

CONCERNING AGGLUTININS FOR *TREPONEMA* PALLIDUM.*

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The relation of *Treponema pallidum* to syphilis has been conclusively proven by Noguchi, who reproduced lesions of syphilis in the testicles of rabbits and in the eyebrows of monkeys by implantation of a pure culture of *Treponema pallidum* isolated from a syphilitoma of a testicle of the rabbit due to inoculation with material from a human lesion. Schereschewsky,¹ in 1909, was probably the first to show that spirochetes may be grown artificially, although in 1907 Levaditi, Yamanouchi, and others found that *Treponema pallidum* could be kept alive in a celloidin sac containing monkey serum, placed in the peritoneal cavity of a monkey. Later, in 1909 and 1910, Mühlens² and Hoffmann³ claim to have grown *Treponema pallidum* in Schereschewsky's gelatinized horse serum medium and to have purified a culture in horse serum agar. Early attempts at inoculation were unsuccessful, although at a later period Hoffmann reported a successful inoculation. The lesion, however, was doubtful, the culture grew without the presence of fresh tissue and produced a strong putrefactive odor, thus resembling the morphological and biological characters of *Treponema microdentium* as described by Noguchi. In 1910 Bruckner and Galasesco⁴ reported the successful inoculation of rabbits with impure cultures of *Treponema pallidum* grown in gelatinized ascitic fluid in which the original syphilitic tissue was still present. Noguchi⁵ has also cultivated

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¹ Schereschewsky, J., *Deutsch. med. Wchnschr.*, 1909, xxxv, 835, 1260, 1652.

² Mühlens, P., *Deutsch. med. Wchnschr.*, 1909, xxxv, 1261; *Klin. Jahrb.*, 1910, xxiii, 339.

³ Hoffmann, *Ztschr. f. Hyg. u. Infektionskrankh.*, 1911, lxviii, 27.

⁴ Bruckner, I., and Galasesco, P., *Compt. rend. Soc. de biol.*, 1910, lxviii, 684.

⁵ Noguchi, H., *Jour. Exper. Med.*, 1911, xiv, 99; 1912, xv, 90, 201.

Treponema pallidum directly from human lesions, and the definite characteristics of his cultures as compared with pure cultures of other spirochetes isolated by him, together with the successful inoculation of monkeys and rabbits, and confirmatory evidence afforded by specific complement fixation and luetin reactions, prove conclusively the etiological relation of *Treponema pallidum* to syphilis.

Until the present time studies of the antibodies of *Treponema pallidum* have been chiefly confined to the syphilitic antibody concerned in the complement fixation reaction of syphilis. The object of the present communication is to record briefly a study of agglutinins for *Treponema pallidum* (Noguchi) in experimental and in human syphilis.

METHOD.

Culture.—The culture of *Treponema pallidum* used in this study was obtained from Dr. Noguchi. The spirochete grows well in ascitic agar with rabbit kidney and fulfills all the cultural and morphological characteristics described by him. It is readily grown in a fluid medium of horse serum bouillon with kidney extract and this medium was selected for the agglutination reactions. In this medium rich cultures of motile spirochetes are secured in two weeks, showing twenty to forty organisms per field when examined by dark-field illumination. In no instance was coagulation of the horse serum or ascitic fluid found to occur. Motility is best seen in young fluid medium cultures, and practically all phases of longitudinal division have been noted.

Immune Sera.—Injections of ascitic agar and serum bouillon cultures into the testicle and scrotal tissue of rabbits failed to develop a syphilitic lesion. The culture, however, was isolated in October, 1910, and it is probable that *Treponema pallidum* quickly loses its pathogenicity in artificial culture media. The rabbits, however, as will be seen later, showed the presence of agglutinin and an amboceptor.

Another series of rabbits was immunized by giving intravenously increasing doses of young unheated fluid cultures. At the end of two months ten cubic centimeters were given at one injection, and in no instance have the animals shown ill effects. The sera of these animals were found to have a high agglutinin content.

Human Sera.—Nineteen sera and one cerebrospinal fluid from patients giving a history of syphilis and yielding a positive Wassermann reaction were studied; also ten sera and two cerebrospinal fluids giving a negative history and negative Wassermann reaction. In no instance were agglutinins found in as low dilutions as 1:20.

Technique.—The macroscopic test was employed. Cultures of *Treponema pallidum* were grown in serum bouillon for four weeks, thoroughly shaken, and centrifuged for five minutes. The result was a culture of good density showing twenty to forty spirochetes per field and free from clumping. One cubic centimeter was used in each test, the serum being diluted in one cubic centimeter of sterile saline solution, the total volume, therefore, being two cubic centimeters. By using this amount of culture a sufficient number of spirochetes were present to yield unmistakable agglutination in positive reactions.

All the sera were unheated in dilutions varying from 1:20 to 1:1,280. After reactions were set up the tubes were incubated for two hours at 37° C. and then placed in the refrigerator over night, final readings being made at the end of twenty-four hours.

In every instance a culture control and controls with normal rabbit and human sera were set up at the same time and in no instance were there any evidences of agglutination or sedimentation.

In most of the tests a drop of the supernatant fluid was examined by dark-field illumination at the time the results were read, and in every instance the controls and negative reactions showed the presence of a large number of spirochetes, a few still motile, and all free from clumping. In a few instances two or three spirochetes were found in clumps, but there was no sedimentation and this was not considered as an evidence of agglutination. In all positive reactions a drop of the sediment examined by dark-field illumination showed the presence of large clumps of spirochetes which were in most instances non-motile, although a few motile organisms were to be found at the edge of a clump, or entirely free. In the higher dilutions where agglutination was incomplete, free active spirochetes were found in the supernatant fluid and clumps in the sediment.

One of the human sera was unfortunately infected with *Bacillus*

subtilis, and a small amount of sediment was readily noted in the test-tubes. An examination by dark-field illumination quickly showed motile non-clumped spirochetes and revealed the cause of the pseudoreaction.

The agglutinated spirochetes stained well with the Giemsa stain and did not show any distinct evidence of disintegration.

RESULTS.

(a) *Rabbit Serum*.—Two rabbits were given injections into the testicular tissue of 0.1 of a cubic centimeter of a culture removed from the vicinity of the piece of kidney in a three weeks old ascitic agar culture rich in spirochetes. The animals did not develop any evidence of syphilis. The serum of one showed agglutination in dilution of 1:20 four weeks after inoculation, the second rabbit agglutinating in dilutions up to 1:40.

Three rabbits received the same dose into the testicular tissues at the same time, and at the end of four weeks another dose of one cubic centimeter of a rich serum bouillon culture subcutaneously in the scrotal tissues. Four weeks later the serum was tested and found to agglutinate in dilutions varying from 1:160 to 1:320. None of the rabbits developed lesions of syphilis, one showing a firm nodule at the site of inoculation but free of spirochetes and probably due to non-specific inflammation.

Of additional interest are the results of complement fixation reactions, especially with spirochetal culture antigens. Further study⁶ of this subject has been made, but it will suffice to include here a brief record of results with the sera tested for agglutinin. Salt solution and alcoholic extracts of *Treponema pallidum* were used; also, for controls, extracts of sterile media and media inoculated with other organisms. Specific reactions were observed especially with the salt solution extract of the pallida, the spirochetes having been grown in fluid culture and washed free of all media before extraction. Noguchi⁷ demonstrated the presence of specific amboceptor in the sera of rabbits immunized with a culture, using a salt

⁶ Kolmer, J. A., and Williams, W. W., A Study of Complement Fixation in Syphilis with *Treponema pallidum* Antigen, *Jour. Med. Research*, 1913 (in press).

⁷ Noguchi, H., *Jour. Am. Med. Assn.*, 1912, lviii, 1163.

solution extract of the pallidum culture in ascitic agar as antigen. Rabbits suffering with acute syphilitic orchitis yielded negative reactions. Craig and Nichols⁸ using alcoholic extracts of pallidum culture also secured negative reactions.

The Wassermann reactions were performed with four different lipoidal extracts. With rabbit sera the results must be regarded as doubtful because the positive results cannot be regarded as specific reactions, for, as will be seen later, normal rabbit serum has been found to yield positive Wassermann reactions under certain conditions.

The following table gives the results of the complement fixation and agglutination reactions.

TABLE I.

No.	Dose of culture.	Complement fixation reaction.		Agglutination reactions.								
		Wassermann reaction.	Spirochete reaction.	1:20	1:30	1:40	1:60	1:80	1:160	1:320	1:640	
14	0.1 c.c. into testicle	-	+++	+	-	-	-	-	-	-	-	-
15	0.1 c.c. into testicle	-	++	+	+	+	-	-	-	-	-	-
16	0.1 c.c. into testicle 1.0 c.c. three weeks later into scrotal tissues	±	+++	+	+	+	+	+	+	+	+	-
17		-	+++	+	+	+	+	+	+	+	+	-
18		-	+++	+	+	+	+	+	±	-	-	-

Three rabbits were gradually immunized by the intravenous administration of increasing doses of unheated rich serum bouillon cultures until ten cubic centimeters could be given at one time. All the animals withstood the reactions well. The sera showed agglutination in high dilutions.

TABLE II.

No.	Dose of culture.	Complement fixation reaction.		Agglutination reactions.								
		Wassermann reaction.	Spirochete reaction.	1:20	1:30	1:40	1:60	1:80	1:160	1:320	1:640	1:1280
34	Intravenous	-	+++	+	+	+	+	+	+	-	-	-
35	Intravenous	-	++++	+	+	+	+	+	+	+	+	+
36	Intravenous	++	++++	+	+	+	+	+	+	+	+	+

⁸ Craig, C. F., and Nichols, H. J., *Jour. Exper. Med.*, 1912, xvi, 336.

Controls.—The fresh sera of four rabbits did not show any evidence of agglutination in dilution as low as 1:20. All four yielded negative complement fixation reactions with spirochetal antigens. One yielded a positive Wassermann reaction and after an examination of a large number of normal rabbits we have found a certain proportion to yield a positive Wassermann reaction with certain antigens.⁹

(b) *Human Sera.*—Nineteen sera from cases of secondary and tertiary syphilis yielded negative agglutination reactions. All the sera gave a positive Wassermann reaction. The reactions with the spirochetal antigens were in most cases weaker and more inconstant.

TABLE III.

Serum.	Diagnosis.	Complement fixation reaction.		Agglutination reaction in dilutions of 1:20 to 1:640.
		Wassermann reaction.	Spirochete antigen.	
R. L.	Chancre 5 yrs. ago; some treatment	++++	—	All negative.
F. B.	Secondary syphilis	+++	++	All negative.
T. D.	Secondary syphilis	++	—	All negative.
F. D.	Tertiary syphilis	++	++	All negative.
I. G.	Tertiary syphilis	++++	++	All negative.
I. T.	Chancre 3 mos. ago	++++	—	All negative.
W. B.	Secondary syphilis	++	—	All negative.
J. B.	Secondary syphilis	+++	—	All negative.
W. T.	Secondary syphilis	++	+	All negative.
J. R.	Tertiary syphilis	++++	+++	All negative.
C. B.	Tertiary syphilis	++++	+	All negative.
M. T.	Tertiary syphilis	+	—	All negative.
R. W.	Secondary syphilis	++++	—	All negative.
G. W.	Tertiary syphilis	+	+	All negative.
T. C.	Tertiary syphilis	++	—	All negative.

⁹ Kolmer, J. A., and Casselman, A. J., Concerning the Wassermann Reaction with Normal Rabbit Serum, *Jour. Med. Research*, 1913 (in press).

TABLE III.—Continued.

Serum.	Diagnosis.	Complement fixation reaction.		Agglutination reaction in dilutions of 1:20 to 1:640.
		Wassermann reaction.	Spirochete antigen.	
C. N.	Tertiary syphilis	++++	++	All negative.
C. S.	Tertiary syphilis	+++	++	All negative.
W. H.	Suspicious sore	—	—	All negative.
B. T.	Tertiary syphilis	+++	+	All negative.

Ten sera from persons giving a negative history, negative complement fixation reactions, and free of all clinical evidence of syphilis were also tested and yielded negative agglutination reactions in dilutions of 1:20 to 1:640.

(c) *Cerebrospinal Fluid*.—Three specimens were examined. One yielded a positive Wassermann reaction but none were tested with the spirochetal antigens. None showed the presence of a demonstrable quantity of agglutinin.

TABLE IV.

Spinal fluid.	Diagnosis.	Wassermann reaction.	Agglutination reactions in dilutions of 1:20 to 1:640.
J. Q.	Disseminated cerebrospinal sclerosis	++++	All negative.
J. B.	Pseudobulbar palsy	—	All negative.
M. B.	Hemiplegia	—	All negative.

CONCLUSIONS.

1. There is no demonstrable amount of agglutinin for *Treponema pallidum* (Noguchi) in normal human and normal rabbit serum in dilutions as low as 1:20.

2. Agglutinins for *Treponema pallidum* are readily produced in young rabbits by the administration of pure cultures of living spirochetes.

3. There is no appreciable amount of agglutinin for *Treponema pallidum* culture used in the sera of secondary and tertiary syphilis or in the cerebrospinal fluid of tertiary syphilis in dilutions of 1:20 to 1:640.

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