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Teaching Materials and Services

News and Notes
The Lepra Evaluation Project (LEP), an epidemiological study of leprosy in northern Malawi. II: Prevalence rates

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Summary Prevalence data obtained during a population survey carried out by the Lepra Evaluation Project (LEP) in Karonga District in Northern Malawi (Central Africa) are presented and analysed. Three different prevalence measures are presented: of individuals with current clinical leprosy who are likely to benefit from (further) antileprosy treatment (the 'clinical' prevalence rate), of individuals with either current clinical leprosy or residual signs only (the 'visible' prevalence rate), and of individuals with any physical or historical evidence of present or past leprosy (the 'cumulative' prevalence rate). Effects of past treatment and leprosy control efforts come to light in the difference between the 'visible' rate and the 'cumulative' rate and indicate that about 61% of the leprosy patients in this area who have received antileprosy treatment in the past, from the Lepra Control Project, are now without remaining signs of clinical leprosy. Past BCG vaccination campaigns and active case finding through school surveys appear to have affected the current age and sex patterns of the disease. Prevalence rates are higher among females than males in the older age groups. The paper demonstrates how the observed pattern and extent of leprosy are a function of the prevalence measure used.

Introduction

Estimation of the number and proportion of individuals with leprosy in a population is important but difficult. Most of the prevalence information available to health ministries and recorded in the literature is drawn from control programmes. Such statistics are heavily influenced by methods and policy changes in case detection, diagnosis, registration, duration of treatment and release from control, and may be misleading.¹

The alternatives to routine control programme data are estimates derived from specially organized surveys. Despite their cost, large scale surveys have been carried out in a number of countries in recent decades, providing descriptions of leprosy prevalence in the Philippines,²

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Thailand, Burma, South India and West Africa. In general these surveys have indicated that the prevalence rate of leprosy in endemic areas increases with age up to a plateau at 20–40 years, after which it may fall. Prevalence rates are generally reported to be higher among males than among females, in particular for multibacillary disease. Beyond these generalities, the published data suggest that the pattern of leprosy varies greatly between different areas of the world in terms of its overall frequency and its distribution by age, sex and clinical classification. The implications of this variation, and to what extent it may reflect biological differences between populations, or methodological differences in case definitions or in the designs of the several studies, are unclear.

A major problem in any epidemiological investigation is that of case definition. What is the 'leprosy' whose prevalence is being measured? In a separate publication we have discussed the problem of defining a case in terms of deciding whether or not an individual has, or has had, disease attributable to Mycobacterium leprae. But that is not the only definition problem in measuring the

![Figure 1](image_url)

**Figure 1.** Relationship between different prevalence measures discussed in this paper. The sizes of the circles is not related to actual rates in Karonga District. The possibility of 'persisters' (dormant *M. leprae*) in individuals with residual signs of leprosy only and in individuals without remaining evidence of past clinical leprosy has been ignored in this schematic presentation.
prevalence of leprosy. Ideally we would like to distinguish four different prevalence measures: (1) of infection (implying the presence of living \textit{M. leprae} in the body); (2) of current clinical disease (implying that an individual is infected and that the infection has progressed to a stage where it can be detected clinically, and confirmed if necessary histopathologically or bacteriologically); (3) of visible disease (implying the presence of (clinical) signs attributable to current infection or residual signs of past infection with \textit{M. leprae}); and (4) of cumulative disease (including all individuals who have ever in their lives manifested clinical leprosy).

Figure 1 shows the relationship between these four different prevalence measures. The first is at present unobtainable, in the absence of sensitive and specific methods for detecting \textit{M. leprae} infection.\textsuperscript{8} Investigators are thus forced to concentrate upon the pattern of clinical and/or residual signs of leprosy only. Unfortunately, very few publications are clear even on this distinction, and on whether 'cured' or 'burnt out' individuals are included as prevalent leprosy cases or not. The situation is exacerbated further by the continued confusion over 'indeterminate leprosy' cases, which are often included in prevalence measures though the very diagnosis may be in doubt.\textsuperscript{9}

The problem of estimating and describing the pattern of leprosy remains important both for operational reasons in guiding leprosy control and for research purposes of understanding its natural history. In this paper we present and discuss the prevalence of leprosy based on data from the Lepra Evaluation Project (LEP) in Malawi. In so doing we explore different definitions of prevalent leprosy, in order to draw attention to their implications for describing the amount and pattern of leprosy in a community.

\textbf{Methods}

The methods of the LEP are described in detail in a separate publication.\textsuperscript{10} In this context we note that it began as a total population survey and was carried out in Karonga District, Northern Malawi. More than 112,000 people were interviewed between 1979 and 1984, representing virtually the entire population in all but the southernmost tip of the District.\textsuperscript{10} Leprosy patients were identified by paramedical workers (Leprosy Control Assistants, LCAs) who examined more than 97\% of those interviewed for skin lesions, enlarged nerves and disabilities. All untreated leprosy suspects and suspected relapses were also examined by the project Medical Officer (JMP), but patients already on treatment were examined by JMP as a matter of routine only if they were still clinically active. Biopsies were taken from 95\% of all newly found suspects, examined by Dr A C McDougall and reported in standardized format.\textsuperscript{11} In addition to information collected during the LEP survey, we have had access to all records of the LEPRO Control Project (LCP) which was active in Karonga District since 1973.\textsuperscript{12} In order to analyse the prevalence of leprosy in this population we have categorized all past and present cases according to four different sets of criteria: (1) diagnostic certainty; (2) classification; (3) current status; and (4) period of ascertainment. These categories are described below:

\section{Diagnostic certainty}

All individuals with any evidence of leprosy were assigned to one or another of four groups: (i) a 'narrow' (certain leprosy) group in which the overall level of certainty of the diagnosis leprosy and of the classification is extremely high; (ii) a 'middle' (probably leprosy) group, in which we expect that a high proportion of the individuals included are or were indeed cases of clinical leprosy but accept that a small proportion whose clinical signs were not in fact attributable to \textit{M. leprae} infection will also be included; (iii) a 'wide' (possibly leprosy) group containing only a small proportion of individuals with present or past clinical leprosy; and (iv) an 'out' group, in which the leprosy diagnosis is totally discarded. Individuals in this group can thus be considered to have or have had no evidence of clinical leprosy.
The method for assigning individuals to one or other of these groups is described elsewhere. Only ‘narrow’ (N) and ‘middle’ (M) group cases are included in the present analysis.

2 Classification

For the purpose of this analysis, all cases are classified as either paucibacillary or multibacillary on the basis of clinical diagnosis, which in turn included slit-skin smear information whenever available. Given our evidence, that there has been a tendency in Malawi to classify patients with bilateral deformities too far towards the lepromatous end of the spectrum we have included borderline (Madrid) and BB (Ridley–Jopling) cases in the paucibacillary group in this analysis. Thus our division is similar but not identical to the one recommended by the 1981 WHO Study Group.

3 Current status

We have used the following criteria in order to define the different prevalence measures discussed in the introduction (see Figure 1). Clinical prevalence implies one or both of the following: a positive BI in slit-skin smears or biopsy at the time of first examination by the LEP, or ‘clinically active’ skin lesions. (‘Clinically active’ implies that signs of inflammation were present.)

The visible prevalence rate includes, in addition to the above, all individuals with residual signs (i.e. clinically inactive skin lesions and/or typical disabilities) attributable to past infection with M. leprae.

Finally, the cumulative prevalence rate includes, in addition to the above, all individuals with a credibly documented history of leprosy in the past. The creation of this prevalence rate thus required a critical review of all past records of the LCP in Karonga District.

4 Ascertainment

Cases could be separated according to three distinct modes of ascertainment: (i) those who were originally diagnosed as leprosy patients by some institution or service other than Lepra before being registered by the Lepra Control Project (LCP) or being ‘rediscovered’ by the LEP; (ii) those who first self-reported to or were found during school surveys by the LCP between 1973 and June 1981 and who had not received antileprosy treatment elsewhere before. (After June 1981 the LCP stopped registering patients independent of the LEP); (iii) those who were first found or self-reported to the LEP during the 1979–84 survey and who neither were known to the LCP nor had a history of having received antileprosy treatment elsewhere before. The large majority of these patients were found by active case finding. In addition to clinical examination findings by a Medical Officer, we also have biopsy results for approximately 95% of the individuals in this group and can be more confident about both the diagnosis and the classification of leprosy in these individuals than in individuals ascertained previously.

Results

Frequency distributions of claimed years of onset of disease are shown for each ascertainment group in Figure 2. This shows that most patients first ascertained by an institution other than the LCP or the LEP reported onset of disease in the fifties and sixties, while most who were first registered by the LCP had onset of disease in the seventies. A large proportion (201/622 = 32.3%) of patients newly found during the 1979–84 LEP survey did not know the year of onset, but most claimed that onset occurred in the late seventies or early eighties.

Table 1 shows the breakdown by sex and clinical classification of cases included in each ascertainment group. The multibacillary proportion was by far highest among patients who first
Distribution of time of onset by mode of ascertainment

Figure 2. Frequency distributions of years of onset of leprosy (as remembered by the patients) by ascertainment group: (1) patients first ascertained by some organization other than LEPR A; (2) patients first ascertained by the LCP, 1973–81; (3) patients first ascertained by the LEP, 1979–84.
Table 1. Distribution of leprosy patients by classification, sex and ascertainment group. This table includes all individuals with current or past clinical signs attributable to infection with *M. leprae* (certainty groups N and M), Karonga District, Malawi, 1979-84

<table>
<thead>
<tr>
<th>Ascertainment group</th>
<th>Males</th>
<th>Females</th>
<th>% Females</th>
<th>% Females among paucibacillary patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Paucibacillary leprosy</td>
<td>Multibacillary leprosy</td>
<td>Paucibacillary leprosy</td>
<td>Multibacillary leprosy</td>
</tr>
<tr>
<td>'elsewhere'</td>
<td>215 (69%)</td>
<td>96 (31%)</td>
<td>239 (84%)</td>
<td>45 (16%)</td>
</tr>
<tr>
<td>by LCP</td>
<td>355 (93%)</td>
<td>24 (7%)</td>
<td>430 (96%)</td>
<td>16 (4%)</td>
</tr>
<tr>
<td>by LEP (excluding incidence cases)</td>
<td>235 (95%)</td>
<td>12 (5%)</td>
<td>373 (99%)</td>
<td>2 (1%)</td>
</tr>
<tr>
<td>Totals</td>
<td>785</td>
<td>132</td>
<td>1042</td>
<td>63</td>
</tr>
</tbody>
</table>
Table 2. Age specific prevalence rates (per 1000 individuals examined) of clinical leprosy among males and females in Karonga District, Malawi, 1979–84

<table>
<thead>
<tr>
<th>Age at examination</th>
<th>Total examined</th>
<th>Paucibacillary leprosy</th>
<th>Multibacillary leprosy</th>
<th>All leprosy</th>
<th>Total examined</th>
<th>Paucibacillary leprosy</th>
<th>Multibacillary leprosy</th>
<th>All leprosy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>cases</td>
<td>rate</td>
<td>cases</td>
<td>rate</td>
<td>cases</td>
<td>rate</td>
<td>cases</td>
</tr>
<tr>
<td>0-4</td>
<td>9702</td>
<td>2</td>
<td>0.2</td>
<td>0</td>
<td>—</td>
<td>2</td>
<td>0.2</td>
<td>0</td>
</tr>
<tr>
<td>5-9</td>
<td>8915</td>
<td>13</td>
<td>1.5</td>
<td>0</td>
<td>—</td>
<td>13</td>
<td>1.5</td>
<td>0</td>
</tr>
<tr>
<td>10-14</td>
<td>6916</td>
<td>17</td>
<td>2.5</td>
<td>0</td>
<td>—</td>
<td>17</td>
<td>2.5</td>
<td>0</td>
</tr>
<tr>
<td>15-19</td>
<td>5355</td>
<td>23</td>
<td>4.3</td>
<td>2</td>
<td>0.4</td>
<td>25</td>
<td>4.7</td>
<td>0</td>
</tr>
<tr>
<td>20-24</td>
<td>3713</td>
<td>15</td>
<td>4.0</td>
<td>2</td>
<td>0.5</td>
<td>17</td>
<td>4.6</td>
<td>0</td>
</tr>
<tr>
<td>25-34</td>
<td>5209</td>
<td>47</td>
<td>9.0</td>
<td>9</td>
<td>1.7</td>
<td>56</td>
<td>10.8</td>
<td>0</td>
</tr>
<tr>
<td>35-44</td>
<td>4512</td>
<td>42</td>
<td>9.3</td>
<td>7</td>
<td>1.6</td>
<td>49</td>
<td>10.9</td>
<td>0</td>
</tr>
<tr>
<td>45-59</td>
<td>5046</td>
<td>33</td>
<td>6.5</td>
<td>9</td>
<td>1.8</td>
<td>42</td>
<td>8.3</td>
<td>0</td>
</tr>
<tr>
<td>&gt;60</td>
<td>2762</td>
<td>15</td>
<td>5.4</td>
<td>4</td>
<td>1.4</td>
<td>19</td>
<td>6.9</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>52130</td>
<td>207</td>
<td>4.0</td>
<td>33</td>
<td>0.6</td>
<td>240</td>
<td>4.6</td>
<td>58003</td>
</tr>
</tbody>
</table>
received treatment from an institution or service other than the LCP (31% for males and 16% for females). In addition it can be seen that the sex ratio varies considerably between ascertainment groups. The female proportion among all patients is lowest for the group ascertained elsewhere (48%) and highest for those ascertained by the LEP (60%). Among paucibacillary patients these percentages were 53% and 61% respectively.

Table 2 and Figure 3 show the prevalence rates of current clinical paucibacillary and multibacillary leprosy, attributable to present infection with *M. leprae*, by age and sex. The prevalence rates of current clinical paucibacillary leprosy rise to a peak in the 35–44 year age group among the males, but continue to rise with age in females. Thus there is little difference between the sexes up to the age of 25–34, while at older ages the prevalence rates of paucibacillary leprosy are higher for females. In addition, dips in the prevalence rates for paucibacillary leprosy can be seen in the 20–24 year age group among males and the 15–19 year age group among females. For current multibacillary disease the prevalence rates are only a fraction of those for current paucibacillary disease and are higher among males than among females.

The prevalence rates of visible leprosy (i.e. either current clinical signs of leprosy or else residual signs only) are shown in Figure 4 and Table 3. Among males the age-specific prevalence rates of visible paucibacillary leprosy rise to a peak of 27.0 per 1000 in the 45–59 year age group. Among females the corresponding rate reaches its peak of 34.8 per 1000 in the oldest age group, over 60 years of age. Again, rates for visible multibacillary disease are much lower than those for visible

![LEP 1979-1984 'clinical' prevalence](image_url)

**Figure 3.** Prevalence rates of current clinical leprosy by type, sex and age at examination by the LEP in Karonga District, Malawi, 1979–84. Vertical bars indicate 95% confidence intervals.
Prevalence rates of leprosy

Figure 4. Prevalence rates of visible leprosy by type, sex and age at examination by the LEP in Karonga District, Malawi, 1979–84. Vertical bars indicate 95% confidence intervals (offset so as not to overlap).

Paucibacillary disease and three times as high for males as for females. Table 3 presents the visible rates by age and sex for visible paucibacillary and multibacillary leprosy combined. Table 3 also shows that the majority of older male patients were ascertained by an institution other than the LCP or the LEP. For older female individuals this proportion was only one third. Nearly all young (e.g. 5–14-year-old) patients were, in contrast, first ascertained by the LEP.

Figure 5 shows the age-specific cumulative prevalence rates of leprosy (including all individuals with current clinical signs, residual signs or a history of either). Though the rates are higher, the trends are similar to those of visible disease in Figure 4, except for ‘bulges’ in paucibacillary disease among the 15–24-year-old males and females. With regard to the mode of ascertainment it can be seen from Table 4 that the majority of older males were first ascertained ‘elsewhere’ but that this proportion was again much smaller for females. Approximately two thirds of the individuals in the 15–24 year age group were first ascertained by the LCP. This is appreciably higher than the proportion of individuals in this age group ascertained by the LCP and included in the visible rates (Table 3). This is particularly conspicuous for females.

Figure 6(a) and (b) show all three prevalence rates for paucibacillary and multibacillary leprosy together for males (Figure 6(a)) and for females (Figure 6(b)). They show again the large difference between the prevalence rates of clinical and visible leprosy and the relatively smaller difference between visible and cumulative leprosy at all except the very young ages.

Table 5 shows the relationship between visible and cumulative leprosy by age and sex, with
### Table 3.
Visible prevalence statistics by age and sex, Karonga District, Malawi, 1979–84. For each age and sex the number and percentage (in parentheses) of leprosy cases ascertained by each mode are shown, and the prevalence rate per 1000. Denominators for these prevalence rates are in Table 2.

<table>
<thead>
<tr>
<th>Age at examination by LEP</th>
<th>Ascertained elsewhere</th>
<th>Ascertained by LCP</th>
<th>Ascertained by LEP</th>
<th>Total cases</th>
<th>Visible prev. rate per 1000</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-4</td>
<td>0</td>
<td>0</td>
<td>3 (100%)</td>
<td>3</td>
<td>0.3</td>
</tr>
<tr>
<td>5-9</td>
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<td>13 (100%)</td>
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<td>1.5</td>
</tr>
<tr>
<td>10-14</td>
<td>0 (3%)</td>
<td>9 (28%)</td>
<td>22 (69%)</td>
<td>32</td>
<td>4.6</td>
</tr>
<tr>
<td>15-19</td>
<td>2 (4%)</td>
<td>17 (38%)</td>
<td>26 (58%)</td>
<td>45</td>
<td>8.4</td>
</tr>
<tr>
<td>20-24</td>
<td>5 (12%)</td>
<td>22 (51%)</td>
<td>16 (37%)</td>
<td>43</td>
<td>11.6</td>
</tr>
<tr>
<td>25-34</td>
<td>19 (19%)</td>
<td>35 (35%)</td>
<td>45 (45%)</td>
<td>99</td>
<td>19.0</td>
</tr>
<tr>
<td>35-44</td>
<td>62 (45%)</td>
<td>22 (16%)</td>
<td>54 (39%)</td>
<td>138</td>
<td>30.6</td>
</tr>
<tr>
<td>45-50</td>
<td>109 (62%)</td>
<td>18 (10%)</td>
<td>48 (27%)</td>
<td>175</td>
<td>34.7</td>
</tr>
<tr>
<td>&gt;60</td>
<td>49 (60%)</td>
<td>12 (15%)</td>
<td>20 (25%)</td>
<td>81</td>
<td>29.3</td>
</tr>
</tbody>
</table>

| Total                     | 247 (39%)             | 135 (21%)         | 247 (39%)         | 629         | 12.1                     |

<table>
<thead>
<tr>
<th>Ascertained elsewhere</th>
<th>Ascertained by LCP</th>
<th>Ascertained by LEP</th>
<th>Total cases</th>
<th>Visible prev. rate per 1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>1 (7%)</td>
<td>13 (93%)</td>
<td>14</td>
<td>1.6</td>
</tr>
<tr>
<td>0</td>
<td>9 (21%)</td>
<td>34 (79%)</td>
<td>43</td>
<td>6.5</td>
</tr>
<tr>
<td>0</td>
<td>17 (45%)</td>
<td>21 (55%)</td>
<td>38</td>
<td>7.5</td>
</tr>
<tr>
<td>7 (15%)</td>
<td>14 (30%)</td>
<td>26 (55%)</td>
<td>47</td>
<td>10.7</td>
</tr>
<tr>
<td>20 (16%)</td>
<td>33 (27%)</td>
<td>69 (57%)</td>
<td>122</td>
<td>16.1</td>
</tr>
<tr>
<td>68 (36%)</td>
<td>47 (25%)</td>
<td>76 (40%)</td>
<td>191</td>
<td>26.1</td>
</tr>
<tr>
<td>68 (35%)</td>
<td>38 (19%)</td>
<td>91 (46%)</td>
<td>197</td>
<td>35.0</td>
</tr>
<tr>
<td>31 (34%)</td>
<td>16 (17%)</td>
<td>45 (49%)</td>
<td>92</td>
<td>37.6</td>
</tr>
</tbody>
</table>

| Total | 194 (26%) | 175 (24%) | 375 (50%) | 744 | 12.8 |
Discussion

The analyses presented in this paper have attempted to address a widespread problem in leprosy endemic areas—the distortion of present disease patterns by past treatment programmes. In this context it should be noted that all of our measures are functions of historical circumstances and thus that their application may reveal very different patterns in different areas of the world. Without the antileprosy treatment first given by Government Health Services and other institutions and later, since 1973, by the Lepra Control Project, the clinical and the visible prevalence rates would most likely have been higher, and the cumulative prevalence rate would have been lower, than observed here. In addition, in the total absence of historical information the visible and the cumulative rates would have been identical. Even now they are very similar in shape (Figure 6(a) and (b)).

Our cumulative prevalence rates of leprosy are undoubtedly underestimates of the total
Table 4. Cumulative prevalence statistics by age and sex, Karonga District, Malawi, 1979–84. For each age and sex, the number and percentage (in parentheses) of leprosy cases ascertained by each mode are shown, and the prevalence rate per 1000. Denominators for these prevalence rates are in Table 2.

<table>
<thead>
<tr>
<th>Age at examination by LEP</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ascertained elsewhere</td>
<td>Ascertained by LCP</td>
</tr>
<tr>
<td>0-4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5-9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10-14</td>
<td>2 (4%)</td>
<td>30 (56%)</td>
</tr>
<tr>
<td>15-19</td>
<td>3 (4%)</td>
<td>56 (66%)</td>
</tr>
<tr>
<td>20-24</td>
<td>10 (10%)</td>
<td>74 (74%)</td>
</tr>
<tr>
<td>25-34</td>
<td>36 (22%)</td>
<td>82 (50%)</td>
</tr>
<tr>
<td>35-44</td>
<td>76 (42%)</td>
<td>50 (28%)</td>
</tr>
<tr>
<td>45-59</td>
<td>126 (60%)</td>
<td>37 (18%)</td>
</tr>
<tr>
<td>&gt;60</td>
<td>58 (54%)</td>
<td>30 (28%)</td>
</tr>
<tr>
<td>Total</td>
<td>311 (34%)</td>
<td>359 (39%)</td>
</tr>
</tbody>
</table>
Figure 6(a) Prevalence rates of leprosy among males by age at examination and type of prevalence measure, for paucibacillary and multibacillary leprosy combined, in Karonga District, Malawi, 1979–84. (b) Prevalence rates of leprosy among females by age of examination and type of prevalence measure, for paucibacillary and multibacillary leprosy combined, in Karonga District, Malawi, 1979–84.
Table 5. Percentages of leprosy patients first treated by LCP who were without clinical signs of leprosy at the time of examination by LEP staff during the 1979-84 survey

<table>
<thead>
<tr>
<th>Age at examination by LEP</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4</td>
<td>(0/0)</td>
<td>100-0%</td>
</tr>
<tr>
<td>5-9</td>
<td>(0/0)</td>
<td>85-7%</td>
</tr>
<tr>
<td>10-14</td>
<td>70-0%</td>
<td>(21/30)</td>
</tr>
<tr>
<td>15-19</td>
<td>69-6%</td>
<td>(39/56)</td>
</tr>
<tr>
<td>20-24</td>
<td>70-3%</td>
<td>(52/74)</td>
</tr>
<tr>
<td>25-34</td>
<td>57-3%</td>
<td>(47/82)</td>
</tr>
<tr>
<td>35-44</td>
<td>56-0%</td>
<td>(28/50)</td>
</tr>
<tr>
<td>45-59</td>
<td>51-4%</td>
<td>(19/37)</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>60-0%</td>
<td>(18/30)</td>
</tr>
<tr>
<td>All ages</td>
<td>62-4%</td>
<td>(224/359)</td>
</tr>
</tbody>
</table>

Proportions of individuals who ever manifested clinical signs of leprosy, insofar as it is likely that a number of individuals contracted self-healing forms of the disease which never came to the attention of either the LCP or the LEP. Such underestimation should have been cumulative over time; and thus should be particularly great for older age groups. The degree of underestimation should also be considerably greater for paucibacillary than for multibacillary forms of the disease, given that multibacillary forms of leprosy are unlikely to self-heal without leaving residual signs. The high proportion of multibacillary disease (Table 1) in the past is therefore considered to be an artefact due in part to misclassification and in part to the mode of detection of these cases. In addition there has been a rise in the proportion of females among patients over time (Table 1) which may indicate that in the past self-reporting was less common among females than among males. If a substantial proportion of their lesions self-healed then our cumulative rates also selectively underestimate the true rates in older females. Although we recognize the cumulative prevalence rates to be underestimates, we did not attempt to investigate histories further, as we were sceptical of the reliability of such information. Nevertheless, the measure is of considerable interest as evidence that more than 4% of males and more than 5% of females in this population manifest clinical leprosy lesions at some time in their lives if they live to the age of 60.

Our prevalence rates of visible leprosy might have been inflated slightly by the inclusion of middle (probable) group patients, in a proportion of whom the clinical signs may not have been due to past or present infection with M. leprae. This overestimation is likely to be small however, since the majority of all patients were in the narrow (certain) group (54% overall) and we have presented evidence elsewhere that the diagnostic specificity among middle group patients ascertained by the LEP is high. Furthermore, the inclusion of a few false positives should have been compensated by the omission of a few genuine leprosy cases allocated into the wide (possibly leprosy) group.

Among males there is a fall in the clinical, visible and cumulative prevalence rates of paucibacillary leprosy after age 60 (Figures 3, 4 and 5). No such fall is seen among females, where the visible and cumulative rates are highest among those over 60 years of age. The reason for these differences between the sexes is not obvious. Possibilities include higher selective mortality and/or emigration among males, but we have no data to support or refute either hypothesis. In addition one could speculate that because the majority of older males spent many years of their lives outside the District, working in the mines in Tanzania, Zambia, Zimbabwe and South Africa, the incidence rates were lower among them than among females, who usually remained in Karonga District throughout their lives.
Apart from the prevalence rates of visible and of cumulative leprosy we have also tried to estimate the prevalence rate of clinical leprosy. This prevalence rate is likely to be an underestimate in so far as a proportion of apparently inactive paucibacillary leprosy lesions might nevertheless contain viable *M. leprae*. Such cases are now included only in the visible prevalence rate. In spite of this problem we believe this rate provides a more appropriate and useful indicator of the actual need for antileprosy treatment facilities than does the prevalence rate of visible leprosy. The majority of ‘patients’ included in the visible prevalence rate are unlikely to benefit from (further) specific antileprosy treatment—though they might of course be in need of physiotherapy, ulcer care or social support. If tests become available which can recognize the presence of *M. leprae* infection it should become possible to determine this prevalence rate of clinical leprosy more accurately. Such tests would hopefully also be able to recognize the presence of dormant *M. leprae* (persisters) in individuals with residual signs only or in individuals with no remaining signs. Such individuals would then be included in the infection prevalence group, on the size, age and sex trends of which we can only speculate at present.16

It is of interest that the prevalence rates of clinical leprosy rise consistently with age except among males aged 20–24 and among females aged 15–19. These inconsistencies may be biologically significant. Two possible explanations offer themselves:

1. The LCP had an active case-finding programme among school children between 1974 and 1979. Unfortunately, the exact number of new leprosy patients found during those school surveys is not known. However, the number was probably substantial (Table 4). The present prevalence rates of clinical leprosy might thus be low among 15–19 and 20–24 year old individuals because leprosy was diagnosed and treated at an early stage in individuals in this age group when they were attending school.

2. BCG has been widely used in Karonga District since the mid-seventies both in mass campaigns among school children and in under-five clinics. Because mass campaigns were discontinued in the late seventies the prevalence rate of BCG scars was highest among males aged 15–24 years (76·5%) and among females aged 15–19 years (74·2%) at the time of the survey. This observation, together with our evidence of at least 50% protective efficacy of BCG against leprosy in this area,17 suggests a causal relationship between BCG and the dip in prevalence rates among females and males respectively. However, these dips are not seen in the cumulative rates, where the rates seem more to ‘bulge’ for these age groups. The answer to this puzzle might be that those who had no signs of leprosy left at the time of examination by the LEP had onset of disease around the time of BCG vaccination or before, while most of these found with current clinical signs of leprosy had onset of disease after the introduction of BCG into the District. Only among the latter can one expect an effect of BCG. The bulge among the former could thus be due to overdiagnosing during school surveys or diagnosing of early self-healing forms during school surveys. Alternatively, it might be that the BCG vaccinations precipitated mild self-healing forms of leprosy among vaccines before the long term protective effect became evident.18,19

We believe it is useful to describe the prevalence of leprosy using three different prevalence rates rather than only one. Only 43% (593/1367) of individuals with visible signs of leprosy, skin lesions and/or disabilities had (active) clinical disease, presumably requiring antileprosy treatment, at the time of being examined by the LEP. The prevalence of clinical disease is more relevant to treatment facility needs than is the prevalence of visible leprosy. On the other hand, the cumulative prevalence rate (including those with well-documented histories of clinical leprosy) gives a more realistic impression of the extent of leprosy in the past and of the achievements of a control project. Presumably largely due to treatment, only about 40% of leprosy patients treated exclusively by the LCP in Karonga District are left with any signs of leprosy. The extent of disability among these individuals is currently under investigation.

In view of our findings that only two-fifths of all individuals with visible signs of leprosy had clinically active disease or were found with *M. leprae* in slit-skin smears or biopsies, and would thus benefit from (further) antileprosy treatment, we wonder how many of the world’s 10–12 million
leprosy 'cases'\textsuperscript{20} are really leprosy patients in the sense that they require specific antileprosy treatment rather than physiotherapy, ulcer care, or social and economic support.

Acknowledgments

The authors wish to thank the Government of the Republic of Malawi for their interest in the Lepra Evaluation Project. Without their support and the co-operation from the people in Karonga District the project would not have been possible.

The Lepra Evaluation Project staff worked hard for many years to obtain the data analysed in this publication but the authors would also acknowledge the Lepra Control Project staff, in particular Mr P Nankhuni and Mr K Mughandira, for making available all information on previous LCP patients.

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References

\begin{enumerate}
\item Burgess PJ, Fine PEM, Ponnighaus JM, Draper C. Serological tests in leprosy. The sensitivity, specificity and predictive value of ELISA tests based on phenolic glycolipid antigens and the implications for their use in epidemiological studies. \textit{Epidemiol Infect}, in press.
\item Boerrigter G, Ponnighaus JM. 10 years' leprosy control work in Malawi (Central Africa)—1, Methods and outcome after treatment. \textit{Lepr Rev}, 1986; 57: 199–220.
\item Ridley DS, Jopling WH. Classification of leprosy according to immunity—a five-group system. \textit{Int J Lepr}, 1966; 34: 255–73.
\item Committee appointed by His Excellency the Governor of Nyasaland. Report of an Inquiry into Emigrant Labour, 1935.
\end{enumerate}
Epidemiological study of leprosy in Malwani suburb of Bombay

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Summary The present investigation was undertaken to study epidemiology of leprosy in Malwani, a western suburb of Bombay, which has a population of 63,321. A total of 691 cases were detected in a 4-year follow-up period between April 1979 and April 1983. The prevalence rate in schoolchildren was 13.88% and the peak incidence occurred in the age group 10–19 years. In this study, the females predominated the males with the male to female ratio being 1:1.33. The disease was found to be more prevalent in the low socioeconomic group and in overcrowded families. Extremities were most commonly affected. A large number of cases occurred in contacts of infectious lepromatous patients. The exact reasons for this could not be ascertained from this rather small sample. It could be related to droplet infections or skin contact.

Introduction

Though leprosy was the first human disorder shown to be due to a microbe identified 100 years ago, it still continues to be a major public health problem, mostly in the developing countries of the world. It is estimated that there are 12–13 million leprosy patients in the world. The majority of them residing in Africa and Asia. Among individual nations, the largest number of leprosy patients (about 4 million) reside in India, where the estimated incidence is about 20,000–30,000 per year.1

In India, the prevalence of disease varies from place to place—the highest being in certain regions of Orissa, Tamil Nadu, Andhra Pradesh and Maharashtra. In Northern India, the prevalence is rather low. About 15–20% of patients are infectious, that is suffering from multibacillary forms of the disease.1 One of the distressing facts is that it affects pre-schoolchildren. The city of Bombay has a very high prevalence rate (13 per 1000). The large scale migration of people from outside in a way contributes to this high prevalence. In this expanding city, the suburbs and rural areas are getting urbanized at a very fast rate. One such area is Malwani with a population of 63,321 located in the western suburbs of Greater Bombay. Malwani has a well organized community health centre, developed by the KEM Hospital and Seth G.S. Medical College of Bombay. A number of preventive programmes have been undertaken including one for leprosy eradication. The aim of the present study is called the baseline information on the leprosy scene at Malwani, so that subsequent affects of control measures can be properly assessed. The present study was conducted over a 4-year period, April 1979 to April 1983.
Materials and methods

The leprosy cases were registered through: (1) Mass house-to-house survey (a mass survey was done at the beginning of the programme); (2) school survey (a mass survey was done in July 1979 and repeated every year for new admissions. Suspected cases were also referred during regular school health check-ups); (3) household contact survey of the index cases (the contacts were examined once every 3 months); (4) referrals from health centres, dispensaries and general practitioners; and (5) self-reported cases having any sign and symptom of leprosy.

All the suspected cases during the mass survey and school survey were referred to the clinic. At the clinic they were examined clinically and bacteriologically. Once the diagnosis was made, the case history and examination findings of the patients were recorded on special case sheets, and an index number was given to each patient.

Doubtful cases were recorded on observation card and followed up at 3-month intervals. They were registered as regular patients after confirmation of the diagnosis.

Classification of the leprosy was done clinically and grouped according to Indian classification: (i) non-lepromatous; (ii) borderline; and (iii) lepromatous type. In the cases of borderline and lepromatous types, slit smears were obtained from the skin lesions and ear lobule.

Results and discussion

Table 1 shows the number of leprosy cases detected during various surveys.

The prevalence rate in the general population of Malvani is 10·91/1000. A high prevalence rate in the slums of North Bombay is also recorded in another study.2

The prevalence rate in school is 13·81/1000 which is higher than the general prevalence rate of the population. The higher prevalence rate in school is because a majority of cases (53·11%) are below 19 years (Table 2). The lepromatous type of cases are not seen in school as the majority—(89·13%) of total lepromatous cases—are above 19 years (Table 2), or lepromatous cases may not be attending school. In the study area the majority of schools belong to the Municipal Corporation. A higher incidence of leprosy in Municipal Schools has also been reported in other studies.3–5 In this study 21·56% of cases are detected through school surveys, so school surveys are a major tool in leprosy control programmes.

The peak incidence is observed in the age group 10–19 years. The other workers have reported the peak incidence in the age group 20–29. No explanation can be offered, at the moment, for these differences. The incubation period of leprosy is very long, so an extensive surveillance programme

Table 1

<table>
<thead>
<tr>
<th>Mode of detection</th>
<th>T</th>
<th>B</th>
<th>L</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Mass survey</td>
<td>229</td>
<td>41·86</td>
<td>38</td>
<td>38·78</td>
</tr>
<tr>
<td>School survey</td>
<td>130</td>
<td>23·77</td>
<td>19</td>
<td>19·39</td>
</tr>
<tr>
<td>Contact survey</td>
<td>50</td>
<td>9·14</td>
<td>6</td>
<td>6·12</td>
</tr>
<tr>
<td>Referrals and self-reported</td>
<td>138</td>
<td>25·23</td>
<td>35</td>
<td>35·71</td>
</tr>
<tr>
<td>Total</td>
<td>547</td>
<td>100</td>
<td>98</td>
<td>100</td>
</tr>
<tr>
<td>Percentage</td>
<td>79·16</td>
<td>14·18</td>
<td>6·66</td>
<td>100·00</td>
</tr>
</tbody>
</table>

Total number of schoolchildren examined: 10,789.
The prevalence rate in school: 13·81/1000.
Table 2. Prevalence of leprosy in different age groups among male and female cases and types of leprosy

<table>
<thead>
<tr>
<th>Age Group</th>
<th>T (Male)</th>
<th>Female</th>
<th>B (Male)</th>
<th>Female</th>
<th>L (Male)</th>
<th>Female</th>
<th>Total (Male)</th>
<th>Female</th>
<th>Total (Male)</th>
<th>Female</th>
<th>Total (Male)</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-9</td>
<td>64</td>
<td>29-77</td>
<td>68</td>
<td>20-48</td>
<td>8</td>
<td>15-09</td>
<td>6</td>
<td>13-33</td>
<td>0</td>
<td>0-00</td>
<td>1</td>
<td>5-56</td>
</tr>
<tr>
<td>10-19</td>
<td>93</td>
<td>43-26</td>
<td>96</td>
<td>28-92</td>
<td>19</td>
<td>35-86</td>
<td>8</td>
<td>17-78</td>
<td>3</td>
<td>10-71</td>
<td>1</td>
<td>5-56</td>
</tr>
<tr>
<td>20-29</td>
<td>15</td>
<td>6-98</td>
<td>64</td>
<td>19-28</td>
<td>5</td>
<td>9-43</td>
<td>10</td>
<td>22-22</td>
<td>2</td>
<td>7-14</td>
<td>4</td>
<td>22-22</td>
</tr>
<tr>
<td>30-39</td>
<td>23</td>
<td>10-70</td>
<td>16</td>
<td>18-07</td>
<td>3</td>
<td>5-66</td>
<td>11</td>
<td>24-45</td>
<td>7</td>
<td>25-10</td>
<td>4</td>
<td>22-22</td>
</tr>
<tr>
<td>40-49</td>
<td>7</td>
<td>3-25</td>
<td>24</td>
<td>7-23</td>
<td>7</td>
<td>13-21</td>
<td>6</td>
<td>13-33</td>
<td>6</td>
<td>21-43</td>
<td>4</td>
<td>22-22</td>
</tr>
<tr>
<td>50-59</td>
<td>9</td>
<td>4-19</td>
<td>11</td>
<td>3-31</td>
<td>8</td>
<td>15-09</td>
<td>3</td>
<td>6-67</td>
<td>5</td>
<td>17-86</td>
<td>3</td>
<td>16-66</td>
</tr>
<tr>
<td>60 or more</td>
<td>4</td>
<td>1-86</td>
<td>9</td>
<td>2-71</td>
<td>3</td>
<td>5-66</td>
<td>1</td>
<td>2-2</td>
<td>5</td>
<td>17-86</td>
<td>1</td>
<td>5-56</td>
</tr>
<tr>
<td>Total</td>
<td>215</td>
<td>100</td>
<td>332</td>
<td>100</td>
<td>53</td>
<td>100</td>
<td>45</td>
<td>100</td>
<td>28</td>
<td>100</td>
<td>18</td>
<td>100</td>
</tr>
</tbody>
</table>

Mean (years) T: 17.9 ± 14.8, B: 22.8 ± 17.9, L: 27.9 ± 19.2, Total: 33.2 ± 18.2
Mean (years) SD: 17.9 ± 17.9, 20.0 ± 15.2, 24.0 ± 15.5
Sex ratio: T: 647/1000 (Significant), B: 1177/1000 (Not significant), L: 1555/1000 (Not significant), Total: 749/1000 (Not significant)

Epidemiological study of leprosy, Bombay
may show peak incidence of infection in an earlier age group. The mean age of infection in females is higher than in males in the non-lepromatous type of infection.

The male to female ratio is 1:1:33. Other studies quoted earlier show the predominance of male cases over female cases. The female cases are seen more where lepromatous cases are less (in this study lepromatous cases are only 6-6% of the total cases) (Table 1). This confirms the findings of another study.6

The male:female ratio in lepromatous cases is 1·56:1, and in borderline the ratio is 1·18:1. The Marshall's study shows the same pattern.8 In non-lepromatous the females predominate over males.

The male:female ratio in the Malwani population is 1·16:1.8 So there is no significant predominance of either sex in the general population. There is practically no sex difference up to the age of 19 years as there are fewer infectious cases below 19 years of age.

There are several factors which influence the sex predominance in the endemic area. The main factor causing the sex difference is opportunity for contact and practically no difference is noted when the opportunity for contact remains the same.

The higher female ratio can be explained on the basis that males are at work during the day time, while the mother or older females remain in contact with children at home (53·11% of cases are below the 19 years age group).

Until it can be shown that the lepromatous rate is greater in males in childhood, it can be assumed that this sex difference may be due to the difference in the susceptibility.5

### Table 3. Religion wise distribution of leprosy cases

<table>
<thead>
<tr>
<th>Religion</th>
<th>Leprosy cases</th>
<th>Malvani population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hindu</td>
<td>331</td>
<td>36093</td>
</tr>
<tr>
<td>Muslim</td>
<td>318</td>
<td>23428</td>
</tr>
<tr>
<td>Christians</td>
<td>39</td>
<td>3102</td>
</tr>
<tr>
<td>Others</td>
<td>3</td>
<td>698</td>
</tr>
<tr>
<td>Total</td>
<td>691</td>
<td>63321</td>
</tr>
</tbody>
</table>

* Z, 4·8046 p < 0·001 significantly less.
† Z, 2·6234 p < 0·05 significantly less.
‡ Z, 0·8740 p > 0·05 not significant (no difference).

### Table 4. Distribution of cases according to the type of dwelling

<table>
<thead>
<tr>
<th>Type of dwelling</th>
<th>Leprosy cases</th>
<th>Malvani population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zopadapatti</td>
<td>463</td>
<td>40557</td>
</tr>
<tr>
<td>Chawls</td>
<td>221</td>
<td>21852</td>
</tr>
<tr>
<td>Individual tenements</td>
<td>7</td>
<td>912</td>
</tr>
<tr>
<td>Total</td>
<td>691</td>
<td>63321</td>
</tr>
</tbody>
</table>

* Z, 1·4954 p > 0·05 not significant.
† Z, 1·2887 p > 0·05 not significant.
The prevalence of leprosy is greater among the Muslim population. Both the communities live in the same socioeconomic settings, however, the average size of Muslim families is larger than Hindu families.9 (This was also observed in our contact study.) Overcrowding could be a very important factor and may be responsible for a higher prevalence in Muslims (see Table 3).

There is practically no significance in the type of dwelling (see Table 4). This is why all the slums of Bombay have different prevalence rates,12 though the main structure of residence in the slum is zopadapatti.

The majority of the cases belong to the low income group (Rs. 0–50) (see Table 5). The average per capita income per month is Rs. 62/- whereas the lowest income per capita per month is Rs. 70/- in the general population of India.10 There is usually an attempt to correlate socioeconomic status to the incidence of leprosy. However, the former can not be a cause, as unemployment due to disease and disability keeps these families in the low income group.

Unemployment is 17·22% (see Table 6). This is because of disability produced by the disease and also because once the patient’s diagnosis is known he is removed from his job. The exact number of the patients removed from their jobs due to disease or disability is not known, for that long term follow-up is required.

The majority of patients show a single lesion (see Table 7). Also in school surveys most of the children show a single lesion. Often the single lesion remains in the silent form and the patient

### Table 5. Distribution of cases according to per capita income per month

<table>
<thead>
<tr>
<th>Monthly income (Rupees)</th>
<th>Cases</th>
<th>Malvani population*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>0–50</td>
<td>327</td>
<td>47·32†</td>
</tr>
<tr>
<td>51–100</td>
<td>283</td>
<td>40·96</td>
</tr>
<tr>
<td>101–250</td>
<td>70</td>
<td>10·13</td>
</tr>
<tr>
<td>More than 250</td>
<td>11</td>
<td>1·59</td>
</tr>
<tr>
<td>Total</td>
<td>691</td>
<td>100</td>
</tr>
</tbody>
</table>

* Z. 11·808 p < 0·001 (highly significant).
Average income per month: Rs. 62/- per capita (patient).
Average income per month: Rs. 99·05 per capita (Malvani population).

### Table 6. Occupation of cases

<table>
<thead>
<tr>
<th>Occupation</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unskilled worker</td>
<td>111</td>
<td>16·06</td>
</tr>
<tr>
<td>Skilled worker</td>
<td>33</td>
<td>4·77</td>
</tr>
<tr>
<td>Clerical cadre</td>
<td>27</td>
<td>3·91</td>
</tr>
<tr>
<td>Trader</td>
<td>42</td>
<td>6·08</td>
</tr>
<tr>
<td>Student</td>
<td>169</td>
<td>24·46</td>
</tr>
<tr>
<td>Housewife</td>
<td>190</td>
<td>27·50</td>
</tr>
<tr>
<td>Unemployed</td>
<td>119</td>
<td>17·22</td>
</tr>
<tr>
<td>Total</td>
<td>691</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 7. The distribution of cases according to the number of lesions

<table>
<thead>
<tr>
<th>No. of lesions</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Single lesion</td>
<td>157</td>
<td>57.04</td>
<td>240</td>
</tr>
<tr>
<td>More than one lesion</td>
<td>139</td>
<td>46.96</td>
<td>155</td>
</tr>
<tr>
<td>Total</td>
<td>296</td>
<td>100</td>
<td>395</td>
</tr>
</tbody>
</table>

Table 8. The distribution of lesions according to the sites of lesion in the cases having a single lesion

<table>
<thead>
<tr>
<th>Sites of lesion</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Arm</td>
<td>47</td>
<td>29.95</td>
<td>71</td>
</tr>
<tr>
<td>Leg</td>
<td>39</td>
<td>24.84</td>
<td>50</td>
</tr>
<tr>
<td>Thigh including hip and buttock</td>
<td>32</td>
<td>20.38</td>
<td>33</td>
</tr>
<tr>
<td>Back</td>
<td>15</td>
<td>9.55</td>
<td>48</td>
</tr>
<tr>
<td>Pectoral region</td>
<td>5</td>
<td>3.18</td>
<td>8</td>
</tr>
<tr>
<td>Epigastric region</td>
<td>5</td>
<td>3.18</td>
<td>9</td>
</tr>
<tr>
<td>Face</td>
<td>14</td>
<td>8.92</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>157</td>
<td>100</td>
<td>240</td>
</tr>
</tbody>
</table>

ignores it. Many cases are also detected during the general examinations of patients in hospitals who have been admitted for other complaints. That is why mass survey school surveys and health education forms an effective tool for identification of cases, which is a pre-requisite for understanding control programmes.

Only the site where the lesion appears first is of significance. In the case of multiple lesions, it would be difficult to find out from the patient the exact site of the first lesion, hence the patients having a single lesion were selected for such analysis (see Table 8). The commonest sites are arm, leg, thigh and buttocks. These are the sites in the body which receive maximum trauma, friction and inflammation, and these sites also have more skin-to-skin contact. However, there is variation among the sites in different age groups and in different regions. No explanation can be offered, at the moment, for these regional differences.

The epigastric and pectoral regions show very few lesions as these are well-protected areas of the body.

Out of the five deformities of the eyes, three were of the lagophthalmos type and two were of blindness, which was caused by persistent iridocyclitis and exposure keratitis due to paralysis of the Vth cranial nerve (see Table 9). Chronic iridocyclitis is a common phenomenon which can lead to blindness in borderline lepromatous cases.

The observation period of this study was 4 years. As the incubation period of leprosy is uncertain, long-term observation is required to find out the impact of transmission of the disease among household contacts. The prevalence rate of 19.30/1000 among contacts was found to be significantly higher than the general prevalence rate of 10.91/1000 for Malvani.

The incidence of infectivity is greater among the contact cases of lepromatous and borderline
Table 9. Type of deformity in male and female cases

<table>
<thead>
<tr>
<th>Type of deformity</th>
<th>Male</th>
<th></th>
<th>Female</th>
<th></th>
<th>Total</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Eye</td>
<td>3</td>
<td>4.12</td>
<td>2</td>
<td>3.58</td>
<td>5</td>
<td>3.88</td>
</tr>
<tr>
<td>Hand</td>
<td>27</td>
<td>36.98*</td>
<td>28</td>
<td>50.00*</td>
<td>55</td>
<td>42.64</td>
</tr>
<tr>
<td>Foot</td>
<td>33</td>
<td>45.21†</td>
<td>20</td>
<td>35.71†</td>
<td>53</td>
<td>41.08</td>
</tr>
<tr>
<td>Hand and Foot</td>
<td>10</td>
<td>13.69</td>
<td>6</td>
<td>10.71</td>
<td>16</td>
<td>12.40</td>
</tr>
<tr>
<td>Total</td>
<td>73</td>
<td>100</td>
<td>56</td>
<td>100</td>
<td>129</td>
<td>100</td>
</tr>
</tbody>
</table>

* Z, 0.98 \( p > 0.05 \) not significant.
† Z, 1.76 \( p > 0.05 \) not significant.

Table 10. Distribution of contact cases in different types of index cases

<table>
<thead>
<tr>
<th>Type of index cases</th>
<th>No. of contacts examined</th>
<th>T</th>
<th>B</th>
<th>L</th>
<th>Total</th>
<th>Incidence of infectivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>43 (86)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>43 (71-67)</td>
<td>1.70</td>
</tr>
<tr>
<td>T</td>
<td>2530</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>450</td>
<td>7 (14)</td>
<td>5 (83.33)</td>
<td>1 (25)</td>
<td>13 (21-67)</td>
<td>2.87</td>
</tr>
<tr>
<td>L</td>
<td>129</td>
<td>0 (0)</td>
<td>1 (16.67)</td>
<td>3 (75)</td>
<td>4 (6-66)</td>
<td>3.10</td>
</tr>
<tr>
<td>Total</td>
<td>3109</td>
<td>50 (100)</td>
<td>6 (100)</td>
<td>4 (100)</td>
<td>60 (100)</td>
<td>1.93</td>
</tr>
</tbody>
</table>

Figures in brackets indicate percentages.

cases than the non-lepromatous type, indicating that contacts of lepromatous cases are at higher risk (see Table 10). The contacts of lepromatous cases are fewer as the majority of lepromatous cases stay alone and away from their families. The contact case findings are similar to the findings of Doull (1961).15

Lepromatous cases are not seen among contacts of non-lepromatous cases. Three lepromatous cases are recorded among the contacts of lepromatous ‘Index case’. This indicates that the disease is infectious and proves the concept of contact transmission.

Conclusions

The epidemiological study of leprosy in Malvani has shown a few minor differences in the pattern of leprosy in comparison with studies elsewhere.

These are: (a) reversal in sex ratio, the male and female ratio is 1:1.33; (b) peak incidence of disease is in a younger age group of 10–19 years; (c) the proportion of lepromatous cases is (6.6%) less than figures for all India; and (d) the deformity rate (18.67%) was found to be lower than elsewhere.

References


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**TEACHING MATERIALS AND SERVICES**

*Hansenologia*; *Dermatologia Tropical*; *textbook in Portuguese*

This excellent book in Portuguese, written by Dr Sinesio Talhari and Dr Rene Garrido Neves, both from Brazil, deserves further publicity. The edition we have is dated 1984, but it may have been revised. It is a strongly bound paperback of about 100 pages, covering all basic aspects of leprosy (but note that the use of 'Hansenology' and similar derivatives is important in their country) in a most competent and up-to-date manner. It is profusely illustrated in black and white, and colour pictures. In view of the scarcity of good material in Portuguese, this book deserves serious attention and wider distribution, including Angola, Mozambique, Cape Verde and Guinea Bissau. Enquiries to Dr Sinesio Talhari, Servicio de Dermatologia, Faculdade de Medicina, 69 000, Manaus, Amazonas, Brasil.

**WHO Training in leprosy**

This document; WHO/CDS/LEP/86.2 (English language) was produced by WHO in 1986 and is available on application to the Department of Publications, WHO, 1211 Geneva 27, Switzerland. It was written as a collaborative effort between Miss P J Neville, Education and Training Secretary, the Leprosy Mission International, London; Dr W Felton Ross, Medical Adviser, American Leprosy Missions Inc., USA; the Leprosy Unit, Division of Communicable Diseases, WHO, Geneva. The four main sections cover: introduction, training health personnel in leprosy, teaching and training considerations, teaching/learning materials on leprosy.

**Self-instructional packages in medical education**

The September 1986 issue of the Newsletter from the School of Medical Education, the University of New South Wales, P.O. Box 1, Kensington, NSW, 2033, Australia carries an interesting article on the above subject by May Wong. Such packages are defined in the opening paragraph as 'collections of learning items that have been designed to guide the learner in a structured manner through one or more learning tasks. The learning materials are organized in such a way that they provide stimuli, learning activities [responses and practice] feedback and assessment and allow the learners to function with little or no intervention from the instructors.'

(This publication merits further study. In the absence of experienced and credible teachers for the subject of leprosy in most endemic areas, this approach may have much to commend it.—*Editor.*)
Chromosome aberrations and sister chromatid exchanges (SCEs) in peripheral blood lymphocyte cultures of untreated leprosy patients

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Division of Human Genetics, Department of Anatomy, St John’s Medical College, Bangalore-560 034, India

Accepted for publication 6 December 1987

Summary The frequencies of various chromosome aberrations and sister chromatid exchanges (SCEs) were studied in blood lymphocyte cultures of untreated leprosy patients. The frequency of chromosome aberrations was significantly higher in lepromatous ($P < 0.001$) and tuberculoid ($P < 0.02$) groups in comparison with that of controls. The frequency of SCEs was found to be within normal range in the tuberculoid group whereas in lepromatous group, a significant ($P < 0.001$) increase was observed. The findings indicate a probable correlation between the form of leprosy and chromosome damage.

Introduction

A variety of physical and chemical agents as well as living organisms like viruses are known to cause chromosome aberrations,$^{1-4}$ and also to induce SCEs.$^{5-7}$ There is however, very little information on the relationship between bacterial infections and chromosomal aberrations. In spite of the worldwide incidence of leprosy, which is a chronic bacterial disease caused by Mycobacterium leprae, there is as yet no report of its effects on the genome of the patient. The in vitro and in vivo studies of the effect of antileprosy drug(s) that have been carried out,$^{8-10}$ have not ruled out the role of $M. leprae$ as an additional clastogenic agent. The present study has, therefore, been designed to assess the effects of $M. leprae$ in causing chromosomal damage in lymphocyte cultures of untreated leprosy patients. Further, the frequencies of SCEs and chromosome aberrations in different types of leprosy have also been studied.

In our heterogeneous lymphocyte cultures, we have utilized just sufficient concentration of 5-Bromodeoxyuridine (BrdU 5 $\mu$g/ml), needed to make sister chromatid differentiation observable$^{11}$ as it is extremely important in chromosome mutation studies to use the minimal concentration of the thymidine analogue so that the level of SCEs is kept at its minimum.$^{12}$

Materials and methods

PATIENT SELECTION

The patients selected were classified by clinical symptoms and bacterial index (BI) according to the Ridley–Jopling scale.$^{13}$ The tuberculoid leprosy (TT) and the borderline tuberculoid leprosy (BT)
patients were included in the paucibacillary group. The borderline lepromatous (BL) and the lepromatous leprosy (LL) patients were considered under the multibacillary group.

Both groups consisted of 10 patients each, the paucibacillary group of four females and six males (age range 12–55 years), and the multibacillary group of one female and nine males (age range 32–65 years). These patients had been carefully selected after ascertaining that they had never undergone treatment for leprosy and apparently had no other disease. Ten age-and-sex-matched normal individuals served as controls. These individuals had had no medication and had not suffered from any viral infections in the immediate 3 months prior to this investigation.

LYMPHOCYTE CULTURES

 Peripheral blood lymphocyte cultures were set up according to the method of Arakaki and Sparkes, with slight modifications. Heparinized whole blood (0.3 ml) was added to 5 ml of culture medium (TC 199, GIBCO, USA) supplemented with 20% heat inactivated human AB serum, phytohemagglutinin (PHA: DIFCO M) and antibiotics (penicillin and streptomycin). 5-Bromodeoxyuridine (BrdU, Sigma, USA) was added at the final concentration of 5 μg/ml at the time of culture initiation and the cultures were grown at 37 °C for 48 h and 72 h for analysis of aberrations and SCEs respectively. The cultures were protected from light exposure during incubation. To arrest cells at metaphase, 4 μg/ml colchicine was added to the cultures 2 h before harvesting. The cells were treated with hypotonic solution (0·075 M KCl) and fixed in 3:1 methanol-acetic acid. Heat-dried chromosome preparations were stained with the Hoechst–Sunlight–Giemsa method. In order to have a correct estimate of aberrations, it is essential to score aberrations only in first division cells. The use of 5-Bromodeoxyuridine (BrdU)—labelling and Hoechst–Sunlight–Giemsa staining in this study enabled us to select only first division cells. One hundred first cycle metaphases from each subject were scored for spontaneous chromosome aberrations. ISCN 1978 criteria were used for the identification and quantification of various types of chromosomal aberrations. Twenty to fifty second cycle metaphases from cultures grown for 72 h were scored for SCEs from every subject.

Results

CHROMOSOME ABERRATIONS

Variations in the frequency of aberrant metaphases were found in different individuals within the same group. In the paucibacillary group, the range of aberrant metaphases were 2–13%, 2–6% in 8 patients, while 2 patients showed a large increase (13%). In the multibacillary group, the percentage of aberrant metaphases ranged from 4 to 13%; 4 to 8% in 7, 10% in 2 and 13% in 1 patient.

Controls, in contrast, had a low frequency of aberrant metaphases ranging from 1 to 3% only. The difference in the frequency of aberrant metaphases was found to be significant ($P < 0.02$) in the paucibacillary group and highly significant ($P < 0.001$) in the multibacillary group when compared with that of controls. The incidence of chromatid gaps and breaks was found to be highest in the multibacillary group and lowest in the control group. Chromosome aberrations other than breaks and gaps were found in low frequency (see Table 1).

SISTER CHROMATID EXCHANGES (SECS)

The results obtained are presented in Table 2. The frequency of SCEs in blood lymphocytes of the untreated paucibacillary group was $7.82 \pm 0.98$ which is comparable to the normal range of SCEs ($7.16 \pm 0.69$) observed in controls. In the multibacillary group, a significantly higher ($P < 0.001$) frequency of SCEs was obtained ($11.04 \pm 1.76$).
Chromosome aberrations and SCEs in untreated leprosy patients

Table 1. Frequency of chromosomal aberrations in lymphocytes of untreated leprosy patients and controls

<table>
<thead>
<tr>
<th>Category</th>
<th>No. of subjects/metaphases</th>
<th>Abnormal metaphases (%)</th>
<th>± SD</th>
<th>Breaks</th>
<th>Csb (%)</th>
<th>Ctb (%)</th>
<th>Gaps</th>
<th>Csg (%)</th>
<th>Ctg (%)</th>
<th>Other aberrations (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paucibacillary</td>
<td>10/1000</td>
<td>5.7†</td>
<td>±0.00</td>
<td>4.00</td>
<td>1.2</td>
<td>0.0</td>
<td>2.8</td>
<td>1.8</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>Multibacillary</td>
<td>10/1000</td>
<td>7.7†</td>
<td>±2.62</td>
<td>1.7</td>
<td>0.4</td>
<td>2.5</td>
<td>3.9</td>
<td>2.0</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>10/1000</td>
<td>2.3</td>
<td>±0.67</td>
<td>0.0</td>
<td>0.0</td>
<td>1.9</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td></td>
</tr>
</tbody>
</table>

Csb, chromosome break; Ctb, chromatid break; Csg, chromosome gap; Ctg, chromatid gap.
* Others include acentric fragments, dicentric chromosomes, double minutes, terminal chromatid deletions, chromatid exchanges and metaphases with a few decondensed chromosomes.
† Significant in Student's 't' test P < 0.02.
‡ Significant in Student's 't' test P < 0.001.

Table 2. Frequencies of sister-chromatid exchanges (SCEs) in lymphocytes of untreated leprosy patients and controls

<table>
<thead>
<tr>
<th>Category</th>
<th>No. of individuals studied</th>
<th>No. of cells analysed</th>
<th>SCEs/cell ± SD</th>
<th>Range of SCEs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paucibacillary</td>
<td>10</td>
<td>200</td>
<td>7.82 ± 0.98</td>
<td>2-19</td>
</tr>
<tr>
<td>Multibacillary</td>
<td>10</td>
<td>270</td>
<td>11.04 ± 1.76</td>
<td>2-25</td>
</tr>
<tr>
<td>Controls</td>
<td>10</td>
<td>225</td>
<td>7.16 ± 0.69</td>
<td>1-17</td>
</tr>
</tbody>
</table>

* Non-significant in Student's 't' test P > 0.05.
† Significant in Student's 't' test P < 0.001.

Discussion

The commonly used drug in leprosy therapy, dapsone (4,4' diaminodiphenylsulfone, DDS), is known to cause chromosomal damage when added directly to lymphocyte cultures of healthy individuals. However, it was not possible to detect such an increased frequency of aberrations when chromosomal preparations from leprosy patients undergoing dapsone therapy were analysed. These controversial results from previous studies indicated the need to investigate the possible causative agents separately to demonstrate each factor in the aetiology of chromosome aberrations. The present study, is, to our knowledge, the first documentation of the observation that significantly higher frequencies of structural chromosome aberrations and SCEs occur in untreated leprosy patients and implies that, bacteria like M. leprae may cause genetic damage. This is in good agreement with the earlier observations that Mycoplasmas can induce chromosome aberrations and SCEs by infecting lymphocytes in cultures. Furthermore, the increased frequency of chromosomal aberrations and SCEs in the multibacillary group relative to the paucibacillary group and the controls, as well as the significant increase of chromosomal aberrations but not of SCEs in the paucibacillary group compared to that of controls, demonstrates the possible existence of association between the severity of this disease (i.e. form of leprosy) and proportional increase in the extent of DNA damage. The presence of chromatid type aberrations is greater than the chromosome type, indicating that the damage might have occurred during the S-phase (DNA-synthesis) or the G2 period of the cell cycle. This also suggests that the agents like bacteria are effective during synthetic phase of the cell cycle.
The role of DDS as a mutagenic agent is not ruled out by our study. In fact, it is possible that these 2 agents may act synergistically in treated patients, but that their mechanism of action may be different.

At the present time, the mechanism of action of M. leprae in causing chromosome aberrations and SCEs is not clearly understood. Several factors may be involved, resulting in, increased frequency of SCEs. One of the important factors is variation in lymphocyte subpopulations and there is a clear evidence for differences in SCE frequencies in different subpopulations of lymphocytes.11,23

The multibacillary patients have a depressed cell-mediated immunity (CMI).24,25 This depression in CMI could be due to the functionally deficient or functionally defective T-lymphocytes and the changes among T-lymphocyte subsets.26 So the presumably altered lymphocyte profile in multibacillary patients might be considered as one of the causes for the increased basal levels of SCEs in lymphocytes. Nevertheless, it is known that certain bacterial enzymes can act as DNases and may induce lesions in host cell DNA.27 A detailed in vitro study using M. leprae may throw light on the mechanism of bacterial action on chromosomes.

Acknowledgments

This work was financially supported by Rameshwardas Birla Smarak Kosh, Bombay. We thank Dr P Neelamkaval, the staff of the department of Dermatology, St John’s Medical College and the staff of Sumana Halli Rehabilitation Centre, Bangalore, for providing blood samples of the patients. We gratefully acknowledge the advice and critical review of the study proposal by Dr B C Das.

References

Chromosome aberrations and SCEs in untreated leprosy patients


TEACHING MATERIALS AND SERVICES

Gandhi Memorial Leprosy Foundation; training schedule

We are grateful to the Director, Mr S P Tare, for sending information about training courses in 1988. They are essentially under four main headings: paramedical workers (4 months); health education (2 months); medical officers (6 weeks); statistics (21 working days). These take place in Wardha and Chilakalapalli (Andhra Pradesh). Further information; Director, GMLF, Hindinagar Wardha, 442 103, India.

Clinical leprosy by V N Sehgal

We have just received a copy of this book from India, an illustrated second edition published by Jaypee Brothers, Medical Publishers, P.O. Box 7193, G-16, EMCA House, 23/23B, Ansari Road, Daryaganj, New Delhi 110 002, Price 45 rupees. The *International Journal of Leprosy* reviewed it as follows: 'The text of this book deals with different clinical facets of the disease, which are essential for the undergraduate and also postgraduate students. They are based on didactic lectures and clinical demonstration on leprosy, which the author has been imparting to students for the past two decades. . . . It may be useful also to practising physicians and leprosy field workers in developing countries in particular.'

KLEP; Karigiri Leprosy Education Programme

We have just received from Dr C J G Chacko, Head, Branch of Laboratories, SLRTC, Karigiri 632 106, India, this excellent 'Technical Manual for Smear Personnel'. It is, ' . . . a step-by-step instruction manual, written primarily for technicians and paramedical workers in developing countries where leprosy is endemic. The contents of the book has arisen out of interactions we have had with technicians and trainees, who face many problems in control programmes without adequate guidance and support. The book has been written in simple English (the vocabulary is Grade I in Chapters 1 and 2) since the majority of our target audience have difficulty in comprehending English. In addition, a page-by-page glossary is provided at the end of the book. '

(The to our knowledge, this is the fifth publication on the subject of slit-skin smears in leprosy in recent years and the time may be approaching for a 'consensus' meeting. In that case, this version form KLEP merits serious attention: it may be one of the best so far produced.—*Editor*.)

TALC teaching set; leprosy in childhood

Since its development in the early 1970s, this set of 24 colour transparencies on leprosy, accompanied by a written description and questionnaire, has sold 5469 copies. A follow-up analysis some years ago indicated that each set is seen by about 80 students in the first year after its receipt. It is now to be revised and brought up to date and a new version should be available later in 1988, or early in 1989.
TALC; Primary eye care
Also from TALC (Teaching Aids at Low Cost, P.O. Box 49, St Albans, Herts AL 1 4AX, England), this set of colour transparencies has been produced by Victoria Sheffield of Helen Keller International Incorporated, New York, and Felicity Savage King of the Institute of Child Health in London. There are 24 colour transparencies of high quality, accompanied by a written text, including notes for teachers. There is also a full size ‘C chart’ for testing vision and a useful glossary.

These sets are only two examples of a wide range of teaching-learning colour transparency sets available from TALC on almost every important aspect of medical care in developing countries, with particular emphasis on childhood. Full information about costs, postage, etc, can be obtained from the above address. Most sets with text cost only a few pounds sterling and there are considerable reductions for applicants working in developing countries. The quality of transparencies and written information is uniformly high and these sets, by any standards, are extremely good value for money.

OXFAM; packs of teaching-learning materials on leprosy
These packs of 8 items of teaching-learning material (English language) are still available from the Health Unit, OXFAM, 274 Banbury Road, Oxford OX2 7DZ, England, but stocks are running low; only about 100 remain. Since this service started in 1982, over 800 packs have been distributed, but there is now evidence that demand is falling and when present stocks are sold out, distribution will stop, probably towards the end of 1988.

Dermatology of black skin. Pocket atlas for dermatologists, 1986
This is a small, pocket size, hardback book of 111 pages, plus index, ‘... not just intended for dermatologists, but for all medical and paramedical staff looking after black patients’. The object is to describe and illustrate the clinical signs of skin diseases on the black skin and to emphasize the differences, compared to white skin. Infectious and parasitic disease have been omitted on the grounds that their features would require a separate volume, but an exception has been made in the case of leprosy, because of its obvious importance in black patients.

The booklet has been translated from the French by a consultant dermatologist, Dr Andrew Pembroke, King’s College Hospital, London. The authors are André Bassett (Strasbourg, France), Bernard Liautaud (Port-au-Prince, Haiti) and Bassirau Ndiaye (Dakar, Senegal). Published in England by Oxford University Press. The section on leprosy has some excellent clinical pictures and is for the most part accurate and helpful in its text. However, there are misleading statements about the distribution of leprosy in the world on page 76 and the WHO drug regimens on page 77, where it is stated that the regimens are based 'on combination therapy with rifampicin, ethionamide and clofazimine,' (they are in fact, based on the use of dapsone and rifampicin for paucibacillary cases and on dapsone, rifampicin and clofazimine for multibacillary cases, ethionamide being recommended in the treatment of multibacillary leprosy only in those cases who cannot tolerate or accept clofazimine). The patient in Fig 118 is described as 'untreated lepromatous leprosy' but in fact the appearances are strongly suggestive of the histoid form, and the statement relating alopecia to leonine facies in the same Figure is misleading. Despite these criticisms on one subject, the book is likely to be of practical value and the illustrations are of very high quality. Apply: any medical bookseller, or branch of Oxford University Press, Oxford, New York, Delhi, Singapore, Cape Town.

This is a strongly bound paperback, published by Churchill Livingstone and written by dermatologists (J D Wilkinson, S Shaw and D A Fenton) from High Wycombe and London. It has 153 pages and no fewer than 245 colour pictures of commonly occurring and important dermatological conditions, mainly UK, Europe and America-orientated, without attempting to include tropical dermatology. The explanatory text is brief but informative. The pictures are of unusually high quality. Although virtually all the patients are light-skinned, this booklet could be of practical value to doctors and paramedical workers in many parts of the world; the pictures are so well chosen that they have an almost instant message which should greatly assist diagnosis and management. Apply: any medical bookseller or branch of Churchill Livingstone: Edinburgh, London, Melbourne & New York.

International courses on leprosy, Fontilles, Spain
We are grateful to the Medical Director, Dr Terencio de las Aguas, Sanatorio de Fontilles, Fontilles, Alicante, Spain for sending details of the XXV International Course on Leprology for Doctors, organized by the Sanatorio San Francisco de Borja de Fontilles and Sovereign Military Order of Malta, with the collaboration of the School of Dermatology in the University of Valencia and the Ministry of Health. The course runs from 7 to 12 November 1988. Another course for health assistants (‘auxiliares sanitarios’) will be held from 10 to 29 October 1988. Apply to the Director as above.
The effects of chemotherapy on antibody levels in lepromatous patients

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Summary We compare whole cell ELISA antigens (*Mycobacterium leprae*) and two specific antigens: PG-I, phenolic glycolipid I of *M. leprae* and M-BGG, a synthetic antigen representing the terminal sugar of PG-I for their effectiveness in detecting antibody during chemotherapy. By the end of the 1st year of treatment, antibody levels to M-BGG had declined by 42% of the initial ELISA values, by the end of the 2nd year by 61% and at the end of 3 years by 68%. Declines of similar magnitude were seen with the other antigens. We examined these sera by RID for changes in levels of IgG, IgM and IgA antibodies. The levels of IgG remained abnormally high throughout the 3 years of antimicrobial therapy. The serum levels of IgM and IgA antibodies remained at the upper limits of normal range. The decline seen with antibody to *M. leprae* antigens was not reflected by a similar decline of serum immunoglobulin levels. Thus, application of ELISA monitoring during the course of treatment may be valuable in measuring the effectiveness of chemotherapy.

Introduction

With the emergence of ever increasing numbers of dapsone resistant *M. leprae* infections, the importance of monitoring chemotherapy has been established. Chemotherapy has been further compromised by poor compliance of patients (especially by light-skinned patients treated with clofazimine), the necessity of daily treatment, lack of drug delivery and by toxic side-effects. Newer chemotherapeutic agents such as rifampicin may alleviate some of these problems, but careful monitoring is still critically important. A sensitive assay which can detect quantitatively anti-*M. leprae* antibodies could reflect the bacterial load and hence the emergence of resistant mutants and/ or lack of adherence to a drug regimen. Changes in patients' sera may be early indicators of changes in disease status. To that end, we have been examining leprosy patients' sera by ELISA (enzyme-linked immunosorbent assay) with both natural and semisynthetic antigens for their efficacy in detecting antibody during treatment of lepromatous patients. Melsom *et al.* using a solid phase radioimmunoassay showed gradual changes in IgG and IgA antibody reactivity against antigen 7 and a smaller decline in specific IgM activity during the initial 2–4 years of therapy in lepromatous
patients. Similar declines in antibody levels with continuing chemotherapy in dapsone-responsive patients have been noted by others. We compare a whole cell ELISA antigen (M. leprae) and 2 specific ELISA antigens (PG-I, phenolic glycolipid I of M. leprae and M-BGG, a synthetic antigen representing the terminal sugar of PG-I which is conjugated to bovine gamma globulin) for their effectiveness in monitoring chemotherapy of lepromatous patients from the Leonard Wood Memorial Leprosy Research Center, Cebu, PI. As noted by others, the serodiagnosis of leprosy has been complicated by the extensive cross-reactions between mycobacterial species. The effects of chemotherapy on antibody levels in sera from 41 lepromatous individuals treated with dapsone over a 5-year period was examined by ELISA. We also examined these patients' sera immunoglobulin levels by radial immunodiffusion for quantitative changes in IgG, IgM and IgA antibodies.

Methods

ELISA

The ELISA used in this report was an indirect assay with a 0·05 ml suspension of antigen dried onto ‘U’ bottom microtiter plates. Antigen coated wells were blocked to prevent nonspecific binding by adding 0·075 ml of 5·0% goat serum in phosphate buffered saline, pH 7·2 (PBS) and incubating overnight at 4°C. The remainder of the assay was carried out as previously described.5,6

ANTIGEN PREPARATION

The Immulon II plates (Dynatech Laboratories, Alexandria, VA, 22314) were coated with antigen in a volatile buffer. The whole cells, M. leprae, used as ELISA antigen, were suspended in a volatile coating buffer at a concentration of 0·04 absorbance units. The volatile coating buffer used was 0·01 M, pH 8·2 ammonium acetate carbonate. The purified antigen, phenolic glycolipid I (PG-I) of M. leprae was prepared by sonicating the PG-I (100 µg/ml) in the volatile buffer for 30 s three times with a 3 mm probe.7,8 This suspension was then diluted to a concentration of 40 µg/ml with the same buffer. The third antigen was a neoglycoprotein, which contained the monosaccharide, 3,6-di-O-methyl glucopyranose, linked to bovine gamma globulin (M-BGG) and represented the terminal sugar of the phenolic glycolipid-I antigen of M. leprae.9 The M-BGG was diluted from the concentrated solution to 1:4000 (0·78 µg/ml) or 1:4500 (0·69 µg/ml) and mixed thoroughly. Each of the 3 antigens was coated on microtiter plates by adding 0·05 ml of antigen suspension to each well. The plates were then incubated overnight at 37°C to dry the antigen onto the plate. The antigen coated plates can then be stored for several months at room temperature. M. leprae, PG-I and M-BGG were provided under NIH contract by Dr P Brennan, Colorado State University.

SERA

In Cebu, sera were collected and stored every 4 weeks starting on admission and up to 6 months. Subsequently, blood withdrawal was performed every 6 months up to the 24th month and then once a year up to the end of the study on the 5th year.

CASE DATA

Sera from this study was collected from patients who participated in the Joint Chemotherapy Trial (JCT) conducted by Leonard Wood Memorial Center for Leprosy Research in Cebu, Philippines in collaboration with Sasakawa Memorial Health Foundation Tokyo, Japan and the Department of Health, Philippines. Sera of patients from 2 chemotherapeutic protocols (1a and 1c) of the joint chemotherapy study were used. Patients in group 1a received dapsone 100 mg/day, 6 times per week for 5 years and rifampicin 1·2 g once on admission. Patients in group 1c received dapsone 100 mg/
day, 6 times per week for 5 years, and clofazimine 100 mg three times per week for the first 24 weeks and rifampicin 1·2 g once on admission. The patients were newly diagnosed previously untreated lepromatous cases free of any serious intercurrent disease and screened by chest X-ray to be free of tuberculosis and of ENL on admission to the trial, all were clinically lepromatous and histologically LL with mean bacterial index of 4·59 ± 0·4. A total of 38 patients were admitted over a 3-year period, sequentially and randomly allocated to either 1a or 1c. There were 17 males with ages 12–56 years old (average age 25·47) on admission to group 1a and 17 males 12–55 years old (average age 22·41) and 3 females 16–22 years old (average age 18·33) for group 1c. Eight patients in 1a and 8 patients in 1c dropped out during the course of the drug trial. Preliminary evaluation did not show any significant difference between the 2 therapy regimens (1a vs 1c) in terms of clinical improvement, bacteriologic reduction and occurrence of reaction states. Because of the low number of patients in each treatment group, the varying length of patient participation within the trial and lack of observable differences in patient recovery between the therapy regimens, the data from the 2 groups were combined unless otherwise stated.

**RADIAL IMMUNODIFFUSSION**

The radial immunodiffusion procedure was used for the quantitation of IgG, IgM and IgA. The prepared kits were from Helena Laboratories (Beaumont, Texas). The immunoglobulin reference standards were standardized against the World Health Organization Reference Standard.

![Figure 1. This figure represents the pooled data from patients treated by both therapeutic protocols (1a & 1c). Each dot represents the average of paired ELISA values for each serum. The ELISA values have been adjusted to represent a percentage of the patients ELISA value at the start of treatment. The bars indicate mean values. The neoglycoprotein antigen used contained the monosaccharide, 3,6-di-O-methyl glycopyranose, linked to bovine gamma globulin (M-BGG), representing the terminal sugar of the phenolic glycolipid-I antigen of *M. leprae*. The 1st year of chemotherapy resulted in a 43% decline in ELISA values. As treatment continued an additional decline in median ELISA values was observed of 18% in the 2nd year and 7% in the 3rd year. Thus resulting in an over all decline of 68% from initial values after 3 years of treatment.](image-url)
Results

EFFECT OF THERAPY ON SPECIFIC ANTIBODY LEVELS IN LEPROMATOUS PATIENTS

Figure 1 shows the decline in ELISA values of M-BGG over 4 years of chemotherapy of lepromatous patients. By the end of the 1st year of therapy, antibody levels from 22 patients to the synthetic antigen M-BGG had declined to 58% (+/− 8·17% with 95% confidence limit using Student’s t distribution) of the initial ELISA values. By the end of the 2nd year, the mean ELISA values from 19 patients had dropped to 39% (+/− 6·3% with 95% confidence limit) of the initial values and at the end of 3 years, the mean for 18 patients had reached 31·8% (+/− 6·1% with 95% confidence limit) of the starting values. This is a mean decline of 68% over a 3-year period after the initiation of therapy.

Tables 1 and 2 present ELISA data demonstrating individual patients sera reactivity to the 3 antigens over the course of treatment with the 2 protocols (1a & 1c). Table 1 shows the decline of antibody titer to the synthetic antigen M-BGG, the semisynthetic antigen PG-I and whole cell antigen from M. leprae with treatment. In the 4 patient examples, there were significant declines demonstrated with all 3 antigen preparations over a 3-year period. There were also declines in the bacterial indices. For example, patient number 019 had an initial M-BGG OD of 0·70 and after 1 year of chemotherapy this value had dropped to 0·28; after 2 years to 0·17 and after 3 years to 0·10.

Table 1. Use of M-BGG, PG-I and M. leprae in serial testing of sera from treated lepromatous patients*

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Date of bleedings</th>
<th>M-BGG</th>
<th>M. leprae</th>
<th>PGI</th>
<th>Bacterial Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>035</td>
<td>13-02-81</td>
<td>0·86</td>
<td>0·82</td>
<td>0·78</td>
<td>4·3</td>
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<td>0·61</td>
<td>0·71</td>
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<td></td>
<td>16-08-82</td>
<td>0·51</td>
<td>0·43</td>
<td>0·53</td>
<td>4·2</td>
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<td></td>
<td>25-02-83</td>
<td>0·43</td>
<td>0·30</td>
<td>0·42</td>
<td>4·5</td>
</tr>
<tr>
<td></td>
<td>25-02-84</td>
<td>0·36</td>
<td>0·36</td>
<td>0·44</td>
<td>3·7</td>
</tr>
<tr>
<td>045</td>
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<td>0·25</td>
<td>0·79</td>
<td>0·37</td>
<td>3·8</td>
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<td>0·48</td>
<td>0·14</td>
<td>4·0</td>
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<tr>
<td></td>
<td>10-01-83</td>
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<td>0·13</td>
<td>4·0</td>
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<td>3·0</td>
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<td>007</td>
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<td>1·10</td>
<td>1·10</td>
<td>4·0</td>
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<td></td>
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<td>0·26</td>
<td>0</td>
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<td>1·54</td>
<td>0·87</td>
<td>4·7</td>
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<td></td>
<td>25-05-81</td>
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<td>0·73</td>
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<td>1·26</td>
<td>0·40</td>
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<td>0·87</td>
<td>0·20</td>
<td>2·0</td>
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<td>23-05-83</td>
<td>0·10</td>
<td>0·75</td>
<td>0·19</td>
<td>0·8</td>
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<td></td>
<td>22-05-84</td>
<td>0·10</td>
<td>0·73</td>
<td>0·16</td>
<td>0·4</td>
</tr>
</tbody>
</table>

* Chemotherapy was conducted at the Leonard Wood Foundation facility in Cebu, Philippines in conjunction with the Sasakawa Memorial Health Foundation. Chemotherapeutic protocol ‘1a’: dapsone, 100 mg six times per week for 5 years; rifampicin, 1·2 g once on admission.
Chemo therapy effects on antibody levels

Table 2. Use of M-BGG, PG-I and \textit{M. leprae} in serial testing of sera from treated lepromatous patients*

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Date of bleedings</th>
<th>Mean A492nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M-BGG</td>
<td>\textit{M. leprae}</td>
</tr>
<tr>
<td>022</td>
<td>04-11-80</td>
<td>1·01</td>
</tr>
<tr>
<td></td>
<td>04-02-81</td>
<td>0·91</td>
</tr>
<tr>
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<td>02-08-82</td>
<td>0·29</td>
</tr>
<tr>
<td></td>
<td>02-08-83</td>
<td>0·16</td>
</tr>
<tr>
<td></td>
<td>02-08-84</td>
<td>0·14</td>
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<tr>
<td>056</td>
<td>22-01-82</td>
<td>0·60</td>
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<td>02-08-82</td>
<td>0·30</td>
</tr>
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<td></td>
<td>04-02-83</td>
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<td>055</td>
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<td>02-08-82</td>
<td>0·43</td>
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<td>03-05-83</td>
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</tr>
<tr>
<td></td>
<td>02-05-84</td>
<td>0·09</td>
</tr>
</tbody>
</table>

* Chemotherapy was conducted at the Leonard Wood Foundation facility in Cebu, Philippines, in conjunction with the Sasakawa Memorial Health Foundation. Chemotherapeutic protocol 'lc': dapsone, 100 mg six times per week for 5 years; clofazimine, 100 mg three times per week for 24 weeks; rifampicin, 1·2 g once on admission.

The values for \textit{M. leprae} were: 1·54, 1·26, 0·87 and 0·75. For PG-I the values declined as follows: 0·87, 0·40, 0·20, and 0·19. The bacterial index also showed decline: 4·7, 3·3, 2·0, 0·8. The decline in ELISA values was most rapid with the M-BGG and PG-I antigens.

Table 2 shows the decline in titer with the same antigens after similar chemotherapy but with the addition of clofazimine, 100 mg three times per week for 24 weeks. Again there were significant declines with the 3 antigens and in the bacterial indices. A representative patient, number 022, showed a drop in OD for M-BGG from 0·91 to 0·14 after 3 years of chemotherapy and a drop from OD 0·56 to 0·28 for \textit{M. leprae} and 1·08 to 0·21 for PG-I. The bacterial index in the same patient dropped from 3·2 to 2·0. Table 3 shows data from both JCT 1a and 1c from a 3-year period of treatment. At the end of 3 years of chemotherapy, the antibody levels as detected by M-BGG had declined by 68%, PG-I by 64% and \textit{M. leprae} by 56% there was no significant difference found between JCT 1a and 1c in terms of decline of antibody levels to the leprosy antigens. Our ELISA did reveal more antibody decline with M-BGG and PG-I than with \textit{M. leprae}.

Comparison of declines in specific antibody and bacterial index during the course of treatment

Table 4 compares the decline of antibody levels as detected by M-BGG and the decline in bacterial
Table 3. Percent decline in antibody levels with JCT 1a, 1c treatment regimens as measured by M-BGG, PG-1 and * {\textit{M. leprae}} ELISA*

<table>
<thead>
<tr>
<th>Antigen</th>
<th>1 yr (n = 22)</th>
<th>2 yr (n = 19)</th>
<th>3 yr (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-BGG</td>
<td>45% (21–84)†</td>
<td>56% (17–90)</td>
<td>68% (48–94)</td>
</tr>
<tr>
<td>PG-1</td>
<td>42% (9–82)</td>
<td>55% (15–88)</td>
<td>64% (2–95)</td>
</tr>
<tr>
<td><em>{\textit{M. leprae}}</em></td>
<td>38% (5–72)</td>
<td>51% (2–86)</td>
<td>56% (5–81)</td>
</tr>
</tbody>
</table>

* JCT 1a regimen: dapsone, 100 mg six times per week for 5 years; rifampicin, 1·2 g once on admission.

JCT 1c regimen: JCT 1a and clofazimine, 100 mg 3 times per week for 24 weeks.

† Mean % decline (range).

Table 4. Effect of treatment on lepromatous patients as measured by decline in ELISA antibody and Bacterial Index (BI)*

<table>
<thead>
<tr>
<th>Years of treatment</th>
<th>0 (n = 21)</th>
<th>1 (n = 17)</th>
<th>2 (n = 18)</th>
<th>3 (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA†</td>
<td>100‡</td>
<td>55 ± 19</td>
<td>44 ± 13</td>
<td>32 ± 12</td>
</tr>
<tr>
<td>BI</td>
<td>4·7 ± 0·4§</td>
<td>3·9 ± 1·0</td>
<td>2·9 ± 1·3</td>
<td>2·1 ± 1·0</td>
</tr>
</tbody>
</table>

* Chemotherapy was conducted at the Leonard Wood Foundation facility in Cebu, Philippines, in conjunction with the Sasakawa Memorial Health Foundation.

† M-BGG—ELISA anti-IgG am conjugate.

‡ Percent of ELISA at start of treatment.

§ SD.

index with 3 years of chemotherapy. The antibody levels can be seen to decline 68% of starting levels and the bacterial index, was found to decline from 4·7 to 2·1 over the 3-year period.

COMPARISON OF ELISA VALUES AND IMMUNOGLOBULIN LEVELS

Table 5 presents a comparison of ELISA values and immunoglobulin levels over the course of 3 years of treatment of lepromatous patients. The mean levels of IgG and IgM declined with treatment, but remained elevated above the mean of normal sera. The IgG had an initial value of 2716 ± 552, 2506 ± 704 after 1 year of therapy, 2484 ± 822 after 2 years and 2244 ± 804 after 3 years. The IgM before treatment was 259 ± 72 and declined to 214 ± 65 after 1 year, to 207 ± 68 after 2 years and to 195 ± 68 after 3 years of therapy. Although declining slightly, the IgG levels remained far above normal values which indicates that there was chronic hypergammaglobulinemia maintained in the face of treatment. The levels of IgM had moved close to the upper limits of normal
Table 5. Comparison of immunoglobulin levels and ELISA activity over the course of treatment of lepromatous patients

<table>
<thead>
<tr>
<th>Years of treatment</th>
<th>IgG</th>
<th>IgM</th>
<th>IgA</th>
<th>ELISA</th>
<th>Normal values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (n = 21)</td>
<td>1 (n = 17)</td>
<td>2 (n = 18)</td>
<td>3 (n = 15)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2716 ± 552†</td>
<td>2506 ± 704</td>
<td>2484 ± 822</td>
<td>2244 ± 804</td>
<td>1125 (710–1540)†</td>
</tr>
<tr>
<td></td>
<td>259 ± 72</td>
<td>214 ± 65</td>
<td>207 ± 68</td>
<td>195 ± 68</td>
<td>121 (37–204)</td>
</tr>
<tr>
<td></td>
<td>333 ± 173</td>
<td>417 ± 208</td>
<td>386 ± 132</td>
<td>492 ± 177</td>
<td>275 (60–490)</td>
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<tr>
<td></td>
<td>100§</td>
<td>55 ± 19</td>
<td>44 ± 13</td>
<td>32 ± 12</td>
<td>0</td>
</tr>
</tbody>
</table>

* Chemotherapy was conducted at the Leonard Wood Foundation facility in Cebu, Philippines, in conjunction with the Sasakawa Memorial Health Foundation.
† Means SD.
‡ Means (Range).
§ Percent of initial ELISA reading.

Discussion

Regardless of the 2 protocols (Ia & Ic) used for treatment, we found antibody levels in lepromatous sera to both semisynthetic and natural antigens declined with length of treatment. The measurement of declining antibody titers in relation to treatment have been reported by others using different antigens and assays systems. Our report is important because it studies the effects of treatment on antibody levels to both natural and synthetic antigens of \textit{M. leprae} over an extended period of time and compares the specific decline in antibody to \textit{M. leprae} antigens with Bacterial Index and with serum immunoglobulin levels. A significant decline was demonstrable after 1 year of therapy and continued over time (Figure 1 and Table 3). By the end of the 1st year of therapy, antibody levels to the semisynthetic or neoglycoprotein antigen M-BGG had declined to a mean value 55% and median value of 58% of the initial ELISA values. By the end of the 2nd year the median ELISA values had dropped to 39% of the initial values with a mean of 44%. At the end of 3 years the median had reached 32% of the starting values with the mean also at 32%. This is a decline of 68% over the 3-year period after the initiation of therapy. We found that the decline in antibody levels as detected by ELISA correlated well with decline in the bacterial index and such declines continued with length of treatment. Two patients numbers 045 and 035 listed in Table 1, show an increase in BI after 2 years of treatment. Examination of their clinical records of these patients and the remaining patients show continued improvement during this period. Others have reported similar correlations between antibodies and bacterial index. Of the 3 antigens studied in Table 3, the greatest decline in ELISA reactivity with 3 years of treatment was seen with M-BGG (68%) and PG-I (66%). The reactivity to whole cell antigen \textit{M. leprae} declined more slowly (56%) during the
same period. This may have been due to the complexity of the whole cell antigen: presentation of more epitopes and the use of an ELISA conjugate which measures IgG, IgM and IgA reactivity.

We also examined these patients’ sera for quantitative changes in IgG, IgM and IgA antibodies by radial immunodiffusion. There was no correlation between antibody levels and chemotherapy or ELISA values. Within a particular patient there were episodes of abnormally high levels of antibodies interspersed with normal values. Also, a particular patient could have abnormally high values of one class of antibodies and normal values for others. Toul16 also reported elevated immunoglobulins but no correlation with bacillary load or other clinical parameters. While the M-BGG ELISA shows a 68% decline after 3 years of chemotherapy, many of the immunoglobulin levels remain above normal values. Thus, it is unlikely that the decline in ELISA values is merely the result of decline in immunoglobulin levels. We speculate that multidrug therapy, because its improved bacteriocidal activity, may generate a more rapid decline since the long term regimens examined herein were primarily monotherapy with dapsone. We are currently examining this unsubstantiated hypothesis on the effects of multidrug therapy and antibody specific antibody levels to \textit{M. leprae}.

In summary, we found that in lepromatous patients followed over a period of more than 3 years antibody titers to \textit{M. leprae} antigens declined throughout the duration of treatment. This decline in specific antibody also parallels the decline in Bacterial Index (BI). This decline in specific antibody was observed with 3 antigens: both M-BGG and PG-I specific antigens as well as with whole \textit{M. leprae}. Significant decline (> 40%) was demonstrable after 1 year and decline continued with treatment such that by the end of 3 years of treatment antibody levels had declined by > 60% of original levels. In spite of the dramatic decline in antibody against \textit{M. leprae} antigens, no correlation could be established within the patterns of class specific hypergammaglobulinemia observed during the course of treatment. This assay could be useful for monitoring drug therapy and the emergence of drug-resistant bacilli in response to therapy. In spite of the dramatic decline in antibody against \textit{M. leprae} antigens, no correlation could be established within the patterns of class specific hypergammaglobulinemia observed during the course of treatment. It has been noted17 that nonviable mycobacterial ‘skeletons’ can persist well into therapy and present a chronic antigenic stimulus to the host.

Acknowledgments

This research was supported by the Leonard Wood Memorial American Leprosy Foundation, Rockville, Maryland; National Institutes of Allergy and Infectious Diseases Grant (AI 24154), the Pacific Health Research Institute, Honolulu, Hawaii; the Heiser Program for Research in Leprosy, New York, New York; the Sasakawa Memorial Health Foundation, Tokyo, Japan; and the University of Hawaii Office of Research Administration. We thank the Leonard Wood Memorial Staff in Cebu, PI and the Philippine Government, Department of Health for JCT data and sera. In addition, we thank Dr Li Futian of the Shanghai Zun Yi Hospital for his assistance with statistical methods.

References

Many facilities offered by the Centre for Physical Education. The Department of Education of the University of Manchester is virtually unique in that it is able to offer a range of courses studying education from childhood to adulthood, in all its phases, in a single Department. Award-bearing courses are offered at all levels from Certificate, Diploma and Master's Degree, to the Degree of Doctor of Philosophy. Short, non-award bearing courses can also be designed on request.

Supplies of thalidomide from Brasil

In a previous issue of this journal, full details were given of the resumption of production and supply of thalidomide by Chemie Grunenthal in West Germany (Volume 58, Number 3, September 1987). We have also previously published sources for this drug in Brasil and we have recently received a letter from another company, in Sao Paulo, which offers this drug for sale. It is: Tortuga Companhia Zootécnica Agniria, Av. Brigadeiro Faria Lima, 1.409–14° andar, Jardim Paulistano, 01451 – Sao Paulo – SP, BRASIL. Telex (011) 22270 +.

From the current descriptive brochure:

The Department of Education of the University of Manchester is virtually unique in that it is able to offer a range of courses studying education from childhood to adulthood, in all its phases, in a single Department. Award-bearing courses are offered at all levels from Certificate, Diploma and Master’s Degree, to the Degree of Doctor of Philosophy. Short, non-award bearing courses can also be designed on request.

Facilities available include the John Rylands University Library, which houses over 3 million volumes, and two Departmental libraries, one of which contains the largest collection of its kind in Europe. Technical resources include a microcomputing unit and photographic laboratories. A cottage in the heart of the beautiful Lake District provides a base for outdoor pursuits for students interested in sailing and canoeing: one of the many facilities offered by the Centre for Physical Education.

The diversity of the Department's activities engenders a network of national and international contacts in the field of Education, including link arrangements with a number of overseas universities. Its 80 academic and research staff offer a wide range of experience and expertise across many areas from the initial and in-service training of teachers in the UK, to the training of village health care workers in the developing world. Students from 50 countries are currently studying in the Department. The English Language Unit, in the Centre for Adult and Higher Education, offers a range of courses in TEFL and TESOL.

All enquiries to Professor John D Turner, Head, Department of Education, University of Manchester, Manchester M13 9PL, England.
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 3 travel fellowships each year to a maximum value of £1200 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.

The Italian Society of Tropical Medicine

The Italian Society of Tropical Medicine (SIMET), a nonprofit organization, is a scientific society founded in 1983 under the patronage of the Ministry of Foreign Affairs (more precisely the ‘Direzione Generale per la Cooperazione allo Sviluppo’) to promote initiatives on a national level for better knowledge and more information on social problems and public health in developing countries.

The Italian Society of Tropical Medicine has opened: 1, A resource centre provided with scientific, informative and teaching equipment, made up of magazines, books, publications, slides, films, etc., available for the interested public. 2, A magazine ‘La Medicina Tropicale nella Cooperazione allo Sviluppo’ which deals with aspects of health cooperation and is available by subscription or after registration to SIMET. 3, An informative bulletin ‘Cooperazione Sanitaria’ contains summaries of the most recent news, from international magazines, on the health cooperation programmes and also important events such as conferences, congresses, symposia and refresher courses in scientific subjects. 4, A traveller service for all those who go to tropical countries. This service advises on preventative precautions against the most important tropical diseases. 5, An informative service for the doctors, general practitioners or specialists in other disciplines about health precautions for patients travelling to developing countries.

Moreover, the goal of this centre is to promote and spread the knowledge of tropical medicine in the development cooperation on a wide base, through the organization of congresses, round tables, symposia and conferences on specific subjects.

Further information: SIMET, Società Italiana di Medicina Tropicale, Piazzale Ponte Milvio, 20-00191 Roma, Italy. Tel. 3963702

Takemi Program for International Health

The Takemi Program in International Health at the Harvard School of Public Health seeks applications for a limited number of fellowships for research and advanced training on critical issues of international health, especially those relating to developing countries. The program focuses primarily on mobilizing, allocating and managing scarce resources to improve health, and on creating sound strategies for disease control and health promotion. The program brings together graduates of advanced degree programs and mid-career professionals from around the world from diverse disciplines. Appointments begin on September for a ten-month period. For additional information contact: Prof Lincoln C Chen, Director, Takemi Program in International Health, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115, USA.

Socio-economic emancipation and village autonomy, India

The Secretary of the Society for Socio-Economic Emancipation and Village Autonomy, Manika – 822 126, District Palamau, Bihar, India, has written with following information:

'We have been active in this region since November 1982. I take this opportunity to inform you that to date we have trained around 350 tribal families in Tassar silk cocoon rearing, 100 young boys and girls in Tassar silk spinning and weaving. We have recently started a training centre in hog’s hair processing and paint-brush making. Silk and paint-brush production has already started and we are looking for suitable markets for these products.'

Development of voluntary organizations

We are grateful for the following information from Dr V Periaswami in Anakapalle, India:

'In your editorial of Leprosy Review (Vol. 58, No. 2, June 1987 under the heading “Development of Voluntary Organizations” it is stated that the first “Leprosy Asylum” was established in Calcutta early in the nineteenth century followed by another in Varanasi. May I mention that during the life of Guru Arjan Singh (15 4-1563 to 30 5·1606) the 5th Guru in succession of Sikh Gurus, established a “Leper Colony” at Taran Taran in Amritsar District of the present Panjab State, North India. I request that you kindly publish this information as a news item in your esteemed journal. (Ref: Nagpur Times, (Sunday spread) published in India, 26 8 84.)
In vitro methods for determination of viability of mycobacteria: comparison of ATP content, morphological index and FDA-EB fluorescent staining in *Mycobacterium leprae*

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Accepted for publication 1 October 1987

**Summary** Bacilli were purified from the 23 cases of multibacillary type of leprosy. The ATP content of these bacilli was assayed by a firefly bioluminescent technique which is capable of detecting a very small number of cultivable mycobacteria as assessed by colony counts. The ATP content was compared with morphological index (MI) and FDA-EB staining of bacilli from the same specimens. It was observed that when MI was 1% or more, the ATP content/solid bacillus was fairly constant in 15 cases studied. It ranged from $2.02 \times 10^{-15}$ g to $5.60 \times 10^{-15}$ g/solid bacillus (mean $3.46 \times 10^{-15}$ g) and was in the same range as ATP content of viable cultivable mycobacteria. In the same 15 cases, when the green-staining bacilli was considered as 'supposedly viable bacilli', ATP content/green-staining bacillus varied up to 9-fold ($0.22 - 1.98 \times 10^{-15}$ g/green-staining bacillus) and this did not correlate. The percentage of green-staining bacilli (FDA-EB) and solid-staining bacilli (MI) was different in all the cases. In 2 cases with 0% MI in which ATP levels were also zero, 7.5% and 21.5% green-staining bacilli were present which implies that the enzymes responsible for green-staining character may persist for some time after death. Three cases with 0% MI had also 0% green-staining bacilli and zero ATP levels, whereas in another 3 cases with zero MI significantly high levels of ATP were detected. It is inferred that solid-staining bacilli may be the viable bacilli but when MI is 0% (1% or less) sampling error or clumping may be responsible for missing out the solid bacilli in some cases. It is concluded that the ATP content of *M. leprae* appears to be an easy, rapid and sensitive tool for determining the viability for monitoring the therapy. On the other hand MI and FDA-EB staining appear to have their limitations.

**Introduction**

As *Mycobacterium leprae* can not be cultivated in *in vitro* systems, there is a strong need to develop other rapid screening methods for monitoring the effect of therapy as growth in the mouse footpad.
takes a long time. Any reliable indirect method for determining the viability of *M. leprae* would be of great interest for assessing the progress of disease and response to therapy. The percentage of solid-staining bacilli (Morphological Index) has been used for a long time to assess the effect of therapy.\(^{1,2}\) Other studies\(^{3,4}\) postulated that nonsolid forms of bacilli were nonviable whereas elsewhere\(^{5}\) the multiplication of *M. leprae* even from specimens with 0% solid organisms was reported.

All living cells contain adenosine-5-triphosphate (ATP), which is present in fairly constant amounts in each cell type and which is rapidly lost after the cells die. These facts provide the basis of the bioluminescent assay of microbial mass which involves the measurement of light produced as a result of an enzymatic reaction catalysed by firefly luciferase. The bioluminescent assay has already been used to measure the biomass of several organisms.\(^{6,8}\) The ATP content of *M. leprae* has been measured\(^{9-13}\) and recently it has also been reported that *M. leprae* synthesizes its own ATP.\(^{14}\) But all these ATP measurements have been on total *M. leprae* populations and variable results have been reported.\(^{10-13}\) In these reports, no effort was made to estimate the number of viable cells by direct or indirect methods.

A fluorescent-staining procedure incorporating the use of fluorescein diacetate (FDA) and ethidium bromide (EB) has been recently described for *M. leprae*\(^{15}\) and it has been reported that there was significant decrease in the percentage of green-stained bacteria with increased periods of therapy.\(^{15,16}\) Because of varying results reported, it would be of interest to compare these methods in the same specimen from same patient. It would also be important to investigate the factors which could influence the results.

In the present study, ATP content of *M. leprae* from different multibacillary leprosy cases has been measured and its correlation with morphological index (MI) and FDA-EB fluorescent staining from the same specimen has been assessed.

### Materials and methods

#### MATERIALS

Biopsies from 23 multibacillary leprosy cases and 5 healthy controls attending the OPD of our Institute were taken. These patients were in different stages of treatment and on different drug regimens.

*Mycobacterium tuberculosis* H\(_{37}Rv* (TMC 102) and *M. lufu* (obtained from Dr F Portaels, Belgium) were grown in Sauton’s medium\(^ {17}\) and used for studying the effect of purification procedures as well as ATP extraction and assay techniques.

#### PROCEDURES

**A. Standardization of procedures**

*M. tuberculosis* H\(_{37}Rv* (TMC 102) and *M. lufu* were grown in Sauton’s medium and log phase growth were harvested. Suspensions were prepared and used for studying the effect of purification and extraction procedures. The purification procedure described by Dhople & Storrs\(^ {10}\) was used. For decontamination 2% (0.5 M) and 4% (1 M) NaOH was used with 20, 30 and 60 min of incubation period at room temperature. For ATP extraction, (i) trichloroacetic acid (TCA) extraction technique,\(^ {18,19}\) (ii) tris boiling method,\(^ {18,19}\) and (iii) chloroform boiling method\(^ {20}\) were compared.

For estimating the correlation between ATP content and viable numbers, the mycobacterial suspensions were diluted in buffered Tween and were plated on Lowenstein Jensen medium.
B Processing of biopsies

(i) Biopsies were homogenized as described in another study.\textsuperscript{10}

(ii) The suspensions after following the modified decontamination procedure,\textsuperscript{10} were used for ATP extraction. From the final suspensions, duplicate smears were prepared on circular slides and processed as described in steps (iii) and (iv). In the decontamination procedure 2\% NaOH (0.5 M) finally was used as this gave optimum results. ATP was extracted by the Tris-EDTA boiling method as this method gave the optimum and reproducible results in our experimental system.

(iii) From the suspensions, the duplicate smears were prepared on circular slides and stained with Ziehl-Nielsen staining and counting was done by the method of McRae & Shepard.\textsuperscript{21} Morphological Index (MI) was calculated on these smears by the technique of McRae & Shepard.\textsuperscript{21} From the total counts and \% solid-staining bacilli (MI), the population of solid bacilli was calculated.

(iv) The other set of smears was stained with fluorescein diacetate (FDA) and ethidium bromide (EB) and examined under the fluorescent microscope by the technique of Kvach \textit{et al.}\textsuperscript{15} From the total counts and \% green-staining bacilli, the green-staining bacillary population was calculated.

(v) ATP was assayed in a Lumitran L-3000 ATP Photometer (New Brunswick Inc. USA) by using the method of Lehtokari \textit{et al.}\textsuperscript{19} One minute integrated ATP counts were taken and concentration were calculated using the standard ATP from LKB.

(vi) After measuring the ATP content in the total bacillary population, ATP content/solid bacillus and ATP content/green-staining bacillus were calculated for each sample as following:

\[
\text{ATP content/solid bacillus} = \frac{\text{Total ATP content}}{\text{Number of solid-staining bacilli estimated in step (iii)}}
\]

\[
\text{ATP content/green staining bacillus} = \frac{\text{Total ATP content}}{\text{Number of green-staining bacilli estimated in step (iv)}}
\]

Results

The results are detailed in Tables 1–4.

**CORRELATION BETWEEN VIABLE COLONY COUNTS/ATP CONTENT**

The viable number of bacilli were determined by colony counts after diluting the suspension in buffered Tween and these correlated directly with ATP levels in \textit{M. tuberculosis} and \textit{M. lufu}. Using this procedure, it was possible to detect $< 100$ viable mycobacteria. This ATP content was found to be reproducible in 3 sets of observations even with 10–100 viable organisms. The ATP content/viable cell was found to be: $2·0–3·4 \times 10^{-15}$ g (\textit{M. tuberculosis}) and $3·5–5·8 \times 10^{-15}$ g (\textit{M. lufu}) respectively.

**EFFECT OF PURIFICATION PROCEDURE**

Except for prolonged incubation with 4\% NaOH (1 m), no other steps in the purification procedure of Dhople & Storrs\textsuperscript{10} were found to interfere with the ATP levels in \textit{M. tuberculosis}, \textit{M. lufu} and human-derived \textit{M. leprae}. The results are detailed in Table 1. It was found that ATP levels significantly fell down when the incubation with 4\% (1 m) NaOH was done for 30 min or more whereas 2\% NaOH (0.5 m) did not affect the mycobacterial ATP even after 1 hr of treatment. This 2\% (0.5 m) NaOH treatment was sufficient to remove the host derived ATP completely. Based on these observations we have used 0.5 m NaOH in our study.
Table 1. Effect of 4% (1 M) NaOH and 2% NaOH (0.5 M) on ATP levels in different mycobacteria. ATP levels (g)*/10^6 bacilli

<table>
<thead>
<tr>
<th>Time of incubation</th>
<th>M. tuberculosis</th>
<th>M. lulu</th>
<th>M. leprae</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h</td>
<td>2.60 x 10^{-9}</td>
<td>2.85 x 10^{-9}</td>
<td>1.57 x 10^{-9}</td>
</tr>
<tr>
<td>20 min</td>
<td>4.27 x 10^{-9}</td>
<td>3.94 x 10^{-9}</td>
<td>2.99 x 10^{-9}</td>
</tr>
<tr>
<td>30 min</td>
<td>1.65 x 10^{-10}</td>
<td>1.55 x 10^{-10}</td>
<td>1.07 x 10^{-10}</td>
</tr>
<tr>
<td>1 h</td>
<td>3.10 x 10^{-9}</td>
<td>2.85 x 10^{-9}</td>
<td>3.00 x 10^{-9}</td>
</tr>
<tr>
<td></td>
<td>5.30 x 10^{-9}</td>
<td>4.80 x 10^{-9}</td>
<td>5.10 x 10^{-9}</td>
</tr>
<tr>
<td></td>
<td>2.50 x 10^{-10}</td>
<td>2.70 x 10^{-10}</td>
<td>2.40 x 10^{-10}</td>
</tr>
</tbody>
</table>

* Mean: based on 3 observations.

ATP EXTRACTION PROCEDURES

We have compared TCA extraction method, chloroform heat method and Tris-EDTA boiling method for extraction of ATP from M. tuberculosis, M. lulu and human-derived M. leprae. It was observed that TCA extraction method extracted the maximum levels of ATP from each of the mycobacteria tested. Considering it was (100%), the efficiency of other methods was calculated. Finally Tris-EDTA boiling method was used in our study because of its convenience and optimum results. The results are detailed in Table 2.

Table 2. Efficiency (%) of different methods for ATP extraction based on two observations

<table>
<thead>
<tr>
<th></th>
<th>M. tuberculosis</th>
<th>M. lulu</th>
<th>M. leprae</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCA extraction method</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Chloroform-heat method</td>
<td>86</td>
<td>80</td>
<td>76</td>
</tr>
<tr>
<td>Tris-EDTA boiling method</td>
<td>98</td>
<td>96</td>
<td>98</td>
</tr>
</tbody>
</table>

ATP LEVELS, MI AND FDA-EB STAINING OF MATERIAL PROCESSED FROM BIOPSIES

The results are summarized in Tables 3 and 4.

It was observed that: (i) MI of patients who were in different stages of therapy varied from 0 to 9%; (ii) the percentage of green-staining bacilli by FDA-EB staining in these smears varied from 0 to 45%; (iii) the percentage of green-staining bacilli and solid-staining bacilli (MI) were different in all cases. Not only were the absolute values different, but there was no apparent relationship between MI and percentage of green-staining bacilli; (iv) the host ATP in the skin tissues could be completely removed by the purification procedure used in the study; (v) when the MI was 1% or more, the ATP content per solid bacillus was fairly constant in 15 of the cases studied. It ranged from 2.02 x 10^{-15} g to 5.60 x 10^{-15} g/solid bacillus with mean 3.46 x 10^{-15} g; (vi) when the green-staining bacilli were considered as 'Supposedly viable bacilli', the ATP content/green-staining bacillus from different multibacillary cases showed huge variations. The ATP content/green-staining bacillus ranged from 0.22 x 10^{-15} g to 1.98 x 10^{-15} g among different cases. However in 9/15 cases, the ATP content/green-staining bacillus ranged from 0.22 x 10^{-15} g to 0.66 x 10^{-15} g; (vii) three cases with 0% MI,
Table 3. The ATP content per solid-staining bacillus and per green-staining bacillus in multbacillary patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>MI (%)</th>
<th>Green-staining by FDA-EB (%)</th>
<th>ATP content/solid bacillus* (g)</th>
<th>ATP content/green-staining bacillus† (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 RB</td>
<td>9·0</td>
<td>38·0</td>
<td>$3·80 \times 10^{-15}$</td>
<td>$0·92 \times 10^{-15}$</td>
</tr>
<tr>
<td>2 AC</td>
<td>8·0</td>
<td>45·0</td>
<td>$4·48 \times 10^{-15}$</td>
<td>$0·89 \times 10^{-15}$</td>
</tr>
<tr>
<td>3 BH</td>
<td>5·5</td>
<td>20·0</td>
<td>$5·60 \times 10^{-15}$</td>
<td>$1·33 \times 10^{-15}$</td>
</tr>
<tr>
<td>4 BS</td>
<td>5·0</td>
<td>33·5</td>
<td>$3·81 \times 10^{-15}$</td>
<td>$0·66 \times 10^{-15}$</td>
</tr>
<tr>
<td>5 AF</td>
<td>5·0</td>
<td>18·0</td>
<td>$3·60 \times 10^{-15}$</td>
<td>$1·05 \times 10^{-15}$</td>
</tr>
<tr>
<td>6 BS</td>
<td>4·5</td>
<td>24·5</td>
<td>$2·24 \times 10^{-15}$</td>
<td>$0·44 \times 10^{-15}$</td>
</tr>
<tr>
<td>7 KC</td>
<td>4·5</td>
<td>13·0</td>
<td>$5·38 \times 10^{-15}$</td>
<td>$1·98 \times 10^{-15}$</td>
</tr>
<tr>
<td>8 IP</td>
<td>5·0</td>
<td>15·0</td>
<td>$2·60 \times 10^{-15}$</td>
<td>$0·62 \times 10^{-15}$</td>
</tr>
<tr>
<td>9 MH</td>
<td>4·0</td>
<td>30·0</td>
<td>$2·10 \times 10^{-15}$</td>
<td>$0·22 \times 10^{-15}$</td>
</tr>
<tr>
<td>10 NZ</td>
<td>3·5</td>
<td>21·5</td>
<td>$2·24 \times 10^{-15}$</td>
<td>$0·44 \times 10^{-15}$</td>
</tr>
<tr>
<td>11 LA</td>
<td>3·0</td>
<td>19·5</td>
<td>$3·90 \times 10^{-15}$</td>
<td>$0·66 \times 10^{-15}$</td>
</tr>
<tr>
<td>12 AM</td>
<td>3·0</td>
<td>10·0</td>
<td>$3·58 \times 10^{-15}$</td>
<td>$1·10 \times 10^{-15}$</td>
</tr>
<tr>
<td>13 KA</td>
<td>2·5</td>
<td>14·0</td>
<td>$2·69 \times 10^{-15}$</td>
<td>$0·44 \times 10^{-15}$</td>
</tr>
<tr>
<td>14 KC</td>
<td>2·5</td>
<td>22·0</td>
<td>$2·02 \times 10^{-15}$</td>
<td>$0·22 \times 10^{-15}$</td>
</tr>
<tr>
<td>15 PD</td>
<td>1·0</td>
<td>15·0</td>
<td>$3·90 \times 10^{-15}$</td>
<td>$0·22 \times 10^{-15}$</td>
</tr>
</tbody>
</table>

* Mean, $3·4 \times 10^{-15}$ g/solid bacillus.
† Mean, $0·8 \times 10^{-15}$ g/green-staining bacillus.

Table 4. ATP content and percentage of green-staining bacilli in patients who were having 0% MI*

<table>
<thead>
<tr>
<th>Slide No.</th>
<th>Patient</th>
<th>MI (%)</th>
<th>% green-staining bacilli (%)</th>
<th>ATP content/bacilli†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BU</td>
<td>0·0</td>
<td>0·0</td>
<td>$0/1·8 \times 10^{5}$</td>
</tr>
<tr>
<td>2</td>
<td>GR</td>
<td>0·0</td>
<td>0·0</td>
<td>$0/1·5 \times 10^{7}$</td>
</tr>
<tr>
<td>3</td>
<td>PR</td>
<td>0·0</td>
<td>0·0</td>
<td>$0/0·9 \times 10^{5}$</td>
</tr>
<tr>
<td>4</td>
<td>GA</td>
<td>0·0</td>
<td>7·5</td>
<td>$0/11·6 \times 10^{6}$</td>
</tr>
<tr>
<td>5</td>
<td>MH</td>
<td>0·0</td>
<td>1·5</td>
<td>$0/1·8 \times 10^{5}$</td>
</tr>
<tr>
<td>6</td>
<td>RA</td>
<td>0·0</td>
<td>6·5</td>
<td>$1·7 \times 10^{-13}$ g/2·6 $\times 10^{6}$</td>
</tr>
<tr>
<td>7</td>
<td>SL</td>
<td>0·0</td>
<td>5·0</td>
<td>$2·8 \times 10^{-13}$ g/6·8 $\times 10^{5}$</td>
</tr>
<tr>
<td>8‡</td>
<td>TA</td>
<td>0·0</td>
<td>7·5</td>
<td>$5·6 \times 10^{-12}$ g/6·3 $\times 10^{5}$</td>
</tr>
</tbody>
</table>

* Mean, biopsies at serial Nos 1–7 were negative for growth in a normal mouse footpad.
† Mean, total bacilli.
‡ Mean, results of mouse footpad inoculation not available.

also had 0% green-staining bacilli and zero ATP levels (Table 4); (viii) in the cases with 0% MI, ATP levels were also zero in 5 cases. In 3 other cases with zero MI very high levels of ATP were detected (Table 4); (ix) in 2 cases with 0% MI, in which the ATP levels were also zero, 7·5% and 21·5% green-staining bacilli were present in the smears (Table 4).

Discussion

ATP content of M. leprae has been measured and reported previously.9–12 Similarly the ATP content
of cultivable mycobacteria has been reported earlier.\textsuperscript{10,11,12} We have observed in this study, that ATP content of cultivable mycobacteria directly correlates with viable counts and it is a sensitive, stable, constant index of viability. In the present study it has also been observed that when the ‘solid-staining bacillus’ is supposed as a viable bacillus, its ATP content is reasonably constant and then this ATP content is in the same range as other viable cultivable mycobacteria studied by us and reported previously.\textsuperscript{11} ATP content of \textit{M. leprae} as observed by us and also reported by others\textsuperscript{11-13} is higher than the levels reported in other studies.\textsuperscript{9,10} This could be due to technical differences, e.g. purification and extraction procedures etc. as seen in the present study. It has been reported previously that ATP content of \textit{M. leprae} correlates with viability in mouse footpad.\textsuperscript{23} In the present study, ATP content has been found to be a stable index of viability in cultivable mycobacteria as determined by colony counts and solid-staining bacillus in \textit{M. leprae} has the similar ATP content in 15 different cases when this is supposed as index of viability. It may thus be inferred that solid-staining bacilli are the viable bacilli as postulated by others.\textsuperscript{4,24,25}

The calculation of MI appears to have practical difficulties. The growth in mouse footpad has been reported even in cases with zero MI.\textsuperscript{5} Also in the present study in 3 of the 8 cases with zero MI, very high ATP levels were detected, which means that there were a significant number of viable bacilli in these specimens. These observations could mean that while the solid-staining bacilli may indeed be the ‘viable bacilli’ as postulated earlier, yet the MI as \textit{per se} is a poor index of viability as seen in another study\textsuperscript{9} and also as observed in the present study. It is likely that due to sampling of a few hundred bacilli and clumping, the solid bacilli are missed resulting in false zero readings. Though the MI may have a limited role in determining the effect of therapy it does not appear to be a reliable index of viability for monitoring the therapy especially when 0% values are recorded. Thus the important limitation of MI is its low sensitivity even though it appears to be a good measure of viability. On the other hand, the ATP content of \textit{M. leprae} populations especially when it is decreasing or has reached zero, can be used as a reliable index of determining the viability even when the viable mycobacteria are very few. Thus it will be a sensitive tool for determining the viability of \textit{M. leprae} from clinical specimens particularly for monitoring the effect of therapy.

Apparently the MI and green-staining populations by FDA-EB staining detect different populations as seen in the present study and as reported by another study.\textsuperscript{16} The figures for both these indices from the same specimen are significantly different (Table 3). It has been reported that the percentage of green-staining bacilli decreases with treatment.\textsuperscript{15,16} If the green-staining bacilli are taken as ‘index of viability’, no correlation in the ATP content of green-staining bacilli from different cases was observed. ATP content/green-staining bacillus varied up to 9-fold in the 15 cases in which ATP content/solid bacillus ranged from 2.02 to 5.6 × 10\textsuperscript{-15} g (approximately a 2.8-fold variation). FDA-EB staining may have some role in monitoring the effect of therapy like MI, but it appears that it may not directly correlate with viability. In 2 cases the ATP levels were zero yet significant percentage of green-staining bacilli were present. It appears that the enzymes responsible for the green-staining character by FDA-EB staining take some time to degenerate after death whereas ATP decays very fast and thus will correlate better with viability as reported earlier\textsuperscript{23} and as observed by us in case of cultivable mycobacteria. It may be mentioned that 7/8 cases with 0% MI in the present study were negative for growth in normal mouse footpad. Two of them who were negative in mouse footpad had significantly high ATP levels (Table 4). It would be of interest to follow patients on MDT and compare these parameters with multiplication in the immunosuppressed experimental hosts as normal mouse may miss small numbers of viable organisms as reported elsewhere\textsuperscript{26} and as seen in the present study.

It is concluded that ATP content of \textit{M. leprae} from leprosy cases appears to be an easy, rapid tool for determining the viability and thus can be a more sensitive tool for monitoring the effect of therapy than the conventional MI and FDA-EB staining which appear to have their limitations.
References

Small grants programme for development of appropriate technology

Grants for work on assays to diagnose diseases prevalent in developing countries are now available through a project known as DiaTech (Diagnostic Technology for Community Health). The grants, awarded to qualified investigators at public and private institutions (both profit and non-profit) around the world, will support various research-related activities dealing with these diagnostic assays—including development of reagents, test kit design, field evaluation, personnel training, manufacture, introduction, distribution, and impact evaluation. DiaTech can cofund projects with other granting agencies. All of the awards, which carry stipends ranging up to US$300,000 over a two-year period, are subject to peer review.

The DiaTech project is administered by the Program for Appropriate Technology in Health (PATH), a nongovernmental non-profit organization, under a cooperative agreement with the United States Agency for International Development (AID).

DiaTech is managed both by PATH personnel and by additional professional staff members from Johns Hopkins University, the University of Maryland, the Thai Red Cross Society, and other institutions. A technical advisory group composed of distinguished scientists evaluates research and development proposals for funding and monitors work in progress.

The DiaTech project’s basic aim is to better the health of people in developing countries through improvement of available and appropriate assays for diagnosing infectious diseases.

Further information about DiaTech grants, including specific guidelines for preparing proposals, may be obtained by writing or calling the Program Administrator/DiaTech, PATH, 4 Nickerson Street, Seattle, Washington 98109-1699, USA (Tel. 206-285-3500).

New joint funding venture: UNDP/World Bank/TDR

The UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases (TDR) and the Rockefeller Foundation (RF) announce a new joint funding venture to promote laboratory, clinical and field research on major tropical diseases in countries where these diseases are endemic. The purpose of this venture is to involve and link institutions of quality in the application of recent advances and knowledge in biomedical sciences, epidemiology and social sciences to the development, testing and application of new ways of preventing, treating and controlling these diseases.

Partners in research and training

To achieve this goal, TDR and RF will support the establishment of partnerships between two or more groups and/or institutions that can, by pooling their resources, provide both the expertise and facilities required as well as the unique research context and field research opportunities which exist in endemic areas. Partnerships are envisaged between groups that together are equipped to apply modern science and technology to these problems and have demonstrated potential to assimilate and apply these technologies in vulnerable target populations in tropical disease-endemic countries.

How to apply

Applications are invited from research groups and/or institutions that have identified one or more counterpart groups and/or institutions with whom they currently have or have had contacts. One or more of the partners must be located in a local institution in a tropical disease-endemic country. The partner institutions should submit jointly a letter of intent, not exceeding three pages, which should: describe the goals of joint activities and the unique contribution and resources of each partner; outline the proposed research and research training activities; briefly summarize previous experiences; and indicate the nature and estimated cost per partner of the total support requested. Letters of intent must be received by no later than 1 December 1987.

Partner institutions that are selected, in December 1987 by a committee of experts, as candidates for support will be requested to submit a detailed proposal, not exceeding 15 pages, by 1 March 1988. Instructions for the final proposal will be provided upon notification of selection. Funds awarded for this joint venture will be based on comparative costs of research and cooperative activities and are expected to be in the range of US$ 40,000—120,000 per partner per year, for a period of up to five years, subject to annual review of progress.

Where to apply

All letters of intent should be sent to both funding agencies, one copy addressed to Director, TDR, and one copy to the Director of Health Sciences, RF, at the addresses given below:

Director, UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases (TDR), World Health Organization, 1211 Geneva 27, Switzerland or Director of Health Sciences, 1133 Avenue of the Americas, New York, N.Y. 10036, United States of America.
The influence of antimycobacterial chemotherapy on delayed hypersensitivity skin-test reactions in leprosy patients

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Summary Skin tests using purified protein derivative (PPD) and Rees’ skin test antigen (RSTA), a soluble extract of *Mycobacterium leprae*, were performed in 53 treated leprosy patients, 52 newly diagnosed untreated leprosy patients and 78 household contacts of untreated leprosy patients in northern Bangladesh. In addition, a small group of 20 leprosy hospital workers and a further group of 50 indigenous subjects with no known exposure to leprosy were studied.

Untreated paucibacillary and multibacillary patients showed significantly fewer positive reactions than comparable groups of treated patients to both PPD and RSTA. It appears from these results that treatment of leprosy patients is associated with enhanced ability to produce a delayed-type hypersensitivity response to mycobacterial antigens. The mechanisms underlying this phenomenon may include both general and specific suppression of antimycobacterial delayed-type hypersensitivity. The household contacts and indigenous subjects showed similar skin test responsiveness, but virtually all of the hospital workers responded to both PPD and RSTA. The implications of these results for studies of immunity in leprosy patients are discussed.

Introduction

Treatment of leprosy patients, particularly those with borderline leprosy, often leads to exacerbations of the disease known as reversal reactions which are characterized by neuritis and inflammatory changes in the skin lesions. Untreated patients with nonpolar leprosy are susceptible to downgrading reactions which are accompanied by an increase in the bacillary index. In both types of reaction nerve damage occurs and the prevention or early treatment of such exacerbations is an important aspect of patient management. The clinical signs can be correlated with histological and *in vitro* immunological changes which suggest alterations in delayed-type hypersensitivity (DTH). However, there has been relatively little investigation *in vivo* of the relationship between DTH and treatment using skin tests.
In 1962, Guinto & Mabalay performed an extensive study of Mantoux testing in leprosy patients. They showed that lepromatous patients had a lower response rate to PPD than tuberculoid patients or healthy controls. In addition, they noted that 'tuberculoid cases in reaction, bacteriologically positive, gave a tuberculin rate approximating to that of the lepromatous group, while that of the nonreactional cases resembled that of healthy controls'. Many of these recently admitted patients might now be classified as having borderline leprosy with downgrading reaction, so these findings are in keeping with the results of in vitro studies of DTH. Recently 'New Tuberculins' have become available which contain a higher proportion of specific antigens allowing skin test reactivity to mycobacteria to be ascertained with greater specificity. We have used a new tuberculin derived from \textit{M. leprae}, known as Rees' skin test antigen (RSTA) or Leprosin A (Batch CD19), to test reactivity to \textit{M. leprae}. The standard Mantoux test was also performed since purified protein derivative (PPD) of \textit{M. tuberculosis} contains a large proportion of common mycobacterial antigens in addition to \textit{M. tuberculosis} specific antigen.

\textbf{Materials and methods}

\textbf{SUBJECTS}

The study was performed at the HEED Leprosy Hospital, in the Sylhet region of northern Bangladesh, during the first 3 months of 1986. Untreated patients were obtained during population survey or presented to the local control team as self-referrals: treated patients were hospital inpatients. As well as leprosy patients, household contacts of untreated patients, hospital patients and a randomly selected group of indigenous subjects were studied. A total of 253 subjects were included in the study. All gave verbal informed consent for the tests, the results of which were made available to the medical officers in charge of leprosy control in the area. The numbers of subjects in each group are shown in Table 1, together with their age and sex distribution. The ethnic origin of the subjects was similar in all of the study groups. Clinical examination of all the subjects was performed by IAC and local leprosy control teams. In addition, biopsies were taken from the lesions of 40 untreated and 15 treated leprosy patients using a 4 mm skin punch biopsy. Leprosy patients were classified on both clinical and histological grounds using the Ridley–Jopling classification: the numbers in each of the patient groups are shown in Table 2. Untreated patients were newly diagnosed and had not received any treatment prior to skin testing. The treatment status of the treated patient group varied from a few days to many years, but 38 out of the 53 treated patients (72\%) had received less than 6

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Male:female ratio</th>
<th>Mean</th>
<th>SD</th>
<th>% &lt; 15 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>New patients</td>
<td>52</td>
<td>1:0:86</td>
<td>33.1</td>
<td>11.9</td>
<td>5.8</td>
</tr>
<tr>
<td>Treated patients</td>
<td>53</td>
<td>1:0:20</td>
<td>34.1</td>
<td>10.5</td>
<td>0</td>
</tr>
<tr>
<td>Household contacts</td>
<td>78</td>
<td>1:0:95</td>
<td>23.4</td>
<td>15.0</td>
<td>35.9</td>
</tr>
<tr>
<td>Hospital contacts</td>
<td>20</td>
<td>1:0:82</td>
<td>35.2</td>
<td>11.1</td>
<td>0</td>
</tr>
<tr>
<td>Control group</td>
<td>50</td>
<td>1:0:79</td>
<td>24.9</td>
<td>8.1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>253</td>
<td>1:0:68</td>
<td>28.9</td>
<td>12.9</td>
<td>12.2</td>
</tr>
</tbody>
</table>
Antimycobacterial chemotherapy influence in leprosy patients

Table 2. Number of leprosy patients according to treatment status and Ridley-Jopling classification

<table>
<thead>
<tr>
<th>Class</th>
<th>TT</th>
<th>BT</th>
<th>BB</th>
<th>BL</th>
<th>LL</th>
<th>Idt</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>New patients</td>
<td>4</td>
<td>23</td>
<td>1</td>
<td>9</td>
<td>8</td>
<td>7</td>
<td>52</td>
</tr>
<tr>
<td>Treated patients</td>
<td>0</td>
<td>29</td>
<td>1</td>
<td>9</td>
<td>14</td>
<td>0</td>
<td>53</td>
</tr>
</tbody>
</table>

months therapy. Recently treated patients were receiving multiple drug therapy (MDT, WHO regime), while many of those treated for longer than 2 years had been recently changed from dapsone monotherapy to MDT. Of the 53 treated patients, 44 (83%) had some disability, while only 22 of the 52 untreated patients (42%) were disabled. Three groups of healthy subjects were tested: household contacts of untreated leprosy patients, hospital workers, and a group of indigenous ‘controls’ with no household leprosy contacts. Since they live in an endemic area, most of these ‘control’ subjects will inevitably have had some exposure to *M. leprae* and were included for comparison with the household contacts who were more likely to have had recent exposure. The age and sex distribution of the groups is broadly comparable, although it should be noted that the household contact group contained a high proportion of subjects under 15 years old and that most of the treated patients examined were male.

SKIN TESTING

Skin tests were performed using 2 reagents, PPD (1:1000 dilution, Evans Medical, Middlesex, UK) and RSTA (Batch CD19), a new tuberculin containing soluble protein antigens of armadillo-derived *M. leprae*. In each subject, 0·1 ml of each reagent was injected intradermally on the volar aspect of opposing forearms. The injection site was ringed with a ball-point pen and the skin tests were read at 72 h. Only 10 subjects (4%) failed to return for skin test reading (2 household contacts, 2 hospital contacts, 1 new patient, and 5 ‘control’ subjects). On 25 occasions it was necessary for 1 of 3 readers other than IAC to read the result. Measurement of positive responses was performed using the ‘ball pen’ method developed by Sokal. This has the advantage that it is easily taught to other readers and has good reproducibility compared with other techniques. Results are expressed as average diameters: those less than 5 mm were taken as negative and the observations were analysed using a chi-squared test with Yates correction.

Results

The results for the paucibacillary patients, graded TT-BT or Idt by the Ridley–Jopling classification, are shown in Figure 1. There are significantly more negative reactions to PPD among the untreated patients than there are in the treated group (*p* < 0·02). Although there is a difference in the number of positive reactions to RSTA in the paucibacillary patients, this does not achieve statistical significance. If the indeterminate patients are removed from the paucibacillary group, the PPD results still show a statistically significant difference (*p* < 0·02) between the untreated (57·7% positive, *n* = 26) and treated paucibacillary patients (89·7% positive, *n* = 29). Of the 7 indeterminate patients, 4 were PPD positive and 4 were RSTA positive.

The multibacillary patients (BB-LL) show many fewer positive reactions to PPD in the untreated group (*p* < 0·05) and their diameter is less than the positive responses shown by the treated multibacillary patients (Figure 2). The RSTA results show a virtually complete lack of
Figure 1. Skin test results for paucibacillary leprosy patients. The dotted line represents 5 mm induration, below which tests were regarded as negative.

Figure 2. Skin test results for multibacillary leprosy patients. The dotted line represents 5 mm induration, below which tests were regraded as negative.
reaction among the untreated multibacillary patients (only 2 positive), but more than half of the treated multibacillary patients react \( (p < 0.01) \).

The results of the skin tests performed on the healthy subjects are shown in Figure 3. The number of responders to both PPD and RSTA are similar amongst the household contacts and the indigenous 'control' group. Although slightly fewer household contacts show positive responses to RSTA, this difference is not statistically significant. However, the diameter of the positive responses to RSTA amongst the household contacts is greater than those of the 'control' subjects. It was notable that all of the hospital contacts responded to PPD and that all but 2 responded to RSTA. The household contacts and indigenous 'controls' showed a significantly higher response rate to RSTA than untreated multibacillary patients (both \( p < 0.01 \)), but this difference disappears after treatment. There is no statistically significant difference in the response to RSTA between household contacts or indigenous 'controls' and paucibacillary patients. Although a smaller percentage of untreated multibacillary patients respond to PPD than either 'control' subjects or household contacts, this does not achieve statistical significance. Higher response rates to PPD are found in both paucibacillary and multibacillary treated patients than in household contacts \( (p < 0.02 \) and \( p < 0.05 \) respectively), but there is no difference between the response of treated patients and household contacts to RSTA. Comparison of treated patients with the indigenous 'control' subjects shows no statistical difference in their PPD or RSTA responsiveness, although the treated patients have a slightly higher response rate to PPD than the 'control' subjects.

Few side-effects were noted by the patients or healthy subjects, although several of the hospital contacts and one family contact complained of pyrexial reactions. No necrotic reactions were encountered, although several giant reactions greater than 40 mm average diameter occurred in response to both reagents. Some of the larger reactions were tender, but none required treatment and all had resolved in 1–2 weeks.
Discussion

PPD contains large amounts of common mycobacterial antigen (groups i and ii) and some specific (group iv) antigen of *M. tuberculosis*.

In contrast, new tuberculin contains less of the common antigens (groups i and ii) and are rich in specific antigen (group iv). Thus RSTA contains mainly specific *M. leprae* derived antigen. Paucibacillary patients (BT-TT) show good cell-mediated immunity to *M. leprae* in their lesions and they show strong responses in lymphocyte stimulation tests to *M. leprae*. Yet despite such responses, they still develop the disease and BT patients will often downgrade on the Ridley–Jopling scale if they are not treated. Such downgrading has previously been associated with decreased skin test reactivity to PPD. Our observations show that untreated paucibacillary patients have a decreased skin test response to both PPD and RSTA despite their apparently good intralesional immunity to *M. leprae*. This may reflect partial suppression of the effector mechanism of DTH to common mycobacterial antigens.

Consistent with this explanation, the percentage of paucibacillary patients with positive skin tests to RSTA is greater in the treated group, while the area of induration amongst the responders is not significantly different. Thus specific DTH to *M. leprae* appears to exist in both treated and untreated paucibacillary patients, and the difference between the two groups may reflect general suppression of DTH to mycobacteria in untreated patients. This contrasts with the multibacillary group in whom both the percentage of responders and the diameter of the positive responses is greater in the treated group, perhaps reflecting the emergence of specific DTH to *M. leprae* as well as increased DTH to common mycobacterial antigen. It should be noted that only 1 of the untreated patients was classified LLp on histology: the remainder were LLs or BL. In Indonesia, where most lepromatous patients are of polar type, RSTA has been shown to produce few positive responses in treated lepromatous patients. Our results suggest that RSTA skin tests can be positive in subpolar lepromatous patients, particularly if they have received treatment.

A large number of possible mechanisms have been suggested for the immune suppression which occurs in leprosy, particularly the lepromatous form. Many of the mechanisms postulated are not mutually exclusive and our data suggests that both general and specific suppression may coexist. Both abnormal antigen presentation and/or the production of suppressor lymphocytes could produce specific effects, while nonspecific suppression of DTH could be produced by humoral factors. Sequestration of antigen-reactive lymphocytes in lesions might also produce suppression of skin test reactions. The relative importance of these mechanisms at different times during the course of the disease may also vary.

The healthy subjects included in this study show a number of interesting comparisons. The striking reactivity to both reagents shown by the hospital contacts probably reflects their constant exposure to mycobacteria, but these subjects also have a considerably better standard of living than the other groups. Similar enhanced responsiveness to RSTA and new tuberculin has been seen in healthy hospital workers in East Java.

It is interesting to note the slightly diminished responsiveness of the household contacts compared with the indigenous ‘control’ groups. This does not reach statistical significance and may reflect the larger number of young subjects in the former group, but it may be that some of the household contacts, those most at risk of contracting leprosy, are immunologically compromised as suggested by Strickland. The small differences in PPD reactivity between the treated patients and the household contacts can also be explained by this mechanism and by differences in immunological experience of mycobacteria.

There was no obvious relationship between treatment duration and the diameter of PPD or RSTA responses: this may reflect the small number of patients in the study or the time scale of reversal of immune suppression. Dapsone has been shown to reduce the incidence of reversal reactions in leprosy patients, but the mechanism of action is not clear and the effect of MDT has not been documented. The situation is complicated by the lack of objective methods of diagnosing reversal reactions in leprosy, although muscle function testing is useful in detecting neuritis.
Our observations suggest that treatment of leprosy patients with multiple drug therapy enhances DTH to both common and specific mycobacterial antigens. It is important that studies of immunity in leprosy patients take account of the possible effect of treatment on their results and give adequate details of the treatment status of their patients. We consider untreated patients to be preferable for most investigations of the pathogenesis of the immunological deficits in leprosy. Nevertheless, since many chemotherapeutic agents used in leprosy have effects on the immune system and cause a radical alteration in the bacillary load, complimentary studies of treated patients are necessary in order to plan optimal treatment regimes. We recognize that retrospective studies are difficult to interpret unambiguously and that there are some differences between the untreated and treated groups (e.g. disability status) which may have influenced our results. We therefore hope to perform a prospective study of skin testing with PPD and RSTA in leprosy patients over the next few years.

Acknowledgments

The work in Bangladesh was funded by the award of the Becton Dickinson Travelling Scholarship to IAC by the Royal College of Pathologists. IAC was supported by LEPRA. The authors are most grateful to Dr M S Islam, Dr I H Cochrane, Dr T Ali, Mr K Skillcorn, and Mr S Halda for their assistance in Bangladesh.

References


NEWS AND NOTES

TDR; invitations to consider research projects in leprosy

In the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases 'TDR News' of Autumn 1987, Number 24, pages 6 and 7 carry invitations to scientists and doctors to participate in certain areas of research. Included in the 'six diseases', proposals for work in leprosy are listed as follows:

- development and validation of seroepidemiological methods, e.g. specific tests for antibody/antigen detection, for Mycobacterium leprae using molecular probes and for cell-mediated immune (CMI) responses both in vivo and in vitro;
- development of prophylactic vaccine(s): development of candidate subunit structures, establishment of effective vectors for immunization, identification of CMI-inducing antigens/epitopes, production of recombinant and/or synthetic vaccine candidates;
- identification of means to overcome pathogenic host responses: development of human and animal model systems, identification of genetic markers of disease, investigation of lymphocyte subsets and their repertoires and the role of lymphokines;
- identification of methods to prevent and control nerve damage: investigations of nerve damage in humans and animal models, determination of the role of CMI in nerve damage, identification of the function of cells/antigens in tissue lesions;
- identification of means for better use of existing drugs: testing of new combinations in animal models, conduct of clinical trials in multibacillary patients using newer regimens;
- assessment of needs for improved therapy: study of relapses after cessation of treatment, identification of risk factors for relapse, survey of rifampicin-resistant leprosy;
- identification of new drugs for leprosy: selection from inventory and/or design of new compounds, development of new microbial screens, conduct of animal studies for antileprosy activity, conduct of short-term clinical trials in humans;
- identification of new drugs for treatment of leprosy reactions: development of models for screening drug activity, testing of active drugs for pharmacological and mutagenic effects, toxicity and structure/activity relationships;
- evaluation of immunotherapeutic methods: conduct of immunoreactivity trials, study of immunotherapy combined with chemotherapy.

Studies on the mode of action of, and resistance to antibiotics, Oxford, UK

In the Oxford Medical School Gazette, Vol. XXXVIII, No 2, Michaelmas 1987, the Department of Microbiology and the Public Health Laboratory of the John Radcliffe Hospital, Oxford, report stated: 'A major determinant of how effective an antibiotic is against a particular bacterium is whether the antibiotic reaches its site of action within the cell sufficiently rapidly to halt growth. Drs Wright Nichols and Mary Slack are working on this topic. Current projects concern; how bacterial metabolism stimulates the uptake of amino-glycoside antibiotics, the interplay between beta-lactamases and outer membrane penetration of beta-lactam antibiotics (penicillins and cephalosporins) in Gram negative bacteria; and analysing quantitatively the diffusion of antibiotics into multicellular bacterial films or biofilms that can form on catheters and other medical implants.'

Handbook of leprosy; W H Jopling; ELBS edition

It is perhaps not fully appreciated that copies of this Handbook, published by William Heinemann Books Ltd, (22 Bedford Square, London WC1B 3HH; Telephone 01-637-331), are available in an English Language Book Society edition at a price of only £3.25 sterling. Full information of the ELBS system was printed in this journal in March 1988, page 43. Order through the usual book trade channels either from the publisher or the publisher's agent or from such United Kingdom exporters as Abacus Books Ltd (Abacus House, Speldhurst Road, Tunbridge Wells, Kent TN4 0HU), Collet's Holdings Ltd (Denington Estate, Wellingborough, Northants NN8 2QT) or Gemini Book Distribution Ltd (Vale Road, Tonbridge, Kent TN9 1TD). When ordering please quote the International Standard Book Number (ISBN), which is 0433 17568 0.

Medical Education Newsletter, Dundee, Scotland

This Newsletter from the Centre for Medical Education, University of Dundee, Dundee DD1 4HN, Scotland, UK, is available to bona fide applicants in the field of medical education, to whom it may well be invaluable. The most recent received in Oxford, Number 38, is in fact more than a newsletter in the usual sense of the word: it is an impressive document of 31 pages, full of practical information on: distance learning; educational technology; AIDS education; communication skills for medical students; assessing clinical competence and postgraduate education. The Centre also runs courses on various aspects of medical education throughout the year; further information from the above address.
Unmyelinated nerve fibres in leprosy.  
A qualitative and quantitative study of sural nerve biopsies in 2 cases of lepromatous leprosy

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& G KLINGMÜLLER†§

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Accepted for publication 10 November 1987

Summary Since morphometric analysis of unmyelinated nerve fibres is lacking in leprosy literature, they were investigated in sural nerves of 2 lepromatous cases. Despite normal or even increased total numbers of unmyelinated axons, tentative differentiation in not-yet-myelinated axons and genuine unmyelinated fibres or their regenerates according to associated Schwann cells and fibre calibres revealed a mild to severe loss of genuine unmyelinated fibres. A predominant involvement, surpassing loss of genuine myelinated fibres, could not be stated in either case. Quantification of degenerating unmyelinated fibres, denervated Schwann cell complexes, and Schwann cell nuclei—both of the unmyelinated type—is also presented and discussed.

Introduction

Since a loss of pain and thermal sensibility with concomitant involvement of peripheral autonomic functions constitutes one of the first symptoms in leprosy and remains a characteristic clinical feature even in later stages of the disease, unmyelinated nerve fibres deserve special interest. Accordingly, qualitative alterations have been described by several authors, studying nerve biopsies of leprous patients by electron microscopy or using animal models of the disease. But so far quantitative analysis of unmyelinated fibres is completely missing. There are only short remarks mostly concerning 'loss', 'reduction', 'decrease' or 'depletion', rarely even an 'increase' or a 'prominence' is mentioned. Here, we present the results of qualitative and, more interestingly, quantitative electron microscopic studies of the unmyelinated fibre population in 2 treated cases of the lepromatous spectrum. Detailed leprological data and preliminary morphometrical results of case 1 have been published previously.

§ Professor Klingmüller has died since the submission of this paper.

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Unmyelinated nerve fibres in leprosy. A qualitative and quantitative study of sural nerve biopsies in 2 cases of lepromatous leprosy

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Summary Since morphometric analysis of unmyelinated nerve fibres is lacking in leprosy literature, they were investigated in sural nerves of 2 lepromatous cases. Despite normal or even increased total numbers of unmyelinated axons, tentative differentiation in not-yet-myelinated axons and genuine unmyelinated fibres or their regenerates according to associated Schwann cells and fibre calibres revealed a mild to severe loss of genuine unmyelinated fibres. A predominant involvement, surpassing loss of genuine myelinated fibres, could not be stated in either case. Quantification of degenerating unmyelinated fibres, denervated Schwann cell complexes, and Schwann cell nuclei—both of the unmyelinated type—is also presented and discussed.

Introduction

Since a loss of pain and thermal sensibility with concomitant involvement of peripheral autonomic functions constitutes one of the first symptoms in leprosy and remains a characteristic clinical feature even in later stages of the disease, unmyelinated nerve fibres deserve special interest. Accordingly, qualitative alterations have been described by several authors, studying nerve biopsies of leprous patients by electron microscopy or using animal models of the disease. But so far quantitative analysis of unmyelinated fibres is completely missing. There are only short remarks mostly concerning 'loss', 'reduction', 'decrease' or 'depletion', rarely even an 'increase' or a 'prominence' is mentioned. Here, we present the results of qualitative and, more interestingly, quantitative electron microscopic studies of the unmyelinated fibre population in 2 treated cases of the lepromatous spectrum. Detailed leprological data and preliminary morphometrical results of case 1 have been published previously.

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Materials and methods

The relevant clinical features of the 2 cases are summarized in Table 1. After informed consent whole sural nerve biopsies in both patients and in a 41-year-old control\textsuperscript{17,18} were performed each at the ankle level. The specimens were fixed in 3·9% glutaraldehyde with 0·05 M phosphate buffer (pH 7·6), postfixed in 2% osmium tetroxide, and embedded in epoxy. Ultrathin sections were stained with lead citrate, and examined by a PHILIPS 400 electron microscope (NS Philips, Eindhoven, The Netherlands).

Morphometric analysis of unmyelinated fibres was performed on nonoverlapping consecutive electron micrographs (initial magnification $\times$ 6000, final magnification $\times$ 21,000) of well-orientated areas of at least 4 cross-sectioned fascicles (further details see Table 2). Small rounded profiles with or even without mesaxon—if located eccentrically or otherwise incompletely wrapped by Schwann cell processes—were considered as unmyelinated axons, when (1) a larger number of microtubules was present compared with the respective Schwann cell cytoplasm, and/or (2) when there was greater electron density of the surrounding membrane compared to that of Schwann cell plasmalemm, and/or (3) when rounded profiles exhibited lesser electron density than Schwann cell cytoplasm.\textsuperscript{19,20} In cases of doubt whether a structure was an axon or a Schwann cell process viewing the negatives through an ocular lens ($\times$ 9) was sometimes helpful to make the decision. Remaining questionable structures were not regarded as axons. Before measuring and counting the axons, the associated Schwann cell complexes were separated into 2 categories—controlled by montages of low power view electron micrographs ($\times$ 4725)—according to the criteria of Ochoa & Mair:\textsuperscript{21} (1) unmyelinated type, characterized by smaller units, built up by plate-like processes; and (2) myelinated type, characterized by larger complexes, built up by more bizarre Schwann cell processes, or complexes complementarily arranged to complexes with remyelinating axons. Since in case 1 differentiation was blurred by focal Schwann cell swelling, which rendered the criterium of

<table>
<thead>
<tr>
<th>Case and biopsy number</th>
<th>Age, sex, and geographical origin</th>
<th>Type of leprosy*</th>
<th>Duration of leprosy symptoms</th>
<th>Duration of specific treatment</th>
<th>Clinical features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1 N38/82</td>
<td>64 years male Cambodian</td>
<td>Lepromatous</td>
<td>18 months</td>
<td>2 weeks (?)</td>
<td>Slight facies leontina. Smooth cutaneous nodules symmetrical over whole body. Radial nerves slightly enlarged. Reduction of pain and thermal sensibility† slightly in skin lesions, markedly over extremities, here patchy symmetrically, including sural nerve area. Muscle stretch reflexes well preserved. Positive syphilis reactions in serum.</td>
</tr>
<tr>
<td>Case 2 N39/82</td>
<td>37 years female Vietnamese</td>
<td>Lepromatous</td>
<td>About 4 years</td>
<td>7 months</td>
<td>Extensive erythematous macules. Radial nerves enlarged. Slight atrophy and paresis of hand and forearm muscles. Loss of pain and thermal sensibility† symmetrically over distal extremities including sural nerve area. Atrophic skin of feet. Muscle stretch reflexes well preserved. Chronic persistant hepatitis B.</td>
</tr>
</tbody>
</table>

* Classified according to clinical data and results of Lepromin-A-Test, nasal mucosal scraping, skin and nerve biopsies with special staining for acid-fast bacteria.
† Sensory testing by classical neurological methods.
Unmyelinated nerve fibres in leprosy

size less liable, a revision of previously communicated results\textsuperscript{22} was undertaken by complete re-evaluation of case 1 with introduction of a third category: 'questionable myelinated type'. The associated unmyelinated axons were measured and counted separately using a MOP-videoplan (Kontron Electronica D-8057 Eching). Denervated Schwann cell complexes (more than 2 Schwann cell processes) and Schwann cell nuclei, both belonging to the unmyelinated type, were counted likewise.

As unmyelinated axons in Schwann cell complexes of the myelinated type most probably represent not-yet-myelinated sprouts of regenerating formerly myelinated fibres, and as an excess of miniature axons in Schwann cells of the unmyelinated type, presumably represent regenerating sprouts of original unmyelinated fibres, the total density/mm\textsuperscript{2} or number per nerve of unmyelinated axons does not reflect the original unmyelinated fibre population.\textsuperscript{23} According to the proposals of Ochoa & Mair\textsuperscript{21} we therefore corrected the total values (1) by subtraction of all unmyelinated axons in Schwann cells of the myelinated type and in case 1 questionable myelinated type, and (2) by further subtraction of the excess (more than 21\% of miniature unmyelinated axons (≤0.8 \textmu m diameter)). The total count per nerve was calculated by extrapolation to the whole nerve area.\textsuperscript{19} Findings concerning myelinated fibres and endoneurial area were derived from a parallel still unpublished study. Attempting to elucidate a probable preferential loss of unmyelinated fibres, in contrast to previous presentations\textsuperscript{22} their corrected density was compared to the density of the presumably genuine myelinated fibre population. For the histograms, myelinated as well as unmyelinated fibre diameters were derived from fibre areas.

Results

CASE 1

Semi thin and ultrathin sections revealed a well-preserved endoneurial architecture with many thinly remyelinated fibres (Figure 1). Solid and degenerated bacteria were seen in numerous macrophages (foam cell types and in many Schwann cells almost invariably associated with unmyelinated fibres) (Figure 1(a) and (b)). Here, the bacterial structures always occurred within an electron lucent halo which was often membrane-bound, exhibiting the characteristics of a phagosome, not infrequently with an electron-dense excentric rim. In some fascicles, Schwann cells were swollen, their cytoplasm exhibiting a watery appearance. Only rarely was it densely packed with filaments. Schwann cells with bacterial structures mostly contained normal-looking unmyelinated axons. Uncharacteristic qualitative changes occurred only rarely, e.g. swollen axoplasm, loss, proliferation or abnormal grouping of microtubules, neurofilament dissolution, proliferation of membraneous organelles. Many miniature axons were visible mostly in Schwann cell complexes of the unmyelinated type. Some axons were seen in an excentric position, on one side only covered by a basement membrane. Others situated more centrally occurred abutted to each other with incomplete wrapping by Schwann cell processes. Rarely, even completely isolated axon-like structures (not counted) were found, only surrounded by the basement membrane.

Quantitative analysis of myelinated fibres revealed a still normal density/mm\textsuperscript{2} (7076) and number per nerve (6818). However, an increase of smaller (≤7 \textmu m) and decrease of larger calibres was obvious (Figure 2). Density/mm\textsuperscript{2} and number per nerve of unmyelinated axons were increased, when compared with the younger control (Table 2) and age-related values of the literature.\textsuperscript{19,21,24} But numerous unmyelinated axons were found in Schwann cells of the myelinated or questionable myelinated type (Table 2). The histogram revealed an impressing shift towards smaller calibres and a widening of the spectrum beyond 2-4-\textmu m diameter fibres (Figure 2) compatible with segmental demyelination or even regeneration of not-yet-myelinated axons. After the correction (see Materials and methods) density/mm\textsuperscript{2} and number per nerve of presumed genuine unmyelinated fibres were much lower (Table 2) but still in normal range. Likewise the percentage of degenerating
Figure 1. (a) Representative endoneurial area of case 1. Note phagosomes with bacterial structures in Schwann cell complexes associated to unmyelinated fibres and the presence of Schwann cell complexes of the myelinated type, now harbouring only unmyelinated axons (†). (b) Bacterial remnants lying free in Schwann cell cytoplasm. Case 1. Bars (a) 5 μm; (b) 1 μm.
axons in Schwann cells of the unmyelinated type (1.4%; control 0.5%) did not definitively exceed normal values. A slight increase of denervated Schwann cell complexes of the unmyelinated type (5444/mm²) — compared to age-matched controls of the literature (3700/mm²) — was compatible with a mild but, considering the enlargement of endoneurial area in our case, definite loss of original unmyelinated fibres. The ratio between the corrected densities of unmyelinated and myelinated fibres was near normal and therefore did not indicate a preferential loss of genuine unmyelinated fibres (Table 2). There was no disproportionate increase in Schwann cell nuclei of the unmyelinated type: 71% compared to 88% in age-related control of the literature.

CASE 2

Semithin and ultrathin sections exhibited a severe reduction of the endoneurial parenchyme and an increase of collagen masses (Figure 3). The heavily decreased myelinated fibres were constantly

![Figure 2. Histograms of (left) myelinated fibres (MF) and (right) unmyelinated fibres (UF) with respective total densities per mm² endoneurial area. Above, control; middle, case 1; below, case 2. Grey areas of the columns represent number of UF in Schwann cell complexes of the unmyelinated type, light areas in those of unquestionable myelinated type.](image-url)
Table 2. Results of morphometric analysis of unmyelinated fibres with corrections according to Ochoa and Mair.\textsuperscript{22} For details see text. (UF, unmyelinated fibres; MF, myelinated fibres.)

<table>
<thead>
<tr>
<th></th>
<th>Area evaluated (mm$^2$)</th>
<th>Total count of UF</th>
<th>Density per mm$^2$</th>
<th>Number per nerve</th>
<th>Density per mm$^2$</th>
<th>Number per nerve</th>
<th>Density per mm$^2$</th>
<th>Number per nerve</th>
<th>Density per mm$^2$</th>
<th>Number per nerve</th>
<th>UF/MF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>0.0339</td>
<td>1666</td>
<td>49,029</td>
<td>47,229</td>
<td>1052</td>
<td>30,959</td>
<td>29,822</td>
<td>1,044</td>
<td>27,270</td>
<td>14,58</td>
<td>3.1/1</td>
</tr>
<tr>
<td>Case 2</td>
<td>0.0367</td>
<td>835</td>
<td>22,727</td>
<td>27,251</td>
<td>32</td>
<td>871</td>
<td>1,044</td>
<td>24</td>
<td>653</td>
<td>7.1/1</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.0352</td>
<td>1685</td>
<td>47,869</td>
<td>30,592</td>
<td>1502</td>
<td>42,670</td>
<td>27,270</td>
<td>1458</td>
<td>41,420</td>
<td>26,471</td>
<td>5.9/1</td>
</tr>
</tbody>
</table>

* Correction by subtraction of UF in Schwann cell complexes of MF-type.
† Of questionable MF-type; n.d. = not done.
Figure 3. (a) Representative endoneurial area of case 2 in respect to unmyelinated fibres. Severe reduction of parenchyma, the majority of residual Schwann cell complexes are of the myelinated type (+). Schwann cell complexes of the unmyelinated type are scanty and mostly devoid of axons. (b) Typical Schwann cell complex of the myelinated type. Note regressive alterations of some unmyelinated axons, the presence of miniature axons, abnormal location of axons, and incomplete wrapping by Schwann cell processes (inset). Case 2. Bars (a) 3 μm, (b) 1 μm, inset 0.3 μm.
small and thinly remyelinated. Bacterial remnants in Schwann cells—here mostly as lysosomal residual bodies—and in some scattered macrophages were only present in moderate number. The majority of unmyelinated axons were found in Schwann cell complexes of the myelinated type (Figure 3(a) and (b)). There were many Schwann cell complexes of either type devoid of axons. On the other hand the normal ratio of about 1:1·5 between Schwann cell complexes and associated unmyelinated axons\(^{21,23}\) was often decreased, reaching values between 1:11 and 1:17. Qualitative alterations of unmyelinated axons and their Schwann cells were similar to those in case 1 but more numerous (Figure 3(b)). In three instances bacteria-like degraded electron dense material was seen inside degenerating unmyelinated axons.

Quantitative evaluation revealed severely reduced density/mm\(^2\) (379) and number per nerve (454) of myelinated fibres. Unmyelinated axons exhibited a still normal total density/mm\(^2\) and number per nerve (Table 2) when compared with our control of similar age and respective values of the literature.\(^{19,21}\) However, the histogram impressively demonstrates the increase of smaller diameters and the high incidence of unmyelinated axons associated to Schwann cells of the myelinated type, including the presence of obviously demyelinated or regenerating not-yet-myelinated large axons (Figure 2). Accordingly, after the correction the density/mm\(^2\) and number per nerve of presumed original unmyelinated fibres were extremely low (Table 2). The percentage of degenerating axons in Schwann cells of the unmyelinated type was increased to 38%. There was a relatively high number of denervated Schwann cell complexes of the unmyelinated type (572/mm\(^2\)). The ratio between the corrected densities of unmyelinated and myelinated fibres was even increased (Table 2), suggesting a slight predominance of myelinated fibre loss. There was no disproportionate increase in Schwann cell nuclei of the unmyelinated type (27%) compared to the control of similar age (66%).

Discussion

Considering the prerequisites of overt leprosy, it is surely difficult to find cases without any further pathological condition and therefore ideally suited for quantitative evaluation. But as lues latens seropositiva in case 1 is without importance for the nervous system, and chronic persistent hepatitis B in case 2 causes at best a subliminal demyelination,\(^{25}\) there is little probability of considerable competitive nerve involvement. Another disadvantage may be seen in the lack of suitable control values from persons of similar origin. But considering the range of respective data available, this may be not the most difficult point in this study.

The qualitative alterations of unmyelinated axons and associated Schwann cells in our cases, all unspecific in nature, paralleled previous observations in cases with lepromatous and borderline lepromatous leprosy of man and in infected mice.\(^{3,4,7,8,10,12,26}\) The abnormal localization of many small unmyelinated axons has been already demonstrated.\(^{11}\) Comparable findings—most probably indicating regeneration—have been described and/or illustrated by various authors.\(^{23,27-30}\) Focal swelling of Schwann cells—presumably of toxic-infective origin—may be in part identical with the description of ‘bulky’ or ‘hypertrophic’ Schwann cells.\(^{15,26}\) These qualitative alterations give some hint towards unmyelinated fibre damage, but currently, quantitative evaluation represents the only reliable method to inquire about their involvement.\(^{23}\) Concerning the quantitative assessment of unmyelinated fibres two main problems arise: (1) the identification of unmyelinated axons; and (2) the classification in ‘real’ unmyelinated fibres and not-yet-myelinated axons according to type of associated Schwann cell complexes. The respective morphological criteria have been mentioned initially. The recognition of regenerating unmyelinated fibres depends on fibre calibre and numerical relations (see Materials and methods). Accordingly, the main result of the present study is the quantitative confirmation of involvement of unmyelinated fibres in lepromatous leprosy.

In case 1 with the slightest degree of functional impairment we noted a high number of unmyelinated axons. An increase has been suspected by Dastur \textit{et al.}\(^{15}\) in early stages of lepromatous leprosy and is known from other polyneuropathies as well,\(^{19,20,23}\) suggesting
Unmyelinated nerve fibres in leprosy

regeneration after primary degeneration. The tentative correction of unmyelinated fibres by subtraction of all obviously nongenuine ones could not confirm a clear cut pathological reduction of original unmyelinated fibres. Fibre loss was only deducible from a slight increase in denervated Schwann cell complexes and the abnormal left-shifted histogram, reflecting the high part of regenerating unmyelinated fibres and confirming the observation of Dastur & Porwal, who were impressed by clusters of very small unmyelinated axons in an otherwise slightly affected leprosy nerve. The marked peak of 0.3–0.6 µm diameter fibres obviously comprises a considerable amount of not-yet-myelinated axons deducible from the type of associated Schwann cells, thus indicating a marked primary loss of myelinated fibres as well, not expected by their mere quantitative assessment.

In case 2 with an advanced stage of lepromatous leprosy and moderate sensory-motor polyneuropathy the number of unmyelinated axons is likewise still in normal range for this age group. However, their high incidence in Schwann cells of the myelinated type reflects the large amount of not-yet-myelinated axons after previous Wallerian degeneration and segmental demyelination of myelinated fibres. Accordingly, the corrected values for original unmyelinated fibres are unequivocally severely reduced. Damage and loss of unmyelinated fibres are indicated by the relatively high presence of degenerating unmyelinated axons and of denervated Schwann cell complexes of the unmyelinated type as well. Presumably frustrane regeneration may be derived from the occurrence of Schwann cell complexes harbouring numerous (up to 17) unmyelinated axons.

In both cases the ratio between the presumably original populations of unmyelinated and myelinated fibres could not support a predominant involvement of unmyelinated fibres, although according to various observations in man and mice the earliest cells to harbour bacilli are Schwann cells associated with unmyelinated fibres and the earliest lesions were observed in unmyelinated fibres and their Schwann cells. Apparently, involvement of myelinated fibres, especially smaller calibres which likewise conduct pain and thermal sensibility, will soon follow and quickly reach an equal (case 1) or even higher degree (case 2).

Viable bacteria and granular bacterial remnants were common in case 1 with the shortest previous systematical treatment. They were preferentially located in Schwann cells of the unmyelinated type as already stressed by others. There seems to be a tendency to more membrane-bound residual structures—typical phagosomes—in our treated case 1 when compared to the illustrations of the literature concerning untreated cases. Moreover, phagosomes obviously exhibited electron dense membranes more often, especially when harbouring bacterial remnants with advanced degeneration, as also underlined by Job. In our case 2, who has been treated for several months, bacterial remnants were scarce and mostly more severely degraded. Bacterial remnants in unmyelinated axons, observed by some authors were seen only in case 2 in rare instances. Therefore, bacterial spread via unmyelinated axons may be of minor importance in the pathogenesis of lepromatous leprosy.

Acknowledgments

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References


Long-term prothionamide compliance: a study carried out in India using a combined formulation containing prothionamide, dapsone and isoniazid

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Summary A comprehensive study of the self-administration of prothionamide is described in which over 2000 urine samples were collected from some 60 South Indian patients over a 2-year period. Prothionamide (350 mg) was prescribed for daily self-administration as the commercially available combined formulation ‘Isoprodi an’ that also contains dapsone and isoniazid. Drug ingestion was monitored by testing the samples qualitatively and quantitatively for the presence of the isoniazid metabolites acetylisoniazid and isonicotinic acid, and for dapsone together with its diazotisable metabolites.

About a third of the patients suffered from moderate or severe gastrointestinal side-effects attributed to prothionamide but no hepatic toxicity was encountered, whether or not treatment was supplemented with monthly supervised doses of rifampicin. The results obtained using the different urine-test methods correlated well and it was concluded that overall just over half the prescribed doses had been ingested. Although enormous variations in individual patient compliance were demonstrated, there was a continuous spectrum of drug taking and patients could not be simply grouped into good or poor compliers.

Older patients took their prescribed treatment less regularly. The compliance of patients who suffered from severe gastrointestinal side-effects was markedly impaired and improved when daily thioamide treatment was replaced by dapsone. The proportion of positive urine tests among samples collected at the patients' monthly clinic visits was similar to those collected by means of surprise home visits.

It was concluded that if prothionamide is used as an alternative to clofazimine in the multidrug treatment of lepromatous leprosy its compliance should be monitored using an isoniazid-marked formulation.

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† Dedicated to the memory of Han Huikeshoven
Introduction

It is recommended that multibacillary patients who find clofazimine skin pigmentation unaccept­table should be treated with daily self-administered doses of dapsone (100 mg) and ethionamide or prothionamide (250–375 mg) plus monthly supervised doses of 600 mg rifampicin for at least 2 years. Experimental studies of the 2 thioamides in the mouse footpad and investigations of their pharmacokinetics in man indicate that their clinical potencies are likely to be very similar. The decision as to which one to employ would therefore appear to depend on their relative acceptability, toxicity, availability and cost.

Both thioamides were widely used for the treatment of pulmonary tuberculosis prior to the introduction of rifampicin. Gastrointestinal side-effects were common, although prothionamide appeared to be somewhat better tolerated. However, the daily dosages used (500–1000 mg) were higher than that recommended for leprosy. Little is known of the acceptability and side-effects of the thioamides when given in lower dosages, although recent reports of their hepatotoxicity when combined with rifampicin give cause for concern.

Experimental studies in the mouse footpad have demonstrated that when ethionamide was administered once a week its bactericidal activity was abolished. Regular drug ingestion will therefore be essential to ensure that treatment failures caused by the multiplication of rifampicin-resistant Mycobacterium leprae are prevented.

We recently reported a pilot cross-over study of ethionamide and prothionamide compliance among leprosy outpatients in Hyderabad, India. About three-quarters of the prescribed thioamide doses were ingested, and daily doses of 125 mg ethionamide, and 125 or 250 mg prothionamide were of similar acceptability to the patients. Furthermore, prothionamide and dapsone could be given together in a single daily capsule without compromising the dapsone compliance of the patients.

Although these results were encouraging, only 12 patients were studied and 2 of the patients (both heavy drinkers) developed jaundice. We therefore concluded that ethionamide or prothionamide could not be confidently recommended for the treatment of multibacillary leprosy until more extensive investigations of their compliance and hepatic toxicity had been undertaken. This paper reports such an investigation.

Methods

TREATMENT OF PATIENTS AND DESIGN OF THE STUDY

Choice of patients

Sixty patients (44 male, 16 female) who lived within an 8 km radius of the Dholpet Leprosy Research Centre, had received treatment at the Centre for at least a year, were judged to be likely to continue treatment for at least 2 years more, and were willing to allow clinic staff to visit their homes were enrolled in the study. Their ages ranged from 13–65 years (mean 37 years), they weighed from 20–96 kg (mean 46 kg) and they had been previously treated for an average of 4 years, primarily with dapsone monotherapy. Forty were lepromatous (23 LL and 17 BL), 1 borderline and 19 tubercu­loid (13 BT and 6 TT).

The study was not a controlled clinical trial in a therapeutic sense since all patients were prescribed the same total net treatment. Furthermore, during the course of the study all patients were given at least 12 supervised monthly doses of rifampicin to ensure that the vast majority of any remaining viable leprosy bacilli would be rapidly killed.

Initial run-in phase

All patients were treated for 12 weeks with self-administered dapsone therapy (one 100 mg tablet daily).
Cross-over phase

The 60 patients were then assigned to 3 consecutive 24-week treatment schedules consisting of: (A) daily self-administered prothionamide (350 mg) plus dapsone (100 mg) plus isoniazid (350 mg) using 2 tablets of the commercial combined formulation 'Isoprodian'; (B) the same treatment supplemented by monthly supervised doses of 600 mg rifampicin; and (C) daily self-administered dapsone (one 100 mg tablet) plus monthly supervised doses of 600 mg rifampicin.

Ten patients were assigned by random allocation to each of the 6 possible sequences (ABC, ACB, BAC, BCA, CAB and CBA) in which the 3 regimens could be administered.

Final phase

All the patients continued treatment for a further 24 weeks with daily self-administered dapsone plus monthly supervised rifampicin (regimen C).

MANAGEMENT OF SIDE-EFFECTS

Patients who experienced gastrointestinal side-effects attributed to prothionamide treatment were given short courses of antacid or antiemetic treatment, reassured and encouraged to continue treatment. However, if their symptoms persisted, and the patient objected strongly to continuing Isoprodian treatment, it was replaced by daily dapsone plus monthly rifampicin. Any patient who developed jaundice or serum glutamic transaminase levels in excess of 90 units/litre was to be immediately withdrawn from the study.

SUPERVISION OF TREATMENT, GIVING OF TEST ISOPRODIAN DOSES AND COLLECTION OF BLOOD AND URINE SAMPLES

The patients were encouraged to visit the clinic once every 4 weeks when they were seen by the doctor and any side-effects that might have been attributable to the treatment recorded. The monthly rifampicin dose was swallowed, a urine sample collected and tablets of Isoprodian or dapsone issued for the following month for ingestion first thing each morning. A second urine sample was obtained each month by means of a surprise home visit using a randomized home visiting schedule. Blood samples were taken on alternate clinic visits for routine transaminase estimations.

On a single prearranged occasion during the initial run-in period, each patient was visited in their home, a urine sample was collected and a test dose of Isoprodian (2 tablets) was swallowed in place of that day's dapsone tablet. Further urine samples were then collected after 24, 48, 72 and 96 h.

ANALYTICAL PROCEDURES

Transaminase estimations

Serum glutamic pyruvic transaminase levels (SGPT) were determined in Hyderabad by a modification of the Reitman & Frankel method using reagents purchased from Ortho Diagnostic Systems, Bombay.

URINE ANALYSES

Aliquots of urine were preserved with a crystal of thymol and stored at 0–4°C until shipment by air (without refrigeration) to London for analysis. The regularity with which patients self-administered
their prescribed Isoprodian treatment was assessed by testing urine samples with a combination of qualitative and quantitative methods for the presence of the isoniazid metabolites acetylisoniazid, isonicotinic acid and isonicotinylglycine; and for dapsone and its diazotisable metabolites. Dapsone estimations were initiated about half-way through the study to enable Isoprodian's ingestion to be compared with that of dapsone in the final phase of the study, when evidence from the acetylisoniazid and isonicotinic acid urine tests began to suggest that many patients were taking Isoprodian very irregularly.

All the analyses were carried out without reference to the origin of the specimen. Samples were tested qualitatively for acetylisoniazid by a minor modification of the Eidus & Hamilton procedure, in which 0.1 ml aliquots were reacted on white porcelain tiles with 0.1 ml 10% aqueous potassium cyanide and 0.2 ml 10% aqueous chloramine-T and the formation of a pink/brick-red colour noted within a few minutes. These tests were also carried out in Hyderabad. Isonicotinic acid and isonicotinyl glycine were detected qualitatively and estimated quantitively (as 'apparent' isonicotinic acid) by a modification of the König procedure. Highly positive samples gave an intense blue colour within 15–30 min. Among weakly positive or negative samples, the intake of nicotine could be judged according to the colours of the resultant reaction products.

Creatinine concentrations were determined by the alkaline picrate procedure, while dapsone and its diazotisable metabolites were estimated using a modification of the Bratton & Marshall method, and individual 'dapsone/creatinine (D/C) ratios' were calculated for each urine sample. The proportion of dapsone doses that had been ingested (P) was calculated by comparing the mean D/C ratios of appropriate groups of test urines (T) with the mean for samples from patients when they were fully compliant (C), after allowing for the contribution from blank urine (B) (P = T - B / C - B). 'C' and 'D' were estimated as 70.6 and 6.3, respectively, by averaging the D/C ratios of sets of urine samples obtained from patients while they were being treated with Isoprodian that were either consistently positive for acetylisoniazid or consistently negative for isonicotinic acid.

Acid-labile isoniazid and acetylisoniazid concentrations were determined using picryl sulphonic acid and cyanogen chloride, respectively.

The significance of apparent differences in the proportions of prescribed drug doses ingested by different groups of patients was analysed statistically using chi-squared tests for percentages of positive and negative urine tests, and t-tests for D/C ratios.

Results

**DRUG ACCEPTABILITY**

*Treatment response*
As anticipated the clinical and bacteriological status of all the patients steadily improved during the 2-year period of the study. Thus of the 21 LL or BL patients who were initially smear-positive, 7 were smear-negative at the end of the investigation and the mean BI of the remaining 13 patients had fallen from 3.3 to 1.9 (1 patient absconded).

*Absence of hepatic toxicity*
There was no evidence of liver damage in the study; jaundice was not encountered and transaminase (SGPT) levels never exceeded 36 units/litre (mean 9 U/l).

*Gastrointestinal side-effects*
The prevalence and severity of the gastrointestinal side-effects attributed to prothionamide when the patients were treated with Isoprodian are summarized in Table 1. Side-effects were considered to
be mild when nausea or giddiness lasted for an hour or so after drug ingestion and subsided without the need for symptomatic treatment. They were graded as moderate when nausea, giddiness or occasional vomiting required treatment with antacids and/or antiemetics and described as severe when they also included anorexia, persisted throughout most of the day, and could not be totally controlled by antacids and antiemetics.

Two of the 60 enrolled patients refused to continue in the study because of the nausea experienced when they were given their test Isprodian doses during the run-in phase. Moderate, severe or acute gastrointestinal side-effects were experienced by 22 (37%) of the patients (Table 1), were more common among the women (10 of 16) than among the men (12 of 44) \((p=0.01)\) as previously demonstrated, and led to Isprodian being replaced by dapsone plus monthly rifampicin in 7 patients. Three of the 4 other patients who subsequently defaulted may have done so because of the side-effects they had experienced. The severity of gastrointestinal side-effects appeared not to be related to the ages of the patients. There were no other adverse reactions.

**Table 1.** Acceptability of Isprodian treatment and prevalence of gastrointestinal side-effects (numbers of patients)

<table>
<thead>
<tr>
<th>Side-effects*</th>
<th>No.</th>
<th>Defaulted</th>
<th>Isprodian stopped†</th>
<th>Completed the study</th>
<th>Good compliance‡*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute §</td>
<td>2 (2)</td>
<td>2 (2)</td>
<td>15 (2)</td>
<td>9 (1)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>15 (2)</td>
<td></td>
<td>22 (4)</td>
<td>11 (3)</td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>23 (4)</td>
<td>1</td>
<td>8 (2)</td>
<td>5 (1)</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>10 (3)</td>
<td>1 (1)</td>
<td>6 (3)</td>
<td>1 (1)</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>10 (5)</td>
<td>2 (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>60 (16)</td>
<td>6 (4)</td>
<td>7 (3)</td>
<td>47 (9)</td>
<td>26 (6)</td>
</tr>
</tbody>
</table>

* For definitions see text.
† At least 3 of first 5 acetylisoniazid urine tests positive.
‡ Replaced by dapsone plus monthly rifampicin.
§ Refused to continue in the study after ingesting the test Isprodian dose.
¶ Female.

---

**Table 2.** Proportion of positive acetylisoniazid and isonicotinic acid urine-test results prior to and following the ingestion of test doses of Isprodian

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>No. samples</th>
<th>Acetylisoniazid test positive (%)</th>
<th>Isonicotinic acid test positive (%)</th>
<th>I/C ratios* Geometric mean (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>54</td>
<td>1 (2)</td>
<td>0 (0)</td>
<td>0.27 (0.05–0.98)</td>
</tr>
<tr>
<td>24</td>
<td>58</td>
<td>55 (95)</td>
<td>58 (100)</td>
<td>3.3 (12–147)</td>
</tr>
<tr>
<td>48</td>
<td>56</td>
<td>3 (5)</td>
<td>52 (92)</td>
<td>3.3 (0.7–24)</td>
</tr>
<tr>
<td>72</td>
<td>58</td>
<td>0 (0)</td>
<td>33 (57)</td>
<td>0.82 (0.29–3.3)</td>
</tr>
<tr>
<td>96</td>
<td>58</td>
<td>0 (0)</td>
<td>9 (16)</td>
<td>0.47 (0.22–1.2)</td>
</tr>
</tbody>
</table>

* μg ‘apparent’ isonicotinic acid/mg creatinine.
Table 3. Proportion of positive acetylisoniazid and isonicotinic acid urine-test results

<table>
<thead>
<tr>
<th>Prescribed treatment</th>
<th>Mode sample collection</th>
<th>No. samples</th>
<th>Acetylisoniazid test positive (%)</th>
<th>Isonicotinic acid test positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily dapsone ± monthly RMP</td>
<td>H + C</td>
<td>878</td>
<td>0 (0)</td>
<td>3 (0·3)</td>
</tr>
<tr>
<td>Daily Isoprodian (Regimen A)</td>
<td>H</td>
<td>197</td>
<td>115 (58)</td>
<td>146 (74)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>272</td>
<td>140 (51)</td>
<td>177 (65)</td>
</tr>
<tr>
<td></td>
<td>H + C</td>
<td>469</td>
<td>255 (54)</td>
<td>323 (69)</td>
</tr>
<tr>
<td>Daily Isoprodian + monthly RMP (Regimen B)</td>
<td>H</td>
<td>187</td>
<td>108 (58)</td>
<td>133 (71)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>241</td>
<td>129 (54)</td>
<td>160 (66)</td>
</tr>
<tr>
<td></td>
<td>H + C</td>
<td>428</td>
<td>237 (55)</td>
<td>293 (68)</td>
</tr>
<tr>
<td>Daily Isoprodian ± monthly RMP (Regimens A and B)</td>
<td>H</td>
<td>384</td>
<td>223 (58)</td>
<td>279 (73)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>513</td>
<td>269 (52)</td>
<td>337 (66)</td>
</tr>
<tr>
<td></td>
<td>H + C</td>
<td>897</td>
<td>492 (55)</td>
<td>616 (69)</td>
</tr>
<tr>
<td>First 24 weeks†</td>
<td>H + C</td>
<td>403</td>
<td>237 (59)</td>
<td>287 (71)</td>
</tr>
<tr>
<td>Second 24 weeks†</td>
<td>H + C</td>
<td>360</td>
<td>199 (55)</td>
<td>234 (65)</td>
</tr>
</tbody>
</table>

* H, home visit; C, clinic visit.
† Results from those patients from whom at least 5 samples were collected during each period.

Table 4. Alternative methods of estimating the proportion of Isoprodian doses ingested

<table>
<thead>
<tr>
<th>Method</th>
<th>No. samples</th>
<th>Basis of calculation</th>
<th>Overall proportion doses ingested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylisoniazid</td>
<td>897</td>
<td>Percentage positive tests*</td>
<td>55%</td>
</tr>
<tr>
<td>I/C ratio</td>
<td>445</td>
<td>Mean test ratio—blank</td>
<td>52%</td>
</tr>
<tr>
<td>D/C ratio</td>
<td>445</td>
<td>Mean compliant ratio—blank</td>
<td>52%</td>
</tr>
</tbody>
</table>

I, apparent isonicotinic acid (µg/ml); C, creatinine (mg/ml); D, dapsone plus diazotisable metabolites (µg/ml).
* Criteria based on results obtained with test doses of Isoprodian (Table 2).

DRUG INGESTION

Collection of urine samples and creatinine estimations

A total of 2130 urine samples were collected in the study, 99% before 2 pm, that is within 8 h of the time patients had been advised to swallow their dapsone or Isoprodian tablets. Forty-four of the samples with creatinine concentrations of less than 0·1 mg/ml were discarded since such samples give inherently unreliable urine-test results.
Table 5. Changes in patient compliance on switching from daily Isoprodian to daily dapsone treatment (numbers of patients)

<table>
<thead>
<tr>
<th>Compliance</th>
<th>Isoprodian treatment</th>
<th>Worse</th>
<th>None</th>
<th>Better</th>
<th>Much better</th>
<th>Dapsone treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excellent</td>
<td>14</td>
<td>2</td>
<td>12</td>
<td>—</td>
<td>—</td>
<td>20</td>
</tr>
<tr>
<td>Fair</td>
<td>6</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>—</td>
<td>9</td>
</tr>
<tr>
<td>Poor</td>
<td>8</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Negligible</td>
<td>6</td>
<td>—</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>All</td>
<td>34</td>
<td>2</td>
<td>21</td>
<td>4</td>
<td>7</td>
<td>34</td>
</tr>
</tbody>
</table>

* For definitions, see text.

Table 6. Correlation between estimates of Isoprodian compliance based on independent evidence for isoniazid and dapsone ingestion

<table>
<thead>
<tr>
<th>Acetylisoniazid test</th>
<th>+</th>
<th>+</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isonicotinic acid test</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dapsone/creatinine ratios (μg/mg)</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10</td>
<td>144</td>
</tr>
<tr>
<td>10–30</td>
<td>59</td>
</tr>
<tr>
<td>&gt; 30</td>
<td>242</td>
</tr>
<tr>
<td>All</td>
<td>445</td>
</tr>
</tbody>
</table>

* Numbers of samples.

Noting the presence or absence of nicotine metabolites when samples were found to be negative for isonicotinic acid provided evidence that the great majority of samples had been correctly labelled. Thus only 3% of the urine samples from the 24 patients who did not smoke or chew tobacco appeared to contain nicotine metabolites as compared with 98% of those from the 36 patients who did.

Reliability and interpretation of the urine-test procedures used to monitor the ingestion of the isoniazid component of the combined Isoprodian formulation

Only 4 false-positive acetylisoniazid or isonicotinic acid results were obtained on testing more than 900 samples collected from the patients while they were not on Isoprodian treatment (first lines of Tables 2 and 3). After the supervised ingestion of 2 tablets of Isoprodian, reliably positive results were obtained for about 24 h by the acetylisoniazid method and 48 h by the isonicotinic acid procedure (Table 2). By 48 h, almost all samples gave negative results when tested for acetylisoniazid, indicating that the percentage of positive acetylisoniazid results from the study...
urines should give an approximate estimate of the proportion of ingested Isoprodian doses. An appreciable proportion of the urine samples obtained up to 96 h after ingestion of the supervised Isoprodian doses gave positive results when tested by the much more sensitive isonicotinic acid procedure and all the 492 study samples giving positive acetylisoniazid tests (Table 3) gave deep blue/purple colours when tested for isonicotinic acid.

Quantitative estimates of the ratios of isonicotinic acid plus isonicotinylglycine (as 'apparent' isonicotinic acid) to creatinine (I/C) among the samples obtained pretreatment and 24, 48, 72 and 96 h after giving the test Isoprodian doses are summarized in Table 2. There was no evidence that the wide range of individual I/C ratios at the different times represented consistent individual patterns of isoniazid metabolite excretion. Indeed, the most likely cause of such a variation, whether patients were rapid or slow acetylators of isoniazid, was excluded by determining their acetylator phenotype from the ratios of acetylisoniazid/acid-labile isoniazid among study samples giving positive qualitative acetylisoniazid results.

**Regularity of Isoprodian ingestion**

The results of the acetylisoniazid and isonicotinic acid tests are summarized in Table 3. The proportions of positive results among samples collected by means of surprise home visits were slightly higher than among those samples collected in the clinic (p = 0.1 for acetylisoniazid and 0.025 for isonicotinic acid tests, respectively using pooled data from regimens A and B). Compliance was not influenced by supplementing Isoprodian treatment with monthly supervised doses of rifampicin, and did not decline significantly with time. The estimates of the proportion of Isoprodian doses ingested according to the percentage of positive acetylisoniazid tests, urinary isonicotinic acid/creatinine and dapsone/creatinine ratios are shown in Table 4.

**Comparison of Isoprodian and dapsone compliance**

There were sufficient data to compare the compliance of 34 of the patients after changing from Isoprodian to dapsone treatment (Table 5). For this comparison 4 arbitrary grades of compliance

![Figure 1.](image-url)
were used; excellent, fair, poor and negligible, according to whether the mean D/C ratios of their urine samples were more than 40, 20–40, 10–20, or less than 10 μg/mg, respectively. Patients in these 4 categories would have ingested on average about 90%, 30%, 10% and fewer than 2%, respectively, of their prescribed drug doses. Compliance improved significantly when patients were switched from Isoprodian to dapsone, equivalent to an increase in the overall proportion of prescribed doses ingested from 48% to 60%. Furthermore, it was particularly noteworthy that, of the 7 patients whose compliance had most markedly improved when changing from Isoprodian to dapsone treatment, 4 had previously experienced severe gastrointestinal side-effects.

**Regularity of Isoprodian self-administration: correlation between the results obtained by different urine tests**

The excellent correlation between the dapsone and isoniazid metabolite findings of individual samples are summarized in Table 6. Thus 97% of the 144 samples with D/C ratios of less than 10 μg/mg, indicating that dapsone had not been ingested within the previous 4 days, gave negative results when tested for both acetylisoniazid and isonicotinic acid. Similarly, 95% of the 242 samples with D/C ratios of more than 30 μg/mg were positive for both the isoniazid metabolites.

**Individual variation in patient compliance**

One of the most striking findings of the investigation was the enormous interindividual variation in patient compliance (Figures 1 and 2). Considering patients from whom at least 10 samples were analysed, 12 provided samples giving more than 80% positive acetylisoniazid tests in contrast with 10 other patients with fewer than 20% positive results (Figure 1(a)). Similarly considering the isonicotinic acid tests (Figure 1(b)), in 18 patients more than 90% of their urine samples gave positive results, while fewer than 30% of the samples from 8 other patients were read as positive. However, patients could not be grouped merely into good and poor compliers; there was a continuous spectrum of compliance. This spectrum was confirmed (Figure 2) when the proportions of ingested Isoprodian doses were calculated from the I/C ratios of the samples.
### Table 7. Influence of age and gastrointestinal side-effects on compliance

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Number patients</th>
<th>Total number samples</th>
<th>% Samples positive for</th>
<th>% Doses ingested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>acetylisoniazid</td>
<td>Isonicotinic acid</td>
</tr>
<tr>
<td>Age Less than 30</td>
<td>16 (15)†</td>
<td>255 (105)</td>
<td>65} 62</td>
<td></td>
</tr>
<tr>
<td>30-40</td>
<td>21 (17)</td>
<td>297 (146)</td>
<td>60} 72</td>
<td></td>
</tr>
<tr>
<td>Over 40</td>
<td>21 (20)</td>
<td>345 (194)</td>
<td>43} 57</td>
<td></td>
</tr>
<tr>
<td>p value (over 40 vs 40 or less)</td>
<td>&lt;0·001</td>
<td>&lt;0·001</td>
<td>&lt;0·001</td>
<td>&lt;0·001</td>
</tr>
<tr>
<td>Side-effects‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>15 (15)</td>
<td>274 (153)</td>
<td>63} 76</td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>23 (23)</td>
<td>378 (192)</td>
<td>52} 58</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>10 (7)</td>
<td>151 (60)</td>
<td>65} 74</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>10 (6)</td>
<td>94 (40)</td>
<td>27} 46</td>
<td></td>
</tr>
<tr>
<td>p value (severe vs none to moderate)</td>
<td>&lt;0·001</td>
<td>&lt;0·001</td>
<td>&lt;0·001</td>
<td>0·07</td>
</tr>
</tbody>
</table>

* I/C: apparent isonicotinic acid/creatinine method; D/C: dapsone/creatinine method.
† For D/C method.
‡ For definitions see text.

**Factors affecting compliance**

Patients aged over 40 ingested Isoprodian less regularly than younger patients (Table 7) and the severity of gastrointestinal side-effects had a marked influence on compliance. Thus patients with severe symptoms ingested only about half as many Isoprodian doses as the other patients. A multiple regression analysis of the results obtained from the 47 patients from whom at least 10 urine samples had been analysed demonstrated that compliance declined with both increasing age and previous length of treatment, but was not influenced by their disease classification and sex, or whether or not they smoked or chewed tobacco. Compliance appeared to be related to the mg/kg dosage of thioamides given, since it improved with increasing body-weight.

**Discussion**

### Regularity of Isoprodian Ingestion

The proportion of prescribed Isoprodian doses ingested within the 24 h prior to collecting the urine samples was estimated to be 55%, 51% or 52% according to whether the acetylisoniazid, isonicotinic acid/creatinine or dapsone/creatinine methods, respectively were employed (Table 4). The agreement between the results obtained by these three independent methods was very encouraging and it may therefore be confidently concluded that just over half the prescribed Isoprodian doses were ingested. This is a significantly smaller proportion \( (p < 0·001) \) than that of the ethionamide and prothionamide doses estimated to have been ingested (73%) in the earlier pilot study.\textsuperscript{16} The most likely explanation for the different levels of compliance in the two studies is the enormous individual variability in patient compliance with the consequence that a larger proportion of better compliers may fortuitously have been included among the 12 patients included in the pilot study.
The overall compliance of the patients who did not experience gastrointestinal side-effects (about 55%) is quite typical of that found in previous studies carried out in Africa and Asia, when dapsone was administered alone.\textsuperscript{28,29} The excellent acceptability of isoniazid makes it most unlikely that its inclusion in Isoprodian tablets has an adverse influence on compliance.

**INDIVIDUAL VARIABILITY IN PATIENT COMPLIANCE**

Almost all previous studies of dapsone compliance among leprosy patients (reviewed\textsuperscript{28,29}) have only analysed single urine samples from individual patients. The results of this study clearly show that the drug self-administration of leprosy patients does not separate into 2 clear-cut modes (Figures 1 and 2); therefore, it is impossible to assess overall compliance on the basis of a single urine test. Previous compliance studies carried out among tuberculosis patients also provided evidence of a continuous spectrum in the individual regularities of drug self-administration.\textsuperscript{30-33} Further evidence for a continuous spectrum of compliance has recently come from a completely new approach to measuring drug taking\textsuperscript{34} in which the use of pilocarpine eye drops to reduce intraocular pressure\textsuperscript{35} or an experimental aerosolized medication to control asthma,\textsuperscript{36} was monitored electronically by means of microswitch devices.

**COLLECTING URINE SAMPLES FOR COMPLIANCE MONITORING**

Organizing and making surprise home visits to collect urine samples is very time consuming for the staff, and a nuisance for the patients. An important result of this study was the finding that the proportions of positive urine samples collected during the surprise home visits were similar to those of samples collected in the clinic (Table 3). This suggests that the impending occasion of the clinic visit does not appreciably alter the daily pattern of individual compliance. The much more conveniently obtained clinic specimens can therefore be employed without risk of biasing the outcome of compliance investigations. This finding is in accord with the only comprehensive study of this question ever undertaken in the whole field of compliance research—an investigation of the taking of isoniazid and p-amino-salicylic acid (PAS) by Chinese tuberculosis outpatients in Hong Kong.\textsuperscript{33}

**ADVERSE SIDE-EFFECTS**

The lack of evidence of hepatic toxicity was a welcome finding, in view of the experience of other groups of workers with combinations of prothionamide, rifampicin and dapsone\textsuperscript{9-12} and the 2 cases of jaundice encountered in the pilot study. Although gastrointestinal effects may be a less serious manifestation of thioamide toxicity than liver damage, they were nevertheless extremely important. Such side-effects were almost entirely responsible for the fact that about a quarter of the enrolled patients failed to complete the study (Table 1). Furthermore, the presence of severe gastrointestinal side-effects appears to have greatly discouraged the taking of prothionamide, as evidenced by the much poorer compliance of patients with such symptoms (Table 7), and the marked improvement in their self-medication when they were transferred to dapsone treatment (Table 5). As a consequence, it was concluded that only 1 of the 10 patients who suffered from severe gastrointestinal side-effects completed the study having taken sufficient Isoprodian for unequivocal therapeutic benefit (Table 1).

**IMPLICATIONS FOR THE TREATMENT OF LEPROMATOUS PATIENTS WITH THIOAMIDE-CONTAINING REGIMENS**

The results of this study indicate that daily prothionamide should only be prescribed when its compliance can be monitored. This can be conveniently achieved using isoniazid formulations such
as those previously prepared for the pilot compliance study with 6 mg isoniazid per tablet or capsule and testing urine samples by the isonicotinic acid method,\textsuperscript{16} or, as in the current study, employing the commercial combined formulation Isoprodian and the Eidus & Hamilton acetylsalicylic acid tile-test method.\textsuperscript{19}

Since variations in individual patient compliance appear to be continuous (Figures 1 and 2), several urine samples need to be tested from each patient in order to obtain a reasonable assessment of their drug taking. An analysis of the acetylsalicylic acid and dapsone urine-test results from the current study demonstrated that the testing of 5 urine samples per patient should be sufficient for this purpose and that when 3 of the first 5 urine samples collected during the Isoprodian treatment phase gave positive acetylsalicylic acid tests, enough prothionamide and dapsone had been ingested to be certain of therapeutic benefit. In the current study it was concluded that as a result of either poor compliance or discontinuation of Isoprodian treatment because of gastrointestinal intolerance, only 26 (43\%) of the 60 patients originally enrolled into the study had ingested sufficient Isoprodian for unequivocal therapeutic benefit (Table 1).

Although attempts to exhort poorly compliant patients to take their treatment more regularly should not be discouraged, past experience suggests that they are rarely likely to succeed. Since supervised intermittent treatment with the thioamides will almost certainly be ineffective,\textsuperscript{15} one can only recommend that poorly compliant patients be treated with regimens containing the maximum amount of supervised treatment. Such a regimen is regimen A in the THELEP-sponsored Field Trials being conducted in South India where 600 mg doses of clofazimine and rifampicin are given under supervision on 2 consecutive days each month and self-administered daily dapsone treatment is supplemented by injections of 225 mg acedapsone once every 2 months.\textsuperscript{57}

Acknowledgments

We wish to thank Saarstickstoff Fatol, Schiffweiler, West Germany for the gift of the Isoprodian tablets which was kindly arranged through the courtesy of Professor E Freerksen. The home visits were undertaken by Mrs Deveena Prabhaker, Mr Daniel Jones and Mr Syed Yousuff Jani. We are also grateful to Mr Shyam Rao who measured the serum transaminase levels and carried out the acetylsalicylic acid urine tests in Hyderabad, and would like to thank Dr J M H Pearson and Professor L Levy for their many helpful comments. Dhoolpet Leprosy Research Centre is managed by Victoria Hospital, Dighpalii, in collaboration with the Medical Research Council (of Great Britain), and also receives support from the British Leprosy Relief Association (LEPRA).

References


NEWS AND NOTES

Monoclonal antibodies to *Mycobacterium leprae*

As part of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, the Scientific Working Group on Immunology of Leprosy (IMMLEP) organized a workshop in June 1984 to characterize the specificity and reaction patterns of several murine monoclonal antibodies to *Mycobacterium leprae*.

Coded aliquots of 22 monoclonal antibodies that had been generated in six different laboratories had previously been sent to seven laboratories for independent analysis by a variety of methods, including enzyme-linked immunosorbent assay (ELISA), radioimmunoassay, electrophoretic and/or immunoblotting techniques, crossed immunoelectrophoresis and indirect immunofluorescence. The results, as agreed upon by the international group of investigators participating in the workshop, have been presented in detail elsewhere, and are summarized here.

Of the 22 monoclonal antibodies tested, 10 were found to be specific for *M. leprae*, with another 10 antibodies cross-reactive for one or more of the 25 mycobacterial species tested. The remaining two monoclonal antibodies were found to be specific for a protein of relative molecular mass 95,000 present in normal armadillo liver homogenates.

These monoclonal antibodies should prove extremely useful in the development of new methods for the immunodiagnosis of leprosy, in the identification of immunogenic *M. leprae*-specific gene products produced using recombinant DNA techniques, and finally in helping to characterize those *M. leprae* antigens that are important for the stimulation of cellular immunity.

Subject to available reserves, aliquots of the *M. leprae*-specific monoclonal antibodies will be made available to qualified investigators by IMMLEP, upon receipt of a short (one-page) summary of the experiments to be carried out with the antibodies. Requests should be addressed to Leprosy, World Health Organization, 1211 Geneva 27, Switzerland.

Gandhi Memorial Leprosy Awards 1988

From the *Hindustan Times*, New Delhi, January 30, ‘President R. Venkataraman today called individuals, voluntary organizations and government to work together to consolidate the achievements made till now in the field of leprosy eradication, and carry forward the initiative through ‘to a dynamic programme of detection, treatment and rehabilitation.’

Speaking at the International Gandhi Memorial Leprosy Award Function, he said, while we have made great improvements in case of detection systems, we must move towards active and thorough coverage of villages. Detection cannot be left to voluntary reporting. Likewise, in the area of treatment there is need for continuous research on the causes and cure of leprosy and camp studies on the results so far achieved.

The President gave away the International Gandhi Memorial Award for Leprosy instituted by the Gandhi Memorial Leprosy Foundation consisting of Rs 1,000,000 in cash, a medallion and a citation to Professor Thachakkadu Natesa Jagadisan from India and Dr Ma Haide from China. The award is given in recognition of distinguished service in the field of leprosy for not less than 10 years and contribution to any aspect of leprosy work, resulting in amelioration of sufferings of leprosy patients and in their assimilation in society as normal and useful members.

THELEP International Workshop on Chemotherapy Research of Leprosy

The Chemotherapy of Leprosy (THELEP) Scientific Working Group of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases organized in November 1986 in Osaka, Japan, in collaboration with the Sasakawa Memorial Health Foundation, an International Workshop on Experimental Chemotherapy of Leprosy. Because additional clinical and field trials were required to improve the treatment of leprosy, and because there is a need to strengthen the research capability of prospective centres for such research through training, the THELEP Steering Committee organized, as a follow-up to the Osaka Workshop, an International Workshop on Chemotherapy Research of Leprosy from 1 to 15 December 1987 at the All Africa Leprosy & Rehabilitation Training Centre (ALER T), Addis Ababa, Ethiopia. The objectives of the Workshop were: 1, to improve the knowledge of the participants through a systematic review of the modern concepts of chemotherapy and the methods of clinical research and field trials; and 2, to promote research activities in chemotherapy of leprosy.

Seventeen senior medical doctors from nine leprosy endemic countries participated in the Workshop as 'trainees'. Twelve doctors or scientists from both the THELEP Scientific Working Group and ALERT served as faculty members. The Workshop was indeed an intensive training course which covered all fields of chemotherapy of leprosy as well as related areas such as leprosy reactions, nerve damage, neuritis and their treatment. The evaluation of the Workshop indicates that it was successful. *Source*: Dr Ji Baohong, Secretary, THELEP Steering Committee, WHO, Geneva, Switzerland.
Comparative histology of skin and nerve granulomas in leprosy patients

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Accepted for publication 16 September 1987

Summary Biopsies were taken from infiltrated lesions and thickened nerves in 23 patients with leprosy. The lesions were histologically graded and the histological features semiquantitated and compared at the 2 sites. No significant difference in the overall histological picture in the skin and the nerve was seen. Two features seen more in nerve granulomas were caseation and a higher granuloma fraction, neither of which was thought to have any significant bearing on the comparative immunohistological grading at the 2 sites.

Introduction

The histological basis for classifying a leprosy patient along the immunohistological spectrum are the changes seen in the skin biopsy. The histologic grading thus arrived at correlates well with the clinical, bacteriological and immunological changes seen in most patients.

The criteria used for histologic grading also include changes in the dermal nerve twigs. Lesions in the larger nerves are however not considered for purposes of classification. The histological changes in the larger nerve trunks reveal a spectrum similar to that seen in the skin. Recent studies further suggest that the immunological grading in the nerve is in fact lower than that in the skin in the same patient. This observation has serious implications with regard to classification, initial therapy and subsequent release from control of leprosy patients. A prospective study comparing the histological changes seen in the skin and in nerve lesions in leprosy patients across the spectrum was conducted with a view to examining this aspect of leprosy pathology in greater detail.

Material and methods

Twenty-two patients were selected from the Leprosy Clinic at the Dermatology Department, Safdarjang Hospital, New Delhi. Only cases with thickened nerves and obvious skin lesions were selected as this would make it more likely that there would be well-developed lesions at both sites so that histological comparison could be properly made. The nerves biopsied were subcutaneous branches such as the superficial peroneal, sural, anterior branch of the ulnar cutaneous and index branch of the radial cutaneous nerve.
In each case a 1.5-cm long segment of nerve and an incisional skin biopsy were taken for study. The tissues were fixed in 10% buffered formalin, processed for paraffin embedding and 5-micron thick sections cut and stained with H & E and Fite-Faraco stains.

The 22 patients were clinically classified as TT-2, BT-14, BB-1, BL-1, LL-3 and Indeterminate-1. The duration of the disease varied from 6 months to 15 years. Only 1 patient of BL leprosy had received specific therapy for leprosy with DDS for a period of 2 years while the remaining 21 patients did not give any history of past treatment with antileprotic drugs. No patient was in reaction at the time of biopsy.

Results

Table 1 shows the concordance or discordance between skin and nerve lesions along the spectrum.

All 14 cases showing concordance at the 2 sites also had well-developed granulomas at both sites and the salient histopathological features in each case were semiquantitated and a comparison made between the lesions at the 2 sites. In the BT cases (Table 2), the nerve showed a higher granuloma fraction and more caseation and the skin showed a larger number of giant cells in the lesions while the other histological features were similar at both sites. In the LL and BL cases (Table 3), a higher BI was seen in 2 of the 4 cases while here too the histological features and the inflammatory cell types seen at both sites were similar.

Eight cases showed a discrepancy between the classification at the 2 sites. The breakdown of these cases (Table 4) shows features of indeterminate leprosy or nonspecific changes in 6 skin and 2 nerve lesions while the corresponding lesion in each case showed granuloma classifiable as BT, TT or BB.

Table 1. Distribution of cases along the spectrum with either concordance (Sk=N) or discordance (Sk=/N) between the skin and the nerve lesions

<table>
<thead>
<tr>
<th>Hist. Grading</th>
<th>TT</th>
<th>BT</th>
<th>BB</th>
<th>BL</th>
<th>LL</th>
<th>Ind.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin=Nerve</td>
<td>9</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Skin=/Nerve</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 2. Comparison of the salient histological features of the Skin/Nerve (S/N) granulomas in the BT cases with well-developed granulomas at both sites

<table>
<thead>
<tr>
<th>No.</th>
<th>GF</th>
<th>Epitheloid cell</th>
<th>Lymphocyte</th>
<th>Giant cell</th>
<th>Caseation</th>
<th>BI</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2/5</td>
<td>5/5</td>
<td>5/4</td>
<td>5/0</td>
<td>0/0</td>
<td>0/0</td>
<td>BT/BT</td>
</tr>
<tr>
<td>2</td>
<td>2/4</td>
<td>5/5</td>
<td>5/4</td>
<td>0/0</td>
<td>0/5</td>
<td>0/0</td>
<td>BT/BT</td>
</tr>
<tr>
<td>3</td>
<td>2/5</td>
<td>5/5</td>
<td>4/4</td>
<td>3/0</td>
<td>0/3</td>
<td>0/0</td>
<td>BT/BT</td>
</tr>
<tr>
<td>4</td>
<td>2/5</td>
<td>5/5</td>
<td>4/4</td>
<td>3/3</td>
<td>0/3</td>
<td>0/0</td>
<td>BT/BT</td>
</tr>
<tr>
<td>5</td>
<td>2/4</td>
<td>5/5</td>
<td>3/3</td>
<td>0/0</td>
<td>0/4</td>
<td>0/0</td>
<td>BT/BT</td>
</tr>
<tr>
<td>6</td>
<td>3/4</td>
<td>5/5</td>
<td>5/5</td>
<td>4/4</td>
<td>0/5</td>
<td>0/0</td>
<td>BT/BT</td>
</tr>
<tr>
<td>7</td>
<td>1/3</td>
<td>5/5</td>
<td>4/2</td>
<td>2/2</td>
<td>0/0</td>
<td>0/0</td>
<td>BT/BT</td>
</tr>
<tr>
<td>8</td>
<td>4/4</td>
<td>5/5</td>
<td>4/4</td>
<td>3/0</td>
<td>0/3</td>
<td>0/0</td>
<td>BT/BT</td>
</tr>
<tr>
<td>9</td>
<td>3/5</td>
<td>5/5</td>
<td>4/4</td>
<td>0/3</td>
<td>0/3</td>
<td>0/0</td>
<td>BT/BT</td>
</tr>
<tr>
<td>10</td>
<td>3/4</td>
<td>5/5</td>
<td>5/5</td>
<td>3/3</td>
<td>0/3</td>
<td>0/0</td>
<td>TT/TT</td>
</tr>
</tbody>
</table>

Total 26/46 55/50 46/41 30/15 0/29 1/1
Discussion

The histological features in the skin and in the nerve lesions in the same patient has been a point of interest for pathologists working with leprosy for some time now. While some workers in this field record a consistent discordance between lesions at the two sites with the nerve lesion showing more bacilli and a lower, i.e. towards LL, histological grading in most cases, others are of the opinion that the lesions are of similar histological grading at both sites. Thus, in one study a significant discordance was found in 21 out of 36 patients with the nerve showing a lower grading in the majority of cases. Similarly, in a recent study discordance in 35 out of 37 borderline cases was found with the nerve again showing a lower histological grading in most patients. On the other hand, in a study of 153 nerve biopsies from Nepal it was observed that in the 133 cases where abnormal findings were seen in the nerve, the skin was also abnormal and that ‘the type (classification) showed agreement at the two sites in every instance’.

Concordance in skin/nerve histology in 14 out of the 22 cases studied was seen in the study under report. It was further seen that well-developed granulomas were present at both sites in these 14 cases only. The remaining 8 cases where discordance was recorded between the skin and the nerve all had features of either indeterminate type disease or of nonspecific inflammation at either site.

Histological comparison of the granulomas at both sites in the first group of 14 cases brought out differences in the amount of caseation, number of giant cells, granuloma fraction and in the number of bacilli. The significance of these differences in terms of local immunity is however not clear. Caseation in leprosy lesions is known to occur almost exclusively in the nerve in TT and BT leprosy lesions. Anecdotal reports of caseation occurring in lepromatous nerves have not been substantiated in any recent study. Again, immunologically speaking, caseation indicates a state of increased hypersensitivity and perhaps a higher immune status as is indicated by the larger number of giant cells. The higher GF in the nerve recorded in the present study has been described in a recent report as the increased extent and severity of the nerve lesion. It is quite possible that both these features are caused by the anatomical differences in the target organs. Thus, while the tougher collagen of the dermis may limit the granuloma spread in the dermis, the relatively softer nerve
funiculi may allow easier spread of the granuloma up to the limits of the perineurium accounting for a higher GF. Similarly, caseation in the nerve may represent necrosis in the nerve after obliteration of the blood supply by the infiltrating granuloma. The higher BI in the nerve in 2 of the 4 multibacillary cases is also a well-established pathological observation. In both cases in the present study the histological picture at both sites showed concordance and no inference regarding a lower immunological state in the nerve could be drawn in this group either.

All 8 cases with discordance between the skin and nerve lesions did not show granuloma formation at either site in this study. In 6 of these patients the nerve showed well-developed granuloma formation and the skin had features of either indeterminate leprosy or of nonspecific inflammation, while in 2 cases BT granulomas were present in the skin and the nerve showed only inflammatory infiltrates without any bacilli. Thus, discordance was seen when incomplete granuloma formation at either site precluded proper classification along the histological spectrum. This difference could be explained by either slower granuloma development or quicker granuloma resolution at either of the 2 sites. Since both phenomena are related to the local immune response in the skin or in the nerve, the above observations do suggest a difference in the immune status between the 2 sites in this group of 8 cases. The difference, however, is not consistent, with the nerve being lower in 6 cases and higher in the remaining 2 cases.

The results of the present study show that there was complete agreement in the histological grading of the skin and the nerve lesion in the 14 cases with developed granulomas at both sites while discordance was seen in 8 cases where granuloma formation was not evident at one of the 2 sites. This is in contrast to earlier reports on the comparative morphology of the skin/nerve lesions which describe a greater degree of discordance with nerves showing a persistently lower immunological status. Since the site of nerve biopsy in both the earlier reports and the present one was the same, other explanations for this difference have to be looked for. The patients chosen for this study had infiltrated patches and thickened nerves and so they all had disease of a moderately advanced status. This in turn would suggest that with progression of the disease, granulomas of similar histological grading and BI are formed in the skin and in the nerve by the immunological mechanisms inspite of a heavier bacillary load or a lower histological grading at either site in the initial stages of the disease.

In conclusion, this study shows that in developed leprosy lesions the skin biopsy findings represents the histological grading in the nerve as well and still remains the most convenient and reliable means of classifying a patient along the immunohistological spectrum of the disease. In earlier lesions there appears to be a dichotomy between the immunohistological grading at the 2 sites which needs to be explored in greater detail in studies on a larger population of patients before the relevance of such a skin/nerve dichotomy to the overall immune status and prognosis of leprosy patients can be understood.

References

Obituary

MONINA G MADARANG

The Leonard Wood Memorial (American Leprosy Foundation) is grieved by the passing of Mrs Monina G Madarang on 8 January 1988.

She was Administrative Director of the Cebu, Philippines facility as well as an excellent laboratory technician. Mrs Madarang received a BSc in Pharmacy from the University of San Carlos, Cebu City, Philippines and an MBA from the University of Philippines, Cebu City, Philippines. She also studied at Mercy Hospital of Medical Technology, San Diego, California and Marquette University, Milwaukee, Wisconsin.

In her 24 years of employment with the Memorial, she held a variety of duties. Her wisdom and knowledge of Leonard Wood Memorial procedures in dealing with International leaders made many lasting friendships and created workable cooperation and collaboration in leprosy projects. She received specialized training in many phases of leprosy projects. She received specialized training in many phases of leprosy work and applied the new skills in furthering our research.

Truly committed to the leprosy cause, often giving far more than duty demanded, her caring, dedication and loyalty is truly a loss to the Memorial.

ANN DONNELLY
Sir,

In previous issues of this Journal, attention has been drawn to the development of a plastic model of the well-known portable McArthur microscope. In the preface to the Users Handbook, published by the Eritrean Relief Association Public Health Programme (BCM Box 865, London WC1V 6XX), one of the introductory paragraphs reads as follows:

'The Eritrean Relief Association, a British Registered Charity, inaugurated an extensive public health programme in 1981 as part of its attempt to provide a framework for longer term development in its programme area, where the population have been afflicted by war for over 20 years and for the last five years by a severe drought. In May 1982 a decision was taken in the Eritrean Public Health Programme (EPHP) that a considerable input of microscopes and microscopy skills would be required in order to change disease patterns in the areas of Eritrea where the programme was operative. Since this involved approximately 200 villages at the time, a project for purchase of this number of microscopes was drawn up. A large number of instruments were reviewed, and the design made initially by Dr John McArthur in 1932 was chosen as the most suitable. The first commercially available instrument appeared in 1933, since which time it has been refined and added to. EPHP took responsibility for redesigning it in plastic.'

During 1987, I was fortunate enough to be given a years' leave of absence from the University of Sheffield Medical School and to gain financial support from LEPRA, to work in Uzuakoli Leprosy Settlement, Uzuakoli, Imo State, Nigeria, where I had the opportunity to try out the plastic microscope for leprosy smears. It was undoubtedly of value in teaching. The medical officer in charge used it in lectures and I found it useful in teaching laboratory assistants. The resolution of bacilli in skin smears was excellent and the image generally brighter than with the standard bench microscope we were using.

There were however a few problems:

1. The springs which hold the slide to the stage have to be tight, but this means that it is difficult to make small movements with the fingers; changing fields tends to be jerky and coarse. In fact it is only too easy to move the slide so that the smear goes out of view. If the slide is highly positive, this does not matter so much, but with low bacteriological indexes, false results may occur.
2. When using the oil immersion lens, because the slide is inverted, it is difficult to position the oil accurately and to ensure that the lens can be moved into position without dispersing it.
3. With the standard slide, smears are positioned lengthways along the slide. When attempting to read a smear at one or other end, there is a tendency for the slide to slip off the platform. These are however relatively minor criticisms, all of which can be overcome. The low cost and simplicity of this instrument, together with its small size and portability, are all attractive features.

Letters to the Editor

THE PORTABLE, PLASTIC McARTHUR MICROSCOPE FOR THE EXAMINATION OF SKIN-SMEARS IN LEPROSY

would be interesting to know if it has indeed proved valuable in Eritrea or elsewhere. Have others found that it has advantages over currently available, low-price bench microscopes?2  

T LONGMORE

Medical School  
University of Sheffield  
Beech Hill Road  
Sheffield S10 2RX

References

2 Cheesbrough M. Laboratory equipment—where are the tools to do the work? Br Med J, 1984, 288: 1978–82.

FIELD DETECTION OF EARLY NEURITIS IN LEPROSY

Sir,

The article by Dr Fritschi (Lepr Rev, 1987, 58, 173–7) on the early detection of neuritis in a field situation is very timely. We must accent the need to identify the acute problems that, if adequately treated, can be reversed, and to remember that silent neuritis is not uncommon.

He did not accent the necessity of recording the baseline evaluation on the first examination of the patient, or the need for adding a comment on each subsequent visit regarding any change, or no change, in muscle strength. Patients may not realize they have a minimal muscle weakness for some period before a paraesthesia attracts their attention. If the baseline has not been recorded it may be assumed that the paraesthesia is the first sign of a neural deficit, and everyone is disappointed when recovery does not occur with appropriate therapy, because it was 'too late'.

For three years our unit has been taking ‘baseline’ records and keeping monthly records of changes. We have detected a number of silent neuritis and have had the satisfaction of seeing recovery in the majority of them.

In testing for neuritis we seek the first signs of deficit.

With the ulna nerve the first motor sign detectable is the inability to adduct the little finger to the ring finger (3rd palmar interossel). Full loss of function of this muscle may occur but the patient may still be able to assume the ‘Indian Dance Position’ in which the little finger is not adducted. There may be a total loss of adduction of the little finger without atrophy of the hypothenar, muscles or loss of their function.

With the radial nerve the first muscle to show weakness is frequently the extensor digiti communis. This can best be tested with the PIP joints flexed and the patient extending the MCP joints. In the ‘Indian Dance Position’ it is possible for the lumbricals to extend the PIP joints of the fingers and if the EPL is affected the median muscles of the thenar eminence may extend the thumb IP joint, so masking the early weakness of these muscles.

The easiest rapid test for common peroneal function is to ask the patient to walk on his heels—any weakness of peroneal or anterior tibial muscles is rapidly displayed by the inability to hold the forefoot up—yet he may be able to assume the positions described in the article.

Sensory variations are less easy to assess, but if we really wish to prevent the development of deformity we just start making baselines and keeping regular records of muscle power changes.

GRACE WARREN & JOLENE K TOMLAN

The Leprosy Mission  
Christian Hospital  
Manorom, Chainat  
Central Thailand
FIELD DETECTION OF EARLY NEURITIS IN LEPROSY

Sir,


After our patients’ hands have been checked for muscle weakness, the examiner runs forefinger and thumb of each hand over the patient’s little fingers simultaneously comparing the stickiness of the two fingers.

This delicate test gives a reliable indication—sometimes the earliest sign—of ulnar neuritis. If positive, relative dryness will be found on one side due to early diminution of sweating. This should be recorded immediately and followed up by a full functional check-up, and of course steps taken to prevent progression of the neuritis.

A similar test could be applied to the feet. While stroking the foot in Fritschi’s last procedure, a mental note is made of the relative dryness of the two sides. In this case dryness of the sole could indicate an early tibial palsy.

Once again the dryness, when found, is recorded and appropriate action taken.

J R HARRIS

The Leprosy Mission
50 Portland Place
London W1N 3DG

[Our reproduction of figure 6 on page 175 of Dr Fritschi’s publication was perhaps somewhat short of optimum quality; to check the sensory area of the posterior tibial nerve, the examining finger should stroke the lateral part of the sole of the foot. The lateral border of the foot is supplied by the sural nerve. Editor.]

THALIDOMIDE IN ERYTHEMA NODOSUM LEPROSUM (ENL)

Sir,

An adult male patient from Bosé, Ikom Local Government Area, Cross River State, Nigeria, presented himself to us at Moniaya Leprosy Hospital, on 20 March 1985. He reported that the first lesion had appeared on the right cheek in 1984, about one year before. At the time of reporting he had erythematous nodules on the upper and lower limbs. Both ulnar nerves were enlarged and tender. He was classified as having borderline lepromatous (BL) leprosy.

Smears taken on 21 March 1985, were reported as follows: +1, +4, +2, +1. He was started on multidrug therapy (MDT) with rifampicin, dapsone and clofazimine. On 9 May 1985, he suffered from a severe reversal reaction, and because of severe neuritis, he was given steroid therapy. On 24 May 1985 it was reported that the lesions were still active. They were on the thighs and lower limbs. On 21 June smears were repeated: −, −, +, +, +, −, − (6 sites).

I saw him for the first time on 22 January 1986. He was suffering from an ENL reaction. For four days he had tender nodules on the arms and legs, and he complained of severe pain around the left elbow. On examination I found impaired function of the left ulnar nerve (3/5), the left median nerve (4/5) and the left radial nerve (4/5). I started him on another course of steroids, i.e. six months of dexamethasone, beginning with 4 mg daily and reducing the dose monthly. Because of the severe pain the left arm was splinted and he was given analgesics. Both ulnar nerves were enlarged and tender, graded 1/3 (right) and 0 (left), (normal = 3)

On 26 February he was having agonizing pain. I made a diagnosis of ulnar nerve abscess and took him to theatre for incision of the nerve sheath, eased the pain; the nerve has not been tender since.

On 6 April he suffered from another ENL reaction. He had pyrexia and ulcerating nodules on the arms and legs. He was given increased doses of lamprene, chloroquine and panadol, and the
steroids were increased to maximum dosage again. There was little improvement. A week later he was still suffering from the severe ENL reaction. Bullae appeared and we wondered if this could have been due to any of the drugs. Eventually the reaction subsided, only to be followed by another month later.

At this stage the right ulnar nerve function became markedly impaired. Impaired function of both median nerves and both peroneal nerves became apparent. He developed marked sensory loss in the feet. He continued to suffer much pain and was subject to repeated episodes of ENL.

To try to locate an underlying cause for the repeated ENL reactions, a few laboratory tests were done: Hb, 10/g; thick drop, malaria—nil/filaria—nil; stool and urine, normal.

He continued to suffer intensely until a limited supply of thalidamide was obtained and on 23 June he began a course: 200 mg BD for 7 days; then 100 mg BD for 7 days; and then 100 mg daily for 7 days.

On 5 July a full review was carried out. The pain had almost completely gone. The skin was clear of nodules. He was feeling very much better. At this stage he was on thalidamide 100 mg daily and the steroids were being reduced.

He was receiving intensive physiotherapy. He was a cooperative patient and diligently worked at the exercises. His main complaint at that time was stiffness of the hands. By now there was marked impairment of function of both ulnar nerves, the right median nerve and the left peroneal nerve. He also has insensitive feet. This impairment of function seemed to happen under our very eyes and there seemed so little we could do for him in the face of the repeated ENL reactions.

However we have no doubt about the remarkable effect that the thalidamide had. It resulted in immediate relief and heralded the beginning of the slow healing process. Unfortunately, as we reduced the thalidamide, ENL nodules reappeared and so we increased the dose again. Then our supply ran out. Three weeks later he had another ENL reaction. It was not quite as bad as previous episodes but he did have pyrexia and painful nodules. Now he is back on thalidamide and we plan to continue this treatment for a few months. He is now free from complaint.

I would be most interested in any comments, criticisms on our management, or advice that anyone would care to give, for I would certainly not like to see another patient suffer in this way.

CECILY BOURDILLON

Medical Missionaries of Mary
PO Box 183
Moniaya—Ogoja
Cross River State
Nigeria

CORTICOSTEROID-INDUCED ACTIVATION OF CHRONIC ULCERATION IN LEPROSY

Sir,

The immune suppressive effect of corticosteroids is well known and widely used in the management of reversal reactions in leprosy. The danger of provoking spread of tuberculosis by such medication is commonly recognized, but the potential dangers from plantar ulcers, without frank discharge of pus or other signs of active infection, are frequently ignored. That this can have disastrous results is illustrated by two cases seen at ALERT, Addis Ababa, in neither of whom was there any evidence of active, progressive infection.

Case 1. A borderline lepromatous patient in reversal reaction received corticosteroids in spite of chronic forefoot ulceration, which was considered indolent and inactive. Two weeks after the start of corticosteroid treatment the right foot became swollen and pus seeped from the previously inactive ulcer. Surgical consultation was requested. The radiogram showed bunching of the dorsal surfaces of talus and of navicular, but no plantar displacement of the latter. There was capsular
new bone formation at the first metatarso-phalangeal joint and periosteal new bone formation of
the shafts of the second, third and fourth metatarsal bones with sclerosis. These changes were
interpreted as the result of chronic forefoot ulceration, probably with infective changes of the first
metatarso-phalangeal joint, while the changes of the metatarsal shafts are considered to be due to
toxic damage from the ulceration. In addition there was erosion and lysis of the distal and proximal
phalanx and of the metatarso-phalangeal joint of the great toe. These were considered to be fresh,
progressive changes, undoubtedly provoked by the administration of corticosteroids.

Case 2. A borderline tuberculoid patient in reversal reaction received corticosteroids in spite of
chronic midfoot ulceration, which was considered indolent and inactive. Within 2 weeks of the
introduction of corticosteroids, the foot became swollen with discharge of pus from the ulcer and
several sinuses. Surgical consultation was requested. The radiogram showed two sclerotic remnants
of metatarsal shafts, indicating longstanding bone changes from loss of sensation. Otherwise, the
picture was one of violently spreading infection throughout the whole foot, including the ankle
joint.

Eventually both patients required major, ablative surgery, midfoot amputation, respectively,
below the knee amputation under heavy antibiotic cover.

Ideally no patient with any evidence of secondary infection, even the so-called chronic, inactive
ulceration, should receive corticosteroids, but since plantar ulceration is such a common feature of
leprosy, this is obviously an impossible demand.

However, all patients who are considered for corticosteroids should be carefully examined and
watched for secondary infection. If possible, surgical consultation and intervention should be
requested before corticosteroid treatment is instituted.

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LEPROMA OF THE METAPHYSIS

Sir,

A 20-year-old Ethiopian with slit-skin smears positive for acid-fast bacilli presented with a
clinical diagnosis of polar lepromatous leprosy and an acute, hard swelling of the right elbow
region.

The radiogram was interpreted as an osteoclastoma. A drill biopsy was taken to confirm this but
unexpectedly the tumour was found to be a lepromatous granuloma, containing many acid-fast
bacilli, both intact and broken and also many globi. Under continued treatment for leprosy the
tumour regressed as did the skin manifestations.

Lepromata of cancellous bone are well-known, particularly of the fingers in relation to the
proximal interpalangeal joints. So far only one report of leproma of cortical bone has appeared1 and
it is of interest that in both cases the identical misdiagnosis was made initially.

Leproma of bone should be suspected whenever a cystic lesion is found in a bacilliferous patient.
Since the leproma can be expected to regress under medical treatment, no specific treatment is
indicated, except support of the region to avoid collapse of the bone, until satisfactory healing has
taken place.

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Reference

NONSEPTIC TARSAL DISINTEGRATION IN LEPROSY

Sir,

Bone changes in leprosy have been described as the result of direct invasion of the skeletal structures with lepromatous granulomata, or secondary infection, or as absorption related to loss of sensation. Characteristically the major joints remain intact and this is ascribed to the preservation of proprioceptors in leprosy, even in the total absence of other forms of sensation. This is completely different from syphilis, where the proprioceptors are usually lost (e.g. tabes dorsalis), and the major joints typically are affected.

This letter describes two patients with documented borderline lepromatous leprosy who also had marked loss of sensation, including joint sense.

**Patient 1.** A 50-year-old male patient with well treated, though still skin-smear positive, borderline lepromatous leprosy presented for treatment of a severely swollen foot. No evidence of diabetes, syphilis or rheumatoid disease was found.

Radiologically the foot was completely disorganized with total disruption of the ankle joint and the midtarsal joints with erosion of the adjacent joint surfaces. There was lysis of the navicular and cuneiform bones and the cuneiform bone with some new bone formation of a hazy nature. There was concentric absorption of some of the metatarsal bones and of two of the remaining phalanges. Aspiration of the copious fluid produced no growth on culture and this was later confirmed during surgery.

The extremity was completely anaesthetic almost to the knee, with loss of vibration and joint position sense. No evidence of past or present plantar ulceration was seen.

**Patient 2.** A 40-year-old female patient with well treated, though still skin-smear positive, borderline lepromatous leprosy presented with a chronically swollen foot. No evidence of diabetes, syphilis or rheumatoid disease was found.

Radiologically the talo-navicular, talo-calcaneal and calcaneo-cuboid joints were disrupted and there was new bone formation of hazy character.

The extremity was completely anaesthetic up to midcalf, with loss of vibration and joint sense. No evidence of past or present plantar ulceration was found.

Both patients had faithfully worn correctly designed shoes, which had obviously protected the feet against ulceration, but failed to compensate for loss of deep and proprioceptive sensation. Both parties required major, ablative surgery. I would be interested to hear of other cases and to know if sensory neurological testing has a predictive value in such cases?

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RIFAMPICIN MONOTHERAPY IN PAUCIBACILLARY LEPROSY

Sir,

It was interesting to read the results of treatment of paucibacillary (PB) leprosy with ten weekly doses of rifampicin (*Lepr Rev* 1987, 58: 349–58). However, I do not think that it is prudent to use rifampicin monotherapy even in PB leprosy patients for the following reasons:

1. The threshold of $10^6$ organisms for the natural occurrence of drug resistant mutants is applicable to *Mycobacterium tuberculosis* and we do not really know whether the analogy is applicable to *M. leprae*.
2. The said threshold is for the drugs against which resistant develops in a stepwise fashion and not for the drugs against which it develops in a single step. The resistance of *M. leprae* against rifampicin develops as a single step process. It has developed earlier than that against dapsone.
After the unfortunate experience of dapsone monotherapy and case reports of rifampicin resistant leprosy, \textsuperscript{2,3} rifampicin monotherapy appears to be unjustified even in PB leprosy patients. If resistance to rifampicin becomes ubiquitous as has happened with dapsone, we will lose the most potent antileprosy drug available to us today.

A H PATKI

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India

References


FIELD DIAGNOSIS OF EARLY LEPROSY

Sir,

I would like to comment on Dr Smith’s paper \textit{(Lepr Rev, 1987; 58: 141–8)} which describes the use of a questionnaire of 20 case histories. The diagnosis of leprosy is based on the presence of at least one of the three cardinal signs of anaesthesia, thickening of peripheral nerves at the sites of predilection and the finding of acid-fast bacilli. The 20 cases reported give no details of skin-smear results and diagnoses are made in the absence of any of the cardinal signs, e.g. case history 3 and 18. I note also that the location of the hypopigmented patches influences the diagnosis in two similar cases, when lesions are on the face (case 1) the response is ‘suspect’ while when on the buttocks (case 18) ‘affected’ is comparatively preferred.

I disagree with the diagnosis in case 1, since there is a history of contact with an infectious case ‘affected’ would be the possible correct diagnosis. I also question the diagnoses in cases 1, 8 and 12 where the sex of the child seems to influence the decision and I would disagree with the diagnosis in cases 13 and 19 which I find confusing.

However, despite my cautionary comments I do appreciate the attention that this paper gives to this much neglected area of leprosy.

B KULKARNI

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India

REPLY—FIELD DIAGNOSIS OF EARLY LEPROSY

Sir,

I have read Dr Kulkarni’s comments carefully and I welcome the opportunity to reply.

I wholeheartedly agree with Dr Kulkarni that this is indeed a much neglected area in leprosy and it has thus been with some trepidation that I have attempted to tackle the subject of the field diagnosis of early leprosy.

In defence of the ‘standard’ diagnoses used in the case histories I would point out that the majority of the 79 field workers who completed the questionnaire agreed with the standard
responses in 17 out of the 20 cases. However, the majority need not be correct and Dr Kulkarni’s reminder of the cardinal signs of leprosy is important. Yet he also from his comments seems willing to positively diagnose leprosy in the absence of any of the cardinal signs (Case 1). This raises the whole issue of the place of the cardinal signs in the diagnosis of early leprosy. It is often our least experienced leprosy staff who are left with these difficult decisions on diagnosis of early disease—attempting to get the right balance between missing true cases and overdiagnosis and overtreatment. This has also implications for the validity of regional comparisons of the prevalence of leprosy. Dr Kulkarni makes an interesting point about the factors which influence the decision, e.g. sex of the subject, history of contact, and the site of the lesion. This opens up areas for further operational research. I would be interested in hearing from anyone else who has used the 20 case histories.

W C S SMITH

Department of Community Medicine
Ninewells Hospital and Medical School
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ARE BACTERIAL COUNTS ON SLIT-SKIN SMEARS IN LEPROSY AFFECTED BY PREPARING SLIDES UNDER FIELD CONDITIONS?

Sir,

Several years ago we carried out simple trials on more than 300 patients/slides over a 6-month period. It was our finding that no difference was found if the slides were dried in the sunlight or shade if drying time was never longer than 5 minutes. We did find a difference when the slides were not stored in a lightproof container after fixation and up to the staining period. The main difference that we encountered was the difference in the quality of smear collection between skin-smear technicians who receive longer more intensive training and work continually with the same work and that of paramedics who received what we consider very limited training experience in collecting smears. We later also checked the readability of slides after staining and stored in light and non-lightproof containers with oil left on the slide and slides washed. There appeared to be no significant difference between the latter but the results were marked if they had not been stored in airtight lightproof containers. The collection of the smear is what we find needs continual supervision and monitoring.

ELIAZAR T ROSE

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Leprosy Control and Field Work

The diagnosis and management of early leprosy; in Persian
This well-known booklet by the late Dr S G Browne has been translated into Persian by Dr Hossein Tabatabai, Leprosy Organisation of Iran, Nejatollahi Av., 189, Tehran 15, Iran. (Because of the use of Arabic script, we may have mistakenly referred to this translation as being in the Arabic language in a previous number of this journal.) This is surely a most commendable initiative; translations of this booklet, written in highly readable terms by an eminent leprologist, would be of great value in other languages, including areas where leprosy is even more significant than it is in Iran. Editor.

Medical laboratory manual for tropical countries
Medical laboratory manual for tropical countries, by Monica Cheesbrough, is a guide to diagnostic techniques. Volume I (clinical parasitology and chemistry) costs £8.95 (airmail) or £7.75 (surface); volume II (microbiology) costs £4.45 (airmail) or £3.85 (surface). A plastic-coated, colour wall chart with large, vivid photographs of 15 helminth eggs and larvae is also available. It costs £3.30 (airmail) or £2.25 (surface). These and other items may be obtained from: Tropical Health Technology, 14 Bevills Close, Doddington, Cambridgeshire, PE15 0TT, England.

New postgraduate fellowships in epidemiology
TDR announces the introduction of a new category of research training grant to provide postgraduate on-site training in field research projects for suitably qualified health or health-related professionals from developing countries.

Training will be geared to the development of advanced epidemiological skills needed for the design and conduct of clinical and community trials and other epidemiological investigations. Support for related disciplines—entomology, ecology, biostatistics and social sciences—will also be available. These grants, generally covering a two- to three-year period, will be awarded on a yearly basis, subject to satisfactory performance and availability of funds.

Eligibility. Candidates for these grants should have adequate post-graduate training in epidemiology or the related disciplines mentioned above. Candidates must also have a stable employment position in their own country, preferably within a health research or training institution or disease control programme. Preference will be given to candidates who plan a career in epidemiological research in their home country.

How to apply. Interested scientists should write for the official application forms, which may be submitted throughout the year. Applicants may themselves suggest a suitable ongoing field research project for their training or they may ask TDR to help them identify an appropriate project. Grantees are expected not only to participate in the activities of the main project to which they are assigned, but also, during the first year of
training, to formulate a research project of their own, preferably linked to the main project. When circumstances dictate, TDR will consider support for operational expenses connected with the grantee’s research activities.

All requests for further information and application forms should be addressed to: Dr R H Morrow, Jr, Secretary, Scientific Working Group on Epidemiology, World Health Organisation, 1211 Geneva 27, Switzerland.

News and Notes

British Association for the Advancement of Science—150th Annual Meeting, Oxford 1988

This Association was established in 1831 and its aims are: 1. to enhance public understanding and awareness of science and technology and their impact on society; to help people to recognize science as part of our culture and heritage and to share in a scientific approach to solving problems; and 2. to increase public support for science, and to defend the public interests of science.

The Association’s work is multidisciplinary, covering all the natural, social and applied sciences. It provides a forum for debate and a focus for educational activities for people of all ages and experience. An independent body, its membership is open to all. The Annual Meeting is the largest general scientific meeting held in Britain. Open to all, its programmes of several hundred talks, exhibitions and demonstrations form a festival of science that attracts several thousand participants each year.

The Scientific Programme covers: physics; chemistry; geology; biological sciences; geography; economics; engineering; anthropology and archaeology; medical sciences; psychology; agriculture and forestry; education; sociology; general; and mathematics. Core topics will include: evolution; molecular electronics; education and science; the challenge for British science; and biotechnology. Lectures will range over such subjects as: The death of the dinosaurs; physics in the home; geology and society; protein engineering; recreation and leisure; the prospects for full employment; robotics and automation; local archaeology; medical imaging; brain localization; the agricultural policy of the EEC; are teachers professionals? and class and public policy.

The Association also has a Youth Section and almost two-thirds of its work involves young people. There will be a great deal to interest younger scientists in the main programme as well as in a special day of events for BAYS (British Association Youth Section). Many delegates will wish to attend the Mason Conferences, during which specialists will describe the latest advances in their various fields. These will be held, among others, by the Institute of Scientific Information, the Institute of Biology, the Royal Society of Chemistry Food Chemistry Group and the Royal Astronomical Society.

For further information contact: Dr D. Morley, British Association for the Advancement of Science. Fortress House, 23 Savile Row, London W1X 1AB.


Full details of the sessions have already been printed in this journal; see Number 1, 58, 1986.

The Congress Location and Hotel Accommodation; The 13th ILA Congress will be held in The Netherlands Congress Centre, The Hague, The Netherlands, from 11–17 September 1988. Hotel accommodation will be provided in several price categories ranging from ca. Dfl. 50,- to Dfl. 250,- and more. Congress Bureau: For all information concerning the congress, please contact the Congress Bureau: QLT Convention Services, Keizersgracht 792, 1017 EC Amsterdam, The Netherlands. Tel. +31 (0)20-26 1372, Tlx. 31578 inter nl att qlt. This Meeting is co-sponsored by the World Health Organization.

XIIth International Congress for Tropical Medicine and Malaria, September 1988

This congress will be held in the International Congress Center RAI in Amsterdam from 18–24 September 1988, immediately after the International Leprosy Congress (above).

Information can be obtained at: Organisatie Bureau, Amsterdam, Europaplein 12. 1078 GZ Amsterdam, The Netherlands. Tel. +31 (0)20-440807, Tlx. 13499.

Meeting of the International Society of Dermatology, Oxford, September 1988

A joint meeting of this Society with the International Society of Dermatopathology will take place in Oxford, UK, 4–8 September, 1988. There will be at least two sessions on leprosy, including histopathology, together with exhibits and demonstrations, one of which will come from the Wellcome Institute of Tropical Medicine in London. Further details, Mrs Christine Cherry, Department of Dermatology, the Slade Hospital, Headington, Oxford, OX3 7JH, England.
The highly effective antileprosy drug with anti-inflammatory properties

For the prevention\(^2\) and treatment\(^3\) of lepra reactions (ENL)

Suitable for use in combined regimens for the prevention and treatment of dapsone-resistance in lepromatous and dimorphous forms of leprosy\(^4\)

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<td>Indications: Lamprene, employed in combination with dapsone and rifampicin (&quot;Rimactane&quot;), serves as treatment for multibacillary forms of leprosy, such as lepromatous (LL), borderline lepromatous (BL), and mid-borderline (BB) leprosy, as well as erythema nodosum leprosum (ENL). Combined chemotherapy is necessary in order to prevent the emergence of resistant strains of M. leprae. <strong>Dosage:</strong> Adults (of approx. 60 kg body weight): for the treatment of multibacillary leprosy (LL, BL, BB) the WHO (World Health Organisation) recommends the following dosage schedule: Lamprene: 300 mg once a month under surveillance + 50 mg once a day as self-medication. Rifampicin: 600 mg once a month under surveillance. Dapsone: 100 mg once a day as self-medication. This threefold combination should be administered for at least 2 years and, whenever possible, until such time as the skin smears become negative. If the patient develops ENL, the treatment with dapsone and rifampicin should be continued as before, whereas the dosage of Lamprene should be raised to at the most 300 mg per day. These high daily doses must not be given for longer than 3 months. Children: Children should receive lower doses adapted to their body weight. Administration: The capsules should be taken at mealtimes or together with milk. Contra-indication: Known hypersensitivity to clofazimine. <strong>Precautions:</strong> Leprosy patients suffering repeatedly from abdominal pains and diarrhoea, as well as those with liver or kidney damage, should if possible not be treated with Lamprene. Treatment with daily doses of Lamprene exceeding 100 mg should not be continued for longer than 3 months, and during this time the patient should be kept under medical supervision. If gastro-intestinal symptoms develop during the treatment, the dosage should be reduced or the interval between doses prolonged. In the event of persistent diarrhoea or vomiting, the patient should be hospitalised. <strong>Pregnancy and lactation:</strong> As in the case of any form of drug therapy, Lamprene should be employed with caution during pregnancy, especially in the first 3 months. Clofazimine crosses the placental barrier and causes temporary discoloration of newborn infants. The active substance also passes into the breast milk. <strong>Unwanted effects:</strong> The following side effects have been observed: Reddish to dark-brown discoloration of the skin and of the leprosous lesions, particularly in pale-skinned patients at sites exposed to light. Discoloration of the hair, conjunctiva, cornea, and lacrimal fluid, as well as of sweat, sputum, urine, and faeces. This discoloration is reversible, although in the case of the skin it often does not disappear completely until some months after the cessation of treatment. Dryness of the skin, ichthyosis, pruritus, photosensitivity, acneform eruptions, and non-specific skin rashes. Nausea, vomiting, abdominal pains, diarrhoea, anorexia, loss of weight, and eosinophilic enteropathy. <strong>Storage:</strong> Protect from heat and moisture. Packs: 100 capsules of 50 mg or 100 mg. Further information is available on request.</td>
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