

STUDIES ON THE ANTIGENIC COMPOSITION OF GROUP A  
HEMOLYTIC STREPTOCOCCI

III. TYPES WITH SEROLOGICALLY IDENTICAL M BUT DISTINCT T ANTIGENS:  
TYPES 10 AND 12\*†

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Since the classification of hemolytic streptococci into groups (1) and the further subdivision of group A, the chief pathogens for man, into serological types (2), there have been certain difficulties in interpreting the results of the type differentiations. The demonstration of two type-specific components, designated as M and T (3), in most strains of group A streptococci has made it possible to explain many of these. A knowledge of the relationship of these two antigens in the respective types is therefore important, particularly in making possible more accurate epidemiological studies of human streptococcal infections.

Group A hemolytic streptococci are generally in the matt phase when isolated from human infections. This variant is characterized by the presence of the M antigen, which is associated with virulence of the organism. The glossy variant, on the other hand, is rarely, if ever, isolated from active infections. It is avirulent for mice, and also appears degraded in that the M substance is absent or present in only minute amounts. Usually glossy, avirulent streptococci and the matt variants both contain the more recently recognized type-specific substance, T, which is apparently not related to virulence.

The M antigen, protein in nature (4), can be separated from the bacterial cells, in the matt phase, and demonstrated in properly prepared bacterial extracts by the precipitin test. The corresponding antibodies to this substance appear to be responsible for the type-specific protection in animals and usually

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agglutinate homologous type matt organisms. The T antigen, on the contrary, has not so far been obtained in cell-free extracts and can be demonstrated only by the agglutinin test. Antibodies to the T substance may often agglutinate either the matt or glossy variant, and in some types, are responsible for the type-specific agglutination reaction. These antibodies apparently do not exhibit protective action.

In most of the epidemiological studies of human streptococcal infections reported thus far, the slide agglutination technique, described by Griffith, has been employed. Many investigators using this method of typing have experienced difficulty with cross reactions, and several (2, 5-11) have noted consistent cross reactions existing among certain types. Moreover, in our experience typing results with the agglutination method sometimes disagreed with those obtained by the precipitin technique. Many of the cross reactions, as well as differences in the results obtained by the two methods for typing, may be explained on the varying relationship of the two antigens, M and T, present in many strains of group A hemolytic streptococci (12).

The present report is concerned with the relationship of the M and T antigens in types 10 and 12. It is based on precipitin, agglutination, and mouse protection tests with unabsorbed and reciprocally absorbed antisera obtained from rabbits immunized with the matt forms of types 10 and 12. Since preliminary tests indicated that type 10 and type 12 matt strains contained similar, if not identical M antigens, and unrelated type-specific T antigens, it seemed important to investigate in more detail the specific relationship existing between these two types. In addition to the standard type strains studied, twenty-three other type 10 or 12 strains collected from various sources were tested to ascertain whether the type-specific antigenic composition of these was similar to one or the other of the representative type strains.

#### *Materials and Methods*

Strain N.Y. 5, recovered by Dochez from a patient with scarlet fever, was used here, as in Griffith's laboratory, as the representative type 10 strain. It has been maintained on culture media and in the dried state (13) in this laboratory for many years; and although used as the glossy variant, proved to contain a small amount of M protein. The matt virulent variant, designated as E 14, was derived from strain N.Y. 5, by animal passage.

Strain S.F. 42, recovered originally from a patient with scarlet fever, was taken as the representative type 12 strain. It was obtained from Dr. F. Griffith in an avirulent, glossy phase and has been maintained in this laboratory on culture media and in the dried state. The matt virulent variant was derived from strain S.F. 42 by mouse passage.

In Table III, the other type 10 and type 12 strains tested are also listed and their sources designated.

The methods of preparing antisera (14) and M extracts (15) were those previously

described. The antisera were absorbed with streptococci killed by heating for 30 minutes at 56°C. Three parts of undiluted serum were mixed thoroughly with one part of packed bacteria and the mixture was incubated at 37°C. for 30 minutes. The serum was then removed after centrifugation at high speed; and merthiolate, one part to 10,000 parts of serum, was added. In certain instances reabsorptions, using four to five parts of serum to one part of packed bacteria, were necessary.

The techniques used in the precipitin (15) and agglutinin (3) tests are described elsewhere. Suspensions for the agglutinin tests were prepared from cultures grown in modified Todd-Hewitt broth (16) at room temperature for 12 to 18 hours. The bacteria were separated from the cultures by centrifugation and resuspended in the desired concentration in fresh broth.

The Rockefeller Institute strain of mice, 3 to 4 weeks old, and weighing about 20 gm., was employed for all protection tests. One-half cc. of antiserum was injected intraperitoneally into each mouse 12 to 18 hours before intraperitoneal inoculation with the test culture. The latter consisted of progressive dilutions, in modified Todd-Hewitt broth, of blood broth cultures incubated for 12 to 15 hours at 37°C.

#### EXPERIMENTAL

Preliminary tests showed that the type 12 matt variant, obtained by mouse passage, could not be differentiated from the type 10 matt strain by the precipitin test (M-anti-M reaction): but with properly absorbed antisera, these two types were readily identified by the agglutinin reaction.

The results of the agglutinin and precipitin tests made with type 10 and type 12 matt antisera are recorded in Table I. A type 14 matt virulent strain and the homologous type 14 antiserum were used as heterologous type controls throughout. No significant cross reactions were observed between the heterologous control strain and the representative type 10 and 12 strains or their respective matt antisera.

It is clear from Table I, that M extracts prepared from the matt variants of type 10 and type 12 reacted equally well with the antisera of either type. The results of the agglutinin tests agreed with the results of the precipitin reactions as demonstrated by the cross agglutination between the matt variants of types 10 and 12. With each type strain, however, in contrast to the precipitin reaction, the agglutinin test was more marked with its homologous type antiserum due to the additional type-specific T-anti-T reaction. The type 10 glossy variant contained a small amount of M protein as demonstrated by the positive precipitin tests with both type 10 and 12 matt antisera and the cross agglutination with type 12 matt antiserum. The type 12 glossy strain on the other hand contained no demonstrable M protein by the precipitin test and gave a positive agglutinin reaction only with the homologous type antiserum.

The reciprocal absorption experiments recorded in Table I show that the M antibodies of both types 10 and 12 antisera were equally well absorbed by the matt strains of either type 10 or type 12. Absorption with the homolo-

gous type matt variants removed both M and T antibodies as demonstrated by the negative precipitin and agglutinin tests for the matt and glossy forms of both types. However, absorption with the heterologous type 10 or type 12 matt strain removed only the M antibodies as shown by the negative precipitin

TABLE I  
*Reciprocal Absorption Experiments, Types 10 and 12*  
*Agglutinin and Precipitin Reactions\**

Antiserum prepared against matt variant		Strain used for suspensions and M extracts							
		Type 10 matt	Type 10 glossy	Type 12 matt	Type 12 glossy				
Type	Treatment of antisera	Results of immunological reactions							
		Agglu- tinin	Preci- pitin	Agglu- tinin	Preci- pitin	Agglu- tinin	Preci- pitin	Agglu- tinin	Preci- pitin
10	Not absorbed	++++	+++	++++	++	+++	+++	++++	±
	Absorbed with type 14 matt	++++	++	++++	++	++	++	-	-
	“ “ “ 10 “	-	±	-	-	-	-	-	-
	“ “ “ 10 glossy	++	+	±	-	++	±	-	-
	“ “ “ 12 matt	++++	±	++++	-	-	-	-	-
	“ “ “ 12 glossy	++++	++	++++	++	++	-	-	
12	Not absorbed	+++	++++	++	++	++++	++++	++++	-
	Absorbed with type 14 matt	+++	+++	++	++	+++	+++	+++	-
	“ “ “ 10 “	-	-	-	-	+++	-	+++	-
	“ “ “ 10 glossy	++	+	+	+	+++	+	+++	-
	“ “ “ 12 matt	-	-	-	-	-	-	-	-
	“ “ “ 12 glossy	++	++	++	++	+++	++	-	

For the agglutinin tests, 0.5 cc. amounts of the bacterial suspension were added to serial dilutions of the antiserum. The final dilutions of the antiserum ranged between 1:20 and 1:1280.

For the precipitin tests, M extracts undiluted and in dilutions of 1:4 and 1:16 were set up with a constant amount of the antiserum.

++++ to - indicates estimates of the degree of reaction in the agglutinin and precipitin tests.

A type 14 matt strain and its homologous antiserum were used as a heterologous type control for the precipitin and agglutinin tests. No significant cross reactions were observed. Also normal serum and broth or saline controls were included in every experiment. These controls were invariably negative.

\* For details of technique, see Methods.

and cross agglutination reactions with the matt variants and the positive agglutinin tests with the homologous type matt and glossy forms. An exception to this, probably due to a technical error in failing to absorb the antiserum completely, was found when the precipitation tests with type 10 M extract were performed with the lots of type 10 antisera absorbed respectively with the type 10 and 12 matt variants. On the other hand, absorption of the type 10 and type 12 antisera with the homologous type glossy strains removed only

TABLE II  
*Reciprocal Absorption Experiments, Types 10, 12, and 14 Passive Protection Tests in Mice*

Type	Antiserum prepared against matt variant	Treatment of sera	Mice inoculated with*															
			Type 10 matt culture			Type 12 matt culture			Type 14 matt culture			Type 14 matt culture						
			10 <sup>-1</sup>	10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-6</sup>	10 <sup>-7</sup>	10 <sup>-8</sup>	10 <sup>-9</sup>	10 <sup>-10</sup>	10 <sup>-11</sup>	10 <sup>-12</sup>	10 <sup>-13</sup>	10 <sup>-14</sup>		
10	Not absorbed	Absorbed with type 10 matt	D1 D1	D1 D1	D1 D1	D1 D1												
			D1 D1	D1 D1	D1 D1	D1 D1												
	Absorbed with type 10 matt	Absorbed with type 10 glossy	D3 S	D3 D3	D3 D3	D3 D3	D3 S	D3 S	D3 S	D3 S								
			D3 S	D3 D3	D3 D3	D3 D3	D3 S	D3 S	D3 S	D3 S								
12	Not absorbed	Absorbed with type 12 matt	D1 D1	D1 D1	D1 D1	D1 D1												
			D1 D1	D1 D1	D1 D1	D1 D1												
	Absorbed with type 12 matt	Absorbed with type 12 glossy	D2 D2	D2 D2	D2 D2	D2 D2	D2 D2	D2 D2	D2 D2	D2 D2								
			D2 D2	D2 D2	D2 D2	D2 D2	D2 D2	D2 D2	D2 D2	D2 D2								
14	Not absorbed	Absorbed with type 14 matt	D1 D1	D1 D1	D1 D1	D1 D1												
			D1 D1	D1 D1	D1 D1	D1 D1												
	Absorbed with type 14 matt	Absorbed with type 14 glossy	D2 D2	D2 D2	D2 D2	D2 D2	D2 D2	D2 D2	D2 D2	D2 D2	D2 D2	D2 D2	D2 D2	D2 D2	D2 D2	D2 D2	D2 D2	D2 D2
			D2 D2	D2 D2	D2 D2	D2 D2	D2 D2	D2 D2	D2 D2	D2 D2	D2 D2	D2 D2	D2 D2	D2 D2	D2 D2	D2 D2	D2 D2	D2 D2
Controls (no serum)			D1 D1	D1 D1	D1 D1	D1 D1												
Normal rabbit serum (merthiolate 1:10,000)			D1 D1	D1 D1	D1 D1	D1 D1												

Same lots of absorbed sera used for protection tests that were used for serological reactions in Table I and Table III.

Blank indicates no test. S indicates survival for at least 2 weeks. D with numeral indicates death within that number of days.

\* For details of technique, see Methods.

the T antibodies as demonstrated by the positive precipitin and agglutinin reactions with the matt variants of both types 10 and 12 and the negative agglutinin tests with the homologous type glossy forms. Absorption with the heterologous type 10 or 12 glossy strain, however, failed to remove either the M or T antibodies as shown by the positive precipitin and agglutinin tests with the matt variants of both types and the positive agglutination reactions with the homologous type glossy and matt forms. Following absorption with the type 10 glossy strain the M antibody titer of both type 10 and type 12 antisera was reduced because this strain, presumably degraded from the matt form in which phase it was originally isolated, still contained some M substance.

From the results of the precipitin and agglutinin tests, the type 10 and 12 strains appeared to contain serologically identical M antigens and unrelated type-specific T antigens. It is now known that virulent strains elaborate relatively large amounts of M substance, and that sera with good type-specific protective capacity have high anti-M precipitin titers. On the other hand, there is no evidence as yet to indicate that the T antibody is concerned with protection. A series of experiments was, therefore, performed to determine whether antisera prepared against the matt variants of these two types would exhibit cross protective action.

In Table II are recorded the results of mouse protection tests made with unabsorbed and reciprocally absorbed type 10 and type 12 antisera prepared against the matt variants. The antisera used in these experiments were from the same lots employed in the serological tests summarized in Table I; and the same type 14 matt virulent strain and its homologous antiserum again were used as the heterologous type controls. It is evident from Table II that the types 10 and 12 antisera, both before and after absorption with the heterologous type 14 culture, protected equally well when tested with the matt virulent strain of either type. It is also apparent that cross absorption of these antisera with the matt variants was as effective in removing the protective action as was absorption with the homologous matt variant. The type 10 antiserum, after absorption with either the homologous type 10 or type 12 matt strain, still showed some protective action when tested with the homologous type 10 virulent matt culture. This is in accord with the fact that the M antibodies were not completely removed from this antiserum, as mentioned above, following absorption with either the homologous type 10 matt or type 12 matt strain, as is shown by the slightly positive precipitin (M-anti-M) reactions in Table I. In contrast to the loss of the protective capacity of the type 10 and type 12 matt antisera by absorption with either matt variant, this action was not appreciably affected by absorption with the glossy form of either type. The results of these experiments show that the protective capacity of these antisera was parallel with their M antibody contents but could not be correlated with their type-specific T antibody components.

TABLE III  
*The Serological Identification of Strains Belonging to Types 10 and 12*

Strain	Source	Agglutinin reaction* Antibody content of sera				Precipitin reaction* Antibody content of sera	
		Type 10 anti-T	Type 12 anti-T	Type 10 anti-M	Type 12 anti-M	Type 10 anti-M	Type 12 anti-M
Type 10 representative strains							
N.Y. 5	Scarlet fever, Dr. A. R. Dochez, type 10 glossy‡	+	-	±	+	+	+
E 14	N.Y. 5 after animal passage, type 10 matt	+	-	+	+	+	+
Other type 10 strains							
8115	Scarlet fever, isolated in Rumania, Dr. F. Schwentker	+	-	-	-	±	±
8186	" " " " " " " " "	+	-	-	-	±	±
10295	" " " " " " " " "	+	-	-	-	-	-
11275	" " " " " " " " "	+	-	-	-	-	-
AW17B	Dr. Anna Williams	+	-	-	-	-	-
AW56	" " " "	+	-	-	-	-	-
AW57	" " " "	+	-	-	-	-	-
Type 12 representative strains							
S.F. 42	Scarlet fever, Dr. F. Griffith, type 12 glossy	-	+	-	-	-	-
T 12	S.F. 42 after animal passage, type 12 matt	-	+	+	+	+	+
Other type 12 strains							
671 Pata.	Rheumatic fever subject, Dr. A. Kuttner	-	+	+	+	+	+
610 Esk.	" " " " " " " "	-	+	+	+	+	+
10RS14	" " Rockefeller Institute Hospital	-	+	±	±	+	+
R 2	Carrier, U. S. Navy	-	+	+	+	+	+
R 29	" " " "	-	+	+	+	+	+
R 97	" " " "	-	+	+	+	+	+
R 102	" " " "	-	+	+	+	+	+
F200C	Septicemia, Dr. Perrin Long	-	+	±	+	+	+
C152	Rheumatic fever, Haverstraw, New York	-	+	+	+	+	+
650 Pa.	" " subject, Dr. A. Kuttner	-	+	-	+	+	+
686 Bell.	" " " " " " " "	-	+	-	+	+	+
C 186	" " " " " K. Dodge	-	+	-	+	+	+
R 63	Carrier, U. S. Navy	-	+	-	+	+	+
815 Chi.	Rheumatic fever subject, Dr. A. Kuttner	-	+	-	-	-	-
Wilson 868	Wound infection, Dr. L. Colebrook	-	+	-	-	-	-
F203B	Acute nephritis, " B. Seegal	-	+	-	-	-	-

+ indicates positive reaction.

- indicates negative reaction.

Blank indicates no test.

\* For details of technique, see Methods and Table I.

‡ N. Y. 5 strain contained some M protein, although it was used as the representative type 10 glossy.

#### *Analysis of Twenty-Three Additional Strains*

After demonstrating by agglutinin and precipitin reactions and passive protection tests that the representative type 10 and type 12 strains contained serologically identical M antigens, but unrelated type-specific T antigens, it became important to determine whether other strains previously classified as one of these two types contained the same M and T antigenic components.

Therefore all available strains previously classified into one or the other of these two types were tested. The results are recorded in Table III. The antisera used for the agglutinin reactions were from the same lots of absorbed sera employed in the previous experiments. The precipitin tests were done with other lots of absorbed type 10 and 12 antisera, which had been used for routine typing.

On the basis of the T agglutinogen, seven of the twenty-three strains were identified as type 10, and the remaining sixteen as type 12. With the exception of two, which contained small amounts of M substance, demonstrable only by the precipitin test, all of the type 10 strains were glossy variants. On the other hand, only three of the 16 type 12 strains were glossy forms. This may be correlated with the fact that the majority of the type 12 matt strains had been only recently isolated from patients, while most of the type 10 strains had been kept in the laboratory for some time. Four of the strains identified as type 12 gave positive precipitin reactions with both type 10 and 12 antisera and agglutinated in the type 12 anti-M serum, but failed to give positive agglutinin reactions with the type 10 anti-M serum. This apparent discrepancy is probably due to the difference in the M antibody content of the antisera used for testing. The type 10 anti-M serum, used for the agglutinin test, was of much lower titer than the other antisera, as indicated above, because some of its M antibody was removed by absorption with the type 10 glossy strain which contained some type 10-12 M protein. The twenty-three additional strains tested, therefore, have antigenic compositions similar to one or the other of the matt or glossy representative type 10 or type 12 strains.

#### DISCUSSION

In this study a series of experiments is reported showing the antigenic relationship of type 10 and type 12 group A hemolytic streptococci. The representative type 10 strain, N.Y. 5, and type 12 strain, S.F. 42, employed here, were the same as those used by Griffith (2). Suspensions and M extracts prepared from both the matt and glossy variants of these two types and a heterologous control type 14 matt strain were used in agglutinin and precipitin tests with unabsorbed and reciprocally absorbed antisera prepared against the homologous types 10, 12, and 14 matt cultures. The results of these experiments demonstrated that the representative type 10 and type 12 matt strains contained serologically identical M antigens but unrelated type-specific T antigens. Passive protection tests done with lots of the same unabsorbed and reciprocally absorbed antisera employed in the agglutination and precipitin reactions confirmed the results of the serological tests. In addition, each of twenty-three other strains previously classified as type 10 or type 12, collected from various sources, have been shown to have an antigenic composition similar to one or the other of the two representative types.

Although several workers (2, 5-11) have called attention to cross reactions



among certain types, we have not encountered any mention previously of difficulty in differentiating between types 10 and 12 except for a recent report by Krumwiede (17). Griffith who was able to obtain a sharp distinction between these two types by the slide agglutination technique, as well as other workers who have employed this method, probably used typing antisera of low anti-M and high anti-T titer prepared against the glossy variant.

The recognition now of the two specific antigens, M and T, present in most matt forms of group A streptococci, makes possible a rational explanation of many cross reactions observed by the agglutinin technique among those strains containing M or T substances characteristic of more than one type. With such relationships existing between these two antigens, both of which may be responsible for agglutination, it is obvious that anyone employing the agglutinin method for the typing of streptococci will encounter cross reactions, unless methods are used in preparing antisera so that they contain only M or T antibodies. Moreover, the results of typing tests in different laboratories will vary, unless antisera with identical antibody content are used by all investigators. Most of these difficulties, however, are obviated by employing as a typing technique (18) the precipitin method which depends entirely on the reaction of the single antigen M with its homologous antibody. Even when two types contain identical M antigens, as appears to be the case with types 10 and 12, the lack of distinction by the precipitin test is of relatively little importance from the practical point of view, since the M substance is associated with virulence and its corresponding antibody is related to type-specific immunity.

The application of typing streptococci to the management of streptococcal infections in the hospital, makes it desirable, therefore, to rely on the M-anti-M reaction. At the present time, it is common practice in hospitals to regard streptococcal infections, such as scarlet fever, as a single disease. It is now known that scarlet fever, as well as erysipelas, puerperal fever, tonsillitis, etc., may be caused by any one of a number of antigenically unrelated types of group A streptococci (2, 19-21). Moreover it has recently been shown that the great majority of secondary, purulent complications occurring late in scarlet fever are due to cross infections with another type of streptococcus from a patient in the same ward (22, 23). It would, therefore, be desirable to put together in the same room only those patients whose diseases are caused by the same type, as determined by the M-anti-M reaction. From this point of view, there is no reason to consider type 10 and type 12 as separate types, and it appears unnecessary to separate individuals suffering from infections due to these two types, for immunity to one probably confers immunity to the other.

#### SUMMARY AND CONCLUSION

1. In this study a series of experiments showing the antigenic relationship of type 10 and type 12 group A hemolytic streptococci is reported. Agglu-

tinin, precipitin, and passive protection tests with unabsorbed and reciprocally absorbed antisera were employed to show that representative type 10 and 12 strains contain serologically identical M antigens but unrelated type-specific T antigens.

2. Twenty-three other strains of group A hemolytic streptococci previously classified as either type 10 or type 12, collected from various sources, were shown to have an antigenic composition similar to one or the other of the two representative type strains.

3. The relationship of the two specific antigens, M and T, must be considered when any method employing the agglutinin reaction for the typing of group A hemolytic streptococci is used.

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