STUDIES ON THE LEPROMIN TEST
III. INFLUENCE OF TUBERCULOSIS CONTACT AND OTHER FACTORS ON THE SIZE OF THE LEPROMIN REACTION

D. L. LEIKER, M.D.

Rotterdam, Netherlands[1]

Most leprologists are convinced of an immunologic relationship between *M. leprae* and *M. tuberculosis*. However, not always is a satisfactory correlation between lepromin and tuberculin reactions to be found. One of the reasons for an unsatisfactory correlation may be found in technical errors connected with the lepromin test (2). Similar and even more important difficulties have to be overcome when testing with tuberculin.

Of the many factors influencing the size of the tuberculin reaction, the following are of major importance:

1. *Batch of tuberculin.*—Tuberculins of different origin may differ in strength. Before using a new type of tuberculin, it should be tested in the field.

2. *Storage.*—Stock solutions, provided that they are kept cool and in the dark, usually keep rather well, but after dilution the strength diminishes rapidly, especially in warm climates. For scientific purposes fresh solutions should be made daily and be kept cool during patrol.

3. *Adsorption to glass.*—In bottles filled only partly, the adsorption of tuberculoproteins to the glass wall may become an important source of error.

4. *Leakage of syringes.*—A large proportion of new syringes, even those from well-known manufacturers, show a not-to-be-neglected leakage along the piston. In the survey reported in this article, only syringes with a leakage less than 0.3 cc. after a pressure of 5.5 kgm. per square centimeter for 6 minutes, were used.

5. *Injection and reading techniques.*—It has been shown that the difference between readings of trained and untrained workers is often considerable. Only after thorough training it is possible to inject constantly about the same volume of tuberculin in the same level of the dermis.

A combination of these several factors may influence the results of the test considerably, and this is of major importance in countries where nonspecific reactions to tuberculin are common.

In many countries where leprosy is endemic, unknown factors are responsible for reactions to tuberculin which cannot be explained by infection with *M. tuberculosis*. The influence of these factors increases with the age of the individuals. The size of these reactions may become so large that it is impossible to distinguish between specific and nonspecific reactions in size-frequency distribution histiograms of tuberculin reactions, as can be done in countries where the influence of nonspecific factors is unimportant.

The size-frequency distribution histiogram of reactions to 5 TU of PPD in 2,607 inhabitants of the Wandamen Bay, Fig. 1, shows some bimodal configuration, but it is difficult to separate negative and positive reactions accurately. It seems that the division must be made somewhere between 10 mm. and 14 mm.

---

[1] Formerly Chief, Division of Leprosy Control, Netherlands New Guinea.

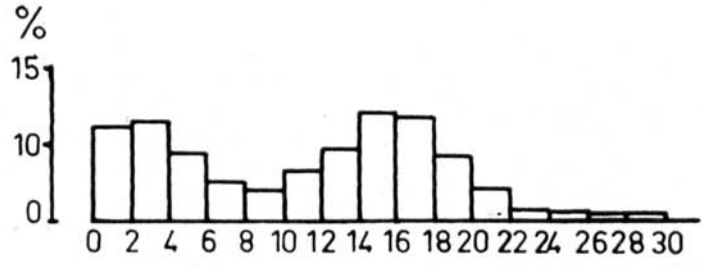

FIG. 1. Frequency-distribution of histiogram of reactions to PPD, 5 TU, in 2,067 individuals in the Wandamen Bay area.

From the histiograms of very young children, Fig. 2, it is concluded that reactions of 14 mm. or more are certainly specific. The influence of nonspecific factors increases rapidly with age, and in adults the border between specific and nonspecific reactions becomes completely inconspicuous.

To collect more evidence, Wijsmuller, chief of the Division of Tuberculosis Control, Netherlands New Guinea ([4]), suggested that human and avian tuberculin be injected simultaneously. This suggestion was based upon the hypothesis that in individuals infected with a human strain of the tubercle bacillus, the homologous tuberculin would give stronger reactions than the heterologous tuberculin. The results are shown in

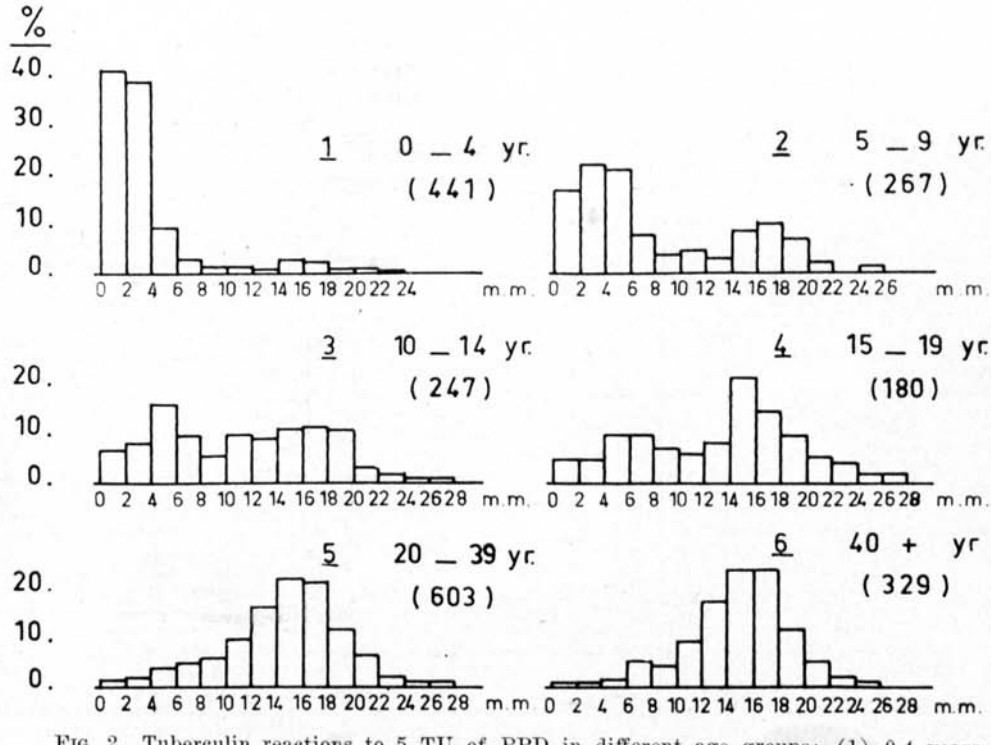

FIG. 2. Tuberculin reactions to 5 TU of PPD in different age groups: (1) 0-4 years, (2) 5 9 years, (3) 10-14 years, (4) 15-19 years, (5) 20-39 years, and (6) 40-or-more years.

TABLE 1. *Correlation diagram of the size of reactions to human and avian tuberculin in 293 people.*

Size of Reactions to 5.T.U. Avian P.P.D.	Size of Reactions to 5.T.U. Human P.P.D. in m.m.													Total
	0-1	2-3	4-5	6-7	8-9	10-11	12-13	14-15	16-17	18-19	20-21	22-23	24-25	
0-1	32													32
2-3	17	47	1											65
4-5	3	23	14	1	1									42
6-7	0	5	12	5	2	1								25
8-9	1	0	5	7	2	1								16
10-11		1	2	5	3	2	1	3		1				18
12-13				2	5	3	5	4	3	2	4	1		29
14-15				1	2	4	5	5	4	1	1	3		26
16-17					0		1	3	3	2	0	1		10
18-19					1		2	3	4	2	3	2	1	18
20-21									1	3	0	2	3	9
22-23											0	0	0	0
24-25											1	1	1	3
Total	53	76	34	21	16	11	14	18	15	11	9	10	5	293

Table 1. Most individuals with a reaction to human tuberculin larger than 14 mm. showed a smaller reaction to avian tuberculin, whereas in people with a reaction to human tuberculin smaller than 14 mm. the reactions to avian tuberculin are larger.

This result was considered as supporting former findings that only reactions of 14 mm. or more are caused by previous contact with *M. tuberculosis*. It was not possible to define this border more accurately. It is still possible that some of the reactions between 10 mm. and 14 mm. are caused by tuberculosis contact. As the percentage of such reactions was small, I have not included them in the following calculations.

The effect of tuberculosis contact on lepromin reactivity is demonstrated in Fig. 3. In very young children with negative PPD reactions, the lepromin reaction is usually negative. Some weakly positive reactions are seen. The cause of these reactions is uncertain. It may be that leprosy contact has played a role. Some small children reacted strongly to lepromin. These strong reactions were only seen in members of families in which there were cases of leprosy.

On examination, no symptoms of leprosy were found in these children. After a follow-up period of four years they were still free from leprosy symptoms. Probably these children were infected with *M. leprae*, but have shown no visible symptoms or only very inconspicuous, transitory, childhood lesions and have developed a high degree of immunity.

In the young PPD-positive children the number of positive lepromin reactions is considerably higher than in the PPD-negative group of the same age. Here is clear evidence of an influence of tuberculin positivity on lepromin reactivity. However, the average size of reactions in these children is much smaller than in adult people with increasing age. A gradual increase with age in the size of reactions, in PPD-negative as well as in PPD-positive individuals, is seen.

A high percentage of adult people with negative PPD reactions show strongly positive lepromin reactions. As this was also seen in villages which were free, or nearly free, from leprosy, other factors besides *M. tuberculosis* and *M. leprae* must exist. It is probable that other acid-fast bacilli, such as those living on animals or in soil, are responsible. The influence of these factors is rather slow and weak compared with the effect of *M. leprae* and *M. tuberculosis*, which speaks in favor of organisms which are not pathogenic to human beings or which have only a distant antigenic relationship to *M. leprae*. Many adults who have been in contact with these factors for many years still show an increase in the size of the lepromin reaction after contact with *M. leprae*, *M. tuberculosis* or after BCG vaccination.

Finally, some adult people react only weakly to lepromin, and a few do not react at all, in spite of contact with these hypothetical acid-fast organisms and in spite of tuberculosis contact. Some people remain lepromin negative after repeated BCG vaccination. It is highly prob-

able that if these people are infected with *M. leprae,* lepromatous or borderline symptoms will appear. It seems that these people are unable to develop a sufficient degree of resistance.

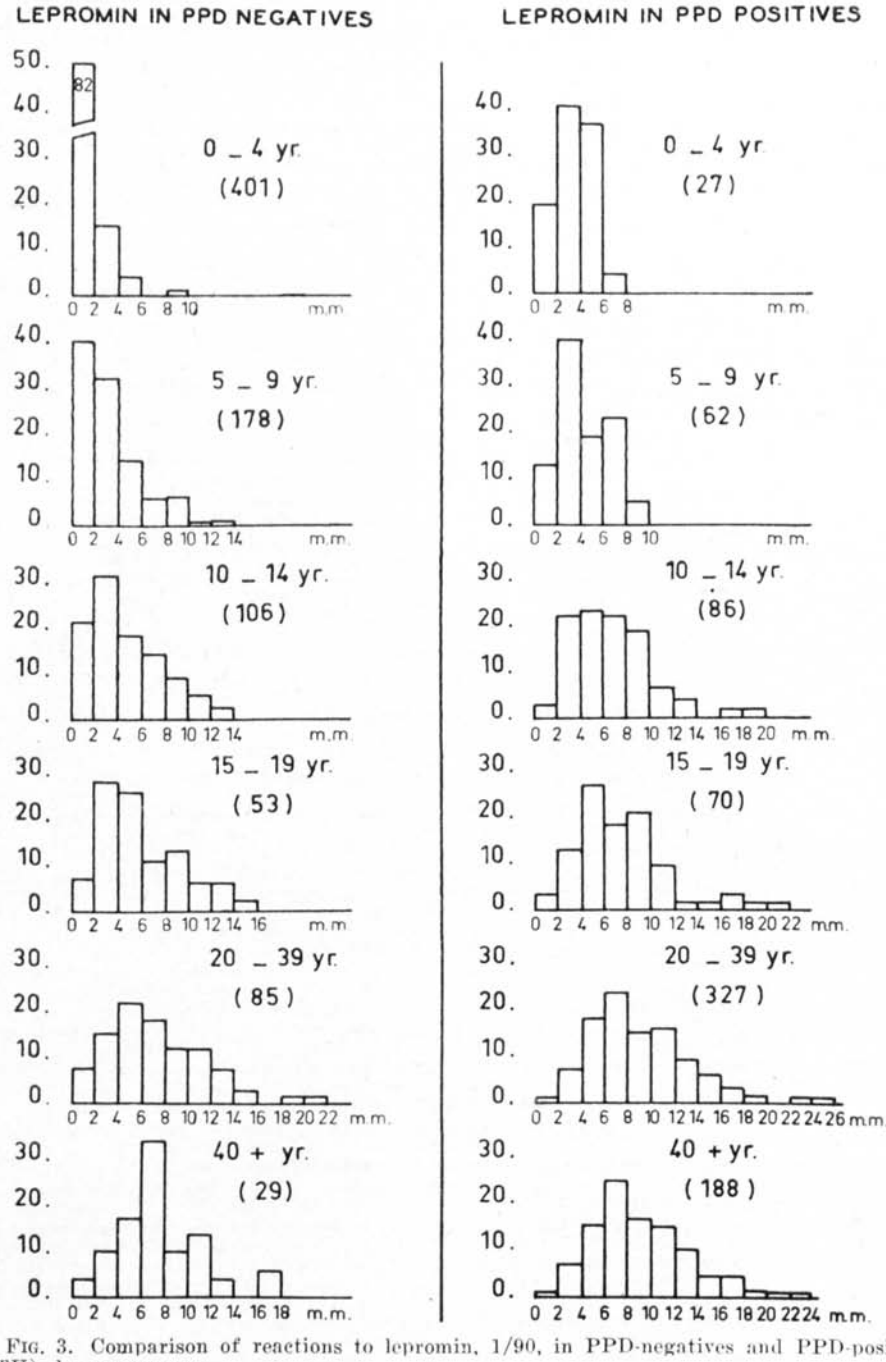

FIG. 3. Comparison of reactions to lepromin, 1/90, in PPD-negatives and PPD-positives (5 TU), by age groups.

Tuberculosis contact, BCG vaccination, and contact with other acid-fast bacilli produce an increase in the size of the lepromin reaction, but the increase is not the same in everybody. Some people do not react at all to lepromin. Rotberg ([3]) has suggested that in these people an endogenous factor (an "N" or "natural" factor) is lacking. If this is true, this factor must be a quantitative one, as the maximal size of the lepromin reaction is different in each individual. If we assume that this factor is also hereditary, much in the epidemiology of leprosy can be explained. There is not only a difference in susceptibility between populations, but it is highly probable that the fact that the incidence of leprosy in some families is higher than in others can be explained by increased exposure to *M. leprae* alone.

However, some clinical symptoms are difficult to explain by assuming one endogenous, quantitative and hereditary factor only. A tuberculoid lesion may heal in the center, but for a long time progress at the margin and then heal completely. The margin of borderline lesions remains rather well defined and great parts of the body remain free of infiltration, even when the disease has progressed far toward the lepromatous form. In borderline cases it is often seen that the site of a former lesion is transformed into an immune area, even when progressive bacilliferous infiltrations appear around the immune area and elsewhere, and even when the disease has developed far to the lepromatous side. In these cases the endogenous factor is clearly not sufficient to arrest the disease, but there is evidence of a definite local resistance to the infection. To explain such findings one has to assume that local factors, connected with the skin tissues, play a role in resistance to *M. leprae*. It is not probable that the hypothetical "N factor" can be influenced by tuberculosis contact, BCG vaccination, etc., but this might be possible in respect to the other factors. Not only theoretically, but also in practice, I have found evidence that previous tuberculosis contact gives some protection against leprosy in part of the population ([1]), probably only that part which possesses the endogenous factor that enables them to develop some degree of immunity.

SUMMARY

In performing the tuberculin test, especially in countries with a high prevalence of nonspecific tuberculin reactions, several technical factors are of great importance with respect to accuracy of the results.

In every country or area the border between specific and nonspecific reactions must be defined before drawing conclusions about the relation between tuberculin and lepromin reactions. If this is done properly it is evident that the effect of infection with *M. tuberculosis* on the size of the lepromin reaction is important.

It is not permitted to draw conclusions about resistance to leprosy from a negative lepromin reaction, when the tuberculin reaction is not known. The size of the lepromin reaction is limited by young age.

Besides *M. leprae* and *M. tuberculosis,* other acid-fast bacilli have an influence on the size of the lepromin reaction, but the influence is slower and weaker. The hypothesis of Rotberg that an endogenous factor ("N factor") is necessary to enable an individual to develop resistance to *M. leprae* would explain much about the epidemiology of leprosy, if this factor is a quantitative and hereditary one. In addition to this factor one has to assume one or more local tissue factors to explain such clinical findings as the "immune area" (Wade).

Theoretically it is improbable that tuberculosis contact or BCG vaccination has any effect on the N factor. In practice, however, evidence was found that previous tuberculosis contact gives some protection against leprosy in part of the population, probably only in those people who possess the endogenous factor which enables them to develop some degree of immunity.

RESUMEN

Al ejecutar la prueba de la tuberculina, sobre todo en los paises que muestran una elevada incidencia de reacciones tuberculínicas anespecíficas, varios factores técnicos revisten mucha importancia con respecto a la exactitud de los resultados.

En todo país o zona hay que definir el borde entre las reacciones específicas y las anespicíficas antes de sacar conclusiones acerca de la relación entre las reacciones a la tuberculina y la lepromina. Si se hace esto dibidamente, resulta evidente que es importante el efecto de la infección con *M. tuberculosis* sobre el tamaño de la reacción a la lepromina.

No es permisible sacar conclusiones acerca de la resistencia a la lepra a base de una reacción negativa a la lepromina, cuando no se conoce la reacción a la tuberculina. El tamaño de la reacción a la lepromina está limitado por una edad tierna.

Además del *M. leprae* y del *M. tuberculosis,* otros bacilos ácidorresistentes afectan el tamaño de la reacción a la lepromina, pero el influjo es más lento y más débil. La hipótesis de Rotberg de que es necesario un factor endógeno ("factor N") para capacitar a un individuo para manifestar resistencia al *M. leprae* explicaría mucho con respecto a la epidemiología de la lepra, si dicho factor fuese cuantitativo y hereditario. Aparte de dicho factor, hay que presuponer la existencia de uno o más factores histológicos locales que expliquen hallazgos clínicos, tales como el de la "zona inmune" (Wade).

Teóricamente, es improbable que el contacto con la tuberculosis o la vacunación con BCG ejerzan efecto alguno sobre el factor N. No obstante, en la práctica se encontraron pruebas de que el contacto anterior con la tuberculosis suministra alguna protección contra la lepra en parte de la población, probablemente sólo en los individuos que poseen el factor endógeno que los capacita para crear alguna inmunidad.

RESUMÉ

Lorsque l'on pratique le test à la tuberculine, surtout si cela a lieu dans des régions où la proportion de réactions non spécifiques à la tuberculine est notable, il est fort important de prendre en considération certaines données techniques susceptibles d'agir sur l'exactitude des résultats.

Dans chaque pays, dans chaque région, la délimitation entre réactions spécifique et non-spécifique doit être définie avant que des conclusions puissent être tirées quant á la tuberculine et la réaction á la lépromine. Si cela est fait de manière adéquate, il devient évident que l'infection par le *Myc. tuberculosis* intervient pour une part importante dans la grandeur de la réaction à la lépromine.

D'une réaction négative à la lépromine, il n'est pas licite de tirer des conclusions concernant la résistance à la lèpre, lorsque la réaction à la tuberculine n'est pas connue. Les dimensions de la réaction à la lépromine sont restreintes par le jeune âge.

Outre *M. leprae* et *M. tuberculosis*, d'autres bacilles acido-résistants ont une influence, plus faible et plus lente néanmoins, sur les dimensions de la réaction à la lépromine. Rotberg a émis l'mypothèse qu'un facteur endogène (facteur N) serait requis pour permettre à un individu de développer une résistance à l'égard de *M. leprae*. Ce facteur, s'il était quantitatif et héréditaire, expliquerait pas mal de choses dans le domaine de l'épidémiologie de la lépre. Outre ce facteur, il faut en outre, pour expliquer des phénomènes cliniques tels que la "zone d'immunité" de Wade, assumer l'hypothèse de l'existence d'un ounde plusieurs facteurs tissulaires locaux.

Théoriquement, il est improbable que le contage tuberculeux ou la vaccination par le B.C.G. ait une quelconque action sur le facteur N. En pratique cependant, il a été clairement constaté qu'un contage tuberculeux antérieur confère une certaine protection contre la lépre à une partie de la population, probablement aux individus qui possèdent le facteur endogène leur permettant de développer un certain degré d'immunité.

REFERENCES

1. Leiker, D. L. Epidemiological and immunological surveys in Netherlands New Guinea. Leprosy Rev. **31** (1960) 241-259.
2. Leiker, D. L. Studies on the lepromin test. I. The influence of the bacillary and tissue components in dilutions of lepromin. Internat. J. Leprosy **29** (1961) 157-167.
3. Rotberg, A. Some aspects of immunity in leprosy and their importance in epidemiology, pathogenesis and classification of forms of the disease. Based on 1529 lepromin tested cases. Rev. brasileira Leprol. **5** (1937) Sp. No. pp. 45-97.
4. Wijsmuller, G. Personal communication.