STUDIES ON THE BIOLOGY OF STREPTOCOCCUS.

IV. The Occurrence of Streptococcus scarlatinæ in Convalescence and in the Complications of Scarlet Fever.

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Recent studies of the etiology of scarlet fever have demonstrated conclusively that a group of hemolytic streptococci which possess specific antigenic and peculiar toxic properties is associated with the angina of scarlet fever.

Strains of streptococci from cases of scarlet fever have been agglutinated by Dochez and Bliss (1–3), Tunnicliff (4, 5), Gordon (6), and by Stevens and Dochez (7), who have found that a streptococcus giving characteristic agglutination with scarlatinal immune sera is present in from 65 to 85 per cent of all cases of scarlet fever during the acute stages of the angina. These streptococci have not only been cultured from the throat during the acute stages of the disease but have been obtained from wounds in cases of surgical scarlet fever, and have been recovered from milk which was apparently responsible for milk-borne epidemics. Tunnicliff and Bliss have been able to obtain streptococci which agglutinated with their sera from convalescent cases of scarlet fever as well as from the attendants in scarlet fever pavilions. Since these agglutination reactions have been carried out, Dick and Dick (8) have demonstrated that the characteristic rash and angina may occur in a certain percentage of people if the fauces are inoculated with pure cultures of streptococci obtained from scarlatinal sources. More recently Dochez and Sherman (9, 10) have produced a serum of distinct therapeutic value by the immunization of horses with living streptococci. This serum not only causes the rash to disappear when injected intramuscularly but regularly induces local blanching if injected intracutaneously within 48 hours after the appearance of the rash (11). Dick and Dick (12) have since obtained skin reactions with diluted filtrates of streptococcus cultures in a certain percentage of individuals who have not previously had scarlet fever. Recent convalescents from scarlatina do not show this reaction. The reaction is neutralized by serum from cases of convalescent scarlet fever and is absent in persons who have been given subcutaneous

inoculations of filtrate. According to Zingher (13), who has repeated their work, the reaction is analogous in many respects to the intradermal Shick test.

The identification of this group of hemolytic streptococci which are so intimately associated with scarlet fever is of great epidemiologic importance. It is possible that they may be identified by means of skin reactions with filtrates and specific neutralization with scarlatinal serum, but the identification of a great number of strains in this manner would be an impractical procedure. At the present time the most practical method by which we may identify this group of hemolytic streptococci associated with scarlet fever is by means of the agglutination reaction. In the literature reviewed in the previous paragraph, Bliss and Tunnicliff point out that streptococci which agglutinate with scarlatinal immune sera occasionally occur in the throats of convalescent cases. Bliss is of the opinion that in the majority of instances streptococci disappear from the throat at about the end of the 2nd week. He has found, however, that they may persist over much longer periods and that carriers may be responsible for small epidemics of contact cases. Since in all probability a number of sporadic cases of scarlatinal infection which occur are return cases resulting from contact with recent cases of scarlet fever, the occurrence of these agglutinable strains of streptococci in the throat and in the complications late in convalescence is of importance. We have employed the agglutination reaction for further study of the distribution of these streptococci under these conditions.

Materials and Methods.

During the winter of 1922–1923 we were able to obtain cultures from a series of patients at Willard Parker Hospital. These cultures were obtained that we might study the occurrence of streptococci in the acute cases and in complications in scarlatina, and determine the percentage of cases in which streptococci were still present at the end of the prescribed period of isolation.

The cultures on the acute cases were obtained between the 3rd and 6th days of illness and were made in the usual manner on blood agar. Rabbits were inoculated with the first five strains isolated and with a Baltimore type scarlatinal strain which

we were able to obtain from Bliss, until their sera gave a 4 plus agglutination reaction with the antigenic strains in dilutions of 1:1,280. The animals were inoculated with increasing amounts of whole broth culture over periods of 4 days with intervals of 4 days between the courses of inoculation. After the fourth course the sera were usually suitable for agglutination. The actual technique of the tests has previously been described by Dochez, Avery, and Lancefield (14), and by Bliss (3). We found that whole broth cultures grown at room temperature were more suitable for agglutination than washed cultures grown at 37.5° (15), but with this exception their technique has been carefully followed. None of the six sera which were prepared agglutinated hemolytic streptococci from pyogenic infections. Many of the cases from which we obtained strains early in the disease were cultured a second time just prior to their discharge from the hospital. The majority of the cultures in the complications were made in cases in which the bacteriology of the throat had been studied.

The results of the agglutination reactions with the strains isolated have been arranged in Tables I to III. The cases have been arranged consecutively in the order in which the cultures were obtained. Table I includes the first forty cases which were cultured between the 3rd and 6th days of the disease. The series of cultures on the convalescent cases, including cultures on many of the positive cases in Table I and additional cases which were cultured when they were discharged from quarantine, is found in Table II. Table III summarizes the agglutination reactions with strains occurring in the purulent exudate in adenitis, rhinitis, and mastoiditis.

Discussion of Bacteriologic Data.

An analysis of the cultures made on the group of acute cases in Table I confirms the work previously reported by other authors. In this group of cases we find hemolytic streptococci present in 87.5 per cent of the throats. This figure is a trifle lower than similar figures previously reported, especially those in reports stating that hemolytic streptococci are to be found in every case of scarlet fever early in the disease. We believe, however, that since all of these cultures were made between the 3rd and 6th days after the onset of the angina, in all probability the throats in the milder cases were already free from the streptococcus. Bliss has already pointed out that in the majority of cases streptococci are not found in cultures made between the 10th and 14th days. 74.3 per cent of these strains isolated were agglutinated

TABLE I.

Agglutination Reactions with Strains of Streptococci from Acute Scarlatinal Throats.

Case No.	Sera.					
	1	2	3	4	5	B1
1	+++	++++	+++		+++	++
2	+++	++	++		+++	+++
3	++	++++	++		++	++++
4	++++	++++	++		+++	+++
5	+++	++++	+++		++	++++
6	++	+++	±		+++	+++
7*						
8*						
9	++	+++	++		++	++
10	++	±	+		+++	++++
11		++	++		+++	++
12	++++	++++	++++		++++	++++
13†						
14	+	+++				++
15	+++	++++	++		+++	±
16	+	++			+	±
17*						
18	++	+++				++
19	++	++			++	+++
20	++	+++			++	+++
21	++	++++			+	+++
22	±	++			++	+++
23	−	±		−	−	−
24	−	−		−	−	−
25	−	++		+	++	+++
26		+++		++		++
27		−		±		−
28		++		+++		+++
29		−		−	−	−
30		−		−	−	−
31*						
32		+++		++	++	+++
33		++		++++	++	±
34†						
35		−		−	±	−
36†						
37		+++		+	++	+++
38*						
39		++		++	++	+++
40		+++		+++	+++	++++

* Streptococci not found.

† Strain granular and inagglutinable.

by type immune sera, so that the cultures from 65 per cent of the cases show hemolytic streptococci definitely belonging to one biological group. Since this study was made, this group of streptococci has been further identified through Type Serum B1, as belonging to a very widely spread group of streptococci always associated with scarlet fever and occurring in widely separated districts. Strains falling in this group have been cultured from milk and have been obtained from cases of scarlet fever occurring in Denmark, Chicago, San Francisco, Baltimore, and in smaller epidemics in other cities in the United States. We have since prepared a toxin apparently identical with the substance obtained by Dick and Dick with representatives of this group.

The strains which were not agglutinated represent by far the minority of streptococci found in these throats. Some of the strains were granular and unsuitable for agglutination reactions. Five of the strains did not agglutinate with any of the sera which we prepared. They may possibly represent a second and less important group of *Streptococcus scarlatinæ*, but from an analysis of Tables II and III a certain number of them are undoubtedly pyogenic strains not primarily associated with the original infection. It is to be expected from the commonness with which streptococci are found in the throat in other infectious diseases, and the frequency of secondary streptococcus infections of the respiratory tract in measles, that secondary infection with non-scarlatinal streptococci would occur in scarlet fever.

After a period of 30 days quarantine, nineteen of the cases in Table I were cultured a second time. All of the nineteen had had streptococci corresponding to the type strains in the earlier throat cultures; eight of the cases still showed streptococci of this group in the cultures made when they left the hospital. Five of the strains recovered were not agglutinated with our sera. We consider this evidence that a mixture of scarlatinal and non-scarlatinal strains occurs in a certain percentage of the cases of scarlet fever. These cases along with nineteen additional convalescents cultured are included in Table II.

In the entire series of thirty-eight cases cultured at the end of the prescribed period of quarantine, nearly half, 47.3 per cent, were carriers of hemolytic streptococci. We were able to agglutinate 55 per cent

TABLE II.

Agglutination of Strains of Streptococci from the Throat in Convalescent Cases of Scarlatina.

Case No.	Day of disease.	Tonsils.	Streptococci present.	Sera.		
				2	4	B1
1	33	+	+	++++	+++	+++
2	34	++	+	±	−	−
4	30	−	−			
6	30	+++	+	++	+++	+++
9	29	++	+	+++		+++
10	35	+	−			
12	32	−	−			
14	30	+	+	++	±	++
15	30	++	−			
16	27	+++	+	++	++	++
18	29	++	+	−		−
19	28	−	−			
21	30	−	+	++++		++++
22	34	++	+	−		−
25	32	+	−			
26	32	+++	+	−		−
32	30	++	+	+++	+++	+++
33	35	++	+	−		−
40	33	++	+	+++	+++	++
41	29	+	−			
42	30	+	+	++++	+++	+++
44	30	+	−			
45	30	−	−			
46	28	+++	+	+++	++	++
47	32	+++	+	−	−	−
48	31	+	−			
50	34	−	−			
51	29	+++	+	++	++	++++
53	30	+	−			
54	32	+++	+	−	−	±
55	27	+	−			
56	28	+	−			
57	27	−	−			
58	30	+	−			
59	28	−	+	±	±	−
60	29	+	−			
61	28	−	−			
62	30	++	++		−	−

of these strains, so that of the total number of cases studied in this group, 29 per cent were carriers of streptococci similar to those isolated

TABLE III.

Agglutination of Strains of Hemolytic Streptococci from Acute Complications of Scarlatina.

Case No.	Source of strain.	Sera.		
		2	4	B1
14	Throat.	++		++
	Otitis.	+++		++
15	Throat.	++++		++
	"	−		−
	Otitis.	+++		+++
23	Throat.	−	−	−
	Otitis.	++	+	++
26	Throat.	+++	++	++
	Rhinitis.	−	±	−
28	Throat.	++	+++	+++
	Adenitis.	++++	++	+++
	Rhinitis.	−	−	−
33	Throat.	++	++++	±
	Adenitis.	++	+++	++
43	"	−		−
49	Mastoiditis R.	−		−
	" L.	−		−
52	"	±		±
63	Otitis.	−		−
64	"	+++		++
65	Empyema.	−		−

from the throats in 65 per cent of the acute cases. Many of the cultures were repeated on 2 or 3 succeeding days. The streptococci were

usually obtained from the crypts of the tonsils. In Table II we have indicated the degree of tonsillar inflammation by plus signs. One plus indicates an apparently normal tonsil and additional plus signs have been used to indicate degrees of inflammation resulting from the angina of the scarlet fever. With the exception of one case, tonsils were present in all of the scarlatinal carriers. This observation is similar to that of Nichols (16) who found that the tonsils were usually the source of continual throat infection in the hemolytic streptococcus carrier state.

The bacteriologic study of the complications which occurred among the 65 cases confirms our belief of secondary invasion with common pyogenic strains of hemolytic streptococci. The results of the throat cultures and cultures from the exudate in these complications are found in Table III. The complications studied occurred late in convalescence. In five of the cases streptococci agglutinating with scarlatinal type serum were present in the throat when the complication occurred. Some of the complications occurring among these cases were apparently due to the scarlatinal strain found in the throat, and in other instances the streptococcus recovered was an extraneous pyogenic strain. In Case 15 an agglutinable and a non-agglutinating strain were obtained in the same throat culture. The complications in the later cases (Nos. 43, 49, 52, 63, 64, and 65) occurred within an interval of a week following the admission of an acute mastoid infection among a group of twenty patients isolated in an annex of the hospital. Only one of the seven strains isolated from these complications could be identified with the group which we had previously obtained. It is our opinion that these non-agglutinating strains occurring in cases from which scarlatinal strains can be isolated represent secondarily invading streptococci, and that the complications in scarlet fever may be caused either by the original scarlatinal strain or by pyogenic strains.

CONCLUSIONS.

1. A group of streptococci which agglutinate with scarlatinal immune sera has been isolated from the throats of 65 per cent of cases of scarlet fever during the 1st week of the disease.

2. Strains of hemolytic streptococci belonging to this group have been isolated from the throats of patients at the termination of quarantine (30 days).

3. Hemolytic streptococci are found most frequently in the throat in convalescent patients in whom the tonsillar inflammation has not entirely subsided.

4. The complications occurring in scarlet fever may be due to the original scarlatinal strain or may be the result of a secondary infection with pyogenic strains of streptococci.

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