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Zone Phenomenon in Complement Fixation. Relation to Greater Sensitivity of Ice Box Type of Wassermann Reaction.

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Two hypotheses were recently presented for the explanation of the mechanism of zoning occurring in complement fixation—one by Levine,¹ the other by Eagle.² The two hypotheses rest on principles which possess no element in common. Therefore, I undertook a critical study of the subject matter. In this paper are presented in brief the results of the study of the theory of Eagle.

The latter can be briefly summarized as follows: Serum in concentrations greater than 1:25 effects an inhibition of complement fixation through the adsorption of fixation-preventing substances presumably present in negative serum.³ These inhibiting substances are adsorbed by the antigen-antibody complexes after they had become fully organized. The adsorption is more intense the more negative a serum is, and corresponds roughly to the concentration of the serum protein. Hence, in weakly positive serums a more pronounced fixation of complement will occur in the higher serum dilution, and hence, the zone phenomenon. This same inhibition, Eagle states, is less marked at lower temperatures, and hence, in an indirect manner it is also responsible for the greater sensitivity of the icebox test.

Experimental studies were carried out as follows: (1) Complement and amboceptor titrations in the presence of high concentrations of negative serum; (2) complement fixation tests with Kahn antigen precipitated from positive and negative reactions⁴ in

TABLE I.

The effect of the addition of some negative serum on the results of the complement titration.

Cc. of 1-50 complement		0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50	0.55	0.60
Diluent	Control tit'n	*											
	saline only	100%	100%	100%	100%	90%	90%	50%	50%	25%	10%	10%	5%
	Plus 0.25 cc. negat. serum	100%	100%	90%	80%	50%	25%	25%	25%	5%	5%	Tr.	None

* Percentages indicate amount of unhemolyzed red cells.

¹ Levine, B. S., *J. Inf. Dis.*, 1931, **48**, 189.

² Eagle, H., *J. Exp. Med.*, 1931, **53**, 615.

³ Eagle, H., *J. Lab. Clin. Med.*, 1933, **18**, 725.

⁴ Levine, B. S., *J. Lab. Clin. Med.*, 1929, **14**.

the presence of high negative serum concentrations; (3) the effect of the removal of the native antishoop amboceptor from negative serum and the bearing of this procedure on the assumption that the results of Eagle's original experiments were influenced by natural amboceptor.

TABLE II.

The effect of hemolysing and non-hemolysing serum on the results of the quantitative complement-fixation reaction.

Cc. of positive serum		0.50	0.25	0.125	0.0625	0.0313	0.0156	0.0078	0.0039
Diluent	Saline	4*	4	4	4	4	2	1	Trace
	Serum A	4	4	4	4	2	1	Trace	None
	Serum B	4	4	4	3	2	2	2	1

* The numerals indicate degree of hemolytic inhibition in conventional terms. Serum A—hemolytic, Serum B—non-hemolytic.

TABLE III.

The effect of negative serum on the results of the quantitative complement-fixation reaction after the absorption of the native amboceptor.

Cc. of positive serum		0.50	0.25	0.125	0.0625	0.0313	0.0156	0.0078	0.0039
Diluent	Saline	4	4	4	4	4	2	1	Trace
	Serum A ₁	4	4	4	4	4	3	2	1
	Serum B ₁	4	4	4	4	4	3	2	2

The results yielded by this critical study warrant the following conclusions: There is no evidence indicating the normal presence in negative serum of substances which inhibit the fixation of the complement by the antigen-antibody complexes either before or after they have become fully organized. It is erroneous to assume that some hypothetical substances are adsorbed by the antigen-antibody to a lesser degree at lower temperatures. One of the characteristics of adsorption in general is the fact that it progresses more slowly but reaches a higher maximum at lower temperatures.⁵ It is precisely this characteristic of adsorption as a physicochemical phenomenon that is responsible to a large degree for the greater sensitivity of the icebox Wassermann. The validity of the quantitative Wassermann technic, although subject to some qualifications, is not affected by the results of Eagle's experiments. The latter were probably due to the native hemolytic properties of the negative serum used by that author. There is no discernible relationship between the cause of the zone phenomenon and that of the greater sensitivity of the icebox Wassermann. Hence, the hypothesis of Eagle is not tenable.

⁵ Ware, J. C., *The Chemistry of the Colloidal State*. John Wiley and Sons, Inc. New York, 1930.