THE RESULTS OF A MODIFIED MIDDLEBROOK-DUBOS HEMAGGLUTINATION TEST IN LEPROSY; 261 CASES

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There is continuing interest in the diagnostic potentialities of the hemagglutination test for tuberculosis described by Middlebrook and Dubos in 1948 (6) and modified by Scott and Smith in 1950 (12). This test was initially described as a useful tool to aid the clinician in the diagnosis and management of tuberculosis (9, 10, 12). Middlebrook's emphasis has been on its usefulness in studying various immunologic processes in relation to tuberculosis.

In December of 1950 this hospital supplied sera from 35 lepromatous patients to Dr. Leslie C. Murphy of the Brooke Army Medical Center (8), who was employing the Middlebrook test in diseases other than tuberculosis in an effort to evaluate its specificity. He found titers varying between 1:2 to 1:512 in the Carville sera. Results of this test in leprosy patients have also been reported by Levine *et al.* in 1951 and 1952 (4, 5), and by Ahuir *et al.* of Spain (1). In Japan (3) Tajiri, Kawaguchi, Fukuda, and Arai have also reported on the test in leprosy.

The present study was made primarily in order that the results of the hemagglutination test might be recorded in a larger group of leprosy patients than has been tested heretofore. A second objective was to observe any changes in titer which might occur in a small group of patients studied repeatedly during the course of a year, in different stages of the disease process and during various treatment regimens.

MATERIALS AND METHODS

The hemagglutination test was applied to sera from 261 leprosy patients in whom tuberculosis had been excluded on the basis of chest roentgenograms, sputum examinations (concentration method), and culture of the concentrated sputa. With 240 patients a single test was made; three or more tests were made during a period of one year on 21 other patients in different treatment groups. There was a control group of 15 persons without leprosy.

On the basis of the clinical, immunologic and histopathologic findings the cases in this series were classified as follows: (*a*) lepromatous, 221 cases, of which 169 were clinically active and bacilli were found, and 52 were clinically quiescent and bacilli not found; and (*b*) tuberculoid, 40 cases, of which 12 were reactional and bacilli found, and 28 were clinically quiescent and bacilli not found.

The procedure employed was the Scott-Smith (10) modification of the Middlebrook-Dubos test. The antigen was Lederle 4X tuberculin.[1] Freshly-drawn sheep's blood furnished the erythrocytes used. All tests were completed within 48 hours from the time the blood was drawn.

[1] Concentrated tuberculin used in all the tests was supplied by Lederle Laboratories, Pearl River, New York.

RESULTS

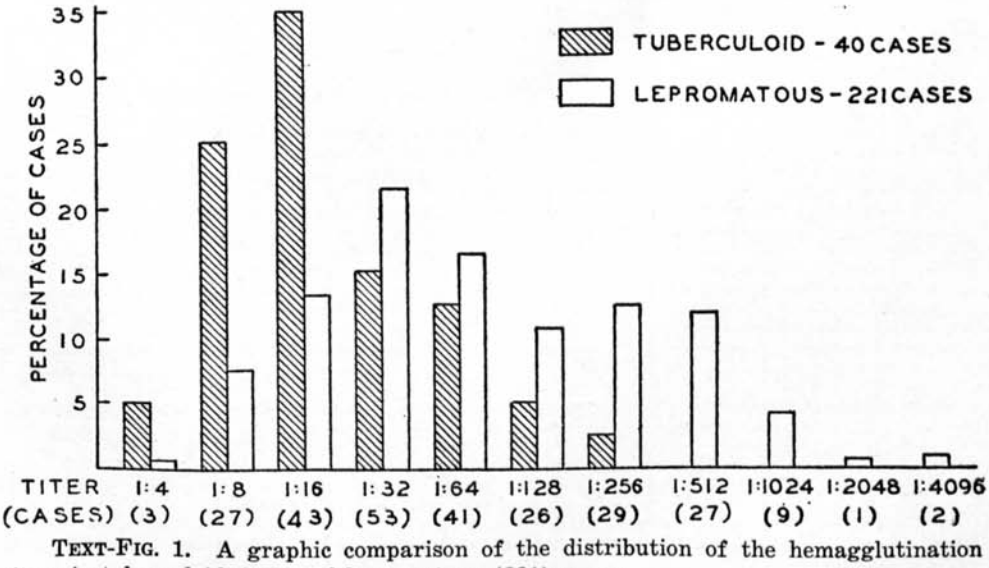
Controls.—The sera of the control group did not show hemagglutination titers higher than 1:8.

Patients.—Table 1 shows the results obtained in the single tests of 261 cases of leprosy. It will be noted that a total of 231 cases (88.5%) had titers higher than 1:8, the highest obtained in the control group. Further analysis shows that of the 40 tuberculoid cases, 28 (70%) had titers above 1:8, while of the 221 lepromatous cases 203 (91.8%) had titers above those found in the control series.

TABLE 1.—*Hemagglutination titers in tuberculoid and lepromatous leprosy; 261 cases.*

Type of leprosy	No. of cases	Number of cases with titers of:										
		1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4296
Tuberculoid	40	2	10	14	6	5	2	1	0	0	0	0
Lepromatous	221	1	17	29	47	36	24	28	27	9	1	2
TOTAL	261	3	27	43	53	41	26	29	27	9	1	2

A comparison of the titer distributions in the tuberculoid and lepromatous groups is shown graphically in Text-fig. 1. The case groups are too small for the percentages to have much statistical validity; the purpose of the graph is simply to emphasize the marked differences between the two type-groups.



TEXT-FIG. 1. A graphic comparison of the distribution of the hemagglutination titers in tuberculoid (40) and lepromatous (221) cases.

In Table 2 the results are further subdivided according to the apparent activity of the leprous process in the two types. The clinically active lep-

TABLE 2.—*Hemagglutination titers in bacteriologically positive and negative cases of leprosy; 261 cases.*

Type of leprosy	Bacilli	No. of cases	Number of cases with titers of: 1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4296
Lepromatous (Quiescent)	Neg.	52	1	12	11	13	3	2	2	6	1	1	0
Lepromatous (Active)	Pos.	169	0	5	18	34	33	22	26	21	8	0	2
Tuberculoid (Quiescent)	Neg.	28	2	5	10	6	5	0	0	0	0	0	0
Tuberculoid (Reactional)	Pos.	12	0	5	4	0	0	2	1	0	0	0	0
TOTAL		261	3	27	43	53	41	26	29	27	9	1	2

TABLE 3.—*Changes in the hemagglutination titer on repeated tests performed over a period of one year: Group 1, cases treated with sulfone or thiosemicarbazone; Group 2, cases treated with isoniazid.*

No.	Date of test	Titer	No.	Date of test	Titer
Group 1A, active lepromatous cases (11)					
2052	8/1951	1:512	2010	6/1951	1:16
	11/1951	1:512		8/1951	1:64
	1/1952	1:256		11/1951	1:128
	3/1952	1:256		1/1952	1:128
				3/1952	1:512
1019	9/1951	1:512			
	11/1951	1:64	401	6/1951	1:32
	1/1952	1:16		11/1951	1:128
	3/1952	1:16		4/1952	1:256
	5/1952	1:16			
			1765	6/1951	1:32
1509	6/1951	1:512		8/1951	1:32
	8/1951	1:512		10/1951	1:32
	11/1951	1:256		12/1951	1:32
	1/1952	1:256		2/1952	1:32
	3/1952	1:128		4/1952	1:32
823	6/1951	1:256	785	3/1952	1:256
	8/1951	1:128		2/1952	1:256
	11/1951	1:64		8/1952	1:256
	1/1952	1:64			
	3/1952	1:32	2104	6/1951	1:256
				8/1951	1:256
1441	6/1951	1:64		10/1951	1:256
	9/1951	1:64			
	11/1951	1:32	1914	7/1951	1:16
	1/1952	1:16		9/1951	1:16
	3/1952	1:16		11/1951	1:16
				1/1952	1:16
				3/1952	1:16
				5/1952	1:16

TABLE 3—Continued.

No.	Date of test	Titer	No.	Date of test	Titer
Group 1B, quiescent lepromatous cases (3)					
1958	6/1951	1:256	1864	10/1951	1:64
	8/1951	1:64		12/1951	1:64
	11/1951	1:16		1/1952	1:64
				3/1952	1:64
1729	6/1951	1:8		5/1952	1:64
	8/1951	1:8			
	10/1951	1:8			
	12/1951	1:8			
	2/1952	1:8			
	4/1952	1:8			
Group 2, active lepromatous cases (6)[a]					
1964	6/1951	1:128	2175	3/1952	1:16
	3/1952	1:128		10/1952	1:128
	7/1952	1:512		1/1953	1:512
	1/1953	1:1024			
			2062	3/1952	1:8
2078	3/1952	1:32		10/1952	1:32
	10/1952	1:128		3/1953	1:128
	1/1953	1:512			
			2173	3/1952	1:8
1361	3/1952	1:64		10/1952	1:8
	10/1952	1:512		3/1953	1:8
	1/1953	1:1024			
2168	3/1952	1:64			
	10/1952	1:256			
	1/1953	1:2048			

[a] Also one quiescent tuberculoid case (the last in the section).

romatous cases with bacilli showed the highest percentage (91.1) of titers above the normal range; the clinically quiescent lepromatous cases showed high titers in 75 per cent of the cases. Of the clinically quiescent tuberculoid group without bacilli, 75 per cent showed titers above 1:8, but in no case did the titer exceed 1:64. The small group of bacteriologically positive reactional tuberculoid cases showed the lowest proportion (58.3%) of titers exceeding 1:8, but some of them were relatively high (1:128 or 1:256).

The changes of titer observed in the 21 cases tested repeatedly during a period of 12 months are shown in Table 3. The cases are tabulated as of two groups, the first of which—14 cases—received sulfone drugs (12) or thiosemicarbazone (2), while the second—7 cases—received isoniazid (Marsilid). The number of tests per case varied from 3 to 6, with an average of about 4.5.

In Group 1, the titers decreased in 6 cases, remained essentially unchanged in 6, and increased in 2. Clinical and bacteriologic improvement

was noted in the cases in which the titer decreased. There was no apparent clinical change in those which showed stationary titers. The two patients whose titers increased died later of amyloidosis. Of the 7 cases in which isoniazid (Marsilid) was being employed, 6 were active lepromatous and 1 was quiescent tuberculoid. All of the former showed rising titers; in 4 of them there was progression of the disease, but in the other 2 the leprous process continued quite unchanged.

DISCUSSION

Middlebrook (7) has described the high degree of specificity of the hemagglutination reaction. He has stated, "The release of the antigens which stimulates the production of the antibodies responsible for the hemagglutination reaction ceases soon after the bacilli stop multiplying." He has felt that a highly positive reaction can occur only in active tuberculosis with increasing numbers of bacilli, and has said that the lesions not associated with actively reproducing *M. tuberculosis* do not offer a stimulus for the formation of hemagglutination antibodies. Dubos (2) has also mentioned that the height of hemagglutination titer indicates the number of multiplying organisms.

This study of 261 cases of leprosy without evidence of tuberculosis has shown that hemagglutination titers are above normal in high percentages of cases. Clinically active lepromatous leprosy with large numbers of bacilli showed a significantly higher proportion of high titers than did the quiescent lepromatous or tuberculoid cases.

The results of the test in a small series of cases studied repeatedly over a period of a year indicate that there is some correlation between the clinical course of leprosy and changes of titer. If further observations substantiate these findings, this test may prove to be an aid in the evaluation of new therapeutic agents or treatment schedules. Although it is recognized that the Middlebrook-Dubos test is not specific, it does appear that infection with *M. leprae* stimulates the appearance of antibodies or something which causes agglutination of red cells sensitized with tuberculin.

SUMMARY

The Middlebrook-Dubos hemagglutination test (Scott-Smith modification) was performed on sera obtained from 261 leprosy patients in whom tuberculosis had been excluded. A single test was made in 240 patients; three or more tests were made during a period of one year in 21 other patients who represented different treatment groups—sulfones, thiosemicarbazone, or isoniazid.

Of the 261 cases, 231 (88.5%) had hemagglutination titers higher than the highest (i.e., 1:8) found in the control group. Of a group of 169 clinically active lepromatous cases with bacilli, 91.1 per cent had titers above the normal range. Of a group of 52 lepromatous cases which

were clinically quiescent and in which bacilli were not found, 75 per cent had titers above normal. Of the 40 tuberculoid cases, 28 (70%) revealed titers above 1:8, the highest reached was 1:256, as compared with 1:4296 in the lepromatous group. The graph shows the marked difference between the lepromatous and tuberculoid cases as regards the distribution of the titers.

Serial hemagglutination tests made over a period of one year on 21 cases under therapeusis showed decrease of titer in 6 cases, no change in 7, and increase in 8. The disease progressed in the 8 cases in which an increase in titer was noted. Clinical and bacteriological improvement was noted in six cases in whom the titer decreased.

RESÚMEN

La prueba de aglutinación de hematíes de Middle Brook-Dubos segun modificación de Scott-Smith, se ejecutó en el suero de 261 pacientes leprosos no tuberculosos. La prueba se hizo una sola vez en 240 pacientes, 3 o mas veces durante un período de un año en 21 pacientes quienes representaban differentes grupos bajo tratamiento con sulfonas, thiosemicarbazone y isoniazid.

De los 261 casos, 88.5% (231) tuvieron niveles de aglutinación mas altos (1:8) que los del grupo testigo. De un grupo de 169 casos lepromatosos activos el 91.1% demostraron niveles sobre el normal. De un grupo de 52 casos lepromatosos inactivos, el 75% demostró niveles sobre el valor normal. De 40 casos tuberculoides, el 70% (28) demostraron niveles sabre 1:8, el mas alto fué 1:256 en comparación con 1:4296 en el grupo lepromatoso. La curva (véase el texto) demuestra la gran diferencia entre los casos lepromatosos y los tuberculoides en relación a la distribución a los niveles de la hemaglutinación.

Se practicó hemaglutinación seriada por un período de un año en 21 casos bajo tratamiento, de los cuales 6 tuvieron niveles mas bajos, 7 no demostraron cambio alguno y hubo aumento en 8. La enfermedad progresó en los 8 casos que demostraron aumento en hemaglutinación. Hubo mejoría clínica y bacteriológica en los 6 casos en los cuales la hemaglutinación disminuyó.

REFERENCES

1. Ahuir, M. R., Torrella, E. and Garcia, M. A. La reacción de hemagglutinación de Middlebrook-Dubos en la lepra. Rev. Leprol. Fontilles 3 (1952) 105-114.
2. Dubos, R. J. The hemagglutination test in tuberculosis. Minutes of the Ninth Streptomycin Conference (Veterans' Administration), April, 1950, p. 222.
3. Japanese Leprosy Association Meeting, April 6-7, 1952 (Tajiri, Kawaguchi, Fukuda, Arai). Internat. J. Leprosy **20** (1952) 541.
4. Levine, M. Hemagglutination of tuberculin sensitized sheep cells in Hansen's disease (leprosy). Proc. Soc. Exper. Biol. & Med. **76** (1951) 171-173.
5. Levine, M., Chung-Hoon, E. K., Ichirui, E., Arakaki, J. and Beatty, M. Serological response in various types and stages of Hansen's disease (leprosy) to tuberculin-sensitized sheep red blood cells. Internat. J. Leprosy **20** (1952) 201-212.
6. Middlebrook, G. and Dubos, R. J. Specific serum agglutination of erythrocytes sensitized with extracts of tubercle bacilli. J. Exper. Med. **88** (1948) 521-528.
7. Middlebrook, G. The hemagglutination test in tuberculosis. American Rev. Tuberc. **62** (1951) 121 (editorial).
8. Murphy, L. G. Personal communication.

9. ROTHBARD, S., DOONEIFF, A. S. and HITE, K. E. Practical application of a hemagglutination reaction in tuberculosis. Proc. Soc. Exper. Biol. & Med. **74** (1950) 72-75.
10. ROTHBARD, S. A serological test in tuberculosis. Experimental and clinical observation. Med. Clin. North America **35** (1951) 603-614.
11. SCOTT, N. B. and SMITH, D. T. A simple modification of the Middlebrook and Dubos hemagglutination test for serum antibodies to products of tubercle bacilli. J. Lab. & Clin. Med. **35** (1950) 303-307.
12. SMITH, D. T. and SCOTT, N. B. Clinical interpretation of Middlebrook-Dubos hemagglutination test. American Rev. Tuberc. **62** (1950) 121-127.