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### HEMAGGLUTINATION OF TUBERCULIN SENSITIZED SHEEP CELLS IN HANSEN'S DISEASE (LEPROSY). (18427)<sup>1</sup>

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The hemagglutination test of Middlebrook and Dubos (1), as modified by Scott and Smith (2), provides a relatively readily available laboratory tool for studying the serological response of patients infected with tubercle bacilli. The following brief report indicates that this technic may be especially applicable for a serological response in Hansen's disease.

*Materials and methods.*—The technic employed for determination of hemagglutination titers was that described by Scott and Smith except that 0.25% sensitized sheep red cell suspensions were employed; the reacting volume was 1.0 ml compared with 0.8 ml in their report; and the initial test titer was 1:4. Some observations were also made on the effect of agitating the reacting mixture in a Kahn shaking machine for 15 minutes, then centrifuging and immediately reading, on the agglutination titer as compared with the technic of Middlebrook and Dubos and Scott and Smith which consists of incubation for 2 hours at 37°C followed by standing overnight before reading. There was an excellent correlation between the two methods of determining the hemagglutination titers, and in our hands the centrifuge method was easier to read, but the titers reported herein are those obtained by the previously described technic.

Specimens from 96 tuberculous patients were obtained from the Leahi Tuberculosis Hospital in Honolulu and sera from 105 Hansen's disease patients were obtained at the Hansen's Disease Hospital (Hale Mohalu) in Pearl City, Oahu, Hawaii. The latter included 80 individuals in the active phases of the disease, all of which are bacteriologically positive, and 25 individuals who had

<sup>1</sup> Reprinted from Proc. Soc. Exper. Biol. and Med. 76 (1951), 171-173.

TABLE 1. Agglutination of Tuberculin Sensitized Sheep Red Cells by Sera from Tuberculosis and Hansen's Disease Patients and Blood Donors.

Source of specimens	Leahi* T.B. hospital (96 patients)	Blood bank donors (40 individuals)	Hansen's disease patients	
			80 active cases (bact. pos.)	25 released cases (bact. neg.)
Agglutination titer	% of specimens			
Neg. 1:4	11.5	75.0	1.3	24
1:4	11.5	15.0	8.7	32
1:8	11.5	7.5	13.7	16
1:16	28.1	—	17.5	16
1:32	14.6	2.5	11.3	4
1:64	17.7	—	17.5	—
1:128	5.2	—	11.3	8
1:256	—	—	8.7	—
1:512	—	—	6.2	—
1:1024	—	—	2.5	—
1:2048	—	—	1.3	—

\* Clinical status of activity of disease in these patients undetermined at this time.

been released as arrested cases, and all of which were bacteriologically negative. (Only 3 of the Hansen's disease patients were also T.B. cases—2 were bacteriologically positive for Hansen's and negative for T.B. organisms and one negative for both.) A control group of 40 specimens was obtained from blood donors at the Blood Bank of Hawaii.

*Results.*—As may be seen from the accompanying table and graph, the low titers of the control group and the frequently very high titers in Hansen's disease patients, who are bacteriologically positive, stand out in marked contrast. The lower intermediate titers of bacteriologically negative Hansen's disease patients, in whom the disease has been arrested, are also very striking. Thus, titers of 1:4 or less were obtained in 90% of blood donors as compared with only 10% in Hansen's disease patients who were bacteriologically positive, but in 56% of released, bacteriologically negative, patients.

Only one (2.5%) of the 40 control specimens gave a titer of over 1:8, and only 2 (8.0%) of the arrested, bacteriologically negative, Hansen's disease patients had titers over 1:32. In contrast to this, 5 (5.2%) of the tuberculosis sanatorium and 9 (11.3%) of the active, bacteriologically positive, Hansen's disease patients showed agglutination titers of 128 and in addition 15 (18.7%) of the Hansen's disease cases had titers of 256 to 2048.

To cogitate on the possibility that the acid-fast bacteria associated with tuberculosis and Hansen's disease might have common antigenic components is, of course, not surprising; but to verify such an hypothesis, in the absence of pure cultures of the latter, was not feasible by the generally employed procedures. The hemagglutination technic of Middlebrook and Dubos provides an indirect approach toward an answer to this hypothesis. The marked serological response to antigens present in tuberculin\* was as unanticipated as it is striking. This tool (agglutination of tuberculin sensitized red cells) opens up new vistas for study of that disease of antiquity whose etiological agent still defies cultivation.

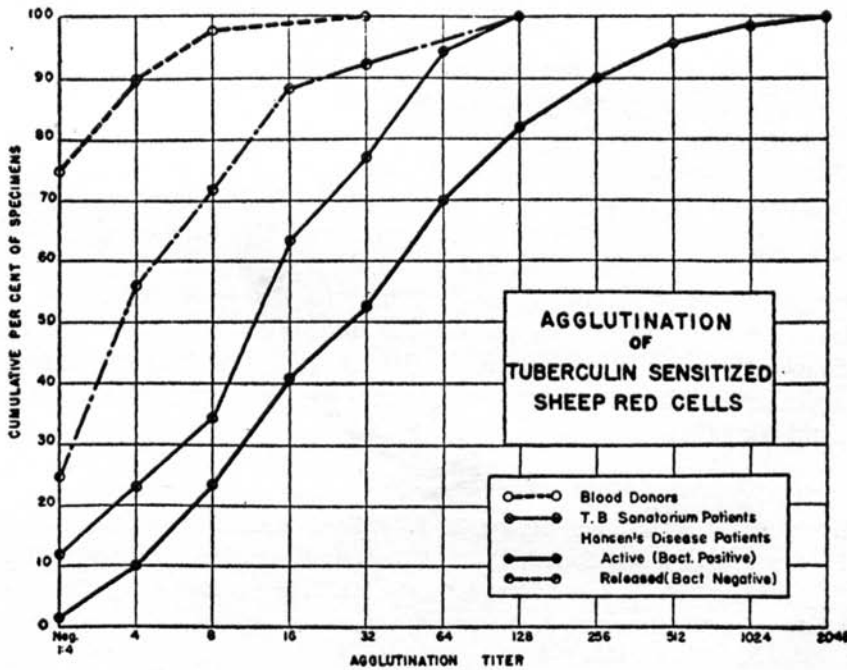


FIG. 1.

It seems evident that *Mycobacterium tuberculosis* and *Mycobacterium leprae* possess common antigens which stimulate production of common agglutinins in human sera. This does not necessarily indicate a corresponding immunological response. However, the possibility that such a relationship may exist (it

\* Appreciation is expressed to Dr. H. D. Piersma of the Lederle Laboratories for supplying us with the 4X tuberculin employed in these studies.

does, for example, with respect to various strains of *Salmonella*, as indicated by Longfellow and Luipold (3) and Levine et al. (4) raises an intriguing conjecture as to the efficacy of B.C.G. vaccination of children of Hansen's disease patients as a protective measure.

On the basis of observations that B.C.G. vaccination induces a positive lepromin test, Chaussinand (5) suggests that it would be of interest to determine whether a preventive action against leprosy is also produced. The data presented, herein, on the serological response of Hansen's disease patients to antigens in tuberculin, serve to augment the view that observations on the heterologous immunological effects of B.C.G. vaccination, with particular reference to Hansen's disease, are worthy of careful consideration.

*Summary.*—The hemagglutination titers of active, bacteriologically positive, cases of Hansen's disease were significantly higher than those of arrested, bacteriologically negative, cases of this disease, and frequently much higher than those encountered in the active cases of tuberculosis examined. The immunological response against antigenic components of the tubercle bacillus elicited by infection with Hansen's disease appears to be very marked and distinct. The inability to grow *Mycobacterium leprae* has naturally hampered serological studies of Hansen's disease. The hemagglutination technic of Middlebrook and Dubos provides a laboratory tool which may be very useful in future studies of this entity.

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