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# AN EVALUATION OF THE USEFULNESS OF DIFFERENT DIAMETERS OF SKIN REACTIONS ELICITED BY *MYCOBACTERIUM LEPRAE* ANTIGENS

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SUMMARY. The advantages of the transverse, vertical and mean diameters for the measurement of skin reactions with *M. Leprae* antigens were evaluated. It was decided that the vertical and mean diameters offered no improvement over the transverse diameter and hence the use of the latter is recommended.

## INTRODUCTION

When testing for cell mediated immune responses with skin tests, the induration reaction produced is usually of a round or oval shape. With tuberculin reactions elicited by the Mantoux tests, the authoritatively recommended reading is of the palpated transverse diameter (1). However, with skin testing, different workers have also used (or recommended), other dimensions in the reading of skin tests: those that are, or have been used, or could be used in the reading of skin tests in man and animals include the transverse, vertical and mean diameters, the surface area and increase of skin thickness. The measurement of the increase of skin thickness is a practice usually limited to use with animals—and in thin and highly sensitive human skin, this procedure is likely to cause unacceptable pain.

Thus, in the reading of skin tests with antigens of *Mycobacterium leprae*, different workers have used different dimensions in the measurement of evoked skin reactions. The Leprosy Congresses in Madrid (2) and in Tokyo (3) recommended reading the diameter (presumably the transverse); the latter also recommended that, "When lesions are not round, two measurements should be made and averaged". The W.H.O. Expert Committee in its First Report (4) and in the Second Report (5) made similar recommendations. In the examination of published papers in English with Lepromin skin tests it was found that there has been no uniformity in the reading of results. Of 36 papers (published between 1934 and 1986) reporting results with Mitsuda reactions, one each used the transverse and greatest diameter ; 6, the mean or average diameter ; 15, read a "diameter", uncertain which ; and 13 made no mention at all of which measurement was read. Of 12 papers reporting results with the Fernandez reaction, one used the transverse diameter; 3, the mean or average diameter; 5, a diameter, uncertain which ; and 3 made no mention of the measurement read. An antigen introduced recently for skin testing in leprosy research, the Soluble Protein Antigen (SPA), does not have an extensive body of literature available for such examination.

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In evaluating the results of skin tests with *M. leprae* antigens the trend has been to classify results as positive or negative. While such classification may have advantages if uniformly applied in the clinical setting, it may not be as useful in the epidemiological setting, where the exact measurement of readings in mms and the graphical presentation of the distribution of readings would yield more information. Such presentations would be also of value in the comparison of data from different workers, provided the readings made were the same. Thus it is of importance that there be uniformity in the readings made by different workers. Such uniformity has been achieved with tuberculin testing (1) but unfortunately is not seen in the reported results of skin tests with *M. leprae* antigens.

An examination of the literature on skin testing with mycobacterial antigens did not reveal any scientific investigation or data analysis made to decide which diameter of skin reaction measurement would give the best results. In this paper is presented an analysis of the different diameters which could be used in the evaluation of skin test results evoked by *M. leprae* antigens.

## MATERIALS AND METHODS

The results presented here are from skin tests carried out on three population groups in different localities, in two geographically different areas. They consisted of adults (12 years of age and above ), of both sexes, and included both the BCG vaccinated and non-vaccinated. The characteristics of the populations and test methodologies have been described in detail elsewhere (6,7). Briefly the methodology used was as follows. The test antigens used were Lepromin A (with a bacillary content of 3 or $4 \times 10^7$ bacilli/ml) and a soluble protein antigen of *M. leprae* (SPA) with a protein content of 10 $\mu$g/ml.

The antigens were adminstered intradermally using standard techniques, on the volar aspect of the forearm at different sites. The reactions were read, for Fernandez reactivity at 48 hours, and SPA reactivity at 72 hours, both as the maximum transverse and vertical diameters palpated, and Mitsuda reactivity at 28 days as the maximum transverse and vertical diameters of the nodule palpated. The transverse ($a$) and vertical ($b$) diameters, and the mean $\frac{(a+b)}{2}$ diameter, were used in analysis in this study. The surface area was excluded, as both the mean and surface area ($\pi \times \frac{a}{2} \times \frac{b}{2}$) are expressions of functions of the transverse and vertical diameters, and therefore the decision was to use the mean diameter alone, with the more manageable smaller values.

## RESULTS

The simplest way of examining the relationships between transverse and vertical diameters would be to look at bivariate frequency distributions. Such distributions for whole populations with Fernandez, Mitsuda and SPA reactivities are presented in Tables 2 and 3. An examination of this data showed that the reactions tended to be oval with the larger reactions, commonly with the vertical diameter exceeding the transverse diameter. This change is more marked with Fernandez and SPA reactions and not as prominent or hardly seen with Mitsuda reactions, which tended to be smaller than the former (Table 4). There was a close correlation between the transverse and vertical diameters with all three types of reactivity (Table 5).

**TABLE 1. Bivariate frequency distribution of transverse and vertical diameters of Fernandez reactions in the whole population tested.**

| Transverse \ Vertical | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 10 | | | | | | | | | | | | | | | | | | | | | | | | |
| 1 | 46 | 18 | | | | | | | | | | | | | | | | | | | | | | | |
| 2 | 1 | 25 | 19 | 3 | | | | | | | | | | | | | | | | | | | | | |
| 3 | | 1 | 27 | 18 | | | | | | | | | | | | | | | | | | | | | |
| 4 | | | 3 | 25 | 32 | | | | | | | | | | | | | | | | | | | | |
| 5 | | | | 6 | 14 | 25 | | | | | | | | | | | | | | | | | | | |
| 6 | | | | | | 24 | 30 | 2 | | | | | | | | | | | | | | | | | |
| 7 | | | | | | | 16 | 21 | 9 | | | | | | | | | | | | | | | | |
| 8 | | | | | | | 4 | 6 | 14 | 4 | | | | | | | | | | | | | | | |
| 9 | | | | | | | | 9 | 24 | 2 | | | | | | | | | | | | | | | |
| 10 | | | | | | | | | 26 | 13 | 4 | | | | | | | | | | | | | | |
| 11 | | | | | | | | | 6 | 10 | 7 | 1 | | | | | | | | | | | | | |
| 12 | | | | | | | | | | 4 | 10 | 1 | | | | | | | | | | | | | |
| 13 | | | | | | | | | | 1 | 2 | 2 | 2 | 2 | 1 | 1 | | | | | | | | | |
| 14 | | | | | | | | | | | 1 | 1 | | | | 1 | | | | | | | | | |
| 15 | | | | | | | | | | | 2 | | | | | 1 | 1 | | | | | | | | |
| 16 | | | | | | | | | | | | 1 | | | | 1 | | | | | | | | | |
| 17 | | | | | | | | | | | | 1 | | | | | 1 | | | | | | | | |
| 18 | | | | | | | | | | | | | 1 | | | | | | | | | | | | |
| 19 | | | | | | | | | | | | | | | | | | | | | | | | | |

**TABLE 2.** **Bivariate frequency distribution of transverse and vertical diameters of Mitsuda reactions in the whole population tested.**

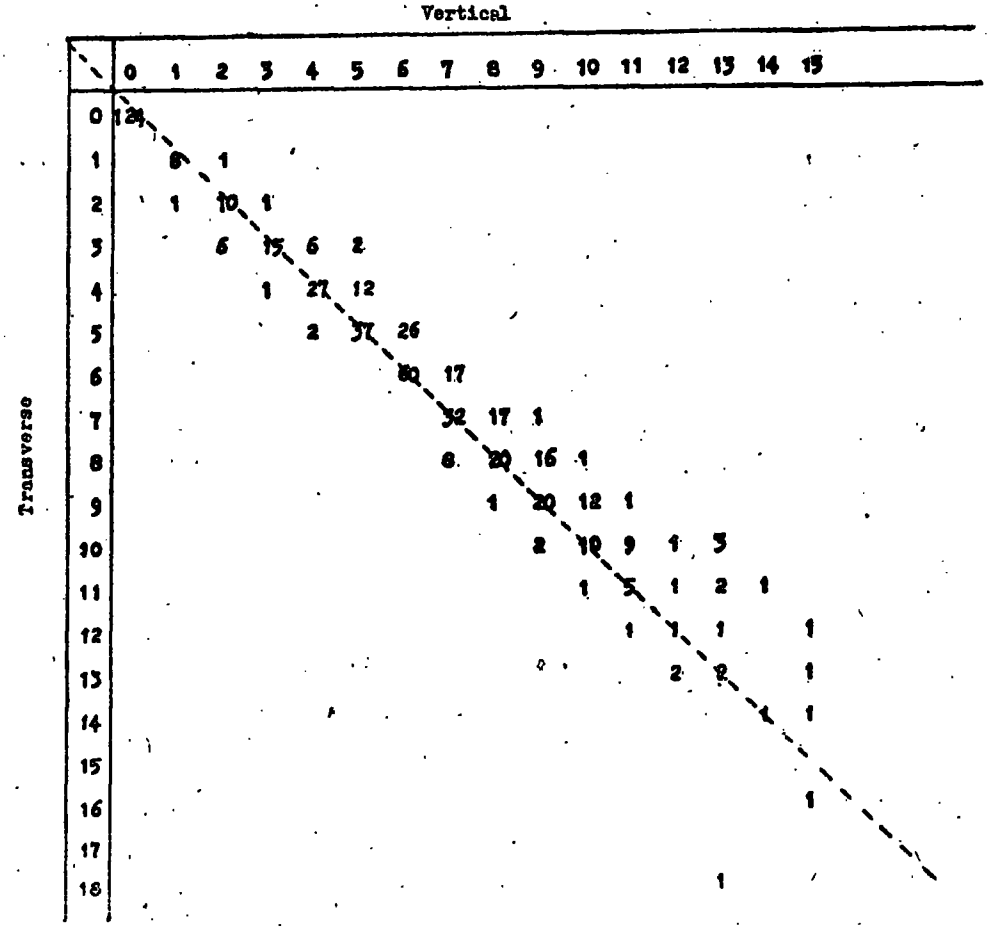

**TABLE 3. Bivariate frequency distribution of transverse and vertical diameters of SPA reaction in the whole population tested.**

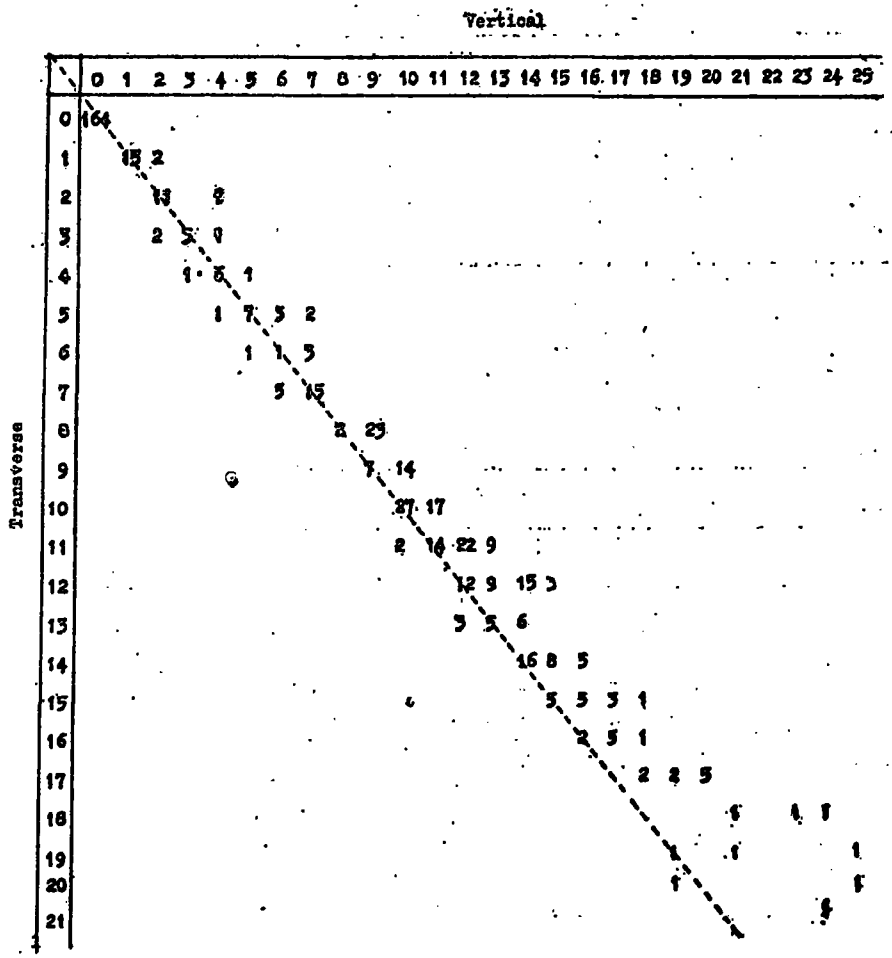

TABLE 4. Relationship of transverse and vertical diameters of different sized groups, with different types of reactivity

| Transverse reaction sizes of | Type of reactivity | Percentage reactions | | |
|---|---|---|---|---|
| | | Where transverse and vertical diameter are equal | Where transverse is greater than vertical diameter | Where transverse diameter is less than vertical diameter |
| ⩽ 5 mm. | Mitsuda | 63 | 6 | 31 |
| | Fernandez | 52 | 4 | 44 |
| | SPA | 75 | 7 | 18 |
| 6—10 mm. | Mitsuda | 61 | 5 | 34 |
| | Fernandez | 39 | 2 | 59 |
| | SPA | 45 | 5 | 50 |
| ⩾ 11 mm. | Mitsuda | 47 | 32 | 21 |
| | Fernandez | 16 | 5 | 79 |
| | SPA | 32 | 4 | 64 |

TABLE 5. Correlation between transverse and vertical diameters with different types of reactivities

| Type of reactivity | Coefficient of correlation (r) | Probable error | Confidence interval |
|---|---|---|---|
| Fernandez (n—647) | 0.75 | 0.01 | 0.71, 0.78 |
| Mitsuda (n—533) | 0.9876 | 0.0006 | 0.9852, 0.9896 |
| SPA (n—504) | 0.81 | 0.01 | 0.78, 0.83 |

(n—number tested and read)

The major purpose of doing a skin test in an infectious disease is to distinguish between those who are "reactors" ("positives") and "non-reactors" ("negatives"). This could be best visualised by examining which measurement would give the best separations, viz, transverse or vertical diameters, or the mean. Frequency distributions of each with different cohorts of tested individuals (from differing localities and of differing BCG vaccination status) were therefore constructed in graphic form (Figs 1a—f, 2a—f and 3a—d). The distributions in general tended to be bimodal with a mode of non-reactors (on the left) and another of reactors (on the right). They showed that, with Mitsuda reactivity, the distributions of transverse, vertical and mean diameters showed no differences at all, while with Fernandez and SPA reactivity, there were in a few instances, a shift of the distribution of reactors to the right, of vertical and mean diameters, showing the presence of larger readings. The reaction size for the differentiation of negative or non-reactor from positive or reactor, would be between the non-reactor and reactor components or of the distribution. In order to examine this, enlarged and more detailed graphical representations of the area of the frequency distributions between 0 to 6 mm for Fernandez reactivity and Mitsuda reactivity, and 0 to 10mm for SPA reactivity were constructed (Figs 4a—f, 5a—f, 6a—d). These however, did not reveal any significant differences between the transverse, vertical, and mean reaction diameters, in the reaction distributions, that may affect this differentiation.

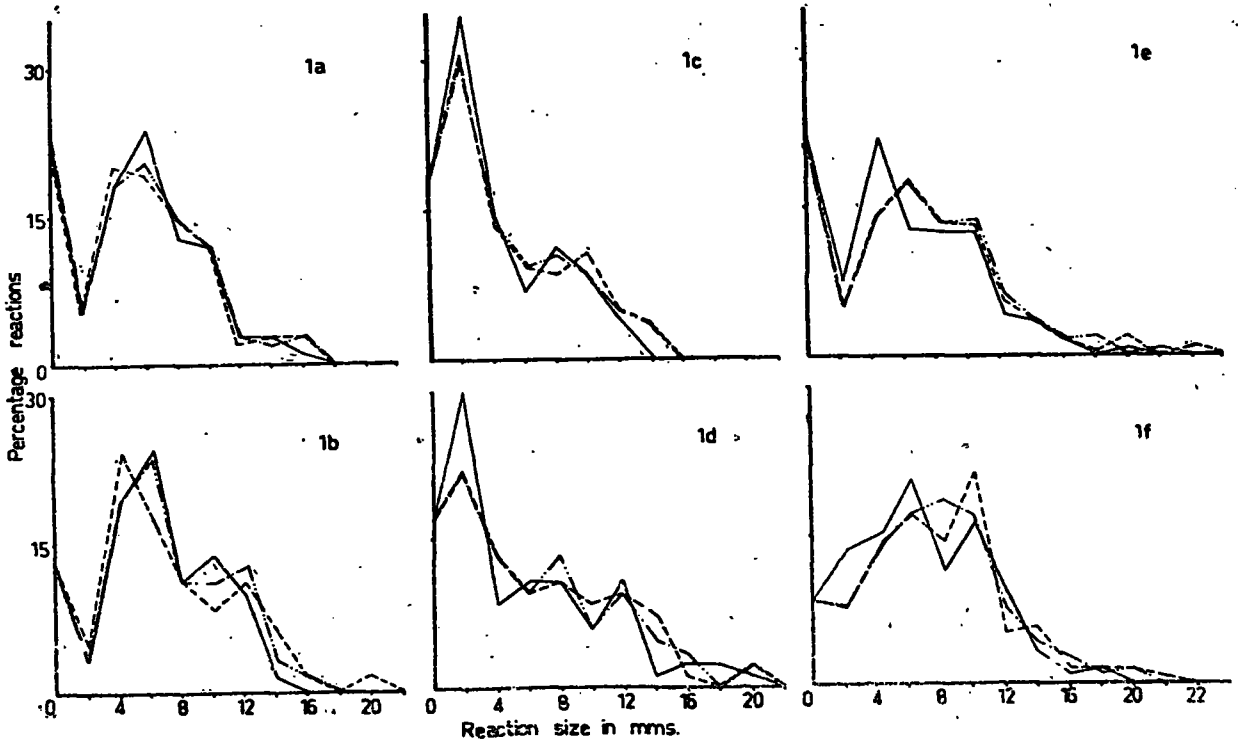


**Figure 1.** Frequency distribution of Fernandez reactions with different diameters (transverse ——— vertical — — — —, mean —·—·—) in the

(*a*) BCG – ve at Pedro
(*b*) BCG + ve at Pedro
(*c*) BCG – ve at Mahagastota
(*d*) BCG + ve at Mahagastota
(*e*) BCG – ve at Galagedara
(*f*) BCG + ve at Galagedara

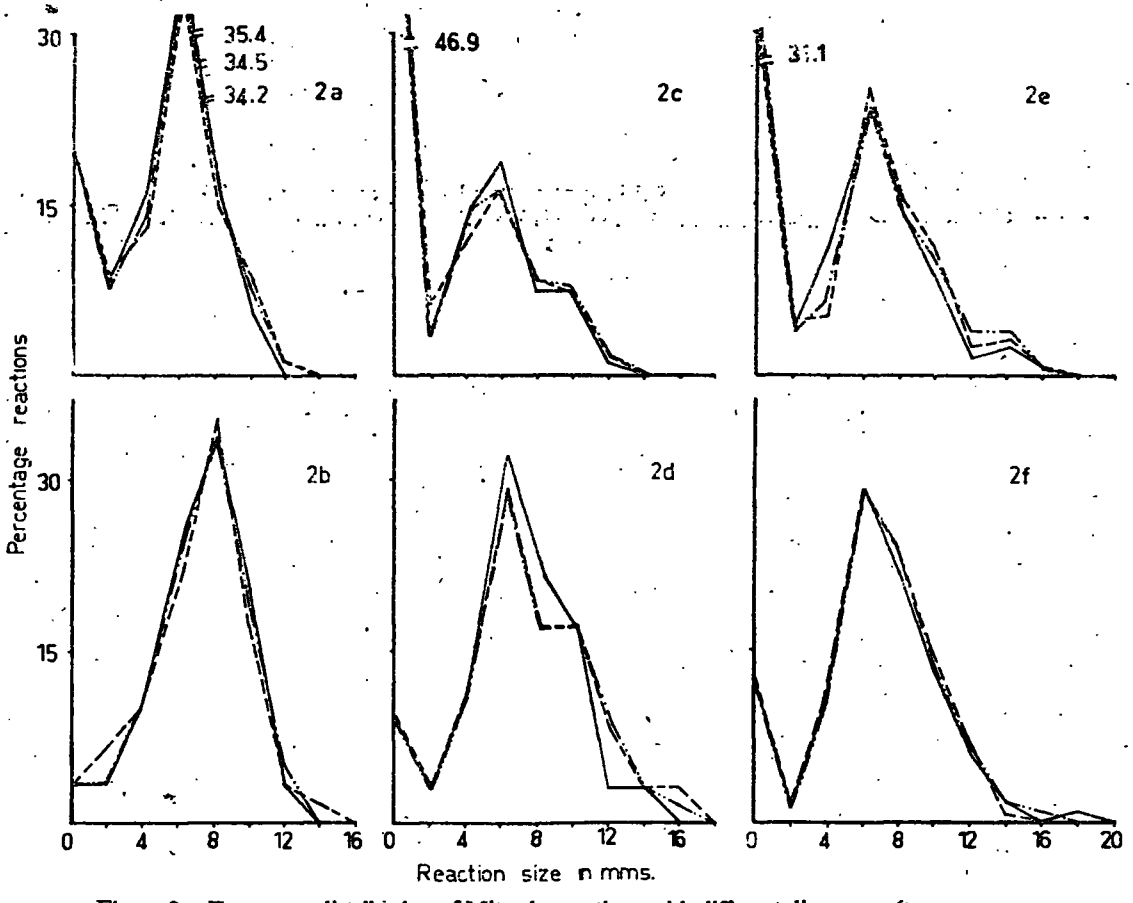


**Figure 2.** Frequency distribution of Mitsuda reactions with different diameters (transverse———— vertical — — — —, mean —·—·—) in the

(*a*) BCG – ve at Pēdro
(*b*) BCG+ve at Pedro
(*c*) BCG – ve at Mahagastota
(*d*) BCG+ve at Mahagastota
(*e*) BCG – ve at Galagedara
(*f*) BCG+ve at Galagedara

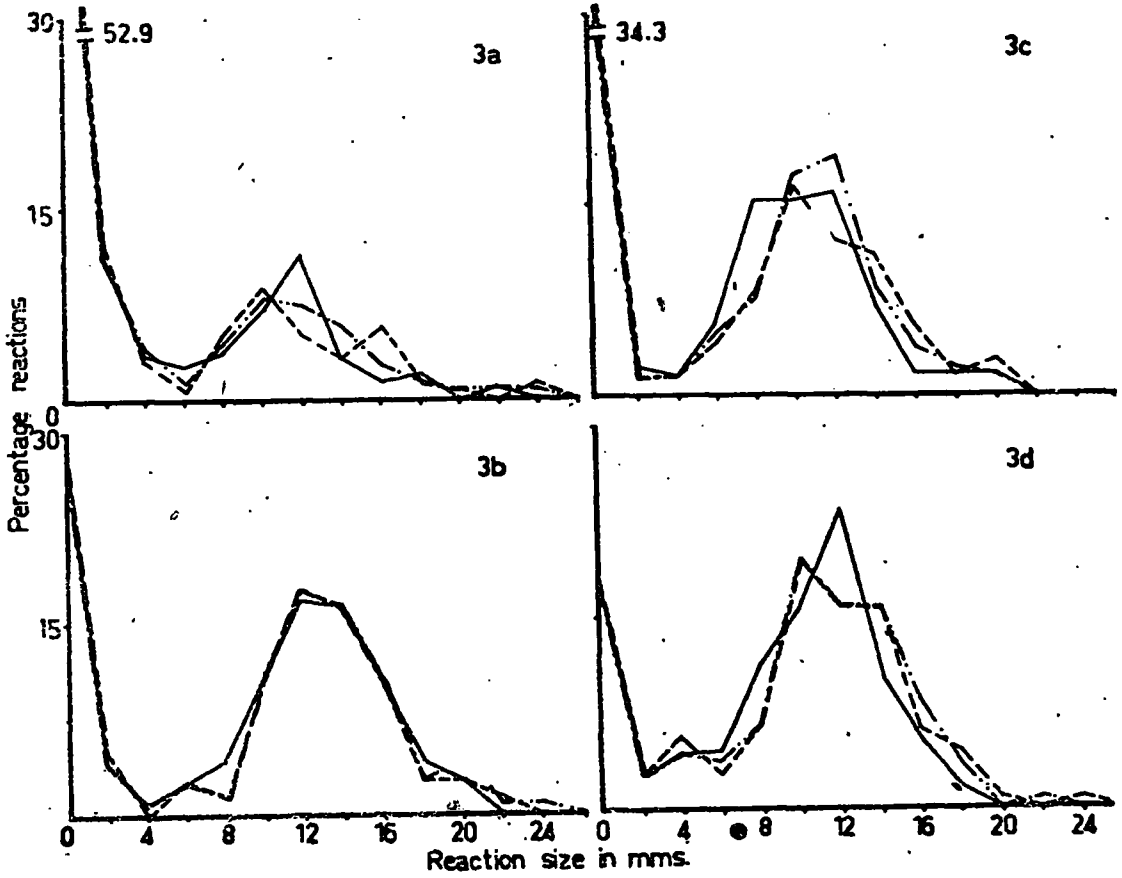


Figure 3. Frequency distribution of SPA reactions with different diameters (transverse———— vertical — — — —, mean —·—·—) in the

(*a*) BCG – ve at Mahagastota
(*b*) BCG + ve at Mahagastota
(*c*) BCG – ve at Galagedara
(*d*) BCG + ve at Galagedara

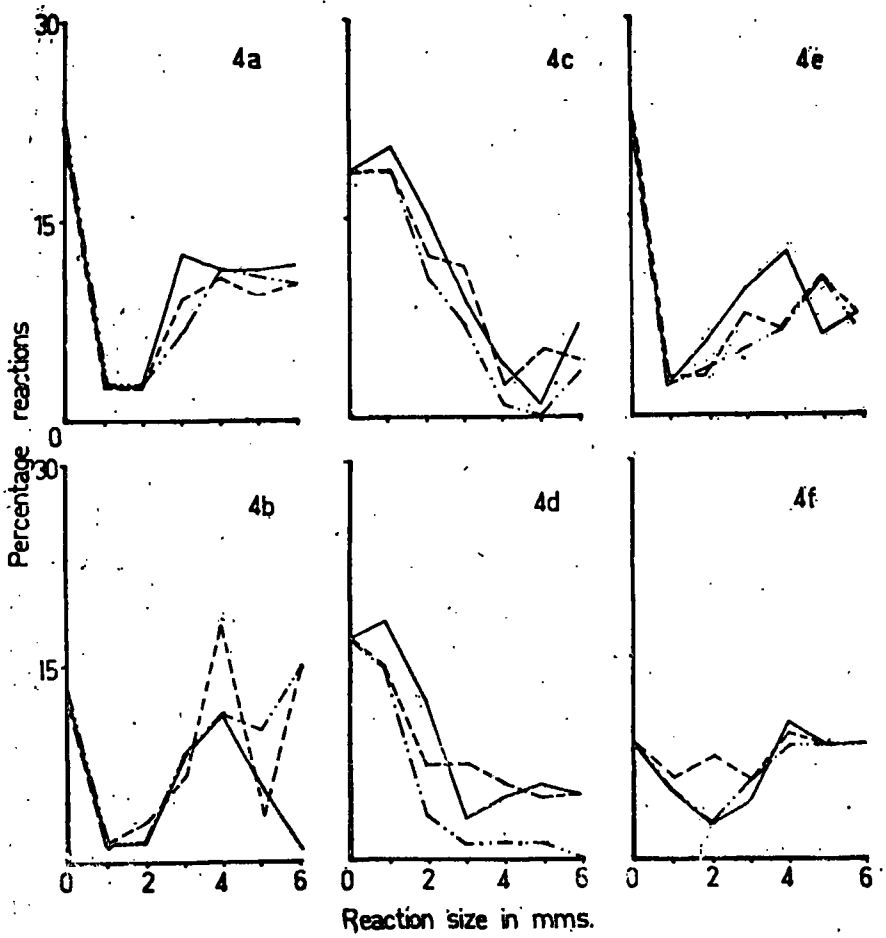


Figure 4. Frequency distribution of Fernandez reactions of the smaller sizes (0—6 mm), with different diameters (transverse ———, vertical — — — —, mean —·—·—)

(*a*) BCG – ve at Pedro
(*b*) BCG + ve at Pedro
(*c*) BCG – ve at Mahagastota
(*d*) BCG + ve at Mahagastota
(*e*) BCG – ve at Galagedara
(*f*) BCG + ve at Galagedara

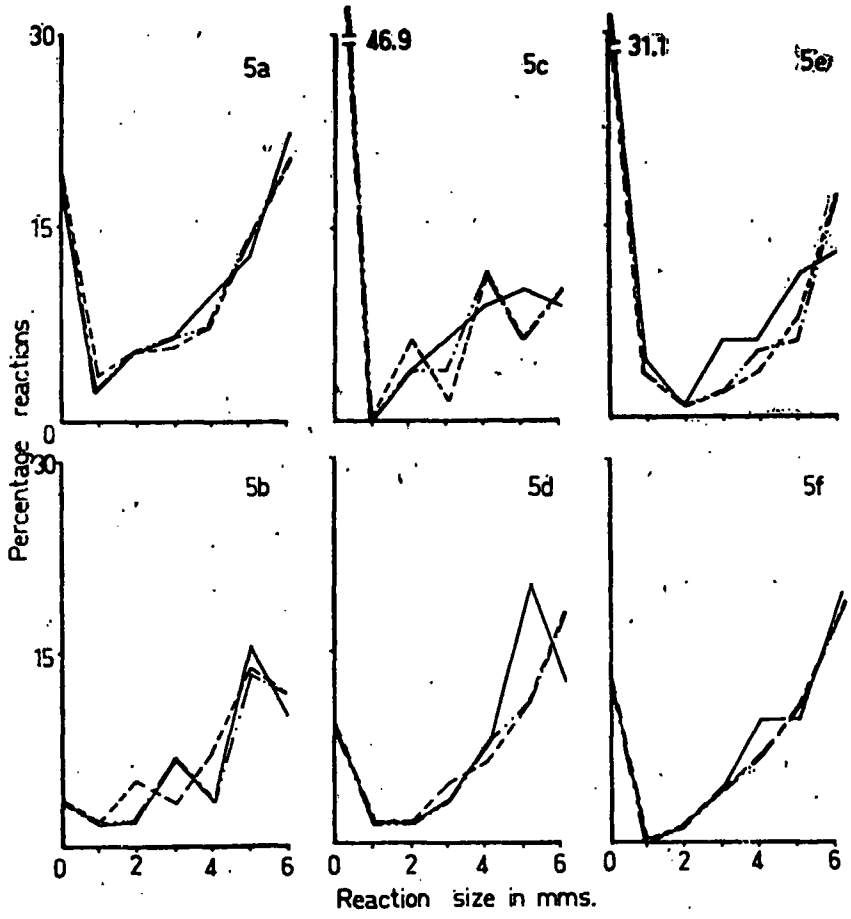


**Figure 5.** Frequency distribution of Mitsuda reactions of the smaller sizes (0—6mm), with different disameters (transverse———, vertical — — — —, mean —·—·—) in the

(*a*) BCG – ve at Pedro
(*b*) BCG+ve at Pedro
(*c*) BCG – ve at Mahagastota
(*d*) BCG+ve at Mahagastota
(*e*) BCG – ve at Galagedara
(*f*) BCG+ve at Galagedara

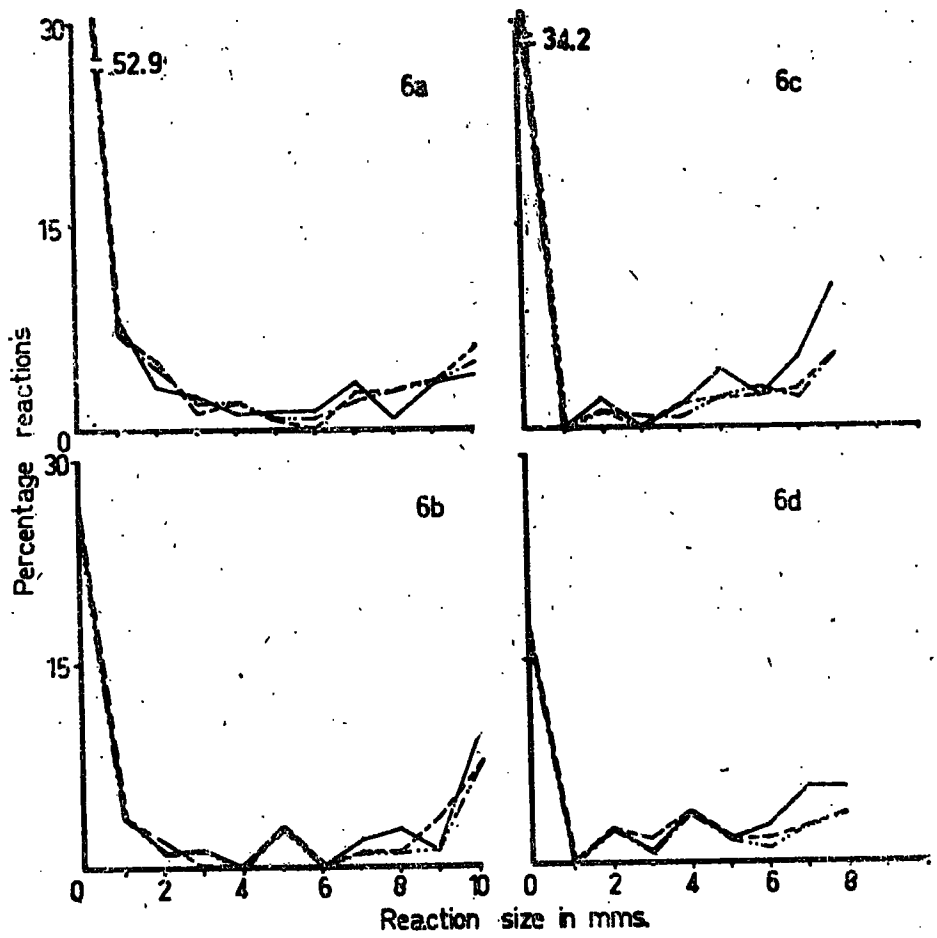


Figure 6. Frequency distributions of SPA reactions of the smaller sizes (0—10 mm and 0—8), with different diameters (transverse ———, vertical — — — —, mean —·—·— ) in the

(*a*) BCG – ve at Mahagastota (*b*) BCG + ve at Mahagastota
(*c*) BCG – ve at Galagedara (*d*) BCG + ve at Galagedara

## DISCUSSION

The results presented here all indicate that, with Fernandez and SPA reactivity, with the larger reaction sizes, the vertical diameters tended to be larger than transverse diameters. The use of the vertical diameter, however, did not in any way contribute to a better interpretation of the skin test result, or to a better differentiation between "negative" and "positive". Further, the use of the mean reaction size did not in anyway provide any improvement over the use of the transverse diameter alone. Thus, the effort spent in calculation of the mean was not worthwhile. With Mitsuda reactivity, the reactions tended to be rounded and smaller and hence the difference between transverse and vertical was not noticeable.

In conclusion, we recommend that the measurement of the transverse diameter alone would be adequate in the reading of skin reactions with *M. leprae* antigens, as is the practice with tuberculin reactions.

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