A CLINICO-SEROLOGIC STUDY OF LEPROSY
I. RESULTS OF SEROLOGIC TESTS FOR SYPHILIS, INCLUDING THE *TREPONEMA PALLIDUM* IMMOBILIZATION TEST

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For many years it has been known that leprosy patients are prone to give positive reactions in the serologic tests for syphilis (STS) in common use. Badger (1) reviewed the literature on the subject up to 1931. Since that time, several other studies have been made (2-5, 10, 12, 13, 15), almost all of them showing that reactions occur frequenty in STS's in the sera of patients with leprosy, some of which reactions have been presumed to be due to the leprosy itself.

The present clinico-serologic study was undertaken to observe further the reactivity of sera from patients with leprosy in various serologic tests for syphilis using different types of antigens, including a treponemal antigen. To this end the following tests were chosen: (*a*) the quantitative Kahn Standard, representing tests with lipoidal antigens; (*b*) the Kolmer (cardiolipin) quantitative[2] test, representing complement-fixation tests with cardiolipin-lecithin antigens; and (*c* & *d*) the VDRL and Rein-Bossak slide flocculation quantitative tests, representing modern slide flocculation tests with cardiolipin-lecithin antigens. The *Treponema pallidum* immobilization (TPI) tests, which demonstrates a kind of antibody due to syphilis different than the other tests (6, 9), was also included. The results obtained with the various tests were compared with each other and with clinical information concerning the patients.

METHODS AND MATERIAL

Laboratory methods.—The serologic tests for syphilis listed above were performed at the Venereal Disease Research Laboratory, as described in the Manual of Serologic Tests for Syphilis (7). The TPI test was performed at the same laboratory with the Nelson and Mayer technique (9) as modified by Portnoy, Harris and Olansky (14). The serum protein determinations were performed at the U. S. Public Health Service Hospital at Carville, according to the method of Wolfson, Cohn, Calvary and Ichiba (16).

Clinical material.—All of the patients in this study were at the Carville hospital, and they were unselected. The number studied, 224, comprises more than one-half the population of this institution; 204 were lepromatous, only 20 tuberculoid. Clinical

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[2] Referred to hereinafter as "Kolmer-C."

information regarding them, collected by the staff of the institution, consisted of national origin; age; sex; history of lesions and/or treatment of syphilis; the duration, type and activity of leprosy; findings of *M. leprae* in the lesions; treatment of the patient; and diseases complicating the leprosy process. This clinical information was compared with our laboratory findings. Analyses of certain of the clinical factors and associated laboratory results are presented.

Of the 81 patients from the United States, 65 were white and 16 were Negro. There were 75 Mexicans. Of the 37 from the Caribbean area, 12 were from Puerto Rico, 20 from the Virgin Islands, 4 from Cuba, and 1 from the British West Indies. Of the 27 from the Pacific area, 2 were from Hawaii, 10 from the Philippines, 13 from China, and 1 each from Japan and Korea. Of the 5 from southern Europe, 4 were from Greece and 1 was from Spain. Of the 224 patients, 151 were males and 73 were females.

RESULTS

The reactivity of the sera from these patients in the various serologic tests performed (STS and TPI) are compared in Table 1. A few sera that were of insufficient quantity to test with all procedures or that gave anticomplementary reactions, were omitted from the tables, except in Tables 2 and 5. All sera not negative in the tests were considered as reactive. The findings ranged from reactions in dilutions of less than 1:1[3] to strongly positive reactions, all occurring in the quantitative tests with the exception of the TPI test which was performed only qualitatively. In the TPI test, positive and doubtful reactions were considered reactive.

Reactivity of the tests in these serums, in order of frequency, was found to be 63.4 per cent with the Kolmer-C, 52.7 per cent with the Kahn Standard, 51.8 per cent with the Rein-Bossak, 46.9 per cent with the VDRL slide, and 11.2 per cent with the TPI test.

Regarding the comparison in Table 1 of the total results of the lipid-antigen tests with those obtained with the TPI test, it will be seen that of the 199 TPI-negative patients, 117 (58.8%) reacted in the Kolmer-C, 99 (49.7%) in the Kahn Standard, 94 (47.2%) in the Rein-Bossak, and 86 (43.2%) in the VDRL test. Among the 25 TPI reactive sera, the Kolmer-C reacted in all; the Rein-Bossak, in 22 (88.0%); and the Kahn and VDRL tests, in 19 (76.0%).

The closest agreement with the reactive TPI-test results was therefore obtained with the Kolmer-C test. In the TPI-negative group, however, the greatest disagreement with the TPI-test results was found also with the Kolmer-C tests, with less disagreement occurring with the other STS tests.

In the other sections of Table 1, the data on the cases by type, lepromatous or tuberculoid, are given. It can be seen that in the TPI-negative group there is a definite tendency for the lepromatous patients to be more seroreactive in the STS's than the tuberculoid patients.

In Table 2, 16 leprosy patients with history of syphilitic lesions (10) and/or treatment for syphilis (6) are listed, with the pertinent findings

[3] Reactions in less than 1 dilution in the quantitative Kahn test were considered negative, according to the author's method of interpretation of test results (7).

applying to each patient. None of the other TPI-reactive cases had any such history.

TABLE 1.—*Comparison of the results of the Kahn, Kolmer, VDRL, and Rein-Bossak tests and of the TPI test on 224 blood specimens, all cases and by type.*

STS tests	TPI test					
	Reactive		Negative		Total	
	No.	Per cent	No.	Per cent	No.	Per cent
All cases						
Kahn						
Reactive	19	76.0	99	49.7	118	52.7
Negative	6	24.0	100	50.3	106	47.3
Kolmer						
Reactive	25	100.0	117	58.8	142	63.4
Negative	0	0	82	41.2	82	36.6
VDRL						
Reactive	19	76.0	86	43.2	105	46.9
Negative	6	24.0	113	56.8	119	53.1
Rein-Bossak						
Reactive	22	88.0	94	47.2	116	51.8
Negative	3	12.0	105	52.8	108	48.2
Total, by TPI reactivity	25	11.2	199	88.8	224	100.0
Lepromatous cases (202)						
Kahn						
Reactive	18	81.8	98	53.8	116	56.9
Negative	4	18.2	84	46.2	88	43.1
Kolmer						
Reactive	22	100.0	117	64.3	139	68.1
Negative	0	0.0	65	35.7	65	31.9
VDRL						
Reactive	18	81.8	86	47.3	104	51.0
Negative	4	18.2	96	52.7	100	49.0
Rein-Bossak						
Reactive	19	86.4	92	50.5	111	54.4
Negative	3	13.6	90	49.5	93	45.6
Total, by TPI reactivity	22	10.8	182	89.2	204	100.0
Tuberculoid cases (20)						
Kahn						
Reactive	1	33.3	1	5.9	2	10.0
Negative	2	66.7	16	94.1	18	90.0
Kolmer						
Reactive	3	100.0	0	0.0	3	15.0
Negative	0	0.0	17	100.0	17	85.0
VDRL						
Reactive	1	33.3	0	0.0	1	5.0
Negative	2	66.7	17	100.0	19	95.0
Rein-Bossak						
Reactive	3	100.0	2	11.8	5	25.0
Negative	0	0.0	15	88.2	15	75.0
Total, by TPI reactivity	3	15.0	17	85.0	20	100.0

It can be seen that a history of syphilitic lesions and/or treatment for syphilis was found in some of these patients in the presence or absence of reactivity in any of the serologic tests used, including the TPI test. It could not, then, be determined by TPI results which individual patients

TABLE 2.—*Leprosy patients (16, all males) with history of syphilitic lesions and/or treatment for syphilis.*[a]

Age	Lesions	Treatment[b]	Type	Kahn	Kolmer–C	VDRL	Rein-Bossak	TPI
51	Primary, 1923	None known	L	1	8	4	2	+
29	Primary (?) '38	1938-1939	T	—	1	—	<1	+
39	Primary (?) '40	None known	L	2	8	4	4	+
29	Secondary, '43	1943, Pn.	L	16	8	4	2	+
55	Primary, 1926	As., Bi.	L	16	1	<1	<1	±
62	Primary, 1913	None known	L	8	2	1	1	—
52	Primary, 1922	1922-1926	L	—	QNS	—	—	—
58	Primary, 1932	Adequate	L	1	AC	—	—	—
45	Primary, 1940	Treated	T	—	—	QNS	—	—
48	Primary, 1933	6 As, 1933	L	—	2	—	—	—
45	—	1937, adequate	L	32	8	4	4	+
39	—	1940, As., Bi	L	—	2	<1	1	±
39	—	1945-1948	L	8	8	1	1	—
18	—	5 As.	L	1	—	2	1	—
78	—	Inadequate	L	2	8	8	4	—
44	—	Inadequate	L	2	2	2	1	—

a Certain cases in which there was insufficient serum for all tests (QNS), or which were anticomplementary (AC), are included in this table.

b One case recorded as having been treated with penicillin (Pn), others with arsenic (AS) or arsenic and bismuth (Bi).

had lipid reactive antibody (reagin) as detected by the STS's, due only to leprosy, or to syphilis, or both, or to neither disease. However, it is believed that TPI-reactive patients are likely to have, or to have had, syphilis,[4] although TPI negativity does not exclude this possibility. It has been demonstrated especially that patients with syphilis, treated during the early stages of this disease, may be found with some frequency to have no immobilizing (TPI) antibody demonstrable at various periods of

[4] No history of other treponematoses, e.g., yaws, pinta, or bejel, was obtained in this group.

time after this treatment (11). The treatment may have been based on positive serologic tests, which may have been reactive due either to syphilis or leprosy.

Analysis has been made of both the TPI-reactive and the TPI-negative groups with respect to histories of syphilitic lesions and/or treatment for syphilis. Of the 25 positive patients, 5 (20%) had positive histories and 5 (20%) had had treatment. Of the 199 negative patients, only 5 (2.5%) had positive histories, and 8 (4.0%) had had treatment.

In Table 3 the leprosy classification is related to the patterns of seroreactivity in the various STS tests and to the TPI results. This demonstrates the numerous patterns of seroreactivity found in the 224 patients in whom results were obtained in all these tests. Any degree of reactivity in any of the quantitative tests (STS) used is expressed as +. A tendency is found here for the patients with lepromatous leprosy to have greater seroreactivity in all the tests, whether or not the TPI test

TABLE 3.—*Patterns of reactivity in STS and TPI test in relation to leprosy classification.*

Results of the STS tests				Results of the TPI test, by type					
				Lepromatous		Tuberculoid		Total	
Kahn	Kolmer-C	VDRL	Rein-Bossak	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
+	+	+	+	16	66	1	0	17	66
+	+	—	+	2	4	0	0	2	4
+	+	—	—	0	14	0	0	0	14
+	—	+	+	0	9	0	0	0	9
+	—	+	+	0	1	0	0	0	1
+	—	—	—	0	4	0	1	0	5
—	+	+	+	1	6	0	0	1	6
—	+	+	—	1	1	0	0	1	1
—	+	—	+	0	2	2	0	2	2
—	—	+	+	0	3	0	0	0	3
—	+	—	—	2	24	0	0	2	24
—	—	+	—	0	1	0	0	0	1
—	—	—	+	0	1	0	2	0	3
—	—	—	—	0	46	0	14	0	60
Totals				22	182	3	17	25	199

was reactive. Seventeen (68%) of the 25 TPI-reactive patients were found to be reactive in all the other tests used, although 66 (33.2%) of the 199 TPI-negative patients also reacted in all the other tests.

In Table 4 the serologic (STS) results in the TPI-negative patients are analyzed, first with respect to the age groups, and second with respect to the duration of leprosy in years. There is seen little or no tendency to more or less reactivity in relation to age in these patients. TPI-reactive individuals were excluded since their inclusion would weight the figures toward the older age groups, considering reactions in the TPI test to be objective evidence for syphilis, past or present.

TABLE 4.—*Serologic results of the Kahn, Kolmer—C, VDRL, and Rein-Bossak tests among 199 TPI negative leprosy patients compared to (a) age of patient, and (b) duration of the disease.*

Age (years)	No. cases	Kahn		Kolmer-C		VDRL		Rein-Bossak	
		Pos.	Per cent	Pos.	Per cent	Pos.	Per cent	Pos.	Per cent
0-10	1	0	0.0	0	0.0	0	0.0	1	100.0
11-20	8	4	50.0	6	75.0	3	37.5	3	37.5
21-30	35	18	51.4	20	57.1	11	31.4	12	34.3
31-40	42	24	57.1	26	61.9	18	42.9	22	52.4
41-50	42	20	47.6	24	57.1	20	47.6	21	50.0
51-60	39	19	48.7	23	59.0	20	51.3	21	53.8
60+	32	14	43.8	18	56.2	14	43.8	14	43.8
Duration									
0-5	41	22	53.7	25	61.0	16	39.0	17	41.5
6-10	47	28	59.6	32	68.1	23	48.9	25	53.2
11-15	37	18	48.6	24	64.9	15	40.5	18	48.6
16-20	36	19	52.8	19	52.8	20	55.6	21	58.3
21-25	4	3	75.0	3	75.0	3	75.0	3	75.0
26-30	22	6	27.3	8	36.4	7	31.8	6	27.3
31+	12	3	25.0	4	33.3	2	16.7	4	33.3

On the other hand, there is seen a tendency for these reactions to be fewer in the cases of longer duration, especially those over 25 years. However, the relative numbers of patients of longer duration are not strictly comparable to those of the other groups.

An analysis has also been made of the results obtained in the STS's in the 199 TPI-negative patients, expressed in frequency of titers.[5] Suffice it to say that the Kahn Standard and Kolmer-C tests gave higher-titered

[5] To conserve space these data are not included here.

results more frequently than the two other tests. Reactions in this group occurring in dilutions of less than 1 in the quantitative tests were more frequent in the Rein-Bossak test than in the Kolmer-C or VDRL test. As has been said, reactions in such low dilutions in the quantitative Kahn test were considered negative.

Further analysis has revealed no relationship between reactivity in the serologic tests and the relative numbers of *M. leprae* found in the patients' lesions. Likewise, no relationship could be found between seroreactivity and the clinical assessment of the activity of the leprous process.[5] Since almost all of the patients were under treatment with a sulfone-type drug, no comparisons could be made relative to the effect of current therapy on seroreactivity.

In Table 5, the patients with the principal diseases complicating

TABLE 5.—*The principal complications in the patients studied, and the results of the various tests.*

Complication[a]	Age	Sex	Type[b]	Syphilis	Kahn	Kolmer -C	VDRL slide	Rein-Bossak	TPI
Amyloidosis 4+	39	M	L	+[c]	8	8	1	1	—
Amyloidosis 4+	26	M	L	—	—	—	+	—	—
Amyloidosis 3+	39	M	L	—	—	—	—	—	—
Amyloidosis 2+	57	F	L	—	—	—	—	—	—
Amyloidosis 2+	34	F	L	—	1	—	2	1	—
Amyloid nephrosis	30	M	L	—	2	8	1	<1	—
Am., P. tbc. (arrested)	60	M	L	—	—	—	—	—	—
P. tbc. (arrested)	52	M	L	—	128	QNS[e]	8	8	+
P. tbc. (arrested)	32	M	L	—	—	—	—	—	—
P. tbc. (arrested)	58	M	L	—	16	8	2	<1	—
P. tbc. (arrested)	41	M	L	—	1	Neg	2	1	—
P. tbc. (moderate)	35	M	L	—	16	40	2	2	—
P. tbc. (moderate)	85	M	L	—	—	40	<1	—	—
P. tbc. (moderate)	53	M	L	—	—	—	—	—	—
P. tbc. (advanced)	47	M	T	—	—	QNS[e]	—	—	—
P. tbc. diabetes	54	M	T	—	—	—	—	—	—
P. tbc. (moderate), diabetes	45	F	L	—	32	8	—	—	—
Diabetes	63	M	L	—	2	1	—	—	—
Diabetes	52	F	L	—	—	8	—	—	—
Diabetes	43	M	L	—	—	—	—	—	—
Diabetes	41	M	T	—	—	—	—	—	—
Diabetes	66	F	L	—	—	—	—	—	—
Diabetes	34	M	T	—	—	—	—	—	—
Diabetes	55	M	L	+[d]	16	1	<1	<1	±
Diabetes	45	M	L	—	—	1	—	<1	—
Diabetes	49	F	L	—	1	—	<1	1	—
Carcinoma of esophagus	57	M	L	—	—	—	—	—	—
Carcinoma of lung	40	M	L	—	4	8	—	<1	—

a P. tbc. = pulmonary tuberculosis.
b Referring to the type of leprosy, lepromatous and tuberculoid.
c Treated 1945-1948.
d Primary 1926.
e These cases are included in this table and Table 2.

leprosy are listed. It is noted that amyloidosis, diabetes, and carcinoma seemed to have no effect on the serologic test results. There appeared to be more increase of seroreactivity in the presence of pulmonary tuberculosis complicating leprosy.

TABLE 6.—*Serum proteins in relation to STS and TPI results.*

Group	No. of cases	Median						Range					
		Prot. total	Globulins				A/G ratio	Prot. total	Globulins				A/G ratio
			Tot.	α	β	γ			Tot.	α	β	γ	
TPI— STS—	20	6.1	2.6	0.9	0.8	0.9	1.3	4.6–7.5	1.5–4.0	0.2–1.6	0.2–1.7	0.4–2.0	0.5–2.9
TPI— STS+	42	6.3	2.7	0.7	0.8	0.9	1.4	5.4–7.6	1.3–4.6	0.1–2.7	0.1–2.2	0.3–2.0	0.3–3.3
TPI+ STS+[a]	22	6.2	2.6	0.8	0.9	0.9	1.4	5.4–7.2	1.8–4.4	0.2–1.4	0.4–2.7	0.3–1.9	0.6–2.2
Normal range								6.7–7.2	3.1–3.35	0.7–1.46	0.66–1.25	1.05–1.35	1.15–1.19

a All TPI-positive cases were positive in 1 dilution or over in one or more of the STS tests. There were no TPI-positive but STS-negative specimens in this study.

The serum-protein determinations—i.e., total proteins, total globulin, alpha, beta, and gamma globulins, and the albumin-globulin ratio—are related to the STS and TPI test results in Table 6. The cases used in this comparison are those with positive results of one dilution or more titer in any test of the former type, those reactive in the TPI test, and those in which there was no seroreactivity. No relationship was found between seroreactivity of any kind and the various serum protein determinations.

SUMMARY AND CONCLUSIONS

The results obtained in a clinico-serologic study of leprosy patients in the U. S. Public Health Service leprosarium at Carville are presented. The data show that reactions in the Kahn Standard, Kolmer-C, VDRL slide and Rein-Bossak tests, and also the TPI test, are encountered in patients who have no clinical or historical evidence of treponemal infection. The reactivity rates in these tests and the serum-protein determinations are presented and compared in relation to each other and to available clinical information concerning the patients.

Reactivity rates of the various tests employed in the 224 patients were as follows:

Kolmer-C quantitative.....63.4 per cent
Quantitative Kahn Standard.....52.7 per cent
Rein-Bossak slide flocculation quantitative.....51.8 per cent
VDRL slide flocculation quantitative.....46.9 per cent
Treponema pallidum immobilization (TPI).....11.2 per cent

Reactivity in the STS is related to the history of syphilitic lesions and/or treatment for syphilis, and to positive results with the TPI test. In the TPI-negative patients, seroreactivity was greater in those with lepromatous leprosy than the tuberculoid type. Seroreactivity in the STS's (in TPI-negative patients) bears no demonstrable relationship to the age of the patient, but seems to diminish somewhat with prolonged duration of the disease. No relationship could be found between seroreactivity and the relative numbers of *M. leprae* found in the lesions, or clinical assessment of activity of the leprous process. Since almost all of the patients were under sulfone treatment, no information concerning therapy in relation to seroreactivity was obtained.

In leprosy patients with complicating amyloidosis, diabetes, or carcinoma no significant relation to seroreactivity was found. There seemed to be some increase in the presence of complicating pulmonary tuberculosis.

No relation was found between reactivity in any of the tests and the findings regarding serum total proteins, total globulins, alpha, beta, or gamma globulin, or the albumin-globulin ratio in this group of leprosy patients when comparisons were made between seroreactive and nonreactive specimens.

RESÚMEN Y CONCLUSIONES

Se presentan los resultados de un estudio clínico-serológico en pacientes leprosos en el leprosario del U. S. Public Health Service en Carville, Louisiana. Los datos demuestran reacciones positivas a las pruebas de Kahn, Kolmer-C, VDRL, Rein-Bossak y TPI, en pacientes que no tienen evidencia clínica de sípilis. Se presentan los resultados de éstas y de las determinaciones de proteínas del suero. Las reacciones positivas en 224 pacientes fueron como sigue:

Kolmer-C	63.4 %
Kahn (cuantitativo)	52.7 %
Rein-Bossak	51.8 %
VDRL	46.9 %
TPI	11.2 %

No hubo relación entre la sero-reacción y el número de *M. leprae* en las lesiones o la evaluación clínica del estado leproso. No hubo relación entre la sero-reacción y complicaciones como amiloidosis, diabetes o cancer. No hubo relación entre la sero-actividad en ninguna de éstas pruebas y las proteínas del suero, las globulinas, alfa, beta o gama, ni con la relación albumina-globulina, en éste grupo de pacientes leprosos.

REFERENCES

1. BADGER, L. F. Significance of positive Wasserman and Kahn reactions in leprosy. Pub. Health Rep. **46** (1931) 957-970.
2. FAGET, G. H. and Ross, H. Evaluation of positive Kolmer and Kahn tests in leprosy. Ven. Dis. Inform. **25** (1944) 133-137.
3. HAZEN, H. H., PARRAN, T., SANFORD, A. H., SENEAR, F. E., SIMPSON, W. M. and VONDERLEHR, R. A. The occurrence in leprosy of positive serodiagnostic tests for syphilis. Ven. Dis. Inform. **17** (1936) 253-259.
4. ISHIBASHI, T. Beiträge zur Serologie der Lepra, mit besonderer Berücksichtigung der für die Komplementbindungsreaktion bei Lepra wirksemen Antigene.

Nebst serologischen Untersuchungen an Seren von mit säurefesten Bazillen immunisierten Kaninchen. Tohoku L. Exper. Med. **30** (1937) 287-314.

5. KVITTINGEN, J., CUTLER, J. C., GUEVARA, J. A., MCCULLOUGH, J. C., ROSE, E., FORD V., TAMPI, R. B., SUKUMAR SEN, LAKSHWIR, KAHN, J. and DASS, P. Serological tests for syphilis in lepers. Tendency for various antigens to give false-positive reactions. Bull. World Health Organ. **5** (1952) 481-504.
6. MAGNUSON, H. J. and THOMPSON, F. A., JR. Treponemal immobilization test of normal and syphilitic serums. J. Ven. Dis. Inform. **30** (1949) 309-320.
7. MANUAL OF SEROLOGIC TESTS FOR SYPHILIS. Supplement No. 22 to J. Ven. Dis. Inform., 1949.
8. NELSON, R. A., JR. Changing concepts in the serodiagnosis of syphilis: Specific treponemal antibody versus Wasserman reagin. British J. Ven. Dis. **28** (1952) 160-168.
9. NELSON, R. A., JR. and MAYER, M. M. Immobilization of *Treponema pallidum in vitro* by antibody produced in syphilitic infections. J. Exper. Med. **89** (1949) 369-393.
10. OBERDOERFFER, M. J. Beitrag zur Frage der unspezifisch positiven Luesreaktionen bei Lepra. Dermat. Wchnschr. **111** (1940) 794-796.
11. OLANSKY, S., HARB, F. W., WOOD, C. E. and RAMBO, D. S. Intramuscular Chloromycetin (chloramphenicol) in the treatment of venereal disease. American J. Syph. **37** (1953) 253-258.
12. PARRAN, T., HAZEN, H. H., MAHONEY, J. F., SANFORD, A. H., SENEAR, F. E., SIMPSON, W. M., and VONDERLEHR, R. A. Preliminary report on the Washington serology conference. Ven. Dis. Inform. **23** (1942) 161-194.
13. PORTNOY, J. and GALVEZ, R. A. Clinical and serologic studies with reference to syphilis in Guatemala, Central America. III. Studies of comparative performance of the Kahn, Kolmer, Mazzini and VDRL slide test among leprosy patients. American J. Syph. **36** (1952) 566-570.
14. PORTNOY, J., HARRIS, A. and OLANSKY, S. Studies of the *Treponema pallidum* immobilization (TPI) test. I. The effect of increased sodium thioglycollate and complement. American J. Syph. **37** (1953) 101-105.
15. SOULE, M. H. The Wassermann reaction and the Kahn test in leprosy. Internat. J. Leprosy **3** (1935) 181-194.
16. WOLFSON, W. Q., COHN, C., CALVARY, E. and ICHIBA, F. Studies in serum proteins. V. A rapid procedure for the estimation of total protein, true albumin, total globulin, alpha globulin, beta globulin and gamma globulin in 1.0 ml. of serum. American J. Clin. Path. **18** (1948) 723-730.