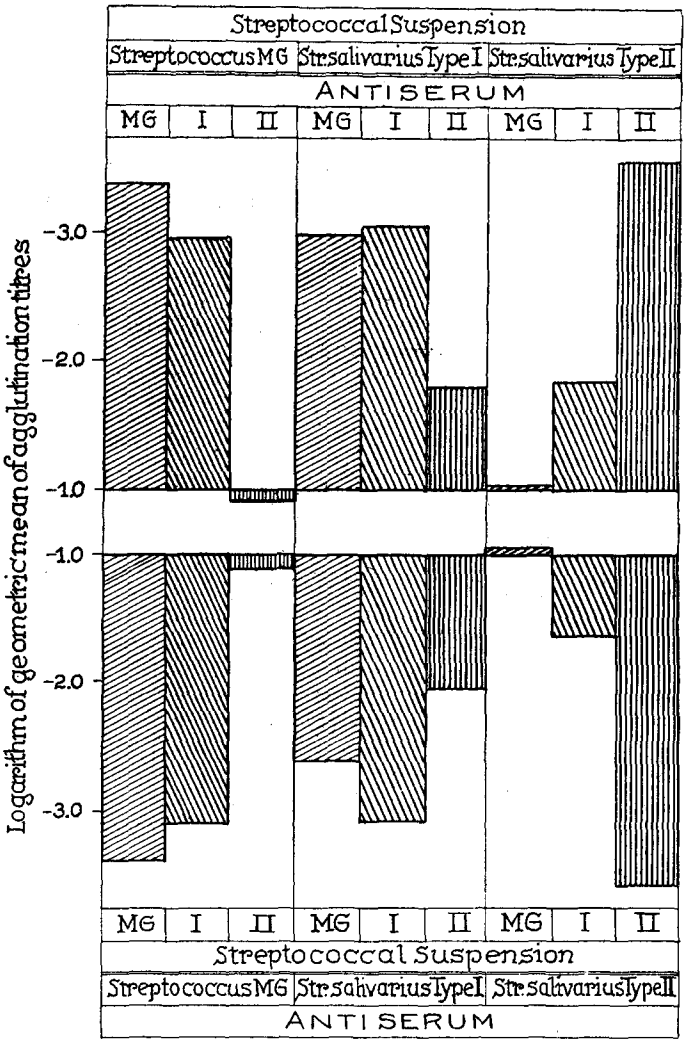

FIG. 1. Agglutination of suspensions of streptococcus MG, *Str. salivarius* type I, and *Str. salivarius* type II by rabbit antiserum against each of these streptococci. The figure shows the logarithm of the geometric means of the agglutination titres observed.

Cross-Agglutination Tests with Absorbed Sera.—The antigenic relationship between streptococcus MG and *Str. salivarius* type I was further investigated by cross-agglutination tests with immune sera absorbed, as described above, with soluble substance (method B) obtained from each streptococcus. The results of a typical experiment are shown in Table II. It will be seen that the

control antistreptococcus MG serum agglutinated the homologous microorganism and *Str. salivarius* type I in serum dilutions of 1:1280 and 1:160, respectively. After absorption with homologous soluble substance, the agglutination

TABLE II

Results of Cross-Agglutination Tests with Streptococcus MG and Str. salivarius Type I, and Absorbed Immune Rabbit Sera

Rabbit serum		Strepto-coccal suspen-sion	Serum dilution								
Against	Absorbed with		1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560
Str. MG	Saline	MG‡	4	4	4	4	4	4	3	1	0
		I§	4	4	4	4	3	0	0	0	0
	SS*, str. MG	MG	3	0	0	0	0	0	0	0	0
		I	2	0	0	0	0	0	0	0	0
	SS, *Str. sal.* I	MG	4	4	4	4	3	0	0	0	0
		I	3	0	0	0	0	0	0	0	0
Str. salivarius type I	Saline	I	4	3	3	3	3	3	2	2	0
		MG	4	4	4	4	2	0	0	0	0
	SS, *Str. sal.* I	I	0	0	0	0	0	0	0	0	0
		MG	4	4	0	0	0	0	0	0	0
	SS, str. MG	I	4	3	2	2	2	2	0	0	0
		MG	4	3	0	0	0	0	0	0	0
Normal serum	Saline	MG	0	0	—	—					
		I	0	0	—	—					
	SS, str. MG	MG	0	0	—	—					
		I	0	0	—	—					
	SS, *Str. sal.* I	MG	0	0	—	—					
		I	0	0	—	—					

* SS = soluble substance
‡ MG = streptococcus MG
§ I = *Str. salivarius*, type I

titer against both streptococci was only 1:10. After absorption with the heterologous soluble substance the agglutination titers were 1:160 and 1:10 respectively. The control anti-type I *Str. salivarius* serum in dilutions of 1:1280 and 1:160, agglutinated the homologous and heterologous microorganisms, respectively. After absorption with the homologous soluble substance these titers were reduced to <1:10 and to 1:20, respectively. After

absorption with the heterologous soluble substance the titers were 1:320 and 1:20, respectively. It appears evident that in each case absorption with homologous soluble substance greatly reduced the titer of both homologous and heterologous agglutinins. On the other hand, absorption with heterologous soluble substance, while it removed most of the heterologous agglutinins, only moderately reduced the titer of homologous agglutinins. These results suggested that the capsular substances of streptococcus MG and *Str. salivarius* type I were immunologically related but also possessed antigenic components which were distinct for each species.

Cross-Quellung Tests.—It was stated above that *Str. salivarius* type I showed capsular swelling when mixed with antistreptococcus MG serum. It was found that the converse was also true, and that anti-type I *Str. salivarius* serum caused capsular swelling of streptococcus MG. The effect of absorption of each variety of antiserum with each variety of soluble substance (method B) was tested by the capsular swelling technique. The results of cross-*Quellung* tests with control and absorbed immune sera are shown in Table III. It will be noted that each of the control immune sera produced capsular swelling of both the homologous and the heterologous streptococci. Following absorption with the homologous soluble substance neither immune serum was capable of causing capsular swelling with either the homologous or the heterologous microorganism. However, following absorption with the heterologous soluble substance, both immune sera were capable of producing capsular swelling of the homologous but not of the heterologous streptococcus. The capsular swelling of the homologous microorganism produced by each immune serum after absorption with the heterologous soluble substance was indistinguishable from that produced by the control immune serum. These results provided further evidence which indicated that the capsular antigens of streptococcus MG and *Str. salivarius* type I were related serologically but were nonetheless distinct. It is noteworthy that the results obtained in cross-*Quellung* tests with immune sera absorbed with these soluble substances were closely analogous to those obtained in cross-agglutination tests with immune sera treated in a similar manner.

Cross-Precipitation Tests.—It was stated above that the soluble substance extracted from either streptococcus MG or *Str. salivarius* type I formed precipitates when mixed with homologous immune rabbit sera. The immunological relationship of these streptococci has been further studied by cross-precipitation tests with each variety of immune serum and with these sera after absorption with soluble substance obtained by the water extraction of each streptococcus. The results of a typical experiment using the capillary technique are shown in Table IV. It will be seen that the control antistreptococcus MG serum formed a precipitate when mixed with both the homologous and heterologous soluble substances in dilutions of 10^{-6} and 10^{-5}, respectively. The

TABLE III

Results of Cross-Quellung Tests with Streptococcus MG and Str. salivarius Type I, and Absorbed Immune Rabbit Sera

Rabbit serum		Streptococcus	
Against	Absorbed with	MG	I
Str. MG	Saline	++++‡	++++
	SS*, str. MG	0§	0
	SS, *Str. sal.* I	++++	0
Str. salivarius type I	Saline	++++	++++
	SS, *Str. sal.* I	0	0
	SS, str. MG	0	++++
Normal serum	Saline	0	0
	SS, str. MG	0	0
	SS, *Str. sal.* I	0	0

* SS = soluble substance

‡ ++++ = definite capsular swelling

§ 0 = no capsular swelling

TABLE IV

Results of Cross-Precipitation Tests with Absorbed Immune Rabbit Sera and Soluble Substances Obtained from Streptococcus MG and Str. salivarius Type I

Rabbit serum		Soluble substance						
		SS, str. MG dilution			Saline control	SS, *Str. sal.* I dilution		
Against	Absorbed with	10^{-4}	10^{-5}	10^{-6}		10^{-4}	10^{-5}	10^{-6}
Str. MG	Saline	4	3	2	0	3	2	0
	SS*, str. MG	0	0	0	0	0	0	0
	SS, *Str. sal.* I	2	1	0	0	±	0	0
Str. salivarius type I	Saline	1	±	0	0	2	1	0
	SS, *Str. sal.* I	0	0	0	0	0	0	0
	SS, str. MG	0	0	0	0	2	±	0
Normal serum	Saline	0	0	0	0	0	0	0
	SS, str. MG	0	0	0	0	0	0	0
	SS, *Str. sal.* I	0	0	0	0	0	0	0

* SS = soluble substance

quantity of precipitate formed was much greater in the homologous as compared to the heterologous combinations. This same immune serum, after absorption with homologous soluble substance, did not form precipitates with

either soluble substance. After absorption with heterologous soluble substance the serum formed only a trace of precipitate with this substance but formed a definite precipitate with homologous soluble substance in a dilution of 10^{-5}. Similar results were obtained with the anti-type I *Str. salivarius* serum. The control serum formed precipitates when mixed with either homologous or

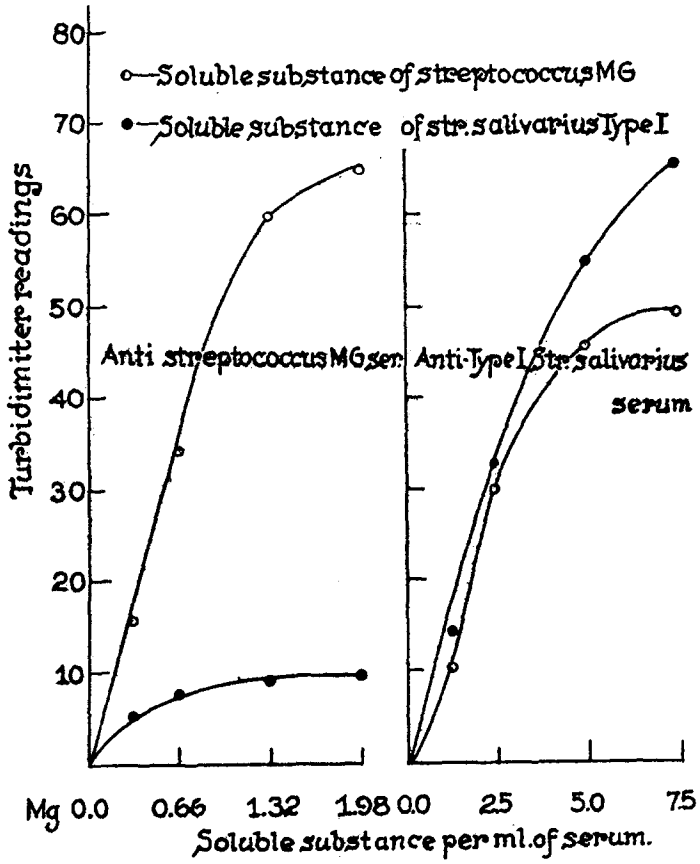

FIG. 2. Precipitation of soluble substances obtained from streptococcus MG and from *Str. salivarius* type I by rabbit antisera against each of these streptococci.

heterologous soluble substance in dilutions of 10^{-5}. The quantity of precipitate formed was again more abundant in the homologous combination. After absorption with the homologous soluble substance this serum did not form a precipitate with either soluble substance. After absorption with the heterologous soluble substance no precipitate was formed in the heterologous mixtures but a precipitate was formed with the homologous soluble substance in a dilution of 10^{-5}.

The difference in the amounts of precipitate formed in the homologous and heterologous mixtures was measured by the quantitative turbidimetric method described above (2). Immune rabbit sera against each variety of streptococcus were mixed with varying amounts of each variety of soluble substance (method A) and turbidimetric readings were recorded after a suitable interval. The results obtained are presented graphically in Fig. 2. It will be seen that in the case of each immune serum the amount of precipitate formed was greater in the mixtures containing the homologous soluble substance. In the case of the antistreptococcus MG serum the maximum quantity of precipitate formed with the homologous soluble substance was 6.5 times greater, and with the anti-type I *Str. salivarius* serum 1.4 times greater, than the maximum quantity of precipitate formed with the heterologous soluble substance.

It seems evident that the results observed in cross-precipitation tests were analogous to those observed both in the cross-agglutination and in the cross-*Quellung* tests. Taken together the results of these various tests constituted strong evidence that the soluble substances extracted from streptococcus MG and *Str. salivarius* type I, although immunologically related, were not antigenically identical.

Cross-Complement Fixation Tests.—The serological relationship of streptococcus MG to *Str. salivarius* type I has been further investigated by cross-complement-fixation tests. The soluble substances obtained from each variety of streptococcus, by both alkali and water extraction, were used as antigens and each variety of immune rabbit serum was tested. Complement was fixed in each case in high dilution of the homologous serum-antigen mixtures and also, but to a lesser degree, in the heterologous mixtures. The results were similar with soluble substance prepared by each method. These observations confirmed those made in agglutination and precipitation tests and showed immunological similarity but not antigenic identity of the soluble substances obtained from streptococcus MG and *Str. salivarius* type I. An insufficient number of complement-fixation tests were carried out with absorbed sera to justify reporting the results at this time.

DISCUSSION

The available evidence (2) indicates that, with the exception only of *Str. salivarius* type I, streptococcus MG is serologically distinct from each of a wide variety of other microbial species including type-specific pneumococci, β hemolytic streptococci, and certain other non-hemolytic streptococci. It was found that *Str. salivarius* type I, like streptococcus MG, also possesses a capsular structure and that its capsule too is composed largely of a nitrogenous polysaccharide. The results of cross-serological tests with streptococcus MG and *Str. salivarius* type I indicate clearly that these two different non-hemolytic streptococci are immunologically related, and yet are antigenically distinct.

It seems evident that the immunological relationship between these two microorganisms is the result of certain similarities in their capsular polysaccharides.

It was shown in a previous paper (1) that streptococcus MG possesses numerous cultural characteristics which are widely different from those of *Str. salivarius* and there seems to be adequate evidence for thinking that they are members of distinct and different species of non-hemolytic streptococci. The fact that streptococcus MG and *Str. salivarius* type I are immunologically related although not antigenically identical can hardly be considered as evidence against this point of view. It is well established that a number of other distinctly different microbial species also possess polysaccharide antigens which are immunologically related. Some noteworthy instances in which apparently coincidental antigenic similarities between different microorganisms have been described previously are the following: pneumococcus type II and a variety of other microorganisms, including Friedländer's bacillus type B (4), a strain of *Saccharomyces cerevisiae* (5), *Past. cuniculicida* (6), and *Leuconostoc mesenteroides* (7); pneumococcus types XX or XII and *Leuconostoc mesenteroides* (7); pneumoccus type I and a strain of *Escherichia coli* (mucoid) (8); various types of pneumococcus and *H. influenzae* types A, B, or C (9, 10); as well as *Rickettsia prowazecki* and *B. proteus* 0X19 (11).

SUMMARY

The results of studies on the immunological relationship between streptococcus MG and *Streptococcus salivarius* type I are described. Evidence is presented to show that *Streptococcus salivarius* type I, like streptococcus MG, possesses a capsular polysaccharide antigen. Similarities in the capsular polysaccharides of these two different species of non-hemolytic streptococci appear to be responsible for their immunological relationship.

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