TREPONEMAL IMMOBILIZATION TESTS IN LEPROSY*

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The treponemal immobilization (TPI) test is said to be the most reliable test for treponemal infection at present available. This paper reports an attempt to check its reliability in five consecutive series of leprosy sera originating from the Eversley Child Sanitarium and the Central Luzon Dispensary, Cebu, Philippines. These sera were obtained through the courtesy of the late Dr James A. Doull, Medical Director, Leonard Wood Memorial, Washington D.C. Three series (1, 2, 3) were investigated at the Chemische Fabrik Promonta and the Institute of Hygiene, Hamburg University, and the other two (4 and 5) at the Institute of Hygiene, Kiel University.

Material and Methods

Table I presents all the sera examined. TPI tests were performed on 618 out of 1,699 sera (36·4 per cent.) and specimens from 420 patients were examined an average of four times with standard serological tests for syphilis (STS) and an average of 1·76 times with the TPI test. The battery of STS consisted of three complement-fixation reactions (original Wassermann reaction (OWaR), cardiolipin-WaR (CWaR), pallidation with Reiter-antigen (PR)) and two flocculation tests (VDRL and Meinicke clarification test II (MKR)).

The sera were drawn five times: immediately and after 24, 48, 72, and 96 weeks, respectively. Serum

from every patient was examined by the TPI test at least once. Additionally, a number of sera was chosen at random for TPI tests in every series besides those which had given positive or doubtful TPI tests in the previous series. Besides those giving positive or doubtful results in the TPI test, sera were also selected for another TPI test when the STS results were positive or doubtful in the preceding series. By this procedure some reactive TPI tests were discovered, which would otherwise have remained hidden. Different results in Series 1, 2, and 3 on the one hand and Series 4 and 5 on the other were to be expected for the following reasons:

(1) As the techniques employed were not quite identical, the results would differ more or less, even in a "perfect" state of reproducibility.

(2) The interval of time would cause manifold fluctuations. This holds for the TPI test as well as for the STS.

Except for routine treatment with sulfone (DDS), camoquine, and Ciba 1906, no antibiotic therapy was given which could have influenced the reactions.

Results

No further discussion of the TPI-negative patients who were investigated on two or more occasions is needed, as no clinical signs of active or burnt-out yaws or syphilis (which is very rare in the Philippine Islands) have been discovered in these patients in spite of repeated clinical examinations; the absence of any kind of treponematoses can therefore be taken for granted.

TABLE I
FIVE SERIES OF SERA FROM PATIENTS WITH LEPROMATOUS LEPROSY

Series	Week when Blood was Drawn	No. of Sera	Additional Sera	No. of Patients	TPI Tests	
					No.	Per cent.
1	1	321	321 + 0	321	321	100
2	24	373	279 + 94	+ 94	92	33
3	48	351	349 + 2	+ 2	71	20·3
4	72	337	334 + 3	+ 3	74	22·2
5	96	317	317 —	—	60	19·1
Total		1,699	1,600 + 99	420	618	36·5

* Received for publication January 4, 1967.

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The results of the investigations with STS (Ruge, Fromm, Fühner, and Guinto, 1960) have confirmed that the TPI test is usually more sensitive than the STS, and that those STS which are not accompanied by reactive TPI tests are—with a few exceptions—false-positive reactions which often decrease or disappear after adequate treatment of leprosy. For these reasons the number of TPI tests discussed will be limited.

Altogether 189 TPI tests were performed on sera from fifty patients who had shown at least one positive or doubtful TPI test in one of these five series. Their distribution is shown in Table II.

TABLE II
FIFTY CASES SHOWING POSITIVE/DOUBTFUL
TPI TESTS

Group	Number of Cases	Number of Examinations	Number of TPI Tests
A	19 (3ys)	5	95
B	14 (ys)	4	56
C	7 (ys)	3	21
D	7 (ys)	2	14
E	3 (ys)	1	3
Total	50 (7ys)	3.76 times (average)	189

(ys) = cases of burnt-out yaws clinically diagnosed; one clinically doubtful case could be verified serologically.

72 pairs of reactive TPI tests were obtained from these fifty patients. The surprisingly good agreement between these two groups indicates that the techniques employed were comparable (Table III).

For comparison, the results of the battery of five STS tests and the TPI test are given in Table IV. Altogether 940 STS tests were performed, but all cases which were classified as non-specific or inconclusive have been omitted (see below). In this way 856 STS and 179 TPI tests remain for evaluation. For convenience all five STS are taken together in Table IV.

TABLE III
REACTIVE TPI TESTS

Series 4 and 5	Series 1, 2, and 3			
	+	±	—	
+	31			
±	1	2	2	
—	4	11	21	
	36	13	23	72

Agreement $54/72 = 75$ per cent.
Partial Agreement $1/72 = 1.4$ per cent. } = 76.4 per cent.

As is usual in lepromatous leprosy, the number of anticomplementary (ac) sera was much higher than that in the general population. The influence of rises in the γ globulins (γG , γA , and γM) on the frequency of anticomplementary reactions has already been clearly demonstrated (Matthews and Trautman, 1965; Ruge, 1966; Schierz, 1964; Vargues, Moraud, and Gonthier, 1964).

The best agreement between STS and TPI was observed in TPI/VDRL flocculation tests (71.2 per cent.) and TPI/PR CFR tests (66.2 per cent.), but on the whole this conformity was much less than that shown in Table II where TPI is compared with TPI, and it is to be noted that the so-called "partial agreement" is greater between STS/TPI than between TPI/TPI (5.3 to 10.3 per cent.: 1.4 per cent. only). Table IV shows that, among the CFR, the PR reaction agreed best with the TPI, followed by CWaR and OWaR; the VDRL agreed even better with the TPI, whereas the MKR appeared less sensitive.

How many of these fifty patients with lepromatous leprosy were also infected with treponematoses?

(1) In six patients (5 males, 1 female) signs of burnt-out yaws were discovered. Four cases presented positive STS and TPI tests at all examinations. Three cases had altogether six negative TPI

TABLE IV
AGREEMENT OF STS WITH TPI TESTS IN 44 SPECIFIC CASES WITH 856 TESTS

Reaction	Agreement		Partial Agreement		Total		Anticomplementary	
	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.
OWaR	77/156	49.4	14/156	9.0	91/156	58.4	16/172	9.2
CWaR	84/150	56.0	13/150	8.7	97/150	64.7	20/170	11.8
PR	93/157	59.2	11/157	7.0	104/157	66.2	15/172	8.6
VDRL	112/170	65.9	9/170	5.3	121/170	71.2		
MKR	88/172	51.2	19/172	10.5	107/172	61.7		

A total of 189 TPI tests were performed, of which 172 could be compared with STS. From these TPI sera one toxic serum and sixteen TPI tests originating from so-called "non-specific" (4) and "inconclusive" (2) cases have been deducted (189 - 1 - 16 = 172).

In CFR, the ac sera have been deducted, two CWaR tests were missing.

In the flocculation tests, two VDRL tests were missing.

tests (1 three times; 1 twice; 1 once). Among the corresponding thirty STS there were three positive flocculation tests (VDRL 4, VDRL 2, MKR 1) and three ac sera.

A seventh case (male) was clinically doubtful. At first the TPI test was negative, but after the third examination it had risen to 95 per cent. and the STS had become positive (serological relapse). The clinical diagnosis was thus confirmed.

(2) This group includes all the other 43 patients with positive or doubtful STS and TPI tests. It is divided into five sub-groups according to the number of tests performed on each single patient.

(a) *Sixteen patients were examined five times*

Five presented positive reactions to all tests. In ten the TPI and/or STS showed a slow decline with certain variations. Case 299 illustrates this (see Appendix).

Case 299: L₁₋₂, Bacilli in smears + + +, aged 14, male.

Examination No.	1	2	3	4	5
STS	—	OWaR 1	—	CFR 2	—
TPI (per cent.)	78	47	44	4	0

A transient relapse was also observed (Case 375).

Case 375: L₂, Bacilli in smears + +, aged 45, female.

Examination No.	1	2	3	4	5
STS	—	OWaR 4,	—	OWaR 4,	—
		CWaR 4		PR 1	
TPI (per cent.)	0	60	52	0	0

The sixteenth patient was thought to show a non-specific reaction and his clinical course was improving (Case 238).

Case 238: L₁, Bacilli in smears +, aged 35, male.

Examination No.	1	2	3	4	5
STS	VDRL 1	—	—	—	—
TPI (per cent.)	45	9	33	4	14

(b) *Thirteen patients were examined four times*

Eight were positive throughout. Three showed gradually disappearing reactivity in the TPI and STS, and there were also two serological recurrences (similar to No. 375, sub-group a).

(c) *Six patients were examined three times*

Four were positive and two had to be considered as non-specific (Case 475) and inconclusive (Case 128) respectively.

Case 475: L₂, Bacilli in smears + +, aged 32, male.

Examination No.	1	2	3	4	5
STS	—	CWaR 3	—	—	serum
TPI (per cent.)	0	no TPI	22	0	coagulated

Case 128: L₂, Bacilli in smears + +, aged 39, male.

Examination No.	1	2	3	4	5
STS	—	—	—	CFR 4	ac
TPI (per cent.)	0	no TPI	no TPI	0	40

Case 128 probably shows the beginning of a serological relapse preceded by a positive CFR and followed by a doubtful TPI, or the non-specific reactions may

be due to the worsening clinical status, accompanied by greater alterations in the serum producing anticomplementary results. The picture should therefore be regarded as inconclusive.

(d) *Six patients were examined twice*

Four are regarded as specific, and two (Cases 112 and 248), who were negative in the beginning, showed a doubtful TPI test in the fifth series. The latter results are considered to be non-specific, as the clinical status was worsening and the anticomplementary sera occurring in Case 112 favoured the explanation of major alterations in the serum.

Case 112: L₂₋₃, Bacilli in smears + + +, aged 10, male.

Examination No.	1	2	3	4	5
STS	—	—	ac	serum	ac
				coagulated	
TPI (per cent.)	0	no TPI			26

Case 248: L₁, Bacilli in smears + + +, aged 40, male.

Examination No.	1	2	3	4	5
STS	—	—	—	—	OWaR
					missing
TPI (per cent.)	0	no TPI	no TPI	no TPI	22

(e) *Two cases were examined once*

Case 1230: L₂, Bacilli in smears + + + +, 12, male.
Series 3 only TPI 91 per cent. and 100 per cent.; all other tests negative. Series 1, 2, 4, and 5 missing.

The patient was considered to have treponemal disease, although all other reactions were negative and clinical evidence of treponemal infection was absent.

Case 208: L₂₋₃, Bacilli in smears + + +, 45, male.
Series 1 PR 1, TPI 29 per cent.

This should be regarded as inconclusive because all following tests were missing.

In summary, Sub-groups (a) to (e) comprise six doubtful cases: four of them are certainly non-specific, whereas two are inconclusive.

The distribution of the 44 cases of treponematoses (seven burnt-out yaws with clinical signs, 35 latent yaws without clinical evidence, two latent syphilis (clinical diagnosis: bubo)) among these 240 patients with lepromatous leprosy is given in Table V.

TABLE V
CASES OF TREPONEMATOSIS

Age (yrs)	Sex		Treponematoses			
			Male		Female	
	Male	Female	No.	Per cent.	No.	Per cent.
—15	31	16	5(1)	16.1	1	6.3
—25	124	52	10(1) [1]	8.0	4(1)	7.7
—35	77	27	5(1) [1]	6.5	3	11.1
—45	45	19	9(3)	20.0	3	15.8
>45	16	13	2	12.5	2	15.4
Total	293	127	31(6) [2]	10.6	13(1)	10.4

() = Clinical signs of burnt-out yaws.
[] = Latent syphilis.

Discussion

Among the 42 cases of burnt-out or latent yaws, the following facts have been observed:

Series 1, 2, and 3

There were fluctuations between the first and second readings of the same TPI test; for instance, first reading 25 per cent., second reading 72 per cent., and, more rarely, *vice versa*.

Nine patients out of ten in whom these differences were found belonged to the more seriously infected group with L_2 Bac +++ or L_3 Bac +++++; only one suffered from L_1 Bac +.

In all, there was a marked but not significant preponderance of those with heavier leprotic involvement (*i.e.* $L_1, L_{1-2} : L_2, L_3 = 1 : 2.4$ (122 : 298)) compared with the total number of patients.

Here the increased serum globulin, especially γ globulin (IgG, IgA, and IgM) certainly plays an important role; the investigations of Planchet (1958) and Rollier and Planchet (1957) have shown that the number of false-positive reactions observed in STS increases as the albumin/globulin ratio decreases, *i.e.* with rising quantities of globulins. That this also applies to TPI tests in a certain degree can be concluded from the above results.

The same holds true for many of the anticomplementary (ac) reactions observed; the rate of false positive and ac sera was significantly lower after prolonged treatment with DDS, camoquine, and Ciba 1906 (Ruge, 1966).

Holst (1964) discovered eight positive TPI and six cardiolipin reactions among 32 lepromatous leprosy sera from Madras, three of which were not associated with positive TPI tests. Since four of these cardiolipin tests disappeared after prolonged heating at 56°C., he presumed that they were false positives. This idea was supported by the fact that many false-positive reactions with cardchol (a lecithin-free cardiolipin antigen) diminished or disappeared after this heating procedure. Holst and Weis Bentzon (1965) later showed that syphilitic sera originating from different stages of the disease also behaved differently after prolonged heating. Since no information is given about the treatment of these patients with DDS, etc., which considerably reduces false positive cardiolipin reactions, no conclusion can be drawn.

In Ethiopia, Buck and Mayer (1964) examined the sera of treated lepromatous and tuberculoid leprosy patients with the fluorescent treponemal antibody (FTA) test, the VDRL, and the rapid plasma reagin (RPR) test with the antigen suspended in choline chloride. This apparently suppressed most of the false-positive reactions in

lepromatous leprosy (62 cases: FTA 30.5 per cent.; VDRL 47.0 per cent.; RPR 30.5 per cent. On the other hand, however, it appears surprising that the number of positive FTA tests was more than twice as high in tuberculoid leprosy as that found with the VDRL and RPR respectively (46 cases: FTA 21.3 per cent., VDRL 8.3 per cent., RPR 9.2 per cent.). However, this deficiency of VDRL and RPR was not verified by Buck and Spruyt (1964).

This situation is still more complicated by using various cardiolipin antigens of different chemical composition. Makleit, Réthy, and Richter (1964/5) examined such antigens by thin layer and gas chromatography and found many which were not suited for demonstrating syphilitic reagins. Julian, Portnoy, and Bossak (1963) succeeded in producing false positive TPI tests in goats injected with homogenates of normal rabbit testes. They do not deny that this may also happen in testing human sera, since it must be considered that traces of material from rabbit testes always adhere to the treponemata prepared for the TPI test. Boiron, Basset, Faye, and Mallet (1964) reported positive TPI tests in a goat and a monkey in Central Africa in a region of endemic syphilis, but did not discuss their findings in detail. Since only a few TPI tests had been performed on a series of 176 different animals (108 cattle, 18 goats, 12 sheep, 38 monkeys), sera from which gave 32 (18 per cent.) positive reactions with cardiolipin antigens, no conclusions can be drawn. It may therefore be possible under certain unfavourable conditions to obtain such false-positive tests, especially with leprosy sera, because patients with severe forms of leprosy have very abnormal sera (Matthews and Trautman, 1965; Mayama, 1965).

In this connexion it appears worthwhile to note that the first traces of specific serum changes in rabbits can be observed on the sixth or seventh day after infection with *T. pallidum*, the non-specific inflammation caused by the injection itself having been demonstrated a few days earlier (Ezold and Naumann, 1965).

Series 4 and 5

Certain fluctuations in the TPI test were also found in this group. Three TPI tests in Series 4 (Cases 157, 326, 347) suddenly turned negative among sixteen and thirteen positive cases of subgroups (a) and (b), although previous and subsequent tests were positive. Since these fluctuations occurred on different examination days, they were genuine and not due to chance. All cases were heavily infected (*i.e.* L_2/L_3 Bac +++/++++), but

no other plausible reasons could be found to explain these transitory negative reactions, which did not extend to the STS.

Case 157: L_{2-3} , Bacilli in smears ++, aged 41, male.

Examination No.	1	2	3	4	5
STS	4	VDRL 1	PR 3,	4	STS 4,
			VDRL 4		CWaR ac
TPI (per cent.)	95	92	96	4	95

Case 326: L_3 , Bacilli in smears + + + +, aged 63, male.

Examination No.	1	2	3	4	5
STS	—	CWaR 2	CWaR 1,	OWaR 1,	ac
			VDRL 1	PR 1,	
				VDRL 1	
TPI (per cent.)	65	92	77	15	70

Case 347: L_2 , Bacilli in smears ++, aged 21, male.

Examination No.	1	2	3	4	5
STS	missing	—	—	—	—
TPI (per cent.)	missing	93	100	8	50

How many of these fifty patients with lepromatous leprosy can be regarded as giving specific reactions?

(1) There have to be deducted seven cases of yaws, in which the clinical diagnosis could be confirmed serologically.

(2) At least four cases are to be deemed non-specific, and two (with some reservation) as inconclusive.

This means that, among 420 patients with lepromatous leprosy, besides the seven cases of yaws, there were another 37 cases infected with treponematoses—35 yaws (22 males, 13 females) and 2 latent syphilis (males)—a total of 44 cases (10.5 per cent.).

The specificity of the TPI test in lepromatous leprosy (122 cases of L_1/L_2 and 289 of L_2/L_3), will be 87–92 per cent. under the conditions prevailing, supposing that a series of repeat tests has been done. The chance of regarding a single TPI test among these patients as specific would be less than 87 per cent.

The calculation can be made in various ways, since three cases of burnt-out yaws were TPI-negative, but partly STS-positive or doubtful. These three cases can be deducted (4/47) or retained in the total number (4/50). The same applies to the inconclusive cases: there will be 6/47 or 6/50 (4 non-specific + 2 inconclusive).

This study of the behaviour of STS and TPI tests proves that it will be better not to start mass investigations until all the patients in question have been adequately treated and clinical and serological improvement has occurred, even if some cases are lost who have become TPI- and STS-negative in the meantime.

The serum alterations are more obvious in the STS than in the TPI test, except for the PR which gave only one doubtful and one positive reaction in these two inconclusive tests. These false PR reactions are most probably due to an antibody against lipopolysaccharide which is also found in normal sera (Pillot, Betz, Colombani, and Ripault, 1965) and is more frequently encountered in syphilitic sera (Bekker, de Bruijn, and Miller, 1966) and in highly altered lepromatous leprosy sera. Pallida-antigen consists of ultrasonically disrupted Reiter treponemes (*i.e.* it still contains lipopolysaccharides attached to the sheath and proteins situated in the body).

Summary

Among 420 patients (293 males, 127 females) with lepromatous leprosy from the Philippine Islands, 44 (10.5 per cent.) were found to be simultaneously infected with treponematoses. The 44 patients included seven cases of burnt-out yaws with clinical residua, two of latent syphilis (clinical diagnosis: bubo), and 35 of burnt-out yaws without clinical manifestations.

The investigations were performed in five consecutive series at intervals of 24 weeks. An average of 1.76 TPI tests and four STS—comprising three complement-fixation tests (original WaR; cardiolipin WaR; Reiter-antigen complement-fixation) and two flocculation tests (VDRL; Meinicke flocculation test II)—was done per patient. Males and females were equally affected (31 : 13 = 10.6 : 10.4 per cent.).

I am especially indebted to Prof. H. Gärtner, Director of the Hygiene-Institute, Kiel, for performing the tests of Series 4 and 5.

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Le test TIT chez les lèpres

RÉSUMÉ

44 cas (10·5 pour cent.) de tréponématoses ont été découverts entre 420 Philippinois—293 hommes, 127 femmes—souffrant de lèpre lépromateuse. On a trouvé

7 cas de "burnt-out" pian présentant des résiduels cliniques, 2 cas de syphilis latente (diagnostique clinique : bubon), et 35 cas de pian latent sans signes cliniques.

Les investigations sérologiques ont été faites par cinq séries consécutives à distance de 24 semaines chacune. Le chiffre des réactions exécutées par patient montait en moyenne à 1·76 TIT et 4 réactions standard comprenant chaque fois: BW original, BW cardioline, Pallida réaction (souche Reiter), et deux réactions de flocculation, c'est à dire VDRL et Meinicke II (clarification). L'index de l'infection tréponématique était presque identique entre les deux sexes: 10·6 et 1·04 pour cent.