A TYPE-SPECIFIC PROTEIN FROM PNEUMOCOCCUS*

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Though the presence of protein antigens in pneumococcus has long been recognized, type specificity within this species has been defined by capsular carbohydrates. Much of the work on protein antigens of pneumococcus has dealt with a nucleoprotein fraction described by Avery and his coworkers (1), and there is general agreement that this material exhibits species specificity. In quantitative studies of pneumococcus R-anti-R systems, however, Heidelberger and Kabat (2) obtained results indicating the existence of a minor type-specific protein antigen in a rough variant of pneumococcus type II (D39R), and later Heidelberger (3) suggested that the results permitted envisaging the possible existence, in non-encapsulated pneumococci, of an antigen that possesses type specificity.

In the hemolytic streptococci of Lancefield group A, in contradistinction to pneumococcus, protein antigens play a primary rôle in the determination of type specificity (4). Of these protein antigens, the M substance isolated by Lancefield, which belongs to the class of alcohol-soluble proteins, is of especial importance. Proteins of this class have been found in several bacterial genera (5) and their presence in pneumococcus has been noted heretofore (6). The present studies are concerned with the properties and type specificity of such alcohol-soluble M proteins of pneumococcus and with the demonstration of their marked similarity to the M proteins of group A streptococci.

Materials and Methods

Preparation of Anti-M Protein and Anti-C Carbohydrate Sera.—Sera were prepared according to the method employed by Goebel and Adams (7) for the preparation of pneumococcus C carbohydrate antisera. Griffith rough variants of the pneumococci studied were incubated overnight in neopeptone broth at 37°C., collected by centrifugation in the cold (4°C.), and resuspended in a volume of normal saline one-tenth that of the original culture. The vaccine was heated then at 100°C. for 5 minutes. Rabbits weighing 2 to 3 kilos were immunized daily for 6 days by intravenous injection of 1 cc. of the vaccine and were bled from the heart on the 6th day following the final injection. Two to four courses of immunization resulted usually in the production of satisfactory sera which possessed antibodies against the somatic C carbohydrate of pneumococcus as well as against the type-specific M protein. An antipneumococ-

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cus type XIX horse serum prepared by the New York City Department of Health and possessing a high titer of anti-C carbohydrate antibody was also employed.

Preparation of M Protein Extracts.—Extracts of pneumococci were made according to the method of Swift, Wilson, and Lancefield (8). To obtain a heavy growth of organisms for extraction, 1 per cent glucose was added to neopeptone broth cultures of pneumococci after incubation overnight and the acid formed on subsequent incubation was neutralized intermittently with 3 N NaOH. The organisms were collected by centrifugation in the cold in an angle centrifuge prior to extraction. To the packed cells, sufficient N/5 HCl was added to adjust the pH to 2.0–2.4. The acid suspension was heated in a boiling water bath for 15 minutes, cooled, centrifuged, and the supernatant decanted. To the clear supernatant, a drop of 0.01 per cent phenol red was added and the solution was adjusted to neutrality with N/2 NaOH. The cloudy solution was centrifuged again and the resultant clear supernatant was used for testing. The extract thus obtained contains the M protein, the somatic or C carbohydrate, and if an encapsulated organism is employed, the capsular carbohydrate as well.

Partial Purification of M Protein.—Two methods were employed to separate the M protein from C carbohydrate present in the cell extracts described. The addition of 1 to 2 volumes of 95 per cent ethyl alcohol to the neutralized extract precipitated the M protein and left most of the somatic carbohydrate in solution. This could be precipitated by the addition of 4 to 5 volumes of alcohol. The precipitates were collected by centrifugation and were readily soluble in normal saline.

The M protein was purified partially also by the picrate method that Dodds and Dickens (9) employed for the purification of insulin. To 2 volumes of cell extract was added 1 volume of saturated picric acid solution. The precipitate was collected by centrifugation and extracted with 2 volumes of 70 per cent aqueous acetone. To the acetone extract were added an equal volume of water and saturated picric acid. The second precipitate was collected and treated with 1 to 2 volumes of acid alcohol (95 per cent C_2H_5OH, 3 volumes, 3 N HCl, 1 volume). The acid alcohol extract was added to 20 volumes of cold acetone from which the hydrochloride of the M protein precipitated in flocculent particles after several hours. The precipitate was collected by centrifugation and was readily soluble in normal saline.

Precipitin and Agglutination Tests.—Precipitin tests were carried out either in small test tubes or in capillary tubes according to the method of Swift, Wilson, and Lancefield (8). With the test tube technique, 0.3 cc. of varying dilutions of cell extract was layered over 0.3 cc. of diluted serum containing 2 parts of serum and 3 parts of normal saline. The tubes were incubated for 1 hour at 37°C. and the reactions read after standing overnight in the ice box. With the capillary method, undiluted serum was used and the reactions read after standing for 16 hours at room temperature. Agglutination tests were carried out with a constant amount of vaccine suspended in normal saline or in saline buffered with M/50 phosphate buffer pH 7.2 and with serial dilutions of serum. Tests were read after 1 hour's incubation at 37°C. and overnight incubation at 4°C.

Strains of Pneumococcus.—In the work reported in this paper and in the following one (10), the naming of the colonial variants of pneumococcus employed is attended with certain difficulties. Two nomenclatures for the colonial variants of pneumococcus are already extant. That of Griffith (11) includes only the encapsulated form designated by the letter S (smooth) and the more widely known of the unencapsulated forms designated by the letter R (rough), selected by growing encapsulated cells in antiserum directed against the capsular polysaccharide. This terminology has enjoyed general use in the description of pneumococcal strains. In 1934, Dawson (12) reported the existence of a second unencapsulated variant of pneumococcus, and to bring the nomenclature of pneumococcal colonial forms into conformity with that of other bacterial species, he suggested that encapsulated forms be designated by the letter M (mucoid), the previously described unencapsulated form by the letter S (smooth), and the newly described unencapsulated form by the letter R (rough) by virtue of its distinctive colon-

ial morphology and autoagglutinable properties. There have been few reports concerning all three colonial variants of pneumococcus and the terminology of Dawson has not been used widely. It is unfortunate that the nomenclatures of Griffith and of Dawson employ the same symbols to designate different colonial forms, for inevitable confusion is created thereby. In the strains to be reported, variants designated smooth by Griffith and mucoid by Dawson will be referred to as encapsulated forms. The more generally recognized unencapsulated variant known as rough in Griffith's nomenclature and smooth in Dawson's terminology, will be designated by the term Griffith rough, and the less widely studied unencapsulated variant described first by Dawson will be described by the term Dawson rough. This terminology, while admittedly unsatisfactory, should minimize ambiguity. A more satisfactory nomenclature awaits a better understanding of colonial variation. Appended below is a list of the strains of pneumococci used in the present studies. Encapsulated strains are grouped by capsular type and unencapsulated strains are described immediately after the parent strain.

Capsular Type I

I-SV1: an encapsulated strain carried for many years in the laboratory.
I-SV1-P86: an encapsulated, sulfonamide-resistant strain derived from I-SV1 in 1938.
I-192R: a Griffith rough strain derived from I-SV1 and carried for many years in the laboratory.
I-192RD: a Dawson rough strain derived from I-192R in 1948.
I-Rack: an encapsulated strain carried for several years in the laboratory.
I-Moody: an encapsulated strain carried for several years in the laboratory.
I-JHHA: an encapsulated strain recovered from a brain abscess in Baltimore in 1948.
I-JHHAR: a Griffith rough variant derived from strain I-JHHA.
I-JHHB: an encapsulated strain isolated from a patient with lobar pneumonia in Baltimore in 1948.

Capsular Type II

II-D39S: an encapsulated strain carried for many years in the laboratory.
II-R36A, II-R36T, and II-R36NC: three colonial variants of the Griffith rough strain II-R36 derived from II-D39S.
II-R36ND: a Dawson rough variant derived from II-R36NC in 1948.
II-BW: an encapsulated strain isolated from a patient with lobar pneumonia in New York in 1948.
II-BWR: a Griffith rough variant derived from strain II-BW.
II-BG and II-BS: two encapsulated strains isolated from patients with lobar pneumonia in New York in 1948.
II-Rat: a strain isolated from a rat during an epizootic in Baltimore in 1947.
II-RatR: a Griffith rough variant derived from strain II-Rat.
II-JHH: an encapsulated strain isolated from a patient with lobar pneumonia in Baltimore in 1948.

Capsular Type III

III-A66 and III-SV3: encapsulated strains carried for many years in the laboratory.
III-A66R2: a Griffith rough variant of strain III-A66.
III-L and III-H: two encapsulated strains isolated from throat cultures in New York in 1948.
III-Rack: an intermediate encapsulated strain carried for several years in the laboratory.

Capsular Type VIII

VIII-H: an encapsulated strain carried for several years in the laboratory.
VIII-R13: a Griffith rough variant derived from strain VIII-H.
VIII-RIH: an encapsulated strain carried for several years in the laboratory.
VIII-Romano: an encapsulated strain isolated from a patient with lobar pneumonia in New York in 1948.
VIII-Marino: an encapsulated strain isolated from a patient with meningitis in New York in 1948.

Capsular Types IV, V, VII, IX, XA, XIV, XVII, XIX, XXIVA

All these strains are strains of encapsulated organisms carried in the laboratory for several years.

EXPERIMENTAL

Type Specificity of Anti-M Protein Sera.—Sera were prepared by immunizing rabbits by the method described in the preceding section with heat-killed vaccines of Griffith rough pneumococcal strains I-192R, I-JHHAR, II-R36T, II-BWR, III-A66R2, and VIII-R13 derived respectively from pneumococci of capsular types I, I, II, II, III, and VIII. These sera, when tested before absorption with extracts of homologous and heterologous pneumococci, gave heavy precipitates with the homologous extracts and also of varying but lesser amount with heterologous extracts. By absorbing the sera with the mass growth of one or more strains of heterologous Griffith rough organisms, it was possible to remove the anti-C carbohydrate and other species-specific antibodies and also cross-reacting anti-M protein antibodies. In addition, to insure the absence of antibody against the capsular carbohydrate of the parent strain, the sera were absorbed with 0.1 volume of a 1:10,000 solution of capsular carbohydrate. Such absorbed sera, when tested by either the capillary or test tube precipitin technique, reacted specifically with cell extracts of the homologous strain (Table I). The type-specific designations of the M proteins studied are recorded in Arabic numerals below the names of the pneumococcal strains employed in making anti-M sera. Subsequent reference to anti-M sera will be to sera absorbed with the strains listed in column 2 of Table I unless otherwise noted.

Three additional facts of interest are recorded in Table I. Although strains I-192R and I-JHHAR were both derived from organisms of capsular type I, they possess different M proteins. A similar difference in antigenic composition is manifested by the two strains II-R36T and II-BWR both derived from organisms of capsular type II. In addition, there is recorded a cross-reaction between the sera and extracts of strains I-JHHAR and II-BWR. When the two sera are absorbed reciprocally, precipitating antibodies for both homologous and heterologous strains are removed. It appears, therefore, that organisms of the same capsular type may be associated with different M proteins and that

organisms of different capsular type may possess the same or closely similar proteins. There is shown also in Table I the absence of serological relationship between the M proteins of pneumococci of capsular types III and VIII. This finding is of interest in view of the known immunological relationship of the capsular polysaccharides of these two types.

TABLE I

Type Specificity of Pneumococcus Anti-M Protein Sera as Demonstrated by Capillary Precipitin Tests

Antiserum prepared against strain	Antiserum absorbed with strains	Extract						Pneumococcus C carbohydrate 1:50,000	Homologous capsular SSS 1:50,000	Saline control
		I-192R	I-JHHAR	II-R36A	II-BWR	III-A66R2	VIIIR13			
I-192R Type 1M	III-A66R2 VIII-R13	++++	−	−	−	−	−	−	−	−
I-JHHAR Type 2M	I-192R II-R36A VIII-R13	−	++	−	++	−	−	−	−	−
II-R36T Type 2'M	I-192R III-A66R2 VIII-R13	−	−	++++	−	−	−	−	−	−
II-BWR Type 2M	II-R36T III-A66R2 VIII-R13	−	++	−	++	−	−	−	−	−
III-A66R2 Type 3M	I-192R II-R36A	−	−	−	−	++++	−	−	−	−
VIII-R13 Type 8M	I-192R	−	−	−	−	−	++	−	−	−

In Table II are shown the reactions of the sera listed above with extracts of 34 pneumococcal strains including 13 capsular types and their derivatives. All the strains of capsular types III and VIII examined reacted specifically with antisera prepared against a Griffith rough variant derived from the laboratory strain of the homologous capsular type. Strains of capsular type I and II, on the other hand, showed heterogeneity of their M protein component. Two different proteins were found associated with each of these capsular types and in addition, strains of both types I and II were encountered that failed to react with antisera against any of these proteins.

Relation of M Protein to Colonial Variation.—The relation of M protein to colonial variation is shown also in Table II. This type-specific component

was present in the cells of both encapsulated and Griffith rough colonial variants. Its presence in quantity in the latter colonial form distinguishes it from the M protein of group A β-hemolytic streptococci, for little or no type-specific protein is found in the glossy variant of streptococcus which has been considered to be the colonial analogue of the Griffith rough pneumococcus (13). Further degraded rough colonial variants of pneumococcal strains I-192R and II-R36NC, obtained by the method of Dawson, were studied also for the presence of M protein. Although the component could not be detected occasionally in Dawson rough variants derived from pneumococcus I-192R, consistently deficient cultures were not obtained.

Capillary Precipitin Reaction

Antiserum (absorbed as in Table I)	I 192R	I SV1 P86	I 192RD	I Rack	I Moody	I JHHA	I JHHB	II D39S	II R36A	II R36N	II R36ND	II BW
I-192R (Type 1M)	++++	++++	±	++++	−	−	+++	−	−	−	−	−
I-JHHAR (Type 2M)					−	++	−					
II-R36T (Type 2'M)	−	−		−	−	−		++++	++++	++++	++++	−
II-BWR (Type 2M)									−			+++
III-A66R2 (Type 3M)	−	−		−	−	−		−	−	−	−	−
VIII-R13 (Type 8M)	−	−		−	−	−		−	−	−	−	−

Species Specificity of Pneumococcus C Carbohydrate.—In all the pneumococcal strains tested, regardless of colonial morphology, the presence of the species-specific C carbohydrate could be demonstrated. For this purpose, the use of the Lancefield extract fraction precipitated by concentrations of alcohol between 70 per cent and 83 per cent and redissolved in normal saline proved most suitable (Table III). Although solutions prepared in this manner reacted occasionally with C-absorbed anti-C rabbit sera, the reactions were always less intense than with unabsorbed sera. No cross-reactions of pneumococcus C carbohydrate were noted with sera containing antibodies against the C carbohydrates of β-hemolytic streptococci of Lancefield's groups A, B, C, D, E, F, G, H, and L; nor were reactions noted between pneumococcus C carbohydrate antisera and extracts of *Streptococcus MG*, *Streptococcus salivarius*, and a strain of *Streptococcus mitis*. Antisera prepared in either the horse or the rabbit can be used to test for the presence of C carbohydrate and the test appears to be a useful aid in the recognition of unencapsulated pneumococci.

Chemical Properties of Pneumococcus M Protein.—That the type-specific substance present in unencapsulated as well as in encapsulated pneumococci is a protein similar in its properties to those of the M protein of group A streptococci

(14) is revealed by the following data. The M protein can be extracted from pneumococcal cells by hot acid solutions in which it remains soluble. It is soluble in acid ethyl alcohol but is precipitated by ethyl alchol from neutral solutions. The serological reactivity of the material is destroyed by proteolytic enzymes as revealed in the following experiment:—

To 12.5 cc. of an extract of pneumococcus I-SV1 was added 12.5 cc. of 95 per cent ethyl alcohol. The precipitate was collected by centrifugation, redissolved in 6 cc. of acid saline, and the pH adjusted to 1.95. To two 2 cc. aliquots of the solution were added respectively 2 cc. of active and of heat-inactivated 2 per cent solutions of commercial crude pepsin, pH 2.5. After incubation for 2 hours at 37°C., the solutions were neutralized with 1 N NaOH and

l M Extracts and Anti-M Sera

Extract type

Kat-K	II JHH	III A66	III SV3	III Rack	III L	III H	IV	V	VII	VIII H	VIII Marino	VIII Romano	VIII RIH	IX	XA	XIV	XVII	XIX	XXIVA
·	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
·	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
·	–																		
·	–	++++	+++	+++	+++	+++	–	–	–	–	–	–	–	–	–	–	–	–	–
·	–	–	–	–	–	–	–	–	±	++	+++	+++	+++	–	–	–	–	–	–

cleared by centrifugation. Serological reactions of the materials treated in these ways are shown in Table IV.

The serological activity of the type-specific substance is lost almost completely after 2 hours' exposure to an active solution of pepsin. Similar results are obtained with the use of commercial crude trypsin.

Like the M proteins of group A streptococci, the type-specific proteins of pneumococci form acetone-soluble picrates. When the procedure of picrate precipitation outlined in a preceding section is carried out, partially purified preparations of M protein of high serological activity can be obtained from extracts of encapsulated or of Griffith rough variants. One such preparation, when examined with the ultraviolet spectrophotometer, contained approximately 98 per cent protein, 2 per cent nucleic acid. Whether or not pneumococcus M protein is antigenic when treated by these methods has not yet been determined.

Agglutinating Properties of Pneumococcus M Protein Antisera.—M protein antisera which have been absorbed with heterologous Griffith rough strains show selective agglutination of Griffith rough variants of the homologous strain (Table V). When such a serum is absorbed further with a solution of ho-

TABLE III

Precipitin Reactions of Ethanol-Precipitated Fractions of Lancefield Extracts of Pneumococci with Pneumococcus Anti-C Carbohydrate Rabbit Antiserum before and after Absorption with C Carbohydrate

Serum	Extract dilution (final)	Extract																Controls	
		I SVI	I 192R	I 192RD	II D39S	II R36A	IV	V	VII	IX	XA	XII	XVII	XIX	XX	XXIVA	C carbohydrate*	Saline
Pneumococcus anti-C rabbit serum	1:2	++	++	+	++	+++	++	+++	+++	+++	+++	+++	++	++	++	++	++++	−
	1:20	+	+		±	++	±	±	+	++	+	+	++	++	±	+	+++	
C-absorbed pneumococcus anti-C rabbit serum	1:2	+	−	−	−	++	−	−	−	+	−	−	−	−	−	−	−	−
	1:20	−	−		−	−	−	−	−	−	−	−	−	−	−	−	−	

* Pneumococcus C carbohydrate solution 1:25,000 diluted 1:2 and 1:20. Extract fraction precipitated by concentrations of alcohol between 70 and 83 per cent. Precipitin reactions performed with test tube technique.

TABLE IV

Effect of Pepsin on M Protein of Pneumococcus M Precipitin Reactions

Extract I-SVI	Type 1 M rabbit antiserum
Untreated extract	++++
Pepsin-treated extract	±
Inactive pepsin-treated extract	+++±
Saline control	−

Precipitin reactions performed with test tube technique.

TABLE V

Agglutination of Griffith Rough Pneumococci by Pneumococcus Type 1 M Rabbit Antiserum

Vaccine used in agglutination test	Antiserum* dilution									Saline control
	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	1:10,240	
I-192R	++	++++	++++	++++	++++	++++	++++	+++	−	−
II-R36A	++	+	−	−	−	−	−	−	−	−
III-A66R2	++	+	+	−	−	−	−	−	−	−

* Antiserum prepared against strain I-192R and absorbed with strains II-R36A and III-A66R2.

mologous M protein purified partially by the picrate method so that it gives no longer a positive precipitin reaction, it agglutinates the homologous strain less well than previously but to a higher titer still than the heterologous strains.

Whether this finding indicates that the treatment of the M protein has altered it appreciably from its native state, the incomplete absorption of M antibody or the presence of an additional type-specific agglutinin cannot be stated at this time. M protein antisera do not agglutinate encapsulated variants of the homologous strain nor do they give rise to a quellung reaction with homologous encapsulated or unencapsulated organisms.

TABLE VI

Loss of Agglutinability of Pneumococcus I-192R by Homologous Anti-M Serum Following Exposure to Pepsin

Vaccine used in agglutination reaction	Antiserum* dilution									Saline control
	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5160	1:10,240	
Pepsin-treated cells 2 hrs. at 37°C.	+	−	−	−	−	−	−	−	−	−
Inactive pepsin-treated cells 2 hrs. at 37°C.	++++	++++	++++	++++	++++	+++	+++	+	−	−

* See footnote to Table V.

TABLE VII

Disappearance of M Protein from Pneumococcal Cells Following Exposure to Pepsin M Precipitin Reactions with Cells of Pneumococcus I-192R

Extract from:	Antiserum *vs.* pneumococcus I-192R
Pepsin-treated cells 2 hrs. at 37°C.	−
Inactive pepsin-treated cells 2 hrs. at 37°C.	++++

Capillary tube technique.

Effect of Proteolytic Enzymes on the M Protein of Intact Pneumococcal Cells.—As is true of the M proteins of group A streptococci (15), those of pneumococcus are susceptible in the intact cell to the action of proteolytic enzymes. When heat-killed pneumococcal cells are subjected to the action of commercial crude pepsin, their agglutinability by homologous anti-M serum is almost completely lost and no M protein can be demonstrated in extracts made from cells so treated (Tables VI and VII). The morphology of the cells and their reaction to the Gram stain are unaltered by exposure to the enzyme under these conditions.

Lack of Protective Effect of Pneumococcal M Protein Antisera.—M protein antisera appear not to have an important effect upon the virulence of homologous encapsulated pneumococci. Mice inoculated intraperitoneally with 1 cc. of anti-type 1 M serum 24 hours before intraperitoneal injection with a

10^{-7} dilution of a fully virulent culture of pneumococcus I-SV1 failed to survive longer than mice injected with normal rabbit serum or than untreated controls (Table VIII).

Absence of Cross-Reactions among Pneumococcal and Streptococcal M Proteins.—Because of the similar chemical properties of the type-specific M proteins of pneumococci and of group A streptococci, extracts of pneumococcus strains I-192R, II-R36A, III-A66R2, and VIII-R13 were tested with antisera against group A streptococci types 1, 3, 5, 6, 14, 17, 18, 19, 23, 24, 26, 28, 30, 31, 36, and 42 and absorbed sera against the same pneumococcus strains were tested with extracts of group A β-hemolytic streptococci types 1, 2, 3, 4, 5, 11, 12, 13, 14, 15, 17, 19, 22, 23, 24, 26, 28, 29, 30, 31, 33, 36, 37, 41, 43, 44, and 46. No cross-reactions were noted.

TABLE VIII

Failure of Anti-M Serum to Protect Mice Infected with Homologous Encapsulated Pneumococci

Strain of pneumococcus	Amount of culture injected	Serum injected	D/S*
	cc.		
I-SV1	10^{-7}	1 cc. type 1 M rabbit antiserum	12/0
I-SV1	10^{-7}	1 cc. normal rabbit serum	12/0
I-SV1	10^{-7}	None	12/0
I-SV1	10^{-8}	None	5/1

Serum injected intraperitoneally 24 hours before inoculation intraperitoneally with pneumococcus culture. Type 1 M antiserum prepared against strain I-192R, absorbed with C carbohydrate.

* D/S, ratio of dead to surviving mice.

DISCUSSION

The present studies reveal the hitherto unrecognized type specificity of a protein antigen of pneumococcus. This antigen is present in both encapsulated and unencapsulated colonial variants and unlike the analogous type-specific protein of several groups of streptococci (12), it is found in most cultures of Dawson rough variants. The M proteins of pneumococcus are an important factor in determining the antigenic heterogeneity of unencapsulated members of this species. All their chemical properties that have been studied so far appear similar to those of the M proteins of group A streptococci. The proteins of both species are soluble in hot 0.2 N acid, and in acid ethyl alcohol and the members of both groups form acetone-soluble picrates. The M proteins of both species appear to be located at or near the surface of the cell because they are destroyed by the action of proteolytic enzymes which do not produce concomitant morphological changes, and in both species they function as agglutinogens and precipitinogens. Despite these many similarities, no cross-reac-

tions have been noted yet between pneumococcal and streptococcal M proteins but it does not seem unlikely that such may be found.

Unlike the M proteins of group A streptococci, the M proteins of pneumococcus appear to play a negligible rôle in the determination of pneumococcal virulence. Evidence of this fact may be drawn from the inefficacy of anti-M serum in protecting the white mouse against infection with encapsulated pneumococci of the homologous type. Further evidence of this negative correlation will be reported in the following paper (10).

Examination of a number of strains of pneumococci of the same capsular type shows that the same capsular carbohydrate may be associated in different strains with dissimilar M proteins. This observation suggests that the synthesis and functions of the capsular carbohydrate and M protein may be unrelated. It creates also a taxonomic problem. Because the same or closely similar proteins seem to be present in organisms of the same capsular type isolated recently, it is suggested tentatively that the protein occurring in such organisms be given the Arabic number corresponding to the Roman numeral designating the capsular type. Proteins found subsequently to be associated with the same capsular carbohydrate may be designated by prime, double prime, or triple prime notations in the order of their identification. The association of different M proteins with pneumococci of the same capsular type is a finding of potential interest in the study of pneumococcal disease, for this phenomenon may provide a tool for the recognition of dissimilarity of organisms of the same capsular type which manifest differences in epidemiological behavior.

The presence of the M protein in pneumococcus provides an additional feature of similarity between members of this species and the streptococci. In addition, it has been shown that the C carbohydrate of pneumococcus can be used for identification of this species in a manner similar to that employed in the classification of certain of the streptococci. These observations lend support to those who would classify pneumococcus in the genus *Streptococcus* rather than under the separate generic name *Diplococcus*.

SUMMARY

The isolation and characterization of a type-specific M protein from pneumococcus are described. This protein is similar chemically in all respects studied to the M proteins of group A streptococci. No immunological cross-reactions have been observed, however, between M proteins of the two species.

Strains of capsular type I pneumococcus have been encountered which contain different M proteins. The same is true for capsular type II pneumococcus. It is apparent, therefore, that the capsular polysaccharides and M proteins can vary independently of each other.

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