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The sensitization of children by opportunist mycobacteria in Lagos, Nigeria

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SUMMARY

Groups of school children aged 6–14 years were tested with PPDS and one of the following five antigens simultaneously, namely PPDA, PPDF, PPDG, PPDPL and PPDY. PPDS was prepared from the human tubercle bacillus, and the others were prepared from *M. avium*, *M. fortuitum*, the 'Gause' organisms, *M. marinum* and *M. kansasii* respectively.

It was observed that there was some increase in induration size to PPDA, PPDG, PPDL, PPDS and PPDY with increase in age, while *M. fortuitum* gave a preponderance of small reactions with no increase of size with age. It was also observed that there was evidence of cross sensitization between PPDS and all the other antigens, particularly in those who had negative or doubtful reactions.

INTRODUCTION

In recent years, considerable interest has been shown in the opportunist acid-fast bacilli recovered from persons with clinical or radiological evidence of disease (MacCallum, Tolhurst, Buckle & Sissons, 1948; Tarshis & Frisch, 1952; Buhler & Pollak, 1953). Beer & Davis (1965) reported that of 2852 cultures of mycobacteria isolated in Lagos in the course of routine examinations for pulmonary tuberculosis, 6% were diagnosed as opportunist mycobacteria and that these were often *M. fortuitum*.

Ogunbi (1969) in a preliminary report on isolation of mycobacteria from patients attending chest clinics in Lagos recorded a 4% incidence of opportunist mycobacteria.

Their incidence in the isolates of all mycobacteria from the Yaba Federal Laboratory in 1965 was about 4%, which is similar to the figure of 4·23% found in the W.H.O. survey in Nigeria (W.H.O. 1957).

Davis & Ogunbi (1967) in a preliminary survey of 737 adults aged 15–25 years in Lagos showed that reactions to PPDs of mycobacteria other than *M. tuberculosis* were very common in Lagos. The population tested showed a high frequency of sensitization by *M. tuberculosis* and this probably accounted for some of the

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Table 1. *Reagents used, and numbers of children tested with each*

Reagent	Prepared from	Children tested	BCG immunization		
			Yes	No	Not known
PPDS	*M. tuberculosis*	4261	1342	2449	470
PPDA	*M. avium*	1228	369	667	192
PPDF	*M. fortuitum*	1108	409	596	103
PPDG	'Gause' organism	512	143	297	72
PPDPL	*M. marinum*	411	64	328	19
PPDY	*M. kansasii*	1002	357	561	84

results. They concluded that scotochromogens and nonphotochromogens were important human sensitizers in Lagos.

MATERIALS AND METHODS

A total of 4261 children between the ages of 6 and 14 were skin tested with two reagents. One reagent was PPDS in each case and the other was one of five other PPDs prepared from different mycobacterial species. Details of the reagents used, numbers of children receiving each, and incidence of BCG immunization in each group, are shown in Table 1. It is seen in the table that 470 children were not sure whether they had had BCG or not, and statistical analysis has shown that the exclusion of these children did not in any way affect the results reported here.

The antigens were supplied as pre-diluted solutions in a strength of 0·0001 mg protein per 0·1 ml containing 1% phenol as a preservative but not containing Tween 80 as a stabilizer.

The tests were performed by injection of 0·1 ml intradermally on the volar surfaces of opposite forearms; the results were read after 48 h.

RESULTS

The mean age for boys was 9·5 years and for girls 9·7 years. The mean weight for boys was 27·9 kg and for girls 27 kg. 31·5% of the children had had BCG, while the rest of them either had had no BCG or were not sure whether they had had BCG or not.

The indurations were graded according to the standards of the American Thoracic Society (1971) and this is considered to be widely accepted internationally. Thus those with PPDS readings below 5 mm diameter are negative, those between 5 and 9·9 mm are doubtful and those of 10 mm or more are definitely positive for tuberculosis.

Table 2 shows the results in two different age groups, also divided according to BCG immunization status. Table 3 shows the results for the two sexes in children who had been immunized with BCG. In table 4 are shown the results for those who had not received BCG, divided into those giving negative, doubtful and positive reactions to PPDS.

Table 2. *Percentages of children, in two age groups, with and without BCG immunization, showing different diameters of induration*

	Aged 6–10 years						Aged 11 years or more					
	No BCG Induration diam. (mm)			BCG Induration diam. (mm)			No BCG Induration diam. (mm)			BCG Induration diam. (mm)		
Reagent	0–4	5–9	10+	0–4	5–9	10+	0–4	5–9	10+	0–4	5–9	10+
PPDS	86·4	4·8	8·8	85·3	4·7	10	72·8	9·9	17·3	75	6	19
PPDA	89·2	6·3	4·4	87·4	8·4	4·2	57·8	27·1	15·1	65	30	5
PPDF	93·7	4·8	1·4	96·2	3	0·8	83·1	5·3	1·6	94	6	0
PPDG	87·6	6·2	6·2	78	10	12	60·3	25·8	13·9	57	29	14
PPDPL	93·9	4·1	2	83·5	9·3	7·2	83·5	9·3	7·2	89	8	3
PPDY	88·7	4·9	6·4	90	9	4	66·6	23·9	9·4	79	15	6

Table 3. *Percentages of boys and girls previously immunized with BCG showing different diameters of induration*

	Boys			Girls		
Reagent	0–4 mm	5–9 mm	10+mm	0–4 mm	5–9 mm	10+mm
PPDS	82·9	5·8	11·4	83·7	4·1	12·2
PPDA	84·2	11·3	4·5	83	12·9	4·1
PPDF	95·8	4·2	0	95·9	2·8	1·4
PPDG (age 6–10 only)	83·3	11·1	5·6	76·6	9·4	14·1
PPDPL	90	2·5	7·5	70·8	16·7	12·5
PPDY	86·6	8·7	4·7	89·9	6·3	3·8

Table 4. *Percentages of non-BCG immunized children reacting to other reagents, grouped according to their reaction to PPDS*

	Diameter of reaction to PPDS								
	0–4 mm (78·7 %)			5–9 mm (7·7 %)			10+mm (13·6 %)		
Diameter of reaction to agents below ...	0–4	5–9	10+	0–4	5–9	10+	0–4	5–9	10+
PPDA	80·4	14·8	4·8	33	45	22	19	36	45
PPDF	95·9	3·9	0·2	76·5	17·6	5·9	87·2	10·2	2·6
PPDG	96·4	1·8	1·8	17	50	33	38	24	38
PPDPL	96·4	3·2	0·4	74·1	18·5	7·4	23·3	37·2	39·5
PPDY	93·6	5·6	0·8	35·8	51·6	12·6	11	43·3	45·7

DISCUSSION

With the exception of PPDF, all the reagents showed an increase of sensitization to the different mycobacteria with increasing age. This was most marked in each case amongst those who had not received BCG (Table 2). The increase with age was greater amongst the intermediate (5–9 mm) reactions with reagents PPDA, PPDG and PPDY, and amongst the larger reactions with PPDS. The most likely explanation of the increase in percentage reactors is increased contact with the mycobacterial species from which the reagents were prepared.

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For PPDF, there was generally a preponderance of small-sized reactions, with no increase in induration size with age, indicating that this antigen was not a common source of sensitivity in the population. This agreed with some work done by Davis & Ogunbi (1967) among Lagos school children where *M. fortuitum* was found not to be an important human sensitizer in Lagos. It was not, however, consistent with the results of a culture study by Beer & Davis (1965) where *M. fortuitum* was commonly encountered.

Possible explanations for the disparity between the PPDF results and the earlier work on the isolation of *M. fortuitum* were that the organisms isolated were *M. chelonei* since this was not differentiable from *M. fortuitum* in 1965, or that the PPD was prepared from *M. fortuitum* of a serotype different from that commonly found in Nigeria.

Table 3 shows the results for BCG recipients divided according to sex. It can be seen that there is little difference between the reactions to most reagents, but there is a preponderance of large reactions to PPDG and PPDPL amonst the girls. However, because of the distribution of children tested the comparison between the sexes for PPDG can only be made for the ages 6–10 years.

In human vaccination programmes it is common practice to judge vaccines in terms of percentage of the vaccinated population that become tuberculin reactors. Palmer & Long (1966) found that if *M. fortuitum* is judged in these terms, it would not be regarded as a satisfactory organism for use as a vaccine. They found that only a small percentage of animals previously inoculated with *M. fortuitum* had tuberculin reactions and these were small in size.

The effect of BCG (shown in Table 2) as measured by sensitization to PPDS was small. One reason for this may be that since some of the children had BCG about 10 years ago the sensitivity due to BCG could be waning. Also, the BCG vaccine could have deteriorated before it was administered or the technique of giving BCG could have been faulty. All these factors would give a variable effect on the results. Therefore, the results for those who had *no* BCG were analysed separately in order to get a true picture of sensitization, uncomplicated by BCG vaccination. This is in agreement with L. B. Edwards (pers. comm.) who stated that once BCG is introduced into a community, it becomes difficult to assess the effects of sensitization by opportunist mycobacteria.

Studies of cross reactivity made between PPDS and the other reagents (see Table 4) amongst children not immunized with BCG show a definite correlation between size of reaction to PPDS and to each of the others, although with PPDF the correlation is only seen amongst the small and intermediate sized reactions. Thus amongst the children producing small sized (negative) reactions to PPDS, less than 5% produce large size (10 mm+) reactions to other reagents, and amongst those producing large reactions to PPDS about 40% produce large reactions to the other reagents. In general there was evidence of cross reactions between PPDS and the other antigens which was better seen among the negative or doubtful reactions to PPDS. In contrast to this, among the presumed specific reactions to PPDS, there was much less evidence of cross reactions.

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