A SIMPLE PROCEDURE FOR THE IDENTIFICATION OF NONSYPHILITIC REACTIONS IN SEROLOGIC TESTS FOR SYPHILIS IN LEPROSY PATIENTS [1]

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It has long been known that serologic tests for syphilis (S.T.S.) which utilize lipid antigens may show with sera from leprosy patients positive reactions in the absence of syphilitic disease, past or present (1-3). Due to the nationwide campaign against venereal disease in the last decade, the incidence of clinical syphilis has been markedly reduced. This has resulted in an apparent increase in the number of nonsyphilitic reactors in S.T.S. Because of the reduction in the incidence of clinically recognizable syphilis, greater reliance has been placed on the results obtained with the S.T.S. It is evident that improvement in the specificity of the S.T.S. is highly desirable.

Attempts have been made to improve specificity by purification of the antigen (4) and by the development of differential or verification procedures (5-11). Although a relative improvement in specificity has been attained by the use of purified antigens, positive reactions unrelated to syphilis are still observed (12-14). In addition, the supposed merits of the various verification procedures have not been substantiated (2, 15-19).

With the presentation of the *Treponema pallidum* immobilization (T.P.I.) test (20) another important tool is provided for the study of syphilis and the other treponematoses. If technical difficulties can be overcome, it is conceivable that this test, or some other one utilizing the treponema, may eventually replace the S.T.S. However, this goal may be in the distant future. For this reason it seems desirable to continue studies designed to improve further the specificity of the currently used S.T.S.

Several years ago, while studying means for increasing the specificity of the serologic test for syphilis, an interesting effect of choline chloride on them was noted which suggested its trial in the differentiation of syphilitic and nonsyphilitic reactors. Since a high incidence of positive reactions in S.T.S. has been observed in leprosy (14, 21-24), it appeared advisable first to determine the suitability of a differential method based on the use of choline chloride using the serums of leprosy patients before beginning a protracted study of nonsyphilitic reactions due to other causes.

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The purpose of this report is to describe a relatively simple differential procedure, and to present the results obtained with it in sera of leprosy patients as well as in syphilitic and normal individuals. Determination of the specificity of this new procedure was made by consideration of the clinical findings and the results obtained with the T.P.I. test. A more complete presentation of clinical and serologic findings in a study of leprosy will be made in the future.

MATERIALS AND METHODS

1. *Treponema pallidum* immobilization test: This test was performed as modified by Portnoy, Harris and Olansky ([25]).

2. VDRL slide flocculation test: This test was performed as described in the Manual of Serologic Tests for Syphilis ([26]).

3. Differential procedure using choline chloride: (*a*) General equipment and glassware: As for the VDRL slide flocculation test.

(*b*) Antigen: An alcoholic solution containing 0.03% cardiolipin, 0.9% cholesterol, and sufficient purified lecithin to produce the present (1953) level of the VDRL slide test reactivity.[2]

(*c*) Buffered saline: As for the VDRL slide flocculation test.

(*d*) 0.9% saline: Dissolve 900 mgm. of dry sodium chloride (C.P.) in 100 cc. of distilled water; to be prepared daily.

(*e*) Choline chloride solution: A stock 40% solution prepared by dissolving 20 gm. of choline chloride [3] in 50 cc. of 0.9 per cent saline. This stock solution has been kept for as long as a year at room temperature without detectable change in serologic behavior. For use in the differential procedure, dilute 1 part of the chloride stock solution with 3 parts of saline to yield a 10% solution of choline chloride.

(*f*) Preparation of serum: Clear serum is obtained by centrifugation of whole clotted blood. The serum is heated at 56°C. for 30 minutes. Sera which are to be tested more than four hours after being initially heated should be reheated at 56°C. for 10 minutes.

(*Note*: In this study many of the sera were tested after storage for varying periods of time at –20°C. or at 5°C. There has been no indication that storage affects the type of reaction obtained in the differential procedure.)

(*g*) Preparation of antigen suspensions: (1) Pipette 0.4 cc. of buffered saline to a 30 cc. round glass or screw-capped bottle.

(2) Add 0.5 cc. of antigen (from the lower half of a 1.0 cc. pipette graduated to the tip) directly onto the saline while rotating the bottle on a flat surface, continuously but gently.

(*Note*: Antigen is added drop by drop, but rapidly, so that approximately 6 seconds are allowed for 0.5 cc. antigen. The pipette tip should remain in the upper third of the bottle, and rotation should not be vigorous enough to splash saline onto the pipette.)

(3) Blow the last drop of antigen from the pipette, without touching the pipette to the saline.

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[2] The differential procedure has been developed using the present formula for the VDRL slide test as basic antigen. Should the reactivity level of this antigen be changed in the future, it would still be necessary to employ the current (1953) formula, or else make adjustments in the choline chloride concentration.

[3] Sources of choline chloride: Merck; Nutritional Biochemical Corporation; Eastman Kodak.

(4) Add 4.1 cc. of buffered saline from a 5 cc. pipette.

(5) Continue rotation of the bottle 10 more seconds.

(6) Place the top on the bottle and shake vigorously for approximately 10 seconds.

(*Note*: Twice this amount of antigen emulsion may be prepared at one time in a 30 cc. bottle if desired. A 10 cc. pipette should then be used for delivering the 8.2 cc. volume of saline. If larger quantities of antigen emulsion are required, more than one mixture should be prepared. These aliquots may then be pooled and tested.)

(7) Transfer equal volumes of the suspension to each of two 3 x 1 inch centrifuge tubes. No less than 2.0 cc. nor more than 5.0 cc. should be placed in any one centrifuge tube.

(8) Centrifuge at 2500 r.p.m. for 15 minutes in an International Equipment Company No. 1 centrifuge.

(9) Remove the tubes from the centrifuge and invert them carefully to pour off the turbid supernatant fluid.

(10) Wipe the walls of the tubes with a piece of gauze or cotton to remove excess liquid.

(11) To one tube, add an amount of 0.9% saline equal in volume to the initial suspension centrifuged. Call this Antigen I. To the other tube, add an amount of 10% choline chloride equal in volume to the initial suspension centrifuged. Call this Antigen II. Suspend both suspensions by shaking and/or drawing up and expelling from the syringe and needle used for dispensing these suspensions. The suspensions may be kept in the centrifuge tube or transferred to other suitable containers.

(12) Dispensing the antigen suspensions: A separate syringe with needle is employed for dispensing each suspension. From 1 cc. of each suspension, 45 drops, plus or minus 2, should be obtained. In practice, it has been found that an 18-gauge needle with the bevel cut off will deliver drops of the proper size. Each suspension should be checked daily for drop size prior to use. The syringes and attached needles are kept in the antigen suspension when not in use.

(13) Preliminary checking of the antigen suspensions I and II: Known syphilitic, normal, and nonsyphilitic reacting serums tested. Serial two-fold dilutions of the syphilitic and nonsyphilitic sera are made in 0.9% saline. Tests are made, as described under item 14, with the undiluted serum and with dilutions prepared of the syphilitic and nonsyphilitic reacting sera, as well as with the undiluted normal serum. Distinctive patterns should be obtained with the syphilitic and nonsyphilitic reacting sera, and no reaction should be obtained with the normal serum. (In this study, a number of T.P.I.-negative leprosy sera which had given the nonsyphilitic type of reaction in the differential procedure were pooled and used for the nonsyphilitic reacting control serum).

(14) Differential procedure: (*a*) Pipette 0.05 cc. of the heated serum into each of 2 rings of a paraffined ringed glass slide. (*b*) Add 1 drop (1/45 cc.) of Antigen I to one aliquot of the serum, and 1 drop (1/45 cc.) of Antigen II to the other aliquot of the serum. (*c*) Rotate for 4 minutes on a mechanical rotator at 180 r.p.m., as described for the VDRL slide test. (*d*) Read tests microscopically, using 100× magnification, immediately after rotation. (*e*) Record the results as follows:

Appearance of clumps	*Record as*
No clumping or very small clumps	—
Small clumps	1
Moderate-sized clumps	2
Moderately-large clumps	3
Large clumps	4

Interpretation of results with the differential procedure.—1. When the following

results are obtained in qualitative testing, interpretation can be made without resorting to quantitative testing:

Antigen I	*Antigen II*	*Interpretation*
4, 3, 2, 1	—	Nonsyphilitic reaction (NS)
4	1	Nonsyphilitic reaction (NS)
—	—	No reaction (NR)

2. When results are obtained other than those just described above, quantitative tests should be made using serial two-fold dilution of the serum in 0.9% saline, beginning with 1:2. Record results, add the numerical values obtained with each antigen, obtain the differences in the sum of numerical values of Antigen I minus Antigen II, and interpret as follows:

(*a*) Syphilitic type of reaction (S): This type of reaction is characterized, in general, by an equal or greater reactivity of Antigen II as compared to Antigen I. For this reason, the difference in the sums of numerical values obtained with Antigen I minus Antigen II will either be zero or a minus value. Exceptions to this statement are those instances, also considered to be of syphilitic type, where Antigen I is slightly more reactive than Antigen II, i.e., where the difference in the sum of the numerical values obtained with Antigen I minus Antigen II is plus 1 or plus 2. Therefore, in the syphilitic type of reaction, the sum of numerical values obtained with Antigen I is plus 2 or less than the sum of numerical values obtained with Antigen II. Examples:

Antigen I	*Sum of numerical values*	*Antigen II*	*Sum of numerical values*	*Difference* [a]
1,– – –	1	3,– – –	3	minus 2
3,1,– –	4	3,1,– –	4	0
4,3,– –	7	3,1,– –	5	plus 2
4,4,1,–	9	4,4,4,1,–	13	minus 4

[a] Difference in sum of numerical values of results obtained with Antigen I minus results obtained with Antigen II.

(*b*) Nonsyphilitic type of reaction (NS): This type of reaction is characterized by a lower reactivity of Antigen II as compared to Antigen I. For this reason, when the differences in the sum of numerical values obtained with Antigen I minus the sum of numerical values obtained with Antigen II is *plus 3 or more*, the reaction is considered to be of the nonsyphilitic type. Examples:

Antigen I	*Sum of numerical values*	*Antigen II*	*Sum of numerical values*	*Difference* [a]
4,4,4,–	12	4,1,– –	5	plus 7
4,4,1,–	9	2,1,– –	3	plus 6
4,4,4,3,–	15	3– – –	3	plus 12
4,1,– –	5	2– – –	2	plus 3

[a] Differences in sum of numerical values of results obtained with Antigen I minus results obtained with Antigen II.

RESULTS

The results obtained with this differential procedure in presumed normal individuals who were seronegative in the VDRL slide flocculation test are presented in Table 1. With 83 T.P.I.-negative sera and one T.P.I.-positive serum, Antigens I and II were nonreactive.

TABLE 1.—*Results of the differential procedure and the T. P. I. test in normal seronegative individuals.*[a]

T.P. I./b	Number of patients	Antigen/c I	Antigen/c II	Interpretation/d	Clinical
Negative (—)	83	—	—	NR	Normal
Positive (+)	1	—	—	NR	Normal

a Negative in the VDRL slide test.
b T. P. I results expressed as: negative (—); doubtful (±); positive (+).
c Titers expressed as described under "methods."
d NR, no reactions; S, syphilitic reaction; NS, nonsyphilitic reaction.

Table 2 presents the results obtained with the differential procedure and T.P.I. tests in 141 nonleprous patients who had clinical or historical evidence of syphilis. The results obtained with these two tests with sera of 87 patients with early, symptomatic syphilis are presented in Table 2A. The syphilitic type of reaction in the differential procedure was obtained in 64 of the 87 cases, 19 were nonreactive, and 4 gave the nonsyphilitic type of reaction.

In Table 2B are presented results obtained with 54 sera of patients with late and late latent syphilis. In all instances, the T.P.I. was positive. A syphilitic type of reaction was obtained in the differential procedure in 53 of the patients; in one instance no reaction was obtained.

Also in Table 2, section C, are the results obtained in the performance of the differential procedure with euglobulin fractions of 12 sera from patients with clinical syphilis. The purpose of this presentation will be discussed later.

TABLE 2.—*Results of the differential procedure and the T.P.I. test in* (A) *early syphilis, in* (B) *late and late latent syphilis, and in* (C) *with euglobulin fractions.*

Antigen I	Antigen II	Interpretation/*a*	T.P.I.	Clinical/*b*
A. *Early syphilis*				
—	—	NR (13)	—	S-10, No Rx
—	—	NR (14)	—	S-10, Rx '53
—	1	S	—	S-10, No Rx
1	1	S (3)	—	S-10, Rx '53
5	4	S	—	S-10, No Rx
9	9	S	—	S-10, Rx '53
9	13	S	—	S-10, No Rx
13	16	S	—	S-10, Rx '53
15	17	S	—	S-10, No Rx
15	15	S	—	S-20, No Rx
21	21	S	—	S-20, Rx '53
—	—	NR	±	S-10, No Rx
1	1	S	±	S-10, Rx '53
2	8	S	±	S-10, No Rx
17	17	S	±	S-20, Rx '53
20	23	S	±	S-20, Rx '53
23	25	S	±	S-20, Rx '53
24	25	S	±	S-10, No Rx
25	28	S	±	S-20, No Rx
30	29	S	±	S-20, No Rx
—	—	NR	+	S-10, No Rx
—	9	S	+	S-10, No Rx
2	4	S	+	S-10, No Rx
3	8	S	+	S-10, No Rx
4	6	S	+	S-10, No Rx
4	9	S	+	S-10, No Rx
5	8	S	+	S-10, No Rx
9	14	S (2)	+	S-10, No Rx
12	13	S	+	S-10, No Rx
16	19	S	+	S-10, No Rx
17	19	S	+	S-10, No Rx
17	21	S	+	S-10, No Rx
20	23	S	+	S-10, No Rx
21	25	S	+	S-10, No Rx
23	26	S	+	S-10, No Rx
25	28	S	+	S-10, No Rx
27	29	S	+	S-10, No Rx
28	29	S (2)	+	S-10, No Rx
29	32	S	+	S-10, No Rx
—	1	S	+	S-10, Rx '53
13	13	S	+	S-10, Rx '53
20	20	S	+	S-10, Rx '53
20	21	S (2)	+	S-10, Rx '53
21	24	S	+	S-10, Rx '53
24	24	S (2)	+	S-10, Rx '53
24	25	S	+	S-10, Rx '53
25	25	S	+	S-10, Rx '53
25	28	S	+	S-10, Rx '53
28	31	S	+	S-10, Rx '53
18	23	S	+	S-20, No Rx
23	26	S	+	S-20, No Rx
24	24	S	+	S-20, No Rx
32	32	S	+	S-20, No Rx
1	4	S	+	S-20, Rx '53
20	20	S	+	S-20, Rx '53
20	21	S	+	S-20, Rx '53
21	23	S	+	S-20, Rx '53
24	25	S	+	S-20, Rx '53
25	27	S	+	S-20, Rx '53
25	28	S (2)	+	S-20, Rx '53
33	36	S	+	S-20, Rx '53
21	18	NS	—	S-10, No Rx
5	1	NS	+	S-20, Rx '53
16	13	NS	+	S-20, Rx '53
21	17	NS	+	S-20, Rx '53

Antigen I	Antigen II	Interpretation/*a*	T.P.I.	Clinical/*b*
B. *Late and late latent syphilis*				
—	1	S	+	S-40, No Rx
1	2	S	+	S-40, No Rx
1	3	S	+	S-40, No Rx
15	20	S	+	S-40, No Rx
16	18	S	+	S-40, No Rx
25	30	S	+	S-40, No Rx
—	1	S	+	S-40, Pt Rx
4	8	S	+	S-40, Pt Rx
20	29	S	+	S-40, Pt Rx
—	1	S	+	S-40, Rx '32
—	1	S	+	S-40, Rx '51
—	1	S	+	S-40, Rx '38
—	1	S	+	S-40, Rx '41
—	1	S	+	S-40, Rx '43
—	1	S	+	S-40, Rx '47
—	1	S	+	S-40, Rx '36
—	1	S	+	S-40, Rx '53
—	1	S	+	S-40, Rx
—	1	S	+	S-40, Rx
—	2	S	+	S-40, Rx '47
—	2	S	+	S-40, Rx '49
—	3	S	+	S-40, Rx '50
—	3	S	+	S-40, Rx '39
—	3	S	+	S-40, Rx '30
1	4	S	+	S-40, Rx '49
1	4	S	+	S-40, Rx
2	3	S	+	S-40, Rx
4	10	S	+	S-40, Rx
5	10	S	+	S-40, Rx
10	14	S	+	S-40, Rx
12	16	S	+	S-40, Rx
13	15	S	+	S-40, Rx '34
13	16	S	+	S-40, Rx '51
21	22	S	+	S-40, Rx
21	25	S	+	S-40, Rx
22	25	S	+	S-40, Rx
25	26	S	+	S-40, Rx
32	39	S	+	S-40, Rx
33	38	S	+	S-40, Rx
1	4	S	+	CVS, No Rx
1	4	S	+	CVS, No Rx
2	4	S	+	CVS, No Rx
16	24	S	+	CVS, No Rx
—	—	NR	+	CVS, Rx '52
1	4	S	+	CVS, Rx '50
19	25	S	+	CNS, No Rx
—	1	S	+	CNS, Rx '20
—	2	S	+	CNS, Rx '43
—	4	S	+	CNS, Rx '43
1	4	S	+	CNS, Rx '42
2	7	S	+	CNS, Rx '35
3	12	S	+	CNS, Rx '50
25	28	S	+	CNS, Rx '52
31	34	S	+	CNS, Rx '50
C. *With euglobulin fractions/c*				
25	29	S	+	S-10, No Rx
28	29	S	+	S-10, No Rx
20	20	S/*d*	—	S-10, No Rx
10	14	S	+	S-10, Rx '53
—	4	S	+	S-10, Rx '53
29	29	S	±	S-20, No Rx
13	20	S	+	S-10, Rx '53
17	20	S/*d*	+	S-20, Rx '53
29	32	S	+	S-20, Rx '53
17	21	S	+	S-20, Rx '53
—	6	S	+	CNS, Rx '35, Rx '41
21	24	S	+	CNS, Rx '52

a Numbers in parentheses indicate the numbers of cases of the particular kind.

b S-10, primary syphilis; S-20, secondary syphilis; S-40, late latent syphilis; CNS, central nervous system syphilis; CVS, cardiovascular syphis. Rx denotes treatment dates indicated when known; Pt Rx denotes partial, or inadequate treatment.

c The tests with Antigens I and II were made with euglobulin, as explained in the text.

d These cases appear as NS (nonsyphilitic reaction) in Table 2A.

(Note: For reasons of space, several notations in Part B indicating when those cases were primary have had to be deleted.—EDITOR.

In Tables 3 and 4 the results obtained with the differential procedure and the T.P.I. tests in 255 leprosy sera are presented. Table 3 gives the results obtained with 14 sera of leprosy patients with clinical or historical evidence of syphilis. It can be seen that of 6 T.P.I.-positive sera, 4 gave the syphilitic type of reaction with the differential procedure, whereas 2 gave nonsyphilitic reactions. Of five sera giving negative T.P.I. findings, four gave no reaction and one gave a nonsyphilitic type of reaction with the differential procedure. Of three doubtful T.P.I. specimens, two gave syphilitic reactions and one gave no reaction with the differential procedure.

TABLE 3.—*Results of the differential procedure and the T. P. I. test in leprosy patients with clinical or historical evidence of syphilis.*

Antigen I	Antigen II	Interpretation	TPI	Clinical/*a*
—	—	NR	—	S-40, Rx '40 (S-10 '40?)
—	—	NR	—	S-40, Rx '22 (S-10 '22)
—	—	NR	—	S-40, Rx '33 (S-10 '33)
—	—	NR	—	S-40, Rx '32 (S-10 '32)
—	—	NR	±	S-40, Rx '40
—	1	S	±	S-40, Rx '26 (S-10 '26)
11	11	S	±	S-40, Rx '13 (S-10 '13)
1	4	S	+	S-40 (S-10 '26)
—	2	S	+	S-40, Rx '38 (S-10 '38)
10	3	S	+	S-40, Rx? '37
6	9	S	+	S-40, Rx? '40
4	1	NS	+	S-40, No Rx (S-10 '23?)
4	1	NS	+	S-40, Rx '43 (S-20 '43)
3	—	NS	—	S-40, Rx '45

a S-10, primary syphilis; S-40, late latent syphilis. Rx denotes treatment, dates indicated when known.

Table 4 shows the results obtained with 241 sera of leprosy patients without evidence of syphilis. It was found that 21 of these sera reacted with the T.P.I. From Table 4A it can be seen that with the differential procedure the syphilitic type of reaction was obtained with 15 of these sera, the nonsyphilitic type in 3, and no reaction in 3.

TABLE 4.—*Results of the differential procedure in leprosy patients without clinical or historical evidence of syphilis, the T. P. I. reactions positive* (A), *and negative* (B).

A. T. P. I. reaction positive			B. T. P. I. reaction negative			
Antigen I	Antigen II	Interpretation	Antigen I	Antigen II	Interpretation	No. of patients
—	—	NR	4	—	NS	30
—	—	NR	3	—	NS	8
—	—	NR	2	—	NS	5
—	1	S	1	—	NS	3
—	2	S	4	1	NS	4
—	2	S	10	—	NS	2
—	3	S	20	8	NS	2
1	3	S	9	3	NS	1
1	3	S	15	3	NS	1
2	6	S	12	5	NS	1
2	10	S	20	6	NS	1
4	9	S	20	5	NS	1
12	14	S	17	3	NS	1
13	19	S	16	3	NS	1
14	19	S	8	—	NS	1
17	19	S	16	4	NS	1
17	15	S	8	5	NS	1
22	26	S	20	6	NS	1
5	2	NS	11	4	NS	1
4	1	NS	8	—	NS	1
2	—	NS	—	—	NR	153
						220—Total

There were 220 T.P.I.-negative sera in the leprosy group. The results obtained with them are presented in Table 4B. There were 153 which gave no reaction in the differential procedure; the remaining 67 gave the non-syphilitic type of reaction.

In Tables 5 and 6 are presented comparisons of reactivity of the VDRL slide test and Antigens I and II in syphilitic (nonleprous) and leprous (syphilitic and nonsyphilitic) sera, in relation to the T.P.I. results. The significance of these findings will be discussed.

TABLE 5.—*Comparison of reactivity of VDRL slide test, Antigen I and Antigen II in syphilitic (nonleprous) sera.*

Results	VDRL	Antigen I	Antigen II
T. P. I. negative (29 specimens)			
Reactive	13 (44.8%)	12 (41.4%)	13 (44.8%)
Nonreactive	16 (55.2%)	17 (58.6%)	16 (55.2%)
T. P. I. positive (112 specimens)			
Reactive	98 (87.5%)	87 (77.7%)	109 (97.3%)
Nonreactive	14 (12.5%)	25 (22.3%)	3 (2.7%)

TABLE 6.—*Comparison of reactivity of VDRL slide test, Antigen I and Antigen II in leprosy sera.*

Results	VDRL	Antigen I	Antigen II
T. P. I. negative (225 specimens)			
Reactive	65 (28.9%)	68 (30.2%)	17 (7.6%)
Nonreactive	160 (71.1%)	157 (68.8%)	208 (92.4%)
T. P. I. reactive (30 specimens)			
Reactive	18 (60.0%)	21 (70.0%)	25 (83.3%)
Nonreactive	12 (40.0%)	9 (30.0%)	5 (16.7%)

DISCUSSION

No attempt will be made to suggest a mechanism for the action of the differential procedure at this time. However, the phenomenon appears to bear some relationship to both the Kahn and the Neurath verification tests. If choline chloride is behaving like sodium chloride, then the inhibition observed may be due to a high salt effect. Some experiments have been made substituting equimolar sodium chloride for choline chloride. Essentially similar degrees of inhibition were observed with nonsyphilitic leprosy sera, but the coarseness of the negative reactions made the interpretation of negative results difficult when sodium chloride was used as an inhibiting agent. With respect to the Neurath test, it is conceivable that choline, as part of the lecithin molecule, may be showing similar effects to those reported by Volkin (27) using human serum or beef heart lecithin.

In considering the specificity of the differential procedure in normal and syphilitic individuals, specimens from carefully documented cases were selected. It was found that all 84 presumed normal individuals, negative with the VDRL slide test, were nonreactive in the differential procedure.

It is of interest to note that the T.P.I. test is often nonreactive in early syphilitic infection (Table 2A), as has been previously reported (29-32). The discrepancies then between the syphilitic type of reaction in the differential procedure and the negative results with the T.P.I. test in these patients may not indicate error of either test, but may be dependent upon the procedure used to indicate the presence of different antibodies appearing at different times in the disease process and the individual antibody response.

In the 87 patients having early clinical syphilis without leprosy, there were 4, all in the early syphilis group, who gave the nonsyphilitic type of reaction. When it was feasible these specimens and others were fractionated according to the method of Neurath, and tests were made with the euglobulin fraction using the differential procedure. The results, shown in Table 2C, indicate that syphilitic-type reactions were obtained with all sera tested, including two specimens from syphilitic individuals that gave the nonsyphilitic type of reaction with whole serum. This would suggest, as Neurath pointed out (11), that proteinaceous substances, by themselves nonreactive, may influence the serologic findings.

In 54 patients with late and late latent syphilis (Table 2B), it was noted that there was agreement between the T.P.I. test results and the differential procedure in all but 1; in that instance there was no detectable lipid reactive antibody (reagin).

One of the criticisms of the Kahn verification test has been that the type of reaction obtained seemed to be related to the titer of the serum (6, 16, 18). Thus, low-titered sera tended to give the biologic false positive type of reaction, whereas high-titered sera tended to give the syphilitic type. Kahn (28) did not question these findings of Scott and associates (16), but personally noted that high-titered sera from patients with lepromatous leprosy gave the biologic false positive type of reaction, whereas low-titered sera of syphilitic patients gave the syphilitic pattern. No evidence for a relationship of syphilitic and nonsyphilitic type reactions to height of titer was found using the differential procedure described in this report.

One of the methods used for increasing the specificity of S.T.S. has been to reduce the sensitivity. In doing this, the efficiency of the test to detect syphilis is decreased. In order to show that the sensitivity of the differential procedure in syphilis is at a high level in relation to the VDRL slide test, comparative data are presented in Table 5. It can be seen that the numbers of T.P.I.-reactive sera reacting with Antigen II is higher than with either the VDRL slide test or Antigen I. There was

97.3 per cent agreement between the T.P.I. reactions and the differential procedure in the syphilitic patients. It can be seen, also, that the reactivity level of the test procedure using the antigen suspension containing choline chloride is as high, or higher, than the VDRL slide test in syphilis. (The interpretation of a syphilitic type of reaction is dependent upon the greater, or equal, reactivity of Antigen II as compared with Antigen I.)

In considering the findings with 255 specimens from leprosy patients, it was found that only 14 had clinical or historical evidence of syphilis (Table 3); an additional 21 were reactive in the T.P.I. test (Table 4A). Reactivity with the T.P.I. test was, for the purposes of this report, presumed to be evidence for syphilis, past or present. Accordingly, the lipid reactive antibody (reagin), when present in the T.P.I. reactive patients, was considered as probably due to syphilis. The T.P.I. test was thus employed in this study as an objective tool in assessing the specificity of the differential procedure.

It was found that in 30 T.P.I.-reactive specimens, 21 gave the syphilitic type of reaction with the differential procedure, 4 gave no reaction, and 5 gave the nonsyphilitic type of reaction. In the 5 nonsyphilitic reactors in the differential procedure, it is conceivable that the type of reaction observed might have been due to a relatively high ratio of nonsyphilitic lipid-reactive antibody to syphilitic lipid-reactive antibody. Experimentally, it was found that a mixture of pooled, nonsyphilitic reactive leprosy sera and pooled, syphilitic reactive nonleprosy sera would give the nonsyphilitic type of reaction with the differential procedure when the ratio of nonsyphilitic serum was very high in relation to the syphilitic serum.[4]

It was noted that with 220 sera that were negative in the T.P.I. test (Table 4B), all reactions obtained with the differential procedure were either nonsyphilitic in type or negative (no reaction).

In Table 4 a comparison of reactivity of the VDRL slide test and Antigens I and II of the differential procedure with the leprosy sera is made. In Table 4A, the qualitative results obtained with 225 T.P.I.-negative specimens show that Antigen II, containing choline chloride, is strikingly less reactive (7.6%) than either the VDRL slide test (28.9%) or Antigen I (30.2%). (The interpretation of a nonsyphilitic type of reaction is dependent upon the reduced reactivity of Antigen II as compared with Antigen I.) This is in marked contrast to the findings in Table 5. The results obtained with nonleprous, T.P.I.-positive sera indicated that Antigen II was again highly reactive as in the syphilitic, nonleprous group.

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[4] The pool of nonsyphilitic (T.P.I.-negative) leprosy serum (NS) gave a positive end-point of 1:1 when diluted in a negative serum pool. The syphilitic (T.P.I.-positive) nonleprosy pool (S) gave a positive end point of 1:8 when diluted in the negative serum pool. The nonsyphilitic type of reaction in the differential procedure was obtained with a mixture of (NS) and (S) only when the ratio of (NS) to (S) was 20:1 or greater.

It is evident that no single verification procedure has thus far been able to distinguish all types of nonsyphilitic reactors. However, there can be no doubt that there are differences in the serologic behavior of the lipid-reactive antibodies in syphilis and in leprosy. Methods which present different serologic profiles or aspects of reactive antibodies ultimately may lead, not only to a better understanding of the nature and significance of these antibodies, but also to a practical means for arriving at a more highly specific serologic test for syphilis.

The differential procedure described in this report is not recommended for general use. Studies are in progress to determine the behavior of the test in other disease conditions.

SUMMARY AND CONCLUSIONS

1. A simple procedure for the differentiation of syphilitic and nonsyphilitic reactions obtained in serologic tests for syphilis with leprosy sera is described.

2. The behavior of this procedure with sera of normal individuals, syphilitic patients, and patients with leprosy (with and without evidence of syphilis) is presented.

3. With antigen suspensions containing choline chloride, sera of syphilitic patients (with or without leprosy) show an augmentation of seroreactivity; nonsyphilitic reactive leprosy sera show a diminution of seroreactivity.

4. On the basis of the clinical and historical evidence, a good correlation was obtained between the differential procedure described and the results of the T.P.I. test in normal persons and in syphilitic, nonleprous individuals.

5. The differential procedure compared favorably with the T.P.I. test in leprosy patients with and without clinical or historical evidence of syphilis.

6. A comparison of reactivity levels of the differential procedure, the VDRL slide test, and the T.P.I. test is presented and discussed.

RESÚMEN Y CONCLUCIONES

1. Se describe un procedimiento simple para la diferenciación de reacciones sifilíticas y no sifilíticas en sueros de pacientes leprosos.

2. Se presenta los resultados de éste procedimiento en suero de individuos normales, de pacientes sifilíticos y de pacientes leprosos con y sin sífilis.

3. Con antigeno conteniendo "Choline Chloride" el suero de pacientes sifilíticos con y sin lepra demuestra aumento en la sero reactividad; Suero de pacientes leprosos no sifilíticas demuestra disminuición en ea seroreactividad.

4. De acuerdo con evidencia clínica hubo buena correlación entre el procedimiento descrito y los resultados de la prueba T.P.I. en personas normales y en individuos sifilíticos no leprosos.

5. El procedimiento diferencial comparó favorablemente con la prueba T.P.I. en pacientes leprosos con y sin evidencia clínica de sífilis.

6. Se presenta una comparación de los niveles de reactividad entre los pruebas diferencial, la prueba V.D.R.L., y la de T.P.I.

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