THE FORD ROBERTSON AND COLQUHOUN MODIFICATION OF THE MEINICKE CLARIFICATION REACTION COMPARED WITH THE HARRISON-WYLER WASSERMANN AND THE STANDARD KAHN REACTIONS: TOGETHER WITH A NEW METHOD OF NOTATION

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Introduction

Within the last fifteen years clarification tests, because of their relative technical simplicity and ease of reading, have gained increasing favour with those responsible for the routine serology of syphilis. While the Wassermann and Kahn tests, together with the other precipitation tests, require a colloid system composed of beef-heart extract and cholesterol, the clarification tests, of which the prototype was described in 1922 by Meinicke, make use of an admixture of beef-heart extract and balsam of Tolu. Details of the Meinicke Clarification Reaction (M.K.R.I.), the sixth of Meinicke's syphilitic antigens, were published in 1929, and the test showed up well at the Montevideo Conference in 1930. For the purpose of securing additional sensitivity he subsequently (1932) introduced a modification containing Victoria blue which he designated the M.K.R.2.

In Europe these antigens are valued highly among flocculation tests, although both were shown to be at a disadvantage on account of the natural acidity of the balsam of Tolu, having sodium carbonate incorporated in the saline dilutions for the specific purpose of neutralising this excess. In their quest for a simplification of the Meinicke tests, Ford Robertson and Colquhoun first removed the excess of free benzoic and cinnamic acids by adsorption on to light magnesium carbonate. Being further stimulated by a desire to improve specificity, they prepared a purer and more stable heart extract by making use of anhydrous extractives of "Analar" purity. Because of the added stability they claimed that this antigen could be more accurately standardized than had been possible with those of Meinicke. Employing this antigen and a simplified technique, Ford Robertson and Colquhoun published their method in 1939; it proved an immediate boon to serologists in this country, who were cut off in wartime from a supply of Meinicke's antigen.

It is our aim to show the practical value of this clarification test, which deserves to be better known. Previous reports on the value of the test (F.R.C.) have been given by Colquhoun, Kyles, and Rannie (1945), Bessemans and others (1947), and McMenemey (1947).

Technique.—Details of the technique may be obtained by consulting the original paper written by Ford Robertson and Colquhoun.

Reading of Results

Ford Robertson and Colquhoun distinguished four degrees of positivity as shown by clarification and one phase graded as doubtful; but we employ a numerical notation, based on the following degrees of clarification:

Complete clarification	3	} positive
Partial clarification with transparency	2	
Partial clarification without transparency	1	} doubtful
No change		
		negative

Three tubes are used in the test and results are recorded in numerals. For example:

Reaction strongly positive (prozone): 223; 123; 013

It will be noted that in this type of reaction the tube on the right shows complete clarification whilst those on the left of it exhibit decreasing degrees of clarification in the weakest reactions (see Fig. 1):

Reaction positive: 333; 322; 222.

All three tubes show complete or partial clarification with transparency, the greatest amount of clarification being present in the tubes on the left:

Reaction weakly positive: 310; 300; 200
Doubtful reaction: 100; 110

The tubes on the left show partial clarification with no transparency:

Negative reaction: 000
No changes take place in any of the tubes.

Transparency may be tested by holding the tubes against a white background on which is a black horizontal line 1 mm. thick. Alternatively the tubes can be held up to the window; transparency is established if the cross bar can be seen through the tube.

Principal F.R.C. Groupings in Relation to Positive and Negative Wassermann and Kahn Reactions

In Fig. 2 are analysed the results obtained from samples of blood sent into a general hospital laboratory for Wassermann tests by hospital and outside doctors and they also include proven cases of syphilis under treatment in a venereal disease clinic. The principal F.R.C. groups are arranged in an increasing order of positiveness reading from left to right and against them are compared the results obtained with the Harrison-Wyler Wassermann Reaction (H.W.), the Richardson (1940) supplement tube of the Harrison-Wyler Wassermann (H.R.) and the standard Kahn (K.R.). So far as the W.R. is concerned results showing fixation of 5 MHD of complement are expressed in black and those fixing 3 MHD in the heavily hatched area above. Light hatching implies a doubtful result (imperfect fixation of 3 MHD). It will be noted that as the scale figures of the F.R.C. test increase there is also a general correlation of increasing positiveness on the part of the W.R., a correlation which in our opinion justifies a numerical classification being employed for the F.R.C. It should be added that the findings in groups 210 and 310 have been merged and also those of groups 321 and 330. A similar correlation obtains with the F.R.C. and the K.R. and to a less extent with the F.R.C. and the R.T. With regard to the K.R. a strong (++) positive is shown in black, a (+) positive in the heavily hatched area, whilst a doubtful is expressed in light hatching. The protocols on which Fig. 2 is based are shown in Table I.

These results show that an F.R.C. reading of 310 or less is more likely to coincide with negatives in the Wassermann and Kahn, whereas a reading of 320 or over is more likely to coincide with positives. It is noteworthy that 3.3 per cent. of all the negative Wassermanns occurred in the group 333, whilst 1.4 per cent. of the positive W.R.'s fell into group 100; the first of these results suggests that the FRC is rather more sensitive than the Wassermann and the second seems to illustrate the fact—generally agreed upon—that although reliable flocculation tests tend to be more sensitive than technically good Wassermanns, occasionally the latter give unequivocal positive reactions when the former are negative. It is evident, therefore, that low F.R.C. values should not be dismissed as “probably negative” until they have been carefully studied in the light of the clinical findings, for it is possible for a reading as low as 100 to be significant: indeed this is no unusual finding in a treated case.

Analysis of Discrepancies between Wassermann and F.R.C. Reactions in a Series of 12,180 Parallel Tests

For the purpose of this comparison a two plus and a one plus Wassermann (W.R.) have been taken together. In the F.R.C. the figure of 300 was regarded as the minimum level of positivity. In studying the discrepancies only first tests results have been analysed: progress tests on cases of syphilis under treatment have been assigned either to “positive” or “negative agreements.” Positive agreements were noted in 1,764 (14.48 per cent.), negative agreements in 10,319 (84.72 per cent.), and disagreements in 97 (0.80 per cent.). The 97 patients whose blood gave a conflicting result in the two tests were followed up, and in 61 of them it was possible either to confirm or refute the diagnosis of syphilis. Of these the W.R. alone supplied the diagnosis in 20, and the F.R.C. in 19. The W.R. was misleading on 9 occasions and the F.R.C.

TABLE I

COMPARISON OF F.R.C. READINGS WITH THOSE OBTAINED WITH WASSERMANN, RICHARDSON, AND KAHN REACTIONS

F.R.C.		Harrison-Wyler					Richardson				Kahn				
Reading	Total notation units	No. in group	% Positive ++	% Positive +	% Doubtful	% Negative	No. in group	% Positive	% Doubtful	% Negative	No. in group	% Positive ++	% Positive +	% Doubtful	% Negative
100	1	107	8.4	1.9	12.2	77.6	60	15.0	8.3	76.7	87	3.5	5.8	28.7	62.1
200	2	150	10.0	2.0	21.3	66.7	107	24.3	25.2	50.5	126	9.5	7.9	35.7	46.8
210 } 300 }	3	176	17.0	6.8	34.1	42.1	121	38.0	28.9	33.1	171	12.3	15.8	38.6	33.3
310	4	210	35.2	11.4	27.6	25.7	129	48.8	26.4	24.8	191	27.8	23.6	33.0	15.7
320	5	169	53.3	11.2	21.3	14.0	107	68.2	19.6	12.2	153	38.6	27.5	28.1	5.9
321 } 330 }	6	176	63.6	4.0	17.1	15.3	109	82.4	10.3	7.3	155	48.4	17.4	24.5	9.7
331	7	64	75.0	4.7	10.9	9.4	29	82.8	6.9	10.4	65	67.7	10.8	13.9	7.7
332	9	76	84.2	2.6	5.3	7.9	37	81.1	5.4	13.5	70	75.7	11.4	8.6	4.3
333	9	334	92.8	3.0	1.8	2.4	210	95.7	2.4	1.9	294	80.3	10.9	5.8	3.1

FIG. 1.—Four tests have been set up in the rack. No. 1 is negative (000); Nos. 2, 3, and 4 are all positive (300, 320, and 332 respectively).

FIG. 2.—Correlation between F.R.C. and other tests. In this histogram the F.R.C. test is compared with (1) the Harrison-Wyler Wassermann reaction, (2) the Richardson supplement tube technique, and (3) the standard Kahn reaction. It will be seen that as the numerical scale of the F.R.C. ascends, there is an increasing positivity with the Wassermann and Kahn and a similar but less perfect correlation with the Richardson.

TABLE II
ANALYSIS OF AGREEMENTS AND DISCREPANCIES BETWEEN THE WASSERMANN AND F.R.C.

	Clarification test	Agreements		Disagreements						
		F.R.C. series	Posi- tive	Nega- tive	Total	Verified				Non- Verifi- able
						W.R.		F.R.C.		
						Correct	Wrong	Correct	Wrong	
Total numbers ..	12,180	1,764	10,319	97	20	9	19	13	36	
% of total		14.48	84.72	0.80	0.16	0.07	0.16	0.11	0.30	
% of total positives excluding negative agreements ..		96.74		5.22	1.05	0.46	1.05	0.70	1.96	

in 13. These results are set out in percentages in Table II.

Those in whom, for one reason or another, it proved impossible either to confirm or refute the diagnosis of syphilis have been classified as "non verifiable." In some of the discrepancies the cause may have been the presence of a transient (non specific) reagin, and a repeat test, had one been available, might have revealed a negative agreement. It will be noted that in cases of syphilis which provided a discrepancy in the two tests the W.R. and F.R.C. were about equally valuable in supplying the diagnosis; in about one half, the W.R. succeeded when the F.R.C. failed, and *vice versa*. Expressed in another way, false negatives occurred on 20 occasions with the F.R.C. and on 19 with the W.R. The F.R.C. on the other hand gave false positive readings more often than did the Wassermann. The observed agreement between the tests was 99.114 per cent., and the specificity of both Wassermann and F.R.C. was 99.89 per cent.

The following are selected examples of discrepancies encountered in patients on their first attendance.

EXAMPLES IN WHICH THE WASSERMANN SUPPLIED THE DIAGNOSIS AND THE F.R.C. FAILED

Case 1.—A girl aged 16 years (W.R. ++, and F.R.C. 000) had a snail-track ulcer in the mouth, and facies typical of congenital syphilis. She responded to anti-syphilitic treatment.

Case 2.—A woman aged 26 years (W.R. ++, F.R.C. 000, and K.R. ++) had tertiary syphilides on the arms, and these were said to have recurred after a short course of mercury. The possibility of the F.R.C. having been positive before treatment cannot be excluded, but in our experience it is unlikely to have been so.

Case 3.—A woman aged 25 years (W.R. ++, F.R.C. 000, and K.R. ++) was a gipsy with condylomata vulvæ and other manifestations of secondary syphilis.

EXAMPLES IN WHICH THE F.R.C. SUPPLIED THE DIAGNOSIS AND THE WASSERMANN WAS NEGATIVE

Case 4.—A woman aged 54 years (W.R. negative, F.R.C. 321, K.R. doubtful) had general paresis. Neither patient nor relatives could supply a satisfactory history of previous treatment, and there is no record of the cerebrospinal fluid.

Case 5.—A woman aged 61 years (W.R. negative, F.R.C. 300, K.R. negative) had quite exceptional scarring of the fauces thought clinically to be syphilis. She denied having had previous treatment.

Case 6.—A man aged 78 (W.R. negative, F.R.C. 330, K.R. negative: on repeat weakly positive) had an ulcer of the tongue and leukoplakia.

The following three cases show the value of a persistent positive F.R.C. in the diagnosis of late forms of syphilis.

Case 7.—A man aged 50 years (W.R. negative, F.R.C. 321, K.R. negative) had tabes dorsalis developing after trauma. He admitted having had treatment for syphilis twelve years previously.

Case 8.—A man aged 48 years (W.R. negative, F.R.C. 013 ("prozone" phenomenon)) had a bizarre pustular rash thought to be syphilis. He had been treated for syphilis twenty-one years previously.

Case 9.—A woman aged 24 years (W.R. negative, F.R.C. 310) had interstitial keratitis and "suggestive teeth." She remembered having had injections as a child.

CASES IN WHICH THE F.R.C. WAS THOUGHT TO BE WRONG

Case 10.—A woman aged 31 years (W.R. negative, F.R.C. 320) at a routine pregnancy test was found to have no evidence of syphilis. She had had four healthy children.

Case 11.—A man aged 26 years (W.R. negative, F.R.C. 333, K.R. negative) was suffering from disseminated sclerosis.

Case 12.—A man aged 27 years (W.R. negative, F.R.C. 320) had a lesion on the nose thought to be lupus vulgaris. There was nothing to suggest syphilis.

Case 13.—A woman aged 37 years (W.R. negative, F.R.C. 320, K.R. negative) had uræmia complicating carcinoma of cervix.

Case 14.—A man aged 39 years (W.R. negative, F.R.C. 330, K.R. negative) had an ulcer on the leg, but syphilis remained unconfirmed.

The following cases from an earlier series with the M.K.R.2 are quoted as showing both the value of a clarification test in unmasking old syphilis and the need for an adequate case history.

Case 15.—A man aged 50 years (W.R. negative, M.K.R.2, 3333) had characteristic symptoms of general paralysis of the insane with a positive Wassermann reaction in the cerebrospinal fluid. This case is an example of how the blood Wassermann, usually because of previous although inadequate antisyphilitic treatment, is sometimes negative in this disease (Merritt and Fremont-Smith, 1937 say in 19 per cent. of cases). For this reason, when neurosyphilis is suspected the diagnosis should never be rejected on the finding of one negative blood test; in these cases the cerebrospinal fluid should be examined at the same time.

Case 16.—A man aged 49 years (W.R. negative, M.K.R.2 3333) had classical pernicious anæmia, but on questioning he admitted having had a penile chancre twenty-nine years before.

Experiences with the F.R.C. Test in a Venereal Disease Clinic

The results of the W.R., F.R.C., and K.R. tests were compared in 182 cases of untreated syphilis (not all of which have been included in Section 6).

Of 54 cases of primary syphilis, the W.R., F.R.C., and K.R. were all negative in 4, the diagnosis being established by means of dark-ground microscopy. Of the remainder, all tests were positive in 34, the W.R. alone in 4, the F.R.C. alone in 7, and the K.R. alone in 1; the W.R. and F.R.C. were positive and the K.R. negative in 4. It will be seen, therefore, that while blood reagin was developing, the F.R.C. tended to be positive before the W.R. In our hands the K.R. for the purpose of early diagnosis proved less reliable than the W.R.

To discover the exact details of an inoculation was not often possible; in 28 instances, however, this was recorded with reasonable accuracy. Among these it was noted that whereas one or more tests might be positive within three weeks, or even earlier, after inoculation they might equally well be negative in the tenth week. In general, however, observations indicated that a change from negative to positive was likely between the fourth and seventh weeks. Stokes (1939) is substantially in agreement

in finding that serological reactions were positive in 35 to 50 per cent. of cases of primary syphilis by the end of the second week: from this time on there was a steady increase, until by the end of the eighth week 80 per cent. or more cases were sero-positive.

In an attempt to assess the relative values of the three tests in primary syphilis marks were assigned, a maximum of three for each test on the first occasion of the blood's being examined. The result was that the F.R.C. scored 66, the W.R. 56, and the K.R. 45.

Of 68 cases of untreated secondary syphilis, all tests gave positive agreement in 61. The W.R. was negative, and both F.R.C. and K.R. were weakly positive in one patient* while in another the W.R. and F.R.C. were positive and the K.R. negative: in five instances, however, the F.R.C. was negative and the W.R. and K.R. positive. One further example of a false negative F.R.C. was noted in a case of latent syphilis.

In a series of 150 cases of syphilis under treatment, the W.R. and F.R.C. became negative at the same time in 50.0 per cent., both tests remained positive in 20.7 per cent., the F.R.C. remained positive when the W.R. was negative in 4.7 per cent., and the W.R. remained positive when the F.R.C. was negative in 2.0 per cent. The F.R.C. showed a higher *pari passu* positivity compared with the W.R. in 22.7 per cent. and in 30 cases the greater sensitivity resulted in persistence after the W.R. had become negative. This is a well-known feature of the Meinicke tests and was noted in the F.R.C. by Colquhoun, Kyles, and Rannie (1945). The value of such additional sensitivity to residual syphilitic reagin has sometimes been questioned: it is of interest, therefore, to note that in 12 patients this continuing positivity presaged a serological relapse in which the W.R. sometime later participated. Vogelsang (1938) noticed the tendency with the M.K.R.2 and recommended treatment until all tests reverted to negative.

The F.R.C. as a Presumptive Test for Blood Donors

The F.R.C. was employed as a "screen" test on 21,587 occasions, many of the volunteers attending regularly: they came from two counties. County A produced eight bloods which subsequently gave the full serological reactions for syphilis out of 10,122 donations (incidence 0.0794 per cent.).

* A patient with secondary syphilis and a negative W.R. is so unusual as to justify comment (while Bernard (1935) stressed the rarity of the occurrence, Moore and Kemp (1926) found negative W.R.'s in 1.29 per cent. of 618 patients with early secondary syphilis, although 9.23 per cent. showed incomplete fixation of complement). The patient (Case 17) was 22 years old, and at the time of her attendance her fiancé, a soldier, had syphilis. Seven days after a provocative dose of 0.45 g. of nearsphenamine both W.R. and K.R. became strongly positive and the F.R.C. 321.

County B produced nine positive bloods out of 11,465 donations (incidence of 0.0795 per cent.); these nine positives were picked out of a preliminary screen of thirty-three, the notation 100 being regarded as the screen threshold (by comparison notation 300, in conformity with the recommendations of Ford Robertson and Colquhoun (1939) has been taken as the diagnostic threshold in Table II).

Discussion

The F.R.C., when compared with the Wassermann, would seem to share with the M.K.R.2 that advantage of added sensitivity which has been described by Bessemans and Asaert (1935), Vogel-sang (1938), d'Ignazio and Calabrese (1938), Barritt (1939), Cuvier and Fournier (1945) and Colle (1946).

The improved sensitivity of clarification as against complement fixation tests rests mostly on their tendency to become positive earlier and to persist after treatment: in fact an oversensitive antigen may fail to become negative even after adequate treatment. In its ability to detect the presence of non-specific reagin, the F.R.C. would seem to fare as well as the others in common usage. It occasionally produced a false positive in pregnancy (Case 10 for instance), but this lack of specificity is common to most tests.

Reference has already been made to the fact that of 178 cases of proven and untreated syphilis the F.R.C. was negative on six occasions when the Wassermann and Kahn were positive. False negative results are really extreme examples of the prozone phenomenon due to the inhibitory effect of a strong syphilitic reagin upon the Tolu balsam colloidal system. This phenomenon was well known in both M.K.R.1 and M.K.R.2, it being fully recognized by Meinicke. Colle (1946) has drawn attention to its practical disadvantage, and its mechanism has been studied by Dunlop and Sugden (1934), Mackie and Anderson (1937), and Ford Robertson and Colquhoun (1940). However, under a further analysis of the data obtained by Colquhoun, Kyles, and Rannie (1945), Ford Robertson (personal communication) found no false negative in 385 untreated cases of syphilis, but in 0.51 per cent. of them inhibition of a considerable degree (that is, readings of 001). In their selected series of cases of untreated syphilis unequivocal prozone reactions were as high as 7.19 per cent. as compared to 0.84 per cent. in a general hospital series of 4,384 cases: three false negatives, however, were noted in this latter series, and three instances of marked inhibition of the order of 001 and 101. More recently, however, Larson (1948) working at Tacoma, U.S.A., has met with no instance of a

false negative F.R.C. in a series of 10,000 comparisons with the Kahn. One example of inhibition was of special interest (Case 18), for after a single dose of 0.45 g. of neoarsphenamine a negative F.R.C. became positive (331) one week later; the Wassermann and Kahn were positive on both occasions. The patient had given birth three months previously to a child who died soon afterwards of congenital syphilis. She was symptomless on examination and was diagnosed as suffering from latent syphilis.

It seems unlikely that these instances of total inhibition are related to the group of "prematurely negative" Wassermann reactions referred to by Moore and Kemp (1926). According to these authors those who, in the stage of secondary syphilis, fail to give a completely positive Wassermann are suffering from an inherent lack of resistance and are liable to "recurrence of a secondary type." In reference to sero-negative cases of active syphilis, Kahn (1942) says that not only may an excess of micro-organisms or antigen interfere with the production of antibodies but these antigens may combine *in vivo* with them: in either case there are no available antibodies to make a positive serum test possible. But the cases in the present series which had total inhibition in the F.R.C. have not been inhibited in the Wassermann or Kahn; neither have they shown a tendency to relapse. Moreover, one of the inhibitory sera was found to contain no less than 80 units of reagin as determined by a quantitative test recently described by Ford Robertson*. It seems unlikely, therefore, so far as the F.R.C. is concerned, that a low reagin content of the blood is the cause of these sero-negative examples of secondary syphilis; some agency inherent within the test must be sought. The discriminating factors would appear to be the cholesterol and the balsam of Tolu functioning as electrolyte-sensitive lipid-coated particles. This is apparent because it has been established that cardiolipin, instead of ordinary heart extract, functions equally well with the cholesterol system of Kahn and the balsam of Tolu of Meinicke. It appears, therefore, as if the variable and inconsistent components of reagin may be detected by one or other antigen system if used routinely and interpreted wisely by the pathologist. To quote Hinrichsen (1941), false negative results in flocculation tests are apt to occur in the presence of excessive amounts of reagins: this accounts for our noticing the phenomenon especially in cases of secondary syphilis, when one would expect a high reagin content.

* Personal communication. Dr. Ford Robertson described the method in a communication to the Research and Clinical Section of the Royal Medico-Psychological Association in February, 1946.

Short of the phenomenon of complete inhibition, partial inhibition may be met with, and again it indicates the presence of a very strong reagin.* We have found it in 0.70 per cent. of those F.R.C.'s which showed clarification in one or more tube, but only in 0.51 per cent. did we get a reading of 003. By contrast, in our experience, the figure of 0003 was not an uncommon result with the M.K.R.2.

Kolmer, quoted by Hinrichsen (1941), remarks that there is no doubt that some cases of syphilis may give false negative readings by all methods now employed. Nevertheless, the phenomenon of complete inhibition in a test for syphilis is highly undesirable, and care must be exercised, especially if the test has to be used alone as a screen for blood donors: in fact it would be desirable to use a more sensitive antigen as well as an antigen serum mixture which will be unaffected by the presence of excessive reagin. But a supersensitive test often defeats its own end, for not only may it incriminate an innocent serum but it may miss one heavily loaded with syphilitic reagin.

In common with other tests, therefore, the F.R.C. should not be used alone. But while it occasionally overlooks a serum charged with inhibitory amount of reagin it may succeed in demonstrating an early infection which may be missed by the Wassermann.

In evaluating a serological test it is customary to pay special attention to the range of doubtfuls. Moore (1936) says that as the false doubtful can be disturbing to the clinician, its occurrence must be rated a partial failure of the test. Schoek (1940) in fact advocated a greater assessment penalty on tests which give false doubtfuls than credit for true doubtfuls; doubtfuls amounted to 7.15 per cent. of those F.R.C. readings which were not fully negative; doubtfuls and weak positives together totalled 42 per cent. The corresponding figures for the Kahn and Wassermann were 31.3 per cent. and 48.7 per cent., and 21.7 per cent. and 25.7 per cent. It was noted that, whereas a case of syphilis resolving under treatment might give a doubtful result with both Wassermann and Kahn, it tended to give a weak positive with the F.R.C.

Because it is clear that, in an all round assessment, the M.K.R.2 and F.R.C. tests are at least equal in value to the precipitation and complement fixation tests, it would seem desirable in choosing two routine tests to employ a cholesterol colloid system for one and a Tolu system for the other. This is

all the more desirable because of the evasive, if not illusory, nature of syphilitic reagin, which suggests qualitative variants. Undoubtedly the Wassermann is the pick of the tests employing a cholesterolised antigen, if only for the reason that it differs from others in making use of the principle of complement fixation. It is suggested, therefore, that the Wassermann and F.R.C. are well suited as a pair of routine tests and that the Kahn, both because of its general excellence and speed, is the third test of choice.

The particular merit of the F.R.C. is its simplicity. It has been our practice to prepare the clarification test the evening before the day arranged for the Wassermann test, and to read the results first thing in the morning; the Wassermann tests are then completed; and the standard Kahn, which is employed as the third test, is ready an hour or two later. The F.R.C. is easy to read. There can be no doubt about the reading since, as compared with the Kahn and other precipitative tests, there can be no personal differences of interpretation. A disadvantage of all clarification tests, however, is that it takes time for the precipitate to settle. Sixteen to twenty hours is a convenient time because often a test can be set up one evening and read the next morning. Satisfactory readings can, however, be made at eight hours and, with experience, at four hours; but then it is being read as a precipitation rather than as a clarification test.

The Copenhagen conference of 1923 laid it down that pathologists who are concerned in systematic serodiagnosis should work in collaboration with the clinician. In the last two decades the tendency, especially on the continent, has been to centralize these tests, with the result that the serologist may not be in touch with the doctor in charge of the case. We regard this as a mistake, for as the sensitivity of the flocculation tests increases it becomes more and more important for the serologist to be in a position to help his clinical colleague in the interpretation of the results. The pathologist who reads the tests, provided he has adequate particulars of the case, is the one who is most competent to interpret equivocal findings in terms of the pathology of syphilis; but the ultimate responsibility for acting on the report belongs, of course, to the clinician. "A laboratory procedure," says Vogelsang (1938), "is a good hand-maid to the clinician, but a bad master when it supersedes clinical experience." The cases that have been cited illustrate difficulties of interpretation which are most satisfactorily solved when the pathologist and clinician have ample opportunities of consultation for which stereotyped "hints on the interpretation of blood tests" are to be regarded as a poor substitute.

* Dr. Ford Robertson reports that since the introduction in 1946 of a quantitative test these prozone reactions have been found to be associated with a high reagin content of the serum (from 80 to 320 units) as expressed in dilutions of serum.

Conclusion and Summary

The F.R.C. test has been found reliable for the serodiagnosis of syphilis. The sensitivity, with certain reservations, and specificity are considered to be favourable and to compare well with other established methods, while its simplicity is a strong point in its favour.

A numerical method of notation is suggested, and evidence is adduced to show that it provides an effective semiquantitative index either of the amount of reagin present or the severity of the infection.

Because of its different colloid basis it is recommended that the F.R.C. be used for routine serodiagnosis together with the Wassermann reaction, the two together providing greater accuracy of diagnosis than either alone: the two tests, in fact, are complementary and adequate. When one or other gives a positive result it is sound practice to adopt as a third test a precipitation method which employs a cholesterolised antigen, the Kahn test probably being ideal.

In primary syphilis the F.R.C. may become positive before the Wassermann reaction; in treated syphilis the F.R.C. may remain positive after the Wassermann has returned to negative, but in 40 per cent. of such cases the persistence of the F.R.C. has predicted a Wassermann relapse.

The phenomena of inhibition and the problem of the false negative reaction are discussed.

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