# SEROLOGICAL RESPONSE IN VARIOUS TYPES AND STAGES OF HANSEN'S DISEASE (LEPROSY) TO TUBERCULIN-SENSITIZED SHEEP RED BLOOD CELLS

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To cogitate on the possibility that the acid-fast bacteria responsible for tuberculosis and those associated with Hansen's disease might have common antigenic components is, of course, but natural; but to verify such an hypothesis in the absence of pure cultures of the leprosy organism is not readily feasible. The hemagglutination technique of Middlebrook and Dubos, it was thought, might serve as a convenient laboratory tool for ascertaining whether or not sera from Hansen's disease patients, who are not suffering from tuberculosis, contain appreciable quantities of antibodies against tuberculin antigens.

## MATERIALS AND METHODS

The technique employed for the determination of hemagglutination titers was that described by Middlebrook and Dubos (5) as modified by Scott and Smith (6), excepted that to conserve antigen 0.25 per cent sensitized sheep red cell suspensions were employed; the reacting volume was 1.0 ml. in place of 0.8 ml. as in their technique, and the initial test dilution was 1:4.

Sera were obtained from 321 Hansen's disease patients at the Hansen's Disease Hospital (Hale Mohalu) at Pearl City and the Hansen's Disease Settlement at Kalaupapa, and from 109 tuberculosis patients at the Leahi Tuberculosis Hospital in Honolulu. A control group consisted of sera obtained from 75 individuals who were accepted as blood donors by the Blood Bank of Hawaii.

## RESULTS

*Hemagglutination titers of blood donors, tuberculous and Hansen's disease patients.*—In Table 1 and Text-fig. 1 are shown the hemagglutination titers obtained with the several lots of sera used. The low titers of the control group and the frequently very high titers among the Hansen's disease patients stand in marked contrast. Thus, whereas only 4 per cent of the blood-donor group had titers higher than 1:8, and only

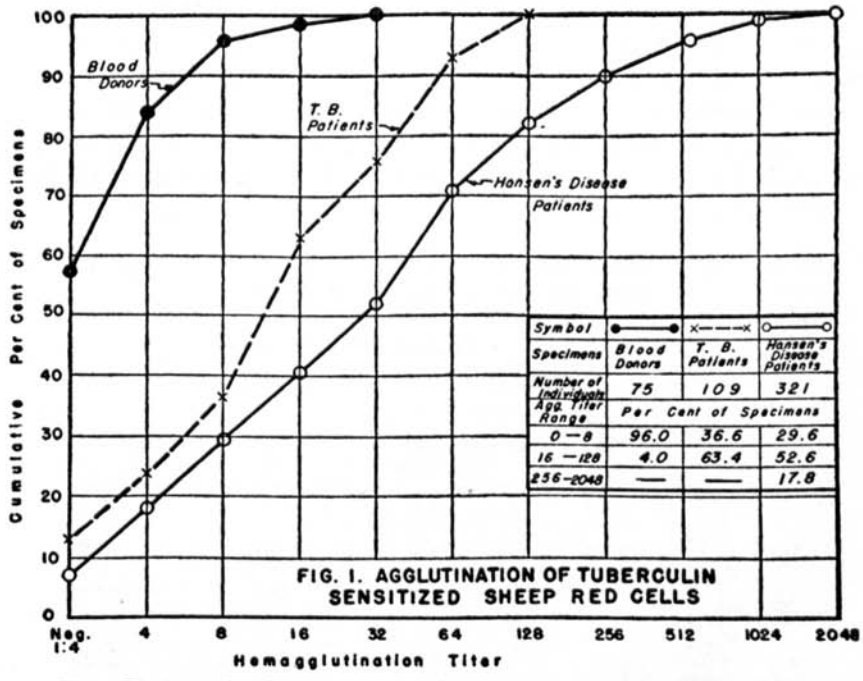
6.4 per cent of the tuberculous patients gave titers of 1:128, no less than 29.3 per cent of the Hansen's disease patients had titers of the latter degree or higher, 17.8 per cent of them being in the range from 1:256 to 1:2048. In this connection it should be pointed out that only 11 of the 321 Hansen's disease patients were tuberculous at the time the serum specimens were collected, and only 23 others had at sometime been, or were suspected of being, tuberculous. The hemagglutination titers of the sera of this group of patients cannot, on the basis of available clinical records, be attributed to simultaneous tuberculosis infection. They must, therefore, be considered incident to a serological response induced by infection with the leprosy bacillus.

TABLE 1.—*Agglutination of tuberculin-sensitized sheep red cells by sera from blood donors and from tuberculosis and Hansen's disease patients.*

| Agglutination titer | Per cent with given titer | | |
|---|---|---|---|
| | Blood donors (75) | Tuberculosis patients (109) | Leprosy patients (321) |
| Negative/*a* | 66.7 | 12.8 | 7.5 |
| 1:4 | 17.3 | 11.0 | 11.2 |
| 1:8 | 12.0 | 12.8 | 10.9 |
| 1:16 | 2.7 | 26.7 | 11.2 |
| 1:32 | 1.3 | 12.8 | 11.5 |
| 1:64 | ---- | 17.5 | 18.4 |
| 1:128 | ---- | 6.4 | 11.5 |
| 1:256 | ---- | ---- | 7.8 |
| 1:512 | ---- | ---- | 5.6 |
| 1:1024 | ---- | ---- | 3.5 |
| 1:2048 | ---- | ---- | 0.9 |

*a* In 1:4 dilution.

*Hemagglutination titers in various types of Hansen's disease.*—Leprologists recognize two distinct "types" of the disease, lepromatous and tuberculoid. Cases which cannot be classified as falling in either of these types are tentatively allocated to




TEXT-FIG. 1. Agglutination of tuberculin-sensitized sheep red cells by sera from blood donors (controls), tuberculosis patients, and Hansen's disease patients.

an "indeterminate group"; some of these may later be reclassified as one of the specific types, whereas others remain indeterminate throughout their course. Some of the differential characteristics of the two types are indicated below (adopted from the manuscript of a monograph by Dr. H. L. Arnold, Jr.).

| *Feature* | *Lepromatous* | *Tuberculoid* |
|---|---|---|
| Acid-fast organisms in lesions | Always demonstrable | Difficult to find when case is not in reaction |
| Evidence of nerve damage in skin lesions of early cases | Rare | Always demonstrable |
| Resistance to progression of disease | Generally slight | Generally marked |
| Course if not treated | Progressively downhill (occasionally spontaneous regression and healing) | Generally self-limiting |
| Mitsuda (lepromin) reaction | Negative | Positive |
| False Kahn and Kolmer reactions | Frequent | Rare |

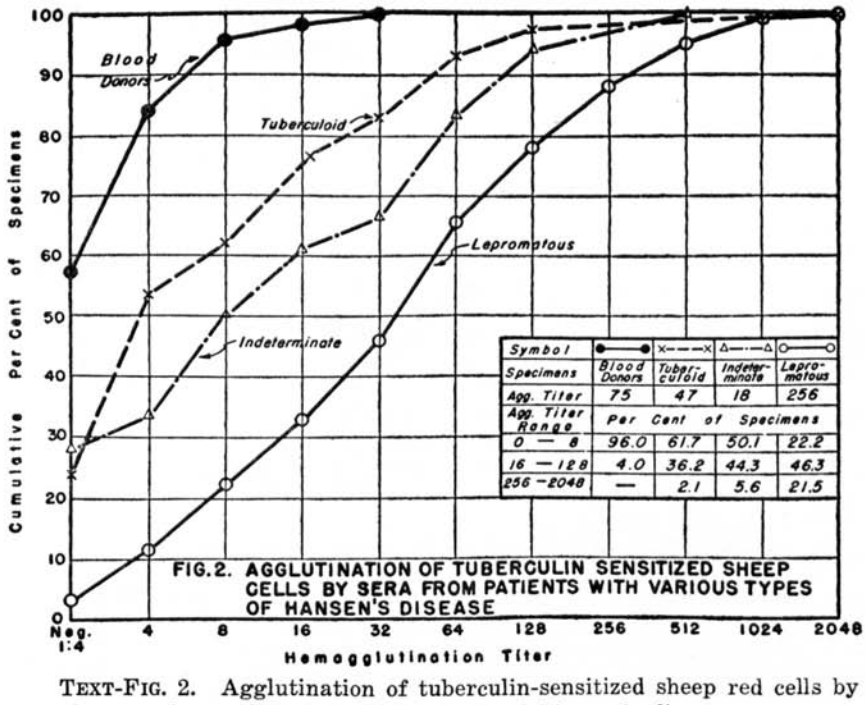
In Table 2 and Text-fig. 2 are shown the hemagglutination titers for 256 lepromatous, 47 tuberculoid and 18 "indeterminate" Hansen's disease patients, and the control group of 75 blood donors. It is apparent that the titers are prone to be high in the lepromatous cases; 21.5 per cent were in the range of 1:256 to 1:2048, as compared with only 2.1 per cent of the tuberculoid patients.

TABLE 2.—*Agglutination titers of sera from patients with various types of Hansen's disease and blood donors.*

| Agglutination titer | Per cent with given titer | | | |
|---|---|---|---|---|
| | Blood donors (75) | Type of leprosy | | |
| | | Tuberculoid (47 cases) | Indeterminate (18 cases) | Lepromatous (256 cases) |
| Negative/*a* | 66.7 | 23.4 | 27.8 | 3.1 |
| 1:4 | 17.3 | 29.8 | 5.6 | 8.2 |
| 1:8 | 12.0 | 8.5 | 16.7 | 10.9 |
| 1:16 | 2.7 | 14.9 | 11.0 | 10.6 |
| 1:32 | 1.3 | 6.4 | 5.6 | 12.9 |
| 1:64 | ---- | 10.6 | 16.7 | 19.9 |
| 1:128 | ---- | 4.3 | 11.0 | 12.9 |
| 1:256 | ---- | ---- | ---- | 9.8 |
| 1:512 | ---- | ---- | 5.6 | 6.6 |
| 1:1024 | ---- | ---- | ---- | 4.3 |
| 1:2048 | ---- | 2.1 | ---- | 0.8 |

*a* In 1:4 dilution.

The low-titer group (1:8 or less) comprised only 22.2 per cent of the lepromatous cases as compared with 51.7 per cent of the tuberculoid ones and 96 per cent of the blood-donor group. At the time the specimens were taken, therefore, the tuberculoid group of patients did not show a very marked response to the tuberculinized antigen, the titers frequently being within the range of normal individuals. It should be said, however, that a large proportion of the tuberculoid cases were arrested ones, so that subsequent studies on the relation of the stage of the disease to the hemagglutination titers must be




TEXT-FIG. 2. Agglutination of tuberculin-sensitized sheep red cells by sera from patients with the various types of Hansen's disease.

awaited for final evaluation of this serological test in the tuberculoid type of leprosy.

*Relation of hemagglutination titers to bacteriological findings.*—It is the experience of leprologists that acid-fast bacilli are practically always demonstrated easily in lepromatous cases during the early and subsequent active stages of the disease, but that in tuberculoid cases it is extremely difficult to find these bacteria and they may not be encountered throughout the course of the disease. It was, therefore, thought that a comparison of the hemagglutination titers for bacteriologically positive and negative cases might be of interest. In Table 3 is shown the distribution of the Hansen's disease cases with respect to the bacteriological findings in the several types of the disease at the time the serum specimens were obtained.

All of the 220 positive bacteriological results shown in the table were obtained among active cases, whereas 99 of the 101 bacteriologically negative cases were arrested ones, the patients being on temporary or permanent release. All of the 44 lepromatous cases which are now negative had previously been positive. In contrast to this, the presence of bacilli had never been

TABLE 3.—*Distribution of Hansen's disease cases with respect to type and bacteriological findings.*

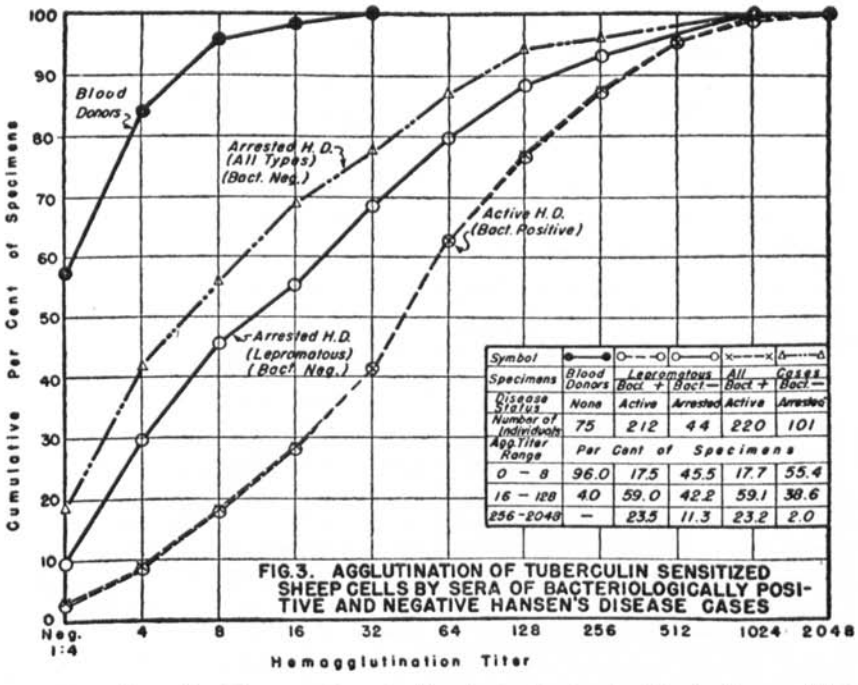
| Type of the disease | Acid-fast bacilli | | | |
|---|---|---|---|---|
| | Positive | | Negative | |
| | No. | Per cent | No. | Per cent |
| Lepromatous (256 cases) | 212 | 82.9 | 44/*a* | 17.1 |
| Tuberculoid (47 cases) | 4 | 8.5 | 43/*b* | 91.5 |
| Indeterminate (18 cases) | 4 | 22.1 | 14/*c* | 77.9 |
| TOTAL (321 cases) | 220/*d* | 68.6 | 101/*e* | 31.4 |

*a* All were previously bacteriologically positive.
*b* Acid-fast microorganisms never found in 21 cases.
*c* Acid-fast microorganisms never found in 8 cases.
*d* All active, neutral or regressing cases.
*e* Includes 99 arrested and 2 active (tuberculoid) cases.

TABLE 4.—*Agglutination titers of bacteriologically positive and negative cases of Hansen's disease, and of blood donors.*

| Agglutination titer | Per cent with given titer | | | | |
|---|---|---|---|---|---|
| | Blood donors (75) | All leprosy cases/*a* | | Lepromatous cases/*a* | |
| | | Positive (220) | Negative (101) | Positive (212) | Negative (44) |
| Negative/*b* | 66.7 | 2.2 | 18.8 | 1.9 | 9.1 |
| 1:4 | 17.3 | 5.9 | 22.8 | 5.7 | 20.5 |
| 1:8 | 12.0 | 9.6 | 13.8 | 9.9 | 15.9 |
| 1:16 | 2.7 | 10.5 | 12.9 | 10.8 | 9.1 |
| 1:32 | 1.3 | 12.7 | 8.9 | 12.8 | 13.6 |
| 1:64 | ----- | 22.3 | 9.9 | 21.7 | 11.4 |
| 1:128 | ----- | 13.6 | 6.9 | 13.7 | 9.1 |
| 1:256 | ----- | 10.5 | 2.0 | 10.8 | 4.5 |
| 1:512 | ----- | 8.2 | ----- | 8.0 | ----- |
| 1:1024 | ----- | 3.6 | 3.0 | 3.8 | 6.8 |
| 1:2048 | ----- | 0.9 | 1.0 | 0.9 | ----- |

*a* All bacteriologically positive cases "active," including neutral and regressed; all bacteriologically negative cases arrested.
*b* In 1:4 dilution.




| Symbol | ●—● | ○- - -○ | ○——○ | ×- - -× | Δ- - -Δ |
|---|---|---|---|---|---|
| Specimens | Blood Donors | Lepromatous Bact. + | Lepromatous Bact. − | All Cases Bact. + | All Cases Bact. − |
| Disease Status | None | Active | Arrested | Active | Arrested |
| Number of Individuals | 75 | 212 | 44 | 220 | 101 |
| Agg. Titer Range | Per Cent of Specimens | | | | |
| 0 − 8 | 96.0 | 17.5 | 45.5 | 17.7 | 55.4 |
| 16 − 128 | 4.0 | 59.0 | 42.2 | 59.1 | 38.6 |
| 256-2048 | − | 23.5 | 11.3 | 23.2 | 2.0 |

FIG. 3. AGGLUTINATION OF TUBERCULIN SENSITIZED SHEEP CELLS BY SERA OF BACTERIOLOGICALLY POSITIVE AND NEGATIVE HANSEN'S DISEASE CASES

TEXT-FIG. 3. The results of the tests in bacteriologically positive ("active") cases and bacteriologically negative ("arrested") ones, all types and lepromatous.

demonstrated in the lesions of 21 of the 43 tuberculoid cases and 8 of the 14 indeterminate ones which are now negative. At the time of the study there were only 4 tuberculoid and 4 indeterminate cases which were positive—a number too small to warrant comparison of the relation of bacteriological findings to titers for these types of the disease. Consideration will therefore be restricted to (*a*) the entire group and (*b*) the lepromatous type of cases, the hemagglutination titers of which are shown in Table 4 and Text-fig. 3.

Considering the whole group of cases, the contrast between the titers among the bacteriologically positive and negative subgroups is quite striking. Thus, only 17.7 per cent of the positive individuals showed titers of 1:8 or less, as compared with 55.4 per cent of those which were negative; furthermore, 23.2 per cent of the positive group, compared with only 6 per cent of the negative one, had titers of 1:256 or greater.

Considering the lepromatous cases, a similar relationship is found between the bacteriological findings and hemagglutination titers. Thus, only 17.5 per cent of the 212 bacteriologically

positive, active cases showed low titers (1:8 or less) as compared with 45.5 per cent of the 44 negative, arrested cases. Titers of 1:256 or more were encountered among 23.5 per cent of the positive, active cases as compared with 11.3 per cent among negative, arrested cases. Indication of a shift to lower titers as the cases become arrested and bacteriologically negative is indicated, as may be seen from Text-fig. 3.

*Relation of hemagglutination titers to the stage of activity of the disease.*—It is customary, at the Hansen's disease hospitals in Hawaii, to recognize various stages in the course of the disease, as indicated in the following tabulation:

| *Stage* | *Condition* |
|---|---|
| Active | Skin and nerve lesions present; fever and toxemia in severe cases (may be absent in mild cases). |
| Neutral | Stationary (occasionally slow progression); generally definite evidence of regression. |
| Regressed | Disease markedly regressed; approaching quiescent stage. |
| Arrested | Disease arrested, quiescent; patient eligible for release; bacteriologically negative. |

The hemagglutination titers of the sera from the patients in the various stages of the disease thus defined—active, neutral, regressed and arrested—are shown in Table 5 and Text-fig. 4. In general, there seems to be a shift towards higher titers as the active stage subsides, followed by a decrease in the titers as the cases regress and finally reach the stage of arrest. At that time the titers are frequently within the range of those observed for normal individuals.

Considering the titer range 1:256 to 1:2048, it will be noted that 20.5 per cent of the active cases and 28.7 per cent of cases in the early quiescent or neutral stage, but only 8.5 per cent of those in which the disease had definitely regressed and 6 per cent of the arrested ones, fell into this high titer group.

The proportions of individuals showing low titers (1:8 or less) were 12.9 per cent of the active and 15.4 per cent of the neutral cases, rising to 29.6 per cent of those in the regressed stage and 55.6 per cent of those in which the disease has become arrested to a point where the patients had been released or were considered eligible for release.

It should be emphasized, however, that these findings are on a group of cases as they exist today, and that inferences regarding changes in the agglutination titers must necessarily

be subject to verification by observations on a series of cases throughout their course.

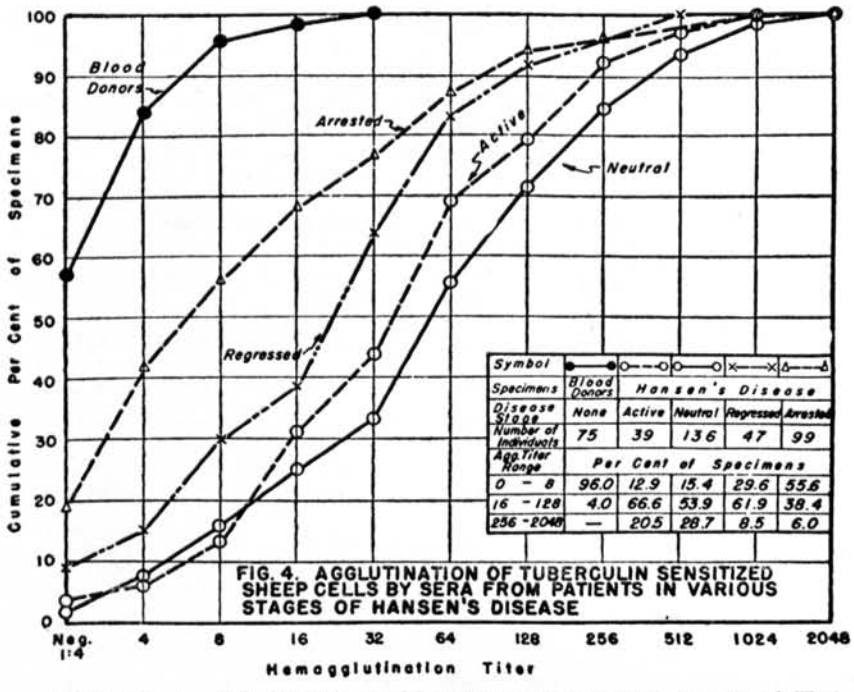
TABLE 5.—*Agglutination titers of sera from patients in various stages of Hansen's disease, and from blood donors.*

| Agglutination titer | Per cent with given titer | | | | |
|---|---|---|---|---|---|
| | Blood donors (75) | Hansen's disease, stages | | | |
| | | Active (39) | Neutral (136) | Regressed (47) | Arrested (99) |
| Negative/*a* | 66.7 | 2.6 | 0.7 | 8.5 | 18.2 |
| 1:4 | 17.3 | 2.6 | 6.6 | 6.4 | 23.2 |
| 1:8 | 12.0 | 7.7 | 8.1 | 14.7 | 14.2 |
| 1:16 | 2.7 | 17.9 | 9.5 | 8.5 | 12.1 |
| 1:32 | 1.3 | 12.8 | 8.1 | 25.6 | 9.1 |
| 1:64 | ----- | 25.6 | 22.1 | 19.3 | 10.1 |
| 1:128 | ----- | 10.3 | 16.2 | 8.5 | 7.1 |
| 1:256 | ----- | 12.8 | 13.2 | ----- | 2.0 |
| 1:512 | ----- | 5.1 | 8.8 | 8.5 | ----- |
| 1:1024 | ----- | 2.6 | 5.2 | ----- | 3.0 |
| 1:2048 | ----- | ----- | 1.5 | ----- | 1:0 |

*a* In 1:4 dilution.

## DISCUSSION

It is evident from the data presented that *Mycobacterium tuberculosis* and *M. 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—as it does, for example, with respect to various strains of Salmonella, as indicated by Longfellow and Luipold (4) and by Levine *et al.* (2)—raises an intriguing question as to the efficacy of BCG vaccination as a possible protective measure for children of Hansen's disease patients. In this connection it may be pointed out that leprologists affirm that individuals who give a positive Mitsuda lepromin reaction are relatively resistant to leprosy, and it has been repeatedly reported that BCG vaccination induces lepromin positivity. It




| Symbol | | | | | |
|---|---|---|---|---|---|
| Specimens | Blood Donors | Hansen's Disease | | | |
| Disease Stage | None | Active | Neutral | Regressed | Arrested |
| Number of Individuals | 75 | 39 | 136 | 47 | 99 |
| Agg. Titer Range | Per Cent of Specimens | | | | |
| 0 - 8 | 96.0 | 12.9 | 15.4 | 29.6 | 55.6 |
| 16 - 128 | 4.0 | 66.6 | 53.9 | 61.9 | 38.4 |
| 256 - 2048 | — | 20.5 | 28.7 | 8.5 | 6.0 |

TEXT-FIG. 4. The results of the tests in the various stages of Hansen's disease, active, neutral, regressed and arrested.

is not surprising, therefore, that Chaussinand (1) and others have suggested that it would be of interest to determine whether a preventive effect against leprosy could be induced by BCG vaccination, and that work on that line is being carried on in some places.

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 BCG vaccination, with particular reference to Hansen's disease are worthy of being made.

## SUMMARY

Hemagglutination titers against tuberculin-sensitized sheep red cells were determined on the sera of 321 Hansen's disease patients and 109 tuberculosis patients, a control group of 75 blood donors being used for comparison. *M. tuberculosis* and *M. leprae* stimulate the production of common agglutinins in human sera, and are therefore presumed to possess common antigens. This serological test provides a laboratory tool which

opens up new vistas for the study of this disease of antiquity, whose etiological agent has so long defied cultivation.

Sera from lepromatous cases which were bacteriologically positive frequently showed much higher titers than were obtained with sera from active tuberculosis cases. Titers of sera from arrested lepromatous, tuberculoid and indeterminate cases, bacteriologically negative, had significantly lower titers, approaching those of normal individuals.

The marked serological response against the antigens in tuberculin induced by infection with Hansen's disease, coupled with observations that BCG vaccination induces the development of positive Mitsuda lepromin reactivity and that lepromin positivity is associated with resistance to leprosy, raises the hope that BCG vaccination of exposed children, particularly of Hansen's disease patients, may exert a protective action against this disease.


## RESÚMEN

Se determinó el nivel de hemaglutininas contra hematies de ovejas sensibilizadas a la tuberculina en el suero de 321 enfermos de la enfermedad de Hansen y de 109 enfermos tubérculos, y de 75 testigos normales. *M. tuberculosis* y *M. leprae* estimulan la producción de aglutininas comunes en el suero humano, y por tanto se presupone que contienen antígenos comunes. Esta prueba de laboratorio provee medios de explorar nuevos horizontes en el estudio de ésta enfermedad antigua, cuyo agente etiológico ha desafiado el cultivo hace tanto tiempo. Sueros de pacientes lepromatosos bacteriológicamente positivos, frecuentemente tuvieron títulos mas elevados que los sueros de pacientes con tuberculosis activa. Los títulos de los sueros de pacientes lepromatosos arrestados, tuberculoides e indeterminados, bacteriológicamente negativos, fueron significativamente másbajos, acercandose a los de los sueros normales.

La marcada reacción contra los antígenos de la tuberculina inducida por la infección de Hansen, conjuntamente con las observaciones que la vacuna BCG induce el desarrollo de una reacción positiva a la lepromina (Mitsuda) y que la positividad a la lepromina está asociada con resistencia a la lepra, dá esperanzas de que la vacunación BCG de niños expuestos a la lepra, especialmente de padres leprosos, ejerza una acción protectiva contra ésta enfermedad.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