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SPECIAL ARTICLE

Quality control of skin smear services in leprosy programmes: preliminary experience with inter-observer comparison in routine services

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Summary A description of a systematic approach to periodical re-examination of samples of skin smears for leprosy taken in the routine services is given.

Procedures and recordings are described in detail and examples given of test runs in Tanzania and Ethiopia.

Scoring of quality control results is done against three indicators. The effect of the application of various criteria is shown in the two test runs. The exercise was experienced as stimulating and quite revealing.

Introduction

With the introduction of multiple drug therapy (MDT) regimens, the bacteriological assessment of patients by means of skin smears has become more important. The result of a skin smear examination can be decisive for the choice of the treatment regimen and for the duration of treatment.

In many programmes both the quantity and quality of skin smear examinations need to be considerably improved.

There is need for regular and systematic quality control of the skin smear services.

The quality and validity of bacteriological information in routine services depend on: 1, choice of the sites from which skin scrapings are taken; 2, the way in which the skin is cut/slit and the tissue scraped; 3, spreading of the material on the glass slide; 4, fixation of the specimen on the slide; 5, quality of the reagents used

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for the staining; 6, appropriateness of the staining procedures; 7, skill and accuracy of the person performing the staining; 8, quality of the microscope (strength of optics, cleanliness, brightness of the source of light, etc.); 9, skill and accuracy of the person who does the microscopic reading; 10, reporting of skin smear results from the laboratory, and accurate recording on the individual patient record card.

In the leprosy control programmes of Tanzania and Ethiopia some preliminary experience has been gained with a method of quality control based on inter-observer comparison of skin smear slides sampled from routine services. The method assesses eight (Nos. 2–9) of the ten aspects mentioned above which influence the quality of the bacteriological information.

Method

Laboratories were given instruction to preserve, in an appropriate box, all examined skin smear slides, positives and negatives, for up to 3 months* after examination.

1 SAMPLE SELECTION

Once every three months† a sample of 6 slides is taken for re-examination by a reference reader. As laboratory technicians seldom travel, selection is usually done by a (regional) leprosy control supervisor (LCS). The slides of the sample are documented on a 'Form for Quality Control of Skin Smear Examinations' (Figure 1) made out in duplicate, and the two sheets are marked A and B.

Selection of slides is done from the laboratory register book. Three positives and three negatives are selected. The method of selection is described on the back of the form, together with all further handling instructions.

In column VI the BI results found in the Service Laboratory are filled in on sheet B but not on sheet A: one BI value per smear.

Sheet B remains with the LCS.

Sheet A, without BI values in column VI, is taken or sent, together with the sample slides, to the Reference Laboratory.

2 REFERENCE LABORATORY

The Reference Laboratory Technician (RLT) re-examines the smears and gives judgements on the quality of smearing in column II, and of staining in column III.

* The staining of smears kept for periods longer than 3 months may fade so that a reference reader would not be able to see as many bacilli as were visible when the specimen was fresh.

† Because it is not feasible to carry out this procedure four times a year, in Tanzania's National Programme the target has now been set at twice a year.

Possible gradings are: good, fair or poor. In column IV the RLT writes a comment for all smears or stainings of poor or fair quality, preferably with suggestions for improvement.

The result of the quantitative assessment of the AFB seen is entered in column V: one BI value for each smear.

3 COMPARISON

When the RLT has completed the re-examination, sheet A is handed to the LCS who, preferably in the presence of the RLT, copies the BI-values found in the Service Laboratory (Column VI of sheet B) onto sheet A. The BI values in column VI are then compared with those of column V and differences entered in the appropriate spaces of column VII (lower, same or higher).

All entries on sheet A in columns II, III and IV are also to be copied onto sheet B. The totals of the various findings are calculated and entered in the bottom line of both sheets A and B.

Sheet A then remains at the Reference Laboratory so that the performance of the Service Laboratory Technician concerned can be monitored over a period of time by comparison of the scores of subsequent examinations.

4 FEEDBACK TO THE SERVICE LABORATORY

Sheet B, together with the six slides, is taken by the LCS, to the Service Laboratory, where all findings and comments of the Reference Laboratory are then discussed with the Service Laboratory Technician concerned. Where results differ significantly the technician is advised to re-examine the smears concerned. Possible causes of the difference or imperfection may then be identified and corrected. The LCS may have to re-instruct the person who takes the smears how to avoid blood, how to spread the specimen properly on the glass-slide, how to fix the smear properly, etc.

Suggestions for improvement should already have been indicated by the RLT in column IV of the form. For example, the Technician 'simply' may have to increase his/her accuracy, or to correct the staining method (e.g. filter the carbolfuchsine before use), or the microscope may need cleaning, etc.

In cases of major discrepancies or persistent errors the Service Laboratory Technician may need re-training, preferably at the Reference Laboratory.

5 MONITORING AT HEADQUARTERS

Finally the LCS sends a summary of the scores of each Service Laboratory Technician–quoting the totals found in the bottom lines of the forms—to the headquarters of the programme (e.g. Ministry of Health), so that the programme coordinator is kept informed of quality control efforts and of results achieved.

FORM FOR QUALITY CONTROL OF SKIN SMEAR EXAMINATIONS	Sheet: *

Name Service Lab.:	code	Total number of slides of last 8 weeks from which sample was taken (all read by same examiner)
Name Examiner:		Number of slides in last 6 months examined by this same examiner
Position/designation: - Lab. Auxilliary ☐ - Lab. Assistant ☐ - Lab. Technician ☐	District:	Total number of leprosy slides handled in this laboratory during the last 6 months (Period:)
	Province:	

Sample	Assessment at the REFERENCE LABORATORY								SERVICE LABORATORY							
I	II			III			IV	V	VI	VII						
Slide identification	Quality of smearing			Quality of staining			Comment or Advice	Ref. Lab. BI	Serv. Lab. BI	LOWER			SAME	HIGHER		
	good	fair	poor	good	fair	poor				⩾ 3	2	1	0	1	2	⩾ 3
taken by **																
taken by																
taken by																
taken by																
taken by																
taken by																
Totals:							Smears read:									
								Score in %								

** taken by: write here the initials of the person who took the specimen.

Sample taken from service lab. on date ☐

Sample arrived at reference lab. on date ☐

Despatch of form and sample from reference laboratory on date ☐

This sample of slides was taken by/is to be returned to:

Name:

Position:

Address:

* At time of sample selection this form is to be filled in DUPLICATE. Sheets to be marked as A and B. For instructions see the back of this form.

INSTRUCTIONS FOR INTER-OBSERVER COMPARISON OF SKIN SMEAR EXAMINATIONS

1 TWO SHEETS

This form is to be made out in duplicate. The 2 sheets are to be marked A and B in the right top corner.
In column I the identification numbers of the selected slides and the initials of the makers are entered on both sheets.

2 SAMPLE SELECTION

The selection of the slides for re-examination is done on the basis of the laboratory register, usually by a leprosy control supervisor (LCS) preferably not by laboratory staff.

2.1 Of one examiner

Slides to be selected in one sample should preferably have been stained and examined all by one laboratory technician.
If more persons examine leprosy smears a separate sample may be taken and separate forms filled for each examiner.

2.2 Sampling of negatives and positives

The sample should consist of six slides. Three with negative smears and three with a variety of positives (which will probably also contain some negative smears). Ideally the sample should contain some smears found to be highly positive (BI = 6, 5 or 4), some moderately positive (BI = 2 or 3) and some with BI = 1.

2.3 Representative for the period

The 6 slides should be chosen from the total examined during the last 8 weeks, taking a few from the slides examined during the early, middle and late parts of that period.

3 FURTHER HANDLING

After sample selection at the Service Laboratory and checking of the slide numbers (entered in column I) the BI results are filled in column VI on sheet B but *not* on sheet A. Sheet A goes with the slides to the Reference Laboratory.
Sheet B remains with the LCS.

4 RECORDING AT THE REFERENCE LAB

4.1 Quality of smearing and of staining

The Reference Laboratory Technician (RLT) will re-examine the smears and fill the columns II and III on sheet A with crosses for either good, fair or poor. In column IV the RTH must write a comment for all smears or stainings of poor quality. Preferably an *advice* should be given on how to improve.

4.2 BI-value

In column V the BI value found for each smear is entered.

4.3 Comparison of Service Lab with Reference Lab

When the RTH has completed the re-examination, sheet A with completed columns will be handed to the LCS who will, preferably in the presence of the RTH, copy the BI-values found in the Service Lab (found in VI of sheet B) on sheet A and then compare the BI values in column VI with those of column V.
Differences are entered in the appropriate subcolumns of VII (lower, same or higher). All other entries on sheet A (columns II, III and IV) are also to be copied on sheet B. Sheet A then remains with the RLT.

5 FEEDBACK TO SERVICE LAB

Sheet B together with the slides is to be taken, by the LCS, to the Service Lab, where all findings are then discussed with the Service Lab personnel concerned. In case of considerable difference it is advisable that the technician examines those smears again. Appropriate action for improvement should be taken where and when necessary.
In case the smear taking was not satisfactory this must be taken up with the person who took the smears.

6 REPORTING FROM REGION TO MINISTRY

Of all comparisons done during 6 months the RLT will make a summary report for the LCS, giving for each Service Lab in the region the total 'scores' of the bottom line of sheet A. This should be done in a table, with all districts mentioned. The LCS is requested to check this report, to make further comments where appropriate and to send one copy of the report to the central coordinator of the programme.

Figure 1.

Results and changes in scores are reviewed periodically so that supervisory activity and/or re-training activity can be directed accordingly.

Findings and comments

1. QUALITY OF SMEARING AND STAINING

In Tanzania the regional leprosy control supervisors (LCS) and the reference laboratory technicians (RLT) of eight regions were asked to try out this method of quality control.

The preliminary results reported here are based on sheets B which were returned to headquarters by three of the participating regions.

Judgements on quality of smearing and quality of staining have been summarized in Table 1. In Tanzania the present routine is to take 3 smears per patient. A sample of 6 slides thus consists of 18 smears.

Criteria for good, fair and poor had not been given, but as the RLTs are themselves practical teachers of AFB microscopy, it was assumed that they were prepared and capable of making such judgements. Scores per sheet showed that

Table 1. Quality of smearing and quality of staining. Scores on samples of 18 smears per district, for 12 districts (A–M) in 3 regions of Tanzania.

		Smearing (+ fixation)			Staining		
Region	District	Good	Fair	Poor	Good	Fair	Poor
I	A	3		15	3	3	12
	B	7	4	7	7	1	10
	C	0	4	14		18	
	D	8	9	1		9	9
	E	11	5	2	9	6	3
II	F	9	9		6	12	
	G	6	11	1	4	12	2
	H	10	6	2	6	11	1
III	J	14	3	1	14	3	1
	K	14	4		17	1	
	L	14	4		15		3
	M	12	4	2	12	4	2
Total scores		108	63	45	93	80	43
		50%	29%	21%	43%	37%	20%

judgements on both smearing and on staining had been made separately for each smear.

The reference readers did not show any tendency to overscore the vague category of 'fair'. Although the judgements are arbitrary and cannot be standardized, the authors believe that the scores given provide useful feedback for both the persons concerned and for programme management. Only after repeated use of the same method will it be clear as to what extent these exercises lead to improvements.

The method is vulnerable to personal bias.

The RLT may be inclined to give many scores 'good' because he/she has trained and supervised the technicians.

The samples are not presented anonymously. Even if the names of the workers concerned were not filled in on sheet A, it is likely that they could be identified by means of the slide identification numbers. Therefore, if an RLT were to be personally biased towards a certain worker (in favour or against), this could influence the judgements given in columns II, III and IV. Because criteria cannot be standardized, and because of the possibility of personal bias, it is not considered valid to use the scores for comparing the performance of various districts or regions.

One RLT had not given any comments in column IV, but the two others had given quite a variety of comments or advice for improvements: 'don't decolourize too much, smear too thick, smear too thin, blood!' etc.

The totals of scores per sample at the bottom of each sheet (Figure 1) offer an easy means of monitoring trends in the change of quality per technician.

In Ethiopia, results were similar.

2. DIFFERENCES IN BI-VALUES

Comparison of results in the three regions in Tanzania, showed considerable differences in the BI-values of Service Laboratories and Reference Laboratories. On several occasions smears read in the Service Laboratory as BI = 1 were given BI = 5 by the Reference Laboratory Technician (RLT). A summary of the differences found is given in Table 2. How to judge these results? What degree of difference should be considered acceptable?

It is not difficult to decide that in samples D, E and J (in Table 2) the two readings showed far too much difference.

It is probably also not difficult to agree that samples F, H and K show differences so minute that the correlation of these readings should be considered quite satisfactory.

But how is one to decide, in such a complex series of scores, where to draw the line between acceptable and unacceptable?

Various possibilities were explored. Here, a construction is presented in which the correlation is judged by three indicators.

Table 2. Differences of BI-values. BIs in twelve Service Laboratories, compared to those of Reference Laboratories were: lower, same or higher.

District	No. of smears	Lower		Same			Higher	
		$-\geqslant 3$	-2	-1	0	$+1$	$+2$	$+\geqslant 3$
A	18			1	10	3	1	3
B	18	2	3	3	7	2	1	
C	14*	1	1	3	7	2		
D	18	5	1	4	6	2		
E	18	4	1	2	7	3	1	
F	18			4	14			
G	18			4	9	5		
H	18			1	14	3		
J	17*	8	4	2	3			
K	18			1	14	2	1	
L	18		3	3	12			
M	18	3	1	4	8	2		
Totals	211	23	14	32	111	24	4	3
Proportion				15%	53%	11%		
	100%	18%			79%		3%	

* Four smears of sample C and one of sample J were not examined as these specimens had either been washed off or were considered of too poor quality to be examined.

(a) Proportion of full correlation. In one project (where BI readings were made by very experienced examiners) the proportion of full correlation (i.e. readings 'same' or difference 0) was 75%.

For the Tanzania test run a criterion of $\geqslant 50\%$ full correlation was applied.

(b) If a difference of 1 mark BI to either side is considered to be of little significance and therefore acceptable, one might also consider the three central columns together ($-1, 0, +1$) to represent 'a measure of correlation' between the two readers. This proportion should be much larger than 50%, e.g. 80%.

(c) If the differences in a sample are acceptable according to criteria 1 and 2, confirming that at least 80% of all differences remain within the range of the three central columns, it is still important whether the remaining scores are in the next nearest columns of 2 marks difference, or represent readings of 3 or more marks difference. Those further deviations are given a heavier weight in the assessment by the third indicator for correlation: variance.

Variance is the sum (Σ) of the square values of the differences (D^2) divided by the number of observations (N):

$$\text{Variance} = \frac{\Sigma D^2}{N}.$$

Of each sample of smears the variance of the observations of the one reader compared to the readings of the reference reader can thus be calculated:

Example. In sample A (see Table 2) the variance of 18 observations was:

$$\text{Variance} = \frac{1^2+(10\times 0^2)+(3\times 1^2)+(1\times 2^2)+(3\times 3^2)}{18}$$

$$= \frac{1+0+3+4+27}{18} = \frac{35}{18} = 1{\cdot}944.$$

The above-mentioned three indicators of correlation have been applied on the findings given in Table 2 and results are shown in Table 3. For variance, a value of less than 1 was adopted as a criterion.

In Ethiopia the test run was conducted within the laboratory of ALERT, where large numbers of skin smear specimens are examined both from hospital patients and field patients. In the first 9 months of 1984 as many as 12,000 slides were examined. The laboratory has 12 technicians who deal with skin smears. A periodical re-examination of slides, picked randomly from the boxes of the

Table 3. Judgements on correlation according to various criteria

District	I Same ≥ 50%		II CC* ≥ 80%		III Variance < 1		Conclusion
A	10/18	+	14/18	INS	1·944	–	
B	7/18	INS†	12/18	–	2·166	–	
C	7/14	+	12/14	+	1·286	INS	
D	6/18	INS	12/18	–	3·056	–	
E	7/18	INS	12/18	–	2·722	–	
F	14/18	+	18/18	+	0·222	+	+
G	9/18	+	18/18	+	0·500	+	+
H	14/18	+	18/18	+	0·222	+	+
J	3/17	INS	5/17	–	5·294	–	
K	14/18	+	17/18	+	0·389	+	+
L	12/18	+	15/18	+	0·833	+	+
M	8/18	INS	14/18	–	2·056	–	
12	7/12		6/12		1·700	5/12	5/12 42%

* CC = the number of BI-readings falling in the three Central Columns (between the vertical lines in Table 2).

† INS = insufficient correlation when found for the first time. In subsequent columns indicated with a minus (–).

various technicians, was carried out for 11 readers. This paper gives results of seven readers.

The reference reader was the Head of the Laboratory (TN).

Technicians were coded, A, B, C to G. Samples usually consisted of 6 slides with 4 smears each. For each technician four, five or six samples were examined. In the case of technician F, only two samples were taken. Findings on 30 samples with a total of 681 smears examined are given in Table 4. Table 4 shows deviations that are much less than those in Table 2. Of the 681 examinations in Ethiopia, the reference reader fully agreed with the BI values of as many as 455 smears (67%).

The bottom line of totals indicates that 93% of the readings remained within the three central columns, that is, the range of not more than 1 BI mark difference to either side (0 ± 1).

For the 211 smears of Tanzania districts (Table 2) these proportions were 53% and 79% respectively. However, it was to be expected that seven laboratory technicians working as colleagues together in one laboratory of a leading referral and teaching hospital should produce better standardized readings than others working on their own in 12 different district hospitals.

In the further columns of Table 4, the correlation was assessed by the same three criteria as those applied in the test run in Tanzania (Table 3). Samples E1 and E2 are the only ones failing on all three criteria. Sample G2 is an interesting one. Its deviation is acceptable according to the rather tough third criterion, but fails on the seemingly mild first criterion of getting the same BI-value in at least half of the smears (same $\geqslant 50\%$). The third criterion for correlation, the variance, particularly 'punishes' deviations beyond 1 BI mark and this works out progressively for every further mark because of the squaring of the differences. By this criterion, samples A1, B1 and F2 have insufficient correlation or, in other words, too much deviation.

For the sake of sample A1, the block for BI-values in Table 4 has been given an extra column on each side. This was to show the one smear that had been read with a difference of 4 BI marks. For the calculation of the third criterion it makes a lot of difference whether the deviation is 3 or 4 or 5 BI marks, namely D^2 becoming either 9 or 16 or 25!

In Table 4 the second criterion ($cc \geqslant 80\%$) does not disqualify any of the 30 samples that had not already been declared insufficient by the first criterion. With all three criteria applied, 6 out of the 30 samples had too much deviation. The final correlation is then 24/30 or 80%.

Discussion

1. FEASIBILITY OF THE METHOD

Requirements: boxes to store slides; proper instructions and tools for durable

Table 4. Quality control of skin smear reading in 30 samples of 7 technicians of ALERT, Ethiopia. Differences of BI-values compared to Reference Reader. Correlation of the two readings assessed by 3 criteria.

Service reader and sample nr	BI-Values									Nr of smears	Correlation			
				Central columns							I	II	III	
	−4	−3	−2	−1	0 same	+1	+2	+3	+4		Same ≥ 50%	CC ≥ 80%	Variance < 1	Conclusion
A 1			1	4	11	2			1	19	11/19 +	17/19 +	1·368 INS†	
A 2			1	4	18		1			24	18/24 +	22/24 +	0·500 +	+
A 3				1	16	4	1	1		23	16/23 +	21/23 +	0·783 +	+
A 4				2	19	2				23	19/23 +	23/23 +	0·174 +	+
B 1			4	6	12	2				24	12/24 +	20/24 +	1·000 INS	
B 2				7	13					20	13/20 +	20/20 +	0·350 +	+
B 3			1	1	19	2	1			24	19/24 +	22/24 +	0·458 +	+
* 4				2	19		3			24	19/24 +*	21/24 +	0·583 +	+
C 1			2	7	14	1				24	+	+	0·667 +	+
C 2			2	4	17	1				24	+	+	0·542 +	+
C 3			1	8	12	2				23	+	+	0·609 +	+
C 4				5	14	4				23	+	+	0·391 +	+
C 5					24					24	+	+	0·000 +	+
D 1			2	2	16	3				23	+	+	0·565 +	+
D 2				3	20	1				24	+	+	0·167 +	+
D 3		1	1		13	5				20	+	+	0·900 +	+
D 4				5	19					24	+	+	0·208 +	+
E 1		1	6	6	9	2				24	9/24 INS	−	1·708 −	
E 2			4	8	9	2	1			24	9/24 INS	−	1·250 −	
E 3			1	5	15	3				24	+	+	0·500 +	+
E 4				2	19	3				24	+	+	0·208 +	+
E 5				1	13	6				20	+	+	0·350 +	+
E 6			1	1	19	1				22	+	+	0·273 +	+
F 1				6	12	5	1			24	+	+	0·625 +	+
F 2			2	3	13	4	1	1		24	+	+	1·000 INS	
G 1				4	19	1				24	+	+	0·208 +	+
G 2			1	10	7	1				19	7/24 INS	+	0·789 +	
G 3				9	14	1				24	+	+	0·417 +	+
G 4		1		5	14	2				22	+	+	0·727 +	+
G 5					16					16	+	+	0·000 +	+
Totals		3	30	121	455	60	9	2	1	681	27/30	28/30	0·584 25/30	24/30
Proportion		5%		18%	67% 93%	9%		2%		100%				80%

* The calculations of the correlation criteria I and II have only been shown for the first 8 samples, but these were thereafter, in case of positive results, left out to keep the table more easily readable.

† INS = insufficient correlation when found for the first time, in subsequent columns indicated with a minus (−).

slide identification (diamond pencil); suitable forms (e.g. of the format shown in Figure 1); and appropriate explanation of purpose and method.

If these requirements are met, the method of quality control described above is feasible. It is crucial that personnel concerned should experience quality control

as a necessary routine procedure of support and assistance, rather than as a threat.

Programme managers must ensure that personnel concerned have been properly trained and instructed in all procedures of skin smear examination, before any attempt is made to introduce this kind of quality control. (See the 10 steps mentioned in the Introduction.)

A refresher course for laboratory technicians is a very appropriate occasion for the introduction of the method. It may be helpful to find out whether quality control by means of inter-observer comparison is done with regard to any other tests in the medical laboratories concerned. If this is not the case the leprosy programme may have to pioneer.

2. USEFULNESS

This method of quality control will prove to be useful if, with subsequent testing, the results improve and then can be maintained at a certain level. The preliminary tests reported here cannot yet provide a measure of usefulness. Although the results of consecutive tests for some of the technicians at ALERT showed improvement (e.g. C1→C5 and E1→E6), this experience is considered to be too limited to draw conclusions regarding its impact on the quality of work.

3. WHO PROVIDES A STANDARD BI READING?

A further limitation of the method is that the reference reader who sets a standard may very well have some 'deviation' in his/her readings. Therefore, it is to be recommended that the various reference readers of a programme from time to time have their readings checked against a common standard.

There are various ways of doing this, for example arrange another inter-observer comparison again between the RLTs, either during a national seminar or refresher course, or by mail. It might also be possible* to provide sets of standard smears with known BI values that can be distributed for a systematic testing of the reference readers.

4. WHICH INDICATORS FOR CORRELATION?

In the assessment of these two test runs in Tanzania and Ethiopia the choice of criteria applied was arbitrary. The authors certainly do not assume that these are the only possible, or the best, criteria.

Generally, two steps are needed to set criteria: (a) choose indicators; and (b) choose criteria for each indicator.

* The Royal Tropical Institute, Amsterdam, recently made one batch of 125 slides (625 smears) of known BI values. These smears were made from homogenous suspensions of various skin biopsies. Regular production of 'standard smears' may be considered.

In these test runs, the following three indicators were used: 1 proportion of BI values 'same' or 'difference zero'; 2 proportion of BI values in three central columns 'CC' or 'difference 0 and +/−1'; and 3 the variance† of the readings = the sum of squares of differences divided by number of observations.

$$\text{Variance} = \frac{\Sigma D^2}{N}.$$

One might wonder why *three* indicators are proposed. Would variance alone not have been an acceptable indicator?

The indicators 'proportion same' and 'proportion in central columns' are presented first because these values are immediately *visible* on the form and are therefore expected to be immediately understood and accepted by all concerned.

It is not known to what extent 'variance' will be acceptable in field programmes. If, as in Tables 2 and 3, a large proportion of the samples already show insufficient correlation by mild criteria of the first two indicators, it may not always be necessary to apply the third indicator.

5. CRUCIAL VALUE BI = 2, FALSE POSITIVES, FALSE NEGATIVES

We considered for some time a possible fourth indicator, which would take into account the occurrence of false negatives and false positives round the crucial values BI = 1 and BI ⩾ 2, because the difference between BI = 1 and BI = 2 can be decisive for the important distinction paucibacillary or multibacillary, with all its consequences for cost and duration of treatment. However, introducing a distinction of false positives and false negatives appeared to be too complicated* at this stage of the project. It may become relevant only after considerable improvement, measured by the present three indicators, has been achieved.

Because of the importance of BIs (0,) 1 and 2, Laboratory Technicians should be instructed that, when specimens contain only a few bacilli, at least *one hundred* microscopic fields should always be examined and all bacilli found should be reported: e.g. BI = 1 (4 bacilli/100 fields) and BI = 2 (27 bac/100 flds).

In situations where finding a few more or less bacilli would categorize a patient as either paucibacillary or multibacillary, e.g. BI = 1 (9 bac./100 flds) as compared to a BI = 2 (11 bac./100 flds), it should be a rule that the slide is re-examined and preferably new smears are taken aiming at the sites most likely to be positive.

6. WHICH CRITERIA

The criteria for each of the three indicators were also chosen arbitrarily. We used:

† Some readers might prefer, instead of the variance, the in statistics more often used 'standard deviation'.

* In the LEPRA Programme of Malawi this criterion is already in use!

for (1) 50%, for (2) 80%, and for (3) the variance to be below 1. However, it is quite possible that a programme manager chooses other criteria, e.g. for (1) 60%, for (2) 90% and for (3) variance $<0{\cdot}7$. With the latter, 8 more of the 30 samples of ALERT (see Table 5) would have been picked out as insufficient, so that the overall correlation would have been only (16/30 =) 53%. Comparison of Tables 4

Table 5. Quality control of skin smear reading. Assessment of correlation in the same 30 samples as in Table 4. By same indicators, but with more strict criteria.

Service reader and sample nr.	BI-Values									Nr. of smears	Correlation			
				Central Columns							I	II	III	
	−4	−3	−2	−1	0 same	+1	+2	+3	+4		Same ≥ 60%	CC ≥ 90%	Variance < 0·7	Conclusion
A 1			1	4	11	2			1	19	11/19 INS†	17/19 −	1·368 −	
A 2			1	4	18		1			24	18/24 +	22/24 +	0·500 +	+
A 3				1	16	4	1	1		23	16/23 +	21/23 +	0·783 INS	
A 4				2	19	2				23	19/23 +	23/23 +	0·174 +	+
B 1			4	6	12	2				24	12/24 INS	20/24 −	1·000 −	
B 2				7	13					20	13/20 +	20/20 +	0·350 +	+
B 3			1	1	19	2	1			24	19/24 +	22/24 +	0·458 +	+
* B 4				2	19		3			24	19/24 +*	21/24 INS	0·583 +	
C 1			2	7	14	1				24	INS	+	0·667 +	
C 2			2	4	17	1				24	+	+	0·542 +	+
C 3			1	8	12	2				23	INS	+	0·609 +	
C 4				5	14	4				23	+	+	0·391 +	+
C 5					24					24	+	+	0·000 +	+
D 1			2	2	16	3				23	+	+	0·565 +	+
D 2				3	20	1				24	+	+	0·167 +	+
D 3		1	1		13	5				20	+	+	0·900 INS	
D 4				5	19					24	+	+	0·208 +	+
E 1		1	6	6	9	2				24	INS	−	1·708 −	
E 2			4	8	9	2	1			24	INS	−	1·250 −	
E 3			1	3	15	3				24	+	+	0·500 +	+
E 4				2	19	3				24	+	+	0·208 +	+
E 5				1	13	6				20	+	+	0·350 +	+
E 6			1	1	19	1				22	+	+	0·273 +	+
F 1				6	12	5	1			24	INS	+	0·625 +	
F 2			2	3	13	4	1	1		24	INS	−	1·000 −	
G 1				4	19	1				24	+	+	0·208 +	+
G 2			1	10	7	1				19	INS	+	0·789 −	
G 3				9	14	1				24	INS	+	0·417 +	
G 4		1		5	14	2				22	+	+	0·727 INS	
G 5					16					16	+	+	0·000 +	+
Totals		3	30	121	455	60	9	2	1	681	20/30	24/30	0·584 21/30	16/30
Proportion		5%		18%	67% 93%	9%		2%		100%				53%

* The calculations of the correlation indicators I and II have only been shown for the first 8 samples, but were thereafter left out, for the sake of readability.

† INS = insufficient correlation when found for the first time, in subsequent columns indicated with a minus (−).

and 5 gives an impression of the effects of the various criteria. When for the category 'same', a proportion of 60% had been required, Table 5 shows that of ALERT's 30 samples, four (C1, C3, F1 and G3) would have been disqualified by this first criterion, while the differences remained within acceptable range according to the two other indicators/criteria.

Applied to the findings of 12 district laboratories in Tanzania, the stricter criteria used in Table 5 would have disqualified two more samples, so that only the three samples F, H and K would have 'passed' as sufficient.

Conclusion

Although indicators and criteria are thus still a matter for discussion, it can be concluded that in our preliminary experience this inter-observer comparison of skin smears was found to be a stimulating exercise, revealing differences in BI reading of which the personnel concerned were not aware. More experience with the method will be needed to produce evidence of its value in routine services.

Acknowledgment

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Lepr Rev (1985) **56,** 193–198

A spot test for detection of antibodies to phenolic glycolipid I

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Summary A novel spot test for detection of antibodies to phenolic glycolipid I of *Mycobacterium leprae* has been developed. This test uses antigen-coated filter strips with a simple non-quantitative visual readout. Results of the spot test were in good agreement with those obtained using a standard microtitre ELISA test for antibodies with the new test being slightly less sensitive than the standard method. The spot test may be useful as a field test for serodiagnosis of leprosy in areas where microtitre plates, spectrophotometers and multichannel pipettes are not readily available.

Introduction

Several groups have recently described tests for detection of antibodies directed to the major phenolic glycolipid of *Mycobacterium leprae*[1, 2, 3] which contains a trisaccharide portion unique to these organisms.[4, 5] Sera from leprosy patients contain antibodies which specifically recognize the phenolic glycolipid with antibody levels increasing from the tuberculoid to the lepromatous pole of the leprosy spectrum.[1 2, 3] Control sera and sera from patients with other mycobacterial infections are negative in these tests[1 2, 3] and the possible use of such assays for the early detection of leprosy has been discussed.[2, 6]

The native glycolipid is a highly hydrophobic molecule and a variety of strategies have been employed to prepare aqueous suspensions suitable for the coating of the antigen to plastic microtitre plates and use in enzyme-linked immunosorbent assays (ELISA). These strategies include sonication[2] or detergent solubilization[1] of the intact lipid, and partial hydrolysis of the lipid to produce the more polar deacylated form of the molecule.[3] Synthetic antigens containing the antigenically important terminal di-methyl glucose residue[7, 8] have

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also been prepared and shown to be highly effective substitutes for the natural glycolipid in ELISA tests.[9]

A common feature of all of these assays is the use of microtitre systems employing disposable plastic multiwell plates. While such systems are extremely convenient for processing large numbers of samples, the plastic ware, multichannel pipettes and spectrophotometers required for the assays are often unavailable in the areas in which leprosy is endemic. We have therefore developed an alternative test for antibodies to phenolic glycolipid which does not utilize microtitre plates and gives a simple qualitative visual readout.

Materials and methods

PREPARATION OF STRIPS

Polysulphone membranes were supplied as HT-200 Tuffryn® Membrane Filters by Gelman Sciences Inc., Ann Arbor, Michigan. Membranes were cut into suitably-sized strips for the particular size of test tube selected for the assay. We have used either short strips (1 cm × 0·5 cm) or long strips (5 cm × 0·5 cm) with identical results. Phenolic glycolipid I was purified from infected armadillo tissue as described previously[3] and dissolved in hexane at a concentration of 50 micrograms per ml. A single 2 microlitre drop was applied to the end of each filter strip (100 ng glycolipid per spot) and allowed to dry for a few minutes at room temperature. Strips stored for 2 months at 0°C or 37°C showed no loss in reactivity.

SPOT TEST

Strips were rinsed briefly with phosphate buffered saline pH 7·2 (PBS) and then immersed in tubes containing 1 ml of PBS with 1% (v/v) normal goat serum (Gibco Labs., Grand Island, NY) and 5 microlitres of human serum samples. With long strips, only the end of the strip with the glycolipid spot was immersed. Strips were incubated with antibody for 1 hr at 37°C and then washed 4 times (5 min each wash) with 1·5 ml of PBS per tube. Peroxidase-conjugated goat anti-human immunoglobulins (IgA + IgG + IgM) (Cappel Laboratories, Cochranville, Pa) was diluted 1:2,000 in PBS containing 1% normal goat serum and 1 ml was added to each tube. After 1 hr at 37°C, strips were again washed 4 times with PBS. Peroxidase substrate was prepared by dissolving 3,3′-diaminobenzidine (Sigma Chemical Co., St Louis, Mo) in 0·1 M citrate buffer pH 5 (0·5 mg/ml) and adding hydrogen peroxide to a final concentration of 0·03%. Strips were incubated with substrate solution for 10 min at room temperature and then washed with water and examined for colour formation.

MICROTITRE ELISA

An ELISA test using the deacylated form of the phenolic glycolipid coated to microtitre plates was performed as described previously[3] except that serum samples were used at a dilution of 1:200 and the peroxidase substrate (o-phenylenediamine) was prepared in citrate buffer pH 5 rather than in PBS.

Results

When polysulphone strips were incubated with serum samples containing antibodies to phenolic glycolipid a spot of colour was seen at the point of application of the antigen. According to the intensity of the colour reaction, results were given a grading of: '−' no reaction, '+' faint spot, '++' distinct spot, '+++' dark spot. Figure 1 shows an example of 12 sera graded according to this approximate scale.

CONTROL SERA

Sera from 20 normal individuals living in Seattle were negative by the microtitre ELISA with deacylated phenolic glycolipid ($A_{492} < 0{\cdot}10$) and were also found to

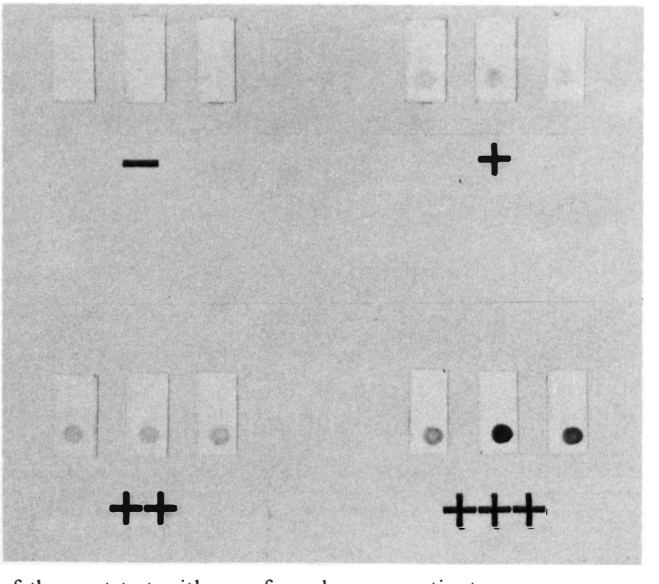

Figure 1. Results of the spot test with sera from leprosy patients.

Serum samples from 12 leprosy patients with different levels of antibodies to phenolic glycolipid I were assayed in the spot test as described in the text. Results were graded according to an approximate scale from '−' to '+++' as shown. The antibody levels (A_{492}) recorded for the same sera in the microtitre ELISA were (left to right): '−' 0·00, 0·02, 0·10; '+' 0·17, 0·19, 0·24; '++' 0·49, 0·57, 0·65; '+++' 0·88, 1·16, 1·18.

be negative in the spot test. Twenty-nine sera from tuberculosis patients were also tested and found to be negative in both tests.

PATIENT SERA

Eighty sera from leprosy patients with A_{492} readings in the microtitre ELISA ranging from 0 to 1·28 were assayed by the spot test and the results graded as described above. Figure 2 shows the spot test grading plotted against the absorbance in the microtitre ELISA. A good correlation between the 2 tests was observed. Sera which were negative in the spot test had absorbance values ranging from 0 to 0·19 with a mean value of 0·08 (standard deviation 0·06). Sera rated '+' had a mean of $0{\cdot}29 \pm 0{\cdot}14$; '++' $0{\cdot}57 \pm 0{\cdot}18$; and '+++' $0{\cdot}97 \pm 0{\cdot}21$. An absorbance reading of less than 0·10 is considered negative in the microtitre ELISA[3] and all such sera were found to be negative in the spot test. All of the sera with A_{492} of 0·20 or above were positive in the spot test. Of 17 sera with A_{492} 0·10 to

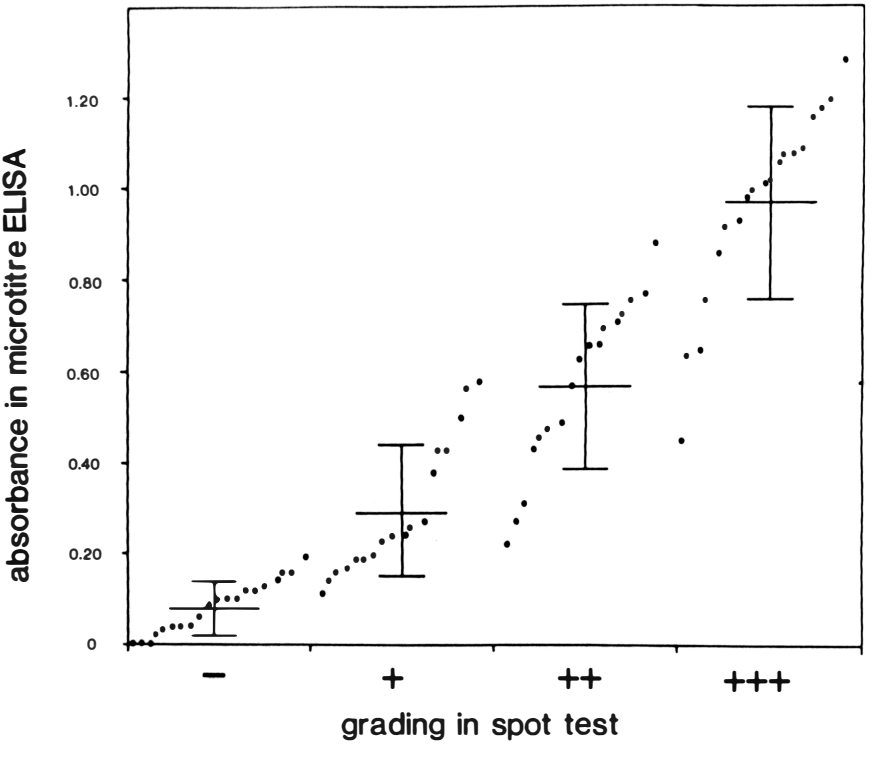

Figure 2. Comparison of the spot test and microtitre ELISA.

Eighty serum samples from leprosy patients were assayed at a dilution of 1 : 200 in the spot test and in the microtitre ELISA. Results are plotted as the A_{492} reading in the microtitre ELISA against the approximate grading in the spot test. Horizontal lines show the mean (and standard deviation) of A_{492} readings for the sera graded in each category of the spot test.

0·19, 7 were positive ('+') by the spot test. Thus 10 sera which were considered positive by the microtitre ELISA were rated negative by the spot test.

VARIATIONS IN THE ASSAY PROCEDURE

While all of the results presented here represent those with serum samples assayed using the standard conditions described above, it was found that comparable results could be obtained using alternative procedures. Incubation of samples at room temperature (20–25°C) was as effective as incubation at 37°C and tap water could be substituted for PBS during the washing steps. Plasma prepared from blood collected in the presence of heparin gave identical results to serum from the same individual. Whole blood either diluted directly into PBS or dried on filter paper and then resuspended in PBS could also be used in the assay. Antibodies to phenolic glycolipid could also be detected in saliva samples using the spot test.

Discussion

The spot test described here differs from previous tests for antibodies to phenolic glycolipid in that it does not require the use of disposable microtitre plates. The solid support used for binding the glycolipid is a polysulphone membrane which was selected on the basis of its low level of background binding of IgM antibodies.[10] The test tubes used for carrying out reactions can be washed and reused for further assays with fresh antigen strips. This feature of the test may make it convenient for use in areas where microtitre equipment is not available.

The results of the test are read by looking for a coloured spot against a white background. For a test involving a non-quantitative visual readout, it was found to be easier to distinguish a coloured spot rather than to judge differences in formation of a soluble coloured product such as that used in the microtitre ELISA tests. In addition, results of this test were not affected by any differences in non-specific binding of serum antibodies to the solid support since any such interactions result only in a darkening of the background colour which is readily distinguished from binding to the antigen spot.

The results of the test were in good agreement with those of the standard microtitre ELISA although sera with very low positive antibody levels (A_{492} $< 0{\cdot}20$) were not uniformly detected as positive in the spot test. This observation suggests that the spot test is less sensitive than the microtitre ELISA. Large scale screening of household contacts of leprosy patients in Mexico and in Sri Lanka suggests that these low positive antibody levels are common amongst individuals exposed to *M. leprae* but having no evidence of clinical disease.[6] The spot test may therefore be less useful in detection of exposure to *M. leprae* but will be useful for identification of individuals with higher antibody levels associated with the development of the lepromatous form of leprosy.

It is not envisaged that the spot test will replace standard microtitre ELISA tests for antibodies to phenolic glycolipid. These tests have clear advantages from the point of view of conducting large scale epidemiology studies of leprosy exposure and infection. In small scale field studies, however, the simpler and more flexible spot test may provide a useful alternative for workers who do not have appropriate facilities for conducting the microtitre tests.

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The action of deoxyfructose serotonin on intracellular bacilli and on host response in leprosy

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Summary The drug deoxyfructose serotonin has already shown anti-leprosy activity according to *in vitro* tests on bacilliary suspensions, in the mouse footpad and in a pilot clinical trial. In a group of further tests in our Institute, activity against intracellular bacilli has been obtained both in macrophages from LL patients and bacilli within Schwann cells in organized nerve culture. *In vitro* tests also show enhanced lymphocyte macrophage interaction promoted by the drug with infected macrophages suggesting a possible enhancement of cellular immune response. Some protection *in vivo* against sciatic nerve damage by *Mycobacterium leprae* has also been shown in mice.

Introduction

Deoxyfructose serotonin, a derivate of serotonin, was first shown to be active against *Mycobacterium leprae* using a rapid *in vitro* test for drug response.[1] We used the technique in which bacilli were incubated for 9 days in the presence and absence of the drug and subsequently tested for metabolic viability by pulse labelling with ^{3}H DOPA.[2] Positive results were also obtained in the mouse footpad.[3, 4] Rees has shown that 20 mg/kg body weight and 2 mg/kg by continuous oral administration completely inhibits multiplication of *M. leprae* in the mouse footpad. 0·2 mg/kg is without effect. He has found that the minimum inhibitory dose is 2 mg/kg both for DDS sensitive and DDS resistant strains of *M. leprae*; some toxicity tests indicated extremely low toxicity. General de Medicine Saint André therefore treated 7 patients in Bamako, 4 of these were polar LL cases.[5] A good clinical response was obtained. This was confirmed independently

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by two leading leprologists who visited Bamako during the trial. Extensive chronic toxicity tests have now been carried out according to the internationally accepted protocol. Absence of toxic and side effects have been shown up to 200 × the clinical dose. Further clinical trials are planned.

In view of the possible clinical importance of this drug, some further studies have been carried out at the foundation for Medical Research. A number of techniques are in daily use for basic studies in leprosy. We decided to submit the drug deoxyfructose serotonin to some of these routine tests in the hope that the results might give some leads for the further work in the development of drugs related to molecules found in nerve tissue. In view of the well-known affinity of *M. leprae* for nerve tissue, a unique characteristic amongst the mycobacteria, such drugs may eventually play an important role in leprosy treatment.

Materials and methods

MACROPHAGE CULTURES

Macrophages obtained from peripheral blood of lepromatous leprosy patients were cultured in Leighton tubes according to the method of Birdi *et al.*[6] and Salgame *et al.*[7] The macrophages were incubated for 7 days in Eagle's minimum essential medium containing 40% human AB serum, the medium being changed every 48 hr. The high proportion of macrophages was identified by non-specific esterase staining.

Leprosy bacilli were obtained from LL patients' skin biopsies by the method of Ambrose *et al.*[1] 5×10^6 Bacilli were added per live culture for 24 hr and ingested bacilli then removed by washing. A suspension of 1% sheep erythrocytes, sensitized with goat anti-sheep erythrocyte antibody, was added for 30 minutes at 37°C under 5% CO_2. Cultures were washed, fixed in glutaraldehyde and stained with Fite-Neelsen acid fast staining to identify *M. leprae*. Control cultures without *M. leprae* were tested by rosetting. Uninfected cultures were also treated with 15 μg per culture of deoxyfructose serotonin for 24 hr and then tested by rosetting.

Another group was incubated for 24 hr with the drug, infected with *M. leprae* for a further 24 hr, washed with drug containing medium and incubated for a further 24 hr. A further set of infected cultures was maintained for 7 days before rosetting. The infected cultures treated with drug were washed 24 hr after infection and the drug removed before further incubation.

LYMPHOCYTE–MACROPHAGE INTERACTION

Peripheral blood was obtained from lepromatous leprosy patients. The leuco-

cytes were separated and passed through a Ficoll-Triosil gradient to obtain peripheral blood monocytes.

$5–10 \times 10^6$ Peripheral blood monocytes were placed in 1 ml volume in Leighton tubes with coverslips. Live *M. leprae* was added as an antigen at a dose of 5×10^6 bacilli per tube. Macrophages adhered to the coverslip and the tubes were incubated for 16–18 hr at 37°C in 5% CO_2.

Cultures were terminated and fixed in glutaraldehyde. They were stained; 100 adhering macrophages were counted on the coverslip and those showing 20 or more lymphocytes attached were recorded.

Control cultures and cultures treated with 15 μg/ml of deoxyfructose serotonin were compared.

SCHWANN CELL CULTURES

(a) Culture methods

Sensory ganglia of 1- to 3-day-old Swiss white mice were removed under sterile conditions. The ganglia were treated with 0·25% trypsin in calcium and magnesium free phosphate buffered saline. Adult rat tail collagen in 1% acetic acid was dialysed against distilled water. A few drops were used to coat coverslips and polymerized in ammonia vapour for 5 min. The rinsed ganglia were placed on the collagen coated coverslips; they were incubated in a small volume of 70% Eagle's minimum essential medium containing 0·6% glucose, 20% foetal calf serum, 10% embryo extract and 100 IU/ml of penicillin.[8]

Schwann cells could be identified by several methods. They were closely associated with nerve fibres in the collagen matrix. Neurofibroblasts grew predominantly on the glass below. When unattached to nerve fibres, Schwann cells became, after a short time, bipolar with extremely long and thin pseudopodia, whether in high or low density cultures. Neurofibroblasts exhibited one or more fan-like leading pseudopodia in low density culture. They only became bipolar due to contact inhibition at high density.

Schwann cells when not attached to nerve fibres divided slowly reaching a peak only after 2 weeks in culture. Neurofibroblasts divided rapidly from the commencement of culture. Cytosine arabinoside and fluoro-deoxyuridine at 10^{-5} M concentration much reduced the number of neurofibroblasts.

When Schwann cells associated with nerve fibres, basement membrane could be demonstrated in the electron microscope. After the second week of culture myelin secretion by the Schwann cells could be demonstrated by Sudan black B staining.

(b) Labelling

2 μCi/ml of ^{3}H thymidine (specific activity 8·9 Ci/mM) and 2 μCi/ml of ^{3}H leucine

(specific activity 7·9 Ci/mM) were in each case added for 24 hr. Cultures were washed, fixed and coated with Ilford K5 nuclear emulsion, they were subsequently examined as autoradiographs both under high power transmission microscopy and with a polarizing vertical illuminator to identify the silver grains unambiguously.

(c) Infection with M. leprae

Suspensions of *M. leprae* prepared in the same way as for infection of macrophage cultures at $5–8 \times 10^7$ bacilli per culture were added to 1–2 week old Schwann cell cultures for 72 hr. Cultures were incubated for a total of 28 days. All cultures were fed on alternate days, being of course maintained at 100% humidity in 5% CO_2.

In the cultures both DNA and protein synthesis were estimated in separate cultures by thymidine and leucine labelling followed by autoradiography. This test was carried out during the proliferative phase of Schwann cell activity, prior to myelin secretion.

Uninfected cultures treated with 10 μg/ml of deoxyfructose serotonin were also subsequently pulse labelled both with thymidine and with leucine in separate experiments.

Cultures infected with *M. leprae* were set up in parallel sets.

One group after infection for 72 hr and washing was fixed and Fite–Neelson acid fast stained; the other set was maintained for a further 28 days and fixed.[9] A parallel group was treated with 10 μg/ml of deoxyfructose serotonin for 28 days then fixed and stained. A total of 10 sets of experiments with parallel control and drug treated cultures were carried out.

SCIATIC NERVE OF MICE

The technique previously described to study nerve damage in the radial cutaneous nerve of patients[11] was used for the mouse sciatic nerve investigations.

Ten Swiss mice were inoculated in the mouse footpad with *M. leprae* obtained from a skin biopsy of an LL patient. Footpad harvests were carried out at 6, 7, 8, 10, 11 and 12 months post inoculation.

A similar group of uninfected mice was fed with 20 mg/kg body weight daily of deoxyfructose serotonin by oral administration.

A third group of uninfected mice with *M. leprae* was similarly fed with 20 mg/kg body weight of the drug.

At harvest, paraffin sections of the sciatic nerve were prepared. Sections were stained with TRIFF and observed under high power in the light microscope. Ultra-thin sections were examined in the transmission electron microscope.

EXPERIMENTAL RESULTS

1. Macrophage cultures

(a) Rosetting. Results are shown in Figure 1 for 4 separate sets of experiments.

On the left of Figure 1(a) and (b) (M0) is shown the percentage of rosetting for macrophages of LL patients before treatment. After 24 hr infection with live *M. leprae* there is a marked fall (IM0).

Uninfected cultures treated for 24 hr with 15 μg per culture of deoxyfructose serotonin maintain *no alteration* in percentage rosetting. (Heat killed *M. leprae* also produced no fall in rosetting.) *M. leprae* infected cultures treated with 15 μg per culture of deoxyfructose serotonin show a return to the percentage level of rosetting for uninfected cultures, Figure 1(b) (IM0 + DFS 24 h).

Infected cultures maintained for a further 7 days and rosetted on the 7th day maintain a low level of rosetting, Figure 1(a) IM0 cultured 7 days.

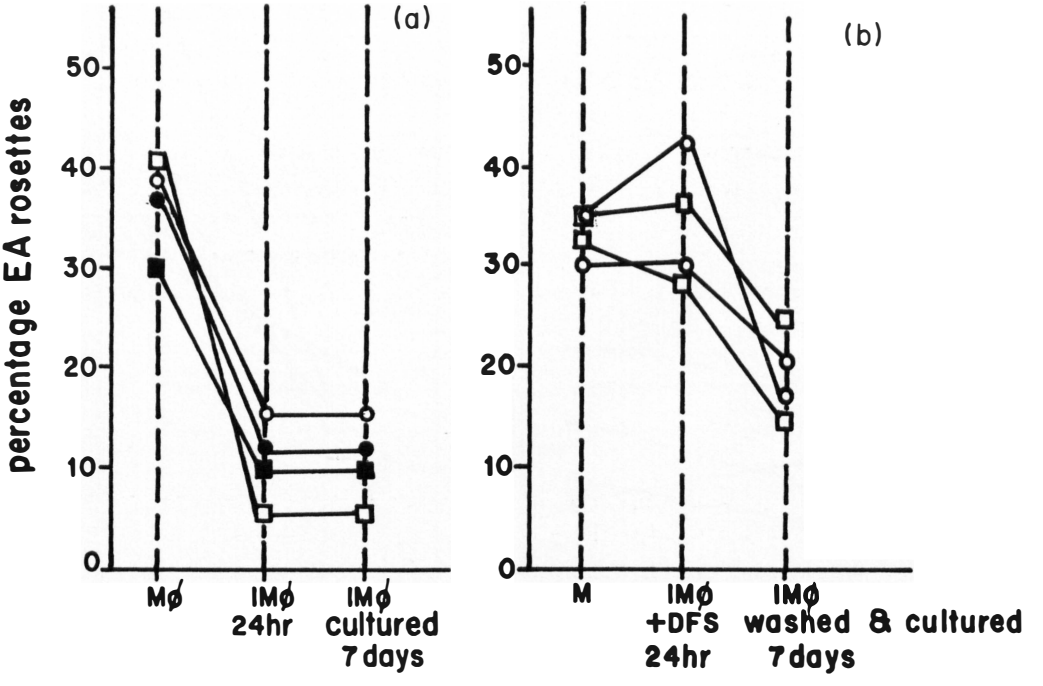

Figure 1. (a) Effect of infection with *M. leprae* on percentage of EA rosetting of macrophages. M0, level of rosetting before infection with *M. leprae*; IM0 24 hr, level of rosetting after infection of macrophages with *M. leprae*; IM0 7 day, level of rosetting after further culture for 7 days. (b) Effect of DFS on rosetting of macrophages infected with *M. leprae*. M0, level of rosetting of control macrophages treated with DFS (20 μg/ml); IM0 & DFS 24 hr, level of rosetting of macrophages infected with *M. leprae* and treated with DFS; IM0 washed 7 days, level of rosetting after washing to remove DFS and culture for a further 7 days.

With drug treated cultures washed after 24 hr drug treatment and maintained and rosetted after a further 7 days the percentage rosetting falls as shown in Figure 1(b) (M0 washed and cultured 7 days).

The experiments therefore show that drug treatment causes the live bacilli to behave like dead bacilli, although this condition is not maintained after washing away the drug.

(b) Lymphocyte-macrophage interaction. In the case of LL macrophages infected with *M. leprae*, the level of rosetting is extremely low, Figure 2. Both fresh and heat killed bacilli give about the same percentage rosetting. This is not appreciably changed by rifampicin treatment.

On the other hand treatment of infected cultures with levamisole leads to an enhanced lymphocyte-macrophage interaction. Treatment with 15 μg per culture of deoxyfructose serotonin has a similar effect.

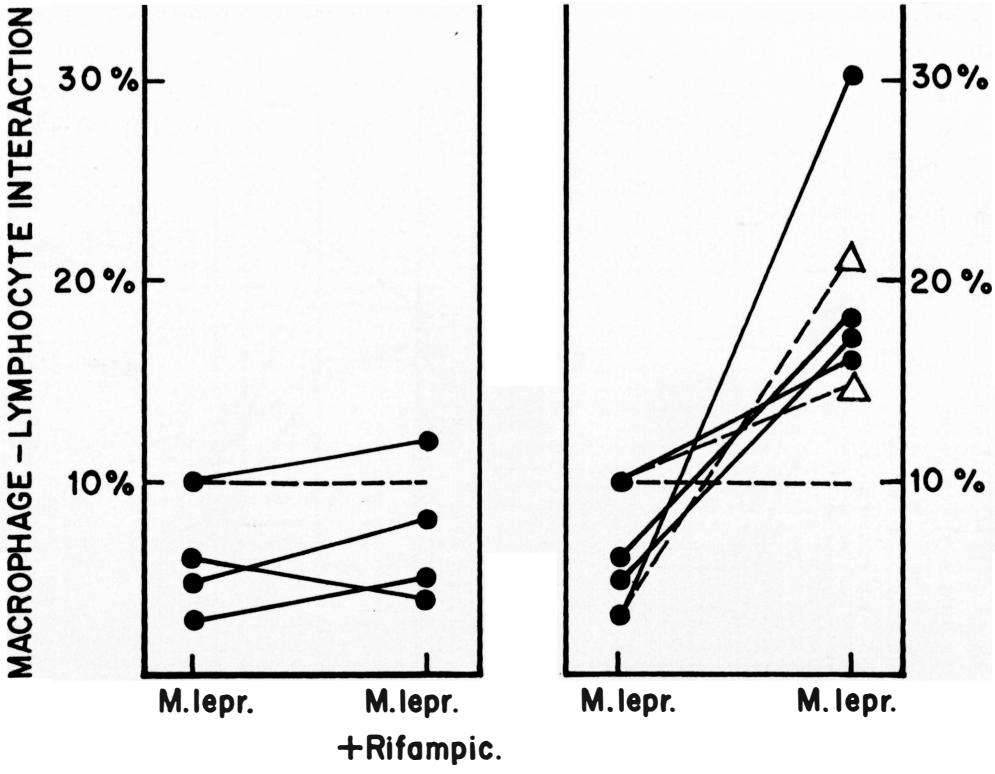

Figure 2. Interaction between lymphocytes sensitized to *M. leprae* antigen and autologous macrophages infected with *M. leprae*. (a) Ordinate indicates the percentage of macrophages showing adhering lymphocytes. *M. leprae,* percentage of interaction with macrophages infected with *M. leprae*; *M. leprae* and rifampicin, the same after treatment with 2 μg/ml rifampicin. (b) Effects of deoxyfructose serotonin and levamisole on interaction. *M. leprae*, percentage of interaction with macrophages with *M. leprae*; ●——, *M. leprae* infected + 15 μg/ml DFS; △– – –, *M. leprae* infected + levamisole.

2. Schwann cell cultures

In Figure 3 are shown histograms for counts before and after 28 days in culture of numbers of bacilli per Schwann cell in 1 out of 10 similar experiments. Only cells containing 1 or more bacilli were counted at day 0 because only such cells can exhibit multiplication of *M. leprae*. The histograms show cells grouped in the range 1–10, 10–20, etc., bacilli per cell. Counts for day 0 and day 28 are shown.

In Figure 4 are shown two similar sets of infected cultures incubated for 28 days and two sets of these cultures treated with 10 μg/ml of deoxyfructose serotonin throughout the 28 days of incubation. Set (a) and Set (b) are out of 10 of the separate experiments giving similar results. Set (a) and Set (b) were counted by different observers.

3. Mouse sciatic nerve

The results are shown in Table 1. DFS alone was without effect on mouse sciatic nerve histology, as compared with controls.

Considerable protection against nerve damage due to mouse footpad infection was shown by the mice fed 20 mg/kg body weight orally of deoxyfructose serotonin.

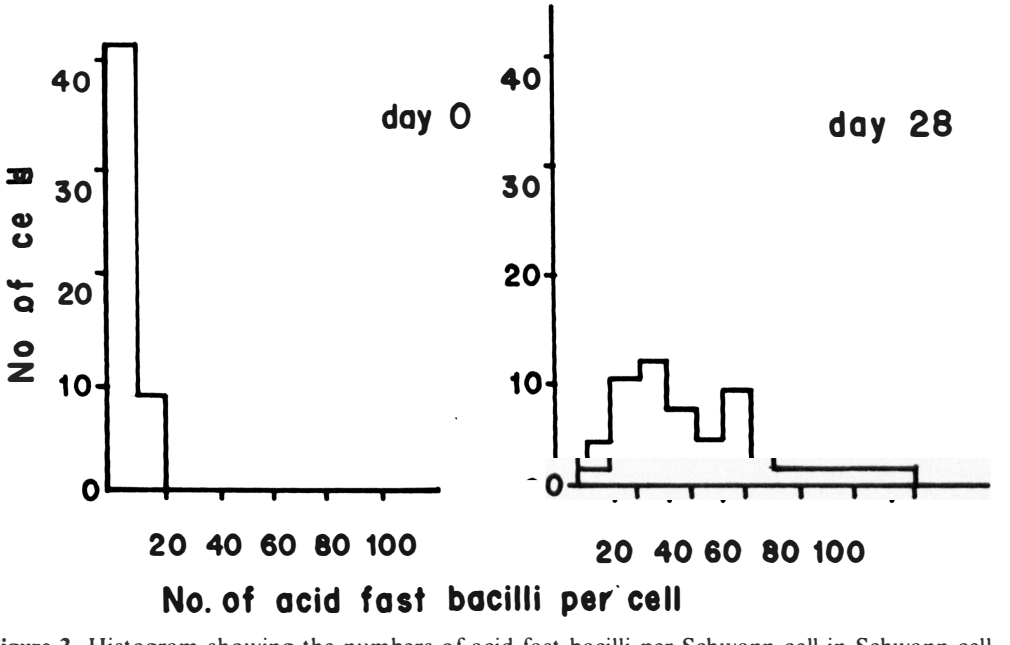

Figure 3. Histogram showing the numbers of acid fast bacilli per Schwann cell in Schwann cell culture infected with *M. leprae* and after further culture for 28 days. The number of bacilli per Schwann cell are grouped within the ranges 0–10, 10–20 etc. The ordinate shows the number of cells counted with bacilli numbers in each range.

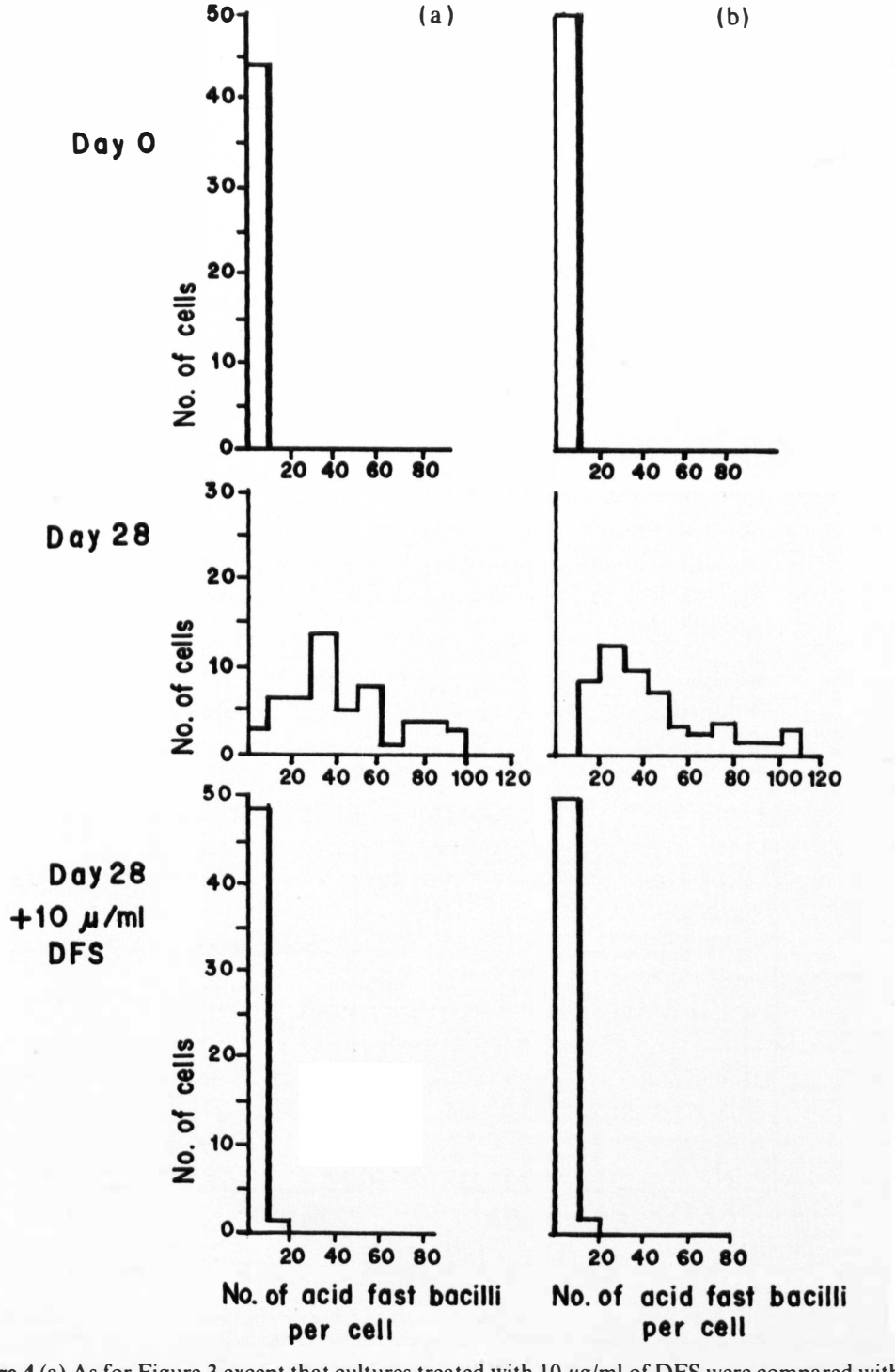

Figure 4 (a) As for Figure 3 except that cultures treated with 10 μg/ml of DFS were compared with control cultures at 28 days, showing complete arrest of proliferation of acid fast bacilli within Schwann cells at 28 days. (b) A similar experiment counted by a different observer. (A total of 10 reproduceable experiments were carried out.)

Table 1. Foot-pad counts/the sciatic nerve damage in DFS treated mice

Months —post	*M. leprae* only		*M. leprae* + DFS		DFS only
	AFB/FP	Nerve damage	AFB/FP	Nerve damage	Nerve damage
6th	2×10^4	—	—	—	Nil
7th	$7{\cdot}3 \times 10^4$	—	—	—	—
10th	$1{\cdot}53 \times 10^5$	Unmyelinated fibre changes (++) Demyelination (±)	$5{\cdot}33 \times 10^4$	Unmyelinated fibre changes (+) Demyelination (±)	Nil
11th	$5{\cdot}9 \times 10^5$	Not done	$2{\cdot}67 \times 10^4$	Not done	Nil
12th	$2{\cdot}35 \times 10^6$	Unmyelinated fibre changes (+++) Demyelination (+)	$2{\cdot}57 \times 10^4$	Unmyelinated fibre (+) Demyelination	Nil Nil

Discussion

The results obtained both with rosetting of macrophages and culture within Schwann cells indicate that deoxyfructose serotonin has an effect on intracellular bacilli. In the case of the macrophage cultures, 24 hr treatment brings the rosetting level up to the value for heat killed bacilli. The fall which follows after washing may be due to the drug having a bacteriostatic action, but 24 hr is a short period of exposure and long term cytotoxicity cannot be excluded. Lymphocyte-macrophage interaction, as described in these experiments is expected to arise from the presentation of leprosy antigen at the macrophage surface. The result showing a similar type of enhancement as that obtained with levamisole suggests that deoxyfructose serotonin also enhances the capacity of the macrophage to process leprosy antigen and liberate it at the macrophage surface, i.e. it possibly enhances the cellular immune response.

There is complete inhibition of replication of *M. leprae* within the cytoplasm of the Schwann cells by 10 μg/ml of deoxyfructose serotonin, whereas the same concentration is without effect either on DNA synthesis or protein synthesis by Schwann cells in the proliferative phase. The fact that the bacilli do not disappear from Schwann cells after 28 days exposure to drugs, although proliferation is arrested, may also be due to a bacteriostatic effect. But Schwann cells do not have a normal phagocytic function unlike macrophages. They may not possess the apparatus to rid themselves completely of moribund bacilli. The protective action of the drug against damage to the sciatic nerve in mice infected with *M. leprae* is also encouraging.

It has already been shown that the suppression of DNA synthesis in proliferative Schwann cells by live *M. leprae* is not shown by heat killed *M. leprae* or by fresh related mycobacteria.[10] These include ICRC-C44, and a group of cultivable acid fast strains provided by Professor L. Kato. This again brings home

the importance of looking for specific features of *M. leprae* which lead to nerve damage. So far drug design for leprosy, in the absence of a suitable culture technique has been based on drug series already effective against other mycobacteria. With deoxyfructose serotonin we have a drug which is specific in its action against *M. leprae*, being inactive against other mycobacteria tested. Specificity of uptake by *M. leprae* is also shown with ^{3}H DOPA.[12] In the search for new and more effective drugs for leprosy treatment, it should surely be in the direction of compounds related to nerve tissue products and metabolites that efforts need to be directed.

Acknowledgments

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Mycobacterium vaccae and immune responses: implications for leprosy control

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Summary *Mycobacterium vaccae*, common in some tropical environments, may have a beneficial effect on the incidence of leprosy by acting as a natural vaccine, or have a real or apparent harmful effect by interfering with the protection afforded by BCG vaccination. We are using an animal model to assess these possibilities. The results reported here show that a strain of *M. vaccae* isolated from Ugandan mud can evoke a significant immune response in mice sensitized subcutaneously or orally. Spleen cells from such mice responded equally well *in vitro* to *M. vaccae* and BCG as measured by two independent assays. Good responses were observed for at least 3 months after oral exposure to *M. vaccae* even though no viable organisms could be detected in the organs at this time showing that persistence of *M. vaccae* is not necessary for expression of sensitization. These experiments support the idea that people who become sensitized to *M. vaccae* or certain other environmental mycobacteria might be expected to show some resistance to leprosy. However, BCG vaccination might appear ineffective in that many individuals would already be sensitized to antigens common to all mycobacteria.

Introduction

The presence of *Mycobacterium vaccae*, a non-pathogenic organism common in certain environments especially those in tropical countries, may have important implications for the incidence of leprosy and its control. The suggestion has been made that *M. vaccae* may act as a natural immunogen.[1] The evidence in support of this view relies largely on skin test positivity in certain communities towards an extract prepared by sonication of *M. vaccae* (vaccin) and the incidence of leprosy in these communities.[1] Further, the pattern of these delayed skin responses to vaccin is similar to that to extracts of *M. leprae* (leprosin A) in that the responses of contacts of leprosy patients to leprosin A often correlate closely with their

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responsiveness to vaccin[2],[3] and in leprosy patients themselves anergy to leprosin A often correlates with anergy to vaccin.[4]

It has been suggested[1] that superimposing BCG vaccination in Uganda upon a presumed natural vaccination resulting from the exposure of the population to *M. vaccae* or other fast-growing mycobacteria in the environment provided an additive protective effect and was responsible for the 80% efficacy of the BCG vaccination trial there against leprosy.[5] In areas of Burma, however, where a BCG vaccination trial was only 20% effective,[6] the synergistic effect of such organisms was postulated to have been negated as a result of exposure to another environmental mycobacterium, *M. scrofulaceum*.[1] The possibility also exists that the presence of mycobacteria such as *M. vaccae* in leprosy endemic areas where BCG vaccination has appeared to fail may have affected the results because BCG vaccination provides no additional benefit above that provided by the 'natural' immunogen. Such an effect was shown to occur when mice or guinea-pigs were exposed to certain atypical mycobacteria (such as *M. avium*), as well as being vaccinated with BCG, in experimental tuberculosis.[7,8] An alternative possibility (as yet uninvestigated) is that *M. vaccae* might interfere with the host's natural immunity to leprosy and/or responses to the BCG vaccine.

To date, animal studies have generally argued against a significant role for *M. vaccae* as they have suggested that the species is poorly immunogenic[9,10] with a very limited ability to protect mice from a challenge with viable *M. leprae*.[11] However, these studies used a limited representation of the strains of *M. vaccae* and strain differences within a species of mycobacteria have been shown to produce very different immune responses in the murine host.[12] We report here some studies on the sensitization of mice with a strain of *M. vaccae* isolated directly from the mud of the BCG vaccination trial area in Uganda.

Materials and methods

MICE

Eight- to 12-week-old female CBA/Ca mice bred at St Mary's Hospital Medical School were used. Animals to be injected subcutaneously (s.c.) with the bacilli were conventionally fed and housed and given tap water to drink *ad libitum*. For these experiments no measures were taken to reduce their exposure to other mycobacteria. Animals to be fed with the bacilli were suckled conventionally but placed into 80-mice sterile isolator units (Olac 76, Bicester, Oxon) and offered sterile food and distilled water *ad libitum*, immediately on weaning. The animals were maintained in this environment throughout the experimental period to inhibit their exposure to other mycobacteria.

MYCOBACTERIA

Mycobacterium vaccae (strain R877R)

We used a stable rough variant of a strain isolated from Ugandan mud by Dr J Stanford, Middlesex Hospital Medical School, London. Bacilli were grown on Sauton agar at 37°C for 2–4 weeks. Suspensions were prepared in sterile water and the total concentration of organisms was estimated by nephelometry. The viability of the suspensions determined retrospectively by colony counts on Sauton agar was $< 5\%$.

Mycobacterium bovis, BCG

For injection, 50-dose ampoules of lyophilized BCG (batch C390; Glaxo Laboratories Ltd, Greenford, Middlesex) containing $1{\cdot}4 \times 10^8$ total (7×10^7 viable) organisms were reconstituted with 0·45 ml sterile water.

For use in *in vitro* assays, BCG was grown in bulk in glycerol-free medium (Glaxo Laboratories Ltd). The organisms were harvested and total numbers determined as for *M. vaccae*. Suspensions of both organisms were heat-killed by incubation at 70°C for 1 h.[13] The dead organisms were washed in tissue culture medium and resuspended to the desired concentration before use.

ADMINISTRATION OF MYCOBACTERIA TO THE MICE

Subcutaneous route

Mice were injected s.c. in the right rump with 0·2 ml mycobacterial suspension. Each mouse received a total of 10^8 *M. vaccae* (2×10^6 viable) or 6×10^7 BCG (3×10^7 viable).

Oral route

To investigate the effect of feeding the mice with *M. vaccae*, 8-week-old mice in isolator units were given sterile distilled water deliberately contaminated with *M. vaccae*. The mice were fed for 3 continuous weeks, the water replaced with a freshly contaminated preparation each week. The average concentration of *M. vaccae* in the water over the 3 weeks was 10^8 total (2×10^6 viable) organisms per ml water. Age- and sex-matched mice maintained in a separate isolator unit under identical conditions but without administration of the mycobacteria were used as controls.

DETECTION OF VIABLE MYCOBACTERIA IN MOUSE TISSUES

Samples of spleen, liver and lung were homogenized in 5 ml of distilled water (2

min, Colworth Stomacher 80). Duplicate samples (20 μl) of homogenate were spread onto Middlebrook 7H10 agar plates and after incubation at 37°C for 1–2 weeks the plates were examined for the presence of *M. vaccae* colonies.

TISSUE CULTURE MEDIUM

RPMI 1640 supplemented with 0·024M-sodium bicarbonate, 0·0147M-HEPES, 10 units/ml gentamicin, and foetal calf serum (FCS; Flow Laboratories, Irvine, Scotland) at 1 or 5% according to the assay, was used throughout.

SPLEEN CELL SUSPENSIONS

Three mice from each experimental group were killed by cervical dislocation and their spleens removed aseptically. The spleens were teased into tissue culture medium and a pooled single cell suspension prepared. The viability of the suspensions was usually about 80% as determined by trypan blue exclusion.

IN VITRO PROLIFERATION ASSAY

The purpose of this assay was to evaluate (^{3}H)-thymidine incorporation (lymphocyte proliferation) stimulated by a mycobacterial challenge *in vitro*. The assays were performed in 96-well, round-bottomed assay plates (Sterilin Ltd, Middlesex, England) with at least 3 wells for each experimental condition. Spleen cells (either 2×10^6 or 5×10^6 nucleated cells/ml) were cultured with heat-killed mycobacteria (10^4–10^8/ml) in a total volume of 100 μl/well. The medium was supplemented with 1% FCS. The assay plates were incubated at 37°C for 4 or 5 days in a humidified 5% CO_2-in-air atmosphere. For the last 16 h of culture 2 μl medium containing 1 μCi of (^{3}H)-thymidine (Amersham International, Amersham, England) was added. (The labelled thymidine had been previously diluted with non-radioactive thymidine to the very low specific activity of 50 mCi/mmol.) Cultures were harvested using an automatic cell harvester (Skatron, Norway) and the amount of incorporated ^{3}H determined with a Packard 'Tricarb' liquid scintillation counter (Packard Instruments Ltd, Berks., England). The background (^{3}H)-thymidine incorporation in the absence of heat-killed mycobacteria by spleen cells from immunized animals was usually greater than that from unimmunized animals (see Table 1). The data are expressed therefore in the figures as the difference between the arithmetic mean counts per minute (cpm) in the replicate wells containing spleen cells plus heat-killed organisms and similar wells without organisms (Δ cpm).

IN VITRO PRIMARY PLAQUE-FORMING CELL ASSAY

As a further measure of the immunological status of the spleen cells from the

Table 1. *In vitro* proliferative response to heat-killed *M. vaccae* of spleen cells from animals injected subcutaneously with *M. vaccae*

	Cpm of (^{3}H)-thymidine[a] incorporated into spleen cells[b] cultured with heat-killed *M. vaccae* (organisms/ml)[c]			
Spleen cells from	0	10^6	10^7	10^8
M. vaccae injected mice[d]	247 ± 15	666 ± 203 (419)[e]	730 ± 63 (483)	700 ± 106 (453)
Control mice	152 ± 15	172 ± 18 (20)	255 ± 66 (103)	242 ± 71 (90)

[a] mean ± standard deviation of replicate assays.
[b] 2×10^6 cells/ml.
[c] 5-day culture.
[d] 2×10^6 viable, 10^8 total *M. vaccae* injected subcutaneously 42 days previously.
[e] Δ cpm. Underlined values are significantly enhanced ($P < 0{\cdot}05$ or better).

immunized mice, their ability to generate primary (IgM) haemolytic plaques (1°-PFC) *in vitro* in the presence of killed mycobacteria was assessed using sheep erythrocytes (SE) as antigen. The assay was performed in Linbro 24-well, flat-bottomed assay plates (Flow Laboratories, Irvine, Scotland). 10^7 nucleated spleen cells were cultured with 4×10^5 prewashed SE (Tissue Culture Services, Berks., England) and 10^6 to 10^8 heat-killed mycobacteria in a total volume of 2 ml. The medium was supplemented with 5% FCS. The assay plates were incubated for 5 days at 37°C in 5% CO_2-in-air after which time the number of 1°-PFCs per culture (10^7 spleen cells) was determined by the method of Cunningham & Szenberg.[14] The results in the figures represent the arithmetic mean from at least 3 replicate wells.

STATISTICAL ANALYSIS

A two-tailed Student's *t* test was used to compare (a) the responses of spleen cells from the immunized animals cultured with and without heat-killed organisms, and (b) the responses of the spleen cells from immunized and unimmunized animals in the presence of the same concentration of heat-killed organisms. Our criterion for a statistically significant enhanced response due to the *in vitro* challenge was that a significant difference should be found in both comparisons. Significant responses are indicated in the table and figures ($P < 0{\cdot}05$ or better).

Results

1 SUBCUTANEOUSLY ADMINISTERED MYCOBACTERIA

Persistence of M. vaccae *in mouse tissue after injection*

No viable *M. vaccae* could be detected in the granuloma, spleen, liver or lung 35 days after the s.c. injection.

In vitro *proliferative responses of spleen cells from mice injected s.c. with* M. vaccae *or BCG*

The optimal conditions for this assay varied with factors such as the batch of CBA mice used, the time after injection and the degree of responsiveness of cells from uninjected, control mice to mycobacterial antigens (see Table 1). For this reason experiments were set up with multiple assay conditions and the results for the 'immune' spleen cells were compared with those of the control cells determined under the same assay conditions. The exact conditions used are defined in the figure legends.

Responses to the injected organism. Spleen cells from mice injected s.c. 42 days previously with *M. vaccae* incorporated more (^{3}H)-thymidine in the presence of heat-killed *M. vaccae* (HKMV) than in its absence and also when compared with

Table 2. *In vitro* proliferative response of spleen cells from mice fed *M. vaccae* in their drinking water

Spleen cells from	Cpm of (^{3}H)-thymidine[a] incorporated into spleen cells[b] cultured[c] with (organisms/ml)[c]:					
	Heat-killed *M. vaccae*			Heat-killed BCG		
	0	10^6	10^8	0	10^7	10^8
M. vaccae fed mice[d]	457 ± 111	1208 ± 172 (751)[e]	606 ± 139 (149)	364 ± 115	845 ± 258 (481)	442 ± 85 (78)
Control mice	340 ± 56	379 ± 117 (39)	724 ± 47 (384)	525 ± 227	415 ± 98 (−110)	301 ± 108 (−224)

[a] Mean ± standard deviation of triplicate assays.
[b] 5×10^6 cells/ml.
[c] 5-day culture.
[d] Mice given water contaminated with 2×10^6 viable (10^8 total) *M. vaccae*/ml per week for 3 continuous weeks, finishing 104 days previously.
[e] Δ cpm. Underlined values are significantly enhanced ($P < 0{\cdot}05$ or better).

cells from unimmunized control animals (Table 1). This enhanced proliferative response was also evident when the data were expressed as Δ cpm (Table 1). This result shows that the response was due to the *in vitro* challenge and dependent upon the prior sensitization of the cells *in vivo*, thereby indicating the presence of memory lymphocytes. The enhanced response was significant at concentrations of HKMV between 10^6 and 10^8 per ml. Similar responses could be detected with spleen cells taken from immunized mice at all times tested between 21 and 70 days after the s.c. injection although the responses were greatest between days 40 and 60. In some experiments a decline in the response to the highest concentration of HKMV was marked (data not shown). For comparative purposes the data obtained when spleen cells taken 42 days after s.c. BCG were challenged *in vitro* with various concentrations of heat-killed BCG (HKBCG) are shown (Figure 1). The responses are comparable although the decline in responsiveness to the higher concentrations of HKBCG is more dramatic in this particular experiment.

Cross-reacting responses. Mycobacterium vaccae and BCG have one group of serologically detectable antigens in common.[15] We therefore investigated how

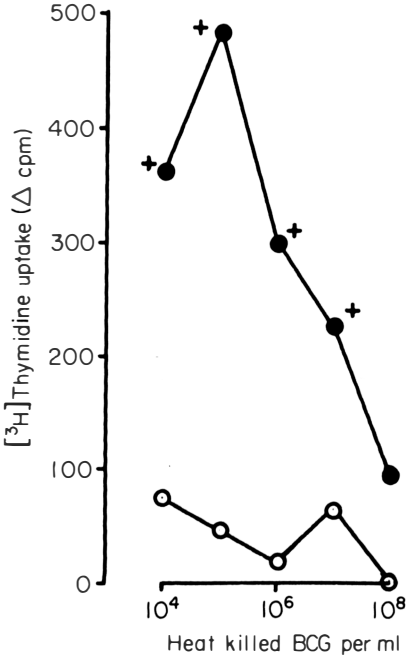

Figure 1. *In vitro* proliferative response of spleen cells to the mycobacterial species used for *in vivo* priming. Three CBA mice per group were each immunized with a single subcutaneous injection of 3×10^7 viable (6×10^7 total) BCG. Forty-two days later replicate cultures containing 2×10^5 pooled spleen cells and heat-killed BCG were set up. Culture duration was 4 days. Closed circles denote responses of spleen cells from immunized animals; open circles denote responses of uninjected control animals; + denotes statistically significantly enhanced responses.

much cross-reactivity could be detected by the sensitized splenic lymphocytes. Substantial cross-reactivity could be detected when either *M. vaccae*-sensitized spleen cells were challenged *in vitro* with HKBCG (Figure 2a) or BCG-sensitized spleen cells were challenged with HKMV (Figure 2b). Figure 2 shows the *in vitro* proliferative responses 21 days after the s.c. injection; the cross-reactivity could be detected at all time points tested between days 21 and 70 (data not shown). As with the proliferative responses to the sensitizing organism the degree of enhancement was reduced with the highest concentrations of heat-killed organism used.

Primary splenic plaque-forming cell responses in vitro

Earlier experiments have shown that the 1°-PFC response of spleen cells to SE *in vitro* is enhanced in the presence of added HKBCG when the mice have been

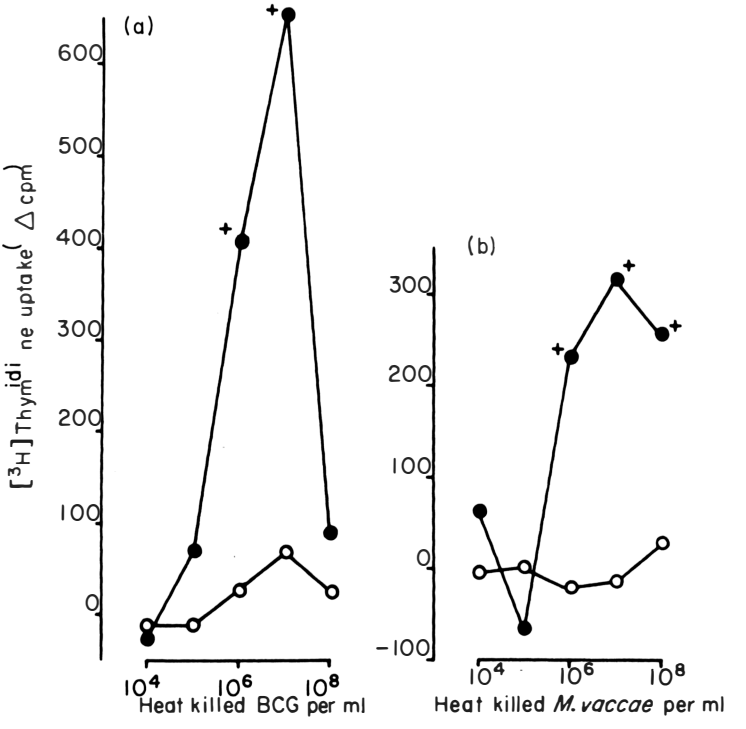

Figure 2. *In vitro* proliferative cross-reacting response of spleen cells to the alternative mycobacterial species. Three CBA mice per group were each immunized with a single subcutaneous injection of (a) 2×10^6 viable (10^8 total) *M. vaccae* or (b) 3×10^7 viable (6×10^7 total) BCG. Twenty-one days later replicate cultures containing 2×10^5 pooled spleen cells and either (a) heat-killed BCG or (b) heat-killed *M. vaccae* were set up. Culture duration was (a) 5 days and (b) 4 days. Closed circles denote responses of spleen cells from immunized animals; open circles denote responses of cells from uninjected control animals; + denotes statistically significantly enhanced responses.

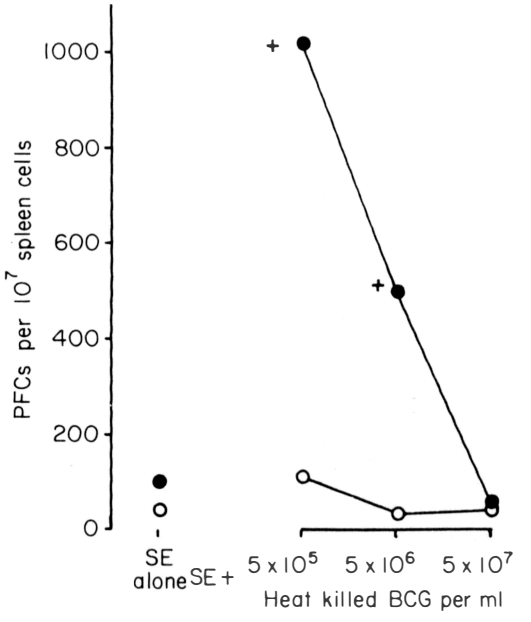

Figure 3. Enhancement of the number of primary plaque-forming cells (1°-PFCs) to sheep erythrocytes (SE) in *M. vaccae*-sensitized spleen cell suspensions challenged *in vitro* with HKBCG. Three CBA mice per group were each immunized with a single subcutaneous injection of 2×10^6 viable (10^8 total) *M. vaccae*. Forty-two days later replicate cultures containing 1×10^7 pooled spleen cells were incubated with 4×10^5 SE, alone or with heat-killed BCG for 5 days. Values shown are the mean numbers of 1°-PFCs per 10^7 spleen cells taken from immunized animals (closed circles) or uninjected control animals (open circles). + denotes statistically significantly enhanced responses.

previously injected with BCG intravenously[13] or s.c. (Swinburne, Brown and Brown; manuscript in preparation). In this study we investigated whether such an enhancement occurred after s.c. injection of *M. vaccae*. When cultured *in vitro* with HKBCG, spleen cells from mice injected s.c. 42 days previously with *M. vaccae* gave markedly enhanced 1°-PFC responses when compared with cells from uninjected control animals (Figure 3). The enhancement was greatest with the lowest concentration of HKBCG used (5×10^5/ml) and declined as the concentration was increased to 5×10^7/ml. As with the proliferative responses this enhancement was dependent upon the prior sensitization of the spleen cells *in vivo* and the presence of HKBCG *in vitro* demonstrating an anamnestic response to common antigens. Similar responses were detected if HKMV was used *in vitro* and responses to both species could be detected throughout the experimental period (21–70 days after injection—data not shown).

2 ORALLY ADMINISTERED MYCOBACTERIA

Persistence of M. vaccae *in mouse tissues after feeding*

When the spleen, liver and lungs of the mice fed with *M. vaccae* were examined

104 days after the feeding was stopped no viable *M. vaccae* were detected in any of the tissues.

In vitro *proliferative responses of spleen cells from mice fed* M. vaccae

Spleen cells from mice fed *M. vaccae* 104 days previously and incubated with 10^6 HKMV/ml gave significantly enhanced proliferative responses both above background (no HKMV) and compared with cells from control mice incubated with 10^6 HKMV/ml (Table 2). A similar response was elicited using 10^7 HKBCG/ml. Cells from the control mice gave a significantly enhanced response to 10^8 HKMV/ml compared to background (^{3}H)-thymidine incorporation (no HKMV). This was presumably a result of exposure of the mice to mycobacterial antigens before their isolation and has been a common observation with cells from our experimental animals. The response to HKMV by such cells is invariably higher than that to HKBCG: Table 2 shows the same cell suspension gave no response to HKBCG.

Discussion

These experiments show that this strain of *M. vaccae* isolated from a BCG vaccination trial area (in Uganda) can generate an immune response in mice comparable to that of Glaxo BCG, after either subcutaneous injection or oral administration of the bacilli, as detected by the presence of memory lymphocytes in the spleen. The anamnestic response to the subcutaneous injection was detected using two separate assays, both of which involve macrophage–T lymphocyte interactions with the mycobacterial antigens. The overall magnitude of the response was influenced by the quantity of heat-killed organisms (antigen) used *in vitro*. Similar results were obtained when the mice were given *M. vaccae* orally although only the proliferation assay was used in these experiments. It should be emphasized that the low levels of (^{3}H)-thymidine incorporated by the proliferating spleen cells reflect the low specific activity of (^{3}H)-thymidine label used and not a poor proliferative response. This low specific activity was chosen to minimize possible fluctuations in (^{3}H)-thymidine incorporation due to the well-documented synthesis and release of non-radioactive thymidine by the macrophages in the cultures.[16]

Other workers using a different strain of *M. vaccae* have reported that the *M. vaccae* sensitizes guinea-pigs and mice only poorly as measured by a delayed footpad swelling response to cytoplasmic extracts of *M. vaccae* or whole bacilli.[10,17] Good responses were obtained only if the *M. vaccae* was incorporated in Freund's adjuvant.[17] Interestingly, although Shepard, van Landingham & Walker[11] were unable to detect a delayed-type hypersensitivity (DTH) response in mice sensitized with *M. vaccae* and challenged with a soluble extract of *M. leprae*,

these mice did show both a swelling of the lymph node draining the sensitization site and also a limited protective immunity against viable *M. leprae*. Their findings are consistent with the observations of others that DTH responses are dissociable from protective immunity.[18,19] Using the same strain of *M. vaccae* as used here, Rook & Stanford[20] detected good footpad swelling responses to vaccin in BALB/c mice sensitized s.c. with live *M. vaccae*. This difference in results reported by the various groups may reflect strain differences between the *M. vaccae* isolates which were demonstrated to exist by Watson *et al.*[17]

In immunoprecipitation tests using rabbit antisera and sonicates of bacilli, *M. vaccae* and BCG appear to share only the common (group 1) mycobacterial antigens.[15] Cross-reactions detected in our study may therefore be due to antigens common to all mycobacteria. (The medium on which the mycobacteria were grown is non-antigenic.) The cross-reactivity is particularly relevant as evidence is now accumulating that certain epitopes on the common mycobacterial antigens are responsible for evoking a protective immune response in the host.[3, 15, 21]

M. vaccae has been considered as a potential candidate for a leprosy vaccine.[9, 11, 17, 22] However, in animal models, *M. vaccae* has ranked a poor third behind *M. leprae* itself and BCG.[11] This ranking may be due to selection of an inappropriate strain of *M. vaccae*. Further, the incentive to pursue studies on *M. vaccae* has been reduced by the observation that other species of mycobacteria need to persist in the tissues to induce protection against pathogenic mycobacteria in mice.[12] It has subsequently been assumed, but not proven, that the same requirement would be necessary for *M. vaccae*, a species reported to persist very poorly.[10] In our experiments the *M. vaccae* was observed not to persist *in vivo* although the presence of memory lymphocytes in the spleen was detected 104 days after feeding with *M. vaccae* had stopped. We have also detected memory lymphocytes at earlier times when no *M. vaccae* was detected in the tissues (data not shown). This indicated that persistence of viable *M. vaccae* was not a requirement for the development or persistence of such memory lymphocytes. Whether or not these are T lymphocytes that also recognize *M. leprae* antigens is currently being investigated.

The observation that *M. vaccae* can sensitize mice as effectively as Glaxo BCG supports the view that the apparent failure of BCG vaccination against leprosy in certain areas may be because the population is already sensitized. The relationship of those responses to protective immunity is currently being studied.

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Lepr Rev (1985) **56**, 221–227

Serum demyelinating factors and adjuvant-like activity of *Mycobacterium leprae:* possible causes of early nerve damage in leprosy

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Summary The role of antibody was investigated by (a) subcutaneous injection using whole serum obtained from 7 leprosy patients, and (b) intraneural injection using immunoglobulins from 8 randomly chosen leprosy patients in random bred Swiss white mice. Three out of 15 samples showed positive demyelination.

The adjuvant like activity of *Mycobacterium leprae* was studied for its role in the causation of primary nerve damage of Swiss white mice. Animals were injected subcutaneously with 20×10^6 live and heat killed *M. leprae* with and without normal sciatic nerve extract. Biopsies of the sciatic nerves were performed 1 and 3 months after injection. Degenerative changes in the non-myelinated fibres were observed at the first month in the sciatic nerves of mice injected with live and heat-killed *M. leprae* along with nerve extract.

Introduction

Our previous studies on early leprosy cases, contacts of leprosy patients and sciatic nerves of foot-pad inoculated mice revealed a characteristic sequence of nerve damage which was typical of leprosy. Such pathological changes were observed in nerves remote from the site of infection even in the absence of acid fast bacilli or inflammatory cells.[1–3].

This raised the possibility of the presence of circulating factors directed against nerve components. Such a hypothesis was consistent with the fact that Mycobacteria exerting adjuvant like action gave rise to auto-antibodies.[4]

Our objectives in Part I of this study were therefore to detect serum demyelinating factors, if any, in leprosy patients. Part II of this study consisted of assessing changes in mouse sciatic nerve that could be brought about by a subcutaneous injection of normal mouse sciatic nerve extract and *M. leprae.*

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Materials and methods

PART I SERUM DEMYELINATION STUDIES

(a) Subcutaneous injection using whole serum

Sera from 7 randomly chosen leprosy patients (4BT, 3BL) were injected subcutaneously into the right and left flanks above the thigh region of normal, random bred Swiss white mice (6 weeks old) in a total dosage of 0·5 ml. Each sample was injected into 2 mice. Biopsy of both the sciatic nerves was performed 1 month after the injection.

(b) Intraneural injection using immunoglobulin

In another series of experiments, immunoglobulins obtained by ammonium sulphate precipitation from sera of 8 randomly selected leprosy patients (4BT, 4BL) were injected intraneurally into the right sciatic nerves of normal Swiss white mice in a dosage of 0·5 μl using a glass micropipette. Biopsies of both the sciatic nerves were collected at the end of 1 week.

The binding of antibody to nerve was also checked *in vitro* using an indirect fluorescent system consisting of sequential application of test serum and FITC labelled Protein A.

Subcutaneous and intraneural injection of human AB serum and normal saline served as controls.

PART II ADJUVANT LIKE ACTIVITY OF *M. LEPRAE*

The experiments were designed as indicated in Table 1.

Table 1. Various combinations of inocula and the sciatic nerve biopsy interval in the mice

Inocula	Number of mice studied at 1st month	3rd month
A 25×10^6 viable *M. leprae*	2	Nil
B 25×10^6 heat killed *M. leprae*	2	Nil
C 25×10^6 viable *M. leprae* + 10% nerve extract in PBS	2	(2+2)*
D 25×10^6 heat killed *M. leprae* + 10% nerve extract	2	(2+2)*
E Nerve extract alone (10% in PBS)	2	Nil

* Two mice for sciatic nerve biopsy and 2 mice used for collection of blood.

M. leprae was derived from homogenized skin of untreated bacilliferous patient. Inocula of 0·1 ml containing 25×10^6 *M. leprae* with or without nerve extract, was injected subcutaneously into one flank of random bred Swiss white mice (1–2 months old). For each set of experiments the same batch of infecting organism was used.

To detect serum demyelinating factors, serum was collected by cardiac puncture at the 3 month period from 2 animals injected with 25×10^6 viable *M. leprae* + 10% nerve extract in PBS and 2 animals injected with 25×10^6 heat-killed *M. leprae* + 10% nerve extract in PBS (Inocula C & D in Table 1). Each serum sample was injected intraneurally as previously described in the right sciatic nerves of 2 mice.

Sciatic nerve biopsies of both parts of this study were processed for electron microscopy. The sections were examined primarily at the semi-thin level and wherever necessary at the ultra-thin level using a JEOL 100S TEM.

Observations

PART I SERUM DEMYELINATION STUDIES

(a) In the first set of experiments where whole serum was used, 1 (from an untreated BT patient) out of 7 serum samples produced patchy segmental demyelination of 20–30% of fibres (Figure 1(a) and (b)) in all the 4 nerves obtained from 2 mice. The changes were predominantly perivascular. These mice had also shown signs of hind leg weakness.

(b) In the second series 2 out of 8 samples revealed similar patchy demyelination in the injected sciatic nerves. No pathology was seen in the contralateral uninjected nerve of the same animal. Of the two positive samples one belonged to a borderline lepromatous bacillary skin negative patient treated for 16 years; the second was obtained from a bacillary positive borderline lepromatous patient treated for 2 years.

Serum from the above mentioned BT patient and the latter BL patients showed positive binding activity to normal mouse nerve by fluorescence.

In the control group no demyelinating activity was detected in subcutaneous injections, however in intraneural inoculations, mild axonal degeneration was noted at the site of injection.

PART II ADJUVANT LIKE ACTIVITY OF *M. LEPRAE*

A large subcutaneous dose of 25×10^6 viable and heat-killed *M. leprae* (inocula A & B, Table 1) did not reveal any pathological changes in the sciatic nerves of mice at 1 month. However in combination with normal mouse sciatic nerve extract (inocula C & D) significant degenerative changes were seen in the unmyelinated

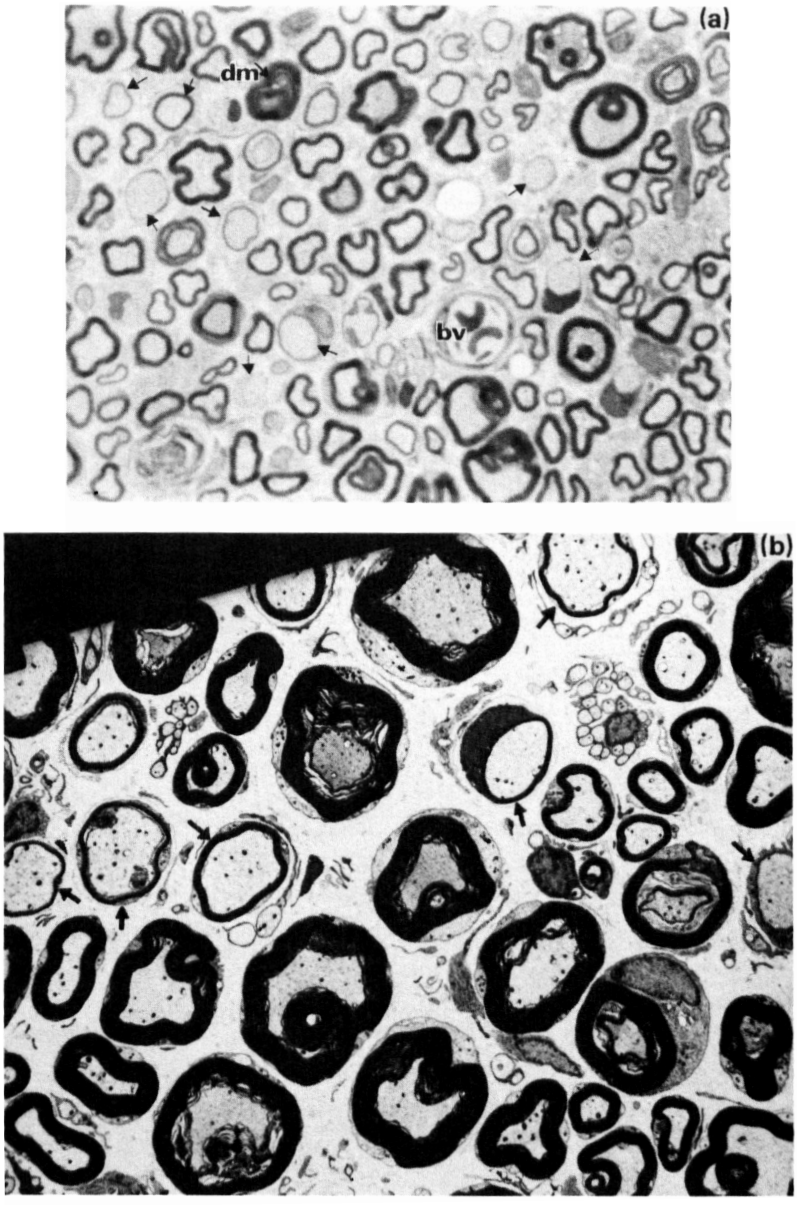

Figure 1. (a) Demyelination of fibres seen in the sciatic nerve of a mouse injected subcutaneously with 0·5 ml of serum obtained from an untreated borderline tuberculoid patient. Biopsy was obtained 1 month after injection. Number of demyelinated axons (arrows) are seen. There is good clearance of myelin debris and most of the fibres show thin rim of myelin (arrow heads) suggesting remyelination. One micron thick, transverse semi-thin section stained with toludine blue. dm, degenerated myelin; bv, blood vessel.
(b) Part of the nerve shown in (a) at ultrastructural level showing a number of demyelinated fibres (arrows) with remyelination. × 2000.

fibres (Figure 2). Contrary to expectations the damage did not extend to myelinated fibres at 3 months; however the initial changes in non-myelinated fibres persisted although to a lesser degree.

Serum obtained at 3 months from the mice injected with inocula C & D failed to demonstrate any demyelinating activity when injected intraneurally into normal Swiss white mice sciatic nerves. Nerve extract (inoculum E) by itself was also unable to induce such changes.

Discussion

Since mycobacteria are known to exert adjuvant like activity[5] it is expected that in a disease like leprosy, autoimmune reactions to peripheral nerve components could be generated. Since myelin basic protein (MBP) is known as a potent antigen, one would favour it as the target component. However, a recent study[6] and an earlier one[7] could not trace any anti-MBP antibodies in leprosy patients; the latter group however recorded the presence of anti-axonal antibodies.

The first part of our study detects a demyelinating antibody, though its exact specificity remains unknown. Antibody mediated demyelination analogous to Experimental Allergic Neuritis (EAN) was reported[8] for the first time without the use of adjuvants via intraneural injection of EAN serum. Using this same

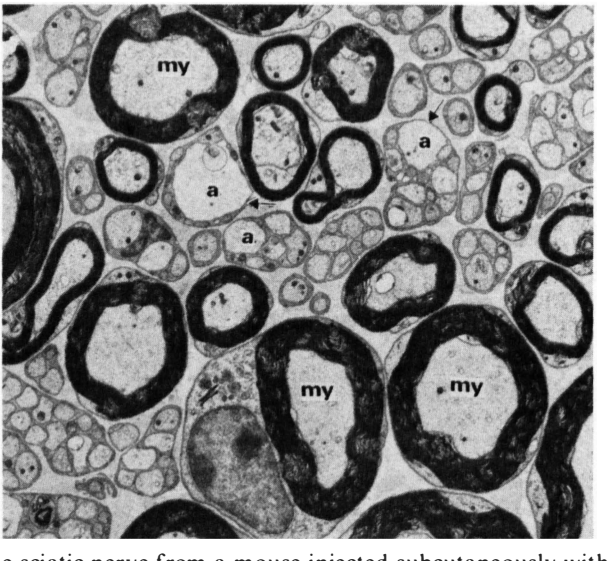

Figure 2. Part of the sciatic nerve from a mouse injected subcutaneously with 20×10^6 viable *M. leprae* + 10% whole nerve extract. Biopsy obtained after 1 month. Number of unmyelinated axons (a) shows degenerative changes like disintegration of neurotubules and filament giving watery appearance to the axoplasm. There is also abnormal Schwann cell axon contact (arrows). Myelinated fibres (my) are well preserved. Electron micrograph. ×4000.

technique we were successful in transmitting demyelinating factor from the patient's serum to a normal mouse in the absence of any presensitizing event.

In the subcutaneously injected mice demyelination was seen predominantly in the perivascular regions suggesting that the factor could have leaked through the blood vessels. The results implied that disseminated neural damage could occur in spite of localized infection and could persist even after prolonged therapy. It would be interesting, however, to know whether demyelination involves the participation of any inflammatory cells and secondly whether it is a single stage process. These observations may also explain the early pathological changes of leprosy patients in nerves as described previously by us.[1, 2] The low frequency of finding a serum factor positive for demyelinating activity may be attributed to catabolic processes and periodic variation in serum antibody concentration.

Our results in the second series of experiments confirm the postulates of another study,[9] that the earliest change in leprous neuropathy is due at least partly to an autoimmune reaction against neural antigens. Unlike their experiments where granulomatous hypersensitivity was manifested in cutaneous nerves, our experiments using *M. leprae* as adjuvant demonstrated degenerative changes in the nonmyelinated fibres in a major nerve trunk away from the site of inoculation. It was interesting to note that these changes were identical to those seen in the unmyelinated fibres of foot-pad inoculated mice between 4th and 8th post-inoculation month.[3]

The two interesting features of this early change are that it requires no presensitization for its expression nor is it necessarily linked to sequential segmental demyelination. The inability to detect progressive nerve damage at 3 months is hard to explain. Perhaps it is a dose effect of the concentration of *M. leprae*/sciatic nerve extract used; hypersensitivity being brought on with larger doses. Segmental demyelination and ensuing inflammation can perhaps occur only when there is systemic sensitization of *M. leprae* leading to a break in the blood nerve barrier.[10]

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Lepr Rev (1985) **56,** 229–238

Presence of soluble, *Mycobacterium leprae*-derived antigen in the inflammatory exudate of reactional lepromatous leprosy

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Summary By immunofluorescence techniques, immunocomplexes deposition in the wall and periphery of dermal blood vessels have been demonstrated in 8 leprosy-reaction lesions (4 ENL, 4 Lucio's phenomena). Two additional ENL lesions were negative for the presence of immunocomplexes with anti-IgM, IgG, IgA, C3 and C1q antisera. The 10 leprosy reaction lesions, however, were positive for the presence of *Mycobacterium leprae*-derived soluble antigen. This antigen, visualized with a potent human anti-*M. leprae* antiserum, was often found in and around the dermal blood vessels showing vasculitis and always in the macrophages (Virchow's cells) present in the leprous granulomas. This finding was independent of the presence of intact or fragmented *M. leprae* in those locations.

The role of mycobacteria derived material in the genesis of type-2 leprosy reactions is discussed.

Introduction

From previous work in our Department on immunological abnormalities in patients with reactional lepromatous leprosy, a considerable incidence of circulating immune complexes (CIC) has been found to be associated with the presence of erythema nodosum leprosum (ENL) and Lucio's phenomenon.[1–3] In one study, a group of patients suffering from diffuse lepromatous leprosy complicated by

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Lucio's phenomenon showed severe impairment in several of their immunological parameters. [2-3] Their levels of total serum proteins were elevated mainly due to an increase in the alpha-2 and gammaglobulin fractions. All of the immunoglobulin classes were elevated but the IgG and IgM classes were the most altered. The complement components C3 and C4 were normal and the 50% haemolytic complement activity was only slightly elevated. The C-reactive protein and rheumatoid factors tests were positive in around 50% of the cases. Thirty-three percent of the patients had circulating immune complexes and all of them had circulating antimycobacterial antibodies.

In general, the patients with reactional lepromatous leprosy showed low numbers of T-lymphocytes (E-rosettes) and, in some cases, B-lymphocytes (EAC-rosettes) were increased. A great majority of patients gave negative leucocyte inhibition factor (LIF) tests with lepromin as the antigen. The LIF tests with PPD and the response to the intradermal injection of PPD and other antigens were comparable both in lepromatous and normal groups.

We have extended our studies on reactional lepromatous leprosy and in this paper we present our results on the presence of soluble immune complexes and antigen at the lesion sites and suggest a role for these materials in the genesis of reactional type-2 leprosy lesions.

Materials and methods

PATIENTS

Six lepromatous patients with ENL and 4 patients with Lucio's phenomenon were studied. They were patients attending regularly at the Centro Dermatológico Pascua (Mexico City) for medical care, or residents of the leprosarium 'Hospital Dr P López' (Zoquiapan, México). All the patients were adult males or females having an old leprosy infection, and they were under conventional anti-leprosy (but not anti-reactional) treatment (DDS) at the time of the study. Five other, age-matched, non-reactional lepromatous patients were also included in the study as a control group. Within this group, 3 patients were under DDS-treatment for over 3 years, one had just begun treatment and the last one was an untreated case.

SKIN BIOPSIES

They were taken with a 5 mm-wide circular punch in a manner deep enough to include all of the dermis and part of the subjacent fatty tissue. Each biopsy was divided into two halves. One half was formalin-fixed and processed for routine histology, and the other was included in 'tissue-teck', frozen on dry ice, and sectioned thereafter as described below.

STAINING OF TISSUE SECTIONS

Five microns-thick tissue sections were prepared in a cryostat (Tissue Teck II, Miles), fixed for 10 min in cold acetone, washed 3 × with phosphate-buffered saline (PBS) and stained with fluoresceinated anti-human IgA, IgM, IgG, C3, C1q, or anti-*Mycobacterium leprae* gamma globulin (see below). Except for the fluoresceinated anti-C1q (which was previously prepared in goat in our laboratory) and anti-*M. leprae* (to be described here) antibodies, all of the other immunoreagents were purchased from Hyland and Ortho (Behring). Staining of tissue sections pretreated for 60 min with an enzyme mixture containing 8 mg egg lysozyme (Sigma L6876) and 8 mg wheatgerm lipase (Sigma L3001) per 40 ml of 0·06 M phosphate buffer, pH 6·8, was performed according to conventional procedures.

FLUORESCEINATED HUMAN ANTI-*M. LEPRAE* ANTIBODY

Gammaglobulins (HGG) were precipitated from a pool of sera from lepromatous patients that were strongly positive in the counterimmunoelectrophoresis (CIE) test[4] with a *M. lepraemurium* extract as the antigen. HGG were precipitated with ammonium sulphate at 1/3 of saturation, exhaustively dialysed against saline borate solution, pH 8·6 (H_3BO_3 0·309 g; $Na_2BO_4O_7 \cdot 10\ H_2O$ 0·477 g; NaCl 8·3 g, and water to 1000 ml), and labelled with fluorescein isothiosyanate (FITC) according to Nairn.[5] Briefly, to each 4 ml of a 2% protein solution, 1·0 ml of 0·2 M Na_2HPO_4, pH 9·0, were added. The pH in the mixture was adjusted to 9·5 with 0·1 M $Na_3\ PO_4$ and the volume brought to 8·0 ml with 0·145 M NaCl. After incubation for 60 min at room temperature in a dark place, the reaction was stopped by cooling the mixture in an ice-bath for a few minutes. Free fluorescein was separated by chromatography through a G-25 Sephadex column (2 × 25 cm), eluting with 0·01 M phosphate buffer, pH 7·2. Labelled protein was collected, dialysed against the above buffer and applied to a DEAE- cellulose column. DEAE chromatography was performed according to Wood[6] using a 2·6 × 13 cm column per each 200 mg of protein. To separate fractions optimally labelled from those under- or over-labelled, elution with 0·01 M, 0·03 M and 0·05 M phosphate buffer, pH 7·2, was performed. Most of our FITC-labelled protein (495/280 ratio = 0·72) was eluted with 0·01 M phosphate buffer, although a considerable amount of conjugate (495/280 ratio = 0·83) was eluted with 0·3 M phosphate buffer. Both fractions, however, showed absence of non-specific staining ability and were highly brilliant under ultraviolet light. The FITC-labelled fraction used in the present experiments was that eluted with 0·01 M phosphate buffer and had a protein concentration of 13 mg per ml. To use, it was diluted 1:5 with saline phosphate buffer, pH 7·2 (NaCl, 0·85 g; Na_2HPO_4, 1·07 g; $NaH_2PO_4 \cdot 1H_2O$, 0·35 g, water to 1000 ml). The antibody specificity of this FITC-labelled fraction was demonstrated by treating mouse liver, spleen, kidney, heart, brain, striated muscle and skin tissue sections, 5 microns-thick, with the antibody preparation for 60 min.

Results

SPECIFICITY OF ANTI-*M. LEPRAE* ANTIBODY

When treatment of normal mouse liver, spleen, heart, brain, striated muscle and skin tissue sections with our anti-*M. leprae* preparation was performed, no staining was observed except for the weak, natural self fluorescence of collagen. On the contrary, a very bright specific fluorescent staining was observed associated to the granulomatous collections in the livers and spleens of mice bearing a 3 month infection with *M. lepraemurium* (Figure 1). The same antibody preparation did stain enzyme-treated smears of purified *M. lepraemurium* suspensions.

IMMUNOHISTOLOGIC STUDY OF BIOPSIES

Immunofluorescence studies in 8 leprosy reaction biopsies (4 ENL, 4 Lucio's phenomena) indicated the presence of immunocomplexes in the walls of the dermal blood vessels or in their periphery (Figure 2). In two cases of ENL we were unable to demonstrate deposition of immunocomplexes in or around the dermal vessels which, however, presented vasculitis of variable intensity and other inflammatory changes including oedema and infiltration of PMN leucocytes. In these two cases the results were negative for the presence of IgM, IgG, IgA, C3 and C1q, despite that fluorescent antisera from different sources were used. These negative results in regard to the demonstration of immunocomplexes in the lesions could possibly be explained assuming that the biopsies were taken from old or healing lesions rather than from early ones. At this time, immunocomplexes could well be phagocytosed and degraded. Alternative explanations are possible as we will underline below. Others[7] have also been unable to demonstrate immunocomplexes in the vessel walls or in the granulomatous infiltrates of 19 biopsies of skin lesions of patients with borderline lepromatous (10) or lepromatous (9) leprosy showing a recent reaction.

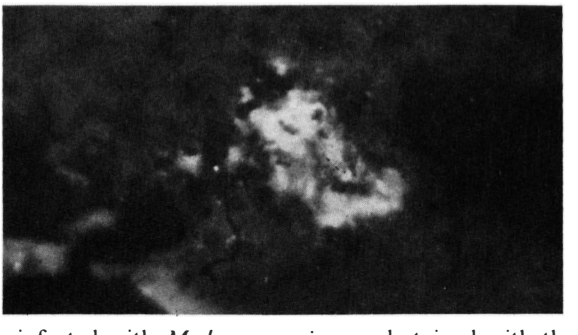

Figure 1. Mouse liver infected with *M. lepraemurium* and stained with the fluorescein-labelled human anti-*M. leprae* immunoglobulin. The specific staining of hepatic macrophages found in a small leprous granuloma is shown (ca. ×600).

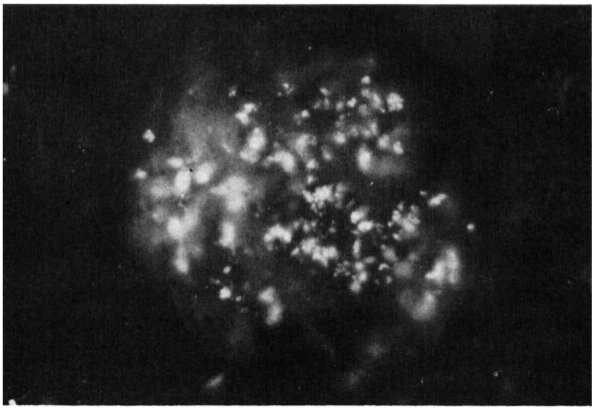

Figure 2. Deposition of immune complexes in a dermal blood vessel of a Lucio's phenomenon as visualized through their reaction with a fluoresceinated rabbit anti-human IgG antiserum. Notice the fluorescent material filling and obstructing the light of the small vessel (× 480).

An interesting observation was the absence of demonstrable *M. leprae* within the inflammatory cells in some cases showing severe vascular lesions. On the other hand, we have observed in all of the biopsies taken from both ENL (6) and Lucio's phenomenon (4), the presence of intracellular soluble mycobacterial antigen.

A granular or homogeneous pattern of fluorescent reaction was consistently observed in the cytoplasm of macrophages and PMN leucocytes around the affected blood vessels and within macrophages and Virchow's cells of the granulomas, this indicating the presence of *M. leprae* soluble antigen (Figures 3–5). This finding was independent of the identification of acid-fast bacilli by the Fite–Faraco staining method. In several cases, the fluorescent tissue was composed of PMN and macrophages full of bacillary fragments. Many cells in the infiltrate showed morphological evidences of degeneration.

Within the control group, a close correlation between the presence of acid fast bacilli and a positive fluorescent anti-mycobacterial test was observed. Two cases with abundant *M. leprae*-laden Virchow's cells gave a positive fluorescent anti-mycobacterial test. The remaining 3 cases contained lesser amounts of Virchow's cells, most of them free of bacilli, and they were negative for the presence of soluble mycobacterial material. In the absence of leprosy reaction, control cases were negative in the anti-C1q fluorescent test.

Discussion

Erythema nodosum leprosum (ENL) and erythema necrotisans or Lucio's phenomenon, are manifestations of the type 2 leprosy reactions.[8]

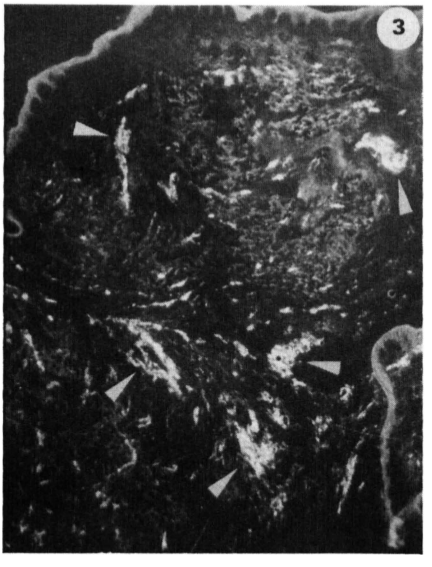

Figure 3. A section of a leprosy reaction type ENL stained with the fluoresceinated human anti-*M. leprae* antibody. A great number of macrophages containing intact or fragmented *M. leprae* or *M. leprae*-derived antigens is seen throughout the dermal lesion both affecting blood vessels and in the leprous granulomas (Virchow's cells) (arrowheads) (× 125).

ENL appears in nearly 50% of lepromatous patients under treatment although it appears also in patients without it. It appears less frequently in subpolar or borderline lepromatous leprosy but it does not appear in the other types of leprosy.

From the clinical point of view, ENL typically presents as collections in the 'normal' skin of small nodules, slightly raised, tender, erythematous, warm and recurrent, that blanch under finger pressure. These nodules last for some days (usually 3 to 7), then vanish leaving no trace unless they reappear on or adjacent to a previous lesion. In this case, they disappear leaving a bluish and fibrotic scar of the thickened skin. Because of their dermal and hypodermal localization, the nodules cannot be rolled under the skin. Occasionally, several isolated lesions fuse with each other and even more seldom they become necrotic and suppurative. This happens especially on the extensor surfaces giving origin to a form of necrotising erythema nodosum.

The Lucio's phenomenon or erythema necrotisans is another form of type 2 leprosy reaction. This is a complication of the diffuse lepromatous leprosy of Lucio and Latapi but it can appear in some cases of advanced nodular lepromatous leprosy, especially when such cases are under irregular treatment or without it. Although this type of leprosy reaction is frequent in patients from Mexico and Central American countries, it is not limited to them and isolated cases of Lucio's phenomenon have been reported in other parts of the world. The lesions appear in the skin as erythematous spots, tender or slightly indurated,

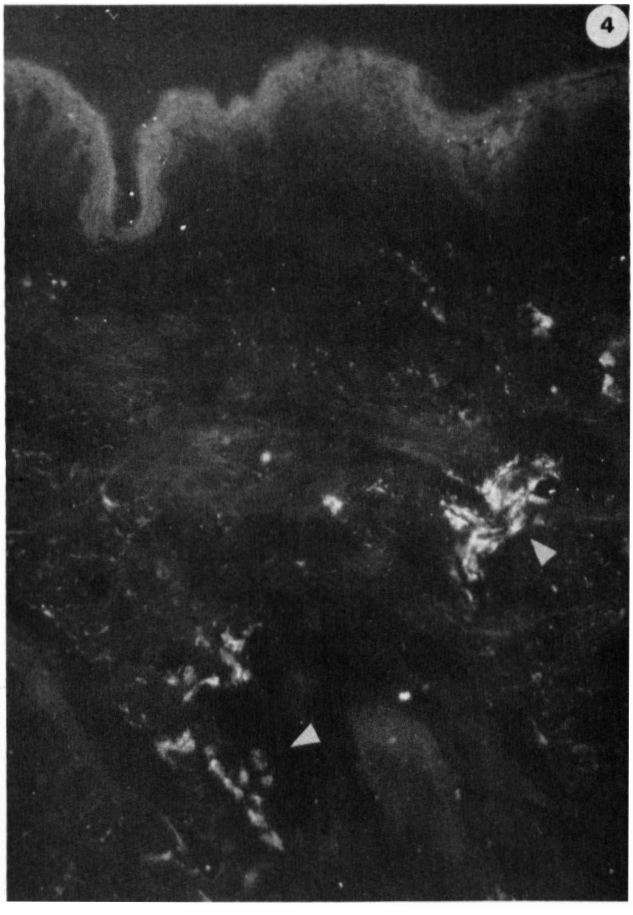

Figure 4. A tissue section from a Lucio's phenomenon stained with a fluoresceinated anti-*M. leprae* antibody, showing the presence of soluble *M. leprae*-derived antigen in the papillar and reticular dermis. Notice the fluorescent material in and around dermal blood vessels and in the Virchow's cells of the lepromatous granuloma (arrowheads) (× 120).

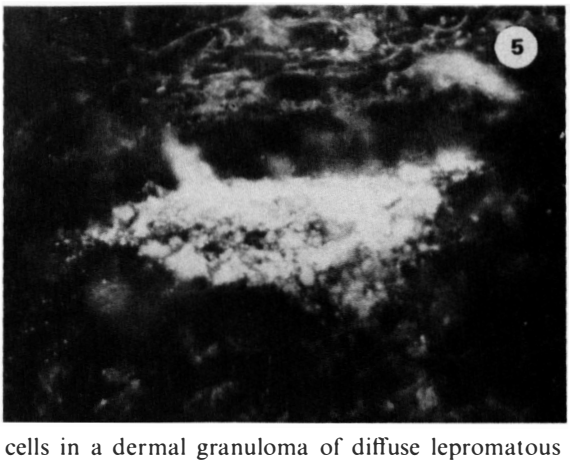

Figure 5. Virchow's cells in a dermal granuloma of diffuse lepromatous leprosy with Lucio's phenomenon stained with a fluoresceinated human anti-*M. leprae* antibody (× 480).

painful, that soon become purpuric and finally necrotic (some of them become ulcerated) leaving an irregular or stellate crust of dark brown colour which eventually comes off leaving an hypo-or hyperpigmented atrophic scar. Although the lesions appear fundamentally on the limbs (and those of the legs are the most severe and long lasting), they may appear on other parts of the body.

The factors responsible for these type 2-leprosy reactions are not known for sure, and because of this, multiple etiologies have been considered. Although there is no definite proof of it, it is most probable that leprosy reactions are a manifestation of the host immune reactivity. It is also possible that massive destruction of bacteria due to the host defensive mechanisms, to the effect of antileprosy drugs, or to other causes, can also constitute a highly toxigenic and therefore injuring mechanism. The 'toxic etiology' of the leprosy reactions can accompany their 'immunological etiology'. This type of leprosy reaction is triggered by very diverse factors: pregnancy, successful vaccination, abrupt climatological changes (higher incidence during the cold or rainy months of the year), intercurrent infections, physical or mental stress, anaemia, diverse parasitosis, etc.[9]

The histologic study of leprosy reactions type 2 in both ENL and Lucio's phenomenon, has revealed the existence of an inflammatory process that complicates the lepromatous skin histology. The inflammatory exudate, essentially made up of PMN leucocytes and variable numbers of lymphocytes and plasma cells, extends through the whole dermis and accumulates around the blood vessels and in the vicinity of the foamy histiocytes of the granulomatous tissue. In general, polymorphs are free of intact bacilli but they may contain fragmented bacillary material. There is an intense vasculitis, sometimes with fibrinoid necrosis and endothelial thickening of the deep vessels, as well as capillary necrosis in the more superficial lesions. Some oedema may be observed in the tissue adjacent to the injured vessels. In ENL, the inflammatory changes (vasculitis and cellular infiltrate) affect, principally, the deep dermis and hypodermis. The affected vessels are those of medium calibre and the changes are, mainly endothelial thickening. In Lucio's phenomenon, the inflammatory changes affect the whole dermis. The epidermal lesions are secondary to the lesions that affect the dermal and hypodermal blood vessels. In this case, small and medium calibre vessels are affected. They often present thickening, obstruction and necrosis.[10]

Such a type of inflammatory infiltrate and vasculitis resembles the histology of the experimental Arthus reaction. In the Arthus' reaction, the observed changes have been explained on the basis of a type III hypersensitivity reaction[11] which is triggered by the presence of immunocomplexes after their deposition on the vascular endothelia. Complement and PMN also participate. The above-mentioned resemblance stimulated several researchers to look for elements of the immune response in the lesions that characterize this type of leprosy reactions. With the aid of immunofluorescent techniques, granular deposits of IgG and IgM

immunoglobulins and C3 have been found in 50% to 60% of the studied ENL biopsies.[11, 12] The same biopsies that were positive for immunoglobulins were so for C3 complement, and in 35% of the biopsies, mycobacterial antigen was detected by its reaction with an undiluted goat antiserum to *Mycobacterium tuberculosis*.[13] In a more recent study, Ridley & Ridley,[14] using an anti-BCG antibody, have also shown the presence of mycobacterial antigen (in the form of immunocomplexes) both extracellular and within neutrophils and macrophages in skin biopsies of patients with ENL.

Granular deposition of IgG and IgM immunoglobulins and complement (C3 and C1q) have been detected on the walls of the dermal blood vessels and in perivascular areas in the biopsies from 2 patients with Lucio's phenomenon.[15]

Apart from these findings, the immunological etiology of the leprosy reaction type 2, finds some support in the demonstration of a higher incidence of circulating immunocomplexes in lepromatous patients undergoing an ENL or Lucio's reaction.[1, 16] Very often, a leprosy reaction of ENL or Lucio's phenomenon type does not appear alone but associated with some of the following systemic manifestations: fever, adenopathy, arthralgias, neuritis, asthenia, iridocyclitis, epididymo-orchitis and some others. These systemic manifestations are analogous to those found in serum sickness which is known to be associated to the presence of circulating immunocomplexes. Other relevant associations with type 2 leprosy reaction are the high incidence of mixed cryoglobulinemia,[14] alterations in the complement levels, often with elevation of C2[17] and C3,[11] the elevated concentrations of IgG euglobulin,[18] and glomerulonephritis.[19, 20]

Based on our observations (see Results), we suggest that *apart* from the immunocomplexes deposition, other factors could participate in the triggering of the injuring type-2 leprosy reactions. One of those factors could be the massive bacterial load (intact or degraded bacilli) inside the tissue PMN and macrophages that leads to the disruption of these cells releasing chemotactic, hydrolytic and other inflammatory factors that eventually cause vasculitis. The role of macrophage secretory products in chronic inflammatory processes has already been clearly established[21] and there is also the possibility that glycolipid and peptidoglycan components of mycobacterial walls also trigger release of mediators, without causing cell death. Additionally, immunocomplexes-mediated tissue damage would also account for the vasculitis that characterizes these type-2 leprosy reactions.

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Leprosy in children aged 0–14 years: report of an 11-year control programme

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Summary A Leprosy Control Programme has been in operation in the entire twin island country of Trinidad and Tobago for 11 years. During the 11 years of the Programme, the number of new cases of leprosy diagnosed in children aged from 0 to 14 years decreased from 65 patients in the third year of the Programme to 3 in both the 10th and 11th years of operation. The epidemiology of leprosy in Trinidad and Tobago during these 11 years is described.

The success of this Programme is credited to: 1, the decisions by the Government of Trinidad and Tobago in 1968 to close the leprosarium and to set up a Leprosy Control Programme; 2, the recruitment and training of qualified personnel; 3, active patient identification and aggressive treatment and follow-up of infectious patients; and 4, the assistance of a vibrant voluntary organization in providing socio-economic assistance for patients and in educating the public.

Introduction

An aggressive outpatient approach to the control of leprosy was implemented in Trinidad and Tobago in early 1971. After a sharp rise in the new case rate for the first 3 years of the programme, the new case rate steadily fell in the following 8 years both in children, aged 0–14 years, and in adults (15 plus). In this paper we will look at the former group and at various related parameters.

Method

PATIENT IDENTIFICATION

Patients were diagnosed by clinical examination, an AFB positive skin smear, and/or a biopsy, except in patients who had only facial lesions.

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The patients were classified according to the criteria of Ridley & Jopling.[1]

Tuberculoid (TT) patients were treated with 4,4-diaminodiphenylsulphone (dapsone) 1–2 mg/kg/day. Medication was continued for 18–24 months after the skin lesions disappeared and the resolution of neuritis. They were considered cured after receiving this medication.

Indeterminate (II) patients were treated the same as above but were given dapsone for 3 additional years after their disease became inactive instead of for 18–24 months.

Borderline tuberculoid (BT) and bacteriologically negative borderline (BB) patients were treated the same as above but were given dapsone for 5 and 10 additional years respectively, after their disease became inactive.

In the first 2 years of the programme, skin smear positive patients were initially treated with 2 drugs: dapsone 1–2 mg/kg/day and clofazimine (lamprene) 1–2 mg/kg/day. Beginning in 1973 rifampicin (rimactane) 10–20 mg/kg/day was added to the other drugs. The maximum daily dose of rifampicin was 600 mg. All 3 drugs were given for the initial 3 months. Clofazimine and dapsone were continued until the patients' skin smears were negative. Beyond this point dapsone alone was given, in borderline (BB) patients for 10 years, in borderline lepromatous (BL) patients indefinitely, and in lepromatous (LL) patients indefinitely.

Results

PATIENTS IDENTIFIED

A total of 275 patients with leprosy were identified in children aged 0–14 years in the first 11 years of the programme (Figure 1). Whereas 144 (52%) of the patients were identified in the first 3 years of the programme (1971–1973) only 17 (6%) were identified in the last 3 years of the programme (1979–1981). During each of the 10th and 11th years of the programme only 3 new patients were identified, representing an 88% reduction in the number of new patients when compared with the first year when 26 patients were identified.

TYPE OF DISEASE

Of the 275 patients, 199 (72%) were classified as tuberculoid, and an additional 51 (19%) were classified as borderline tuberculoid. The 9 (3%) classified as indeterminate were also skin smear negative, thus 94% fit in the paucibacillary group. Only 16 (6%) were in the multibacillary group (BB, BL, and LL). See Table 1.

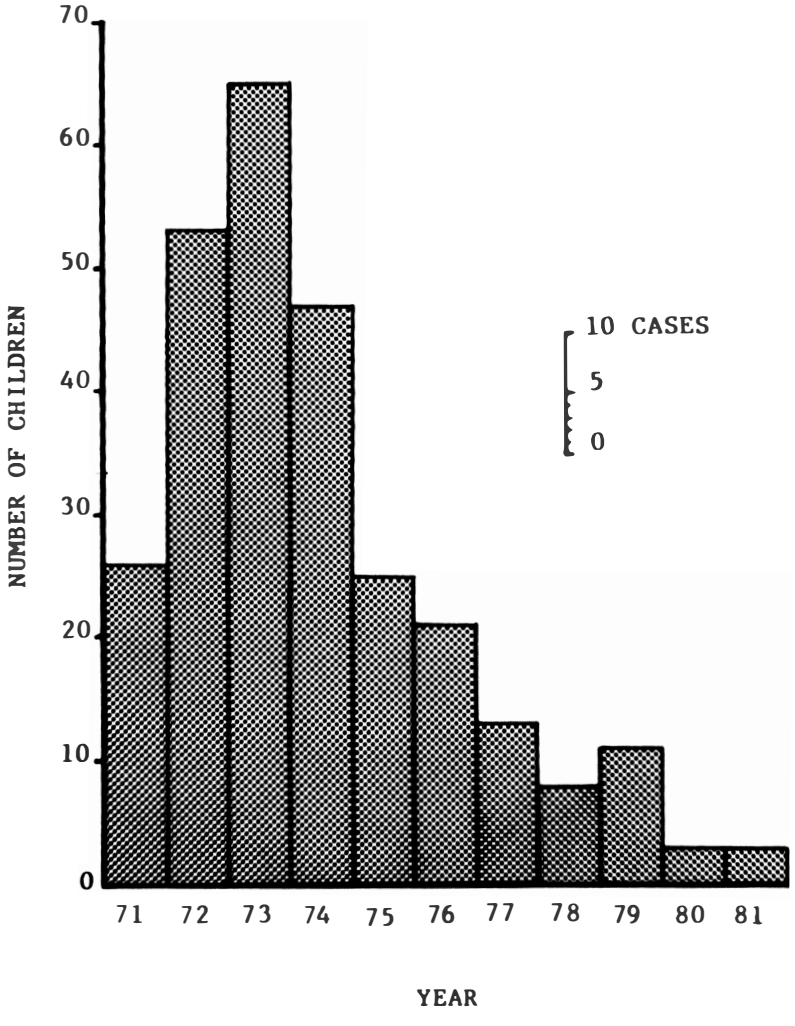

Figure 1. Newly-diagnosed leprosy in 0–14 year olds, 1971–1981, in Trinidad and Tobago.

AGE, SEX, AND ETHNIC ORIGIN

The age and sex of the patients at the time of diagnosis is shown in Table 2. There was no significant difference in the number of boys or girls per 10,000 population affected (Chi-square = 1·46). Thirty-one (11%) of the patients were children less than 5 years of age, whereas 141 (51%) were aged 10–14 years. The case detection rate was approximately four times higher in those aged 10–14 years than in those children less than 5 years. The youngest patient was 16 months old.

The ethnic origin of the patients (Table 3) shows that 49% of the patients were East Indian, 40% Negro, 11% mixed and less than 1% other. This varies from what would have been expected if cases had occurred in proportion to the percentage of the population in each ethnic group (Chi-square = 12.99, with 2 d.f.,

Table 1. Classification of leprosy in children 0–14 years of age 1971–1981 in Trinidad and Tobago

Type	Number of patients Male	Female	Total	% of total patients
II	7	2	9	3
TT	101	98	199	72
BT	30	21	51	19
BB	0	4	4	1
BL	4	3	7	3
LL	5	0	5	2
Total	147	128	275	100

Table 2. Leprosy in children 0–14 years, by age and sex at diagnosis 1971–1981 in Trinidad and Tobago

Age (years)	Number of patients Male	Female	Total	% of total patients	Case detection rate/10,000* population
0–4	12	19	31	11	3
5–9	50	53	103	37	8
10–14	85	56	141	51	11
Total	147	128	275	100	8

* Based on estimated 1975 mid-year population aged 0–14 years.

$P < 0{\cdot}01$). The expected number of cases by ethnic group would be Negro 118, East Indian 110, mixed 39, and other 8.

GEOGRAPHICAL LOCATION

The overall rate of leprosy for the 11 years of the Programme was 8/10,000 population aged 0–14 years. Figure 2 shows the case detection rate per 10,000 population aged 0–14 years for areas of Trinidad and Tobago. The overall rates varied from zero to 27/10,000 during the 11-year period. In County St George East 2, the area with the highest rate, the case detection rate was more than four times the average for the rest of the country (6/10,000).

CHARACTERISTICS OF PATIENTS WITH POSITIVE SKIN SMEARS

Overall 7% of the patients aged 0–14 had positive smears. Whereas 100% of the BB, BL and LL patients had positive smears, only 8% of BT and 0% of TT and II

Table 3. Leprosy in children 0–14 years of age by ethnic origin 1971–1981 in Trinidad and Tobago

Ethnic origin	Number of patients*	Percentage of total patients	Percentage in total population
East Indian	136	49	40
Negro	109	40	43
Mixed	29	11	14
Other	1	0	3
Total	275	100	100

* Chi-square = 12·99 with 2 d.f., $P < 0{\cdot}01$.

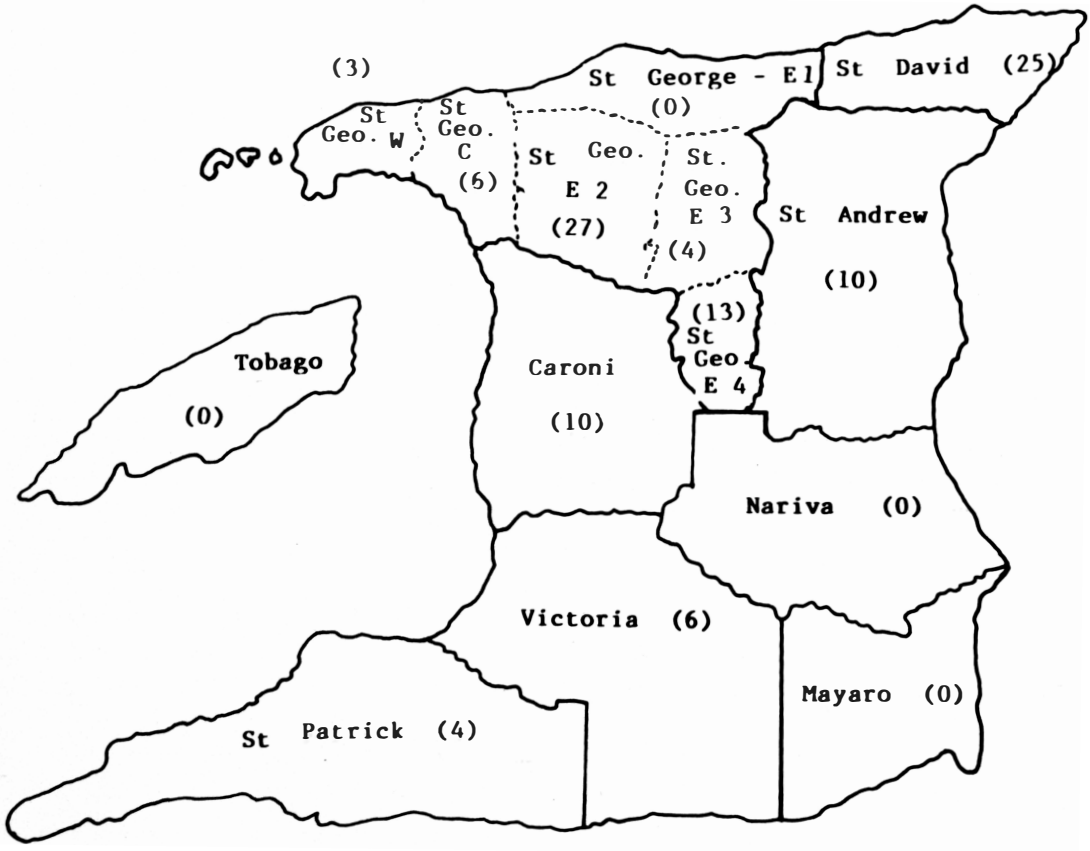

Figure 2. Map of Trinidad and Tobago showing case detection rates for leprosy per 10,000 children aged 0–14 years for the 11-year period, 1971–1981.

patients had positive smears. All BT patients with positive smears were aged 5 years or above. Only 1 child under age 5 had a positive skin smear, and only 3 children under age 10 had positive smears (Table 4). Of the 20 children with positive skin smears at diagnosis, 10 (50%) were smear negative in 2 years, 14 (70%) were negative in 4 years, 17 (85%) were negative in 6 years and 18 (90%) were negative 8 years after diagnosis. The 2 still positive at the end of the study had been diagnosed in 1979.

DISABILITIES

At the time of diagnosis 4 children (aged 8, 12, 13, and 14 years) had disabilities. Their types were TT, BT, BB, and BT respectively. None of the children who regularly took their medication developed any disabilities while they were on the register. One child, age 6, who did not take her medication regularly developed disabilities 5 years after diagnosis.

STATUS OF PATIENTS AT END OF ELEVEN YEAR PERIOD

The status of patients as of 31 December 1981, is shown in Table 5. One hundred and twenty patients had completed their therapy and were considered cured. Nine migrated. Only 3 patients were lost to follow-up. Of those patients still on the register on 31 December 1981, 74 were considered to have inactive disease and were continuing their therapy as described previously. Sixty-seven patients were considered to have active disease and were continuing therapy. Up to December 1981, LL and BL patients remained on the register, and on medication, until death (unless they emigrated), as up to that time our policy was that they must take medication for life. As of 1 January 1982, we began following the World Health Organization's recommendations regarding short-term, partially-supervised multidrug therapy, which rapidly altered our register. Only 2 children still needed treatment and remained on the register as of 31 December 1983.

Table 4. Age and type of leprosy in children age 0–14 years who had AFB positive skin smears 1971–1981 in Trinidad and Tobago

Age	Number with (+) smears	Type of leprosy					
		II	TT	BT	BB	BL	LL
0–4	1	0	0	0	0	1	0
5–9	2	0	0	1	0	1	0
10–14	17	0	0	3	4	5	5
Total	20	0	0	4	4	7	5

Table 5. Status as of 31 December 1981 of 275 children diagnosed as having leprosy during the 11-year period 1971–1981 in Trinidad and Tobago

Status	Number
On register:	141
Active leprosy	67
Inactive leprosy	74
Off register:	132
Cured	120
Migrated	9
Lost	3
Refused treatment after becoming inactive	2
Total	275

Discussion

In 1965 and again in 1970, the World Health Organization Expert Committee on Leprosy estimated that the number of leprosy cases worldwide was approximately 10·8 million.[2] By 1977, this same Committee estimated that the number of cases had increased to 12 million.[3]

Despite the increase in worldwide cases, control programmes have reduced the prevalence and incidence of leprosy in some countries. In Thailand, where a programme has been in existence more than 20 years, a 70% reduction in leprosy prevalence from 13·4/1,000 was shown in a random sample survey taken 10 years after the programme was begun. Similar reductions have occurred in Burma,[4] and Upper Volta.[5]

Although random sampling surveys have not been carried out in Trinidad and Tobago, other statistics suggest that major reductions in disease prevalence and incidence have occurred. In 1971 there were 1632 known leprosy patients on the register for a rate of 16/10,000 population. At the end of 1981 there were 763 known leprosy patients on the register for a rate of 7/10,000 or a reduction of 53%.[6] The number of identified cases of leprosy patients in the entire Programme paralleled the number of cases identified in children aged 0–14 years. In 1971, 70 patients were detected, in 1973, 130 were detected, and in 1981 only 30 patients were detected. The number of cases detected in 1981 represents a 59% reduction as compared with 1971 and a 78% reduction when compared with 1973.

A census survey of primary and secondary school children in County St George East 2, the area with the highest rate and number of leprosy cases in children, was undertaken in 1974 and again in 1982. In 1974, 13 cases were identified. In 1982 only 3 children were identified as new cases.

Based on information which the WHOECL had from various control programmes around the world, they recommended that 75% or more of lepromatous and borderline cases (multibacillary) should be on treatment for a control programme to be successful.[7] Of the 382 multibacillary patients on the Leprosy Register in Trinidad and Tobago at the end of 1981, 359 (94%) were under treatment. At the end of December, 1981, 341 (89%) were bacteriologically negative. Of the 20 children who were originally bacteriologically positive, 18 were bacteriologically negative by the end of 1981. The 2 remaining bacteriologically positive children were diagnosed in 1979.

The decrease in leprosy cases in Trinidad and Tobago is thought to be due to the institution of a comprehensive, integrated Hansen's Disease Control Programme that emphasized case detection and vigorous follow-up and treatment of cases. It could be argued that the decrease in leprosy might have occurred without the control programme, due to other factors such as the improving socio-economic conditions in the country during the past decade. Although this is possible it is unlikely, based on experience with leprosy in other parts of the world.[8]

The ability to decrease leprosy prevalence from 1632 (16/10,000) to 763 (7/10,000) over an 11-year period suggests that a reduction in the prevalence of leprosy is possible even when the initial prevalence rate is relatively low. It suggests also that having 90% of known lepromatous and borderline patients on chemotherapy is effective in reducing the spread of disease and suggests that the WHOECL criteria are valid.[9]

The epidemiology of leprosy in children during the 11 years of the Programme suggests that the case-detection rate of disease in children may drop more quickly than the case-detection rate in adults as the disease is being brought under control. The reasons for this are not known. As the group of individuals making up the 0–14 year age group changes about 7% per year (newborns replacing the 14-year olds who become 15 years old), a major decrease in transmission would very quickly result in a decreased case-detection rate in children as an indicator of the leprosy situation in an area.

EPIDEMIOLOGY OF LEPROSY IN TRINIDAD AND TOBAGO

The finding that 94% of the patients had paucibacillary disease is consistent with the reports of others who have shown a low rate of lepromatous disease in children.[10, 11] The similar number of cases for boys and girls is consistent with what would be expected.[12, 13]

Epidemiological studies have not reliably shown a predilection for African or

Indian races to have a difference in their incidence rates for disease. Among our children, however, there is a relative over-representation of the East Indians.

In comparing the rates of leprosy in children in different areas of Trinidad and Tobago, it is useful to note that although the rate in the country may be only 8/10,000 for the 11 years (or 0·7 patients/ 10,000/year), the rates in a local area may be three times higher.

The finding of only 7% of children with positive smears, especially in the 0–10 year age group is consistent with the finding that most of these children had TT or BT disease. A corollary to this is that it is rarely useful to perform a skin smear on children younger than age ten.[14]

The experience of the HDCU of having no deformities occur in children while on Hansen's Disease Register if they were taking their medication regularly is in agreement with the WHO Expert Committee on Leprosy which notes that deformity is not an inevitable or necessary part of leprosy. Its occurrence indicates some defect in the strategy of leprosy diagnosis and treatment. In a well-conducted leprosy control programme, almost no leprosy patients on first diagnosis will be suffering from some deformity attributable to neglected disease.[15] Evaluation of the Programme shows that only 3 (1%) patients were lost to follow-up. Similar control programmes should be possible in other countries where leprosy transmission is continuing.

Conclusion

What made this Programme successful? The role of the individuals who initiated interest in the problem of leprosy was extremely important. Without their help, surveys probably would not have been done, and official recognition of the problem might not have occurred.

The strong support given by the government in providing personnel, financial resources and in accepting a new approach to leprosy control was vital.

Strong support from a voluntary organization helped reduce fear among the public and encouraged early identification and treatment of leprosy.

Teaching medical and nursing students, plus training public health and hospital personnel made a major positive impact over the years.

A strong emphasis on early case finding, adequate treatment and good case holding were crucial in breaking the cycle of transmission of disease.

The decision of the government to go from a programme of isolation to a programme of integration was a key factor to bringing leprosy under control. With the old system, undetected cases were spreading disease so that new cases continued to occur. Patients were isolated causing them to lose financial security, families and community status. With an integrated approach patients can lead a normal life, maintaining their jobs, families and community status. With the former fears related to isolation removed and with an emphasis on early

detection, new multibacillary cases remain a source of infection in their communities for only a few months instead of for years.

Acknowledgments

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Lepr Rev (1985) **56,** 249–253

Malignancy in chronic ulcers in leprosy: a report of 5 cases from Northern Nigeria

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Summary Five patients, 2 of the usual variety of squamous cell carcinoma and 3 of verrucous carcinoma are reported. Four patients had BT type of leprosy and 1 had LL type of leprosy. Interestingly, one of the verrucous carcinomas occurred over the palm which is a rare site.

Introduction

Published accounts of malignant change in leprosy ulcers are rare and the literature records mostly case reports. In a brief period of 2 years, 5 such patients were detected at the Leprosy Control and Research Centre, Zaria, Nigeria.

Patients

During the period May 1982 to April 1984, 5 patients with chronic ulcers over hands and feet with a malignant transformation were treated at the Leprosy Control and Research Training Centre, Zaria, Nigeria. These 5 black African males form the basis of this report.

Case 1. A 45-year-old male shepherd with BT leprosy gave a history of recurrent ulceration over the left heel for the past 20 years. It became persistent during the past 2 years. The heel showed a 15 cm diameter hypertrophic ulcer without any regional adenitis. Histology of the ulcer revealed a verrucous carcinoma. The patient is doing well to date following a below-knee amputation done 16 months ago.

Case 2. A 60-year-old trader with BT leprosy had persistent ulceration of the

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left heel for 10 years. It became hypertrophic in the past 3 years. The ulcer measured 6 × 8 cm and there were a few small non-tender lymphnodes in the groin. Biopsy of the ulcer showed squamous cell carcinoma. A below-knee amputation was done 17 months ago and he is doing well.

Case 3. A 43-year-old male with LL leprosy had a 12 cm diameter hypertrophic ulcer of the middle of the sole of his right foot for 10 years. There were a few small non-tender lymphnodes in the right groin. Biopsy of the ulcer showed squamous cell carcinoma. A below knee amputation was done. One month following the amputation the patient died with a large ulcerating mass in his right groin.

Case 4. A 70-year-old male with BT leprosy presented with a 4 × 5 cm hypertrophic, hyperaemic ulcer of his left palm of 4 years duration (Figure 1). The ulcer was said to have developed after a 15 kilometer bicycle ride and was persistent. Rubbery tender nodes were palpable in the left axilla. Biopsy of the ulcer revealed verrucous carcinoma (Figure 2). A below-elbow amputation was done 3 months ago and the patient is well up to the time of writing.

Case 5. A 55-year-old male having BT leprosy reported with a 14 × 8 cm ulcer on the postero-lateral aspect of his left heel of 2 years duration. It started as a hard nodule 2 years ago and the patient pricked it thinking it was a thorn blister. It had been growing since then. The ulcer was hypertrophic with nodular margins overhanging the edges, projecting 3 cm above the level of the surrounding skin. Multiple intracutaneous nodules over the lateral aspect of the left leg, presumably secondary deposits, were also present (Figure 3). The left groin showed a 8 × 6 cm clump of lymph nodes. Biopsy of the ulcer revealed squamous cell carcinoma

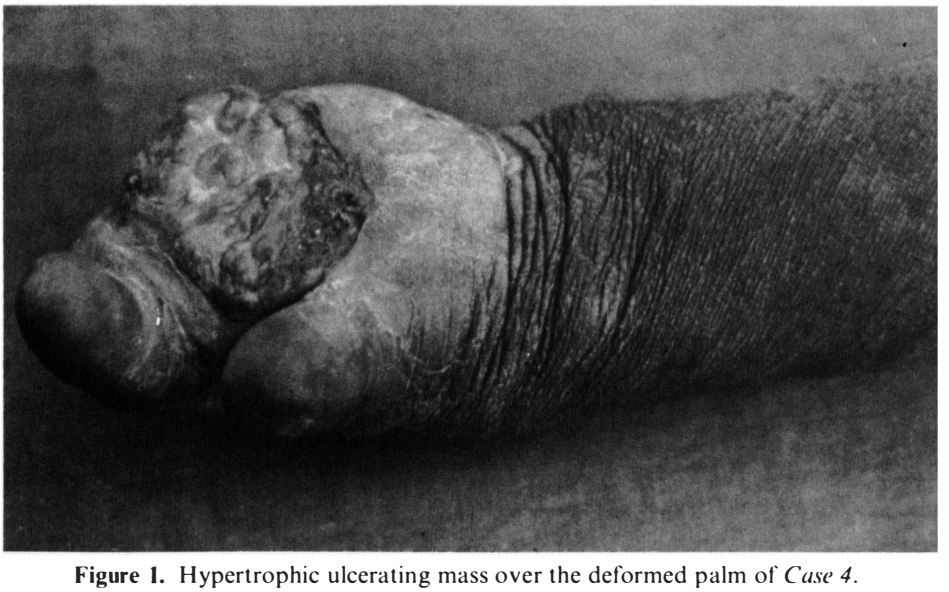

Figure 1. Hypertrophic ulcerating mass over the deformed palm of *Case 4.*

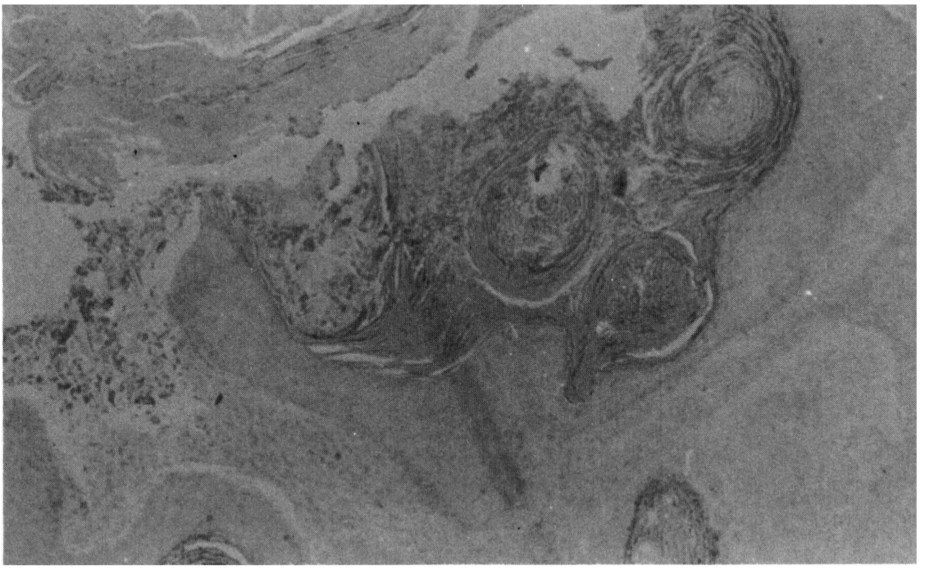

Figure 2. Biopsy of the same patient as in Figure 1, shows large cysts filled with keratin flakes in the malignant squamous epithelium: verrucous carcinoma. (HE × 40).

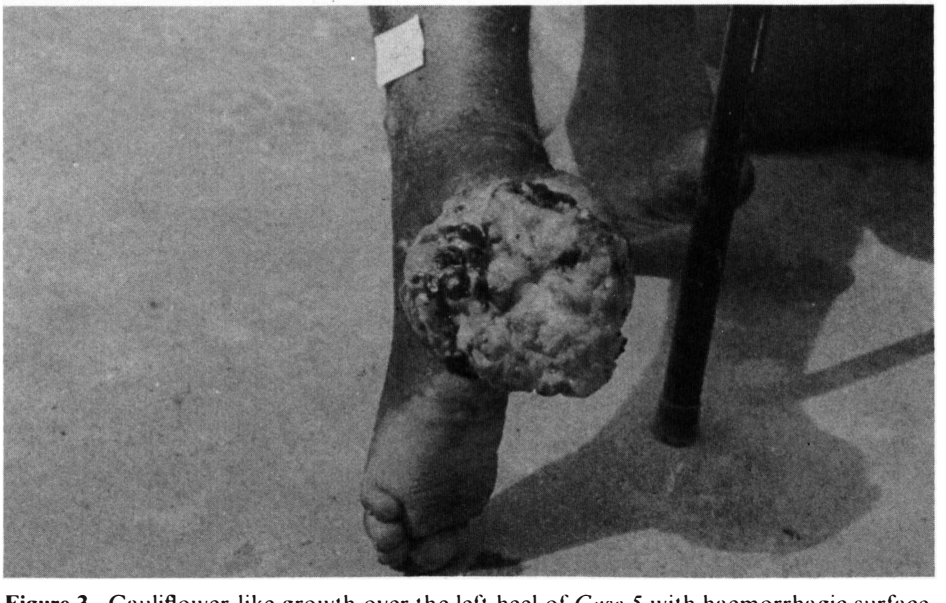

Figure 3. Cauliflower-like growth over the left heel of *Case 5* with haemorrhagic surface.

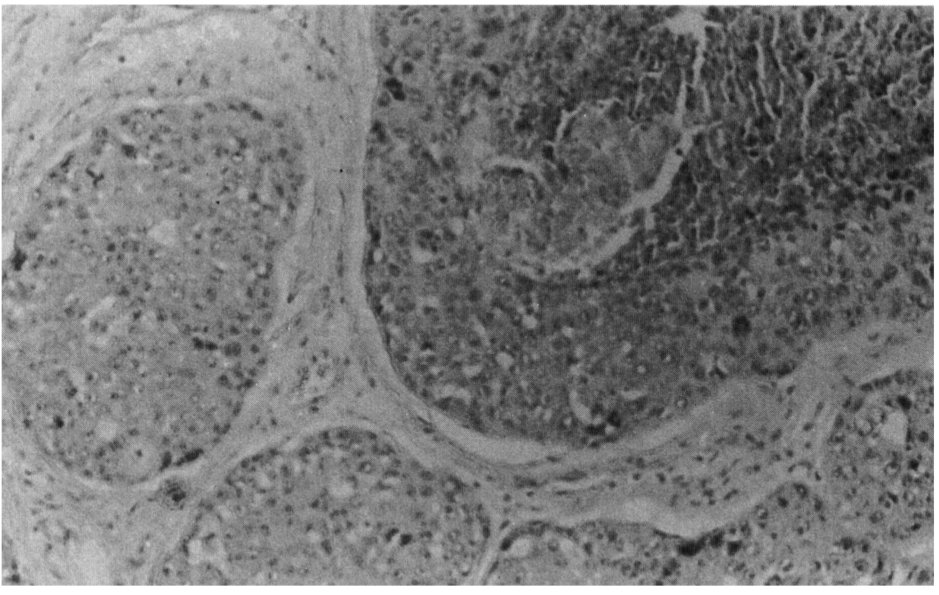

Figure 4. Multiple islands of malignant squamous epithelial cells infiltrating deep into the connective tissue, *Case 5* (HE × 400).

(Figure 4). A palliative left below-knee amputation was done 9 weeks prior to the compilation of this manuscript and the patient is well so far.

Discussion

Squamous cell carcinoma is a frankly malignant epithelial tumour arising from epidermal cells. It is more common in males in the older age group. There are many predisposing factors like solar irradiation, leucoplakia, Bowen's disease, burn scars, xeroderma pigmentosa etc, including chronic ulcers of the skin.

Leprosy predisposes to some malignancies[1] but cancer development in chronic trophic ulcers is rare.[2 4] Though chronic trophic ulcers over hands and feet are very common in leprosy, this malignant transformation is so rare as to be still presented as single case reports.[5]

The usual squamous cell carcinoma merits no special histological description. Verrucous carcinoma is its variant with very slow growth, poor invasive nature and good prognosis. Histologically it is characterized by large keratin filled cysts.

Among the 5 cases currently being reported, 4 were plantar ulcers. The one found on the palm is distinctly rare and we were not able to find such a report in literature. All the cases reported to the hospital after several years of persistent ulceration. The fact that the duration was long and 3 out of 4 were verrucous type, indicates that the prognosis is good. Depressed cell-mediated immunity in *Case 3*

with LL leprosy might have contributed both to the development of the carcinoma and early death due to metastasis.

Detection of 5 cases in this centre in a brief period of 2 years indicates that it is not so rare. One should specifically look for it in chronic ulcers so that treatment can be given early and adequately.

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Obituary

MITSUGU NISHIURA MD
1920–1985

Dr Mitsugu Nishiura, an emeritus professor of Kyoto University, died in New Delhi from a cerebral haemorrhage on 18 January 1985. He was studying at the Central JALMA Institute for Leprosy in Agra where he had been sent by the Japanese International Cooperative Agency. It had been a long-cherished desire of his to devote himself to study at JALMA, Agra on his retirement from Kyoto University.

Professor Nishiura was born in Kobe on 4 March 1920. After graduating from the Faculty of Medicine, Kyoto University, in March 1946, he worked in the Department of Dermato-urology, Kishiwada Municipal Hospital, and then in 1952 he started to work as an assistant in the Leprosy Research Laboratory of the Department of Dermatology, Kyoto University. In 1959, he visited the Indian National Cancer Centre in Bombay to study abroad for 1 year under a Rockefeller fellowship and received a Doctorate of Medicine later that year. In 1960, he became a professor in the Faculty of Medicine, Kyoto University, and continued his medical career as a head of the Leprosy Research Laboratory until he became an emeritus professor upon his retirement in 1983.

Professor Nishiura published many excellent studies on the pathology of leprosy, and above all, the results of his study on the application of electron microscopy to the bacteriology and pathology of leprosy won worldwide recognition. He was given the Sakurane Prize by the Japanese Leprosy Association in 1962 and the Seto Prize by the Japanese Society of Electron Microscopy in 1976. Furthermore, his technological studies of electron microscopy led to the production of a freeze replication apparatus of his own design, and contributed to the diffusion of the freeze-etching technique.

Professor Nishiura took great interest in giving medical students the opportunity to learn the clinical practice of leprosy, since the Leprosy Research Laboratory was one of the few national facilities where outpatients and inpatients with leprosy could be treated. This contributed to the diminishing prejudice against leprosy on the part of medical doctors and students, leading to many doctors becoming skilled in this field, and who are now actively conducting studies on leprosy.

Professor Nishiura visited Thailand, India, Cambodia, Bangladesh and Venezuela where he participated in international cooperative studies with native researchers. It is specially significant that he had stayed in the Central JALMA Institute for Leprosy and he had been studying as well as teaching his juniors since the faculty was constructed by the Japan Leprosy Mission for Asia (JALMA). He also contributed to the smooth transfer of its control from JALMA to the Indian government.

His memory will be kept alive by his friends, and also by all those who benefited so greatly from his advice or his assistance in training.

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Lepr Rev (1985) **56**, 256–257

Domiciliary and Field Work

Ethiopia; *Manual for multiple drug therapy, 1983*

This manual was produced by the Leprosy Documentation Service (INFOLEP) in Amsterdam in December 1983. The main text is only 20 pages long, but there are extensive appendices. The chapter headings read: 1 Introduction; 2 Categories of patients; 3 Need for release from treatment (RFT) before MDT is introduced; 4 Criteria for release from treatment of patients who have already been treated for many years; 5 Diagnosis and classification of new patients; 6 Registration of new and old patients for MDT; 7 The multiple drug regimens; 8 Procedures during treatment; 9 Slit-skin smears; 10 Side-effects of the drugs and complications; 11 Duration of the treatment; 12 Follow-up after Release from Treatment; 13 Retreatment; 14 Reporting; and 15 Evaluation of the completion of MDT.

The Appendices are: I Leprosy Control Patient Record Card with instructions; II The Leprosy Treatment Register; III The Central Register; IVa Quarterly Report Leprosy Patients; IVb Annual Report Leprosy Patients, V Quarterly Report of Patients on MDT; VI Six-monthly Cohort Report on the Completion of Treatment of Paucibacillary patients; VIIa Six-monthly Cohort Report on the Completion of Treatment of Multibacillary Patients; VIIb Annual Report on the Completion of Treatment of Multibacillary Patients; VIIIa Taking and fixing of slit-skin smears; VIIIb Skin Smears Services Request and Result list; VIIIc Quality Control Skin Smears Service; IX Account of receipts and issues of leprosy drugs; X Transfer and Referral of Leprosy Patients; XI Possible side-effects of Leprosy Drugs; XII Relapse, Reaction and Reactivation; XIII List of code numbers of Regions and Awrajas.

This manual which was one of the first of its kind, is also one of the best; it is extremely well thought-out and detailed and it bears the hallmarks of a combined authorship of considerable experience. In the light of new knowledge gained during the implementation of MDT since 1983, we understand that new versions, for both the National Leprosy Control Programme and the ALERT Leprosy Control Programme in the Shoa Province, are under consideration. Enquiries to Leprosy Documentation Service (INFOLEP), Royal Tropical Institute, Mauritskade 63, NL 1092, AD, Amsterdam, The Netherlands.

Teaching and learning in leprosy. Leprosy Documentation Service, Amsterdam

This well-produced, ring-bound volume is a guide to the material available on this topic. The Introduction reads: 'An attempt has been made to give as broad a picture as possible with respect to language, subject aspects of leprosy, types of printed material, and groups of leprosy workers (ranging from research workers to junior health staff).'

Also included is a description of the ILA Workshop on Teaching and Training held in New Delhi in 1984; recommended materials; planning and production of individual items of teaching/learning materials; and a list of materials that are available in some 28 languages. Price: $5.00

Further details are available from: Leprosy Documentation Service (INFOLEP), Royal Tropical Institute, Mauritskade 63, 1092 AD Amsterdam, The Netherlands.

Vice-President of India visits remote leprosy centre

The Vice-President of India Mr R Venkataraman's visit to Kasturba Kushta Nivaran Nilayam, Malavanthangal, South Arcot District marked 40 years of leprosy relief and control service. In his welcoming address Professor Jagadisan told of how the prevalence of leprosy has dropped in the last four decades 48 per thousand to 5 per thousand. He went on to say that now the incidence of leprosy was much lower the Institution is being transformed from a leprosy centre to a rural health and development centr primary health care, prevention of blindness, eye camps and dental care being the essential components. The facilities, once only for leprosy patients, are now being opened to non-leprosy boys and girls, e.g. spinning, weaving and tailoring training units, furthering the integration of leprosy patients with others of the community.

Dr Raghavendra Row Memorial Teaching Programme in Leprosy, 1985. Acworth Leprosy Hospital

The one-day programme for this year held in Bombay was devoted to the teaching of the pathology of leprosy to a small group of pathology post-graduate students. It was held at the Department of Pathology, Grant Medical College, and Dr C J G Chacko was the guest teacher.

A special feature of the programme was that after being introduced to the basis of the immunopathologic classification of leprosy each participant was presented with a set of histology slides of skin and nerve for individual study and evaluation. The teachers then demonstrated features of note and answered questions. Dr R V Bapat also spoke briefly about recent developments in the immunology and microbiology of leprosy.

The participants were asked to evaluate (anonymously) the day's programme. All of them expressed their satisfaction with the plan of teaching and said that they had benefited from the histology teaching.

The suggestions made included: (a) an extended programme of 2 days, (b) more participants, (c) practicals in staining of

bacilli in sections and smears, and (d) attention to leprosy lesions in other tissues. Many felt that a concentrated pathology teaching programme should be made an annual event.

Books by post, Intermediate Technology Development Group

This organization, an independent charity, aims 'to help people work themselves out of poverty by providing information, advice and assistance on the choice of appropriate technologies. For these technologies to be accessible to those who need them most, they must be relatively small, cheap and simple to use. They must make the best use of readily available local skills, and resources, and minimize the demands on scarce and imported resources.'

Leaflets about the work of ITDG are available on request. In the 1984–5 *Books by Post Catalogue* some of the titles included under 'Health' are: *Auxiliaries in primary health care, Helping health workers learn, Low cost aids, and Primary child care.*

Intermediate Technology Development Group Ltd, 9 King Street, Covent Garden, London WC2E 8HW, United Kingdom.

Centre for Educational Development in Health, Arusha, Tanzania

This Centre came into being in October 1982 as a result of the Ministry of Health of Tanzania and voluntary agencies recognizing a need to establish a mechanism which would ensure that investments and activities would really contribute to primary health care, which the Ministry has adopted as their key strategy for obtaining WHO's goal of Health for All by Year 2000. The functions of the Centre include: 'development of curricula, production of books, teaching materials and aids, training of teachers, research, and the organization of short courses and seminars to provide continuing education for teachers and health workers of all categories'.

The Centre offers a one-year Diploma of Health Personnel Education course for all teachers of the health professions. The emphasis is on the practical implications of how to provide good and relevant training for health workers in primary health care settings.

For further details of this and other courses and services please write to: The Principal, Ministry of Health, Centre for Educational Development in Health, Arusha, (CEDHA), PO Box 1162, Arusha, Tanzania.

Instant slides

Polaroid have now developed a system which produces mounted 35 mm slides within minutes of photographing a subject. Use any of the following films, colour, high contrast black and white, or continuous tone black and white, together with a developing pack (one is issued with every film).

For further details please write to: Customer Service, 35 mm, Polaroid (UK) Ltd, Ashley Road, St Albans, Herts AL1 5PR, England.

Leprosy lesions in skins of different colours, TALC

This 24-slide set (2 lesions shown on each slide) provides a wide spectrum of types of leprosy lesions, which are basically typical of the various key points on the Ridley–Jopling spectrum, as seen in different coloured skins. The colours range from pale Chinese to dark Indian. There is also an accompanying tape which provides a clinical description of the various lesions seen in leprosy. This slide set is perhaps most useful to those working in South East Asia and is also a supplement to the TALC sets on 'Leprosy and childhood' and 'Classification of leprosy'.

For full details of these and other publications (slide sets, books and flannelgraphs) write to: Teaching Aids at Low Cost, PO Box 49, St Albans, Herts AL1 4AX, United Kingdom.

Formation of Karnataka State Anti-Leprosy Council, India

This body has been formed to promote anti-leprosy work in the State of Karnataka. It is their experience that stimulation of such work comes from independent bodies. The Council will be actively working in the leprosy field but will work through existing agencies. It will therefore not be a burden to the Government or Voluntary Agencies. The President Dr M S Nilkanta Rao has issued a letter giving the reasons for forming the council, and its primary object; 'To improve the anti-leprosy work in the State—both governmental and voluntary—by all scientific means.'

Further details are available from the President, Karnataka State Anti-Leprosy Council, No. 16 Hutchins Road, Bangalore-560 005, India.

Histopathology Services for Developing Countries

For the last 15 years the Department of Histopathology at St Thomas' Hospital has provided a free, postal, diagnostic service for a number of hospitals, both government and mission, in developing countries. It was originally envisaged that the need for such services would decrease as they were built up locally. For a variety of reasons, differing from country to country, this has not happened and the need is still there and likely to continue. To meet these problems and to provide histopathological expertise in parasitic, communicable and other tropical disease in the UK a consultant histopathologist post has been created jointly with the London School of Hygiene and Tropical Medicine and University College Hospital Medical School. This post has been filled by the appointment of Dr S B Lucas who has spent 2 of the last 4 years in this unit and who is keen to maintain or increase diagnostic services, including leprosy histopathology. Specimens should be sent to Dr S B Lucas, Department of Histopathology, School of Medicine, University College London, University Street, London WC1. (Tel: 01-387-9300.)

Lepr Rev (1985) **56,** 258–259

Reports, News and Notes

Dr Ross St C. Barnetson joins the Editorial Board of *Leprosy Review*

It is with very great pleasure that we record the acceptance by Ross Barnetson of an invitation to join our Editorial Board. Prior to his appointment as consultant dermatologist in the Department of Dermatology in the Edinburgh Royal Infirmary, he worked for several years on the teaching and clinical staff of the All Africa Leprosy and Rehabilitation Training Centre (ALERT) in Addis Ababa and has contributed extensively to studies on the immunological and clinical aspects of this disease. He brings a rare combination of expertise in dermatology, leprosy, immunology, clinical work and teaching, which will surely be of the greatest value to this Journal. *Editor*

Luxembourg Award to Pierre Van den Wijngaert, General Secretary of ILEP

On the occasion of the recent meeting of the International Federation of Anti-Leprosy Associations (ILEP) in Luxembourg, the Minister of State, Mr Jacques Santer presented Mr Pierre Van den Wijngaert with the 'Ordre du Mérite du Grand-Duché de Luxembourg' in recognition of his long and distinguished contribution to the fight aginst leprosy. Mr Wijngaert was founder of the Belgian association 'Les Amis du Père Damien' and he has been General Secretary of ILEP since its foundation in 1966. The Editorial Board of *Leprosy Review* congratulates Mr Van den Wijngaert on this richly deserved award and wishes him many more happy years of work with ILEP.

Teaching and learning materials on leprosy from The Leprosy Mission (International), London

In her Report for 1984, Miss P Jane Neville, Education and Training Director, summarizes the activities of the 'Teaching and Learning' Distribution Service 1982–84 and draws attention to the fact that during 1984 a total of 11,026 items were despatched from London to various leprosy-endemic areas overseas. Fifty countries were contacted during the year, bringing the grand total of countries contacted to date to 106. There are 20 items on the TLMI list of materials and a checklist is provided indicating their suitability for doctors, health programme planners, health educators, shoe workshop managers, junior health workers, supervisors, senior health workers, laboratory technicians, physiotherapy technicians, medical students and surgeons. Some items are free, others are to be paid for. Sea mail is used free of charge, but there is an extra charge for despatch by air mail, if this is requested. Enquiries to: Teaching and Learning Materials, The Leprosy Misson (International), 50 Portland Place, London W1N 3DG, England—*but please note that workers in India* should apply for the printed book list and prices to The Leprosy Mission, CNI Bhavan, 16 Pandit Pant Marg, New Delhi 110 001, India.

LEPRA Prize Essay Competitions for Medical Students in the UK and India

In the United Kingdom, the subjects offered for 1984 were 'Monoclonal antibodies and Recombinant DNA technology; present and future use in leprosy and tuberculosis' or 'Leprosy will be most expediently controlled by the use of fully integrated programmes which make use of the primary health care approach'. The first prize was awarded to Mr Michael Seckl of University College Hospital, London for an entry of exceptional quality on monoclonal antibodies and DNA. This manuscript has been submitted for possible publication in the medical press and the award will be made at the Annual General Meeting of LEPRA in London in May 1985. The subjects chosen for the UK competition in 1985 are 'The relation between allergy and immunity in leprosy' or 'Leprosy will be controlled by an anti-leprosy vaccine in conjunction with chemotherapy, not by improvement in socio-economic conditions.' Information has, as usual, been distributed to all 31 universities with a medical school in the UK.

In India, at St John's Medical College, Bangalore, no entry of sufficient merit was received in 1984, but the prize is to continue in 1985 with the subjects—'Ulceration of the foot in leprosy' or 'Leprosy will be controlled by an anti-leprosy vaccine in conjunction with chemotherapy, not by improvement in socio-economic conditions.' Further details: Dr Ciaran Kelly, LEPRA, Fairfax House, Causton Road, Colchester CO1 1PU.

Core Journals in Dermatology

This A4 document of about 14 pp, described as 'The fastest current awareness abstracting Service' is published by Elsevier Science Publishers in collaboration with the Excerpta Medica Database, and supported by Glaxo. It deals with dermatology (only) and covers material of relevance in 5 leading general journals (*Annals of Internal Medicine, British Medical Journal, Journal of the American Medical Association, Lancet* and *New England Journal of Medicine*) and 14 specialist journals (*Acta Dermato-Venereologica, Annales de Dermatologie et de Venereologie, Archives of Dermatological Research, Archives of Dermatology, British Journal of Dermatology, British Journal of Venereal Diseases, Clinical and Experimental Dermatology, Contact Dermatitis, Dermatologica, Hautarzt, International Journal of Dermatology, Journal of the American Academy of*

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Dermatology, Journal of Cutaneous Pathology and Journal of Investigative Dermatology). Abstracts are published 4–6 weeks after receipt of the journal. It is issued monthly at an annual subscription which varies between US $50 and $100, depending on individual or institutional status. Apply to Elsevier Science Publishers, Journals Division, PO Box 211, 1000 AE Amsterdam, The Netherlands.

Medical Research Charities in the UK; Handbook.

This '*Handbook of the Association of Medical Research Charities*', dated May 1984, gives details of the activities and main interests of the major medical research charities and provides a forum for their regular meetings. These give members an opportunity 'to discuss strategy and organisation and to hear from visiting speakers about trends and developments of significance for medical research'. The annual income of the 35 members amounted to £128,000,000 in 1983, of which £77,000,000 was spent on research, but the Introduction reminds the reader that many charities in the UK are not members of this Association and that the overall provision of funds for medical research are considerably greater. Copies are obtainable from The Association of Medical Research Charities, The Development Trust for the Young Disabled, Royal Hospital and Home for Incurables, West Hill, Putney, London, SW15 3SW.

Heiser Program for Research in Leprosy, 1986

Once again this programme has sent details of the awards which are offered. These fall under the headings of post-doctoral research fellowships, research grants and visiting research awards. The Scientific Advisory Committee includes Maclyn McCarty, W Lane Barksdale and Barry Bloom. Applications and enquiries to Mrs Barbara Hugonnet, Director, Heiser Program for Research in Leprosy, 450 East 63rd Street, New York, New York 10021, USA.

Robert Cochrane Fund for Leprosy

The fund, in memory of the contribution of the great leprologist Robert Cochrane, is administered by the Royal Society of Tropical Medicine and Hygiene. It is to be used to finance up to 2 travel fellowships each year to a maximum value of £1000 each.

The intention is to enable leprosy workers to travel for practical training in field work, or in research, or to enable experienced leprologists to travel in order to provide practical clinical training in a developing country. There is no restriction on the country of origin or destination providing the above requirements are fulfilled.

Application forms are available from the Society and must be received by the Society at least 6 months ahead of the proposed trip. All applications must be sponsored by a suitable representative of the applicant's employer or study centre, and agreed by the host organization. A 2 page report on the travel/study should be submitted to the Society within 1 month of the recipient's return. Apply: The Administrator, Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London W1N 4EY.

XVII World Congress of Dermatology, Berlin, 1987

We have received preliminary information about this Congress which will be held in Berlin from 20 to 25 September 1987. The main headings of the programme are: special lectures; advances in dermatology; symposia; workshops; courses; free communications; case presentations; informal discussion groups; poster communications; scientific exhibitions; audio-visual communications; scientific film sessions; update educational sessions; question and answer sessions. Further information from Professor Dr C E Orfanos, General Secretary, Department of Dermatology, University Medical Centre, Steglitz, Hindenburgdamm 30, D-1000, Berlin 45, Germany.

XIII International Leprosy Congress, The Hague, Netherlands, 1988

The President and Secretary of the International Leprosy Association are happy to announce that the XIIIth International Leprosy Congress will be held at the Hague, Netherlands from 11 to 17 September 1988. The Pre-Congress Workshops will be held on 8, 9 and 10 September 1988. The Inauguration of the Congress has been tentatively fixed for the evening of 11 September 1988 and the Scientific Sessions will start on 12 September. The concluding session will be on the forenoon of 17 September 1988.

Mr H E M De Bok of the Netherlands Leprosy Association is making the arrangements for the Congress and the first Information Brochure will be sent to you by September 1985.

If you have any suggestions, please contact: Dr R H Thangaraj, Secretary—ILA, No. 5 Amrita Shergill Marg, New Delhi 110003, India.

XXVIth IUAT World Conference on Tuberculosis and Respiratory Diseases, Singapore, 4–7 November 1986

The Conference is open to members and non-members of the IUAT. The programme will cover the fields of *tuberculosis* and *non-tuberculous respiratory diseases*, including smoking-related issues, from the clinical, immunological, physiological, diagnostic, therapeutic, preventive and epidemiological points of view, as well as aspects related to *action programmes* and their assessment, training of personnel, related diseases such as those due to non-tuberculous mycobacteria and problems of *delivery of services* (such as primary health care, compliance, community mobilization).

There will be 6 morning plenary sessions, afternoon parallel sessions, poster sessions, sunrise seminars and several workshops. Official languages will be English and French, Spanish interpretation is being considered.

Abstracts for proposed presentations should be sent to: IUAT Secretariat, 3, rue Georges Ville, 75116 Paris, France. Abstract forms will be distributed by the end of March 1985 to all IUAT members. Non-members may obtain copies by writing to us in Paris. *Proposals must concern original work in progress.*

The IUAT world conferences are the only international forum for tuberculosis, and in respiratory disease the IUAT is working out a programme of research, training and action for the promotion of respiratory health.

For practical information, kindly write to: Secretariat of the XXVIth IUAT World Conference on Tuberculosis and Respiratory Diseases, c/o SATA, 267 Cantonment Road, Singapore 0208, Republic of Singapore.

Lepr Rev (1985) **56**, 260–261

Letters to the Editor

HOW INFECTIOUS IS SECONDARY DAPSONE-RESISTANT LEPROSY?

Sir,

There is a growing view, supported especially by work in South India, that dapsone resistance does not pose such a serious threat to leprosy control as has been believed. One question which has to be anwered unequivocally concerns the infectivity of secondary dapsone-resistant leprosy and, as far as I can ascertain, this has not been investigated. It is well known, of course, that skin smears from active lesions in a case of dapsone-resistant relapse show both BI and MI strongly positive, whereas those from clinically normal skin are generally negative, thus supporting a hypothesis that the total bacillary load is probably less than in an untreated lepromatous case. The frequency of positive nasal smears and nose blows, which would be more generally acceptable as an indicator of infectivity, has, however, not been recorded.

One study[1] investigated the bacteriology of the nose in 62 lepromatous patients treated for varying periods with dapsone monotherapy. Nasal smears were examined from a total of 49 cases but in only one was the MI positive. Dapsone sensitivity was not investigated in this series.

I have myself reported infiltration and nodules of the palate and laryngeal involvement in patients with clinical evidence of secondary dapsone resistance.[2] In these cases, however, nasal smears were not done.

In my opinion, there is a need for a well-planned study to determine the proportion of secondary dapsone-resistant cases which excrete viable *Mycobacterium leprae* from their nasal mucosa and thus constitute a potential source of transmission of disease. Such a study should be linked to an investigation of the probable index case in all patients with proven primary dapsone resistance.

H W WHEATE

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Sutton
Surrey, SM2 5JE

References

[1] Barton RPE, Rees RJW, McDougall AC and Ellard GA. The nose in lepromatous leprosy, bacteriological and histopathological studies of patients treated with dapsone monotherapy for varying periods of time. *Int J. Leprosy*, 1982; **50**: 58–67.

[2] Wheate HW. Dapsone-resistant leprosy as seen in Africa. *Quaderni di Cooperazione Sanitaria*—Health Co-operation Papers, 1981; **1**: 41–6.

TUBERCULOID LEPROSY AT THE SITE OF A DOG BITE

Sir,

Although it is well known that skin lesions of sarcoidosis sometimes appear in and around scars ('scar sarcoidosis'), it is less well known that skin lesions of leprosy may originate in scars. Leprosy workers seem to have made little note of this interesting phenomenon, if reports in the literature are any criterion, and the only published photograph with which I am acquainted is given in Jopling & Harman[1] in which a lesion of tuberculoid leprosy is shown involving 3 vaccination

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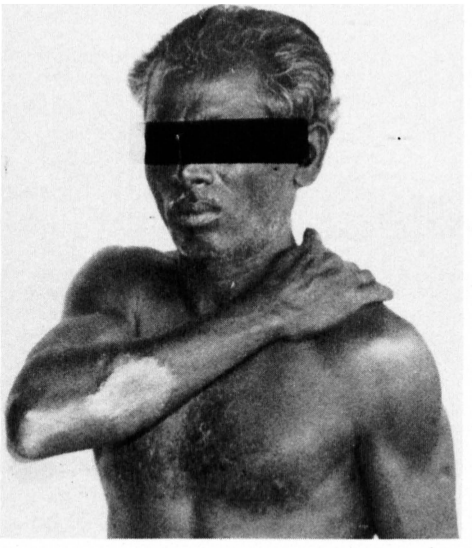

Figure 1

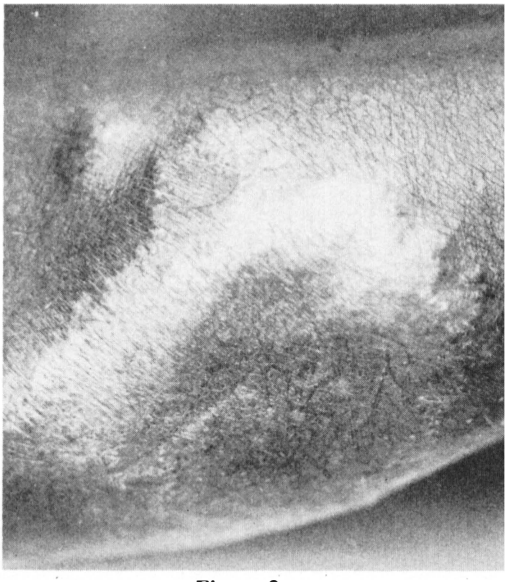

Figure 2

scars. These authors state: 'leprosy lesions of all types tend to surround scars'. My interest in this subject has been aroused by the following case history:

A 45-year-old male reported at the Skin Clinic of the Western Railway Hospital, Bombay, with a hypopigmented skin lesion on the dorsum of his right forearm. The lesion measured 4 × 4 in., was flat in the centre and raised at the periphery, was anaesthetic and virtually hairless although situated in a hairy region of skin (Figures 1 and 2). No AFB were found in skin smears, and histology was diagnostic of tuberculoid leprosy. The patient stated that he had been bitten on that same forearm by a street dog 4 years ago, and the skin lesion appeared 6 months ago as a hypopigmented patch in the scar, subsequently enlarging to its present size. On examination, the scar could be seen in the central region of the lesion (Figure 2).

As leprosy has never been described in dogs, the question of *Mycobacterium leprae* having been introduced into the patient's skin does not arise, but there is a distinct possibility that at some time in the future a case of leprosy resulting from animal bite may be reported. I have in mind the report of Lumpkin *et al.*[2] on leprosy developing in 5 native-born Texans who handled wild armadillos, added to the fact that naturally-acquired leprosy has been found in the chimpanzee and the Mangabey monkey.

M P GARG

Western Railway Hospital
Maratha Mandir Road
Bombay 400 008
India

References

[1] Jopling WH, Harman RRM. Leprosy. In: Rook A, Wilkinson DS, Ebling FJG, eds. *Textbook of Dermatology*. Oxford: Blackwell, 1979: 749–65.

[2] Lumpkin LR, Cox GF, Wolf JE. Leprosy in five armadillo handlers. *J Am Acad Dermatol* 1983: 899–903.

Lepr Rev (1985) **56**, 262–263

Book Reviews

Leprosy in Malaysia: past, present and future. A Joshua-Raghavar, published from Sungai Buloh, Selangor, West Malaysia, 1983.

This is a book of 22 pages, plus substantial appendices, written by a secondary school teacher in Malaysia, who himself contracted leprosy in 1932. It is a detailed and comprehensive account of leprosy in Malaysia, including: Introduction; The Early Period up to World War II; The Era of Segregation; Developments in the Post World War II Period; Leprosy Control; Post-War Developments in the Social and Economic Fields; the Present; the Future; Human Aspects and Appendices. There are excellent illustrations in black and white and an extensive bibliography. In the UK, copies are available on application to Dr R J W Rees, Clinical Research Centre, Watford Road, Harrow, Middlesex, HA1 3UJ.

Leprosy in the Light Skin; an illustrated manual. D L Leiker and E Nunzi, published by Associazione Italiana 'Amici di Raoul Follereau', Organizzazione per la Cooperazione Sanitaria Internazionale, OCSI, Via Borselli, 4, 40135, Bologna, Italy. Received 1985.

This is a superbly produced hardback book of 199 pages, with over 300 colour prints covering virtually all aspects of leprosy on the skin. The written text covers definition, bacteriology, epidemiology, immunology, symptomatology, diagnosis, classification, reactions, complications, differential diagnosis, treatment, education of patients, contact examination, prophylaxis, employment of patients. There is also a selected list of references and useful addresses. The colour photography and the quality of reproduction are extremely high. This book should be of great value to leprologists and dermatologists dealing with leprosy in North America, Europe, China, the Far East, Philippines and other parts of the leprosy-endemic world where leprosy has to be recognized in patients with light skin. Enquiries to the above address. Price $30 (US) to include air mail postage.

Hansenologia; Dermatologia Tropical. Sinesio Talhari and Réne Garrido Neves, published in Manaus, Brasil, 1984.

This is a strongly-bound paperback of 102 pages plus bibliography, containing a large number of prints, mainly in black and white, but also in colour. It is written in Portugese only, with the intention of '. . . helping general practitioners, students of medicine and even dermatologists in the diagnosis and treatment of leprosy'. Sinesio Talhari is titular professor of dermatology in the 'Fundacao Universidade do Amazonas' and René Neves is professor of dermatology in the 'Universidade Federal Fluminense' in Niteroi in the State of Rio de Janeiro. The numerous black and white plates are of high quality and illustrate most aspects of the disease extremely well. This text should be easily understood by Spanish-speaking readers and it will surely be of the greatest value in South America, and perhaps in other parts of the world also. Price $12.00 (US). Apply to Clinica de Dermatologia Tropical, Av. Japurá 572, 69.000, Manaus-Am-Brasil, South America.

Leprosy in Africans. W K Jacyk, published by the German Leprosy Relief Association, D-8700, Wurzburg 11, Dominikanerplatz 4, West Germany, 1983.

This is a strongly-bound paperback booklet of 52 pages with an equal number of very good quality colour prints showing leprosy on the African skin, together with some pictures of conditions other than leprosy by way of differential diagnosis. There is no formal text, but each picture has a brief description. The excellent photographs were originally taken mainly for teaching purposes in the Department of Medicine in Ahmadu Bello University, Zaria/Nigeria.

It should be carefully noted that there are a few errors in the captions:

Page 16: the number 16 and the description are correct, but the photograph should be that shown on page 35.
Page 35: reverse situation; the number and title are correct, but the correct photograph should be that shown on page 16.
Page 32: the photograph is upside down.

Apply to DAHW at the above address.

Management of Paralytic Deformities in Leprosy. J G Andersen and J W Brandsma, All Africa Leprosy and Rehabilitation Training Centre, PO Box 165. Addis Ababa, Ethiopia, received 1984. Price $10.00 (US), including mailing.

This is a strongly-bound paperback of 109 pages written by two highly experienced workers in ALERT on a matter of great importance. Their aim, as explained in the Introduction

has been to produce an introduction to reconstructive surgery as it applies to the sequels of peripheral nerve damage in leprosy.' The primary target groups are trainee surgeons and physiotherapists. The main headings include: Nerve damage in leprosy; Surgical considerations and priorities; Paralytic deformities of the face; Physiotherapy for paralysed eyelid muscles; Paralytic deformities of the hand; Secondary effects of ulnar and median paralysis; Exercises for the paralysed hand; Handsurgery pre-operative assessment; Paralytic deformities of the foot; Pre-operative Physio-

therapy. There is a selected bibliography. Enquiries to the above address.

Surgical Reconstruction and Rehabilitation in Leprosy. E P Fritschi, Schieffelin Leprosy Research and Training Centre, Karigiri, North Arcot District, Tamil Nadu, India, Pin. 632 105.

This is a strongly-bound paperback of 320 pages, including index, by the Director and Consultant Surgeon of the SLRT, published by The Director for Southern Asia, The Leprosy Mission, New Delhi, India. This is a second edition, dated 1984, the first having been in 1971. The chapter headings include: General principles of surgical reconstruction in leprosy; Peripheral neuritis in leprosy; The examination of the hand; The restoration of finger function; The management of miscellaneous contractures and deformities of the fingers; The restoration of thumb function—pinch and grasp; The triple nerve lesion; The surgery of facial deformity; Miscellaneous surgical conditions; The foot; Ulcers of the foot and their sequelae; Footwear for anaesthetic feet; Physiotherapy and Rehabilitation. The price in India is Rs 45 (approximately $3.00 US). Available from Director for Southern Asia, The Leprosy Mission, 4th Floor, Sheetla House, 73 74 Nehru Place, New Delhi 110 019.

Guidelines for Tuberculosis Control Programmes in Developing Countries. An OXFAM Memorandum. OXFAM Practical Guide No. 4. Paul Shears, the OXFAM Health Unit, 1985.

This is a strongly-bound paperback of 59 pages, including 7 appendices, produced by the OXFAM Health Unit, 274 Banbury Road, Oxford OX2 7DZ, at £1.50 per copy. The Introduction draws attention to the fact that a review of most tuberculosis control programmes in developing countries shows that we are currently achieving less than 50% success in treating patients with tuberculosis, and are even less successful in reducing the overall prevalence of the disease. This low success rate is attributed to difficulties in implementation at the district and village level and this manual has been written 'to assist heath workers and project leaders at this level, working in conjunction with the national TB control programme.' The 16 chapter headings are as follows: Introduction; Difficulties of TB control; Some examples from the field; Basic pathology and epidemiology of TB; Principles of control; Involving the community; Establishing the size of the TB problem in your community; Planning an appropriate TB control programme; BCG vaccination programme; Case-finding; Principles of drug treatment; Problems of drug treatment; Resistance, default or poor compliance; Monitoring and evaluating the programme; TB programmes in special situations; TB in children and non-pulmonary TB in adults; The role of development programmes in reducing the TB problem; Funding TB control programmes. There are also appendices on methods of tuberculin surveys, sputum microscopy, drug dosages, drug adverse effects, drugs for resistant cases, measuring drug compliance, useful addresses and further reading.

This booklet fills a gap which has been outstanding for many years and it will surely be invaluable to those responsible for tuberculosis control in the field of developing countries. Apply to OXFAM at the address above.

A. C. McDOUGALL

New approaches to the identification of parasites and their vectors. B N Newton and F Michal. Tropical Diseases Research Series 5, Schwabe, Basel, 1984.

This book is the proceedings of a symposium on the application of biochemical and molecular biology techniques to problems of parasite and vector identification held in November 1982. The parasites covered are the causative organisms of 5 of the diseases which fall within the mandate of the UNDP/World Bank/WHO special programme namely malaria, African and American trypansosmiases, filariasis, (including onchocerciasis), the leishmaniases and schistosomiasis. Leprosy is the 6th disease in the programme but was not covered in this symposium. It was felt by the participants that better methods of characterizing organisms which are morphologically identical but which differ quite markedly in their epidemiological significance are needed. It was emphasized that the various sophisticated technologies currently being used in research laboratories must be made applicable for use in the field. The techniques covered include enzyme electrophoresis, the use of monoclonal antibodies and DNA probes, the study of polytene chromosomes of dipteran vectors of disease and the biochemical analysis of cuticular hydrocarbons of insect vectors.

It is customary when introducing work on parasite or vector taxonomy to take it as axiomatic that correct classification is fundamental to efficient control. However, there is a shortage of examples where fine distinctions in taxonomy have actually led to a change in what control programmes can do. There may even be a tendency for the enthusiasm of laboratory biologists for their techniques to lead them to produce answers before finding out whether the questions matter. A useful directory to the questions in this field which do matter is given by G S Nelson. For example, he refers to the need for a better method for identifying malarial sporozoites in mosquitoes (which will become crucial in the field testing of transmission-blocking vaccines) and the monoclonal antibody method now becoming available to ease this problem. With regard to onchocerciasis, the papers by Nelson and Meredith both underline the importance of the difference between the blinding form of the worm *Onchocerca volvulus* which occurs in the West African savannah and the much less harmful parasite in the forest zone which nevertheless appears identical by normal microscopic methods. The distinction is crucial to the strategy of the current onchocerciasis control programme and in his chapter Omar reports a histochemical method by which the 2 forms of worm can apparently now be distinguished.

Rapid progress in parasite taxonomy is currently taking place and it is a pity that there was such a long delay in publishing these proceedings.

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1. Browne, S. G.: Lepr. Rev. *37*, 141 (1966)
2. Azulay et al.: Lepr. Rev. *46* (Suppl.), 99 (1975)
3. Schulz, E. J.: Lepr. Rev. *42*, 178 (1972)
4. Yawalkar, S. J., Vischer, W. A.: Lepr. Rev. *50*, 135 (1979)

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Further information is available on request.

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