

COMPLEMENT DEVIATION IN SCARLET FEVER WITH
COMPARATIVE STUDIES OF THE WASSER-
MANN AND NOGUCHI SYSTEMS.*

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Scarlet fever is a specific infectious disease of unknown etiology, running a definite course and conferring a specific immunity in the great majority of cases. This report is part of a series of investigations concerning the immune bodies, which it is believed must occur in the blood of persons having recovered from this infection.

Complement deviation tests were first undertaken with scarlet fever sera not with the hope of studying these hypothetical immune bodies, but to ascertain if the antigen prepared from luetic liver then in use would unite with something in the serum, deviate the complement, and thus render the sero-diagnosis of syphilis less reliable.

Much and Eichelberg first investigated the subject and examined the sera of twenty-five cases of which ten gave deviation or were "positive." The antigen employed by them—a watery extract of luetic liver—was evidently defective and their results misleading, but as the report appeared at a time when the Wassermann test was attracting attention, it stimulated interest because of its bearing on the serum diagnosis of syphilis. Later reports of other investigators showed considerable variation.

Of thirty-three cases examined by Jochmann and Töpfer only one case was positive, and this one proved negative at a later examination. Halberstaedter, Müller and Reiche examined ten cases, using several antigens prepared from luetic livers. The various antigens gave different results: five sera were weakly positive with one and negative with another. The remaining five were all negative. Boas and Hauge studied sixty-one cases; half before the disappearance of the rash and the other half at other periods. The antigen used was Michaelis' alcoholic extract of normal human heart. In three cases there was inhibition of hemolysis. In one, a girl nine years of age, who presented no evidences of syphilis, hemolysis occurred on the fiftieth day of illness. Another test fourteen days later proved negative. They concluded, "that complement-fixation in scarlet fever occurred so rarely and disappeared so rapidly after the illness that it can in no way hinder the great practical diagnostic worth of the Wassermann reaction."

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Bruck and Cohn examined twenty-eight cases, using as antigen eight different alcoholic extracts of syphilitic livers and one alcoholic extract of guinea-pig heart. Seven sera were positive with the syphilitic liver antigens and one with the heart antigen. The remaining twenty sera were negative. Meier tested fifty-two cases with a watery extract of luetic liver. In only one case was a slight positive reaction secured. Schleissner examined twenty cases with an alcoholic extract of guinea-pig heart as antigen and found all negative. Seligmann and Klopstock tested thirty cases by Michaelis' method. Their cases fell into two groups. The first fourteen yielded negative results. Cases 15 to 17 were weakly positive; cases 18 to 30 were strongly positive. Their results led them to state that complement deviation was of aid in the diagnosis of scarlet fever. Yet they must have suspected their antigens, because they say: "If an alcoholic extract can suddenly give a positive reaction with normal serum it is a fact of great theoretical and practical importance. . . ." Hoehne tested fifty-three sera from thirty-seven cases with an alcoholic extract of syphilitic liver. All were negative. Fifteen patients were examined twice and one three times to exclude a positive reaction late in the disease. Haendel and Schultz examined forty-eight cases in the same manner. Seven were positive and forty-one negative. Thirty-one of these sera were tried with a watery extract of scarlet fever liver and twenty were found positive. Fraenkel and Much studied twelve cases in the same way. Five yielded positive results; three were during the first week of the scarlet fever. Zeissler tested forty-two cases with an alcoholic extract of human heart. The cases were in the first to the forty-fourth day of the disease. As three cases gave positive reactions the author agreed with the statement of Much and Eichelberg that scarlet fever is more likely than any other disease to yield positive Wassermann reactions. Fua and Koch, who studied fifty-seven cases with an alcoholic extract of guinea-pig heart, found fourteen with positive, and forty-three with negative reactions. Finally, Hecht, Lateiner and Wilenko examined 105 sera, of which ninety-four were taken during life and eleven after death. In fourteen cases, the reaction was done twice. The ages of the patients varied from two to forty years; the examinations embraced all stages of the disease. As antigens, they used alcoholic extracts of guinea-pig heart and scarlet fever liver. Of the ninety-four sera from living patients they observed complement deviation in one case with the heart antigen and none with the scarlet fever liver antigen. The one positive result was in a case of severe nephritis. Of the eleven sera secured from patients after death, two were positive, in both of which nephritis was present. They conclude that complement fixation occurs in only a few sporadic cases of scarlet fever, that an antigen of scarlet fever liver is not specific, as Haendel and Schultz believed, and that the practical value of the Wassermann test in syphilis is not impaired by a few positive results in scarlet fever.

Apparently all of the work upon complement deviation in scarlet fever according to the Wassermann technique has been done by German investigators. Noguchi examined sera from sixty-two cases of scarlet fever according to his special technique with two doubtful positive and sixty negative reactions. The results secured

by the German observers varied from 40 per cent. positive reactions by Much and Eichelberg and 53 per cent. positive reactions by Seligmann and Klopstock to completely negative results by Schleissner, Hoehne, and others. Haendel and Schultz secured twenty-four positive reactions with thirty-one sera examined when using an extract of scarlet fever liver, and believed they had discovered an important fact, while Hecht, Lateiner and Wilenko after examining 105 sera with negative reactions in 102, concluded that such an extract was not specific and acted as an extract of guinea-pig heart or luetic liver. It is probable that many of these investigators secured false reactions by using faulty antigens—antigens that were themselves anticomplementary.

EXPERIMENTAL PART.

Sera from 250 cases of scarlet fever, representing all stages of the disease, were examined according to the following plan:

1. According to the Wassermann system with the following antigens:

- (a) An alcoholic extract of luetic liver.
- (b) An alcoholic extract of scarlet fever liver (from a malignant case of six days' duration).

2. According to the Noguchi system with:

- (a) Active serum.
- (b) Inactive serum.

The two following antigens were used with the active and inactivated serum of each case:

- (a) An alcoholic extract of luetic liver.
- (b) Aceton insoluble lipoids.

3. Because of the very important relation which the anti-sheep hemolysin normally present in human blood bears to the Wassermann system, the serum of each case was examined quantitatively for the presence of this hemolysin.

The 250 cases examined can be analyzed as follows:

Scarlet fever simplex.....	235 cases.
Scarlet fever malignant.....	13 cases.
Scarlet fever from burns of body.....	1 case.
Scarlatiniform serum rash.....	1 case.

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 the cases was secured at the following periods of the disease:

During the 1st week.....	58 cases.
During the 1st to 2d week.....	55 cases.
During the 2d to 3d week.....	32 cases.
During the 3d to 4th week.....	31 cases.
During the 4th to 5th week.....	28 cases.
During the 5th to 6th week.....	29 cases.
During the 6th to 7th week.....	11 cases.
During the 7th to 8th week.....	2 cases.
During the 8th to 9th week.....	4 cases.

Thirty-three patients had in addition to scarlet fever various complications, as follows:

Adenitis—cervical	3 cases.
Diphtheria—tonsillar	5 cases.
Nephritis—acute diffuse	7 cases.
Otitis media—suppurative	10 cases.
Pertussis	1 case.
Pharyngitis—pneumococcic	2 cases.
Pneumonia—broncho	3 cases.
Vincent's angina	2 cases.

The patients varied in age from one to thirty-five years.

WASSERMANN SYSTEM.

Technique.—Serum was inactivated at 55° C. for twenty-five minutes and used in amounts of 0.2 c.c. The antigens of luetic and scarlet fever liver were made after the method of Michaelis and Lesser, and every week or ten days were examined for exact antigenic value and for anticomplementary and hemolytic action in amounts of at least three antigenic units. The amount of luetic liver antigen used varied between 0.08 and 0.1 c.c.; the scarlet fever liver antigen between 0.25 and 0.3 c.c.; of anti-sheep hemolysin (rabbit serum) 0.002 c.c. represented two units. The pooled serum of two or more guinea-pigs was used as complement after titration for exact strength. Syphilitic and normal serum controls were used, with and without antigen. Serum, complement, antigen, and 0.85 per cent. salt solution up to 5 c.c. were mixed and incubated for one hour at 37° C.; 2 units of hemolysin and 1 c.c. of a 5 per cent. suspension of sheep corpuscles were then added and the whole incubated for two hours more, after which the tubes were placed in the refrigerator for twenty-three hours and the results noted.

Results.—(a) With alcoholic extract of luetic liver used as antigen: there were five, or 2 per cent., positive reactions out of the 250 sera tested. In these five cases positive reactions also resulted with scarlet fever liver antigen and with active and inactivated serum of the Noguchi system. The following are the five positive cases:

No. 1, Case 38, Mrs. B. A., 30 years. Fourteenth day of scarlet fever. After fading of the rash, she developed a peculiar eruption over the body a few days before these tests were made. History of luetic infection negative. All reactions were absolute or + + + +, according to Citron's method of recording. No other complications.

No. 2, Case 87, R. H., male, 11 years. Thirteenth day of scarlet fever. Poorly nourished; no definite evidences of syphilis. All reactions slightly positive. No complications.

No. 3, Case 135, E. S., female, 6 years. Thirteenth day of scarlet fever. No evidences of syphilis. All reactions slightly positive. No complications.

No. 4, Case 150, M. D., female, 14 years. Twenty-eighth day of scarlet fever; sickly and undersized. All reactions moderate in degree. No complications.

No. 5, Case 160, Mrs. B. S., 23 years. Fortieth day of scarlet fever. History unsatisfactory but apparently negative. All reactions of moderate degree. No complications.

(b) With an alcoholic extract of scarlet fever liver used as antigen: the 250 sera were tested with this antigen. The antigen itself was tested with a large number of normal and syphilitic sera and was found to be antigenic in dosage from 0.25 to 0.3 cubic centimeter, and free of anticomplementary and hemolytic action. The results were practically identical with those resulting from the use of the luetic liver antigen. Deviation of complement resulted in six cases, or 2.4 per cent. These included the five cases given above with the addition of case 89, which also gave positive reactions with active and inactivated serum, according to the Noguchi system.

Case No. 89, M. H., male, 5.5 years. Eighth day of malignant scarlet fever. No definite evidences of luetic infection. No complications beyond the severe streptococcic angina of scarlet fever.

It is impossible to express any opinion as to whether any or all of these cases were syphilitic. The fact that in all the cases the other reactions were likewise positive lends some color to the probability of their being syphilitic, especially case 38. However, since but 2 per cent. of the scarlet fever cases were found to deviate complement with the regular Wassermann technique, we regard this reaction in scarlet fever *per se* as being uniformly negative.

As was expected, the antigen of scarlet fever liver acted in the same manner as that of the luetic liver. Hence our results agree with those of Hecht, Lateiner and Wilenko.

NOGUCHI SYSTEM.

Technique.—With active serum one drop (0.02 c.c.), and with inactivated serum four drops (0.08 c.c.) from a capillary pipette were used. Of the two antigens employed, one was an alcoholic extract of luetic liver in a dose of about 0.08 c.c., and the second of acetone insoluble lipoids, 0.1 c.c. of a 0.3 per cent. emulsion being used in each test. The antigens were examined every week or two for antigenic value, and hemolytic and anticomplementary effects. Anti-human hemolysin was used in such strength that 0.05 c.c. represented two units.¹ For complement, the pooled serum of two or more guinea-pigs in 40 per cent. strength was used after titration. The dose was usually about 0.1 c.c. Positive and negative controls with and without antigen were set up. Serum, antigen, complement, and 0.85 per cent. salt solution to make the total 1 c.c. were incubated at 37° C. for one hour. Then two units of hemolysin and 0.1 c.c. of a ten per cent. suspension of washed human corpuscles were added and the mixture was reincubated for two hours. Tubes were then placed at room temperature for an hour, and the results noted.

Results.—(a) With active serum there were sixteen, or 6.4 per cent. positive reactions. Five of these were with the five cases yielding positive Wassermann results. In five, or 2 per cent. of the cases (nos. 31, 50, 75, 81, 107), the reaction was positive with active serum alone, and negative with inactivated serum by both the Noguchi and Wassermann systems. In six cases the reactions were positive with both the active and inactivated serum. In other words, with the Noguchi system there occurred eleven more positive reactions than with the Wassermann system, five of these being with active serum alone.

(b) The first ninety of these reactions were made with alcoholic extract of luetic liver used as antigen, and it is well to note that four of the positive reactions which were obtained with active serum were made with this antigen. The remaining 140 cases were tested with an antigen of acetone insoluble lipoids from which but one positive reaction with active serum resulted.

¹I am indebted to Dr. A. P. Hitchens and Dr. J. T. Ullom, who kindly gave me good hemolysin at a time when the sudden death of several rabbits would have stopped the work for a few weeks.

Noguchi has emphasized the danger of securing positive results with normal serum when using such an antigen as an alcoholic extract of liver, due to the fact that nucleoproteids, pepton, and various decomposition products of proteids in general are able to fix complement. The antigen of acetone insoluble lipoids, which is perfectly antigenic and neither anticomplementary nor hemolytic, at least in an amount of four antigenic units, has given entirely satisfactory results.

None of the eleven cases yielding positive results with the Noguchi system alone showed any evidences of syphilis, although it is, of course, a difficult matter to exclude the possibility of hereditary taint. From the fact that only one out of 140 cases, or 0.72 per cent., deviated complement with active serum in the presence of a satisfactory antigen of acetone insoluble lipoids, and in view of the fact that normal active serum will, in a small percentage of cases, deviate complement, I am inclined to conclude that in scarlet fever *per se* the Noguchi reaction is uniformly negative.

One of the main objections to the Wassermann system is the fact that there is present normally in human serum varying amounts of anti-sheep hemolysin. This is of great importance because an excess of the hemolysin can obscure a result by producing hemolysis in the presence of syphilitic antibodies up to a certain limit, even though less than one unit of complement be present. Practically all investigators have observed this effect, but very few have expressed their results in accurate terms. The 250 sera were tested by us for anti-sheep hemolysin, 0.1 cubic centimeter of undiluted guinea-pig serum being used in each test for complement.

Technique.—0.2 c.c. of inactivated serum, plus 0.1 c.c. of fresh guinea-pig serum, plus 1 c.c. of a 5 per cent. suspension of washed sheep corpuscles, plus 4 c.c. of 0.85 per cent. salt solution were employed. The incubation period was at 37° C. for one hour, followed by sixteen hours in the refrigerator, after which the results were noted.

Results.—Complete hemolysis occurred in 229 cases, or 88.1 per cent., and partial hemolysis in twenty-one cases, or 11.9 per cent. The amount of complement, 0.1 cubic centimeter of guinea-pig serum, was employed because this is the usual dose recommended, although serum from different animals varies in its complement

content. It emphasizes the necessity of careful titration of complement, for an excess of this factor, up to a certain limit, will give hemolysis with a decreased amount of hemolysin. The presence of anti-sheep hemolysin in normal human serum probably accounts for the negative Wassermann reaction with serum containing small amounts of syphilitic antibody. On the other hand, normal active serum will give a positive Noguchi reaction. For these reasons, we are led to agree with Kaplan that a Wassermann and Noguchi reaction should be performed with each serum—the one acting as a control upon the other.

CONCLUSIONS.

The Wassermann reaction in scarlet fever *per se* is uniformly negative. The antigen of scarlet fever liver yields practically the same results as that of luetic liver, and both fail to deviate complement with scarlet fever antibodies.

The Noguchi reaction in scarlet fever is practically negative. Sixteen cases, or 6.4 per cent. of 250 cases, were positive when active serum was used; with inactivated serum, but eleven, or 4.4 per cent., remained positive. Five of these eleven cases were also positive with the Wassermann system. In other words, sixteen cases, or 6.4 per cent., were positive according to the Noguchi system with active or inactivated serum or both, whereas with the Wassermann system only 2 per cent. were positive.

The presence of anti-sheep hemolysin normally in human serum is one of the main disturbing factors in the Wassermann system; for this reason, complement and hemolysin (made by immunization of rabbits) require careful titration. A positive Wassermann reaction usually indicates the presence of syphilitic antibodies, and a negative Noguchi reaction, their absence, and both systems should be used in the examination of all cases.

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