# THE WASSERMANN AND MEINICKE KLÄRUNGS REACTIONS (ORIGINAL M.K.R. II AND FORD ROBERTSON-COLQUHOUN MODIFICATION) IN THE DIAGNOSIS OF SYPHILIS.

## AN ANALYSIS OF 3,284 PARALLEL TESTS.

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NICOLE and Fitzgerald's assumption in 1932 that the Wassermann test had at last passed to rest in the limbo of historically interesting experiments can hardly be said to be correct, and the test still remains to-day as the standard method for the sero-diagnosis of syphilis.* At the same time the steadily increasing specificity of flocculation tests and their value as ancillary procedures cannot be denied, and the accepted diagnostic ideal is a parallel examination by one of these and by the Wassermann test.†

One of the most commonly used flocculation tests in mental hospital practice is the Meinicke Klärungs reaction (either the original or the M.K.R. II method), the Kahn test being in more general use elsewhere. For spinal fluids, Meinicke and Hagen-Ambrock's "blue-spot" modification is useful, although its efficiency is marred by the fact that a positive result depends upon the absence rather than the presence of the blue spot. In some hospitals the flocculation tests have entirely superseded the Wassermann test, and it is pertinent to inquire how close a correlation between the two obtains.

Since 1934 the Wassermann and Meinicke (M.K.R. II) reactions of the serum

* The scientific supplement to the Board of Control's Report two years later shows that 41 mental hospitals (including the Central Pathological Laboratory of the L.C.C. Mental Hospitals) were using the Wassermann reaction, and that during the year 18,364 tests were done. It is significant that the test was done in all hospitals in which a pathologist was available. The Meinicke and Kahn tests were used in 14 and 16 hospitals respectively, the Sachs-Georgi in two and the Sachs-Witebsky and the Sigma in one hospital each.

† The term "flocculation test" is here used in a general sense to imply a test in which a visible change occurs in a mixture of serum and antigen.

of all new admissions to this hospital have been determined as a routine, and at the same time the reaction of the spinal fluid has been determined in cases of known or suspected cerebral syphilis. It is upon the results of these examinations that this paper is based.

## MATERIAL.

In all, 6,962 tests have been made during the period under review (Table I), and of these, 3,284 parallel examinations by the two methods are available for analysis.

TABLE I.

| Test. | Blood. | Spinal fluid. | Total. |
|---|---|---|---|
| Wassermann test | 3,382 | 296 | 3,678 |
| Meinicke test (M.K.R. II) | 2,575 | 227 | 2,802 |
| ,, ,, (F.R.C.) | 452 | 30 | 482 |
| Total | 6,409 | 553 | 6,962 |

The majority of the Meinicke tests have been made by the M.K.R. II method with the commercial antigen. Recently, Ford Robertson and Colquhoun (1939) have described a new Meinicke antigen, and we have been able to test this on a parallel series of 482 specimens. This method is referred to as the M.K.R. (F.R.C.) in the text.*

## METHODS.

### *Wassermann Reaction.*

The original Wassermann technique which was used in this hospital employed 1½ m.h.d. of complement for the control tube and 2 and 3 m.h.d.'s for the test proper. At this time Burroughs Wellcome antigen was used exclusively. When necessary a quantitative test was set up, using a series of tubes in which the complement was increased by 2 m.h.d. The inherent disadvantage of this method lies in the abnormally high percentage of doubtful reactions in the 2 m.h.d. tube. Unless the complement-absorbing capacity of the antigen is accurately determined before use more than 1 m.h.d. may be absorbed, and incomplete hæmolysis will result in the 2 m.h.d. tube. The tendency, of course, is for false positive readings to be made. This method was used for 2,952 tests.

To overcome this difficulty the method was changed a year ago to the modified M.R.C. No. 1 method (Wyler, 1929), in which 3 m.h.d. (and 2 m.h.d. if necessary) are used for the control, and 3 and 5 m.h.d. for the test proper. At the same time we prepared a sensitive human heart antigen with a low complement-absorbing capacity. The unit volume—0·11 ml.—which Wyler adopted was, of course, quite arbitrary, and determined by the ease with which the dropping pipettes could be calibrated for this volume. By trial and error we made nozzles which would deliver 0·1 ml. of antigen and saline, and we have used this unit throughout. Seven hundred and twenty-six tests have been made by this method.

For convenience, the results obtained by the first method have been correlated with Wyler's notation :

++ . Complete fixation of 5 m.h.d. of complement.
\+ . Complete fixation of 3 m.h.d. of complement.
± . Partial fixation of 3 m.h.d. or fixation of 2 m.h.d. in the original method.
– . Complete hæmolysis throughout.

Anti-complementary sera (17—0·48%) have been omitted.

* Saethre and Breteville-Jensen (1938) and Borgen and Natvig (1938) have both described refinements of the Meinicke technique which improve its specificity. These are being tested.

*Meinicke Klärungs Reaction (M.K.R. II).*

For this test we have used the commercial antigen (*Adler-Apotheke, Hagen i.W.*) and the macro-test method described in Appendix III of the Report and Recommendations of the Pathology, Bacteriology and Bio-Chemistry Sub-Committee of the Royal Medico-Psychological Association on the Standardization of the Wassermann Reaction for the Use of Mental Hospital Laboratories (Ford Robertson, 1931). The notation which we employed was:

| | | |
|---|---|---|
| ++++ | . | Complete clarification of all four tubes or of the fourth tube only. |
| +++ | . | Complete clarification of the first three tubes. |
| ++ | . | Complete clarification of the first two tubes. |
| + | . | Clarification in the first tube only. |
| — | . | No clarification in any tube. |

The "blue-spot" modification was set up in parallel in a number of spinal fluids. For these it is slightly more sensitive than the original method, but the number which we have tested is too small to be of statistical significance. The two methods have been combined in this analysis.

*Meinicke Klärungs Reaction (F.R.C. Modification).*

Through the courtesy of Dr. W. M. Ford Robertson we were able to test his new antigen for some months prior to the publication of the method. Using the F.R.C. antigen the technique is essentially similar to the original method, but there are important and time-saving differences:

(1) Only three tubes are used.

(2) The sodium carbonate is omitted, and the three antigen emulsions are made with 3%, 1·5% and 1% sodium chloride solutions, one volume of the antigen being mixed with 10 volumes of each salt concentration for the test.

(3) The antigen-saline mixture is made at 56° C. (an approximation to this temperature is sufficient, the thermal range for sensitivity being extremely wide). The ripening period, so necessary with the original method, is omitted.

The test is set up as follows:

| Tube. | A. | B. | C. |
|---|---|---|---|
| Serum (not inactivated) | 0·3 | 0·1 | 0·1 ml. |
| Antigen-salt mixture | 0·5 | 0·5 | 0·5 ,, |
| Strength | (3%) | (1·5%) | (1%) |

The readings are made in eighteen hours at room temperature. If necessary the test may be expedited by centrifugation, as in the M.K.R.Z. method. In reading the results we have employed a numerical system of notation, in which, in any tube—

| | | |
|---|---|---|
| 4 | . | Complete clarification. |
| 3 | . | Almost complete clarification with only a trace of haze. |
| 2 | . | Partial clarification with a definite haze. |
| 1 | . | Commencing clarification with much haze. |
| 0 | . | No clarification. |

With this test, any degree of clarification occurring in the first tube may be met with in the second and third tubes, the strong positive reaction being represented by complete clarification in all three tubes (444). The strongest reaction of all may show a zone phenomenon in which the second and third tubes are completely clear, but there being only a partial clarification in the first tube (2/3 44). With strongly positive sera the results are often readable in two hours, and it is at this time when the zone phenomenon is most noticeable. By the following day all the tubes are usually clear. A reaction of the (100) type is extremely doubtful and,

I think, probably negative. It occurs, at all events, most commonly in patients with a negative Wassermann test, and who have no clinical signs or possible history of syphilis. In this analysis it has been taken as negative (*vide infra*).

## RESULTS.

### 1. *Wassermann Reaction.*

The Wassermann reactions of 3,678 specimens have been determined, 3,382 being sera and 296 spinal fluids. The grading of the results is shown in Table II.

TABLE II.—*Wassermann Reaction.*

| Reading. | Blood. | | Spinal fluid. | | Total. | |
|---|---|---|---|---|---|---|
| | Number. | %. | Number. | %. | Number. | %. |
| ++ | 400 | 80·8 | 130 | 90·2 | 530 | 82·9 |
| + | 32 | 6·5 | 3 | 2·0 | 35 | 5·5 |
| ± | 63 | 12·7 | 11 | 7·8 | 74 | 11·6 |
| Total + | 495 | 14·6 | 144 | 48·6 | 639 | 17·4 |
| − | 2,887 | .. | 152 | .. | 3,039 | .. |
| Total | 3,382 | .. | 296 | .. | 3,678 | .. |

The incidence of syphilis among the admissions to the hospital during the five-year period under review is shown by the positive blood examinations—14·6%. Of these, approximately 83% showed strongly positive reactions in either the blood or spinal fluid, while about 12% gave reactions of the weak type. This is the frequency of grading to be expected in mental hospital practice where recent infections are uncommon, and is, of course, different to that obtaining in a V.D. clinic. The weak positive reactions represent the old arrested or treated cases of the disease, while the strongly positive ones are evidence of an active cerebral lesion of the disease.

### 2. *Meinicke Klärungs Reaction* (*M.K.R. II*).

A total of 2,802 parallel Meinicke and Wassermann tests is available for analysis in this section (Tables III and IV).

The observed agreement between the tests in this series is 97·9%; the agreement is closer when the Wassermann is negative than when it is positive (Table IV), and is considerably better with blood than with spinal fluids. This improved correlation with blood is a general observation with the Meinicke test.

TABLE III.—*Comparison of M.K.R. II and W.R. Tests.*

(1) Observed agreement.

| Reading. W.R. | M.K.R. | Blood. | Spinal fluid. | Total. | %. |
|---|---|---|---|---|---|
| ++ | + | 313 | 95 | 408 | |
| + | + | 22 | 2 | 24 | |
| ± | + | 36 | 2 | 38 | |
| Total | | 371 | 99 | 470 | |
| − | − | 2,159 | 114 | 2,273 | |
| Agreement | | 2,530 | 213 | 2,743 | 97·9 |
| ++ | − | 3 | 3 | 6 | |
| + | − | 8 | .. | 8 | |
| ± | − | 17 | 6 | 23 | |
| Total | | 28 | 9 | 37 | |
| − | + | 17 | 5 | 22 | |
| Disagreement | | 45 | 14 | 59 | 2·1 |

TABLE IV.—*Agreement of M.K.R. II and W.R. Tests.*

(1) Observed agreement.

| | Blood. | % | Spinal fluid. | % | Total. | % |
|---|---|---|---|---|---|---|
| W.R. − | 2,176 | 99·2 | 119 | 95·8 | 2,295 | 99·1 |
| M.K.R. II − | 2,159 | | 114 | | 2,273 | |
| W.R. + | 399 | 93·0 | 108 | 91·6 | 507 | 92·7 |
| M.K.R. II + | 371 | | 99 | | 470 | |
| Total tests | 2,575 | 98·2 | 227 | 93·8 | 2,802 | 97·9 |
| Total agreement | 2,530 | | 213 | | 2,743 | |

*Discrepancies.*

In this series there are 59 discrepancies between the M.K.R. II and Wassermann tests. Of the bloods which showed a complement deviation of 5 m.h.d. with a negative Meinicke reaction, one was an old serum, and one, with complete fixation of 10 m.h.d., gave a partial clarification in the first tube after two consecutive negative Meinicke tests. Both were clinically cases of general

paralysis of the insane. Apart from the two cases described by McMenemey,* one of the spinal fluids with a positive Wassermann reaction gave a negative M.K.R. II reaction but a positive " blue-spot " test. This decreased sensitivity of the original Meinicke reaction with spinal fluids is well known, and it was for this reason that Meinicke and Hagen-Ambrock introduced the " blue-spot " modification.

In two other cases showing a negative Meinicke reaction the serum has repeatedly fixed 3 m.h.d. of complement, although the test has been done in parallel with a number of different antigens. In these two cases there are no superficial clinical signs of syphilis, and they are being investigated in greater detail. Two further cases showed a positive Wassermann at one time, which was negative when repeated later; the Meinicke was persistently negative. In these two cases one must consider the Wassermann test to be at fault.

The greatest source of discrepancy lies in the weak W.R. readings (±). Ten of these were extremely doubtful, showing only a partial fixation of 2 m.h.d. of complement (7 bloods, 3 spinal fluids). The fact that a number tended to occur on the same day is suggestive of excessive antigen-complement absorption, and in each case the true reading was probably negative. In 7 other cases the W.R. was subsequently shown to be negative, and again, in these 17 cases, one may consider the fault to have lain with the Wassermann reaction.

The 22 cases in which the Meinicke was positive but the Wassermann negative include 4 in which the former was extremely doubtful, and 3 in which there was only a partial clarification of two tubes. Ten sera showed clear-cut reactions of the (++++) or (+++) grades, without any clinical signs of syphilis. These may represent the true " Meinicke-fast " type.

Properly, one should compare the results of the two tests as they are actually determined, but as there are mutual errors it is necessary to correct each for its own discrepancies. The true correlation between the two, and the residual

* W. M. McMenemey (Pathologist to the Hospital from 1934–38) has described the earlier discrepancies in the Supplement to the Board of Control Report for the year 1935–36. The cases which he described are:

(a) *Blood.*—Two males, both typical general paralytics, with blood W.R. + and M.K.R. II (complete fixation of 2 m.h.d. only). Two others (one feeble-minded and one psychasthenic) showed a W.R. + (weak; less than 2 m.h.d.) with a negative M.K.R. II. Of these, the Wassermann test appears correct in the first two and at fault with the second two.

On the other hand, one case of G.P.I. showed a positive W.R. and M.K.R. II in the spinal fluid, while the blood showed a positive M.K.R. II but a negative W.R. and Kahn test.

(b) *Spinal fluid.*—The W.R. was positive and the M.K.R. II negative in four cases of general paralysis of the insane, one of which at least was active. In another case of cerebro-spinal syphilis the same combination occurred in the spinal fluid, although both tests were positive in the blood. In a sixth case the spinal fluid showed a weak positive (2 m.h.d.) with a negative M.K.R. II, both tests being negative in the blood. This man was known to have been infected twelve years previously and to have been treated successfully. In all these cases the Wassermann exhibits a greater sensitivity than the M.K.R. II test.

In one case of chronic meningitis a previously negative W.R. in the spinal fluid became weakly positive shortly before death without alteration in the M.K.R. II reading. There was no history of syphilitic infection.

In two cases the spinal fluid W.R. was positive, while the M.K.R. II was negative. The modified " blue-spot " test was positive in each case.

The proper adjustment for these cases has been made in Tables V and VI.

error of the one, is shown in Tables V and VI, in which the appropriate corrections have been made.

TABLE V.—*Comparison of M.K.R. II and W.R. Tests.*

(2) Adjusted correlation.

| Reading. | | Observed. | | | Adjusted. | | |
|---|---|---|---|---|---|---|---|
| W.R. | M.K.R. | Blood. | Spinal fluid. | Total. | Blood. | Spinal fluid. | Total. |
| + | + | 371 | 99 | 470 | 373 | 100 | 473 |
| − | − | 2,159 | 114 | 2,273 | 2,175 | 117 | 2,292 |
| Agreement | | 2,530 | 213 | 2,743 | 2,548 | 217 | 2,765 |
| + | − | 28 | 9 | 37 | 17 | 5 | 22 |
| − | + | 17 | 5 | 22 | 10 | 5 | 15 |
| Disagreement | | 45 | 14 | 59 | 27 | 10 | 37 |

With this adjustment there still remain 37 cases in which there is a discrepancy between the two tests, and in all, this adjustment having been made according to the clinical state and history of the patient, the Wassermann proved itself the more sensitive and specific of the two. In the adjusted series there were 22 cases in which the Meinicke would improperly have excluded a syphilitic infection (0·78%).

TABLE VI.—*Agreement of M.K.R. II and W.R. Tests.*

(2) Adjusted agreement.

| | Blood. | % | Spinal fluid. | % | Total. | % |
|---|---|---|---|---|---|---|
| W.R. − | 2,185 | 99·5 | 122 | 95·8 | 2,307 | 99·4 |
| M.K.R. II − | 2,175 | | 117 | | 2,292 | |
| W.R. + | 390 | 95·7 | 105 | 95·2 | 495 | 95·6 |
| M.K.R. II + | 373 | | 100 | | 473 | |
| Total tests | 2,575 | 98·9 | 227 | 95·6 | 2,802 | 98·7 |
| Total agreement | 2,548 | | 217 | | 2,765 | |

3. *Meinicke Klärungs Reaction (Ford Robertson-Colquhoun Modification).*

Here the available material is much smaller, and any conclusions which may be drawn from this series must, of necessity, be more tentative in character. Moreover the method has purposely been put to its strictest test. There are 482 parallel tests for comparison with this antigen, the observed correlation being shown in Tables VII and VIII.

TABLE VII.—*Agreement of M.K.R. (F.R.C.) and W.R. Tests.*

| Reading. W.R. | M.K.R. | Blood. | Spinal fluid. | Total. |
|---|---|---|---|---|
| ++ | + | 49 | 11 | 60 |
| + | + | 4 | 1 | 5 |
| ± | + | 7 | 3 | 10 |
| | | — | — | — |
| Total | | 60 | 15 | 75 |
| − | − | 385 | 11 | 396 |
| | | — | — | — |
| Agreement | | 445 | 26 | 471 |
| | | — | — | — |
| ++ | − | .. | 1 | 1 |
| + | − | 3 | .. | 3 |
| ± | − | 2 | 1 | 3 |
| | | — | — | — |
| Total | | 5 | 2 | 7 |
| − | + | 2 | 2 | 4 |
| | | — | — | — |
| Disagreement | | 7 | 4 | 11 |

TABLE VIII.—*Agreement of M.K.R. (F.R.C.) and W.R. Tests.*

| | Blood. | % | Spinal fluid. | % | Total. | % |
|---|---|---|---|---|---|---|
| W.R. − | 387 | 99·5 | 13 | 84·6 | 400 | 98·9 |
| M.K.R. − | 385 | | 11 | | 396 | |
| W.R. + | 65 | 92·3 | 17 | 88·2 | 82 | 91·5 |
| M.K.R. + | 60 | | 15 | | 75 | |
| Total tests | 452 | 98·5 | 30 | 86·7 | 482 | 97·7 |
| Total agreement | 445 | | 26 | | 471 | |

In examining these figures it is important to realize that the antigen used for the spinal fluids was the same as that used for the bloods. Ford Robertson and Colquhoun have shown that the alteration in pH of the spinal fluid which normally occurs on standing exerts a stabilizing effect upon the colloid-electrolyte system which may result in false readings. They have, therefore, prepared a special antigen with a lower lipoid content for spinal fluids. We have not yet had an opportunity of testing this antigen to any extent, but it is being prepared. I have no doubt that the greater percentage discrepancy between the tests with spinal fluids is due to this fact, and properly the correlation in this series should be taken from the blood figures alone. Here the parallelism

between the two tests is almost identical with that observed with the commercial antigen.

The antigen was tested against the serum of 23 cases of active pulmonary tuberculosis. In every case it was specifically negative.

*Discrepancies.*

In this series there are 11 discrepancies between the two tests. The majority of these consist of old treated cases of cerebral syphilis. Two male general paralytics showed a blood Wassermann reaction of the (++) degree (complete fixation of 3, and partial fixation of 5 m.h.d.). In one of these the M.K.R. was completely negative, while in the other the test showed a (100) reading. This latter reading has been taken as negative throughout this analysis, although in a case such as this it might be considered to be positive. Another case of G.P.I. and a congenital syphilitic idiot gave Wassermann reactions of (+) and (±) degree in the blood respectively, together with negative M.K.R. readings. In one case which is still being investigated there are no clinical signs of syphilis, although the blood W.R. shows complete fixation of 3 m.h.d. of complement. The M.K.R. reading was negative. Of the two cases in which a negative W.R. coincided with a positive M.K.R. in the blood, one was a treated syphilitic ; provocative injections of N.A.B. failed to produce a positive Wassermann reaction, although the M.K.R. remained persistently positive. This case may represent a " Meinicke-fast " condition. The second case was of a woman who had no clinical signs of syphilis but a slightly doubtful early history. On two successive weeks the blood W.R. was negative and the M.K.R. of the (400) degree. Following a provocative injection of N.A.B. the W.R. was weakly positive after six days (incomplete fixation of 3 m.h.d. by the M.R.C. No. 1 method), while the M.K.R. was less strong (200). A week later the W.R. was again negative and the M.K.R. remained at the (200) grade. Here the M.K.R. may be taken as being more sensitive, and it is probable that she had had an early infection.

This antigen was originally adjusted for sensitivity, and it is a general rule that sensitivity can only be increased at the expense of specificity. In practice it was found to be rather too sensitive, and this was decreased with a concomitant increase in its specificity. As we have used both antigens this may explain the number of false reactions which we have observed (although this is no greater as a percentage than with the commercial antigen). There is, however, another possible explanation of these false reactions.

In Tables IX and X are shown the actual readings of the Meinicke tests in the positive cases, both in the agreement and disagreement series and for both the antigens. The immediate significance of these tables is in demonstrating for each antigen the relative frequency with which one has to make a diagnosis upon a clarification in one tube only of the set. With the original M.K.R. II

the frequency of single-tube reactions was 6·5%. With the new antigen a single-tube reaction occurred in one half of all the positive cases, and of these, 10 were of the weak type (200). Moreover it is significant that all the false positive reactions were of this type.

TABLE IX.—*Observed Readings with the Meinicke Klärungs Reaction.*

(1) M.K.R. II.

| M.K.R. reading. | W.R. Positive. | W.R. Negative. |
|---|---|---|
| ++++ | 299 | 3 |
| +++− | 112 | 7 |
| ++−− | 19 | 5 |
| +−−− | 12 | 2 |
| −−−+ | 17 | 1 |
| −−++ | 4 | .. |
| −+++ | 3 | .. |
| ±±±−* | 3 | 4 |
| | 469 | 22 |

Single-tube reactions, 32 (6·5%).

* Doubtful reactions.

The most common source of technical error in flocculation tests is the use of dirty glassware.* Contamination may result in either an excessive stability of the electrolyte-colloid system or in its precipitation, and to a certain extent this probably depends upon the direction in which the pH is shifted; to a certain extent this is overcome by the buffering action of the serum in the test proper. This possibility is guarded against by careful technique, but the possibility of atypical tube phenomena cannot be entirely excluded. With the original M.K.R. II the method itself guards against this to a certain extent, for in 93·5% of positive reactions there is a precipitation in more than one tube. The same cannot be said for the new antigen, however, for only 50% of the positive reactions are duplicated in one or more tubes in the series.

This single-tube reaction may be turned into a two- or three-tube reaction by the use of different electrolyte strengths without altering the lipoid content of the antigen. In a small series of cases I have used 3·5%, 2·5% and 1·5% sodium chloride solutions for making the antigen emulsion, following the standard technique in all other respects. By this means a reaction which would

* The commonly recommended acid-bichromate mixture for cleaning Meinicke tubes is not the best. Immediately after use they should be washed out with cold tap-water and immersed in 10% hydrogen peroxide overnight. They are then washed with hot water and soap, rinsed clean with cold tap-water, run through distilled water and dried. If they are dried in a hot-air oven the temperature should not be above 110° C.

TABLE X.—*Observed Readings with the Meinicke Klärungs Reaction.*

(2) M.K.R. (F.R.C.).

| M.K.R. reading. | W.R. Positive. | W.R. Negative. |
|---|---|---|
| 444 | 4 | .. |
| 443 | 2 | .. |
| 440 | 1 | .. |
| 433 | 2 | .. |
| 432 | 4 | .. |
| 431 | 4 | .. |
| 430 | 2 | .. |
| 421 | 1 | .. |
| 410 | 2 | .. |
| 400 | 21 | .. |
| 344* | 1 | .. |
| 332 | 2 | .. |
| 323 | 1 | .. |
| 321 | 1 | .. |
| 310 | 3 | .. |
| 300 | 9 | 2 |
| 223 | 1 | .. |
| 222 | 2 | .. |
| 221 | 1 | .. |
| 200 | 8 | 2 |
| 132 | 1 | .. |
| 123 | 1 | .. |
| 121 | 1 | .. |
| | 75 | 4 |

Single-tube reactions, 38 (50·6%).

* Zone reaction strongly syphilitic.

normally be of the (400) grade becomes (440) without apparent loss of specificity. This, together with the effect of increasing the volume of serum in the second tube, leaving the electrolyte concentration unaltered, requires further investigation.

A further difficulty occurs with the interpretation of the (100) reaction. In one case, recorded as a discrepancy, this type of reading occurred with a positive Wassermann (5 m.h.d.). Here it might be considered positive. But the interpretation of a result should not depend upon circumstances (although it too often does), and if this degree of clarification is to be taken as positive

on one occasion, it should be so taken at all times. If this reaction is to be positive the correlation is bad, for one would have to include a further 11 patients with negative Wassermann reactions, and in whom no clinical signs or possible history of syphilis existed, yet who showed Meinicke reactions of this type. This reaction has, I think, been properly classed as a negative.

It is significant that in one of the cases mentioned above, the M.K.R. was of the (300) degree when repeated a week later. Similarly in another frank general paralytic the blood Meinicke was negative while the Wassermann and both tests in the spinal fluid were strongly positive. Repeated a week later the M.K.R. in the blood became positive (400). The only explanation of these cases appears to be an anomalous tube phenomenon whereby the colloid-electrolyte system became abnormally stable. Had the salt concentration in the antigen emulsions been such as to produce a two- or three-tube precipitation in place of the single tube which occurred, these anomalies would not have taken place and the correlation would have been proportionately better. With such a variation, this new antigen would possess a specificity on a par with the Wassermann reaction.*

### 4. *Combined Series.*

The correlation for the combined series is given in Tables XI and XII, the latter being adjusted to a complete Wassermann specificity. In the adjusted series the agreement between the Wassermann and Meinicke reactions is 98·5%. On blood alone it is nearly 99%. The correlation in the observed series, with which one is properly concerned in practice, is 97·9% in 3,284 cases.

## Discussion.

To a certain extent the final analysis of these figures is statistically inaccurate, for the results of two separate Wassermann techniques are compared with the results given by two different Meinicke methods. However, the correlation between the two is good, and shows approximately two discrepancies in every hundred tests. In all there were 70 observed discrepancies, of which 44 were of the W.R. + : M.K.R. − type and 26 in the reverse direction. The correlation is worst with spinal fluids, this being a general observation of all workers; over 60% of the disagreements with spinal fluid were false negatives.

* It is not really practicable to determine the *sensitivity* of either of these tests, as opposed to their *specificity*, in a mental hospital. This is properly done on new, untreated infections in a V.D. clinic. An old case which has been adequately treated will in all probability have a negative W.R., although the serum may, in some cases, become "Meinicke-fast". A case in which the mental symptoms are directly referable to a luetic infection will show a positive W.R. in the spinal fluid irrespective of the M.K.R. Menninger and Bromberg (1935) found a positive W.R. in 100% of neurosyphilitics (500 cases of asymptomatic neuro-syphilis, tabes, general paralysis, tabo-paresis and meningo-vascular syphilis). It is with this aspect that one is properly concerned in a mental hospital.

TABLE XI.—*Agreement between M.K.R. and W.R. Tests.*

(3) Observed agreement (combined).

| W.R. | M.K.R. | Blood. | Spinal fluid. | Total. |
|---|---|---|---|---|
| ++ | + | 362 | 106 | 468 |
| + | + | 26 | 3 | 29 |
| ± | + | 43 | 5 | 48 |
| | Total | 431 | 114 | 545 |
| − | − | 2,544 | 125 | 2,669 |
| | Agreement | 2,975 | 239 | 3,214 |
| ++ | − | 3 | 4 | 7 |
| + | − | 11 | .. | 11 |
| ± | − | 19 | 7 | 26 |
| | Total | 33 | 11 | 44 |
| − | + | 19 | 7 | 26 |
| | Disagreement | 52 | 18 | 70 |

TABLE XII.—*Agreement between M.K.R. and W.R. Tests.*

(2) Adjusted agreement (combined).

| | Observed. | | | | | | Adjusted. | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Blood. | %. | Spinal fluid. | %. | Total. | %. | Blood. | %. | Spinal fluid. | %. | Total. | %. |
| W.R. − | 2,563 | 99·3 | 132 | 94·7 | 2,695 | 99·0 | 2,572 | 99·5 | 135 | 95·0 | 2,707 | 99·3 |
| M.K.R. − | 2,544 | | 125 | | 2,669 | | 2,560 | | 128 | | 2,688 | |
| W.R. + | 464 | 95·1 | 125 | 91·2 | 589 | 92·5 | 455 | 95·2 | 122 | 94·3 | 577 | 95·0 |
| M.K.R. + | 431 | | 114 | | 545 | | 433 | | 115 | | 548 | |
| Total tests | 3,027 | 98·3 | 257 | 93·1 | 3,284 | 97·9 | 3,027 | 98·9 | 257 | 94·6 | 3,284 | 98·5 |
| Agreement | 2,975 | | 239 | | 3,214 | | 2,993 | | 243 | | 3,236 | |

It is well known that a weak false positive reaction most commonly occurs with the Wassermann test, particularly when the method employed involves the use of a 2 m.h.d. tube for the test proper. These reactions are not met with so frequently with Wyler's modified M.R.C. No. 1 method, and in fact, only 4 of the 726 sera tested by this method had to be corrected for this error. By adjusting for these false reactions the correlation between the tests is even better, and one may expect a discrepancy between the two but once in every hundred tests.

The Wassermann reaction is a test which may be adjusted to any level of sensitivity at will and at the time of the test. Moreover it may be used quantitatively. It is a general observation that the complement-fixation tests are more sensitive in congenital syphilis and appear positive sooner in primary infections than the flocculation tests. Ford Robertson's and Colquhoun's antigen has purposely been adjusted for sensitivity, and it is said for it that it will react with early infections even sooner than the Wassermann test. It is not possible from the nature of mental hospital patients to investigate this claim, but the results with sera from V.D. clinics should be of interest.

At the other end of the scale, the Wassermann tends to become negative with treatment more quickly than the Meinicke, and in some cases a positive Meinicke may persist long after the patient is clinically cured (the "Meinicke-fast" condition). For this reason the Meinicke tests might be considered as an index of cure, while the Wassermann might be taken as the standard for diagnosis.

The Meinicke reaction, together with the other flocculation tests, is a qualitative test, and as such is simple and convenient for routine use. It may be set up by any laboratory assistant, and its interpretation is rarely a matter of difficulty. On the other hand, the Wassermann test is essentially quantitative in character, and this inherent advantage offsets the greater labour entailed in setting up the test. The two, however, still remain complementary, and there can be no doubt that it is desirable for them to be made in parallel on every specimen which is examined.

The occurrence of a positive Meinicke reaction with a negative Wassermann test calls for the further investigation of the patient and, if necessary, the use of provocative injections of N.A.B. The reverse combination is not of such importance. A weak positive W.R. may be a false reaction, and will almost certainly disappear if repeated, for these false positives do not persist. If stronger, with complete fixation of 3 or 5 m.h.d. of complement, the patient should be considered as being or having been infected, even in the presence of a negative flocculation test.* If this latter is repeated it will commonly become positive, the first result being due to an abnormal stabilization of the colloid-electrolyte system. It is the possibility of this latter combination, and its actual occurrence in practice, which must prevent the Meinicke reaction from being employed to the sole exclusion of the Wassermann test in the diagnosis of syphilis.

## Summary.

1. The Wassermann and Meinicke reactions have been compared in a series of 3,284 tests. The observed correlation between the two was 97·9%, and after adjustment 98·5%.

* "Wassermann-fast" sera are known. In our experience they are not so common as "Meinicke-fast" sera.

2. A new Meinicke method described by Ford Robertson and Colquhoun has been tested in 482 cases. The correlation between this and the Wassermann test with serum is of the same degree as with the original method. The use of the antigen prepared for blood in spinal fluid tests leads to an excessive number of false reactions. A special antigen is used for spinal fluids.

3. With this method one half of the positive reactions occur in one tube only. It is shown that this is a source of error, and it is suggested that, by altering the concentration of salt in the antigen emulsions, or the volume of serum in the tubes, the reaction may be turned into a two- or three-tube one with a corresponding increase in accuracy.

4. There are no grounds for abandoning the Wassermann reaction in sole favour of the Meinicke test in the diagnosis of syphilis.

I wish to thank the Medical Superintendent, Dr. G. W. Shore, for permission to use the material, and Dr. W. H. McMenemey, Pathologist to the Radcliffe Infirmary, Oxford, for the use of his figures obtained while at this hospital. I am very grateful to Dr. W. M. Ford Robertson for generous supplies of the F.R.C. antigen. I have also to thank my assistants, Mr. H. G. Johnson and Mr. R. F. Lane, for their excellent work in making so many of these tests.

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## Note upon the Foregoing Paper.

By W. M. Ford Robertson.

I wish, firstly, to express my appreciation and thanks to Dr. Thomas for having carried out with manifest care this series of tests with the F.R.C. antigen. The fact, too, that he has been able to correlate the results with a large series of Meinicke tests has added to their value.

The evaluation of discrepancies between the Wassermann and the flocculation tests is usually one of difficulty, as it depends on clinical diagnosis and the reliance placed on one or other test. In framing our observations we have no personal knowledge of the cases, and from considerable experience