A STUDY OF THE AGGLUTININS IN HUMAN SERA FOR HEMOLYTIC STREPTOCOCCI FROM SCARLET FEVER.

BY

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INTRODUCTION AND REVIEW OF LITERATURE.

An association of hemolytic streptococci and scarlet fever has long been noted. Recently, Dick and Dick,[1] Tunnicliff,[2][3][4] Bliss,[5][6] Gordon,[7] Dochez,[8] and others seem finally to have established a hemolytic streptococcus as the etiological factor of the disease.

In the course of these studies many immunological experiments have been performed. Agglutination reactions have been widely studied with varying degrees of success. Marmorek[9] (1895) was the first to produce an antistreptococcal serum but it exhibited only feeble and irregular agglutinating properties. Moser and von Pirquet[10] (1902) first noted that streptococci from scarlet fever were different from those found in other diseases. These workers produced an immune agglutinating serum in horses which seemed specific for the streptococci in dilutions of 1-1000 and 1-64,000. Later (1903) they seemed to be unable to confirm these results.[11] Meyer[12] (1902) was unable to differentiate the streptococci from scarlet fever from those of pyogenic infections by immune sera. Rossiwald and Schick[13] (1905) confirmed in general the specificity of the agglutination reaction. However, Salge[14] (1902), Salge and Hasenknopf[15] (1903), Aronson[16] (1903), and Neufeld[17] (1903), the latter with immune rabbit serum, all failed to find that agglutination reactions would differentiate scarlet fever streptococci from the streptococci from other sources. Weaver[18] (1904) found that of the streptococci cultivated from scarlet fever cases some were agglutinated by almost all scarlatinal serum in dilutions varying from 1-160 to 1-4000. Serum from cases of lobar pneumonia and erysipelas also agglutinated his scarlatinal streptococci so he concluded that the agglutination test was not specific and therefore valueless as a means of diagnosis. Evidence was thus piling up on two aspects of the problem of the specificity of hemolytic streptococci in scarlet fever. In 1905 Jochmann[19] weighed both sides of the question and after this it was rather taken for granted that the relation of hemolytic streptococci to scarlet fever was only a secondary one. Reudiger's work[20] (1906) was the last for about a decade. He did agglutination tests between the streptococci from throat cultures of scarlet fever cases and immune sheep sera but found that hemolytic streptococci from erysipelas and tonsilitis were agglutinated in almost as high dilutions as the scarlet fever strains.

Attempts to differentiate groups of hemolytic streptococci by the complement fixation reaction[21][22] and very recently by precipitin tests[23] were all unsuccessful.

In 1919 the invaluable work of Dochez, Avery, and Lancefeld[24] in developing a method of the differentiation of the various biological types of hemolytic streptococci by the agglutination reaction (verifying this by protection experiments) gave the impetus for new experimentation. Hamilton and Havens[25] (1919) confirmed the existence of seriological groups of Streptococcus pyogenes, using 110 strains gathered during the influenza epidemic. Nakayama[26](1919), though he found considerable similarity in results of cross agglutination tests with streptococci from scarlet fever, could not secure decisive results in absorption tests. Havens[27](1919) found 93% of his 293 strains of hemolytic streptococci were differentiated into three groups. Gordon[7](1921) continuing earlier studies, also divided hemolytic streptococci into three groups by absorption tests. One group he called Streptococcus scarlatinae. Tunnicliff[2][3][4] in very detailed studies (1920-22) proved the specific nature of the hemolytic streptococcus in relation to scarlet fever by cross agglutination tests and successful absorption tests. Dochez and

Bliss[8](1920) confirmed this specificity by agglutination and cross agglutination tests of streptococci isolated from throats of scarlet fever patients and streptococci from non-scarlatinal origin. Bliss[5](1920) found 80% of the scarlet fever strains were agglutinated by immune sera and none by antisera made from antigens of non-scarlatinal origin. The same author in detailed studies of the various antigenic relations of streptococci isolated from scarlet fever cases confirmed the results noted above by agglutination and absorption tests with ten different immune sera.[6] He found, in addition, that serum from patients convalescent from scarlet fever agglutinated weakly or not at all the homologous strains of hemolytic streptococci, depending upon the time during convalescence at which the serum was taken. Using Bliss's technique, Stevens and Dochez[28](1923) tested for the specificity of the agglutination reaction, finding that 74% of the strains isolated from acute cases of scarlet fever and 55% of the strains from convalescents were agglutinated by anti-scarlet fever streptococcus serum. Some of the strains used by these authors were of European origin. They also performed careful absorption tests with specific absorption with antigenic and homologous strains.[29] Later, (1924) they studied the agglutination reactions of strains isolated at various periods of convalescence.[30] Herrold and Tunnicliff[31] (1924), by concentrating human serum with ammonium sulphate found serum from convalescents, particularly after the 18th day, agglutinated scarlet fever streptococci but not streptococci from other sources.

It was thought that it would be interesting to investigate the presence of agglutinins for scarlet fever streptococci in the sera from an average group of healthy individuals. It is with this object in view that the experiments described below were performed. The following points have been taken into consideration, tentatively accepting the etiological role of the hemolytic streptococcus in scarlet fever as a working hypothesis.

1. The presence or absence of specific agglutinins for scarlet fever streptococci in human serum.

2. A complete history of the individual tested, including scarlet fever (detailed), other acute infectious diseases, tonsilitis (detailed), and hypersensitiveness.

3. The presence of agglutinins in the sera of individuals who have not had scarlet fever. These are to be called hypothetical normal agglutinins, keeping in mind that with the wide spread occurrence of all streptococcus infections, normal agglutinins would be difficult to differentiate.

4. The degree of agglutination found in tests with sera from individuals who have had scarlet fever

and sera from individuals who have not had scarlet fever.

5. The dilution in which maximum agglutination is found.

6. A history of tonsilitis and the presence of agglutinins.

7. A history of scarlet fever in parents before the birth of the individuals tested compared with the presence or absence of agglutinins in the serum of the individual.

8. Specificity of agglutinins found as evidenced by the ability of all sera tested to agglutinate streptococci from non-scarlatinal origin.

METHOD

Some difficulty has been experienced by all workers in getting the streptococci to grow in such a manner as to give a uniform suspension suitable to use in agglutination tests. The phosphate broth of Dochez, Avery, and Lancefeld[24] and used recently by Bliss and by stevens and Dochez, was found to give insufficient growth in eighteen to twenty hours. Variations of this broth were now made. Finally combining the suggestion of the use of calcium carbonate as the buffer as adapted by Reudiger[20] from Hiss,[32] the use of little or no NaCl by Dochez, Avery, and Lancefeld,[24] and the use of not over 0.2% sugar concentration (Bliss[6]), the following broth was adopted for use in all of the experimental work done: a meat infusion broth containing 1% peptone, 0.1% dextrose, no NaCl, and adjusted to a pH of 7.6 before the last sterilization. Streptococci were grown in large 50 cc. hard glass tubes, each containing about 30 cc. of the above broth. In order that calcium carbonate particles would not be present to confuse or hinder the agglutination reaction the use of C. P. powdered calcium carbonate was discontinued and thoroughly washed pieced of crystalline rock calcium carbonate were used instead. These were merely dropped into each tube before the final sterilization. By a heavy inoculum (one cubic centimeter of a good broth culture in one of the above tubes) a very heavy suspension of the streptococci was secured in an 18020 hour old culture. Growth was always at 37°C. Transfers were usually made daily, innoculating new tubes from the old before the latter were killed. Purity of cultures was routinely tested by allowing the last drop from the pipette with which a new tube had been inoculated to run on a blood or serum agar slant. The organisms grew nicely on meat infusion, 5% serum agar slants. The addition of tomato juice to broth or agar seemed to facilitate growth.[33]

For agglutination tests whole broth cultures exposed for one hour at 55°C. were used. Polyvalent antigens were prepared by mixing equal volumes of the killed broth cultures.

Individuals to be tested were bled from finger punctures, the blood being drawn up into a capillary pipette and put immediately into a small, dry fermentation tube. Five to seven tenths of a cubic centimeter of blood was readily secured in this way. By running a platinum wire around the edge of the tube after clotting and allowing the tubes to stand 20-24 hours, clear serum could easily be drawn off. This proved such a simple and effective method of securing serum it might prove of practical value in the routine running of Widal tests, etc.

Dilutions of the sera were made in plain extract broth, pH 7.4. Five tenths cubic centimeter of serum dilution and five tenths cubic centimeter of bacterial suspension were put in small round bottomed hemolytic tubes (10.0 cm. x 0.8 cm.) and incubated at 55°C for one hour. Readings were taken immediately and at the end of 24 hours in the icebox. Negative controls were run with every eight tubes set up. Positive controls were not run with all the agglutination tests as the titre of the rabbits being immunized was not up. However, in every set of tests run, one serum which would cause agglutination of the organisms was always used. Agglutinating titre of 1-3200 for scarlet fever streptococci was built up in young rabbits by successive intravenous injections of killed whole cultures and later injections of heavy suspensions, secured by centrifugalizing the whole cultures.

RESULTS

Agglutination of Streptococci from Scarlet Fever by Human Sera.

Agglutination tests were run on 188 college students using the technique just described. A polyvalent antigen of two strains of hemolytic streptococci was used, (#404 and #405) cultures of which had been kindly supplied by Dr. F. W. Mulsow of the University of Iowa. Both cultures gave positive Dick tests in susceptible individuals. Almost no trouble was experienced with spontaneous agglutination of these strains. This is interesting when compared with the recurrent clumping of the hemolytic strains of non-scarlatinal origin noted later. Eight agglutination tests were run against only one of the scarlet fever strains because of accidental contamination of the other.

In comparing readings made upon immediately removing the tubes from the water bath and those made at the end of 24 hours in the icebox there were 27 cases of showing a general increase in the degree of agglutination and 20 showing a decrease. The decrease is not as marked generally. In only two cases was there a complete absence of agglutination in the tubes which, on the first reading, had shown some agglutination. In eight cases, which were negative on the first reading, positive agglutination was visible as the end of 24 hours. The other cases cited were slight changes in the amount of agglutination. With the exception of this slight increase in the degree of agglutination in readings taken after 24 hours the negative readings were always given precedent. Unless definite clumping could be observed, the tube was read as negative and all records made as doubtful positives have been transcribed as negatives. It might be stated that Norton,[34] experimenting with the technique of streptococci agglutination, found no changes in readings from one and one-half hours after incubation to over night.

The following is a summary of the results of the agglutination tests. Of the entire group 28.6% of the sera agglutinated scarlet fever streptococci with 71.4% showing no signs of agglutination. In relation to the history of the disease, Table I is given.

T A B L E I.

Personal History of Scarlet Fever in the Individual Compared with the Results of the Agglutination Test.		
Agglutination	With History of Scarlet Fever	No History of Scarlet Fever
Positive	33.3%	27.2%
Negative	66.7%	72.8%

Study of the Positive Agglutination Tests.

Further study of the individuals whose serum agglutinated the scarlet fever streptococci is most interesting. Full data on these cases is reocrded in Tables II and III.

T A B L E II.

Results of Positive Agglutination Tests from Individuals with a History of Scarlet Fever.

Case Number	Age	Sex	Scarlet Fever History of Tested Individual: Severity	Complications: Kidney	Ear	Glands	Heart	Lung	Sore Throat	Rash	Quarantine	Age When Had It	Number of Years Ago	Of his Family: Parents	Others	Tonsilitis: Severity	Recurrence	Tonsilectomy	Infections, Acute Diseases: Erysipelas	Rheumatism	Pneumonia	Nephritis	Appendicitis	Measles	Diphtheria	Chicken Pox	Mumps	Hyper-sensitiveness: Hay Fever	Asthma	Food Poison	Poison Ivy	Agglutination: 1-20	1-40	1-80	1-160
9	20	M	-	-	-	-	-	-		+	+	14	6	-	-	+	+	-	-	-	-	-	-	+	-	+	+	-	-	-	+	-	Trace		
22	22	F	?	-	-	-	-	-	-	+	-	17	5	-	-	-	-	+	-	-	-	-	-	+	-	+	-	-	-	-		-	+	+++	+
40	22	F	-	-	-	-	-	-	-	+	+	12	10	-	Bro and Sist	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	+	++++	++++	+++	+++
43	22	F	-	-	-	-	-	-	-	+	-	1	21	-	Sist			+	-	-	-	-	-	+	-	+	+	-	-			-	+	-	-
64	21	M	-	-	-	-	-	-	-	+		14	7			-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	++	++	++
81	19	M		-	-	-	-	-	-	++	+	15	4	M	-	-	-	+	-	-	-	-	-	+	-	+	+	-	-	-	-	-	+++	++	±
92	20	F		-	+	-	-	-	-	+	+	13	7	-	B	-	-	-	-	-	+	-	-	+	-	+	-	-	-	-	-	-	Trace		
104		F	++	-	-	-	-	-		+	+		12	-	-	-	-	-	-	-	+	-	-	+	-	+	-	-	-	-	-	++	+++	++++	+
126	24	F		-	-	-	-	-		+	+	12	12	?	?	-	+	-	-	-	-	+ Ch	-	+	-	++	-	-	-	-	+++	-	++	+++	++
144	20	M	+ twice	-	-	-	-	-		+	+	1	19	-	B	+	+	+	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	+	-
176	20	M	-	-	-	-	-	-		+	+	7	13	-	B		+	+	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	+	-
191	18	M	+	-	-	-	-	-		+	-	9	9	M	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	+	±	-
195	18	M	-	-	-	-	-	-		+	+	6	12	-	B	-	-	+	-	-	-	-	-	+	-	+	+	++	+	-	+	+	++	++	+
197	22	M	-	-	-	-	-	-		+	+	7	15	-	B	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+
35		M								+		?	?																				Trace		

T A B L E III

Results of Positive Agglutination Tests from Individuals with no History of Scarlet Fever.

Case Number	Personal History: Age	Sex	Tonsilitis: Severity	Recurrence	One Attack	Tonsilectomy	Acute Infectious Disease: Erysipelas	Arthritis	Pneumonia	Nephritis	Appendicitis	Measles	Diphtheria	Chicken Pox	Mumps	Hyper-Sensitiveness: Hay Fever	Asthma	Food Poison	Poison Ivy	Agglutination Dilutions: 1-20	1-40	1-80	1-160	Family Scarlet Fever	Contact Scarlet Fever
1	53	M	-	-		+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	±	+++	+	?	+
5	20	F	-	-		+	-	-	+	-	-	+	-	+	-	-	-	-	-	++	+	±	-	-	-
12	28	M	-	-	+	+	-	-	-	-	-	+	-	+	+	-	-	+	+	-	++	+++	-	-	+
17	23	M				-	-	-	-	-	-	+	-	-	-	-	-	-		++	++	++	-	-	-
19	23	M	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	-	-	-	-	-
20	22	F	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	+	±	-	-	-
23	24	M		+	-	+	-	-	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-
24	73	M	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	±	++	+	+	-	-	+
25	24	M	-	+	-	+	-	-	-	-	-	+	-	-	-	-	-			-	+	+	-	-	-
26	21	M	-	-	-	-	-	-	+	-	-	+	-	+	+	-	-	-	-	++	++	+	-	S	+
41	21	M	±	+	-	-	-	-	-	-	-	+	-	-	+	-	-	+	-	-	+	±	-	-	-
42	21	F	-	-	-	+	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	-	M	-
45	29	F	-	-	++	+	-	+	-	-	-	+	-	+	+	-	-	-		-	-	+	+	?	-
65	25	F	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	++	++	+	-	-	-
68	21	F	-	-	++	+	-	-	-	-	-	+	-	+	-	+	-	-	-	+	-	-	-	-	-
69	20	M	-	-	+	+	-	-	-	-	-	+	-	+	-	-	-	-		+	++	+++	++	F	-
80	24	F	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-					B&S	-
83	20	M	-	-	+	+	-	-	-	-	-	-	-	+	-	+	-			-	+	++	-	-	-
97	21	M	-	+	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	++	++	++	++	-	-
105	29	F	-	-	-	+	-	-	+	-	-	+	-	+	+	-	-	-		-	+	-	-	?	++
125	23	M	-	-		+	-	-	+	-	-	+	-	-	-	+	-	-	-	-	+	±	-	-	-
127	19	M	-	-		-	-	-	+	-	-	+	+	+	+	-	-	-	+	+++	+++	-	-	-	?
128	19	M	+	✓		-	-	-	-	-	-	+								-	+++	++	+	-	-
129	19	M	-			+	-	-	-	-	-	+	-	-	-	+	-			-	++	++	+	B	-+
130	20	M	-	-	-	-	-	-	-	-	+	+	-	+	+	-	-	-		++	+++	+++	-	M	-
131	20	M	+	+		+	-	-	-	-	-	+	-	-	+	-	-	-	-	+		+++		-	-
139	18	M	+	+	+	-	-	-	-	-	-	+	-	+	-	-	-	-		-	++	+	++	-	-
143	18	F	-	+	-	-	-	-	++	-	-	++	-	-	-	-	-	+	+	++	++	+	-	-	+
147		F	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	++	++	++		
148	19	M	±	+	-	-	-	-	-	-	-	+	-	+	+	-	-	-		-	0	+	++	-	-
155	27	M	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	+	-	++	+++	+	-	-
167	26	F	+	+	+	+	-	-	+	-	-	+	-	+	+	-	-	-		+	-	-	-	-?	-
170	20	M	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	±	++	±	+	?	-
171	25	M	+	+		+	-	-	-	-	-	+	±	+	+	-	-	-	?	-	-	+++	±		
192	21	M	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	-	-	+	-	+	+
194	19	M	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	+	-	-	+	-	-	+
202	20	M	±	+	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	+	++++	++	?	-
203	22	M	±	±	-	+	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	++	-	-
200	22	M	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	+	++	++	-	-

At first glance the percentage of positive agglutination tests coming from individuals of no history of scarlet fever, 27.2%, seems very high when compared to that from individuals with a positive scarlet fever history, 33.3%. The former would be the percentage of individuals having hypothetical normal agglutinins. In the literature we find almost no record of normal agglutinins to streptococci, though normal agglutinins to other forms have long been observed. For this reason a closer study was made of this group.

Nine individuals or 23% had come in close and direct contact with the disease, through quarantine with other members of the family having the disease, two contacts in army hospitals, and one in repeated contacts in professional nursing. In three cases had one of the parents had scarlet fever in infancy. Thus, counting only those people who seem tohave had no connection with scarlet fever only 18.9% are found to have hypothetical normal agglutinins instead of 27,2%. Undoubtedly others have come in some contact with the disease so that making any difference in the interpretation of results because of this may not seem warranted.

The relation of agglutinating titres to histories was next considered. A close study of the slightly positive reactions was made. The percentage of agglutination tests from positive and negative histories are recalculated on the basis of these studies. A summary is given in the following table:

TABLE IV

Percentage of Individuals with Positive and Negative Scarlet Fever Histories Having Various Degrees of Agglutination.

HISTORY	AGGLUTINATION TESTS			
	All Reactions	++, +++ & ++++ Reactions	+++ ++++ Reactions	++++ Reactions
Personal Scarlet Fever History	33.3%	22.2%	17.8%	11.2%
No Personal Scarlet Fever History	27.2%	18.2%	8.2%	2.0%
No Personal Scarlet Fever History nor History of Contact	18.9%	11.9%	4.3%	0.0%

Normal agglutinins to scarlet fever streptococci were first indicated by Moser and von Pirquet,[10] who found that normal serum but rarely agglutinated the streptococci they isolated from scarlet fever. They found three cases out of 28 tested that did agglutinate their organisms. Convalescent serum was found by Bliss[6] to agglutinate specific streptococci if taken after the 18th day. He could not secure any agglutination by serum collected in 4 to 6 days of convalescence. Herrold and Tunnicliff,[31] by concentrating the sera, secured more positive results, for 25% of the sera they collected before the 11th day and 92% of the sera they collected after the 18th day agglutinated scarlet fever streptococci but not streptococci from other sources. Unconcentrated serum in a 1-10 dilution and serum from a diphtheria patient failed to agglutinate the scarlet fever strains. No other work seems to have been done on human agglutinins to scarlet fever streptococci.

Normal agglutinins to other streptococci have been noted in a few instances. Rosenow and Gray,[35] studying the pleomorphic streptococci from epidemic poliomyelitis, found some normal individuals with agglutinins for these organisms. However, all their normal individuals had been in a region where poliomyelitis had been in an epidemic form during the time the study was made, some definitely admitting a close contact with the disease. Their use, even though carefully controlled, of the streptococcus antigens stored in the icebox for months seems questionable. The results of 137 agglutination tests performed with 27 antigens and serums from 41 individuals are summarized in the table below, copied in part from their work. The comparison with immune human sera from convalescents is to be noted.

Total incidence of upper limit of Agglutination of Poliomyelitis strains by normal and immune human sera.

Dilution	Serum	
	Normal Percentage	Immune Percentage
1:1	10	12
1:10	21	29
1:100	12	23

Agglutination by immune serum continues up to 1:1,000,000. Sherwood and Downs,[36] studying pleomorphic streptococci, found that seven out of thirteen persons had normal agglutinins to various strains of Streptococcus salivarious, and four persons out of seven to one strain of Streptococcus mitus. Kohlmer, Trist, and Yagle[37] in their study of the specific bacterial antibodies present in the blood serum of influenza patients found that neither these nor their nine healthy control individuals had any agglutinating bodies.

Thus, in view of the few instances in which normal agglutinins have been noted for streptococci of any kind, the findings in this paper would seem to be of interest. Referring to Table IV, it will be seen that at least 2% of the individuals who had no personal history of scarlet fever were found to have definite agglutinating bodies in their blood. The more marked difference between individuals who had had the disease and those who had not, when only the three and four plus readings are considered, is significant.

Dilution in Which the Greatest Degree of Agglutination Took Place.

The following table shows a comparison of the maximum agglutinating in a given titration to the dilution in which it occurs, expressed in the percentage of all the cases in which positive agglutination was found.

TABLE V

Comparison of Degree of Agglutination and Dilution in Which It Occurred.

Dilution	Result of Agglutination.				
	+	++	+++	++++	All Reactions
1- 20	6%	4%	0%	0%	10%
1- 40	14%	8%	6%	2%	30%
1- 80	12%	8%	18%	4%	42%
1-160	4%	14%	0%	0%	18%

There is, of course, a great variation in the tests, for the maximum agglutination in a given titration occurs in any one of the four dilutions. The 1-80 dilution showed regularly the greatest degree of agglutination. In 82% of all the cases agglutination was lost by the time the final concentration of the serum was 1-160. The usual diagnostic value of the 1-40 dilution is interesting to recall here. Few workers have demonstrated the agglutinins they have found for streptococci in high dilutions. Weaver[18] stated that scarlatinal serum agglutinated the organisms he isolated from scarlet fever cases in a dilution of 1-4000. His work was done before any differentiation of streptococci was made and so his organisms may not all be hemolytic streptococci. No agglutination was demonstrated by Herrold and Tunnicliff[31] by unconcentrated scarlet fever serum of 1-10 dilution nor by concentrated scarlet fever serum in dilutions higher than 1-20 and 1-40. Bliss[6] found scarlet fever serum agglutinating specific streptococci in dilutions of 1-32 and 1-64. Tunnicliff[38] demonstrated agglutinins for the diplococci she presupposes to be the causal organism of measles in dilutions of 1-8 in serum from convalescents taken eight days after the eruption. Rosenow and Gray,[35] working with streptococci from epidemic poliomyelitis and Sherwood and Downs[36] with their pleomorphic streptococci (S. salivarius and S. mitus) found true normal agglutinins in dilution as high as 1-100.

Comparison Based upon the Number of Years since the Individual Had Scarlet Fever.

It was thought, particularily inview of the time element found in demonstrating agglutinins in the serum from convalescents (as pointed out by Bliss[6] and by Herrold and Tunnicliff[31]) that it would be interesting to compare the number of years since the individuals had had scarlet fever with the presence of agglutinating antibodies in his blood. The following is a tabulation of the results from 41 individuals.

TABLE VI

Percentage of Positive and Negative Agglutination Compared to the Number of Years since the Individual Had Scarlet Fever.

	Number of Years since Individual Had Scarlet Fever						
	1- 5	5-10	10-15	15-20	20-25	25-30	Average
Of all Individuals	12.1%	34.0%	27.3%	7.2%	17.0%	2.4%	12
Individuals Whose Serum Gave Positive Agglutination	14.3%	42.9%	28.6%	7.1%	7.1%	0%	10.8
Individuals Whose Serum Gave Negative Agglutination	11.1%	29.6%	26.0%	7.4%	22.2%	3.7%	13.3

It will be seen that the majority of people in this group had the disease from five to ten years ago. It is this group of individuals which has the greatest percentage of agglutinins. The very marked decline of the presence of antibodies after the 15th year following the disease is to be noted. Herrold and Tunnicliff(31) found that the serum from one individual who had had scarlet fever two years previously did not agglutinate scarlet fever streptococci. This seems to be the only reference to such work in the literature.

A Relation of the Influence of Heredity to Antibody Content.

The possibility of transmitted immunity has long been discussed. Newly born infants are supposed to be passively immune to scarlet fever for five or six months. Zingher,(39) basing his conclusions on results of Dick skin tests and a study of the antitoxic content of blood from the umbilical cord of the placenta, demonstrated some positive placental transmission (uterine) of antitoxin and found infants somewhat immune up to one year of age. The presence or absence of agglutinins correlated with the histories of scarlet fever in the parents should prove interesting in this work. However, only four cases were found in which the parents were known to have had the disease before the birth of the individual tested. Three of these cases (Numbers 42, 69, and 139) had agglutinins in their blood. In tow of these had the mothers had scarlet fever and in the other the father. Case 187 had no agglutinins.. The father had had the disease in infancy. No conclusions could be warranted from so small a group.

The Relation of a History of Tonsilitis to the Presence of Agglutinins in Human Serum.

Seventeen out of thirty-nine individuals of a negative scarlet fever history but whose blood was found to have some agglutinins for scarlet fever streptococci reported tonsilectomies, indicating that they had bad tonsils. Most of these gave a previous history of tonsilitis. One gave a record of severe recurrent attacks pf tonsilitis. This makes a total of 50%. In addition four gave histories of one severe attack of sore throat with fever and head ache and in one case gastro-intestinal disturbances. In cognizance of the observation that mild attacks of scarlet fever occur without exanthema and if hemolytic streptococci be accepted as the etiological factor of scarlet fever it is possible that these four individuals may have had light attacks of the disease. However, they seem not to have been in known contact with the disease.

The sera from these individuals showed no tendency to agglutinate hemolytic streptococci from non-scarlatinal origin.

Study of the Negative Agglutination Tests.

The following points are of interest when the histories of individuals who had no agglutinins for scarlet fever streptococci in their serum are studied.

Of the individuals showing negative agglutination reactions but who have histories of scarlet fever only 16% stated that the disease occurred in anything but a mild form. The 84% described mild attacks, usually without any complications following.

Of the individuals who gave histories of not having had scarlet fever we find 15% who have had some definite contact with the disease.

Agglutination of Streptococci from Non-Scarlatinal Origin by Human Serum.

Out of the 188 individuals tested 157 were tested to discover if their serum would agglutinate streptococci from non-scarlatinal origin. Titrations were made at the same time as the one against the scarlet fever streptococci and with a similar technique. However, as indicated before, these strains spontaneously agglutinated. One finally had to be discarded completely. Half of the tests were made with a polyvalent antigen of hemolytic streptococci from a case of peritonitis (#401) and a hemolytic streptococcus from a case of blood poisoning (#303) and the other half with an antigen made from the peritonits strain (#401) and a hemolytic streptococcus from a necropsy from a case of carcinoma of the larynx and lobar pneumonia. (#440).

Only one of the 157 individuals tests agglutinated the organisms of non-scarlatinal origin. This serum had not agglutinated scarlet fever streptococci. The individual showed no history of tonsilitis, scarlet fever, erysipelas, rheumatism, pneumonia, nephritis, appendicitis, endocarditis, or diphtheria, but had had measles, chicken pox, and mumps.

DISCUSSION

A general consideration of the foregoing results would seem to suggest the following:

The hemolytic streptococci now presumed to be the etiological faction of scarlet fever is more widely prevalent than has formerly been considered and is possibly associated with many common streptococcus infections. The presence of specific agglutinins in the serum of unimmunized individuals might substantiate this supposition. It is to be remembered, of course, that the exact nature of these normal agglutinins is unknown. Wells,[40] summarizes the status of our knowledge as follows: "Whether these 'normal' agglutinins * * * are the same as the agents which produce the reaction in much more highly diluted immune sera, is not known. Possibly they represent non-specific antibodies that arise by natural immunization to bacteria and other foreign proteins that enter the body from the intestines, for they are not present early in life. Landsteiner and others believe that they are not specific and are more resistant than the antibodies that result from immunizing."

The type of immunity resulting from streptococcus infections is still under investigation. The immunity has often been considered as only relatively transient. Gay,[41] in recent studies, says it is a local tissue immunity. Though agglutinins are most assuredly formed in many diseases it has been doubted what part these antibodies play in protecting the body against infections. The action of antistreptococcal serum has seemed to be due largely to its opsonic properties.[42] Bull[43] has found that agglutinins aid in phagocytosis. Work of the Dicks indicates that a toxin produced by the scarlet fever streptococci is responsible for the manifestations of the disease and that antitoxin capable of neutralizing the toxic effects of the toxin is produced by artifical immunization[44] and in the course of the disease.[45] A skin susceptibility test analogous to the Shick test for diphtheria has been devised by them. It seems that it would be interesting to subject the individuals tested in our series of experiments to such skin tests and compare the results obtained. Such a study of the relative occurrence of antitoxins and agglutinins in a given subject might throw some light on the question of a unitarian view of antibodies. Such studies have been performed and the results will be given in a paper by Sherwood and Baumgartner. The presence of specific agglutinins in fairly high concentrations as observed in these researches indicates that some streptococcus infection has occurred with a subsequent response on the part of the body. These agglutinating bodies may aid, as suggested by Bull, in the phagocytic mechanism and not in an antitoxic mechanism.

SUMMARY AND CONCLUSIONS

Agglutinins for streptococci isolated from acute cases of scarlet fever are found in the blood of apparently healthy and normal individuals.

28.6% of the sera from 188 individuals agglutinated scarlet fever streptococci. 29.6% of the positive agglutinating sera came from individuals who had had scarlet fever previously.

Of the 7.4% whose sera did not agglutinate the scarlet fever streptococci 77.6% had not had the disease.

33.3% of the individuals who had had scarlet fever had agglutinating bodies for scarlet fever streptococci in their blood.

Counting only the three and four plus agglutination reactions 8.2% and couting only the four plus reactions 2.0% of the individuals whose sera agglutinated the scarlet fever streptococci had not had scarlet fever. 50% of this group had a history of tonsilitis and tonsilectomies.

Agglutinins for scarlet fever streptococci were demonstrated in three out of four cases in which the parents had had scarlet fever Before the birth of the individual and the individual had not had the disease.

Maximum agglutination occurred in the serum dilution 1-80.

Agglutination seemed to be specific since only one individual was found whose serum agglutinated streptococci from non-scarlatinal origin.

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