STUDIES ON THE LEPROMIN TEST
I. THE INFLUENCE OF THE BACILLARY AND TISSUE COMPONENTS IN DILUTIONS OF LEPROMIN

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In preparing lepromin by the Mitsuda-Hayashi method a certain quantity by weight of bacillus-rich leproma tissue is suspended in a certain quantity of saline solution, the coarser particles then being removed by passing through a surgical-gauze filter. If there are no variations of the porosity of the filters used at different times, it may be assumed that the quantity of tissue elements in such lepromins will be more or less constant, at least if the characteristics of the tissues used for different batches are reasonably similar. The quantity of the tissue element will increase proportionately with the use of filters of coarser gauge. The density of bacilli in such lepromins is of course variable, even if one uses—as one should—only tissues rich in bacilli. Therefore it is not justified, without further control, to apply to such lepromins the criteria for positivity which were adopted by the VI International Congress at Madrid, in 1953.[2]

Although the percentage of positive reactions in tuberculoid patients does give an impression of the strength of the lot of lepromin being tested, the number of weakly-positive reactions in tuberculoid patients is often so large that retesting with another, slightly weaker, lepromin may give an important shift in the percentages of positive and negative reactions. It is therefore advisable to grade lepromin reactions in terms of millimeters of palpable infiltration, and not to speak of "positive" or "negative" reactions. Publications speaking of percentages in those terms are difficult to evaluate, and as the lepromins used in different studies are often variable to a not unimportant degree, differences in the results reported can often be explained by the use of lepromins which are not comparable. Besides, the technique of the lepromin test easily leads to errors. These errors will not seriously affect the results in persons reacting strongly, but they can be of major importance in weak reactors.

With some lots of lepromin there forms a sediment of tissue elements and conglomerates of bacilli, and only after vigorous shaking can a suitable suspension be obtained again. Moreover, a greasy, bacillus-rich material may attach itself to the glass wall at the surface of the suspension, and it can be dispersed in the suspension again only by energetic shaking.

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[2] The minimal size of the reaction nodule to be read as positive is 3 mm., as F. Hayashi had proposed and the Second Pan-American Leprosy Conference (1946) had agreed. First Report of the WHO Expert Committee on Leprosy recommended 4 mm. as the minimum for positivity.

When injecting lepromin, relatively strong pressure has to be put on the piston of the syringe. It has been shown that many new syringes, even of well-known firms, show leakage along the piston. To inject an accurate dose of 0.1 cc. of lepromin in a certain level of the skin considerable skill is required. In reading the results a margin of error of 1-2 mm. must be taken into account, even with trained investigators. A combination of some of these errors can be highly important at the border area between positive and negative reactions.

In preparing lepromin according to the modified technique of Wade ([9]), a single layer of a nylon bolting-cloth filter of a given fine mesh, or gauge, is always used. The lepromin is standardized by comparison of smears, the density of bacilli in a smear made by spreading the material picked up by a standard wire loop over a standard surface area being compared with the density of the same quantity of material of a standard lepromin, tested in practice, spread over a similar surface area. Then the new lepromin is further diluted until the bacillary density is about the same as that of the standard lepromin. Although this method is not entirely accurate, either, the results are very useful in practice in every respect.

By dilution, however, the quantity of the tissue element in a lepromin suspension becomes variable. The lepromin contains less tissue elements in proportion to the use of more strongly positive lepromatous tissues.

Hayashi ([6]) and Dharmendra ([3]), among others, saw no reaction to bacillus-free tissue suspensions. On the contrary, however, Davey ([2]) and de Faria ([4]) sometimes noted weakly positive reactions to such suspensions. In 1956 attention was drawn again to the occurrence of reactions to normal skin suspensions by the investigations of Kooij and Gerritsen([7]). They saw weakly "positive" reactions in tuberculoid leprosy patients, but not in lepromatous patients. Moreover, Floch ([5]) stated that reactions to highly diluted lepromin are strengthened by adding an extract of normal skin to the suspension.

Kooij and Gerritsen arrived at the far-reaching hypothesis that the reactions to lepromin are only foreign-body reactions, in which the bacilli as well as the tissue element act as foreign bodies, and that it is not justified to assume an immunologic relationship between leprosy and tuberculosis by means of the lepromin reaction.

PRESENT INVESTIGATIONS

The investigations of Kooij and Gerritsen were restricted to a limited number of leprosy patients. It therefore seemed desirable to extend this investigation to healthy persons of different age groups, comparing the results with different dilutions of normal skin suspension with those obtained with lepromin dilutions, with respect to frequency, size, and speeds of reactions. In one experiment a suspension

of an "atypical" ("unidentified") mycobacterium was compared with lepromin and the normal skin suspension in a group of lepromatous patients, together with a comparison of reactivity to the atypical bacillus in tuberculin-positive and tuberculin-negative patients. The results of this work are reported here.

To begin with, a skin preparation was made by suspending 1 gram of autoclaved normal skin in 15 cc. phenol-saline solution, and filtering it 3 times through Wade's nylon filter. Of this 1/15 suspension, containing more tissue elements than lepromin of the usual strength, 0.1 cc. was injected intradermally in 98 healthy persons. The results are shown in the first part of Table 1.

TABLE 1.—*Reactions, read three weeks after the tests, to* (a) *0.1 cc. of a 1/15 normal skin suspension, and* (b) *0.1 cc. of a 1/150 lepromin dilution, in 98 healthy persons.*

Size of reactions (mm.)	Age group (years)						
	0-4	5-9	10-14	15-19	20-39	40+	Total
Normal skin suspension, 1/15							
0–1	15	3	2	2	5	3	30
2–	6	2	3	9	31	7	58
4–	–	–	–	1	5	1	7
6–	–	–	–	–	2	–	2
8–	–	–	–	–	–	1	1
10–	–	–	–	–	–	–	–
Total	21	5	5	12	43	12	98
Lepromin dilution, 1/150							
0–1	18	2	4	2	3	2	31
2–	1	3	1	3	11	4	23
4–	1	–	–	4	13	4	22
6–	1	–	–	2	5	1	9
8–	–	–	–	1	5	–	6
10–	–	–	–	–	4	–	4
12–	–	–	–	–	2	1	3
Total	21	5	5	12	43	12	98

An important number of "positive" reactions in the sense of the Madrid classification were seen. The table shows that 10 out of the 98 subjects—but only 1 of the 43 who were less than 20 years of age—gave 4 mm. reactions or larger. The observations of Kooij and Gerritsen in patients were thus confirmed in healthy persons as well.

In the same group of persons, 0.1 cc. doses of lepromin diluted to 1/150 (1 gm. of tissue to 150 cc. phenol-saline) were also injected. Although the quantity of tissue in this lepromin presumably amounts to only about one-tenth of that of the normal skin suspension, and the per-unit number of bacilli in the lepromin dilution was considerably

smaller than that in lepromins of usual strength, it can be seen from the second part of Table 1 that the reactions to this diluted lepromin were much stronger than those with the concentrated skin suspension. No less than 44 of the 98 persons (45%) gave 4 mm. or larger reactions. From these findings it is concluded that with lepromins the tissue element plays a smaller part in the reactions than the leprosy bacilli.

In a second group of (29) healthy persons, 0.1 cc. doses of a 1/30 normal skin suspension and of a 1/30 lepromin were injected. The results are shown in Table 2.

TABLE 2.—*Three-week reactions to* (a) *0.1 cc. of 1/30 normal skin suspension, and* (b) *0.1 cc. of 1/30 lepromin, in 29 healthy persons.*

Size of reactions (mm.)	Age group (years)					
	0-4	5-9	10-14	15-19	20+	Total
Normal skin suspension, 1/30						
0–1	7	2	1	1	2	13
2–	2	2	2	–	4	10
4–	–	1	1	–	4	6
6–	–	–	–	–	–	–
8–	–	–	–	–	–	–
10–	–	–	–	–	–	–
Total	9	5	4	1	10	29
Lepromin, 1/30						
0–1	3	–	–	–	–	3
2–	2	–	1	–	–	3
4–	2	2	2	1	2	9
6–	2	2	1	–	3	8
8–	–	–	–	–	3	3
10–	–	1	–	–	1	2
12–	–	–	–	–	–	–
14–	–	–	–	–	1	1
Total	9	5	4	1	10	29

The normal skin suspension caused no less than 6 reactions of the 4 mm. grade—none larger—among 29 persons of whom 23 gave reactions of that size or larger to the lepromin. It is concluded that the tissue element in lepromins of usual strength plays a part which cannot be totally neglected, and that in some persons it may cause weakly "positive" reactions.

In the third group of (141) healthy persons, 0.1 cc. doses of a 1/90 normal skin suspension and of a 1/90 lepromin dilution were injected. From the first part of Table 3 it can be seen that this diluted lepromin still gives many satisfactorily strong reactions. (The table shows that 74, or 52 per cent, gave reactions measuring 4 mm. or more.) This

finding has been confirmed by an investigation with this lepromin in 124 tuberculoid patients, of which 87 per cent showed reactions measuring 3 mm. or more.

TABLE 3.—*Three-week reactions to* (a) *a 1/90 dilution of lepromin, and* (b) *to a 1/90 normal skin suspension, in 141 healthy persons.*

Size of reactions (mm.)	Age group (years)						
	0-4	5-9	10-14	15-19	20-39	40+	Total
Lepromin dilution, 1/90							
0–1	27	5	–	1	1	1	35
2–	10	3	3	6	10	–	32
4–	8	1	5	11	10	5	40
6–	1	1	–	2	18	6	28
8–	–	–	–	1	1	–	2
10–	–	–	–	1	1	2	4
Total	46	10	8	22	41	14	141
Normal skin suspension, 1/90							
0–1	46	9	6	12	30	9	112
2–	–	1	2	10	11	5	29
4–	–	–	–	–	–	–	–
6–	–	–	–	–	–	–	–
8–	–	–	–	–	–	–	–
10–	–	–	–	–	–	–	–
Total	46	10	8	22	41	14	141

From the second part of Table 3 it appears that the tissue component in a 1/90 normal tissue suspension is not entirely without effect. If this finding can be applied to the reactions to lepromin itself, the tissue elements thereof can have only a minor effect, but should not be entirely ignored. Further dilution of 1/90 lepromin for testing is certainly possible, but due to many possibilities of making errors, more difficulties would arise in the evaluation of the weak reactions. If one wants to minimize the influence of the tissue component, it is recommended to start with strongly-positive lepromatous material, thoroughly cleansed of tissue extraneous to the lepromas themselves, and to dilute the lepromin suspension a little more than usual.

The question remains whether the tissue element is of major importance for the evaluation of the result of the lepromin test. Morphologically, the course of the reactions to the normal skin suspension does not differ essentially from the course of the lepromin reactions. The impression has been gained, from the periodic readings shown in Table 4, that the normal-skin reaction reaches its maximum size somewhat more quickly than the lepromin reaction does, but this has to be verified with a larger material.

Rutgers ([8]) believes that the lepromin reaction is accelerated in tuberculin-positive compared with tuberculin-negative persons. Consequently, the tuberculin reactions of the subjects have to be taken into account in the composition of groups for normal skin reactions as well.

TABLE 4.—*Progression of reactions in healthy persons to* (a) *1/30 and* (b) *1/90 concentrations of normal skin suspensions, and to* (c) *1/30 and* (d) *1/90 concentrations of lepromin; readings at 10, 21 and 28 days.*

Time of reading (days)	Size of reaction (mm.)											
	0	1	2	3	4	5	6	7	8	9	10	>10
Normal skin suspensions, 1/30												
10	1	3	5	11	6	-						
21	8	5	6	2	2	3						
28	17	1	4	2	2	–						
Normal skin suspension, 1/90												
10	48	12	18	2	1	–						
21	62	7	10	2	–	–						
28	73	–	6	2	–	–						
Lepromin, 1/30												
10	–	3	–	2	3	5	6	4	1	–	2	–
21	2	1	1	2	3	5	2	5	1	2	–	–
28	3	–	1	2	5	1	3	4	3	1	–	2
Lepromin dilution, 1/90												
10	18	8	12	13	13	4	9	3	1	–	–	
21	19	3	6	13	16	7	7	7	1	–	2	
28	24	–	5	12	16	8	7	5	1	1	2	

In our material, of which a part of the test persons had been tested with 5 TU of PPD, we noticed that on the average the tuberculin-positive test persons showed a stronger reaction to normal skin than the tuberculin-negative persons. Also, the reactions to normal skin were on the average stronger in adults than in the younger age groups.

When a correlation diagram of the reactions to lepromin and to normal skin is made (Table 5), a definite correlation in size appears to exist between the two reactions. This has also been noticed in the reactions of a group of tuberculoid patients. In a group of adult lepromatous patients with lepromin reactions of less than 3 mm., no reactions more than 2 mm. to 1/30 normal skin suspension were seen. Some borderline patients with lepromin reactions of 4-5 mm. size (lepromin 1/30) showed normal-skin reactions of 1-2 mm.

Although the possibility that lepromatous patients can react to very concentrated skin suspensions or fractions of normal skin is not yet definitely excluded, it nevertheless does not seem improbable that

there is an antigenic relationship between the leprosy bacillus and components of certain of the tissue elements.

TABLE 5.—*Correlation diagram of reactions to normal skin suspension 1/15, and to lepromin 1/20 in healthy persons.*

Lepromin	Normal skin suspension (mm)							
	0	1	2	3	4	5	>6	Total
0–1	10	14	7	–	–	–		31
2–		5	14	4	–	–		23
4–		1	9	3	1	–		14
6–			6	11	3	1		20
8–			3	2	1			6
10–				2	–		1	4
12–				1	1		1	3
Total	10	20	39	23	6	1	2	101

The experience that children generally react more weakly to lepromin and to the normal skin suspension than do adults does not speak in favor of the concept that here we have to do with foreign-body reactions. It is not clear, either, how for example a previous tuberculosis infection or a BCG vaccination could intensify a foreign-body reaction. If this were the case, one would expect that such an organism would react more strongly to other, nonacid-fast, bacilli too, which has not been proved in practice.

That the leprosy bacillus in lepromin acts as a foreign body becomes improbable when we consider the fact that lepromatous patients do react to injections with other acid-fast bacilli.

In a group of 96 lepromatous patients, 0.1 cc. of a suspension of a strain of acid-fast bacillus "875" was injected. This "875" bacillus was morphologically identical to *M. leprae,* but it could be grown on Sabouraud agar at room temperature, and therefore it differs from both *M. leprae* and *M. tuberculosis.* This suspension had been standardized according to the method of Wade, so that the bacillary density was about the same as that of the 1/30 lepromin which was injected simultaneously in the same patients, together with a dose of the 1/15 normal skin suspension. The results are shown in Table 6, in which the reactions to the "875" suspension are shown in total and are also divided according to the tuberculin sensitivity of the patients.

In all of the lepromin-negative lepromatous patients there were seen strong reactions to the "875" suspension. The reactions in tuberculin-positive patients were, on the average, stronger than in the tuberculin-negatives, on the order of 10.6 mm. *vs* 6.5 mm. If the lepromin reaction were only a foreign body reaction, it would be difficult to understand why these patients reacted to the foreign body "875," but not to the foreign body "*M. leprae.*"

It seemed that the lepromatous patients did react somewhat more

TABLE 6.—*Reactions to suspension (1/30) of "Bacillus 875," total and in tuberculin-positive and -negative groups, compared with reactions to 1/15 normal-skin suspension and 1/30 lepromin, in a group to 96 adult lepromatous patients.*

Antigen	Reaction size (mm.)										Total
	0-1	2-3	4-5	6-7	8-9	10-11	12-13	14-15	16-17	18-19	
"875" 1/30 (total)	0	5	14	14	24	21	11	3	3	1	96
"875" 1/30 PPD positive (5 TU)	0	0	2	4	13	17	10	3	3	1	53
"875" 1/30 PPD negative (5 TU)	0	5	12	10	11	4	1	–	–	–	43
Normal skin 1/15	84	11	1	–	–	–	–	–	–	–	96
Lepromin 1/30	51	41	4	–	–	–	–	–	–	–	96

weakly to the "875" suspension than did healthy persons of similar age groups and comparable tuberculin sensitivity. The differences were small, however, and the groups were not large enough to establish this observation with certainty. This might nevertheless indicate that *M. leprae, M. tuberculosis,* the "875" bacillus, and also normal skin, have one or more components in common, and that it is typical for the lepromatous patient to miss the ability to react to this component and thus to destroy the bacillus.

Another objection to considering the lepromin reaction as an indication of resistance against leprosy has lately been raised by Collier (1). He believes the nonreacting of lepromatous patients to lepromin to be a consequence of the presence of large numbers of bacilli which would cause an "allergic paralysis." Although this explanation seems attractive, serious objections to it must be made. There is no complete parallel between the size of the lepromin reaction and the number of bacilli present in the body. When a series of early indeterminate-group patients is tested with lepromin it is found that many of them already show positive reactions, but some of them are negative and remain negative afterwards too. The latter will develop into the lepromatous type if no adequate treatment is given. In this early indeterminate phase the number of bacilli is still small, in spite of which fact—according to Collier's thesis—an allergic paralysis must already have been induced. Among the early indeterminate cases with weakly positive lepromin reactions it is not uncommon to find some patients who will in the near future show a conspicuous reactional tuberculoid form of leprosy, with temporary rather strongly-positive smears. Often these patients become spontaneously negative, whereas the lepromin reaction has grown more strongly positive. Here the relatively large number of bacilli evidently has not caused an allergic paralysis.

Furthermore, the early lepromatous patient is always lepromin negative, while borderline cases which already have progressed far to the lepromatous side may still show distinct if weak positive reactions. In the latter cases the quantity of bacilli present is many times larger than that in the former cases, but nevertheless no allergic paralysis has manifested itself.

During a survey with lepromin and tuberculin I sometimes found adult, PPD-positive persons giving no reaction whatever to lepromin, with leprosy symptoms on examination, but after two years or so the first symptoms of lepromatous leprosy were detected. It can be assumed that at the moment of examination the leprosy infection must already have been present, but at that time the number of bacilli was very small in any case and insufficient to produce an allergic paralysis.

There is a definite parallel between the size of the lepromin reaction, clinical symptoms, histologic picture, and the course of the disease in untreated patients, but not between lepromin reaction and quantity of bacilli. Although some investigators report conversion of lepromin reactions from positive to negative during the development of the lepromatous type, and others tell of conversions from negative to positive in some lepromatous patients after arrest of the disease, such information is scarce and does not correspond with the experience of many other leprologists who have never seen such phenomena.

On examination of the patients in some leprosaria I found that all who showed a weakly positive lepromin reaction after prolonged treatment were clearly not purely diffuse lepromatous, or had shown unmistakable borderline symptoms.

Similar patients often show weakly positive reactions also during the active, strongly-positive phase of the disease, which makes a conversion improbable. Especially with these patients with weakly positive reactions the type of lepromin and the technique of the test play an important part; on repeating the test a difference in reaction of some millimeters can easily be found, and one would be inclined to assume a conversion.

As long as the arguments against the relationship between the lepromin reaction and resistance against leprosy infection are not more convincing than they are now, it seems wise to maintain the statement of the Madrid congress that "a positive lepromin reaction is an expression of a certain amount of resistance to *M. leprae,* directly proportionate to the degree of positivity."

SUMMARY

Lepromins of different origin may differ materially with respect to the numbers of bacilli and the amounts of tissue elements they contain. Emphasis is laid on the importance of giving in reports technical

data about lepromin used, and to expressing the size of the reactions observed in millimeters of infiltration, instead of speaking of "positive" and "negative" reactions. Thus comparison of the results of different workers would be made possible.

The influence of the tissue elements on the lepromin reaction is evaluated. The findings of Kooij and Gerritsen that normal skin suspensions may produce "positive" reactions in leprosy patients has been confirmed in healthy people. A correlation in size was found between normal skin reactions and lepromin reactions. However, the influence of the tissue element in lepromin can be neglected if a lepromin of usual strength, prepared from bacillus-rich tissue, is slightly diluted.

The hypothesis of Kooij and associates that the bacilli in lepromins act as a foreign body is doubted, because intradermal injection of a suspension of a strain of acid-fast bacilli ("875") morphologically identical to but different from both *M. leprae* and *M. tuberculosis*, produces strong reactions in lepromatous patients. It is difficult to see why these patients do not react to the "foreign body *M. leprae*," as they do to the "foreign body 875." Acceptance of a specific factor in the lepromin reaction is more in agreement with the facts.

The hypothesis of Collier that the lepromin negativity of lepromatous patients can be explained on the ground of an allergic paralysis, produced by the presence of large numbers of bacilli, is not in agreement with the findings in the different types of leprosy.

RESUMEN

Las leprominas de distinta procedencia pueden discrepar decididamente con respecto al número de bacilos y la proporción de elementos histológicos que contienen. Se recalca la importancia de ofrecer en los informes datos técnicos acerca de la lepromina usada y de expresar el tamaño de las reacciones observadas en milímetros de infiltración, en vez de hablar de reacciones "positivas" y "negativas". De este modo, sería posible comparar los resultados obtenidos por distintos técnicos.

Se justiprecia el influjo de los elementos histológicos sobre la reacción a la lepromina. Los hallazgos de Kooij y Gerritsen de que suspensiones de piel normal pueden producir reacciones "positivas" en leprosos han sido confirmados en sujetos sanos. Se ha observado una correlación del tamaño entre cutirreacciones normales y reacciones a la lepromina. No obstante, puede desatenderse el influjo del elemento histológico en las leprominas si se diluye ligeramente una lepromina de la concentración habitual, preparada de tejido rico en bacilos.

Se pone en duda la hipótesis de Kooij y colaboradores en el sentido de que los bacilos de las leprominas obran como cuerpo extraño, porque la inyección intradérmica de una suspensión de una cepa de bacilos ácidorresistentes ("875"), morfológicamente idéntica pero distinta tanto del *M. leprae* cuanto del *M. tuberculosis*, produce reacciones intensas en los enfermos lepromatosos. Es difícil comprender por que esos enfermos no reaccionan al "cuespo extraño *M. leprae*" lo mismo que al "cuerpo extraño 875". La aceptación de un facto específico en la reacción a la lepromina concuerda mejor con los hechos.

La hipótesis de Collier de que cabe explicar la negatividad de los enfermos lepromatosos a la lepromina a base de una parálisis alérgica producida por la presencia de grandes cantidades de bacilos no conviene con los hallazgos obtenidos en las distintas formas de lepra.

RESUMÉ

Des lépromines de sources diverses peuvent différer par leurs contenus respectifs en bacilles et en éléments tissulaires. L'auteur souligne qu'il est important de préciser dans les rapports les spècifications techniques de la lépromine utilisée. Il recommande d'exprimer l'étendue des réactions observées en millimétres d'infiltration, plutôt que d'indiquer sommairement "réaction positive" ou "réaction négative". La comparaison des résultants obtenus par différent chercheurs serait ainsi rendue possible.

L'intervention des éléments tissulaires dans la réaction à la lépromine est discutée. La possibilité d'obtenir des réactions "positives" par l'injection de suspensions de peau normale, signalée par Kooij et Gerritsen chez des malades de la lèpre, est confirmée chez des sujets sains. Une corrélation a été constatée entre les dimensions obtenues par la réaction à la peau normale et celles observées avec la lépromine. On peut néanmoins négliger cette intervention des éléments tissulaires présents dans les differentes especès de lépromine, si celle qui est utilisée, d'une activité normale et préparée à partir de tissus riches en bacilles, est légèrement diluée.

L'hypothèse que les bacilles présents dans la lépromine agissent comme des corps étrangers, émise par Kooij et collaborateurs, est mise en question. En effet, il a été remarqué que l'injection intradermique d'une suspension d'une souche de bacilles acido-résistants ("875"), identiques morphologiquement mais cependant distincts de *M. leprae* et de *M. tuberculosis*, entraîne de fortes réactions chez les malades lépromateux. Il est malaisé de comprendre pourquoi ces malades ne réagissent pas au "corps étranger *M. leprae*", alors qu'ils réagissent au "corps étranger 875". Il est davantage en accord avec les faits d'accepter l'intervention d'un facteur spécifique dans la réaction à la lépromine.

L'hypothèse suggérée par Collier pour expliquer l'absence de réaction à la lépromine chez les lépromateux, qui se base sur un bloquage de l'allergie causé par une grande abondance de bacilles, ne correspond pas aux observations reccueillies dans les différents types de lèpre.

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