

A STUDY OF COMPLEMENT FIXATION IN SYPHILIS
WITH SPIROCHÆTA CULTURE ANTIGENS.*

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It is our purpose in this communication to give the results of some work upon complement fixation in syphilitic and other sera, using specific antigens prepared from pure cultures of *Spirochæta pallida*, *Spirochæta pertenuis*, and *Spirochæta microdentia*. The results are compared with those obtained with the same sera and a stock antigen used in our routine Wassermann tests, this antigen consisting of an alcoholic extract of syphilitic fetal liver.

It is now recognized by most authorities that the Wassermann reaction in syphilis is not a true antigen-antibody reaction, but depends upon certain lipotropic substances produced in the tissues of syphilitics during the progress of the disease. The researches of Landsteiner, Müller, and Pötzl (1), and Porges and Meier (2) demonstrated that alcoholic extracts of normal as well as syphilitic tissues served as antigens, while those of Noguchi and Bronfenbrenner (3) proved that the active principles in the antigens used in the Wassermann test were present in the lipoidal substances found in extracts from both syphilitic and non-syphilitic tissues of man and other animals.

The success attained by Noguchi and others in obtaining *Spirochæta pallida* in pure culture gave rise to the hope that an antigen prepared from such cultures would be efficient in complement fixation in syphilis and would place the test upon the basis of a true antigen-antibody reaction. It is obvious that if such a specific antigen could be obtained the value of the Wassermann test, great

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as it is at present, would be much enhanced, as the positive reactions observed frequently in leprosy and frambesia, as well as those observed more rarely in a few other diseases, as carcinoma, malaria, and trypanosomiasis, would be eliminated. A few attempts in this direction have been made by others, but owing to the fact that the *Spirochæta pallida* has only recently been obtained in pure culture, the literature is very limited.

In 1909, Schereschewsky (4), working with impure cultures of *Spirochæta pallida*, obtained complement fixation with syphilitic sera, but he also obtained fixation with some of the control antigens. His work is unsatisfactory because the reactions occurred with the control antigen and also because of the uncertainty that exists regarding the organisms present in his mixed cultures. As one of us (Craig (5)) has shown, certain strains of staphylococci and streptococci, when growing in normal human serum, produce substances which give a positive reaction with the Wassermann test, and it is not improbable that some of the positive results obtained by Schereschewsky may have been due to lipoidal substances produced by bacteria growing in the horse serum media which he used in his cultures. At any rate, his results were suggestive and undoubtedly stimulated research along these lines.

The most important contribution to this subject is that of Noguchi (6) published in 1912, in which he details his complement fixation experiments with antigens prepared from aqueous extracts and emulsions of *Spirochæta pallida* prepared from the testicles of infected rabbits and from pure cultures. He endeavored to determine whether sera giving a positive reaction with the stock lipoidal antigen would also react with these specific antigens. He found that in certain cases of treated syphilis or in those in which the infection had existed for a very long time without symptoms, the specific antigens gave a positive reaction when the lipoidal antigen gave a negative or a very weak reaction, and that with the antigen made from a pure culture of *Spirochæta pallida* no reaction occurred in the serum from a case of leprosy, although extracts of both syphilitic and normal rabbit testicles gave a positive reaction with the same serum. Our own results with syphilitic rabbit testicle antigen will be discussed later.

Noguchi's conclusions are as follows.

1. The Wassermann reaction is caused by the lipotropic substances in sera but not by the antibodies which combine specifically with the pallida antigen.
2. The fixation produced by the culture pallida antigen with certain syphilitic sera is caused by the specific antibodies contained in the latter and may constitute a specific diagnostic method for syphilis.
3. The fixation caused by testicular extracts behaves like the culture pallida antigen in the majority of cases, but when the sera (syphilitic or leprosy) contain abundant lipotropic substances, they may give a Wassermann reaction as well, which is not the case with the culture pallida antigen.
4. In the serum of rabbits with active syphilitic orchitis there is no indication of the presence of a sufficient amount of antibodies for the pallida antigen, although it gives a strong Wassermann reaction.

Noguchi regards the reaction with the pallida antigen as an index of the resistance of the patient to the infection, and states: "We have in the Wassermann reaction a fair measure of the activity of the infecting agent, and now we will have in the pallida fixation reaction a gauge for the defensive activity of the infected host."

In our work we have intentionally used alcoholic instead of watery extracts for the following reasons. (1) We desired to test the practical value of a specific antigen, and it is well known that alcoholic extracts are much more stable than watery extracts or emulsions. (2) We wished to compare our results with those obtained from our stock antigen which is an alcoholic extract. (3) It is generally recognized that an alcoholic extract of a syphilitic fetal liver is superior to that of non-syphilitic tissues because it apparently contains both the non-specific lipoids and the specific substances from the spirochætæ; hence alcohol would be suitable for the extraction of specific substances from the cultures.

As will be seen, our work shows that complement fixation in syphilis can be obtained in certain sera with an alcoholic antigen prepared from pure cultures of *Spirochæta pallida*, but it also shows that similar results can be obtained by using similar antigens prepared from cultures of other spirochætæ. We are not prepared to say definitely that these reactions are specific in the sense of being group reactions, but the evidence points in this direction. At any rate more work must be done before the use of specific culture antigens can be put on a practical basis.

Material and Technique.—In these experiments were used 51 syphilitic sera and 54 non-syphilitic sera. Of the latter 38 were from patients suffering from diseases other than syphilis, and 16 from normal individuals. In addition, 11 rabbit sera were employed, making a total of 116 sera tested. The sera were all inactivated by heating to 55 to 56° C. for one half hour just before they were tested.

The *stock antigen* was an extract of syphilitic fetal liver in ten parts of absolute alcohol, evaporated to one third of its volume, and diluted with nine parts of normal salt solution. This antigen was titrated to determine its anticomplementary qualities, and with a known syphilitic serum to determine the unit necessary to produce inhibition of hemolysis.

The *culture antigens* were prepared from cultures obtained through the kindness of Dr. Noguchi, pure cultures of the spirochætæ being grown on the media recommended by him, consisting of one part of ascitic fluid and two parts of a weakly alkaline agar to which a piece of sterile rabbit kidney was added. Of each spirochæta, two tubes containing rich growths, of one and two months, respectively, were selected. The oil was poured off and the tube filed and broken. The agar column was removed, the upper or uninfected portion was cut away, and the tissue picked out. The remaining medium was shaken up with ten times its weight of absolute alcohol and was extracted for ten days, being shaken frequently. The extract was then filtered, the filtrate was evaporated to one third its volume, and was titrated in a dilution of 1 to 10 with normal salt solution. The antigens thus obtained were all titrated to determine their anticomplementary qualities and, in addition, the pallida antigen was titrated against a positive syphilitic serum.

All the antigens in this series of experiments, including the stock antigen, were used in a dose of 0.1 of a cubic centimeter of a 1 to 10 dilution.

For complement fresh guinea pig serum was used, while for amboceptor the serum of rabbits immunized to human red blood corpuscles was employed. The blood suspension consisted of a 1 per cent. suspension of human red blood corpuscles in normal salt solution.

As a control on the extract of cultures of the spirochætæ, an alcoholic extract of the media employed in growing the spirochætæ was prepared in the same manner. These media, of course, had not been used for cultivation purposes.

Results with Normal Sera.—Sera from sixteen normal individuals were tested with the stock antigen, and with pallida, pertenuis, and microdentia antigen, but all of them gave negative results. In not a single instance was there the least trace of a reaction in these sera. It is therefore evident that none of these antigens react with normal blood serum. In each test 0.8 of a cubic centimeter of inactivated serum was used.

Results with Serum from Diseases Other than Syphilis.—Sera from thirty-eight patients suffering from diseases other than syphilis

were tested, a negative result being obtained in all but one case; this was diagnosed as arthritis and gave a plus result (i. e., an inhibition of at least 50 per cent.) with the microdentia antigen. This one positive result prevents our stating that none of these antigens react with diseases other than syphilis, but it is our opinion that it will be found that a positive reaction will not be obtained in non-syphilitic diseases, and that this one positive reaction was due to some accidental cause, perhaps to the contamination of the serum with bacteria. In each test 0.8 of a cubic centimeter of inactivated serum was used.

Results with Serum from Syphilitic Patients.—Fifty-one sera from as many syphilitic patients were tested, 0.8 of a cubic centimeter of the inactivated serum being used in each test. In recording these tests the sign ++ means absolute inhibition of hemolysis; the sign +, at least 50 per cent. of inhibition; and the sign +—, weaker degrees of inhibition. The sign — means total lack of inhibition, while the sign 0 means that no test was made. The results of the tests with syphilitic sera are shown in table I.

An analysis of table I is interesting. No positive results were obtained with the antigen from the uninoculated culture, a fact which proves that there is nothing in the medium used for cultivation which produces the reaction. A large percentage of the sera that gave positive results with the stock antigen also reacted with the pallida antigen, no less than twenty-seven of the thirty-six positive cases, or 75 per cent., reacting with both antigens; although, as a rule, the reactions were weaker with the pallida than with the stock antigen. A large percentage of sera that were positive with the stock antigen were also positive with the pertenuis and microdentia antigen.

An interesting point is that while in general the reactions were weaker with the specific antigens than with the stock antigen, there occurred several instances in which the reactions with the specific antigens were stronger. Thus with sera 11 and 19 the stock antigen gave a plus-minus reaction, while the pallida antigen gave a double plus reaction; while in sera 22 and 29 the stock antigen gave a plus-minus and the pallida antigen gave a plus reaction. In serum

38 the stock antigen gave a negative reaction while the pallida gave a plus reaction.

TABLE I.

Complement Fixation with Stock Antigen and Specific Spirochæta Antigens in Human Syphilitic Sera.

| Serum No. | Stock antigen. | <i>S. pallida</i> antigen. | <i>S. pertenuis</i> antigen. | <i>S. microdentia</i> antigen. | Control antigen. | Remarks. |
|-----------|----------------|----------------------------|------------------------------|--------------------------------|------------------|-------------------------------------|
| 1 | ++ | ++ | o | o | - | Secondary, early. |
| 2 | ++ | + | o | o | - | Primary. |
| 3 | ++ | + - | + - | o | - | Secondary; 2 doses salvarsan. |
| 4 | ++ | - | o | o | o | Latent, 18 yrs.; 4 doses salvarsan. |
| 5 | ++ | + | + | + | - | Latent, 4 yrs.; 2 doses salvarsan. |
| 6 | ++ | - | + - | - | - | Latent, 3 yrs.; 3 doses salvarsan. |
| 7 | ++ | - | - | o | - | Tertiary, 10 yrs. |
| 8 | ++ | - | o | o | - | Secondary, 9 mos. |
| 9 | + | + | o | o | o | Secondary, early. |
| 10 | + | + | o | o | - | Primary. |
| 11 | + | ++ | o | o | - | Primary, 2 wks. |
| 12 | + | + | o | o | o | Latent, 2 yrs. |
| 13 | + | - | o | + - | o | Primary, 2 wks. |
| 14 | + | - | o | + | - | Primary, 4 wks. |
| 15 | + | + | o | + | - | Secondary, early. |
| 16 | + | + | - | - | - | Secondary, 1 yr. |
| 17 | + | - | o | + | - | Tertiary. |
| 18 | + | + | + - | + | - | Secondary. |
| 19 | + | ++ | o | + | - | Tertiary; duration unknown. |
| 20 | + | + - | + - | + - | - | Latent; duration unknown. |
| 21 | + - | + - | o | o | - | Latent, 3 yrs. |
| 22 | + - | + | o | o | - | Primary, 3 wks. |
| 23 | + - | + - | o | o | o | Secondary, early. |
| 24 | + - | - | - | - | - | Primary, 2 wks. |
| 25 | + - | - | - | o | - | Epilepsy. |
| 26 | + - | + - | o | + - | - | Arthritis, syphilitic. |
| 27 | + - | + - | o | + - | o | Latent, 2 yrs. |
| 28 | + - | + - | o | + - | - | Latent, 2 yrs. |
| 29 | + - | + | o | + | - | Latent, 1 yr. |
| 30 | + - | + - | - | + - | - | Secondary, 1 yr. |
| 31 | + - | ++ | ++ | + | - | Latent; duration unknown. |
| 32 | + - | + - | o | + | - | Latent, 3 yrs. |
| 33 | + - | + - | + - | - | - | Secondary, 3 mos. |
| 34 | + - | + | + - | + - | - | Latent, 4 yrs. |
| 35 | + - | + - | - | + - | - | Latent, 6 yrs. |
| 36 | + - | + - | + - | - | - | Secondary, early. |
| 37 | - | + - | - | + - | - | Latent, 2 yrs. |
| 38 | - | + | + | + - | - | Latent, 1½ yrs.; 4 doses salvarsan. |
| 39 | - | + - | - | - | - | Latent, 6 yrs. |
| 40 | - | + - | - | o | o | Primary, 2 wks. |

Each serum was also tested without antigen, as is usual in the Wassermann test.

As regards the reactions given with the antigen prepared from pure cultures of *Spirochæta pallida*, seventeen sera gave the same result as with the stock antigen, four gave weaker reactions, six gave stronger reactions, and four gave positive results in sera in which the stock antigen gave negative reactions. However, the large proportion of negative reactions (seven out of twenty) in sera giving a double plus or plus reaction with the stock antigen demonstrates the superiority of the latter antigen in the diagnosis of syphilis.

Of the four sera giving a negative reaction with the stock antigen, one gave a plus reaction with the pallida antigen while three gave a plus-minus reaction with the same antigen. All these cases had been treated and the one giving a plus reaction with the pallida antigen had previously given a double plus reaction with the stock antigen, but later had been given four doses of salvarsan.

There were eighteen sera tested with the antigen prepared from pure cultures of *Spirochæta pertenuis*. Of these eight gave a negative result; one in a serum giving a plus reaction with the stock antigen, four in sera giving a plus-minus reaction with the stock antigen, and three in sera giving a negative reaction with that antigen. In general the results obtained with the pertenuis antigen were similar to those obtained with the pallida antigen.

There were twenty-five sera tested with the antigen prepared from pure cultures of *Spirochæta microdentia*. Of these only six gave a negative reaction. Of the negative sera, five gave reactions with the stock antigen, although in three of them the reaction was only a plus-minus one. Of the positive sera, eleven gave the same results with the microdentia antigen as with the stock antigen, while in three sera the reaction with the microdentia was stronger than with the stock antigen. In three sera the reaction with this antigen was weaker than with the stock antigen.

Fifteen of the sera were tested with all the antigens, and the results are shown in table II. They are of interest because they strongly suggest the group nature of the reaction.

In four of the sera the results were the same with all the culture antigens, but in the remainder they varied, the pallida antigen

giving reactions more nearly like those obtained with the stock antigen, while the results obtained with the microdentia and pertenuis were strikingly similar, although in its results the microdentia antigen approached the pallida nearer than did the pertenuis.

TABLE II.

Complement Fixation in Fifteen Syphilitic Sera Tested with All the Antigens.

| Serum No. | Stock antigen. | <i>S. pallida</i> antigen. | <i>S. pertenuis</i> antigen. | <i>S. microdentia</i> antigen. | Control antigen. | Remarks. |
|-----------|----------------|----------------------------|------------------------------|--------------------------------|------------------|------------------------------|
| 1 | ++ | + | + | + | - | Latent, 4 yrs. |
| 2 | ++ | - | - | - | - | Latent, 3 yrs. |
| 3 | + | + - | + - | + - | - | Latent; duration unknown. |
| 4 | + - | - | - | - | - | Primary, 2 wks. |
| 5 | + | + | - | - | - | Secondary, 1 yr. |
| 6 | + | + | + - | + | - | Secondary; duration unknown. |
| 7 | + - | + - | - | + - | - | Secondary, 1 yr. |
| 8 | + - | ++ | ++ | + | - | Latent; duration unknown. |
| 9 | + - | + - | + - | - | - | Secondary, 3 mos. |
| 10 | + - | + | + - | + - | - | Latent, 4 yrs. |
| 11 | + - | + - | - | + - | - | Latent, 6 yrs. |
| 12 | - | + - | - | + - | - | Latent, 2 yrs. |
| 13 | - | + | + | + - | - | Latent, 1 ½ yrs. |
| 14 | - | + - | - | - | - | Latent, 6 yrs. |
| 15 | - | + - | - | o | o | Primary, 2 wks. |

Each serum was also tested without antigen, as is usual in the Wassermann test.

Results Obtained in the Various Stages of Syphilis.—Table III gives the results obtained with the different antigens in the various stages of syphilis, and while the cases are, perhaps, too few in number to justify any radical conclusions, it is evident that the culture antigens give reactions in every stage of the disease, although more positive reactions are obtained in latent cases.

The occurrence of the reaction with the culture antigens in the primary stage of syphilis is of importance, for if we believe that these are true antigen-antibody reactions, it must be admitted that the antibodies occur in the patient's serum very early in the disease, for in no less than five of the thirteen sera from patients in the primary stage of the disease, the pallida antigen gave a positive reaction. In the secondary stage ten of the thirteen sera tested gave positive reactions with the pallida antigen, and in the latent stage (including both early and late latent cases) fifteen of the twenty-one sera gave positive reactions.

TABLE III.
Complement Fixation in Various Stages of Syphilis with Stock Antigen and Specific Spirochata Antigens.

| Serum No. | Stock antigen. | <i>S. pallida</i> antigen. | <i>S. pertennis</i> antigen. | <i>S. microdentia</i> antigen. | Control antigen. | Remarks. | |
|------------------|----------------|----------------------------|------------------------------|--------------------------------|------------------|----------|---------------------------------|
| Primary stage. | 1 | ++ | + | o | o | - | Duration 3 wks. |
| | 2 | + | + | o | o | - | Duration 2 wks. |
| | 3 | + | ++ | o | o | - | Duration 2 wks. |
| | 4 | + | - | o | + - | o | Duration 2 wks. |
| | 5 | + | - | o | + | - | Duration 4 wks. |
| | 6 | + - | + | o | o | - | Duration 3 wks. |
| | 7 | + - | - | - | - | - | Duration 2 wks. |
| | 8 | - | + - | - | o | o | Duration 2 wks. |
| | 9 | - | - | o | o | - | Duration 10 days. |
| | 10 | - | - | o | - | - | Had salvarsan. |
| | 11 | - | - | - | - | - | Duration 8 days. Had salvarsan. |
| | 12 | - | - | o | - | o | Duration 5 days. Had salvarsan. |
| Secondary stage. | 1 | ++ | ++ | o | o | - | Early. |
| | 2 | ++ | + - | + - | o | - | 2 doses salvarsan. |
| | 3 | ++ | - | o | o | - | Duration 9 mos. |
| | 4 | + | + | o | o | o | Early. |
| | 5 | + | + | o | + | - | Early. |
| | 6 | + | + | - | - | - | Duration 1 yr. |
| | 7 | + | + | + - | + | - | Early. |
| | 8 | + - | + - | o | o | o | Early. |
| | 9 | + - | + - | - | + - | - | Duration 1 yr. |
| | 10 | + - | + - | + - | - | - | Duration 3 mos. |
| | 11 | + - | + - | + - | - | - | Early. |
| | 12 | - | - | - | o | o | 2 doses salvarsan. |
| | 13 | - | - | o | - | - | Duration 2 mos. |
| Tertiary stage. | 1 | ++ | - | o | o | - | Duration 10 yrs. |
| | 2 | + | - | o | + | - | Duration unknown. |
| | 3 | + | ++ | o | + | - | Duration unknown. |
| | 4 | - | - | o | - | o | 1 dose salvarsan. |
| | 5 | - | - | - | - | - | Duration unknown. |
| Latent stage. | 1 | ++ | + - | o | o | o | Duration 18 yrs. |
| | 2 | ++ | + | + | + | - | Duration 4 yrs. |
| | 3 | ++ | - | - | - | - | Duration 3 yrs. |
| | 4 | + | + | o | o | o | Duration 2 yrs. |
| | 5 | + | + - | + - | + - | - | Duration unknown. |
| | 6 | + - | + - | o | o | - | Duration 3 years. |
| | 7 | + - | + - | o | + - | o | Duration 2 yrs. |
| | 8 | + - | o | + - | + - | - | Duration 2 yrs. |
| | 9 | + - | + | o | + | - | Duration 1 yr. |
| | 10 | + - | ++ | ++ | + | - | Duration unknown. |
| | 11 | + - | + - | o | + | - | Duration 3 yrs. |
| | 12 | + - | + | + - | + - | - | Duration 4 yrs. |
| | 13 | + - | + - | - | + - | - | Duration 6 yrs. |
| | 14 | - | + - | - | + - | - | Duration 2 yrs. |
| | 15 | - | + | + | + - | - | Duration 1 1/2 yrs. |
| | 16 | - | + - | - | - | - | Duration 6 yrs. |
| | 17 | - | - | o | - | o | Duration 2 yrs. |
| | 18 | - | - | o | + - | o | Duration 3 yrs. |
| | 19 | - | + - | - | + - | - | Duration 6 yrs. |
| | 20 | - | - | - | o | o | Duration 3 yrs. Had salvarsan. |
| | 21 | - | - | - | o | o | Duration 6 yrs. Had salvarsan. |

The highest percentage of reactions with the pallida antigen occurred in the secondary stage of syphilis. The patients from whom the serum was obtained, with two exceptions, presented active lesions of the disease.

Results with Rabbit Serum.—We have used the serum from a rabbit suffering from a syphilitic orchitis; the serum from three rabbits that had recovered from a syphilitic orchitis; the serum from two that had recovered from frambesia; and the serum from four normal rabbits. Table IV gives our results in these tests.

TABLE IV.
Complement Fixation with Stock Antigen and Specific Spirochæta Antigens in Rabbit Sera.

| Rabbit No. | Stock antigen. | <i>S. pallida</i> antigen. | <i>S. pertenuis</i> antigen. | <i>S. microdentia</i> antigen. | Control antigen. | Remarks. |
|------------|----------------|----------------------------|------------------------------|--------------------------------|------------------|----------------------------|
| 79 (1) | ++ | — | — | — | — | Active syphilitic lesions. |
| 79 (2) | ++ | — | — | — | — | Active syphilitic lesions. |
| 77 (1) | — | — | — | — | 0 | Recovered from syphilis. |
| 77 (2) | — | — | — | — | 0 | Recovered from syphilis. |
| 75 | — | — | — | — | — | Recovered from syphilis. |
| 85 (1) | — | — | — | 0 | — | Recovered from yaws. |
| 85 (2) | — | — | — | — | — | Recovered from yaws. |
| 1 | — | — | — | 0 | — | Normal rabbit. |
| 2 | — | — | — | 0 | — | Normal rabbit. |
| 84 | — | — | — | 0 | — | Normal rabbit. |
| 86 | — | — | — | 0 | 0 | Normal rabbit. |

Each serum was also tested without antigen, as is usual in the Wassermann test. The quantity of rabbit serum used was 0.1 and 0.2 c.c., inactivated.

The important fact shown by this table is that no reactions occurred in any of the sera except in rabbit 79, presenting an active syphilitic orchitis, and that this serum was positive only with the stock antigen. In this respect our results confirm those of Noguchi, who also found that his pallida antigen did not react with rabbit serum even though the animal presented active lesions of the infection.

Experiments with Rabbit Testicle Antigen.—Over a year ago we experimented with alcoholic antigens made from the testicles of rabbits suffering from syphilitic orchitis and yaws, and our results were published by Nichols (7). We found that while such antigens gave positive results with certain syphilitic sera, they also reacted

with certain normal sera, as well as with sera from other diseases. In addition we found that the antigen prepared from the testicles of rabbits inoculated with syphilis gave positive results with the sera of rabbits inoculated with yaws, and *vice versa*. As we found that antigens prepared from normal rabbit testicles gave similar results we concluded that these reactions were in no sense specific.

Discussion of Results.—The results obtained with the pallida antigen approach closely those obtained with the stock antigen, but were generally weaker, and in some undoubted syphilitic cases, where the stock antigen gave strong reactions, the pallida antigen gave a negative result. It is evident that the pallida antigen gives more uniform results than either of the others, but there is no great difference between them in the majority of the sera tested.

The results obtained with the microdentia antigen are almost identical with those obtained with the pallida antigen, a rather interesting observation when one remembers that this spirochæta resembles *Spirochæta pallida* more closely morphologically than any other so far described, except *Spirochæta pertenuis*. It is not surprising that the pallida and pertenuis should both give reactions, but the reaction of the microdentia is more difficult to explain, as this spirochæta is not pathogenic for animals.

The large number of positive reactions in secondary cases showing active lesions is difficult to reconcile with the theory that complement fixation in syphilis with specific antigens is a gauge of the resistance of the patient, if one accepts absence of visible symptoms as indicating resistance.

In considering the results given above, the origin and history of the cultures naturally come in question. The pallida strain used was grown by Noguchi from the infected testicle of a rabbit and came originally from mucous patches of a marked secondary case of syphilis. The culture was pathogenic for rabbits soon after inoculation. The pertenuis strain was also grown from the testicle of a rabbit after passage through a monkey, and came originally from a characteristic case of yaws in a colored soldier returning from the Philippines. The microdentia strain was isolated by Noguchi from peridental material in a child. This strain was not pathogenic for animals. It produces a distinctive odor of putrefaction in cul-

tures, and it is morphologically distinctly finer than either the pallida or the pertenuis. There is no doubt in our minds regarding the identity of the cultures.

It seems to us that these reactions with specific antigens may be explained in either of the following ways:

1. That they are non-specific reactions and are due to lipoid substances produced in the media during the growth of the organisms. The failure to react with normal sera and rabbit sera speaks against this conclusion, but the point can be settled by further work on other organisms such as refringens, vibrios, and bacteria.

2. That they are specific group reactions. It is not surprising if some group reaction exists between the pallida, the pertenuis, and even the microdentia, for the microdentia, while in other respects much farther removed from the pallida than the pertenuis, resembles the pallida more closely morphologically than any other spirochætæ. The specific action of salvarsan upon these spirochætæ also argues some connection between them.

It is believed that these experiments suggest that the complement fixation reaction obtained in syphilis with antigens made from pure cultures of the spirochætæ is a group reaction, for it is very difficult to explain the results in any other way. Further work should be done with spirochætæ farther removed from the pallida (e. g., *Spirochæta recurrentis* and *Spirochæta refringens*), and with various species of bacteria.

As regards the practical value of alcoholic extracts of pure cultures of *Spirochæta pallida* in the diagnosis of syphilis, it is evident from our results that they cannot be depended upon, for many cases of undoubted syphilis, giving a double plus or plus reaction with our stock syphilitic liver antigen, gave absolutely negative results with the culture antigens. For diagnostic purposes, therefore, the alcoholic extracts of pure cultures of *Spirochæta pallida*, instead of possessing advantages over an alcoholic extract of fetal syphilitic liver, possess a much weaker complement fixation power and a negative reaction is often encountered in patients giving a positive reaction with the Wassermann test.

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