

Tests with three antigens in leprosy-endemic and non-endemic areas *

J. CONVIT,¹ M. E. PINARDI,² F. ARIAS ROJAS,³ I. GONZÁLES,⁴ G. COREY,⁵ J. J. ARVELO,⁶ & H. MONZÓN⁷

A study comparing the 48-h and 30-day reactions produced by three antigens was made in areas of low and high leprosy endemicity in Venezuela and in areas of Chile, a non-endemic country. The antigens used for the intradermal tests were standard Mitsuda antigen, supernatant from standard Mitsuda antigen, and PPD. The results indicate that there is no difference in the Mitsuda reaction of persons living in areas of high or low endemicity, but they show a statistically significant difference between the reactions in persons who live in endemic areas and those of persons living in a country where the disease has not been described. The difference in the Fernandez reaction obtained with the supernatant was not statistically significant between the two population groups in the endemic country, but was highly significant when comparing the endemic and the non-endemic countries.

In 1916 Mitsuda (4) prepared an antigen by grinding lesions obtained from patients with lepromatous leprosy in saline and then sterilizing this suspension by autoclaving. This antigen, when injected in patients with lepromatous leprosy, did not evoke any type of response, but when injected in patients with tuberculoid leprosy and in a certain percentage of indeterminate and borderline patients it produced, 30 days later, a nodule varying in size from 3 to 4 millimetres. In some cases there was necrosis and the positive reactions almost always left a noticeable scar on the person tested. Later it was discovered that almost 98% of normal persons tested with this antigen gave a positive response, which was generally slightly less intense than in those with tuberculoid leprosy.

In 1940 Fernandez (2) described another response produced by this antigen, which appeared 48 h after the injection of the antigen in those with tuberculoid leprosy and in a certain number of normal persons who had been in close contact with persons with leprosy.

The Mitsuda antigen has been interpreted as a measure of the capacity of a certain individual to evoke an immunological response towards the infecting agent that produces leprosy. It is generally accepted that almost all patients with lepromatous leprosy give a negative response to Mitsuda antigen, whereas those with tuberculoid leprosy and most normal persons, by giving a positive reaction, show that they have the capacity to respond immunologically towards the leprosy bacillus. In the case of normal persons this antigen is used to determine host defence potential, and when persons living in close contact with patients are found to be persistently negative they are kept under close observation and even, in some countries, given preventive treatment against leprosy.

The Fernandez reaction has been interpreted by some workers as evidence of previous contact with the leprosy bacillus, even though other investigators feel that it could also be caused by other mycobacteria such as *Mycobacterium tuberculosis*.

Although the Mitsuda reaction has been studied extensively, in countries both endemic and non-endemic for leprosy, most of these studies were done before the present methods for the standardiza-

* From the PAHO Center for Training and Research in Leprosy and Related Diseases, Instituto Nacional de Dermatología, Caracas, Venezuela. This work was supported with funds from the World Health Organization through its IMMLEP Programme, and by Grant 016 of the Consejo Nacional de Investigaciones Científicas y Tecnológicas, Caracas, Venezuela.

¹ Director.

² Auxiliar Docente.

³ Jefe de la Cátedra de Medicina Preventiva, Universidad Central de Venezuela, Caracas.

⁴ Profesor de Dermatología, Facultad de Medicina, Universidad de Chile, Santiago de Chile.

⁵ Médico Epidemiólogo, Ministerio de Sanidad, Chile.

⁶ Médico Jefe del Departamento de Rehabilitación.

⁷ Médico Adjunto del Departamento de Dermatología Sanitaria.

tion of lepromin had been developed, and there are therefore no data on the number of bacilli in the antigens used.

The Fernandez reaction is not widely known and has been poorly evaluated in relation to epidemiological studies of general population groups. Therefore, its specificity to leprosy is still to be defined.

With a view to obtaining further data on the Mitsuda and Fernandez reactions by means of a standardized antigen, a survey was carried out in 3 communities differing in their epidemiological characteristics. The principal aims of this study were to determine whether the Fernandez reaction is specific for leprosy, and to determine its level of agreement with the Mitsuda reaction.

MATERIALS AND METHODS

The areas chosen for the study were as follows:

Highly endemic area. This area was represented by the village of El Tesoro, Barinas State, southeastern Venezuela. The prevalence of the disease at the moment of the investigation was 3.33%, there being 10 patients of which 6 were lepromatous or borderline and 4 tuberculoid or indeterminate. This village corresponds to a semidispersed rural zone with a population of 300, 245 of which were studied. Their distribution by age and sex is shown in Table 1.

Table 1. Population tested in El Tesoro distributed by age and sex.

Age group (years)	Male	Female	Percentage of total
0-4	12	18	12.3
5-9	39	24	25.7
10-14	16	22	15.5
15-24	19	18	15.1
25-34	10	14	9.8
35-44	12	13	10.2
45-54	6	3	3.7
55-64	6	6	4.9
65-74	1	3	1.6
75-84	1	2	1.2
total	122 (49.8%)	123 (50.2%)	100.0

Table 2. Population tested in El Corozo distributed by age and sex

Age group (years)	Male	Female	Percentage of total
0-4	9	17	14.7
5-9	33	19	29.4
10-14	20	13	18.6
15-24	8	11	10.7
25-34	1	20	11.9
35-44	4	5	5.1
45-54	3	7	5.6
55-64	3	1	2.3
65-74	1	2	1.7
75-84	-	-	-
total	82 (46.3%)	95 (53.7%)	100.0

Low endemic area. This area was represented by the village of El Corozo, also in Barinas State. At the time of the investigation it had a population of 250; there was only one case of leprosy, diagnosed as indeterminate, and a prevalence of 0.4%. In this village 177 persons were tested; their distribution by age and sex is shown in Table 2. Barinas State occupies fifth place in the prevalence scale for leprosy in the country, with a prevalence of 0.31%.

Non-endemic area. A rural zone of Chile, a country in which no autochthonous cases of leprosy have been diagnosed, was chosen. A group of 635 persons was studied; their distribution by age and sex is shown in Table 3.

Antigens

1. for the 48-h test we used a supernatant prepared by centrifuging standard lepromin (160×10^6 acid-fast bacilli per ml) for 2 h at $192\,000\text{ g}$, and filtering the supernatant through a $0.45\text{-}\mu\text{m}$ Millipore membrane.

2. Complete standard lepromin with a concentration of 160×10^6 bacilli per ml.

3. Purified protein derivative of mammalian tuberculin (PPD) with a concentration of 2 units, obtained from the Statens Serum Institut, Copenhagen.

All three antigens were injected simultaneously in amounts of 0.1 ml, in the volar surface of the

Table 3. Population tested in Chile distributed by age and sex

Age group (years)	Male	Female	Percentage of total
0-4	-	-	-
5-9	45	94	21.8
10-14	76	97	27.3
15-24	238	61	47.0
25-34	2	5	1.1
35-44	-	1	0.15
45-54	3	8	1.8
55-64	-	5	0.85
65-74	-	-	-
75-84	-	-	-
total	364 (57.3%)	271 (42.7%)	100.0

forearm. Antigens 1 and 2 were injected in the right forearm, antigen 3 in the left forearm.

For the sake of uniformity all the injections were given by the authors themselves, using disposable syringes and needles. The tests were read at 48 h and at 30 days. The diameter of each reaction was read in millimetres and recorded.

Reactions (erythema and infiltration) were grouped according to the following scale for the Fernandez reaction and the tuberculin test:

10-15 mm in diameter: 1+
 16-20 mm in diameter: 2+
 > 21 mm in diameter: 3+

The scale for reading the Mitsuda test was as follows:

5-7 mm in diameter: 1+
 8-10 mm in diameter: 2+
 > 11 mm in diameter: 3+

When there was necrosis, the letter N was added to the result.

RESULTS

Fernandez reaction

Table 4 shows the percentage of positives obtained with the test, which maintains a close correlation with the endemicity of the area. Thus, the smaller percentage, 3.5, corresponds to children in Chile (non-endemic area) and the maximum value, 43.5%, corresponds to the children of El Tesoro (highly endemic area).

Table 4A. Distribution of reactions and percentage positivity in children 0-14 years of age

Test		Chile	El Corozo	El Tesoro
Mitsuda	neg.	25	1	3
	1 +	136	13	20
	2 +	76	36	20
	3 +	75	61	88
	% +	92.0	99.1	97.7
Fernandez	neg.	301	84	74
	1 +	11	18	28
	2 +	0	3	17
	3 +	0	6	12
	% +	3.5	24.3	43.5
PPD	neg.	283	92	79
	1 +	19	12	30
	2 +	9	4	13
	3 +	1	3	9
	% +	9.3	17.1	39.7

Table 4B. Distribution of reactions and percentage positivity in adults 15-84 years of age

Test		Chile	El Corozo	El Tesoro
Mitsuda	neg.	19	0	1
	1 +	141	4	3
	2 +	81	15	17
	3 +	82	47	93
	% +	94.1	100.0	99.1
Fernandez	neg.	311	36	63
	1 +	10	13	25
	2 +	1	6	12
	3 +	1	11	14
	% +	3.7	45.4	44.7
PPD	neg.	232	42	72
	1 +	52	11	28
	2 +	15	4	11
	3 +	24	9	3
	% +	28.2	36.4	36.8

Table 4C. Statistical significance of the difference between the percentage of positives to the Fernandez and PPD tests

	Age group (years)	Percentage of positives		Difference ^a
		Fernandez	PPD	
Chile (n=635)	0-4	-	-	-
	5-14	3.5	9.3	S
	15-24	3.6	26.7	S
	25-34	0.0	57.1	-
	35-44	0.0	100.0	-
	45-84	6.2	37.5	NS
	total	3.6	18.9	S
El Tesoro (n=245)	0-4	30.0	10.0	NS
	5-14	47.5	48.5	NS
	15-24	54.0	43.2	NS
	25-34	45.8	41.6	NS
	35-44	48.0	36.0	NS
	45-84	28.5	25.0	NS
	total	44.1	38.4	NS
El Corozo (n=177)	0-4	7.6	0.0	-
	5-14	29.4	22.4	NS
	15-24	63.1	52.6	NS
	25-34	42.8	33.3	NS
	35-44	44.4	33.3	NS
	45-84	29.4	23.5	NS
	total	32.2	24.3	S

^a S=significant, NS=non-significant at the 5% level.

In healthy adults approximately the same phenomenon is seen. The smaller value, 3.7%, corresponds to the non-endemic zone of Chile and, even though an inversion of values is seen (45% in the area of low endemicity and 44.7% in the area of high endemicity) is not large enough to be significant. The difference between adults and children in the endemic areas can be explained easily by the much greater mobility of the adults, and therefore by the increased risk of being infected by *M. leprae*.

It is also important to point out that the 3.5% of positive reactions obtained in children in Chile were all weak (1+) reactions (Table 4A), and of the 3.7% positivity found in adults 3.1% corresponded to weak reactions. Only one reaction was classified as strong (3+)—i.e., 0.3% of the total number of tests in 323 healthy adults studied (Table 4B).

Analysis of narrow age-groups in Chile reveals a correlation between Fernandez and PPD in some of them but not in others (Table 4C). In the 45-84-year age-group the difference is not significant, whereas in the 25-34 and 35-44-year groups all the Fernandez tests were negative while 78.5% of the PPD tests were positive. However the small number of persons in these last two age-groups restricts the significance of this finding.

It is important to emphasize that in El Tesoro and El Corozo, the difference is not significant in all age-groups, as can be seen from Table 4C.

The statistical significance of the results obtained can be seen in Table 5. This seems to indicate that the Fernandez reaction can be considered near-specific for leprosy. We feel that once we have purified this antigen further and isolated the active fraction we could have a test specific for leprosy. Work of this nature is in progress in our laboratories at present.

The possibility that the values obtained in both adults and children in the endemic areas of Venezuela can be due to a cross reaction to *M. tuberculosis* appears unlikely when Tables 4A, 4B, and 4C are analysed. In the non-endemic area in Chile the PPD test in adults was 28.2% positive whereas the percentage of positive Fernandez tests was only 3.7, which seems to show that sensitivity to *M. tuberculosis* does not influence the positivity of the Fernandez test.

What is important is that in comparing positivity to the Fernandez test in children and adults in the area of high endemicity, El Tesoro, the crude numbers are basically the same. This could be explained by the nature of the sensitization towards *M. leprae*; it is possible that this sensitization is limited either to domiciliary foci and their surroundings or by limiting factors of the population itself.

Table 5. Statistical significance of differences between percentages of positivity

Test	Chile-El Corozo		Chile-El Tesoro		El Corozo-El Tesoro	
	children	adults	children	adults	children	adults
Mitsuda	S ^a	S	S	S	NS	NS
Fernandez	HS	HS	HS	HS	HS	NS
PPD	S	NS	HS	NS	HS	NS

^a S=significant, HS=highly significant, NS=non-significant at the 5% level.

Mitsuda reaction

The Mitsuda reaction, which was read 30 days after the injection of the antigen, was positive in the three communities studied, with percentages that varied from 92 in Chile to 100 in El Corozo (Table 4).

This high percentage of positivity in all the groups, even in the non-endemic area of Chile, undoubtedly reflects a non-specific response or cross-sensitization. The possibility of sensitization by the test itself has also to be considered. Nevertheless, the difference between the 92% positivity seen in Chile and the 100% seen in El Corozo and El Tesoro is statistically significant and must be interpreted as a direct influence of *M. leprae*. The results obtained in the areas of low and high endemicity do not show a statistical significance between the two villages or between children and adults (Table 5).

Another possible explanation for the high number of positive Mitsuda reactions in both Chile and Venezuela is that they might be due to the high potency of the antigen used (160×10^8 bacteria/ml), which could produce a certain percentage of false positive reactions. Unfortunately, owing to administrative and technical problems, it was not possible in this instance to take biopsies from the reactions to study them histologically. We are carrying out further work in this respect.

PPD test

The response to PPD in healthy children followed the same pattern as for the Fernandez reaction.

However, there was a noticeable difference between the Fernandez reaction and the PPD test in the adult population (Table 4) of non-endemic areas, which would favour the specificity of the Fernandez reaction. The statistical significance of the PPD test between the groups in non-endemic and endemic areas, as well as between the two groups in the endemic area, are highly significant among children but not in adults; this could be explained by the response to PPD in children being partially influenced by exposure to *M. leprae*.

CONCLUSIONS

There are no important differences in the positive response to the Mitsuda antigen between healthy children and healthy adults in the areas of high and low endemicity in Venezuela.

There is a statistically significant difference in the response to the Mitsuda antigen between the endemic areas and the non-endemic area, both in children and in adults, which might be due to a specific sensitization or to the influence of a cross-reaction with *M. tuberculosis*.

The Fernandez reaction seems to be near-specific both in children and in adults. This specificity is clearly demonstrated in this investigation by the high statistical significance of the difference between endemic and non-endemic areas.

The response to PPD of children living in areas endemic for leprosy might be at least partially influenced by exposure to *M. leprae*.

RÉSUMÉ

ESSAIS DE TROIS ANTIGÈNES DANS DES RÉGIONS À LÈPRE ENDÉMIQUE
ET DANS DES RÉGIONS SANS ENDÉMICITÉ LÉPREUSE

Une étude comparant les réactions provoquées par trois antigènes après 48 heures et après 30 jours a été réalisée au Venezuela, dans des régions à forte et à faible endémicité lépreuse, et au Chili, pays où la lèpre n'est pas endémique. Les antigènes utilisés pour les épreuves intradermiques étaient l'antigène Mitsuda standardisé, un sur-nageant de cet antigène, et un dérivé protéinique purifié. On n'a pas constaté de différences de réaction à l'antigène Mitsuda chez les habitants des régions à forte ou à faible endémicité, mais les différences de réaction ont été statis-

tiquement significatives lorsque l'on a comparé les résultats chez des habitants de régions à forte ou à faible endémicité à ceux d'habitants d'un pays indemne de la maladie. Les différences de résultats dans le cas de la réaction de Fernandez (surnageant) ne sont pas statistiquement significatives si l'on compare les populations des régions de forte endémicité à celles des régions de faible endémicité, mais elles sont très significatives si l'on compare les populations des pays où la lèpre est endémique aux populations des pays indemnes de la maladie.

REFERENCES

1. CUMMINS, S. L. & WILLIAMS, E. M. *British medical journal*, **1**: 702-703 (1934).
 2. FERNANDEZ, J. M. M. *International journal of leprosy*, **8**: 1-14 (1940).
 3. HAYASHI, F. *International journal of leprosy*, **1**: 30-39 (1933).
 4. MITSUDA, K. *Japanese journal of dermatology and urology* (1916).
 5. RABELLO, F. E. JR. *Revista brasileira de leprologia*, **5**: 465-479 (1937).
 6. RODRIGUEZ, J. N. *International journal of leprosy*, **6**: 11-32 (1938).
 7. STEIN, A. A. & STERPERIN, M. I. *Nederlandsch tijdschrift voor hygiene, microbiologie en serologie*, **1**: 209-218 (1934).
 8. STEIN, A. A. & STERPERIN, M. I. *Urologic and cutaneous review*, **38**: 860-863 (1934).
-