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The effect of desert conditions on the reactivity of Libyan schoolchildren to a range of new tuberculins

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SUMMARY

This study was carried out to investigate the effect of desert conditions on the pattern of delayed hypersensitivity to mycobacteria in school children aged 6–10 and 11–18 years. A new range of tuberculins prepared from ultrasonic lysates of living mycobacteria belonging to 12 different species was employed. Three centres were chosen for study, a sea port and two desert towns differing greatly from each other. The results obtained were compared with those of a previous study using the same reagents in Kenya.

As expected both the range of mycobacterial species to which the children reacted, the rate of acquisition of specific hypersensitivity with age and the total percentage of children reacting to individual reagents differed from centre to centre. The harsh desert conditions of Ajdabia produced the least, and the proximity of the people's dwellings to those of their farm animals in Kufra produced the most positive reactors to essentially environmental species. The greatest number of reactors to our Tuberculin were found in Benghazi where the cosmopolitan urban conditions probably lead to a high contact with open cases of tuberculosis. As assessed by skin test reactivity, immunization with BCG in Libya was much less effective than in Kenya. The interpretation of the differences between the results from the different test centres and between those for Libya and Kenya are discussed.

INTRODUCTION

Previous studies using tuberculins prepared by ultrasonic disruption of various species of mycobacteria have been carried out in East Africa where they have shown a high level of specificity. They have been used in studies of leprosy (Paul, Stanford & Carswell, 1975) and *Mycobacterium ulcerans* infection (Stanford, Revill, Gunthorpe & Grange, 1975), and have also been used to measure the effect of BCG immunization and of geographical locality on both school children (Paul, Stanford, Misljenovic & Lefering, 1975) and adults (Stanford *et al.* 1976). In principle the effect of BCG was greatest amongst school children and that of geographical locality amongst adults. Unexpectedly high incidence of reactivity

Table 1. *The skin test reagents used, the abbreviations employed in the figures, the number of tests performed with each reagent and the overall percentage of positive reactors* (5 *mm. or more*)

Skin test reagent	Abbreviation	Total no. of tests	Total % +ve
PPD (RT23)	RT	1564	40
Tuberculin	T	1128	44
Aviumin	A	767	30
A*-in	A*	773	38
Gordonin	Go	718	42
Ranin	R	759	33
Duvalin	D	616	8
Chelonin	C	881	25
Nonchromogenicin	No	671	13
Vaccin	V	683	13
Neoaurumin	Ne	758	30
Gilvin	Gi	624	15
Flavescin	F	832	26

Total number of children tested 3919
Total number of tests performed 10774

to environmental mycobacteria was found among the school children coming from the desert regions of Lodwar in Kenya (Paul *et al.* 1975) and Karamoja in Uganda (not published). To investigate this observation further the present study has been carried out in a different latitude on children living at the edge of and deep within the deserts of Libya. The particular regions studied are Ajdabia, Benghazi and Kufra in Cyrenaica.

Libya has had a considerable tuberculosis problem in recent years. It is thought that there was a definite increase in the prevalence of the disease during and after the second world war. A survey carried out in 1959 by the World Health Organization in Cyrenaica found a prevalence of active disease of 1·83%. However, the campaign against tuberculosis has been successful and figures for the whole country show a decline in the incidence of active disease per thousand from 2·3 in 1967 to 0·6 in 1973 (Newman, 1959; Kanter, 1967; Libyan Ministry of Health, 1974).

The prevalence of leprosy has been assessed to be about 3 per 1000 and the Libyan Department of Health estimates the incidence to be less than 0·3 per thousand. No other mycobacterioses are known to occur (Libyan Ministry of Health, 1975).

MATERIALS AND METHODS

Reagents

The majority of reagents were of the same batches previously used in East Africa, with the addition of one new reagent. This was Flavescin prepared from *Mycobacterium flavescens* (strain NCTC 10271). Table 1 lists the reagents used and gives some information about them. The concentration of the reagents, based on

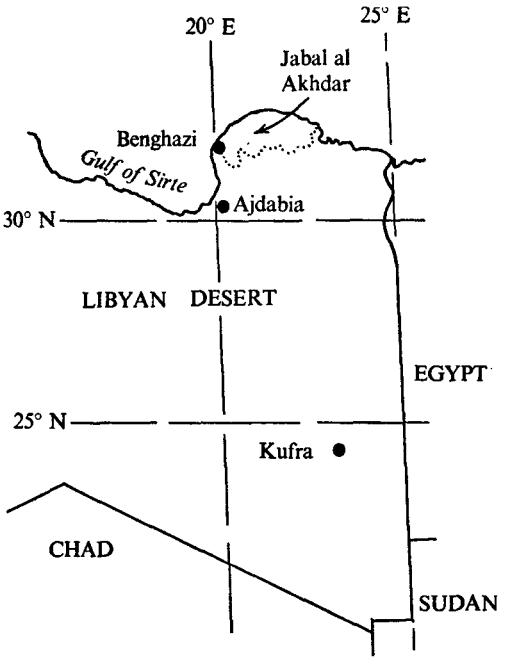

Fig. 1. Map of the Eastern half of Libya (Cyrenaica) showing the positions of the three test centres in relation to major geographical features.

a chemical assay (Lowry, Rosebrough, Farr & Randall, 1951) was 2 μg. protein/ml. (i.e. 0·2 μg./0·1 ml. dose). All reagents were prepared in a borate buffer containing Tween 80 as previously described. PPD (RT23) containing 0·4 μg. protein/ml was obtained from Statens Seruminstitut, Copenhagen.

Persons tested

These were children attending schools in Ajdabia, around Benghazi and in Kufra oasis (Fig. 1). To make the study comparable with the earlier one in Kenya, approximately equal numbers of children aged from 6 to 10 and from 11 to 18 years were tested with most reagents. Information obtained from each child included name, age and sex; BCG status was established by inspection of scars. Numbers of children tested with each reagent are shown in Table 1.

Test Procedure

All tests were performed by the intradermal method, by injecting 0·1 ml. of each reagent into the volar aspect of the forearm using 'Gillette scimitar' 1 ml. disposable tuberculin syringes and 20 gauge needles; 2 reagents were administered at the same time (one on each forearm) to children in junior schools (aged 6–12), and 4 reagents were administered (two on each forearm) to those in senior schools (aged 12–18). All tests were read after 72 hr. by measuring both longitudinal and transverse diameters of induration and recording the mean. As in our previous studies, 5 mm. induration or more has been taken as 'positive'.

Administration of reagents was arranged as far as possible to cover all

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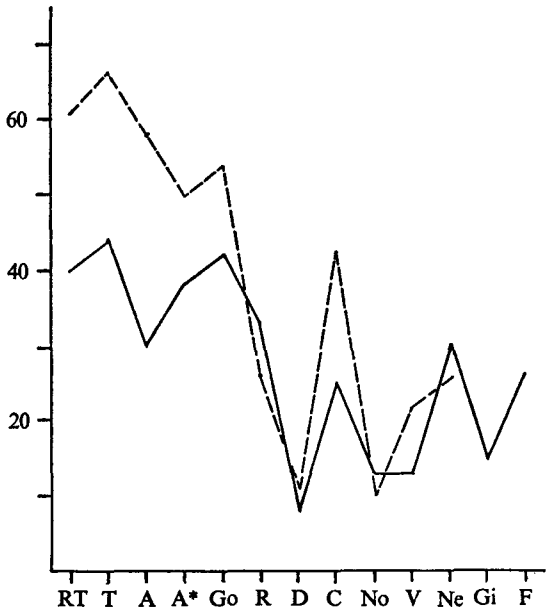

Fig. 2. The total percentages of positive reactors to each skin test reagent for Libya (———) and Kenya (— — —).

combinations at some point in the study. This was particularly applicable to Flavescin which had not been used before.

RESULTS

The collected results for all the children tested, expressed as percentages of positive reactors, are listed in Table 1 and shown diagrammatically in Fig. 2. The results vary from 40 to 45% for PPD (RT23), our Tuberculin and Gordonin to less than 10% for Duvalin.

The results for those who had or had not received BCG immunization, separated into age groups 6–10 and 11–18, are shown in Table 2 and expressed diagrammatically in Fig. 3. It can be seen that the effect of BCG is considerable on the results for PPD, Tuberculin, Aviumin, Gordonin and Nonchromogenicin, increasing the percentage of persons reacting to these reagents by about 20. The effect was almost negligible on the results for Duvalin, Chelonin, Vaccin, Neoaurumin, Gilvin and Flavescin. Increasing age most affected response to A*-in, Gordonin, Neoaurumin and Flavescin and had least effect on Duvalin, Nonchromogenicin and Vaccin.

The results for individual test centres are shown in the left hand columns of Table 3 and diagrammatically in Fig. 4. The greatest amount of reactivity to all reagents with the exceptions of PPD, Tuberculin and Vaccin were recorded from Kufra and with the exception of PPD the least response to the reagents was found in Ajdabia. Where sufficient children were tested for the results to be meaningful they are separated according to age group and BCG status for each test centre in the right hand columns of Table 3. It can be seen that the picture

Table 2. *The percentage of positive reactors to the different reagents amongst schoolchildren grouped according to age and immunization with BCG*

	Age 6–10			Age 11–18			All ages (mean %*)		
Reagents	No BCG	BCG	Both (mean %*)	No BCG	BCG	Both (mean %*)	No BCG	BCG	Both
RT23	6	30	18	30	53	41·5	18	41·5	30
Tuberculin	11	36	23·5	31·5	50	41	21	43	32
Aviumin	6·5	28	17	25	40	32·5	16	34	25
A*-in	19	38	28·5	58	51	54·5	38·5	44·5	41·5
Gordonin	4·5	26·5	15·5	33	57	45	19	42	30·5
Ranin	12	19	15·5	31	45	38	21·5	32	27
Duvalin	2	11	6·5	5	11	8	3·5	11	7
Chelonin	12	20	16	30	30	30	21	25	23
Nonchromogenicin	5	15	10	2	24	13	3·5	19·5	11·5
Vaccin	3	14	8·5	15	13·5	14	9	14	11·5
Neoaurumin	3·5	21	12	58	43	50·5	31	32	31·5
Gilvin	2·5	13	8	19	21	20	11	17	14
Flavescin	6·5	14	10	39·5	38	39	23	26	24·5

	Percentage of children immunized with BCG		
	Ajdabia	Benghazi	Kufra
Age 6–10	69	66	87
Age 11–18	97	84	89

* In these columns where mean percentages are shown this is done to correct for the disproportionate number of children who had received BCG in each age group indicated at the bottom of the table.

of highest reactivity in Kufra and lowest reactivity in Ajdabia is true for each of the subgroups.

The percentage of children immunized with BCG was 82% in Ajdabia, 77% in Benghazi and 88% in Kufra; the figures for the separate age groups at each of the test centres are given in Table 2.

An additional small survey was carried out on adult males working on the Kufra project, but largely originating in Benghazi, Tripoli and the Sudan. Of 180 persons tested with RT23, 75% produced positive reactions as compared with 89% of 80 persons tested with Tuberculin. Smaller numbers were tested with Chelonin and Gilvin producing 57 and 42% of positive reactions respectively.

DISCUSSION

The results present numerous interesting facets particularly with regard to immunization with BCG, the effect of increasing age and differences between the results for the different test centres. Additionally comparisons can be made between results for Libya and those previously obtained for Kenyan school children (Paul, Stanford, Misljenovic & Lefering, 1975).

Assuming a total percentage of positive reactors to individual skin test reagents above 20% to be significant, then the results shown in figure 2 indicate

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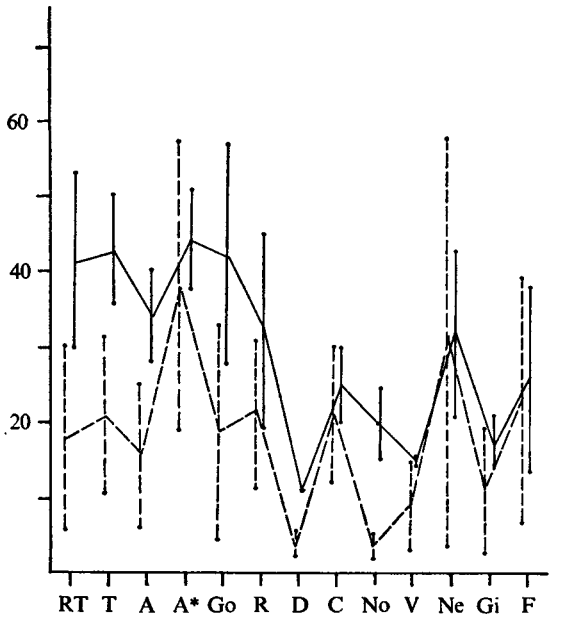

Fig. 3. The percentages of positive reactors to each of the skin test reagents for children from all centres grouped according to their BCG status. The solid line shows the results for those immunized with BCG and the broken line shows the results for those who have not received BCG. The vertical lines link the results for children aged 6–10 years (lower end) and aged 11+ (upper end).

Table 3. *Percentages of positive reactors to the different reagents for the separate test centres*

	Total results			Age 6–10						Age 11–18					
				No BCG			BCG			No BCG			BCG		
Reagents	Aj	B	K	Aj	B	K	Aj	B	K	Aj	B	K	Aj	B	K
RT23	41	38	39	7	.	5	30	.	30	.	15	45	60	45	53
Tuberculin	31	56	41	10	12	.	22	43	44	.	33	30	37	72	41
Aviumin	18	34	46	0	13	.	8	31	46	.	25	.	23	43	54
A*-in	14	45	71	5	13	39	11	33	70	.	33	83	15	63	76
Gordonin	28	45	69	0	9	.	7	46	.	.	33	.	47	52	72
Ranin	20	35	54	0	24	.	4	19	34	.	31	.	26	44	66
Duvalin	0	8	24	0	4	.	0	12	21	.	5	.	0	9	24
Chelonin	15	26	38	7	17	.	11	16	34	.	30	.	18	28	44
Nonchromogenicin	3	18	28	0	7	.	3	17	26	.	2	.	4	26	42
Vaccin	5	19	17	0	6	.	4	22	17	.	15	.	6	21	.
Neoaurumin	13	29	64	0	7	.	6	26	31	.	22	94	17	37	75
Gilvin	1	22	30	0	5	.	0	14	25	.	19	.	1	30	33
Flavescin	2	33	50	0	13	.	0	30	13	.	19	60	3	43	68

The total percentages of positive reactors for all children tested are shown in the first three columns. Where there are sufficient numbers of tests the results are shown according to age and BCG immunization in the remaining columns.

Aj, Ajdabia; B, Benghazi; K, Kufra.

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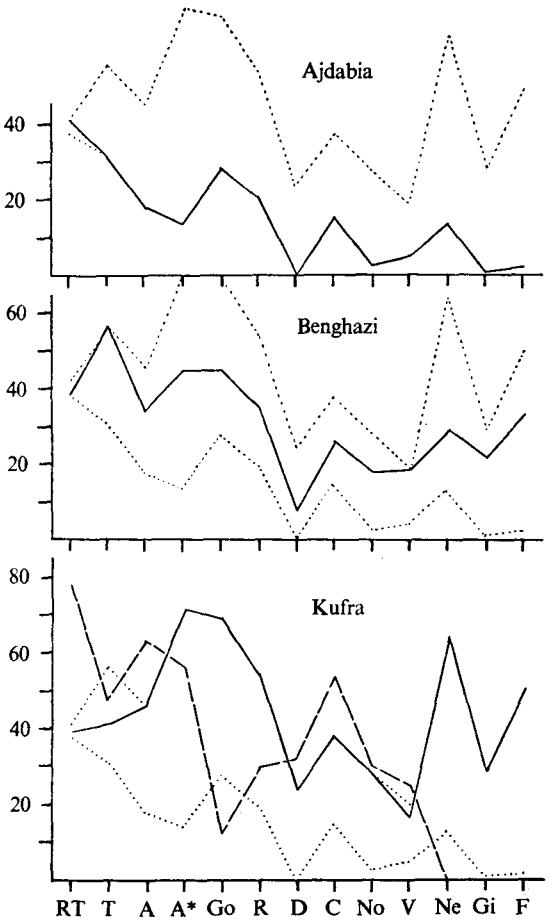

Fig. 4. The percentages of positive reactors to each of the skin test reagents at each centre (solid line). The dotted lines indicate the maximum and minimum percentages positive achieved in any one centre. In the diagram for Kufra the extra line (— — —) shows the results obtained from Lodwar in Kenya.

immunologically effective contact with *Mycobacterium tuberculosis*, *M. avium*, the so far unnamed organism referred to as 'A*' and *M. gordonae* of the slow growing subgenus and with *M. fortuitum* (from which Ranin is produced), *M. chelonei*, *M. neoaurum* and *M. flavescens* amongst fast growing species. Fig. 2 also shows the collected results of tests on Kenyan children (broken line). In Kenya 1½ times as many children have delayed hypersensitivity to *M. tuberculosis*, *M. avium*, and *M. chelonei*. There are also a considerably greater number of reactors to 'A*', *M. gordonae* and *M. chelonei* in Kenya than in Libya. Explanations of these differences should be sought in the differing effectiveness of BCG immunization schedules, differing contact with environmental mycobacteria, differing prevalences of mycobacterioses and differing capacities to react to mycobacterial antigens in the two populations.

The simplest groups of children to consider are those who had not received immunization with BCG and the results for these are shown as mean percentages of positive reactors for the two age groups in each country in Figure 5.

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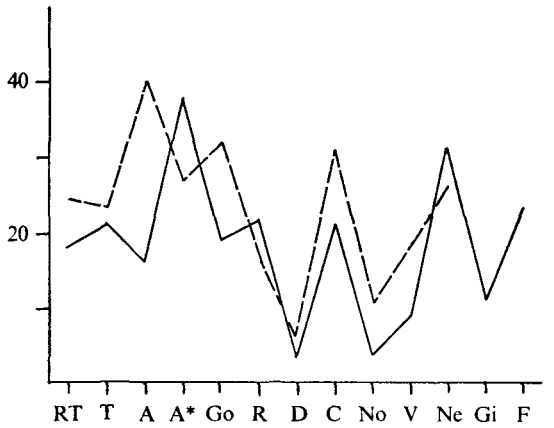

Fig. 5. The percentages of positive reactors to each of the skin test reagents among those not immunized with BCG from Libya (———) and Kenya (— — — —).

Superficially the results look rather similar, but there are at least twice as many children reacting to Aviumin, Nonchromogenicin and Vaccin and $1\frac{1}{2}$ times as many reacting to Gordonin and Chelonin in Kenya than in Libya. There are rather more children reacting to A*-in, Ranin and Neoaurumin in Libya than in Kenya. This is a reflexion of the differing distribution of mycobacterial species in the environment which is discussed further below.

In both Libya and Kenya BCG is administered without preselection of tuberculin (PPD) negative children. In view of this, the effect of BCG on reaction to the different skin test reagents can only be assessed in the nonreacting portion of the population. Thus the percentage increase in positive reactors to each antigen attributable to immunization with BCG equals

$$\frac{(\%\text{ positive reactors immunized minus }\%\text{ positive reactors unimmunized}) \times 100}{\%\text{ negative reactors unimmunized}}.$$

Based on this calculation the effect of BCG on the patterns of skin test reaction in both countries is shown in figure 6.

BCG has two well known effects:

(1) Induction of specific delayed hypersensitivity to *M. tuberculosis* and its variants and protection from infection with these organisms.

(2) An adjuvant effect boosting subsequent delayed hypersensitivity reactions to other antigens administered within a certain time of immunization. (In our case these other antigens were not deliberately administered, but came from casual contact with mycobacteria in the environment).

When the results shown in Fig. 6 are considered in relation to these two effects it can be seen that BCG has been much more effective in Kenya than in Libya in its effect on specific Tuberculin and PPD (RT23) sensitivity and in its boosting effect on sensitivity to slow growing species (*M. avium*, *M. sp.* '*A**' and *M. gordonae*), although this also depends on their frequency in the environment. However, it would be wrong to assume that the protective effect against tubercu-

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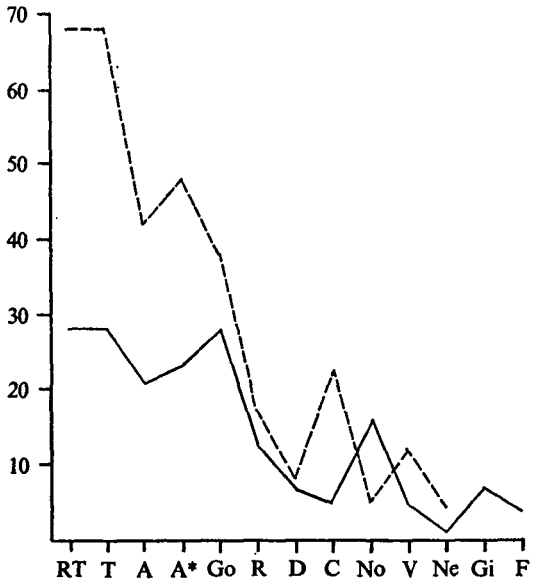

Fig. 6. The increase in percentage of positive reactors to each of the skin test antigens attributed to immunization with BCG in Libya (———) and Kenya (– – –). The results shown are based on the calculation:

$$\text{Effect of BCG} = \frac{(\%\ \text{+ve reactors immunised minus}\ \%\ \text{+ve reactors unimmunized})}{\%\ \text{-ve reactors unimmunized}} \times 100.$$

losis is concordant with these differences in delayed hypersensitivity. Sensitivity to fast growing species is much less enhanced and is similar in both countries. Thus both BCG immunization and distribution of environmental mycobacterial species are factors responsible for the differences between the results for Libya and Kenya seen in Fig. 2.

The reasons for the different performance of BCG in Libya and Kenya so far as demonstrable delayed hypersensitivity is concerned are hard to pinpoint. In both countries both Japanese and British (Glaxo) BCG vaccines have been in use and administrative methods would appear to have been too similar to offer any ready explanation. An investigation by one of us (A.W.) of the viable counts of samples of Glaxo vaccine waiting for distribution in Tripoli indicates their full potency. However, studies on samples of vaccine recovered from the field have yet to be performed. An analysis of BCG administration records in Benghazi is expected to provide information on which children received which vaccine and when this is available further light may be shed on the problem.

The effect of increasing age in relation to the effect of BCG immunization of the Libyan children is illustrated by Fig. 3. Where particular species are common in the environment there should be a marked increase with age in the percentages of children reacting to the specific reagents in both BCG immunized and non-immunized groups. Based on this criterion effective contact with *M. tuberculosis*, *M. avium*, '*A**', *M. gordonae*, *M. fortuitum*, *M. neoaurum* and *M. flavescens* might be

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considered common and such contact with *M. duvalii*, *M. nonchromogenicum* and *M. vaccae* uncommon amongst Libyan school children.

The situations of the test centres all differ considerably (Fig. 1). Ajdabia is a small town a few miles inland from the Eastern shores of the Gulf of Sirte. It is a very hot and dry place in the process of changing from rural to urban conditions. Its importance lies in its trade, which comes largely along the old caravan routes from the interior, and the town is a major distributing centre for goods from the South Sahara and the Sudan. Except for a small amount of corn growing and the fattening of animals on alfalfa brought 500 miles across the desert from Kufra, there is little agricultural activity. At the domestic level, some families still live in primitive conditions with their few grazing animals, but an increasing number live in modern houses and they have new schools, hospitals and shopping centres.

Benghazi, which is the chief city of Cyrenaica, lies at the western extremity of a hilly fertile area, called the Jabal al Akhdar. It is a major sea port and commercial town. Most of the children tested at this centre came from the town itself or from nearby agricultural villages.

Kufra oasis is the site of a big experiment in reclamation of desert by irrigation. The chief crop is alfalfa, which is used locally to feed large herds of sheep and goats, and is sent across the desert to other centres such as Ajdabia. It is also an important trading post with the Sudan. The lakes of the oasis are salty but the well water used for irrigation is fresh as also is the water collecting in shallow holes dug even in the proximity of the lakes. Most people live in palm thatched dwellings amongst the sand dunes that surround Kufra town, the latter being built of mud brick houses. The inhabitants live with their goats and chickens in close contact with their environment.

These three test centres were chosen because of their differing conditions, one a littoral urban area, and two quite different desert areas. It can be seen from Fig. 4 how the results obtained relate to the different centres.

If one accepts a mean result for PPD and our Tuberculin, then children in Ajdabia react less to every reagent than do children in the other centres. With the exceptions of PPD and our Tuberculin and marginally of Vaccin, the greatest number of reactors to each reagent were found in Kufra. The results from Benghazi were intermediate except for Tuberculin to which there was the greatest number of reactors, and Vaccin to which there were 2% more reactors than in Kufra.

The very low incidence of reactivity of children in Ajdabia to most reagents indicates that their environment contains few mycobacteria. Only Gordonin, Tuberculin and RT23 elicit reactions in more than 20% of children (Fig. 4). When these results are further analysed (Table 3 and Fig. 7) they provide most interesting information. The specificity of the skin test reagents and the effect of BCG immunization almost freed of the complicating factor of continual exposure to small doses of mycobacterial antigen from the environment during the period of maximum adjuvanticity of BCG is beautifully illustrated in the results for the younger age group. Perhaps a parallel can be drawn between the child living in the 'mycobacteria-free' Libyan desert and the Eskimo child of the 'mycobacteria-free'

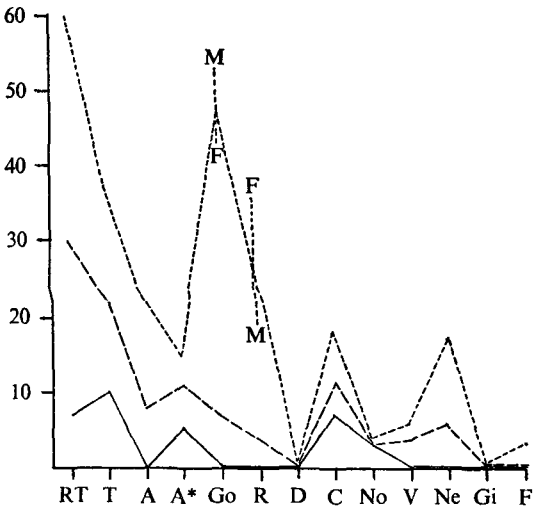

Fig. 7. The percentages of positive reactors to each of the skin test reagents in Ajdabia divided according to age and BCG status. Aged 6–10 unimmunized (———) aged 6–10 immunized (— — — —) aged 11+ immunized (- - - - - - - - -).

frozen north. Perhaps also this parallel might be extended to indicate that the short period of protection from tuberculosis provided by BCG among Eskimo children (Wilson, Galbraith & Grzybowski, 1973) will be found to apply also to children in Ajdabia. The effect of increasing age in Ajdabia also illustrates the small amount of contact with freeliving mycobacteria, although unfortunately we have sufficient results for BCG vaccinated children only in the upper age group. The appearance of significant numbers of reactors to Gordonin and Ranin in the older age group suggests either that this is acquired during visits to places outside Ajdabia such as Benghazi, where reaction to these reagents is common, or it is acquired as a result of different occupations or activities as the children grow older. If this were the case sex differences would be expected in the results for Gordonin and Ranin. As shown in Fig. 7, there is a considerable preponderance of positive reactors to Ranin amongst girls (35% as compared with 19% for boys) and a slight, probably insignificant, preponderance of reactors to Gordonin amongst the boys (52% as compared with 44% for girls).

The most notable feature of the results from Benghazi is the high percentage of positive reactors to our Tuberculin relative to RT23, the results for which are similar to those of the other centres (Table 3 and Fig. 4). If these results are correct, the most likely explanation of the high response to our Tuberculin is its greater sensitivity to small doses of antigen acquired by frequent contact with cases of tuberculosis associated with urban surroundings. This explanation is supported by the great increase in numbers of Tuberculin reactors with age. However, an alternative explanation might be based on the use of different BCG vaccines in different regions from time to time. An analysis of positive reaction sizes to both our Tuberculin and RT23 makes any explanation of the Benghazi results based on cross reactivity of either reagent with other mycobacteria most

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unlikely. Our small survey of adults also showed a preponderance of reactors to our Tuberculin (89%) over reactors to RT23 (75%). This high incidence of reactivity gives an indication of the commonness of contact with tuberculosis.

The pattern of reaction found at Kufra in the heart of the Sahara is most remarkable for its high percentages of positive reactors to A*-in, Gordonin, Ranin, Chelonin, Neoaurumin and Flavescin, making it directly comparable with Lodwar in the Turkana desert of Kenya. Not only are the results for the reagents just mentioned the highest recorded in Libya, but the results for A*-in, Gordonin, Ranin and Neoaurumin are also higher than those recorded in any of the townships studies in Kenya (Fig. 4). Thus the difference found in Kenya between the results for the desert townships of Lodwar and Marsabit recurs even more strongly between the results for the desert towns of Kufra and Ajdabia in Libya. Ajdabia is similar to Marsabit not only in the aridity of its environment but also in the trading and cereal growing bases for its commercial existence. On the other hand Kufra and Lodwar depend heavily on animal husbandry and the people share their homes and live in close proximity with their livestock.

The species of mycobacteria encountered in Kufra are not the same as those at Lodwar (see Fig. 4) and their effects on PPD positivity are very different. In Lodwar 30% more people reacted to PPD (RT23) than to our Tuberculin whereas in Kufra the results for these two reagents are within 2% of each other. The disparity in Lodwar was considered to be due to cross-reactivity of PPD with delayed hypersensitivity to *M. avium*, recorded as a high level of Aviumin positivity (62%). In Kufra Aviumin positivity is 46% and the large percentages of reactors to A*-in (71%), Gordonin (69%), Ranin (54%), Neoaurumin (64%) and Flavescin (50%) do not appear to have increased reaction to PPD. The results from Lodwar also differ from those from Kufra in respect of Gordonin, Neoaurumin (both low in Lodwar) and Chelonin (high in Lodwar). Soil samples were collected in both Lodwar and Kufra and when their analyses are completed it will be interesting to seek correlations with the skin test results.

The mechanism by which close contact with ruminant animals could produce a considerable increase in responsiveness to environmental mycobacteria has not been investigated. It is probable that the few organisms in the environment are ingested by the animals, concentrated in their intestines where they may well multiply and are defaecated into the byre at night. Once in a warm, moist atmosphere protected from direct sunlight the multiplication of mycobacteria would be enormously enhanced in the presence of acidic urine-soaked straw. Dust and moist particles emanating from the animal's quarters, which often constitute an integral part of human dwellings, could prove a very potent source of contact with these mycobacteria.

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REFERENCES

KANTER, H. (1967) *Libya—A Geomedical Monograph*. Berlin/Heidelberg: Springer Verlag.

LIBYAN MINISTRY OF HEALTH (1974). *The Tuberculosis Control Programme of the Libyan Arab Republic*.

LIBYAN MINISTRY OF HEALTH (1975). *The Leprosy Control Programme of the Libyan Arab Republic*.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**, 265–75.

NEWMAN, R. (1959). Report of Tuberculosis Prevalence Survey (EM/TB/56). World Health Organization, Alexandria.

PAUL, R. C., STANFORD, J. L. & CARSWELL, J. W. (1975). Multiple skin testing in leprosy. *Journal of Hygiene* **75**, 57–68.

PAUL, R. C., STANFORD, J. L., MISLJENOVIC, O. & LEFERING, J. (1975). Multiple skin testing of Kenyan schoolchildren with a series of new tuberculins. *Journal of Hygiene* **75**, 303–13.

STANFORD, J. L., REVILL, W. D. L., GUNTHORPE, W. J. & GRANGE, J. M. (1975). The production and preliminary investigation of Burulin, a new skin test reagent for *Mycobacterium ulcerans* infection. *Journal of Hygiene* **74**, 7–16.

STANFORD, J. L., PAUL, R. C., PENKETH, A., THURLOW, S., CARSWELL, J. W., BARKER, D. J. P. & BAROT, S. (1976). 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. *Journal of Hygiene* **76**, 205–14.

WILSON, J. M., GALBRAITH, J. D. & GRZYBROWSKI, S. (1973). Tuberculosis in Eskimo children. *American Review of Respiratory Disease* **108**, 559–64.