A STUDY OF COMPLEMENT FIXATION IN SYPHILIS WITH TREPONEMA ANTIGENS.*

JOHN A. KOLMER, M.D., WILLIAM WHITRIDGE WILLIAMS, M.D., AND ERNEST E. LAUBAUGH, M.D.

(*From the Laboratory of Experimental Pathology, University of Pennsylvania, Philadelphia.*)

It was originally supposed by Wassermann, Neisser and Bruch and their co-workers that complement fixation in syphilis was due to a direct union of specific antigen and an antibody in the nature of an amboceptor. With their aqueous extract of syphilitic tissues this was probably partially true. Later it was found that suitable alcoholic extracts of syphilitic tissues would serve as "antigen" and the antigenic properties of Treponema pallidum were considered to be soluble in alcohol. But the work of Landensteiner, Pötzl, Porges, and Meier and particularly that of Noguchi and Bronfenbrenner showed quite conclusively that extracts of normal tissues would serve equally well as antigen in so far as could be demonstrated by complement-fixation experiments. Thus, the so-called antigen was shown to be essentially a lipoidal preparation and the reaction entirely different from the original conceptions. And the true nature of Wassermann's reaction is still unknown, although the total of research in this subject would indicate that Treponema pallidum as well as, to a lesser extent, Spirocheta pertenuis and Bacillus lepræ produce in their host a reactionary product "a reagin" in the nature of a ferment, which in the presence of a suitable lipoid inactivates complement, the process being better known as complement deviation or fixation.

Therefore, workers in this field anxiously awaited the isolation of Treponema pallidum in pure culture when it would be possible to work with a specific antigen, determine the nature of the true syphilitic antibody and possibly establish a complement-fixation test specific for syphilis.

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In 1909 Schereschewsky[1] grew a spirochete in impure culture which however proved non-pathogenic for animals. Of this culture he prepared an antigen and tested two hundred and six serums securing positive results in the majority. He does not give any detail in his report and from the fact that he worked with an impure culture the results cannot be regarded as satisfactory.

In 1912 Noguchi,[2] having cultivated Treponema pallidum and proven its identity by reproducing lesions of syphilis in the lower animals, made an important contribution to this subject. Using aqueous extracts and emulsions of Treponema pallidum prepared from the testicles of infected rabbits and from pure cultures he found that long standing or treated cases of syphilis yielded positive reactions with the spirochete antigens, whereas with the lipoidal extracts the reactions were negative. In primary and secondary syphilis the reactions with spirochete antigens were uniformly negative, while with the lipoidal antigens uniformly positive. Rabbits with acute syphilitic orchitis yielded negative reactions with the spirochete antigens, whereas rabbits immunized over a period of time with pure cultures of Treponema pallidum yielded positive reactions. He did not find the antigenic principle of the treponema soluble in alcohol and advises the sole use of an aqueous extract. As a result of his work he concluded that the syphilis reaction with lipoidal extracts as "antigen" serves to indicate the degree of activity of Treponema pallidum as depends upon the amount of reactionary substance produced, whereas with the pallidum antigen one secures an index of the defensive activity of the infected host, because the reaction with spirochete antigen is a direct union of true antigen and a true antibody.

Craig and Nichols,[3] using alcoholic extracts of pure cultures of Treponema pallidum, Spirocheta pertenuis and Spirocheta microdentia, applied complement-fixation reactions with one hundred and sixteen serums as follows: fifty-one from syphilitic persons; thirty-eight from patients with disease other than syphilis; sixteen from normal persons

and eleven from rabbits. The results with all three antigens were quite similar although, in its results the microdentia antigen approached the pallidum nearer than did the pertenuis. The results obtained with the pallidum antigen approached closely those obtained with the stock alcoholic extract of syphilitic liver, but were generally weaker, and in some undoubted syphilitic cases where the stock antigen gave strong positive reactions, the pallidum antigen gave a negative result. Of thirteen serums of primary syphilis no less than five were positive; of thirteen in the secondary stage ten were positive and in the latent stages, fifteen of twenty-one serums gave a positive reaction. After securing similar results with all three antigens they believe that there may be a specific group reaction but conclude that the practical value of alcoholic extracts of pure cultures of Treponema pallidum, as compared to lipoidal extracts, is limited and cannot be depended upon.

The objects of our investigation were mainly fourfold:

(1.) To study specific complement-fixation with pallidum antigens using serums of patients suffering with non-syphilitic diseases and with serums of patients in the various stages of syphilis.

(2.) To investigate complement-fixation with serums of immunized animals with specific pallidum antigens.

(3.) To study the specificity of pallidum culture antigens as controlled by antigens of sterile culture media and cultures of Bacillus typhosus and Spirillum choleræ.

(4.) To make a comparative study of aqueous and alcoholic extracts of cultures as antigens.

Materials. — Serums: The following two hundred and eighty-nine serums were used:

(1.) Eighty-six of persons giving positive Wassermann reactions and in most instances a clear history of luetic infection.

(2.) Seventy-nine of persons yielding negative Wassermann reactions and a negative history of infection.

(3.) Four serums of normal persons.

(4.) Serums of eight rabbits immunized by the intravenous injection of increasing doses of serum bouillon cultures of Treponema pallidum over periods of time varying from two weeks to three months with weekly injections.

(5.) Serums of two rabbits immunized by the intravenous injection of pure cultures of Bacillus typhosus and Spirillum choleræ.

(6.) Serums of ten normal rabbits as controls over the rabbits immunized with Treponema pallidum.

(7.) Serums of over one hundred normal rabbits as controls of the Wassermann reaction with normal rabbit serum. This work was done with the aid of Dr. A. J. Casselman and the results given in a separate report.

Cultures: (1.) The culture of Treponema pallidum was received through the kindness of Dr. Noguchi. It grows well in ascites kidney agar and ascites or serum kidney bouillon and shows all the characteristics described by him.

(2.) The cultures of typhoid and cholera have been in our laboratory for several years, being used for diagnostic agglutination, production of bacterins, immune serums, etc.

Antigens: These were prepared as follows:

1. Aqueous extract of washed spirochetes: The treponema pallidum was grown anaërobically in large tubes of ascites and serum kidney bouillon for periods varying from four to six weeks. At the end of this time good rich growths were secured. Two hundred cubic centimeters of these fluid cultures were carefully pipetted off so as to avoid the kidney and thoroughly centrifugalized. The sedimented spirochetes were then shaken with sterile salt solution and again centrifugalized. This washing was repeated once more. Examination of the remaining sediment showed beautiful clumps of the organisms. These were then suspended in twenty cubic centimeters of sterile salt solution and .4 per cent phenol added. In this way we had an

antigen rich in treponema. This emulsion was then shaken with glass beads for twenty-four hours and titrated.

1a. Alcoholic extract of washed treponema: This antigen was prepared in exactly the same way as above except that the washed organisms were suspended in twenty cubic centimeters of absolute alcohol; shaken for twenty-four hours; placed in the incubator at 37° C. for seven days and titrated.

2. Aqueous extract of washed typhoid bacilli: Culture was grown on four tubes of slanted agar-agar for forty-eight hours; washed off with sterile salt solution; filtered; centrifugalized; washed twice; bacilli suspended in twenty cubic centimeters of saline plus .4 per cent phenol; shaken for twenty-four hours and titrated.

2a. Alcoholic extract of washed typhoid bacilli: This antigen was prepared in the same manner as above, except that the washed bacilli were suspended in twenty cubic centimeters of absolute alcohol; shaken for twenty-four hours; extracted in the incubator at 37° C. for seven days and titrated.

3. Aqueous extract of washed cholera spirilla: This antigen was prepared in the same manner as the aqueous extract of washed typhoid.

3a. Alcoholic extract of washed cholera spirilla: This antigen was prepared in the same manner as the alcoholic extract of washed cholera.

4. Aqueous extract of ascites kidney agar pallidum culture: Cultures from two to six weeks of age were examined and those selected showing the best growths in the agar column. The oil was poured off; the tube cut and the column of ascites agar between the kidney and the oil removed with particular care not to include the kidney or the oil. This material was weighed; ground with fine sand and powdered glass; mixed with five volumes of sterile saline solution; heated to 60° C. for one hour; .4 per cent phenol added; shaken for twenty-four hours with glass beads; filtered and titrated.

4a. Alcoholic extract of ascites kidney agar pallidum culture: This antigen was prepared in exactly the same

manner except that five volumes of absolute alcohol were added; shaken with glass beads for twenty-four hours; extracted in the incubator at 37° C. for a week; filtered and titrated.

5. Aqueous extract of sterile ascites kidney agar: This antigen was prepared for a culture control in exactly the same manner as the aqueous extract of ascites agar pallidum culture.

5a. Alcoholic extract of sterile ascites kidney agar: This antigen was prepared in exactly the same manner as the alcoholic extract of ascites kidney agar pallidum culture.

6. Aqueous extract of ascites kidney agar typhoid culture: The culture of typhoid was grown in ascites kidney agar for two weeks (aërobically); examined for purity and an antigen prepared in the same manner as the aqueous extract of ascites kidney pallidum culture.

6a. Alcoholic extract of ascites kidney agar typhoid culture in the same manner as the alcoholic extract of ascites kidney agar pallidum culture.

7. Aqueous extract of ascites kidney agar cholera culture: This antigen was prepared in the same manner as the aqueous extract of typhoid culture (No. 6).

7a. Alcoholic extract of ascites kidney agar cholera culture: Prepared in the same manner as the alcoholic extract of typhoid culture (No. 6a).

In the Wassermann reactions with all of the human and rabbit serums at least three of the following lipoidal antigens were used at the same time. All of these antigens have been in use for some time and are of proved value.

1. Alcoholic extract of syphilitic liver.
2. Acetone extract of syphilitic liver.
3. Cholesterinized alcoholic extract of beef heart.
4. Acetone-insoluble lipoids of beef heart (Noguchi).

Technic. — Hemolytic system, etc.: The sheep system was used throughout. Washed sheep's corpuscles were made up in a two and one-half per cent suspension; the fresh

serum of the guinea-pig was diluted 1 : 20 and used as complement in dose of one cubic centimeter; the anti-sheep hemolysin was titrated each day and one hemolytic dose used with the human serums and two doses with the rabbit serums. All serums were heated to 55° C. for one-half hour and used in amounts of .1 to .2 cubic centimeter. Serum, antigen, and complement were incubated for one hour; amboceptor and corpuscles added; incubated for an hour and a half and placed in the refrigerator over night, readings being made and recorded the next morning.

Antigen titration: This is a matter of great importance because a general rule must be adopted and used equally with all antigens and all serums. Therefore we are including a table of the anticomplementary titrations and the antigenic doses we employed of the various antigens. Practically all of the experiments and titrations in this work were repeated twice. We accepted as our antigenic dose about one-quarter that of the anticomplementary. The aqueous extracts were diluted 1 : 10 and the alcoholic 1 : 4 with saline solution.

TABLE I.

Anticomplementary titration of culture antigens.

Dose of Antigen.	Aq. Extr. Washed Spirocheles.	Alc. Extr. Washed Pallidum.	Aq. Extr. Washed Typhoid.	Alc. Extr. Washed Typhoid.	Aq. Extr. Washed Cholera.	Alc. Extr. Washed Cholera.	Aq. Extr. Pallidum Culture.	Alc. Extr. Pallidum Culture.	Aq. Extr. Sterile Media.	Alc. Extr. Sterile Media.	Aq. Extr. Typhoid Culture.	Alc. Extr. Typhoid Culture.	Aq. Extr. Cholera Culture.	Alc. Extr. Cholera Culture.
	1.	1a.	2.	2a.	3.	3a.	4.	4a.	5.	5a.	6.	6a.	7.	7a.
	1:10	1:4	1:10	1:4	1:10	1:4	1:10	1:4	1:10	1:4	1:10	1:4	1:10	1:4
0.1 cc.	H	H	H	H	H	H	H	H	H	H	H	H	H	H
0.2 "	H	H	H	H	H	H	H	H	H	H	H	H	H	H
0.4 "	H	SIH	H	H	H	H	H	H	H	H	H	H	H	H
0.8 "	II	MIH	H	SIH	H	SIH	H	H	SIH	H	MIH	SIH	MIH	H
1.0 "	H	MIH	SIH	MIH	H	MIH	SIH	H	MIH	H	IH	MIH	IH	H
2.0 "	SIH	IH	MIH	IH	SIH	IH	IH	MIH	IH	MIH	IH	MIH	IH	IH
Antigenic dose used,	0.8 cc.	0.1 cc.	0.5 cc.	0.2 cc.	0.5	0.2 cc.	0.3 cc.	0.5 cc.	0.2 cc.	0.5 cc.	0.2 cc.	0.2 cc.	0.2 cc.	0.5 cc.

H, complete hemolysis; SIH, slight inhibition of hemolysis; MIH, marked inhibition of hemolysis; IH, complete inhibition of hemolysis.

Controls. — The controls were the same as we use in the routine Wassermann reaction: a serum control with each serum in maximum dosage; antigen, complement, hemolytic and corpuscle controls.

Results with serums from diseases other than syphilis. — Serums from seventy-nine persons suffering with diseases other than syphilis were tested with three to four stock lipoidal extracts and with aqueous and alcoholic extracts of washed pallidum culture and ascites agar pallidum culture, a negative result being obtained in each instance. The serums of four normal individuals not included in this series were likewise tested and yielded negative reactions. In each test .2 cubic centimeter inactivated serum was used, being the same amount as we use routinely in conducting the Wassermann reaction.

Results with serum from syphilitic patients. — Eighty-six serums from as many syphilitic patients were tested. The great majority of these, all but two, were in the secondary, tertiary, or latent stages as our serums were obtained from the wards of the Philadelphia General Hospital and not from an out-patient clinic. The results are given in Table II. Each serum was tested with at least three lipoidal extracts as the degree of fixation varies with the different lipoids. Thus the serum of a treated or long standing case may react quite feebly with one antigen and strongly with the other two. These differences are probably not dependent upon the antigenic qualities of the antigen but apparently upon different affinities of the lipoidophilic antibody for the lipoidal substances in the antigen. The records as given in the table are the results of the strongest reaction, the others being omitted for brevity. In recording the results the sign $++++$ means total inhibition of hemolysis; the sign $+++$ at least seventy-five per cent inhibition; the sign $++$ fifty per cent inhibition; the sign $+$ twenty-five per cent inhibition; $\pm$ means a doubtful reaction, and the sign — total and complete hemolysis. The sign O means not tested. The serums are arranged according to the stage of infection and not in the order in which they were examined.

TABLE II.

Complement fixation with stock antigens and pallidum antigens in human syphilitic serums.

Serum No.	Stock Antigen.	Aq. Extr. Washed Pallidum. 1.	Alco. Extr. Washed Pallidum. 1a.	Aq. Extr. Pallidum Culture. 4.	Alc. Extr. Pallidum Culture. 4a.	Aq. Extr. Control. 5.	Alc. Extr. Control. 5a.	Remarks.
1.	++++	−	−	−	−	−	−	Primary chancre of vulva.
2.	++++	−	−	−	−	−	−	Primary chancre of lip.
3.	++	−	−	−	−	−	−	Secondary.
4.	++++	−	o	−	o	−	o	"
5.	++	o	++	o	++	o	−	"
6.	++++	o	++	o	++	o	−	"
7.	++++	o	−	o	−	o	−	"
8.	++++	−	−	−	−	−	−	"
9.	++++	o	o	+	±	−	±	"
10.	++++	−	−	−	−	−	−	"
11.	++++	−	−	−	−	−	−	"
12.	−	o	o	+	±	−	±	"
13.	+++	o	o	±	+	±	−	"
14.	++	−	−	−	−	−	−	"
15.	++	−	−	−	−	−	−	"
16.	++++	++	−	+	−	−	−	"
17.	++++	−	−	−	−	−	−	"
18.	++++	−	−	−	−	−	−	"
19.	+++	−	−	−	−	−	−	"
20.	++++	−	−	−	−	−	−	"
21.	++++	+	−	+	−	−	−	"
22.	++++	+	−	+	−	−	−	"
23.	++++	+	−	±	−	±	−	"
24.	++++	++	−	+	−	−	−	"
25.	++++	−	o	−	o	o	o	"
26.	+++	−	−	−	−	−	−	Tertiary.
27.	+++	−	−	−	−	−	−	"
28.	++++	+	o	+	o	±	o	"
29.	++++	+	−	+	−	−	−	"

TABLE II. — *Continued.*

Serum No.	Stock Antigen.	Aq. Extr. Washed Pallidum. 1.	Alco. Extr. Washed Pallidum. 1a.	Aq. Extr. Pallidum Culture. 4.	Alc. Extr. Pallidum Culture. 4a.	Aq. Extr. Control. 5.	Alc. Extr. Control. 5a.	Remarks.
30.	++++	+	−	+	−	±	−	Tertiary.
31.	++++	+	−	−	−	−	−	"
32.	++++	+	−	+	−	−	−	"
33.	+++	−	−	−	−	−	−	"
34.	++++	+	−	+	−	−	−	"
35.	+++	−	−	−	−	−	−	"
36.	++++	−	−	−	−	−	−	"
37.	++++	o	o	+++	−	−	−	"
38.	+++	o	o	−	−	−	−	"
39.	++	o	o	++	−	++	±	"
40.	+	o	o	+	±	−	±	"
41.	+++	o	o	++	++	−	±	"
42.	++++	o	o	+++	++	−	±	"
43.	++++	o	o	+	−	−	−	Cerebro-spinal fluid. Tertiary.
44.	++++	o	o	+++	++	−	−	Tertiary.
45.	++++	o	o	++	+	−	−	"
46.	+++	−	−	−	−	−	−	"
47.	±	−	−	−	−	−	−	"
48.	++++	o	++	o	++	o	o	"
49.	+	−	−	−	−	−	−	"
50.	++++	−	−	−	−	−	−	"
51.	+++	o	++	++	+	−	−	"
52.	++++	o	±	o	±	o	±	"
53.	++	−	−	−	−	−	−	"
54.	++++	−	−	−	−	−	−	"
55.	++++	−	−	−	−	−	−	"
56.	++++	−	−	−	−	−	−	"
57.	+++	−	o	−	o	−	o	"
58.	+++	o	o	−	o	−	o	"
59.	+	o	o	−	o	−	o	"
60.	++++	o	o	+	o	−	o	"
61.	++++	o	o	+	o	−	o	"

TABLE II. — *Concluded.*

Serum No.	Stock Antigen.	Aq. Extr. Washed Pallidum. 1.	Alco. Extr. Washed Pallidum. 1a.	Aq. Extr. Pallidum Culture. 4.	Alc. Extr. Pallidum Culture. 4a.	Aq. Extr. Control. 5.	Alc. Extr. Control. 5a.	Remarks.
62.	++++	o	o	—	o	—	o	Tertiary.
63.	++++	o	o	+	o	±	o	"
64.	++++	o	o	+	o	—	o	"
65.	+++	o	o	—	o	—	o	"
66.	++++	o	o	—	o	—	o	"
67.	++++	o	o	+	o	—	o	"
68.	++	o	o	+	o	—	o	"
69.	+	o	o	+	o	—	o	"
70.	++++	o	o	—	o	—	o	"
71.	++++	o	o	+	o	—	o	"
72.	++++	o	o	+	o	—	o	"
73.	+	o	o	—	o	—	o	"
74.	++++	o	o	—	o	—	o	"
75.	++++	o	o	—	o	—	o	"
76.	++++	o	o	—	o	—	o	"
77.	+++	o	o	—	o	—	o	"
78.	++++	o	o	+	o	—	o	"
79.	+++	o	o	—	o	—	o	"
80.	++++	o	o	+	o	±	o	"
81.	++++	o	o	+++	o	—	o	"
82.	++	+	—	+	—	—	—	"
83.	+	±	—	±	—	—	—	"
84.	++++	++	+	+++	+	—	±	Hereditary syphilis.
85.	+++	+	o	+	o	—	o	Hereditary syphilis.
86.	+++	—	o	—	o	—	o	Hereditary syphilis.

An examination of this table shows the following:

1. In all instances the degree of fixation with pallidum antigens was much less than with the stock antigens.

2. In no instance was the degree of fixation with the pallidum antigens as strong as with the stock antigens.

3. Of eighty-five serums from all stages of the disease in which there were positive reactions with the stock antigens, forty-two serums (or 49.4 per cent) yielded negative reactions with the pallidum antigens.

4. In one case (No. 12) of secondary syphilis treated over two months with inunctins of mercury, our aqueous pallidum extract gave a positive result, whereas with the stock antigens the results were negative.

5. In most instances the aqueous extracts were better antigens than the alcoholic extracts.

6. Substances may be extracted from sterile culture media such as was used in growing the pallidum culture, which will fix a small amount of complement with the pallidum antibody. It will be noted that one-quarter the anticomplementary dose was used following the general rule adopted (see Table I.). Therefore these results can hardly be attributed to an anticomplementary action of the antigens. This subject will be later dealt with in more detail.

7. In looking over this table one may gain the impression that our results with lipoidal extracts were practically one hundred per cent positive. This is due to the fact that we are including here only those cases in which the history, clinical condition and Wassermann reaction clearly indicate the presence of syphilitic infection.

Results obtained in the various stages of syphilis. — Tables III. and IV. give a resumé of the reactions with the various pallidum antigens in the secondary and tertiary stages of syphilis:

TABLE III.

Complement fixation in the secondary stage of syphilis with pallidum antigens.

Antigens.	Number Serums Tested.	No. Positive.	No. Negative.	No. Doubtful.	Per Cent Positive.
1. Aq. Extr. washed pallidum.....	17	5	10	0	29.3
1a. Alc. Extr. washed pallidum.....	18	2	15	0	11.1
4. Aq. Extr. pallidum culture media	20	6	10	2	30.0
4a. Alc. Extr. pallidum culture media,	21	3	16	2	14.3
5. Aq. Extr. sterile media.....	20	0	18	2	0
5a. Alc. Extr. sterile media.....	21	0	19	2	0

TABLE IV.

Complement fixation in the tertiary stage of syphilis with pallidum antigens.

Antigens.	Number Serums Tested.	No. Positive.	No. Negative.	No. Doubtful.	Per Cent Positive.
1. Aq. Extr. washed pallidum.....	22	7	14	1	31.8
1a. Alc. Extr. washed pallidum.....	23	2	20	1	8.7
4. Aq. Extr. pallidum culture media	56	27	28	1	48.2
4a. Alc. Extr. pallidum culture media,	32	6	24	2	18.7
5. Aq. Extr. sterile media.....	56	1	51	4	.17
5a. Alc. Extr. sterile media.....	32	0	27	5	0

Both primary cases of syphilis yielded negative reactions with the pallidum antigens, but with only two serums we

cannot express any opinion relative to complement fixation with specific antigens in the primary stage of syphilis. In the secondary stage about thirty per cent and in the tertiary stage about forty-eight per cent reacted positively with the aqueous extracts of pallidum. The number of positive reactions with the alcoholic extracts were considerably less. Of three patients from the Children's Hospital suffering with hereditary syphilis, two reacted positively and the degree of reaction in one was particularly strong.

Results with rabbit serums: We have used the serums of ten rabbits immunized with pure cultures of Treponema pallidum. These injections were given over a varying period of time, starting with one cubic centimeter of serum bouillon culture, the final dose being ten cubic centimeters.

Rabbit 14 received six injections; Rabbit 15 received six injections; Rabbit 16 received five injections; Rabbit 17 received six injections; Rabbit 18 received six injections; Rabbit 34 received four injections; Rabbit 35 received four injections; Rabbit 36 received four injections; Rabbit 71 received one injection; Rabbit 72 received two injections.

The rabbits were bled from the ear, serums inactivated and used in .1 cubic centimeter amount. Table V. gives the results of fixation tests. The stock antigen used was a cholesterinized alcoholic extract of beef heart which we found very sensitive and highly antigenic. Five normal rabbits' serums were used as controls. Serum and antigen controls as usual.

TABLE V.

Complement fixation with immune rabbit serum and pallidum antigens.

No. Rabbit Serum.	Stock Antigen.	Aq. Extr. Washed Pallidum. 1.	Alc. Extr. Washed Pallidum. 1a.	Aq. Extr. Pallidum Cul. Media. 4.	Alc. Extr. Pallidum Cul. Media. 4a.	Aq. Extr. Sterile Cul. Media. 5.	Alc. Extr. Sterile Cul. Media. 5a.
14.	+++	++++	−	++++	++++	+++	++
15.	++	++++	−	++++	+++	++	−
16.	−	+++	−	−	−	−	+
17.	−	++++	−	++++	++++	++	++
18.	+	++++	−	++++	++++	+	++
34.	−	++++	−	++++	++++	+	−
35.	++	++++	−	++++	++++	++	+
36.	−	++++	−	++++	++++	+	+
71.	++	+	−	+	+++	−	±
72.	++	+++	−	+++	+++	+	−
Normal. 1.	−	−	−	−	−	−	−
Normal. 2.	+	−	−	−	−	−	−
Normal. 3.	+	−	−	−	−	−	−
Normal. 4.	−	−	−	−	−	−	−
Normal. 5.	−	−	−	−	−	−	−

An examination of these results indicates the following:

1. Serums of rabbits immunized with pure cultures of the Treponema pallidum give marked complement fixation with pallidum antigens.

2. The fact that only one of these immune serums fixed complement with an alcoholic extract of washed pallidum would indicate that none or a small amount of antigenic substances can be obtained from the pallidum itself by alcoholic extraction. However, an alcoholic extract of pallidum in culture media (ascites kidney agar) yielded an antigen as strong as the aqueous extract. This would seem to indicate that Treponema pallidum elaborates a substance or substances in the culture media which is extractable in alcohol and of antigenic value.

3. Fixation with aqueous and alcoholic extracts of sterile culture media occurred. This is difficult to explain. Antigen controls without serum were always put up as a routine practice and were always completely hemolyzed. It seems as if the pallidum antibody is not specific for the pallidum in complement fixation tests but will inactivate complement (fixation) with antigenic substances in ascites kidney agar extractable by salt solution and alcohol. This non-specific fixation was also observed in working with antigens of typhoid and cholera bacilli.

4. The results of complement fixation with these immune serums and a stock antigen are less easily interpreted because we have found that normal rabbit serum will fix complement in certain instances with lipoidal antigens. After studying over a hundred normal rabbits in this manner we conclude that the injections of Treponema pallidum stimulated the production of small amounts of lipoidophilic reagin in addition to the more specific pallidum antibody.

5. Apparently there are no substances in normal rabbit serum which fix complement with pallidum antigens. This also agrees with the results of Craig and Nichols.

Experiments concerning the specificity of the pallidum antibody in complement fixation tests. — The serums of two rabbits immunized with typhoid and cholera organisms were used, also five normal rabbit serums as controls. The typhoid immune serum agglutinated the bacilli in dilution 1 : 32000 and the cholera in dilution 1 : 1500. These serums were tested with all of the pallidum antigens and antigens of washed bacilli. Likewise all of the serums of the rabbits immunized with Treponema pallidum were tested with antigens of the typhoid and cholera bacteria. Fifteen human serums were tested with the antigens in like manner: twelve serums from syphilitic persons and three serums from persons suffering with diseases other than syphilis. The results are shown in Table VI.:

TABLE VI.

Complement fixation reactions with immune serums and typhoid cholera antigens.

Serum No.	Stock Antigen.	Aq. Extr. Washed Typh. Bac. 2.	Alc. Extr. Washed Typh. Bac. 2a.	Aq. Extr. Washed Chol. Bac. 3.	Alc. Extr. Washed Chol. Bac. 3a.	Aq. Extr. Typh. Cul. Med. 6.	Alc. Extr. Typh. Cul. Med. 6a.	Aq. Extr. Chol. Cul. Med. 7.	Alc. Extr. Chol. Cul. Med. 7a.	Remarks.
14.	+++	+	−	+	−	++	−	+++	±	Rabbit immunized with T. pallidum.
15.	++	±	−	±	−	+++	+	+++	−	Rabbit immunized with T. pallidum.
16.	−	+	−	+	−	−	−	−	+	Rabbit immunized with T. pallidum.
17.	−	+	−	+	−	+++	±	+++	+++	Rabbit immunized wtth T. pallidum.
18.	+	−	−	−	−	+++	−	+++	++	Rabbit immunized with T. pallidum.
34.	−	±	+	−	±	+++	−	++	−	Rabbit immunized with T. pallidum.
35.	++	+	+	+	+	+++	±	+++	++	Rabbit immunized with T. pallidum.
36.	−	++	−	++	−	+++	−	+++	−	Rabbit immunized with T. pallidum.

71.	++	−	−	−	−	+	−	−	+	Rabbit immunized with T. pallidum.
72.	++	+	+	+	+	+++	−	+++	+	Rabbit immunized with T. pallidum.
1.	−	−	−	−	−	−	−	−	−	Normal rabbit.
2.	+	−	−	−	−	−	−	−	−	" "
3.	+	−	−	−	−	−	−	−	−	" "
4.	−	−	−	−	−	−	−	−	−	" "
5.	−	−	−	−	−	−	−	−	−	" "
22.	+	−	−	+	−	−	−	+	+	Rabbit immunized with cholera spirilla.
27.	−	+	−	−	−	+	−	−	−	Rabbit immunized with Typh. Bac.
1.	++++	−	−	−	−	−	−	+	++	Secondary syphilis.
2.	+++	−	−	−	−	−	−	−	−	Tertiary "
3.	++++	−	−	−	−	+	−	−	−	" "
4.	++	−	−	−	−	−	−	+	−	Secondary "
5.	++++	−	−	−	−	−	−	−	−	" "
6.	++	−	−	−	−	±	±	+	−	" "

TABLE VI. — *Continued.*

Serum No.	Stock Antigen.	Aq. Extr. Washed Typh. Bac. 2.	Alc. Extr. Washed Typh. Bac. 2a.	Aq. Extr. Washed Chol. Bac. 3.	Alc. Extr. Washed Chol. Bac. 3a.	Aq. Extr. Typh. Cul. Med. 6.	Alc. Extr. Typh. Cul. Med. 6a.	Aq. Extr. Chol. Cul. Med. 7.	Alc. Extr. Chol. Cul. Med. 7a.	Remarks.
7.	++++	—	—	—	—	—	—	—	—	Primary syphilis.
8.	++++	—	—	—	—	+	—	+	±	Tertiary "
9.	++++	—	—	—	—	+	+	±	—	" "
10.	++++	—	—	—	—	—	—	—	—	" "
11.	++++	—	—	—	—	—	—	—	—	" "
12.	—	—	—	—	—	—	—	—	—	Rheumatism.
13.	—	—	—	—	—	—	—	—	—	Pneumonia.
14.	—	—	—	—	—	—	—	—	—	Lead poisoning.

By comparing with Table V. it will be noted:

1. The antibody resulting from injecting rabbits with pure cultures of Treponema pallidum will fix complement with antigens of typhoid and cholera organisms.

2. This non-specific fixation of complement with pallidum antibody and typhoid cholera antigens is noted by observing that the degree of fixation with the culture antigens is slightly greater than with the antigens of sterile media (Table V.).

3. By using aqueous extracts of pure washed cultures of typhoid and cholera this fixation is noted to be independent of culture media or substances produced therein during the growth of the bacilli.

4. Alcoholic extracts of pure washed cultures of typhoid and cholera organisms did not give complement fixation with these immune serums. It has been already noted that but a small amount of the antigenic substances of pure washed spirochetes were found extractable by alcohol.

5. Serums of human cases of syphilis did not fix complement with the aqueous or alcoholic extracts of pure washed cultures of typhoid and cholera, but gave some slight and irregular fixation with the antigens of these organisms in the culture media. These results may have been due to substances extracted from the culture media independent of the bacilli.

Thus it appears that the treponema antibody is not absolutely specific for an antigen of the treponema itself but apparently has some affinity for substances extractable from sterile culture media as well as cholera and typhoid bacteria. This is especially apparent with the serums of rabbits immunized with pure cultures of the treponema and practically negligible with serums of luetic persons. From the fact that Treponema pallidum is now well known to produce in its host a substance, generally called the "syphilis antibody," which has as its chief characteristic an affinity for lipoids, it was thought possible that these results were to be explained, at least partially, by ascertaining if lipoidal substances were present in the organisms and culture media used for antigens. Accordingly pure washed cultures of Treponema pallidum,

Bacillus typhosus and Spirillum choleræ as well as three weeks' old cultures of these organisms in ascites kidney agar and also sterile ascites kidney agar, were ground with powdered glass; extracted with absolute alcohol for four days at 37° C., evaporated; residue extracted with ether; evaporated; residue extracted with petroleum ether and evaporated. The residue was then examined qualitatively for phosphorus by Prof. Alonzo Taylor with the following results:

1: Washed pallidum; positive. 2: Washed typhoid bacilli; positive. 3: Washed cholera spirilla; positive. 4: Pallidum ascites agar culture; positive. 5: Typhoid ascites agar culture; doubtful. 6: Cholera ascites agar culture; positive. 7: Plain sterile media; doubtful.

Only ten to twenty grams of the culture media was extracted and it is entirely likely that a definite result would have been secured with larger amounts. The finding of phosphorus under these conditions indicates quite conclusively the presence of a lipoid, and the presence of a lipoid offers at least one explanation: that the non-specific reactions with the human serums and sterile culture media and the rabbit immune serums with antigens of culture media as well as typhoid and cholera organisms are due to the presence of a separate pallidum antibody having an affinity for lipoids in general, such as the Wassermann reaction in human syphilis depends upon, and which inactivates (fixes) complement with the lipoid present in these bacilli as well as in sterile culture media. Why this is especially true of the rabbit immune serums we are at present unable to state. One may consider it due to the lipoidophilic substance normally present in rabbit serum and responsible for positive Wassermann reactions with normal serum, but this cannot be the explanation because normal rabbit serum yielded negative results with these antigens.

SUMMARY.

A full summary having been given with each division of the study, only a brief recapitulation is given here under the four heads of the investigation:

1. Serums of normal persons and normal rabbits as well as serums of persons suffering with diseases other than syphilis and yielding negative Wassermann reactions, do not contain substances capable of fixing complement with pallidum antigens.

About fifty per cent of serums from persons in all stages of syphilis and giving positive Wassermann reactions and luetic histories reacted negatively with the pallidum antigens. Two cases of primary syphilis were negative; of twenty-three cases of secondary syphilis, thirty per cent reacted positively; of fifty-eight cases of tertiary syphilis, forty-eight per cent were positive, as were two of three cases of hereditary syphilis. In every instance the degree of fixation with the pallidum antigen was much less than with the stock lipoidal extracts and in only one case of secondary syphilis treated with mercury was the reaction positive with the pallidum and negative with the stock antigens. The reactions with the pallidum antigens are weak and too inconstant to be of routine practical value.

2. Serums of rabbits immunized with pure cultures of Treponema pallidum yielded strong reactions with the pallidum antigens. As controlled by the examination of one hundred normal rabbits these serums also give positive reactions with stock lipoidal extracts.

3. Many of the human serums and all the rabbit immune serums were likewise tested with control antigens of sterile culture media, pure washed cultures of typhoid and cholera bacteria as well as cultures of these organisms in the same culture media as used in the cultivation of Treponema pallidum. With the human serums a few doubtful reactions were obtained with the antigens of sterile media and more marked reactions with the typhoid and cholera culture media antigens. The rabbit immune serums not only reacted strongly with these antigens but likewise yielded weak reactions with antigens of washed typhoid and cholera bacteria. From the fact that a lipoid was demonstrated in these antigens it may be that the non-specific reactions were due to the usual union of lipoid and lipoidophilic antibody, although we are

unable to explain at present why this is especially true with the rabbit immune serums.

4. From the fact that the reactions with alcoholic extracts of pure washed treponema were uniformly negative it is apparent that the antigenic principle of the treponema is not readily abstractable in alcohol, the aqueous extracts being preferable in complement fixation reactions.

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