

THE VALUE OF ABSORPTION METHODS IN THE WASSERMANN TEST *

C. H. BAILEY, M.D.

NEW YORK

It is conceded by most observers that in working with a hemolytic system it is advisable to use known amounts of both amboceptor and cells. Wassermann's original method for the diagnosis of syphilis makes use of 1 c.c. of a 5 per cent. suspension of sheep corpuscles with just twice the amount of amboceptor necessary to hemolyze these cells. Since the discovery of the existence of an antisheep amboceptor in some human serums it has been a question whether this additional amount of amboceptor might not be sufficient to produce hemolysis in conjunction with a small residue of complement not fixed in the first stage of the reaction. If this should occur negative results would thus be obtained in syphilitic cases.

The recognition of this possibility has given rise to several modifications of the Wassermann reaction. The best-known of these is probably that of Noguchi. He maintained¹ that "Wassermann's original method is subject to an error arising from the presence in human serum of a varying amount of natural amboceptor capable of being reactivated by guinea-pig's complement." He found experimentally that four units of antisheep amboceptor prevent entirely the detection of one unit of syphilitic antibody. The modification of the Wassermann technic devised by him has, among other advantages, that of avoiding this danger by the use of a hemolytic system consisting of human blood cells and the serum of a rabbit immunized against them.

Several other methods of obviating this source of error have been suggested which still make use of sheep corpuscles as in the original Wassermann method. That of Bauer,² in which each serum is tested for antisheep amboceptor and artificial immune serum added only to those which show an insufficient amount of natural antisheep amboceptor to give complete hemolysis with the amounts of sheep cells and complement used in the Wassermann reaction, will of course be efficient in those cases in which the human serum contains just enough amboceptor to give complete hemolysis. As, however, some serums contain many times this amount of natural amboceptor, with these the source of error still remains.

*From the Pathological Department of St. Luke's Hospital, F. C. Wood, M.D., Director.

1. Noguchi: *Jour. Exper. Med.*, 1909, xi, 392.

2. Bauer: *Sem. med.*, 1908, xxviii, 429.

Jacobaeus³ proposed absorbing the sheep amboceptor from human serum by incubating the serum, after the addition of sheep cells at 37 C. for one-half hour; then centrifuging off the cells and proceeding with the Wassermann reaction according to the regular technic. In a series of 257 cases he obtained about 10 per cent. more positives by this method than without absorption. He asserts that complementoid is also removed by this method, thus giving it the advantage of a modification introduced by Wechselmann⁴ in which complementoid is removed by digesting the inactivated human serum with barium sulphate. Bauer⁵ had previously tried the same procedure which Jacobaeus employed, but discarded it on account of its making the serum antihemolytic. He declared that this property was much increased by the addition of liver extract, thus causing negative serums to give positive reactions.

S. Mintz,⁶ using this method in a series of thirty-eight cases, obtained thirty positive reactions against twenty-five without absorption. The serums which reacted positively were all syphilitic.

I have tried this method of amboceptor absorption on 305 serums regardless of the amount of natural antisheep amboceptor present. The results of the Wassermann reaction on each of these serums has been compared with the result of the reaction on the same serum with the natural antisheep amboceptor present.

Only fifty-three of the 305 serums contained one or more units of natural antisheep amboceptor in 0.2 c.c., that is, sufficient to hemolyze completely 1 c.c. of a 5 per cent. suspension of sheep corpuscles in the presence of one unit of complement. In seventy serums there was no trace of antisheep amboceptor in 0.2 c.c. In the remaining 182, antisheep amboceptor was present, but in a quantity not sufficient to produce complete hemolysis.

The technic employed to remove the antisheep amboceptor from the human serum was as follows: To 0.5 c.c. of the patient's serum, after inactivation, was added 2 c.c. of 0.85 per cent. salt solution and 0.1 c.c. of sheep cells. After shaking, the mixture was incubated at 37 C. for twenty minutes. The cells were then centrifuged off and the supernatant fluid used in the Wassermann reaction, 0.5 c.c. of the diluted serum being used in the tube with antigen and 1 c.c. in the control tube without antigen, the amount of antigen, complement, etc., being correspondingly reduced to one-half the usual quantity. The remainder of the fluid was used to test the completeness of the amboceptor absorption. In about

3. Jacobaeus: *Ztschr. f. Immunitätsforschung*, Orig., 1911, viii, 615.

4. Wechselmann: *Ztschr. f. Immunitätsforschung*, 1909, iii, 524.

5. Bauer: *Berl. klin. Wehnschr.*, 1908, xiv, 834.

6. S. Mintz: *Ztschr. f. Immunitätsforschung*, 1911, ix, 29.

one-quarter of the cases a sufficient amount of the patient's serum was used to test for remaining antishoop amboceptor in a full cubic centimeter.

In all but three of the 305 serums, removal of the sheep amboceptor was complete. In each of these 0.2 c.c. showed a faint trace of hemolytic power for sheep cells still present. One additional serum showed very slight hemolytic power remaining in a full cubic centimeter but none was demonstrated in 0.2 c.c.

So far as the removal of antishoop amboceptor from human serum is concerned, we may conclude that the method is practically always efficient. The objection to the method is that inhibitory bodies are in some way produced by this process ("Sachs-Friedberger phenomenon") which considerably slow hemolysis, there sometimes being a trace of inhibition at the end of an hour in the control tubes and in negative serums. The difference in reaction between positive and negative serums is, however, clear-cut, the inhibitory action not being sufficiently marked to render the method impracticable as a means of avoiding any error which may be due to the presence of natural antishoop amboceptor.

Rossi⁷ asserts that incubation at 0 C. for twenty minutes is as efficient in absorbing the amboceptor as incubation at 37 C., while by this method the inhibitory phenomenon does not appear. His method is to add 0.5 c.c. of sheep red blood corpuscles to 1.5 c.c. of the patient's serum, both having been previously cooled to 0 C. This mixture is kept at this temperature for from twenty to thirty minutes, then rapidly centrifuged, and the serum drawn off with a pipette. In a series of sixty syphilitic cases Rossi obtained fifty positives by the Wassermann reaction and fifty-six positives after absorption.

This method of absorption, as well as that at 37 C., was tried on 195 of the above 305 serums. In a portion of these the Rossi technic was followed in detail. With the remainder the technic was the same except that serum and cells were mixed in the proportions used for absorption at 37 C. This method was found equally efficient in absorbing the antishoop amboceptor. So far, however, as the avoidance of inhibitory action is concerned, it was unsuccessful, there being little if any difference in this regard between the two methods.

The results of the Wassermann reactions on the 305 serums with natural antishoop amboceptor still present and on the same serums after the amboceptor had been completely removed is shown in Table 1. The results of the Wassermann reaction following absorption at 0 C. did not differ in any particular from those following absorption at 37 C.

7. Rossi: *Ztschr. f. Immunitätsforschung*, 1911, x, 321.

TABLE 1.—WASSERMANN REACTION ON 305 SERUMS BEFORE AND AFTER REMOVAL OF NATURAL ANTISHEEP AMBOCEPTOR

Wassermann Reaction	Positive	Doubtful Positive	Doubtful Negative	Negative
On serums with natural antisheep amboceptor..	103	16	2	184
On serums after removal of antisheep amboceptor	104	18	1	182

Those cases are classed as positive which give complete inhibition in the tube containing 0.2 c.c. of patient's serum and antigen, with complete hemolysis in the control tube containing 0.4 c.c. of patient's serum without antigen. Those in which there was a slight trace of hemolysis in the tube with antigen and complete hemolysis in the control, and those which, with complete inhibition in the tube with antigen, showed a trace of inhibition in the control, are classed as doubtful positive. Those with partial hemolysis with antigen or partial inhibition in the control are classed as doubtful negative; all others as negative.

As is shown by Table 1, there was a difference in results in but three of the 305 cases. The cases in which the reaction differed were as follows:

1. *Pyorrhœa Alveolaris*.—No history of syphilis obtainable. The serum contained 5 units of antisheep amboceptor. Wassermann reaction, with antisheep amboceptor present, negative; after removal of antisheep amboceptor, doubtful positive.

2. *Syphilitic Laryngitis*.—Primary lesion fourteen years ago. Treatment previous to Wassermann reaction not known. The serum contained over 3 units of antisheep amboceptor, the exact amount not being determined. Wassermann reaction with antisheep amboceptor present, doubtful negative; after removal of antisheep amboceptor, positive.

3. *Tabes Dorsalis*.—No history of syphilis. Serum contained 2 units of antisheep amboceptor. Wassermann reaction, with antisheep amboceptor present, negative; after removal of antisheep amboceptor, doubtful positive.

TABLE 2.—CLASSIFICATION OF 423 WASSERMANN REACTIONS DONE ON SERUMS WITHOUT AMBOCEPTOR ABSORPTION

	Positive	Doubtful Positive	Doubtful Negative	Negative
Syphilis—				
Primary	6	1	0	1
Secondary, untreated	20	0	0	1
Secondary, treated	7	0	1	4
Tertiary	54	11	1	10
Latent	24	9	1	26
Congenital	2	1	0	2
General paresis	1	0	0	0
Tabes	5	2	1	2
Diseases possibly of syphilitic origin—				
Aneurysm	6	2	0	0
Aortic insufficiency	9	0	0	5
Facial paralysis	1	0	0	2
Chronic inflammations of the eye.....	7	2	0	13
Diseases not diagnosed clinically as syphilitic	1	2	3	177

It will be noted that in none of these cases was the variation in results a difference between a frank negative and a frank positive reaction, thus differing considerably from the results reported by Jacobaeus and other observers. It will be seen from Table 2, in which are classified the last 423 Wassermann reactions done on serums without amboceptor absorption, that the figures are similar to those reported by other laboratories using the Wassermann method. The 305 cases on which absorption methods were tried are among this number. The three cases classed under "Diseases not diagnosed clinically as syphilitic" which gave positive or doubtful positive reactions require special mention.

Rheumatoid Arthritis.—Wassermann was positive; no history of syphilis obtainable. Patient complained of pain and swelling in her right great toe of three weeks' duration, and pain in right ankle and left arm for one week. On entrance toe was swollen, somewhat red and tender. There was slight tenderness over inner aspect of left tibia. Temperature was normal. During the five weeks in hospital, both elbows, and several phalangeal joints were involved. Patient was on mixed treatment eleven days, potassium iodid being continued nineteen days longer without improvement.

Gelatinous Carcinoma of Rectum.—Wassermann was doubtful positive. The patient denied lues and the past history was not suggestive. Diagnosis was made from section of excised portion of tumor.

Lymphosarcoma of Tonsil.—Wassermann was doubtful positive. This woman gave a history of one miscarriage, one child born dead at term, one child dead at 22 months (cause not known). She had two living children. Otherwise there was nothing suggestive in the past history. Diagnosis was made from section of excised portion of tumor.

In reporting results of the Wassermann reaction it is unnecessary to give in detail the technic employed, the method being so well known. There are, however, certain points which, it seems to us, should be mentioned. In the reactions reported here the guinea-pig serum was always titrated and care taken never to use as much as 2 units, as will very frequently be done if 0.1 c.c. is used. The antigen used in nearly all of the 305 cases was an acetone insoluble fraction of beef heart prepared as recommended by Noguchi.⁸ The quantity used in the reaction was 0.01 c.c. This antigen was not hemolytic or anticomplementary in four times this amount and had high antigenic properties. Titrated against four positive serums, it gave with one complete inhibition in one-tenth the quantity used, with a second complete inhibition in one-thirteenth, and with the other two complete inhibition in one-twentieth the quantity used.

As the results obtained by the amboceptor absorption methods vary considerably from those reported by other observers, experiments were done to test the effect of the introduction of artificial amboceptor on the

8. Noguchi: Serum Diagnosis of Syphilis, Ed. 2, 1911. Phila.

Wassermann reaction done with the above antigen. Three serums were selected which were frankly positive with this antigen, and Wassermann reactions were done on each of these after the addition of 5, 10, and 15 units of artificial antisheep amboceptor. Reactions were also done on the same serums without the addition of artificial amboceptor and after the addition of 5, 10 and 15 units, using an amount of antigen which by titration with each serum contained 2 units of antigen for that serum. Each serum contained natural antisheep amboceptor but in an amount less than 1 unit. This was not removed.

Serum 1 contained 2 units of syphilitic antibody. Wassermann reactions were positive with both strong and weak antigens. With the addition of 5, 10 and 15 units of amboceptor the reactions were doubtful or negative with both antigens, but the inhibition was greater with the stronger antigen.

Serum 2 contained 18 units of syphilitic antibody. Wassermann reactions were frankly positive with the strong antigen, even with the addition of 5, 10 and 15 units of amboceptor. With the weak antigen the serum gave a frankly positive reaction when artificial amboceptor was not added. With 5 units of amboceptor the reaction was doubtful, with 10 and 15 units, negative.

Serum 3 contained more than 25 units of syphilitic antibody. Wassermann reactions were frankly positive with both antigens with 15 units of antisheep amboceptor present.

CONCLUSIONS

From the above cases and experiments I conclude that it is possible for antisheep amboceptor in human serum to affect the Wassermann reaction, but that when an antigen of high titer is used this is possible only with serums of very low antibody content and several units of antisheep amboceptor. As these two conditions, in my experience, occur but rarely in practical work, I feel that, when a strong antigen is used, the importance of antisheep amboceptor in human serum as a cause of negative reactions in syphilitic cases is not great. As a routine procedure, the absorption of amboceptor is unnecessary. Its removal is, however, advisable from serums which give a negative or doubtful reaction, and which contain a large amount of antisheep amboceptor. This is easily accomplished by digestion with sheep cells. It is immaterial whether this is done at 0 C., 37 C., or at room temperature.