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Serological Findings in Leprosy

An Investigation into the Specificity of Various Serological Tests for Syphilis *

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In serological tests for syphilis, leprosy sera often give biologically false positive reactions. These may be due to the presence of non-specific elements—for example, the ubiquitous lipid antibodies—in the leprosy sera; or they may be the result of errors in technique or unfavourable working conditions in the laboratory. This paper presents the results of an investigation in which several hundred sera from lepers were submitted to four of the so-called "standard" serological tests for syphilis (STS), using either cardiolipin or crude lipid antigens; to a complement-fixation test using as antigen a suspension of Reiter treponemes (PR test); and to the Treponema pallidum *immobilization (TPI) test. The investigation was carried out in a moderate climate and in technically well-equipped laboratories.*

It was found that the number of biologically false positive reactions was not as high as had been expected in the light of previous investigations. It was discovered, moreover, that it was the lipid antigens that were mainly responsible for the non-specific reactions, since both the PR and the TPI test showed a far greater specificity than any of the STS. But the TPI test, though highly specific, is also technically very complicated and therefore not suitable for use in regions where technical facilities are lacking. The authors consider that, in such regions, the simpler PR test will give sufficiently accurate results in the serodiagnosis of treponematoses. It must, however, be recognized that even the treponemal tests are not capable of differentiating between syphilis and yaws infections.

It is well known that sera from lepers quite often give biologically false positive reactions to serological tests for syphilis (Edmundson et al., 1954b; Bokkenheuser & Kooij, 1957; Kvittingen et al., 1952; Rollier et al., 1955a, 1955b; Ruge, 1955; Zarco & Chan, 1958. But since there is a great variation in the proportions of such reactions discovered, the supposed frequency of non-specific standard tests for syphilis might perhaps be too high.

Through the courtesy of Dr James A. Doull, Medical Director, Leonard Wood Memorial Foundation, Washington, D.C., USA, we had the opportunity of investigating several hundred lyophilized leprosy sera which were sent from two leprosaria in Cebu, Philippines. This island has, according to Rodríguez (1957), a leprosy prevalence rate of 2.37 per thousand, which is more than twice as high as the rate estimated for the whole archipelago.

MATERIAL AND METHODS

Altogether, 327 sera were examined. These sera were drawn from 318 patients (males and females) suffering from lepromatous leprosy, which is known to be responsible for most of the cases of biologically false positive reactions (Edmundson et al., 1954b; Portella & de Almeida, 1952; Rollier et al., 1955a, 1955b; Ruge, 1955) and from 9 persons who were

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borderline cases. All the patients gave a negative Mitsuda test; 112 had not previously undergone any sulfonamide treatment, 74 had received up to 3.5 g of sulfonamide, and 132 had received 3.5 g or more (on the average, 56 g).[1] No influence of previously administered antibiotics could, in practice, be detected in the results of the seroreactions in question.

The lyophilized sera dissolved readily in double-distilled water and could be investigated without difficulty. The complement-fixation tests on these sera were carried out with cardiolipin antigen, with alcoholic human-heart extract antigen, and with Pallida antigen (a commercially available carbolized suspension of the Reiter strain of *Treponema pallidum* (H. Reiter—personal communication, 1959; Mulzer & Nothaas, 1928), which was treated with ultrasonic waves) (Ruge 1956b). The technique used in the complement-fixation tests was a modified Kolmer technique, with the shortened binding-time in the ice-box recommended by Jacobsthal, which has been previously described by Gaehtgens (1937), Führer (1952), Führer & Gaehtgens (1954), and Fromm (1954).

The following serological tests for syphilis were investigated in our study: the cardiolipin Wassermann reaction (CWR); the original Wassermann reaction (WR); the Pallida reaction (PR); the Meinicke clarification test (MKR II); the cardiolipin microflocculation test (VDRL); and the *Treponema pallidum* immobilization test (TPI test.) The MKR II test was carried out according to the technique of Schlesmann (1930, 1932), the VDRL test according to the modified test-tube technique of Harris and his colleagues (US Federal Security Agency, 1949) and the TPI test according to the technique described by Meinicke (1953). Since the TPI test is considered to be highly specific, we used it as a standard and evaluated our results on the assumption that it was 100% reliable.

RESULTS

Of the 327 sera from 318 patients with lepromatous leprosy and 9 borderline cases, 31 (9.5%) were found to be reactive to the TPI test (see Table 1).

[1] Since 3.5 g was the average quantity of sulfonamide received during a month's course of treatment, the majority of the group of patients who received on the average 56 g of the drug (i.e., 16 times the amount) had been undergoing treatment for more than one year.

TABLE 1

PRESUMABLY SPECIFIC REACTIONS TO SEROLOGICAL TESTS FOR SYPHILIS AMONG 327 LEPROSY SERA [a]

Number of sera	TPI	CWR	WR	PR	MKR II	VDRL
6	6/0	6 Neg.	6 Neg.	6 Neg.	6 Neg.	6 Neg.
13	13/0	8/3 1 AC 1 Neg.	3/4 1 AC 5 Neg.	8/1 1 AC 3 Neg.	5/4 4 Neg.	8/0 3 Neg. 2 ND
6	0/6	6 Neg.	6 Neg.	6 Neg.	6 Neg.	6 Neg.
6	0/6	2/1 3 Neg.	2/1 3 Neg.	1/3 2 Neg.	1/0 5 Neg.	1/1 4 Neg.

[a] Numerator = positive cases (++++ or +++); TPI test, 51-100%.
Denominator = weekly positive or doubtful cases (++ or +); TPI test, 20-50%.
Neg. = negative cases; TPI test, 0-19%.
AC = anticomplementary serum.
ND = not done (quantity of serum insufficient).

It can be seen from Table 1 that the TPI test gave a higher total of positive, weakly positive and doubtful reactions (19 positive and 12 weakly positive or doubtful) than any of the other tests (STS [2] and PR). Twelve of the 31 sera were reactive only to the TPI test (6 positive and 6 weakly positive or doubtful). Some of the remaining 19 TPI-reactive sera (13 positive and 6 weakly positive or doubtful) were also reactive to one or more of the other tests.

Thus, out of a total of 327 sera, 19 (5.8%) gave positive reactions to the TPI test and 12 (3.7%) gave weakly positive or doubtful reactions. According to the assumption mentioned above, these 31 sera represented 31 cases of treponematosis (syphilis and/or yaws). Evaluated by this criterion, the other tests were less sensitive than the TPI test.

Of the 327 leprosy sera, 47 (14.4%) gave a negative result in the TPI test, but showed some reaction or were anticomplementary in one or more of the other tests. The detailed results of these 47 cases, which were regarded as giving biologically false positive reactions, are shown in Table 2.

It can be seen from Table 2 that 13 of the 47 TPI-negative sera gave strong positive reactions, and 34 gave weakly positive or doubtful reactions, to one or more of the other tests. Most of these were

[2] Standard tests for syphilis (in the present investigation, the CWR, WR, MKR II and VDRL tests).

TABLE 2

PRESUMABLY NON-SPECIFIC REACTIONS TO SEROLOGICAL TESTS FOR SYPHILIS AMONG 327 LEPROSY SERA [a]

Number of sera	TPI	CWR	WR	PR	MKR II	VDRL
		Positive cases (++++ or +++)				
13	13Neg.	3/0 4 AC 6 Neg.	5/0 4 AC 4 Neg.	0/0 4 AC 9 Neg.	3/3 7 Neg.	1/0 12 Neg.
		Weakly positive or doubtful cases (++ or +)				
34	34Neg.	0/9 3 AC 22 Neg.	0/2 3 AC 29 Neg.	0/1 3 AC 30 Neg.	0/13 21 Neg.	1/13 20 Neg.

[a] For explanation, see footnote to Table 1.

isolated reactions; in only ten cases did a serum sample react to two or more tests—for example, VDRL +++ positive, combined with a weakly positive anticomplementary reaction; or CWR ++++ positive, combined with WR ++++ positive.

In all, 54 positive, weakly positive or doubtful reactions and 21 anticomplementary reactions occurred among these 47 sera. On the basis of the negative TPI tests, the 54 positive, weakly positive or doubtful reactions would have to be classified as false positive. But the individual tests contributed rather differently to these false positive reactions. Thus, MKR II was reactive in 19 sera, VDRL in 15, CWR in 12, WR in 7 and PR only in 1. Evidently, the complement-fixation test using treponemal antigen —i.e., the PR test—showed the lowest degree of false positive reactivity.

In Table 3, the distribution of the positive and weakly positive or doubtful reactions between the sexes and among the different age-groups of the 318 cases of lepromatous leprosy is presented.

From Table 3, the following rates of specific and non-specific (false positive) reactions encountered among the different age-groups and between males and females can be obtained:

(*a*) *Reactive only to TPI test*

Out of 219 sera from males, 9 (4.1%) were reactive only to the TPI test, while out of 99 sera from females, 2 (2.0%) were only TPI-reactive.

(*b*) *Reactive to TPI test and to some STS and/or PR*

Of the 219 sera from males, 15 (6.8%) were reactive to the TPI test and to one or more of the other tests,

TABLE 3

ANALYSIS OF SEROLOGICAL REACTIONS OF 318 PATIENTS WITH LEPROMATOUS LEPROSY BY SEX AND AGE-GROUP [a]

Age-group (years)	No. of cases	No. of sera reactive to:		Non-specific reactions
		TPI test only	TPI test and to some STS and/or PR	
		MALES		
0-15	23	—	3/0	0/3 1 AC/0
16-20	43	1/0	—	2/8
21-25	44	1/1	1/0	1/5 1 AC/0
26-30	33	0/1	0/1	0/1 AC
31-35	24	0/1	1/2	1/3
36-40	21	1/2	1/0	0/1
41-45	17	—	3/2	—
Over 45	14	1/0	1/0	0/1
Total	219	4/5 (4.1%)	10/5 (6.8%)	4/21 (11.4%) 2 AC/1 AC (1.4%)
		FEMALES		
0-15	10	—	—	1/0
16-20	22	—	—	0/4 1 AC/0
21-25	17	—	0/1	1 AC/1 AC
26-30	13	0/1	1/0 0/1 AC	0/2
31-35	12	—	1/0	0/3
36-40	5	—	1/0	1/0
41-45	12	—	—	2/0
Over 45	8	1/0	—	1/1 0/1 AC
Total	99	1/1 (2.0%)	3/1 (4.0%) 0/1 AC (1.0%)	5/10 (15.2%) 2 AC/2 AC (4.0%)
		MALES AND FEMALES		
0-15	33	—	3/0	1/3 1 AC/0
16-20	65	1/0	—	2/12 1 AC/0
21-25	61	1/1	1/1	1/5 2 AC/1 AC
26-30	46	0/2	1/1 0/1 AC	0/2 0/1 AC
31-35	36	0/1	2/2	1/6
36-40	26	1/2	2/0	1/1
41-45	29	—	3/2	2/0
Over 45	22	2/0	1/0	1/2 0/1 AC
Total	318	5/6 (3.4%)	13/6 (6.0%) 0/1 AC (0.3%)	9/31 (12.6%) 4 AC/3 AC (2.2%)

[a] For explanation, see footnote to Table 1.

while of the 99 sera from females, 4 (4.0%) showed TPI reactions and some STS and/or PR reactions.

(*c*) *Specific reactions in relation to age-group*

Out of 98 sera from patients (males and females) up to 20 years of age, 4 (4.0%) showed a specific serological reaction (i.e., were reactive to the TPI test); of the 143 sera from patients aged 21-35 years, 13 (9.1%) gave a specific serological reaction, and of the 77 sera from patients aged 36 years and over, 13 (16.9%) gave such a reaction.

(*d*) *Non-specific reactions in relation to sex*

Of the 219 sera from males, 25 (11.4%) showed non-specific (false positive) reactions, while of the 99 sera from females, 15 (15.2%) gave such reactions.

(*e*) *Non-specific reactions in relation to age-group*

Of the 98 sera from patients up to 20 years of age, 18 (18.4%) showed non-specific (false positive) reactions, while of the 143 sera from patients aged 21-35 years 15 (10.5%), and of the 77 sera from patients aged 36 years and over 7 (9.1%), gave such reactions.

(*f*) *Anticomplementary reactions*

Of the 219 sera from males, 3 (1.4%) showed an anticomplementary reaction, while of the 99 sera from females, 5 (5.1%) were anticomplementary.

The distribution of the specific and non-specific reactions observed among the different clinical and bacteriological stages of lepromatous leprosy is set out in Tables 4 and 5.

Table 4 presents the serological findings in the "clinical" group; the classification of the clinical stages is based on the criteria recommended at the International Congress of Leprosy in 1953 in Madrid. The observations collected in the "bacteriological" group (Table 5) are arranged in respect of the quantity of *Mycobacterium leprae* discovered—i.e., +, ++, +++, ++++. To facilitate comparison, the two tables have been printed side by side.

Since segregation of the cases by sex would produce an incomprehensive picture—quite apart from the fact that the groups are rather small—only the total figures are shown in Tables 4 and 5. It can be seen that the cases giving specific reactions are distributed relatively equally among the groups in both tables, the proportion varying only from 7.5% to 16%. On the other hand, the percentage of non-specific reactions in Table 5 seems to show a peak in the +++ group, though the differences between the groups did not prove to be statistically significant.

DISCUSSION

In considering the specificity of the TPI test, we should take into account Fribourg-Blanc's (1957) well-founded and critical observations. He points out that the reactions to the TPI test only have to be qualitatively classified as positive, weakly positive or negative. A certain number of treponemes may be immobilized by some factors in the serum which are not of specific origin; while some others may be damaged or die when preserved in the basal medium, as is usually the case with other bacteria, too.

TABLE 4

DISTRIBUTION OF SPECIFIC AND NON-SPECIFIC SEROLOGICAL REACTIONS OF 318 PATIENTS, ACCORDING TO CLINICAL STAGE OF LEPROMATOUS LEPROSY [a]

Clinical stage	Number of cases	Number of: non-specific reactions	Number of: specific reactions
1	43	1/4	1/3
1-2	40	1/4 1 AC/1 AC	3/0 0/1 AC
2	146	5/14 1 AC/2 AC	8/7
2-3	26	1/3	2/2
3	63	1/6 2 AC/0	4/0

[a] For explanation, see footnote to Table 1.

TABLE 5

DISTRIBUTION OF SPECIFIC AND NON-SPECIFIC SEROLOGICAL REACTIONS OF 318 PATIENTS, ACCORDING TO BACTERIOLOGICAL STAGE OF LEPROMATOUS LEPROSY [a]

Bacterio-logical stage	Number of cases	Number of: non-specific reactions	Number of: specific reactions
+	48	1/3 0/1 AC	1/3
++	74	1/8 1 AC/0	7/2 0/1 AC
+++	125	6/16 3 AC/2 AC	6/6
++++	71	1/4	4/1

[a] For explanation, see footnote to Table 1.

However, in an adequate medium this immobilization due to different factors will never exceed 15-20% of the total number of treponemes counted in the darkfield examination. All reactions above this percentage may be regarded as specific reactions. According to Fribourg-Blanc and other authors, a more exact result can be obtained only by quantitative titration — a procedure which cannot be adopted in routine diagnostic work owing to the rather delicate and complicated technique involved. In accordance with the above-mentioned considerations, in our investigation all cases showing an immobilization rate below 20% have been regarded as negative, all those with a rate of 20% to 50% as weakly positive or doubtful, and all those with a rate of 51% to 100% as positive.

(a) Presumably non-specific (false positive) reactions

In the material examined the percentage of cases presenting non-specific reactions is rather low in comparison with the figures obtained in other investigations in the past decade. We found only 47 false reactors among the 327 sera tested—i.e., 14.4%. In two sera an anticomplementary reaction was obtained in the complement-fixation tests in combination with positive flocculation tests (MKR II ++ in one case and VDRL +++ in the other).

As usual, because of the impossibility of carrying out control tests on each serum separately, the flocculation tests gave about twice as many positive reactions as the complement-fixation tests. In the latter tests, a number of sera (7, or 2.1%) gave anticomplementary reactions; similar findings have previously been described by other investigators and may be regarded as typical for leprosy sera (Lomuto, 1954; Ruge, 1955, 1956a; Shively & Kuhns, 1950).

The 327 sera were tested with 5 different antigens, so that in all 1635 reactions were examined; of these, only 54 (3.2%) were found to be non-specific. Of the 981 complement-fixation reactions examined, 21 (2.2%) were observed to be anticomplementary.

It is noteworthy that the PR test gave only one non-specific reaction, and even that was only doubtful (+). This excellent result confirms our previously reported personal investigations and those of other authors that the PR, using treponemal antigen, represents a highly specific test for syphilis.

The non-specific reactions that occur in leprosy sera seem to be closely associated with alterations of the sera which are especially marked in cases of lepromatous leprosy. Electrophoretic and chemical investigations have demonstrated lipoproteinaemia, conversion of the albumin-globulin rate, irregular balance of the various globulin fractions, etc. (Ciacco, 1955; Contreras et al., 1954; Garcia-Pérez, 1952; Gómez-Orbaneja et al., 1953; Hoxter et al., 1951; Mauzé & Arnaud, 1954; Pozzo & Hofmann, 1955; Miguel et al., 1954; Rollier et al., 1955a, 1955b; Tarabini, 1957; de Vita & Bini, 1958). A relation between the conversion of the albumin-globulin rate and non-specific serological reactions has been shown by Fühner (1949).

Of these serum alterations, changes in the lipoproteins probably play the most important role, by influencing the results of the lipid reactions to a greater or lesser extent. This would explain the relatively high proportion of non-specific reactions occurring in the standard tests for syphilis using lipid antigens and would also explain the very small number of such reactions met with in the PR test, which is serologically especially active in respect of the treponemal protein fraction, as has been proved by Gaehtgens (1937), Fühner (1957), Fromm (1954, 1955a, 1955b), and Jeney et al. (1957).

Lindau & Laurell (1952) and Laurell (1955), using electrophoresis, were able to demonstrate that the lipoproteic fractions even of normal negative sera gave positive reactions in the WR test after they had been isolated. These authors consider that this effect is due to the lack of some " inhibitors ", which regularly occur in normal sera and prevent them from giving positive reactions. By their experiments they were able to throw further light on the findings of the Neurath verification test. Since great abnormalities are not infrequently encountered in leprosy sera, it may be quite possible that here—besides other alterations—the normal " inhibitor " is missing; thus the prevalence of many non-specific reactions would be explained.

With regard to the frequency of these non-specific reactions in leprosy sera, it has to be emphasized, however, that the 5 antigens employed in our battery produced very different degrees of non-specificity. If every unit of positivity of every non-specific reaction discovered is estimated as one point (i.e., +=1 point, ++=2 points, etc.), the following values for the degree of non-specificity of the five tests are obtained: PR, 1; VDRL, 18; CWR, 22; WR, 22; MKR II, 34. Only the value for the PR test differs significantly from the other values.

(b) Presumably specific reactions

It is surprising that 12 sera (6 positive and 6 weakly positive or doubtful) should have reacted only to the

TPI test. These sera are irregularly distributed among the age-groups, as can be seen from Table 3. Nineteen TPI reactions (13 positive and 6 weakly positive or doubtful) were combined with reactions to one or more of the other tests (STS and PR). Since the prevalence rate of syphilis among the rural population is rather low—less than 1% (R.S. Guinto, personal communication)—and most of the lepers come from villages, the majority of these 31 cases has to be attributed to yaws, assuming that the positive reactions are, in fact, absolutely specific for treponemal infections.

Among the 327 cases only five gave a history of suspected yaws. Three of these five sera were positive in the TPI test, STS and PR, one showed a reaction only to MKR II, and one was negative in all tests. In addition, 10 of the 327 lepers—8 males and 2 females—offered an anamnesis of abscesses and lymph-nodules in the groins, probably of venereal origin, but not necessarily syphilitic. One of these cases gave a positive TPI reaction and another a positive TPI reaction combined with weakly positive complement-fixation reactions. These two cases could therefore be regarded as truly specific. This small number would agree with the above-mentioned rather low incidence of syphilis among the villagers.

The number of presumably specific reactions appears to be rather too high in comparison with the clinical findings, but the following points have to be taken into consideration in interpreting the serological findings.

1. It is well known that, in syphilis, positive TPI reactions generally persist for much longer than do PR and STS reactions, and that, unlike the latter, they are apparently not usually influenced by treatment. Since yaws is the "mild sister" of syphilis, it may be justifiable to assume that, in this treponematosis too, the same or at least very similar conditions apply.

2. In the light of a report by Cruz,[1] the prevalence rate of active yaws in Cebu and the surrounding islands must be estimated to be at least 1-2%. In mass surveys which covered 1 297 463 people in the neighbouring islands of Samar and Leyte, Cruz found 44 458 cases of active yaws (3.4%) that were undergoing treatment.

According to the experience of Huan-Ying Li & Soebekti (1955) in Indonesia, which is not very far from the Philippines, cases of active yaws are associated with a certain proportion of cases of latent yaws and contacts. The two last-mentioned groups can be discovered only by serological investigation. The two workers found, for instance, that 1-2% of active cases were associated with 6-8% of latent cases, and that the greater the number of active cases, the smaller the ratio of active to latent cases. Since the conditions of rural life, etc. prevailing in the Philippines are similar to those in Indonesia, it is justifiable to assume that the situation with regard to yaws is also comparable, in which case the relatively high proportion of specific reactions obtained in our study could be plausibly explained.

The same trend is apparent in the paper by McFadzean, McCourt & Wilkinson (1957) from Gambia. For instance, among 127 clinically healthy people, randomly selected from villages where treponematoses were not prevalent, these authors discovered 22 persons (17%) with positive TPI reactions; on the other hand, among "healthy" people from villages where treponematoses were common, the TPI-positive rate was 47%.

In 1959, in a personal letter to one of us (H.G.S.R.) Dr A. E. Wilkinson made the following comment:

"Out of 338 sera examined, 11 gave positive and 5 doubtful TPI tests where the STS were negative or equivocal. Some of these patients had late lesions which might be attributable to past treponemal infection, but many were asymptomatic. I think the finding of immobilizing antibody indicated previous treponemal disease, which may well have been "burnt out" by the time the patients were examined, as both yaws and siti are largely infections of childhood and some treatment may have been given in the past. In most instances where we found a positive TPI test the STS were also positive, usually to a high titre. As you know, there have been large numbers of immigrants coming to this country from the West Indies in recent years. These are presenting us with many serological problems as they are frequently found to have positive STS (and TPI tests) with no clinical evidence of syphilis or yaws, but sometimes a history of some treatment in childhood. I think old yaws is probably the explanation in many cases, but without a test which will differentiate syphilis from yaws, we can never be certain whether this is really so."

Of course, the above-mentioned examinations will have to be verified on a larger scale.

Similar findings have been reported from Liberia by da Cruz Ferreira & Sternberg (1956), who discovered among 737 "healthy" persons 46.6% positive reactors to the VDRL test. Pagès et al.

[1] Unpublished working document WHO/VDT/174, 1955.

(1959) and Pautrizel (1956) also recorded similar observations in Africans, pointing out that among them the treponematoses often took a hidden course and did not present any clinical signs. This finding provides further evidence that a rather high proportion of the so-called "mute infections" (*stumme Infektionen*) well known to be prevalent in smallpox, measles, typhus, and other diseases (Reiter, 1925, 1959) occurs also in the treponemal diseases.

3. It is generally agreed that among selected groups of sick people—for example, tuberculosis and leprosy patients—syphilis and yaws are more prevalent than they are in the general population (Heymann, 1955). Ruge (1955) found this to be true in Egypt, where the syphilis rate among tuberculous patients was 5.1%, among lepers was 3.8%, and among the general population was 3.0%.

In addition, it must be borne in mind that yaws and leprosy cannot always be diagnosed properly on clinical grounds, since atypical lesions of the two diseases may resemble each other. Davey (1957), in his interesting note, has especially stressed this point and enumerated the parallels between yaws and leprosy (chronic nature of the disease, spread by contagion, prevalence in rural regions, high incidence among children and poorer communities, adverse influence of uncleanliness, abundance of flies, and lack of clothing, etc.). It is therefore quite possible that there were some hidden double infections among the cases from which our material was drawn.

4. The higher proportion of positive TPI reactions obtained (31 as compared with 13 PR and 14 STS) might perhaps be attributable to the use of living and virulent treponemes. Since there are marked differences in the clinical picture of syphilis and yaws in human beings, and certain, though more insignificant, differences in rabbits and hamsters (Hill & Gordon, 1954; Guimarães, 1955; McLeod & Magnuson, 1952; Turner & Hollander, 1957), it is possible that there are also certain differences in the immunological picture. Proof of this assumption has been provided by the investigations of Guimarães (1946) and Medina (1954), who demonstrated that yaws could sometimes be transmitted to cured syphilitics and syphilis to cured cases of yaws. On the other hand, Magnuson et al. (1956) showed that volunteers with cured syphilis could be reinfected with *T. pallidum* although their sera still gave positive reactions to STS and TPI tests. Thus there is no clear proof of the theory that living *T. pallidum* would indicate the presence of antibodies better than other antigens, especially in the case of yaws infections. But there is, at any rate, a definite difference in the antibodies demonstrated by the different tests: the immobilizing antibodies react in the TPI test, the treponemal protein antibodies react in the PR test, and the ubiquitous lipid antibodies react with the lipid antigens in the STS. In this connexion, it is of interest that Ross (1959) observed among 250 lepers in Carville, La., USA, where yaws does not exist, only 10 (4%) with positive TPI reactions and only the same number with positive reactions to the Reiter protein complement-fixation test. The agreement between the results of these two treponemal reactions was as close as 96%.

5. Last, but not least, it must be strongly emphasized that, like all biological tests, even the TPI test sometimes gives false positive reactions, as has already been pointed out by Touraine (1953), Bekker & Onvlee (1955a, 1955b), Edmundson et al. (1954a), Harmsen & Fromm (1957), Fegeler (1957), Miller et al. (1957), and Fromm & Meyer-Rohn (1959), and therefore its specificity should not be overestimated. With regard to false positive TPI reactions in leprosy sera, the observations published by Charpy (1953), Floch et al. (1953), Lazzaro & Sapuppo (1958), Lissia (1956), Rossetti et al. (1958), Touraine (1953) and other authors should not be overlooked.

In this connexion, special attention should be given to the observations of Edmundson et al. (1954b), who examined at Carville the sera of 224 lepers—204 lepromatous and 20 tuberculoid cases—with STS (Kolmer complement-fixation test with cardiolipin antigen, Kahn standard test, Rein-Bossak test and VDRL slide test) and TPI tests. Among these 224 lepers, who presented careful clinical information, 16 were found to have a history of syphilitic lesions and/or treatment for syphilis, but none presented a history of any other treponematoses—for example, yaws, pinta, or bejel.

It is interesting to note that in these 16 syphilitic cases one or other or all of the STS were weakly positive, except in two cases, where neither the Kolmer nor the VDRL slide test could be performed since the quantity of serum available was not sufficient. On the other hand, although 25 of the 224 sera reacted to the TPI test, only 7 TPI reactions (5 positive and 2 doubtful) were observed in the group of 16 syphilitic lepers. No explanation is

given for the additional 18 TPI-reactive cases, none of whom had any history of treated or untreated treponematoses.

A simple analysis of the above facts might indicate that the TPI test should be regarded as non-specific in, say, 12 of the 18 additional reactive cases— i.e., in 5.4% of all the cases (224) examined.

Furthermore, if we compare the findings of Nelson (1952), of Edmundson et al. (1954b) and of Cannefax et al. (1959) in the sera of lepers from Carville, the following results are obtained:

	TPI-reactive cases	
	number	*%*
Nelson (1952): 70 cases	12 *	17.1
Edmundson et al. (1954b): 224 cases	25	11.2
Cannefax et al. (1959): 248 cases:	8 **	4.8

* 11 positive, 1 doubtful
** 8 positive

The differences between the three groups are statistically significant. Even if the three batches of sera examined came from different groups of patients, it seems rather odd that the percentage of presumptive syphilitic lepers should have decreased from 17.1% to 4.8% in a period of only seven years. Since there is no special mention in these published papers of the presumed cause of these unexpected TPI reactions among lepers, no plausible explanation of these differences can be given. But it can at least be said that the decrease will depend *inter alia* on the sensitivity of the TPI test with regard to certain leprosy sera and possibly on the test technique employed.

If the above considerations and calculations are applied to the investigations presented in this paper, it would appear that 8 of the 12 additional TPI reactions in our study are to be considered non-specific—i.e., 2.4% of all the sera examined could be said to have given non-specific reactions. Out of the total of 31 TPI-reactive cases, therefore, 23 could be assumed to have given specific reactions; and of these 23 one case could be definitely attributed to syphilis (see page 798). Thus, 22 cases (6.7% of all the cases examined) would have to be attributed to yaws, which would mean that to the 5 cases of anamnestic yaws (burnt-out clinical cases) 17 cases of latent (burnt-out) or contact yaws would have to be added. Thus, the ratio of active yaws to latent cases and contacts would be 1: 3, which is in close agreement with the findings of Huan-Ying Li & Soebekti (1955) in Indonesia mentioned earlier.

In addition, it must be stressed that it is not always possible to detect technical errors in the performance of the TPI test at the first examination; sometimes a second or even a third examination has to be carried out in order to demonstrate mistakes in technique. The reproducibility of this rather delicate and complicated test is by no means unquestionable, as has been proved by Attili (1958), Bellone & Bonelli (1956), Boncinelli et al. (1957), Borel (1956), Bossak et al. (1957), Browne et al. (1959), Edmundson et al. (1954a), Ehrmann (1956, 1957), Fromm & Hippius (1958), Fromm & Meyer-Rohn (1959), Lebeuf (1956), Nielsen (1957), Olansky et al. (1953), Portella & Thompson (1957), Ranque & Depieds (1957), Thiers et al. (1958), Wheeler et al. (1954), Zaffiro et al. (1958), Zuccarini (1958) and others.

We have had the opportunity to reinvestigate almost the complete series of cases presented in this study after they had received sulfonamide treatment for 24 and for 48 weeks. At this re-examination there were some marked discrepancies in the results of the TPI tests, and we are not sure whether this poor reproducibility is due entirely to technical errors or whether some other factors have come into play. To reiterate: it should always be recognized that biological reactions will not—and cannot be expected to—give 100% reproducible and reliable results. We shall report in greater detail on our findings on re-examination of the cases in a later paper.

* * *

Just after this paper had been submitted for publication, Dr H. Reiter published an interesting article on his strain of *Treponema*,[1] from which it can clearly be seen that the so-called " Reiter treponeme " was originally a genuine *Treponema pallidum*.

Also since the preparation of this paper, another interesting publication has appeared,[2] in which are presented the results of a comprehensive and extremely instructive serological survey, carried out in 14 different laboratories on 1200 sera of various types—normal, biologically false positive, from syphilitics, from lepers, and from pinta and yaws cases. This survey proves in a convincing manner that it is not possible to obtain complete (100%) agreement in the results, even under the best working conditions. This applies to *all* serological tests—i.e., to STS, to the *Treponema pallidum* complement-fixation test, *and* to the TPI test.

[1] Reiter, H. (1960) *Die med. Welt*, p. 712.

[2] *Serology Evaluation and Research Assembly (SERA) 1956-1957*, Washington, D.C. (US Public Health Service Publication No. 650).

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RÉSUMÉ

Il est connu que les sérums de lépreux donnent souvent des réactions faussement positives dans les tests sérologiques de diagnostic de la syphilis et d'autres tréponématoses. Ces réactions s'expliquent par la présence d'éléments non spécifiques, tels que les anticorps lipidiques, ou par des erreurs de technique, dans des conditions de travail insuffisantes.

Les auteurs ont étudié 327 échantillons de sérums de lépreux, provenant des Philippines. Ils les ont soumis aux tests de diagnostic de la syphilis — tests classiques avec antigènes cardiolipidiques ou lipidiques bruts (STS), test de fixation du complément avec *Treponema pallidum* souche de Reiter (PR), et test d'immobilisation du tréponème (TPI). Ces épreuves ont été effectuées dans des laboratoires bien équipés, en climat tempéré.

On a obtenu 54 réactions faussement positives, chiffre inférieur à celui que l'on attendait, d'après de précédentes recherches. Elles se répartissent comme suit: 19 avec le test de clarification de Meinicke, 15 avec le test VDRL, 12 avec le Wassermann-Cardiolipine, 7 avec le Wassermann original et 1 avec PR. Cette dernière épreuve est celle qui présente le degré le plus faible de non-spécificité.

Bien que la syphilis ou le pian n'aient pas été cliniquement diagnostiqués parmi les sujets examinés, le nombre des réactions positives spécifiques STS, PR, TPI, a été assez élevé. On peut l'expliquer par le fait que le test TPI reste plus longtemps positif que les autres tests, et n'est guère influencé par le traitement (qu'il s'agisse de syphilis ou de pian). Le pian sévit dans certaines îles à raison de 1-2% de la population, sans parler des cas latents et des contacts que révèle la sérologie. Il est reconnu aussi que le pourcentage d'infections syphilitiques ou pianiques est plus élevé dans un groupe de malades (lépreux ou tuberculeux) que dans l'ensemble de la population. D'autre part, le pian et la lèpre ont de nombreux symptômes communs, leurs lésions atypiques se ressemblent, et ils ne peuvent être distingués par le seul examen clinique.

La proportion plus élevée de réactions TPI obtenues (31, contre 13 PR et 14 STS) peut s'expliquer par l'emploi de tréponèmes vivants et virulents, et le fait que ce test met en évidence des anticorps différents des anticorps lipidiques et protéiniques peut indiquer des différences immuno-sérologiques entre le pian et la syphilis.

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