

THE PRESENCE OF ANTAGONISTIC SUBSTANCES IN THE
BLOOD SERUM IN EARLY AND LATE SYPHILIS
AND IN PARESIS AND TABES.*

MARTHA WOLLSTEIN AND R. V. LAMAR.

NEW YORK.

The appearance of the work of Wassermann, Neisser and Bruck,¹ and of Wassermann, Neisser, Bruck and Schucht² describing a positive serum reaction in syphilis by means of Wassermann's modifications of the Bordet-Gengou method of complement binding, gave an impetus to the study of serum reactions in syphilis, paresis and tabes, and of the diagnostic value of the results obtained. The reaction of Wassermann, Neisser and Bruck is based in principle on the specific binding of complement, such as occurs, for instance, in the presence of an immune precipitin containing serum and the corresponding precipitinogen. In the reaction the antigen is represented by an extract of the liver of a syphilitic fetus, and the antibody is contained in the blood serum of a syphilitic patient or in either the blood serum or cerebrospinal fluid, or both, of a paretic or tabetic patient.

To carry out a test for the reaction one mixes in test tubes definite quantities of the liver extract and the inactivated blood serum (or cerebrospinal fluid) and adds complement. After standing one hour in the incubator to allow for the binding of the complement, an inactive immune hemolytic serum, with its corresponding corpuscles, is added and the tubes are returned to the incubator, where they remain for two hours. At the end of this length of time they are taken out and examined for evidences of hemolysis. A final opinion, however, as to the presence and degree or absence of hemolysis is best deferred until after the tubes shall have remained over night in the ice chest. In the case of a positive reaction, the evidence that complement has been bound and thus diverted from the hemolytic amboceptor of the immune serum is afforded by the fact that hemolysis has not occurred.

As in other hemolytic experiments, perhaps even to a greater extent than in those, it is essential that a number of control tests be made. For example, in addition to the customary system controls, the extract and serum should be tested separately for both hemolytic and anti-hemolytic

* From the Rockefeller Institute for Medical Research, New York.

1. *Deutsche med. Wchnschr.*, 1906, xxxii, 745.

2. *Ztschr. f. Hyg. u. Infektionskrankh.*, 1906, lv, 451.

effect, and together, without the addition of complement, for hemolytic effect. Furthermore, it should be shown that the liver extract with normal or non-syphilitic serum does not bind complement, etc.

As regards the nature of the substances on whose interaction the binding of complement depends, there exist certain differences of opinion. Without discussing the question at length, it may be said that Wassermann believed in the beginning that the substances were directly related in origin to *Spirochæta pallida*. Later he held that the reaction is only a serum diagnosis of some substance which was formerly unknown to us, and which stands in some relation to the syphilitic infection. Citron,³ on the other hand, while fully confirming the specificity and diagnostic value of the reaction, believes that the nature of the substances can not be determined definitely until the spirochete has been grown in pure culture. Nevertheless, he suggests that during the course and in consequence of the syphilitic infection certain products of cell metabolism may lead to auto-immunization, and that it may be to these substances, with their corresponding antibodies, that the reaction is due. According to Levaditi and Yamanouchi,⁴ the antigen may be represented by sodium taurocholate and glycocholate, and to a less extent by lecithin. Finally, Weil and Braun⁵ believe that some substance besides lipoids may be responsible for the reaction, inasmuch as they obtained it with extracts of liver, from which the lipoids had been removed by repeated shaking with petroleum ether.

Notwithstanding the doubt which surrounds the cause and nature of the Wassermann reaction in syphilis, its value as a diagnostic means would seem to have been confirmed by the observations of Detre,⁶ Citron,⁷ Marie and Levaditi,⁸ Meier,⁹ Michaelis and Lesser,¹⁰ and many others. In considerably more than 1,000 cases of syphilis in all stages, in paresis and in tabes a positive reaction has been obtained in approximately 80 per cent. of the number. While with normal and non-syphilitic serum the occurrence of the reaction has been reported only once, namely, in frambesia tropica, by Blumenthal.¹¹ This last result is less surprising when we consider the similarity which yaws bears to syphilis.

3. Berl. klin. Wehnschr., 1907, xliv, 1370.

4. Compt. rend. Soc. de Biol., 1907, lxiii, 740.

5. Wien. klin. Wehnschr., 1908, xxi, 151.

6. Wien. klin. Wehnschr., 1906, xix, 619.

7. Deutsche med. Wehnschr., 1907, xxxiii, 1165.

8. Ann. de l'Inst. Pasteur, 1907, xxi, 138.

9. Berl. klin. Wehnschr., 1907, xlv, 1636.

10. Berl. klin. Wehnschr., 1908, xlv, 301.

11. Deutsche med. Wehnschr., 1908, xxiv, 258.

As has been pointed out, this reaction is concerned mainly, at least from a clinical standpoint, with the demonstration of an antibody. To be sure Wassermann, Neisser, and Bruck¹ drew attention to the fact that the ability to demonstrate antigen in the body fluids of syphilitics would be of considerable importance in diagnosis and treatment. They even succeeded in doing so in a few instances in which they used as an antibody-containing fluid the serum of apes immunized with syphilitic material. Usually, however, the attempt has failed; possibly, they suggest, on account of the difficulty of obtaining a sufficiently strong immune serum.

Recently Fornet, Schereschewsky, Eisenzimmer and Rosenfeld,¹² by means of a direct method, as opposed to the above, which may be considered as an indirect one, claim to be able to show the presence of either syphilitic antigen or antibody in the blood serum. The method is essentially that advocated by Ascoli in carrying out the usual precipitin test. On bringing into contact the serum from a patient with early syphilis and that from a paretic or tabetic, Fornet observed a delicate ring of cloudiness at the zone of contact. The ring never occurred when either pathologic serum in the test was replaced by a normal serum. The cloud is due, according to Fornet, to the precipitate which results from the interaction of "lues-precipitinogen," arising directly from the spirochetæ themselves, and its corresponding "lues-precipitin." Inasmuch as the production of precipitin demands the preceding occurrence within the organism of precipitinogen, Fornet concludes that it is precipitinogen which is present in early syphilis and precipitin in later conditions as in paresis and tabes. In a few instances he found precipitinogen in paresis and tabes, i. e., the serum from one paretic or tabetic gave a ring with the serum from another. This apparent discrepancy is explained by the assumption that in the instances where precipitinogen was present the syphilitic process was still active within the body.

Assuming the correctness of the observations and deductions of Fornet and his associates, it would appear that complement would be bound if added to a mixture of serum derived from a patient with early syphilis (presumably containing precipitinogen) and serum derived from a paretic or tabetic (presumably containing precipitin), since it is always bound in the presence of a specific precipitin reaction. Indeed, in view of the extreme delicacy of the binding reaction (Neisser and Sach's modification of the forensic precipitin test), it might be expected to reveal the presence of quantities of precipitinogen in syphilis too minute to produce

12. München med. Wchnschr., 1907, liv, 1471; Deutsche med. Wchnschr., 1907, xxxiii, 1679.

a visible precipitate. Consequently, in our attempts to show the presence of antagonistic substances in the two varieties of sera, we have used both the methods of complement deviation and precipitation.

Material was placed at our disposal through the kindness of Dr. Moore of the Manhattan State Hospital and of Drs. Mitchell and Eckert of the City Hospital. The sera of nineteen individuals were tested in various combinations. From patients with secondary syphilis with active manifestations there were seven specimens; from tertiary syphilis (gumma), one specimen; from paresis, four specimens; tabes, one specimen, and from normal persons, six specimens. In the binding experiments, the technic of Wassermann and his pupils was followed, except that the liver extract was replaced by serum from a patient with secondary syphilis. The results of these tests were uniformly negative, as is shown in Table 1:

TABLE 1.

				Result.
Serum M., secondary syphilis	+	Serum W. A., paresis		Negative
Serum J., secondary syphilis	+	Serum W. A., paresis		Negative
Serum V., secondary syphilis	+	Serum M., tertiary syphilis		Negative
Serum J. S., secondary syphilis	+	Serum R., tabes		Negative
Serum J. S., secondary syphilis	+	Serum R. B., paresis		Negative
Serum J. S., secondary syphilis	+	Serum T. C., paresis		Negative
Serum J. T., secondary syphilis	+	Serum W. P., paresis		Negative
Serum M., secondary syphilis	+	Serum J., normal		Negative
Serum W. A., paresis	+	Serum J., normal		Negative
Serum V., secondary syphilis	+	Serum M., normal		Negative
Serum M., tertiary syphilis	+	Serum M., normal		Negative
Serum J. S., secondary syphilis	+	Serum C., normal		Negative
Serum R., tabes	+	Serum C., normal		Negative
Serum J. S., secondary syphilis	+	Serum G., normal		Negative
Serum R. B., paresis	+	Serum G., normal		Negative
Serum T. C., paresis	+	Serum G., normal		Negative

The sera were employed in quantities of 0.2 c.c. and 0.1 c.c. In each experiment, the following control tests were made:

TABLE 2.

Serum 1.	Serum 2.	Complement guinea pig.	Immune rabbit serum, anti-sheep.	Sheep's corpuscles, 5 per cent.	Result in terms of hemolysis.
0.3	1	—
...	0.3	1	—
0.2	0.1	...	0.002	1	—
0.3	...	0.1	0.002	1	+
...	0.3	0.1	0.002	1	+
...	...	0.1	0.002	1	+
...	...	0.1	1	—
...	1	—
...	0.002	1	—

The results with the precipitate reaction in contrast to the above were not uniform, but quite variable and indecisive. Nine tests were made with serum from patients with secondary syphilis (supposed to

contain precipitinogen) and serum from patients with tertiary syphilis, paresis and tabes. Of these, three were positive and six negative. Normal serum was tested with the serum from patients with secondary syphilis six times, with three positive and three negative results; with one serum from tertiary syphilis, with positive result; with one serum from tabes with negative result, and with three sera from paresis, with one positive and two negative results. Twice the serum from one patient with secondary syphilis was tested against the serum from another patient in the same stage of syphilis, with one positive and one negative result. And, finally, one normal serum was tested in the undiluted state with its own dilutions of one to five and one to ten. Table 3 shows the inconstancy of the results.

TABLE 3.

Serum S. secondary syphilis.			Serum T C. paresis.			Serum R. B. paresis.			Serum G. normal.			Result. After.	
Native.	1 to 5.	1 to 10.	Native.	1 to 5.	1 to 10.	Native.	1 to 5.	1 to 10.	Native.	1 to 5.	1 to 10.	2 hours.	16 to 20 hours.
X	:	:	X	:	:	X	:	:	X	:	:		
X	:	:	:	:	:	:	:	:	:	:	:		
:	X	:	X	:	:	:	:	:	:	:	:	+	+
:	X	:	:	:	:	:	:	:	:	:	:		
X	:	:	:	X	:	:	:	:	X	:	:		
X	:	:	:	:	:	:	X	:	:	:	:	+	+
X	:	:	:	:	X	:	:	:	:	X	:		
X	:	:	:	:	:	:	:	X	:	:	:	+	+
X	:	:	:	:	:	:	:	:	:	:	X	+	+
:	:	:	X	:	:	X	:	:	:	:	X	+	+
:	:	:	:	:	:	:	:	:	:	:	:	+	+
:	:	:	X	:	:	X	:	:	X	:	:	+	+
:	:	:	:	:	:	:	:	:	X	X	:	+	+
:	:	:	:	:	:	:	:	:	X	:	X	+	+

In employing the test, it is absolutely necessary that the sera be perfectly clear. On account of the delicacy and fineness of the rings, a certain amount of practice is required for a correct reading of results. Sometimes in our experiments the rings appeared within two hours, sometimes not until the following morning, and sometimes those which appeared within two hours persisted until the following morning and sometimes not. With two exceptions the cloud appeared only when one or the other serum was employed in the diluted state. A temperature of 37° C. seemed to have no effect on the reaction. While not attempting to explain the occurrence of the rings, whether they are merely optical phenomena or

real precipitates of some undetermined substance, we feel unjustified in believing that they are necessarily the result of a specific reaction between lues-precipitinogen and lues-precipitin.

Recently Plaut, Heuck, and Rossi¹³ have reported results with the precipitate reaction which are quite similar to those described above. With the combination of serum from syphilitic patients and serum from paretics they reported six positive and ten negative results; with serum from syphilitic patients and normal serum, four positive and four negative results; with normal serum and serum from paretics, six positive, five negative. Two experiments, in which the serum of one patient with secondary syphilis was tested with the serum from another patient with secondary syphilis, gave negative results. Of two tests with serum from one normal person and serum from another normal person, one was positive and one negative. Finally, when one normal serum was tested with its own dilutions there were three positive results and one negative result.

They believe that the ring of a positive reaction is an actual precipitate, but of what nature they are not prepared to say. The difference in the density of the two media appeared to have an influence upon the occurrence of the reaction, since in their tests a positive result was never obtained when both sera were used undiluted. Although considering it possible that by means of precipitation a specific reaction for syphilis may be found, they conclude that in its present form the precipitate reaction has no place in diagnosis.

CONCLUSION.

Neither by the complement-binding method nor by the method of precipitation as at present carried out is it possible to show the presence of antagonistic substances in the blood serum of patients in the secondary stages of syphilis on the one hand and in the tertiary stage on the other, or in such parasyphilitic affections as paresis and tabes.

Rockefeller Institute for Medical Research.

13. Münch. med. Wchnschr., 1908, lv, 66.