A STUDY OF STREPTOCOCCUS ANTIBODIES IN SCARLET FEVER WITH SPECIAL REFERENCE TO COMPLEMENT FIXATION REACTIONS *

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Owing to the fact that scarlet fever has long been noted a contagious disease, it is not surprising that early and persistent attempts were made to discover its exact cause. Many years before the development of bacteriology, investigators believed that a specific contagium was present and searched for it, mainly in the blood, with their crude magnifying lenses.

In 1869 Hallier considered the micrococcus which he claims to have seen in the blood corpuscles of scarlet fever patients to be the cause. From then until 1884, when Loeffler isolated a streptococcus from the throat of a scarlet fever patient, various cocci, bacilli and "bodies" were discovered in the skin, blood and urine, brought forward as the solution of the problem, received no confirmation, and dropped into oblivion.

In 1885 Powers noted the presence of scarlet fever among the consumers of milk from the Hendon farm, England. The udders of the cows were found ulcerated. Klein, delegated to make bacteriological examinations of the cows, isolated streptococci. Later he secured streptococci from scarlet fever patients and considered these organisms analogous to those secured from the cows. Certain individual characteristics were ascribed to these streptococci, and Klein believed a streptococcus to be the specific organism of scarlet fever.

Since then Fiessinger, Dowson, Berge, Limone, Baginsky and Sommerfeld have either positively or tentatively regarded streptococci as the cause of the disease. Gordon, Pearce, Hektoen, Schamberg, Gildersleeve, Bergey and I have noted the close relation of streptococci to scarlet fever. In 1889 Class of Chicago described a large Gram-negative diplococcus found in scarlet fever cases and which he considered the exact cause. His work has not received uniform confirmation, although it is certainly true that his diplococcus occurs in a large number of scarlet fever patients.

In 1887 Pfeiffer described protozoa-like bodies in the blood of scarlet fever patients, and in 1904 interest in this phase of the subject was renewed by the researches of Mallory, who described certain bodies,

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*Read before the joint meeting of the New England, New York and Philadelphia Pediatric Societies, New York, Nov. 9, 1911.

Cyclaster scarlatinalis, which he believed might be the cause of the disease, in the skin of scarlet fever patients.

The latest contribution to the bacteriology of scarlet fever was made by Vipond,¹ who describes the specific organism of scarlet fever, secured from the enlarged lymphatic glands, as being a rapidly growing, spore-forming, acid-producing bacillus. Bouillon cultures of this organism are claimed to have produced the disease in monkeys and rabbits. In my own² bacteriological investigation of the glands in twenty-six cases of scarlet fever, I failed to find in any case any organism resembling those described by Vipond. In nine of these cases streptococci were found.

As will be noted, streptococci have received most attention in researches in scarlet fever. Practically all the work was concerned in establishing the frequency with which streptococci occurred in this infection and whether or not they possessed such morphological and biological characters as would enable one to identify them from the broad class of streptococci and thereby establish their exact relation to scarlet fever. Since 1905 interest has been renewed in this subject by the reports of Russian physicians concerning the efficacy of prophylactic injections of streptococcus bacterins (vaccines) in scarlet fever. Gabrickevsky,³ probably the first to advocate the practice of streptococcus immunization, while not expressing himself definitely as believing a streptococcus to be the cause of scarlet fever, certainly believes that it bears a very close etiological relation to the disease. He prepares his vaccine from cultures of streptococci secured at autopsy of scarlet fever patients and kills the emulsion by heating for one hour at 60 C. So far as I am able to ascertain, the method has not been in practice outside of Russia. If streptococcic immunization does absolutely prevent scarlet fever, then a streptococcus not only bears a close relation to the disease but may be considered the specific cause.

Confident that scarlet fever streptococci do not possess any specific cultural or morphological characteristics, I began to study the antibodies of a number of different cultures from various sources with two main objects in view:

1. To determine more accurately, if possible, by such study the relation of streptococci to scarlet fever than is possible by morphological and biological studies alone.

2. To determine if the method of streptococcus immunization as practiced in Russia can be supported by laboratory investigation.

1. Vipond, A. E.: Scarlet Fever. Preliminary Note of its Specific Micro-organism, Arch. Pediat., 1911, xxviii, No. 7, p. 564.

2. Kolmer, J. A.: A Note on the Bacteriological Examination of Lymphatic Glands in Scarlet Fever, Am. Jour. Dis. Child., 1911, ii, 329.

3. Gabrickevsky: Streptococcic Vaccines and Their Application in Scarlatina. Russk. Vrach., 1905, iv, 941.

ORGANISMS AND IMMUNE SERUMS

In all of the following experiments the same cultures and immune serums were used. Accordingly reference will be made to culture and serums by number only. For instance, Antigen 1 means that it was made of *Streptococcus* Culture 1, and Immune Serum 1 means that it was produced by injections of *Streptococcus* Culture 1, and so on through opsonins and agglutinins.

No. 1.—*Streptococci* secured from scarlet fever patients. A horse was immunized (Immune Serum 1) by three injections of streptococci killed by exposing to 60 C. for one hour; the next two injections were of attenuated organisms after exposure to 50 C. for thirty minutes, and the last injection preceding bleeding was of living cultures. All injections were subcutaneous.

No. 2.—A streptococcus secured by blood-culture from a case of scarlet fever. A rabbit was immunized (Immune Serum 2) by six intravenous injections, at intervals of a week, of organisms exposed to 60 C. for one hour.

No. 3.—A streptococcus from a case of septicemia, obtained from Dr. A. P. Hitchens. A rabbit was immunized (Immune Serum 3) by six intravenous injections of organisms exposed to 60 C. for one hour.

No. 4.—A streptococcus, isolated by Fillin from a case of septicemia, and obtained from the American Museum of Natural History, New York. A rabbit was immunized (Immune Serum 4) by six intravenous injections of organisms exposed to 60 C. for one hour.

No. 5.—A streptococcus isolated from a non-scarlatinal tonsillitis. A rabbit (Immune Serum 5) was immunized by six intravenous injections of organisms exposed to 60 C. for one hour.

The injections were given every seven days and the serum tested from time to time by bleeding from the ear. At the end of seven weeks the rabbits were killed and serum stored in sterile tubes in the refrigerator without the addition of preservatives. Normal horse serum was secured from two healthy animals and normal rabbit serum from six large healthy rabbits.

COMPLEMENT FIXATION REACTIONS

It is reasonable to suppose that immune bodies exist in the blood of persons having recovered from scarlet fever, as this disease is specifically infectious, runs a definite course and confers a specific immunity in the great majority of cases.

In 1904 Besredka⁴ applied complement fixation reactions to the study of horses immunized with streptococci and found that the various serums produced complement fixation only with the strain of streptococci used

4. Besredka: Le sérum antistreptococcique et son mode d'action, *Ann. de l'Inst. Pasteur*, 1904, xviii, 363.

in producing them. He concluded that we have in the fixation reaction a means of studying and differentiating streptococci. Fresh emulsions of living organisms were used as antigens. In the same year Besredka and Dopter⁵ applied the reaction to the study of scarlet fever but without positive results. Three years later Foix and Mallein⁶ applied the reaction to the study of twelve cases of scarlet fever and found positive results in ten. Nine controls, including serums from four cases of erysipelas and one of puerperal sepsis, yielded negative results. Uppenheim⁷ tried complement fixation reactions with two strains of streptococci furnished by Paltauf, with Moser's antistreptococcus serum. The results were negative, and while he does not give any details of his technic, he concludes that Moser's serum did not contain streptococcus antibodies. Castex,⁸ using a fresh emulsion of apparently one strain of streptococcus, applied the reaction in the study of four cases of erysipelas, five cases of scarlet fever and a case each of streptococcus abscess, puerperal fever, pleurisy and arthritis, securing positive reactions in all but three cases of scarlet fever. He expresses his belief in the unity of the streptococcus. Sommerfeld,⁹ using a fresh emulsion of streptococci as antigen, found no fixation in scarlet fever. Livierato studied eighteen cases of scarlet fever and found all positive with an antigen of scarlet fever streptococci and negative with antigens of staphylococci, diplococci, *Bacillus typhosus*, etc. Swift and Thro,¹⁰ after studying three strains of streptococci, three strains of diplococci and one of *Staphylococcus aureus*, found that immune bodies specific for different strains of streptococci, can be demonstrated by means of the complement fixation test. To these investigators is also due great credit for working out a satisfactory method of preparing bacterial antigens.

Since it is apparently possible for one not only to detect streptococcus antibodies in serum, but also to differentiate streptococci by complement fixation reactions, it was considered advisable to study scarlet fever serums and determine in what percentage of cases streptococcus antibodies could be found. Although it is possible to find streptococci in a large per-

5. Besredka and Dopter: Du rôle des streptocoques au cours de la Scarlatine, Ann. de l'Inst. Pasteur, 1904, xviii, 373.

6. Foix, C., and Mallein, E.: La réaction de Bordet et Gengou vis à vis du streptocoque dans la scarlatine, Presse méd., 1907, xv, 777.

7. Uppenheim, A.: Ueber Komplementbindung bei Scharlach, München. med. Wehnschr., 1909, lxi, 2471.

8. Castex, M. R.: Recherches cliniques sur la présence d'anticorps streptococcies dans les serums des malades atteints de streptococcies diverses, Presse méd., 1909, xvii, 324.

9. Sommerfeld, P.: Ueber Komplementablenkung bei Scharlach, Arch. f. Kinderh., 1909, i, 38.

10. Swift, H. F., and Thro, W. C.: A study of Streptococci with the Complement Fixation and Conglutination Reactions, THE ARCHIVES INT MED., 1911, vii, 24.

centage of scarlet fever cases, yet streptococci may be found in from 60 to 70 per cent. of healthy throats (Ruediger) and therefore this organism may be perfectly harmless in a certain proportion of scarlet fever patients. This part of the study may be considered under two heads:

1. To apply the principles of complement fixation in the differentiation of streptococci after experimental immunization of animals.
2. To study scarlet fever serums with suitable antigens in order to detect streptococcus antibodies and ascertain if these antibodies are specific.

TECHNIC

1. *Complement*.—Fresh guinea-pig serum titrated for exact strength. One unit was used in each tube.

2. *Hemolysin*.—The serums of rabbits immunized to sheep corpuscles. The hemolytic unit was determined each time before setting up the tests and was so diluted that 1 c.c. of the dilution contained two units.

3. *Corpuscle Suspension*.—Defibrinated sheep's blood washed three times with 0.85 per cent. salt solution and made up to a 5 per cent suspension with saline; 1 c.c. of this was used in each tube.

4. *Immune or Normal Rabbit Serum*.—The serum was heated to 55 C. for twenty-five minutes and used in increasing amounts.

5. *Antigens*.—The preparation of these is most important. Fresh emulsions are usually so highly anticomplementary in comparison to their antigenic value as to be useless. I have used practically the same method devised by Swift and Thro. The secret consists in drying the organisms at the lowest possible temperature and preferably in a vacuum over calcium chlorid or sulphuric acid.

The steps in the procedure were as follows:

1. Cultures were grown on slanted gelatin agar in large bottles for four days.
2. Growths were washed off carefully with 0.85 per cent. saline solution and examined to make sure that the culture was pure.
3. The emulsion was strained, in case fragments of the media came away.
4. The emulsions were thrown down in a high speed electric centrifuge.
5. The bacteria in the bottom of the centrifuge tube were dried in a cold room over calcium chlorid.
6. Tubes were plugged and paraffined and put away in the refrigerator until all serums were in readiness for the fixation tests.
7. The dried organisms were ground in a mortar with 0.85 per cent. saline solution containing 0.5 per cent. phenol in the proportion of about 0.05 gm. to 1 c.c. of the saline.

8. The emulsions were shaken with glass beads for forty-eight hours and then centrifuged to clearness. The supernatant fluid was stored in stained glass bottles in the refrigerator. Such antigens were found to keep about one week. The dried organisms may be kept for months and the antigen made up as needed.

Complement, antigen and serum were mixed and the total quantity brought up to 5 c.c. by the addition of 0.85 per cent. salt solution. Tubes were incubated at 37 C. for one hour and then two units of hemolysin and 1 c.c. of the 5 per cent. suspension of corpuscles added. Tubes were incubated for two hours and then placed in the refrigerator for twenty-three hours, when results were noted.

The exact anticomplementary and fixing strengths of the antigens were determined in the manner shown in Table 1.

TABLE 1.—DETERMINATION OF ANTICOMPLEMENTARY AND FIXING POWER OF THE DIFFERENT STREPTOCOCCUS ANTIGENS

ANTICOMPLEMENTARY POWER—

Amt. of Serum, c.c.	Amt. of Antigen, c.c.	Antigen 1	Antigen 2	Antigen 3	Antigen 4	Antigen 5
....	.06	C.H.*	C.H.	C.H.	C.H.	C.H.
....	.08	C.H.	C.H.	C.H.	C.H.	C.H.
....	.1	C.H.	C.H.	C.H.	C.H.	C.H.
....	.15	C.H.	C.H.	C.H.	C.H.	C.H.
....	.18	C.H.	C.H.	S.I.H.	C.H.	S.I.H.
....	.2	S.I.H.	S.I.H.	M.I.H.	S.I.H.	M.I.H.
....	.3	M.I.H.	M.I.H.	C.I.H.	M.I.H.	C.I.H.

FIXING POWER—

Immune Serum:

.2	.06	S.I.H.	C.H.	S.I.H.	C.H.	C.H.
.2	.08	M.I.H.	S.I.H.	S.I.H.	C.H.	S.I.H.
.2	.09	I.H.	M.I.H.	M.I.H.	C.H.	M.I.H.
.2	.1	I.H.	M.I.H.	M.I.H.	S.I.H.	I.H.
.2	.12	I.H.	I.H.	I.H.	S.I.H.	I.H.
.2	.15	I.H.	I.H.	I.H.	M.I.H.	I.H.
.2	.2	I.H.	I.H.	I.H.	M.I.H.	I.H.

Normal Serum:

.2	.06	C.H.	C.H.	C.H.	C.H.	C.H.
.2	.08	C.H.	C.H.	C.H.	C.H.	C.H.
.2	.09	C.H.	C.H.	C.H.	C.H.	C.H.
.2	.1	C.H.	C.H.	C.H.	C.H.	C.H.
.2	.12	C.H.	C.H.	C.H.	C.H.	C.H.
.2	.15	C.H.	C.H.	S.I.H.	C.H.	C.H.
.2	.2	S.I.H.	S.I.H.	I.H.	S.I.H.	M.I.H.

*C.H. indicates complete hemolysis; S.I.H. slight inhibition of hemolysis; M.I.H. marked inhibition of hemolysis; I.H. complete inhibition of hemolysis.

It will be noted that the immune body content was fairly pronounced in all serums except from Rabbit 4, in which it was rather low. Having determined the anticomplementary and antigenic units of each antigen and the amount of antibody in each immune serum we were ready for the most important step of all, to determine if the antibody is specific for the streptococcus used in producing it. To determine this, it was necessary

to try out each immune serum with each antigen separately and note the results, as shown in Tables 2, 3, 4, 5 and 6.

TABLE 2.—FIXING POWER OF SERUM 1

Amt. of Serum, c.c.	Antigen c.c.	Strepto- coccus 1	Strepto- coccus 2	Strepto- coccus 3	Strepto- coccus 4	Strepto- coccus 5
.05	0.1	S.I.H.	S.I.H.	C.H.	C.H.	C.H.
.08	0.1	M.I.H.	S.I.H.	C.H.	C.H.	C.H.
0.1	0.1	I.H.	M.I.H.	C.H.	C.H.	S.I.H.
0.15	0.1	I.H.	I.H.	S.I.H.	S.I.H.	S.I.H.
0.2	0.1	I.H.	I.H.	S.I.H.	M.I.H.	M.I.H.

It will be noted (Table 2) that complement fixation is most marked with the scarlet fever streptococcus antigens 1 and 2.

TABLE 3.—FIXING POWER OF SERUM 2

Amt. of Serum, c.c.	Antigen c.c.	Strepto- coccus 1	Strepto- coccus 2	Strepto- coccus 3	Strepto- coccus 4	Strepto- coccus 5
.05	0.1	S.I.H.	S.I.H.	C.H.	C.H.	C.H.
.08	0.1	S.I.H.	S.I.H.	C.H.	C.H.	C.H.
0.1	0.1	M.I.H.	M.I.H.	C.H.	C.H.	C.H.
0.15	0.1	M.I.H.	M.I.H.	S.I.H.	C.H.	S.I.H.
0.2	0.1	I.H.	I.H.	M.I.H.	S.I.H.	S.I.H.

Table 3 shows again that complement fixation is most marked with the scarlet fever streptococcus cultures. It is important to note in these tables that the tendency to specificity of serums and antigens is noted only in the higher dilutions of serum.

TABLE 4.—FIXING POWER OF SERUM 3

Amt. of Serum, c.c.	Antigen c.c.	Strepto- coccus 1	Strepto- coccus 2	Strepto- coccus 3	Strepto- coccus 4	Strepto- coccus 5
.05	0.1	S.I.H.	S.I.H.	S.I.H.	C.H.	C.H.
.08	0.1	M.I.H.	M.I.H.	M.I.H.	C.H.	C.H.
0.11	0.1	I.H.	I.H.	I.H.	S.I.H.	C.H.
0.15	0.1	I.H.	I.H.	I.H.	M.I.H.	S.I.H.
0.2	0.1	I.H.	I.H.	I.H.	I.H.	M.I.H.

It will be noted that the fixing power of Serum 3 (Table 4) with its own antigen (3) was no greater than with Antigens 1 and 2. In this respect it did not show any specific action although with Antigens 4 and 5 fixation resulted only with lower dilution of serum.

TABLE 5.—FIXING POWER OF SERUM 4

Amt. of Serum, c.c.	Antigen c.c.	Strepto- coccus 1	Strepto- coccus 2	Strepto- coccus 3	Strepto- coccus 4	Strepto- coccus 5
.05	0.1	C.H.	C.H.	C.H.	C.H.	C.H.
.08	0.1	C.H.	C.H.	C.H.	C.H.	C.H.
0.1	0.1	C.H.	C.H.	C.H.	C.H.	C.H.
0.15	0.1	C.H.	C.H.	C.H.	S.I.H.	C.H.
0.2	0.1	S.I.H.	S.I.H.	S.I.H.	M.I.H.	C.H.

The antibody content of Serum 4 (Table 5) was quite low, so that fixation occurred only in low dilution. It proved, however, to be slightly specific in nature.

TABLE 6.—FIXING POWER OF SERUM 5

Amt. of Serum, c.c.	Antigen c.c.	Strepto- coccus 1	Strepto- coccus 2	Strepto- coccus 3	Strepto- coccus 4	Strepto- coccus 5
.05	0.1	C.H.	C.H.	C.H.	C.H.	C.H.
.08	0.1	C.H.	C.H.	C.H.	C.H.	S.I.H.
0.1	0.1	S.I.H.	S.I.H.	C.H.	C.H.	M.I.H.
0.15	0.1	S.I.H.	S.I.H.	S.I.H.	C.H.	I.H.
0.2	0.1	M.I.H.	M.I.H.	S.I.H.	S.I.H.	I.H.

The immune body in Serum 5 (Table 6) showed its specific character in the higher dilutions of serum.

Of interest here is the complement fixation reactions with polyvalent streptococcus serum (Table 7) sent me by Dr. A. P. Hitchens, director of the Mulford Biological Laboratories. The horse, No. 349, had been immunized by subcutaneous injections of streptococci from various sources, including scarlet fever streptococci which I sent to Dr. Hitchens.

TABLE 7.—FIXING POWER OF POLYVALENT SERUM

Amt. of Serum, c.c.	Antigen c.c.	Strepto- coccus 1	Strepto- coccus 2	Strepto- coccus 3	Strepto- coccus 4	Strepto- coccus 5
.05	0.1	C.H.	C.H.	S.I.H.	C.H.	M.I.H.
.08	0.1	C.H.	C.H.	I.H.	C.H.	I.H.
0.1	0.1	S.I.H.	S.I.H.	I.H.	C.H.	I.H.
0.15	0.1	S.I.H.	S.I.H.	I.H.	S.I.H.	I.H.
0.2	0.1	S.I.H.	S.I.H.	I.H.	M.I.H.	I.H.

It will be noted in Table 7 that fixation is best marked with Streptococcus 5, isolated from a case of streptococcic tonsillitis; then with Streptococcus 3, a culture sent me by Dr. Hitchens. The serum also contained antibodies for scarlet fever streptococci as demonstrated by the reactions with 1 and 2. This would tend to show that each streptococcus produced its own antibody.

COMPLEMENT FIXATION WITH SCARLET FEVER SERUMS

Serums from 107 patients with scarlet fever were examined and these cases may be analyzed as follows:

Scarlet fever simplex	104 cases
Scarlet fever malignant.....	3 cases

During the first week or ten days of scarlet fever, the rash is present and begins to fade; during the second week desquamation is usually well marked and is completed by the sixth or seventh week. Serum from these patients was secured at the following periods of the disease:

Week of Disease	Cases	Week of Disease	Cases
First	38	Fourth to fifth	6
Second	31	Fifth to sixth	4
Second to third	12	Sixth to eighth	3
Third to fourth.....	13		

All of these serums were examined with an antigen of Streptococcus 1. The technic was the same as that already given, only emphasis is again laid on the importance of accurate titration of complement and amboceptor.

Results: Serums from twelve cases, or 11.2 per cent., yielded positive reactions as follows:

Week of Disease	No. Positive	Week of Disease	No. Positive
First	4	Fourth	2
Second	2	Sixth to seventh	2
Third	2		

Eight of these positive serums yielded negative reactions with the regular Wassermann system of luetic liver antigen. Six of the above positive serums were tried out with the other three streptococcus antigens, yielding negative results with the higher dilutions of serum.

Of interest here is the work of Much and Eichelberg,¹¹ who applied the complement fixation reaction to scarlet fever serums with antigen of luetic liver, not with the purpose of studying the hypothetical immune bodies of scarlet fever, but to ascertain if the antigen used in the diagnosis of syphilis would unite with something in the serum, deviate complement, and thus render the serodiagnosis of syphilis less reliable. Their report stimulated considerable work with widely different results. In June, 1911, I reported¹² the result of the study with the Wassermann and Noguchi systems of 250 cases of scarlet fever in all stages of the disease with uniformly negative results. A large number of these same serums were used in this streptococcus work.

CONCLUSIONS

I believe that the following deductions are correct:

1. A streptococcus produces a specific antibody up to a certain limit. The specific nature of these antibodies is seen by complement fixation reactions in high dilutions between a streptococcus immune serum and its antigen. With low dilutions, however, the specific nature of the antibody is not brought out.

11. Much and Eichelberg: Die Komplementbindung mit wässerigen Luesextrakt bei nichtsyphilitischen Krankheiten, Med. klin., 1908, iv, 671.

12. Kolmer, J. A.: Complement Deviation in Scarlet Fever with Comparative Studies of the Wassermann and Noguchi Systems, Jour. Exper. Med., 1911, xiv, 235.

2. Not only do scarlet fever streptococci tend to produce their own specific immune serum, but likewise streptococci causing septicemia and those causing non-scarlatinal angina, etc., tend to produce their own immune serums. Therefore it does not seem any more justifiable to claim a specific streptococcus for scarlet fever than it does to make such a claim for a specific streptococcus causing septicemia and other conditions.

3. Finding but 11.2 per cent. positive reactions in scarlet fever tends to show that streptococcus infection in scarlet fever severe enough to produce immune bodies is not so common as is generally believed. One of the three toxic or malignant cases yielded a positive reaction. These severe cases are frequently due to an overwhelming effect of the true scarlet fever virus, whatever that may be, without secondary streptococcus infection.

OPSONINS

Since it has been practically proved that a streptococcus is not the cause of scarlet fever it is difficult to understand how immunization with streptococci can prevent that disease. We can understand, however, that such immunization may be of value in preventing or mitigating secondary streptococcic infections which are present in at least 11 per cent. of scarlet fever patients and responsible for severe primary lesions and complications. Likewise, it is probable that such protection would result by an increase in opsonins against streptococci. The question of method, however, relative to production of opsonin is very important, and accordingly this study had two main objects in view.

1. To ascertain if the streptococco-opsonic index can be raised by the injection of heat-killed streptococci. Since the Russian physicians advise two or three doses of vaccine as a prophylactic, my special object was to determine if this number of injections was sufficient to raise the opsonic index.

2. To ascertain whether the opsonins produced by the different strains of streptococci were specific in nature.

The opsonic index of normal serum is low for streptococci from all sources. Bordet, in a study of antistreptococcic serum, says that he believes that streptococci exert a negative chemotactic influence and that the administration of the serum removes this influence and stimulates phagocytosis. Banks¹³ and Tunncliff¹⁴ have shown that early in scarlet fever the streptococco-opsonic index is usually below normal, that it rises rather sharply with the decline of fever and amelioration of symptoms and then slowly falls to normal during convalescence. If the injection of

13. Banks: Variations in Scarlet Fever of the Opsonic Power of Streptococci, *Jour. Path. and Bacteriol.*, 1907, xii, 113.

14. Tunncliff, R.: The Streptococco-Opsonic Index in Scarlatina, *Jour. Infect. Dis.*, 1907, iv, 304.

streptococcus vaccine can prevent streptococcus infection, then it seems reasonable to suppose that it does so by increasing the quantity of opsonins against streptococci and that this could be demonstrated experimentally. Simonds¹⁵ was able to raise the streptococco-opsonic index of rabbits by injecting heat-killed streptococci. Weaver and Tunnicliff were not able to do this with heat-killed organisms but were successful after injecting emulsions killed in 25 per cent. solution of galactose. In the Russian method of preventive inoculation the vaccines are killed by heat and if it is true that vaccines do not increase opsonins it is difficult to understand how protection is brought about.

TECHNIC AND RESULTS

The same cultures and immune serums were used as in the complement fixation tests. Cultures showing a tendency to grow in the conglomerate masses in bouillon were trained to grow diffusely by daily subcultures in glucose + 0.5 bouillon. The emulsions were thoroughly broken up with glass beads prior to use in opsonin work.

The four rabbits were bled from the ear one week after the third injection of streptococci and the opsonic indices shown in Table 8 obtained.

TABLE 8.—OPSONIC INDICES AFTER THREE INJECTIONS OF STREPTOCOCCI

Immune Serum	Streptococcus 2	Streptococcus 3	Streptococcus 4	Streptococcus 5
Rabbit 2	0.9	0.6	0.6	0.7
Rabbit 3	0.5	1.0	0.6	0.8
Rabbit 4	0.2	0.3	1.1	0.3
Rabbit 5	0.65	0.6	0.5	0.9

It will be noticed by Table 8 that the quantity of opsonin is slightly raised in 3 and 4. The next table (Table 9) is composed of the streptococco-opsonic indices after six injections, the horse having been bled freely from the jugular vein and the rabbits killed. These examinations were made within thirty-six hours after bleeding and repeated at the end of seventy-two hours.

TABLE 9.—OPSONIC INDICES AFTER SIX INJECTIONS OF STREPTOCOCCI

Immune Serum	Streptococcus 1	Streptococcus 2	Streptococcus 3	Streptococcus 4	Streptococcus 5
Horse Serum 1	3.2	3.2	1.1	1.0	1.1
Rabbit 2	1.2	1.2	0.6	0.8	0.9
Rabbit 3	0.6	0.6	1.6	0.8	0.9
Rabbit 4	0.2	0.2	0.37	1.4	0.32
Rabbit 5	0.8	0.8	0.85	0.7	1.7
Polyvalent Serum ..	2.8	2.8	3.3	4.1	3.6

It will be noted (Table 9) that the horse serum contains the largest quantity of opsonin. This serum also gave beautiful complement fixation

15. Simonds, J. P.: The Effect of the Injection of Killed Streptococci on the Streptococco-Opsonic Index of Normal Rabbits, Jour. Infect. Dis., 1907, iv, 595.

reactions and has proved distinctly curative in severe cases of scarlet fever treated in the wards of the Philadelphia Hospital for Contagious Diseases. The quantity of opsonin has been increased in all animals by the additional three doses of streptococci. The polyvalent serum, the same as used in the complement fixation tests, shows a high opsonin content for all strains of streptococci, especially the non-scarlatinal strains.

CONCLUSIONS

1. It is possible to raise the streptococco-opsonic index by the injection of organisms killed by an exposure to 60 C. for one hour. This increase is very slight with three or less administrations and becomes apparent only after a relatively large number of injections.

2. The increase in the quantity of opsonin after three injections is so slight as to make the likelihood of establishing an immunity against streptococcic infection very dubious.

3. The opsonins were more or less specific toward the strains used in the process of immunization.

AGGLUTININS

Streptococci stimulate the production of agglutinins but the quantity produced varies within wide limits. Investigation tends to show that such agglutinins are not specific in character and for this reason agglutination reactions are of little value in the study of streptococcus infections. The reports of investigations, however, are far from agreeing on this point and since I worked on this problem with much interest I thought it would not be amiss to record my results.

The first studies in agglutination of streptococci by immune serums were made, according to Weaver,¹⁶ by Van de Velde, who found anti-streptococcus serum to have weak agglutinating power over homologous streptococci but none over other strains. Moser and von Pirquet, Meyer, Wlassjenski and Salve found agglutinins present in immune serums and considered them more or less specific in nature. Krauss and Aronson secured negative results. The work of the latter needs special mention because of its more thorough character. He immunized horses with cultures of streptococci from scarlet fever, rheumatism and otitis media and after careful agglutination tests with the immune serums insists that in no case is one able to differentiate streptococci by the most careful work. Weaver also made an excellent report on the agglutination of streptococci. Cultures from various sources, including scarlet fever, phlegmon, puerperal sepsis, endocarditis, etc., were used. Serums from various diseases were tested. It was found that scarlet fever streptococci were agglutinated

16. Weaver: Agglutination of Streptococci, Especially Those Cultured from Cases of Scarlatina, by Human Sera, *Jour. Infect. Dis.*, 1904, i, 91.

by the serums from pneumonia, erysipelas, typhoid fever and puerperal sepsis and that agglutination between scarlet fever streptococci and scarlet fever serum is in no way specific and cannot be of any value in diagnosis.

In my investigation I used the same five immune serums as were used in the complement fixation and opsonin work. These serums were five and six days old when the final agglutination reactions were set up. They were kept in a refrigerator without the addition of preservatives. In addition to the agglutination reactions with the five immune serums, the serums of thirty-two scarlet fever patients were examined.

TABLE 10.—SHOWING RESULTS OF AGGLUTINATION OF STREPTOCOCCI BY IMMUNE SERUMS

Strepto- coccus	IMMUNE SERUM 1							
	Dilutions							
	1/10	1/15	1/20	1/25	1/30	1/40	1/100	1/140
1	+	+	+	+	+	+	+	—
2	+	+	+	+	+	+	+	—
3	+	+	+	+	+	—	—	—
4	+	+	+	—	—	—	—	—
5	+	+	+	+	+	—	—	—
IMMUNE SERUM 2								
1	+	+	+	—	—	—	—	—
2	+	+	+	—	—	—	—	—
3	+	+	—	—	—	—	—	—
4	+	+	+	+	—	—	—	—
5	+	+	+	—	—	—	—	—
IMMUNE SERUM 3								
1	+	+	+	—	—	—	—	—
2	+	+	+	—	—	—	—	—
3	+	+	+	+	+	—	—	—
4	+	+	—	—	—	—	—	—
5	+	+	+	—	—	—	—	—
IMMUNE SERUM 4								
1	—	—	—	—	—	—	—	..
2	—	—	—	—	—	—	—	..
3	+	—	—	—	—	—	—	..
4	+	+	+	—	—	—	—	..
5	+	—	—	—	—	—	—	..
IMMUNE SERUM 5								
1	+	+	—	—	—	—	—	..
2	+	+	—	—	—	—	—	..
3	+	+	+	+	—	—	—	..
4	+	—	—	—	—	—	—	..
5	+	+	+	+	—	—	—	..
POLYVALENT SERUM								
1	+	+	+	+	—	—	—	..
2	+	+	+	+	—	—	—	..
3	+	+	+	+	+	+	—	..
4	+	+	—	—	—	—	—	..
5	+	+	+	+	—	—	—	..

TECHNIC AND RESULTS

Cultures of streptococci were subcultured daily in glucose bouillon + 0.5 phenolphthalein until they grew diffusely. The macroscopic technic

was used throughout. Dilutions from 1 to 10 up were employed. The tubes were kept in the incubator at 36 C. for two hours and examined with the aid of a magnifying glass. Final readings were made at the end of twenty-four hours.

Table 10 gives in detail the results of these agglutination tests with the various immune serums and different cultures of streptococci.

Examination of Table 10 shows: (1) The quantity of agglutinin produced was in most instances small in amount. The highest values were found with the horse serum, a result of prolonged immunization with dead, attenuated and finally living cultures. (2) The reactions were inconstant and quite variable. (3) Agglutinins for streptococci are not specific and hence of no value in the differentiation of streptococci.

STREPTOCOCCUS AGGLUTININS IN SCARLET FEVER

Serums from the thirty-two patients examined may be classified as follows:

Scarlet fever simplex	31 cases
Scarlet fever malignant	1 case

These cases included all stages of the disease.

Results: Agglutination of scarlet fever streptococci occurred with four serums in dilutions up to 1-to-40. All of the remaining serums yielded absolutely negative results. The four positive serums also agglutinated the other streptococci, but in lower and various dilutions. In no case was a high degree of agglutination demonstrable.

CONCLUSIONS

1. Streptococcus agglutinins are produced slowly by experimental immunization and the quantity is quite variable.
2. These agglutinins are apparently non-specific in character and of no value in differentiating streptococci.
3. They were demonstrated in 12.5 per cent. of scarlet fever patients but only in comparatively low dilutions of serum.
4. There was no relation between agglutination and complement fixation antibodies or between agglutinins and opsonins.

THE RELATION OF STREPTOCOCCI TO SCARLET FEVER

A great deal has been written under this heading. In the first place I agree with those who claim that streptococci found in scarlet fever patients, whether in the throat, in suppurative lesions, or in the blood, cannot be differentiated from streptococci found normally in the throat or in septic conditions by morphological or cultural characteristics. Frequently cultures from scarlet fever cases are apparently different, but these differences have been temporary and easily broken up. While com-

plement fixation tests have shown a specific antibody for scarlet fever streptococci, this is true only in high dilutions of serum. And further, the same specific relation is readily demonstrated between streptococci causing septicemia and streptococci causing non-scarlatinal sore throat, etc., and their immune serums. Hence, if we are to claim a specific streptococcus for scarlet fever a similar claim may be made for every other streptococcus infection and thus to claim a specific streptococcus for endocarditis and another for puerperal sepsis, etc., seems to be out of the question. It is true that streptococci are found in a goodly percentage of scarlet fever cases. We are confident, however, that many so-called streptococcus anginas would be found on closer examination to be pneumococcic. My percentage of streptococcus blood infections in scarlet fever is somewhat lower than that obtained by others. One fact is certain: not all severe, septic and fatal cases of scarlet fever are due to streptococci. Antistreptococcus serum, the same horse serum (Serum 1) as used in these experiments, has given good results in certain instances. Patients extremely sick with the true toxemia of scarlet fever are not benefited by it at all. Opsonins have been found somewhat specific for the particular culture responsible for their formation but I am not yet prepared to conclude from this that a certain strain of streptococcus causes this condition and another that condition. I believe that the true scarlet fever poison lowers the resistance of the patient to streptococcus infection and this is substantiated by noting the low streptococco-opsonic indices during the first week of scarlet fever. How this is brought about is impossible as yet to explain. A similar relation may be noted by the tendency of the diphtheria patient to contract scarlet fever. We believe that the streptococcus found in scarlet fever belongs to the general class of streptococci and is modified in some way during the course of the infection so as to give it a somewhat specific antibody and occasionally temporary cultural characteristics. The same is true of streptococci causing septicemia and simple sore throat. How these changes are brought about constitutes an interesting field for further investigation.

STREPTOCOCCUS IMMUNIZATION

The reports of Russian physicians concerning the efficacy of injections of streptococcus vaccine as a preventive of scarlet fever are on the whole quite convincing. But the disease is so protean in character, is disseminated so frequently in such an inexplicable and confusing manner, so little is really understood concerning the infection, that one must review these enthusiastic reports with healthy skepticism. I immunized 350 persons¹⁷ according to the Russian method and while that number is far

17. Kolmer, J. A.: The Relation of Streptococci to Scarlet Fever with Active Immunization by Means of Streptococcus Bacterins, *Pennsylvania Med. Jour.*, 1912, xv, No. 5.

too small to base an opinion on, yet it was observed that the method was without apparent value. Since experimental work does not, in my opinion, establish the identity of a specific streptococcus in scarlet fever, and from the fact that streptococci are found only in a proportion of scarlet fever cases and in many of these is apparently harmless, and since experimental work does not show an increase in opsonin after at least three injections of streptococcus vaccine sufficient to establish a good degree of immunity, I do not believe that streptococcus immunization prevents scarlet fever, although it is possible that it may prevent or mitigate the severity of secondary streptococcus infections.

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