

A STUDY OF COMPLEMENTS USED IN THE WASSERMANN REACTION

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The serums commonly used as complement in the Wassermann reaction are those of guinea-pig and of man. That the complement content of serums varies has repeatedly been shown and Browning and McKenzie¹ found that complements vary in fixability.

Complements usually deteriorate rapidly and several workers sought by artificial means to prevent such deterioration. Noguchi dried it on filter paper, others kept it in a frozen state and more recently Ramy² proposed sodium acetate as a preservative for complement.

The use of human complement has been advocated by Hecht,³ Tschernogubow,⁴ Fleming,⁵ Emery,⁶ von Dungern,⁷ Gurd,⁸ Noguchi⁹ and others.

In this report the following points are considered:

1. Fixability and complement content of fresh guinea-pig serum.
2. Fixability and complement content of fresh human serum.
3. Noguchi's recent method compared with our regular method.
4. The effect of normal human serum on syphilitic human serum.
5. The effect of normal guinea-pig serum on syphilitic human serum.
6. The rate at which syphilitic serum is altered by normal serum.
7. Deterioration of guinea-pig complement in the refrigerator.
8. Sodium acetate as a preservative for complement.
9. Glycerol as a preservative for complement.

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¹ Ztschr. f. Immunitätsforsch. u. exper. Therap. 1909, 2, p. 459.

² Jour. Am. Med. Assn., 1917, 69, p. 973.

³ Wiener klin. Wchnschr., 1909, 22, p. 338.

⁴ Deutsch. med. Wchnschr., 1909, 35, p. 668.

⁵ Lancet, 1909, 1, p. 1512.

⁶ Lancet, London, 1910, 179, p. 732.

⁷ Münch. med. Wchnschr., 1910, 57, p. 507.

⁸ Jour. Infect. Dis., 1911, 8, p. 427.

⁹ Jour. Am. Med. Assn., 1918, 70, p. 1157.

METHOD

In our regular method the serum to be tested is heated to about 55.5 C. for 30 minutes and is mixed with an equal volume of sterilized glycerol. Six test tubes are used and each receives 0.2 cc of serum-glycerol mixture. Complement is used in quantities of 0.2 cc of a 1:5, 1:10 and 1:20 dilution. Of antigen, alcoholic extract of human heart muscle, the largest dose that is no longer anticomplementary, is used and is diluted so that the test dose is contained in 0.2 cc. Human corpuscles are well washed, the washed blood corpuscles are made up into a 2.5% suspension in salt solution (0.9%), and the test dose is contained in 0.2 cc of the suspension. Hemolytic amboceptor is prepared in rabbits and is used in doses of 1 unit per test tube. The term unit is applied to the smallest quantity which with 0.2 cc of 1:10 dilution of complement in the presence of 0.2 cc of serum-glycerol mixture completely dissolves the test dose of blood corpuscles in one hour. The total quantity in each test tube is 1 cc, first incubation is in the refrigerator for 5 hours and second incubation in the incubator for one hour. After the tubes have stood at room temperature for from 1-2 hours the results are read and recorded.

TEST 1

Ten guinea-pig serums, Serums 1-10, inclusive, were tested on 12 syphilitic human serums. All complement serums were used singly and in mixtures of 5 serums each. The quantity of amboceptor was kept uniform throughout.

TABLE 1
COMPARISON OF TEN GUINEA-PIG COMPLEMENTS

Number of Serum	Number of Complement	Sex of Guinea-Pig	Amboceptor per Tube Unit	Readings*						Results
				Antigen Tubes			Control Tubes			
				1	2	3	1'	2'	3'	
1	1	M	1g	+	0	0	+	+	±	Strongly positive, 5+.
	2	F	1g	+	tr	0	+	+	±	Strongly positive, 4+.
	3	M	1g	+	0	0	+	+	tr	Strongly positive, 4+.
	4	F	1g	+	0	0	+	+	±	Strongly positive, 5+.
	5	M	1g	+	0	0	+	+	±	Strongly positive, 5+.
	1-5	M and F	1g	+	0	0	+	+	±	Strongly positive, 5+.
	6	F	1g	+	tr	0	+	+	±	Strongly positive, 4+.
	7	M	1g	+	tr	0	+	+	±	Strongly positive, 4+.
	8	F	1g	+	tr	0	+	+	±	Strongly positive, 4+.
	9	M	1g	+	±	0	+	+	±	Strongly positive, 3+.
2	10	F	1g	+	tr	0	+	+	±	Strongly positive, 4+.
	6-10	M and F	1g	+	tr	0	+	+	±	Strongly positive, 4+.
	1	M	1g	+	±	0	+	+	±	Strongly positive, 3+.
	2	F	1g	+	±	0	+	+	±	Strongly positive, 3+.
	3	M	1g	+	±	0	+	+	0	Weakly positive, 1+.
	4	F	1g	+	+	0	+	+	±	Moderately positive, 2+.
	5	M	1g	+	+	0	+	+	±	Moderately positive, 2+.
	1-5	M and F	1g	+	+	0	+	+	±	Moderately positive, 2+.
	6	F	1g	+	+	0	+	+	±	Moderately positive, 2+.
	7	M	1g	+	+	0	+	+	±	Moderately positive, 2+.
	8	F	1g	+	+	0	+	+	±	Moderately positive, 2+.
	9	M	1g	+	±	0	+	+	±	Strongly positive, 3+.
	10	F	1g	+	±	0	+	+	±	Strongly positive, 3+.
	6-10	M and F	1g	+	+	0	+	+	±	Moderately positive, 2+.

* Explanation: In all tables + = complete hemolysis; ± = hemolysis between 50% and 100%; tr = hemolysis less than 50%; 0 = no hemolysis.

TABLE 1—*Continued*
COMPARISON OF TEN GUINEA-PIG COMPLEMENTS

Number of Serum	Number of Complement	Sex of Guinea-Pig	Amboceptor per Tube Unit	Readings*						Results
				Antigen Tubes			Control Tubes			
				1	2	3	1'	2'	3'	
3	1	M	1g	+	0	0	+	+	±	Strongly positive, 5+.
	2	F	1g	+	tr	0	+	+	+	Strongly positive, 5+.
	3	M	1g	+	tr	0	+	+	0	Moderately positive, 2+.
	4	F	1g	+	±	0	+	+	±	Strongly positive, 3+.
	5	M	1g	+	tr	0	+	+	±	Strongly positive, 4+.
	1-5	M and F	1g	+	tr	0	+	+	±	Strongly positive, 4+.
	6	F	1g	+	±	0	+	+	±	Strongly positive, 3+.
	7	M	1g	+	±	0	+	+	±	Strongly positive, 3+.
	8	F	1g	+	±	0	+	+	±	Strongly positive, 3+.
	9	M	1g	+	±	0	+	+	±	Strongly positive, 3+.
4	10	F	1g	+	tr	0	+	+	±	Strongly positive, 4+.
	6-10	M and F	1g	+	±	0	+	+	±	Strongly positive, 3+.
	1	M	1g	+	tr	0	+	+	±	Strongly positive, 4+.
	2	F	1g	+	+	0	+	+	+	Strongly positive, 3+.
	3	M	1g	+	tr	0	+	+	tr	Strongly positive, 3+.
	4	F	1g	+	tr	0	+	+	±	Strongly positive, 4+.
	5	M	1g	+	tr	0	+	+	±	Strongly positive, 4+.
	1-5	M and F	1g	+	tr	0	+	+	±	Strongly positive, 4+.
	6	F	1g	+	0	0	+	+	±	Strongly positive, 5+.
	7	M	1g	+	0	0	+	+	±	Strongly positive, 5+.
5	8	F	1g	+	0	0	+	+	±	Strongly positive, 5+.
	9	M	1g	+	0	0	+	+	±	Strongly positive, 5+.
	10	F	1g	+	0	0	+	+	±	Strongly positive, 5+.
	6-10	M and F	1g	+	0	0	+	+	±	Strongly positive, 5+.
	1	M	1g	+	±	0	+	+	±	Strongly positive, 3+.
	2	F	1g	+	+	tr	+	+	+	Moderately positive, 2+.
	3	M	1g	+	tr	0	+	+	tr	Strongly positive, 3+.
	4	F	1g	+	tr	0	+	+	±	Strongly positive, 4+.
	5	M	1g	+	±	0	+	+	±	Strongly positive, 3+.
	1-5	M and F	1g	+	±	0	+	+	±	Strongly positive, 3+.
6	6	F	1g	+	tr	0	+	+	±	Strongly positive, 4+.
	7	M	1g	+	tr	0	+	+	±	Strongly positive, 4+.
	8	F	1g	+	tr	0	+	+	±	Strongly positive, 4+.
	9	M	1g	+	tr	0	+	+	±	Strongly positive, 4+.
	10	F	1g	+	0	0	+	+	±	Strongly positive, 5+.
	6-10	M and F	1g	+	tr	0	+	+	±	Strongly positive, 4+.
	1	M	1g	+	±	0	+	+	±	Strongly positive, 3+.
	2	F	1g	+	+	0	+	+	+	Strongly positive, 3+.
	3	M	1g	+	tr	0	+	+	tr	Strongly positive, 3+.
	4	F	1g	+	tr	0	+	+	±	Strongly positive, 4+.
7	5	M	1g	+	tr	0	+	+	±	Strongly positive, 4+.
	1-5	M and F	1g	+	tr	0	+	+	±	Strongly positive, 4+.
	6	F	1g	+	tr	0	+	+	±	Strongly positive, 4+.
	7	M	1g	+	tr	0	+	+	±	Strongly positive, 4+.
	8	F	1g	+	0	0	+	+	±	Strongly positive, 4+.
	9	M	1g	+	0	0	+	+	±	Strongly positive, 5+.
	10	F	1g	±	0	0	+	+	±	Strongly positive, 6+.
	6-10	M and F	1g	+	0	0	+	+	±	Strongly positive, 5+.
	1	M	1g	±	0	0	+	+	±	Strongly positive, 5+.
	2	F	1g	+	tr	0	+	+	+	Strongly positive, 5+.
8	3	M	1g	+	0	0	+	+	0	Strongly positive, 3+.
	4	F	1g	±	0	0	+	+	±	Strongly positive, 6+.
	5	M	1g	±	0	0	+	+	±	Strongly positive, 6+.
	1-5	M and F	1g	±	0	0	+	+	±	Strongly positive, 6+.
	6	F	1g	+	0	0	+	+	±	Strongly positive, 5+.
	7	M	1g	+	0	0	+	+	±	Strongly positive, 5+.
	8	F	1g	+	tr	0	+	+	±	Strongly positive, 4+.
	9	M	1g	+	tr	0	+	+	±	Strongly positive, 4+.
	10	F	1g	+	tr	0	+	+	±	Strongly positive, 4+.
	6-10	M and F	1g	+	tr	0	+	+	±	Strongly positive, 4+.

TABLE 1—Continued
COMPARISON OF TEN GUINEA-PIG COMPLEMENTS

Number of Serum	Number of Complement	Sex of Guinea-Pig	Amboceptor per Tube Unit	Readings						Results	
				Antigen Tubes			Control Tubes				
				1	2	3	1'	2'	3'		
8	1	M	1 g	+	tr	0	+	+	±	Strongly positive,	4+.
	2	F	1 g	+	±	0	+	+	+	Strongly positive,	4+.
	3	M	1 g	+	tr	0	+	+	tr	Strongly positive,	3+.
	4	F	1 g	+	tr	0	+	+	+	Strongly positive,	4+.
	5	M	1 g	+	tr	0	+	+	±	Strongly positive,	4+.
	1-5	M and F	1 g	+	tr	0	+	+	±	Strongly positive,	4+.
	6	F	1 g	+	tr	0	+	+	±	Strongly positive,	4+.
	7	M	1 g	+	±	0	+	+	±	Strongly positive,	3+.
	8	F	1 g	+	+	0	+	+	±	Moderately positive,	2+.
	9	M	1 g	+	±	0	+	+	±	Strongly positive,	3+.
9	10	F	1 g	+	tr	0	+	+	±	Strongly positive,	4+.
	6-10	M and F	1 g	+	±	0	+	+	±	Strongly positive,	3+.
	1	M	1 g	+	0	0	+	+	±	Strongly positive,	5+.
	2	F	1 g	+	tr	0	+	+	±	Strongly positive,	5+.
	3	M	1 g	+	tr	0	+	+	tr	Strongly positive,	3+.
	4	F	1 g	+	0	0	+	+	+	Strongly positive,	5+.
	5	M	1 g	±	0	0	+	+	±	Strongly positive,	6+.
	1-5	M and F	1 g	±	0	0	+	+	±	Strongly positive,	6+.
	6	F	1 g	+	tr	0	+	+	±	Strongly positive,	4+.
	7	M	1 g	+	tr	0	+	+	±	Strongly positive,	4+.
10	8	F	1 g	+	±	0	+	+	±	Strongly positive,	3+.
	9	M	1 g	+	tr	0	+	+	±	Strongly positive,	4+.
	10	F	1 g	+	0	0	+	+	±	Strongly positive,	5+.
	6-10	M and F	1 g	+	0	0	+	+	±	Strongly positive,	5+.
	1	M	1 g	+	tr	0	+	+	±	Strongly positive,	4+.
	2	F	1 g	+	tr	0	+	+	±	Strongly positive,	5+.
	3	M	1 g	+	0	0	+	+	tr	Strongly positive,	4+.
	4	F	1 g	tr	0	0	+	+	±	Strongly positive,	6+.
	5	M	1 g	±	0	0	+	+	±	Strongly positive,	6+.
	1-5	M and F	1 g	±	0	0	+	+	±	Strongly positive,	6+.
11	6	F	1 g	±	0	0	+	+	±	Strongly positive,	6+.
	7	M	1 g	+	0	0	+	+	±	Strongly positive,	5+.
	8	F	1 g	+	0	0	+	+	tr	Strongly positive,	4+.
	9	M	1 g	+	0	0	+	+	±	Strongly positive,	5+.
	10	F	1 g	±	0	0	+	+	±	Strongly positive,	6+.
	6-10	M and F	1 g	±	0	0	+	+	±	Strongly positive,	6+.
	1	M	1 g	+	0	0	+	+	±	Strongly positive,	5+.
	2	F	1 g	+	0	0	+	+	±	Strongly positive,	6+.
	3	M	1 g	±	0	0	+	+	±	Strongly positive,	5+.
	4	F	1 g	0	0	0	+	+	tr	Strongly positive,	8+.
12	5	M	1 g	tr	0	0	+	+	±	Strongly positive,	8+.
	1-5	M and F	1 g	±	0	0	+	+	±	Strongly positive,	6+.
	6	F	1 g	tr	0	0	+	+	±	Strongly positive,	8+.
	7	M	1 g	±	0	0	+	+	±	Strongly positive,	6+.
	8	F	1 g	±	0	0	+	+	±	Strongly positive,	6+.
	9	M	1 g	±	0	0	+	+	±	Strongly positive,	6+.
	10	F	1 g	tr	0	0	+	+	±	Strongly positive,	8+.
	6-10	M and F	1 g	tr	0	0	+	+	±	Strongly positive,	8+.
	1	M	1 g	+	tr	0	+	+	±	Strongly positive,	4+.
	2	F	1 g	+	tr	0	+	+	+	Strongly positive,	5+.
13	3	M	1 g	+	0	0	+	+	0	Strongly positive,	3+.
	4	F	1 g	±	0	0	+	+	tr	Strongly positive,	5+.
	5	M	1 g	+	0	0	+	+	±	Strongly positive,	5+.
	1-5	M and F	1 g	+	0	0	+	+	±	Strongly positive,	5+.
	6	F	1 g	+	tr	0	+	+	±	Strongly positive,	4+.
	7	M	1 g	+	tr	0	+	+	±	Strongly positive,	4+.
	8	F	1 g	+	±	0	+	+	±	Strongly positive,	3+.
	9	M	1 g	+	tr	0	+	+	±	Strongly positive,	4+.
	10	F	1 g	+	0	0	+	+	±	Strongly positive,	5+.
	6-10	M and F	1 g	+	0	0	+	+	±	Strongly positive,	5+.

Table 1 shows the reactivating power and the fixability of the 10 complements. The results obtained varied greatly. Complement 3 showed poor fixability and poor reactivating power. Serum 2 gave 3+ with Complements 1 and 2 and 1+ with Complement 3. Poor fixability was not uniform with all the serums because Serum 5 fixed Complement 3 better than it did Complement 2. The mixture of 5 complements gave a fair average of the results obtained with the complements singly. There was no difference between complement from male and from female guinea-pigs.

TEST 2

Five human complements (Complements 1-5, inclusive) were tested on Human Serums 7-12, inclusive. These were the same serums that were used in Test 1. The complements were used in doses of 0.2, 0.1 and 0.05 c c per test tube. Noguchi⁹ recommends 0.1 c c of human complement per test tube.

TABLE 2
COMPARISON OF HUMAN COMPLEMENTS

Number of Serum	Number of Complement	Sex of Guinea-Pig	Amboceptor per Tube Unit	Readings*						Results	
				Antigen Tubes			Control Tubes				
				1	2	3	1'	2'	3'		
7	1	M	1 g	+	+	0	+	+	tr	Weakly positive,	1+.
	2	F	1 g	+	+	tr	+	+	+	Weakly positive,	1+.
	3	M	1 g	+	+	tr	+	+	+	Weakly positive,	1+.
	4	F	1 g	+	+	tr	+	+	+	Weakly positive,	1+.
	5	M	1 g	+	+	tr	+	+	+	Weakly positive,	1+.
	1-5	M and F	1 g	+	+	tr	+	+	+	Weakly positive,	1+.
8	1	M	1 g	+	+	0	+	+	tr	Weakly positive,	1+.
	2	F	1 g	+	+	tr	+	+	+	Weakly positive,	1+.
	3	M	1 g	+	+	tr	+	+	+	Weakly positive,	1+.
	4	F	1 g	+	+	tr	+	+	+	Weakly positive,	1+.
	5	M	1 g	+	+	tr	+	+	+	Weakly positive,	1+.
	1-5	M and F	1 g	+	+	tr	+	+	+	Weakly positive,	1+.
9	1	M	1 g	+	+	0	+	+	tr	Weakly positive,	1+.
	2	F	1 g	+	+	tr	+	+	+	Weakly positive,	1+.
	3	M	1 g	+	+	tr	+	+	+	Weakly positive,	1+.
	4	F	1 g	+	+	tr	+	+	+	Weakly positive,	1+.
	5	M	1 g	+	+	tr	+	+	+	Weakly positive,	1+.
	1-5	M and F	1 g	+	+	tr	+	+	+	Weakly positive,	1+.
10	1	M	1 g	+	+	0	+	+	tr	Moderately positive,	2+.
	2	F	1 g	+	+	0	+	+	tr	Moderately positive,	2+.
	3	M	1 g	+	+	0	+	+	+	Moderately positive,	2+.
	4	F	1 g	+	+	0	+	+	+	Moderately positive,	2+.
	5	M	1 g	+	+	0	+	+	+	Moderately positive,	2+.
	1-5	M and F	1 g	+	+	0	+	+	+	Moderately positive,	2+.
11	1	M	1 g	+	+	0	+	+	tr	Moderately positive,	2+.
	2	F	1 g	+	+	0	+	+	+	Moderately positive,	2+.
	3	M	1 g	+	+	0	+	+	+	Moderately positive,	2+.
	4	F	1 g	+	+	0	+	+	+	Strongly positive,	3+.
	5	M	1 g	+	+	0	+	+	+	Moderately positive,	2+.
	1-5	M and F	1 g	+	+	0	+	+	+	Moderately positive,	2+.
12	1	M	1 g	+	+	0	+	+	tr	Moderately positive,	2+.
	2	F	1 g	+	+	0	+	+	tr	Weakly positive,	1+.
	3	M	1 g	+	+	0	+	+	+	Moderately positive,	2+.
	4	F	1 g	+	+	0	+	+	+	Moderately positive,	2+.
	5	M	1 g	+	+	0	+	+	+	Moderately positive,	2+.
	1-5	M and F	1 g	+	+	0	+	+	+	Moderately positive,	2+.

The results obtained with the human complements are shown in Table 2. These were more uniform than those obtained with guinea-pig complement but were much weaker. With a mixture of 5 guinea-pig complements Serum 7 gave 6+ and with a mixture of 5 human complements it gave 1+. Serum 8 gave 4+ and 1+; Serum 9 gave 6+ and 1+; Serum 10 gave 6+ and 2+; Serum 11 gave 6+ and 2+, and Serum 12 gave 5+ and 2+.

TABLE 3
THE TEST AS RECOMMENDED BY NOGUCHI

Number of Serum	Number of Complement	Sex of Person	Amboceptor per Tube Unit	Readings*						Results	
				Antigen Tubes			Control Tubes				
				1	2	3	1'	2'	3'		
7	1-5	M and F	1 g	+	+	±	+	+	±	Negative,	—
8	1-5	M and F	1 g	+	+	±	+	+	±	Negative,	—
9	1-5	M and F	1 g	+	+	±	+	+	±	Negative,	—
10	1-5	M and F	1 g	+	+	±	+	+	±	Negative,	—
11	1-5	M and F	1 g	+	+	tr	+	+	±	Weakly positive,	1+
12	1-5	M and F	1 g	+	+	±	+	+	±	Negative,	—

TEST 3

Noguchi⁶ recently advocated the use of human complement instead of guinea-pig complement with heated human serum. The first incubation he conducts in the water-bath at 37 C. for 30 minutes. In order to show how that method compares with our regular method Serums 7-12, inclusive, were tested. These serums came from patients that were known to be syphilitic.

TABLE 4
THE EFFECT OF NORMAL HUMAN SERUM ON THE SO-CALLED SYPHILITIC ANTIBODY

Number of Serum	Diluent	Comple-ment	Amboceptor per Tube Unit	Readings*						Results
				Antigen Tubes			Control Tubes			
				1	2	3	1'	2'	3'	
7	S. S.	G-P.	1 g	+	+	0	+	+	±	Moderately positive, 2+.
	F. H. S.	G-P.	1 g	+	+	±	+	+	±	Negative, —.
	H. H. S.	G-P.	1 g	+	+	±	+	+	±	Negative, —.
8	S. S.	G-P.	1 g	+	+	tr	+	+	±	Weakly positive, 1+.
	F. H. S.	G-P.	1 g	+	+	±	+	+	±	Negative, —.
	H. H. S.	G-P.	1 g	+	+	±	+	+	±	Negative, —.
9	S. S.	G-P.	1 g	+	+	0	+	+	±	Moderately positive, 2+.
	F. H. S.	G-P.	1 g	+	+	±	+	+	±	Negative, —.
	H. H. S.	G-P.	1 g	+	+	±	+	+	±	Negative, —.
10	S. S.	G-P.	1 g	+	±	0	+	+	±	Strongly positive, 3+.
	F. H. S.	G-P.	1 g	+	+	±	+	+	±	Negative, —.
	H. H. S.	G-P.	1 g	+	+	±	+	+	±	Negative, —.
11	S. S.	G-P.	1 g	+	tr	0	+	+	tr	Strongly positive, 3+.
	F. H. S.	G-P.	1 g	+	+	tr	+	+	tr	Negative, —.
	H. H. S.	G-P.	1 g	+	+	tr	+	+	tr	Negative, —.
12	S. S.	G-P.	1 g	+	+	0	+	+	±	Moderately positive, 2+.
	F. H. S.	G-P.	1 g	+	+	±	+	+	±	Negative, —.
	H. H. S.	G-P.	1 g	+	+	±	+	+	±	Negative, —.

Table 3 shows the results obtained with the method recently advocated by Noguchi. Serums 7, 8, 9, 10 and 12 gave negative results and Serum 11 gave a weakly positive result, 1+. As Table 1 shows, our regular method gave positive results. Serums 7, 8, 9, 10, 11 and 12 gave 6+, 4+, 6+, 6+, 6+ and 5+, respectively.

TEST 4

Having found that human complement gives much weaker positive results than does guinea-pig complement the effect of normal human serum on syphilitic human serum was studied on 6 syphilitic serums. Three portions were taken from each of Serums 7-12, inclusive; one portion of each serum was diluted with an equal volume of salt solution containing 50% glycerol (S. S.), one portion was diluted with an equal volume of glycerolated fresh human serum (F. H. S.) and the other portion was mixed with an equal volume of glycerolated heated human serum (H. H. S.). At about 24 hours after these serums had been mixed these mixtures were tested by our regular method.

Table 4 shows the results obtained with Serums 7, 8, 9, 10, 11 and 12 after they had been diluted with an equal volume of salt solution and with an equal volume of normal human serum. Diluted with an equal volume of salt solution these serums gave 2+, 1+, 2+, 3+, 3+ and 2+, respectively, while all the portions diluted with an equal volume of human serum fresh or heated gave negative results.

TABLE 5
THE EFFECT OF GUINEA-PIG SERUM ON THE SO-CALLED SYPHILITIC ANTIBODY

Number of Serum	Diluent	Comple-ment	Ambo-ceptor per Tube Unit	Readings*						Results
				Antigen Tubes			Control Tubes			
				1	2	3	1'	2'	3'	
8	S. S.	G-P	1g	+	±	0	+	+	tr	Moderately positive, 2+.
	G-P. S. 1	G-P	1g	+	+	±	+	+	±	Negative, —.
	G-P. S. 2	G-P	1g	+	+	±	+	+	±	Negative, —.
	G-P. S. 3	G-P	1g	+	+	±	+	+	±	Negative, —.
9	S. S.	G-P	1g	+	tr	0	+	+	tr	Strongly positive, 3+.
	G-P. S. 1	G-P	1g	+	+	±	+	+	±	Negative, —.
	G-P. S. 2	G-P	1g	+	+	±	+	+	±	Negative, —.
	G-P. S. 3	G-P	1g	+	+	±?	+	+	±	Faintly positive, ±.
10	S. S.	G-P	1g	+	tr	0	+	+	tr	Strongly positive, 3+.
	G-P. S. 1	G-P	1g	+	+	±	+	+	±	Negative, —.
	G-P. S. 2	G-P	1g	+	+	±	+	+	±	Negative, —.
	G-P. S. 3	G-P	1g	+	+	±?	+	+	±	Faintly positive, ±.
11	S. S.	G-P	1g	+	tr	0	+	+	tr	Strongly positive, 3+.
	G-P. S. 1	G-P	1g	+	+	±	+	+	±	Negative, —.
	G-P. S. 2	G-P	1g	+	+	±?	+	+	±	Faintly positive, ±.
	G-P. S. 3	G-P	1g	+	+	±?	+	+	±	Faintly positive, ±.
12	S. S.	G-P	1g	+	tr	0	+	+	tr	Strongly positive, 3+.
	G-P. S. 1	G-P	1g	+	+	±	+	+	±	Negative, —.
	G-P. S. 2	G-P	1g	+	+	±	+	+	±	Negative, —.
	G-P. S. 3	G-P	1g	+	+	±	+	+	±	Negative, —.

TEST 5

The work of Test 4 was repeated with heated guinea-pig serum (G-P. S.) instead of normal human serum as diluent.

The effect of guinea-pig serum on syphilitic human serum is shown in Table 5. Mixed with an equal volume of salt solution Serums 8, 9, 10, 11

and 12 gave 2+, 3+, 3+, 3+ and 3+, respectively, while the same serums mixed with equal volumes of guinea-pig serums gave negative or nearly negative results.

TEST 6

The rate at which the complement-binding power disappears from syphilitic human serum when mixed with normal human or guinea-pig serum was observed on Serums 10 and 11. Three portions were taken of each serum; one portion was diluted with an equal volume of salt solution (S. S.) containing 50% glycerol; one portion was diluted with an equal volume of glycerolated human serum (H. S.), and the other portion was mixed with an equal volume of glycerolated guinea-pig serum. Each portion was tested at the end of 2 hours, 4 hours and 6 hours.

TABLE 6
THE RATE OF DISAPPEARANCE OF COMPLEMENT-BINDING POWER

Number of Serum	Diluent	Time	Amboceptor per Tube Unit	Readings*						Results
				Antigen Tubes			Control Tubes			
				1	2	3	1'	2'	3'	
10	S. S.	2	1 g	+	+	0	+	+	±	Moderately positive, 2+.
	H. S.	2	1 g	+	+	tr	+	+	±	Weakly positive, 1+.
	G.-P. S.	2	1 g	+	+	±?	+	+	±	Faintly positive, ±.
	S. S.	4	1 g	+	+	0	+	+	±	Moderately positive, 2+.
	H. S.	4	1 g	+	+	±?	+	+	±	Faintly positive, ±.
	G.-P. S.	4	1 g	+	+	±	+	+	±	Negative, —.
	S. S.	6	1 g	+	+	0	+	+	±	Moderately positive, 2+.
	H. S.	6	1 g	+	+	±?	+	+	±	Faintly positive, ±.
	G.-P. S.	6	1 g	+	+	±	+	+	±	Negative, —.
11	S. S.	2	1 g	+	±	0	+	+	±	Strongly positive, 3+.
	H. S.	2	1 g	+	+	tr	+	+	±	Weakly positive, 1+.
	G.-P. S.	2	1 g	+	+	tr	+	+	±	Weakly positive, 1+.
	S. S.	4	1 g	+	±	0	+	+	±	Strongly positive, 3+.
	H. S.	4	1 g	+	+	tr	+	+	±	Weakly positive, 1+.
	G.-P. S.	4	1 g	+	+	±?	+	+	±	Faintly positive, ±.
	S. S.	6	1 g	+	±	0	+	+	±	Strongly positive, 3+.
	H. S.	6	1 g	+	+	±?	+	+	±	Faintly positive, ±.
	G.-P. S.	6	1 g	+	+	±?	+	+	±	Faintly positive, ±.

Table 6 shows the rate at which the complement-binding power disappeared from Serums 10 and 11. At the end of 2 hours about 50% of the complement-binding power had disappeared from the portion mixed with normal human serum and about 75% from the portion mixed with guinea-pig serum. At the end of 4 hours the normal human serum had neutralized about 75% of Serum 10 and the guinea-pig serum had completely neutralized it. With Serum 11 neutralization was a trifle slower.

TEST 7

Deterioration of guinea-pig complement was determined in the refrigerator ranging from 10-15 C. Three guinea-pig serums (Serums 11, 12 and 13) were each divided into 2 portions, A and B. Portion A was left on the clot and Portion B was removed from the clot and was kept in a sterile test tube. Each serum was tested singly and the three serums were mixed and the mixture was also tested. The mixture was designated X; X A was mixed

immediately before the test while X B was mixed a few hours after the bleeding; the mixture was kept in the refrigerator and was tested from day to day. In these tests the quantity of amboceptor was kept constant.

TABLE 7
DETERIORATION OF COMPLEMENT IN THE REFRIGERATOR

Number of Complement	Portions A=On Clot B=Off Clot	Age Days	Number of Serum	Readings*						Results	
				Antigen Tubes			Control Tubes				
				1	2	3	1'	2'	3'		
11	A	1	10	±	0	0	+	+	tr	Strongly positive,	5+.
	B	1	10	±	0	0	+	+	tr	Strongly positive,	5+.
	A	2	10	±	0	0	+	+	tr	Strongly positive,	5+.
	B	2	10	±	0	0	+	+	tr	Strongly positive,	4+.
	A	3	10	±	0	0	+	+	0	Strongly positive,	4+.
	B	3	10	±	0	0	+	±	0	Strongly positive,	3+.
	A	4	10	±	0	0	+	+	0	Strongly positive,	4+.
	B	4	10	±	0	0	+	tr	0	Moderately positive,	2+.
	A	5	10	±	0	0	+	±	0	Strongly positive,	3+.
B	5	10	tr	0	0	±	tr	0	Positive (Unfit).		
12	A	1	10	±	0	0	+	+	±	Strongly positive,	6+.
	B	1	10	±	0	0	+	+	±	Strongly positive,	6+.
	A	2	10	±	0	0	+	+	tr	Strongly positive,	5+.
	B	2	10	+	0	0	+	+	tr	Strongly positive,	4+.
	A	3	10	±	0	0	+	+	tr	Strongly positive,	5+.
	B	3	10	±	0	0	+	+	0	Strongly positive,	4+.
	A	4	10	±	0	0	+	+	tr	Strongly positive,	5+.
	B	4	10	±	0	0	+	±	0	Strongly positive,	3+.
	A	5	10	±	0	0	+	+	tr	Strongly positive,	5+.
B	5	10	±	0	0	±	tr	0	Unfit		
13	A	1	10	±	0	0	+	+	±	Strongly positive,	6+.
	B	1	10	±	0	0	+	+	±	Strongly positive,	6+.
	A	2	10	±	0	0	+	+	tr	Strongly positive,	5+.
	B	2	10	±	0	0	+	+	tr	Strongly positive,	4+.
	A	3	10	±	0	0	+	+	0	Strongly positive,	4+.
	B	3	10	±	0	0	+	±	0	Moderately positive,	2+.
	A	4	10	+	0	0	+	+	0	Strongly positive,	3+.
	B	4	10	+	tr	0	±	tr	0	Unfit	
	A	5	10	±	0	0	+	±	0	Strongly positive,	3+.
B	5	10	+	0	0	±	tr	0	Unfit		
X	A	1	10	±	0	0	+	+	±	Strongly positive,	6+.
	B	1	10	±	0	0	+	+	±	Strongly positive,	6+.
	A	2	10	±	0	0	+	+	tr	Strongly positive,	5+.
	B	2	10	±	0	0	+	+	tr	Strongly positive,	5+.
	A	3	10	±	0	0	+	+	0	Strongly positive,	4+.
	B	3	10	±	0	0	+	±	tr	Strongly positive,	3+.
	A	4	10	±	0	0	+	±	tr	Strongly positive,	3+.
	B	4	10	±	tr	0	±	tr	0	Negative (Unfit).	
	A	5	10	±	0	0	+	±	0	Strongly positive,	3+.
B	5	10	±	0	0	±	tr	0	Unfit		

The rate at which these 3 complements deteriorated in the refrigerator is shown in Table 7. Portion A remained fairly constant for 3 days while Portion B of the serums singly and in mixture showed considerable deterioration on the 3rd day. After the 3rd day deterioration of Portion A also became noticeable. Deterioration took place in the fixability of the complement and in the power to reactivate the hemolytic amboceptor.

TEST 8

In accordance with the method described by Ramy,² 3 fresh guinea-pig serums were diluted with a 10% solution of sodium acetate. A few hours after the guinea-pigs had been bled each serum was divided into 2 portions,

A and B. Portion A was left on the clot and no preservative was added. Portion B was pipetted off the clot, was centrifuged and 4 parts of the clear serum were mixed with 6 parts of a 10% solution of sodium acetate in salt solution (9 gm. NaCl per liter). The serums were tested singly and in the form of a mixture of the 3 serums which is designated as X. X A was mixed immediately before testing and X B was mixed immediately after Portion B had been mixed with the sodium acetate solution. The serums were kept in the refrigerator and were tested at short intervals.

TABLE 8
COMPLEMENT PRESERVED WITH SODIUM ACETATE

Number of Comple- ment	Portions A=Not Pres- erved B=Pres- erved	Age Days	Number of Serum	Readings*						Results
				Antigen Tubes			Control Tubes			
				1	2	3	1'	2'	3'	
14	A	1	10	±	0	0	+	+	±	Strongly positive, 6+. Strongly positive, 6+. Strongly positive, 5+. Strongly positive, 6+. Strongly positive, 4+. Strongly positive, 4+. Unfit. Positive. Positive. Positive.
	B	1	10	±	0	0	+	+	±	
	B	2	10	+	0	0	+	+	±	
	A	3	10	±	0	0	+	+	±	
	B	3	10	+	tr	0	+	+	±	
	B	4	10	+	0	0	+	+	tr	
	A	6	10	±	0	0	±	tr	0	
	B	6	10	+	tr	0	+	±	tr	
	B	8	10	+	tr	0	+	±	tr	
	B	11	10	+	tr	0	+	±	tr	
B	16	10	+	tr	0	+	±	tr		
15	A	1	10	±	0	0	+	+	±	Strongly positive, 6+. Strongly positive, 6+. Strongly positive, 5+. Strongly positive, 6+. Strongly positive, 4+. Strongly positive, 4+. Positive. Strongly positive, 3+. Strongly positive, 3+. Strongly positive, 3+.
	B	1	10	±	0	0	+	+	±	
	B	2	10	+	0	0	+	+	±	
	A	3	10	tr	0	0	+	+	tr	
	B	3	10	+	tr	0	+	+	±	
	B	4	10	+	0	0	+	+	tr	
	A	6	10	+	tr	0	+	±	tr	
	B	6	10	+	±	0	+	+	±	
	B	8	10	+	tr	0	+	+	tr	
	B	11	10	+	tr	0	+	+	tr	
B	16	10	±	tr	0	±	±	tr		
16	A	1	10	±	0	0	+	+	±	Strongly positive, 6+. Strongly positive, 6+. Strongly positive, 5+. Strongly positive, 6+. Strongly positive, 4+. Strongly positive, 4+. Positive. Strongly positive, 3+. Strongly positive, 3+. Strongly positive, 3+.
	B	1	10	±	0	0	+	+	±	
	B	2	10	+	0	0	+	+	±	
	A	3	10	±	0	0	+	+	±	
	B	3	10	+	tr	0	+	+	±	
	A	4	10	+	tr	0	+	+	±	
	B	6	10	+	±	tr	+	+	±	
	B	6	10	+	±	0	+	+	±	
	B	8	10	+	±	0	+	+	±	
	B	11	10	+	tr	0	+	+	tr	
B	16	10	+	tr	0	+	±	tr		
X	A	1	10	±	0	0	+	+	±	Strongly positive, 6+. Strongly positive, 6+. Strongly positive, 5+. Strongly positive, 6+. Strongly positive, 4+. Strongly positive, 4+. Strongly positive, 3+. Strongly positive, 3+. Strongly positive, 3+. Strongly positive, 3+.
	B	1	10	±	0	0	+	+	±	
	B	2	10	+	0	0	+	+	±	
	A	3	10	±	0	0	+	+	±	
	B	3	10	+	tr	0	+	+	±	
	B	4	10	+	0	0	+	+	tr	
	A	6	10	+	tr	0	+	+	tr	
	B	6	10	+	±	0	+	+	±	
	B	8	10	+	tr	0	+	+	tr	
	B	11	10	+	tr	0	+	+	tr	
B	16	10	+	tr	0	±	±	tr		

Table 8 shows the results obtained by comparing complement preserved by Ramy's method with complement that was without preservative. On the 1st day after the serums had been obtained Portions A and B gave results that were

identical. On the 3rd day Portion A of each complement and of the mixture X gave 6+ and Portion B gave 4+. On the 6th day Portion A of each serum was practically unfit while Portion B gave 3+. On the 8th day and on the 11th day all Portions B gave 3+ while on the 16th day all were unfit. In the serum mixed with sodium acetate solution complement deterioration began earlier than in the unpreserved serum but it progressed somewhat slower. While in plain serum, Portion A, the complement showed full strength on the 3rd day it had lost one-third of its fixability in the preserved serum, Portion B.

TABLE 9
GLYCEROLATED COMPLEMENT COMPARED WITH NONGLYCEROLATED COMPLEMENT

Number of Comple- ment	Portions A=On Clot B=Off Clot C=Glycer- olated	Age Days	Number of Serum	Readings*						Results
				Antigen Tubes			Control Tubes			
				1	2	3	1'	2'	3'	
17	A	1	10	+	0	0	+	+	±	Strongly positive, 5+. Strongly positive, 5+. Strongly positive, 6+. Strongly positive, 4+. Strongly positive, 4+. Strongly positive, 6+. Strongly positive, 3+. Negative. Strongly positive, 3+. Unfit.
	B	1	10	+	0	0	+	+	±	
	C	1	10	±	0	0	+	+	±	
	A	3	10	+	tr	0	+	+	±	
	B	3	10	+	tr	0	+	+	±	
	C	3	10	±	0	0	+	+	±	
	A	7	10	+	tr	0	+	+	tr	
	B	7	10	+	tr	0	+	tr	0	
	C	7	10	+	±	0	+	+	±	
18	C	10	10	±	0	0	±	±	tr	
	A	1	10	±	0	0	+	+	±	Strongly positive, 6+. Strongly positive, 6+. Strongly positive, 6+. Strongly positive, 5+. Strongly positive, 4+. Strongly positive, 8+. Strongly positive, 3+. Negative. Strongly positive, 5+. Unfit.
	B	1	10	±	0	0	+	+	±	
	C	1	10	±	0	0	+	+	±	
	A	3	10	+	0	0	+	+	±	
	B	3	10	+	tr	0	+	+	±	
	C	3	10	tr	0	0	+	+	±	
	A	7	10	+	tr	0	+	+	tr	
	B	7	10	+	tr	0	+	tr	0	
C	7	10	+	0	0	+	+	±		
19	C	10	10	tr	0	0	±	±	tr	
	A	1	10	±	0	0	+	+	±	Strongly positive, 6+. Strongly positive, 6+. Strongly positive, 6+. Strongly positive, 5+. Strongly positive, 4+. Strongly positive, 8+. Strongly positive, 3+. Negative. Strongly positive, 5+. Unfit.
	B	1	10	±	0	0	+	+	±	
	C	1	10	±	0	0	+	+	±	
	A	3	10	+	0	0	+	+	±	
	B	3	10	+	tr	0	+	+	±	
	C	3	10	tr	0	0	+	+	±	
	A	7	10	+	tr	0	+	+	tr	
	B	7	10	+	tr	0	+	tr	0	
C	7	10	+	0	0	+	+	±		
X	C	10	10	tr	0	0	±	±	tr	
	A	1	10	±	0	0	+	+	±	Strongly positive, 6+. Strongly positive, 6+. Strongly positive, 6+. Strongly positive, 5+. Strongly positive, 4+. Strongly positive, 7+. Strongly positive, 3+. Negative. Strongly positive, 5+. Unfit.
	B	1	10	±	0	0	+	+	±	
	C	1	10	±	0	0	+	+	±	
	A	3	10	+	0	0	+	+	±	
	B	3	10	+	tr	0	+	+	±	
	C	3	10	±?	0	0	+	+	±	
	A	7	10	+	tr	0	+	+	tr	
	B	7	10	+	tr	0	+	tr	0	
C	7	10	+	0	0	+	+	±		
	C	10	10	tr	0	0	±	±	tr	

TEST 9

Glycerol as a preservative for complement was studied on 3 guinea-pig serums, Complements 17, 18 and 19. A few hours after bleeding each serum was divided into 3 portions, A, B and C. Portion A was left on the clot, Portion B was pipetted off the clot and was put into a sterile test tube.

Portion C was pipetted off the clot, was put into a steril tube and 1 part of sterilized glycerol was mixed with 3 parts of serum. All serums were tested singly and in a mixture composed of the 3 serums. The mixture is designated as X. X A was mixed immediately before testing, while X B and X C were prepared a few hours after the guinea-pigs had been bled. All complement serums were kept in the refrigerator until used, and were tested on the 1st, 3rd and 7th days after having been obtained from the guinea-pigs.

Table 9 shows the results obtained with glycerolated complement as compared with nonglycerolated complement. Portion A showed a slight loss of fixability on the 3rd day, Portion B had lost a trifle more than Portion A, and Portion C was bound better on the 3rd day than on the 1st day. On the 7th day Portion A was fixed or bound very poorly, Portion B was not bound at all, while Portion C gave results nearly equal to those obtained on the 1st day. Portions A and B were not tested after the 7th day while Portion C was found to be unfit on the 10th day.

SUMMARY AND CONCLUSIONS

The complements of fresh guinea-pig serum varied greatly in fixability. While one complement gave 6+ with a certain human serum another complement gave only 3+ with the same serum, other things having been equal. Another serum gave 3+ with one complement and 1+ with another complement. This variation in fixability was not due to complement alone when a complement showed poor fixability with a certain human serum the same complement was well fixed by other human serums. A mixture of 5 complement serums gave fairly uniform results. These results show clearly the unreliability of a single complement serum.

Human complement gave much weaker positive results than did guinea-pig complement. Noguchi's recent method of doing the Wassermann reaction gave negative results with serum that was known to be from syphilitic persons and gave 6+ by our regular method. Normal human serum and guinea-pig serum, respectively, were mixed with syphilitic human serum giving 6+ result with the Wassermann reaction; on testing them again 24 hours later negative results were obtained while controls which were diluted similarly with salt solution gave positive results. Neutralization of complement-fixing power by normal human serum or by guinea-pig serum progressed fairly rapidly, 50% was neutralized in 2 hours. At the end of 4 hours neutralization of complement-fixing power was sometimes complete, especially by guinea-pig serum, which was a trifle more effective than the human serum. A neutralization test of this kind may become of value in

detecting syphilitic serum which no longer gives a positive result by the Wassermann reaction alone. Future observations must determine its value.

Guinea-pig complement left on the clot and kept in the refrigerator remained fairly constant for 3 days, while complements which had been removed from the clots deteriorated more rapidly. Deterioration took place in two directions, the complement lost fixability and it lost the power to reactivate the hemolytic amboceptor. Complements with which 6+ was obtained about 24 hours after bleeding gave negative results with the same human serums 6 days later. Fixability disappeared more rapidly than did the power to reactivate hemolytic amboceptor.

Ramy's method of preserving complement by sodium acetate was a total failure in my hands. Fresh guinea-pig serum mixed in proportion of 4:6 with a 10% solution of sodium acetate in salt solution began to deteriorate immediately. In 3 days it lost about 33% fixability while the control portion to which no preservative had been added remained constant. Although deterioration of preserved complement began earlier than of the control it progressed somewhat slower.

Glycerol seemed to prevent deterioration of complement for a few days. In complement preserved by glycerol fixability increased during the first 3 days and on the 7th day it still was nearly equal to the fresh serum.