A CLINICAL ASSESSMENT OF THE TREPONEMAL WASSERMANN REACTION*

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The production of a specific antigen from living, pathogenic *T. pallidum* (Nichols strain) and its use with a complement-fixation technique (Price, 1949) was described early in 1957 (Price and Whelan, 1957). This initial report also presented the results of the treponemal Wassermann reaction (TWR) in 3,279 sera tested serially in parallel with the standard Wassermann reaction (SWR: Price, 1950); in 503 antenatal and 785 "problem" sera of this series, the results of the treponemal immobilization test (TPI) were available to assess the specificity of the TWR.

Price (1958), reporting the results of the TWR in 7,930 sera and 505 specimens of cerebrospinal fluid, concluded that:

- (1) The results of the TWR are reproducible;
- (2) The results are more sensitive and specific than with the use of the standard lipoidal antigen;
- (3) The TWR becomes positive in early syphilis sooner than the TPI;
- (4) The TWR has little advantage over other tests in established and untreated syphilis;
- (5) It is more sensitive in detecting positive reactions in the sera of coloured patients from areas where yaws is endemic than "reagin" tests or TPI;
- (6) There is no statistical difference between the results of the TWR and TPI in non-treponemal reactors;
- (7) The TWR usually becomes negative before the TPI.

The technical and economic advantages of the TWR as compared with the TPI were obvious and Price hoped that the TWR would provide a practical and specific procedure for the routine serological diagnosis or exclusion of treponemal infections. This hope was shared by clinicians, particularly in view of the delay and difficulty arising from the fact that TPI facilities in Britain are essentially confined to the V.D. Reference Laboratory in London. The rarity of fresh syphilitic infections, the downward trend in cases of genuine late syphilis, and the newer knowledge of the possible significance of the chronic

biologic false positive reaction, have made the syphilologist increasingly cautious in interpreting positive reactions with the non-specific "reagin" tests. The decision of this Society to devote two of its meetings in the current session to the serological and clinical assessment of newer serum tests is perhaps significant of the anxiety and sense of frustration felt by venereologists in regard to the serological facilities for the diagnosis of syphilis in Britain.

Clinical Assessment

My own clinical assessment of the value of the TWR is an extremely modest one, based on a very small number of selected cases. These patients have however, been examined and diagnosed personally, an important feature often missing from assessments of serological tests but one inevitably restricting the size of the series studied. The patients have been seen in Manchester, largely at the Royal Infirmary, and fall into two main groups:

- (1) Patients found to have, or to have had, syphilis; in all latent cases the certainty of infection is supported by satisfactory evidence, not solely serological, of infection in sexual or biological contacts, *e.g.* the mothers of undoubted cases of late congenital syphilis.
- (2) Blood donors of the Manchester region.

Both groups are highly selected; in the first place, sera have, with few exceptions, been sent by me to the Reference Laboratory only in difficult cases, and secondly, the tests performed have been selected by the Reference Laboratory. Unfortunately, this has often meant that, in addition to "reagin" tests, only the TWR or TPI has been performed, and thus the number of cases in which the results of *both* tests are available is very small. Ideally, in our present state of knowledge, the performance of any test should be

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judged against that of the TPI which is still considered to be free from the risk of non-treponemal reactions (Nielsen and Reyn, 1956).

Known Syphilis

The data of 120 patients in whom a firm diagnosis of syphilis seemed justified is shown in Table I.

TABLE I
DEFINITE SYPHILIS

Syphilis		Sex		
		Male	Female	Total
Late Acquired...	With physical signs	20	13	33
	Latent	6	17	23
	Mothers of patients with late congenital syphilis	0	14	14
	Total	26	44	70
Late Congenital	Number of patients	23	27	50
	Average age (yrs)	31	30	—
	Range (yrs)	9-50	15-52	—
Grand Total (All Syphilis)		49	71	120

The TWR result is available in 62 of these cases and the TPI in 95. Unfortunately, the results of both tests are known in only 35 patients (Table II).

TABLE II
RESULTS OF TWR AND TPI IN CASES OF KNOWN SYPHILIS

TWR	TPI			
	Positive	Negative	Doubtful	Fluctuating
Positive	16	Nil	Nil	1
Negative	12	4	1	1

Thus, in 35 cases of syphilis, the TWR and TPI were negative in eighteen (51 per cent.) and four (11 per cent.) respectively. Ignoring doubtful or fluctuating results, the TWR agreed with the TPI in twenty (62 per cent.) cases and disagreed in twelve (38 per cent.). In twelve cases (37 per cent.) with positive TPI the TWR was negative, indicating that its sensitivity in late syphilis is inferior to that of the TPI but that its specificity appears to be of a high order. In this group of patients the syphilitic infection was of long standing and the duration of the infection is obviously an important factor in the sensitivity of the tests. In a proportion of these 120 cases of syphilis the duration of infection could be estimated accurately, and in Table III (opposite) the results of serological tests are related to this factor. The persistence of immobilizing antibody after reagin and perhaps other antibodies have diminished is apparent (Figure) especially in the small group of treated patients.

FIGURE.—Positive reactions (per cent.) by duration of infection.

Blood Donors

All blood donations collected in the Manchester region are routinely "screened" serologically, using the Berger Kahn method. Those donations not giving a negative result are then tested with the standard Wassermann reaction and Kahn test in Manchester and, if these results are abnormal, the donor is referred to his own practitioner with the suggestion that the donor should be investigated by me. Under these arrangements I have examined 146 blood donors in the past 5 years, and in 103 cases sera have been sent to the Reference Laboratory in London. The TWR is available in 65 cases and the TPI in 58, but in only twenty blood donors are the results of both tests available. The final diagnoses reached in these 103 blood donors are shown in Table IV.

TABLE IV
DIAGNOSIS AND SEX OF 103 BLOOD DONORS

Diagnosis	Male	Female	Total
Late Congenital Syphilis	16	7	23
Late Acquired Syphilis with Physical Signs	7	3	10
Latent Syphilis	20	25	45
Non-treponemal Reaction	13	12	25
Total	56	47	103

TABLE III
DURATION OF INFECTION IN CASES OF KNOWN SYPHILIS

Type of Infection	Duration (yrs)	Results of Tests																			
		SWR					PPR					TWR					TPI				
		No. of Cases	Pos.	Neg.	D	F	No. of Cases	Pos.	Neg.	D	F	No. of Cases	Pos.	Neg.	D	F	No. of Cases	Pos.	Neg.	D	F
Acquired Un-treated	Under 10	9	77	23	0	0	9	77	23	0	0	1	100	0	0	0	8	75	25	0	0
	10-20	21	81	19	0	0	21	66	29	0	5	13	83	17	0	0	11	100	0	0	0
	21-40	20	40	60	0	0	20	30	65	0	5	13	42	50	0	8	18	79	11	5	5
	Over 40	3	66	33	0	0	3	66	33	0	0	1	0	100	0	0	3	66	33	0	0
Acquired Treated	Under 10	4	75	25	0	0	4	50	50	0	0	1	0	100	0	0	4	75	25	0	0
	10-20	8	75	25	0	0	8	63	37	0	0	6	66	33	0	0	5	100	0	0	0
	21-40	4	25	75	0	0	4	50	50	0	0	2	50	0	0	50	3	100	0	0	0
	Over 40	1	0	100	0	0	1	0	100	0	0	0	0	0	0	0	1	0	100	0	0
Congenital	Under 20	6	50	50	0	0	6	50	50	0	0	2	50	50	0	0	6	63	37	0	0
	20-29	18	94	0	0	6	18	83	11	0	6	11	73	27	0	0	15	87	0	0	13
	30-39	17	76	6	0	18	17	76	12	0	12	7	86	0	0	14	14	86	6	0	6
	Over 39	9	70	30	0	0	9	70	30	0	0	5	80	20	0	0	7	86	14	0	0
All Cases	Under 20	48	80	20	0	0	48	64	34	0	2	23	80	20	0	0	34	81	19	0	0
	21-40	59	66	27	0	7	59	61	32	0	7	33	62	28	0	10	50	84	6	2	8
	Over 40	13	64	36	0	0	13	64	36	0	0	6	66	33	0	0	11	73	27	0	0
	Total ..	120	69	28	0	3	120	63	33	0	4	62	68	27	0	5	95	82	12	1	5

Pos. = Positive.
Neg. = Negative
D = Doubtful
F = Fluctuating } percentages

TABLE V
SERUM REACTIONS AND DIAGNOSIS IN TWENTY BLOOD DONORS

Diagnosis		Results of Tests															
		SWR				PPR				TWR				TPI			
		No. of Cases	Pos.	Neg.	F	No. of Cases	Pos.	Neg.	F	No. of Cases	Pos.	Neg.	F	No. of Cases	Pos.	Neg.	F
Syphilis	Late Congenital ..	4	75	0	25	4	50	25	25	4	75	0	25	4	100	0	0
	Late Acquired with Signs	4	75	25	0	4	75	25	0	4	50	50	0	4	100	0	0
	Latent	8	87	0	13	8	63	25	12	8	37	62	11	8	100	0	0
	Total	16	81	6	13	16	63	25	12	16	50	44	6	16	100	0	0
Non-treponemal Reaction ..		4	50	25	25	4	0	100	0	4	0	100	0	4	0	100	0

The diagnosis and serum reactions in the twenty blood donors in whom the results of both TWR and TPI are available are shown in Table V. Here again the specificity of the TWR is very satisfactory (100 per cent.), but the sensitivity of the TWR is low (44 per cent. negative). It is interesting to note that, in this small group of blood donors, Price's precipitation reaction (PPR) is equally specific and slightly more sensitive. That the PPR is not con-

sistently satisfactory is shown (Table VI, overleaf) in the group of 58 blood donors in whom the TPI result is known; here the PPR was positive in five of eleven patients judged to be examples of a non-treponemal reaction. Table VII (overleaf) shows greater agreement between PPR and TWR, both in TPI-positive and TPI-negative cases, than between standard WR (SWR) and TWR; this arises from the greater sensitivity and lower specificity of the SWR.

TABLE VI
REAGIN TESTS AND TPI IN 58 BLOOD DONORS

Diagnosis		Results of Tests											
		SWR				PPR				TPI			
		No. of Cases	Pos.	Neg.	F	No. of Cases	Pos.	Neg.	F	No. of Cases	Pos.	Neg.	F
Syphilis ..	Late Congenital.. ..	15	93	0	7	15	80	13	7	15	100	0	0
	Late Acquired with Signs ..	5	80	20	0	5	60	40	0	5	80	0	20
	Latent	27	92	4	4	27	89	8	3	27	100	0	0
	Total	47	91	4	4	47	83	13	4	47	98	0	2
Non-treponemal reaction		11	73	18	9	11	45	55	0	11	0	100	0

TABLE VII
SEROLOGICAL AGREEMENT OF SWR, PPR AND TWR IN BLOOD DONORS

Result of TPI Test		Positive		Negative	
		SWR and TWR	PPR and TWR	SWR and TWR	PPR and TWR
Sero-Agreement (Per cent.)	Agreement	44	62	25	100
	Disagreement	56	38	75	—

Antenatal Cases

Of 106 antenatal cases giving abnormal serological results on routine testing, the TWR result is

available in 54 and the TPI in seventy. Again it is unfortunate that the results of both tests are available in only eighteen cases. Syphilis was diagnosed in only 58 (55 per cent.) of the 106 expectant mothers. The diagnoses and serum reactions in the 54 cases in whom the TWR is available, are shown in Table VIII.

Table IX shows the serum reactions and diagnosis in the eighteen antenatal cases in which both TPI and TWR results are available, and Table X (opposite) shows the sero-agreement between these two tests.

In this small group of antenatal cases the high proportion of false-positive reactions with the TWR is disappointing.

TABLE VIII
DIAGNOSIS AND SERUM REACTIONS IN 54 ANTENATAL PATIENTS (PERCENTAGES)

Diagnosis		No. of Cases	Results of Tests (per cent.)								
			SWR			PPR			TWR		
			Pos.	Neg.	F	Pos.	Neg.	F	Pos.	Neg.	F
Syphilis ..	Late Congenital.. ..	7	57	29	14	71	14	14	86	0	14
	Late Acquired with Physical Signs (including Three with Yaws) ..	4	50	50	0	75	25	0	75	25	0
	Latent	15	93	7	0	93	7	0	86	7	7
	Total	26	77	19	4	84	12	4	84	8	8
Non-treponemal Reactions		28	4	96	0	7	86	7	11	86	3

TABLE IX
SERUM REACTIONS AND DIAGNOSIS IN EIGHTEEN ANTENATAL CASES

Diagnosis	No. of Cases	Results of Tests											
		SWR			PPR			TWR			TPI		
		Pos.	Neg.	F	Pos.	Neg.	F	Pos.	Neg.	F	Pos.	Neg.	F
Syphilis*	14	79	14	7	79	14	7	64	14	22	93	0	7
Non-treponemal Reactions	4	0	100	0	50	50	0	75	25	0	0	100	0

*Including one case of late yaws

TABLE X
SERO-AGREEMENT IN EIGHTEEN ANTENATAL CASES
(PERCENTAGES)

Result of TPI Test		Positive (13 cases)	Negative (5 cases)
TWR (per cent.)	Agreement ..	69	20
	Disagreement ..	31	80
Total ..	No. ..	13	5
	Per cent. ..	100	100

“Problem” Cases

In 46 patients with active disease, including such conditions as haemolytic anaemia, rheumatoid arthritis, aneurysm, ulcers, etc., the TWR result is available in 27, the TPI in 35, and both in sixteen cases. These patients were referred to me because routine serological tests for syphilis (STS) had given abnormal results or for the exclusion of sero-negative syphilitic infection. The TWR and TPI disagreed in this difficult group (Table XI) in five cases (31 per cent.).

TABLE XI
SERO-AGREEMENT IN SIXTEEN “PROBLEM” CASES

TPI Result		Positive	Negative
TWR ..	Agreement ..	2	9
	Disagreement ..	2	3
Total ..		4	12

Early Syphilis

The TWR was positive in two cases of secondary syphilis, in one of which the TPI was also found to be positive. In one of these patients the TWR, like the SWR and PPR, had reverted to negative one year after treatment.

In ten cases of primary syphilis, the TPI was positive in two cases (Table XII) and doubtful in a third. Four cases were entirely sero-negative by reagin tests of which the PPR was slower to become positive.

Fluctuating Results

I have been impressed by the fluctuation in the results of both TWR and TPI in patients in whom these tests have been performed more than once within a short period of time (Table XIII). This seems to occur in untreated cases of long-standing syphilis and is, I suppose, to be expected in such patients. One has been familiar in the past with

TABLE XII
TPI IN PRIMARY SYPHILIS

Duration after Development of Chancre (days)	Results of Tests		
	SWR	PPR	TPI
7	Doubtful	—	Negative
7	Negative	Negative	Negative
7	Negative	Negative	Negative
7	Negative	Negative	Negative
17	Positive	Positive	Negative
11	Positive	Positive	Positive 100 per cent.
30	Positive	Positive	Doubtful
12	Negative	Negative	Negative
91	Positive	Positive	Positive
21	Positive	Negative	Negative

TABLE XIII
FLUCTUATING RESULTS WITH TWR AND TPI

Type of Syphilis	Sex	Duration of Infection (yrs)	Results of Tests			
			SWR	PPR	TWR	TPI
Congenital	M	31	+ Neg. +	+ Neg. +	Neg. +	+
Congenital	F	20	+ +	+ +	+	Neg. +
Congenital	F	32	+ +	+ +	Neg. +	
Congenital	M	30	+ Neg. +	+ Neg. +	Neg. +	+
Treated in 1931	M	28	Neg. Neg.	Neg. Neg.	+	+ 88 per cent. S.I.
Congenital	F	25	+ +	+ +	+	+
Congenital	M	29	+ +	Neg. Neg.	+	+
Congenital	M	20	+ +	+ +	+	Neg. +
Congenital	F	33	+ +	+ +	Neg. +	
Late latent	F	?	+ +	+ +	Neg. +	Doubtful 46 per cent. S.I. + 70 per cent. S.I.
Late ..	M	about 40	Neg. Neg. Neg.	Neg. Neg. Neg.	+ +	Neg. Doubtful + 76 per cent. S.I. + 42 per cent. S.I.

similar behaviour by the reagin tests in patients approaching, or fluctuating about, the sero-negative state. These varying results in different specimens of serum are presumably due to fluctuation in antibody level and do not reflect on the reproducibility of the tests on the *same* specimen of serum.

Conclusions

Because of restricted serological facilities, it has been difficult for one venereologist in England to gain enough experience of the TWR and TPI to form any dogmatic conclusions. My own small experience leads me to prefer the TPI to the TWR, as there is no conclusive evidence that the TPI gives rise to false positive reactions and, early syphilis

excepted, fewer false negative reactions seem to occur. In my highly selected material, the TPI gave false negative reactions in 11 per cent. of cases presumed on good evidence to have had syphilis. Whilst recognizing the technical and economic advantages of the TWR, I believe that it is not a substitute for the TPI; in fact, it is little superior to the information provided by the combination of SWR and PPR.

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