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THE INTERFERENCE OF INACTIVE SERUM AND EGG-WHITE IN THE PHENOMENON OF COMPLEMENT FIXATION.[1]

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Is the fixative action of a specific antigen-antibody combination directed only towards the serum constituents known as complement or towards any protein whose physico-chemical properties with regard to the fixation phenomenon are similar to complement? This question needs experimental determination, as the complementary activity of a given specimen of serum has no constant quantitative relationship to the amount of other non-complementary protein constituents contained in the same serum.

If the fixation were directed exclusively towards the active complementary constituents, we could measure quantitatively the fixing capacity of the combination of a given specific antigen and antibody by simply estimating the units of complement fixed. On the other hand, if the fixation is also directed towards the other indifferent proteins simultaneously present in the complement-containing serum, the quantitative estimation of the fixative capacity of the antigen-antibody combination becomes complicated; we cannot measure it by the units of complement fixed, for the fixation is also shared by the other serum components whose exact amounts cannot be ascertained by this method. A great miscalculation of the real fixing capacity must inevitably result when one uses as indicator the complementary activity alone. This source of error is steadily increased by the gradual disappearance of the complementary activity from the serum, while the amount of the other non-complementary constituents not only remains undiminished, but is increased by the gradual conversion of the active complement into an

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inactive complementoid. Before this fundamental relation is established, any attempt to estimate complement fixation quantitatively is premature.

In this communication we present the results which we obtained in studying the effects of various non-complementary proteins upon the complement fixation phenomenon, including the syphilis reaction. In our study we employed, as usual, the antihuman hemolytic system recommended by Noguchi. Every factor concerned in this system is under perfect quantitative control.

THE SYPHILIS REACTION.

In this series the effects of various inactivated or spontaneously deteriorated sera and egg-white were studied. Varying quantities of each serum and of egg-white, in a twenty per cent. dilution in 0.9 per cent. salt solution, were added under three different conditions to the fixing mixture of syphilitic serum and antigen (acetone-insoluble fraction of lipoid from human liver). They were added and incubated (1) before the addition of complement; (2) at the same time as the complement; and (3) after the complement had been fixed. Some tubes without any serum or egg-white were, of course, provided as controls. The amount of syphilitic serum used was 0.02 cubic centimeter for each tube, and this amount was capable of fixing 0.08 cubic centimeter of guinea pig complement in the presence of 0.1 cubic centimeter (four units) of the antigen emulsion. In the case of non-anticomplementary sera, the amount of complement used was uniformly 0.04 cubic centimeter of fresh guinea pig serum. On the other hand, certain deteriorated sera showed a more or less anticomplementary property. This had to be overcome by adding, in addition to the 0.04 cubic centimeter of complement, the exact amount of active complement (guinea pig serum) necessary to neutralize each such specimen. The total volume in each tube was made equal to one cubic centimeter. The incubation period was one hour at 37° C. The first and third series in Table I were twice incubated, as this was required by the nature of the experiments. Then 0.1 cubic centimeter of a 10 per cent. suspension of washed human corpuscles and two units of anti-

TABLE I.

	1st series. Inactive serum added before complement.			2d series. Inactive serum and complement introduced at same time.			3rd series. Complement added before inactive serum.		
	0.1	0.05	0.03	0.1	0.05	0.03	0.1	0.05	0.03
Guinea pig serum No. 1, inactivated by age. . .	C.H.	Mch.H.	Sl.H.	Mch.H.	Md.H.	Tr.H.	No H.	No H.	No H.
Guinea pig serum No. 2, inactivated by age. . .	C.H.	Mch.H.	Sl.H.	Mch.H.	Md.H.	Tr.H.	No H.	No H.	No H.
Guinea pig serum No. 3, inactivated, 56°C. . .	C.H.	Md.H.	Sl.H.	Mch.H.	Md.H.	Tr.H.	No H.	No H.	No H.
Guinea pig serum No. 4, inactivated, 56°C. . .	C.H.	Md.H.	Sl.H.	Mch.H.	Md.H.	Tr.H.	No H.	No H.	No H.
Human serum No. 1, inactivated by age.....	Sl.H.	No H.	No H.	Tr.H.	No H,	No H.	No H.	No H.	No H.
Human serum No. 2, inactivated by age.....	Mch.H.	Sl.H.	No H.	Sl.H.	No H.	No H.	No H.	No H.	No H.
Human serum No. 3, inactivated by age.....	No H.	No H.	No H.	No H.	No H.	No H.	No H.	No H.	No H.
Human serum No. 4, inactivated, 56°C.	C.H.	Mch.H.	Sl.H.	Mch.H.	Sl.H.	No H.	No H.	No H.	No H.
Human serum No. 5, inactivated, 56°C.	Mch.H.	Sl.H.	No H.	Md.H.	Sl.H.	No H.	No H.	No H.	No H.
Sheep serum No. 1, inactivated by age	C.H.			Mch.H.			No H.		
Sheep serum No. 2, inactivated 56°C.	C.H.			Mch.H.			No H.		
Pig serum, inactivated by age.....	C.H.			Mch.H.			No H.		
Horse serum, inactivated, 56°C.	C.H.			Mch.H.			No H.		
Cat serum, inactivated, 56°C.....	C.H.			Mch.H.			No H.		
Dog serum, inactivated, 56°C.....	Mch.H.	Sl.H.	No H.	Sl.H.	No H.	No H.	No H.		
Goat serum No. 1, inactivated by age.... ..	Sl.H.	No H.	No H.	No.H.	No H.	No H.	No H.		
Goat serum No. 2, inactivated, 56°C.	Mch.H.	Sl.H.	No H.	Md.H.	Sl.H.	No H.	No H.		
Chicken serum, inactivated by age.....	C.H.	Mch.H.	Sl.H.	Mch.H.	Sl.H.	No H.	No H.		
Chicken serum, inactivated, 56°C.....	C.H.	Mch.H.	No H.	Md.H.	Tr.H.	No H.	No H.		
Egg-white (20%), unheated	C.H.	Almost C.H.	Md.H.	Almost C.H.	Mch.H.	Sl.H.	No H.	No H.	No H.
Egg-white (20%), heated to 56°C.....	C.H.	Mch.H.	Sl.H.	Mch.H.	Sl.H.	No H.	No H.	No H.	No H.

human amboceptor (rabbit) were added to all tubes, mixed well, and incubated for two hours at 37° C. The results are given in Table I.

The foregoing experiments demonstrate several important points. The first series shows that the addition of various animal sera, from which the complementary property had disappeared either by a spontaneous deterioration or by an artificial inactivation, to the fixing mixture of syphilitic serum and antigen, saturated completely the latter's fixing capacity so that it was no longer capable of fixing the complement of fresh guinea pig serum subsequently introduced. Exceptions were encountered with human and goat sera, which behaved inconstantly. While some of the human sera interfered with the fixation to the same degree as that of most animal sera, others showed but little interference. The serum of the goat was also comparatively less interfering. The most remarkable fact in the series is that the egg-white acted in the same way as most animal sera.

In the second series we observe somewhat less interference of fixation by the sera and egg-white. This is not at all surprising, because of their simultaneous addition with the complement, under which condition the fixing had been directed to both. In the third series there was no hemolysis. It is evident that the subsequent addition of these substances had no reversing effect upon the complement, which had been fixed before they were introduced.

The deductions which may be drawn from these observations are that the fixing capacity of the syphilitic serum and antigen can be saturated not only by active complement-containing sera or inactive sera, but also by an apparently indifferent suspension of proteins (egg-white), so long as their physico-chemical properties are the same. It also shows how erroneous it is to estimate the fixing capacity by the estimation of one biological property (complementary) without reference to the ratio of this biological activity to the other components of the serum serving as complement. For quantitative work with the complement fixation test, the establishment of a standard ratio between the complementary unit and the volume of the serum, becomes essential. In addition to this,

the relation of the fixability of the sera of different species must be considered.

The Complement Fixation by Specific Precipitate (Bordet-Gengou Phenomenon).—Parallel series of experiments with the foregoing were also made with the precipitate produced by the anti-meningococcic horse serum (Flexner and Jobling) and meningococcic extract.

The results obtained were in perfect harmony with those of the syphilis reaction just described; hence protocols are not called for.

The fixability of these sera and egg-white diminished progressively at temperatures above 56° C., and disappeared at 85° C.

Alcohol, which coagulates proteins, removes the interfering property of sera and egg-white.

CONCLUSIONS.

The fixing property of a specific precipitate and of syphilitic serum in the presence of certain antigenic lipoids, can be removed by adding certain non-complementary proteins of blood serum or hen's egg.

This disappearance of the complementary activity in the syphilis reaction, as well as in the true Bordet-Gengou reaction, is a phenomenon which incidentally accompanies the fixation of certain serum constituents, some of which possess a complementary activity. The presence or absence of the complementary property in these protein components does not influence fixation. Whether the disappearance of the complementary activity during the phenomenon of so-called fixation is due to a mechanical precipitation of the molecules through absorption or whether it is due to a physico-chemical alteration of the active molecules, is unknown. It is more probable that a chemical interaction takes place in the case of the syphilis reaction. Certain sera, for example, those derived from man and goat, show a low fixability.

It is interesting to note that the fixability is gradually diminished when these sera and egg-white are heated to a temperature above 56° C., and totally disappears at 85° C. The coagulation of proteins with absolute alcohol or by boiling, destroys their interfering property.

The fact that the fixation is not selectively directed towards complement, has a very important meaning for exact serology. The one-sided accuracy as to the complementary unity is no longer sufficient for quantitative work. Both the complementary and the volumetric unity of a serum serving as the source of complement should be taken into consideration. Besides, the fixability of the sera of various species of animals must also be considered.

From these facts a formula may be derived for deciding the degree of suitableness of a serum.

$$X = K\frac{P}{V}$$

X is the degree of suitableness; K, the species constant for the fixability; P, the complementary activity; and V, the volume of serum. It will be seen that the suitableness is proportional to the fixability constant and the complementary unity, and inversely proportional to the volume of serum employed.

As to what species yields the largest value for X, we refer the reader to our studies published elsewhere.[2]

[2] (1) Variations in the Complement Activity and Fixability of Guinea pig Serum; (2) Comparative Merits of Various Complements and Amboceptors in the Serum Diagnosis of Syphilis, this number of the *Journal of Experimental Medicine.*