Intradermal Tests with Mycobacterial Substances and Normal Tissue Suspensions [1]

D. L. Leiker[2]

The literature of the last two decades reflects much interest in a possible relationship between leprosy and tuberculosis. Several epidemiologic and immunologic studies support the concept of a close relationship between the two. Other studies, however, offer little in favor of this hypothesis. Tests with mycobacterial antigens have given different results in the hands of different workers, and the evidence for a simple antagonism between the two diseases is not convincing.

The outcome of several BCG trials suggests some protective value against leprosy. Most studies, however, leave some doubt as to the validity of comparison between trial and control groups. In a recent, more carefully designed trial in Uganda (2) a statistically significant lower incidence of tuberculoid leprosy was found in children vaccinated with BCG than in controls. No conclusions, however, can be drawn as yet from this study as to the protective value of BCG against more progressive forms of leprosy, as the proportion of these forms in the study was too low to allow statistically significant conclusions.

Unfortunately most immunologic studies on leprosy and tuberculosis suffer from technical errors. Few leprosy workers have adopted the technical criteria for tuberculin testing recommended by the World Health Organization. In most tropical countries other factors, besides tuberculosis, induce nonspecific reactions to tuberculin, up to 14 mm. or even more in diameter, in dosage of 1-5 TU of PPD. It is essential to separate specific and nonspecific tuberculin reactions as accurately as possible. This requires the use of low dosages of PPD, instead of high dosages of less purified tuberculins, and special attention to storage of tuberculin, leakage of syringes, training in injection technic, reading of reactions, etc. The Mantoux technic of intradermal injection is far preferable to the Heaf multipuncture technic, which does not enable the differentiation of reactions of intermediate strength.

In many articles it is presumed, but without proof, that tuberculin reactions 5 or 6 mm. or more in diameter are caused by tuberculosis infections. The results of testing are frequently expressed as positive or negative, without statement of the actual diameter of the infiltration in millimeters. Reassessment of such studies, and comparison with the work of other authors, thus becomes impossible.

In leprosy, the matter is complicated by the use of lepromins that are not reasonably well standardized. Detailed studies by leprosy workers, distinguishing between specific and nonspecific tuberculin reactions, are scarce. Usually in such studies attention is focused on the relationship between tuberculosis and leprosy. No doubt in most areas tuberculosis is one of the most important diseases influencing the epidemiology of leprosy, but it certainly is not the only one and in some areas other mycobacteria may be of greater importance. Little is known about the identity and prevalence of other mycobacteria in endemic leprosy areas. There is evidence that *Mycobacterium ulcerans*, for example, and related mycobacteria, are more widely spread than previously was thought. Even less is known about nonpathogenic mycobacteria. The high frequency of strong lepromin reactions

[1]Received for publication 10 April 1967.
[2]D. L. Leiker, M.D., Dermatologist, Institute of Tropical Hygiene, Amsterdam, The Netherlands.

in areas free from leprosy, and with little tuberculosis, may have to be explained, to some extent, by infection with nonpathogenic mycobacteria.

In this article no attempt is made to review the whole literature on the subject, but a number of studies are selected to illustrate the fact that in leprosy patients there is a common pattern in reaction to various mycobacterial substances.

STUDIES WITH HUMAN TYPE TUBERCULIN

The reports of most authors agree that the frequency of positive tuberculin reactions is lower in lepromatous patients than in healthy controls. Some authors, however, have failed to confirm these findings ([6, 13]). Guinto and Mabalay ([5]) claimed definite proof of a lower frequency of positive tuberculin reactions in lepromatous patients. The matter can still not be regarded as settled, because in none of the studies were specific and nonspecific tuberculin reactions separated.

It is not justified to regard reactions to tuberculin 5 to 6 mm. in diameter, as positive without evidence that these reactions are mainly caused by tuberculosis infection. It has not been established that specific and nonspecific tuberculin reactions are equally influenced by leprosy. They should therefore be assessed separately.

Rutgers ([13]) found reactions of 6 mm. or more to 5 TU of PPD in 70 per cent of 132 healthy persons, and in 82 per cent of 62 lepromatous patients. The group of tuberculoid patients gave 76 per cent positive reactions. Of 88 healthy contacts, however, 89 per cent gave a positive reaction. If the healthy subjects and the healthy contacts are taken together, 80 per cent of 220 healthy subjects were positive, and the difference from the lepromatous patients becomes insignificant. In fact the positive reactions of 6 mm. and more formed a mixed group of specific and nonspecific reactions. If only the very large reactions (>20 mm.), which were doubtless specific, are compared, the prevalence was 44 per cent in the lepromatous group, 3.4 per cent in leprosy contacts and 1.5 per cent in healthy controls. This suggests an increase of specific positive reactions in lepromatous patients, but does not exclude the possibility of a decrease of nonspecific reactions. Rutgers' findings are not necessarily contrary to those of others.

Guinto and Mabalay ([5]) found a significantly lower frequency of tuberculin reactions of 5 mm. or more to 5 TU in lepromatous patients (47% of 206 lepromatous patients as compared with 81% of 233 healthy controls). If, however, the frequencies of 2+ and 3+ reactions, in both groups, including a higher percentage of specific reactions, are compared, they become 35 per cent and 49 per cent, respectively. The fact that the difference becomes smaller suggests that the decrease in frequency of positive reactions in lepromatous patients is due largely to a decrease in the frequency of nonspecific reactions.

Previously Leiker ([9, 10]) had separated specific and nonspecific reactions. Among 30 lepromatous patients the average size of the nonspecific tuberculin reaction to 5 TU of PPD was 6.3 mm., as compared with 7.9 mm. in 192 healthy controls. In a second area, the average nonspecific reaction among 46 lepromatous patients was 3.5 mm. as compared with 5.2 mm. in 220 healthy controls. The corresponding figures found in 21 and 31 tuberculoid patients of the same areas were 6.8 mm. and 4.2 mm., respectively. These figures show a significant decrease in size of nonspecific tuberculin reactions in leprosy patients. Only a slight decrease in the average size of specific tuberculin reactions was found in leprosy patients, but further studies are indicated to confirm this. The matter is complicated by anomalous findings. Lepromatous patients, more often than healthy persons, respond to small dosages of tuberculin with very severe reactions, but, on the other hand, the frequency of failure of response to tuberculin also is increased. Leiker ([10]) found a severe reaction to 5 TU of PPD in 6 (3%) of 220 lepromatous patients, but in only 2 (0.1%) of 2,300 healthy subjects in the same area. In another area the only person with a severe reaction, out of 450 tested, was a lepromatous patient.

Rutgers ([13]) also mentioned severe reactions to 5 TU of PPD in four out of 62 lepromatous patients, but none in 132 healthy persons. Leiker ([10]) found reactions of 0-2 mm. to 5 TU of PPD in 22 (30%) of 76 lepromatous patients, and only 57 (14%) of 399 healthy controls. Rutgers ([13]) stated that 14.5 per cent of 62 lepromatous patients did not respond at all to 5 TU of PPD, as compared with 1.5 per cent of 132 healthy controls. There is no evidence that tuberculosis patients with leprosy fail more frequently to respond to tuberculin with a specifically positive reaction than do tuberculosis patients without leprosy.

It is therefore probable that the increase in frequency of negative or very small reactions in lepromatous patients is to be explained by a moderate depression in the size of the smaller nonspecific tuberculin reactions, rather than by a marked or complete depression of specific reactions. No explanation can be offered for the greater frequency of very severe reactions to tuberculin in lepromatous patients.

TUBERCULINS OF OTHER THAN HUMAN TYPE

McKinley ([12]) compared the response of leprosy patients and healthy controls to various kinds of tuberculin preparations. These preparations included several made from mycobacteria that had been isolated from leprosy patients. Some of the results are summarized in Table 1. Without exception the leprosy patients (the percentage of lepromatous patients was not recorded) showed fewer positive tuberculin reactions than the healthy controls. The difference is small, however, between the groups tested with human type PPD. As tuberculosis was at that time widespread in the Philippines in and outside leprosaria, the results of testing with human type PPD suggest that leprosy had little influence on the specific tuberculin reactions.

Badger *et al.* ([1]) reported 100 per cent of positive reactions to Lleras Acosta antigen in 31 tuberculosis patients, as compared with 55 per cent in 60 lepromatous patients. Tested with the Karlihi strain the same patients showed 90 per cent and 58 per cent reactions, respectively. These studies again agree that the frequency of positive reactions to various tuberculins is lower among leprosy patients than among healthy subjects.

BCG reactions. Rutgers ([13]), reading BCG reactions after one, two and three weeks, found average indurations of 1.0, 1.1 and 0.55 mm., respectively, in 11 lepromatous patients with PPD reactions less than 6 mm. in diameter, and average indurations of 7.3, 6.3 and 9.3 mm. among 18 healthy controls. Among 50 lepromatous patients with PPD reactions of 6 mm. or more, the average indurations after inoculation of BCG were 10.5, 9.3 and 6.9 mm., respectively, and 11.5, 10.3 and 10.4 mm. among 40 healthy controls. In both groups the

TABLE 1. *Reactions of healthy persons and leprosy patients to tuberculin type preparations.*

Tuberculin type preparations	Healthy persons		Leprosy patients	
	No.	% pos.	No.	% pos.
PPD, human	110	90	100	85
PPD, avian	110	87	100	26
M. smegmatis	110	57	100	26
Karlinski, leprosy	110	61	100	26
Daines, leprosy	110	69	100	29
Phipps Ll, leprosy	110	64	100	32
Duval, leprosy	10	100	10	60
M. marianum	10	89	10	30

reaction to BCG was, on the average, smaller in lepromatous patients than in healthy controls. The difference was more marked in the group of nonspecific tuberculin reactions than in the group of specific tuberculin reactions.

Suspensions of other mycobacteria. In experiments with a suspension of dead mycobacteria ("875"), which had been cultivated on Sabouraud medium, Leiker [10] found an average late reaction to this suspension of 10.3 mm. among 53 PPD-positive (specific reaction) lepromatous patients, and 14.3 mm. among 39 PPD-positive healthy controls. Among 48 PPD-negative lepromatous patients the average reaction was 6.1 mm., as compared with 9.3 mm. among 52 PPD-negative healthy controls. The average reactions to "875" suspension among 13 PPD-positive and 23 PPD-negative tuberculoid patients were 12.2 and 8.3 mm., respectively, i.e., figures between those among lepromatous patients and among healthy controls. Again the differences were slightly more marked in the group with nonspecific tuberculin reactions than in the group with specific tuberculin reactions.

Normal tissue suspensions. The Committee on Leprosy Skin Tests in the Philippines [3] reported 11 doubtful and weakly positive reactions to intradermal injection of a concentrated, watery extract of normal spleen, among 191 healthy children, and no reactions in 110 leprosy patients. Lopez de Faria [11] reported positive early reactions to normal skin suspensions in several patients with tuberculoid leprosy, but only a few reactions in lepromatous patients. Among nine tuberculoid patients six showed a late reaction of 4.5–8 mm., and three a reaction of 3 mm. Among six lepromatous patients only one 3 mm. reaction was seen. These findings were confirmed by Kooij and Gerritsen [7], Davey and Drewett [4], and Leiker [8], and more recently by others. Leiker also found that positive reactions to normal skin occurred frequently in healthy persons, that on the average tuberculin reactors presented larger reactions to normal skin than persons not reacting to tuberculin, and that a correlation existed between the size of the reaction and the size of the lepromin reaction.

Lepromin. It is generally agreed that the lepromin reaction is negative in lepromatous patients and moderate, or strongly positive in tuberculoid patients. The reaction varies among healthy subjects, but becomes positive in the majority (about 90-95%) after tuberculosis infection or BCG vaccination. Lepromatous patients, however, are not always completely anergic to lepromin. Guinto (personal communication) was able to induce positive lepromin reactions (not strongly positive) in about 40 per cent of lepromatous patients when lepromin concentrated 5 times or more was used. With less concentrated, but strong lepromin Leiker [10] found an average size of lepromin reaction of 0.97 mm. among 54 patients with diffuse lepromatous leprosy, of 1.47 mm. among 53 patients with nondiffuse lepromatous leprosy, and of 2.76 mm. among 21 patients with borderline lepromatous leprosy (lepromatous with borderline elements).

It is also agreed that the early lepromin reaction is negative in lepromatous patients, but relatively few data are available on the exact size of reactions in lepromatous patients and healthy controls. Rutgers [13] did not find a difference in the size of early lepromin reactions between 62 lepromatous patients and 136 healthy controls, but the average size of nonspecific reactions was larger in leprosy contacts, tuberculosis patients and tuberculoid leprosy patients than in the first two groups. As the lepromin used was weaker than normal as judged from the low percentage of positive reactions in tuberculoid leprosy, the results are not conclusive.

DISCUSSION

The statement that lepromatous patients are anergic to lepromin is not justified. The majority of lepromatous patients show a very weak response to lepromins of slightly more than the usual strength. In many patients a stronger reaction is seen after the use of a more concentrated lepromin. Probably only a small minority of lepromatous patients, i.e., only those with pure, primary, diffuse lepromatous leprosy, are truly anergic to lepromin. It is more nearly correct to say that in the majority of leproma-

tous patients the ability to react to lepromin is markedly decreased.

Lepromatous patients react strongly to suspensions of other mycobacteria than *M. leprae*. The difference in size between the lepromin reactions and the reactions to other mycobacterial suspensions is sufficiently great to serve as an aid in the distinction between *M. leprae* and other mycobacteria. It is not correct to say, however, that lepromatous patients react to suspensions of other mycobacteria in a manner similar to that of nonleprosy patients. In this article it is shown that, on the average, lepromatous patients are less capable of reacting to various mycobacterial suspensions than are healthy controls.

The peculiarity of lepromatous patients is not that they are anergic to lepromin while showing a normal response to suspensions of other mycobacteria, but that they are less capable of reacting to intradermal injection of various mycobacteria, to their products, and to suspensions of normal tissue, than are healthy controls. These facts again bring leprosy somewhat more out of its isolated position and stress the intimate relationship among mycobacterial diseases.

The mechanism of the lepromin reaction is still obscure. It is not a specific reaction, for similar reactions may be evoked by a great variety of agents, including other substances than mycobacteria. Newborn children do not react to lepromin. Apparently previous sensitization is a condition for reactivity. Most children become sensitized when they grow up. In only a minority of persons can it be assumed that *M. leprae* is the sensitizing agent. More frequently the tissues are sensitized by other mycobacteria, e.g., *M. tuberculosis*. The possibility that, apart from mycobacteria, other agents may sensitize the tissues to lepromin cannot yet be excluded. It is of interest that a correlation has been found between the size of reactions to lepromin and that to normal tissue suspensions ([8, 9, 10]). It seems that infection with *M. tuberculosis* sensitizes the skin to normal skin suspensions too. Lepromatous patients show a diminished reaction not only to mycobacterial suspensions but to normal skin suspensions also.

The immunologic findings cannot be explained satisfactorily by a single factor. The findings may be explained, to some extent, by the following hypotheses. First, mycobacteria and normal tissue have one or more common components, but differ quantitatively with respect to these components. Second, the capability of becoming sensitized to these components is determined genetically. Third, only in individuals genetically capable of reacting to these components, will infection with any of the mycobacteria sensitize the tissues to the common components. Fourth, because there are quantitative differences in the genetically determined capability of individuals to react to the component, and because mycobacteria differ quantitatively with respect to this component, persons react in a quantitatively different manner to intradermal tests with mycobacterial suspensions, even after previous challenge with infection by one or more species of mycobacteria.

There is no definite proof of the existence of a genetic factor. The fact that the incidence in certain families is higher than in others frequently can be explained by a greater rate of exposure. However, most leprologists have frequently observed families with a high incidence not only of leprosy patients, but also of progressive types of leprosy, as compared with other families with a comparable number of sources of infections in the households. Exposure rates and susceptibility, however, are difficult to assess separately. It will not be easy to derive conclusive evidence from family incidence studies.

More promising is the study of lepromin reactions in families with and without cases of leprosy. Such studies have already been made, but they were not adequately designed and the results are not conclusive. Even if it is found that the frequency of negative lepromin reactions in children of lepomatous patients is higher than that in children of healthy parents, the existence of a genetic factor is not proven. Some of the healthy parents may be potential lepromatous patients, and, on the other hand, a negative lepromin test in children does not exclude the possibility that the children are

capable of developing a positive lepromin reaction after they have become sensitized by a mycobacterial infection. The lepromin reactions in children of lepromatous patients should be compared with those in children of healthy parents with a strongly positive lepromin reaction, after the healthy parents and the children of patients and of healthy parents have been challenged with a mycobacterial infection, e.g., by BCG vaccination. If, after this challenge, the children of leprosy patients show a significantly lesser response to lepromin than similarly challenged children of lepromin-positive parents, the existence of a hereditary factor can be accepted.

This hypothesis has some important practical consequences. Attempts could be made to identify the common component and to isolate this component from mycobacteria that can be grown easily on culture media. The isolate could perhaps be used as a substitute for lepromin, after standardization. Second, the prospects of developing a vaccine for active immunization against leprosy are not bright. BCG vaccination probably has protective value only for those individuals who are capable of developing resistance to *M. leprae*. The vaccination has a booster effect that may prevent tuberculoid leprosy, but it is unlikely that it will prevent progressive forms of the disease.

Third, if it is true that lepromatous patients are less capable of reacting to a common component of *M. leprae*, the waves of tuberculosis, e.g., in Europe, may not only have killed a large proportion of the tuberculosis-susceptible stock of the population, but at the same time many leprosy-susceptible individuals. It is probable that the process of natural selection by leprosy itself (higher death rate, lesser chances of marriage of lepromatous patients, sterility in males with lepromatous leprosy, etc., resulting in a smaller offspring as compared with that of healthy persons) has played some part in the decline of leprosy in Europe. It is a slow process, however, too slow to explain a marked decline of the disease within a few centuries. Tuberculosis, on the other hand, being a killing disease, could have acted much more rapidly. As tuberculosis and leprosy have become less common diseases in Europe, the susceptible stock of the population may slowly increase again.

SUMMARY

Most patients with lepromatous leprosy are not completely anergic to lepromin. The lepromin reaction is only markedly decreased.

Lepromatous patients show a weaker response not only to lepromin; the reactions to various mycobacterial substances and to normal tissue suspensions also are, on the average, weaker than the reactions of healthy subjects.

The peculiarity of leprosy patients is probably not that they are less capable of reacting to the presence of *M. leprae* in the skin, but that they are less capable of reacting to a component common to mycobacteria in general and possibly normal tissue also.

The size of the reaction to injections of mycobacterial substances could depend first on a genetically determined potential capability of reacting to the common component of mycobacteria, second, on the degree of sensitization to this component, produced by previous infection with one or more species of mycobacteria, and third, on the quantity of the common component in the test material.

This hypothesis suggests first that attempts to identify and isolate the common component, using mycobacteria that can be grown easily on artificial culture media, might result in the discovery of a substitute for lepromin. Second, the prospects for developing a vaccine for active immunization against leprosy are not bright. Probably BCG vaccination has a booster effect only; it may prevent tuberculoid leprosy in potentially resistant persons, but it is not likely that it will prevent progressive forms of leprosy in those individuals who are genetically incapable of developing resistance. Third, the hypothesis of a genetically determined capability of reaction to a common component of mycobacteria offers a better explanation for the decline of leprosy in European countries than other hypotheses. The wave of tuberculosis, a kil-

ling disease, may have markedly reduced the leprosy-susceptible stock of the population.

RESUMEN

La mayor parte de los pacientes con lepra lepromatosa no son totalmente anérgicos a la lepromina. Le reacción lepromínica está solo notablemente disminuída.

Los enfermos lepromatosos muestran una reacción mas debil no solo a la lepromina; las reacciónes a varias substancias micobacterianas y a suspensiones de tejido normal, son también, en promedio, mas débiles que las reacciones de las personas sanas.

La peculiaridad de los enfermos de lepra es probablemente, no que ellos sean menos capaces de reaccionar a la presencia de *M. leprae* en la piel, sino que ellos tienen una capacidad menor de reaccionar frente a un componente común de los micobacteria en general y posiblemente también al tejido normal.

El tamaño de la reacción a inyecciones de substancias micobacterianas puede depender de una capacidad potencial geneticamente determinada de reaccionar al componente común de los micobacteria, segundo, del grado de sensibilización a este componente, producido por inyecciones previas con una o mas especies de micobacteria, y tercero, de la cantidad del componente común en el material ensayado.

Esta hipótesis sugiere, primero que los ensayos para identificar y aislar el componente común, usando micobacteria que pueden desarrollarse con facilidad en medios artificiales de cultivos, podría resultar en el descubrimiento de un substituto de la lepromina. Segundo, las posibilidades de preparar una vacuna para una inmunización activa contra la lepra no son brillantes. Probablemente la vacuna BCG tiene solo el valor de un refuerzo; ella puede prevenir la lepra tuberculoide en personas potencialmente resistentes; pero nada sugiere que ella pueda prevenir formas progresivas de lepra en aquellas personas que son geneticamente incapaces de desarrollar resistencia. Tercero, la hipótesis de una capacidad de reacción geneticamente determinada a un componente común de micobacteria constituye una mejor explicación que otras hipótesis para la disminución de la lepra en los países de Europa. La ola de tuberculosis, una enfermedad mortal, bien puede haber reducido marcadamente la reserva de la población susceptible a la lepra.

RÉSUMÉ

La plupart des malades atteints de lèpre lépromateuse ne sont pas complètement anergiques à la lépromine. La réaction à la lépromine est seulement diminuée de façon notable.

Les malades lépromateux ne montrent pas seulement une réponse plus faible à la lépromine; les réactions à diverses substances mycobactériennes et aux suspensions de tissu normal sont également plus faibles que les réactions des sujets bien portants.

La particularité des malades atteints de lèpre n'est probablement pas qu'ils sont moins capables de réagir à la présence de *M. leprae* dans la peau, mais bien qu'ils sont moins capables de réagir à un constituant commun aux mycobactéries en général, et peut-être aussi au tissu normal.

La dimension de la réaction enregistrée à la suite d'injection de substances mycobactériennes pourrait dépendre d'abord d'une capacité génétiquement déterminée de réagir au consituant commun des mycobactéries, ensuite, du degré de sensibilisation à ce constituant, produit par une infection antérieure par une ou plusieurs espèces de mycobactéries, et enfin, la dimension de la réaction pourrait dépendre en troisième lieu de la quantité du constituant commun présente dans les produits utilisés pour l'épreuve.

Cette hypothèse suggère d'abord que les essais menés pour identifier et isoler le constituant commun, en utilisant des mycobactéries qui peuvent être facilement cultivées sur milieux de culture artificiels, pourraient résulter dans la découverte d'un substitut de la lépromine. Deuxièmement, cette hypothèse suggère que les perspectives de développer un vaccin pour une immunisation active contre la lèpre ne sont pas très brillantes. Il est probable que la vaccination par le BCG ne possède qu'un effet de rappel; elle pourrait prévenir la lèpre tuberculoïde chez des personnes qui seraient potentiellement résistantes, mais il est peu vraisemblable que le BCG puisse prévenir des formes progressives de lèpre chez ceux-là qui sont génétiquement incapables de développer une résistance. Troisièmement, l'hypothèse d'une capacité génétiquement déterminée à réagir à un constituant commun des mycobactèries, fournit une explication meilleure pour le déclin de la lèpre dans les pays européens que ne le font les autres hypothèses. L'envahissement par la tuberculose, maladie fort léthale, pourrait avoir réduit de façon marquée les effectifs de la population qui auraient été susceptibles à la lèpre.

REFERENCES

1. Badger, L. F., Patrick, D. W., Fite, G. L. and Wolfe, D. Leprosy. Two strains of acid-fast bacilli isolated from a case of human leprosy. A comparison with other strains of acid-fast organisms with particular reference to the Lleras bacillus. USPHS Natl. Inst. Hlth. Bull. No. 173, 1940, 1-44.
2. Brown, J. A. K., Stone, M. M. and Sutherland, I. BCG vaccination of children against leprosy; first results of a trial in Uganda. British Med. J. **1** (1966) 7-14.
3. [Committee on Leprosy Skin Tests] Summary report on antigenic skin tests in leprosy. Mon. Bull. Bur. Hlth. (Philippines) **17** (1937) 265-275.
4. Davey, T. F. and Drewett, S. E. Lepromin-like activity of normal skin tissue. Leprosy Rev. **29** (1958) 197-203.
5. Guinto, R. S. and Mabalay, M. C. A note on the tuberculin reaction in leprosy. Internat. J. Leprosy **30** (1962) 278-283.
6. Halle, J. H., Molesworth, B. D., Grove-White, R. J., Sambamurthi, C. M. and Russell, D. A. The relationship and significance of the Mantoux and lepromin reactions in leprosy. Internat. J. Leprosy **23** (1955) 139-147.
7. Kooij, R. and Gerritsen, Th. Positive "lepromin" reactions with suspensions of normal tissue particles. Internat. J. Leprosy **24** (1956) 171-181.
8. 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.
9. Leiker, D. L. Studies on the lepromin test. III. Influence of tuberculosis contact and other factors on the size of the lepromin reaction. Internat. J. Leprosy **29** (1961) 488-495.
10. Leiker, D. L. Studies on the lepromin test. IV. Influence of leprosy on the reactions to lepromin, tuberculin and the "875 bacillus" suspension. Internat. J. Leprosy **29** (1961) 496-501.
11. Lopez de Faria, J. Estudo da reação a lepromina. Rio de Janeiro, Dept. Impre. Nacional, 1951, pp. 1-190.
12. McKinley, E. B. The present status of diagnostic skin tests in leprosy. Internat. J. Leprosy **6** (1938) 33-46.
13. Rutgers, A. W. F. Lepra en tuberculose. Zaandijk, J. Heijnis Tsz., 1956.