STUDIES ON HEMOLYTIC STREPTOCOCCI

IV. STREPTOCOCCUS SCARLATINAЕ

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INTRODUCTION

In the second paper of this series Streptococcus pyogenes was described, and it was stated that among the 120 scarlet fever strains in our collection, 74, or 61.6 per cent, had the characters of that species. Strains from a great variety of other streptococcus diseases also agreed with S. pyogenes. It was stated that another group included only scarlet fever strains. That group will be described in this paper.

NOMENCLATURE

On account of the technical limitations of the early bacteriologists, they were unable to describe strains fully enough to be identified with groups recognized as species many years later. It is logical, however, to apply the name Streptococcus scarlatinae, which Klein gave in 1887 to his scarlet fever strains, to a group which appears to be specific for scarlet fever. (In the earlier paper the reasons were given for not applying the name S. scarlatinae to the S. pyogenes group which, according to our data, appears to be the most common cause of scarlet fever.)

Andrewes and Horder were the first to recognize a distinctive character of a group of streptococci which they observed to be associated particularly with scarlet fever. They described the type as capable of fermenting sucrose, lactose, and raffinose, but not inulin, salicin, coniferin, or mannitol. They considered the
likelihood that this group of organisms was the same as Klein's S. scarlatinae, but rejected the idea on the basis of the frequent incapacity of their streptococcus for growth on gelatin at 20°C. They gave it the name Streptococcus anginosus. Writing before it had been established that streptococci can produce scarlet fever, Andrewes and Horder stated that it seemed likely that if scarlet fever is a streptococcal disease some form of S. anginosus would be found to be the causal agent.

Andrewes and Horder did not offer a clear definition of S. anginosus, for, as the data presented in this paper will show, differences in ability to grow at 20°C are not sufficiently definite to be regarded as a differential test for the classification of scarlet fever strains. Hence, the introduction of the new name S. anginosus for the salicin-nonfermenting group was not justifiable. Since it has been commonly used, however, it must now be regarded as a synonym of S. scarlatinae.

Andrewes and Horder described 25 variants of S. anginosus which differed from their type strain in fermentation reactions. Ten of the variants were capable of fermenting salicin. They found their poorly-defined S. anginosus not only in scarlet fever, but occasionally also in other sore throats, as well as in other diseased conditions. They observed, however, that it is rarely associated with suppuration. According to our data, it appears that Andrewes and Horder would not have found such a wide distribution of S. anginosus in other than scarlet fever and sore throat cases if they had limited the species to strains incapable of fermenting salicin.

Holman defined the species S. anginosus more clearly by limiting it to strains capable of fermenting lactose but incapable of fermenting mannitol or salicin. He confirmed the observation of Andrewes and Horder that few strains of this group are found to be the causative agents in purulent conditions.

The preceding review has shown how the specific name anginosus came into use for the group of streptococci to which, as it now appears, the name S. scarlatinae should apply. For the following reasons the writer accepts the specific name S. scar-
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*latinae* for the group of streptococci associated with scarlet fever and distinguished by inability to ferment salicin:

(1) It was the first specific name given to streptococci associated with scarlet fever; (2) it is a suitable name, implying the specificity for scarlet fever which, as will be shown later, the group possesses; (3) a strain ("N. Y.5") of this group, designated as *S. scarlatinae* in the Catalog of the American Type Culture Collection, is commonly used by the manufacturers for the production of scarlet fever antitoxin, on account of the potency and polyvalency of its toxin.

A number of writers have designated as *S. scarlatinae* any streptococcus which fails to ferment salicin. The errors resulting from relying on fermentation reactions alone for the classification of streptococci were discussed in the preceding paper of this series. It happens, however, that according to our data, although there are other groups of hemolytic streptococci associated with other diseases which fail to ferment salicin, nevertheless among scarlet fever strains failure to ferment salicin is a distinctive character. In our collection of 395 strains of hemolytic streptococci from human disease sources, the strains belonging to the species *S. scarlatinae* are the only ones which fail to ferment salicin, excepting the strains of one small group which disagrees with *S. scarlatinae* in other characters and appears to be incapable of causing scarlet fever, and some of the strains of "minute hemolytic streptococci" of Long and Bliss which, on account of their very slow and sparse growth, would hardly be confused with *S. scarlatinae*. Hence, whenever salicin-nonfermenting scarlet fever streptococci are mentioned in the literature, it is reasonably certain that *S. scarlatinae* is the species under discussion.

There is a question in the mind of the writer as to whether it might not be more logical to regard the group of salicin-nonfermenting strains as a variety or sub-species of *S. pyogenes*. Since, however, bacteriologists generally regard every clearly defined group as a separate species, and since in writing or speaking it is awkward to give species and sub-species designation, the group is referred to as a species in this paper. At any rate the name *scarlatinae* is the valid name for the salicin-nonfermenting group of scarlet fever streptococci, whether the group be regarded as a species or a variety.
THE CHARACTERS OF STREPTOCOCCUS SCARLATINAE

The differential characters of S. scarlatinae are like those of S. pyogenes (see the second paper of this series), excepting that S. scarlatinae does not ferment salicin. The characters of the type strains of the two species are given in table 1. Although the two species are distinguished by only one of the differential tests, this test gives an obviously valid distinction, because inability to ferment salicin is correlated with definite pathogenic properties. Our data show that S. pyogenes is capable of causing not only scarlet fever, but also erysipelas, puerperal fever, and many kinds of acute suppurative diseases, whereas it appears that S. scarlatinae may be specific for scarlet fever, (or sore throat without the rash,) for in our collection of 262 strains of hemolytic streptococci from all kinds of human streptococcus diseases other than scarlet fever, not one agreed with S. scarlatinae.

In addition to the one differential test which distinguishes

<p>| TABLE 1 |
|-------------------|-------------------|-------------------|-------------------|
| Differential characters of the type strains of S. pyogenes and S. scarlatinae |</p>
<table>
<thead>
<tr>
<th>Designation of type strain</th>
<th>Sensitivity to nascent phage</th>
<th>Sensitivity to R/33 filtrate</th>
<th>Fermentation of</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. pyogenes</td>
<td>1168</td>
<td>+ + + -</td>
<td>- + + - + -</td>
</tr>
<tr>
<td>S. scarlatinae</td>
<td>642</td>
<td>+ + + -</td>
<td>- + - + + -</td>
</tr>
</tbody>
</table>

<p>| OTHER CHARACTERS |
|-------------------|-------------------|-------------------|-------------------|</p>
<table>
<thead>
<tr>
<th>Final pH in glucose broth</th>
<th>Growth in bile</th>
<th>Hydrolysis of sodium hippurate</th>
<th>Lysis of human fibrin</th>
<th>Virulence for mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. pyogenes</td>
<td>5.4</td>
<td>-</td>
<td>-</td>
<td>++++</td>
</tr>
<tr>
<td>S. scarlatinae</td>
<td>5.4</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* The methods for determining the characters were described in the first paper of this series.
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S. scarlatinae from S. pyogenes, there are other differences which may be observed when a number of strains of the two species are compared, but they are not sufficiently definite to be useful for the identification of individual strains.

On 10-per-cent-bile blood agar 42.8 per cent of 14 strains of the S. scarlatinae group grew as compared with 17.6 per cent of the 74 scarlet fever strains of the S. pyogenes group.

Nineteen scarlet fever strains of S. pyogenes and thirteen strains of S. scarlatinae were compared as to their ability to dissolve human plasma. The results summarized in table 2 show that, in general, the strains of S. scarlatinae possess weaker fibrinolytic power than the scarlet fever strains of S. pyogenes.

<table>
<thead>
<tr>
<th>TABLE 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comparison of fibrinolytic properties of scarlet fever strains of the species S. pyogenes and S. scarlatinae</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DEGREE OF FIBRINOLYSIS</th>
<th>S. PYOGENES (19 STRAINS TESTED)</th>
<th>S. SCARLATINA (13 STRAINS TESTED)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>number</td>
<td>per cent</td>
</tr>
<tr>
<td>++++</td>
<td>4</td>
<td>21.1</td>
</tr>
<tr>
<td>++</td>
<td>4</td>
<td>5.2</td>
</tr>
<tr>
<td>+</td>
<td>8</td>
<td>42.1</td>
</tr>
<tr>
<td>-</td>
<td>5</td>
<td>26.3</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>5.3</td>
</tr>
</tbody>
</table>

The virulence of S. scarlatinae for mice is low. Among 9 strains which had been maintained in laboratories for years, 7 failed to kill mice in the lowest dilution tested (1:100); 2, including the type strain, killed mice in the 1:1000 dilution of the passage strain.

The observation of Andrewes and Horder that salicin-nonfermenting strains would not grow at 20°C. could not be confirmed. Of twelve strains of S. scarlatinae examined for this character, all grew at 20°C., as did also a similar number of strains of S. pyogenes. No difference could be observed in the rate of growth of the strains of the two species at this temperature.

The serological classification of representative strains of S. scarlatinae has been determined by several investigators. Two
strains of our collection (nos. 642 and 646) were studied serologically by Williams, and also by Griffith. Williams placed them in her agglutinative type 3, and stated that all of her scarlet fever strains of type 3 belonged to the anginosus group, failing to ferment salicin. Griffith placed strains 642 and 646 in his type 10, which appears to be the same as Williams' type 3. It would appear from the work of these two investigators that the strains of the species S. scarlatinae all belong to the same agglutinative type, whereas the strains of S. pyogenes belong to a number of agglutinative types, as was shown in the previous paper.

Dr. Elizabeth Verder, associated with the writer, has studied 10 S. scarlatinae strains and found that they all belong to Lancefield's group A.

THE TYPE STRAIN

It seemed logical to choose for the type strain of S. scarlatinae the one which, on account of its polyvalency and the high potency of its toxin, has been widely distributed among laboratories for the production of scarlet fever antitoxin. Hence, strain 642, isolated many years ago from a case of scarlet fever by Dochez and known in the literature as "N.Y.5," was chosen for the type strain. It has been deposited in the American Type Culture Collection where it was already listed as S. scarlatinae no. 4543.

Strain 642 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, or to lytic filtrate B/563; under the conditions of the test, final pH in glucose broth is 5.4; lactose and trehalose are fermented; salicin, mannitol, and sorbitol are not fermented. Growth occurs on 10-per-cent- but not on 40-per-cent-bile blood agar; sodium hippurate is not hydrolyzed; fibrinolysis of human plasma is weak, occurring between the 3rd and 24th hours or failing; mice are killed in the 10⁻⁴ dilution in a series of rapid passages.

The cells of strain 642 are Gram-positive, and in broth culture they occur in pairs or chains, many of which are long. On infusion agar containing 5-per-cent rabbit blood, after 48 hours' incubation, the colonies of the stock strain are smooth, convex,
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discrete. The largest isolated colonies are about 1 mm. in diameter. A zone of clear hemolysis about 1 mm. wide surrounds the colonies.

THE SOURCES OF S. SCARLATINAE STRAINS

Our collection contains 14 S. scarlatinae strains. They were from the following geographical sources:

From the United States, 5 strains; from Argentina, 3 strains; from Austria, 3 strains; from Hungary, 2 strains; from Russia, 1 strain.

The previous designations of our strains belonging to the species S. scarlatinae which have been studied by other investigators are as follows: Dochez' N.Y. 5 and 53 (American Type Culture Collection nos. 4543 and 4014); Williams' no. 57, type III; Dick IV; Michigan Department of Health no. 322; Andrewes and Christie's "Moscow 81." At least four of these strains are used by various laboratories for the production of antitoxin.

The four strains designated as S. scarlatinae listed in the Catalog of the American Type Culture Collection (third edition, 1934) were studied. As stated above, two were found to have the characters of S. scarlatinae. The other two were found to belong to other streptococcal species.

PREVALENCE OF S. SCARLATINAЕ

Fourteen, or 10.5 per cent of our 133 scarlet fever strains agreed with S. scarlatinae. Williams reported about the same percentage of salicin-nonfermenting strains in her collection. Seven out of 68, or 10.7 per cent of her scarlet fever strains failed to ferment salicin and agreed with her agglutinative type 3. Bliss found no strains which failed to ferment salicin among the 25 scarlet fever strains which he studied.

Apparently S. scarlatinae was not common in England when Griffith collected his strains of streptococci. He found no scarlet fever strains belonging to his type 10, which, as already pointed out, appears to be made up of S. scarlatinae strains. However, he isolated strains of his type 10 from 12 cases in a school outbreak of sore throat.

Although Griffith failed to find S. scarlatinae among the English
scarlet fever strains, it seems to have been prevalent in England three decades earlier, when Andrewes and Horder were so impressed with the frequency of salicin-nonfermenting strains in scarlet fever throats that they predicted that if it would ever be shown that a streptococcus is the etiologic agent in scarlet fever, \textit{S. anginosus}, as they called it, would be found to be the one concerned. Thus, it appears, there was a wave of \textit{S. scarlatinae} infection spread over England in 1906, but this organism was uncommon in England when Griffith collected the streptococci which he studied. In later papers of this series similar waves of other species of hemolytic streptococci will be noted.

Hence, although the geographical sources of our strains show that \textit{S. scarlatinae} is widely distributed, our data and the information in the literature lead to the conclusion that \textit{S. scarlatinae} is not always and everywhere a common cause of scarlet fever.

THE DISEASE SPECIFICITY OF \textit{S. SCARLATINAЕ}

As previously stated, Andrewes and Horder observed a relationship between the presence of \textit{S. anginosus} in the throat and symptoms of scarlet fever. They also noted that \textit{S. anginosus} was only rarely associated with suppuration. They found it in disease conditions other than scarlet fever, but as we have already pointed out, the wider distribution they reported, as compared with the marked disease specificity which we found, can be explained by the fact that Andrewes and Horder considered certain salicin-fermenting strains to be variants and reported them as \textit{S. anginosus}.

It is more difficult to understand why Holman found \textit{S. anginosus} in a variety of disease conditions, for he limited the species to salicin-nonfermenting strains. However, Holman confirmed the observation of Andrewes and Horder that remarkably few strains of this group could be definitely classed as the causative agent in purulent conditions, but that they are frequently associated with scarlet fever.

According to our data, it would appear that \textit{S. scarlatinae} has a disease specificity, for among 262 strains of hemolytic streptococci from human diseases other than scarlet fever, not one
agreed with *S. scarlatinae*. From the observation of Griffith, already quoted, however, and from the following observation of Williams, there seems to be no doubt that *S. scarlatinae* may cause sore throat without a rash. Williams reported that 4 strains of streptococci from cases of septic sore throat during an epidemic were found to agree with *S. anginosus* and with her type 3 agglutinative group. The epidemic occurred among the members of the staff of a contagious disease hospital, and was thought to have started from a case of scarlet fever. No rash developed in the sore throat subjects, all of whom had had scarlet fever, or had been recently immunized with scarlatinal vaccine.

**CAN S. SCARLATINAЕ INFECT COWS?**

None of the strains of *S. scarlatinae* in our collection was from a milk-borne epidemic. The present study is not extensive enough, however, to conclude from these data that *S. scarlatinae* may not infect the cow’s udder. The literature yields no information on this point. Frost, Gumm and Thomas described a milk streptococcus characterized by inability to ferment salicin, which differed from *S. scarlatinae*, however, in its ability to produce a higher degree of acidity in broth culture, and in its ability to hydrolyze sodium hippurate. These characters indicate that their strains were of the animal type.

Minett and Stableforth also described salicin-nonfermenting strains of hemolytic streptococci from cow’s milk. They were peculiar strains, capable of fermenting both trehalose and sorbitol. In their ability to produce acid in sorbitol they differed from *S. scarlatinae*. Smith and Brown and also Jones reported that they found in cow’s milk streptococci incapable of fermenting salicin. Their studies were made before the usefulness of trehalose and sorbitol for the differentiation of human and animal strains had been discovered. It now appears probable that their strains were of the animal type.

**DISCUSSION**

Having classified 61.6 per cent of scarlet fever strains of our collection in the species *S. pyogenes* (see first paper of this series),
and 10.5 per cent in the species *S. scarlatinae*, there remain a considerable percentage of scarlet fever strains which do not agree with either of these two species. They belong to other species which will be described in later papers of this series.

The confusion which resulted from basing the classification of hemolytic streptococci on disease source should be dispelled when disease problems are reconsidered in the light of the newer knowledge of streptococcal classification. The fact that different species of streptococci may be involved in different scarlet fever epidemics offers a possible explanation as to why the character and severity of the disease, the occurrence of secondary infections, and the mortality rates are subject to wide variations in different epidemics.

Considering the observations of the pyogenic property of *S. pyogenes* and the lack of this property in *S. scarlatinae*, it may be expected that complications will be found to occur more commonly in scarlet fever epidemics caused by *S. pyogenes* than in those caused by *S. scarlatinae*. An observation made in the Contagious Disease Hospital, Chicago, by Dack, Woolpert and Hoyne is of interest in this connection. They found that the strains from complicated cases of scarlet fever are more actively fibrinolytic than strains from uncomplicated cases. Their observation is harmonious with our observation that the strains of *S. pyogenes* are more actively fibrinolytic than the strains of *S. scarlatinae*.

So many difficulties attend the classification of hemolytic streptococci according to toxin production that a full understanding of the quality of toxin produced by the several species of scarlet fever strains is impossible at this time. The quality of toxin produced by streptococci is a definite character, however, and a practical method for qualitative determinations on large numbers of cultures will probably be found sometime. The limited data at hand suggest that grouping according to toxin production is correlated with other characters, as was to be expected. If, in the future, any group which is described as a species in the present series of papers is found to include more than one group as deter-
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mined by toxin production, the group would then be further subdivided accordingly.

Although the species *S. scarlatinae* includes only 10.5 per cent of scarlet fever strains, Wadsworth and Coffey found that the antiserum produced by the type strain neutralized the toxin produced by 67.3 per cent of scarlet fever strains. Hence, if the strains of the species *S. pyogenes*, which when judged by our collection includes 61.6 per cent of scarlet fever strains, all produce toxin of the same neutralizing quality, the corollary statement may be made, that the toxin produced by strains of the species *S. pyogenes* must be neutralized by antitoxin produced by “N.Y.5.” As a matter of fact, the sum of the percentages of the scarlet fever strains of the two species in our collection (61.6 + 10.5 = 72.1) gives a figure as close to the percentage of scarlet fever strains found by Wadsworth and Coffey to be neutralized by “N.Y.5” serum as would be expected when two collections are compared with reference to a single character.

There are in our collection of *S. pyogenes* 23 strains, the toxin of which has been studied by other investigators and found to be neutralized by “N.Y.5” antitoxin. Three of the strains were from cases of scarlet fever; ten from erysipelas; two from puerperal fever; two from septic sore throat; two from cases of rheumatism; one from suppurative arthritis; one from pleurisy; and two were from cow's milk isolated during septic sore throat epidemics. The toxin of three of the 23 strains was studied by Eagles; that of two strains was studied by Williams and Gurley; that of two strains was studied by Wadsworth and Coffey. M. V. Veldee of this laboratory studied 16 of the 23 *S. pyogenes* strains, carrying out the neutralization test on the rabbit's ear. The writer is indebted to him for permitting this statement to be made from unpublished data. Dr. Veldee also examined two other *S. scarlatinae* strains (Dick IV and Williams type 3, no. 57) and found that their toxin is neutralized by “N.Y.5” antitoxin.

**SUMMARY**

In our collection of 395 strains of hemolytic streptococci from various human diseases, there is a group of 14 strains, all
from cases of scarlet fever, which is distinguished from *Streptococcus pyogenes* by lack of ability to ferment salicin. The group is described in this paper. From a review of the literature it is concluded that the logical designation for this group is *Streptococcus scarlatinae*. The species name "anginosus" is a synonym.

Agreeing with the findings of other investigators, the data show that *Streptococcus scarlatinae* may cause scarlet fever or sore throat without rash, but that it is rarely, if ever, the cause of other diseases. The strains of this group are weak in their ability to dissolve human fibrin.

REFERENCES


