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The Wassermann Reaction: A More Reliable Technique.

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RECENTLY, Browning, Cruickshank and McKenzie have described a new method of performing the Wassermann reaction. It depends on the fact that a syphilitic serum in the presence of an emulsion of lecithin plus cholesterin absorbs much more complement than in the presence of an equivalent amount of an emulsion of lecithin alone, whereas a negative serum absorbs equal amounts with both emulsions.

The lecithin, which is easily obtained from an alcoholic extract of fresh ox liver, is dissolved in sufficient absolute alcohol to make a 0·75 *per cent.* solution. Saturation of this with cholesterin gives the lecithin-cholesterin solution.(2)

The lecithin emulsion is prepared by adding one part of the 0·75 *per cent.* solution to seven parts of a 0·85 *per cent.* NaCl solution. It has been definitely shown by Sachs and Rondoni (1), and confirmed by Browning and McKenzie (2), that the more turbid the emulsion is, the better is the reaction with a syphilitic serum.

The greatest turbidity is obtained by slow mixing of the lecithin and salt solutions, and this can be accomplished if the salt solution is put in a test-tube and the lecithin solution then allowed to run slowly on to its surface, and the tube gently rotated. Lecithin gives a fairly turbid, uniform emulsion.

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The emulsion of lecithin-cholesterin is prepared in the same way ; it is uniform and very dense.

The emulsion of the crude alcoholic liver extract (used by way of control, *vide* below) is similarly prepared.

In carrying out the Wassermann reaction by the lecithin-cholesterin method, the technique generally is similar to that with the crude alcoholic extract as described by Browning and McKenzie (3).

Two series of tubes are arranged :

(A) Contains 0·6 c.c. of lecithin emulsion + 0·05 c.c. of the serum to be tested, previously heated to 57° C. for half an hour.

(B) Contains 0·6 c.c. of lecithin-cholesterin emulsion + 0·05 c.c. of the serum.

Four sets of controls are always set up :

(*a*) Containing 0·6 c.c. of lecithin emulsion alone.

(*b*) Containing 0·6 c.c. of lecithin cholesterin emulsion alone.

(*c*) Containing 0·05 c.c. of the serum plus 0·6 c.c. of 0·85 *per cent.* NaCl solution.

(*d*) Containing 0·6 c.c. of 0·85 *per cent.* NaCl solution alone for the estimation of the complement dose.

Increasing quantities of complement (fresh guinea-pig's serum) are added to the tubes of each series.

After incubation at 37° C. for one and a half hours, 1 c.c. of a 5 *per cent.* suspension of ox's red blood-corpuscles, previously sensitised with five doses of immune body from the rabbit, is added to each tube. They are again incubated at 37° C., shaken at intervals of twenty minutes, and at the end of one and a half hours the tubes are removed from the incubator. The reading at this stage is found to be practically always the same as the final reading taken after the tubes have stood over-night at room temperature.

The sera, about 150 in all, have been examined simultaneously with emulsions of lecithin, lecithin-cholesterin, and crude alcoholic extract ; and in this way it has been found possible to establish the superiority of the lecithin-cholesterin method.

It is well known that an emulsion of lecithin with a syphilitic serum may cause absorption of complement (a positive Wassermann reaction). But this is the case only with powerfully reacting sera. With weaker sera the lecithin may give no more deviation than with a normal serum (see Table I).

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With the lecithin-cholesterin emulsion, in the presence of a normal serum, the deviation is always practically the same as with the lecithin emulsion (Table II).

In only one out of seventy negative reactions did the lecithin-cholesterin series show the absorption of as much as one dose of complement more than the lecithin series. On the other hand, in the case of positive sera the lecithin-cholesterin emulsion always deviates more complement than the lecithin emulsion (Table II). The amount of this increase varies, but with lecithin-cholesterin in the presence of syphilitic serum there may be five times as much complement absorbed as by lecithin; and the increase tends to be specially well marked where the reaction is weakly positive (Table III).

Thus the reaction depends on the relative amounts of complement absorbed by lecithin and lecithin-cholesterin emulsions, and not upon the absolute amount of complement deviated, as in the original method when a crude alcoholic extract is used.

This obviates to a great extent a difficulty which very frequently arises when the crude extract is used, and which is caused by irregularities in the deviability of complement, depending on individual properties of the complement-containing sera. By the new method, therefore, if the absorption of complement by sera in the presence of lecithin and lecithin-cholesterin is equal, there is an undoubted negative reaction. As before mentioned, an increase of one dose with lecithin-cholesterin is within the limit of a negative reaction. But even a small increase (two or three doses of complement more absorbed by lecithin-cholesterin than by lecithin) is in favour of the serum being positive, and a more marked difference (five doses or more) is conclusive under the conditions stated.

The anti-complementary effect of the emulsions of both lecithin and lecithin-cholesterin is a very constant factor. In practically all cases, both deviate to the same extent and rarely more than two or three doses.

Crude extract emulsion compares very unfavourably with this. Constantly one finds it deviating two to three doses more than the other two and not rarely much more (Table IV). This uniformity is a great advantage which the lecithin-cholesterin method possesses. Because of the excessive anti-complementary effect of the crude extract, about a quarter of

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the experiments were vitiated from a diagnostic point of view so far as this reagent was concerned. Under such circumstances the reaction with an undoubted negative serum may appear positive, whereas the lecithin-cholesterin method shows its certainly negative character (Table V). Accordingly the new method effects a considerable saving in time, since only very rarely does one find the lecithin and lecithin-cholesterin emulsions exercising a marked anti-complementary effect, and even then with negative sera the deviation of complement with both emulsions is equal.

A similar difficulty arises in the original method where the serum by itself exerts a marked deviating effect on complement. Here also the advantage of the lecithin-cholesterin method is apparent. Upwards of 150 sera, mostly from asylum cases, have been examined, many of them being repeatedly tested. In general, five or six sera were examined at the same time, and the importance of always having both negative and positive cases in the series was borne in mind. It is considered a point of especial importance to have a known negative serum as a control in every case where the examination is being made for diagnostic purposes. (It is essential in every instance to estimate accurately the inhibitory effect of all the emulsions and of the sera on complement and also to test the complement dose.)

Sixty-five sera from cases clinically diagnosed as general paralysis were examined, and of these 96 *per cent.* gave a positive reaction. It is interesting to note that the blood-serum of one of these cases gave a negative reaction, whereas a positive reaction was obtained with the cerebro-spinal fluid when both fluids were tested simultaneously.

Of other thirty-five mental cases of diverse types chosen at random, in which no history of syphilis was obtainable, 16 *per cent.* gave a positive reaction. This is a most significant result when one considers that these cases did not clinically fall into the category of syphilitic or parasyphilitic psychoses.

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TABLE I.—*Doses of Complement Required to Cause Just Complete Lysis with—*

	0·6 c.c. lecithin emulsion + ·05 c.c. of serum (57° C.).	0·6 c.c. lecithin-cholesterin emulsion + ·05 c.c. of serum (57° C.).	0·6 c.c. crude extract emulsion + ·05 c.c. of serum (57° C.).	0·6 c.c. lecithin emulsion alone.	0·6 c.c. lecithin-cholesterin emulsion alone.	0·6 c.c. crude extract emulsion alone.	·05 c.c. serum (57° C.) + 0·6 c.c. salt solution.
Negative serum .	10	10	10	3	2	2	3
Positive serum .	8	24	24	3	2	2	2

These two sera were examined at the same time. The table shows that a negative serum in the presence of lecithin emulsion may absorb more complement than a positive serum under the same conditions.

TABLE II.—*Doses of Complement Required to Cause Just Complete Lysis with—*

	0·6 c.c. lecithin emulsion + ·05 c.c. of serum (57° C.).	0·6 c.c. lecithin-cholesterin emulsion + ·05 c.c. of serum (57° C.).	0·6 c.c. crude extract emulsion + ·05 c.c. of serum (57° C.).	0·6 c.c. lecithin emulsion alone.	0·6 c.c. lecithin-cholesterin emulsion alone.	0·6 c.c. crude extract emulsion alone.	·05 c.c. serum (57° C.) + 0·6 c.c. salt solution.
Negative serum .	2	2	2	1	1	1	4
Positive serum .	10	26	36	1	1	1	2

These two sera were examined at the same time. The negative serum absorbed equal amounts of complement in the presence of both lecithin and lecithin-cholesterin emulsions, whereas the positive serum showed a marked increase in the amount of complement absorbed in the presence of the lecithin-cholesterin emulsion.

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TABLE III.—*Doses of Complement Required to Cause Just Complete Lysis with—*

	0·6 c.c. lecithin emulsion + ·05 c.c. of serum (57° C.).	0·6 c.c. lecithin-cholesterin emulsion + ·05 c.c. of serum (57° C.).	0·6 c.c. crude extract emulsion + ·05 c.c. of serum (57° C.).	0·6 c.c. lecithin emulsion alone.	0·6 c.c. lecithin-cholesterin emulsion alone.	0·6 c.c. crude extract emulsion alone.	·05 c.c. serum (57° C.) + 0·6 c.c. salt solution.
Negative serum	3½	3½	3½	1	1	2	½
Positive serum	3½	6½	7½	1	1	2	½

This table shows that the lecithin-cholesterin emulsion in the presence of a weakly positive serum gives a marked increase in the amount of complement absorbed. With the crude abstract the reaction is doubtful.

The negative serum, examined simultaneously, serves as a control.

TABLE IV.—*Amount of Complement Absorbed by Emulsions.*

No. of experiment.	Dose of complement.	0·6 c.c. of lecithin emulsion.	0·6 c.c. of lecithin-cholesterin emulsion.	0·6 c.c. of crude extract emulsion.
I	·0075 c.c.	·01 c.c. just complete lysis	·015 c.c. just complete lysis	·035 c.c. marked lysis
II	·005 „	·025 c.c. just complete	·025 c.c. just complete	·035 c.c. just complete
III	·005 „	·015 c.c. just complete	·01 c.c. just complete	·025 c.c. just complete
IV	·005 „	·02 c.c. just complete	·025 c.c. just complete	·03 c.c. trace of lysis
V	·005 „	·015 c.c. just complete	·015 c.c. just complete	·035 c.c trace of lysis
VI	·006 „	·015 c.c. just complete	·0175 c.c. just complete	·03 c.c. distinct lysis

In Table V a negative serum appears positive in the presence of an emulsion of crude extract because of the marked anti-complementary effect of that reagent. The lecithin-cholesterin method shows its true character. A positive serum, examined at the same time, is shown for comparison.

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TABLE V.—*Amount of Complement Absorbed by—*

	0·6 c.c. lecithin emulsion + ·05 c.c. of serum (57° C.).	0·6 c.c. lecithin-cholesterin emulsion + ·05 c.c. of serum (57° C.).	0·6 c.c. crude extract emulsion + ·05 c.c. of serum (57° C.).	0·6 c.c. lecithin emulsion alone.	0·6 c.c. lecithin-cholesterin emulsion alone.	0·6 c.c. crude extract emulsion alone.	·05 c.c. serum (57° C.) + 0·6 c.c. salt solution.
Negative serum .	·04 c.c. just complete	·04 c.c. complete +	·085 c.c. just complete	·015 c.c. just complete	·0175 c.c. just complete	·03 c.c. distinct lysis	·0175 c.c. just complete
Positive serum .	·035 c.c. just complete	·065 c.c. just complete	·09 c.c. just complete	·015 c.c. just complete	·0175 c.c. just complete	·03 c.c. distinct lysis	·0175 c.c. just complete

Dose of complement = ·006 c.c.

(1) A paper read at the Annual Meeting in Edinburgh, 1910.—(2) For fuller details see Browning, Cruickshank and McKenzie, *Journ. Path. and Bact.*, April, 1910.

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The Presence in Blood Sera of Substances which have an Activating or Inhibitory Effect on the Hæmolytic Properties of Cobra Venom.

By CHARLES JAMES ROSS, B.A., M.B., Ch.B. (From the Western Asylums' Research Institute, Glasgow. Director, Dr. Ivy McKenzie.)

MEDICAL science on the experimental side has been productive of no more interesting or fruitful results than those which have appeared in connection with the study of hæmolysis. The groundwork of this subject was laid by Bordet (1) in 1900 by the discovery of the fact that an immune serum contains a thermostable substance (immune

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