Ceylon J. Med. Sci., 29 (No. 1 June) 1986, pp. 39—52

The Relationship between Reactivities to Lepromin A and Soluble Protein Antigen of Mycobacterium Leprae and Tuberculin

by

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SUMMARY The relationship between reactivity to tuberculin (PPD-RT23) and reactivities to antigens of *Mycobacterium leprae* (Fernandez, Mitsuda and to a Soluble Protein Antigen of *M. leprae*) was studied using three methods. A clear relationship could be demonstrated with two of the methods. A statistically significant correlation could be demonstrated by the method of regression analysis between tuberculin and all three types of reactivities; however the level of correlation (r^2) was unusually low. This study also revealed an increase of all three types of reactivity with BCG vaccination.

INTRODUCTION

The relationship between leprosy and tuberculosis has evoked considerable interest from the early days of leprosy research. The available literature on the subject has been comprehensively reviewed from time to time [7,12,13] at regular intervals indicating the considerable scientific interest in the subject and its importance. However, the gaps in our knowledge in this area are yet considerable. Some early workers wrote that leprosy and tuberculosis are 'antagonistic diseases'[1,4]. Others have noted that patients with lepromatous disease often tended to develop tuberculous disease as well[2]. Further, there is also evidence that the status of hypersensitivity to mycobacterial antigens, or its induction or modification using BCG, may affect the subsequent development of leprosy disease.

The earlier studies have all investigated the relationship between reactivities to lepromin of human origin and tuberculin reactivity, and none appear to have examined the relationship between tuberculin and reactivity to Soluble Protein Antigen (SPA) of *Mycobacterium leprae*, which have now become available for skin tests. In this report is examined the relationship between tuberculin reactivity and reactivity to Lepromin A (of armadillo origin) and that to SPA.

MATERIALS AND METHODS

The tests done and methods of analysis of relationships between reactions were as described in detail elsewhere. [8,10,11] The methodology was as follows:

The method of skin testing used was the standard intradermal technique recommended for tuberculin testing, the skin tests being carried out on the volar aspect of the forearm, at sites 6 - 7 cms. apart. All individuals tested were adults (12 years of age, and above) in three population groups in two geographical areas. The antigens used were (1) Lepromin A (of armadillo origin supplied by Dr. Hastings of the National Hansens' Disease Centre, Carville, U.S.A., through courtsey of the Chief, Leprosy Section, World Health Organisation, Geneva), (2) a Soluble Protein Antigen (SPA) of *M. leprae*, prepared by ultrasonic disruption of the organism, with a protein content of 10 μg/ml (Batch CD19) - (supplied by Dr. R. J.W. Rees of the IMMLEP M. leprae Bank, Harrow, Middlesex, U.K.), and (3) Tuberculin PPD - RT23, 2 t.u. per dose (Staten Serum Institute, Copenhagen, Denmark). The tests were read, with Lepromin A - Fernandez reactivity at 48 hours, and Mitsuda reactivity at 28 days, and SPA and Tuberculin reactivities at 72 hours. The results were read as the maximum transverse diameter of the induration palpable, with Fernandez, SPA and tuberculin reactivities, and of the nodule palpated (observed) with Mitsuda reactivity.

TABLE I. Availability of results at different locations for analysis of the relationships between reactivities

Location of population group	Antigens used in tests	Comparison of reactivities possible	Numbers tested: Total	BCG Status: Negative	BCG Status: Positive
Pussellawa (elevation 900m)	(1) Lepromin A (bacillary content 3×10^7/ml) (2) Tuberculin PPD RT 23 (2 t. u./dose)	(1) Fernandez vs. Tuberculin	256	147	109
		(2) Mitsuda vs. Tuberculin	221	124	97
Nuwara Eliya -Pedro (elevation (1950m)	As above	(1) Fernandez vs. Tuberculin	120	74	46
		(2) Mitsuda vs. Tuberculin	98	56	42
Nuwara Eliya -Mahagastota (elevation 1950m)	(1) Soluble Protein Antigen of M. leprae (SPA, Protein content 10μg/ml) (2) Tuberculin PPD RT 23 (2 t. u./dose)	(1) SPA vs. Tuberculin	87	53	34

Results were therefore available for reactivites elicited by three different antigens in three population groups, in two geographical areas (Table I). The relationships examined here will be that between tuberculin reactivity and Fernandez and Mitsuda and SPA reactivities. Broadly, the relationship between reactivities to antigens of *Mycobacterium leprae* (Lepromin A and SPA) and tuberculin were studied by examination of (a) the correlations between reactivities by the use of regression analysis, (b) the changes in the patterns of frequency distributions of a reactivity, where reactions are classified according to immunological status [whether a "reactor" (or "positive") or 'nonreactor" (or "negative")] of another reactivity and (c) the occurrence of combinations of reactions of different immunological status (as above) with each type of reactivity in the same individual.

In the analysis of reactions, the differentiation of reactions to antigens of *M. leprae* into "non-reactors" ("negative") and "reactors" ("positive") could be made relatively easily on the basis of a simple examination of frequency distributions.[10,11] Such interpretation was difficult with the frequency distributions of tuberculin reactions. Further, it has been suggested that the criteria that may have to be used for classification of "negative" and "positive" tuberculin reactions (to identify specific infections by *Mycobacterium tuberculosis* - analogous to the "reactor" component of distributions of reactions with antigens of *M. leprae*) - may be different in different altitude and geographical zones of Sri Lanka[9]. Thus a reaction size of 6 mm or more may be acceptable for a "positive" reaction - "reactor", at the highest elevation and 10 mm or more at a lower elevation (though with greater error). Therefore, when the immunological status of tuberculin reactions were used in analysis, both these criteria were used in the same type of analysis.

TABLE 2. Bivariate frequency distribution of tuberculin and Fernandez reactions in the same individuals, in the whole population

	REACTION SIZES WITH	FERNANDEZ										
		0	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19-20
TUBERCULIN	0	81	18	23	34	15	5	4	4			
	1—2	1	3	6	8	5		1	1			
	3—4	4	1	7	8	1	1	1				
	5—6	4	1	1	4	3	1					
	7—8	5	1	6	3	3		1	1			
	9—10	4		6	4	5	1	1			1	
	11—12	9		3	4	2	3	2				
	13—14	2		4	5	6	1	2	1			
	15—16	4		1	2	4	5			1	1	
	17—18	5			2	2	2	1	2			
	19—20	1		2	1			1	1	1		

Individuals not included in the Table had reaction sizes - Tuberculin / Fernandez (respectively) of, 22/14 (two reactions), 24/10, 26/10 and 34/22.

Results

In Tables 2, 3 and 4 are presented bivariate frequency distributions of Tuberculin and Fernandez reactivity, Tuberculin and Mitsuda reactivity and Tuberculin and SPA reactivity (in the same individuals) respectively, in the whole population tested.

TABLE 3. Bivariate frequency distribution of tuberculin and Mitsuda reactions in the same individuals, in the whole population

REACTION SIZES WITH		MITSUDA										
		0	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19-20
TUBERCULIN	0	26	6	32	34	40	5	1				
	1—2	2		5	8	6	2					
	3—4		3	2	3	10	3					
	5—6	1		3	5	3	2	2				
	7—8	3	1	1	5	4	4	1				
	9—10	1		2	8	3	3	1				
	11—12	2		2	6	8	3	1	1			
	13—14				4	7	1	3				
	15—16	3		1	6	4	5					
	17—18	2		2	4	3	1					
	19—20			1		2						

Individuals not included in the Table had reaction sizes — Tuberculin/ Mitsuda (respectively) of, 22/8, 22/12, 24/6 (two reactions), 26/6 and 28/4.

TABLE 4. Bivariate frequency distribution of tuberculin and SPA reactions in the same individuals, in the whole population

REACTION SIZES WITH		SPA										
		0	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19-20
TUBERCULIN	0	28	3	1	1	2	4	3	2		1	
	1—2	8	5	1						1		
	3—4			1						2		
	5—6											
	7—8	1			1			1	1			
	9—10											
	11—12	1		1						1		1
	13—14						1	1		1	1	
	15—16	1				1			1			
	17—18									2		
	19—20								1			

Individuals not included in the Table had reaction sizes — Tuberculin/ SPA (respectively) of, 18/24, 22/0, 22/12, 22/14, 24/0 and 24/10.

In Table 5 is presented the correlation betweeen reactivities to antigens of *M. leprae* and tuberculin in the whole population studied, irrespective of location or BCG status. (Fernandez and Mitsuda reactivity in relation to tuberculin could be studied at two locations and SPA with tuberculin in one location only). While all correlations are highly statistically significant ($\alpha \geqslant 0.01$), the strengths of correlations (r^2) were low, the closest correlation being between Fernandez and tuberculin reactivity and least between SPA and tuberculin reactivity.

TABLE 5. Correlation between different types of reactivity to antigens of *M. Leprae* and tuberculin of the whole population

Correlation between	Constant (S.E.)	Coefficient (S.E.)	r^2	F value
Fernandez vs. Tuberculin	2.8044 (0.4004)	0.4517 (0.064)	0.32	48.816**
Mitsuda vs. Tuberculin	2.5695 (0.6927)	0.4581 (0.1122)	0.22	16.661**
SPA vs. Tuberculin	3.634 (0.779)	0.3478 (0.088)	0.15	15.57**

S.E. — Standard error (within parenthesis) ** Statistically significant at an $\alpha \geqslant 0.01$ level

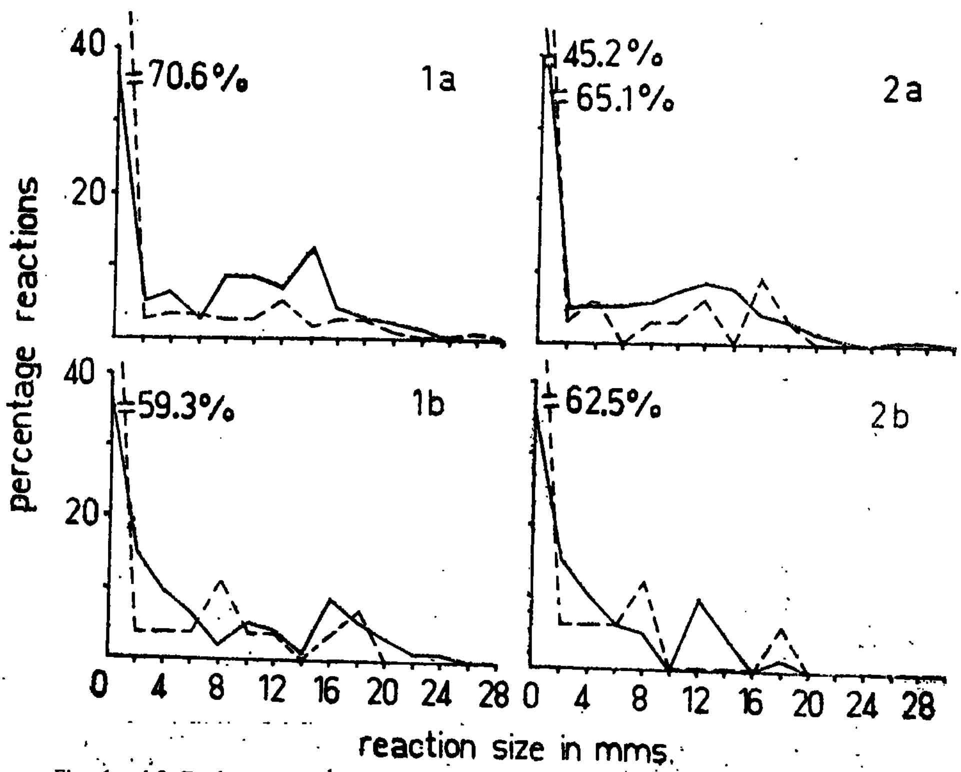
TABLE 6. Correlation between different reactivities to antigens of *M. Leprae* Fernandez and Mitsuda) and tuberculin,considered in relation to non-reactor/reactor status of Fernandez, Mitsuda, and SPA reactivities (levels of significance)

Subset			Fernandez vs. Tuberculin where: F is NR/R	M is NR/R	Mitsuda vs. Tuberculin where: M is NR/R	F is NR/R	SPA vs. Tuberculin+ where: SPA is NR/R
Of whole population		NR	NS	**	NS	NS	NS
		R	**	**	**	**	NS
Effect of BCG status	NR	BCG—ve	*	**	NS	NS	NS
	NR	BCG+ve	NS	**	NS	NS	NS
	R	BCG—ve	**	NS	NS	NS	NS
	R	BCG+ve	**	**	*	*	NS
Effect of location	BCG — ve NR	Pussellawa	NS	*	NS	NS	
	BCG — ve NR	Pedro	NS	NS	NS	NS	
	BCG — ve R	Pussellawa	*	NS	NS	NS	
	BCG — ve R	Pedro	**	NS	NS	NS	
	BCG + ve NR	Pussellawa	NS	*		NS	
	BCG + ve NR	Pedro	NS	**		NS	
	BCG + ve R	Pussellawa	*	**	*	NS	
	BCG + ve R	Pedro	NS	NS	*	*	

+ Results with SPA and Tuberculin were available only for a single location at Mahagastota; NR-Non reactors
R — Reactors; NS — Not significant; * — Significant at ≥ 5% level; ** — Significant at ≥ 1%level.
F — Fernandez reactivity; M — Mitsuda reactivity

In Table 6 is presented a summary of the analysis of correlations (levels of significance) between tuberculin and Fernandez, Mitsuda and SPA reactivity. The level of significance of correlations had been analysed separately in respect of non-reactor and reactor status [10,11] of Fernandez and Mitsuda reactivities and SPA reactivity. The reactor groups of the whole population seem to show more statistically significant correlations than do the non reactor groups. Also, none of the cohorts studied in the correlation between SPA and Tuberculin showed statistically significant correlations. No definitive trends seem to appear in relation to the other correlations of other cohorts studied, i.e. with BCG vaccination status and location.

The second type of analysis carried out was an examination of the variations of the frequency distributions of reactions with changes in immunological status of a determining reactivity (which could be tuberculin, Fernandez, Mitsuda or SPA reactivity) (Figures 1-3). It is noteworthy that in all cases the frequency distributions showed that the essential difference was that the reactor distributions (when compared with the non-reactor distributions) showed a reduction of non-reactors (of the distributions being examined) with an increase of reactors - a "shift to the right". The major exception to this rule seems to be at Pussellawa, with the distributions of Mitsuda reactions which seemed to show no markedly apparent change in pattern of reactions with immunological status of tuberculin reaction.



Figs. 1 and 2. For legend see page 48

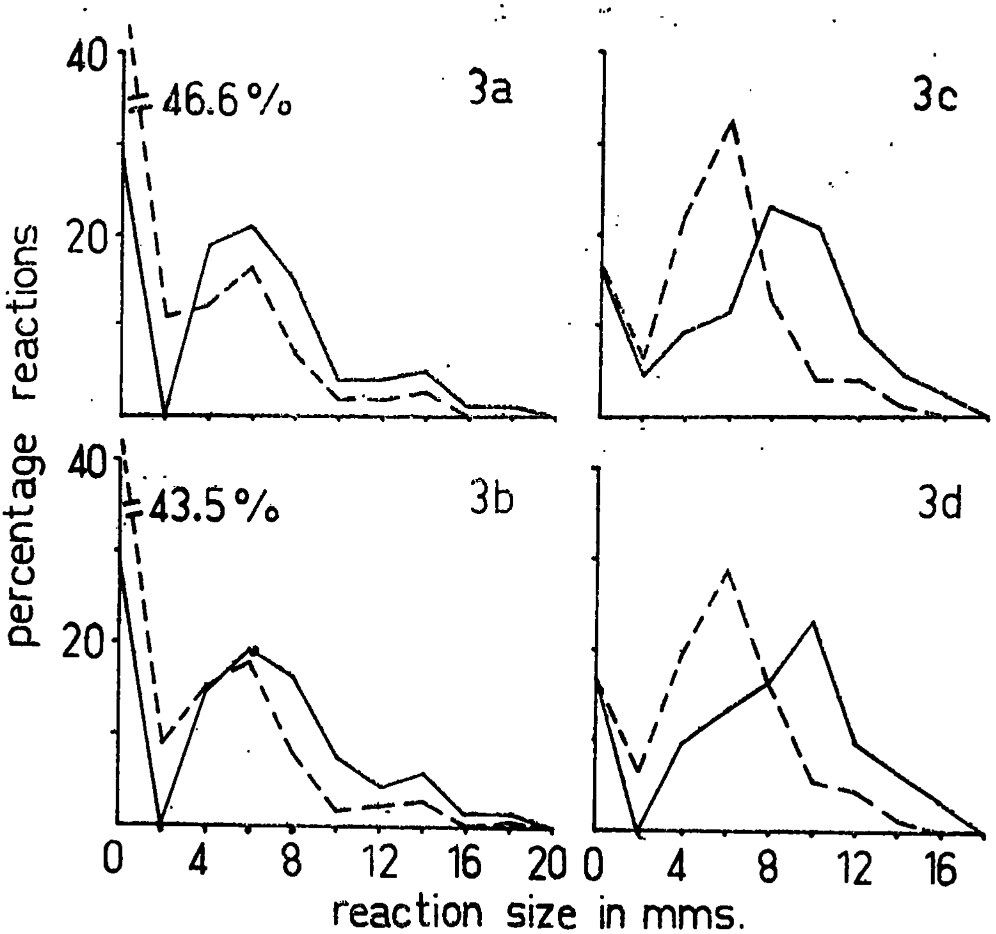


Fig. 3. For legend, see page 48.

Another way in which some investigators have examined the relationship between tuberculin reactivity and lepromin reactivity is to compare the occurrence of "positives" (viz. reactors, and non-reactors) in the same individuals with both. Such evaluations are presented in Tables 7, 8 and 9. The most striking change observed here is the effect of BCG vaccination in markedly increasing the number of reactors with all three reactivities. Apart from this, evaluation of these results do not present definite or specific conclusions regarding the concurrence (or not) of immunological status as assessed with different types of reactivity.

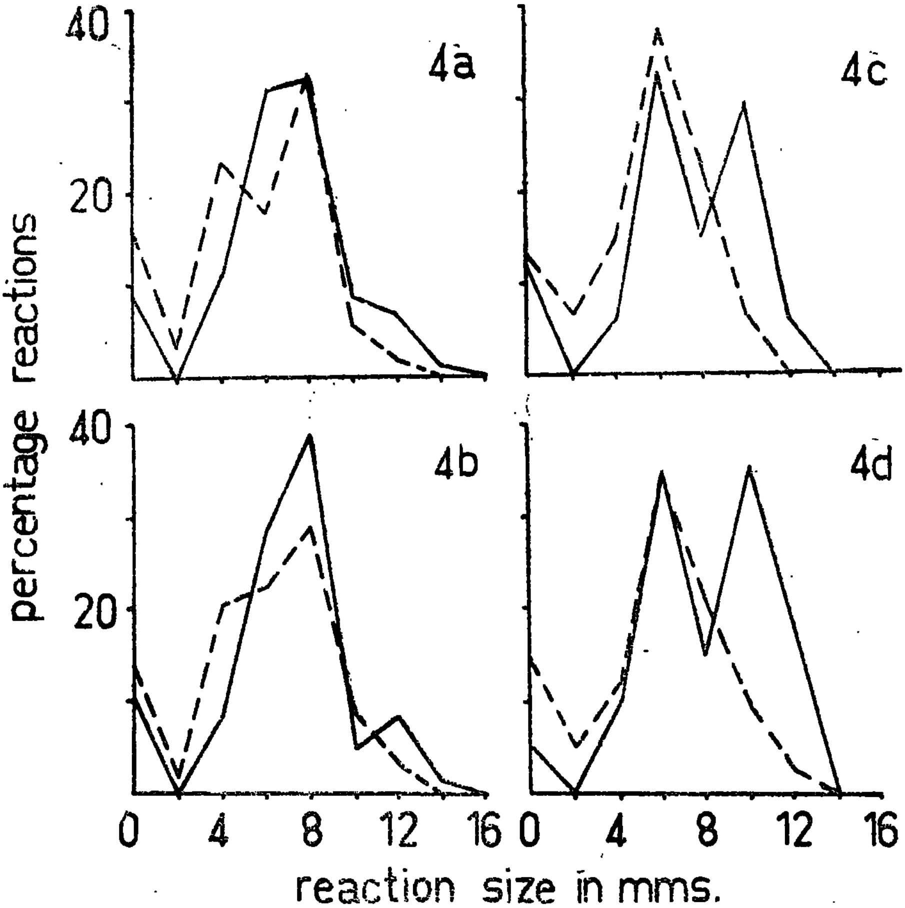


Fig. 4. For legend see page 48.

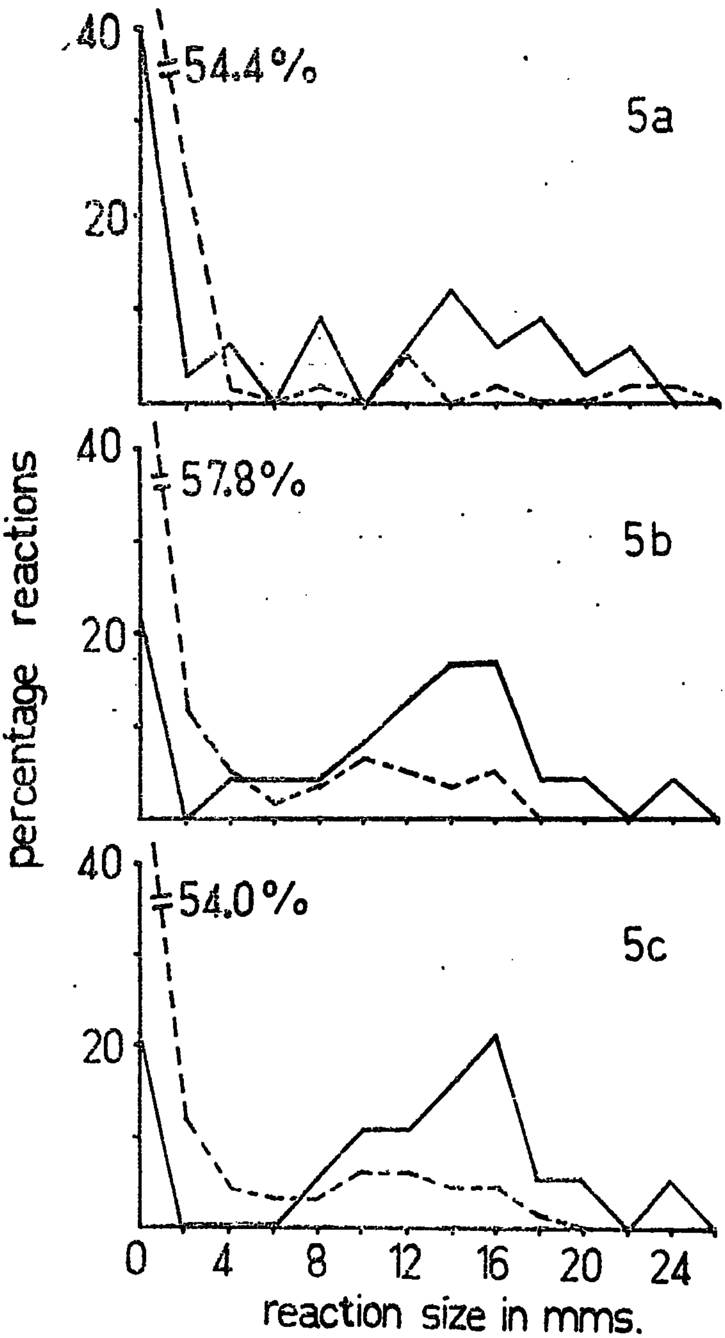


Fig. 5. For legend see page 48.

LIST OF FIGURES

FIGURE 1. Frequency distribution of tuberculin reactions where Fernandez reaction size was $\leqslant$ 2 mm. (--- --- ---) and >2 mm. (————)
(*a*) at Pussellawa
(*b*) at Pedro

FIGURE 2. Ferquency distribution of tuberculin reactions where Mitsuda reaction size was $\leqslant$ 2 mm. (— — —) and $\geqslant$ 3 mm.(————)
(*a*) at Pussellawa
(*b*) at Pedro

FIGURE 3. Frequency distribution of Fernandez reactions where tuberculin reaction size was
(*a*) $\leqslant$5mm. (— — — —), and $\geqslant$6 mm. (————) at Pussellawa
(b) $\leqslant$ 9 mm. (— — — —), and $\geqslant$ 10 mm. (————) at Pussellawa
(*c*) $\leqslant$5 mm. (— — — —), and $\geqslant$ 6 mm. (————) at Pedro
(*d*) $\leqslant$ 9 mm. (— — — —), and $\geqslant$ 10 mm. (————) at Pedro

FIGURE 4. Frequency distribution of Mitsuda reaction where tuberculin reaction size was
(*a*) $\leqslant$ 5 mm. (— — — —), and $\geqslant$ 6 mm. (————) at Pussellawa
(*b*) $\leqslant$ 9 mm. (— — — —), and $\geqslant$ 10 mm. (————) at Pussellawa
(*c*) $\leqslant$ 5 mm. (— — —), and $\geqslant$ 6 mm. (————) at Pedro
(*d*) $\leqslant$ 9 mm. (— — —), and $\geqslant$ 10 mm. (————) at Pedro

FIGURE 5. Frequency distribution (at Mahagastota) of
(*a*) tuberculin reactions where SPA reactions were $\leqslant$ 4 mm. (— — —), and $\geqslant$5 mm. (————)
(*b*) SPA reactions where tuberculin reactions were $\leqslant$ 5 mm. (————), and $\geqslant$ 6 mm. (————)
(*c*) SPA reactions where tuberculin reactions were $\leqslant$ 9 mm. (— — — —), and $\geqslant$ 10 mm. (————)

TABLE 7. Occurrence of individuals with different immunological status with different reactivities (association between Fernandez, Mitsuda and tuberculin reactivities)

Status of tuberculin, Fernandez and Mitsuda reactivities respectively	Percentage individuals: Where tuberculin reactivity was considered non-reactor if reaction size was 5 mm. or less and reactor if 6 mm. or more			Percentage individuals: Where tuberculin reactivity was considered non-reactor if reaction size was 9 mm. or less and reactor if 10 mm. or more		
	BCG - ve	BCG+ve	Total	BCG -ve	BCG+ve	Total
RRR	16	36	22	14	19	16
RRN	3	6	4	2	2	2
RNN	6	.	4	5	.	3
RNR	9	11	10	7	6	7
NNR	16	21	17	18	26	21
NRR	24	17	22	27	34	28
NRN	10	6	9	11	10	11
NNN	16	4	12	16	4	12

R - Reactor; *N* - Non-reactor; *RRR* - reactor to tuberculin, Fernandez and Mitsuda respectively, *NNN*, non-reactor to all three, *RRN* - reactor to Fernandez and tuberculin and non-reactor to Mitsuda respectively and so on.

TABLE 8 Occurence of individuals with different immunological status with different reactivities (association between SPA and tuberculin reactivities)

Status of tuberculin and SPA reactivities respectively	Percentage individuals: Where tuberculin reactivity was considered non-reactor if reaction size was 5 mm. or less and reactor if 6 mm. or more			Percentage individuals: Where tuberculin reactivity was considered non-reactor if reaction size was 9 mm. or less and reactor if 10 mm. or more		
	BCG -ve	BCG +ve	Total.	BCG -ce	BCG+ve	Total.
RR	20	32	25	15	27	19
RN	9	6	8	6	3	3
NR	9	24	15	19	32	24
NN	61	32	52	61	38	52

R - Reactor; *N* - Non-reactor; *RR* - reactor to tuberculin and *SPA* respectively, *NN* - Non-reactor to both, *RN* - Reactor to tuberculin and Non-reactor to *SPA*, *NR* - Non-reactor to tuberculin and reactor to SPA

TABLE 9 Occurnce of individuals with different immunological status to different reactivities–analysis of variability of results

		Percentage Individuals					
		When a tuberculin non-reactor is a reaction of 5 mm. or less, and reactor is 6 mm. or more			When a tuberculin non-reactor is a reaction of 9 mm. or less, and reactor mm. or more		
		BCG-ve	BCG+ve	Total	BCG-ve	BCG+ve	Total
Status of Tuberculin, Fernandez and Mitsuda reactivities together - where status of each							
Agree	R	16	36	34	14	19	29
	N	16	4		17	4	
Disagree	R	18	17	66	14	8	71
	N	50	44		55	69	
Status of Tuberculin and Fernandez reactivities only - where status of each							
Agree	R	18	41	66	16	21	50
	N	16	25		34	29	
Disagree	R	32	11	34	12	6	50
	N	34	23		38	44	
Status of Tuberculin and Mitsuda							
Agree	R	25	48	57	21	25	45
	N	26	9		27	13	
Disagree	R	9	6	43	6	2	55
	N	40	37		44	61	

N and R — here refers to non-reactor or reactor status of tuberculin reactivity

It has been shown that the smaller reaction sizes to tuberculin are due to "non-specific sensitisation" - the cross reaction elicited, due to infection by some other mycobacterium. The problem of nonspecific sensitisation is complex, with probably multiple factors influencing its occurrence. Of tuberculin reactions, the intermediate reatcion sizes (of 2-9 mm) are considered to be largely due to such non-specific reactions. The variation of Fernandez and Mitsuda reaction patterns with such non-specific sensitisation was examined by the comparison of frequency distributions of the latter in those whose tuberculin reactions were (a) ≤ 2, mm (b) 3-9 mm, and (c) ≤ 10 mm. These patterns were examined with cohorts of different BCG status, where numbers permitted, at Pussellawa and Pedro. With Fernandez reactivity, which could be examined in both areas, with increase in tuberculin reaction size, there seemed to be an increase in Fernandez reaction size as well but there was no change which could be said to be peculliar to (or specific to) the non-specific sensitisation group. With Mitsuda reactivity, the comparison was possible only at Pussellawa, where the Mitsuda pattern was similar in all three groups of tuberculin reactivity.

DISCUSSION

The results presented here show a statistically significant, though at a low level(r^2) of correlation, between tuberculin reactivity and reactivity to antigens of *M. leprae*. For correlations between tuberculin and antigens of *M. leprae*,r^2 was somewhat lower than that for correlations between reactivities elicited by antigens of *M. leprae*. The relationships between tuberculin and other reactivities were also confirmed by the examination of frequency distributions of different reactivities in relation to immunological status (non-reactor/reactor) of another type of reactivity. The examination of the concurrence of the immunological status of different types of reactivities however failed to show any definite results except to demonstrate the influence of BCG vaccination status in increasing the occurrence of reactor status. Further it was not possible to show any special influence of "non-specific sensitisation" as demonstrated by tuberculin testing on the patterns of Fernandez and Mitsuda reactivity.

Direct comparison of the data of this survey with that of other workers is not possible, because of differences of the criteria used for evaluation (different criteria used for reading and or delineating "positive" and "negative") and also of the antigens used. However the conclusions reached by different workers have been variable. With "early lepromin reactions" read at 48 hr, as compared with tuberculin reactions, Fernandez[3] reported agreement and disagreement, as regards "positivity" of each type of reactivity, in equal numbers; Kupfer[6] similarly found no correlation. With the late lepromin reaction, a correlation was reported by Kupfer[6] and Doull *et al.*,[2] while Kooij and Rutgers[5] found no correlation.

ACKNOWLEDGEMENTS

This investigation was supported in part by a grant from the Leprosy Section, World Health Organisation, Geneva.

We wish to thank Mr. D.W. Dissanayake, Medical Assistant, Mahagastota State Plantation, Nuwara Eliya, and Ms. M. G. Kusumalatha, Medical Assistant, Trafford Hill State Plantation, Galagedera, for assistance in carrying out this study; and Ms. T.M. Gamage and Ms. M.Wijekoon of the Department of Microbiology, Faculty of Medicine, University of Peradeniya, Peradeniya, for assistance in data analysis.

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