THE SERUM CONSTITUENTS RESPONSIBLE FOR THE SACHS-GEORGI AND WASSERMANN REACTIONS.

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SINCE the Sachs-Georgi or flocculation reaction was first described as a practical serum test for syphilis, the relationship of this reaction to the Wassermann or complement-deviation test has been a subject of some interest to serological workers. It has been supposed that the precipitation or flocculation effect and the complement-deviation reaction are dependent on the same underlying interaction between the syphilitic serum and the lipoid antigen. Undoubtedly the two reactions tend to correspond closely when estimated quantitatively with different sera. On the other hand this correspondence is by no means constant.

In analysing serological reactions, the investigation of the different serum constituents has generally yielded information of some significance. This method has been extensively applied in the study of serum-complement (Liefmann, 1909, Sachs and Altmann, 1909, Browning and Mackie, 1913, 1914, and others).

With a view to ascertaining whether the flocculation reaction was a function of the same serum constituents as the Wassermann effect, a number of sera which reacted positively in both tests, were fractioned by the methods adopted in the analysis of complement, and the flocculating and the complement-deviating effects of the fractions were quantitatively compared with those of the native serum.

A heart-cholesterin antigen was used for both the Wassermann and Sachs-Georgi reactions. In the complement-deviation reaction fixed amounts of antigen dilution and serum were tested with varying doses of guinea-pig's complement; the details of the method employed were as described by Mackie and Rowland (1920). The flocculation test was carried out by a quantitative method in which varying dilutions of serum were tested with a fixed quantity of antigen emulsified in normal saline (1 in 6), *vide* Table I; the mixtures were placed in agglutination tubes, incubated for three hours at 37° C. and then allowed to stand overnight at room temperature (*vide* Mackie, 1921).

When serum is diluted with nine volumes of ice-cold distilled water and carbon dioxide gas is then passed through the mixture, a precipitate is obtained which represents the euglobulin and a portion of the pseudoglobulin of the serum (Browning and Mackie, 1914); the albumin and the remainder of the pseudoglobulin remain in solution. In this way two serum fractions can be obtained, which, as regards complement-containing serum, represent separate components of complement—the so-called "mid-piece" (carbonic-acid-insoluble) and "end-piece" (carbonic-acid-soluble). These fractions have been designated also the "globulin" and the "albumin" fractions, though the former contains only a portion of the globulin and the latter a considerable proportion of globulin as well as albumin.

Syphilitic sera, which had been previously heated at 55° C. for half an hour, were "split" by the carbon dioxide method of Liefmann (1909) and it was found that the activity of the serum in the flocculation reaction depended almost entirely on the carbonic-acid-soluble fraction (Table I), as has been shown by Sahlmann (1921).

Table I. Sachs-Georgi Reaction.

Serum withdrawn 7. 9. 22.

0·4 c.c. varying dilutions of serum or serum fractions +0·2 c.c. cholesterinized heart extract antigen diluted 1 in 6 with normal saline.

Date of test		SERUM DILUTIONS. 1 in 2	1 in 4	1 in 8	1 in 10	1 in 16	1 in 32	1 in 64
8. 9. 22	unheated serum	+ + + +	+ +	+ + +	.	–	–	–
	serum, 55° C.	+ +	+ + + +	+ + + +	.	+ + + +	+ + +	–
11. 9. 22	unheated serum	–	–	–	.	–	–	–
	serum, 55° C.	–	+	+ +	.	+ +	+	–
11. 9. 22	from heated serum carbonic-acid-insoluble fraction (precipitate dissolved in an amount of normal saline equal to 2 vols. of the serum from which it was derived)	–	–	–	.	–	–	–
	carbonic-acid-soluble fraction, representing a 1 in 10 dilution of the original serum	.	.	.	+ + + +	+ + + +	+ + +	–
	Both fractions together	.	.	.	.	+	+	–

In the tables the number of + marks denotes the degree of flocculation; + + + + = complete precipitation, supernatant fluid clear; – signifies absence of flocculation; readings made after 24 hours.

Further experiments were then carried out in which the carbonic-acid-soluble fraction was "split" into pseudo-globulin and albumin by the ammonium sulphate method (according to the technique utilized by Browning and Mackie in the analysis of complement). In this way three serum fractions were obtained: (1) carbonic-acid-insoluble globulin, (2) carbonic-acid-soluble pseudo-globulin, and (3) albumin. It was found that the flocculating effect of the serum was almost entirely a function of the carbonic-acid-soluble pseudo-globulin and that the carbonic-acid-insoluble fraction, as previously noted, was inactive and inhibitory. The albumin was inactive but non-inhibitory. (Table II A shows the results of one of these experiments.)

These three fractions were also studied as regards their Wassermann effects[1] and it was noted that the most active constituent was the carbonic-

[1] The fractions obtained by the ammonium sulphate method were not anticomplementary by themselves.

acid-insoluble globulin, though the carbonic-acid-soluble pseudo-globulin also exhibited a weak reaction; the full effect of the serum was elicited by combining the two globulin components; the albumin was inactive (Table II B). Kapsenberg (1921) has also shown recently by the ammonium sulphate method that the Wassermann reaction depends entirely on the globulin of the serum.

Table II A. Sachs-Georgi Reaction.

Blood withdrawn 14. 9. 22.

0·4 c.c. varying dilutions of serum + 0·2 c.c. of cholesterinized heart extract antigen diluted 1 in 6 with normal saline.

	Serum dilutions ...	1 in 2	1 in 4	1 in 8	1 in 10	1 in 16	1 in 32	1 in 64
	Amounts of pure serum in c.c. represented by these dilutions	0·2	0·1	0·05	0·04	0·025	0·0125	0·0062
Date of test								
15. 9. 22	unheated serum	+ +	+ +	+ +	.	+ + +	+ +	+
	serum, 55° C.	+ + + +	+ + + +	+ + + +	.	+ + +	+ +	+
18. 9. 22	unheated serum	+ +	+ +	+ +	.	+ +	–	–
	serum, 55° C.	–	+ +	+ + + +	.	+ + +	+ +	–

Varying amounts of serum fractions, expressed in terms of the amount of native serum from which they were derived (made up with normal saline to a volume of 0·4 c.c. where the actual quantity to be tested was less than this volume) + 0·2 c.c. of antigen diluted 1 in 6.

	From heated serum	c.c. 0·2	c.c. 0·1	c.c. 0·05	c.c. 0·04	c.c. 0·025	c.c. 0·0125	
18. 9. 22	carbonic-acid-insoluble fraction	–	–	–	–	–	–	.
,,	carbonic-acid-soluble fraction	.	+ + + +	+ + + +	+ + + +	+ + +	+ +	.
,,	both these fractions together	.	.	+	+	–	–	.
19. 9. 22	carbonic-acid-soluble pseudoglobulin	+ + + +	+ + + +	+ + + +	.	+ + +	+ +	.
,,	albumin	.	–	–	.	–	–	.
,,	carbonic-acid-soluble pseudoglobulin + albumin	.	+ + + +	+ + +	.	+ + +	+ +	.
,,	carbonic-acid-soluble pseudoglobulin + carbonic-acid-insoluble globulin	.	–	–	.	–	–	.

Thus, on analysing sera in this way, it has been shown that the fraction mainly responsible for the Wassermann reaction is inactive in the flocculation test and even inhibitory to the active constituent, which is only weakly effective in the complement-deviation test.

By this method of analysis the two reactions appear to be independent and complement-deviation can hardly be regarded as dependent on the formation of a precipitate in the mixture of syphilitic serum and lipoid antigen, for a marked Wassermann effect occurs with a serum fraction which is inactive

Table II B. Results of the Wassermann Reactions carried out with the original serum and the various fractions derived from the heated serum.

Serum is the same as in Table II A.

original serum (55° C.)	deviated	14	doses of	complement
carbonic-acid-insoluble globulin	,,	10	,,	,,
carbonic-acid-soluble fraction	,,	4	,,	,,
carbonic-acid-soluble pseudoglobulin	,,	4	,,	,,
albumin	,,	0	,,	,,
carbonic-acid-insoluble globulin + carbonic-acid-soluble pseudoglobulin	,,	14	,,	,,

The amounts of the fractions used were all strictly comparable with one another and with the amount of serum used in the test.

Table III. Sachs-Georgi Reaction. Representative examples exhibiting the varying behaviour of different specimens of serum.

0·4 c.c. varying serum dilutions + 0·2 c.c. antigen (*vide* Table I).

Serum dilution...		1 in 2	1 in 4	1 in 8	1 in 16	1 in 32	1 in 64
Serum no.							
(1)	unheated	–	–	+	+ + +	+ + +	–
	55° C.	+ + + +	+ + + +	+ + + +	+ + + +	+ + +	–
(2)	unheated	+ + + +	+ +	+ +	–	–	–
	55° C.	+ +	+ + + +	+ + + +	+ + + +	+ + +	–
(3)	unheated	+ +	+ +	+ +	+ + +	+ + +	+
	55° C.	+ + + +	+ + + +	+ + + +	+ + +	+ + +	+ + +
(4)	unheated	+ + +	+	–	–	–	–
	55° C.	+ + + +	+ + + +	+ + +	–	–	–
(5)	unheated	+ + + +	+ + + +	+ + +	+ +	–	–
	55° C.	–	+ +	+ + +	+ +	–	–
(6)	unheated	+ + + +	+ + + +	+ + +	+ +	–	–
	55° C.	+	+ +	+ +	+	–	–

Readings after 24 hours.

in the precipitation test and the full Sachs-Georgi reaction is elicited with a fraction that is only slightly active in the Wassermann test.

Heating of the serum to 55° C. for half an hour is an important factor in intensifying the flocculation effect; on the other hand, in some instances, heated serum may be less active than fresh serum. This is illustrated in Table III (Nos. 5 and 6); such behaviour has been repeatedly observed with different

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specimens of serum. Heating acts apparently by diminishing the inhibitory effect of the inactive globulins (Table IV).

It has also been noted on many occasions with various specimens of serum that the maximum Sachs-Georgi effect may only be obtained with sera from recently withdrawn specimens of blood. Sera kept for a few days tend to show a weaker reaction and marked zone effects (Tables I and II A). This is apparently due to an increased inhibitory effect on the part of the inactive globulin fraction as shown in Table I; thus, the carbonic-acid-soluble fraction is more active by itself than the whole serum four days after withdrawal of the blood, and represents the original activity of the serum from the fresh specimen.

Table IV. Sachs-Georgi Reaction.

0·4 c.c. varying dilutions of serum or serum-fractions + 0·2 c.c antigen dilution (*vide* previous tables).

Serum dilutions ...	1 in 2	1 in 4	1 in 8	1 in 10	1 in 16	1 in 32	1 in 64
Serum							
Unheated	−	−	+	.	+ + +	+ + +	−
55° C.	+ + + +	+ + + +	+ + + +	.	+ + + +	+ + +	−
From unheated serum							
carbonic-acid-soluble fraction diluted 1 in 10	.	.	.	+ + + +	+ + + +	+ + +	+ +
carbonic-acid-insoluble fraction diluted 1 in 2	+	−	−	−	−	−	−
Both fractions together	.	.	.	.	+ +	+ +	−
From heated serum							
carbonic-acid-soluble fraction diluted 1 in 10	.	.	.	+ + + +	+ + + +	+ + +	+
carbonic-acid-insoluble fraction diluted 1 in 2	+	+	−	−	−	−	−
Both fractions together	.	.	.	.	+ + + +	+ + +	+

Readings after 24 hours.

To elicit optimum results therefore in the Sachs-Georgi reaction, serum should be used for the test as soon as possible after removal of the blood and both heated and unheated specimens should be tested. In an older specimen, the maximum effect may be obtained by fractioning with carbon dioxide and testing the carbonic-acid-soluble fraction.

This study of the serum fractions in the Sachs-Georgi reaction has elicited information regarding the mechanism of the reaction which is of considerable theoretic and practical interest.

The inhibitory effect of one serum fraction on the action of another has already been emphasized in reference to other serum reactions (Mackie, 1920) and the practical significance of this effect is also well exemplified in these experiments.

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