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Multiple skin testing of tuberculosis patients with a range of new tuberculins, and a comparison with leprosy and *Mycobacterium ulcerans* infection

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SUMMARY

Four hundred and seventy tuberculosis patients were each skin tested with four of a range of 17 mycobacterial reagents in four countries in all of which tuberculosis and leprosy were endemic. Sixteen of the reagents were new tuberculins prepared from extracts of living mycobacteria disrupted by ultrasonic disintegration and the last was PPD, RT23.

The effect that tuberculosis exerted on the delayed-type skin test response to these antigens was assessed by comparing results for tuberculosis patients with those for Tuberculin positive and Tuberculin negative control populations. Tuberculosis patients on Rifampicin therapy showed no difference in their skin test responses to any of the antigens from those patients on other forms of anti-tuberculosis treatment.

Amongst the normal population it was found that possession of Tuberculin positivity was associated with an enhanced response to all the other mycobacterial antigens with the exception of A*-in which demonstrated a reciprocal relationship with Tuberculin in Burma. It was also noted, in Burma particularly, that sensitization to mycobacterial species other than *Mycobacterium tuberculosis*, especially to the slow growers, plays a role in determining responses to different mycobacterial species.

In tuberculosis patients enhanced skin test responses were also seen but only in those countries, e.g. Libya, where the prevalence of mycobacterial species was low. Where mycobacteria were common, as in Burma, the converse was true and tuberculosis was associated with a diminished skin test response to each antigen. The high prevalence of A*-in positivity in Burma, its reciprocal relationship with Tuberculin there and the results for all the antigens in the tuberculosis patients indicate that the cell mediated skin test response may have a threshold. If this is exceeded the skin test becomes negative so that non-reactors then include those who have been excessively sensitized as well as those who have not been sensitized. Despite this, a greater percentage of tuberculosis patients in each country responded to the specific reagent Tuberculin than did the control populations and their mean

positive induration sizes were consistently larger. Nevertheless, amongst the tuberculosis patients in Burma 13 % were complete non-reactors to Tuberculin and this apparent anergy also applied to the other reagents with which these individuals were tested.

This differs from lepromatous leprosy where the anergic state pertains exclusively to *M. leprae* and a few seemingly closely related species. The breadth of anergy in *M. ulcerans* infection has not been measured but it is known to effect both Burulin and the PPD, RT23.

Just as in leprosy and *M. ulcerans* infection, tuberculosis can be shown to have a disease spectrum here detected by multiple skin testing. The significance of this spectrum and its similarities with and differences from that of the other mycobacterioses is discussed.

INTRODUCTION

For many years tuberculosis has been regarded as an infection in which the cell mediated response is well developed. This is demonstrated by both the histology of the lesions and the greatly enhanced delayed-type skin test response to intradermally administered tuberculin. However, it is being increasingly recognized that there are forms of the disease in which a state of anergy may occur and in which the correlates of cell mediated immunity are depressed.

In this context there may be similarities to be drawn between tuberculosis and the other mycobacterioses, notably those caused by *M. leprae*, and *M. ulcerans*. Studies on the skin test responses to a variety of tuberculins prepared by ultrasonic disruption of the relevant mycobacterial species have already been carried out in a number of countries on the normal populace (Paul, Stanford, Misljenóvic & Lefering, 1975; Stanford *et al.* 1976*a*, *b*), leprosy patients (Paul, Stanford & Carswell, 1975) and patients with *M. ulcerans* infection (Stanford, Revill, Gunthorpe & Grange, 1975).

The present survey has been carried out using this range of new tuberculins in patients infected with *M. tuberculosis* in four countries, in all of which tuberculosis and leprosy are endemic in varying degrees. Assessment of responses attained in tuberculosis patients was made by comparison with those in the normal populations and leprosy patients respectively. This was particularly the case in Burma where the largest and most detailed study was carried out.

The use of the term 'tuberculin' both in a general sense for skin test reagents produced from any mycobacterial species and in a specific sense for the reagent produced from *M. tuberculosis* itself, is confusing. This is particularly the case in the present paper where the word is used in both senses on numerous occasions. The alternative terms, 'sensitin' used by Magnusson (1961) and 'mycobacterin' recently suggested by Runyon (1976) both have disadvantages. In view of this we have continued to use 'tuberculin' with a small initial letter when it is used in the general sense and with a capital 'T' when it is used in the specific sense.

Table 1. *The organisms used for the production of the skin test antigens*

Organism	Source	Collection number	Antigen prepared (with abbreviations used in the text shown in brackets)	
M. tuberculosis			PPD	(RT23)
M. tuberculosis	Clinical isolate	813	Tuberculin	(T)
M. sp. '*A**'	Soil, Uganda	R528	A*-in	(A*)
M. avium	Soil, Uganda	R527	Aviumin	(Av)
M. gordonae	Soil, Uganda	R62, R81	Gordonin	(Go)
M. kansasii	Clinical sputum isolate	8	Kansasin	(K)
M. marianum	Clinical sputum isolate	22	Marianin	(M22)
M. ulcerans	Congolese patients	668, 791	Burulin	(B)
M. xenopi	Xenopus laevis	NCTC 10042	Xenopin	(X)
M. chelonei	Injection abscesses	124; 446	Chelonin	(C)
M. duvalii	Leprosy tissue	NCTC 358	Duvalin	(D)
M. flavescens	Guinea-pig	NCTC 10271	Flavescin	(F)
M. fortuitum	Soil, Uganda	R191, R197	Ranin	(R)
M. gilvum	Isolate from sputum	NCTC 10742	Gilvin	(Gi)
M. neoaurum	Soil, Uganda	R872, R922	Neoaurumin	(Ne)
M. nonchromogenicum	Soil, Uganda	R507	Nonchromogenicin	(No)
M. vaccae	Soil, Uganda	R859, R877	Vaccin	(V)

Slow growing species are shown above and fast growing species below the space.

MATERIAL AND METHODS

The skin test antigens used in this study were prepared from the organisms listed in Table 1. The majority were those employed in our earlier studies with the exception of Marianin, Kansasin and Xenopin used for the first time in Burma and subsequently in a recent small study in Uganda.

The organism called *M.sp.*'*A**' in Table 1 is an, as yet, unnamed slow growing species identified in the Ugandan environment (Stanford & Paul, 1973). The organism appears to resemble *M. avium* in some of its characteristics.

In all four countries the dose of reagents used was 0·1 ml containing 0·2 μg protein. The concentration of reagents was based on a chemical assay (Lowry, Rosebrough, Farr & Randall, 1951) and all reagents other than PPD were prepared in borate buffer containing 0·0005% Tween 80 as previously described. PPD (RT23) was obtained from the Statens Seruminstitut, Copenhagen and contained 0·04 μg protein per skin test dose (2 tuberculin units). Four tests were administered per person, using Gillette Scimitar 1 ml disposable tuberculin syringes and 25 gauge needles, so that each forearm received two reagents intradermally into its volar aspect. Tests were read after 72 h by measuring longitudinal and transverse diameters of induration and recording the mean. As in previous studies 5 mm, or more, induration was taken as a positive reaction.

Area of study and persons tested

The study was carried out in Uganda, Kenya, Libya and Burma. In all 4 countries tuberculosis patients tested were currently receiving in-patient treatment for active disease in tuberculosis hospitals. In Libya 132 in-patients of the 2 tuberculosis hospitals in Cyrenaica were tested, one being in Benghazi and the

other in Cyrene. In Burma 198 patients in the tuberculosis hospitals in Mandalay and Rangoon were studied. 140 Ugandan and Kenyan patients were drawn from a number of general hospitals.

Patients on steroid therapy were excluded from the study because of the known immunosuppressive effect of this treatment. The age, sex, BCG vaccination status, extent of the disease and current antituberculosis medication were recorded for each patient. An analysis of results obtained for those patients on Rifampicin was also made in view of the possible immunosuppressive effect of this drug reported in the literature. (Păunescu, 1970; Nilsson, 1971; Rook, 1973.)

Normal populations similarly tested in these countries were used as controls and have been divided into two groups, those that were Tuberculin positive and those that were Tuberculin negative. They have been selected in this manner rather than according to BCG vaccination status, for reasons that are discussed below. This unavoidably restricted the number included in the control groups to those who were tested with reagents including our Tuberculin.

The age group taken for the normal control population lay between 11 and 40 since the majority of tuberculosis patients came within this range. In Libya no suitable normal adult population was skin-tested so that the normal control group consists of 11–18 year olds drawn from the same area from which the tuberculosis patients came.

The leprosy patients included for comparative analysis were those tested in Burma. They came from the leprosaria in Rangoon and Mandalay and from the World Health Organization BCG leprosy trial area in the neighbourhood of Singu. For the purpose of this study they are divided into three disease classification groups, Tuberculoid (TT), Borderline (BT, BB and BL) and Lepromatous (LL).

RESULTS

Similar results were obtained in Uganda and Kenya and to make their numbers sufficient for analysis they have been combined together under the country grouping designated East Africa. Results for East Africa, Libya and Burma are set out in separate tables (2, 3, 4 and 5).

The numbers of persons tested with a particular antigen within the separate population groups studied, together with the percentage positive reactions to the reagent and the mean positive induration size given in millimetres are shown in these tables. As can be seen from the tables different combinations of antigens were used in the three areas. Unfortunately no antigens prepared from slow growers, other than our Tuberculin or RT23 were tested in tuberculosis patients in Libya.

A preliminary analysis of the mean positive induration sizes showed a difference for all antigens, of only 1–2 mm between the Tuberculin positive and Tuberculin negative groups. When divided according to BCG vaccination status the mean positive induration sizes again did not differ significantly even for Tuberculin and RT23. In Tables 2, 3 and 4, therefore, a single mean positive induration size has been given for each antigen tested in the normal population.

A more detailed analysis of the results obtained for reagents tested in the normal

Table 2. *The number of tuberculosis patients and persons in the normal population tested in East Africa, with the percentage of positive results and mean positive induration sizes for each antigen*

	Normal population				Tuberculosis patients		
	Number tested						
Antigen	Negative	Positive	% positive	M.I.S. (mm)	Number tested	% positive	M.I.S. (mm)
T	167	435	72	15·2	28	93	17·6
RT23	60	51	46	17·1	74	96	21·2
	Number tested		% positive				
	Tuberculin negative	Tuberculin positive	Tuberculin negative	Tuberculin positive			
A*	17	97	18	66	11·3	29	47	11·5
Av	21	178	24	52	12·3	37	53	11·8
B	7	45	0	7	8·2	88	9	7·5
Go	18	45	22	76	10·9	30	74	12·9
K	44	13	2	8	8·0	nt	nt	nt
M22	44	13	0	0	—	nt	nt	nt
X	18	0	0	nt	—	nt	nt	nt
C	19	143	25	65	9·7	28	74	7·6
No	7	11	29	36	9·2	29	32	9·6
Ne	28	46	7	30	9·3	35	14	8·6
R	29	92	17	35	8·8	40	52	11·5
V	62	108	8	19	9·7	31	25	9·0

M.I.S., the mean positive induration size in millimetres; nt, not tested.

population in Burma is shown in Table 6, where persons tested are grouped according to response to our Tuberculin and BCG immunization status. In deriving the combined percentage positive reactors to slow growers and fast growers respectively in each of the groups equal weighting was given to the percentage positive result for each antigen.

Tuberculin positivity is seen to be associated with an increase in positivity to other antigens, whether these were prepared from slow or fast growing mycobacteria. A similar association is not seen with regard to BCG vaccination. It is for this reason that controls in the normal population were divided according to their response to our Tuberculin.

The percentage increase in positive reactions for each antigen in each country attributable to either Tuberculin positivity or active tuberculosis is shown in Table 7. These percentage increases for each antigen were derived using the formula

$$\left(\frac{T\text{ pos} - T\text{ neg}}{100 - T\text{NEG}}\right) \times 100\,\%,$$

where T pos = either the % positive reactors in the Tuberculin positive control group, or the % positive reactors in the tuberculosis patients; T neg = % *positive*

Table 3. *The number of tuberculosis patients and persons in the normal population tested in Libya, with the percentage of positive results and mean positive induration sizes for each antigen*

	Normal population					Tuberculosis patients		
	Number tested			M.I.S.	Number	%	M.I.S.
Antigen	Negative	Positive	% positive	(mm)	tested	positive	(mm)
T	136	219	62	14·4	48	100	20·4
RT23	188	92	33	14·0	50	100	19·4
	Number tested		% positive				
	Tuberculin negative	Tuberculin positive	Tuberculin negative	Tuberculin positive			
A*	61	149	33	63	10·7	nt	nt	nt
Av	18	33	17	70	10·2	nt	nt	nt
Go	57	40	23	82	11·3	nt	nt	nt
C	31	70	35	34	7·9	59	32	9·0
D	13	28	8	25	7·3	94	22	8·5
F	55	94	24	54	8·7	48	42	9·2
Gi	63	99	8	41	8·6	45	56	9·4
Ne	37	65	14	52	8·2	24	71	9·8
No	39	60	3	37	9·3	77	58	10·8
R	60	87	13	64	10·4	92	42	11·4
V	38	62	5	37	8·9	83	53	10·2

M.I.S., the mean positive induration size in millimetres; nt, not tested.

reactors in the Tuberculin negative group; $100 - TNEG$ = % *negative* reactors in the Tuberculin negative control group.

No significant difference was seen in either the percentage of positive reactors or the mean positive induration sizes for any of the reagents tested in those patients who were on Rifampicin therapy, as compared with those on other anti-tuberculosis regimes.

The age distribution of tuberculosis patients tested in the three countries is shown in Fig. 1. They are split by decades from the age of 11 to 70 plus. No significant sex difference was observed.

The patients were also divided according to sex, and BCG immunization status prior to disease. This is shown in Tables 8*a* and *b*. All three countries possessed a greater number of males than females amongst the tuberculosis patients tested but differed in the extent to which they had received BCG. In Libya 40% of these patients were found to have received BCG immunization. Of these, 83% were male and only 17% were female. This difference was found to be statistically significant ($P < 0{\cdot}01$). In Burma and East Africa 15% and 0% respectively had received BCG and no sex difference was observed.

It was found in Libya and Burma that those of the tuberculosis patients who had received BCG were of a much younger average age being 24·9 and 26 years

Table 4. *The number of tuberculosis patients and normal population tested in Burma with the percentage of positive results and mean induration sizes in millimetres for each antigen*

	Normal population					Tuberculosis patients		
	Number tested		% positive					
Antigen	Tuberculin negative	Tuberculin positive	Tuberculin negative	Tuberculin positive	M.I.S. (mm)	Number tested	% positive	M.I.S. (mm)
T	219	206	(48%)		14·2	149	86	20·0
A*	41	37	51	32	11·7	49	27	10·7
Av	47	41	32	44	8·7	51	18	8·0
Go	47	42	51	86	11·8	48	61	15·1
K	17	29	35	69	8·7	49	45	15·4
M22	91	66	74	83	13·2	51	22	12·3
X	9	36	33	97	13·0	nt	nt	nt
C	21	25	43	60	8·8	49	20	8·6
D	23	21	22	43	12·2	49	12	12·3
F	9	36	22	72	9·8	49	18	11·8
Gi	21	25	0	20	9·4	49	4	10·5
Ne	9	36	11	67	9·9	49	29	13·0
No	33	22	3	27	9·8	19	16	14·7
R	34	22	47	73	11·6	49	16	12·5
V	23	21	9	38	7·8	nt	nt	nt

The percentage shown in parentheses for Tuberculin is the percentage positive result for the total (425) number of tests performed with this reagent.

M.I.S., the mean positive induration size in millimetres; nt, not tested.

Table 5. *The number of leprosy patients tested in Burma with the percentage of positive results and mean positive induration sizes in millimetres*

	Number tested			Percentage positive			Mean induration size		
Antigen	Tuberculoid (TT)	Borderline (BT, BB, BL)	Lepromatous (LL)	Tuberculoid (TT)	Borderline (BT, BB, BL)	Lepromatous (LL)	T	B	L
T	2	3	35	nt	nt	63	nt	nt	19·7
Go	90	6	98	50	17	22	9·8	10·0	11·2
M22	53	38	122	60	26	65	11·3	12·5	15·3
X	50	4	48	46	25	54	9·7	9·0	11·3
C	99	16	98	59	50	59	8·7	8·1	7·0
F	52	10	48	35	20	44	9·4	9·5	8·5
No	133	18	100	35	28	12	10·3	9·0	9·8
V	144	29	100	24	7	15	10·0	12·0	8·5

M.I.S., the mean positive induration size in millimetres; nt, not tested.

Table 6. *The results, in Burma, for the skin test responses to antigens derived from slow or fast growing mycobacteria, grouped according to tuberculin response and BCG immunization status*

	Tuberculin positive BCG vaccinated popn		Tuberculin positive BCG unvaccinated popn		Tuberculin negative BCG vaccinated popn		Tuberculin negative BCG unvaccinated popn	
Antigens	Individual % positive	Combined % positive	Individual % positive	Combined % positive	Individual % positive	Combined % positive	Individual % positive	Combined % positive
A*	38	69	29	71	(71)	55	47	46
Av	44		(40)		32		32	
Go	84		(100)		43		63	
K	75		65		(0)		40	
M22	79		94		(81)		70	
X	93		100		100		(25)	
C	62	45	58	57	(33)	8	47	24
D	25		(67)		(0)		36	
F	73		71		(0)		(25)	
Gi	15		25		(0)		0	
Ne	67		67		(0)		(13)	
No	31		(22)		0		5	
R	62		(89)		33		55	
V	25		(56)		(0)		14	

Antigens derived from slow growing mycobacteria are shown above the space, those from fast growing species below the space.

Table 7. *The percentage increase in positive reactions for each of the skin test antigens in each of the countries attributable to either tuberculosis or the possession of tuberculin positivity*

	Libya		East Africa		Burma	
Antigen	Tuberculin positive popn	Tuberculosis patients	Tuberculin positive popn	Tuberculosis patients	Tuberculin positive popn	Tuberculosis patients
A*	70	nt	59	32	−39	−49
Av	64	nt	37	38	18	−21
Go	77	ut	69	67	71	20
K	nt	nt	6	nt	52	15
M22	nt	nt	0	nt	35	−200
X	nt	nt	nt	nt	96	nt
C	−2	−5	53	65	30	−40
D	18	15	nt	nt	27	−13
F	39	24	nt	nt	64	−5
Gi	36	52	nt	nt	20	4
Ne	44	66	25	8	63	20
No	35	57	10	4	25	13
R	59	33	22	42	49	−58
V	34	51	13	21	32	nt

nt, not tested. A negative prefix before a result indicates a percentage decrease.

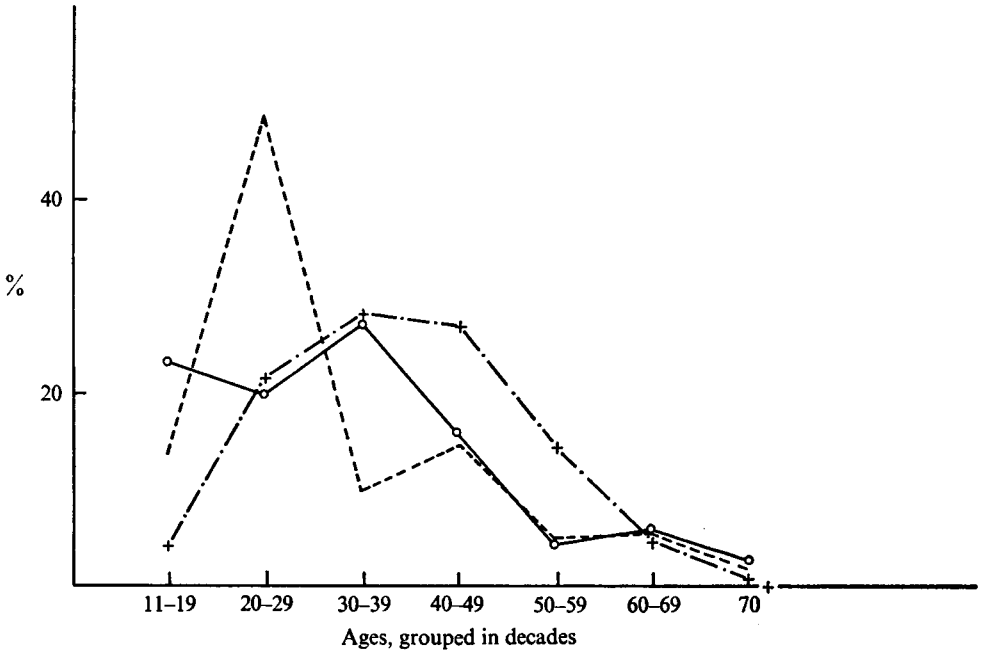

Fig. 1. The age distribution of the tuberculosis patients tested. The solid line represents the 140 tested in East Africa (average age 32·2 ± 15·1 years). The infrequently broken line represents the 198 tested in Burma (average age 38·4 ± 12·3 years) and the frequently broken line depicts the 132 tested in Libya (average age 30·5 ± 14·0 years).

Table 8(*a*). *The total number of tuberculosis patients tested and their sex ratio*

Country	Total no. males	Total no. females	Total no. tested	Sex ratio (M:F)
East Africa	90	50	140	1·8:1
Burma	128	70	198	1·8:1
Libya	96	36	132	2·7:1

Table 8(*b*). *The distribution of BCG immunization amongst the tuberculosis patients tested*

	Number with BCG scar			BCG immunized group as percentage of totals tested (see Table 8*a*)			Average age of BCG immunized group
Country	Male	Female	Total	Male	Female	Total	
East Africa	0	0	0	0	0	0	—
Burma	17	12	29	13	17	15	26·0(±)8·2 yr
Libya	44	9	53	46	26	40	24·8(±)9·7 yr

Table 9. *A comparison of results between groups of antigens from slow and fast growing mycobacteria tested in the normal population and leprosy patients in Burma*

Antigen group		Normal population (%)		Leprosy patients (%)		
		Tuberculin negative	Tuberculin positive	Tuberculoid (TT)	Borderline (BT, BB, BL)	Lepromatous (LL)
Slow growers	Go, M22, X	53	89	52	23	47
Fast growers	C, F	33	66	47	35	52
	No, V	6	33	30	18	15

Slow growers are grouped together above the space, fast growers below it. The latter are divided into two groups (C and F, No and V). Each of the individual antigens shown have been tested in sufficiently large numbers in the five population divisions of the table so that in deriving combined percentage positives equal weighting has been given to each of the individual antigen results.

respectively. This compared with an average age of 41 in Burma and 34 in Libya among patients who had not received BCG.

The respective BCG coverages in the normal population in East Africa, Burma and Libya were estimated as 28, 50 and 84%. This latter figure is unduly high because it was assessed for 11–18 year olds rather than adults for whom the figure would certainly be lower. Similarly, in Burma the figure is that given in the Burmese baseline survey of 1972 and is for the age group 15–19. Very few adults over the age of 30 have received BCG so the figure of 50% is undoubtedly falsely elevated.

Of the tuberculosis patients tested with an *M. tuberculosis* derived reagent 23 failed to produce a positive response. These same people also failed to respond to any of the other reagents with which they were tested. We have no additional information about the three from Kenya, but the 20 from Burma were well documented. These 20 patients comprised 13% of the 149 patients tested with Tuberculin. All were confirmed as being excretors of acid-fast bacilli in their sputa and none were on steroid therapy. Sixteen were males and four were females but this apparent sex difference is not significant since the proportion of males to females was the same amongst all 149 patients tested with Tuberculin. A closer analysis of the 20 anergic tuberculosis patients reveals that four had extensive pulmonary disease, two had miliary disease and two had been diagnosed in the 6 weeks before skin testing and may not have had time to convert to Tuberculin positivity. This leaves 12 patients with no obvious cause for their anergic state and who were not otherwise seriously debilitated. Without exception all were currently on anti-tuberculosis treatment with Isoniazid, Streptomycin, Thiacetazone or Sodium aminosalicylate.

A comparative analysis of results obtained in Burma for three groups of antigens tested in both the normal population and leprosy patients is shown in Table 9.

DISCUSSION

In all the countries of the study tuberculosis and leprosy are endemic, and *Mycobacterium ulcerans* infection is endemic in parts of Uganda. Tuberculosis has presented a considerable health problem in each of the areas necessitating the setting up of national control programmes. The amount of leprosy varies between the countries from a very low incidence of 0·03 % in Libya, to a high incidence approaching 5 % or more in some areas of Burma. The lowest incidence of tuberculosis is found in Libya also, being 0·6 % having fallen from 2·3 % in 1967. East Africa possesses the highest incidence, estimated at 3 % or more whilst Burma lies between these two. The Burmese baseline survey of 1972 (Burma Ministry of Health, 1974) established a figure of 0·3 % based on WHO criteria for diagnosis but this is undoubtedly too low since tuberculosis is the second commonest cause of mortality in Burma. In the district of Burma where our study was performed the incidence of sputum positive cases was 0·68 %. Extrapolation from this would indicate a prevalence of active disease of 1·5 % or higher.

The epidemiology of tuberculosis apparently varied in the countries studied, as can be seen from Fig. 1. The lowest mean age of disease occurred in Libya (30·5 years) and the highest in Burma (38·4 years). In the latter country almost half of the patients were over the age of 40. The graph demonstrates little difference, however, between East Africa and Burma in contrast to Libya where the disease occurred mainly in a much younger age group. This reflects the different distribution of age groups amongst the populations of the different countries as exemplified by the fact that 50 % of Libya's population is under the age of 15 years.

Another notable difference between the tuberculosis patients in the different countries lay in the differing numbers that had received BCG (see Table 8*b*).

The 46 % of male tuberculosis patients in Libya who had received BCG seems remarkably high. Ninety-three % of them were below the age of 40 and formed 59 % of the male patients below this age. In the age group 11–18 years the percentage of tuberculosis patients who had received BCG was even higher at 69 % in comparison with 84 % in the control 11–18 year old population. Among female tuberculosis patients in Libya who had been BCG immunized 95 % were under the age of 30 and constituted 35 % of the total number of female patients below this age. This indicates a very disappointing amount of protection afforded by BCG immunization in Libya which was greater among males than females. This is supported by observations made in our study of Libyan schoolchildren (Stanford *et al.* 1976*b*). Protection may be better in East Africa and Burma as judged by the lower proportions of tuberculosis patients who had received BCG.

Since cultures of organisms were not obtained from most patients we cannot be certain that all cases of clinical tuberculosis were caused by *M. tuberculosis* itself. A number might have been infected with the bovine type and others with *M. kansasii*, *M. xenopi* or *M. avium*. Infections with these other species are unknown in East Africa and are very infrequent in Libya. However, it is possible that they occur in Burma. Even if this is the case it is most unlikely that more than one or two such infections could have been included amongst the patients studied. Never-

theless, it is worth noting that sensitivity to Aviumin is quite common in the normal population in all three countries. Sensitization to Kansasin and Xenopin is frequent in Burma and almost absent in the small group of people tested in Uganda; these reagents have not been tested in Libya.

The results for our Tuberculin and RT23 in the tuberculosis patients and the normal control population are reasonably straightforward. The overall positivity in the control populations of East Africa, Libya and Burma were 78, 62 and 48 % respectively for our Tuberculin compared with 55 and 33 % for RT23 in East Africa and Libya. (RT23 was not tested in Burma.) The large difference of 29 % between our Tuberculin and RT23 in Libya was considered to be due to the detection of waning hypersensitivity to *M. tuberculosis* following BCG. The continuous boosting effect of contact with environmental mycobacteria experienced in most countries is minimal in Libya because of the low numbers of such organisms in the desert. In East Africa the difference in positivity may be attributable to the greater sensitivity of our Tuberculin in detecting contacts of active tuberculosis. Many of the control population in East Africa were staff of hospitals and as such were tuberculosis contacts. This is borne out by the mean positive induration sizes, which were little different from those of the tuberculosis patients.

In active tuberculosis both our Tuberculin and RT23 behave very similarly. In East Africa and Libya figures of, or near to, 100 % positive were achieved. The mean induration size rose by 6 and 5·4 mm for our Tuberculin and RT23 respectively in Libya and by 2·2 mm for both reagents in East Africa above the values for the control populations.

The lack of any demonstrable difference in the skin-tests of those patients on Rifampicin therapy may have been due to the establishment of Tuberculin sensitivity prior to the commencement of treatment.

In Burma, where only our Tuberculin preparation was tested, 86 % of patients were positive and again the induration size increased above that of the normal population by 5·8 mm. The interesting feature of the Burmese tuberculosis patients was the large number (13 % of the total), who were completely negative to our Tuberculin and to any other mycobacterial antigen tested at the same time. These 'anergic' patients, mentioned already in the results, may well form the group that develop disseminated types of disease with a subsequent bad prognosis and high mortality.

When the results for the other antigens are considered (see Tables 2, 3, 4 and 5) it is found that there is great variation between countries. The reasons for this are many and reflect such features as differences in environmental mycobacterial prevalences, different degrees of contact with tuberculosis patients, and the infection artificially induced by BCG. It is also possible that sensitization to mycobacteria other than *M. tuberculosis* may play a role in determining responses to other skin test antigens. This might well occur in Burma, where there are a large number of reactors to a wide range of mycobacterial antigens, especially those derived from slow growing species. For example, Gordonin positivity increased by 60 % in association with Marianin positivity and by 70 % in association with Aviumin positivity (results not shown). It would seem, therefore, that there is a complex

interaction between mycobacteria, one species playing a part in determining the ability to develop sensitization to another.

The importance of Tuberculin positivity is demonstrated in Table 6. Where persons have gained this characteristic either by natural infection or successful BCG immunization, the responses to other mycobacterial antigens are increased. Conversely this increase is not seen in people who remain Tuberculin negative despite having received BCG. Thus the importance of BCG appears to lie in its ability to convert people to Tuberculin positivity and it is only in those persons in whom successful conversion occurs that the enhancing effect on other mycobacteria is realized.

From Table 7 it can be seen that all the mycobacterial antigens tested showed a rise in positivity in the Tuberculin positive normal populations with the exception of Chelonin in Libya which shows no change and A*-in in Burma which shows a decrease. This reciprocal relation between A*-in and our Tuberculin was greatest in the 19–40 age group.

When the antigens are grouped according to their derivation from either slow or fast growers it is found that the increase in positivity in association with Tuberculin positivity is greatest for the slow growers with the exception of *M.sp.*'*A**' in Burma. Their average increase in positivity was 70·5, 55 and 39 % for Libya, East Africa and Burma respectively. Once again it is the reciprocal relation of A*-in with Tuberculin that is responsible for the low overall increase in positivity amongst slow growers seen in Burma. When the results for A*-in are omitted the average rise in positivity to slow growers in Burma becomes 59 %. Differences in the mean positive induration size between the Tuberculin positive and Tuberculin negative groups rarely exceeded 2 mm.

Similarly in the Libyan and East African tuberculosis patients the results showed a generalized increase above those for the Tuberculin negative control populations (see Table 7). In Libya a potentiation was seen in the tuberculosis patients above that seen in the Tuberculin positive control population for those reagents that gave very low responses in the Tuberculin negative control group, e.g. Nonchromogenicin, Vaccin and Gilvin. Conversely Ranin and Flavescin which gave higher percentage positives in the Tuberculin negative population in Libya reached a maximum in the Tuberculin positive population and gave slightly lower, though still elevated, responses in the tuberculosis patients. Results for antigens in both tuberculosis patients and the Tuberculin positive control population tested in East Africa were dependent upon, and both elevated to a similar extent above, the baseline Tuberculin negative values. A reason for the similarity is that many of the control population were staff drawn from hospitals, as previously stated.

In Burma a very different picture emerges. Of all the antigens tested in the tuberculosis patients only 4 show a significant increase over the Tuberculin negative population. From Table 7 these can be seen to be Gordonin, Kansasin, Nonchromogenicin and Neoaurumin, which gave increases of 20, 15, 13 and 20 % respectively. However, they have not attained anywhere near the percentage positivity of their Tuberculin positive control counterparts, but in all four their mean positive induration sizes have increased significantly (see Table 5). In contrast, the other

antigens all showed much reduced responses compared with the Tuberculin negative control population. The greatest decreases were those for Marianin and Ranin which showed reductions of 200 and 58 % respectively. In the case of these antigens the mean positive induration sizes remained unchanged.

There appear to be two possible explanations of these results. One is in continuity with the theory that explains the results in Libya and East Africa; i.e. tuberculosis may serve as an adjuvant to the responses to other mycobacteria but only up to a certain point. This maximum for many of the antigens is achieved in Burma where a very large percentage of the Tuberculin negative population respond to them indicating a very considerable contact with mycobacteria in the environment. Further stimulus, in the form of Tuberculin positivity in the case of A*-in, or of tuberculosis for some of the other reagents, may switch off the cell mediated skin test response and reduce the percentage positivity. This could explain the increased induration sizes together with falling percentage positivities seen for the four antigens previously mentioned (Go, K, No and Ne). To each of them there could be a continuous process of increasing demonstrable sensitization of those not previously reacting or reacting weakly to these reagents. There could also be a switching off of persons who were strongly positive to these reagents before developing tuberculosis. Thus strong reactors might become negative, and weak or non-reactors become strongly positive. In support of this, the overall mean positive induration sizes, whilst not differing much from those of the control population, are larger than those seen in Libya or East Africa. Non-reactors tended to be completely negative rather than to produce weak reactions between 2 and 5 mm.

It is in this context that the apparently contradictory results for A*-in may be explicable. These results for the normal population and tuberculosis patients in the countries studied are shown as histograms in Fig. 2. From this figure there appears to be a sequence. In Libya there is only a small percentage of persons sensitized to *M.sp.'A*'* in the BCG unvaccinated population. Consequently there is a shift to the right in the histogram for the vaccinated population with a marked decrease in the percentage of negative reactors. However, in East Africa and Burma the percentage of reactors to A*-in among the BCG unvaccinated is even higher than amongst the vaccinated in Libya. Both BCG vaccinated and unvaccinated populations in East Africa give similar histograms since there is little difference in Tuberculin positivity between them. However, in Burma the sequence is taken one step further in the BCG vaccinated group. The histogram shows a greater shift to the right and a concomitant increase in mean positive induration size of over 1 mm. There is in addition a separation between the complete negatives and the positive reactors and yet the percentage of complete negatives increases instead of decreasing.

In both East Africa and Burma the histograms for A*-in for the tuberculosis patients demonstrate the same picture except that again the percentage of complete negatives increases. One could, therefore, postulate that for A*-in the switching off of the cell mediated skin test response occurs not only in the tuberculosis patients but also in the Tuberculin positive section of the population if the

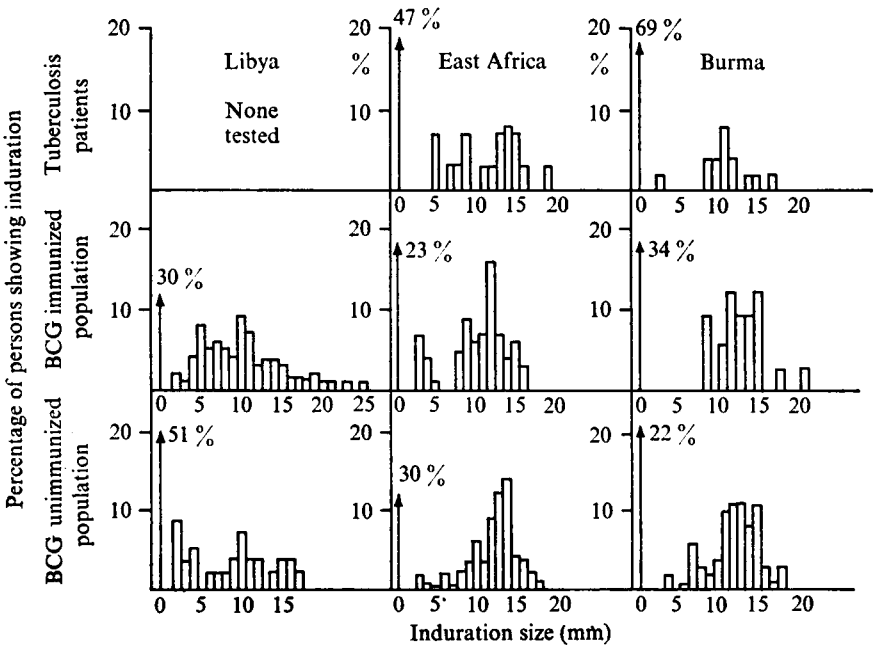

Fig. 2. Diagram showing the individual histograms for A*-in in tuberculosis patients, BCG immunized and BCG unimmunized normal populations. Results in persons divided according to BCG status have been used to enlarge the numbers. Except in Uganda there were greater numbers of Tuberculin positive reactors amongst the BCG immunized than the BCG unimmunized normal populations.

percentage positivity in the Tuberculin negative population is very high. Results for the other antigens treated in a similar way show the same sequence with the exception that the switching off of the cell mediated skin test responses and the resultant increase of percentage of completely negative reactors only occurs in association with tuberculosis, and not with Tuberculin positivity alone.

An alternative explanation for the results obtained in Burma is that tuberculosis occurred in those patients that possessed little sensitization to environmental mycobacteria, particularly to the slow growers and to a lesser extent to the fast growers. Nevertheless, this explanation does not account for the increased responses found in East Africa and Libya.

Of the antigens tested, some amongst the fast growers do not fit easily into any general picture. Thus Nonchromogenicin, for example, showed the same low order of positivity in the Tuberculin negative populations of both Libya and Burma and yet gave differing results in the tuberculosis patients (see Tables 3 and 4). In such cases if the results are to have a rational meaning a further factor may be presumed to be operating. This could be the influence of leprosy to which these fast growers seem in some way to be related (Stanford & Rook, 1976). The high prevalence of leprosy in Burma and the very low prevalence in Libya have been mentioned above.

From Table 9 it can be seen that slow and fast growers react differently in leprosy patients. Grouped results for antigens of slow growers show similar positivity to those of the Tuberculin negative population when tested in tuberculoid and lepro-

matous leprosy patients. In the active, or labile, borderline phase of the disease their responses are depressed in a similar fashion to that found in active tuberculosis in Burma. Results for reagents derived from fast growers, by contrast, can be divided into two groups. There are those such as Chelonin and Flavescin (see Table 9) that behave similarly to the slow growers but at a generally lower degree of positivity, with the exception that they show a raised response in the tuberculoid patients, intermediate between the results for the Tuberculin negative and Tuberculin positive control population. The second category consisting of reagents Vaccin and Nonchromogenicin reacts altogether differently. They show little positivity in Burma in the Tuberculin negative group, a sharp rise in the Tuberculin positive population which is maintained in the tuberculoid leprosy patients, and a subsequent decline in positivity in the borderline patients that remains diminished in the lepromatous patients. This demonstrates the continued loss of the cell mediated skin test response at the lepromatous end of the spectrum for these latter antigens, in common with that found for *M. leprae* itself, in contrast to the comparative recovery of skin test sensitivity to the other fast growers and the slow growing species. The reagents also differ in that some show an increase in their mean positive induration sizes when tested in lepromatous (LL) patients (see Table 5). As yet, we can offer no satisfactory explanation for this finding.

The anergy at the lepromatous end of the Ridley–Jopling scale (Ridley & Jopling, 1966) differs from that seen in the tuberculosis patients in that it pertains almost exclusively to fast growers whilst Tuberculin negative tuberculosis patients are negative to all other mycobacterial antigens. A state of anergy also occurs in *Mycobacterium ulcerans* infection and it might be best studied in this disease. If conclusions can be drawn from experimental infections of the mouse, patients pass through a Burulin positive phase in the latent period before the disease becomes clinically obvious. This is followed by a Burulin negative phase of variable length during which the disease is at its most destructive. At this time there appears to be no immunological recognition of, or reaction to, the highly cytotoxic organisms in the lesions, although there are certainly antigen reactive cells present in lymph nodes. (Rook 1975*a*, *b*).

This anergic phase which may last several months is followed by a period of recovery in which the patients become strongly positive to Burulin. During the Burulin negative phase there is no reaction to the reagent RT23 which becomes positive in the healing phase (Stanford *et al.* 1975). Unfortunately tuberculins produced from other species have not been tested to any extent in this disease.

The end results of anergy in the three diseases are very different. In tuberculosis, anergy, if not already associated with severe debilitation, may well result in such a state with a rapidly fatal outcome. Effective chemotherapy quickly results in a return to skin test positivity. In *M. ulcerans* infection it is probable that every patient with clinical manifestations passes through weeks, or months, of an anergic phase since tissue damage only occurs during this time. Lepromatous leprosy however, though associated with anergy, can persist for years with a high morbidity before death supervenes. Even after an apparent cure, skin test and lymphocyte transformation reactions may not return to normal in this disease.

These features of leprosy together with its predilection for superficial sites in which pathological differences can easily be observed led to the early appreciation of its disease spectrum. In tuberculosis on the other hand, in which a similar spectrum certainly exists, its appreciation has been long delayed partly because of the deep situation of the lesions which effectively removes them from all but roentgenographic observation and partly because of the speed in which patients at the anergic end of the spectrum die. In their turn the different survival times of leprosy and tuberculosis patients in the anergic phases of their diseases reflect the great differences between *M. leprae* and *M. tuberculosis*. The former acts as an inert particle once delayed hypersensitivity to it is ablated and the latter continues to release cord factor and possibly other lethal cytotoxins which can no longer be counteracted as the immune mechanism fails. Almost certainly there is a delay between the disappearance of skin test positivity in tuberculosis and the onset of a truly anergic state, during which time lymph node cells and probably lesion orientated cells continue to contain the disease (Rook, Carswell & Stanford, 1976).

Thus, just as in leprosy where many patients pass years in the various stages of the borderline disease, fluctuating between anergy and allergy, so too do tuberculosis patients sometimes show a flitting Tuberculin positivity which stabilizes as they recover or remains negative as they decline. Without continuing follow up with repeated skin tests on those tuberculosis patients in whom we found our skin tests negative (13 % of those tested in Burma) it is difficult to allocate them to a defined point on the spectrum and equally some of those found positive reactors might be negative on a subsequent test.

It is of interest that in all four countries those tuberculosis patients who had positive skin tests to other mycobacterial antigens had a larger mean positive induration size for Tuberculin. Again without follow up it is difficult to know whether such differences reflect changes along a disease spectrum. However, it is apparent that, as in leprosy, tuberculosis demonstrates a range of immunological variability and that multiple skin testing may play a role in detecting this.

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