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VARIATIONS IN THE COMPLEMENT ACTIVITY AND FIXABILITY OF GUINEA PIG SERUM.[1]

By HIDEYO NOGUCHI and J. BRONFENBRENNER.

(From the Laboratories of the Rockefeller Institute for Medical Research, New York.)

Some time ago one of us (Noguchi) encountered a peculiar action in goat serum. This serum, while fresh and active, remained undeviated by the Wassermann reaction or by any combination of specific antigen and antibody. It was natural, therefore, to inquire whether a similar action might not be found in the sera of other species of animals or in the sera of individuals of a species in which the complement is generally capable of deviation.

Our present paper concerns itself with the quantitative determination of the complementary activity of various sera and their behavior towards the complement deviation phenomenon.

The most important and most widely studied of all complements is that of the guinea pig. Although there are some observations bearing upon the relative complementary activity of different specimens of this serum, there is no work in which quantitative estimations of complementary activity of a large number of specimens with simultaneous determinations on the fixability of each has been carried out. This has been done in our present studies.

We have also studied the complementary activity of the sera of several other species, such as beef, sheep, goat, pig, dog, cat, rabbit, rat, chicken, etc.[2] Some of these sera were tested for their fixability, our purpose being to see whether the complements of any of these could be used for the fixation test.

METHOD OF STUDY.

1. *The Titration of the Activity of Complement.*—The amboceptor derived from rabbits immunized with washed human corpuscles was used in all the

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[2] The results with these sera will be published separately.

TABLE I.

Serial number of guinea pig sera.	Complement activity.							Fixability (20-hour-old serum).		
	Serum left in contact with clot for 20 hours (0° C.).			Serum left in contact with clot for 46 hours (0° C.).			Activity after 46 hours.	Amount of guinea pig serum fixed.	Number of complement units fixed.	Remarks on fixability.
	Hemolysis 100 per cent.	Hemolysis 50 per cent.	Hemolysis 25 per cent.	Hemolysis 100 per cent.	Hemolysis 50 per cent.	Hemolysis 25 per cent.				
1	0.025	0.012	0.006	0.025	0.012	0.006		0.135	5.4	Marked.
2	0.022	0.01	0.005	0.015	0.006	0.0035	Increased	0.12	5.45	Marked.
3	0.022	0.01	0.005	0.025	0.012	0.0055		0.09	4.09	Normal.
4	0.022	0.01	0.005	0.015	0.007	0.0035	Increased.	0.09	4.09	Normal.
5	0.022	0.01	0.005	0.015	0.007	0.004	Increased.	0.09	4.09	Normal.
6	0.022	0.01	0.005	0.022	0.009	0.005		0.147	6.68	High.
7	0.018	0.008	0.004	0.04	0.02	0.01	Marked decrease.	0.085	4.72	Normal.
8	0.018	0.008	0.005	0.022	0.01	0.005	Decreased.	0.09	5.	Normal.
9	0.022	0.009	0.005	0.022	0.01	0.005		0.09	4.09	Normal.
10	0.015	0.0065	0.004	0.013	0.006	0.003		0.09	6.	High.
11	0.017	0.0075	0.004	0.015	0.007	0.0035		0.09	5.3	Marked,
12	0.015	0.007	0.004	0.017	0.0075	0.0035		0.09	6.	High.
13	0.022	0.01	0.006	0.025	0.012	0.005		0.12	5.83	High.
14	0.02	0.008	0.004	0.015	0.006	0.003	Slight increase.	0.09	4.5	Normal.
15	0.022	0.01	0.006	0.015	0.006	0.003	Increased.	0.06	2.54	Highly refractory,
16	0.025	0.012	0.006	0.02	0.01	0.005	Slight increase.	0.09	3.6	Markedly refractory.
17	0.025	0.012	0.006	0.025	0.0121	0.006		0.15	6.	High.
18	0.02	0.01	0.005	0.018	0.008	0.004		0.09	4.5	Normal
19	0.022	0.01	0.005	0.02	0.01	0.005		0.085	3.86	Markedly refractory.
20	0.02	0.008	0.004	0.022	0.009	0.005	Slight decrease.	0.14	7.	High.
21	0.02	0.008	0.004	0.02	0.008	0.004		0.085	4.25	Normal.
22	0.02	0.008	0.004	0.018	0.0065	0.004	Slight increase.	0.09	4.5	Normal.
23	0.018	0.008	0.004	0.015	0.006	0.003	Slight increase.	0.09	5.	Normal.
24	0.02	0.008	0.004	0.022	0.01	0.006	Slight decrease.	0.09	4.5	Normal.
25	0.02	0.008	0.004	0.015	0.006	0.0035	Increased.	0.09	4.5	Normal.
26	0.022	0.009	0.005	0.015	0.006	0.003	Marked increase.	0.03	1.66	Highly refractory.

Serial number of guinea pig sera.	Complement activity.							Fixability (20-hour-old serum).		
	Serum left in contact with clot for 20 hours (0° C.).			Serum left in contact with clot for 46 hours (0° C.).			Activity after 46 hours.	Amount of guinea pig serum fixed.	Number of complement units fixed.	Remarks on fixability.
	Hemolysis 100 per cent.	Hemolysis 50 per cent.	Hemolysis 25 per cent.	Hemolysis 100 per cent.	Hemolysis 50 per cent.	Hemolysis 25 per cent.				
27	0.02	0.008	0.004	0.02	0.008	0.0045		0.09	4.5	Normal.
28	0.018	0.0075	0.004	0.015	0.006	0.004	Slight increase.	0.09	5.	Normal.
29	0.018	0.0075	0 004	0.015	0.006	0.0035	Slight increase.	0.09	5.	Normal.
30	0.035	0.015	0.0075	0.022	0.0075	0.004	Increased.	0.09	2.57	Highly refractory.
31	0.025	0.01	0.0045	0.015	0.006	0.003	Marked increase.	0.09	3.6	Markedly refractory.
32	0.02	0.008	0.004	0.022	0.01	0.006	Slight decrease.	0.15	7.5	High.
33	0.025	0.01	0.005	0.06	0.03	0.015	Marked decrease.	0.24	9.6	Unusual.
34	0.025	0.01	0.004	0.018	0.008	0.005	Increase.	0.	0.	Absolutely refractory.
35	0.025	0.01	0.004	0.022	0.0085	0.0045	Slight increase.	0.075	3.	Highly refractory.
36	0.02	0.008	0.004	0.022	0.009	0.005	Slight decrease.	0.09	4.5	Normal.
37	0.018	0.0075	0.004	0.018	0.007	0.0035		0.105	5.8	Marked.
38	0.027	0.012	0.005	0.04	0.02	0.01	Marked decrease.	0.12	4.44	Normal.
39	0.025	0.01	0.004	0.018	0.008	0.005	Marked increase.	0.12	4.8	Normal.
40	0.025	0.01	0.005	0.022	0.01	0.005	Slight increase.	0.09	3.6	Markedly refractory.
41	0.027	0.012	0.004	0.017	0.007	0.004	Marked increase.	0.09	3.33	Markedly refractory.
Averages	**0.0216**			**0.0209**				**0.098**	**4.64**	

hemolytic experiments. In addition to this, another amboceptor derived from a goat similarly immunized was employed in certain experiments recorded in the latter part of this paper. Unless otherwise stated, however, rabbit amboceptor is referred to.

The amboceptor was used in constant doses. For each tube 0.1 c.c. of a 10 per cent. suspension of washed human corpuscles was used. The complement to be titrated was added to a series of tubes in graduated quantities. The total volume of fluid in each tube was brought up to 1 c.c. by means of the addition of 0.9 per cent. salt solution. The tubes were placed in a water thermostat and incubated for two hours at 37° C.

2. *The Determination of the Fixability of Complement.*—In determining the fixation of complement, the system used was the one introduced by Noguchi for the diagnosis of syphilis. The amount of antihuman amboceptor (rabbit) was uniformly two units for each tube The quantity of washed corpuscles used was 0.1 c.c. of a 10 per cent. suspension. The quantity of complement varied according to the titers of the different sera. The amount of antigen emulsion was 0.1 c.c., which contained about four antigenic units. It was made from the aceton-insoluble fraction of lipoids from a human liver, according to Noguchi's method.[3] In doses of 0.45 c.c. it had no anticomplementary action. The serum was a mixture from several untreated cases of secondary syphilis and was inactivated before use. It had no inherent anticomplementary property in doses of 0.2 c.c., and in the fixation experiments the quantity employed was only one-tenth of this.

The determination of the amount of fixed complement was made by reading, according to the colorimetric method of Madsen, the degree of hemolysis present in a tube where an incomplete fixation had taken place, and then calculating the approximate amount of complement still free. As the exact amount of complement originally employed is known, it is an easy matter to calculate the amount of complement which had been fixed.

The results obtained are given in Table I. In this series of experiments forty-one guinea pigs were examined at the same time.

In studying the results summarized in Table I, several interesting facts may be noticed. It was rather unexpected to find that the complementary activity increased more or less after the sera remained in contact with the clot for forty-six hours, when compared with the activity of the same sera left in contact with the clot for twenty-hours after the bleeding. A few sera, however, became somewhat weaker under the same circumstances. The following analysis gives the results that were obtained.

The average titers for the first (twenty hours) and second (forty-six hours) determinations are nearly the same, being 0.0216 cubic centimeter for the first, and 0.0209 cubic centimeter for the

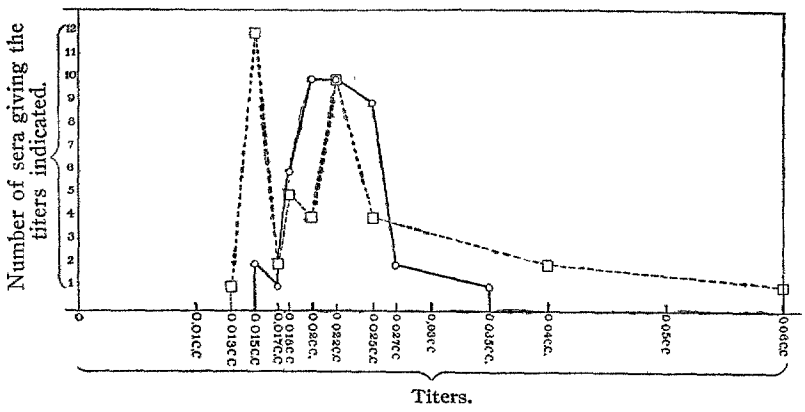
[3] Serum Diagnosis of Syphilis, 1st edition, Philadelphia, 1910, p. 71.

Comparable titers.	Number of sera examined. Determination after 20 hours.	Determination after 46 hours
0.013 c.c.	0	1
0.015 c.c.	2	12
0.017 c.c.	1	2
0.018 c c.	6	5
0.02 c.c.	10	4
0.022 c.c.	10	10
0.025 c.c.	9	4
0.027 c.c.	2	0
0.03 c.c.	1	0
0.035 c.c.	0	0
0.04 c.c.	0	2
0.06 c.c.	0	1
	41	41

second. It will be noticed, also, that the differences between the highest and the lowest titers are much greater with the second determination. To insure greater uniformity in action, it is preferable to employ sera which have been left in contact with the clot for about twenty-four hours.

The titers of these forty-one sera are not absolute; they can be made higher by employing a larger quantity of amboceptor. We sought not absolute, but relative titers in our present experiments, and the following chart shows the frequency with which the same titers were obtained with different sera.

CHART 1.

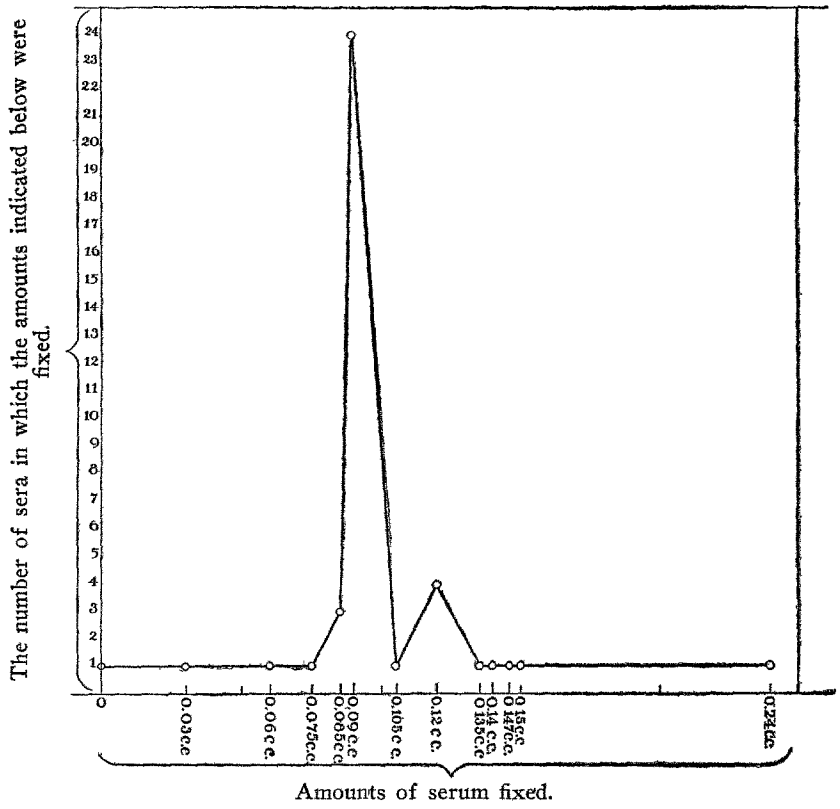


–O– Determination for the sera that remained in contact with the clot for 20 hours (0°C.)
–□– Determination for the sera that remained in contact with the clot for 46 hours (0°.C)

Let us now consider the relative susceptibility of different sera to the fixing phenomenon in the syphilis reaction. Here we have two

Amount of guinea pig serum fixed.	Number of specimens having the titers indicated.	Amount of guinea pig serum fixed.	Number of specimens having the titers indicated.
0.0 (non-fixable)	1	0.12 c.c.	4
0.03 c.c.	1	0.135 c.c.	1
0.06 c.c.	1	0.14 c.c.	1
0.075 c.c.	1	0.147 c.c.	1
0.085 c.c.	3	0.15 c.c.	1
0.09 c.c.	24	0.24 c c.	1
0.105 c.c.	1		
			41

CHART 2.



problems. The first is to determine the absolute quantities of different sera fixed by given constant amounts of syphilitic serum and antigen; and the second is to determine the number of units of complement that have disappeared. The absolute amounts of sera fixed under the same conditions vary considerably with different specimens, but the variation in the majority of the specimens is slight.

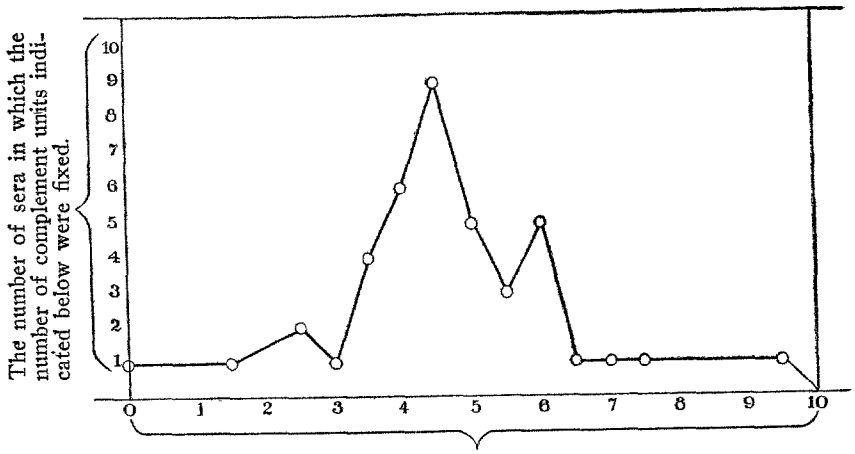
The contrast between the extremes in the fixation of guinea pig serum is most striking. The non-fixable serum (No. 34) showed no abnormal complementary activity, and the highest fixable serum (No. 33) had also the usual titer of activity, although the latter deteriorated with an unusual rapidity during forty-six hours. Another specimen (No. 38) had also shown a rapid deterioration, but there was no abnormality in its fixability.

Taking up the same subject from the standpoint of unity of complementary activity, the following statistics were obtained. In grouping the units, the fractions intermediate between two standard divisions were included in the nearest division. Several arbitrary zones of fixability may be created (see below) in order to enable us to gain a better idea of the occurrence of various degrees of fixability among the sera thus examined. In the zone of normal fixability

Number of units of complement fixed.		
0.	1	Zone of non-fixability, 1 specimen (2.4 per cent.).
1.5	1	Zone of inferior fixability, 8 specimens (19.5 per cent.).
2.	0	
2.5	2	
3.	1	
3.5	4	
4.	6	Zone of normal fixability, 20 specimens (48.7 per cent.).
4.5	9	
5.	5	
5.5	3	Zone of superfixability, 12 specimens (29.2 per cent.).
6.	5	
6.5	1	
7.	1	
7.5	1	
8.	0	
8.5	0	
9.	0	
9.5	1	
	41	

are included the specimens which varied from the average by 0.5 of a unit in either direction.

CHART 3.



The average amount of serum fixed was 0.098 cubic centimeter (see Table I, p. 71) and the average number of units fixed was 4.64 (see Table I, p. 71). In fact, the majority of sera possessed a susceptibility to fixation not far removed from the average.

SUMMARY.

The following conclusions may be drawn from the foregoing series of experiments. The complementary activity varies within a definite limit in different specimens of guinea pig serum. With sera which stood in contact with the clot for twenty hours, the strongest and weakest were in the ratio of 0.015 cubic centimeter to 0.04 cubic centimeter. The former was 2.66 times stronger than the latter. The variation observed with the same series of sera after forty-six hours was still more striking. The strongest was 0.013 cubic centimeter, and the weakest, 0.06 cubic centimeter, that is, the former was 4.6 times stronger than the latter. These findings agree with those made by Massol and Grysez.[4] The variations

[4] Massol and Grysez, *Compt. rend Soc. de biol.*, 1910, lxvii, 588.

were not so marked with the majority of sera. It is noteworthy that a large number of the sera gained in the complementary activity when remaining in contact with the clot for forty-six hours, while some sera became weakened during the same length of time.

The amount of serum *fixed* by given constant quantities of syphilitic serum and antigen varies much more markedly than the variations in their *complementary* activity. One serum failed altogether to be fixed. On the other hand, one sample of serum was so easily fixable that 0.24 cubic centimeter (corresponding to 9.6 complement units of this specimen) disappeared, while the average quantity fixed was only 0.098 cubic centimeter (corresponding to 4.64 complement units). The normal standard of fixability was shown in about 50 per cent. of the specimens examined. If the zone of normal fixability is enlarged in both directions by one unit, the percentage of normal fixability would become 65.8. There is no definite relationship between the complementary activity and the fixability of a given specimen of guinea pig serum.

The facts derived from our present experiments, especially in regard to the exceptions in the fixative quality of this serum, demand the utmost precaution from those intending to employ it for diagnostic purposes, as, for example, in the Wassermann reaction. No quantitative work is possible with the complement fixation reaction unless the experimenter is capable of determining the fixability of the serum in use. One of us (Noguchi) has long realized this source of error, and in order to reduce it he has advised the employment of a mixture of sera from more than two guinea pigs.