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A preliminary study of the effect of contact with environmental mycobacteria on the pattern of sensitivity to a range of new Tuberculins amongst Ugandan adults

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SUMMARY

A range of new Tuberculins prepared from extracts of living organisms belonging to 12 mycobacterial species has been used to assess the effect of BCG immunization and contact with environmental mycobacteria on Ugandan adults. A total of 2,456 tests were carried out on 562 people, 86% of whom came from three areas selected for special study. These areas were chosen on the basis of occurrence of leprosy and *M. ulcerans* infection and on data concerning the distribution of environmental mycobacteria. It was found that the effect of BCG was small compared with that previously observed amongst Kenyan schoolchildren, but that the effect of geographical origin was considerable. There was some correlation between the percentages of reactivity to the reagents and the frequency of mycobacteria in the environment.

INTRODUCTION

Preliminary studies on the mycobacterial flora of the environment in Uganda have been carried out on both grass and mud specimens. The studies on grasses have shown mycobacteria to be most commonly associated with seasonal and permanent swamplands (Barker, Clancey & Rao, 1972) and the studies on mud have related these organisms particularly to areas with surface water pH values between 5·5 and 7 (Stanford & Paul, 1973). Of the districts from which mud samples were taken, the highest isolation rate and the greatest number of different species were recovered from East Bunyoro, Southern Lango and North Busoga.

Few mycobacteria were isolated from mud samples taken from regions where the surface water was alkaline, such as the slopes of the Ruwenzoris in Toro or the semi-desert Karamoja.

Amongst the many mycobacterial strains isolated, *Mycobacterium avium*, *M. nonchromogenicum*, *M. engbaekii*, *M. gordonae*, *M. fortuitum*, *M. vaccae*, *M. neoaurum*, *M. kansasii* and an as yet unnamed distinct species M. 'A*' (temporary taxon) have been recognized. Skin test antigens were prepared from each of these species with the exception of *M. kansasii*, of which only one environmental strain was recovered. Additional skin test antigens were prepared from *M. tuberculosis*, *M. chelonei* and *M. ulcerans*, which are common causes of mycobacterial infection in Uganda, and from *M. duvalii*, an organism originally considered to have some relation with *M. leprae* (Duval & Wellman, 1912; Godal, Myrvang, Stanford & Samuel, 1974). Lastly an antigen was prepared from *M. gilvum* as a result of the observation of G. A. W. Rook (personal communication) that this organism commonly induces transformation of lymphocytes from Kampala Ugandans.

This set of 13 skin test reagents together with PPD (RT23) has been used in this study to test adults from different areas of Uganda in an attempt to assess whether their pattern of sensitivity to mycobacterial antigens reflects the environment modified by the local prevalence of the mycobacterioses. The preliminary studies of Pritchard, Stanford & Paul (1974) indicated differences in reactivity to two of these reagents (Ranin and Gordonin) between cattle herds from the alkaline acacia scrubland of Karamoja and from the acidic swamplands and lush grasslands of Ankole.

MATERIALS AND METHODS

The skin test reagents

The reagents employed in this study were the same as those used in our studies on Kenyan schoolchildren (Paul, Stanford, Misljenovic & Lefering, 1975*a*) with the addition of Gilvin and Burulin. The Gilvin was prepared by the same method used for the other reagents from *M. gilvum* strain NCTC 10742. The Burulin was prepared as described before (Stanford, Revill, Gunthorpe & Grange, 1975), except that its protein was estimated by the method of Lowry, Rosebrough, Farr & Randall (1951).

The full list of reagents, the dosages used and the number of tests carried out are shown in Table 1.

The people tested

A total of 2,456 tests were given to 562 people. Of these, 259 were staff at general hospitals, 116 were staff at leprosaria and 187 were patients in general hospitals. Details obtained from each person tested included name, age, sex, place of origin and BCG status.

Test procedure

In every case the dose used was administered in 0·1 ml. given intradermally by injection into the volar aspects of the forearms. The six reagents used with a dose

Table 1. *Skin test reagents used, numbers of people tested with each dose and total percentage of positive reactors*

Dose	Number of tests with each reagent			% +ve
	0·04 μg.	0·2 μg.	1 μg.	
Reagents prepared from slow-growing species				
PPD RT23	68	—	—	56
Tuberculin (T)	—	193	55	66
Aviumin (A)	—	204	—	35
A*-in (A*)	—	214	—	70
Gordonin (Go)	—	224	55	43
Burulin (B)	—	106	—	10
Reagents prepared from fast-growing species				
Ranin (R)	—	204	—	24
Duvalin (D)	—	171	55	29
Chelonin (C)	—	145	—	70
Nonchromogenicin (No)	—	134	55	24
Lactin (L)	—	139	—	42
Vaccin (V)	—	206	55	17
Neoaurumin (Ne)	—	118	—	15
Gilvin (Gi)	—	—	55	27

Total number of tests 2,456.

of 1 μg. protein were administered simultaneously, three on each arm in the same 55 people. Those tested with the 0·2 μg. protein dose and with RT23 only received two injections into each arm, so arranged that at some point in the study every reagent was paired with each of the others. Tests were read by measuring the longitudinal and transverse diameters of induration at 72 hr. Reactions of 5 mm. or more mean diameter were taken as positive for RT23 and for tests done with the lower concentration of the other reagents (Paul, Stanford & Carswell, 1975*b*; Paul *et al.* 1975*a*). For tests performed with the 1 μg. dose of reagent 7 mm. was taken as positive. This correction was made on the basis of unpublished data on the use of increased concentrations of our reagents and observations made with Burulin (Stanford *et al.* 1975).

RESULTS

The number of tests performed with each reagent at the various dosages, and the total percentage of positive reactors are given in Table 1. The percentage of positive reactors varied from 10 % for Burulin to 70 % for A*-in and Chelonin. Of the people tested, 27 % had previously received BCG immunization, and the results for immunized and non-immunized populations are shown in Fig. 1. Immunization with BCG makes little difference to the percentage positivity to individual reagents. Ages ranged from 10 to 70 years with an average of 27·4 years; the great majority of people were between 16 and 35 years old.

For reasons discussed below, results for three areas of Uganda (Fig. 2) are

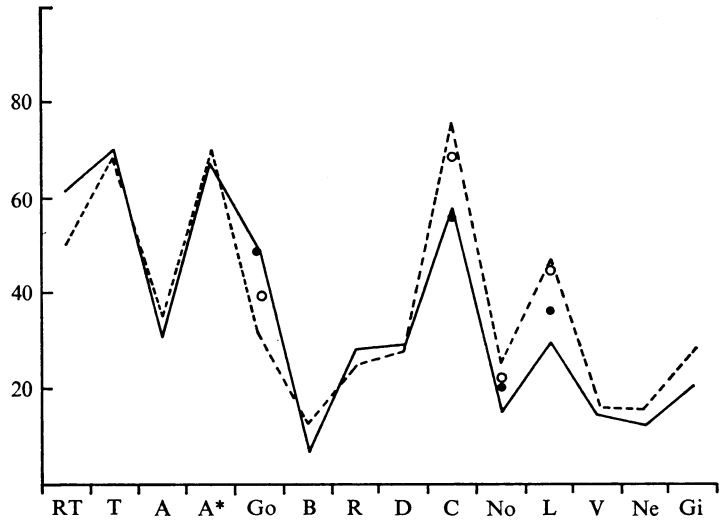

Fig. 1. Diagram showing the percentages of persons producing positive reactions (5 mm. or more) to individual skin test reagents. The solid line links results for all those tested who had received BCG immunization and the broken line links the results for those who had not. Where the two results for individual antigens differed by 10% or more they were corrected for regional differences, and these results are shown as dots for BCG-immunized persons and rings for non-immunized persons.

Key to skin test reagents: RT, P.P.D. (RT23); T, Tuberculin; A, Aviumin; A*, A*-in; Go, Gordonin; B, Burulin; R, Ranin; D, Duvalin; C, Chelonin; No, Nonchromogenicin; L, Lactin; V, Vaccin; Ne, Neoaurumin; Gi, Gilvin.

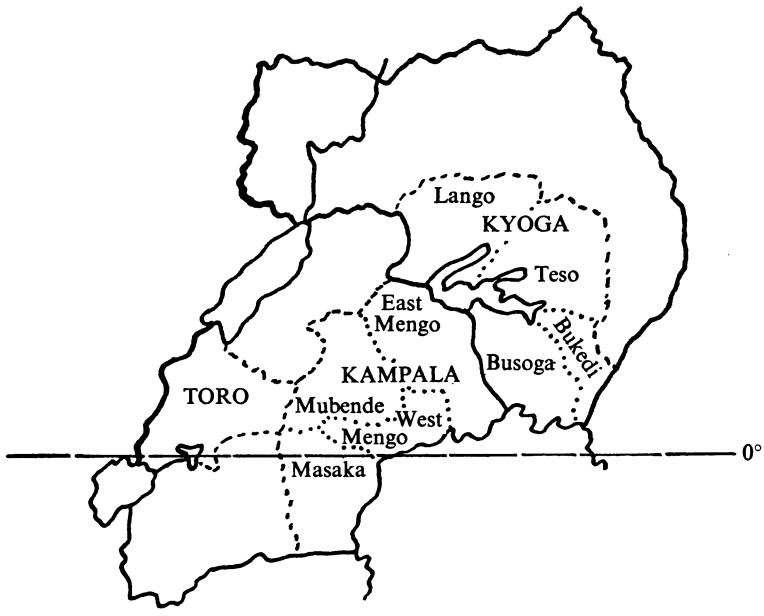

Fig. 2. Map of Uganda showing the three areas selected for special study (capital letters) and the districts of which they are composed.

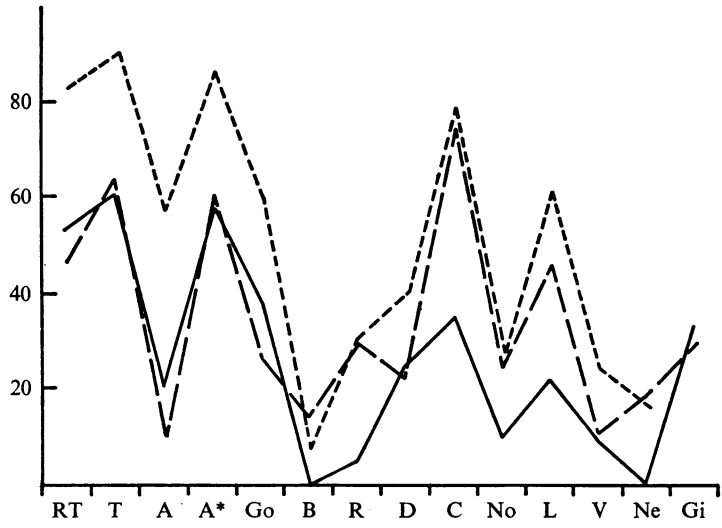

Fig. 3. Diagram showing the percentages of persons from separate areas of Uganda producing positive reactions (5 mm. or more) to individual skin test reagents. The solid line represents those from Toro, the infrequently broken line those from Kampala area and the frequently broken line those from Kyoga area. The reagents used are those listed in the legend to Fig. 1.

Table 2. *Percentages of persons giving positive reactions to the various skin test reagents. The two columns on the left give the results for those who have received BCG and those who have not, whereas the three right-hand columns give the results for people coming from Toro, Kampala and Kyoga regions*

	BCG	No BCG	Toro	Kampala	Kyoga
PPD RT23	61	50	53	47	83
Tuberculin (T)	70	69	61	64	90
Aviumin (A)	31	35	20	10	57
A*-in (A*)	67	69	58	61	87
Gordonin (Go)	49	31	37	27	60
Burulin (B)	7	13	0	14	7
Ranin (R)	28	24	5	30	31
Duvalin (D)	29	28	25	21	40
Chelonin (C)	57	75	35	75	78
Nonchromogenicin (No)	15	25	10	24	28
Lactin (L)	29	47	22	46	61
Vaccin (V)	14	16	9	11	25
Neoaurumin (Ne)	12	16	0	19	17
Gilvin (Gi)	20	28	33	29	—

treated separately. Eighty-six per cent of the tests were carried out on people coming from these three areas. The easternmost area consisting of Lango, Teso, Bukedi and Busoga districts we are calling Kyoga area, the central area of East and West Mengo, Mubende and Masaka districts we are calling Kampala area and the westernmost area consists of Toro district alone. The results for the separate areas, expressed as percentages of positive reactors, are given in Table 2 and in

Fig. 3; there are considerable differences between these results. In general results are highest for Kyoga area and lowest for Toro area. Results for Kampala area are similar to those for Toro with the reagents prepared from slow-growing species (Table 1, upper section) and similar to those for Kyoga area with the reagents prepared from fast-growing species (Table 1, lower section). From Toro area 43% of persons, from Kampala area 16% and from Kyoga area 25% had been immunized with BCG. The age ranges and distribution were similar for each area. From Toro district 7% of persons, from Kampala area 6% and from Kyoga area 21% were known close contacts of leprosy patients.

DISCUSSION

Uganda is an ideal country for this type of study for many reasons. It has a great variety of environmental conditions including mountains, forest, grassland, desert and both acid and alkaline swamplands. All are in well separated areas, but within a few hours drive on good roads from the capital. It has numerous distinct tribal areas, and movement of the population between these areas is not very frequent, except into the larger towns or in the case of certain nomadic herdsmen.

At least four mycobacterioses occur amongst the human population. These are tuberculosis, leprosy, *Mycobacterium ulcerans* infection, and injection abscesses caused by the fast-growing mycobacteria *M. chelonei* and *M. fortuitum*. Tuberculosis occurs in all parts of the country but probably varies in its prevalence from district to district (Morrow in *The Uganda Atlas of Disease Distribution*, 1968). Unfortunately no reliable relevant figures for incidence or prevalence of this disease are available.

According to the surveys made in 1956–59 (Brown in *The Uganda Atlas of Disease Distribution*, 1968) the prevalence of leprosy varies from more than 30 per thousand in some areas to less than 3 per thousand in others. A very detailed map of the prevalence of *M. ulcerans* infection in 1970 has been prepared by one of us (Barker, 1972) and this shows the disease to occur only in certain well-demarcated areas. Mycobacterial injection abscesses have only been recognized in Kampala and in Kamuli, North Busoga, but are probably common in all parts of the country.

Preliminary studies (Stanford & Paul, 1973) which have now been greatly extended show that environmental mycobacteria vary in their frequency from place to place in Uganda, depending at least in part on humidity and the pH of surface water.

Based on the information on distribution of environmental mycobacteria and on leprosy and *M. ulcerans* infection, it has been possible to select areas of Uganda for assessment of the effectiveness of contact with various mycobacteria in the development of specific delayed hypersensitivity. As previously claimed, we believe our skin test reagents have sufficient specificity for this purpose (Paul *et al.* 1975*a*, *b*).

The reasons for the choice of Toro, Kampala and Kyoga areas as defined above and shown in Fig. 2 are listed in Table 3. It can be seen that Toro generally has a high prevalence of leprosy and rather scanty soil mycobacteria. Kyoga area has a

Table 3. *Some of the data used in the selection of the three study areas*

	Areas		
	Toro	Kampala	Kyoga
Environmental mycobacteria			
Isolation rate from mud samples	20%	67%	98%
Isolation rate from grass samples	Not done	34%	56%
Estimated prevalence of leprosy	26/1000	11/1000	23/1000
Known leprosy contact rate in persons tested	7%	6%	21%
Persons tested who had received BCG	43%	16%	25%
Occurrence of *M. ulcerans* infection	Almost nil	Sporadic in some parts	Endemic in some parts

high prevalence of leprosy, and almost every soil sample taken contained free-living mycobacteria. Kampala area has a low prevalence of leprosy, a high prevalence of tuberculosis and a very patchy and variable distribution of environmental mycobacteria. Although *M. ulcerans* infection is very common in some of the western parts of Kyoga area bordering on the river Nile, none of the people tested in this study came from these particular parts. Sporadic cases of the disease occur in Kyoga area both north and south of the main body of Lake Kyoga and in the northern part of Kampala area. With the exception of a tiny focus on the Semliki plain, the disease is absent from Toro.

Fig. 1 indicates the remarkable similarity between results for the BCG-immunized and non-immunized portions of the population as a whole. In fact the similarity is even greater if an allowance is made for the unequal distribution of immunized persons in the three areas specially studied. This is a very different picture from that seen among Kenyan schoolchildren (Paul *et al.* 1975*a*), where there was considerable disparity between immunized and nonimmunized persons particularly in their reactions to the slow-growing mycobacteria.

The effect of different environments, shown in Fig. 3, on the reaction profiles is much greater than that of BCG. In the case of every antigen the proportion of positive reactors was lower in Toro area than in Kyoga area by at least a third and in most cases by more than one-half. The results for Kampala area were similar to those for Toro so far as reactivity to slow growers was concerned, and similar to Kyoga in reactivity to fast growers.

Taking each antigen in turn the results for Tuberculin are essentially the same as those for RT23, although a few per cent higher in every case. It is difficult to explain why reactivity to these reagents should be higher in Kyoga area than in the other areas, since such other evidence as there is does not suggest that tuberculosis is commonest there. Reactivity to Aviumin in Kyoga area is almost three times greater than in the other areas, and reactivity to A*-in and Gordonin is also much the highest in Kyoga area, indicating more frequent contact with these organisms there. The low figures for Burulin reactivity are interesting; its total absence in Toro coincides with the virtual absence of *M. ulcerans* infection there,

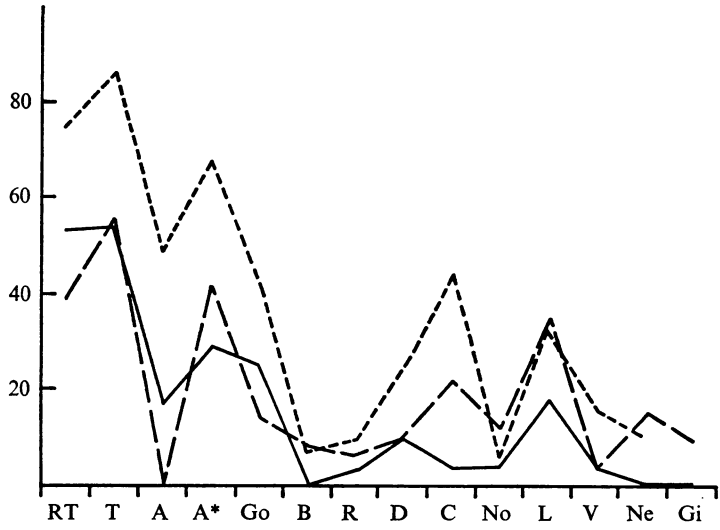

Fig. 4. Diagram showing the percentages of persons from separate areas of Uganda, producing positive reactions (10 mm. or more) to individual skin test reagents. The solid line represents those from Toro, the infrequently broken line those from Kampala area and the frequently broken line those from Kyoga area. The reagents used are those listed in the legend to Fig. 1.

and in the other areas sporadic cases occur but are uncommon. A study carried out with our Burulin in Ibuje county in the extreme West of Lango district, where *M. ulcerans* infection is very common, showed a third of the population reacting to it (P. G. Smith & W. D. L. Revill, personal communication). Reactivity to Ranin and Chelonin, produced from the species implicated in injection abscesses, was virtually the same in both Kampala and Kyoga areas and much less in Toro. Reactivity of about 75% to Chelonin in Kampala and Kyoga areas was unexpectedly high since no strains of *M. chelonei* have been isolated from the environment. However, this is almost certainly due to the great sensitivity of *M. chelonei* to most decontamination methods and the apparent unsuitability of Löwenstein-Jensen or pyruvate egg medium for its isolation. *Mycobacterium duvalii* has never been isolated from any source in Uganda, yet reactivity to Duvalin is between 20 and 40% throughout. The possibility exists that this might be due to some cross-reactivity with *M. leprae* despite our failure to demonstrate this in previous studies (Paul *et al.* 1975*b*). Nonchromogenicin and Lactin, which are both produced from variants of *M. nonchromogenicum*, each produce the same proportion of positive reactions in the three regions, although reactivity to Lactin is double that to Nonchromogenicin – the opposite result was observed among Kenyan schoolchildren (Paul *et al.* 1975*a*). *Mycobacterium vaccae* and *M. neoaurum* have been isolated from samples collected around Lake Kyoga but not from other parts of Uganda. Reactivities to Vaccin of 25% in Kyoga area and only about 10% in Kampala and Toro areas may reflect this; however, these figures might be affected by contact with *M. leprae* (Paul *et al.* 1975*b*). The small amount of reactivity to Neoaurumin in Kampala and Kyoga areas and the lack of any

reactivity in Toro also supports the cultural findings. *M. gilvum* has not been identified in the Ugandan environment and no useful comment can be made on the 30 % of positive reactors to Gilvin found in Kampala and Toro areas. Gilvin was not tested in persons from Kyoga area.

The large numbers of positive reactors to RT23 and Tuberculin, particularly in Kyoga area, might well be thought due to cross-reactivity with other species in view of the small reaction size we have taken as positive. According to many previous studies using RT23, the choice of 10 mm. as the minimum mean diameter of induration accepted as positive would exclude the so-called intermediate size reactions due to non-specific sensitization. That such reactions only play a small part in our results is shown in Fig. 4, which has been constructed using 10 mm. or more as the criterion of positivity. It can be seen by comparison with Fig. 3 that there has been very little change in the shape of the reaction profile. The effect is greater on most of our own reagents than on RT23, because the doses used were selected to give small-sized reactions, essential when multiple tests are to be performed.

With the exception of the results for RT23 and Tuberculin in Kyoga area the results obtained fit in very well with what is known about the distribution of environmental mycobacteria and of mycobacterioses in Uganda. In fact they indicate that an indirect survey of environmental mycobacteria might be made by multiple skin testing of the population very much more quickly and at less cost than a direct bacteriological study. However, some allowance might have to be made for the different degrees of allergenicity shown by different species.

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