

TREPONEMAL IMMOBILIZATION TEST*

RESULTS OF 1,000 OBSERVATIONS

BY

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The interest aroused among venereologists by the reports of Nelson and Mayer (1949) followed by the paper of Magnuson and Thompson (1949) makes it unnecessary to describe the Treponemal Immobilization Test (TPI). Immediately after these publications we tried a few experiments and became convinced that it would take a long time to master the very delicate technique of the test. One of us (A. S.) visited the Johns Hopkins School of Hygiene and Public Health, Baltimore, to get first-hand information about it. Subsequently we were able to perform the test for the first time in Europe† with the original technique (Durel and others 1951a,b), and we now propose to survey the results obtained in the first thousand cases, representing 1,466 tests, many patients having had repeated examinations.

Obviously the present study is only provisional; for many years complement-fixation and flocculation tests have been used on a wide scale and on many points discussion is still going on. To give a definite opinion on the TPI test would be premature and its study must be patiently pursued.

Methods

(1) **TPI Techniques.**—It is not our purpose to deal with the technical aspects of the test since these are described in the papers quoted above. We only mention a few changes in procedure which seem advantageous (Sausse, 1951; Borel, 1952; Durel, Borel, and Sausse, 1952):

Apparatus

- (i) Waring blender superseded by a grinder with interchangeable tubes which can be used for centrifugation.
- (ii) Improved closure of the *T. pallida* extraction flask.
- (iii) Simplified device for the production of an anaerobic atmosphere.

Technical Procedure

- (i) Use of a $\frac{1}{3\sqrt{10}}$ dilution scale.
- (ii) Use of 20 per cent. complement in the reaction mixture.
- (iii) Extraction of *T. pallida* by rapid rocking in the presence of undiluted animal serum to obtain the inocula for infecting rabbits for future tests. This modification is important, as it allows one to obtain abundant inocula, very rich in *T. pallida* and free of testicular tissue. Early acute orchitis is thus obtainable at almost every inoculation without non-specific reactions. The suspension obtained from two orchitic testes is sufficient for the inoculation of six to eight animals.

We used the Gand and Nichols strains of *T. pallidum*; the latter, which was not available in Europe when we began our work, was kindly sent to us by Prof. Turner of Baltimore.

(2) **Source of Sera Tested.**—The sera studied came from our department and from most of the specialized departments in Paris, especially from the Saint-Louis Hospital. We were given the history (usually detailed and precise) of each case in order to try and correlate the clinical, serological, and therapeutic data.

(3) **Recording of Results.**—The TPI results are expressed in terms of *Specific Immobilization* (S.I.) according to the following formula which takes into account the percentage of dead organisms in the control tube:

$$\% \text{ S.I.} = \frac{\% \text{ T.P. motile (Control tube)} - \% \text{ T.P. motile (Test tube)}}{\% \text{ T.P. motile (Control tube)}}$$

We consider the test to be negative (–) when the S.I. is below 20 per cent., doubtful (±) when it is between 20 and 50 per cent., and positive (+) when it is above 50 per cent. A quantitative test can be carried out with sera giving 100 per cent. S.I. The titre is the reciprocal of the serum dilution, giving 50 per cent. S.I. (Nelson and Diesendruck, 1951.)

The results of the Standard Tests for Syphilis (STS) were difficult to express on account of the variety of the reactions used and the diversity of notations. The tests usually carried out at Saint-Lazare Hospital are the Wassermann reaction with Debains antigen and Institut Pasteur cardioliipin antigen, the Kahn and

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Kline tests with Institut Pasteur antigens, and the Rein-Bossak test with Rein antigen. These reactions were performed, if needed, on sera sent from other hospitals for the TPI test.

The lack of relativity between the STS performed at the time of the TPI test and those carried out previously would have greatly reduced the value of the comparison between the TPI and each type of STS. *We shall only indicate for each group of tests, complement-fixation or flocculation, its general behaviour, shown by the symbols +, ±, or -.* The phrase "a few +" means that most of the serum tests were negative except for a weak positive result by one or two techniques. Quantitative results when mentioned are expressed in terms of dilutions (dils) and not in sigma units.

(4) Scheme of Study.—The results with untreated and treated patients are dealt with separately.

A. Untreated Patients.

- (a) Suspected of having syphilis:
 - (i) contacts,
 - (ii) lesions suspected of being syphilitic,
 - (iii) unexplained positive or doubtful serology.
- (b) Known syphilitics.

B. Treated Patients.—These studies include :

- (a) Diagnostic value of test :
 - (i) retrospective diagnosis,
 - (ii) responsibility of syphilis for lesions,
 - (iii) reinfection, and relapse,
 - (iv) therapeutic provocation.
- (b) Behaviour of TPI during different forms of treatment.
- (c) Comparison between TPI and STS.
- (d) Starting phenomena.
- (e) Cerebrospinal fluid.

Results

A. Untreated Patients

(a) *Presumed Syphilitics.*—Syphilis may be suspected in patients who have been exposed to syphilitic infection, who present syphilis-like lesions, or who are found to have a completely or partially positive serology.

(i) *Contacts.*—As cases of direct latent syphilis are quite unusual, serological and clinical observation is sufficient to detect syphilis in a patient who has recently been in contact with a case of florid syphilis. When the exposure has occurred a long time previously, the patient's infection may be expected to have become latent and the TPI is of greater value.

In the few cases shown in Table I the TPI test seemed useless, but this was not actually so. If a plainly positive or negative STS (chiefly when reproducible) is highly significant, unstable results or weakly positive ones are not of similar value. In these cases the decision will be influenced by the TPI as appears in the following condensed case histories :

Case 186 (Dr. Perin).—Healthy-looking woman, blood donor, whose husband had syphilitic aortitis. Patient's serum reactions negative except for a weakly positive Rein-Bossak test with neat serum. TPI 98 per cent. S.I.

Case 207 (Dr. de Graciansky).—Healthy-looking man with no previous history of V.D. Wife's serology strongly positive. Patient's Wassermann reaction negative, flocculation test positive. TPI positive, titre > 1,000.

Case 239 (Dr. de Graciansky).—Healthy-looking man with history of malaria. Wife's serology strongly positive. Patient had negative Wassermann reaction and unstable flocculation test (Kahn + to ++ with serum diluted 1 in 6). C.S.F. negative. TPI 100 per cent. S.I. with blood, titre about 100, 40 per cent. S.I. with C.S.F.

When clinical and serological manifestations are lacking at birth, congenital syphilis may be included in the contact group. Before the use of the TPI such cases were generally submitted to systematic treatment. In ten cases the test allowed the rejection of the diagnosis of syphilis. The following case is of special interest :

Case 114 (Dr. Payenneville).—Syphilis discovered in the mother at the end of pregnancy ; she was given 6 mega units penicillin during the last 10 days of pregnancy and was delivered on 8.2.51. Wassermann reaction + on cord blood. On 28.2.51 the child had a negative Wassermann reaction, showing that the reagins had disappeared rapidly, which is not usually the rule. TPI on this date 9 per cent., on 7.5.51, 6 per cent.

TABLE I
POSITIVE TPI TESTS AMONG CONTACTS

Source of Infection	General Pattern of STS*				
	CFT+ Fl-	CFT- Fl+	Unstable	A few +	CFT- Fl-
Syphilitic consort	2 out of 2	2 out of 2	1 out of 1	1 out of 1	0 out of 8
Syphilitic parent	2 out of 2	—	—	—	0 out of 19

*Owing to the impossibility of summarizing in charts the detailed results of the different serological reactions employed in every laboratory, + and - express the general pattern of complement-fixation or flocculation tests, "a few +" means that most of the STS were negative except for a weak positive result by one or two techniques.

TABLE II
POSITIVE TPI TESTS ON PATIENTS WITH LESIONS SUSPECTED OF BEING SYPHILITIC

Type of Disease suggested by Lesions	General Pattern of STS					
	CFT+ FI+	CFT- FI+	Discrepant	Unstable	A few +	CFT- FI-
Primary syphilis	—	3 out of 3	—	—	0 out of 1	0 out of 6
Secondary syphilis	2 out of 2	—	—	—	0 out of 2	0 out of 7 (2 doubtful results)
Late muco-cutaneous syphilis	4 out of 4	2 out of 2	—	—	—	—
Possibility of late neuro-visceral syphilis	—	0 out of 1	0 out of 1	2 out of 4	0 out of 2	25 out of 26 (1 doubtful result)

(ii) *Patients with Lesions suspected of being Syphilitic.*—The number of positive TPI tests found among this group of patients is shown in Table II. The TPI is chiefly interesting in cases arousing the suspicion of late syphilis as it is known that the usual serum tests may be uncertain, even in recognized syphilis. The following are typical case histories :

Case 270 (Dr. Sigwald).—Ataxia and loss of reflexes in lower limbs since 1939. STS always negative. TPI 16 per cent. in the serum and 7 per cent. in C.S.F. In fact this was a case of familial ataxia. The patient's sister has a similar ataxia with a negative TPI.

Case 763 (Dr. Bolgert).—Tabetic pressure pains in the feet with mild psychic disorder ; knee and ankle jerks absent. C.S.F. showed a colloidal mastic test deflected on the left, albumin 45 mg./100 ml., 48 cells per c.mm. TPI negative in both blood and C.S.F. Vertebral metastases due to oesophageal carcinoma.

Case 905 (Drs Bolgert and Lévy).—Multiple gummata for 18 months. Antituberculous treatment without effect. STS negative. TPI 0 per cent.

A few cases are curious ; had there been more such cases it might give a new impulse to the old discussion about the aetiology of tabes dorsalis and Argyll-Robertson pupil reactions.

Case 804 (Drs Price and Nicol).—Clinically obvious tabes dorsalis ; STS always negative in blood and C.S.F. No previous history of infection and no treatment. TPI 2 per cent.

Case 1029 (Dr. Sigwald).—Ataxia, absent knee and ankle jerks, positive Romberg's sign, vibratory anaesthesia, myosis, weak pupillary reflex. No previous history, no treatment. Negative STS in both blood and C.S.F. C.S.F. albumin 38 mg./100 ml., cells 2.7 per c.mm., Pandy test weakly positive. TPI twice negative.

Case 1106 (Prof. Moreau).—Isolated true Argyll-Robertson pupil reaction. No previous history, no treatment. All reactions negative with blood and C.S.F., TPI negative.

Case 1416 (Prof. Fauvert).—Argyll-Robertson pupil reaction, absent patella reflex. No previous history, no treatment. STS negative, TPI 4 per cent.

(iii) *Doubtful Serological Results.*—The development of systematic serological examinations has increased the number of "surprises" giving a totally or partly positive

result in apparently healthy individuals, or at least in patients without presumptive syphilitic disease. As the TPI detects antibodies quite distinct from reagins, it is extremely helpful for the diagnosis of syphilis in cases with unstable or discrepant results with the STS.

The results are summarized in two tables, Table III positive TPI tests indicating unknown but probable syphilis, and Table IV negative TPI tests showing the possibility of falsely positive serological reactions—"biological false positive" results.

Table III does not need detailed comment ; it shows that the TPI contributed an important element to the diagnosis of syphilis. We are not surprised to find a positive TPI in a case of yaws (13-year-old boy with untreated secondary yaws, totally positive STS, TPI positive to a titre of 390 with the Nichols strain). In the case of "atypical pneumonia" we must admit that, although it was clinically a case of Franconi-Heggli syndrome, the cold agglutinin titre was only 1 in 8. The cases of malaria were quiescent. Four of the seven patients with filariasis had been cured for a long time ; in the only patient with a positive TPI, the filariasis had been cured for 20 years. Only two of the "Various" cases merit mention. One was a patient with jaundice and the other had lymphogranuloma inguinale ; these conditions might be held responsible for causing a biological false positive reaction with the STS, but the TPI showed that this was not so.

The results shown in Table IV need fuller discussion, especially where there is a marked discrepancy between the STS and the TPI. The most interesting observations are summarized below :

Case 856 (Dr. Schneider).—No previous history, no lesions, STS completely negative 2.6.50. Went to the Cameroons and contracted filariasis.

On his return to France, on 20.8.51, Wassermann reaction +, Kolmer test + + +, Kline test + +, Kahn test + +, thymol test, 11 (normal value with Meunier electrophotometer = 20). Blood cholesterol 1.5/2.8.*

2.10.51, TPI negative. Possible explanations of discrepancy are an error in technique, a biological false positive reaction due to filariasis (although the cases in Table III suggest this is not usual), or an incubating syphilis. The patient returned to Africa and could not be kept under observation.

* Cholesterol ester 1.5 g./litre, total cholesterol 2.8 g./litre.

TABLE III
POSITIVE TPI TESTS AMONG PATIENTS WITHOUT SYMPTOMS OF SYPHILIS BUT WITH POSITIVE STS WHICH WERE SUSPECT

Clinical Condition	General Pattern of STS				
	CFT+ FI+	CFT- FI+	Discrepant	Unstable	A few +
Healthy	55 out of 55	9 out of 15 (2 doubtful cases)	7 out of 11	3 out of 3	5 out of 24 (1 doubtful)
Old Yaws	1 out of 1	—	—	—	—
Old Malaria	7 out of 8 (1 doubtful)	0 out of 2	—	1 out of 1	1 out of 2
Atypical Pneumonia	1 out of 1	—	—	—	—
Filariasis (Loa)	1 out of 3 (1 doubtful)	0 out of 2	—	—	0 out of 3
Pregnancy	—	1 out of 1	—	1 out of 2	—
Various	—	0 out of 2	1 out of 3	2 out of 3	0 out of 8

TABLE IV
NEGATIVE TPI TESTS IN SERA FROM PATIENTS WITH PROBABLE BIOLOGICAL FALSE POSITIVE SERUM REACTIONS

Clinical Condition	General Pattern of STS				
	CFT+ FI+	CFT- FI+	Discrepant	Unstable	A few +
Healthy	—	4 (76, 104, 111, 299)*	4	—	18
Old Malaria	—	2 (716, 1382)	—	—	1
Atypical Pneumonia	—	—	—	—	1 (1,000)
Filariasis (Loa loa)	1 (856)	1 (175)	—	—	3
Frequent Herpes	—	1 (34)	—	—	1
Lupus eryth. disc.	—	—	—	—	1
Banti's Syndrome	—	—	1 (154)	—	—
Amoebic Hepatitis	—	1 (489)	—	—	—
Leprosy	—	—	—	—	1
Various	—	—	1	1	5

*Numbers in brackets are those of case histories cited in text.

Case 76.—Healthy subject. Kolmer —, Kline —, MKR2 +++, Rein-Bossak +++. TPI negative.

Case 104.—Healthy subject. Wassermann reaction —, Kahn + + with neat serum, TPI 6 per cent.

Case 111.—Healthy subject. Wassermann reaction —, Hecht —, Kahn + +, Rein-Bossak —, Vernes A = 0, B = 15, TPI 14 per cent.

One month later, Wassermann reaction —, Hecht —, Kahn ±, Rein-Bossak ±, TPI 14 per cent.

Case 299.—Healthy subject. Wassermann reaction —, Hecht —, Kahn + + +, Rein-Bossak + +, TPI 8 per cent.

Case 716.—Old malaria, Wassermann reaction —, Kolmer —, Kline + +, Kahn —, MKR2 + + +, thymol 24, TPI negative.

Case 1382.—Old malaria. On six occasions Wassermann reaction —, Kolmer —, Kahn + + +, MKR2 + + +, thymol 8, TPI 4 per cent.

Case 175.—Old filariasis. Wassermann reaction ±, Kolmer +, Kline +, Kahn + + +, MKR2 +, TPI negative.

Case 34.—Frequent relapse of herpes. Wassermann reaction —, Kahn + +, MKR2 + +, Rein-Bossak + twice, TPI negative.

Case 489 (Dr. Schneider).—Amoebic hepatitis. 29.1.48, Kolmer —, Kline —, Kahn —, thymol 122.5, blood cholesterol 1.4/2.2.

26.4.51, Wassermann reaction —, W.R. (cholesterolized) + + +, Kolmer + +, Kline + + +, Kahn + + +, thymol 169, blood cholesterol 0.85/1.65, TPI 2 per cent.

29.6.51, Wassermann reaction —, W.R. (chol.)—, Kolmer —, Kline —, Kahn —, Rein-Bossak + + with neat serum, TPI negative.

Case 154 (Dr. Price).—Banti's syndrome. 5.9.50, Wassermann reaction + + +, Kahn + + +.

6.9.50, Wassermann reaction —, Kahn + + +. 11.10.50, Wassermann reaction + + +, Kahn + + +, Price precipitation reaction + + +.

14.3.51, Wassermann reaction anticomplementary, P.P.R. + + +, TPI 10 per cent.

Case 1000 (Dr. Siboulet).—Evidence of atypical pneumonia. Cold agglutinin titre 1 in 500, Wassermann reaction ±, Kolmer and Kahn weak positive, TPI negative.

(b) *Known Syphilitic Patients.*—Among the patients recognized as having syphilis, but hitherto untreated, the TPI gave the results shown in Table V.

TABLE V
POSITIVE TPI TESTS AMONG KNOWN BUT UNTREATED
SYPHILITIC SUBJECTS

Clinical Condition	General Pattern of STS		
	CFT+ Fl+	CFT- Fl+	CFT- Fl-
Primary Syphilis ..	5 out of 15 (2 doubtful)	0 out of 2 (1 doubtful)	0 out of 6 (2 doubtful)
Secondary Syphilis ..	15 out of 21 (5 doubtful)	0 out of 1	—
Tertiary Muco-cutaneous Syphilis	2 out of 2	—	—
Neuro-visceral Syphilis..	5 out of 5	2 out of 2	—

B. Treated Patients

(a) Diagnostic Value of Test

(i) *Retrospective Diagnosis.*—Too many physicians establish the diagnosis of syphilis on an insecure basis. If such a patient undergoes intensive treatment, it will never be known whether he really had syphilis or not. Where mild treatment has been given, the finding of a negative TPI together with negative STS may allow one to suspect the original diagnosis of syphilis. Such a doubt may be unwarranted, as cases are known of syphilitic patients "cured" with mild treatment. Two cases may be quoted :

Case 538.—March, 1945, cervical erosion with purulent endocervicitis, supposed to be a chancre, no dark ground examination. Wassermann reaction + + +, Kahn ±, patient jaundiced. Only treatment was three injections of lipobismuth and twelve of HgCN. The STS were negative from 1946–51.

13.4.51, TPI 0 per cent.

There are good reasons for thinking that this patient had a non-syphilitic erosion of the cervix and originally had a biological false positive sero-reaction.

Case 1030.—In 1946 a two-year-old child had a rash of the scalp with a circinate appearance. In addition there was an ogival palatine vault and saddle deformity of the nose. Despite completely negative serum tests 24 injections of lipobismuth were given. The STS were always negative.

28.10.51, TPI 0 per cent.

(ii) *Diagnosis of Suspected Lesions in Syphilitic Patients.*—When a patient who has had syphilis presents neuro-visceral lesions, it is always possible to suspect syphilis as the cause, even though the STS are negative. The TPI, which seems to be more stable than the STS, may be taken into account when making the diagnosis. A TPI was requested in eleven cases with such conditions (optic neuritis, angina pectoris, glossitis, gumma, etc.); in ten the result was negative, allowing the

rejection of the diagnosis of active syphilis. The following two case histories illustrate this :

Case 914 (Prof. Boudin).—History of a chancre in 1923 treated for six months with NAB and bismuth. In September, 1951 there was loss of the critical faculties and myosis. Despite negative STS on three occasions the patient was given 20 mega units penicillin. On 13.11.51 the C.S.F. showed a negative Wassermann reaction, 5.2 cells per cu. mm. in the lumbar and 14 per cu. mm., in the cerebral fluid, albumin 56 mg./100 ml. colloidal mastic test 1120.2212.2206. TPI negative (serum). On 28.1.52 the TPI was 6 per cent. with blood and negative with C.S.F. As far as we are aware such a dose of penicillin would not convert a TPI to negative in a case of GPI. The result of the TPI made one look for another diagnosis ; a frontal lobe tumour was found and removed surgically.

Case 1386 (Dr. de Graciansky).—Primo-secondary syphilis in 1948, weak and irregular treatment with bismuth. In January, 1952, two gummatous lesions of the frontal region developed. STS completely negative, TPI negative. The gummata were probably not syphilitic in origin.

(iii) *Undertreatment, Relapse, and Reinfection.*—Studies on experimental syphilis show that the TPI titre rises slowly in the case of relapse following sub-curative treatment and abruptly after positive re-inoculation (booster effect). It is difficult to obtain data in human syphilis as the TPI is not yet sufficiently widely used for curves of the titre to be available in these cases.

We have seen four examples of a fresh rise in the S.I. after treatment with 4.8 mega units P.A.M., giving the impression that the treatment might have been sub-curative ; in a further case the rise occurred even after treatment with 15 mega units.

Case 30 (Dr. Bolgert).—Secondary syphilis 8.3.49, treated with three injections HgCN and 15 mega units penicillin. Clinically cured but dissociated sero-reactions remained.

13.12.50, Wassermann reaction —, Hecht —, Kahn ±, MKR2 + +.

17.1.51, Rein-Bossak + + + +.

28.3.51, Wassermann reaction —, Hecht —, MKR2 + +, Kline + +, Kahn —.

17.1.51, TPI 64 per cent.

18.2.51, TPI 49 per cent.

10.5.51, S.I. rose to 100 per cent. (titre 14).

Although this titre of 14 is a very low one, this seemed to presage a relapse, and prudently similar treatment was repeated.

10.10.51, TPI 40 per cent.

16.1.52, TPI 6 per cent.

We have also seen a second rise in the S.I. curve in the course of a case of secondary syphilis treated with 34 g. aureomycin. We could not find any case of booster effect suggesting re-inoculation.

(iv) *Therapeutic Provocation.*—Following Milian, some syphilologists think that a latent syphilis may be revealed by the first steps of treatment, being shown by

the positive STS following this "provocation." In the light of the results given by the TPI the truth of such an interpretation may be questioned, and the assumption of a biological false positive sero-reaction may be made on the basis of a conflict between organism and drug. Two illustrative cases are :

Case 1157 (Prof. Degos).—History of chancre in February 1947, STS + + + +. Treatment with NAB and 160 injections bismuth. Negative serology since September 1947.

25.9-1.10.51, re-activation with sulfar. 0.18 g., 0.30 g., 0.42 g.

11.10.51, W.R. —, Hecht —, Kahn —.

21.10.51, W.R. —, Hecht —, Kahn —, V.D.R.L. + +.

1.11.51, W.R. —, Hecht —, Kahn +, V.D.R.L. + + +.

6.12.51, TPI negative.

Case 1158 (Prof. Degos).—History of a chancre in 1920. NAB, Hg, and Bi from 1920 to 1950. Sero-negative since 1933. From 13-17.10.51, sulfar. 0.18 g., 0.30 g., 0.42 g., in order to re-activate. 17.10.51 H⁶H⁴K + + + +. 12.12.51, TPI negative, STS returned to negative.

(b) *Relation between TPI and Treatment.*—The general relation is shown in Table VI, which does not take into account the form of treatment. The expression of results is very difficult because of the numerous treatment schedules in use, especially before the penicillin era. As we are considering only comparative cases it has been necessary to exclude so many cases that the results presented are not sufficiently numerous to be of statistical value. We shall consider only the treatment of primary and secondary syphilis; cases of late syphilis involve still more complex problems.

Six schedules of treatment were studied :

(i) *Neoarsphenamine + Bismuth.*—This group of cases was treated according to the French custom before

the penicillin era, by at least one course of 6-7 g. NAB given over an 8-week period and accompanied or followed by at least four courses each of twelve lipobismuth injections given during one year. The usual dosage is two injections weekly, each of 75 mg. liposoluble Bismuth.

(ii) *Bismuth Only.*—This corresponds to treatment for at least one year with four courses of injections similar to those mentioned above. In many cases the course consists of fifteen injections instead of twelve.

(iii) *Penicillin, less than 15 mega units.*—This group includes cases treated with 2.4, most frequently with 4.8, and rarely with 10 mega units. Treatment has generally been given with a depot form of penicillin (aqueous procaine penicillin, P.A.M.).

(iv) *Penicillin, less than 15 mega units + Bismuth.*—The previous treatment schedule with at least two courses of twelve injections of lipobismuth.

(v) *Penicillin, at least 15 mega units.*—Following the work of Bolgert and Lévy (1947, 1951) a scheme of treatment often used in France comprises three injections HgCN (10 mg. daily), then 15 mega units aqueous or depot penicillin given during a period of 16 days. Many other authors think that mercury can be omitted. We have included in this group all patients who received at least 15 mega units penicillin, with or without mercury.

(vi) *Penicillin, at least 15 mega units + Bismuth.*—Many specialists, especially when treating prostitutes, add a few courses (not less than four) of bismuth.

Table VII (overleaf) gives results correlating the TPI with different types of treatment in early syphilis. In order to eliminate the starting phenomena or "serological inertia" we have only shown the TPI tests done one year after the beginning of treatment. The rare doubtful cases have been omitted. As is well known, the classical serology does not reverse at once when the treatment is completed; the reagins have to

TABLE VI
GENERAL RESULTS IN TREATED SYPHILIS
Number of Positive TPI Tests v. Total Number in Each Group

Clinical Condition		General Pattern of STS					
		CFT+ Fl+	CFT- Fl+	Discrepant	Unstable	A few +	CFT- Fl-
Primary Syphilis	Sero-negative	—	—	—	—	—	0/24
	Sero-positive	27/35 (+ 4 doubtful)	15/23 (+ 2 doubtful)	3/5	2/2	2/8	13/69
Secondary Syphilis	31/35 (+ 2 doubtful)	22/28 (+ 3 doubtful)	1/1	5/6	6/12	13/65 (+ 2 doubtful)	
Latent Syphilis	126/189 (+ 2 doubtful)	54/61 (+ 5 doubtful)	5/6	16/20 (+ 2 doubtful)	18/24	14/32 (+ 1 doubtful)	
Muco-cutaneous Late Syphilis	3/3	2/2	—	1/1	1/1	1/1	
Late Neurosyphilis	19/19	7/7	3/3	1/1	2/2	13/19	
Cardiac or Visceral Late Syphilis	12/12	2/2	—	1/2	1/2 (+ 1 doubtful)	0/4 (+ 1 doubtful)	
Late Congenital Syphilis	12/13	5/6	—	1/1	0.1 (+ 1 doubtful)	2/6 (+ 1 doubtful)	

TABLE VII
RESULTS VERSUS TREATMENT

Number of Negative TPI Tests after 12 months in Cases of Early Syphilis compared with Total Number of Cases in Each Group

Type of Disease		Type of Treatment					
		As + Bi	Bi alone	Penicillin < 15 MU		Penicillin > 15 MU	
				Alone	+ Bi	Alone	+ Bi
Primary Syphilis	Sero-negative ..	3/3	3/3	—	3/3	11/11	—
	Sero-positive	24/55	4/5	—	4/6	11/11	2/3
Secondary Syphilis		22/45	5/10	2/2	3/5	23/32	3/4

be eliminated. This elimination, which depends upon the time needed for destruction of the reagin molecule, cannot be shortened to less than 70 to 80 days (counted from the starting of the curve). We propose to call this phenomenon the period of "serological inertia". A similar inertia exists of course with the TPI :

Case 401 (Dr. G. Lévy).—History of syphilis in 1921, well treated with As and Bi. STS negative until 1950.

31.3.51, new chancre, STS strongly positive.

1-26.4.51, three HgCN (10 mg.) + 15 mega units penicillin. TPI titres, May 22, 1,360 ; June 18, 520 ; July 20, 260 ; September 21, 88 ; January 17, 1952 ; titre 13. STS curve similar.

(c) *Comparison between TPI and STS.*—It may be of interest to compare the general results obtained with the TPI and with the STS. These results are shown in Table VIII and will be discussed later.

With the collaboration of Mlles Gasne and Pierre we tried to form an opinion of the comparative values of the TPI and the Rein-Bossak reaction which Dr. Rein demonstrated to us at the Saint-

Lazare Hospital. Our results, shown in Table IX, indicate that when confidence is placed in the TPI, the sensitivity of the Rein-Bossak test does not too much affect the specificity of this reaction.

(d) *Starting Phenomena.*—In our experience the development of the immobilizing antibody is relatively delayed in syphilis as it appears somewhat later than the reagins. This point must be appreciated in order to interpret the TPI in early syphilis, especially after treatment has been begun. The TPI becomes positive when the level of antibodies reaches a certain threshold. It seems likely that the sensitivity of the test will be increased in the future, thus allowing positive results to be obtained at a lower threshold and hence their detection at an earlier date in the infection. Thus the "staggering" between the TPI and the STS does not have any great significance, as the immobilizing antibodies are elaborated before their detection by the

TABLE VIII
COMPARISON OF TPI TESTS AND STS

STS	Number of Cases	TPI +	TPI ±	TPI -
CFT + FI +	354	315 (88.9% ± 3.6)*	13	26 (7.3% ± 2.6)
CFT - FI +	102	73 (71.5% ± 9)	9	20 (19.6% ± 8)
Discrepant or Unstable	82	55 (67% ± 10)	3	24 (29.2% ± 10)
A few +	84	36 (42.8% ± 11)	3	45 (53.5% ± 11)
CFT - FI -	378	94 (24.8% ± 4.4)	15	269 (71.4% ± 5)
Totals	1,000	573	43	384

*Percentage and ± twice the standard error calculated with respect to the corresponding number in the first column.

TABLE IX
COMPARISON BETWEEN REIN-BOSSAK REACTION AND TPI

Rein-Bossak	++++			+++			++			+			Total		
	+	±	-	+	±	-	+	±	-	+	±	-	+	±	-
CFT + FI +	1	—	1*	1	1	—	3	—	—	—	—	—	5	1	1
CFT - FI +	—	—	—	2	2	1	—	—	1	—	—	—	2	2	2
Unstable or discrepant	—	—	—	1	—	—	—	—	1	—	—	—	1	—	1
A few +	6	—	1*	10	1	3	3	—	3	3	—	2	22	1	9
CFT - FI -	1	—	—	6	—	1	17	2	6	7	1	1	31	3	8

*Probably due to "starting phenomenon".

TPI. We are only reporting what we were able to observe in 1951 with the technique then in use.

Three situations may be described :

(i) *No Elaboration of Detectable Antibodies.*—If treatment is started very quickly, it is possible that there will not have been enough time for immobilizing antibodies to be present at a sufficient level to give a positive TPI. We have had the opportunity of observing this phenomenon in twelve cases of primary chancre and two of secondary syphilis :

Case 430 (Dr. de Graciansky).—25.5.51, chancre and roseola, STS +, Kahn 4 dils. Treated with 6 mega units penicillin.

Kahn May 30, 12 dils ; July 11, 6 dils ; July 25, 4 dils ; August 17, 2 dils.

TPI May 25, 2 per cent. ; June 27, 19 per cent. ; July 25, negative ; September 26, negative.

Case 801 (Dr. de Graciansky).—2.8.51, penile chancre, DG. +, Kahn 4 dils. Treated immediately with 6 mega units penicillin.

8.8.51, Kolmer + + +, Kahn 16 dils.

19.9.51, Kolmer + +, Kahn 2 dils.

20.10.51, Kolmer ±, Kahn —.

TPI August 2, negative ; November 28, 2 per cent.

(ii) *Delayed Elaboration of Antibodies.*—It is pointed out above that, with the techniques that we used, the TPI may fail to become positive when early treatment

intervenes. It is possible that, in the absence of treatment, antibodies might be elaborated, but lag behind the STS. It must be admitted that we have not seen an indubitable example. We indicate this possibility only because it arises from the facts given above and it may be referred to in certain paradoxical cases.

(iii) *“Peak” Phenomenon.*—In cases of syphilis treated early, it frequently happens that the STS show only a brief period of positivity. A similar “peak” may be seen in the TPI. We had two such cases in primary syphilis, and one in secondary syphilis :

Case 417 (Dr. de Lestrade).—25.4.51, Exposure ; 19.5.51, chancre of the penis, D.G. +, STS negative 4 mega units penicillin with bismuth. Remained seronegative.

TPI May 23, 2 per cent. ; June 23, 100 per cent. (titre 14) ; November 19, negative.

(e) *Tests on the C.S.F.*—We have performed 58 TPI tests on the C.S.F. The eighteen positive cases are summarized in Table X.

The forty negative cases were these : 27 with positive TPI in blood (1 syphilitic meningitis, 2 tabes dorsalis, 2 keratitis, 1 ectasia, 2 tertiary syphilis, 1 latent syphilis, 18 old syphilis).

Thirteen with negative TPI in blood (2 nervous disorders, 2 clinical tabes, 1 lesion suggesting

TABLE X
POSITIVE TPI TESTS ON C.S.F.
(In every case the TPI was positive on the patient's serum)

Case no.	Clinical Findings	Treatment	STS Results	
			Serum	C.S.F.
95	Tabes dorsalis	90 MU. penicillin	CFT— FI—	—
196	Tabes, G.P.I.	None	CFT + FI+	+*
215	G.P.I.	Malaria, As, Bi	Discrepant	—
239	Serological doubt	6 MU penicillin	Unstable	—
394	Serological “surprise”	None	CFT+ FI+	—†
634	Tabes dorsalis	As, Bi	CFT— FI—	—
636	Serological “surprise”	As, Bi, penicillin	CFT+ FI+	?
638	Secondary syphilis	15MU penicillin	CFT— FI—	?
642	Serological “surprise”	None	CFT+ FI+	?
845	G.P.I.	As, Bi, penicillin	CFT+ FI+	+
968	? G.P.I.	None	CFT— FI—	—‡
976	Tabes dorsalis	As, 80 MU penicillin	CFT+ FI+	—
1036	Hemiplegia one year after chancre	Insufficient	CFT+ FI+	?
1181	Congenital syphilis G.P.I.	Malaria, As, Bi	CFT— FI—	—
1250	Argyll-Robertson pupils	As	CFT— FI—	—
1314	Tabes dorsalis	Insufficient	CFT— FI—	—
1316	Tabes dorsalis	15 MU penicillin	CFT+ FI+	—
1351	Tabes dorsalis	60 MU penicillin	CFT— FI+	—

*After 15 MU penicillin, C.S.F. Wassermann reaction was negative, but TPI test remained at 100 per cent. S.I.

†After 6 MU penicillin, S.I. fell from 98 per cent. to 15 per cent. in 6 months, blood continuing to give 100 per cent. S.I.

‡Colloidal mastic test of GPI type.

tertiary syphilis, 1 cancerous polyneuritis, 4 old treated syphilis, 3 recently treated syphilis).

The S.I. is lower in the C.S.F. than in the blood. C.S.F. does not seem to keep so well as serum, even when stored in a deep-freeze cabinet.

Discussion

At the present time any discussion about the TPI can only be provisional. Syphilis has too many categories to permit a sufficient number of observations to have yet been made on each class meriting study with the TPI. Notwithstanding the exact and critical studies of Nelson and his associates, surprising results still occur even when technical mistakes, difficult to avoid in such a complex reaction, are excluded. A few years will have to elapse before all the features of this new serological investigation can be determined.

For a true interpretation of the TPI it must be remembered that it does not represent a unique reaction. On the contrary, it puts syphilis, as regards its serology, in line with other infections. It is useful to think that it follows the same laws, particularly concerning immunity and healing. In this respect we quote from an important report on immunity by Teissier, Reilly, and Rivalier (1929):

Healing, desensitization, increasing level of antibodies are the last terms of the process involved in specific proteinotherapy and whose respective ratios may be experimentally changed with time, thus allowing the independence of these three terms to be stated . . .

It is unwise to overestimate the significance of the presence or absence of immobilizing antibodies.

Untreated Patients.—Examination of Tables I-IV shows that the TPI is useful in detecting syphilis in contacts, in patients with a suspicious lesion or whose serological tests give doubtful results, and in cases of possible late syphilis. These last are the most difficult. Among 56 observations Case 856 is an exception because of the finding of strongly positive complement-fixation and flocculation tests with a negative TPI. We have no explanation for it.

If the specificity of the TPI is admitted, which is logical in view of what is known about it, it seems that it detects numerous biological false positive reactions (Table IV). The number of such cases is too small to warrant much comment, but the origin of these biological false positive reactions was rarely found; most patients were healthy at the time of the test and their previous histories were of little significance. To call these STS false reactions obviously implies confidence in the TPI. Although this seems legitimate, *it would be desirable to repeat the test three months later in order to eliminate the*

possibility of technical mistakes or of delayed production of antibodies.

Treated Patients.—The diagnostic significance of the TPI appears in the four groups surveyed: retrospective diagnosis; evaluation of the part played in late disorders by an old history of syphilis; discrimination between relapse and reinfection; interpretation of provocation. It has not the same importance in every case; for example, in retrospective diagnosis, decision is almost a matter of opinion and depends on what is called inadequate treatment. Here it is necessary to be cautious; its practical value is certainly greater when used to decide whether previous syphilis is responsible in cases with cardiac or nervous lesions.

Theoretically the TPI should allow the discrimination of relapse from reinfection, but it will have to be more widely used to become valuable in this respect. Good quantitative serological tests are probably as valuable as the TPI in this connection, but it remains a practical question of the greatest interest. Is it necessary to re-treat a syphilitic patient whose antibody titre is rising? Case 30 quoted above is instructive, for it shows that re-treatment lowered the TPI again. We have not yet enough data to know whether instability of the titre may occur. It would be interesting to know whether a TPI reversed to negative by treatment may rise again without a new infection.

The results given by the TPI in cases of "provocation" seem to raise a serious doubt about the value of this kind of investigation.

The *prognostic importance* of the TPI is far from being established with certainty, because antibodies and healing do not run parallel with each other. Studies on experimental syphilis will certainly be very instructive (Gastinel and others, 1952).

Examination of Table VII shows that, from the point of view of the TPI, treatment with As or Bi has been less satisfactory than treatment with penicillin. It also seems that the TPI remains positive longer in secondary than in primary syphilis. Has this fact any prognostic significance or is it explained by an infection of longer duration, allowing the elaboration of more antibodies? Perhaps it is favourable for the future of the patient, but we will not yet venture to express an opinion.

In comparing the STS with the TPI, Table VIII shows that positive reactions were found with the TPI, and with the complement-fixation and flocculation tests slightly more often than negative reactions with the same three tests, the percentages being 88.9 and 71.4 respectively. In other words, when the specificity of the TPI is admitted, from a

general point of view, the test is slightly more sensitive (17.5 per cent. \pm 3.8) than the STS.

When they give concordant results the two methods of investigation enhance each other's value without, however, solving certain problems. Among the positive cases, what is the meaning of those STS and TPI results which seem irreducible? Are they due to the persistence of the antigen, perhaps in a modified form (granular form of the parasite), perhaps not pathogenic, or to the persistence of antibodies after healing? The latter explanation seems more credible.

If negative and concordant results with the STS are of value in allowing the exclusion of syphilis in a patient suspected of having the disease, the time factor upon which we have already insisted being taken into account, what is their value as proof of cure in a treated patient? In every case, before reaching a conclusion, it is prudent to repeat the test in 3 months to avoid a false negative TPI. Even in these conditions it would be premature to be dogmatic, but when clinical findings are negative, the conjunction of a negative TPI and negative STS strengthens the belief that observation may supersede indefinitely repeated treatment.

The cases of discrepancy are more instructive. With the help of the clinical and therapeutic data we may try to judge whether the STS or the TPI is at fault.

(a) *Probable Fault lying with the STS.*—The "responsibility" of the STS in cases of discrepancy may be supposed to be of three different kinds:

(i) *Imperfect technique.*—It is known that an excess of reagins may cause a negative STS by a zone phenomenon.

(ii) *Error of the STS.*—These are positive sometimes in cases of viral infection or for any other cause of biological false positive reactions.

(iii) *Staggering of the two types of reaction.*

The curve of the STS presents the following general behaviour. In 30–35 days after infection it begins to rise, becoming positive at about 40–45 days. Its further behaviour depends upon treatment. If this is started quickly, the curve falls rapidly but not immediately (serological inertia). If treatment is begun later, it frequently has no effect on the STS, which have become irreducible. A few differences may be observed in the time or the intensity according to the reactions used (earlier positivity of the reactions using fresh serum, Hecht, or Meinicke; persistence of the flocculation reactions, which also show less agreement among themselves than do the complement-fixation reactions). These well-known points have been mentioned only to emphasize that

(1) there are transitional forms between the easily reducible curve of early syphilis and the irreducible curve of latent syphilis of long duration, and

(2) the curve of the TPI presents the same general behaviour, with the same possibilities of rapid reducibility by treatment, of irreducibility, and of intermediate cases.

Despite the curve of the TPI starting later and being more stable than the curve of the STS this "staggering" between the two curves shows causes of discrepancy, without explaining them.

The "responsibility" of the STS for this discrepancy when the titre is rising is rarely established, since, if any discrepancy is observed at the beginning of syphilis, it is due rather to the time needed for the TPI to become positive. On the other hand, when the titre is falling, negative complement-fixation and flocculation tests are frequently found with a positive TPI—in our series, 94 times out of 1,000. Which reaction causes this discrepancy? We think the STS are responsible, since experience with experimental syphilis shows their lability, and the disease may be progressive (gumma, etc.) in some patients with completely negative STS.

In fact the word "responsibility" is used for lack of a better term; it would have some value if the presence of immobilizing antibodies or of reagins meant persistence of the infection, which is by no means proved.

(b) *Probable Fault lying with the TPI.*

(i) *Imperfect technique.*—It may be held that the technique of the test is imperfect because it so often demands the discarding of a whole batch of tests that are thought to be unsatisfactory. This shows the difficulty of performing the test but does not prove it to be imperfect from the theoretical point of view because the error is detected by the control tubes. Our intention is to speak theoretically of intrinsic imperfections. The only one imaginable is the possibility of a false negative reaction due to the obligatory dilution of the serum. This is diluted by the TP suspension and complement, and is only present in the reaction mixture at a concentration of 1 in 10. It might be assumed that a very weakly positive serum might become doubtful or even negative on account of this dilution. In the light of the titres usually observed we may take it that the number of sera unable to support a 1 in 10 dilution is very small, if any.

The possibility of a false negative TPI arising by a zone phenomenon has still to be investigated.

(ii) *Error of the TPI.*—Theoretically the only possible error is the immobilization by the action of antibodies of a treponematosus allied to syphilis, such as yaws. The comparative estimation of the immobilization titres obtained against different strains of treponemata may help to avoid this error.

We were asked by Drs de Graciansky and Grupper to perform TPI tests in patients treated with cortisone, and the first results seem to show that the hormone may disturb the TPI. Our investigations are still in progress.

The TPI may be at variance with the STS when the titre is rising; we have already discussed the starting phenomena: absence, peak, delay, etc. In three treated cases out of the 1,000 investigated we

have found a negative TPI and strongly positive STS. A biological false positive reaction seems unlikely when the STS remain positive for several examinations. May the immobilizing antibodies exceptionally be more labile than reagins under the influence of treatment? May it have been due to an unknown technical error in the TPI?

It is interesting to note that in the eighteen cases with a positive TPI in the C.S.F., the test was also positive in the blood on every case. Table X shows that in thirteen cases out of fifteen the Wassermann reaction was negative in the C.S.F. Among the forty cases with a negative TPI in the C.S.F., the STS were also negative when the tests were carried out at the same time (only seven cases). Among the eighteen cases with a positive TPI in the C.S.F., STS were carried out at the same time on sixteen, with the following results: positive 5, weak positive 2, negative 9.

The case of the doubtful TPI is interesting. Most cases were patients caught in the ascending or descending part of the curve, and the next test showed the direction the curve was taking. In five cases the second result was also doubtful: the history was that of old syphilis, adequately treated late in the infection. This seems to indicate that all the different progressive steps are present during the elaboration of antibodies in an infection modified by treatment.

The unstable TPI test is even more interesting. When tests are repeated without the intervention of treatment, the steadiness of the results is very noticeable, even in quantitative tests. But as is usual in serology, exceptions may occur. Slight variations are unimportant, but a greater instability may be found. An old case of syphilis, for instance, may give a negative TPI at first, and on re-testing 100 per cent. Obviously all our attention was directed to these cases, and generally it was not the test itself which was at fault, but the pathologist. Critical examination of the laboratory reports showed uncertainty at a certain stage of the investigation. On a few occasions, the error was not found and up to the present the result remains unexplained. *This proves that the TPI, a peculiarly delicate biological reaction, must be carried out in collaboration with clinicians who are competent to interpret it, and that it must still be the subject of patient research before being taken into general use.*

In conclusion, it may be asked whether the TPI gives the so-much-needed proof of cure. It is necessary to remember the general biological principle that the presence of antibodies is often, but not always, connected with cure; it is necessary,

therefore, to be prudent. However, in a case of recent syphilis, well treated, when a negative TPI, repeated after an interval of 2 months, is added to negative STS, and clinical evidence of infection is absent, it seems that the patient is secure from relapse. To assume the contrary would involve the possibility of modified parasites, unable to stimulate antibody formation, but capable of reverting to their pathogenic form. The reciprocal assumption is not true; it seems that in syphilis, as in other infections, the elaboration of antibodies may outlast the presence of antigens, and thus a TPI that remains steadily positive is not in itself of prognostic significance. In such a case we try to lower the titre with intensive treatment; if the titre falls, treatment is continued; if it is not affected, the patient remains under observation.

We think the use of the TPI more important for the detection of the false reactions that are daily problems for the clinician. The test has, moreover, another important application; more confidence can be placed in this new serological test than in the STS in research in experimental syphilis. It permits studies that have never been carried out successfully with the usual STS in experimental syphilis, and the knowledge thus obtained will certainly be of value in the study of syphilis in man.

Summary

(1) *Untreated Patients.*—The TPI is of little value in the diagnosis of syphilis in contacts of cases of infectious florid syphilis, as the diagnosis can best be made by methods already available. In cases of latent infection of long duration, however, the TPI may afford the only proof of infection.

The TPI is especially valuable in patients presenting lesions suspected of being syphilitic, particularly where these suggest late or congenital syphilis.

When the STS are frankly positive or negative, similar results are usually obtained with the TPI. It is most useful in interpreting intermediate results obtained with the STS and may detect biological false positive reactions.

(2) *Treated Patients.*—The TPI sometimes gives help in the retrospective diagnosis of a case incorrectly treated as syphilitic.

It has great value in judging the part played by syphilis in patients known to have had the disease who present lesions which may be attributable to it.

Theoretically it may aid discriminating between relapse and reinfection, provided that a series of tests have been carried out, a condition rarely fulfilled at present.

It raises doubts about the therapeutical provo-

cation method, the results of which may only be due to biological false positive reactions.

(3) *Relations to Various Forms of Treatment.*—Penicillin therapy shows a better negative response with the TPI than the older arsenic and bismuth therapy; the sooner treatment is started the quicker the action on the TPI.

The TPI gives a new possibility of establishing a cure when it is twice negative; this is open to question as healing and antibody production are not definitely related. A TPI that remains positive after treatment has no prognostic value.

(4) *Comparison with STS.*—Careful quantitative STS usually but not invariably agree with the TPI. Total positivity of the two types of test is more often seen (88.9 per cent.) than total negativity (71.4 per cent.). Possible causes of discrepancy are discussed.

(5) The TPI shows a few peculiarities in early syphilis: no elaboration of detectable antibodies, delay, "peak" phenomena.

(6) The results of the TPI on the C.S.F. of 58 cases are reported. No positive results were found which were not also positive with the sero-

logical tests. Frequent discrepancies are found with regard to the Wassermann reaction.

We wish to express our thanks to the heads of departments who, recognizing the importance of correlation with clinical findings, have allowed us to report our observations.

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