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

II. Immunological Characteristics of Streptococcus MG*‡

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In the preceding paper (1) evidence was presented to show that various strains of a non-hemolytic streptococcus, designated streptococcus MG, belong to a homogeneous bacteriological group and that the properties of this microorganism appear to characterize it as a distinct and hitherto undifferentiated microbial species. Interest in streptococcus MG was aroused because of the frequency with which strains were isolated from patients with primary atypical pneumonia and because of the development of antibodies against this microorganism in the sera of patients convalescent from the disease (2–4). In the present paper the results of studies concerning the immunological characteristics of streptococcus MG will be described in detail.

Numerous attempts have been made to classify the large group of non-hemolytic streptococci by means of immunological methods. The term "non-hemolytic" will

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be employed, as in the preceding paper (1), to designate all streptococci which do not cause hemolysis, including both indifferent and so called *viridans* varieties.

Sherman (5) has pointed out that in most serological studies these streptococci appear to comprise a very heterogeneous group. In many instances because of similar reactions on blood agar, otherwise dissimilar strains have been grouped together. Most strains however have not been described in sufficient detail for complete or systematic identification. On the other hand, studies of certain bacteriologically similar strains have shown dominant immunological types.

Some authors (6–10) have studied strains of non-hemolytic streptococci which were heterogeneous with respect to their sources and their cultural characteristics. Moreover, these strains did not appear to fall into any clearly defined immunological groups when studied by agglutination, agglutinin-absorption, or complement-fixation techniques. Howell (8) pointed out that, when tested by complement-fixation methods, strains from "definite disease groups—tended toward group specificity." The immunological similarity of different strains of non-hemolytic streptococci associated with certain clinical syndromes has been observed in the case of influenza (11–13), chronic prostatic infection (14), bronchial asthma (15), and dental caries (16).

Kinsella and Swift (17) noted no correlation between the fermentation and immunological reactions of different strains of *Streptococcus viridans* but were able to demonstrate by complement-fixation tests, using antiformin extracts as antigens, and also by agglutination tests that two definite although broad and overlapping immunological groups exist amongst these microorganisms. Hitchcock (18) confirmed these observations and demonstrated by the complement-fixation technique that one of these groups possesses antigens in common with certain hemolytic streptococci, whereas the other group possesses antigens also present in pneumococci. The cross-reactions observed were thought to be dependent upon similarities between the protein or lipoprotein fractions of the various bacterial cells.

Barnes (19) introduced a precipitation or flocculation technique with the supernatant medium for the study of non-hemolytic streptococci. He found that, with few exceptions, a close correlation existed between the flocculation observed and the cultural characteristics of the strains studied. It seems likely, as pointed out by Krumwiede and Valentine (20), that some of the reactions observed represented not precipitation but culture agglutination or "thread" reactions. Barnes showed, however, that extracts of these microorganisms gave specific precipitation reactions when mixed with homologous immune sera.

Hitchcock (21) showed that soluble "residue antigens," non-protein in nature, could be extracted from non-hemolytic streptococci and thought, as the result of precipitation and complement-fixation tests with these antigens, that the non-hemolytic streptococci formed "an antigenically distinct but entirely heterogeneous group." He (22) studied strains of indifferent streptococci isolated from the human throat and found that about 50 per cent of those which fermented inulin belonged to one immunological type. It seems probable that the strains which fermented inulin were *Streptococcus salivarius*.

Solowey (23) studied numerous strains of *viridans* streptococci from subacute bacterial endocarditis, human throats, and teeth. More than 75 per cent of these strains were *Str. salivarius* by Sherman's criteria, and more than 50 per cent of both *Str. salivarius* and unidentified strains fell into two dominant serological groups

when tested by the precipitation method. Sherman, Niven, and Smiley (24) by means of similar techniques found that 40 per cent of the 184 strains of typical *Str. salivarius* belonged to one serological type. They designated these strains as *Str. salivarius* type I, and stated that one available strain of Small's *Streptococcus cardioarthritides* (25) was a typical member of this species and type. These authors also reported a second less common but distinct immunological type of *Str. salivarius* which they designated type II.

It was shown in the preceding paper (1) that streptococcus MG could be distinguished bacteriologically from *Str. salivarius* and that although it was probably related to certain strains of streptococci usually classified as *Str. mitis*, it could be differentiated on the basis of its resistance to bile from any of the strains comprising this heterogeneous group.

Lancefield (26) showed that agglutination of non-hemolytic streptococci by immune sera and precipitation of such sera by a soluble specific substance extracted from the microorganisms are related phenomena. By means of cross-precipitation and cross-complement-fixation tests evidence was obtained that these microorganisms resembled pneumococci in possessing two distinct antigens; a soluble specific substance, probably carbohydrate in nature, and a nucleoprotein which was similar in different strains. Lancefield thought, however, that the *viridans* or non-hemolytic streptococci studied by her differed from pneumococci in that they did not possess a capsule and that the soluble specfic substance was distributed throughout the bacterial cell.

Evidence will be presented below which indicates that streptococcus MG possesses a capsular polysaccharide and that this substance is responsible for certain type specific immunological reactions manifested by this microorganism. It will be shown in the following communication (27) that streptococcus MG is immunologically related to *Str. salivarius* type I but is not related to *Str. salivarius* type II or to any other bacterial species tested.

Methods

Streptococcus MG.—Three strains of streptococcus MG (1, 2) were used in this study. Strains 344 and 9 were isolated from the lung tissues of two fatal cases of primary atypical pneumonia. Strain 462 was isolated from the throat of a patient with an acute upper respiratory infection of undifferentiated character.

R Variants of Streptococcus MG.—Strains 344, 9, and 462 were repeatedly subcultured in broth containing 50 per cent homologous immune rabbit serum. Following twenty serial subcultures in this medium each strain was found to have undergone marked alterations as judged by cultural, biological, and immunological tests. The induced alterations persisted despite numerous subcultures in broth containing no immune serum. The differences between these variants and the parent strains were found to be analogous in many respects to the differences which exist between the non-encapsulated R variants of pneumococci and the encapsulated cells from which they were derived. Consequently these induced variants will be referred to throughout this paper as the R variants of streptococcus MG.

Streptococcus salivarius.—A representative strain, S31A, of *Str. salivarius* type I and a representative strain, S30D, of *Str. salivarius* type II, were also used in this study.[1]

[1] These strains of *Str. salivarius* were kindly supplied by Dr. J. M. Sherman, Laboratory of Bacteriology, College of Agriculture, Cornell University, Ithaca, New York.

Streptococcal Suspensions.—For the immunization of rabbits or for agglutination tests streptococcal suspensions were prepared in the following manner: an 18 hour Todd-Hewitt broth culture of the desired streptococcus was killed by heating at 65°C. in a water bath for 60 minutes. The bacterial cells were then separated from the culture medium by centrifugation and washed three times with 0.85 per cent NaCl. The washed cells were resuspended in a quantity of saline equal to one-tenth the volume of the original culture. The suspensions were stored at 4°C. and remained stable under these conditions for more than 2 months.

Immune Sera.—Rabbits were immunized by the repeated intravenous injection of streptococcal suspensions. Each rabbit was given three courses of four daily injections with a rest period of 3 days between each course. The quantities of suspension used in the first course were 0.1, 0.1, 0.2, and 0.5 cc.; in the second course 0.1, 0.2, 0.5, and 1.0 cc.; and in the third course 0.2, 0.5, 1.0, and 2.0 cc. Serum was obtained 4 weeks after beginning immunization. Frequently a fourth course of injections similar to the third course was given 9 weeks after beginning immunization, and additional serum was obtained about 10 days after its completion. Sera were stored under sterile conditions but without preservative at 4°C.

EXPERIMENTAL

Three representative strains of streptococcus MG were selected from those described in the preceding paper (1) and were studied by a variety of immunological procedures. Induced R variants derived from each of these strains were studied in a similar manner. Representative strains of *Str. salivarius* type I and type II were also studied. The techniques employed and the results obtained with each technique will be described separately.

Agglutination of Streptococcus MG

Agglutination Technique.—The streptococcal suspensions used for antigens were similar in all respects to the streptococcal suspensions employed for the immunization of rabbits. The suspensions were diluted with 0.85 per cent NaCl solution to approximately the turbidity of tube 5 in the McFarland turbidimetric scale. Diluted suspensions were stable when stored at 4°C. for over 2 months. Serial twofold dilutions of unheated serum were made in 0.85 per cent NaCl solution. To 0.2 cc. of each serum dilution was added 0.2 cc. of three times washed streptococcal suspension. Readings were made after the mixture had stood for 18 hours either at room temperature or at 37°C. Variation in the results described below was noted if certain of these conditions were altered.

Reproducible agglutination titres were found to be most readily obtained when the tests were carried out in the manner described above. It was found important to employ (1) three times washed streptococcal suspensions, (2) unheated sera, and (3) reaction temperatures of from 20 to 37°C.

Heat-killed suspensions of strain 344 which had been washed a varying number of times were tested against homologous immune rabbit serum. The results of a typical experiment are shown in Table I. It will be seen that after but one washing the bacterial suspension was not agglutinated by dilutions of immune serum greater than 1:160, whereas after three washings this suspension was agglutinated by a serum dilution of 1:2560. Similar results were obtained with streptococcal suspensions prepared from other strains and tested against their homologous immune sera. In the light of experiments to be described below it seems probable that the increased agglutination titres obtained with repeatedly washed streptococcal suspensions resulted from the removal of soluble antigen present in the original culture medium. As will be shown, this soluble antigen was capable of absorbing agglutinins from immune sera.

The agglutination titres of antisera prepared in rabbits against streptococcus MG. were not significantly altered by heating undiluted sera for 30 minutes at temperatures varying from 55 to 65°C. On the other hand, agglutinins against this streptococcus in the sera of human beings were definitely reduced in titre by heating at the temperatures indicated above. Moreover, the agglutination titres of rabbit antisera against *Str. salivarius* were also markedly affected by heating at similar temperatures. In order to obtain comparable results, therefore, all agglutination tests were carried out with unheated sera.

It was found necessary to control the temperature in carrying out agglutination tests. When either human or rabbit sera were tested at 4°C. it was difficult to make accurate readings, and alterations in titres sometimes occurred. It was found (4) that human sera in low dilution occasionally agglutinated streptococcus MG at 4°C. Reaction temperatures from 20 to 37°C. gave reproducible results and consequently all agglutination tests were carried out at these latter temperatures.

TABLE I

The Effect of Multiple Washings of Streptococcus MG upon the Results of Agglutination Tests with Homologous Immune Rabbit Serum

Rabbit serum against	Streptococcus MG suspension	Serum dilution							
	No. of washings	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560
Str. MG*	1	4‡	4	4	2	0	0	0	0
	3	4	4	4	4	4	4	2	2
Normal serum	1	0	0	0	—				
	3	0	0	0	—				

* Str. MG = streptococcus MG.

‡ 0–4 = degree of agglutination.

— = not tested.

Agglutination tests were carried out under the conditions described above with various strains of streptococcus MG and the sera of rabbits immunized with each strain. The induced R variants of streptococcus MG and antisera against one of the R variants were included in certain tests. The results of a typical experiment are shown in Table II. It will be noted that none of the streptococcal vaccines were agglutinated in the presence of normal rabbit serum. Both strain 344 and strain 9 were completely agglutinated by high dilutions of antisera against either strain, but were agglutinated only by very low dilutions of the antiserum against the R variant. On the other hand, the R variant was agglutinated, although in a distinctly different manner, not only by its homologous serum but also by the antisera against each of the other strains.

Agglutination of streptococcus MG by homologous antisera was characterized by the formation of firm plaque-like masses which fell to the bottom of the tube and left a water-clear supernatant fluid. With low dilutions of immune serum plate-like discs of agglutinated bacteria were often produced. Vigorous agi-

tation did not disperse the agglutinated particles. Distinct prozone effects were frequently observed with low dilutions of high titre antisera against streptococcus MG. These were considerably increased when agglutination tests were carried out at 4°C. Agglutination of the R variants by either homologous antisera or by antisera against the parent strains was typically fragile, soft, and fluffy. The supernatant fluid remained cloudy and the bacterial clumps were

TABLE II

Results of Agglutination Tests with Strains of Streptococcus MG and Homologous Immune Rabbit Sera

Rabbit serum against str. MG	Suspension of str. MG	Serum dilution									
		1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120
Strain 344	Strain 344	4*	4	4	4	4	4	3	1	0	0
	Strain 9	4	4	4	4	4	4	3	2	1	0
	R Variant of strain 344	+‡	+	+	+	+	±	±	±	0	0
Strain 9	Strain 344	3	3	4	4	4	4	4	3	0	0
	Strain 9	3	3	3	4	4	4	4	3	2	0
	R Variant of strain 344	+	+	+	+	±	±	±	0	0	0
R variant of strain 344	Strain 344	0	0	0	0	0	0	0	0	0	0
	Strain 9	2	0	0	0	0	0	0	0	0	0
	R Variant of strain 344	+	+	+	+	+	+	+	+	±	—
Normal serum	Strain 344	0	0	0	0	—					
	Strain 9	0	0	0	0	—					
	R variant of strain 344	0	0	0	0	—					

* 0–4 = degree of firm agglutination.

‡ ±–+ = degree of fragile agglutination.

very easily dispersed by gentle shaking. Usually it was necessary to use a hand lens to read agglutination tests with the R variants.

Antisera against streptococcus MG were tested by the agglutination technique against a variety of other bacterial species. These included strains of staphylococcus, group A β hemolytic streptococcus, non-encapsulated *H. influenzae*, non-encapsulated pneumococcus, type I pneumococcus, *Str. salivarius* types I and II, and numerous other species of non-hemolytic streptococcus. Antisera against streptococcus MG strongly agglutinated representative strains of *Str. salivarius* type I but did not agglutinate *Str. salivarius* type II or any of the other microorganisms tested except for certain strains which were also

agglutinated in the presence of normal serum. As will be shown in the following paper (27) evidence was obtained which indicated that *Str. salivarius* type I was antigenically related to streptococcus MG. In the preceding paper (1) evidence was presented to show that despite immunological similarities, there were numerous and striking biological differences between *Str. salivarius* type I and streptococcus MG.

Capsular Swelling Tests with Streptococcus MG

It will be recalled that all the strains of streptococcus MG described in the preceding paper (1) were selected because each showed capsular swelling, or *Quellung*, in the presence of an immune rabbit serum against one strain. The *Quellung* reaction has been employed, as described below, for the study of the antigenic structure and immunological type specificity of streptococcus MG.

One loop of a young broth culture of streptococcus was thoroughly mixed on a cover slip with a loop of 1 per cent methylene blue and then a loop of immune rabbit serum was added. The culture was mixed under similar conditions with normal rabbit serum. These mixtures, either as hanging drops or in contact with a slide, were observed for capsular swelling.

Although an indistinct halo was commonly observed surrounding the cell bodies of streptococcus MG, a definite capsule was not visible in the presence of normal rabbit serum. When mixed with homologous immune rabbit serum however each strain showed definite capsular swelling similar to, although not so obvious as, that observed with encapsulated pneumococci under analogous conditions. The capsular swelling which resulted when strain 344 was mixed with homologous immune serum was illustrated in the preceding paper (1). Capsular swelling occurred very rapidly and was usually evident immediately after the preparation had been made. It became somewhat more definite with increasing time, however, and was very obvious in preparations which had been allowed to react for a few hours at 4°C.

The induced R variants failed to show any evidence whatsoever of capsular swelling in the presence of homologous rabbit antisera or in immune rabbit sera against either streptococcus MG or *Str. salivarius* type I. Repeated tests under a variety of conditions did not in a single instance give any evidence that the R variants possessed capsules.

The encapsulated form of streptococcus MG was tested against antipneumococcus rabbit sera, type I to type XLIII, and in no instance was capsular swelling observed. Similarly no capsular swelling was observed when it was mixed with anti-type II *Str. salivarius* serum. On the other hand, the sera of rabbits immunized with *Str. salivarius* type I did cause capsular swelling of streptococcus MG. Details of studies concerning the antigenic relationship between streptococcus MG and *Str. salivarius* type I will be given in the following paper (27).

Precipitation Tests with Culture Filtrates of Streptococcus MG

The serological specificity observed in agglutination and *Quellung* tests with streptococcus MG suggested the possibility that this microorganism like pneumococcus might produce a soluble specific substance. Filtrates of broth in which streptococcus MG had been grown were tested for the presence of such a substance.

Preparation of Culture Filtrates.—Strains of streptococcus MG were grown in Todd-Hewitt broth for 18 hours at 37°C. The cultures were centrifuged and the clear supernatant media were filtered through Coors No. 3 candles. The filtrates were checked for sterility by the usual cultural methods, adjusted to pH 7.5, and tested for serological activity by the precipitation technique.

Capillary Precipitation Method.—The technique described by Swift, Wilson, and Lancefield (28) was employed. Serial dilutions of the solutions to be tested were made in 0.85 per cent NaCl solution. Each dilution was mixed with the desired undiluted serum in a capillary tube. The tubes were held for 2 hours at 37°C. and then for 18 hours at 4°C. The presence or absence of precipitation was then recorded.

Filtrates prepared from cultures of streptococcus MG and from R variants of this microorganism were tested against homologous immune rabbit sera as well as against various heterologous immune rabbit sera. The results observed in a typical experiment are recorded in Table III. It will be seen that culture filtrates of strain 344 and strain 9 when diluted 1:32 and 1:8, respectively, yielded precipitates when mixed with anti-strain 344 rabbit serum. It will also be seen that culture filtrates of R variants derived from each of these strains failed to yield precipitates when mixed with this immune serum. Moreover, the culture filtrates of both strains 344 and 9 as well as their R variants failed to yield precipitates when mixed with normal rabbit serum or with immune rabbit sera against either of the R variants. These results indicated that soluble and serologically active material was present in culture filtrates of encapsulated strains of streptococcus MG but was not demonstrable in culture filtrates of R variants derived from these strains.

For purposes of comparison with β hemolytic streptococci so called "M" extracts were prepared from streptococcus MG according to Lancefield's technique (29). These extracts were tested by the capillary method described above. It was found that such extracts did not yield precipitates when mixed with normal rabbit sera, but did yield heavy precipitates in the presence of anti-streptococcus MG rabbit serum. These extracts were tested also with specific rabbit antisera against β hemolytic streptococci of Lancefield's groups A to M. In no instance did precipitation result when the extracts were mixed with any of these group specific antisera. The serologically active material in "M" extracts of streptococcus MG was completely destroyed by further heating at 100°C. in N/10 HCl for 30 minutes but was not affected by similar treatment in N/10 NaOH. In this respect it differed from the M substance of β hemolytic streptococcus which is stable to acid and heat.

Cultural Characteristics of R Variants of Streptococcus MG

The method employed to induce dissociation of strains of streptococcus MG to R variants has been described above. The results of *Quellung* and precipitation tests showed that induced R variants not only possessed no demonstrable capsules but also did not release serologically active material into the culture media in which they were grown. In these respects, as well as in their behavior in agglutination tests, R variants of streptococcus MG were closely analogous to non-encapsulated R variants of pneumococcus.

A study of an R variant of strain 344 by many of the cultural methods described in the preceding paper (1) showed that it resembled the parent strain in

TABLE III

Results of Precipitation Tests with Culture Filtrates of Streptococcus MG and Streptococcus MG Immune Rabbit Sera

Rabbit serum against str. MG	Culture filtrate of str. MG	Dilution of culture filtrate			
		1:2	1:8	1:32	1:128
Strain 344	Strain 344	4*	4	2	0
	R variant of strain 344	0	0	0	0
	Strain 9	4	2	0	0
	R variant of strain 9	0	0	0	0
R variant of strain 344	Strain 344	0	0	0	0
	R variant of strain 344	0	0	0	0
	Strain 9	0	0	0	0
	R variant of strain 9	0	0	0	0
Normal serum	Strain 344	0	0	—	
	R variant of strain 344	0	0	—	
	Strain 9	0	0	—	
	R variant of strain 9	0	0	—	

* 0–4 = degree of precipitation.

almost all respects. The R variant, however, was more susceptible to the bacteriostatic action of methylene blue and had lost its capacity to split esculin. It is of interest that the loss of this latter function was also noted with R variants derived from other strains of streptococcus MG.

As in the case of pneumococcus, induced R variants of streptococcus MG could usually be distinguished from encapsulated strains when grown in liquid medium. The growth of R variants was flocculent, whereas the growth of the encapsulated strains was diffuse with some sedimentation. Unlike pneumococcus, however, individual colonies formed by R variants on the surface of agar plates were indistinguishable from colonies of the encapsulated parent strains. Differences in the colonies produced by the encapsulated cells and the R variants of streptococcus MG could be detected readily, however, when the strains

were cultured in semisolid medium, similar to that employed by Ward and Rudd (30). These authors found that encapsulated strains of β hemolytic streptococcus, when grown in a medium containing 0.2 per cent agar, formed semitransparent feathery colonies, whereas non-encapsulated strains formed opaque compact colonies. Similar differences in the colonial form of encapsulated and non-encapsulated strains of pneumococcus were observed in this medium (30). When encapsulated strains and non-encapsulated R variants of streptococcus MG were grown in a similar semisolid medium striking differences in colonial form were noted. In Fig. 1 are illustrated the compact colonies produced by the R variant, and colonies surrounded by feathery zones characteristic of encapsulated strains.

By the use of this technique it was possible to select colonies formed either by encapsulated streptococci or by R variants in a mixed culture containing both varieties of bacterial cells.

Isolation and Purification of Soluble Specific Substance

A soluble specific substance was extracted from encapsulated strains of streptococcus MG. This substance was found to possess specific serological activity in high degree. The methods of preparation of this substance, and the results of various studies carried out with it are given below.

Method A.—Alkali extracts were prepared in the following manner: Cultures of streptococcus MG grown for 18 hours at 37°C. in Todd-Hewitt broth were centrifuged and the bacterial sediment collected. When large quantities of culture were used a Sharples centrifuge was employed. A 5 per cent suspension of the bacterial cells was then made in distilled water. One-tenth volume of 1N NaOH was added to this suspension and the mixture was heated for 20 minutes in a boiling water bath. After cooling under the tap, phenol red was added and the pH readjusted to neutral. The suspension was then centrifuged and the bacterial sediment discarded. The supernatant fluid was mixed with four volumes of absolute ethyl alcohol. Sodium acetate was then added to a concentration of 1 per cent and the mixture stored overnight at 4°C. The white flocculent precipitate which resulted was separated by centrifugation, drained free of alcohol, and dissolved in a quantity of distilled water equal to one-half the volume of the original suspension. Trichloracetic acid was then added, slowly with stirring, to a final concentration of 2 per cent and the precipitated protein and nucleic acid were separated by centrifugation and discarded. The supernatant, which contained most of the biologically active material, was immediately neutralized by the addition of 50 per cent NaOH and again mixed with four volumes of alcohol in the manner previously described. The resulting precipitate was separated by centrifugation, and redissolved in 50 ml. of distilled water. This solution was placed in a cellophane sack and dialyzed for 1 week at 4°C. against frequent changes of distilled water. The contents of the sack were then filtered through sintered glass and the water-clear filtrate was frozen and dried in a lyophile apparatus. A fluffy white powder was obtained by this procedure which was finally dried to constant weight at 100°C. in an Abderhalden apparatus and stored in a desiccator over calcium chloride. This material will be referred to as soluble substance (method A).

Similar soluble substances were prepared from suspensions of bacterial cells which had been dried by the following procedure. An approximate 5 per cent suspension of the cells in 0.85 per cent NaCl was added slowly and with stirring to twenty volumes of acetone. After

standing for 18 hours at room temperature the cells were separated from the acetone by centrifugation and re-suspended in a small volume of acetone. The acetone was removed by filtration through a sintered glass funnel and traces of water remaining in the cells were removed by washing them on the funnel first with absolute alcohol and then with anhydrous ether. The ether was removed *in vacuo* and the cells were dried in a vacuum desiccator over calcium chloride.

The yields of soluble substance obtained by alkali extraction of the bacterial cells of various strains of streptococcus MG, both before and after drying, were similar and equalled about 20 mg. per gram of cells or 5 liters of the original streptococcal culture.

Method B.—Water extracts were prepared in the following manner: Streptococcal cells which had been dried by the acetone method detailed above were extracted by a procedure much less drastic than the alkali method just described. The dry bacterial cells were evenly suspended in distilled water to a concentration of about 5 per cent. The suspension was allowed to stand overnight at room temperature and then centrifuged. The supernatant fluid was removed and stored at 4°C. and the sedimented cells were extracted a second time with distilled water in the same manner. After centrifugation the sedimented cells were discarded and the supernatant fluid was combined with that from the first extraction and filtered through a Coors No. 3 candle. This filtered solution was then deproteinized by the chloroform method described by Sevag (31). The procedure was repeated until no further precipitate at the interface was obtained. The resulting deproteinized solution was then mixed with nine volumes of absolute ethyl alcohol and crystalline sodium acetate was added to make a concentration of 1 per cent. This mixture was stored at 4°C. for 18 hours. The white flocculent precipitate which appeared was sedimented by centrifugation and the supernatant alcohol was discarded. The precipitate was dissolved in distilled water and dialyzed in a cellophane sack, first against tap water and then against distilled water. A second similar precipitation with alcohol was carried out and the precipitate, after it had been washed several times with absolute alcohol was dissolved in distilled water. This solution was filtered through sintered glass and dried in a lyophile apparatus. The material obtained by this procedure will be referred to as soluble substance (method B).

The yield of soluble substance obtained by water extraction of acetone—dried cells of streptococcus MG was about 20 mg. per gram of bacterial cells.

Precipitation Tests with Soluble Substances

The soluble substance extracted from streptococcus MG was tested for specific serological activity. The capillary precipitation technique was employed and soluble substance (method A) as well as soluble substance (method B) were tested against various immune rabbit sera.

The results obtained in a typical experiment are shown in Table IV. It will be seen that soluble substance prepared either by method A or method B was capable, in a dilution of 1:640,000, of yielding a precipitate when mixed with homologous immune rabbit serum. No precipitate formed when either of these preparations was mixed with immune serum against the R variant of strain 344, anti-type II *Str. salivarius* rabbit serum, or with normal rabbit serum. The precipitate which formed in the presence of homologous immune serum appeared

almost immediately after the mixtures were made. It was very abundant in tubes containing the lower dilutions of soluble substance and had a dense, stringy, and somewhat translucent appearance. When soluble substance was mixed with homologous immune serum in test tubes firm disc-like precipitates were formed which were not broken up by vigorous agitation.

Soluble substance (method A) and soluble substance (method B) were also tested by the capillary precipitation technique against anti-type I to type XLIII pneumococcus rabbit sera as well as group A to M β hemolytic streptococcus antisera. In no instance did a precipitate form with any of these sera. However, when soluble substance extracted from streptococcus MG by either method A or method B was mixed with anti-type I *Str. salivarius* rabbit serum

TABLE IV

Results of Precipitation Tests with Soluble Substance Obtained from Streptococcus MG and Immune Rabbit Sera

Rabbit serum against	Soluble substance str. MG, strain 344	Dilution of soluble substance × 10^4				
		1:1	1:4	1:16	1:64	1:256
Str. MG, strain 344	Method A	4	4	3	1	0
	Method B	4	4	3	1	0
R variant of str. MG, strain 344	Method A	0	0	0	0	0
	Method B	0	0	0	0	0
Str. salivarius type II	Method A	0	0	0	0	0
	Method B	0	0	0	0	0
Normal serum	Method A	0	0	—		
	Method B	0	0	—		

definite precipitation occurred. The results of cross-serological tests with streptococcus MG and *Str. salivarius* type I, will be described in the following paper (27). Evidence will be presented which indicates that the soluble substance of streptococcus MG is immunologically related although not antigenically identical to a similar soluble substance obtained from *Str. salivarius* type I.

Antistreptococcus MG sera diluted 1:100 yielded precipitates when mixed with soluble substance, and dilutions of 1:2,000,000 of soluble substance formed precipitates in the presence of homologous immune serum. As regards specific reactivity at high dilution there appeared to be no difference between soluble substance prepared either by alkali or by water extraction of streptococcus MG.

The results of precipitation tests with soluble substance of streptococcus MG and various immune rabbit sera indicate that with the exception of anti-

type I *Str. salivarius* serum this substance yielded precipitates only in the presence of antisera against encapsulated strains of streptococcus MG. It appears that not only was this substance serologically specific but also that very small amounts were serologically reactive.

Various preparations of soluble substance (method A) and soluble substance (method B) were tested by quantitative methods to determine their relative capacities to produce specific precipitation when mixed with antistreptococcus MG sera.

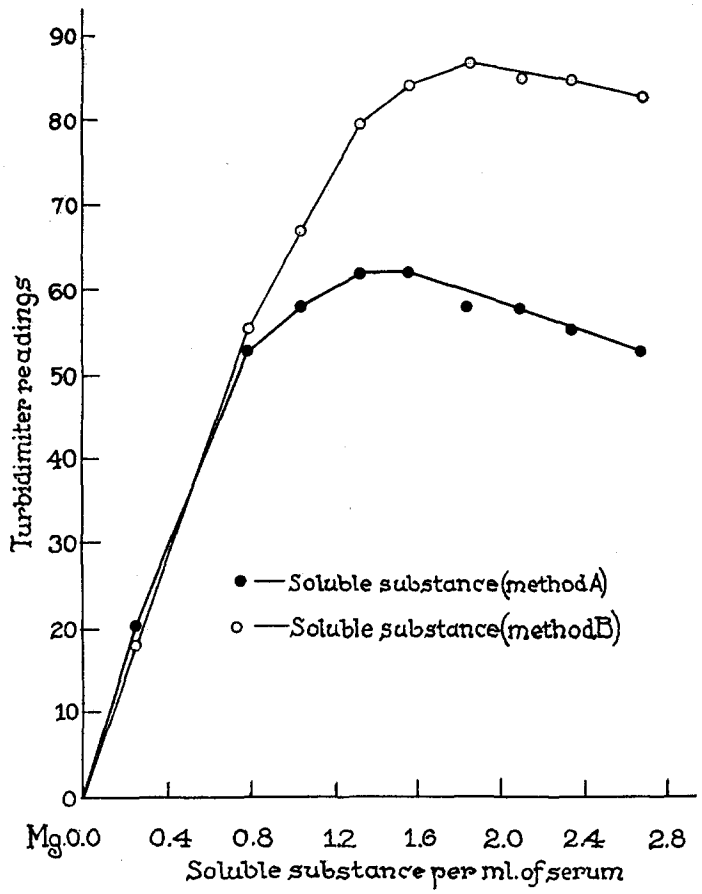
Quantitative Precipitation Method.—A modification of the turbidimetric technique described by Libby (32) was employed. One ml. quantities of a predetermined optimum dilution of immune serum in 0.85 per cent NaCl were distributed in a number of test tubes. To each was added 1 ml. of varying dilutions of soluble substance, in 0.85 per cent NaCl. The mixtures after thorough shaking were held at room temperature, and the degree of turbidity produced in each tube was measured at 5 minute intervals in a photoelectric turbidimeter. The results were recorded, and graphs were plotted from the reading observed for each mixture.

Quantitative precipitation tests employing the turbidimetric method were carried out with three preparations of the soluble substance (method A) obtained from two strains of streptococcus MG and an antiserum against one strain. The serum was diluted so that 0.015 ml. was contained in each 1.0 ml. of solution. The maximum precipitation observed with each of these preparations occurred in the mixtures which contained 0.66 mg. of soluble substance per ml. of antiserum and the quantities of precipitate formed by each preparation were almost identical. The shapes of the curves superimposed upon the observed readings suggested that there were only very slight differences in the specific serological activities of these three preparations. Marked prozone effects were observed with each of the preparations in the regions of antigen excess.

Two preparations of soluble substance obtained by methods A and B, respectively, from strain 344 were tested by the quantitative technique against another homologous immune serum. The results obtained in one experiment are shown graphically in Text-fig. 1. It will be seen that with this antiserum and soluble substance (method A) maximum precipitation occurred in the mixture containing 1.32 mg. of soluble substance per ml. of serum. With soluble substance (method B) maximum precipitation occurred in the mixture containing 1.85 mg. of soluble substance per ml. of serum. It will also be noted that the amount of precipitate formed with soluble substance (method A) as determined by the turbidity readings was approximately 70 per cent of the amount formed with soluble substance (method B).

It will be recalled that soluble substance prepared by alkali extraction (method A) appeared on the basis of specific reactivity at high dilution to be closely similar to soluble substance prepared by water extraction (method B).

However, it seems evident from the results obtained in quantitative precipitation tests that soluble substance (method B) was capable of causing much more complete precipitation of antibody from homologous immune serum than was soluble substance (method A). As will be shown below tests with immune sera



TEXT-FIG. 1. Precipitation of soluble substance obtained from streptococcus MG by alkali extraction (method A) and by water extraction (method B) with antistreptococcus MG immune rabbit serum.

absorbed with soluble substance revealed that material prepared by water extraction was a more complete antigen than similar material prepared by alkali extraction of streptococcus MG. It seems probable that the rigorous chemical procedures employed in the alkali extraction (method A) resulted in partial degradation of the soluble substance possibly by depolymerization or by partial hydrolysis.

Complement Fixation Tests with Soluble Substance

The serological activity of soluble substance obtained from streptococcus MG was tested by the complement-fixation reaction.

Complement-Fixation Technique.—The reagents employed were mixed in the following quantities: soluble substance (1:50,000 to 1:10,000,000) 0.2 ml.; serum (1:5 to 1:625) 0.2 ml.; guinea pig complement, two units contained in 0.5 ml. of saline. Tubes containing these mixtures were incubated for one-half hour at 37°C. after which sensitized sheep cells, consisting of 0.5 ml. of 2 per cent sheep cells and 0.2 ml. of 0.85 per cent NaCl solution containing two units of amboceptor, were added. The tubes were re-incubated for 30 minutes at 37°C. following which readings for hemolysis were made.

TABLE V

Results of Complement-Fixation Tests with Soluble Substance Obtained from Streptococcus MG and Immune Rabbit Sera

Serum		Soluble substance	Serum dilution			
Species	Immunized with	Dilution	1:5	1:25	1:125	1:625
Rabbit	Normal	10^{-5}	0	0	0	0
		10^{-6}	0	0	0	0
		10^{-7}	0	0	0	0
	Str. MG	10^{-5}	4	4	4	0
		10^{-6}	4	4	4	0
		10^{-7}	0	0	0	0
	R. variant of str. MG	10^{-5}	0	0	0	0
		10^{-6}	0	0	0	0
		10^{-7}	0	0	0	0

0 = complete hemolysis.
4 = no hemolysis.

Normal rabbit serum and the serum of rabbits immunized with streptococcus MG, strain 344, as well as the serum of rabbits immunized with the R variant of this strain were tested. The results were similar when soluble substance prepared by either method A or method B was used as antigen. The results of a typical test with soluble substance (method A) as antigen are shown in Table V. It will be seen that complement fixation occurred in mixtures containing soluble substance and antistreptococcus MG rabbit serum. A 1:1,000,000 dilution of soluble substance in the presence of a 1:125 dilution of homologous immune rabbit serum caused fixation of complement. It will also be seen that complement was not fixed in mixtures containing soluble substance and normal rabbit serum or anti-R variant rabbit serum.

Physical and Chemical Tests with Soluble Specific Substance

Soluble substance of streptococcus MG prepared by either method A or B was a fluffy white powder. It was not soluble in 80 per cent alcohol but was readily soluble in water or 0.85 per cent NaCl and was not dialyzable through cellophane. Solutions of one preparation (method B) were tested in an Ostwald viscosimeter. In a dilution of 1:1000 the viscosity was about equal to that of water but in a dilution of 1:100 it was five times that of water.

Several preparations of soluble substance obtained either by method A (alkaline extraction) or by method B (water extraction) from streptococcus MG were subjected to a mumber of qualitative chemical tests. Solutions containing 1 mg. per ml. gave a negative biuret test for protein and a negative Dische diphenyl-amine reaction for desoxyribonucleic acid. The orcinol test (Bial) for pentose or ribonucleic acid was weakly positive. The Molisch test for carbohydrate was strongly positive, even when dilutions of 1:5000 were tested.

The effect of certain enzymes upon the serological activity of soluble substance (method A) was also tested. It was found that under the conditions employed desoxynuclease, desoxyribonuclease, trypsin, and pepsin failed to reduce the specific serological activity of the soluble substance.

Quantitative elementary analyses were carried out on each of three preparations of soluble substance (method A) obtained from two strains of streptococcus MG. It was found that there was considerable variation in the analytical results. Per unit of dry weight C varied between 25 and 32 per cent, H between 5.2 and 5.4 per cent, N between 1.6 and 5.3 per cent, and P between 6.5 and 9.0 per cent.

One preparation of soluble substance (method A) was hydrolyzed for 6 hours at 100°C. with 1N H_2SO_4. The resulting hydrolysate was then tested for the presence of reducing sugars by the copper reduction method of Somogyi. It was found that the hydrolysate contained 78 per cent reducing sugars. The test for glucosamine by the method of Sörensen was positive.

Despite the variations observed on quantitative chemical analysis, the results of the various tests which were carried out strongly suggested that the soluble specific substance isolated from streptococcus MG was a long-chain nitrogenous polysaccharide.

Tests for Antigenicity of Soluble Substance

The capacity of soluble substance extracted from streptococcus MG to stimulate the formation of antibodies was tested in rabbits and in human beings. Four rabbits were each injected with a total of 128 μg. of soluble substance obtained by alkali extraction from strain 344. This was equivalent to the amount of soluble substance extracted from the total quantity (6.4 ml.) of streptococcal suspension employed to immunize rabbits. Two rabbits were injected by the

intravenous route and two subcutaneously. The amount of soluble substance in each injection and the spacing of the injections were analogous to those used for immunization with streptococcal suspensions. Serum was obtained from the rabbits 4 weeks after the beginning of immunization and was tested for antibodies against streptococcus MG by the agglutination and precipitation techniques. It was found that the serum obtained from each of these rabbits failed to agglutinate a suspension of strain 344 and also failed to yield a precipitate when mixed with soluble substance extracted from this strain. Consequently it was concluded that soluble substance extracted from streptococcus MG was not antigenic for rabbits under the conditions tested.

The antigenicity of soluble substance (method A) for human beings was also tested. A single intradermal injection of soluble substance in 0.1 ml. of 0.85 per cent NaCl was given to each of eight normal human beings. Four persons were given 10 μg. and four were given 100 μg. No local or systemic reaction was observed in any instance. Serum was obtained from each person before and 1, 4, 9, and 28 weeks after the injection. These sera were tested for antibodies against streptococcus MG by the agglutination and precipitation techniques described above.

It was found that each of the eight persons who received soluble substance intradermally developed in their serum agglutinins against streptococcus MG and seven also developed precipitins for the soluble substance. None of the sera obtained before the injection of soluble substance was capable at dilutions of 1:10 or more of agglutinating strain 344 and none yielded precipitates when mixed with either soluble substance (method A) or soluble substance (method B) prepared from this strain. In each instance, however, serum obtained 1 week after the injection possessed agglutinins for streptococcus MG and the titres ranged from 1:10 to 1:40. Each of the sera obtained 4, 9, and 28 weeks after the injection also was capable of agglutinating this microorganism. The titres ranged from 1:20 to 1:160 and remained essentially the same for each person throughout the period of observation. Sera obtained 4, 9, and 28 weeks after injection were tested by the precipitation technique against soluble substance extracted from streptococcus MG. It was found that sera from seven persons yielded definite precipitates when mixed either with soluble substance (method A) or soluble substance (method B), while the serum from the remaining person did not form a precipitate with either preparation. The results observed with the sera of the human beings who showed the least and greatest antibody response, respectively, following the injection of either 10 μg. or 100 μg. of soluble substance are recorded in Table VI. The results indicate that the soluble substance obtained from streptococcus MG was antigenic for human beings. In this respect as well as in its lack of antigenicity for rabbits the soluble substance obtained from streptococcus MG resembled the capsular polysaccharide of pneumococcus (33).

Cutaneous Tests with Soluble Substance

It has been shown in the case of pneumococcus that the intradermal injection of capsular polysaccharide in rabbits (34) or human beings (35), immunized with the homologous type microorganism results in a local reaction at the site

TABLE VI

Results of Agglutination and Precipitation Tests with the Serum of Human Beings Given a Single Intradermal Injection of Soluble Substance Obtained from Streptococcus MG

Amount of soluble substance injected	Serum, weeks after injection	Agglutination						Precipitation
		Serum dilution						Dilution of soluble substance
		1:10	1:20	1:40	1:80	1:160	1:320	1:10,000
μg.								
10	0	0	0	0	0	0	0	0
	1	2	0	0	0	0	0	—
	4	3	2	1	0	0	0	1
	9	3	1	1	0	0	0	—
	28	—	—	—	—	—	—	—
	0	0	0	0	0	0	0	0
	1	4	4	4	0	0	0	—
	4	4	4	4	0	0	0	1
	9	4	4	3	1	0	0	2
	28	4	4	1	0	0	0	1
100	0	0	0	0	0	0	0	0
	1	3	2	1	0	0	0	—
	4	3	3	1	0	0	0	3
	9	3	2	0	0	0	0	0
	28	3	2	0	0	0	0	0
	0	0	0	0	0	0	0	0
	1	4	4	4	2	0	0	—
	4	4	4	4	4	3	0	3
	9	4	4	4	4	3	0	1
	28	4	4	4	4	3	0	1

0–4 = degree of agglutination or precipitation.
— = not tested.

of the injection. Rabbits which had been immunized with streptococcus MG were tested for this type of response. Two normal rabbits and two rabbits which had been immunized with strain 344 were injected intradermally with soluble substance (method A). Each rabbit was given at the same time, but at different sites, four injections containing, respectively, 0.8, 4, 20 and 100 μg. of soluble substance in 0.1 ml. or 0.85 per cent NaCl. The immune rabbits had specific agglutinins and precipitins in their serum at the time of the injec-

tions. At 20 hours after the injections control rabbits showed no reaction at the site of injection of the smaller doses and there were only small areas of erythema, 2 to 8 mm. in diameter, about the sites of injection of the larger doses of soluble substance. The immune rabbits, on the other hand, showed at 20 hours after the injections not only definite erythema but also moderate edema and induration which measured 3 mm., 11 mm., and 15 mm. in diameter, respectively, at the sites of injection of 4 μg., 20 μg., and 100 μg. of soluble substance. At 44 hours after the injections these reactions had largely disappeared.

Similar cutaneous tests were carried out with human beings. It was stated above that a single intradermal injection of 10 or 100 μg. of soluble substance produced no local reaction in normal human beings, although it stimulated the production of antibodies against streptococcus MG. When a second intradermal injection of soluble substance was given 1 week after the first injection a definite local reaction occurred. The reaction was characterized by the development of an itching wheal surrounded by an area of erythema 3 to 4 cm. in diameter. It appeared at the site of the second injection about 1 hour after it was given and persisted for about 12 hours. No other symptoms occurred in any of the human beings tested. In some instances an identical reaction occurred in persons who received no second injection. This appeared after an interval of 7 to 10 days at the site of the first injection.

Absorption of Immune Sera with Soluble Substance

It seemed of importance to determine to what extent the antibodies in immune rabbit serum against streptococcus MG could be removed by absorption with soluble specific substance obtained from this microorganism.

Absorption Technique.—Undiluted immune rabbit serum was mixed with an equal volume of 0.2 per cent solution of soluble substance in saline. This concentration was selected because, as is shown above, 2 mg. of soluble substance per ml. of serum was somewhat in excess of the amount required for maximum precipitation of antibody from the immune rabbit sera tested. The mixture of serum and soluble substance was kept at 4°C. for 18 hours and was then centrifuged. The supernatant fluid was removed and tested as described below. In each experiment the immune serum was also mixed with an equal volume of saline and treated in an identical manner.

Specimens of serum from rabbits immunized with strain 344 of streptococcus MG were absorbed in the manner described with soluble substance obtained by alkali extraction (method A) or with soluble substance obtained by water extraction (method B). The absorbed sera were tested against streptococcus MG as well as against soluble substance obtained from this microorganism. The results of typical experiments with absorbed sera are described below.

Agglutination Tests with Absorbed Sera

Antistreptococcus MG serum which had been absorbed with either soluble substance (method A) or soluble substance (method B) was tested to determine its capacity to agglutinate streptococcus MG. The results of one experiment are shown in Table VII. It will be seen that the control unabsorbed specimen which had been mixed with saline had an agglutination titre of 1:1280. Fol-

lowing absorption with 2 mg. of soluble substance (method A) per ml. of serum the agglutination titre was 1:320, whereas after absorption with an identical quantity of soluble substance (method B) the agglutination titre was only 1:10. It appears evident that soluble substance prepared by water extraction (method B) was capable of removing almost all the antibody responsible for agglutination of streptococcus MG from homologous immune serum, whereas that prepared by alkali extraction (method A) absorbed much less antibody under identical conditions. It will be recalled that in quantitative precipitation tests it was found that soluble substance extracted by method B yielded significantly more precipitate with immune serum than did that extracted by method A.

TABLE VII

Results of Agglutination Tests with Streptococcus MG and Absorbed Immune Rabbit Serum

Rabbit serum		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	4	4	4	4	4	4	4	2	0
	SS* (method A)	4	4	4	4	4	3	0	0	0
	SS (method B)	3	0	0	0	0	0	0	0	0
Normal serum	Saline	0	0	0	0	—				

* SS = soluble substance.

Precipitation Tests with Absorbed Sera

Antistreptococcus MG serum following absorption with either soluble substance (method A) or soluble substance (method B) was tested to determine its capacity to form precipitates with soluble substance. The capillary precipitation technique was used. The results of one experiment are shown in Table VIII. It will be noted that the control specimen which was mixed with saline yielded precipitates with soluble substance (method A) and soluble substance (method B) when either preparation was diluted 1:640,000. After absorption with soluble substance (method A) the serum did not yield a precipitate with this preparation but did form a precipitate when mixed with soluble substance (method B). Following absorption with soluble substance (method B) the serum did not form a precipitate in the presence of either preparation. The much more complete absorption of precipitins as well as agglutinins from antistreptococcus MG serum by soluble substances obtained by the gentle water extraction method indicates that preparations treated in this manner were more closely similar to the native antigen of the bacterial cell than were preparations obtained by alkali extraction.

Capsular Swelling Tests with Absorbed Sera

The capacity of the absorbed antistreptococcus MG sera to cause capsular swelling of the homologous microorganism was tested in the usual manner. It

was found that immune serum absorbed with soluble substance (method A) caused capsular swelling of streptococcus MG as marked as that produced by the control serum. Following absorption with soluble substance (method B), however, immune serum produced no demonstrable capsular swelling of this streptococcus. It was found, moreover, that under certain conditions, the development of capsular swelling was inhibited when soluble substance (method B) but not soluble substance (method A) was added directly to the immune serum at the time of making the test.

TABLE VIII

Results of Precipitation Tests with Soluble Substance Obtained from Streptococcus MG and Absorbed Immune Rabbit Sera

Rabbit serum		Soluble substance									
		Prepared by method A					Prepared by method B				
Against	Absorbed with	Dilution × 10^4					Dilution × 10^4				
		1:1	1:4	1:16	1:64	1:256	1:1	1:4	1:16	1:64	1:256
Str. MG	Saline	4	4	3	1	0	4	4	3	1	0
	SS* (method A)	0	0	0	0	0	3	2	1	0	0
	SS (method B)	0	0	0	0	0	0	0	0	0	0
Normal serum	Saline	0	0	—			0	0	—		

* SS = soluble substance.

The results of these tests provide additional evidence that soluble substance obtained by alkali extraction was much less effective in absorbing type specific antibody from antistreptococcus MG serum than was soluble substance prepared by water extraction. Moreover, they suggest that the soluble specific substance extracted from streptococcus MG represents at least one antigenic constituent of the capsular structure of the microorganism.

Complement-Fixation Tests with Absorbed Sera

Antistreptococcus MG serum following absorption with soluble substance (method B) was tested by the complement-fixation technique against this substance in a manner identical to that described above. It was found that under the conditions of these experiments complement-fixing antibodies against soluble substance obtained by water extraction were not demonstrable after absorption of immune serum with this substance.

The results of the various experiments carried out with antistreptococcus MG rabbit serum indicate clearly that the antibodies reactive in agglutination, precipitation, capsular swelling, and complement-fixation tests were largely if not completely removed by absorption with soluble specific substance obtained from the homologous microorganism. The available evidence strongly sug-

gests that antibodies specifically directed against this capsular constituent of streptococcus MG were responsible for the positive serological reactions obtained by each of the techniques mentioned.

DISCUSSION

In the preceding paper (1) evidence was presented which indicates that a non-hemolytic streptococcus, designated streptococcus MG, possesses cultural characteristics which serve to differentiate it from other clearly defined species of non-hemolytic streptococci. Numerous strains of this microorganism, isolated from the respiratory tract of human beings, were found to produce very similar, if not identical, reactions under a wide variety of experimental conditions. The available evidence suggests that the strains studied are members of a homogeneous and hitherto undifferentiated species of non-hemolytic streptococcus. Moreover, it appears that all of the strains investigated thus far belong to a single serological type.

The results of studies described in the present communication as well as the results of certain experiments reported in the preceding paper (1) indicate that streptococcus MG possesses a capsular structure. The antigenic type specificity of this microorganism was found to be dependent upon the integrity of its capsule, since with induced R variants, which possessed no demonstrable capsule, evidence for type specific serological activity was not obtained.

The soluble, and serologically specific substance, present in filtrates of cultures of encapsulated streptococcus MG as well as in extracts of the bacterial cells themselves, was found on chemical study to be largely, if not wholly, carbohydrate in nature. Investigations carried out with a number of purified preparations of this substance yielded results which indicate that the material consists in large measure of a long-chain nitrogenous polysaccharide. No evidence for the presence of this polysaccharide was obtained in similar investigations with non-encapsulated variants of streptococcus MG.

The available evidence suggests that the serologically specific polysaccharide obtained from streptococcus MG is a major constituent of the capsular structure. Moreover it appears that the capsular polysaccharide is responsible for the type specific immunological reactions, demonstrable with this microorganism. This conclusion is strongly supported by evidence obtained from tests with homologous immune serum absorbed with the capsular polysaccharide, which indicates that this substance combined with and removed from the serum the antibodies reactive not only with the capsular polysaccharide but also with the encapsulated cells of streptococcus MG.

There appear to be a number of striking similarities between streptococcus MG and pneumococcus with respect to the cellular structure, the disposition of serologically specific substance, and the chemical nature of the capsular antigen. Despite the fact that these two bacterial species are both culturally and antigenically wholly distinct they apparently possess the following characteristics

in common: (1) the capacity to elaborate a capsule composed in large part of a complex polysaccharide, (2) the capacity to undergo dissociation in the presence of homologous immune serum and as a consequence to develop non-encapsulated R variants, (3) the ability of encapsulated strains but not of R variants to release soluble specific substance, *i.e.* capsular polysaccharide, into the medium in which they are grown, (4) the dependency of type specific immunological reactions upon the antigenic specificity of the capsular polysaccharide, and (5) the ability to synthesize a capsular antigen which, in the intact cell, is antigenic both for rabbits and for human beings, but which, in the form of the isolated capsular polysaccharide, is antigenic for human beings but not for rabbits.

It is noteworthy, too, that the quantitative differences observed in the specific serological activity of preparations of capsular polysaccharide obtained on the one hand by a rigorous procedure, *i.e.* alkali extraction, and on the other by a gentle procedure, *i.e.* water extraction, from streptococcus MG are closely analogous to those previously described with deacetylated and acetyl capsular polysaccharide of pneumococcus type I (36).

Lancefield previously studied the antigenic structure of four representative but culturally dissimilar strains of non-hemolytic streptococci (26). Two distinct antigens, a nucleoprotein, and a non-protein fraction, thought to be carbohydrate, were extracted from the cells of each strain. The nucleoprotein antigens obtained from these strains were immunologically similar, but the carbohydrate antigen was distinct for each strain. This latter antigen was thought to be responsible for the immunological specificity of each strain. In these respects the non-hemolytic streptococci were believed to resemble pneumococcus. No capsules were observed, however, and the strains studied were thought to contain the soluble specific carbohydrate within the cell body rather than at the cell surface.

The striking analogy between streptococcus MG and pneumococcus, both as regards the nature of their antigenic constituents and their cellular structure seems of considerable interest.

SUMMARY

The results of studies on the antigenic structure and immunological specificity of a non-hemolytic streptococcus, designated streptococcus MG, are described. Evidence is presented to show that this microorganism possesses a capsule and that this structure contains a polysaccharide antigen which is responsible for the type specific serological reactions obtained with streptococcus MG.

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EXPLANATION OF PLATE 26

FIG. 1. Colonies of a non-encapsulated R variant strain of streptococcus MG and of the encapsulated parent strain in semisolid medium. In the test tube to the left are shown the compact opaque colonies of the R variant strain and in the test tube to the right the colonies of the encapsulated strain. Note the characteristic semi-translucent, feathery zones surrounding the colonies of the encapsulated strain and the absence of these zones around the R variant colonies. Enlarged about 1½ ×.

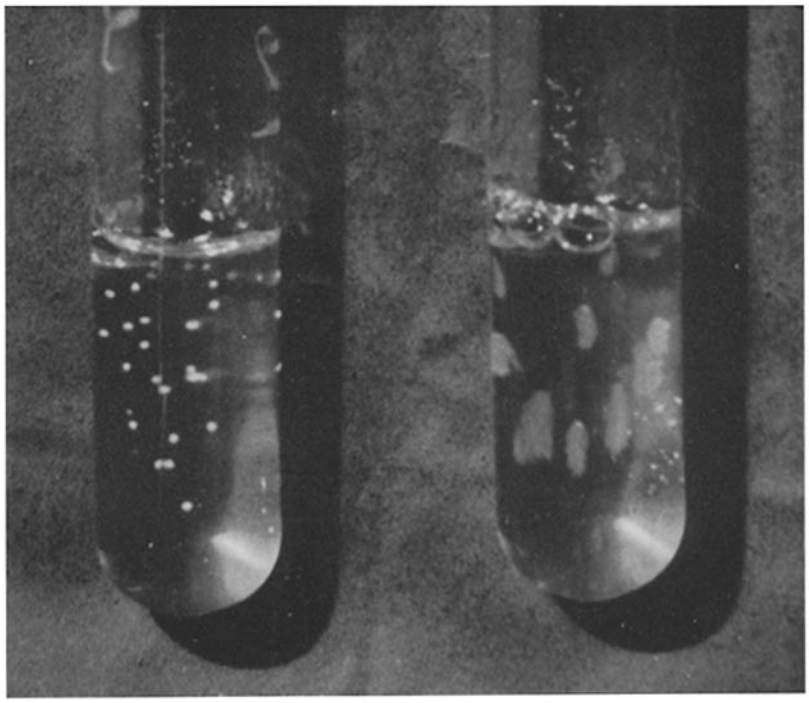

FIG. 1

(Mirick *et al.*: Non-hemolytic streptococcus from respiratory tract. II)