CONCERNING THE WASSERMANN REACTION

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THE intention is to draw attention to (*i*) certain features of the technique, (*ii*) the false reactions, (*iii*) the Wassermann positive rate for Indians, and (*iv*) a new name for the Wassermann reaction, namely, Lecithin Complement Fixation (LCF).

I. Certain features of the technique

1. *The titrated controls.*—The usual positive control put up from a 1 in 5 dilution of a positive serum left over from the last test, or even intentionally collected and kept for some time, is a very poor control. Some positive sera give a complete fixation of the usual one or more MHD of the complement in a 1 in 200 dilution; others can do so in 1 in 5 dilution only. The margin of variation allowed for in this control, 5 to 200, is too wide to be useful in indicating whether the antigen-antibody systems involved in the reaction of complement fixation are working with the usual sensitiveness and specificity on a particular day.

The writer pools and preserves sera fixing 4 MHD of complement with an uncholesterinized antigen (Greval, Das and Sen Gupta, 1938; Greval, Chandra and Das, 1940). A 1 in 50 dilution of the pooled sera gives a complete fixation and a 1 in 100 dilution an incomplete fixation with the usual cholesterinized antigen and the smaller dose of the complement. Sometimes the potency of the serum is greater. The potency is determined by titration. It keeps unchanged until the serum is exhausted and yields repeatable reactions, day after day, for months.

It is pointed out that without the employment of titrated positive controls, the Wassermann reaction of borderline cases of syphilis is not repeatable and therefore not reliable.

The writer is aware of the reported seasonal variability of the Wassermann reaction (Hoverson *et al.*, 1935). He has, however, personal experience of the seasonal variability of the fixability of the complement. The latter variability must be excluded by the titration controls; otherwise an increase or decrease in the reaction of a serum may result from this cause alone; in fact the writer believes that more often than not it does so result.

2. *The complement.*—This reagent has six attributes which affect the reaction of fixation significantly. It may be (*i*) of high titre, (*ii*) of low titre, (*iii*) of optimal titre, (*iv*) cholesterol shy, (*v*) cholesterol fast, and (*vi*) of optimal reaction with cholesterol.

The optimal titre is given by 1 in 40, 50 and 60 according to the scheme of the British Method No. 4 (Medical Research Committee, now Council, 1918). Titres below 40 are low, and above 60 high. Other things being equal, low titre complements are fixed less and high titre complement more than the optimal titre complement.

The optimal reaction with the cholesterol is the one which makes the tubes in the two rows of the titration (with and without antigen) correspond, *i.e.* which ensures that neither more nor less than 1 MHD of the complement will be rendered inert by the cholesterol contained in the antigen. When more than 1 MHD is rendered inert, the complement is cholesterol shy; and when less than 1 MHD is rendered inert, it is cholesterol fast. Other things being equal, the shy complements are fixed more than the fast complements.

The complements displaying the four non-optimal attributes need special adjustments : the number of MHD used is increased or decreased and the cholesterol content of the antigen is raised or lowered. Very briefly, more is used of a high titre and less of a low titre complement than the dose indicated by the titration; less cholesterol is used than in the standard antigen with a shy complement; and either the number of MHD is decreased, or else phenol is added

(*Continued from previous page*)

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to the antigen in the case of the fast complement (Greval, Chandra and Das, *loc. cit.*). With these adjustments, the titrated controls give the same reaction as they do with a complement of optimal titre and reaction. Even when the reaction of the controls falls short of the one expected, a measurement of the departure from the standard reaction is available.

3. *The antigen.*—In the writer's experience, it is not so much the type of the antigen as the quality which makes a significant difference in the fixation of complement. He uses three antigens routinely and four occasionally. They are: (*i*) plain alcoholic heart-extract antigen, (*ii*) McIntosh and Fildes' antigen, (*iii*) Bordet's antigen, and (*iv*) McIntosh and Fildes' antigen phenolized. The first three are used as routine, and the fourth occasionally, for clinically known cases only. The first, second and fourth antigens are made from the same source and third from a standard source.

The plain alcoholic heart extracts vary in their potency in reacting with the kind of serum which provides the titrated controls. In preparing the standard antigen, the extracts must be selected or corrected by concentration, pooled and tested. A reasonably high potency is aimed at and obtained (with a complement of optimal titre and reaction). A good extract fixes 4 MHD of complement with a 1 in 10 dilution of the serum.

McIntosh and Fildes' antigen is made from the plain alcoholic heart extract conforming to the standard. The phenolized antigen is made from the former.

Bordet's antigen is made from pieces of hearts (laid aside for the purpose) which have yielded the standard plain alcoholic extract without concentration. The quantity of this antigen required for the routine test is smaller than that of the others.

specific and differentiates less between new, old, treated and untreated cases; and (*iv*) the cholesterinized alcoholic heart extract phenolized is almost as sensitive yet specific and differentiating.

4. *The rbc suspension.*—Other things being equal, the greater the density of the suspension, the bigger will be the quantity of the complement in 1 MHD, and the less sensitive will be the hæmolytic system. A 5 per cent suspension provides a less sensitive system than a 3 per cent suspension. The density is checked and adjusted if necessary colorometrically (Wyler, 1929; Greval, 1929; Greval, Yesudian and Choudhury, 1930). An unadjusted suspension is quite capable of turning a = reaction into a – reaction and *vice versa*. The error resulting from lack of attention to this point, though significant, is not so serious as those resulting from lack of attention to the complement and the antigen.

The human serum has a natural hæmolytic amboceptor against sheep's red cells. This fact engaged the attention of serologists about twenty years ago. It was feared that some positive human sera might turn negative falsely because of an excess of this amboceptor. Two safeguards were provided: (*i*) the amboceptor was absorbed or (*ii*) human red cell suspension was used. The first safeguard was unnecessary (Greval, 1927), and the second not only provided an inferior hæmolytic system (because of the low titre of the human-rabbit hæmolytic amboceptor) but actually introduced more problems (because of the groups and types) than it solved.

5. *The writer's scheme of a complement fixation test for syphilis.*—

(1) *For serum.*—Five tubes are used as follows:—

	1st tube (serum control)	2nd tube (for +)	3rd tube (also for +)	4th tube (for ++)	5th tube (for +++)
Antigen ..	*nil*	Cholesterinized Bordet.	McIntosh and Fildes	McIntosh and Fildes	Plain alcoholic heart extract.
Complement ..	2 MHD	2 MHD	3 MHD	5 MHD	4 MHD

The procedure of preparing the antigens, has been given in detail previously (Greval, Chandra and Das, *loc. cit.*).

Very briefly, in the test proper, with a 1 in 5 dilution of the unknown sera, (*i*) the plain alcoholic heart extract reacts with strongly positive sera only, designated +++; (*ii*) the cholesterinized alcoholic heart extract reacts with moderate positive and weakly positive sera, designated ++ and + respectively according to the dose of the complement fixed; (*iii*) Bordet's antigen is more sensitive but less

A sixth tube is put up for clinically known cases only. It contains phenolized McIntosh and Fildes' antigen and complement 3 MHD.

For reasons of economy, the test may be split up into two parts: 4th, 5th and 6th tubes are put up only when the 2nd and 3rd tubes show inhibition of lysis of a high degree (+ or a trace of lysis only, designated T).

The results are based on McIntosh and Fildes' antigen and alcoholic heart extract. Cholesterinized Bordet's antigen and phenolized McIntosh and Fildes' antigen, if giving a + reaction,

merely draw attention to a doubtful result or even a negative result with a significant history.

The results are recorded (after the tubes have been in a refrigerator, or at ice-box temperature, overnight, to make sure of traces of hæmolysis) and reported as follows :—

Tubes :—					
1	2	3	4	5	Report
−	−	−	−	−	− Negative.
−	+	+	+	+	+++ Strongly positive.
−	+	+	+/T	−	++ Positive.
−	+	+	±/−	−	+ Weakly positive.
−	+	T	T	−	± Doubtful. Such reactions are not 9/10 to 5/10 positive.
−	+	±	±	−	
−	+	±	−	−	± Doubtful.
−	+	?−	−	−	± Doubtful. Repeated before reporting.
−	+	−	−	−	± Doubtful with significant history. − Negative without significant history.
−	T/±	−	−	−	− Negative.
−	−	T/±	±	−	± Doubtful. A + in tubes 3 and 4 should be retested. T is the likely reaction. A persisting + is duly reported positive.

It will be observed that tube 2 does not count when the plus or minus sign is recorded.

Doubtful reactions or even negative reactions with significant histories are further tested with (*i*) half the dose of the sera and (*ii*) half the dose of the antigen (only McIntosh and Fildes') to exclude paradoxical reactions (less reaction with more reagent).

(2) *For cerebrospinal fluid.*—Cerebrospinal fluid, without inactivation, is also put up with the three antigens in two strengths : (*i*) undiluted fluid, and (*ii*) two volumes of undiluted fluid instead of one volume. The 5 MHD tube is omitted. Inhibition of lysis in the tube containing two volumes of the undiluted cerebrospinal fluid, even when complete, is not accepted as a positive reaction unless there is also a definite inhibition of lysis in the tube containing one volume of undiluted fluid. The results are recorded and reported as follows :—

+++ Strongly positive, when complete inhibition of lysis occurs with the alcoholic heart extract in one or both tubes, subject to the provision concerning two volumes.

++ Positive, when complete inhibition occurs with the McIntosh and Fildes' antigen in both tubes.

\+ Weakly positive, when inhibition is complete with the McIntosh and Fildes' antigen with 2 volumes of the fluid and partial but well marked with 1 volume.

± Doubtful, when partial but well-marked inhibition occurs in one or both tubes with McIntosh and Fildes' antigen.

− Negative, when no inhibition or inhibition of a poor quality occurs with the McIntosh and Fildes' antigen.

The tube put up with Bordet's cholesterinized antigen supports the inhibition of lysis in the corresponding tube put up with the McIntosh and Fildes' antigen.

Paradoxical reactions are excluded.

6. *A complement fixation test for syphilis done with several antigens versus several tests for syphilis.*—The writer suggested in 1939 (Greval, Chandra and Das, *loc. cit.*) that a Wassermann reaction done with several antigens should be preferred to several different reactions done for syphilis. When all is said and done, the flocculation tests have not succeeded in taking the place of the Wassermann reaction. Harrison (1931) 'would not found a diagnosis on a serum test unless the Wassermann reaction was positive'. This statement represents the opinion of most workers even after the issue of the League of Nations' Health Organization Report of the Second Laboratory Conference on the Sero-diagnosis of Syphilis (1928), resolution I of which states regarding the flocculation tests that 'the conference . . . is of opinion that the best of them may be regarded as equal in value to the best of those which depend on fixation of complement (Bordet-Wassermann)'.

II. The false reactions

False positive reactions.—The following conditions have been held responsible for a false-positive reaction (Smith, 1940) :—

'I. Other diseases—leprosy, yaws, malaria, trypanosomiasis, pinta and bejal frequently; septicæmia, endocarditis, pneumonia, tuberculosis, relapsing fever, spotted fever, typhus fever, scarlet fever, infectious mononucleosis, pernicious anæmia, leukæmia, xanthomatosis, severe jaundice and lymphopathea venereum less frequently; others very infrequently.

2. Bacterial growth in serum.

3. Excess of fat and digestive products in serum.

4. Passive transfer from the mother.

5. High barometric pressure increases tendency.

6. Improperly prepared titrated and mixed reagents.

7. Probably some "cured" cases of syphilis'.

Item 6 may be eliminated. It has no place in a standardized and controlled technique.

Attention is drawn to three other conditions : kala-azar (Greval, Sen Gupta and Napier, 1939), lecithinophile eosinophilia (Greval, 1940), and the lecithinophile hepato-gastro-intestinal syndrome (Greval and Sen, 1942).

False negative reactions.—Weak or even negative reactions of the blood in early and late syphilis are well known. What is not so well known is that 'for no apparent cause the bloods of thirty per cent of the patients slowly revert to negative, even though they may show definite signs of syphilis during this period' (Becker, 1937); hence the specially sensitive antigens used for clinically known cases. Even an error on the right side resulting in such cases

being reported 'doubtful' or even 'weakly positive' will suggest to the clinician the need for a thorough examination of the nervous and the cardiovascular system. As a matter of fact 'it is in these cases that persistently weakly positive reactions are found' (Becker).

The writer does not favour a provocative injection which is as likely to accentuate a false reaction as a true one. History, clinical scrutiny, and repetition of the reaction after the subsidence of a passing morbid state are more likely to help. 'One positive test will usually convict a labourer over his denial; two may indict a railroad president or a banker; but I have known three to be insufficient to satisfy a clinician of the "guilt" of a minister' (Becker).

III. Wassermann positive rate for Indians

The writer's figures, much lower than those of previous workers', are as follows (Greval and Sen, *loc. cit.*) :—

Crude rate (found initially) .. 8.7 per cent.
Corrected rate (persisting finally, *without anti-syphilitic treatment*) .. .. 5.3 per cent.

The true latent syphilis rate must be *below* 5.3 per cent in *towns* like Calcutta. *In the country* it must be much lower.

IV. A new name for the Wassermann reaction, Lecithin Complement Fixation (LCF).

The original technique of the reaction was materially different from the present techniques. It depended upon the use of a *restricted quantity of the hæmolytic amboceptor* and an *excess of the complement :* it was a *qualitative test.*. The present-day techniques of most workers in England and America depend upon the use of an *excess of the amboceptor* and a *restricted quantity of the complement :* they provide a *quantitative test.* In America the reaction has been re-named after the serologists who have introduced the modification. The writer suggested in 1940 (Greval, Chandra and Das, *loc. cit.*) that it should be re-named Lecithin Complement Fixation (LCF). The name would be particularly useful in the tropics (and elsewhere too in cases with tropical histories) in allaying the alarm caused by doubtful, '50 to 90 per cent positive' and even positive Wassermann reaction. Diseases other than syphilis can undoubtedly be responsible for the reaction in the tropics.

The active substance in the antigen is presumed to be chiefly lecithin. It may be another allied lipoid, or it may consist of several lipoids. The letter L will represent any of these substances, if necessary.

Summary

1. *Certain features of the technique.*—Titrated positive controls are the only indicators of the day-to-day constancy and comparability of the reaction. The complement has six qualities, four of which make special adjustment with the antigen necessary. The antigen must be standardized with the aid of the titrated controls. The red cell suspension must also be standardized. In the writer's scheme, three antigens are used routinely and four in known cases, and the dose of the antigen and the serum is halved under certain conditions. Complement-fixation tests for syphilis done with several antigens should be preferred to several different tests for syphilis.

2. *The false reactions.*—To the usual list of conditions responsible for false positive reaction should be added (*i*) kala-azar, (*ii*) lecithinophile eosinophilia, and (*iii*) the lecithinophile hepato-gastro-intestinal syndrome. Quite a large percentage of positive cases while still suffering from syphilis turn negative without treatment. Provocative injection is not recommended. Clinical scrutiny and repetition of the doubtful reactions are recommended.

3. *Wassermann positive rate for Indians.*—(*i*) Crude rate (found initially) 8.7 per cent and (*ii*) corrected rate (persisting finally, without anti-syphilitic treatment) 5.3 per cent. The true latent syphilis rate must be *below* 5.3 per cent in towns and much lower in the country.

4. *A new name for the reaction.*—The present day Wassermann reaction is so different from the original reaction that it might as well be re-named Lecithin Complement Fixation (LCF). This name would indicate its basis and also allay alarm in cases with tropical histories.

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