

## AN EVALUATION OF THE NUMERICAL CRITERION FOR "POSITIVE" WITH SKIN TESTS USING MYCOBACTERIUM LEPRAE ANTIGENS

M. R. M. PINTO\*, N. B. ERIYAGAMA\*, S. SIVARAJASINGHAM\*\*, E. M. EKANAYAKE\*\*, THILAKANJALI M. GAMAGE\*

\* Department of Microbiology, Faculty of Medicine, University of Peradeniya, Peradeniya, Sri Lanka.

\*\* Department of Economics, Faculty of Arts, University of Peradeniya, Peradeniya, Sri Lanka.

(Date of receipt : 03 May 1989)

(Date of acceptance : 19 September 1989)

**Abstract:** There has been disagreement regarding the reaction sizes which could demarcate "positive" and "negative" reactions with *Mycobacterium leprae* antigens. In this investigation, positives and negatives were differentiated using statistical methods. The results of this analysis were in agreement with the commonly used reaction size of 3mm and larger being "positive" for Mitsuda reactivity. With Fernandez reactivity there is disagreement with the earlier recommended 10mm reaction size, the conclusion of this study being 3mm or more be considered positive. With reactivity to Soluble Protein Antigen, there is no earlier data and it is suggested that reactions of 3mm or less be considered "negative" and 9mm or more "positive".

### 1. Introduction

In clinical or epidemiological practice, it is common to label a skin test as "positive" or "negative". What is meant here, is that either, there is 'specific' immunological reactivity or nonreactivity, respectively, or that an individual is infected or not infected, (i.e. with an infectious agent, e.g. with a given species of *Mycobacterium*). The demarcation of "positive" (or "reactor" or "infected"), and "negative" (or "nonreactor" or "not infected"), is often not clear and bedevilled by other factors which interfere in the interpretation. In the evaluation of mycobacterial infections there would intrude cross sensitisation by shared or similar antigens, and perhaps reactivities to multiple infections. Therefore in the clinical situation, the decision of the numerical criterion for separation of "positive" and "negative" is made on the basis of which value would give the least error in interpretation. An example in which such a criterion is commonly used, is in the tuberculin test, where in tropical countries, such as Sri Lanka, the cross reaction and multiple infections probably occur commonly.

In skin tests with *Mycobacterium leprae* antigens, different workers have used different numerical criteria of "positivity". Further, the qualitative criteria used, (erythema, swelling and induration), also have been different. Many authorities have not defined the rationale for the use of the criteria they have employed. Latterly, there has been more uniformity, with

the World Health Organisation's Expert Committee<sup>29,30,31</sup> and International Leprosy Congresses<sup>26,27</sup>, setting criteria for the evaluation and interpretation of lepromin tests. However, there has been no attempt to scientifically demarcate between "positive" and "negative" reaction sizes with skin reactions to *Mycobacterium leprae* antigens.

In this report we have attempted to evaluate what reaction size should be used to distinguish between the positive (reactor) and negative (non reactor) with skin tests with *M. leprae* antigens.

## 2. Materials and Methods

The results evaluated here were from skin test surveys using Lepromin A (bacillary content 3 or  $4 \times 10^7$ /ml.) and a soluble protein antigen (SPA) of *M. leprae* (protein content 10 $\mu$ g/ml.). The tests were administered using the standard intradermal skin test technique,<sup>28</sup> in doses of 0.1ml., on the volar aspect of either forearm. The reactions were all read in mm, as the maximum transverse diameter of induration palpated, with the Fernandez reaction, at 48 hrs, and SPA reaction at 72 hrs, and with the Mitsuda reaction, the nodule palpated, at 28 days. The characteristics of the populations tested have been described in detail, elsewhere.<sup>22,23</sup> The results evaluated here are of adults (persons 12 years of age and above) in four different localities in Sri Lanka.

The analysis described here was based on the following observations and assumptions:

- (1) The observation of reaction patterns with the three types of reactivity, showed that, in general, the patterns were bimodal — with modes of reactors and non—reactors.
- (2) The assumption that reactors and non—reactors form biologically (i.e. immunologically) separate populations.
- (3) The assumption that the distribution of reactors was a normal distribution.
- (4) The assumption that the point of intersection of the distributions of reactors and non—reactors, if could be separately drawn, would be the best point of separation of the two (viz. "positive" and "negative"), in the whole unseparated, naturally seen distributions.

Thus the problem here would be to separate out the distributions of reactors and non—reactors from a mixed distribution as occurs in general population groups.

In examining the distributions of reactions of the different types it is seen that there is an area where there is an obvious intermingling of both reactor and non-reactor distributions, while elsewhere in the curve, it is likely that the reactor distribution includes only individuals of that one group.

Thus based on the assumption (3) above, and using reactions in the segment of the curve which probably belongs to the reactor distribution it would be possible to compute, mathematically the hypothetical best fit normal distribution of reactors, for any given type of reactivity using the formula:

$$y_i = \frac{jN}{(\sqrt{2\pi})S} e^{-\frac{1}{2} \left[ \frac{X_i - \bar{X}}{S} \right]^2}$$

(Fitted to the assumed reactor segment of the frequency distribution, where  $y_i$  - ordinate value of the fitted normal curve;  $j$  - class interval;  $S$  - standard deviation (calculated according to  $S = \sqrt{\frac{\sum (X - \bar{X})^2}{N - 1}}$ ;  $X$  - observed reaction size;  $\bar{X}$  - mean of reaction sizes;  $N$  - number of observations;  $0 = 2.718$ ;  $i = 1, 2, \dots$  and so on.

From the latter then it would be possible to deduce the probable distribution of non-reactors. Then, it is possible to obtain in most instances a separation of the cohorts of reactors and non-reactors and evaluate by simple visual inspection which the best point of separation of the two groups would be. In most instances, this would be the point of intersection of the cohorts of reactors (computed) and non reactors (deduced).

It was possible to separate out those tested into several cohorts on the basis of geographical area of residence and BCG vaccination status - both of which may be possible variables affecting the frequency distributions of reactions.<sup>22,23</sup>

### 3. Results

Probable normal distributions of reactors, were computed for eight cohorts each of Fernandez and Mitsuda reactivity and four cohorts of SPA reactivity. From the latter, the probable distribution of non-reactors was deduced. In Figures 1a-h 2a-h and 3a-d are presented the actual distributions of the whole populations, computed probable normal distributions of reactors, and deduced probable distributions of non-reactors with Mitsuda, Fernandez and SPA reactivity. In the Table are presented the possible cut off

reaction sizes obtained from a visual inspection of the computed reactor, deduced non-reactor, and natural total distribution. The point of intersection of the former two distributions could be considered to be the satisfactory reaction size for discriminating between reactors and non-reactors. However, it should be noted that while with some (e.g. Mitsuda reactivity) the separation of reactors and non-reactors, both in the natural distributions, and computed and deduced distributions is clear with others, such demarcations are not as clear.

Table 1: Discriminating reaction size for "positive", with different reaction types, in different population cohorts.

Population cohort	Discriminating size in mm.		
	Fernandez reactivity	Mitsuda reactivity	SPA reactivity
Pussellawa			
BCG -ve	2.5	1	
BCG +ve	1.5	1	
Pedro (Nuwara Eliya)			
BCG -ve	1	1.5	
BCG +ve	1	4	
Mahagastota (Nuwara Eliya)			
BCG -ve	4.5	1	7mm. or lesser
BCG +ve	4.5	1.75	9mm. or lesser
Galagedera			
BCG -ve	1	1.75	4mm. or lesser
BCG +ve	1	1	6mm. or lesser

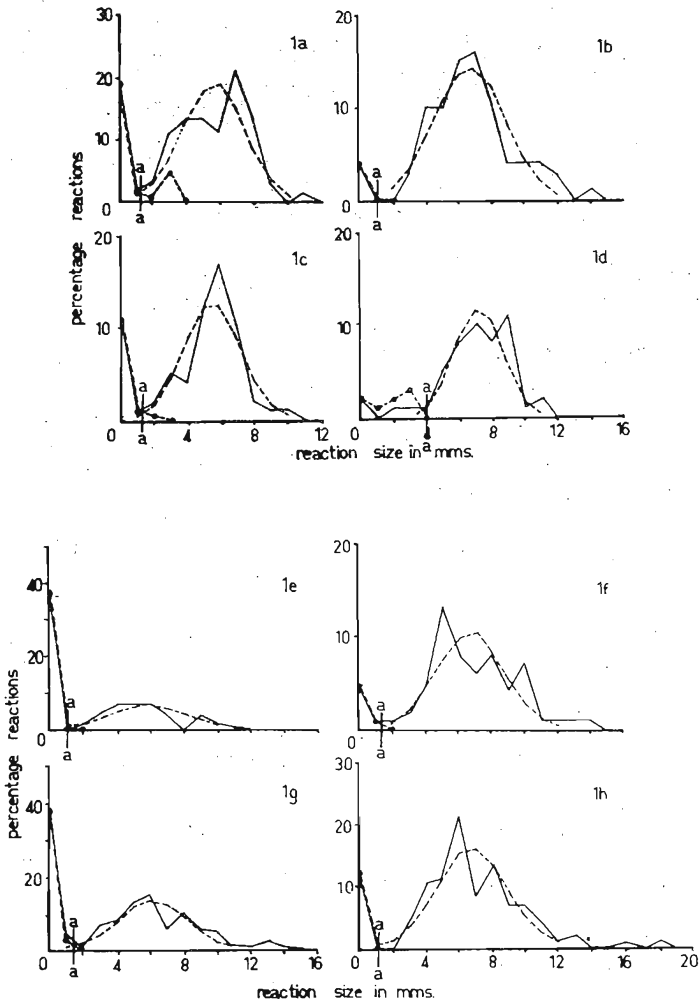


Figure 1: Distribution of Mitsuda reactions in different population cohorts. Actual distribution of whole population, both reactors and non reactors (—), computed distribution of reactors (-----), deduced distribution of non reactors (—0—), 'a' indicates probable point of intersection of distributions of non reactors and reactors.

(a)	Pussellawa	BCG	-ve	(n=110)
(b)	Pussellawa	BCG	+ve	(n=85)
(c)	Pedro	BCG	-ve	(n=66)
(d)	Pedro	BCG	+ve	(n=50)
(e)	Mahagastota	BCG	-ve	(n=77)
(f)	Mahagastota	BCG	+ve	(n=60)
(g)	Galagedera	BCG	-ve	(n=118)
(h)	Galagedera	BCG	+ve	(n=100)

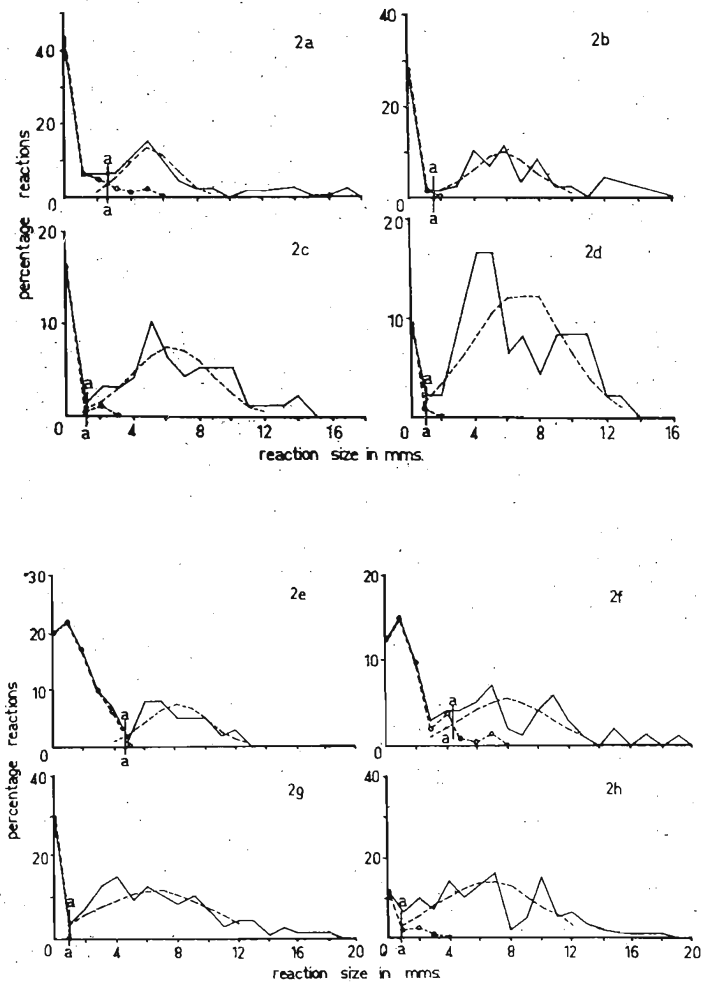


Figure 2: Distribution of Fernandez reactions in different population cohorts. Actual distribution of whole population, both reactors and non reactors (—), computed distribution of reactors (-----), deduced distribution of non reactors (—0—), 'a' indicates probable point of intersection of distributions of non reactors and reactors.

(a)	Pussellawa	BCG	-ve	(n=110)
(b)	Pussellawa	BCG	+ve	(n=85)
(c)	Pedro	BCG	-ve	(n=66)
(d)	Pedro	BCG	+ve	(n=50)
(e)	Mahagastota	BCG	-ve	(n=114)
(f)	Mahagastota	BCG	+ve	(n=84)
(g)	Galagedera	BCG	-ve	(n=131)
(h)	Galagedera	BCG	+ve	(n=111)

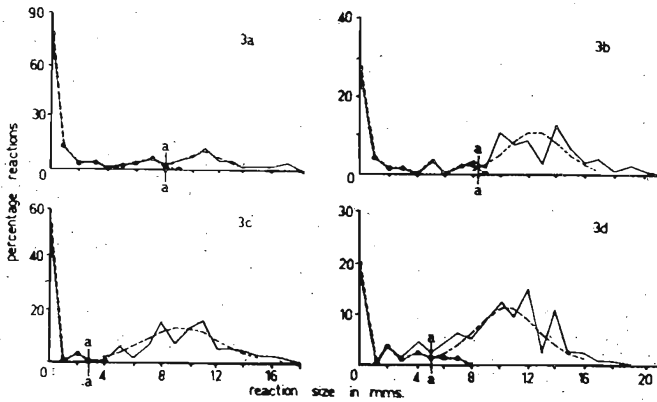


Figure 3: Distribution of SPA reactions in different population cohorts. Actual distribution of whole population, both reactors and non reactors (—), computed distribution of reactors (-----), deduced distribution of non reactors (---0---), 'aa' indicates probable point of intersection of distributions of non reactors and reactors.

- |     |             |     |     |         |
|-----|-------------|-----|-----|---------|
| (a) | Mahagastota | BCG | -ve | (n=114) |
| (b) | Mahagastota | BCG | +ve | (n=84)  |
| (c) | Galagedera  | BCG | -ve | (n=131) |
| (d) | Galagedera  | BCG | +ve | (n=96)  |

#### 4. Discussion

In his original paper, Mitsuda<sup>20</sup> considered a reaction of less than 10 mm "weak", 10–20 mm "moderate" and larger than 20 mm "strong". The criteria used for "positivity" determination has since undergone extensive change. Thus with Mitsuda reactivity recent recommendations are of a smaller reaction size for the demarcation of "positivity". With 19 papers reviewed in this respect, nine used 5 mm<sup>3,5-7,9-12,17</sup> (over half the number of the latter reports were by the same research group), three used 4 mm<sup>1,13,14</sup>, and seven used 3 mm.<sup>2,8,16,18,19,21,25</sup> Thus among research workers 3 mm seems to be a more favoured reaction size used for demarcation). Recent workers have no doubt also been influenced by the decisions of International Leprosy Congresses,<sup>26,27</sup> the most recent WHO Expert Committee<sup>31</sup> to examine this problem, and of an influential text book<sup>4</sup> to recommend this reaction size. On the other hand with Fernandez reactivity, though the available literature is lesser the majority of workers have considered 10 mm and above as "positive" (n = 7)<sup>1,5,8-10,14,20</sup> while one paper considered 5 mm.<sup>15</sup> The two international Congresses<sup>26,27</sup> and the WHO Expert Committee<sup>29</sup> which examined this problem, all recommended 10 mm.

Evaluating our data it is seen that with Mitsuda reactivity, almost any degree of reactivity, if present, is a "positive". Thus a level of 3 mm or more

as recommended, is an acceptable level at which to demarcate a ("reactor" or) "positive". With Fernandez reactivity, the conclusions of this analysis are totally in disagreement with that of previous recommendations. Of eight cohorts six show a clear demarcation at less than 3 mm. Thus 3 mm would seem a satisfactory point of demarcation.

With SPA reactivity, only four cohorts were available for analysis. From these, the definition of the discriminating reaction size of "positive" is not clear. It is seen that the reactors form a distinctive sub-distribution within the total. The demarcation seems to be likely to be at between 3 and 9 mm. Thus the compromise reaction size of 5 mm as the cut off point was considered as the best, being in between. However, another and possibly more valid interpretation is that those with reactions of 3 mm or less be defined as definite "negative" or "non reactor", and with 9 mm or more, as definite "positive" or "reactor", and those of 4 to 8 mm, of doubtful status.

The analysis here has been made on the assumption that each whole population group distribution is of only two components, viz reactors and non-reactors. It should be noted, that this, may not necessarily be so — and that the whole distribution may contain different unrecognised cohorts. For example, in mycobacterial infections, skin testing may evoke a response of cross sensitisation rather than of specific infection. At present it is not possible to identify these with a single skin test. Cross reactivity with the Mitsuda reaction — may lead to definite positivity, and not cause intermediate size reactions which interfere with interpretation. On the other hand cross reactions which interfere with interpretation, producing intermediate sized reactions, may be a possibility with Fernandez and SPA reactivity (considering the nature of these reactions), though probably to a lesser degree than with tuberculin reactivity. The cross reaction and other possible factors which may have influenced reactivity have been ignored in this analysis.

### Acknowledgements

This investigation was supported by grants from the World Health Organisation, Leprosy Section, Geneva, and the University of Peradeniya.

The antigens used in this study were supplied by Dr. R.C. Hastings (Lepromin A) of Carville, Maryland, U.S.A., (through courtesy of the Chief, Leprosy Section, W.H.O., Geneva) and Dr. R.J.W. Ress (SPA), Clinical Research Centre, Harrow, England.

The clerical assistance of Ms. M. Wijekoon of the Department of Microbiology, Faculty of Medicine, University of Peradeniya, Peradeniya, is gratefully acknowledged.

## References

1. AZULAY, R. D. (1947) The Mitsuda test in non-leprosy persons in a nonendemic country. *Int. J. Leprosy* 15 : 264-266.
2. BECHELLI, L. M., HADDAD, N., PAGNANO, P. M. G., NEVES, R. G., MELCHIOR, E. & FRANGAN, R. C. (1980) Double blind trials to determine the late reactivity of leprosy patients and unaffected persons to different concentrations of armadillo lepromin in comparison to human lepromin. *Int. J. Leprosy* 48(2) : 126-134.
3. BEIGUELMAN, M. D., QUAGLIATO, R. & DE CAMARGO, D. P. (1965) Influence of repeated lepromin injections on the Mitsuda skin reaction. *Int. J. Leprosy* 33(4) : 795-799.
4. COCHRANE, R. G. & DAVEY, F. T. (1964) *Leprosy in Theory and Practice*. The Williams & Wilkins Company, Baltimore, P. 623.
5. CONVIT, J., PINARDI, M. E., ARIAS ROJAS, F., GONZALES, I., COREY, G., ARVELO, J. J. & MONZON, H. (1975) Tests with three antigens in leprosy-endemic and non-endemic areas. *Bull. World Health Organ.* 52 : 193-197.
6. DOULL, J. A., GUINTO, R. S. & MABALAY, M. C. (1957) Effect of BCG vaccination, lepromin testing, and natural causes in inducing reactivity to lepromin and to tuberculin. *Int. J. Leprosy* 25(1) : 13-37.
7. DOULL, J. A., GUINTO, R. S. & MABALAY, M. C. (1959) The origin of natural reactivity to lepromin. The association between the Mitsuda reaction and reaction to graded doses of tuberculin. *Int. J. Leprosy* 27(1) : 31-42.
8. DUBOIS, A. (1936) La reaction de Mitsuda (notice Complimentaire) *Bull. Soc. Path. Exot.* 29 : 649-651.
9. FERNANDEZ, J. M. M. (1943) Sensitisation to lepromin in presumably non leprosy individuals. *Int. J. Leprosy* 11 : 15-22.
10. GUINTO, R. S., DOULL, J. A. & MABALAY, E. B. (1955) Tuberculinization and reactivity to lepromin. Association between lepromin and tuberculin reactions in school children in Cordova and Opon, Cebu, Philippines. *Int. J. Leprosy* 23(1) : 32-47.
11. GUINTO, R. S., DOULL, J. A. & MABALAY, E. B. (1955) A note on the lepromin reaction in males and females of the general population of Cordova, Mactan Island, Cebu, Philippines. *Int. J. Leprosy* 23(2) : 131-134.
12. GUINTO, R. S., DOULL, J. A. & MABALAY, E. B. (1955) Mitsuda reaction in persons with and without household exposure to leprosy. *Int. J. Leprosy* 23(2) : 135-138.
13. GUINTO, R. S. & WADE, H. W. (1958) Results of tests with serial dilutions of lepromin in separate groups of normal young children, with a comparison of two lepromins and the standard Dharmendra antigen. *Int. J. Leprosy* 26(4) : 328-345.
14. GUINTO, R. S., MABALAY, M. C. & DOULL, J. A. (1962) Reactivity of children to lepromin and various tuberculins as affected by recent and old BCG vaccination. *Int. J. Leprosy* 30(3) : 284-290.
15. HALE, J. H., MOLESWORTH, B. D., GROVE-WHITE, R. J., SAMBAMURTHI, C. N. & RUSSELL, D. A. (1955) The relationship and significance of Mantoux and lepromin reactions in leprosy. *Int. J. Leprosy* 23(2) : 139-147.

16. HOGERZEIL, L. M. & PRABHUDASS, N. (1978) Delayed hypersensitivity skin reactions to lepromins prepared from *M. leprae* and selected cultivable mycobacteria. *Leprosy in India* 50(4) : 560-565.
17. KOOIJ, R. & GERITSEN, T. H. (1956) Positive "lepromin" reactions with suspensions of normal tissue particles. *Int. J. Leprosy* 24(2) : 171-181.
18. MAYERS, W. M., KVERNES, S. & BINFORD, C. H. (1975) Comparison of reaction to human and armadillo lepromin in leprosy. *Int. J. Leprosy* 43 : 218-225.
19. MILLER, J. W., GANNON, S. C. & CHAN, C. S. P. (1975) Comparison in leprosy patients of Fernandez and Mitsuda reactions using human and armadillo antigens. A double blind study. *Int. J. Leprosy* 43 : 226-233.
20. MITSUDA, K. (1953) On the value of a skin reaction to a suspension of leprous nodules. *Hifuka Heuyoka Zasshi* (1919) 19 : 697-708. *Int. J. Leprosy* 21 : 347-358.
21. MUSTAFA, A. S., & TALWAR, G. P. (1978) Early and late reactions in tuberculoid and lepromatous leprosy patients with lepromins from *Mycobacterium leprae* and five selected cultivable mycobacteria. *Leprosy in India* 50(4) : 566-571.
22. PINTO, M. R. M., ERIYAGAMA, N. B. & PEMAJAYANTHA, V. (1987) Studies of reactivity of some Sri Lankan population groups to antigens of *Mycobacterium leprae*. I. Reactivity to Lepromin A. *Lepr. Rev.* 58 : 105-118.
23. PINTO, M. R. M., ERIYAGAMA, N. B. & PEMAJAYANTHA, V. (1987) Studies of reactivity of some Sri Lankan population groups to antigens of *Mycobacterium leprae*. II. Reactivity to a soluble protein antigen of *Mycobacterium leprae*. *Lepr. Rev.* 58 : 219-226.
24. RATNAM, K. V. (1986) Lepromin skin test in normal people in Singapore - a one year follow up. *Int. J. Leprosy* 54(3) : 479-480.
25. SHARMA, R. C. & SINGH, R. (1978) Comparative study of skin reactions in leprosy patients to *M. leprae* - lepromin and to antigens from cultivable saprophytic mycobacteria. *Leprosy in India* 50(4) : 572-578.
26. Transactions of the VI International Congress of Leprology (1953) Madrid. 101-103.
27. Transactions of the VIII International Congress of Leprology Tokyo, November, 1958, Tofu Kyokai, Tokyo, 1959. 464-465.
28. W. H. O. Standard tuberculin test. World Health Organisation, (1963) W. H. O. /TB/ tech. Guide/3.
29. W. H. O. Expert Committee on Leprosy. First report. Technical report series No. 70. (1953) World Health Organisation, Geneva.
30. W. H. O. Expert Committee on Leprosy. Second report. Technical report series No. 189. (1960) World Health Organisation, Geneva.
31. W. H. O. expert Committee on Leprosy. Fourth report. Technical report series No. 459 (1970) World Health Organisation, Geneva.