New Streptococcal Serotypes Causing Pyoderma and Acute Glomerulonephritis Types 59, 60, and 61

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Three new streptococcal M serotypes, types 59, 60, and 61, have been described. They were first isolated from patients with pyoderma and acute glomerulonephritis (AGN), seen during epidemiological studies in Alabama. A possible antigenic relationship between types 59 and 61 was suggested by their T-agglutination reactions; a more specific T antiserum prepared for type 59 was useful in separating these two types, as well as other strains known to share T antigens 11, 12, and 5/27/44. On the basis of precipitin tests, a common antigenic determinant among types 59, 61, and 49 was suggested. This is of interest in view of the relation between these types and AGN. Type 59 has been relatively more widespread in distribution than type 61, but neither have been related to epidemic AGN. Type 60, first identified as "T-4," thus being related to previously described M types which share this antigen, is of great interest in terms of the epidemiology of AGN. Most strains in our collection were recovered from patients with pyoderma and AGN or from their infected siblings. Recently, the type was found to be prevalent among patients with pyoderma and AGN seen in Trinidad. Data reported in relation to these new types further illustrate the dichotomy in M serotypes common to pyoderma and AGN on the one hand and those types found in collections of patients with pharyngitis on the other hand. The high rate of non-M-typable streptococci among pyoderma collections, encountered earlier, is best explained by lack of suitable reference antisera available for prevalent pyoderma serotypes.

Epidemiological studies of pyoderma and acute glomerulonephritis (AGN) were initiated in this laboratory a decade ago. At that time, little was known regarding M antigens of the common pyoderma streptococci (16, 17), and only one known M serotype, type 49, was associated with AGN following pyoderma (24). Few strains of streptococci could be serotyped with the then available reference M antisera, and antisera for T-agglutination typing were not available from the National Center for Disease Control (NCDC) Streptococcal Laboratory. To pursue definitive epidemiological studies, a collaborative investigation with M. Moody at the NCDC and W. Maxted and M. Parker at the Central Public Health Laboratory (CPHL), Colindale, London, was undertaken. A sizable collection of strains from pyoderma and associated cases of AGN studied in Alabama during the years 1964 through 1966 were examined (4). Most strains were classified only by their T antigens and, with the exception of certain nephritogenic serotypes, the T-agglutination patterns commonly encountered were like those Parker and co-workers described as "impetigo streptococci" (16, 17).

In subsequent studies in our laboratory, the majority of nephritogenic serotypes from pyoderma-associated cases were classified as either M-49 or M-2, the latter having the agglutination pattern 8/25/Imp.19 rather than the T-2 antigen (3). However, a sizable collection of pyoderma strains, including certain ones associated with AGN, remained non-M-typable.

We are now reporting the isolation and characterization of three new streptococcal M serotypes, types 59, 60, and 61, each of which was initially isolated from patients with pyoderma and AGN. Their biological character and epidemiological significance are described.

MATERIALS AND METHODS

Streptococcal antisera and stock strains were obtained from the CPHL, Colindale, London, courtesy of W. R. Maxted and M. T. Parker, and from the Reagents Division, NCDC, Atlanta, Ga. Additional strains or antisera, or both, were obtained from M. Moody of the Streptococcal Laboratory, NCDC; R.
Lancefield, Rockefeller University; L. W. Wannamaker, Minneapolis, Minn.; and G. Stollerman, Memphis, Tenn.

**Streptococcal strains.** Vaccine strains, described in detail below, and other wild-type strains from our collection were isolated during epidemiological studies of pyoderma and acute nephritis. All studies were done in the Department of Pediatrics, University of Alabama in Birmingham, School of Medicine.

Stock M-types from CPHL, Colindale, included: 1 to 3, 5, 6, 9, 11, 12, 14, 15, 17 to 19, 22 to 24, 26, 29 to 31, 33, 36, 37, 39, 43, 46, 47 to 51, plus 3-R, 28-R, and provisional 58 (strain 3890); those from NCDC included: 1 to 6, 8, 9, 11 to 15, 17 to 19, 22 to 27, 29 to 33, 36 to 44, 46, 47, 49, 51, plus new or provisional types 52 to 57; those from R. Lancefield included: 4, 24, 26, 29, 46, 2-R, 48, and 48-R. Miscellaneous strains included recent pyoderm isolates of new types 52 to 54 and type 41 from Wannamaker, plus pyoderm and nephritis isolates found in Trinidad, including several identified as "Trinidad" T-4 and certain others identified only by agglutination at T-8/25Imp.19, from W. R. Maxted.

**Reference antisera: source and type.** From NCDC the M antisera were for types 1 to 6, 8, 9, 11 to 15, 17 to 19, 22 to 33, 36 to 44, 46, 47, 49, 51, plus the new M types 52 to 57. From R. Lancefield the M antisera were for types 2, 4, 24, 26, 29, 24, 46, and 48, plus certain antisera for types possessing an R antigen. From CPHL, Colindale, the M antisera were for types 2, 49, 55, and 58. T antisera initially employed were obtained from CPHL, Colindale. Standard sets of pooled and individual T antisera included: "T" pool, 1, 3, 13, B/3264; "U" pool, 2, 4, 6, 28; "W" pool, 5, 11, 12, 27, 44; "X" pool, 8, 14, 25, Imp.19; "Y" pool, 15, 17, 22, 23, 47; and "Z" pool, 9, 18, 19, and 30. A set for agglutination, prepared with stock strains from CPHL, Colindale, was later provided by NCDC.

**Streptococcal serotyping.** M typing was done by either the capillary precipitin method of Swift et al. (22) or the micro-immunodiffusion recommended by Rotta et al. (20). T-agglutination typing was done by the method of Griffith (6).

Vaccines for immunization and M and T antisera were produced by standard methods as outlined by Moody et al. (13), with certain modifications as currently employed at CPHL, Colindale (recommended by W. R. Maxted, personal communication). These modifications included alteration in absorption times with cross-reacting serotypes and dilution of agglutination antisera to minimize such crosses.

**Bactericidal tests.** Direct and indirect bactericidal tests utilized for determining growth of strains after rotation in human blood and for the detection of type-specific M antibody were performed as described by Lancefield (8). Dilutions used in the bactericidal tests ranged from $10^{-3}$ through $10^{-4}$; simultaneous testing of several heterologous strains, using only the $10^{-4}$ bacterial dilution, was done in some instances. Heated, unabsorbed M antisera were used unless obtained from a reference laboratory. Colony counts were done with a Quebec counter. (In the tables, the number of chains listed as remaining at the end of the test represent the actual colony count.) Laked or partially laked plates represent complete or extensive hemolysis and are so indicated. Antisera enhancing good killing of a $10^{-4}$ strain dilution of an homologous type were considered acceptable.

Vaccine strains and their respective antisera were submitted to three reference laboratories: Rebecca Lancefield, Rockefeller University; CPHL, Colindale, London, and the Streptococcal Laboratory, NCDC. New type numbers 59, 60, and 61 were designated for these types now reported.

**RESULTS**

Each of the three new M serotypes were first encountered during epidemiological studies of pyoderma and AGN. Strains subsequently shown to be examples of types 59 and 61 first attracted attention because of unusual or atypical agglutination reactions, including cross-reactions with certain "pool" antisera (see above for constitution of agglutination-type pools). Their agglutination reactions were sufficiently distinctive to serve as useful markers for sorting them out from other strains. A third new type, type 60, was first identified as a T-4, non-M-typable strain. The association of prototype strains of each of these three new types with pyoderma and AGN prompted the initial effort to define their M antigens. In Table 1, original vaccine strains for each type are listed in relation to year, clinical source, and original T-agglutination reactions by which they were identified. Also shown are bactericidal tests results which served to first identify them as suitable vaccine strains.

**Studies with types 59 and 61.** Type 59 was the first of the three types to be studied extensively. Certain similarities in this type and type 61 prompted a later comparison of the two. The original agglutination pattern of the type 59 vaccine strain was limited to a strong W pool and a weak X pool reaction. The failure to agglutinate with one or more individual T antisera within the W pool, 5, 11, 12, 27, and 44, was surprising in view of the strong pool reaction. The strains were simply labeled as "pools" W++, ++/X+, non-M-typable. However, it was subsequently possible to show, with a separate lot of antisera from CPHL, Colindale, and the new NCDC agglutination antisera, that these type 59 strains agglutinated as T-12 or T-11/12.

Agglutination typing results with the original type 61 vaccine strains revealed cross-reactions with several (T, W, and X) pool antisera, but the W pool reaction was prompt and strongest. The strains could be further T-typed as T-11 after additional trypsinization, which also eliminated the cross-reactions with T and X pool sera. The agglutination characteristics of type
61 strains did serve as a useful marker during early efforts to separate them from "related" T-types which share the T-11 antigen, including certain of those strains later proven to be members of type 59. Some strains of type 61 were also found to agglutinate with the T-9 antiserum from NCDC, resulting in a T-9/11 "agglutination complex." T-agglutination reactions of both new M types 59 and 61 were of further value as epidemiological markers in the detection of strains subsequently typed with the respective M antiserum.

Preparation of antisera: types 59, 60, and 61. Original vaccine strains for all three new serotypes were isolated from children with pyoderma and AGN seen in 1967, and presence of an M antigen was suspected on the basis of their ability to multiply when rotated in the presence of normal human blood (Table 1). Suitable M antiserum were produced with two prototype M-59 strains, but 2829-S is the reference type strain. Strains 2797-S (type 60) and 2998-T (type 61) are reference type strains for the latter two new types.

Type 59 bactericidal and precipitin test. Data from an indirect bactericidal test with the reference type 59 vaccine strain and two homologous antisera are shown in Table 2. Enhancement of killing of the homologous strain was significant at the $10^{-6}$ dilution and complete at the $10^{-4}$ dilution. Reciprocal tests with the two vaccine strains confirmed their being identical.

Unabsorbed antiserum against 2829-S reacted strongly in capillary precipitin and gel diffusion tests with homologous strains, and minor cross-reactions were removed by absorption with T-6 glossy cells. Persistent 2+ cross-reactions remained with three types: type 49, type 2 (T-8/25/Imp.19), and 2998-T, the type 61 vaccine strain. Cross-reacting strains were, in each case, originally isolated from pyoderma lesions of patients with AGN; absorption with type 49 cells removed all crosses.

Indirect bactericidal test results with unabsorbed type 59 antisera, comparing the vaccine strain with various heterologous types, are shown in Table 3. All types found earlier to cross-react in precipitin tests are included; new type 60, though not a cross-reacting strain, was also included. Enhancement of phagocytosis was observed only with the homologous type 59

Table 2. Indirect bactericidal tests with type 59 vaccine strains and homologous antisera

<table>
<thead>
<tr>
<th>Strain</th>
<th>Determinations</th>
<th>No. of chains, with bacterial dilutions of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$10^{-6}$</td>
</tr>
<tr>
<td>2829-S</td>
<td>Inoculum</td>
<td>1,000</td>
</tr>
<tr>
<td>2829-S</td>
<td>End of test</td>
<td></td>
</tr>
<tr>
<td>2829-S</td>
<td>With anti-2829-S</td>
<td>85</td>
</tr>
<tr>
<td>2829-S</td>
<td>With anti-2853-S</td>
<td>100</td>
</tr>
<tr>
<td>2829-S</td>
<td>With M-6 antiserum</td>
<td>PL</td>
</tr>
<tr>
<td>2829-S</td>
<td>With NRS</td>
<td>PL</td>
</tr>
</tbody>
</table>

* PL, Partially laked plate; NRS, normal rabbit serum. M-6 antiserum used as known heterologous control.

Table 1. Description of vaccine strains: growth in normal human blood

<table>
<thead>
<tr>
<th>Vaccine strain*</th>
<th>Designated M type</th>
<th>Original agglutination reactions</th>
<th>Culture</th>
<th>Growth* in rotated normal human blood at bacterial dilutions:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pool Individual T antigen</td>
<td></td>
<td>$10^{-4}$</td>
</tr>
<tr>
<td>2829-S</td>
<td>M-59</td>
<td>W + + + /X</td>
<td>Inoculum</td>
<td>400</td>
</tr>
<tr>
<td>2853-S</td>
<td>M-59</td>
<td>W + + + /X</td>
<td>Growth</td>
<td>L</td>
</tr>
<tr>
<td>2797-S</td>
<td>M-60</td>
<td>U +++++</td>
<td>Inoculum</td>
<td>400</td>
</tr>
<tr>
<td>2990-S</td>
<td>M-60</td>
<td>U +++++</td>
<td>Growth</td>
<td>L</td>
</tr>
<tr>
<td>2798-S</td>
<td>M-61</td>
<td>T/W +++++/X</td>
<td>Inoculum</td>
<td>107</td>
</tr>
<tr>
<td>2998-T</td>
<td>M-61</td>
<td>T/W +++++/X</td>
<td>Growth</td>
<td>PL</td>
</tr>
</tbody>
</table>

* Two vaccine strains of each type were originally isolated; each was first isolated from patients with pyoderma and AGN seen in 1967. Reference type is italicized.

* Laked plate—complete hemolysis; PL, partially laked or extensive areas of hemolysis. The number of chains listed as remaining at the end of the test represent the actual colony count.
TABLE 3. Indirect bactericidal tests with M-59 and heterologous types

<table>
<thead>
<tr>
<th>Strains</th>
<th>No. of chains in inoculum at:</th>
<th>No. of chains at end of test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10^{-9}</td>
<td>10^{-3}</td>
</tr>
<tr>
<td>2829-S (type 59)</td>
<td>2,000</td>
<td>26</td>
</tr>
<tr>
<td>Stock M-9</td>
<td>500</td>
<td>13</td>
</tr>
<tr>
<td>M-12</td>
<td>3,000</td>
<td>77</td>
</tr>
<tr>
<td>M-22</td>
<td>2,000</td>
<td>27</td>
</tr>
<tr>
<td>M-31</td>
<td>PL</td>
<td>58</td>
</tr>
<tr>
<td>M-48</td>
<td>2,000</td>
<td>22</td>
</tr>
<tr>
<td>M-49</td>
<td>2,000</td>
<td>66</td>
</tr>
<tr>
<td>2797-S* (type 60)</td>
<td>3,000</td>
<td>33</td>
</tr>
<tr>
<td>2998-T* (type 61)</td>
<td>2,000</td>
<td>29</td>
</tr>
<tr>
<td>2500-S* (type 2)</td>
<td>2,000</td>
<td>34</td>
</tr>
</tbody>
</table>

a Normal rabbit serum.
b Reciprocal indirect bactericidal tests with type 59; these tests were done on one or more occasions; no cross-bactericidal activity shown.
c Partially laked plate.

strain. As indicated, reciprocal bactericidal tests were done on one or more occasions with six heterologous types, which further confirmed the homogeneity and specificity of type 59 as a distinct new serotype.

Type 61: bactericidal and precipitin test.
The preparation of an M antiserum with both strong homologous precipitin reactions and enhancement of bactericidal activity proved somewhat difficult with type 61. Strain 2998-T was the superior vaccine strain, meeting both above requirements, and is the reference type strain. Indirect bactericidal test results with 2998-T and lots of antisera from three animals are shown in Table 4. Best homologous precipitin reactions were obtained with that antiserum labeled 2998-T (X). After absorption with T-6 cells, cross-reactions occurred with type 49 and new type 59, which were eliminated by absorption with type 49 cells.

The possible significance of cross-precipitin reactions between types 49, 59, and 61 was investigated further in reciprocal indirect bactericidal tests (Table 5). A type 49 antiserum, prepared in our laboratory with strain 2972-T, isolated from a child with pyoderma and nephritis, was employed. This latter strain appeared rich in M protein. No cross-protection between these heterologous types was demonstrated.

These same strains and antisera were examined further in capillary precipitin tests and micro-immunodiffusion. Antiserum absorbed only with T-6 cells gave strong homologous reactions, but two-way heterologous cross-reactions were observed among the three types. A

TABLE 4. Indirect bactericidal test with 2998-T (type 61) and homologous antisera M-6-anti-M-6 as controls

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of chains, with bacterial dilutions of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10^{-9}</td>
</tr>
<tr>
<td>2998-T</td>
<td>1,000</td>
</tr>
<tr>
<td>M-6—End of test</td>
<td></td>
</tr>
<tr>
<td>With anti-2998-T (X)*</td>
<td>122</td>
</tr>
<tr>
<td>With anti-2998-T (Y)</td>
<td>90</td>
</tr>
<tr>
<td>With anti-2998-T (Z)</td>
<td>70</td>
</tr>
<tr>
<td>Stock</td>
<td>1,000</td>
</tr>
<tr>
<td>M-6—End of test</td>
<td></td>
</tr>
<tr>
<td>With anti M-6</td>
<td>50</td>
</tr>
<tr>
<td>With anti-2998-T (X) PL*</td>
<td>250</td>
</tr>
</tbody>
</table>

a X, Y, and Z represent three different rabbits immunized with vaccine strain.
b PL, Partially laked plate.
Table 5. Reciprocal indirect bactericidal test with types 49, 59, and 61

<table>
<thead>
<tr>
<th>Determination</th>
<th>2998-T</th>
<th>2829-S</th>
<th>2972-T</th>
<th>M-6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10^-4</td>
<td>10^-5</td>
<td>10^-6</td>
<td>10^-7</td>
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<tr>
<td>Inoculum</td>
<td>1,000</td>
<td>85</td>
<td>10</td>
<td>430</td>
</tr>
<tr>
<td>End of test</td>
<td>L</td>
<td>3,000</td>
<td>134</td>
<td>L</td>
</tr>
<tr>
<td>Normal rabbit serum</td>
<td>87</td>
<td>8</td>
<td>2</td>
<td>PL</td>
</tr>
<tr>
<td>Anti-2998-T (type 61 antiserum)</td>
<td>PL</td>
<td>2,000</td>
<td>400</td>
<td>10</td>
</tr>
<tr>
<td>Anti-2829-S (type 59 antiserum)</td>
<td>PL</td>
<td>2,000</td>
<td>160</td>
<td>L</td>
</tr>
<tr>
<td>Anti-2972-T (type 49 antiserum)</td>
<td>PL</td>
<td>3,000</td>
<td>200</td>
<td>PL</td>
</tr>
<tr>
<td>Anti-M-6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* M-6 cells (control) were run at one dilution only. L, Laked plate; PL, partially laked plate. Italicized numbers represent homologous results.

Table 6. Indirect bactericidal tests with 2797-S (M-60) and M-type 4

<table>
<thead>
<tr>
<th>Strain</th>
<th>Determination</th>
<th>2797-S (M-60)</th>
<th>2845-T (M-4)</th>
<th>No. inoculated</th>
<th>No. at end of test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No. inoculated</td>
<td>No. at end of test</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2797-S</td>
<td>2845-T</td>
<td>500</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td></td>
<td>With anti-2797-S</td>
<td>With anti-M-4</td>
<td>88</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td></td>
<td>With anti-M-4</td>
<td>With anti-M-6</td>
<td>3,000</td>
<td>3,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>With anti-M-6</td>
<td>With normal rabbit serum</td>
<td>800</td>
<td>PL</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>400</td>
<td>900</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>143</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>900</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td>160</td>
<td></td>
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<td></td>
<td>180</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>160</td>
<td></td>
</tr>
</tbody>
</table>

* PL, Partially laked plate.

type 60 and the previously described new types 59 and 61; no cross-reactions were observed.

Type 60 M-antisem was examined with extracts of all established types including those recently described new types. M-types 4, 24, 26, 29, and 46 are known to share the T-4 antigen. Type 60 did not cross-react with the M antisem of these various types. A minor one-way precipitin cross-reaction was noted between extracts of type 4 and the type 60 antisem, which was easily removed by absorption with type 4 cells. A strong (4+) homologous precipitin reaction remained.

Specificity of agglutination reactions: types 59, 60, and 61. A general correspondence between T antigens and M serotypes was noted with many of the originally described, lower-
numbered types of group A streptococci, but such relationships among pyoderma strains have proven variable, more complex, and occasionally unpredictable. The new M-types 59 and 61 appeared related by T agglutination, to one-another and to several other distinct M serotypes which are agglutinated by such T antisera as 5, 11, 12, 27, or 44. Efforts to further define their T antigens met with some success. An agglutination antiserum for type 59, prepared against 2829-S, proved relatively specific after absorption and dilution (antiserum absorbed with T-6 and M-11 cells, then diluted 1:5, 1:50, and 1:100 as recommended by Maxted). Agglutination reactions with several homologous and heterologous types at different antiserum dilutions are shown in Table 7. Significant cross-reactions occurred only at the 1:5 dilution and were limited to those stock T-vaccine strains included in this table. Minor cross-reactions were noted at 1:5 with certain wild strains, including the type 61 vaccine strain, known to agglutinate with various T antisera common to the W pool (11, 12, 5/27/44). The type 59 agglutination antiserum was subsequently included as a reference antiserum in our laboratory.

Type 60 strains agglutinated briskly with T-4 antisera from both Colindale and NCDC, but this reaction was removed by a 10-fold dilution of the antisera without loss of homologous (T-4, M-4) agglutination reactions. The agglutination antiserum prepared for type 60 (strain 2797-S) proved specific for the homologous type after absorption with T-6 cells and cells prepared with the stock type 4 vaccine strain. Absorption with the latter cells eliminated cross-agglutination reactions with type 4 strains as well as other M serotypes, which either share or have a T antigen closely related to that common to type 4. The type 60 antiserum for agglutination prepared in this manner gave strong homologous reactions and was found to be a useful addition to the battery of reference antisera in use in this laboratory. Attempts to prepare a specific agglutination antiserum for type 61 were unsuccessful.

Prevalence of new types 59, 60, and 61 among patients with pyoderma and AGN. A systematic search for strains representative of

<table>
<thead>
<tr>
<th>Strains</th>
<th>Reference antiserum</th>
<th>T-type 59 agglutination antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Agglutination &quot;pool&quot;</td>
<td>T type</td>
</tr>
<tr>
<td>2728-Sa</td>
<td>W/X</td>
<td>T-11/12</td>
</tr>
<tr>
<td>2853-Sa</td>
<td>W/X</td>
<td>T-11/12</td>
</tr>
<tr>
<td>3167-S</td>
<td>W/X</td>
<td>T-11/12</td>
</tr>
<tr>
<td>2238-S</td>
<td>W/X</td>
<td>T-11/12</td>
</tr>
<tr>
<td>2840-S</td>
<td>W/X</td>
<td>T-11/12</td>
</tr>
<tr>
<td>Colindale stock</td>
<td>W</td>
<td>T-5</td>
</tr>
<tr>
<td>Colindale stock</td>
<td>W</td>
<td>T-11</td>
</tr>
<tr>
<td>Colindale stock</td>
<td>W</td>
<td>T-12</td>
</tr>
<tr>
<td>Colindale stock</td>
<td>W</td>
<td>T-27</td>
</tr>
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<td>Colindale stock</td>
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<td>T-44</td>
</tr>
<tr>
<td>Colindale stock</td>
<td>T</td>
<td>B-3264</td>
</tr>
<tr>
<td>Colindale stock</td>
<td>Z</td>
<td>T-18</td>
</tr>
<tr>
<td>Miscellaneous wild strains</td>
<td>W</td>
<td>T-12</td>
</tr>
<tr>
<td>(Alabama collection)</td>
<td>W</td>
<td>T-11</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td>5/27/44</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td>T-11</td>
</tr>
<tr>
<td></td>
<td>X</td>
<td>Imp.19</td>
</tr>
<tr>
<td></td>
<td>X</td>
<td>25/Imp.19</td>
</tr>
<tr>
<td></td>
<td>X</td>
<td>8/25/Imp.19</td>
</tr>
<tr>
<td></td>
<td>X</td>
<td>8/25/Imp.19</td>
</tr>
<tr>
<td></td>
<td>X</td>
<td>14/49</td>
</tr>
<tr>
<td></td>
<td>U</td>
<td>T-28</td>
</tr>
<tr>
<td></td>
<td>U</td>
<td>T-4</td>
</tr>
<tr>
<td></td>
<td>U</td>
<td>T-4</td>
</tr>
</tbody>
</table>

* Type 59 vaccine strains.
* All remaining stock vaccine strains were negative at 1:5 dilution.
these new types began with a re-examination of our collection from epidemiological studies of pyoderma and AGN accomplished during the years 1966 through 1968. Those strains found M positive are shown in Table 8. Agglutination reactions with reference antisera and the newly prepared agglutination sera are included, and M types are arranged in relation to these results. Ninety strains were agglutinated with the "T-59" antiserum, and 70 of the 90 were proven to be M-type 59. Additional strains not listed which were negative with both the agglutination and precipitin antisera for type 59 included 83 T-12, non-M-typable strains and 47 others identified by agglutination only as members of the 5/27/44 agglutination complex. Type 61 strains were limited to those agglutinated as T-11 or T-9/11. During this 3-year period, type 61 occurred with similar frequency to type 11; no other M type was identified among the strains sharing the T-11 or T-9/11 agglutination reaction. Type 60 strains were limited to those initially identified by agglutination as T-4; they were subsequently shown to be specifically agglutinated by the "T-60" agglutination antiserum. Type 60 strains were far more common than type 4 in this collection.

Additional, previously non-M-typable strains, most isolated from patients with AGN following pyoderma, who were seen either prior to 1966 or in 1969, were also reexamined with these new M antisera. A total of 19 type 60 strains, including 18 isolated prior to 1966, were identified in addition to those shown in Table 8. Of the total of 48 type 60 strains found, 11 were from patients with pyoderma and nephritis and two others were from their sibling contacts. It was the most common of the three new types associated with AGN. Additional strains of both type 59 and type 61 were identified, bringing the total to 72 and 37, respectively, confirmed as members of these types. In all, 24 cases of poststreptococcal nephritis were associated with one or another of these new types between 1964 and 1969.

A sizable collection of strains from studies of both pyoderma and acute pharyngitis are yet to be examined, but preliminary data from studies to date suggest that type 60 occurs more often among patients with pharyngitis than either of the remaining two new types. All three types, however, are most often associated with pyoderma.

**DISCUSSION**

The identification of M antigens among group A streptococci provides precise data for the investigator concerned with clinical and epidemiological studies of streptococcal infections (9, 12). Earlier studies of pyoderma and nephritis provided little information regarding the prevalence of given M serotypes causing such infection (2, 4). Parker and co-workers (16, 17) were the first to classify collections of group A streptococci found in skin lesions. Most strains could be classified only by T agglutination and fell into one of three agglutination complexes: 3/13/B3264, 5/11/12/27/44, or 8/25/Imp.19. Because these strains appeared especially common to pyoderma and were less often found in collections from other clinical sources, they were referred to as "impetigo streptococci."

A renewal of interest in pyoderma and nephritis in the past decade led to extensive epidemiological studies which have resulted in the recognition of at least 10 new streptococcal serotypes, including the three now reported. Most of these are especially common in pyoderma, and several of them are of major importance in nephritis. This impressive number of new M types found among pyoderma collections explains in large part the earlier failure to identify M antigens among pyoderma collections. The relationship of these new M types to the agglutination complexes characteristic of impetigo streptococci is of interest. Types 52 and 53 from Red Lake (23) and type 56 from Memphis (7) are agglutinated by 3/13/B3264 T antisera; types 55, 57 and 58 from Trinidad (15, 18) share the 8/25/Imp.19 complex of antigens; types 59 and 61, now being reported, are the first newly recognized M serotypes from pyoderma collections.
which share certain antigens common to the 5/11/12/27/44 complex. Thus, several new M serotypes have now been identified among each of the common impetigo complexes, and there is no longer doubt that these pyoderma streptococci produce M antigens.

A number of M types are now recognized to share antigens common to the 3/13/B3264 complex. Though infrequently incriminated in acute glomerulonephritis, these various types are most common among patients with uncomplicated impetigo (4, 15, 18, 23). New types 55, 57, and 58 are members of the 8/25/Imp.v9 agglutination complex. Types 55 and 57 have been associated with epidemic nephritis in Trinidad (15, 18, 19), and type 57 has also caused limited cases of nephritis at Red Lake (5). Earlier mention was made of the association of M-type 2 streptococci with pyoderma and nephritis in Alabama and our finding that these strains also share the 8/25/Imp.v9 complex (3). Among the new types found in our population, type 60 is of especial interest because of its relation to nephritis. Type 60 caused nephritis in Alabama as early as 1965. Recently this type has caused epidemic nephritis in Trinidad (being prevalent there in 1968 and 1969) (19) and has also been recovered in large numbers in Israel, where it apparently caused acute pharyngitis (1). Type 60 strains are of further interest because they are agglutinated by T-4 antiserum, in common with several other M serotypes (21). Among those types related in this manner, only type 4 and type 60 are of recognized importance in nephritis.

Both types 59 and 61 were also originally isolated from patients with pyoderma and nephritis and have accounted for a number of cases of nephritis in our population, but neither has yet been associated with epidemic outbreaks of nephritis. The cross-precipitin reactions observed between types 59 and 61 and a major pyoderma-nephritogenic serotype, type 49, attracted our interest. Although these cross-reactions could be eliminated by absorption of the respective antiserum, the observation was nonetheless suggestive that a common antigen may be shared by these types. Present epidemiological data suggest that type 59 is relatively more widespread in distribution than type 61 (R. R. Facklam, personal communication). Epidemiological studies among patients in our population to date indicate that each of the three new types are more common among patients with pyoderma than in those with acute pharyngitis.

Although disadvantages of the T-agglutination method for identifying group A streptococci, especially if used alone, are well recognized (12, 13, 21), the initial characterization of these newly reported serotypes by their T-agglutination reaction was of immense value in first recognizing them and subsequently selecting possible candidates as vaccine strains. The utilization of both type 59 and type 60 agglutination antisera has proven of practical value in continuing epidemiological studies. Maxted et al. (11) demonstrated the value of a specific agglutination antiserum in distinguishing type 49 streptococci from related types which share the T-14 antigen. Thus, greater specificity for the T-agglutination method of serotyping appears feasible and is likely dependent upon the selection of new or more appropriate vaccine strains. Preliminary identification of strains by T-antigen analysis may narrow the range of M antisera required for initial M-typing of wild strains (12-15). More specific agglutination antisera would further enhance the value of this method and could result in more economical and efficient use of M antisera. In addition, such antisera could enhance and expedite epidemiological studies involving large numbers of streptococci.

Cumulative studies of pyoderma and nephritis have made it abundantly clear that the needs for given M antisera within a reference set vary in relation to the site of streptococcal infection being investigated. There is mounting evidence that a clear dichotomy exists between acute pharyngitis and pyoderma insofar as prevalent streptococcal M serotypes causing these infections are concerned (25; H. C. Dillon, Fifth Int. Symp. Streptococcus Pyogenes, in press). Vaccine strains for the earlier established types came largely from sources other than skin and soft tissue infection, with cases of acute pharyngitis and/or scarlet fever being the most frequent sources (13, 23). The identification of new streptococcal serotypes among collections of pyoderma streptococci should lead to the development of reference antisera for both agglutination and precipitin typing particularly suited for pursuing studies of the epidemiology and immunity in pyoderma and nephritis.

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LITERATURE CITED


