

INTERNATIONAL JOURNAL OF LEPROSY

VOLUME 29, NUMBER 2

APRIL-JUNE, 1961

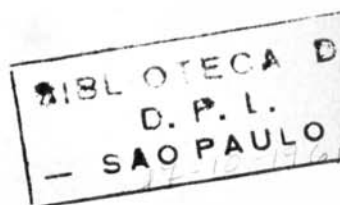
RESULTS OF REPEATED INJECTIONS OF LEPROMIN IN TUBERCULOID CASES

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There can no longer be any doubt that the injection of lepromin in the Mitsuda test—it being in effect a “microvaccination” (Ustvedt)—modifies the immunological status of an individual, as stated in the recommendations on immunology of the Tokyo congress ⁽⁹⁾. Furthermore, a second test dose adds to the effect, at least in normals, so that by that means positive results can be elicited in many persons who were negative to the first one, as was pointed out by one of us editorially ⁽¹⁰⁾, and was most recently shown again by Bechelli ⁽¹⁾, who also showed that large proportions of persons who react weakly (1+) to the first test will show *intensification* of the reaction (to 2+ or more).

The matter of *acceleration* of the reaction in tuberculoid patients—which Fernandez ⁽²⁾ has called the “Olmos Castro phenomenon”—is not as well established since it seems contrary to general experience. Although Mitsuda ⁽⁵⁾ spoke as if the reaction, when positive, reached its maximum after about 15 days, Hayashi ⁽³⁾ said that the reaction should be looked for on the 8th, 16th, and 24th days after the injection, because the late reactions might not appear before the second or third week. In the many studies involving patients which ensued over the years, it was concluded that positive reactions would generally be expected to reach their maxima after about three weeks.¹ More recently, however, evidence has been advanced by certain workers that, while this is generally true of reactions in normal people (controls), the reaction is accelerated in tuberculoid patients, and that in them the maximum is reached in about two weeks.

¹ The pertinent part of the Tokyo Congress report ⁽⁹⁾ reads: “This reaction consists of a nodular induration which usually begins to appear after the first week . . . [and] ordinarily reaches maximum about the third or fourth week. . . . Sometimes the evolution is accelerated and reaches its peak before the third week, while at other times it is delayed, reaching its peak after the fourth week.”

Rutgers⁽⁸⁾ and Kooij and Rutgers⁽⁴⁾, who used the relatively weak Dharmendra antigen, observed acceleration of the reaction in tuberculoid cases. For example, it can be calculated from their figures that while only 13 per cent of the healthy controls had become positive—4 mm. or more—by the 7th day (and 35% by the 14th day), 39 per cent of the tuberculoid cases had reached that stage by the 7th day (and 43% by the 14th day). Acceleration was also seen in the tuberculosis cases tested, and in the leprosy contacts. As for the average measurements, it is shown in a graph that the maximum was reached on the 7th day in the tuberculoid group (54 cases), on the 14th day with the tuberculosis patients (93), on the 21st day with the leprosy contacts (84), and on the 28th day with the healthy controls (99).

Olmos Castro and associates^(6,7) tabulated the results of single tests in a total of 51 tuberculoid cases. Almost all of them showed "erythematous infiltration" on the 2nd day, but by the 7th day the reaction lesions of 31 had changed to "erythematous nodules," although as a rule the maximum was reached on the 14th day.

They also showed that on second testing of 6 normal, reactive persons after a 21-day interval, there was both acceleration and intensification of the reactions, with wide differences among the individuals. The curve of averages shows that the maximum of the late reaction was shifted from 21 days to 14 days. The most conspicuous differences were seen in the early reaction, on the 2nd day. All 6 had been negative at 2 days on the first test (average 3.5 mm., range 2.5-5.0 mm.), but they were all positive on the second occasion. Two of them then averaged 25 mm. (23.0 and 27.5 mm.); the other 4 averaged 11.4 mm. (range 9.5-15.5 mm.).

Contemplation of these reports led us to wonder what effects would be seen with *serially repeated* testing of tuberculoid cases. Would such treatment lead to both sustained intensifying and further accelerating of the reactions? Such changes had been seen in a minority of a small group of normal persons so tested in work done some years ago but as yet only briefly mentioned in print⁽¹⁰⁾. To investigate this matter, albeit on a small scale, was the purpose of the work here reported. The results, not surprisingly, appear to be negative.²

MATERIAL AND METHOD

Fifteen adult tuberculoid cases in the Alupe leprosarium—all of the adult patients who were available at the time—were taken for this experiment. All of them were bacteriologically negative at the time, although three had been positive to a limited degree (bacillus index 0.2, 0.3 and 0.8, 6-smear test) when admitted some months before. All were under sulfone treatment except two, who were receiving DPT (Ciba 1906).

After each injection of lepromin, readings were made after the 2nd, 4th, 6th, 9th, 14th and 21st days, in order to observe when the reactions reached their maximum size. This schedule was modified slightly on occasions when Sundays or holidays interfered, but it is believed no essential errors resulted on that account.

On the 21st day after the first injection a second one was made, with readings spaced as before. The injections were continued in this way to a total of six, the readings totalling a period of 18 weeks. The lepromin used had been prepared (H.W.W.) by the technique published in a WHO committee report⁽¹¹⁾. The test dose, as usual, was 0.1 cc.

All the injections were given on the volar surface of the forearms, starting at the

² The only experimental observation of this sort, to our knowledge, was referred to in a three-line paragraph in the annual report for 1958 of the Hind Kusht Nivaran Sangh (Indian Leprosy Association) (see THE JOURNAL 28 (1960) 498), speaking of work of Figueredo, of Bombay. "Repeated injections of lepromin (Dharmendra type) in progressively increasing doses were found to lower the reaction (early as well as late) in 4 tuberculoid cases."

top near the antecubital region, and alternating sides going down toward the wrist with an interval of 2-3 inches between the sites. The sites were marked by touching the skin nearby with a probe dipped in liquid phenol.

RESULTS

THE MITSUDA REACTION

The results of the tests in terms of the late, or Mitsuda reaction, which was the primary objective of the inquiry, are shown in Table 1.

TABLE 1.—Mitsuda reactions induced in a series of 6 repeated tests done at 3-week intervals in 15 tuberculoid cases, in mm. on the day when the maximum reading was first observed.

Patient	Number of test					
	1st	2nd	3rd	4th	5th	6th
	Max. (mm.) Day	Max. (mm.) Day	Max. (mm.) Day	Max. (mm.) Day	Max. (mm.) Day	Max. (mm.) Day
1. Geor.	9 - 21st	9 - 10th	10 - 14th	8 - 14th	14 - 17th	8 - 8th
2. Nguk.	7 - 21st	8 - 21st	6 - 19th	6 - 14th	7 - 18th	8 - 19th
3. Mawe.	7 - 21st	7 - 21st	7 - 14th	7 - 7th	9 - 19th	7 - 19th
4. Aolo.	7 - 21st	7 - 28th	Leave	Leave	9 - 15th	Left
5. Joha.	10 ^u - 21st	12 - 28th	10 - 14th	11 - 19th	16 ^u - 24th	16 - 11th
6. Paul.	10 ^u - 21st	11 - 21st	12 - 14th	15 - 19th	Ulcerated; quit	
7. Muru.	11 ^u - 21st	12 ^u - 21st	8 ^u - 14th	9 - 14th ^a	11 ^u - 15th	12 - 8th
8. Musi.	7 - 14th	9 - 21st	8 - 14th	6 - 14th	Leave	Leave
9. Mako.	7 - 14th	8 - 21st	Leave	Leave	9 - 19th	7 - 11th
10. Jako.	8 - 14th	8 ^u - 28th	9 ^u - 14th	Leave	11 - 18th	8 - 8th
11. Kany.	9 - 14th	8 - 21st	10 - 14th	9 - 14th	9 - 21st	7 - 17th
12. Anya.	9 - 10th	7 - 21st	10 - 19th	10 - 14th	11 - 14th	7 - 11th
13. Mwat.	11 - 10th	8 - 7th	8 - 14th	9 - 19th	10 - 17th	9 - 17th
14. Aoka.	12 ^u - 10th	12 ^u - 14th	11 ^u - 14th	13 ^u - 14th	13 ^u - 18th	10 ^u - 11th
15. Musa.	8 - 8th	10 - 21st	10 - 14th	8 - 9th	10 - 28th	10 - 14th

^a Tuberculoid reaction.

^u = ulceration.

Scanning the figures of the table as a whole leads to a sense of confusing variations, with no definite or consistently sustained trend regarding either acceleration or intensification of the reactions.

From the results of the first test, which ordinarily is the only one made for determining the immunologic status of a case, it is seen that in 7 of the 15 patients—practically one-half of the total—the maximal reaction was not attained until about the 21st day—i.e., after the 14th day, no readings having been made in the interval. Thus it appears that there was no acceleration of the reaction in these cases, although 3 of them were fairly strong reactors, with ulcerations.

The other 8 cases, however, reached their maximal sizes earlier—4 by the 14th day, 3 by the 10th day, and one as early as the 8th day. This last one, it may be noted, took 21 days to reach maximum after the second test, and varied remarkably throughout the series.

The second tests, instead of showing general intensification of the reaction (beyond 1 mm., which could be within the limit of observational error), did so in only 3 cases, while on the other hand there was a comparable decrease in 2 cases. More striking is the fact that there was usually a delay in reaching maximum. Acceleration was seen in only 2 cases, but a delay of a week or even two weeks in no less than 9 of the cases, including the fastest reactor to the first test. It would seem as though there had been a partial exhaustion of the reactive antibodies in those cases, which took some time to replace.

At the end of the series of injections—ignoring unexplainable fluctuations in the interim—there had been *intensification* of the reactions in material degree in only 2 of the patients (Nos. 5 and 6, the latter of whom withdrew because of the severity of the reaction and ulceration after the 4th test), and of doubtful significance in a third (No. 15).

Acceleration of the final reactions was seen in only 5 instances:

CASE 1: From the 21st day (1st test) to the 8th day (6th test), but 17 days for the 5th test (without intensification).

CASE 4: From the 21st day to the 15th (5th test), (without material intensification).

CASE 5: From the 21st day to the 11th day (6th test)—but 24 days for the 5th—(in this instance there being also intensification).

CASE 7: From the 21st day to 8th day (6th test)—an abrupt change from the previous repeat tests, (without intensification).

CASE 10: From the 14th day to 8th day (6th test), but abruptly down from all previous tests, following a bout of reaction (without intensification).

It happens that all but the last of these cases—the one who had experienced reaction—were among the originally slow (21-day) reactors.

It thus appears that in tuberculoid leprosy cases a considerable proportion may be expected, on first test, to require the usual three-week period for the maximal development of the Mitsuda reaction, even though that may be a strong one with ulceration.

Curiously, of the 8 earlier-reacting cases, all but 2 required on the second test the three-week period, or more, for the maximal reaction—and in no instance was the reaction materially larger than the first one had been.

Actual intensification after the series of tests occurred in only a few patients; in most of them, the infection itself was associated with maximal reactivity of the individuals. Acceleration occurred in more of them, but mostly in those who at the first test had reacted most slowly.

On the whole, it seems that the infected patient, with the tuberculoid form of the disease, cannot be expected to respond to repeated testing with much intensification and/or acceleration of the reactions as normal persons may be expected to do.

THE FERNANDEZ REACTION

In practice the early, or Fernandez, reaction is not given the same importance as the Mitsuda reaction, and since it is difficult to read in dark-skinned persons it is usually not recorded by workers in Africa. However, in about one-half the cases it was possible to make out an erythematous area with an edematous wheal. It is usually impossible to define with any accuracy the area of erythema, but the wheal can be seen and is palpable.

This reaction is usually read as negative, or positive (i.e., an erythematous-edematous area 10 mm. or more in diameter), after 24 or 48 hours. It has been noticed, however, that if almost daily readings are made the reaction in these patients sometimes appears still later and grows larger, taking up to 7 or 8 days to reach its maximum, after which it may decrease and be replaced by the nodular type of reaction. The time of the wheal-like reaction not only varies from patient to patient, but in a given patient from occasion to occasion.

The detailed data on this reaction do not seem worth presentation here. Suffice it to say, first, that reactions to the first test of 10 mm. or more were seen in only 2 cases (Nos. 10 and 14), although 8 mm. reactions were recorded on that occasion for 2 others (Nos. 3 and 11); also a 14 mm. reaction was recorded on the second test for one patient (No. 5) who had been read as negative for the first one—and who never had anything like that reading again.

Apart from the case last mentioned there was none which showed increased sensitization to the stage of positivity during the period of serial testing. In fact, several patients showed a tendency to decrease of sensitivity; the others had not changed materially at the end, although in most of them there had been fluctuations along the way. In none was there the "spectacular enhancement" seen in normal persons subjected to similar serial testing in an older study (¹⁰).

No evidence has been seen that this intervention affected the later course of the disease in these patients, who were all improving under treatment at the time. The bout of lepra reaction experienced in one case (No. 7) cannot be regarded as anything but coincidental.

SUMMARY

In a group of 15 tuberculoid leprosy patients an attempt was made to modify the reactions to lepromin, as can be done in normal persons, by a series of 6 serial injections made at 3-week intervals.

In the first test, practically one-half (⁷) of the patients showed no acceleration of the maximum from the usual three weeks; the period was shortened in the other 8 patients, to two weeks or even less. It appears that among African cases of tuberculoid leprosy a considerable proportion do not show, when first tested, a more rapid Mitsuda response than usual.

An unexpected feature of the observed results was that most of the patients took longer to reach their maxima in the second tests than in the first ones. That was not because the reactions were materially larger; it seems possible that the first tests may have caused partial exhaustion of available reactive antibodies, and that their replacement was somewhat slow.

At the end of the series of tests, in the course of which many of the patients showed considerable degrees of variation, there was little evidence of sustained acceleration of the reaction, and less of sustained intensification. What acceleration there was occurred mainly in the slower (21-day) reactors to the first test.

These results suggest that in infected people with tuberculoid form of leprosy the reactivity to lepromin can at most be only moderately accelerated, when at all, by multiple serial testing, and cannot be much intensified. It is as if their maximal reactivity had in general been attained by the effects of the infection itself.

It is hoped that this investigation may be repeated, on a larger scale, and to include leprosy contacts, in some other part of the world.

RESUMEN

En un grupo de 15 enfermos de lepra tuberculoidea se trató de modificar las reacciones a la lepromina, según puede hacerse en personas normales, con una serie de 6 inyecciones seriadas practicadas a plazos de 3 semanas.

En la primera prueba, prácticamente la mitad (7) de los enfermos no reveló aceleración del máximo de las tres semanas de costumbre; en los otros 8 enfermos se acertó el período a dos semanas y hasta menos. Parece que, entre los casos africanos de lepra tuberculoidea, una proporción considerable no revela, al ser ensayados por primera vez, una reacción de Mitsuda más rápida de lo habitual.

Una característica inesperada de los resultados observados fué que la mayor parte de los enfermos tardó más tiempo en alcanzar el máximo en las segundas pruebas que en las primeras. No sucedió esto porque las reacciones fueren notablemente mayores; parece posible que los primeros ensayos ocasionasen agotamiento parcial de los anticuerpos reactivos disponibles, y que su reemplazo fuera algo lento.

Al terminar la serie de pruebas, en el transcurso de las cuales muchos de los enfermos mostraron grados considerables de variación, había pocos signos de aceleración sostenida de la reacción, y aun menos de intensificación sostenida. La aceleración, si la había fue principalmente en los reactores más lentos (21 días) de la primera prueba.

Denotan estos resultados que, en las personas infectadas con la forma tuberculoidea de la lepra, a lo más no cabe acelerar más que moderadamente la reactividad a la lepromina, si se acelera, con multicomprobación seriada, y tampoco cabe intensificarla mayor cosa. Es como si la reactividad máxima de los enfermos se hubiese alcanzado por los efectos de la infección misma.

Espérase que se repita esta investigación, en mayor escala y comprendiendo casos de contacto leproso, en alguna otra parte del mundo.

RESUMÉ

Dans un groupe de 15 malades atteints de lèpre tuberculoïde, les auteurs ont essayé de modifier les réactions à la lépromine, ainsi qu'il peut être fait chez des sujets normaux,

par une série de 6 injections successives de lépromine administrées à 3 semaines d'intervalle.

Lors de la première épreuve, la moitié pratiquement (7) des malades n'ont pas montré une apparition plus précoce du maximum de la réaction, si l'on se réfère aux trois semaines habituelles. Chez les 8 autres malades, cette période a été raccourcie à deux semaines, ou même moins. Il en ressort que chez des africains atteints de lèpre tuberculoïde une proportion considérable des cas ne témoigne pas, lors d'une première épreuve à la lépromine, d'une réaction de Mitsuda plus rapide que de coutume.

Un phénomène inattendu fut observé. En effet, après la deuxième injection, chez la plupart des malades, la réaction atteignit son maximum plus lentement que lors de la deuxième épreuve. Ce n'est pas cependant que la réponse eutane ait été plus étendue. On peut envisager comme explication de ce résultat, un épuisement partiel des antigènes disponibles après la première injection, suivi de leur renouvellement à une allure relativement lente.

A la fin de cette série d'épreuves, au cours de laquelle d'importantes variations ont été notées chez les malades, il a été constaté que l'accélération de la réaction était peu manifeste. Le renforcement de la réaction est encore moins évident. Pour autant qu'une accélération de la réaction ait pu être notée, elle a été relevée surtout dans le groupe de malades ayant réagi le plus lentement (21 jours) lors de la première épreuve.

Ces résultats suggèrent que chez des malades atteints de la forme tuberculoïde de la lèpre, la réactivité à la lépromine ne peut tout au plus être que modérément accélérée, pour autant qu'elle le soit, par la répétition multiple du test, et qu'elle ne peut pas être renforcée. Tout se passe comme si la réactivité maximale avait généralement été acquise par l'effet de l'infection elle-même.

Les auteurs souhaitent que ces recherches soient répétées dans d'autres parties du monde, sur une plus vaste échelle, et de manière à inclure des contacts de malades atteints de lèpre.

ACKNOWLEDGMENT

The senior author wishes to thank the Administrator, East Africa High Commission, for permission to publish.

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