

STUDIES ON THE ANTIGENIC COMPOSITION OF GROUP A
HEMOLYTIC STREPTOCOCCI

IV. RELATED T BUT DISTINCT M ANTIGENS IN TYPES 15, 17, 19, 23, 30, AND
IN TYPES 4, 24, 26, 28, 29, 46. IDENTIFICATION BY
SLIDE AGGLUTINATION*†§

By WALTER A. STEWART,|| M.D.,

Lieutenant (j.g.), Medical Corps, United States Naval Reserve

REBECCA C. LANCEFIELD, Ph.D.,

ARMINE T. WILSON,|| M.D.,

Lieutenant, Medical Corps, United States Naval Reserve

AND HOMER F. SWIFT, M.D.

(From the Hospital of The Rockefeller Institute for Medical Research)

(Received for publication, September 29, 1943)

Most investigators who have used the slide agglutination technique for typing group A hemolytic streptococci have found particular difficulty in distinguishing certain apparently closely related strains. Griffith, in 1934, described a "group agglutination" among types 15, 17, and 18 (1). Other workers have reported difficulty in distinguishing types included in this study (2-8). By means of the precipitin reaction, however, these types are readily differentiated on the basis of the type-specific M substance.

In this laboratory, agglutination experiments have shown that types 15, 17, 19, 23, and 30 compose a series of related types, and that members of a second series comprising types 4, 24, 26, 28, 29, and 46 are also closely related.

Previous study of the relationship of strain C 203 to type 1 demonstrated that strains which contained distinct M antigens might, nevertheless, give cross agglutination due to a common T antigen (9). This finding suggested that, since the M antigens of the types concerned in the present investigation were distinct, similar T antigens might be responsible for the cross agglutination observed among these types. Experiments were undertaken to test this hypothesis.

* The work described in this paper was done under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and The Rockefeller Institute for Medical Research.

† The Bureau of Medicine and Surgery, Navy Department, does not necessarily undertake to endorse views or opinions which are expressed in this paper.

§ This work was done with the technical assistance of Miss Doris B. Zenger.

|| Member of the United States Navy Research Unit at the Hospital of The Rockefeller Institute for Medical Research.

Materials and Methods

Strains.—The strains used in this investigation are listed and their sources and antigenic composition are given in Table I.

Techniques.—The techniques employed in precipitin and agglutination reactions and in preparation and absorption of sera were the same as previously described (10–12). All serological tests were performed with absorbed sera which had been

TABLE I
Strains

| Type | Strain | Antigenic composition | Original source and designation |
|---|---------|-----------------------|--|
| <i>1. Members of the series 15, 17, 19, 23, 30</i> | | | |
| 15 | T 15 | M and T | Griffith, strain JS 5 |
| 15 | D 19 | “ “ “ | Rockefeller Hospital |
| 17 | T 17 | T | Griffith, strain Beatty |
| 17 | J 17 E | M and T | Coburn, “ R 9 |
| 17 | D 205 | M | Schwentker, “ 6043 |
| 19 | T 19 | “ | Griffith, “ S.F. 73/4 |
| 19 | J 17 D | M and T | Coburn, “ R 3 |
| 19 | S 24 | “ “ “ | Dochez, Avery, and Lancefield, strain S 24 |
| 23 | T 23 | “ “ “ | Griffith, strain Barts 102 |
| 30 | D 24 | M | “ “ Quinn |
| 30 | D 11 | M and T | Dochez, Avery, and Lancefield, strain S 136 (called strain H by Gay) |
| <i>2. Members of the series 4, 24, 26, 28, 29, 46</i> | | | |
| 4 | T 4 | M and T | Griffith, strain Grove Hospital |
| 24 | C 115 | “ “ “ | “ “ G 54 received from Dr. D. Colebrook |
| 24 | C 98 | M | Rockefeller Hospital, strain 22 RS 72 |
| 26 | T 26 | M and T | Griffith, strain Withers |
| 26 | C 179 | M | Rockefeller Hospital, strain 11 RS 50 |
| 28 | D 140 A | M and T | “ “ |
| 28 | T 28 | M | Griffith, strain Small |
| 29 | D 23 | M and T | “ “ Coggins |
| 29 | J 17 B | M | Coburn, “ R 5 |
| 46 | C 105 | M and T | Rockefeller Hospital, strain 20 RS 14 |

tested to make certain that non-type-specific antibodies had been removed. Since trypsin-treated suspensions are especially susceptible to agglutination by non-type-specific antibodies (13), a negative agglutination reaction with such cultures indicated well absorbed serum free of most non-type-specific antibodies. In analyzing the type-specific antigenic composition of the strains studied, sera containing both M and T antibodies were employed, as well as those containing only M antibodies. To obtain these sera rabbits were immunized with heat-killed matt strains. Serum was also prepared which contained T but no M antibodies; this was obtained by immunization with streptococci which had been exposed to proteolytic enzymes.

EXPERIMENTAL

A. The Series Composed of Types 15, 17, 19, 23, and 30.—

1. *Experiments Showing that the M Antigens of These Types Are Distinct.—* Extracts of all matt strains of these types, when tested with serum containing M antibodies, gave strongly positive, type-specific precipitin reactions. Examples of these precipitin reactions are given in Table II.

2. *Experiments Showing that the T Antigens of These Types Are Related.—* The sera which gave the M precipitin reactions shown in Table II were also used for agglutination tests. These sera contained anti-T agglutinins as well as anti-M precipitins. Agglutination reactions performed with these sera and suspensions of the same strains used in the M precipitin reactions showed

TABLE II

M Precipitin Reactions Indicate that Types 15, 17, 19, 23, and 30 Possess Unrelated M Antigens

| Extracts prepared with cultures of | Antisera of types | | | | |
|------------------------------------|-------------------|------|------|------|------|
| | 15 | 17 | 19 | 23 | 30 |
| Type 15 (strain T 15)..... | ++++ | — | — | — | — |
| “ 17 (“ J 17 E)..... | — | ++++ | — | — | — |
| “ 19 (“ J 17 D)..... | — | — | ++++ | — | — |
| “ 23 (“ T 23)..... | — | — | — | ++++ | — |
| “ 30 (“ D 11)..... | — | — | — | — | ++++ |

These antisera contained M antibodies upon which these reactions depended, and also T agglutinins with which no precipitin reactions have been demonstrated. Readings of the precipitin reactions in this and subsequent tables are summaries of results obtained with the following antigen dilutions: undiluted; 1-4; 1-16. They are recorded on a — and ± to +++++ scale.

that all five strains agglutinated well in the antisera for types 15, 17, 19, 23, and 30 (Table III). Members of this series failed to agglutinate in anti-T sera of other types. The cross agglutination noted by Griffith (1) of type 18 with types 15 and 17 and that noted by Plummer (6) and by Bynoe (8) between type 26 and certain members of this series (types 17, 19, and 23) were not observed in these experiments. The fact that similar results were obtained with sera containing T but no M antibodies indicates that the cross agglutination was probably due to T-anti-T reactions.

3. *The Relationship of the T Antigens As Tested by Reciprocal Absorption Experiments.—*In these experiments antisera containing both M and T antibodies as well as those containing only T antibodies were employed. The inter-relationship of the T antigens was found the same in experiments performed with both kinds of antisera; but type-specific agglutination, as well as type-specific precipitin reactions, occurred with the former and obscured the relationships due to T agglutinins. Sera containing T antibodies but no type-specific

antibodies were, therefore, found more useful in studying the relationships of the T substances, and the results of the experiments with these sera were as follows:—

One lot of each serum, from rabbits immunized with trypsin-treated cultures of each type in this series, was absorbed with organisms of a heterologous unrelated type (strain S 43, type 6); and the five absorbed sera were then tested with results, shown in the first section of Table IV, which indicate that cross agglutination by T antibodies occurred. Other lots of the same antisera were then absorbed with a T-containing strain of each type in the series, as indicated in the table, until all antibodies were removed for the strain used for absorption. Agglutination reactions were then performed with all lots of ab-

TABLE III
Agglutination Reactions Indicate that Types 15, 17, 19, 23, and 30 Possess Related T Antigens

| Cultures used in agglutination reactions (M- and T-containing strains) | Antisera of types | | | | | |
|---|-------------------|------|------|------|------|----|
| | 15 | 17 | 19 | 23 | 30 | 18 |
| Type 15 (strain T 15)..... | +++ | +++ | ++ | +++ | +++ | — |
| “ 17 (“ J 17 E)..... | ++++ | ++++ | ++++ | ++++ | ++++ | — |
| “ 19 (“ J 17 D)..... | ++++ | ++++ | ++++ | ++++ | ++++ | — |
| “ 23 (“ T 23)..... | +++ | +++ | +++ | ++++ | +++ | — |
| “ 30 (“ D 11)..... | ++++ | ++++ | ++++ | ++++ | ++++ | — |

These agglutination reactions were performed with antisera containing both M antibodies and T antibodies. Similar results were obtained with antisera containing T antibodies but no M antibodies.

Type 18 serum is included as a representative of anti-T sera of other types which did not agglutinate members of this series.

Readings of the agglutination reactions in this and subsequent tables are summaries of results obtained with a series of serum dilutions ranging from 1-20 to 1-2560. They are recorded on a — and ± to ++++ scale.

sorbed antisera and a strain known to contain T antigen, representative of each type in this series. Table IV shows that strains of types 15, 17, and 23 each removed all agglutinins from antisera for types 15, 17, and 23, and absorbed from type 19 and type 30 antisera all agglutinins reactive with types 15, 17, or 23, but not those specifically reactive with types 19 and 30. In the same way, strains of types 19 and 30 each absorbed all agglutinins from the antisera for types 19 and 30, but only absorbed those agglutinins active against types 19 and 30 from antisera for types 15, 17, and 23, and left in each of these sera antibodies which agglutinated strains of the latter three types. Thus it would appear that these five types have some T factor in common which is the basis of the reciprocal cross agglutination reactions, and in addition types 15, 17, and 23 are related by a common T antigen not present in types 19 and 30. Conversely, types 19 and 30 possess a common T antigen not shared by members of types 15, 17, and 23.

TABLE IV
Reciprocal Absorption Experiment Showing Relationships of T Antigens in This Series of Types T Agglutination Reactions

| Anti-T sera from rabbits immunized with trypsin-treated cultures | | T-containing strains used in agglutination reactions | | | | |
|--|--------------------------------------|--|-----|-----|-----|-----|
| | | Types | | | | |
| | | 15 | 17 | 23 | 19 | 30 |
| Type 15 serum absorbed 5 times | | +++ | +++ | +++ | +++ | +++ |
| " 17 " " 6 " | with unrelated strain (Type 6, S 43) | +++ | +++ | +++ | +++ | +++ |
| " 23 " " 6 " | | +++ | +++ | +++ | +++ | +++ |
| " 19 " " 6 " | | +++ | +++ | +++ | +++ | +++ |
| " 30 " " 5 " | | +++ | +++ | +++ | +++ | +++ |
| " 15 " " 4 " | | with type 15 strain | - | - | - | - |
| " 17 " " 3 " | - | | - | - | - | - |
| " 23 " " 4 " | - | | - | - | - | - |
| " 19 " " 4 " | - | | - | - | +++ | +++ |
| " 30 " " 3 " | - | | - | - | +++ | +++ |
| " 15 " " 4 " | with type 17 strain | - | - | - | - | - |
| " 17 " " 3 " | | - | - | - | - | - |
| " 23 " " 5 " | | - | - | - | - | - |
| " 19 " " 5 " | | - | - | - | +++ | +++ |
| " 30 " " 4 " | | - | - | - | +++ | +++ |
| " 15 " " 5 " | with type 23 strain | - | - | - | - | - |
| " 17 " " 4 " | | - | - | - | - | - |
| " 23 " " 6 " | | - | - | - | - | - |
| " 19 " " 6 " | | - | - | - | +++ | +++ |
| " 30 " " 5 " | | - | - | - | +++ | +++ |
| " 15 " " 5 " | with type 19 strain | +++ | +++ | +++ | - | - |
| " 17 " " 5 " | | +++ | +++ | +++ | - | - |
| " 23 " " 5 " | | +++ | +++ | +++ | - | - |
| " 19 " " 5 " | | - | - | - | - | - |
| " 30 " " 4 " | | - | - | - | - | - |
| " 15 " " 3 " | with type 30 strain | +++ | +++ | +++ | - | - |
| " 17 " " 3 " | | +++ | +++ | +++ | - | - |
| " 23 " " 2 " | | +++ | +++ | +++ | - | - |
| " 19 " " 4 " | | - | - | - | - | - |
| " 30 " " 2 " | | - | - | - | - | - |

These antisera contained no M antibodies because they were obtained from rabbits immunized with trypsin-treated cultures.

4. *Type-Specific Agglutinogens.*—In absorption experiments with sera originally containing both M and T antibodies, from which all T antibodies were absorbed, type-specific agglutinins were demonstrated. In types 15, 17, and

23 the antibodies responsible for both the type-specific agglutination and precipitin reactions appear to be M antibodies. In types 19 and 30, it seems possible that agglutinins other than the M antibodies may also take part in type-specific agglutination.

5. *Strains in This Series Which Contain M but No T Antigen.*—In the second paper of this series, strains containing type-specific M antigen but devoid of the corresponding T substance are described (14). At the present time, strains with this antigenic composition have been observed in types 17, 19, and 30.

6. *Blocking of the Agglutination Reaction.*—The failure of an agglutino-gen to react with its antibody was previously reported in strains of types 1 and 6

TABLE V
Blocking of Agglutination Reactions

| Type 19 strains used in agglutination reactions (contain M and T antigens) | Type 19 antisera: | |
|--|-------------------------|-------------------------|
| | (Contains M antibodies) | (Contains T antibodies) |
| <i>Strain S 24</i> | | |
| Culture grown at room temperature or at 37°C..... | — | +++ |
| <i>Strain J 17 D</i> | | |
| 1. Stock culture..... | ++++ | ++++ |
| 2. Mouse passage culture | | |
| (a) Grown at 37°C..... | ++++ | — |
| (b) Grown at room temperature..... | ++++ | ++++ |

In strain S 24, agglutination due to M antigen is blocked. No method has been found for bringing out agglutination of this strain by M antibodies.

In strain J 17 D, after mouse passage, agglutination due to T antigens is blocked, apparently by an excess of M antigen. Stock cultures or mouse passage cultures grown at room temperature are agglutinated by T antibodies.

(11, 14). In the present series of types, similar phenomena were observed among members of type 19. Two strains, S 24 and C 84 A, were agglutinated by T but not by M antibodies, in spite of the fact that these cultures contained M substance in antigenic form and were able to absorb M antibodies from immune sera. This peculiarity appeared to be a constant characteristic of these two strains.

Whereas blocking of the M antigen occurred in the type 19 strains just described, the reactions of the T antigen were blocked in another member of this type. A culture of strain J 17 D made virulent by passage through mice failed to agglutinate in anti-T serum, although the stock culture, a matt avirulent variant, was agglutinated readily by T antibodies. When the former strain was subjected to slightly unfavorable conditions, it also became agglutinable in anti-T serum. These findings are recorded in Table V.

B. The Series Composed of Types 4, 24,¹ 26, 28, 29, and 46.—

1. Experiments Showing that the M Antigens of These Types Are Distinct.— Precipitin reactions based on a combination of the M antigen with its antibody showed no cross reactions among these types (Table VI). Each serum gave a precipitin reaction only with extracts made from M-containing strains of the homologous type.

2. Experiments Showing that the T Antigens of These Types Are Related.— Sera, prepared with any strain in this series which contained T antigen, agglutinated all other T-containing strains of the series, as summarized in Table VII. Antisera from rabbits immunized with trypsin- or pepsin-treated cultures, which consequently contained T antibodies but no M antibodies, caused cross agglutination to the same degree as sera containing antibodies to both the

TABLE VI

M Precipitin Reactions Show that Types 4, 24, 26, 28, 29, and 46 Possess Unrelated M Antigens

| Extracts prepared with cultures of | Antisera of types | | | | | |
|------------------------------------|-------------------|------|------|------|------|------|
| | 4 | 24 | 26 | 28 | 29 | 46 |
| Type 4 (strain T 4)..... | ++ | - | - | - | - | - |
| " 24 (" C 115)..... | - | ++++ | - | - | - | - |
| " 26 (" T 26)..... | - | - | ++++ | - | - | - |
| " 28 (" D 140 A)..... | - | - | - | ++++ | - | - |
| " 29 (" D 23)..... | - | - | - | - | ++++ | - |
| " 46 (" C 105)..... | - | - | - | - | - | ++++ |

These antisera contained M antibodies upon which these reactions depended, and also T agglutinins with which no precipitin reactions have been demonstrated.

M and T antigens. These agglutination reactions, therefore, are independent of the M-anti-M system and are probably due to the combination of T antibodies with closely related T antigens contained in strains of the different types.

Table VII shows many gradations in the degree of cross agglutination among these types. Weak reactions in a given serum, for example the type 24 strain in type 28 serum, could not be ascribed to a generally low antibody content in that serum because some strains of heterologous type (for instance, types 4 and 46) agglutinated well in it. Comparable analyses show that differences in agglutinability of individual strains could not be the explanation for the varying degrees of cross agglutination, but probably indicate qualitative antigenic differences in related T antigens.

¹ The type originally designated in this laboratory as type C 98 and later as provisional type 45 appears to be the same as Griffith's type 24, and is now designated as type 24. The designation, provisional type 45, has now been dropped and will not be reassigned.

3. *The Relationship of the T Antigens Tested by Reciprocal Absorption Experiments.*—It seemed probable from the results shown in Table VII that types 4, 24, 26, 28, 29, and 46 possess similar trypsin-stable T antigens responsible for the cross agglutination reactions. The relationships of these antigens were studied further by means of reciprocal absorption experiments. Antisera, prepared by immunizing rabbits with heat-killed cultures of strains of each type were absorbed separately with the other strains in the series, and then tested for agglutinins. Absorption with strains T4 (type 4) and C 105 (type 46) removed all antibodies which gave cross reactions with other members of this series (Table VIII). These experiments indicate that all of these types contain

TABLE VII

Agglutination Reactions to Show that Types 4, 24, 26, 28, 29, and 46 Possess Related T Antigens

| Cultures used in agglutination reactions (M- and T-containing strains) | Antisera of types | | | | | |
|--|-------------------|------|-----|------|------|------|
| | 4 | 24 | 26 | 28 | 29 | 46 |
| Type 4 (strain T 4)..... | ++++ | ++++ | +++ | ++++ | ++++ | ++++ |
| “ 24 (“ C 115)..... | ++++ | ++++ | +++ | + | ++ | ++++ |
| “ 26 (“ T 26)..... | ++++ | ++++ | +++ | ++ | +++ | +++ |
| “ 28 (“ D 140 A)..... | +++ | ++ | +++ | ++++ | +++ | +++ |
| “ 29 (“ D 23)..... | ++++ | ++++ | ++ | ++ | ++++ | +++ |
| “ 46 (“ C 105)..... | +++ | ++++ | +++ | ++++ | ++ | ++++ |

All sera were absorbed with a heterologous strain of a type not included in this series. Two kinds of antisera were used: (1) Antiserum containing M and T antibodies was made by immunizing with untreated, heat-killed organisms. (2) Antiserum containing T antibodies but no M antibodies was also made (except for type 28) by immunizing with streptococci which had been digested with trypsin prior to being heat-killed. The agglutination reactions were similar in both cases.

related T antigens since types 4 and 46 are able to absorb T antibodies from antisera prepared with any one of them.

In contrast to the foregoing, attempts to absorb these T antibodies with strains of other types in this series (*viz.* types 24, 26, 28, and 29) were less successful in removing agglutinins. Even though these strains finally absorbed all antibodies for themselves, T agglutinins were left for some of the other members of this series. Strains of types 28 and 29 were especially lacking in ability to absorb T agglutinins for any other strains than themselves, and members of types 24 and 26 were intermediate in this respect between types 28 and 29 and those which absorbed T antibodies easily (types 4 and 46).

Similar absorption of T antibodies was observed when antisera containing no M antibodies (obtained from rabbits immunized with enzyme-treated cultures) were absorbed with strains of this series.

It is noteworthy that those strains in which the T substance occurs in most

highly antigenic form are also most readily agglutinated by T antibodies. Such strains are, in addition, most effective in absorbing T antibodies from immune sera. In contrast to this, those strains with T substance of low antigenicity agglutinate only in low dilutions of T antibodies and absorb these antibodies less readily than strains with highly antigenic T substance.

4. *Type-Specific Agglutinogens*.—After the cross reacting T agglutinins were absorbed from antisera containing both M and T antibodies, type-specific agglutinins remained in the absorbed sera. These agglutinins were probably M antibodies since these sera gave type-specific anti-M precipitin reactions. Antisera, prepared with enzyme-treated cultures, lack M antibodies. When

TABLE VIII
Absorption Experiment Shows that T Antigens in This Series of Types Are Related. Removal of T Antibodies by Absorption with Strain T 4 Agglutination Reactions

| Cultures used in agglutination reactions (M- and T-containing strains) | Antisera absorbed twice with strain T 4 | | | | | |
|---|---|-----|-----|------|------|----|
| | Types | | | | | |
| | 4 | 24 | 26 | 28 | 29 | 46 |
| Type 4 (strain T 4)..... | — | — | — | — | — | — |
| “ 24 (“ C 115)..... | — | +++ | — | — | — | — |
| “ 26 (“ T 26)..... | — | — | +++ | — | — | — |
| “ 28 (“ D 140 A)..... | — | — | — | ++++ | — | — |
| “ 29 (“ D 23)..... | — | — | — | — | ++++ | — |
| “ 46 (“ C 105)..... | — | — | — | — | — | ++ |

The antisera used for this experiment were similar to those used for the experiments presented in Table VII, in that they contained both M and T antibodies. Absorption with strain T 4 removed the T antibodies but left the specific anti-M agglutinins. In other experiments in which sera containing anti-T but no anti-M agglutinins were used, no specific agglutinins were present after absorption with strain T 4.

such antisera were prepared for the types in this series, they contained neither M precipitins nor type-specific agglutinins. This evidence increases the probability that M antibodies are responsible for type-specific agglutination in these types.

Exceptions were observed in the case of types 4 and 28 in which type-specific agglutinins were present but no M precipitins could be demonstrated in antisera after removal of T antibodies. With the type 4 sera employed in these absorption experiments it seems probable that the original low content of M antibodies and the numerous absorptions required to remove the anti-T agglutinins resulted in a non-specific reduction in M antibodies. Although these antibodies were then too dilute to give a precipitin reaction, they were still sufficiently concentrated to agglutinate homologous type strains.

Most type 28 strains were agglutinated type-specifically by sera containing

high concentrations of M antibodies. This agglutination due to M antibodies was, however, blocked in strains of this type containing T as well as M antigens, for example, strain D 140 A. In addition, an unusual type-specific reaction was observed with T antibodies in this type. Although these experiments are still incomplete, antisera have been prepared which agglutinated T-containing

TABLE IX
Comparison of Immunological Reactions of Strains Containing No T Antigen with Those Containing Both M and T Antigens
Agglutination Reactions

| Cultures used in agglutination reactions | Sera of types | | | | | | | | | |
|--|------------------------------------|------|------|------|------|------|--------------------------|------|------|------|
| | Containing both M and T antibodies | | | | | | Containing M antibodies* | | | |
| | 4 | 24 | 26 | 28 | 29 | 46 | 24 | 26 | 28 | 29 |
| 1. Containing M and T antigens | | | | | | | | | | |
| Type 4 (strain T 4)... | ++++ | ++++ | +++ | ++++ | ++++ | ++++ | - | - | - | - |
| " 24 (" C 115)... | ++++ | ++++ | +++ | + | ++ | ++++ | ++++ | - | - | - |
| " 26 (" T 26)... | ++++ | ++++ | +++ | +++ | +++ | +++ | - | ++++ | - | - |
| " 28 (" D 140 A)... | +++ | ++ | +++ | ++++ | +++ | +++ | - | - | ++++ | - |
| Type 29 (strain D 23)... | ++++ | ++++ | ++ | ++ | ++++ | +++ | - | - | - | ++++ |
| " 46 (" C 105)... | +++ | ++++ | +++ | ++++ | ++ | ++++ | - | - | - | - |
| 2. Containing M antigen† | | | | | | | | | | |
| Type 24 (strain C 98)... | - | ++++ | - | - | - | - | ++++ | - | - | - |
| " 26 (" C 179)... | - | - | ++++ | - | - | - | - | ++++ | - | - |
| " 28 (" T 28)... | - | - | - | ++++ | - | - | - | - | ++++ | - |
| " 29 (" J 17 B)... | - | - | - | - | ++++ | - | - | - | - | ++++ |

* These sera were prepared with strains containing M but no T antigen. Therefore they contain M antibodies but no T antibodies, and give type-specific agglutination. Sera of the same antibody content, also giving type-specific agglutination, can be prepared for all types in this series from antisera containing both M and T antibodies by removal of T antibodies with appropriate absorptions. (See Table VIII and Appendix.)

† No strains in types 4 or 46 containing M but no T antigen have as yet been isolated. This accounts for their absence in the second part of this table.

type 28 strains type-specifically and to high titer in spite of the fact that these sera contained no demonstrable anti-M agglutinins or precipitins.

5. *Strains in This Series Which Contain M but No T Antigen.*—The agglutination reactions of strains in this series containing M, but no T antigen are summarized in Table IX. Because of the lack of T antigen, such strains do not agglutinate in anti-T serum and do not give rise to T antibodies when used for immunizing rabbits. The antisera prepared with these strains give type-specific agglutination reactions with M-containing strains of this series: those with T antigens as well as those lacking T antigens.

6. *Blocking of the Agglutination Reaction.*—Strain D 140 A, type 28, in spite

of the fact that it contained both M and T antigens, was not agglutinated by M antibodies in test tube experiments with the usual serum dilutions. With the slide agglutination technique, however, in which the relative concentration of serum is high and the sensitivity of the suspension is also increased by its greater density, this strain is readily agglutinated in anti-M serum.

C. Cross Agglutination Reactions in These Two Series Due to Trypsin-Labile Antigens Other Than the Type-Specific M Substance.—Certain antigen-antibody systems which lead to consistent cross agglutination have been encountered among types 19, 24, and 30 and between types 17 and 30. Some experimental evidence has been obtained which indicates that these particular cross agglutination reactions are due to trypsin-labile antigens which are neither M nor T substances. The nature and exact relationships of these antigens to those already known have not been ascertained.

DISCUSSION

The present report is concerned with the existence of distinct M and related T antigens occurring in each of two series of specific types of group A hemolytic streptococci. The analysis is based on a study of the antibodies induced by certain representative strains.

The eleven types in these series possess distinct M substances. The combination of M antigen with its antibody is the basis of differentiation of these types by the precipitin test, which is independent of the T substance since the latter does not take part in this reaction. It has been found, however, that T substances are responsible for the cross relationships consistently observed in agglutination reactions among the types within these two series. In the series consisting of types 15, 17, 19, 23, and 30, related T antigens were found present in all five types. Additional related T antigens could also be demonstrated in types 15, 17, and 23, on the one hand, and in types 19 and 30 on the other. In the second series, composed of types 4, 24, 26, 28, 29, and 46, such clear relationships among the T antigens were not observed.

A number of these cross agglutination reactions have not been described by other workers in the field. This is probably due to the fact that several of the standard Griffith strains used in the preparation of typing sera do not contain T antigens, upon which this cross reaction depends. For example: Griffith's type 30 strain, Quinn, since it does not possess a T antigen is not agglutinated by the T antibodies induced by other members of this series (types 15, 17, 19, and 23); and antisera prepared with this strain lack the related T antibodies. Probably for the same reason there have been no reports of a cross reaction between type 28 and other members of the series to which it is related. Griffith's original type 28 strain, Coggins, has been shown to lack the T antigen; and this accounts for the success in preparing type-specific serum.

Certain other infrequently reported cross reactions, for example the agglu-

mination of type 4 strains by serum of type 29, occur regularly when the test tube technique is employed. When, on the other hand, the tests are made with the slide agglutination method, the reactions are likely to be negative unless anti-T agglutinins are present in unusually high titer in the type 29 antiserum. These examples are cited to explain the apparent discrepancies between the experiences of many investigators using the slide agglutination technique and the results reported here.

Cross agglutination reactions not due to the usual T antigens were observed between types 17 and 30, and also among types 19, 24, and 30. In contrast to the T antigens which were relatively stable to proteolytic digestion (13), the antigens responsible for these cross agglutination reactions were readily destroyed by tryptic digestion. These complex relationships are briefly mentioned in order to make this account of cross agglutination reactions in these two series as complete as possible.

Since the eleven types included in these two series were distinguished by Griffith (1) by means of the slide agglutination technique, it is apparent that some antigen other than T must have been the basis of his classification of these types. In view of the close agreement that has been observed in most instances between type-specific agglutinin and precipitin reactions, it seems probable that Griffith succeeded in distinguishing these eleven types on the basis of their M antigens. In the case of types 10 and 12, on the other hand, the differentiation originally made by Griffith depended on T antigens, since in these two types the M antigens are so closely related as to be indistinguishable immunologically (16). In certain other types, classification based on either the M or T antigen gives comparable results. In type 1, however, agglutination reactions due to M antibodies do not occur; and the agglutination by which this type was originally differentiated is due to T antibodies (11). With the single exception of strain C 203, strains classified in this type on the basis of the agglutination reaction (due to T antigen) were also placed in this type on the basis of the precipitin reaction (due to M antigen).

Difficulties encountered in distinguishing certain types by means of the agglutination reaction are overcome by ascertaining the antigens with which the types were originally differentiated and preparing antisera which contain only the specific antibodies for the antigens concerned. These substances are, in most instances, M antigens; while the substances responsible for the cross agglutination in the two series here described are usually T antigens.

SUMMARY

1. The occurrence of closely related T antigens in the series composed of types 15, 17, 19, 23, and 30 accounts for most of the cross reactions observed among these types. Similarly T antigens, unrelated to the first series but mutually related, occur in a second series comprising types 4, 24, 26, 28, 29, and 46.

2. Matt variants of each of the eleven types studied possess type-specific M antigens demonstrable either by precipitin or agglutinin reactions.
3. In seven of these types, strains have been encountered which do not possess the T antigen usually associated with the type in question.
4. Procedures are outlined in the appendix for preparing specific antisera for the classification of these types by the slide agglutination technique.

APPENDIX

This appendix describes methods for preparing special antisera by means of which the slide agglutination technique can be used to differentiate the eleven types included in these two series. These sera are also of value in ascertaining the presence or absence of M and T antigens in these strains.

The preparation of satisfactory sera depends largely on immunization with strains of suitable antigenic composition. Due to imperfectly understood cross agglutination reactions, only certain strains of a given type can be successfully used for this purpose. In Table I are given the original sources and antigenic composition of the strains, the use of which is described in detail in Tables X and XI, where the methods of preparing antisera and the outlines of the various procedures employed in each of the two series are summarized. Some of the steps are described in detail below.

A. Antiserum for the Series Composed of Types 15, 17, 19, 23, and 30.—Anti-T serum specific for this series is prepared by immunizing rabbits with trypsin-treated suspensions of the type 19 strain, J 17 D, as shown in Table X, column 2.

In order to remove non-type-specific agglutinins absorptions are performed with a heterologous type not included in this series: *viz.* strain S 43, type 6; and a serum containing the highest titer of anti-T agglutinins is selected for use. This serum agglutinates all T-containing strains in this series; it contains no M antibodies because enzyme-treated streptococci were used for immunization.

B. Type-Specific Antisera.—Matt strains are used for immunization in order to obtain sera which, after suitable absorption, are type-specific. The capacity to produce M substance is often diminished in matt strains by repeated subculturing on artificial media but may usually be restored by serial passage of the cultures through mice (15).

When suitable strains containing type-specific M antigen but lacking T antigen are available, the preparation of satisfactory type-specific sera is greatly facilitated, since absorption with a strain of heterologous type will remove non-type-specific agglutinins. In the absence of such strains those containing both M and T antigens are used for immunization, and the common T agglutinins are removed by absorption with special strains. Since several absorptions are usually necessary to remove these T agglutinins, a procedure which may also reduce the titer of the type-specific agglutinins, only antisera with an initial high titer of M antibodies can be used. The M precipitin test (12) is a reliable index of M antibody content and is independent of T agglutinins, and therefore is employed as a means of selecting sera with a high titer of M antibodies. Examples of these two methods of preparing type-specific antisera are discussed below.

1. With a Strain Containing M but No T Antigen: Type 17.—Rabbits are immunized with a heat-killed culture of the type 17 strain, D 205 (Table X). The antiserum

for use is selected by the M precipitin test and absorbed with any strain of heterologous type, such as strain S 43. The absorbed serum, containing only type-specific antibodies, agglutinates all M-containing type 17 strains (J 17 E) and should not agglutinate T-containing strains of other types in this series, such as T 15 and D 11.

TABLE X

Preparation of Antisera for Classification by Slide Agglutination of Types 15, 17, 19, 23, and 30

| | | Antiserum Specific for series 15, 17, 19, 23, 30 | Antisera Specific for type: | | | | |
|--|---|---|---|------------------------------------|-------------------------|---------------------------|---------------|
| | | | 17 | 15 | 23 | 19 | 30 |
| A. Cultures used for immuniza- tion | 1. Strains..... | J 17 D | D 205 | T 15 | T 23 | J 17 D | D 11 |
| | 2. Preparation of im- munizing suspen- sions..... | Trypsin-treated prior to heat- killing | Heat-killed | | | | |
| | 3. Type antigens in suspensions..... | T | M | M and T | | | (See text) |
| B. Sera | 1. Type antibodies in sera prior to ab- sorption..... | Anti-T aggluti- nins | Anti-M agglu- tinins | Anti-M and anti-T aggluti- nins | | | |
| | 2. M precipitin test prior to absorp- tion..... | Negative | Positive | | | | |
| | 3. Strains used in ab- sorption..... | S 43 | T 17 | D 11 | | | |
| | 4. Antibodies in serum after absorption..... | Anti-T aggluti- nins: specific for series | Anti-M agglutinins: specific for respec- tive type | | | | |
| C. Test of serum for specific agglutinins | 1. Should agglutinate.. | T-containing strains in series 15, 17, 19, 23, 30 | Strains D 205 J 17 E | Strain T 15 | Strain T 23 | Strains T 19 J 17 D | |
| | 2. Should not agglu- tinate..... | Strains not con- taining T of this series | Strains T 15 D 11 | Strains T 17 D 11 | Strains T 17 D 11 | Strains T 17 D 11 | |

2. *With a Strain Containing M and T Antigens.*—Type 15 serum is prepared by immunization with heat-killed cultures of strain T 15. Since this strain contains both M and T antigens, the antiserum contains the corresponding type 15 M antibodies and the T antibodies common to the series. Serum is selected on the basis of the M precipitin test and absorbed with strain T 17. This removes the T agglutinins common to the series, and leaves type-specific M agglutinins for type 15. The antiserum therefore, agglutinates M-containing type 15 strains but not streptococci of other types.

TABLE XI
Preparation of Antisera for Classification by Slide Agglutination of Types 4, 24, 26, 28, 29, and 46

| | | Antiserum Specific for series 4, 24, 26, 28, 29, and 46 | Antisera Specific for type: | | | | | |
|---|---|---|--|------------------------|--------------|--------------|-----------------------|-------------------------|
| | | | 24 | 29 | 46 | 4 | 26 | 28 |
| A. Cultures used for immunization | 1. Strains..... | T 4 | C 115 | D 23 | C 105 | T 4 | C 179 | T 28 |
| | 2. Preparation of immunizing suspensions... | Trypsin-treated prior to heat-killing | Heat-killed | | | | | |
| | 3. Type antigens in suspensions... | T | M and T | | | | M | |
| B. Sera | 1. Type antibodies in sera prior to absorption..... | Anti-T agglutinins | Anti-M and anti-T agglutinins | | | | Anti-M agglutinins | |
| | 2. M precipitin test prior to absorption. | Negative | Positive | | | | | |
| | 3. Strains used in absorption... | S 43 | T 4 | | C 105 | S 43 | | |
| | 4. Antibodies in serum after absorption... | Anti-T agglutinins: specific for series | Anti-M agglutinins: specific for respective type | | | | | |
| C. Test of serum for specific agglutinins | 1. Should agglutinate..... | T-containing strains in series 4, 24, 26, 28, 29, 46 | Strains C 98 C 115 | Strains J 17 B D 23 | Strain C 105 | Strain T 4 | Strains C 179 T 26 | Strains T 28 D 140 A |
| | 2. Should not agglutinate..... | Strains not containing T of this series | Strain T 4 | Strain T 4 | Strain T 4 | Strain C 105 | Strain T 4 | Strain T 4 |

3. *Special Technique Necessary for Type 30.*—The preparation of this serum is dealt with separately and omitted from Table X because the type-specific agglutinins in this type are not well understood and the methods recorded are therefore tentative. Specific serum has, however, been prepared by the following procedure which is recorded to complete the series.

(a) A heat-killed suspension of strain D 11, containing M and T antigens, is used for immunization.

(b) The serum is absorbed with strain J 17 D to remove anti-T agglutinins.

(c) A serum is then selected for use on the basis of its agglutinin titer for type 30.

(d) If cross agglutination occurs with type 17 (D 205) after the series anti-T agglutinins are removed, the serum is absorbed with strain D 205.

(e) The serum is retested and should contain type 30 agglutinins (for strains D 24 and D 11) but neither anti-T agglutinins nor type 17 agglutinins (for strains J 17 D, D 205).

These examples are given in detail since, with the exception of that covering type 30, they illustrate the general methods employed for preparing the antisera used in the slide agglutination technique. Table XI outlines the use of similar methods for preparing antisera for the identification of types 4, 24, 26, 28, 29, and 46.

By employing these antisera in the slide agglutination technique, group A hemolytic streptococci of these particular types can be classified with results similar to those described by Griffith. This type classification is also the same as that obtained with the precipitin technique since both methods are largely based upon reaction of the M antigen with its antibody. Information as to the presence or absence of T antigen also in these streptococci can be obtained by using the anti-T sera described. As already mentioned knowledge concerning the existence of these two antigens in strains isolated might prove very useful in epidemiological investigations.

BIBLIOGRAPHY

1. Griffith, F., *J. Hyg., Cambridge, Eng.*, 1934, **34**, 542.
2. Pauli, R. G., and Coburn, A. F., *J. Exp. Med.*, 1937, **65**, 595.
3. Kodama, T., Ozaki, M., Nishiyama, S., and Chiku, Y., *Kitasato Arch. Exp. Med.*, 1938, **15**, 162.
4. Coburn, A. F., and O'Connell, S., *Proc. Soc. Exp. Biol. and Med.*, 1939, **40**, 645.
5. Rudd, G. V., White, C., and Ward, H. K., *Australian J. Exp. Biol. and Med. Sc.*, 1939, **17**, 25.
6. Plummer, H., *J. Immunol.*, 1941, **42**, 91.
7. Rantz, L. A., *J. Clin. Inv.*, 1942, **21**, 217.
8. Bynoe, E. T., *Canad. Pub. Health J.*, 1943, **34**, 272.
9. Lancefield, R. C., *J. Exp. Med.*, 1940, **71**, 539.
10. Lancefield, R. C., *Proc. Soc. Exp. Biol. and Med.*, 1938, **38**, 473.
11. Lancefield, R. C., *J. Exp. Med.*, 1940, **71**, 521.
12. Swift, H. F., Wilson, A. T., and Lancefield, R. C., *J. Exp. Med.*, 1943, **78**, 127.
13. Lancefield, R. C., *J. Exp. Med.*, 1943, **78**, 465.
14. Lancefield, R. C., and Stewart, W. A., *J. Exp. Med.*, 1944, **79**, 79.
15. Todd, E. W., and Lancefield, R. C., *J. Exp. Med.*, 1928, **43**, 751.
16. Watson, R. F., and Lancefield, R. C., *J. Exp. Med.*, 1944, **79**, 89.