STUDIES ON HEMOLYTIC STREPTOCOCCI

II. STREPTOCOCCUS PYOGENES

ALICE C. EVANS

Senior Bacteriologist, National Institute of Health, Washington, D. C.

Received for publication January 27, 1936

NOMENCLATURE

In 1884 Rosenbach described the streptococcus of wound infections and gave it the specific name *Streptococcus pyogenes*. Later, Klein described a streptococcus which he found associated with scarlet fever, and gave it the specific name *Micrococcus scarlatinae*. Arloing found a streptococcus associated with puerperal fever, and named it *Streptococcus puerperalis*, although in the same lecture in which he gave that name he contended that the streptococcus of puerperal fever is the same as that which may cause erysipelas or wound infections.

Prior to Rosenbach's description of *S. pyogenes*, Fehleisen had demonstrated that a streptococcus is associated with erysipelas. Rosenbach compared Fehleisen's streptococcus of erysipelas with the streptococcus of wounds, and found them different. He gave the name *S. erysipelatis* to Fehleisen's streptococcus. On account of the technical limitations of the early bacteriologists, Rosenbach was unable to describe the streptococci of wounds and of erysipelas fully enough to permit their identification at the present time. Nevertheless it is reasonable to accept his statement that he found differences between the streptococci from the two sources.

The name *S. erysipelatis* was adopted by Birkhaug for the streptococci which he found associated with erysipelas. The writer has studied some of Birkhaug's strains, and found that a few of them fall into a well defined group clearly distinguishable from other hemolytic streptococci. These peculiar Birkhaug
strains have been distributed widely to many laboratories in this and other countries under the name *S. erysipelatis*. Hence it is logical to retain that name for the small distinct group. The description of *S. erysipelatis* will be given in a later paper of this series. The present preliminary discussion was occasioned by the fact that some writers who have considered the nomenclature of streptococci have found it necessary to offer excuses for not recognizing the priority of the specific name *erysipelatis* for the main group of hemolytic streptococci. No such excuse is necessary if the name is applied to another distinct species of streptococcus capable of causing erysipelas.

There has never been described any character which would distinguish the three alleged species *pyogenes*, *scarlatinae* and *puerperalis*. It has been claimed that they possess a disease specificity correlated with agglutinative types. Recent investigators, however, (Williams; Griffith (1935)) are agreed that streptococci from scarlet fever or puerperal fever or erysipelas may fall into the same serological group with streptococci from other disease sources; and that scarlet fever, puerperal fever or erysipelas may be caused by more than one agglutinative type of streptococcus. Hence the system of classification of streptococci based on disease specificity is invalid, and must be replaced by a new system.

Andrewes and Christie arrived at the conclusion that *S. pyogenes* (Rosenbach) is the name that should be adopted for the large group of hemolytic streptococci to which the names *pyogenes*, *scarlatinae* and *puerperalis* have been given. Griffith (1935) accepted the name *S. pyogenes* for those streptococci which produce a zone of hemolysis when planted on the surface of horse blood agar, conform to certain colonial appearances, and are associated with outbreaks of disease in man.

The writer accepts the name *S. pyogenes* for the large group of hemolytic streptococci which includes the majority of strains from scarlet fever and from erysipelas, and also includes almost half of the puerperal fever strains in our collection. It includes a greater percentage of strains from acute suppurative diseases than any other group. The specific characters as defined by
STUDIES ON HEMOLYTIC STREPTOCOCCI

Griffith are not distinctive, however. It was pointed out in the first paper of this series that on account of the variation within a given species, colony appearance is not of much use for classification purposes. Griffith's proposal that the name *S. pyogenes* be limited to hemolytic streptococci pathogenic for man does not offer a distinctive character, because some hemolytic streptococci pathogenic for man differ so widely from *S. pyogenes* that they obviously belong to other species; and on the other hand *S. pyogenes* may attack lower animals as well as man. The latter point will be discussed further on.

It is the purpose of this paper to describe those characters of *S. pyogenes* which differentiate it from other species of streptococci which cause clear hemolysis on blood agar plates.

CHARACTERS OF STREPTOCoccus PYOGENES

The differential characters of *S. pyogenes* useful for routine identification purposes are as follows: Resistance to nascent phage D/693; resistance to lytic filtrate B/563; sensitivity to phages B/563, C/594 and C/646 in the nascent state. (There are quantitative differences in the sensitivity of the various strains to phage C, some being more readily lysed than others, but all *S. pyogenes* strains are at least slightly sensitive to both C/594 and C/646.) Lactose, salicin and trehalose are fermented; sorbitol and mannitol are not fermented.

Other characters are as follows: final hydrogen ion concentration in glucose broth is pH 5.0 or higher; sodium hippurate is not hydrolyzed; a few strains are capable of growth on 10 per cent bile agar, but none grew on 40 per cent bile agar. The optimum temperature is 37°C. Growth occurs more slowly at 20°C.

Of 33 strains of *S. pyogenes* tested, all but one were found to be capable of dissolving human fibrin. There are marked quantitative differences between the strains, as evidenced by the time required for lysis. The data are given in table 1. Tillett reported similar differences in the fibrinolytic activity of the human strains which he tested.

1 The methods for determining the characters were described in the first paper of this series.
Virulence of *S. pyogenes* for mice is generally low, as compared with the majority of animal strains of hemolytic streptococci. Of 21 strains tested, 12 failed to kill mice in doses of 0.01 cc. One exceptional strain killed in a dose of 0.00000001 cc. in the third mouse of a series of rapid passages. There was every degree of virulence between the two extremes. (Streptococci of certain other species which are regarded as highly virulent for mice, kill regularly in doses of 0.00000001 cc.)

Three strains of *S. pyogenes* from cases of erysipelas were tested for ability to produce erysipelas in rabbits. One-tenth cubic centimeter of 24-hour blood broth culture was injected intradermally into the tip of the shaved ear. One culture produced no effect. One produced a rise in temperature to 40°C. on the day following the inoculation, without redness of the ear, excepting at the point of inoculation. The third strain produced a typical erysipelatous lesion. On the day following the inoculation, a large part of the ear was reddened, with slight edema, the affected areas following the lymphatics. The outer portion of the ear, throughout its entire length, was delimited by a characteristic "wall," the margin being sharply marked, elevated and indurated on palpation. The temperature rose to 40.2°C., but there were no other signs of illness. After three days the ear began to heal. Other species of hemolytic streptococci produced erysipelas in rabbits with greater constancy than *S. pyogenes*.

Lancefield reported that the great majority (87.5 per cent) of her strains from human disease sources fell into her serological group A. Edwards, using Lancefield's technique, studied 26 strains from human disease and found that all of them agreed

<table>
<thead>
<tr>
<th>TOTAL NUMBER OF CULTURES TESTED</th>
<th>++++ INHIBITION OF CLOTTING</th>
<th>+++ INHIBITION IN LESS THAN 1 HOUR</th>
<th>++ INHIBITION IN 1 TO 3 HOURS</th>
<th>+ INHIBITION IN 3 TO 24 HOURS</th>
<th>-- NO INHIBITION IN 24 HOURS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>Per cent</td>
<td>Number</td>
<td>Per cent</td>
<td>Number</td>
<td>Per cent</td>
</tr>
<tr>
<td>33</td>
<td>10</td>
<td>30.3</td>
<td>5</td>
<td>15.1</td>
<td>12</td>
</tr>
</tbody>
</table>

TABLE 1

*Time required for lysis of human fibrin by S. pyogenes*
with Lancefield’s group A. From the work of these investigators, therefore, it is obvious that *S. pyogenes*, which includes 46.6 per cent of the strains of our collection from human disease sources, should logically belong to Lancefield’s group A.

Every strain in our collection of *S. pyogenes* which has been grouped serologically by other investigators using Lancefield’s technique, has fallen into her group A. These strains include Lancefield’s scarlet fever strain C 203, Coburn’s rheumatism strain RPH₁, the strain C₅b isolated from a cow associated with the Canton, Massachusetts epidemic of septic sore throat, and the strain K 158C isolated from a rabbit, all studied by Lancefield; two puerperal fever strains “Cripps” and “Watt” studied by Plummer; two strains from abscesses, (“17424” and “17318”) reported by Mickle (1935); and two strains from the throats of food handlers “Jackson” and “Siniscalco,” reported by Mickle. The number of strains which have been grouped according to the precipitin test is not large, but it includes several strains whose histories are suggestive of other precipitin groups. The fact that these strains agreed with *S. pyogenes* according to both phagological and serological reactions, indicates a definite correlation between the two reactions.

**RELATIONSHIP OF S. PYOGENES TO THE ESTABLISHED SEROLOGICAL GROUPS**

It has been shown that *S. pyogenes* is one of the species belonging to Lancefield’s precipitin Group A, which contains the great majority of human pathogenic strains. When grouped according to agglutinin absorption reactions, *S. pyogenes* has been found to include a large number of serological types.

Representative strains belonging to thirteen of Griffith’s (1935) serological types agreed with *S. pyogenes*. They are his types 1, 2, 4, 5, 8, 9, 11, 12, 14, 19, 24, 25 and 26. Griffith’s types 15 and 18, which he found to be not clearly differentiated, each include one strain of our collection which agreed with *S. pyogenes*, and another strain which must be regarded as atypical *S. pyogenes*. The latter group will be discussed in a later paper. Griffith’s type 3 is his only one in which there is a confusion of
the species when compared with the present classification. There are in our collection 5 strains which Griffith placed in his type 3. Two of them agreed with the typical \textit{S. pyogenes}, but the remaining three belonged to three other species. Andrewes and Christie made a detailed serological study of a number of strains belonging to Griffith's types 1, 2, 3 and 4. They readily confirmed the individuality of his types 1, 2 and 4, but they could not confirm his type 3 as a distinct entity. They were of the opinion that the agglutinins which gave cross-reactions within this group were non-specific, group agglutinins. Our results confirm those of Andrewes and Christie in indicating that Griffith's type 3 is not a well defined group, but is made up of strains of various characters.

Of the serological types established by Williams and her collaborators, the strains representing the following types agreed with \textit{S. pyogenes}: scarlet fever types I, sub I, II and IV; erysipelas types I, sub-IIa and sub-IIb.

The scarlet fever strains representing all 6 of Mueller and Klise's serological types agreed with \textit{S. pyogenes}.

The strains from rheumatism representing Coburn and Pauli's groups I, II, III and VI agreed with \textit{S. pyogenes}. The strains of their group V had the characters of another species. There was a discrepancy in the two classifications in regard to the two strains of their group IV, one agreeing with \textit{S. pyogenes}, and the other agreeing with the strains of their group V.

\textbf{TYPE STRAIN}

Strain 1168 was chosen for the type strain, although any one of a large number of strains might have served equally well. Strain 1168 was isolated on April 27, 1935, by Dr. R. E. Otten of the Philadelphia General Hospital, from pus obtained at operation in a case of mastoiditis. The description of the type strain is as follows:

The cells are Gram-positive, and in broth culture they occur in pairs or in chains of short or medium length. On infusion agar containing 5 per cent rabbit blood, after 48 hours incubation, the colonies are smooth and discrete, the largest isolated
colonies being about 1.0 mm. in diameter. The small colonies in crowded areas are convex. The larger colonies have an opaque center and a narrow, more translucent edge, which may be somewhat flattened. A zone of clear hemolysis about 1.0 mm. wide surrounds the colonies.

Strain 1168 is sensitive to phages B/563, C/594 and C/646 in the nascent state; it is not sensitive to D/693 in the nascent state, nor to lytic filtrate B/563; final pH in glucose broth was 5.4; lactose, salicin and trehalose are fermented, mannitol and sorbitol are not fermented. There is no growth on 10 per cent bile blood agar; sodium hippurate is not hydrolyzed; the clotting of human plasma is inhibited; mice were killed in the $10^{-4}$ dilution in a series of rapid passages.

The type strain has been deposited in the American Type Culture Collection.

SOURCES OF HUMAN PATHOGENIC STRAINS OF S. PYOGENES

Of the 373 strains of hemolytic streptococci from human diseases in our collection, 174, or 46.6 per cent agreed with the type strain of S. pyogenes. The diseases with which they were associated were as follows:

Scarlet fever. Of the 120 scarlet fever strains in the collection, 74, or 61.6 per cent agreed with S. pyogenes. They were from the countries of three continents, as follows: From various parts of the United States, 36 strains; from Argentina, 2 strains; from Austria, 1 strain; from China, 1 strain; from England, 16 strains; from Germany, 2 strains; from Hungary, 8 strains; from Italy, 1 strain; from Russia, 5 strains; from Scotland, 2 strains.

The previous designations of those scarlet fever strains belonging to the species S. pyogenes which have been studied by other investigators are as follows:

The strain known as Dick I.

Williams and Gurley's strains: 2(type 1); 55(type sub-I) 114(type 2), and 28(type 4).

Mueller and Klise's strains: M 2(B I); M 9(B II); M 113(B III);
M 18(B IV); M 116(B V); M 173(B V); M 10(B VI).

Griffith's strains: 130(type 1); 22(type 2); 8(type 2); Lewis (type 3);
28(type 4); 4(type 8); Blackmore (type 11); 42(type 12); J.S.5(type 15); 7 İstanbul(type 19); 13(type 26).

Mickle's (1928) strains: 60175 and 60179 from the Avon epidemic, and strains 2422, 2666, 2669 and 2670 from the Thomaston epidemic.

James' strains: S4, S2, Sn and S185.

Smith's strains: S3, and S1o.

Andrewes and Christie's strains: Moscow 41, 45, 52, 61 and 69; R. Clarke, Davy, 39, and St. Louis.

It is to be noted that the well known strains "N.Y.5," "Dick II," "Dick III" and "Dick IV," all of which are commonly used for toxin production by the manufacturers of scarlet fever antitoxin, do not agree with S. pyogenes. They belong to other species, which will be described in later papers of this series. It may be stated, however, that "N.Y.5" and "Dick IV" do not ferment salicin; Dick III does not ferment lactose; and Dick II and Dick III differ phagologically from S. pyogenes.

Erysipelas. Of the 43 erysipelas strains in the collection, 22 or 51.2 per cent agreed with S. pyogenes. Eighteen were isolated in various parts of the United States, 1 in Canada, and 3 in England. Among the S. pyogenes strains from erysipelas in our collection are Spicer, Gonshorek and Spicer's strains E/102, E/103, E/104, E/105, E/106, E/111, and E/114; and strain 769 received from the American Type Culture Collection (listed under the name S. erysipelatis).

Puerperal fever. Of the 62 puerperal strains in the collection, 29, or 46.8 per cent agreed with S. pyogenes. One strain was isolated in New York City; 5 were isolated in Canada; and the remainder were isolated in London. The previous designations of those puerperal fever strains which have been studied by other investigators are as follows:

Meleney's epidemic strain.

Griffith's strains: Franklin (type 5); and Barker (type 14).

Colebrook's strains: Collis, Mills, Aying, Pearce, Herbert, Evans, Richards, Jackman, Patis and Timothy.

Andrewes and Christie's strains: Stevens, Jarvis, Crofts, Toms, Annie Smith, Eldridge, Unstead, Kent, Warrington, Digweed and French.
Rheumatism. Seven, or 43.7 per cent of the 16 strains from cases of rheumatism agreed with *S. pyogenes*. All 7 were strains previously studied by Coburn and Pauli. They are their strains: RC₁, RPH₁ and R₁₂ (Group I); R₁₁ (Group II); R₁₈ (Group III); RG₃ (Group IV); R₁₇ (Group VI).

Acute suppurative diseases. Nineteen, or 36.5 per cent of the 52 strains from acute suppurative diseases (meningitis, peritonitis, empyema, osteomyelitis, mastoiditis, sinusitis, otitis media, cystitis, adenitis and abscesses) agreed with *S. pyogenes*. Two strains were from London and the remainder were from Washington, D. C., Baltimore and Philadelphia.

Septic sore throat. In our collection there are strains from 21 epidemics of sore throat, with more than one strain from several of the epidemics. In an earlier paper (1935) it was reported that the majority of septic sore throat strains differ phagologically from *S. pyogenes*, but that *S. pyogenes* was involved in four of the sore throat epidemics. They were the Canton, Massachusetts epidemic of 1913; the Lee, Massachusetts epidemic of 1928, the Pittsfield, Massachusetts epidemic of 1928 (Lee and Pittsfield are only 10 miles apart), and an epidemic in a public school in England, from which Griffith obtained his strain "Symons" (type 9).

There were no human strains from the Canton epidemic available for this study, but a strain which was isolated from suspected milk agreed with *S. pyogenes*.

The 4 strains, listed as *Streptococcus pyogenes* Rosenbach in the third edition (1934) of the Catalogue of Cultures of the American Type Culture Collection were all studied, with the following results:

Strain 420 (the catalog number) agreed with the characters of *S. pyogenes* as described in this paper. Strain 4444 differed very slightly from typical *S. pyogenes*. The remaining two strains listed as *S. pyogenes* belonged definitely to other species of hemolytic streptococci, as indicated by both phagological and fermentation reactions.
ALICE C. EVANS

STRAINS OF S. PYOGENES ISOLATED FROM COWS ASSOCIATED WITH EPIDEMICS

It is of historic interest that in 1885 Klein, who gave the specific name to the etiologic agent of scarlet fever, traced an epidemic of scarlet fever to the milk of cows on a certain farm.

Armstrong and Parran reviewed the data on milk borne epidemics in the United States to January, 1927, and found that 42 epidemics of septic sore throat and 40 epidemics of scarlet fever were on record. Twelve of the sore throat epidemics were traced to dairy farms with cows suffering with mastitis. There were no data in the records, however, which would show whether the epidemics were caused by S. pyogenes or some other hemolytic streptococcus.

In our collection there are 7 strains from cows which agree with S. pyogenes. All were isolated from milk associated with epidemics, and all were previously studied by other investigators. In table 2 are given the histories of the strains. The findings of the investigators who previously studied these strains are as follows:

The Avon, Connecticut epidemic of scarlet fever (1928) was traced to a cow which, according to Mickle, showed no physical signs of udder infection at the time, but shortly afterwards began to produce milk of a gargety appearance. Mickle (1928) states that Dr. Jones of Princeton, New Jersey and Dr. Blake of New
Haven both studied the milk strains and found that they were like the scarlet fever strains. Two streptococcus cultures from the suspected milk, Nos. 567 and 757, and also a strain from the throat of a milk handler, and a strain from the throat of a patient were available for the present study, all of which were found to agree with *S. pyogenes*.

The Lee, Massachusetts epidemic of sore throat (1928) was traced to a cow which had active mastitis. Robinson and Beckler reported that they found that the strain from the cow and the strains from patients agreed with *S. epidemicus*. Williams and Gurley reported that they received strains of two serological types from the Lee epidemic. The milk strain which they studied was our No. 623. They found that it had the characteristics of scarlet fever strains. Williams and Gurley studied throat strains from the Lee epidemic and found that they agreed with *S. epidemicus*. Our results confirm the conclusions of Williams and Gurley in showing that both *S. pyogenes* and *S. epidemicus* were involved in the Lee epidemic. Two milk strains (623 and 737) and 4 throat strains of our collection agreed with *S. pyogenes*, and two throat strains agreed with *S. epidemicus*.

Strain 773 was isolated from the milk of a cow which had an injured teat and inflamed udder. A sharp outbreak of scarlet fever was traced to this cow. Jones and Little studied the milk strain extensively. They found that it was culturally indistinguishable from scarlet fever strains; that it was serologically related to scarlet fever strains, and that it produced a toxin which was neutralized by scarlet fever antitoxin. Pilot and Stocker also found that it produced a toxin neutralizable by scarlet fever antitoxin and that its toxin differed from that produced by *S. epidemicus*.

Strain 891 (C\(\alpha\)b) was isolated by Smith and Brown from a sample of milk from a dairy herd assumed to be the source of infection in a severe epidemic of tonsillitis in Canton, Massachusetts in 1913. Lancefield classified this strain in her group A.

Tillett studied two of the strains from cows under discussion (Nos. 773 and 891) and found that they are capable of lysing human fibrin.
The scarlet fever epidemic in Plymouth, Massachusetts, in 1929 was reported by Scamman. The ability of the Plymouth strain to produce mastitis in a cow was tested by the writer in collaboration with Dr. W. T. Miller, of the Bureau of Animal Industry, United States Department of Agriculture. The test was made five years after the isolation of the culture. Twenty-four hour infusion broth culture was injected into two quarters of the udder, in doses of 2 and 5 cc. respectively. There was no appearance of mastitis following this inoculation. Two weeks later, the other two quarters were injected with 10 and 15 cc. respectively of 24-hour broth culture. Clinical mastitis developed in the two quarters last inoculated. It gradually subsided, and 7 weeks after the last inoculation, samples of milk from the infected quarters appeared normal, and yielded only a few colonies of hemolytic streptococci. The experiment demonstrated only a mild pathogenicity of the Plymouth strain for the cow.

**TABLE 3**

*Human carrier strains of S. pyogenes*

<table>
<thead>
<tr>
<th>STRAIN</th>
<th>PREVIOUS DESIGNATION</th>
<th>PREVIOUS INVESTIGATORS</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1061</td>
<td>Brewer</td>
<td>Andrews and Christie</td>
<td>From throat of nurse attending puerperal case</td>
</tr>
<tr>
<td>1075</td>
<td>Wright</td>
<td>Andrews and Christie</td>
<td>From throat of doctor during puerperal epidemic</td>
</tr>
<tr>
<td>1196</td>
<td>Jackson</td>
<td>Mickle</td>
<td>Food handler</td>
</tr>
<tr>
<td>1197</td>
<td>Siniscalco</td>
<td>Mickle</td>
<td>Food handler</td>
</tr>
</tbody>
</table>

Four strains from normal human throats agreed with *S. pyogenes*. Their histories are given in table 3. Two were from food handlers without a history of contact with infected subjects. One (No. 1061) was from the throat of a nurse attending a case of puerperal fever. The patient's strain ("Toms" listed under Andrews and Christie's puerperal strains) and the carrier strain were identical. The other carrier strain (No. 1075) was from
the throat of an attending doctor isolated during an epidemic of puerperal fever at Queen Charlotte's Maternity Hospital, London. Three strains isolated from patients were available for this study. (Strains "Warrington," "Digweed" and "French," listed under Andrewes and Christie's puerperal strains). The carrier strain and the three patients' strains were identical.

SUMMARY

According to the data presented, Streptococcus pyogenes Rosenbach as here defined, is the species of hemolytic streptococcus which is most commonly associated with human disease. If our collection is representative, Streptococcus pyogenes causes the majority of cases of scarlet fever and erysipelas, and it causes almost half of the cases of puerperal fever. It is responsible for a comparatively small percentage of epidemics of septic sore throat. The figures for each disease are given in table 4.

TABLE 4
Diseases caused by Streptococcus pyogenes

<table>
<thead>
<tr>
<th>DISEASES</th>
<th>TOTAL NUMBER OF STRAINS FROM HUMAN DISEASE SOURCES</th>
<th>STRAINS OF S. PYOGENES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Per cent</td>
</tr>
<tr>
<td>Scarlet fever</td>
<td>120</td>
<td>74</td>
</tr>
<tr>
<td>Erysipelas</td>
<td>43</td>
<td>22</td>
</tr>
<tr>
<td>Puerperal fever</td>
<td>62</td>
<td>29</td>
</tr>
<tr>
<td>Rheumatism</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>Acute suppurative diseases</td>
<td>52</td>
<td>19</td>
</tr>
<tr>
<td>Epidemic sore throat</td>
<td>24</td>
<td>4</td>
</tr>
<tr>
<td>Other miscellaneous diseases</td>
<td>56</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>373</td>
<td>174</td>
</tr>
</tbody>
</table>
ALICE C. EVANS

KLEIN, E. 1887 On the etiology of scarlatinae. Ibid., 16, 367-414.
MICKLE, FRIEND LEE 1935 Personal communication.
MUeller, J. HOWARD, and KLISE, KATHERINE S. 1933 Jour. Inf. Dis., 52, 139-145.
ROSENBAECH, FRIEDR. JUL. 1884 Mikro-organismen bei den Wund-Infektionen der Menschen. Wiesbaden.
WILLIAMS, ANNA W. 1932 Streptococci in Relation to Man in Health and Disease. The Williams & Wilkins Company, Baltimore.