COMPARISON OF THE MACROSCOPIC READINGS AND MICROSCOPIC FINDINGS OF THE LEPROMIN REACTION [1]

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Since Mitsuda reported, first in 1919 ([7]) and again briefly in 1923 ([8]), on the skin test which later came to be known as the lepromin test, and especially since F. Hayashi ([5, 6]) developed it, a great deal has been written about it. Although there are still uncertainties about some of the features of the late, or Mitsuda, reaction to lepromin, especially its fundamental nature and mechanism, there is no disagreement about the value of that reaction in patients: positivity signifies a certain amount of resistance against the leprosy infection.

The histopathology of the positive late reaction nodule was investigated by Mitsuda himself in three specimens, and—at least in the translation of his original report—he spoke of a "tuberculoid tissue"; and Hayashi ([6]) said that his own findings had been consistent with those of Mitsuda. Since then numerous other authors have investigated the matter, including Schujman ([14]), Rabello and Rotberg ([12]), Nagai ([9]), Tachikawa ([15]), Alayon ([1]), Büngeler and Fernandez ([4]), Nolasco ([10]), Büngeler and Alayon ([3]), Piñeyro Rodriguez ([11]), and especially Yokota ([17]). In general these workers agree that the macroscopically positive late reaction lesion consists histologically of a tuberculoid granuloma. It is interesting to note Schujman's observation that a small number of positive reactions did not show the tuberculoid granuloma microscopically.

In 1956, we ourselves ([13]) examined the reaction lesions of a small number of patients with indeterminate and lepromatous leprosy which had cleared up under sulfone treatment and had been submitted to oral BCG vaccination, and concluded that the clinical reading of the results of the test was not very reliable. Some of the reactions which had been read as doubtful showed the presence of the tuberculoid granuloma, and some positive reaction lesions proved not to be tuberculoid. More recently, Bechelli *et al.* ([2]) also reported that a macroscopically positive reaction nodule does not always correspond to a tuberculoid granuloma. We have now extended our study, on more leprosy patients and several who did not have leprosy.

[1] This paper was prepared for presentation at the VII International Congress of Leprology, held in Tokyo, Japan, November 12-19, 1958, but another paper was chosen for the program.

PRESENT STUDY

Material.—In this study the sites of 94 lepromin tests were biopsied. On clinical, macroscopic reading only 2 of the reactions were negative; 21 were doubtful, 60 were 1+ positive, and 11 were 2+ positive.

The patients from whom these specimens were obtained comprised 41 clinically cured lepromatous cases, 7 tuberculoid cases, 22 indeterminate cases, 7 cases of pulmonary tuberculosis, and 17 healthy contacts. Most of the biopsies were made between 1 and 2 months after the injection of the lepromin.

To increase the accuracy of the microscopic readings over those of single-slide examinations, serial slides were made of all the material. Twelve slides, each having on the average 4 sections of 5 micron thickness, were examined for each specimen. The staining methods were: hematoxylin and eosin, Wade's modification of Fite's second technique for acid-fast bacilli ([16]), and Scarlet R for lipids (frozen sections).

Criteria for readings.—The macroscopic readings followed the criteria adopted by the VI International Congress of Leprology, Madrid 1953:

Negative (−): Absence of all local reaction between the first and fourth weeks.

Doubtful (±): Slight infiltration, difficult to detect, and less than 3 mm. at the point of inoculation.

Weak positive (+): Frank infiltration between 3 and 5 mm. in diameter.

Moderate positive (++): Nodular infiltration, larger than 5 mm. in diameter.

Strong positive (+++): Infiltration with ulceration.

The basis of the histopathologic reading was the presence or absence of the tuberculoid granuloma. We have not concerned ourselves with the quantitative aspect of the positive (tuberculoid) reactions, because we feel that the presence of a tuberculoid granuloma must have the same meaning whether it be big or small.

In our experience with such material we have found in negative reactions three different types of histologic changes: (*a*) simple chronic inflammatory reaction, made up of lymphocytes and histiocytes; (*b*) foreign-body giant-cell granuloma; and (*c*) predominantly fibrotic inflammatory reaction.

RESULTS

The results of the histologic examinations, the findings recorded as positive or negative, are grouped in Table 1 according to the clinical readings. The results of two examinations are given, one result based on a single slide chosen at random, the other result based on 12 serial slides. In the latter examination a total of 9 findings, mostly in 1+ specimens, were changed from negative to positive, as might be expected.

TABLE 1.—*Correlation between the clinical and histologic readings of lepromin reactions; comparison of results of single-slide and multiple-slide reactions.*

Clinical readings	No. of specimens	Histologic findings			
		Negative		Positive	
		Single slide	Multiple slides	Single slide	Multiple slides
Negative	2	2	2	0	0
Doubtful	21	14	13	7	8
Positive, 1+	60	20	13	40	47
Positive, 2+	11	6	5	5	6
Total	94	42	33	52	61

The striking feature of this analysis is the marked disagreement between the positive results in the macroscopic readings and the microscopic findings. Taking into consideration only the negative and posi-

tive reaction specimens, the results with respect to the correlation of the clinical and histologic readings are as follows:

Correlation	*Single slide*	*Multiple slides*
Agreement	47 (64.4%)	55 (75.4%)
Disagreement	26 (35.6%)	18 (24.6%)

If the clinically doubtful reactions are included and considered as positive, which must be the actual interpretation, we then have the following:

Correlation	*Single slide*	*Multiple slides*
Agreement	54 (57.5%)	63 (67.0%)
Disagreement	40 (42.6%)	31 (33.0%)

Table 1 also shows that: (1) Of the 21 doubtful reactions, 8, or 38 per cent, showed the tuberculoid granuloma histologically—microscopic positivity. One of these positives was negative after the single-slide examination.

(2) Of the total of 71 positive-reaction specimens, no less than 18, or 25 per cent, did not show the tuberculoid granuloma. This was the case with 13 of the 60 one-plus specimens (20 after the single-slide examination), and with 5 of the 11 two-plus specimens.

Analysis of our findings with respect to the cases from which the specimens were obtained (not including the 2 negative ones from arrested lepromatous cases), and correlation with the macroscopic readings, is shown in Table 2.

TABLE 2.—*Sources of the 92 specimens of doubtful and positive reactions, and correlations with microscopic findings and clinical readings.*

Source case	No. of cases	Tuberculoid histology	Macroscopic readings		
			±	1+	2+
Arrested lepromatous	39	Present	3	14	0
		Absent	10	10	2
Tuberculoid	7	Present	0	3	2
		Absent	0	0	2
Indeterminate	22	Present	3	13	2
		Absent	2	1	1
Pulmonary tuberculosis	7	Present	0	4	2
		Absent	0	1	0
Healthy contacts	17	Present	2	13	0
		Absent	1	1	0

The numbers of cases are obviously very small for a comparative study with respect to the clinical condition of the cases involved. However, consideration of the results in the arrested lepromatous group, which is the largest one, brings out certain points of interest.

(1) The proportion of specimens from this group which did not show the tuberculoid histology is far higher than the proportion from all the other case groups combined, i.e., 22 of 39 (57%) against only 9

of 53 (17%). If we take only the macroscopically positive specimens, ignoring the doubtful ones, the difference between the proportions is equally high (46% vs 13%).

(2) On the other hand, from the point of view of positivity, of the 26 specimens that were macroscopically positive, no less than 14 (or 54%) showed the tuberculoid histology, as did 3 of the 13 doubtful specimens (23%).

The positive findings confirm those of Rabello and Rotberg and of Nolasco that cases which have been lepromatous may give positive Mitsuda reactions. They are contrary to Rotberg's anergic-margin theory, according to which cases of lepromatous leprosy arise from the "anergic margin" of individuals because of their lack of capacity to produce the tuberculoid granuloma. They also show that the organism of the lepromatous patient, if brought to a good condition, is able to recover the defense reaction as evidenced by the capacity to form the tuberculoid granuloma.

SUMMARY

In extension of previous observations, we have made histologic examinations of the sites of 94 lepromin tests made on patients with arrested lepromatous leprosy and with tuberculoid and indeterminate leprosy, and also several cases of pulmonary tuberculosis, and healthy contacts. Clinically, the reaction sites biopsied were read as negative in 2 instances, doubtful in 21, one-plus positive in 60, and two-plus positive in 11.

Comparison of the histologic findings with respect to tuberculoid granuloma after examination of a single slide and of twelve serial slides shows that positivity may sometimes be missed by the single-slide examination. That happened in 9 instances in this study, mostly in one-plus specimens.

Comparison of the macroscopic readings and the final microscopic findings of the 73 positive reaction lesions shows agreement in 55 instances, or 75.3 per cent, and disagreement in 18, or 24.7 per cent. If the clinically doubtful specimens should be included these figures would be changed somewhat, with more disagreements, but their significance would not be changed.

These results show that the macroscopic reading of the results of the lepromin test is not reliable, if it be considered that the reaction is truly positive only when the tuberculoid picture is produced.

The following points are also emphasized:

(*a*) Of 21 specimens of clinically doubtful reactions, 8 (38%) showed the tuberculoid granuloma.

(*b*) Of the total of 71 specimens of positive reactions, 18 (25%) did not present the tuberculoid granuloma.

(*c*) Of 13 macroscopically doubtful reactions in arrested lepromatous cases, 3 (23%) presented the tuberculoid granuloma.

(*d*) Of the total of 26 positive reactions in arrested lepromatous cases, 14 (54%) presented the tuberculoid granuloma.

These results in arrested lepromatous cases are not in agreement with the idea that cases of lepromatous leprosy necessarily arise in Rotberg's anergic margin, because of an incapacity of those individuals to react to the infection with the relatively resistant tuberculoid granuloma, with the result that they undergo lepromatization.

RESUMEN

Extendiendo observaciones anteriores, se han ejecutado exámenes histológicos de los sitios de 94 pruebas de la lepromina verificadas en enfermos que tenían lepra lepromatosa estacionada y lepra tuberculoidea e indeterminada y también en varios casos de tuberculosis pulmonar y en contactos sanos. Clínicaments, los sitios de la reacción biopsiada se consideraron como negativos en 2 casos, dudosos en 21, positivos de uno más en 60 y positivos de dos más en 11.

La comparación de los hallazgos histológicos con respecto a granuloma tuberculoideo, después del examen de una sola laminilla y de doce laminillas seriadas demuestra que algunas veces puede pasarse por alto la positividad con el examen de una sola laminilla. Así sucedió en 9 casos en este estudio, mayormente en los ejemplares de uno más.

La comparación de las lecturas macroscópicas y de los hallazgos microscópicosa finales en las 73 lesiones de reacción positiva revela acuerdo en 55 casos, o sea en 75.3 por ciento, y desacuerdo en 18, o sea 24.7 por ciento. Si se incluyeran los ejemplares clínicamente dudosos, estas cifras variarían algo, con más desacuerdos, pero no cambiaría su significado.

Muestran estos resultados que la lectura macroscópica de los resultados de la reacción de la lepromina no es fidedigna, si se considera que la reacción es vedaderamente positiva únicamente cuando se produce el cuadro tuberculoideo.

Recálcanse además los siguientes puntos:

(*a*) De 21 ejemplares de reacciones clínicamente dudosas, 8 (38%) revelaron el granuloma tuberculoideo.

(*b*) Del total de 71 ejemplares de reacción positiva, 18 (25%) no manifestaban el granuloma tuberculoideo.

(*c*) De 13 reacciones macroscópicamente dudosas en casos lepromatosos estacionados, 3 (23%) presentaban el granuloma tuberculoideo.

(*d*) Del total de 26 reacciones positivas en casos lepromatosos estacionados, 14 (54%) presentaron el granuloma tuberculoideo.

Estos resultados en casos lepromatosos estacionados no concuerdan con la idea de que los casos de lepra lepromatosa aparecen forzosamente en el borde anérgico de Rotberg, debido a la incapacidad de estos sujetos para reaccionar a la infección con el relativamente resistente

granuloma tubercuoideo, dando por resultado que experimentan lepromatización.

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