A COMPARISON OF SOME NEW FLOCCULATION TESTS FOR THE CEREBROSPINAL FLUID WITH THE WASSERMANN REACTION (M.B.R. II., MODIFIED CITOCHOL AND MODIFIED KISS REACTIONS)*

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In recent years numerous tests have been found of use in detecting syphilitic infection in the blood which might supplement the Wassermann reaction, and in time possibly replace it by reason of their greater simplicity. At the Central Pathological Laboratory of the London County Mental Hospitals the 'Meinicke clarification reaction' (M.K.R.)¹ is now carried out as a routine parallel test to the Wassermann reaction, and has been found to give very reliable confirmatory results.

This test, however, in company with several others has not been found to be nearly so suitable for application to the cerebrospinal fluid. The ideal test should conform to the following rules: It should be (1) sensitive, (2) specific, (3) easy to read, (4) easy to carry out, and (5) the amount of special fluid required should be small. One of us (A.S.P.)² had already worked on these tests at the Deutsche Forschungsanstalt in Munich. There, the Kahn, Sachs-Witebsky and M.K.R. tests were excluded because they were not found to conform well to most of the above conditions, while the M.B.R. II and the citochol reactions were found to give the best results.

In the series of tests under review, therefore, the M.B.R. II was tried, also a modification of the citochol reaction suggested by Professor Sachs which had been successful on the serum but hitherto untried on the spinal fluid, and a modification of the Kiss reaction which appeared to be more successful than the original technique.

The criterion for the estimation of the value of a flocculation test on spinal fluid is comparison with the Wassermann reaction and confirmation by clinical findings. It was observed with the older tests (Sachs-Georgi, Kahn, etc.) that these were nearly always positive when the Wassermann reaction was strongly so. They were, however, generally negative when the latter was weakly positive. Keeping this fact in mind, we endeavoured to obtain material which would contain a fair proportion of cases with weakly

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positive Wassermann reactions. Such material is to be expected from cases of neurosyphilis which have been subjected to malarial, tryparsamide or other forms of therapy. If lumbar punctures are performed some time after completion of a course of treatment all grades of positive Wassermann reactions from + 30 + to negative can be obtained.

MATERIAL

We succeeded in obtaining fluids from 127 cases which had undergone some form of treatment. Our total material consisted of 500 cerebrospinal fluids, composed as follows :—

1. Cases of neurosyphilis (mainly general paralytics and taboparetics) after treatment	127
2. Cases of untreated neurosyphilis	114
3. Cases with no evidence of syphilis of the nervous system ..	259
	—
	500

Among the non-syphilitic material was a large sprinkling of organic nervous disease, e.g. disseminated sclerosis, meningitis, tumours, etc.

Cases with syphilitic disease of the central nervous system (mainly general paralytics) have Wassermann reactions varying in intensity from + 30 + to negative. These numbers were distributed as follows :—

Strong positive Wassermann reaction in fluid	(+ 30)	102
Moderately strong positive Wassermann reaction in fluid	(+ 15)	37
Moderately weak positive Wassermann reaction in fluid	(+ 6)	45
Weak and very weak positive Wassermann reaction in fluid	(+ 3 and + 2)	36
		—
Total positive		220
Total negative		280
		—
Grand total		500
		—

Owing to the scarcity of fluid, not every flocculation test could be carried out in each individual case.

The M.B.R. II reaction was carried out 500 times ; the citochol, 426 times ; the Kiss, 251 times.

In the 220 cases with positive Wassermann reactions the following reactions were carried out in addition to the usual routine test—viz., Wassermann, cells, protein, and Lange : M.B.R. II, 220 times ; citochol 179 times, and Kiss, 118 times. Of the fluids with a negative Wassermann reaction, 280

were tested with the M.B.R. II, 247 with the citochol, and 133 with the Kiss reactions.

NOTES ON THE TECHNIQUE

The Wassermann test was carried out at the Central Pathological Laboratory, using the technique as described by Mann and Partner³ and now accepted as the standard method for mental hospital practice by the Royal Medico-Psychological Association. For the M.B.R. II we made use of the directions published by Professor Müller.⁴

The modified citochol reaction.—In carrying out the modified citochol reaction the technique followed was that described in a personal communication from Professor Sachs, who stated that this modification had been successful on the blood serum, but had not yet been tried on the spinal fluid. The technique is as follows :

1. One part of citochol extract is quickly diluted with two parts of 3 per cent. sodium chloride. After five minutes' standing at room temperature, a further nine parts of the sodium chloride solution are rapidly added. The extract thus diluted 12 times is immediately ready for use.

2. To carry out the test, 0.1 c.c. of spinal fluid, which has been heated to 55° C. for half an hour, is mixed with 0.1 c.c. diluted extract. The test-tubes are then shaken for three minutes, after which 0.5 c.c. of 0.9 per cent. solution of sodium chloride is added. Readings are made immediately and again the following day. A glass magnifying threefold is used. The test-tubes are 16 mm. in diameter and about 8 cm. long. Economy in the amount of fluid put into the test was practised by using half quantities.

Kiss flocculation test.—*Present technique.*—Into two test-tubes (16 mm. × 8 cm.) are placed respectively 1 c.c. of Kiss antigen and 1 c.c. of 10 per cent. sodium chloride solution. These are rapidly mixed together by pouring from one tube to the other. In order to remove the alcohol the mixture is centrifuged, the supernatant fluid poured off and replaced by 1 c.c. of 10 per cent. salt solution. The emulsion is then ready for use.

According to the original technique⁶ 0.2 c.c. of inactivated spinal fluid was added to 0.02 c.c. of the diluted antigen ; but in view of the fact that one of us² had previously obtained better results by the use of 0.01 c.c. of the extract with 0.2 c.c. of fluid these proportions were used with Dr. Kiss's approval.

After adding together the fluid and the reagent the tubes were shaken for five minutes.

If the flocculation appeared immediately after shaking, this was designated strong positive (+++); if after three hours at room temperature, positive(++); if after 20 hours at room temperature, weak positive(+). Cases were designated doubtful which showed an indistinct and scarcely visible flocculation. The reading was made with the aid of a glass of threefold magnification. Reading with the aid of an agglutinoscope did not prove of

any assistance as the antigen of itself displayed a fine precipitation. On this account the reaction was difficult to read and required considerable practice.

SURVEY OF THE RESULTS OBTAINED

The behaviour of the flocculation tests and of the conglobation reaction (M.B.R. II) in relation to the various grades of positive findings of the Wassermann reactions in the cerebrospinal fluid is recorded in the following table :—

TABLE 1

Degree of Positivity of W.R.			+ 30	+ 15	+ 6	+ 3 and + 2
1A						
Percentage positive	M.B.R. II		100·0	86·5	66·7	25·0
" "	Citochol		81·3	44·0	22·9	7·1
" "	Kiss ...		80·1	33·3	17·6	12·5
1B						
" doubtful	M.B.R. II		0·0	5·4	4·4	2·8
" "	Citochol		8·6	4·0	11·5	7·1
" "	Kiss ...		1·4	0·0	0·0	0·0
1c						
" positive and doubtful	M.B.R. II		100·0	91·9	71·1	27·8
" "	Citochol		89·9	48·0	34·4	14·2
" "	Kiss ...		81·5	33·3	17·6	12·5

If we consider table 1A, in which the flocculation tests are compared to the four different strengths of Wassermann reaction, we see that all three flocculation tests are positive in the majority of cases which give a Wassermann reaction of + 30, the M.B.R. II being 100 per cent., whilst the other two tests are only about 80 per cent. positive.

In group II (W.R. + 15) the Kiss and citochol tests have fallen considerably behind in sensitivity, being only about half as sensitive as the M.B.R. II, which in itself was only 86·5 per cent. positive when compared with the Wassermann reaction.

In groups III and IV (W.R. + 6 and + 3 or + 2), the M.B.R. II with 66·7 per cent. and 25 per cent. positive is much superior to the other two reactions. This table shows the superiority of the M.B.R. II over the two other tests employed, and also emphasises the shortcomings of all three reactions when compared with a sensitive Wassermann reaction technique, in the case of weakly positive results.

Table 1B shows the cerebrospinal fluids in which doubtful flocculation tests are compared to positive Wassermann reactions. It has been shown exactly what is meant by 'doubtful' in the reading of each test. The Kiss

reaction showed fewest of these, and the citochol most. In the case of the M.B.R. II the so-called doubtful reactions were, in fact, quite easy to distinguish. In the other two tests they were more difficult.

Table 1c gives a better idea of the true value of the three different tests, and the figures give a much more encouraging idea of their value, especially that of the M.B.R. II.

Regarding the specificity of these reactions, the M.B.R. II gave false positives on three occasions, two of which were normal as far as the central nervous system was concerned, but with positive bloods. The other was a case of disseminated sclerosis.

The citochol gave four false positives, one being a case of cerebral tumour. The Kiss gave one false positive on a normal fluid.

SENSITIVITY OF THE PRESENT WASSERMANN TECHNIQUE

A series of tests such as the present may be used not only to determine the value of the various flocculation reactions but can also be used to compare different techniques of the Wassermann reaction. In the present series the technique employed was that used at the Central Pathological Department of the London County Mental Hospitals.³ It is a technique especially adapted for neurosyphilis. For this purpose relatively large amounts of fluid are used in comparison with the total volume. The figures in the table that follows show how the present technique picks up weak positives, whereas techniques with relatively smaller amounts of fluid fail to do so.

The following table (2) gives the results obtained with the M.B.R. II, citochol and Kiss methods in the previous series, when the Wassermann

TABLE 2

Degree of Positivity of W.R.				Strong	Medium	Weak
2A						
Percentage positive		M.B.R. II	...	95·3	86·1	72·2
"	"	Citochol	...	96·5	68·0	15·0
"	"	Kiss	...	82·9	30·8	0·0
2B						
"	doubtful	M.B.R. II	...	0·0	2·8	5·6
"	"	Citochol	...	1·7	4·3	30·0
"	"	Kiss	...	0·0	0·0	8·0
2c						
"	positive and doubtful	M.B.R. II	...	95·3	88·9	77·8
"	"	Citochol	...	98·2	72·3	45·0
"	"	Kiss	...	82·9	30·8	8·0

technique used was that of the Deutsche Forschungsanstalt at Munich, where relatively smaller quantities of fluid were employed.

When the results obtained with the M.B.R. II in the present series are compared with those in the former series (tables 1c and 2c), it is observed that in the first two columns the results are almost the same, viz., 100.0 per cent. as against 95.3 per cent., and 91.9 per cent. as against 88.9 per cent. In the third column there are 71.1 per cent. compared with 77.8 per cent. In the remaining column, which appears in table 1 only, the figure is 27.8 per cent.

It would appear that the column showing Wassermann reactions of the strength of + 6 in the present series of tests corresponds to the weakest Wassermann reaction obtained in the previous series; and further that the fourth column in table 1, where the Wassermann reaction is expressed as + 3 and + 2, detected a fainter degree of syphilitic change than the test as carried out in the previous series was able to show. In that series there were eight positive M.B.R. II's out of 162 cases of neurosyphilis, where the serological change was not great enough to give a positive result even in the third column, and where, therefore, the Wassermann reaction was recorded as negative. In the present series, there were 11 positive M.B.R. II's out of 220 cases of neurosyphilis, where the change was also not sufficiently pronounced to give a positive result in the third column. Of these 11 cases, however, nine were recorded in the fourth column as + 2 and + 3 positive, and only the remaining two were negative.*

Since then it has been shown that the third column in the present series corresponds pretty closely in sensitivity to the third column in the previous series. It would appear that our weakest strength of Wassermann reaction, as recorded in the fourth column (+ 2 and + 3 positive), includes nearly all those cases which would be too mild to be detected by the previous technique.

Furthermore, with the modified citochol reaction, there were two cases of neurosyphilis where this test was positive and two in which it was doubtful, when the Wassermann reaction was + 2 or + 3, and none in which the latter was negative. Yet in the previous series there were two cases of neurosyphilis with doubtful citochol reactions and a negative Wassermann. Again, with our modified Kiss reaction we had two cases positive when our Wassermann reaction was + 2 or + 3, and none when the latter was negative; but in the previous series there was one case with a negative Wassermann and a doubtful Kiss reaction.

It must be concluded then that the Wassermann technique employed at the Central Pathological Laboratory of the London County Mental Hospitals as the standard method for mental hospital practice is decidedly

* Of these two cases, one was a treated general paralytic, whose fluid contained no excess of cells or protein, the Lange test being negative. The other was clinically a general paralytic, with a vague history of treatment 17 years previously.

more sensitive than techniques such as that employed at Munich, where relatively smaller quantities of spinal fluid are used. Despite this sensitivity, the test remained specific for syphilis. In this series of 500 fluids no false positives were obtained, and in only two cases of neurosyphilis without treatment did a negative result appear. One of these cases was a tabetic with all other tests negative, including the Wassermann reaction in the blood. The second case was clinically one of general paralysis, with excess of cells and protein, a Lange curve of the paretic type and a positive M.B.R. II. The M.B.R. II showed +++ reaction and the citochol $\pm$; the Kiss reaction unfortunately was not done in this case. The blood gave a negative Wassermann reaction and a Meinicke (+++), and there was an uncertain history of treatment for syphilis in 1915.

VALUES OF THE MODIFICATIONS OF THE CITOCHOL AND KISS TESTS

If the second table is compared with the first it will be seen that the modified citochol test is much less efficient than the original, being only positive or doubtful in 89.9 per cent. of the strongly positive Wassermann reactions as against 98.2 per cent. of the original. The Kiss reaction, however, with the present modification approved by Dr. Kiss shows a higher percentage of positive reactions in the fluid where the Wassermann is weak or very weak, than did the original technique (30.1 per cent. as against 8 per cent.). The modification suggested by one of us (A. S. P) is therefore an improvement on the original technique. Nevertheless, the reaction is not nearly so efficient as the M.B.R. II.

DISCUSSION OF RESULTS

The Wassermann reaction as carried out with the present technique remains more sensitive and specific than any of the flocculation tests. This technique was devised especially for cases of neurosyphilis and is more sensitive than those in which relatively smaller amounts of fluid are used.

In a recent publication by J. E. Nicole and E. J. Fitzgerald⁷ it was concluded—though on the basis of only 150 cases—that the Kahn reaction is more satisfactory for the fluid than the Wassermann reaction, while the centrifuge technique of the Müller reaction (157 cases) was found better than the M.B.R. II itself. We found, however, that the Kahn needs too much fluid (3 c.c.), is complicated to carry out, and extremely difficult to read. The M.B.R.Z. was found in preliminary tests to be no more satisfactory, but more complicated to carry out than the M.B.R. II.

We believe that the present technique of the Wassermann test, in which relatively larger amounts of fluid are used than in the technique used by them (Modified Wyler No. 1), is still more sensitive than any of the flocculation tests.

Of the flocculation reactions, the M.B.R. II has been found on the basis of 500 tests to be easy to carry out and to give, even in the so-called doubtful cases, a result which is definite and easy to read. With regard to sensitivity, with the four strengths of Wassermann, it gives results which are 100, 92, 71 and 28 per cent. positive respectively. Only one result was falsely positive out of 500 cases.

The M.B.R. II has one great advantage compared with the Wassermann—it can be used where there is too little fluid for the latter. Where only 0·3 c.c. is available it gives reliable results, while if test-tubes half the size of those described above are used, equally clear results are obtained from as little as 0·15 c.c. This test is therefore well adapted also for animal experiments.

The citochol reaction was more difficult to read and was less sensitive. The Kiss reaction even with the modification suggested was still less useful.

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