Editorial

DRUG COMPLIANCE IN THE TREATMENT OF LEPROSY*

Recent publications in this journal concerning the regularity with which leprosy patients self-administer their dapsone treatment\(^1\-^3\) and of improved techniques for monitoring dapsone compliance\(^4\-^7\) make this an appropriate time to review the findings of previous investigations concerning the regularity of dapsone self-administration and to discuss their implications for the strategy of leprosy treatment.

Investigations concerning the regularity of self-administration in the treatment of other diseases indicates that poor compliance is an extremely widespread phenomenon.\(^8\-^13\) Extensive studies of compliance of tuberculosis patients have been carried out in several Third World countries in situations where leprosy is also endemic using simple colorimetric urine-tests to monitor the ingestion of isoniazid.\(^14\-^19\) In two of these studies\(^17\, 18\) significant correlations were demonstrated between irregularity in drug self-administration, as indicated by the proportion of urine samples giving negative tests, and inadequate therapeutic response. Investigations in India\(^20\) and East Africa\(^21\-^23\) have also demonstrated that regimens that are highly effective when used in controlled clinical trials are often much less effective in routine use, primarily because patients do not continue with their treatment for an adequate length of time. Such evidence concerning the prevalence and therapeutic importance of poor compliance provided the impetus for initiating controlled clinical trials to investigate the efficacy of supervised intermittent regimens and of short-course chemotherapy in the treatment of pulmonary tuberculosis.\(^24\-^6\)

The dapsone/creatinine ratio method for monitoring the ingestion of dapsone

The most satisfactory method of monitoring patient compliance is by means of simple procedures to specifically detect the prescribed drugs or their metabolites


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in the urine. Since dapsone, and most other antileprosy drugs are prescribed for daily self-administration, a highly satisfactory urine-test procedure would be one that gave reliably positive results for up to 12–15 h after the ingestion of a standard daily dose of the drug and consistently negative results within 48 h. With such a test, a negative result would indicate unequivocally that a patient has omitted to ingest at least one of his or her prescribed doses.

Two factors frustrate efforts to devise such a simple test to monitor dapsone self-administration. Firstly, dapsone and its metabolites are eliminated very slowly in the urine at rates that parallel the decline in dapsone serum concentrations. This decline is equivalent on average to a half-life of about 27 h. Secondly, colorimetric procedures for detecting dapsone are unspecific. Thus the most satisfactory colorimetric method for estimating dapsone, in which its aromatic amino groups are diazotized and coupled with N-1-naphthyl-ethylene-diamine, gives colours with a wide range of other compounds containing aromatic amino groups.

For these reasons qualitative urine-tests give positive results for several days after the ingestion of a single therapeutic dose of dapsone and attempts to read tests in such a way as to avoid classifying concentrated dapsone-free urine samples with substantial amounts of natural diazotisable compounds as positive are liable to result in categorizing dilute dapsone-containing samples as negative. The effects of diuresis can, however, be largely overcome by quantitatively determining the ratio of the concentration of dapsone plus its diazotisable metabolites to creatinine in the urine using modifications of the Bratton and Marshall and alkaline picrate colorimetric methods, respectively. Urine samples can be conveniently preserved by the addition of hydrochloric acid or thymol and do not need to be refrigerated.

Studies of dapsone compliance using this method have been reported from Malawi, Ethiopia, India, Burma, Tanzania and Kenya. Most outpatients are prescribed dapsone for daily self-administration. Estimates of their compliance were obtained by comparing the dapsone/creatinine ratios of urine samples collected during their visit to a rural, urban or hospital clinic with those of samples from supervised and non-treated controls, respectively. The samples from the supervised controls were generally collected from hospitalized patients 24 h after they had swallowed at least 4 consecutive supervised daily doses of dapsone of the same denomination as that given to the outpatients. The results of such investigations are summarized in Table 1 and indicate that irregular self-administration of dapsone is very widespread. In every study the dapsone/creatinine ratios of urine samples collected from the outpatients were significantly less than those from the supervised controls indicating that substantial proportions of the dapsone treatment prescribed for and collected by the outpatients were not being ingested. The overall level of self-medication as indicated by the mean dapsone/creatinine ratio of urine samples collected from outpatients could be interpreted in two ways. Firstly, if it is assumed that
the pattern of dapsone ingestion immediately prior to collection of the urine samples is typical of the patients' general compliance, the approximate percentage of prescribed dapsone doses being ingested can be calculated.\textsuperscript{30} Such estimates calculated from the results obtained in investigations carried out in Malawi, Ethiopia, India and Burma are summarized in Table 1. Alternatively, the data can be analysed to provide an approximate estimate of the average length of time since patients had swallowed their last dose of dapsone.\textsuperscript{1}

**Table 1. Investigations of compliance using the dapsone/creatinine ratio method among outpatients prescribed dapsone for daily self-administration**

<table>
<thead>
<tr>
<th>Investigation</th>
<th>Clinic*</th>
<th>Number patients</th>
<th>Doses taken** (%)</th>
<th>Grossly irregular patients** (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blantyre, Malawi (1974)</td>
<td>R</td>
<td>164</td>
<td>53</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Addis Ababa, Ethiopia (1974)</td>
<td>U</td>
<td>89</td>
<td>42</td>
<td>11</td>
<td>31</td>
</tr>
<tr>
<td>Gudiyatham Taluk, India (1976)</td>
<td>R</td>
<td>100</td>
<td>87</td>
<td>11</td>
<td>32</td>
</tr>
<tr>
<td>Chinglepattu, India (1977)</td>
<td>R</td>
<td>90</td>
<td>59</td>
<td>-</td>
<td>33</td>
</tr>
<tr>
<td>Mandalay and Rangoon, Burma (1979)</td>
<td>U and R</td>
<td>455</td>
<td>24</td>
<td>56</td>
<td>34</td>
</tr>
<tr>
<td>Bombay, India (1979)</td>
<td>U</td>
<td>260</td>
<td>-</td>
<td>27</td>
<td>35</td>
</tr>
<tr>
<td>Gudiyatham Taluk, India (1981)</td>
<td>R</td>
<td>125</td>
<td>60</td>
<td>37</td>
<td>1</td>
</tr>
<tr>
<td>Mazuffarpur, India (1981)</td>
<td>R</td>
<td>44</td>
<td>-</td>
<td>25</td>
<td>2</td>
</tr>
<tr>
<td>Dichpalli, India (1981)</td>
<td>R</td>
<td>55</td>
<td>34</td>
<td>47</td>
<td>36</td>
</tr>
</tbody>
</table>

* R = Rural, U = Urban, H = Hospital.

**For definitions see text.

The dapsone/creatinine ratios of many patients were clearly much less than those of the supervised controls and various definitions have been proposed for categorizing patients as being either 'irregular' or 'grossly irregular' in their dapsone self-administration. The proportions of patients classified as grossly irregular in Table 1 are those with apparent dapsone/creatinine ratios within the range found among samples from control subjects not receiving dapsone and varied from 11–56% of those studied. Such grossly irregular patients had probably not swallowed a tablet of dapsone for at least 4 days prior to the collection of the test urine sample. The question as to whether urine samples that have been collected from patients on the occasion of a visit to a clinic to replenish their stock of dapsone tablets are likely to be representative has been discussed previously.\textsuperscript{30} Interestingly, in the one study where urine samples were collected by means of both surprise home visits and during routine clinic attendances, significant differences were not encountered between the results from the two sets of samples.\textsuperscript{1}
Therapeutic implications of poor dapsone compliance

Substantial failure to self-administer prescribed dapsone-treatment results not only from patients failing to ingest the tablets that they have collected but also because a substantial proportion of patients default from treatment altogether.39, 40 Thus a recent study from South India has shown that after their first year's treatment many lepromatous patients become increasingly irregular in collecting their drug supplies and in self-administering dapsone and that such poor compliance may account for the failure of many patients to achieve clinical and bacteriological quiescence despite prolonged periods of dapsone monotherapy.36 However, because a single dose of 100 mg dapsone results in serum and tissue drug concentrations that are approximately 500-fold in excess of its minimal inhibitory concentration against *Mycobacterium leprae* and since the relatively long half-life of dapsone is such that inhibitory concentrations of the drug are probably maintained for about 10 days,28 it is clear that the failure to self-administer dapsone must be extremely prolonged before it results in clinical and bacteriological relapse caused by the renewed multiplication of fully dapsone-sensitive *M. leprae*. Such a conclusion is strengthened by the observation that over the short term the response of a small group of patients treated with a dose of 1 mg dapsone/day was indistinguishable from that routinely observed with standard 50 or 100 mg daily doses.41

The most important therapeutic penalty of poor dapsone compliance is likely to be an enhanced risk that lepromatous patients will relapse after many years of treatment due to the emergence of dapsone-resistant strains of *M. leprae*. It is extremely difficult to prove an association between poor compliance and relapse with dapsone-resistant leprosy many years later because records of out-patient treatment attendance are rarely well documented over long periods and urine-testing to monitor dapsone ingestion has only been introduced relatively recently. Nevertheless, Shepard and his colleagues obtained strongly suggestive evidence indicating that irregular treatment with dapsone is likely to be a major factor encouraging the development of dapsone resistance,42 while Jacobson reached a similar conclusion from his review of the case histories of over 75 patients with dapsone-resistant leprosy found over a 12-year period at the Carville United States Public Health Service Hospital.43 Other evidence for the importance of maintaining high concentrations of dapsone to prevent the subsequent emergence of dapsone resistance comes from studies concerning the epidemiology of dapsone resistance in Malaysia and Ethiopia. In the Malaysian investigation it was found that lepromatous inpatients whose treatment was initiated with injections of solapsone (equivalent to approximately 10 mg dapsone/day) were three times more likely to relapse with dapsone-resistant leprosy than those whose treatment started with dapsone in full dosage.44 In Ethiopia, where standard treatment during the period 1965–74 for outpatients consisted of weekly doses of
Table 2. Decline in the antileprosy activity of a 100 mg dapsone dose with time

<table>
<thead>
<tr>
<th>Days</th>
<th>Dapsone serum concentration (% initial) (µg/ml)</th>
<th>Inhibition M. leprae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S*</td>
</tr>
<tr>
<td>2</td>
<td>29</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>8.5</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>4.5</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>2.5</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>1.5</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>0.2</td>
<td>±</td>
</tr>
</tbody>
</table>

* Fully drug-sensitive.
**Resistant to 0.0001% dapsone in mouse diet.
† Resistant to 0.001% dapsone in mouse diet.

5 or 10 mg dapsone that were gradually increased to a maximum of 200–300 mg over 6–9 months,\textsuperscript{45} the incidence of acquired dapsone resistance was alarmingly high (about 3% per annum) and resistance to dapsone developed more rapidly than in Malaysia.\textsuperscript{46} Standard doses of 50 or 100 mg dapsone taken regularly each day should not only completely prevent the growth of fully sensitive organisms but also that of mutants with low degrees of dapsone resistance,\textsuperscript{47} while substantial interruption of treatment will permit such mutants to multiply. Repeated interruptions of treatment over many years can therefore be expected to result in the step-wise selection of mutants with increasing levels of dapsone resistance until organisms are completely insusceptible to inhibition by the highest tolerated dapsone doses. The bacteriological and pharmacological basis for such selection is set out in Table 2. In constructing this table it has been assumed that a single dose of 100 mg dapsone results in peak serum concentration of about 1.5 µg/ml, that serum and tissue dapsone concentrations then decline at a rate equivalent to a half-life of 27 h and that the minimal inhibitory concentration of dapsone against fully sensitive strains of M. leprae is about 0.003 µg/ml. The evidence for these assumptions and for the excellent tissue penetration of dapsone have been reviewed elsewhere.\textsuperscript{28, 48} It will thus be apparent that if patients are prescribed 100 mg dapsone for daily self-administration, mutants with low degrees of resistance (minimal inhibitory concentration of about 0.03 µg/ml) might start to multiply after the omission of about 6 consecutive daily doses.

More sensitive methods for monitoring dapsone compliance

While the dapsone/creatinine ratio method has demonstrated the ubiquity of the failure of patients to take their prescribed dapsone treatment, the lack of
specificity of the Bratton and Marshall procedure for detecting dapsone and its metabolites, exemplified by the significant blank values of urine samples from untreated subjects, results in the method being insufficiently sensitive to identify omissions of more than about 4 days treatment. However, as indicated by the data set out in Table 2, it is probable that significant therapeutic penalties are likely to be incurred in the long run only when gaps in dapsone self-administration of 6 days or more occur. By this time dapsone serum and tissue concentrations and urinary excretion will have fallen to about 1–2% of that encountered if the prescribed treatment were being regularly ingested. There is therefore a need for more sensitive and specific assay methods to detect dapsone and its metabolites in the urine.

After a single dose of 100 mg dapsone, urinary concentrations of dapsone in excess of about 0.02 μg/ml are maintained for 7 days. High pressure liquid chromatographic or thin layer chromatographic techniques of this order of sensitivity have been developed by several groups of workers but none of these methods is simple enough for ready application to comprehensive studies of dapsone compliance. It is for this reason that the pioneering investigations of Huikeshoven and his colleagues to develop simple enzyme-immunoassays of great sensitivity and specificity for detecting dapsone and its metabolites in body fluids are of such promise.

In the first of their two ‘ELISA’ (Enzyme-linked immunosorbent assay) methods, aliquots of test urine samples and a solution containing a dapsone-specific immunoglobulin coupled to horseradish peroxidase are pipetted into the wells of a microtitre plate coated with a bovine serum albumin dapsone conjugate. After a washing step, the bound peroxidase is visualized using hydrogen peroxidase and 5-amino-salicylic acid, the amount of colour being inversely related to the concentration of dapsone and its metabolites in the test sample. When read by eye the method was capable of detecting concentrations of down to about 0.01 μg/ml dapsone in urine and gave reliably positive results when applied to urine samples collected from 9 of 10 volunteers for 8 days after the ingestion of single doses of 100 mg dapsone.

The second method described on page 125 of this issue, was approximately ten times more sensitive. In this method competition occurs between dapsone and a horseradish peroxidase dapsone conjugate for binding with antibody absorbed onto the microtitre well. When this method was applied to the detection of dapsone in finger prick blood from two volunteers after the ingestion of single 100 mg dapsone doses, consistently positive results were obtained for 5–6 days. Although such a finger prick blood method avoids the complication to the interpretation of urine assays caused by the effects of diuresis, it is likely to be considerably less sensitive since the concentrations of dapsone and its metabolites in the serum are much lower than those in the urine. Such tests are probably also less acceptable to patients. As pointed out by Rook in his letter on page 281, further increases in the sensitivity of these
enzyme-immunoassays are to be anticipated if the peroxidase substrate used in these investigations, 5-amino-salicylic acid, were replaced by the more recently discovered ABTS reagent (2,2'-azino-di-(3-ethyl benzthiazoline-6-sulphonic acid)). The combination of 3-(dimethylamino) benzoic acid and 3-methyl-2-benzo-thiazolone hydrazone hydrochloride monohydrate (MBTH) described by Ngo and Lenhoff might also be investigated.

Improved enzyme immunoassay methods could be used to identify those outpatients whose dapsone compliance was so poor that dapsone urinary concentrations (or dapsone/creatinine ratios to allow for diuresis) were less than 1–2% of those among samples collected from supervised controls receiving daily doses of 100 mg dapsone. Such exceedingly irregular patients, who, as will be seen from Table 2, would be most at risk of relapsing on account of the selection of dapsone-resistant strains of M. leprae, could then have their treatment specifically supplemented with acedapsone injections.

Monitoring the ingestion of other antileprosy drugs

The increasing prevalence of dapsone-resistant strains of M. leprae among lepromatous patients that has arisen as a result of past treatment with dapsone monotherapy has emphasized the importance of treating all new lepromatous patients with combinations of antileprosy drugs. The most promising drugs for use in combination with dapsone or for the treatment of patients with dapsone-resistant leprosy are rifampicin, clofazimine, ethionamide, prothionamide and thiacetazone. The regularity with which such companion drugs are taken is likely to be a major factor in determining their potential value in preventing the emergence of dapsone resistance. An experimental investigation in the mouse foot-pad model has indicated that poor compliance would probably severely impair the efficacy of thiacetazone, since it is an inherently weak bacteriostatic drug, and would compromise the bactericidal activities of ethionamide and prothionamide.

The taking of a standard 600 mg dose of rifampicin can often be detected simply from the characteristic orange/brown colour that it imparts to urine samples collected within 6–8 h of ingestion. Rifampicin can, however, be reliably demonstrated in the urine for at least 12 h using a simple plate diffusion test with Staphylococcus aureus, while an alternative microbiological procedure of far greater sensitivity has also been described.

No method has as yet been described for monitoring the ingestion of clofazimine. Among light-skinned patients the degree of skin pigmentation may provide an indication of the extent to which the drug is being taken, but it is in just such patients that the clofazimine is likely to be least acceptable. A urine-test method for detecting the ingestion of ethionamide has been described by Eidus and Harnanansingh. This method, which depends on the extraction and
concentration of its yellowish sulphoxide metabolite, is also applicable to detecting the ingestion of prothionamide and gives reliably positive results for at least 12 h after the ingestion of 250 mg doses of either drug.\textsuperscript{63, 64}

No satisfactory colorimetric procedure exists for monitoring the ingestion of thiacetazone, but its excretion in the urine can be detected by extracting into chloroform/amyl alcohol and scanning the ultraviolet absorption spectrum of the extract to detect an absorption peak at about 333 m\textmu.\textsuperscript{65} Reliably positive results may be anticipated for about 36–48 h after dosage with 150 mg of the drug.

Combined formulations of rifampicin plus isoniazid, ethionamide plus isoniazid, prothionamide plus isoniazid and thiacetazone plus isoniazid are commercially available for use in the treatment of tuberculosis while a combination of dapsone plus prothionamide plus isoniazid has been developed for use with rifampicin in the treatment of both leprosy and tuberculosis. The use of such isoniazid-containing combinations could considerably facilitate compliance studies concerning the ingestion of these drugs since their ingestion can be readily detected using colorimetric procedures to detect the presence of metabolites of isoniazid in the urine.

The Eidus and Hamilton procedure\textsuperscript{66} for detecting acetylisoniazid is extremely simple. Twelve urine samples can be reacted at a time using a white procelain plate with hemispherical depressions with the dropwise addition of aqueous potassium cyanide and chloramine-T. A positive result is indicated by the appearance of a pink/red colour within 5 min. Urine samples collected after a 300 mg dose of isoniazid, the size of the isoniazid component of most of these combined formulations, would give reliably positive results for about 12 h and be uniformly negative after 48 h.\textsuperscript{64, 67, 68}

The urine-test method for monitoring isoniazid ingestion based on detecting the metabolites isonicotinic acid and isonicotinylglycine\textsuperscript{69} is slightly more elaborate involving the addition of pH 5 acetate buffer and the reagents potassium cyanide, chloramine-T and barbituric acid. A positive result is indicated by the formation of a blue/purple colour within 15–30 min. This method is much more sensitive than the acetylisoniazid procedure and gives reliably positive results for about 48 h after the ingestion of 300 mg isoniazid.\textsuperscript{64} Such an approach was used to monitor the self-administration of thiacetazone in the Ethiopian compliance study reported on page 147.

Such standard therapeutic doses of isoniazid are very well tolerated so that the use of such combined formulations for relatively limited compliance studies poses no unacceptable hazard to the patients. The sensitivity of the isonicotinic acid method for detecting isoniazid ingestion is however so great that it is possible to use minute doses of isoniazid as an innocuous marker for monitoring the self-administration of medicaments for daily self-administration since over 99\% of the urine samples collected within 18 h of ingesting 6 mg isoniazid gave positive results.\textsuperscript{70} Such doses of isoniazid have been incorporated into capsules
or tablets containing dapsone, thiacetazone, ethionamide or prothionamide in preparation for future studies concerning the regularity of their self-administration by leprosy patients.

Supplementing daily self-administered dapsone with supervised intermittent treatment of other drugs

Just as the demonstration of irregular drug self-administration in the treatment of tuberculosis prompted the introduction of supervised chemotherapy, the evidence of poor dapsone compliance in leprosy illustrated by the investigations summarized in Table 1 has encouraged the advocacy of supplementing oral daily doses of dapsone with injections of 225 mg acedapsone given once every 3 months. Such injections, which release on average about 2 mg dapsone into the circulation each day and maintain dapsone serum concentrations in excess of about 0.03 μg/ml would prevent the multiplication of both fully sensitive *Mycobacterium leprae* and mutants with low degrees of dapsone resistance and would significantly augment the dapsone levels of patients whose compliance as defined above was exceedingly irregular.

In view of the extremely rapid and powerful bactericidal activity of rifampicin against *M. leprae* and its high cost, the most advantageous way to supplement self-administered daily dapsone treatment would probably be to give single 600 mg doses of the drug once-monthly to outpatients on the occasion of a clinic visit to collect their stock of dapsone tablets. Supervised monthly doses of clofazimine might be given in a similar manner. However experimental studies indicate that ethionamide, prothionamide and thiacetazone are probably unsuitable drugs for intermittent administration.

G A ELLARD

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Ellard GA, Unpublished results.


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ELISA inhibition technique for the demonstration of sulphones in body fluids

Comparison of two ELISA methods*

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Received for publication 24 October 1980

Summary A new enzyme-linked immunosorbent assay (ELISA) for sulphones is described. The main tool is a dapsone-enzyme conjugate (E-DDS). The technique is compared with the one described earlier, in which the main tool is a specific antibody-enzyme conjugate (E-Ig). The E-DDS-based ELISA is 50% inhibited by as little as 4 ng DDS/ml, i.e. it is 7.5 times more sensitive for DDS than the E-Ig-based ELISA. In both ELISA's other sulphones cross-react with DDS, although the patterns are different. Cross-reactions with sulphone analogues, such as sulphonamides, do not occur. The sensitivity of the new ELISA is not reduced when E-DDS is lyophilized. A possible explanation for the difference in sensitivity of the two ELISA's is given, and the practical applicability of the new technique is discussed.

Introduction

In two previous papers1,2 we described the development of a simple enzyme-linked immunosorbent assay (ELISA) for the demonstration of sulphones in body fluids. The main tool of this inhibition technique is a sulphones-specific antibody-enzyme conjugate (E-Ig). Because of its high sensitivity it can be applied to detect substantial failure in dapsone (DDS) self-administration by leprosy patients (unpublished data).

Recently, basic alterations in the method led to a second ELISA for sulphones, even more sensitive than the first one. The main tool of this new inhibition technique is a DDS-enzyme conjugate (E-DDS). The present paper describes the new ELISA and compares it with the one reported earlier.

*This investigation received support from the Chemotherapy of Leprosy (THELEP) component of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, from the Netherlands Leprosy Relief Association (Nederlandse Stichting voor Leprabestrijding), and from the Italian Leprosy Relief Association (Amici dei Lebbrosi).
Materials and Methods

CONJUGATION OF DDS AND PEROXIDASE

Horseradish peroxidase (10 mg) was conjugated to DDS according to the method of Nakane and Kawaoi. In the second step a saturated solution of DDS in PBS was used. Unconjugated DDS was removed by repeated ultrafiltration, until no DDS could be detected in the PBS filtrate. The concentrated conjugate (E-DDS) was stored at 4°C in a final volume of 2.5 ml.

E-DDS-BASED ELISA INHIBITION TECHNIQUE

Each well of a microtitre tray was incubated with a solution of 2.4 µg sulphones specific Ig in 100 µl carbonate buffer of pH 9.6 (2 h, 56°C). The tray was washed with PBS/Tween as described. Then 50 µl aliquots of specified solutions of sulphones or analogues in normal horse serum containing 0.05% Tween 20 were added to the wells, followed by 50 µl aliquots of a 4 x 10^{-3} dilution of the E-DDS concentrate in PBS/Tween containing 5% normal horse serum (PBS/Tween/Serum). After incubation (30 min, 56°C) the tray was washed and a 5AS/H₂O₂ solution was added as described. However, the reaction was not stopped by addition of NaOH, and readings were done after 2 h using a Titertek Multiskan (Flow Laboratories) at 492 nm (O.D. 492).

E-Ig-BASED ELISA INHIBITION TECHNIQUE

Starting from a coating with BSA-DDS as described, this ELISA was set up in 3 modifications:

(a) using unconjugated sulphones specific Ig, followed by horseradish-peroxidase-conjugated anti-rabbit IgG antiserum in an additional incubation step;
(b) using freshly prepared sulphones specific E-Ig;
(c) using lyophilized sulphones specific E-Ig.

In all modifications 50 µl- aliquots of serial dilutions of a DDS solution were added to the wells as described, using PBS/Tween/Serum as diluent. (For this ELISA 5% serum addition to PBS/Tween gave optimal inhibitions, whereas the sensitivity of the other ELISA appeared to be optimal when 100% serum was used as a solvent for the inhibiting compounds.) Readings were made after 2 h at 492 nm as above.

PRINCIPLES

Figure 1 shows the principles of the 2 ELISA inhibition techniques.
ELISA inhibition technique

Figure 1. Two ELISA inhibition techniques. In both the attachment of the enzyme-conjugate is inhibited by free molecules of sulphones. Symbols: = sulphones specific Ig; O = sulphones; = E-DDS; = DDS conjugated to BSA ( ); = E-Ig.

Figure 2. Dose-response curve for sulphones specific Ig coating in ELISA. From the curve an Ig quantity of 2.4μg per well (x) was chosen for coating in this ELISA.

Results

In preliminary experiments the sulphones specific Ig coating gave an optimal ELISA response, if Ig quantities of 1–10μg/well were used (Fig. 2). Coating in all further experiments with the new ELISA was done using 2.4μg/well. Figure 3 is a dose-response curve of E-DDS in ELISA without inhibition. From this curve an E-DDS dilution of 4×10^{-3} was chosen for the inhibition tests with sulphones and analogues.

The inhibition of the E-DDS ELISA by DDS is illustrated in Fig. 4, together with similar inhibition curves for the E-Ig ELISA. Based on these curves, Table 1 lists the sensitivities of the ELISA’s for DDS, expressed in ng DDS/ml and ng DDS/well used at 50% response. The E-DDS ELISA appears to be about 7.5 times more sensitive for DDS than the E-Ig ELISA when fresh conjugate is used. The new ELISA maintains its high sensitivity after lyophilization of the conjugate, whilst the sensitivity of the old one is 1.7 times reduced by a similar manipulation.
Figure 3 Dose-response curve for E-DDS in ELISA. From the curve an E-DDS dilution factor of $4 \times 10^{-3}$ (x) was chosen for the inhibition tests.

Figure 4 ELISA inhibition by DDS. ○, E-DDS-based ELISA (fresh and lyophilized E-DDS gave identical curves); ●, E-Ig-based ELISA using fresh conjugate (the double antibody modification gave an identical curve); ◼, E-Ig-based ELISA using lyophilized conjugate.

**Table 1.** Sensitivities of ELISA's for DDS

<table>
<thead>
<tr>
<th>ELISA</th>
<th>50% response</th>
</tr>
</thead>
<tbody>
<tr>
<td>ng DDS/ml</td>
<td>ng DDS/well</td>
</tr>
<tr>
<td>E-DDS fresh</td>
<td>4</td>
</tr>
<tr>
<td>E-DDS lyophilized</td>
<td>4</td>
</tr>
<tr>
<td>Double antibody</td>
<td>30</td>
</tr>
<tr>
<td>E-Ig fresh</td>
<td>30</td>
</tr>
<tr>
<td>E-Ig lyophilized</td>
<td>50</td>
</tr>
</tbody>
</table>

Results are taken from the inhibition curves illustrated in Figure 4.
Table 2. Relative sensitivities of ELISA's for sulphones and analogues

<table>
<thead>
<tr>
<th>Compound</th>
<th>cross-reaction (%)</th>
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<tbody>
<tr>
<td></td>
<td>E-DDS</td>
</tr>
<tr>
<td>1. H₂N–○–SO₂–○–NH₂ (DDS)</td>
<td>100</td>
</tr>
<tr>
<td>2. CH₃CONH–○–SO₂–○–NHCOCH₃ (DADDS)</td>
<td>24</td>
</tr>
<tr>
<td>3. H₂N–○–SO₂–○–NHCOCH₃ (MADDS)</td>
<td>7</td>
</tr>
<tr>
<td>4. H₂N–○–SO₂–○–NH₂SO₃K</td>
<td>3</td>
</tr>
<tr>
<td>5. H₂N–○–SO₂–○–NO₂</td>
<td>121</td>
</tr>
<tr>
<td>6. H₂N–○–SO₂–○–NHOH</td>
<td>43</td>
</tr>
<tr>
<td>7. H₂N–○–SO₂–○–</td>
<td>31</td>
</tr>
<tr>
<td>8. ○–SO₂–○ (Diphenylsulphone)</td>
<td>0.8</td>
</tr>
<tr>
<td>9. H₂N–○–S–○–NH₂</td>
<td>0.9</td>
</tr>
<tr>
<td>10. H₂N–○–SO₂NH–○ (Sulphadiazine)</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>11. H₂N–○–COOH</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

The figures indicate the amounts (molarity) needed to reduce the ELISA response to 50% relative to these figures for DDS (= 100% cross-reaction). The figures for the E-Ig-based ELISA are taken from a previous paper.¹

Table 2 gives the sensitivities of the 2 ELISA’s for other sulphones and analogues, relative to the sensitivity of the respective ELISA for DDS. There is a significant difference between the 2 ELISA’s as regards the sensitivity for DDS itself relative to that for the other sulphones. Whereas the E-Ig conjugate has a higher affinity for most other sulphones tested, only one other sulphone inhibits the E-DDS-based ELISA more than DDS. An important characteristic of both systems is that there is no cross-reaction with sulphonamides.

Discussion

From the few reports about ELISA’s on drugs both inhibition techniques might be expected to have equally high sensitivities.⁶ ⁷ It is only partly understood why the modified ELISA for sulphones is more sensitive than the initial one. An explanation for this may be that in the modified method the competition for the antibody is between free sulphones and a conjugate (E-DDS) in which the bridge attaching DDS is different from the one used in the conjugate against which the antibodies were raised. In the initial method the bridges are
homologous. In 1975 Van Weemen and Schuurs\textsuperscript{8} reported on the advantage of heterologous combinations in enzyme-immunoassays. However, introduction of a heterologous bridge to BSA-DDS, with which the free sulphones have to compete in the initial method, has not yet been shown to increase the sensitivity.

Differences in patterns of cross-reactions (Table 2) might be explained in a similar way. What is important is that for the qualitative demonstration of sulphones in body fluids both patterns of cross-reactions are equally acceptable.

The E-DDS ELISA is 7.5 times more sensitive for DDS than the E-Ig ELISA, and 12.5 times more sensitive if lyophilized materials are used. Although there is no need to improve the sensitivity of the initial ELISA urine test (unpublished data),\textsuperscript{2} higher sensitivity means a greater flexibility and a wider applicability to other body fluids.

The only apparent disadvantage of the modified ELISA is that the incubation times are longer. Coating and E-DDS incubation at room temperature require respectively 24 and 4 h. Production costs and shelf life of the reagents of both methods are about the same.

References


8 Van Weemen BK, Schuurs AHWM. The influence of heterologous combinations of antisum and enzyme-labeled estrogen on the characteristics of estrogen enzyme-immunoassays. \textit{Immunochim}, 1975, 12, 667–70.
ELISA inhibition technique for the demonstration of sulphones in body fluids

The use of dried blood on filter paper to monitor leprosy patient compliance*

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Received for publication 24 October 1980

Summary Two enzyme-linked immunosorbent assays (ELISA) for sulphones in body fluids were adapted to measure sulphones in blood dried on filter paper. The more sensitive modification, based on competition between dapsone (DDS) and enzyme-dapsone conjugate, detects sulphone in blood extracts up till 6 days following 100 mg DDS intake. Application to monitor patient compliance is demonstrated, using finger-prick blood from 30 Ethiopian leprosy patients. Results are compared to those in urine, and to statements as regards the last daily dose of 100 mg DDS. Eight negative results were found, and employing serial dilutions of positive controls, this indicated that omissions of more than 5 doses in succession occurred. Practical aspects of the technique are discussed.

Introduction

In previous papers1-2 we introduced an enzyme-linked immunosorbent assay (ELISA) on urines from leprosy patients, to monitor self-medication with dapsone (DDS). The test proved its potential value in Nigeria and Cameroon as a simple and sensitive tool for laboratories using less sophisticated equipment (unpublished data).

However, simple qualitative urine tests are inevitably influenced by diuresis.3

*This investigation received support from the Chemotherapy of Leprosy (THELEP) component of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, from the Netherlands Leprosy Relief Association (Nederlandse Stichting voor Leprabestrijding), and from the Italian Leprosy Relief Association (Amici dei Lebbrosi).
Moreover, in some areas female patients often feel shy to produce a urine sample on request of the leprosy officer. We therefore considered the use of minute quantities of blood as an alternative to urine. It would simplify procedures for field work, if such samples could be collected as spots on filter paper.\textsuperscript{4-5} In preliminary experiments it was found that DDS concentrations in extracts from dried blood spots obtained from volunteers taking standard DDS doses were just high enough to inhibit the ELISA presently used on urine (unpublished results). Apparently, the blood spot ELISA worked, but it was feared that under changing circumstances and in the hands of less experienced workers the detection limit for DDS might be too critical for practical work. Moreover, substantial failure in DDS self-administration would not be distinguished from the occasionally missed dose by this ELISA. Recently, the feasibility of the idea was enhanced by the development of an even more sensitive modification of the ELISA for sulphones.\textsuperscript{6}

The present paper firstly describes the possibilities of the two ELISA's to demonstrate sulphones in dried blood samples from volunteers. Then, an application of the most sensitive test is described, using blood spots on filter paper obtained from leprosy patients in Ethiopia.

Materials and methods

Blood Samples from Volunteers

Each of two healthy volunteers took a single dose of 100 mg DDS. Blood was taken by finger prick (4 × 50 µl) and by venapuncture (5 ml in a heparinized tube, and 5 ml in a tube without heparin), before and 1, 2, 3, 4, 5 and 6 days after the DDS intake. The finger-prick blood was collected in 50 µl heparinized capillary tubes that were emptied on small areas (1.5 cm diameter) of filter paper (Whatman 3). From each venous blood sample four 50 µl aliquots were immediately pipetted (Finn pipette) on similar small areas of filter paper. All filter paper samples were dried in air and stored at room temperature until analysed.

Samples from Patients and Controls

One 50 µl blood sample was collected from each of 30 outpatients of the All Africa Leprosy and Rehabilitation Training Centre (ALERT) at Addis Ababa. They belonged to a group of patients who received special attention for a variety of reasons such as suspected non-compliance with self-medication. The samples were taken when they visited ALERT periodically to collect 4 weekly supplies of 100 mg DDS tablets for daily self-administration. The blood was collected from finger pricks using 50 µl heparinized capillary test tubes, that
were emptied on small areas of filter paper. The papers were dried in air and stored at room temperature. Also, urine samples were collected from the patients and 5 ml aliquots were stored at room temperature after addition of a few grains of thymol. The patients were questioned about the last date of 100 mg DDS intake.

Positive control samples were collected from 6 inpatients of ALERT who took their daily doses of 100 mg DDS under strict supervision. The samples were collected just before a new dose was due to be taken. Blank control samples were collected from each of 6 healthy volunteers who were not taking DDS.

FILTER PAPER EXTRACTION

Each dried blood spot was cut out, slightly folded and dropped into a test tube (1.5 cm diameter). To each tube 500 µl phosphate buffered saline containing 0.05% Tween 20 (PBS/Tween) was added. The tubes were covered with parafilm and left overnight at 4°C. They were slightly shaken before use.

E-Ig BASED ELISA INHIBITION TECHNIQUE

This ELISA was done with freshly prepared sulphones specific E-Ig as described in the previous paper, replacing the 50 µl aliquots of specified solutions of sulphones or analogues by 50 µl aliquots of filter paper blood extracts, and using an E-Ig dilution factor of $7 \times 10^{-3}$. The urine samples, however, were tested with the lyophilized ELISA reagents as previously described, unpublished data.2

E-DDS BASED ELISA INHIBITION TECHNIQUE

This ELISA also was done as described in the previous paper, replacing the 50 µl aliquots of specified solutions of sulphones or analogues by 25 µl aliquots or normal horse serum containing 0.05% Tween 20 plus 25 µl aliquots of filter paper blood extracts, and using an E-DDS solution factor of $1.7 \times 10^{-3}$.

Results

The possible use of the two ELISA's to detect sulphones in different types of filter paper blood extracts was tested on blood samples from two volunteers who took single oral doses of 100 mg DDS. Figure 1 clearly shows that the E-DDS based technique is the most sensitive, which agrees with findings in the previous paper.6 For an analysis of the results, the multi-scanner ELISA readings are listed in Table 1. The data may be summarized as follows:

1. The E-Ig based ELISA is 90–100% inhibited by filter paper blood extracts obtained 1 day after a single dose of 100 mg DDS, and it is about 50–60%
Figure 1. Two ELISA's applied to dried blood extracts from two volunteers who took single doses of 100 mg DDS. Top: E-Ig based ELISA; bottom: E-DDS based ELISA. Even rows are duplicates of foregoing odd rows.

Methods of blood collection: by venapuncture in a tube without heparin (rows 1, 2, 7, 8), by venapuncture in a heparinized tube (rows 3, 4, 9, 10), or by finger prick in a heparinized capillary tube (5, 6, 11, 12).

Times of blood collection: 1(A), 2(B), 3(C), 4(D), 5(E) and 6(F) days after 100 mg DDS doses, and before the DDS intake (G). Lines H show ELISA's on the extraction buffer for control.

inhibited by such extracts obtained 3 days after that dose.

2. The E-DDS based ELISA is 100% inhibited by filter paper blood extracts obtained 1, 2 or even 3 days after a single dose of 100 mg DDS, and it is about 50–60% inhibited by such extracts obtained 5 or even 6 days after that dose.
### Table 1. Multi-scanner readings of two ELISA’s applied to dried blood extracts from two volunteers who took single doses of 100 mg DDS

<table>
<thead>
<tr>
<th>ELISA (basis)</th>
<th>Time (days)</th>
<th>Volunteer A</th>
<th>Volunteer B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>VB*</td>
<td>VBH*</td>
</tr>
<tr>
<td>E-Ig</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>7</td>
<td>7_1</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>E-DDS</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

The numbers are averages of duplicate results. The scanner was set on the optical densities resulting from the blank samples (Figure 1, lines G and H), thus printing these with the matrix numbers 8 or 9.

*VB = venous blood without heparin; VBH = venous blood with heparin; FPH = finger-prick blood with heparin.

3. Heparin does not influence the ELISA’s, but the finger-prick blood samples apparently contain less sulphones than the samples obtained by venipuncture. However, this difference in contents is less than that found between samples obtained on two successive days from the same subject.

These results correspond with preliminary experiments on blood spots obtained from 6 volunteers up to 10 days after taking single doses of 100 mg DDS (unpublished data).

Because of its greater sensitivity, the E-DDS based ELISA was chosen for testing blood spots obtained from 30 Ethiopian leprosy patients. The control samples from the 6 inpatients who took their daily doses of 100 mg DDS under strict supervision, were initially analysed individually and undiluted, and subsequently in serial twofold dilutions after pooling of the extracts. Comparison of test samples with this type of control series gives an indication of what the average negative or positive result implies, basing calculations conveniently on a $T_{1/2}$ (half-life) for DDS of one day. Figure 2 is a photograph of the ELISA on these blood spots. Table 2 presents the multiscanner readings. The positive control series indicates that test samples collected up to 4 days after a last daily dose of 100 mg DDS may result in scanner readings lower than 2. Readings of 2—6 are to be expected if the last dose was taken 5—6 days prior to the sampling, and readings of 7—9 indicate that the last dose was taken longer than 6 days ago.

Apart from this ELISA on blood spots which was done in Amsterdam, also
Figure 2. ELISA on blood spots from 30 Ethiopian leprosy patients and controls. All samples are in duplicate. A, 1–12, are 6 positive control samples; B, 1–12, are twofold serial dilutions (in horse serum with 0.05% Tween 20 addition) of pooled positive controls, from 1:2 (1–2) to 1:64 (11–12); C–G, 1–12, are 30 test samples; H, 1–12, are 6 blank control samples.

Table 2. Multiscanner readings of ELISA on blood spots from 30 Ethiopian leprosy patients and controls

<table>
<thead>
<tr>
<th></th>
<th>1–2</th>
<th>3–4</th>
<th>5–6</th>
<th>7–8</th>
<th>9–10</th>
<th>11–12</th>
<th>types of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>positive controls</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>6</td>
<td>9</td>
<td>positive serial dilutions</td>
</tr>
<tr>
<td>C</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>1</td>
<td>8½</td>
<td>test samples</td>
</tr>
<tr>
<td>D</td>
<td>½</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>½</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>7½</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>½</td>
<td>1</td>
<td>9</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>8½</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>8</td>
<td>7½</td>
<td>7½</td>
<td>7½</td>
<td>8½</td>
<td>7</td>
<td>blank controls</td>
</tr>
</tbody>
</table>

The composition of the table corresponds to Figure 2. Numbers are averages of duplicate results. The scanner was set on the optical densities resulting from the blank controls, thus printing these with the matrix numbers 7–9.

urine samples were tested by ELISA in Addis Ababa. Readings of the latter were done by naked eye. Only 4 of the 30 urine samples were negative. In a previous paper it was shown that urine is positive by ELISA up to 4–10 days after a single dose of 100 mg DDS. This means that positive results may be expected up to 5–11 days after a last daily dose of 100 mg DDS.

The blood and urine ELISA results are analysed in Table 3 in relation to the statements of the patients about the dates of their last DDS intake. Table 3 indicates the following:

1. In 19 patients the ELISA tests and the statements correspond.
Table 3. ELISA analysis of DDS self-administration by 30 Ethiopian leprosy patients

<table>
<thead>
<tr>
<th>Patients numbers</th>
<th>Blood ELISA*</th>
<th>Urine ELISA†</th>
<th>Intake statements‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>4</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>

*Scanner readings ranging from 0–1 were marked +; readings ranging from 7–9 were marked −; other readings did not occur.
†The ELISA on urine was read by naked eye.
‡Statements that the last dose was taken from 0 to 4 days before the sampling are marked +; statements that the last dose was taken more than 6 days before the sampling are marked −; there were no statements that doses were taken on the 5th or 6th day.

2. In 3 patients the ELISA tests disproved the negative statements.
3. In 4 patients the ELISA on blood spots disproved the positive statements, whereas the ELISA on the urine indicated that the last dose could not have been taken longer ago than about 11 days.
4. In 4 other patients both ELISA’s disproved the positive statements, thus indicating that the last dose was probably taken longer ago than 11 days.

Discussion

Both ELISA’s, but especially the one based on E-DDS, appeared to be sensitive enough for work with dried blood spots. In the E-DDS based ELISA an inhibition of 50% was realized using 25 µl filter paper extract of blood obtained 5–6 days after a single dose of 100 mg DDS. It is remarkable that in both ELISA’s finger-prick blood is found to contain a little less sulphones than blood obtained by venapuncture. Since the former type of blood is easily mixed with other tissue fluids, this could mean a confirmation of the recent finding that such fluids contain lower DDS levels than blood. Nevertheless, sulphone levels in finger-prick blood appear to be high enough for detection by ELISA.

As an example of the possible application of this method the examination of 30 blood spots obtained from Ethiopian leprosy patients was described. A choice was made for a semi-quantitative set-up, relating the results to those obtained on serial dilutions of positive control samples. This surely gives more information than the simpler alternative of comparing test samples to undiluted controls only. Those leprologists who are more interested in monitoring the omission of only 1 or 2 daily DDS doses, should make various dilutions of the test samples in order to find out at which dilution a positive sample turns
negative. Conclusions should again be based on average $T_\frac{1}{2}$ values, with the unavoidable restriction that a slow eliminator remains longer positive than a rapid eliminator.

A finger prick is an unpleasant experience. Naturally, also blood obtained by an ear-lobe prick can be used in ELISA. An intrinsic advantage of blood over urine is the avoidance of diuresis fluctuations. An advantage of the filter paper technique is its simple management. Certainly it makes the delivery of samples to a central laboratory much easier.

Acknowledgements

The authors are grateful for the assistance of Ato Wondomagn Mekuria in collecting blood samples, and for the administrative work done by Miss LKMC Niemer.

References


Haemagglutination inhibition technique for the demonstration of sulphones in urine *

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Received for publication 24 October 1980

Summary A haemagglutination inhibition (HI) test for the detection of sulphones in urine is described. The lowest quantity of dapsone (DDS) in urine, detectable by HI is 1–0.1 μg/ml. In urine samples collected from 10 volunteers sulphones are detectable by HI up to 3–6 days after taking single 100 mg DDS doses. The method is less sensitive than the enzyme-linked immunosorbent assay (ELISA), described earlier, but its advantage is that only one incubation and no washing steps are required. This simple and specific test can be used to monitor self-medication of leprosy patients under field conditions.

Introduction

In a series of previous papers1–4, (unpublished data) we described an enzyme-linked immunosorbent assay (ELISA) for the demonstration of sulphones in body fluids. This technique can be applied in 2 modifications to monitor leprosy-patient compliance, using either urine or blood specimens. The test is specific for sulphones, and the sensitivity is high. Since no sophisticated instruments are needed, the method can be used in leprosy endemic areas where laboratory equipment is often scarce.

Yet, due to its washing steps, the ELISA has not the simplicity of a spot test. However, such washing steps are absent in an haemagglutination inhibition (HI) technique, as described for the detection of drugs of abuse5–8 and for the assay of gentamicin.9 A drawback of various haemagglutination techniques is

*This investigation received support from the Chemotherapy of Leprosy (THELEP) component of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical diseases, from the Netherlands Leprosy Relief Association (Nederlandse Stichting voor Leprabestrijding) and from the Italian Leprosy Relief Association (Amici dei Lebbrosi).

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the short shelf life of sheep red blood cells (SRBC), but fixation methods may overcome this problem.\textsuperscript{10-12} Another limitation is the aspecific agglutination caused by substances present in normal urine specimens. However, when proper precautions are taken this aspecific reaction may be avoided.\textsuperscript{6, 7}

Because of its simplicity it was considered worthwhile trying to develop a HI in addition to the ELISA for sulphones. This paper describes a HI that detects sulphones in urine samples from volunteers who took single doses of 100 mg dapsone (DDS).

Materials and Methods

URINE SAMPLES
Aliquots of urine samples collected earlier\textsuperscript{2} for testing the ELISA for sulphones were used. They were from 10 healthy volunteers who took single oral doses of 100 mg DDS. The specimens were collected immediately before the DDS doses were taken and on 14 consecutive days thereafter.

DDS-SENSITIZED SHEEP RED BLOOD CELLS (SRBC-DDS)
Sheep blood was mixed (1:1) with modified Alsever's solution.\textsuperscript{10} The SRBC were separated by centrifugation (10 min, 2000 r.p.m.) and washings (5 x in saline, 2 x in PBS, 0.01 M, pH 7.2). Then they were suspended in a PBS volume equal to the cell volume.

Monodiazotized DDS was prepared by slow addition (0°C) of an aqueous solution of NaNO\textsubscript{2} (17.5 mg/ml) to 124 mg DDS dissolved in 5 ml 1 N HCl, until slight turbidity occurred. The product was diluted 1:50 in PBS, and adjusted to pH 7.0 using 1 N NaOH. One volume SRBC suspension was mixed (room temperature) with 10 volumes of the diazotized DDS solution. The product was shaken (2 min), centrifuged (10 min, 2000 r.p.m.), washed with PBS (10 x) and saline (3 x) and suspended in saline as an 8% suspension. This SRBC-DDS suspension was formalinized as described by Arquilla,\textsuperscript{10} and the stabilized cells were preserved at 4°C as a 50% stock suspension in PBS.

HI DETECTION OF SULPHONES
HI tests were performed in U-shaped wells of microtitre plates (Cooke), using 25 μl aliquots of each of the following reactants:

(1) Supernatant of a test urine sample that was kept overnight at 4°C, diluted 1:10 in PBS;
(2) Specific anti-sulphones Ig stock solution described previously,\textsuperscript{1} diluted 1:320 in PBS, or PBS without Ig for control tests,
Haemagglutination inhibition technique

Figure 1. HI results on urine samples to detect sulphones. Row 1: mixed urine samples containing standard amounts of DDS, respectively 100 (A), 10 (B), 1 (C), 0.1 (D), 0.01 (E), 0.001 (F), 0.0001 (G) and 0 (H) μg/ml. Rows 3, 5, 7, 9 and 11: urine samples from 5 different volunteers, collected respectively before treatment (H), or on day 1 (A), 2 (B), 3 (C), 4 (D), 5 (E), 6 (F) and 7 (G) after taking 100 mg DDS. Even rows are controls for aspecific HA of the samples in preceding odd rows.

(3) SRBC-DDS stock suspension, diluted 1:15 in PBS.

The 25 μl aliquots were pipetted into the wells in this order. Urine and Ig were not preincubated as suggested by others, since in preliminary experiments this did not appear to influence the test. Results were read after 60 min incubation at room temperature. The optimal dilutions of the reactants were found by checkerboard titration.

Results

Figure 1 is a photograph of HI results using urine samples from 5 of the 10 volunteers. Even rows are controls for aspecific HA of the urine samples in preceding odd rows. In none of the 1:10 diluted specimens aspecific HA was detected, whereas in less diluted specimens this phenomenon frequently occurred. The first 2 rows of Figure 1 show results using standard amounts of DDS in urine. The lowest quantity of DDS detectable by HI in these standard samples is 1–0.1 μg/ml urine, i.e. 2.5–0.25 ng/well (wells C1–D1). Also, in most urine samples collected up to 4 days after the 100 mg dose of DDS sulphones could be detected by HI.

Since the same urine samples were analysed previously by the ELISA the results of both tests can be compared. Figure 2 is taken from the ELISA article. The odd rows show the same specimens as the corresponding rows in
Figure 2. ELISA on urine samples to detect sulphones. Odd rows contain the same urine samples as those in Figure 1. Even rows are duplicate tests of samples in preceding odd rows (2 A–H, and 4, 6, 8, 10, 12 H), or samples collected on day 8 (A), 9 (B), 10 (C), 11 (D), 12 (E), 13 (F) and 14 (G) after taking 100 mg DDS.

Figure 1. Row 2 is a duplicate of row 1, and the other even rows show ELISA results on days 8–14 after the taking of 100 mg DDS. Comparison of ELISA and HI results, using standard amounts of DDS in urine, indicates that HI is 10–100 times less sensitive than ELISA. Comparison of results on test samples illustrates that ELISA is positive for about 4 days longer than HI after single DDS dose. This would suggest a sixteen-fold difference in sensitivity, if calculations are based on an average T\textsubscript{1/2} (half-life) for DDS of about one day.\textsuperscript{2} The figures show that both in ELISA and HI, samples from the third volunteer (rows 7 and 8) were positive for longer than those from the others. A similar correspondence was seen in the samples obtained from a volunteer who was an extremely rapid DDS eliminator with an estimated T\textsubscript{1/2} for DDS of 11 h, as described in the ELISA paper.\textsuperscript{2} These samples (not shown in Figs 1 and 2) were negative sooner than average samples in both tests.

In Table 1 the numbers of urine samples positive by the two immunochemical methods are listed, together with the numbers positive by the quantitative DDS/creatinine (D/C) estimation, also reported in the ELISA paper.\textsuperscript{2} The table illustrates that in sensitivity the HI method takes an intermediate position between the ELISA and D/C methods.

Discussion

The sensitivity of the HI test for sulphones is sufficiently high for monitoring leprosy-patient compliance with daily self-administration of 100 mg DDS doses,
Haemagglutination inhibition technique

Table 1. Comparison of ELISA, HI and D/C tests to detect sulphones in urine samples after single oral doses of 100 mg DDS (10 subjects)

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>No. pos. by ELISA</th>
<th>No. pos. by HI</th>
<th>No. pos. by D/C*</th>
</tr>
</thead>
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*Only D/C ratios higher than the mean pretreatment value plus 3 standard deviations were considered positive.

although it lacks the potential of the more sensitive ELISA to distinguish between occasional and more substantial non-compliance. The difference in sensitivity between the 2 tests may be explained partly by the 1:10 dilution of urine samples to be tested in HI. This dilution is essential, for even in 1:8 diluted samples aspecific HA occurred. It is therefore advisable to test control aliquots of each urine sample for this phenomenon. For the same reason it is important to keep the test samples at 4°C for several hours, or preferably overnight, and use the clear supernatant. For the analysis of freshly voided specimens, centrifugation will be needed in order to obtain a supernatant.

The specificity for sulphones is high, since in preliminary experiments no cross-reaction with important sulphone analogues, the sulphonamides, was found. It shares this specificity with the ELISA,¹ ³ The strength of the HI test lies in its rapidity, simplicity and economy. Its advantage over the ELISA is the absence of washing steps, since no separation of reactants is required. Once the materials for the HI test are available it is as simple as a spot test.

Formalized red blood cells, stored as long as 1 year at 4°C, are known to show no significant alteration in their properties.¹⁰ It remains to be investigated whether the conjugation of DDS prior to the fixation, has any influence on this stability. In other HA assays the antigen was conjugated to cells that already were formalinized,¹⁰ ¹² but so far this procedure did not give satisfactory results with DDS. Suspensions of the red cell conjugates for other HA assays are reported to remain stable for many weeks. Such preparations can be used in HA after 1 washing with the diluent. When lyophilized after dilution to the final desired concentration, the sensitized cells can be used directly after
resuspension in distilled water. The stability of the SRBC-DDS is currently being examined. Preliminary results both with resuspensions and lyophilized portions are comparable to those described. We are especially interested in the influence of tropical temperatures, which occur in most leprosy endemic areas. So far it could be shown that both suspensions and lyophilized portions of SRBC-DDS remained in optimal condition when kept at 37°C for 3 days. The stability of the specific Ig is quite satisfactory, as described earlier.

We also examined the influence of elevated temperatures on the test itself. Identical results could be obtained in HI at room temperature, at 37°C and at 56°C. However, an elevation of temperature required the use of a higher concentration of specific anti-sulphones Ig. The optimal incubation time of 60 minutes was similar at varying temperatures.

Based on these and further experiments the test will be adapted to field conditions in leprosy-endemic areas. It is hoped that the combination of immunochemical specificity with spot test simplicity will make the HI a valuable method to monitor DDS self-medication where required.

Acknowledgements

The authors are indebted to the volunteers for taking doses of DDS and giving the materials for this study.

References

Haemagglutination inhibition technique


The self-administration of dapsone by leprosy patients in Ethiopia

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Received for publication 5 February 1981

Summary In a second investigation of the regularity of dapsone self-administration among outpatients in Addis Ababa, the compliance of patients participating in a trial to assess the ability of combinations of dapsone, thiacetazone and rifampicin to prevent relapse with dapsone-resistant leprosy was compared with that of non-trial patients. Despite the considerable additional time spent on encouraging the trial patients to take their treatment regularly, their level of dapsone compliance was similar to that of the non-trial patients. Only about 60% of the 295 outpatients studied appeared to be ingesting their prescribed dapsone treatment regularly and the overall level of dapsone compliance resembled that encountered in the first investigation conducted 6 years previously. The taking of thiacetazone by the trial patients whose dapsone treatment was supplemented with this drug was unsatisfactory. The implications of these findings for the outpatient treatment of lepromatous leprosy are discussed.

Introduction

The treatment of leprosy continues to be based on the long-term daily self-administration of oral doses of dapsone. The regularity with which patients actually ingest their prescribed treatment can be assessed by means of a quantitative urine-test method. In this method the ratio of the concentration...
of dapsone plus its diazotizable metabolites to creatinine in the urine is determined using simple colorimetric methods (‘D/C ratio’) and the D/C ratios of urine samples collected from outpatients self-administering their dapsone treatment compared with those from inpatient controls given the same daily dose of dapsone under strict supervision. When this method was first used to monitor dapsone self-administration in Ethiopia and Malawi it was concluded that only about 50–60% of the outpatients studied were ingesting their prescribed treatment regularly. Subsequent investigations have indicated that poor dapsone compliance is probably a world-wide problem.

During the 6 years since we first assessed the extent of dapsone self-administration by outpatients receiving treatment from the Addis Ababa Leprosy Hospital there has been an alarming spread of dapsone-resistant strains of *Mycobacterium leprae* in the area. This development has highlighted the importance of prescribing lepromatous patients the maximum well-tolerated dosage of dapsone, of supplementing dapsone treatment with other potent anti-leprosy drugs and of encouraging regular self-medication. This paper describes the results of a second investigation of compliance among outpatients in Addis Ababa with the object of assessing whether significant changes have taken place since the previous investigation in the regularity of dapsone self-administration and if drug taking could be improved by educational means.

**Methods**

*Patients and samples*

Urine samples were collected from lepromatous patients who were participating in a trial to assess the ability of combinations of dapsone, thiacetazone and rifampicin to prevent patients relapsing with dapsone-resistant leprosy. Over 800 patients weighing 40 kg and over were randomly allocated to 4 treatment groups. Group A received 1 tablet of 100 mg dapsone daily for 12 months. Group B received 100 mg dapsone plus 150 mg thiacetazone daily for 12 months while in group C this treatment was supplemented with 600 mg rifampicin daily given during months 1 and 7. Group D received 12 months daily dapsone plus daily rifampicin during months 1 and 7. Thiacetazone was given in the form of ‘Thiazina’ tablets (150 mg thiacetazone plus 300 mg isoniazid) since these were readily available and widely used for the treatment of tuberculosis in Ethiopia, and no more expensive than thiacetazone tablets which were hard to obtain.

Patients collected a 4-week stock of medicaments at each visit to the outpatient clinic. In order to encourage patients to take their treatment regularly, the MRC Project staff gave considerable time to health education, discussing each patient’s treatment with him at the time of his entry into the trial. There
was little need to explain how frequent dapsone-resistant leprosy had become; the fact that many patients' disease got worse after years of treatment was common knowledge. Emphasis was therefore laid on the value of the additional treatment which, if taken regularly together with their dapsone tablets, should prevent such an occurrence. Patients were also warned that the additional thiacetazone or rifampicin treatment might sometimes have unpleasant side-effects but they were urged not to prematurely stop treatment in the event of an adverse reaction but to discuss any symptoms with the clinic staff before contemplating changing treatment. At the end of the 12 months all the patients then continued treatment with 100 mg dapsone daily.

Urine samples were collected on the occasion of a clinic visit from 73 patients in the twelfth month of the trial and from another 63 trial patients who had completed their trial treatment 6 months previously. In order to compare the compliance of patients in the study with an appropriate control group, urine samples were also collected from 222 non-trial patients attending the outpatient clinic whose prescribed daily dose of dapsone was also 100 mg. Whenever urine samples were collected, patients were asked whether they still had any remaining tablets of dapsone at home. Urine samples were preserved by the addition of a crystal of thymol and stored refrigerated to prevent undue evaporation prior to their analysis in London.

**Analytical methods**

Urinary concentrations of dapsone plus its diazotizable metabolites (as dapsone equivalents) and creatinine were determined by modifications of the Bratton and Marshall\textsuperscript{10} and alkaline picrate procedures.\textsuperscript{1} Urine samples from the trial patients prescribed Thiazina (Groups B and C) were also tested for the presence of the isoniazid metabolites isonicotinic acid and isonicotinylglycine by the qualitative procedure described by Ellard and Greenfield.\textsuperscript{11}

**Results and Discussion**

The dapsone/creatinine ratios of the urine samples collected from the patients are summarized in Table 1. These ratios have been interpreted by comparison with those obtained in the previous Ethiopian dapsone compliance investigation from untreated controls and from samples collected 24 h after the last of a series of daily doses of 100 mg dapsone given to inpatients under strict supervision.\textsuperscript{2} On the basis of these control results outpatients were considered to be taking dapsone 'regularly' if their D/C ratios exceeded 30 µg/mg. The great majority of these patients had probably ingested a 100 mg tablet of dapsone within the 24 h prior to their visit to the clinic and a substantial proportion of these patients had probably taken their last dose on the morning of their clinic
<table>
<thead>
<tr>
<th>Origin of samples*</th>
<th>No. of subjects</th>
<th>D/C ratios (μg/mg)†</th>
<th>Mean‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls not on dapsone§</td>
<td>27</td>
<td>4.0–10.9</td>
<td>7.0 ± 0.3</td>
</tr>
<tr>
<td>Supervised controls on dapsone§</td>
<td>27</td>
<td>36.9–123</td>
<td>79.7 ± 4.6</td>
</tr>
<tr>
<td><strong>Non-trial patients</strong></td>
<td>222</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taking dapsone regularly*</td>
<td>139 (63%)</td>
<td>30.1–312</td>
<td>98.1 ± 4.5</td>
</tr>
<tr>
<td>Taking dapsone irregularly*</td>
<td>58 (26%)</td>
<td>10.1–30.0</td>
<td>17.5 ± 0.8</td>
</tr>
<tr>
<td>Taking dapsone grossly irregularly*</td>
<td>25 (11%)</td>
<td>5.2–9.9</td>
<td>7.9 ± 0.3</td>
</tr>
<tr>
<td><strong>Patients during trial</strong></td>
<td>73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taking dapsone regularly</td>
<td>43 (59%)</td>
<td>32.4–275</td>
<td>109.1 ± 9.9</td>
</tr>
<tr>
<td>Taking dapsone irregularly</td>
<td>25 (34%)</td>
<td>10.2–28.7</td>
<td>18.3 ± 1.2</td>
</tr>
<tr>
<td>Taking dapsone grossly irregularly</td>
<td>5 (7%)</td>
<td>4.5–9.2</td>
<td>7.2 ± 0.9</td>
</tr>
<tr>
<td><strong>Patients after trial</strong></td>
<td>63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taking dapsone regularly</td>
<td>24 (38%)</td>
<td>32.2–199</td>
<td>116.2 ± 9.5</td>
</tr>
<tr>
<td>Taking dapsone irregularly</td>
<td>28 (44%)</td>
<td>10.1–29.6</td>
<td>17.6 ± 1.3</td>
</tr>
<tr>
<td>Taking dapsone grossly irregularly</td>
<td>11 (17%)</td>
<td>3.9–9.6</td>
<td>7.7 ± 0.6</td>
</tr>
</tbody>
</table>

*For details and definitions see text.
†μg dapsone equivalents per mg creatinine.
‡Mean ± standard deviation of mean.
§Results from previous investigation (Low and Pearson, 1974).

visit since the D/C ratios of about a third of these samples exceeded the maximum value (123 μg/mg) encountered among the controls.

Patients whose urine samples had D/C ratios of 10–30 μg/mg were considered to be taking their dapsone ‘irregularly’. If the D/C ratios of these patients were typical of their long-term pattern of dapsone self-administration, then a comparison of their mean D/C ratios (about 18 μg/mg) with those of the controls2, 3 would suggest that on average these patients were only taking about 15% of their prescribed treatment. Alternatively, by considering the kinetic profile of D/C ratios after oral dosage with dapsone it may be calculated that on average such patients probably took their last dapsone dose some 3–4 days prior to their visit to the clinic.8, 12 Patients with urine D/C ratios of less than 10 μg/mg were considered to be ‘grossly irregular’ in ingesting their treatment. Since their mean D/C ratios did not differ significantly from those of the untreated controls, it is probable that the great majority of these patients had taken no dapsone for at least 5 days before coming to the clinic.

About 60% of the non-trial patients were judged to be self-administering their dapsone regularly, a proportion similar to that found in the original investigation carried out 6 years previously.2 Although the overall level of compliance has therefore probably not changed significantly during this time, the great majority of patients are now being prescribed 100 mg dapsone daily
whereas previously the prescription of 50 or 25 mg daily doses was quite common, so that the overall level of dapsone intake has probably been considerably improved.

Disappointingly, the considerable efforts devoted to encouraging the trial patients to take their treatment regularly appear to have been unsuccessful, at least as judged by the results obtained on the urine samples collected at the end of the trial. Thus the proportions of trial patients considered to be ‘irregular’ or ‘grossly irregular’ in self-administering their dapsone tablets were similar to those encountered among the non-trial patients. Indeed, the compliance of the trial patients 6 months after the end of the trial appeared to be inferior to that of the non-trial patients. Separate analyses (not shown) indicated that the D/C ratios of samples collected from patients who stated that they still had some remaining dapsone tablets at home did not differ significantly from those who said their stock of pills was finished.

Forty-five urine samples collected during the twelfth month of the trial from patients who had been prescribed thiacetazone in the form of daily Thiazina tablets were tested by the isonicotinic acid procedure. Positive results were obtained from 13 of the samples indicating that only about 30% of the patients had ingested a Thiazina tablet within the previous 48 h. It was therefore apparent that the prescribed thiacetazone supplement was being taken very irregularly by many of the patients in the trial, perhaps on account of adverse side-effects experienced by about a quarter of the patients who had been prescribed the drug.

Discussion

The results of this study have confirmed the now well-documented conclusion that although dapsone is a well-tolerated drug, many patients self-administer it irregularly. The failure of our efforts to encourage patients to take their treatment regularly indicates that improving patient compliance is likely to be a difficult task. It may be that many patients have previously been unwittingly encouraged to associate the taking of dapsone with the occurrence of reactions because of the widespread practice of stopping dapsone treatment during bouts of both ENL or upgrading reactions, or it could be that the problem of non-compliance in leprosy had deeper psychological foundations. The many years that lepromatous patients need to continue with their treatment is almost certainly another factor militating against regular drug self-administration, since experience in the treatment of other chronic conditions such as hypertension, schizophrenia and tuberculosis has shown that patient compliance deteriorates with increasing duration of therapy. Furthermore, all previous attempts to improve compliance by educating diabetic, hypertensive or tuberculosis patients about the importance of regularly self-administering their treatment have failed.
Although it is probable that a few missed daily doses of dapsone are unlikely to significantly impair the efficacy of therapy, substantial breaks in drug taking which result in dapsone concentrations in the body falling to levels that permit dapsone-resistant mutants of *M. leprae* to multiply cannot but increase the risk of patients eventually relapsing with dapsone-resistant leprosy. It is therefore likely that a substantial proportion of the patients considered to be self-administering their dapsone grossly irregularly (10% of the total) will eventually incur therapeutic penalties as a result of their poor compliance and that some of those judged to be taking dapsone irregularly (30% of the total) could also be at risk.

The irregularity with which the patients ingested their prescribed thiacetazone was also very disquieting. Mouse foot-pad studies have shown that the activity of thiacetazone against *M. leprae* is essentially bacteriostatic\(^1\) and that its efficacy is substantially impaired if it is given intermittently.\(^2\) Since peak serum concentrations of thiacetazone achieved in man after daily dosage with 150 mg thiacetazone only exceed its minimal inhibitory concentration against *M. leprae* by a factor of about eight-fold\(^3\) and its half-life is about 21 h,\(^4\) it may be anticipated that gaps of as little as 3 or 4 days may seriously impair its efficacy as a companion drug to prevent lepromatous patients relapsing with dapsone-resistant leprosy.

The implications of our findings are two-fold. Firstly, consideration should be given to supplementing self-administered daily dapsone treatment with supervised doses of rifampicin, the most potent bactericidal antileprosy drug. Thus a 600 mg dose of rifampicin might be given once monthly at the time of each clinic visit\(^5\) and, if finances permit, the regimen might be strengthened by giving a second 600 mg dose of rifampicin to be swallowed the following day.\(^6\) Secondly, before any decisions are made to employ companion drugs such as thiacetazone, ethionamide or prothionamide that need to be taken on a daily basis\(^7,8\) for routine treatment, studies should first be carried out to determine the regularity with which they are likely to be self-administered by outpatients. Such compliance studies should be considerably facilitated by the recent discovery of the potentialities of using a minute dose of isoniazid as an innocuous marker for monitoring drug ingestion.\(^9\)

References

Dapsone compliance in Ethiopia


The association of pregnancy and leprosy

I. New cases, relapse of cured patients and deterioration in patients on treatment during pregnancy and lactation — results of a prospective study of 154 pregnancies in 147 Ethiopian women

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Received for publication 26 January 1981

Summary One hundred and fourteen women with leprosy and 33 women without leprosy were studied during 118 and 36 pregnancies respectively. Two healthy controls developed leprosy during the study period: 12 of 25 women with 'cured' tuberculoid leprosy relapsed with new lesions or nerve damage; 46 of 93 women with active tuberculoid or lepromatous leprosy showed increased activity of their leprosy either as a transient phenomenon (21 patients) or due to probable dapsone resistance (28 patients). These occurred chiefly during the third trimestre and are thought to be due to decreased host resistance and increased immunological instability during pregnancy.

Introduction

Pregnancy has long been associated with the first appearance of leprosy or aggravation of the disease.1–3 One study shows 75% of women studied to have developed the first sign of leprosy in association with child bearing, of whom two-thirds had the first signs of leprosy during the puerperium (the first 6 weeks after delivery).4 Suggested reasons for this are hormonal,5 metabolic6 or some suppression of host resistance.4, 7 Suppression of cell-mediated
immunity (CMI) during pregnancy may also be associated with downgrading and a shift toward the lepromatous end of the spectrum. Hence one might expect recovery of CMI following delivery to be associated with upgrading phenomena and reversal reaction. Some patients present with the onset of leprosy and in reaction during the puerperium or first few weeks of lactation. Many of the observations quoted have been based on retrospective studies. This paper presents the results of a prospective study on the effect of pregnancy on leprosy carried out at the Addis Ababa Leprosy Hospital between 1975 and 1978.

Patients and methods

One hundred and forty-seven Ethiopian women were studied during 154 pregnancies. There were 114 women with leprosy (118 pregnancies) and 33 women without leprosy (healthy contacts: HC, with 36 pregnancies). The women who were all from the low socio-economic class lived, for the most part, in the villages surrounding the Addis Leprosy Hospital. They were first seen, for this study, when they presented themselves at the Hospital ante-natal clinic which supplied ante-natal care for registered leprosy patients, wives of leprosy patients and members of staff. Initially the patients studied were those with active tuberculoid leprosy, active lepromatous leprosy with positive skin smears and healthy contacts; later the study group was broadened to include women with ‘cured’ tuberculoid leprosy who had stopped treatment and women with chronic, quiescent lepromatous leprosy with negative skin smears. Selection of the patients within the above general classification was based on their willingness to participate in the study, to deliver their babies in hospital rather than at home and to be seen with their babies for regular assessment, including blood tests, for a period of up to 2 years during lactation.

CLASSIFICATION AND TREATMENT OF MOTHERS

The 114 women with leprosy were classified initially as follows using the scale of Ridley & Jopling:

- Cured tuberculoid and borderline tuberculoid leprosy (released from control) (TT and BT/RFC) 25 (25 pregnancies).
- Active tuberculoid and borderline tuberculoid leprosy (TT and BT) 17 (18 pregnancies).
- Borderline lepromatous leprosy (BL) 40 (41 pregnancies).
- Lepromatous leprosy (LL) 32 (34 pregnancies).

Eighty-two patients were receiving dapsone monotherapy (50–100 mg daily)
but 26 patients (1 BL, the rest BT or TT) were believed cured, had stopped treatment and had been 'released from control' (RFC, see below). Six patients (2 BL, 4 LL) had already developed dapsone-resistant leprosy, as defined\textsuperscript{10} and were receiving clofazimine (4 patients, all LL, 5 pregnancies) or rifampicin plus thiambutosine and dapsone (2 patients both BL). Treatment and supervision of these patients was carried out through the hospital outpatient clinics: 81 patients receiving dapsone monotherapy (18 TT and BT, 36 BL, 27 LL) were supplied with dapsone tablets on a weekly or fortnightly basis by paramedical leprosy workers at hospital or municipality clinics, were referred to hospital clinics for treatment of reactions or other complications of leprosy, and were assessed by a doctor at the hospital 'Review Clinic' every 6 months when routine slit-skin smears were examined, 4 patients (2 BL, 2 LL) receiving dapsone monotherapy 100 mg daily in a chocolate-coated tablet for suspected dapsone resistance\textsuperscript{10} and 7 patients (2 BL, 5 LL) with proven dapsone resistance were seen every 6 months at a special clinic for the treatment of drug-resistant leprosy, routine slit-skin smears were done every 6 months and biopsies were taken annually.

**PATIENTS RELEASED FROM CONTROL (RFC)**

At the start of the study the practice in the hospital for stopping treatment of leprosy was as follows: TT patients were RFC after 2–3 years of treatment with dapsone 50–100 mg daily; BT patients were RFC after 4 or more years of treatment with dapsone 50 mg daily (300 mg weekly) when there had been no clinical evidence of active leprosy for at least 2 years; BL patients were RFC when they had received treatment for 15–20 years and had been skin-smear (bacteriological index: BI) negative with no clinical evidence of active leprosy for 10 years; LL patients continued on treatment for life and hence were not RFC.

The 25 patients classified as TT and BT/RFC were originally classified as TT or BT at the hospital new case clinic. Diagnosis had been made on clinical grounds supported by negative BI but without histological confirmation. Seventeen patients had been diagnosed at the Addis Ababa Leprosy Hospital and 8 at rural clinics or hospitals where they received their initial treatment before being transferred to Addis Ababa. One patient seen first in Addis Ababa and 1 patient coming from a rural clinic had had doubts raised regarding classification of leprosy and had been recorded as being 'LI' and 'BB' respectively on one occasion; on subsequent assessment by a senior hospital leprologist both were recorded as 'BT' on clinical grounds. The duration of stopping treatment ranged from 3 months to 10 years (mean 2.6 years).

**ENTRY OF WOMEN TO THE STUDY: TIMING AND ASSESSMENT**

At the time of entry to the study (Table 1) in addition to full obstetrical assessment, a general physical examination was made and the patient’s leprosy
Table 1. Time of entry to study

<table>
<thead>
<tr>
<th>Histological classification of leprosy</th>
<th>Number of women entering the study</th>
<th>1st trimestre</th>
<th>2nd trimestre</th>
<th>3rd trimestre</th>
<th>Total pregnancies</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TT and BT/RFC</td>
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<tr>
<td>TT and BT/Active</td>
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</tr>
<tr>
<td>BL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LL</td>
<td></td>
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</tr>
</tbody>
</table>

*The number ( ) indicates the number of women entering the study at or after 36 weeks gestation.

HC = healthy contacts; RFC = released from control.

status assessed clinically; skin smears were taken from leprosy patients and a biopsy for histological classification, if it had not already been taken. Subsequent detailed leprosy assessments were made as indicated by the patient’s symptoms and clinical state.

The first group of women admitted to the study were taken in during the third trimestre, several of them at or after 36 weeks gestation. After the first 3 months of the study it was apparent that leprosy deteriorated during pregnancy, thereafter whenever possible, patients were admitted to the study during the first or second trimestre with detailed leprosy assessment at the time of entry and during each following trimestre and at 6-month intervals during lactation, more frequently if indicated.

ROUTINE ASSESSMENT OF STUDY PATIENTS

Women in this study were seen for routine ante-natal care at monthly intervals until 28 weeks gestation, every 2 weeks until 34 weeks and weekly thereafter. In addition to receiving the routine ante-natal care their leprosy status was assessed clinically, complications were recorded, and additional investigations arranged as indicated. They were admitted to hospital for 24-hour collections of urine for oestriol analysis and also for medical, obstetrical or social reasons as necessary. LL patients in particular were admitted to hospital for several weeks prior to delivery to prevent foetal wastage by precipitate delivery at home. As inpatients they received routine ante-natal surveillance but stopped attending outpatient ante-natal clinics (ANC). This factor accounts largely for the reduced attendance at ANC by LL patients (Table 2).

At detailed leprosy assessment the patient’s complaints, state of health and drug treatment were recorded. The patient was then examined in a well-lit room, with inspection and palpation of the skin, peripheral nerves and regional lymph nodes. Clinical drawings were made of the skin lesions, slit-skin smears were taken from 6 sites (both ears and 4 smears from active lesions, or from both ears, elbows and knees when no active lesions were seen; smears were
Table 2. Frequency and timing of assessment during pregnancy

<table>
<thead>
<tr>
<th>Classification of leprosy</th>
<th>No. of women</th>
<th>No. of pregnancies</th>
<th>No. of attendances at ANC (Mean ± SEM)*</th>
<th>Frequency of leprosy assessments†</th>
<th>Frequency of laboratory investigations‡</th>
<th>No. of admissions for obstetrical/medical/social reasons</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC</td>
<td>33</td>
<td>36</td>
<td>5.9 ± 0.5</td>
<td>X1 32 4 X2 X3 X4 X5 X &gt; 5 14 X1 31 4 X2 X3 X4 X5 X &gt; 5 1</td>
<td>X1 8 X2 13 X3 4 X4 8 X5 13</td>
<td>4</td>
</tr>
<tr>
<td>IT and BT/RFC</td>
<td>25</td>
<td>25</td>
<td>6.2 ± 0.5</td>
<td>X1 19 5 1 X2 X3 X4 X5 11 5 X1 17 5 1 X2 X3 X4 X5 1</td>
<td>X1 6 X2 13 X3 3 X4 8 X5 13</td>
<td>3</td>
</tr>
<tr>
<td>TT and BT/Active</td>
<td>17</td>
<td>18</td>
<td>5.1 ± 0.6</td>
<td>X1 15 3 X2 X3 X4 X5 13 3 X1 17 5 1 X2 X3 X4 X5 1</td>
<td>X1 2 X2 4 X3 1 X4 2 X5 3</td>
<td>4</td>
</tr>
<tr>
<td>BL</td>
<td>40</td>
<td>41</td>
<td>5.9 ± 0.5</td>
<td>X1 32 9 X2 X3 X4 X5 19 15 2 X1 17 5 1 X2 X3 X4 X5 1</td>
<td>X1 2 X2 4 X3 1 X4 2 X5 3</td>
<td>4</td>
</tr>
<tr>
<td>LL</td>
<td>32</td>
<td>34</td>
<td>4.5 ± 0.6</td>
<td>X1 16 18 X2 X3 X4 X5 16 13 3 X1 17 5 1 X2 X3 X4 X5 1</td>
<td>X1 1 X2 1 X3 1 X4 1 X5 1</td>
<td>9</td>
</tr>
</tbody>
</table>

*Assessment by doctor (MED).
†First number equals the number of patient-assessments during pregnancy for this special study only; number within ( ) equals the number of patient-assessments for the special study together with the routine 'full clinical assessments' at hospital review clinics and clinics monitoring suspected dapsone resistance.
‡First number equals frequency of laboratory investigations (skin smear for BI and MI/biopsy for histology/biopsy for mouse foot pad tests), singly or in combination; number ( ) equals frequency of laboratory investigations and additional tests (VMT with ST/EMG/skin test with A6), singly or in combination.

HC = healthy contacts (without leprosy); TT and BT/RFC = tuberculoid and borderline leprosy 'released from control', i.e. 'cured'; TT and BT/Active = tuberculoid and borderline tuberculoid leprosy, active; BL = borderline lepromatous leprosy; LL = lepromatous leprosy; ANC = ante-natal clinic.
BI = bacteriological index, MI = morphological index.

1 When it was not possible to perform skin smears for BI or biopsy during pregnancy some were done immediately after delivery, during the puerperium.
2 When skin smears for BI or biopsy had not been done before, some were done for the first time in the study during lactation at follow-up assessments.
taken from the same sites on subsequent occasions unless new lesions had appeared, in which case smears were taken from them) for bacteriological and morphological index (BI and MI).

Biopsies were taken for diagnosis and classification from active lesions or when the disease was quiescent from the buttocks. The biopsies were divided in two and read by two independent leprologists. Patients who were deemed healthy contacts were assessed in the same way as leprosy patients with the exception of the skin biopsy, which was only done if there was a suspicious lesion or nerve enlargement. Clinical classification was undertaken by two independent observers. When a patient was suspected of having developed dapsone-resistant leprosy a biopsy of an active nodule with a positive morphological index was taken and tested in mouse foot pads for dapsone resistance.11

Sensory skin testing (ST)12 and voluntary muscle testing (VMT)13 was done by the physiotherapy department. Nerve conduction velocity (EMG) was estimated in a few patients where it was difficult to decide whether nerve damage was of recent onset.

Skin testing using a standardized purified protein of *Mycobacterium leprae* grown in armadillos (A6) was done during lactation instead of lepromin testing.

The patients' hospital records were reviewed periodically and additional data regarding the initial diagnosis and treatment of leprosy, routine leprosy assessments, complications of leprosy and special investigations not obtained at the study assessments was abstracted and used in the final analysis of results. The frequency of assessment and of laboratory tests and other investigations carried out during pregnancy and lactation is shown in Tables 2 and 3. The total number of special investigations is not shown as tests carried out at the same time, regardless of number, are recorded as one time of testing.

**DIAGNOSIS OF DETERIORATION OF LEPROSY STATUS**

(i) New ‘overt’ cases of leprosy were diagnosed clinically and confirmed by skin smears and biopsy.

(ii) Relapse in RFC patients was diagnosed clinically, by the appearance of new lesions and/or new nerve damage or by positive BI or biopsy showing active leprosy.

(iii) Deterioration in leprosy status of patients receiving treatment was defined as the occurrence of one or more of the following: conversion from negative to positive or rise in the patient’s BI or MI, appearance of new lesions, extension of existing lesions, erythema and oedema of margins or tuberculoid lesions (in the absence of reaction) or increased activity of the lesion as diagnosed by histology.
### Table 3. Frequency of leprosy assessments and investigations during lactation

<table>
<thead>
<tr>
<th>Classification of leprosy</th>
<th>No. of women</th>
<th>Frequency of leprosy assessments*</th>
<th>Not seen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Seen with baby, asymptomatic, no leprosy assessment</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>X1</td>
<td>X2</td>
</tr>
<tr>
<td>HC</td>
<td>36</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(11)</td>
<td>(3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4)</td>
<td>(5)</td>
</tr>
<tr>
<td>TT and BT/RFC</td>
<td>25</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(5)</td>
<td>(11)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>TT and BT/Active</td>
<td>18</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4)</td>
<td>(5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>BL</td>
<td>41</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(7)</td>
<td>(3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>LL</td>
<td>34</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3)</td>
<td>(7)</td>
</tr>
</tbody>
</table>

*First number equals the number of patient-assessments during lactation for this special study only; number within ( ) equals the number of patient-assessments for this special study together with the routine 'full clinical assessments' at hospital review clinics and special clinics monitoring suspected dapsone resistance.

†First number equals frequency of laboratory investigations (skin smears for BL and MI/biopsy for histology/biopsy for mouse foot pad test) singly or in combination; number within ( ) equals frequency of laboratory investigations and additional tests (VMT with ST/EMG/skin test with 'A6') singly or in combination.
Results

HEALTHY CONTROLS (HC)

Of 33 women observed during and after 36 pregnancies, 2 developed leprosy. One asymptomatic woman developed a hypopigmented macule during the third trimester of pregnancy which on biopsy showed indeterminate leprosy. Postpartum the lesion grew in size but the woman had no complications of leprosy. The second woman complained of severe 'rheumatism' at 10 weeks postpartum when she was found to have enlarged nerves which on biopsy showed active BL leprosy. Hypopigmented skin lesions and skin infiltration were apparent by 6 months postpartum.

'CURED' TUBERCULOID AND BORDERLINE TUBERCULOID LEPROSY (TT AND BT/RFC)

Of these 25 patients, 9 relapsed with active leprosy (6 BT, 3 BL) within periods of from 3 months to 3 years after stopping treatment. Eight of the 9 relapses were diagnosed on clinical grounds, 7 were confirmed on biopsy; 3 were BI positive. Five out of the 9 relapses occurred during the third trimester of pregnancy.

In addition 3 patients were considered to have incipient relapse on the evidence of new nerve enlargement or neuritis though skin biopsies and BI were negative.

Ten out of 12 patients relapsed in association with the first pregnancy and 2 during the second pregnancy after RFC. Details of clinical features and investigations are shown in Table 4.

ACTIVE TUBERCULOID LEPROSY (TT AND BT)

Of 18 patients on dapsone monotherapy, 8 had a transient increase in activity of the skin lesions, usually in the third trimester, without any histological evidence of reaction. In 3 cases the lesions appeared more active with raised erythematous margins, in 4 cases there was conversion from BI negative to positive. In 2 cases there was increase in size and number of the skin lesions during lactation.

LEPROMATOUS LEPROSY (BL AND LL)

Sixty-eight women (36 BL, 32 LL) were studied through 71 pregnancies and followed up after delivery. (Four others were assessed only during pregnancy). Increased activity was found in 38 (54%) during pregnancy, puerperium or lactation (in 20 during the third trimester or puerperium). At the time that...
Table 4. TT and BT/RFC cases showing relapse in association with pregnancy

<table>
<thead>
<tr>
<th>No.</th>
<th>Parity</th>
<th>Original diagnosis</th>
<th>Duration of treatment (years)</th>
<th>Years RFC before present pregnancy</th>
<th>Symptoms</th>
<th>Clinical features</th>
<th>Additional tests</th>
<th>Final diagnosis</th>
<th>Timing of relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td>A - relapse cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1 + 0</td>
<td>'T'</td>
<td>7</td>
<td>3/12</td>
<td>—</td>
<td>1 active new macule face; nerves normal</td>
<td>BT Active</td>
<td>2.3</td>
<td>BL</td>
</tr>
<tr>
<td>2</td>
<td>2 + 0</td>
<td>BT</td>
<td>11</td>
<td>1½</td>
<td>Rheumatism</td>
<td>New nodules + + on on legs; nerves normal</td>
<td>BL Active</td>
<td>3 +</td>
<td>BL</td>
</tr>
<tr>
<td>3</td>
<td>5 + 0</td>
<td>BT</td>
<td>14</td>
<td>2½</td>
<td>Inactive skin; nerves normal</td>
<td>Quiescent BT</td>
<td>Not done</td>
<td>0.8</td>
<td>BL</td>
</tr>
<tr>
<td>4</td>
<td>1 + 0</td>
<td>BT</td>
<td>1</td>
<td>3</td>
<td>—</td>
<td>8 new macules (legs and arms); 6 enlarged nerves; new motor and sensory loss</td>
<td>BT Active</td>
<td>0</td>
<td>BT</td>
</tr>
<tr>
<td>5</td>
<td>0 + 1</td>
<td>BT</td>
<td>4</td>
<td>1</td>
<td>'Burning' parasthesiae</td>
<td>EMG active demyelination</td>
<td>BT Active</td>
<td>0</td>
<td>BT</td>
</tr>
<tr>
<td>6</td>
<td>3 + 0</td>
<td>'T'</td>
<td>9</td>
<td>1</td>
<td>—</td>
<td>New macule; face skin reaction neuritis</td>
<td>BT Active</td>
<td>0</td>
<td>BT</td>
</tr>
<tr>
<td>7</td>
<td>1 + 0</td>
<td>BT</td>
<td>8</td>
<td>1½</td>
<td>—</td>
<td>New nodules on face and ears; 3 enlarged nerves</td>
<td>BL Active</td>
<td>0</td>
<td>BT</td>
</tr>
<tr>
<td>8</td>
<td>1 + 0</td>
<td>BT</td>
<td>8</td>
<td>2</td>
<td>Rheumatism</td>
<td>2 new macules face; nerves normal</td>
<td>BT Active</td>
<td>0</td>
<td>BT</td>
</tr>
<tr>
<td>9</td>
<td>4 + 0</td>
<td>BT</td>
<td>5</td>
<td>2*</td>
<td>'Burning' parasthesiae</td>
<td>Active erythematous edge of old macule, face; 3 enlarged nerves</td>
<td>BT Active</td>
<td>0</td>
<td>BT</td>
</tr>
<tr>
<td>B - incipient relapse cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4 + 0</td>
<td>TT/BT</td>
<td>6</td>
<td>4/12</td>
<td>Rheumatism</td>
<td>Skin inactive; 2 enlarged nerves</td>
<td>Old BT</td>
<td>0</td>
<td>BT</td>
</tr>
<tr>
<td>2</td>
<td>3 + 0</td>
<td>BT</td>
<td>7</td>
<td>1½</td>
<td>Rheumatism</td>
<td>Skin inactive; 3 enlarged nerves</td>
<td>'Burnt out' BT</td>
<td>0</td>
<td>BT</td>
</tr>
<tr>
<td>3</td>
<td>2 + 0</td>
<td>BT</td>
<td>10</td>
<td>3*</td>
<td>Rheumatism</td>
<td>Skin inactive; 1 enlarged nerve</td>
<td>Inactive BT</td>
<td>0</td>
<td>BT</td>
</tr>
</tbody>
</table>

*RFC during previous pregnancy. TM = Trimestre; lact = lactation.
increased activity was first observed 17 out of the 38 patients had new nodules (a further 7 developed new skin lesions later); in 34 cases the increased activity was confirmed by a rise in BI, in 4 cases by biopsy only. In 16 cases the increased activity was a transient phenomenon, with fall in BI to less than the pre-pregnancy levels or reversion of the active biopsy to 'LL regressing' during early lactation. However, 6 patients then went on, within 11–15 months, to show a subsequent rise in BI and clinical evidence on relapse during late lactation or the next pregnancy. The phenomenon of transient increase in activity was shown by patients prescribed dapsone monotherapy, dual therapy with dapsone and rifampicin, and clofazimine monotherapy.

**OTHER CLINICAL FEATURES ASSOCIATED WITH INCREASED ACTIVITY OF LEPROSY (BL AND LL)**

The complaint of 'rheumatism' either preceded or accompanied the increased activity of the disease in more than half of the patients. Erythema nodosum
leprosum (ENL) preceded or accompanied the increased activity of the disease in one-half of the patients, occurring for the first time during pregnancy in most cases. In contrast reversal reaction was seen postpartum when recovery of CMI would be expected to occur. New nerve enlargement was observed in one-third of the patients, and neuritis (due to ENL or reversal reaction) was observed in more than half of the patients who had increased activity of the disease, including those who had only a transient rise in BI during pregnancy. Neuritis is discussed in detail elsewhere.\textsuperscript{14}

Twenty-eight patients who were considered to have evidence of dapsone resistance are discussed elsewhere.\textsuperscript{15}

RELAPSE IN ASSOCIATION WITH DOWNGRADING PHENOMENA (BL AND LL)

Six patients (all BL) downgraded from BL to LL, 5 during pregnancy and 1 during lactation. They were diagnosed on histology, but 3 also had clinical evidence of relapse due to dapsone resistance. In addition 3 relapse patients showed, for the first time, the histological features of polar lepromatous leprosy (LL\textsubscript{p})\textsuperscript{16} during the third trimester or immediately after delivery. After delivery 3 of the patients who had downgraded to LL upgraded to BL or BT.

Discussion

Protection and survival of the foetus as an allograft is the result of adaptive maternal responses to pregnancy including transient suppression of CMI.\textsuperscript{17} Suggestive evidence for this is the increased survival time of adult skin homografts on pregnant hosts, especially during the third trimester, compared with non-pregnant hosts;\textsuperscript{18} the depression of tuberculin sensitivity in the third trimester of pregnancy;\textsuperscript{19} the increased severity of certain viral diseases during pregnancy\textsuperscript{20–22} and amelioration of diseases such as rheumatoid arthritis,\textsuperscript{23–24} ulcerative colitis\textsuperscript{25} and sarcoidosis\textsuperscript{26} during pregnancy with deterioration postpartum. The pregnancy-associated alterations of these conditions pertain to cell-mediated immune reactions.\textsuperscript{27}

Host resistance to mycobacterial disease is dependent on CMI and can be measured \textit{in vitro} by the lymphocyte transformation test (LTT). Results of such tests, using phytohaemagglutinin (PHA) and purified protein derivative of tubercle (PPD), indicate suppression of CMI during pregnancy which ceases at delivery or shortly afterwards.\textsuperscript{28–31} It is possible that pregnancy-associated α-macroglobulin plays a part in this process.\textsuperscript{32} In pregnant leprosy patients it is likely that plasma contains suppressive factors in addition to those normally associated with pregnancy, as plasma from mothers with
leprosy had a greater inhibitory effect on their babies’ LLT than plasma from healthy mothers.33

Before the era of chemotherapy it was observed that there was a sex difference in the mortality from tuberculosis: according to the United States Census Bureau Statistics there was a consistently higher death rate in females aged 15–25 years of age from 1900 to 1942.34 It was well recognized that pregnancy had an adverse effect on tuberculosis. In many cases the first sign of tuberculosis was observed soon after parturition and where tuberculosis was already established mortality was increased during later pregnancy and the puerperium,35–39 although with proper sanatorium care throughout pregnancy the danger was greatly diminished if not avoided.40 A similar adverse effect of pregnancy on tuberculosis was observed in cattle41 and experimental animals.42

In leprosy the overall prevalence in men is greater than in women. However, women appear to develop the disease at an earlier age than men. For instance, among leprosy patients in India 50% of the women had developed leprosy by the age of 20 years, compared with 30% of men.2 In Ethiopia as many as 75% of female patients in the studies of the Medical Research Council Leprosy Project had developed leprosy by the age of 20 (M E Duncan & J M H Pearson, unpublished observations). It is tempting to link this early onset with an increased risk of infection and rate of evolution associated with increased endocrine activity during puberty and suppression of CMI in frequent pregnancies during the late teens.

In leprosy where the host resistance is dependent chiefly on CMI, one would expect pregnancy to be associated with (i) the first appearance of leprosy; (ii) relapse in cured patients; and (iii) increased activity of the disease with a tendency to shift towards the lepromatous end of the spectrum and increase in bacillary load. These features were all seen in our study.

(i) NEW CASES

In Addis Ababa the new case rate for the city is 1 per 3,000 population; in the villages surrounding the Leprosy Hospital the rate is higher, 1 per 1,000 population (0.1%). It is therefore significant that of 33 women observed during 36 pregnancies, 2 (5.6%) showed the first sign of disease during the third trimestre or early lactation. Our observation confirmed earlier reports.1–4, 6–7, 43 Women already infected with *Mycobacterium leprae* and incubating the disease show overt leprosy in late pregnancy or early lactation as a result of decreased host resistance of pregnancy.1, 7, 43

(ii) RELAPSE OF ‘CURED’ PATIENTS

The relapse rate in patients with cured TT and BT leprosy in Ethiopia has been reported44 as 5% per annum. A considerable number of patients relapsed because they had been misclassified as BT rather than BB or BL, and thus had
received inadequate treatment prior to stopping therapy. Our observation that 9 ‘cured’ TT and BT patients relapsed with active leprosy (3 as BL and 6 as BT) confirms the above findings. While the original clinical diagnosis had not been in doubt in any of our cases and all were BI negative, none had had histological confirmation. In pregnant women the skin lesions may not be typical of either BT or BL leprosy, thus causing difficulties in clinical classification as happened with two of the patients who relapsed in our study (Table 4). Ideally (to ensure adequate treatment), histological confirmation and classification is recommended in all patients especially women presenting with overt leprosy in association with pregnancy or lactation. It is also possible that the initial ‘BT’ classification in our patients was correct but that 3 of these women who were all parous had downgraded to BL during a previous pregnancy.

Nerve damage was a feature of relapse in BT/RFC patients, 4 out of 8 had nerve damage early. This is the same as is found in early active tuberculoid leprosy (JMH Pearson, unpublished observation). The observation of Naafs (B Naafs, personal communication) that ‘rheumatism’ was a symptom of relapse in these patients was confirmed in this study although we found it was more consistently a symptom of ‘late silent neuritis’.

It has been suggested that pregnancy be regarded as a test of cure of leprosy.43 Seven of the 9 women who relapsed with active leprosy did so during the first pregnancy after stopping treatment and 2 relapsed in the second pregnancy after stopping treatment (Table 4). Thus one pregnancy cannot be regarded as a test of cure and we recommend that all women with ‘cured leprosy’ who have stopped therapy, be carefully assessed during and after all subsequent pregnancies if late nerve damage is to be avoided.

(iii) INCREASED ACTIVITY OF LEPROSY WITH A TENDENCY TO SHIFT TOWARDS THE LEPROMATOUS END OF THE SPECTRUM AND INCREASE IN BACILLARY LOAD

We observed increased activity of their leprosy in just under half of the patients with active TT or BT leprosy (8/18) and in rather more than half (38/71) of the BL and LL patients who were followed up. The very high rate of relapse or deterioration of leprosy status, half of which appeared to be a transient phenomenon, would undoubtedly have been overlooked had these patients not been assessed frequently with the use of routine skin smears and biopsies even in the absence of skin lesions. The importance of carrying out routine skin smears at regular intervals cannot be overemphasized, as it is only by so doing that relapse can be detected early.45–46

The increased activity of the patient’s leprosy recorded in 17 of the 89 patients was diagnosed on the basis of a rise in BI and/or MI or on increased activity at the histological level in women who did not at any time during the study show new or active skin lesions. The timing and transient nature of this
phenomenon was of interest in that it was related to the third trimestre of pregnancy when CMI would be maximally suppressed. A similar observation was made by Browne who refers to a transient non-significant rise in BI which he attributes to hormonal disturbances of pregnancy. However, by having the opportunity to follow up these Ethiopian patients we found that 6 out of 16 lepromatous women who had a transient rise in BI during pregnancy developed the clinical picture of dapsone resistance during the next 15 months (4 with new nerve damage) and 9 others developed new nerve damage during lactation. Thus we feel that a transient rise in BI during pregnancy can no longer be considered as significant.

The conversion to BI positive with increase in size and number of new lesions in BT patients, the tendency to downgrade from BL to LL during pregnancy with upgrading following delivery, and the onset of leprosy with reversal reaction during early lactation are evidence of the increased instability of women with leprosy during pregnancy, especially those classified as borderline.

While further investigation is required to elucidate the mechanisms of the adverse effect of pregnancy on leprosy, the practical implications which should be made widely known to all leprosy workers are:

1. The pregnant woman, because of physiological suppression of CMI most marked during the third trimestre, is especially at risk. If she is a known leprosy contact incubating leprosy, she is most likely to show overt disease either in late pregnancy or during lactation when it may well be complicated by reaction. 'Cured' BT patients run the risk of relapsing with active disease, and in patients receiving treatment for leprosy there is a 50% chance of the disease being aggravated with a shift towards the lepromatous end of the spectrum, increased bacillary load, subsequent risk of ENL and in the puerperium reversal reaction possibly with severe nerve damage.

2. In relapsing RFC patients and those who are developing dapsone-resistant leprosy, with multiplication of viable bacilli during pregnancy, there is a real risk that the foetus may be infected in utero and go on to clinical leprosy in early childhood; furthermore the woman herself will become a risk to her household and the community as she is likely to be infectious.

We therefore recommend:

(I) Health education. Incorporation of this knowledge into health education of women in the reproductive age group. At the same time advice on family planning should be given so that as far as possible pregnancies can be postponed until after the leprosy is well under control.

(II) Increased surveillance.

(i) For women with active leprosy: increased surveillance during pregnancy, (a) to ensure a maximal patient compliance, if possible substituting parenteral for oral dapsone therapy during the first
trimestre if emesis gravidarum is troublesome; (b) routine assessments with skin smears and biopsies, as possible, during the second and third trimester and at 3 and 6 months postpartum, by which time most relapses should have occurred.

(ii) For women with cured leprosy (TT and BT/RFC): clinical assessment with particular attention to peripheral nerves during pregnancy and at 6, 12 and 18 months postpartum. Additional tests, namely skin biopsy, nerve biopsy (if possible), VMT, ST or EMG when relapse is suspected but clinical findings are not diagnostic.

(iii) For healthy contacts, especially of infectious cases: assessment during the third trimester of pregnancy and postpartum, ideally at 3 and 6 months.

(iv) For the child born to a woman who has had an active relapse during pregnancy there is risk of clinical leprosy in early childhood. This is likely to be of the indeterminate type and self healing, particularly in the very young child, and probably occurs more frequently than realized hitherto. Regular inspection at child health care clinics when weighing and measuring the child, naked, provides diagnostic opportunities. A history of lactation should be obtained as anti-leprosy drugs are transmitted through the mother’s milk. (This will be discussed more fully elsewhere.)

(III) Additional anti-leprosy drugs. The question of additional anti-leprosy drugs during pregnancy and lactation requires careful thought and is discussed elsewhere. To prevent relapse in TT and BT/RFC patients, we underline the recommendations made by Touw-Langendijk and Naafs that very careful review of the patient’s initial diagnosis, treatment, progress and date of leprosy ‘inactivity’ be made before the patient is RFC. We add to this a recommendation that no female patient be RFC either during pregnancy or within a year of delivery; all pregnancies and dates of delivery should be noted in the patient’s records. These two recommendations would reduce the number of patients being RFC prematurely. For those already RFC and becoming pregnant, increased surveillance during and after pregnancy and an awareness of the risk of relapse to allow early diagnosis is probably preferable to blind treatment of RFC patients for an empirical period of time during and after pregnancy, which could mask clinical relapse.

Acknowledgements

We thank the staff and patients of the Addis Ababa Leprosy Hospital for their help and co-operation throughout this study. We also thank the staff of the physiotherapy department, under the direction of Miss J Watson and
Mr W Brandsma, for performing Voluntary Muscle Testing and Sensory Skin Testing; Dr B Naafs for measuring nerve conduction velocities; the staff of the Records Department for their help not only in supplying hospital case records but for tracing and recalling defaulting patients for review; and Drs R St C Barnetson, RA Marshall and RJW Rees for advice on the script.

M Elizabeth Duncan was supported for part of the study by a research grant from the British Leprosy Relief Association (LEPRA).

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The association of pregnancy and leprosy

II. Pregnancy in dapsone-resistant leprosy

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Received for publication 26 January 1981

Summary Sixty-seven women with lepromatous leprosy were studied during 70 pregnancies and followed up during lactation; 6 patients were already dapsone resistant and an additional 4 were receiving dapsone 100 mg daily under trial conditions for suspected dapsone resistance. During the study 28 patients including the 4 already suspected of having dapsone resistance relapsed with probable dapsone resistance. While failure in patient compliance was thought to be important in some cases, recurrent pregnancies, by providing periods of physiological suppression of cell-mediated immunity, could well be the factor in causing the progression of dapsone resistance among women.

Introduction

Dapsone-resistant leprosy has become a major problem in Ethiopia. The incidence among patients with lepromatous leprosy in the Addis Ababa area in the period 1973–77 was about 3% per annum¹ and a high prevalence of primary dapsone resistance has also been reported.²

The factors contributing to the development of drug-resistant leprosy are probably much the same as those in tuberculosis. Inadequate dosage, irregular treatment and above all prolonged monotherapy all played a part in the causation of the Ethiopian epidemic of dapsone-resistant leprosy. However, it is possible that other factors, such as the immunosuppression associated with

Present addresses:
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JMH Pearson, Dhoolpet Leprosy Research Centre, Karwan, Hyderabad 500006, AP, India.
pregnancy, could play a part in determining the time at which incipient dapsone-resistant leprosy becomes clinically manifest.

Even in untreated lepromatous leprosy host factors are present which make some contribution towards controlling the infection. Thus, it is almost universally observed that such patients have only some 10% of solidly staining, presumed viable bacilli, the remainder are for the most part fragmented and non-viable, and therefore some host mechanism must be responsible for this process. Such a host factor must either prolong the generation time or (more likely) slow down the increase in bacillary load by killing a considerable proportion of bacilli without assistance by chemotherapy. If this control were lessened, for example as part of the process of immunosuppression during pregnancy, it could be expected that relapse or deterioration of untreated patients would be associated with pregnancy. Similarly, a subpopulation of dapsone resistant Mycobacterium leprae might be expected to show a rapid increase in numbers under these conditions, even if a patient were receiving mono therapy with dapsone.

This paper reports the results of a prospective study of the effects of pregnancy on lepromatous leprosy.

Patients and methods

The patients were all Ethiopian women of the low socio-economic class who lived in the villages surrounding the Addis Ababa Leprosy Hospital. There were 67 women (35 classified as having borderline lepromatous leprosy, BL and 32 with lepromatous leprosy, LL) studied throughout 70 pregnancies. They were all receiving outpatient treatment for leprosy and were first seen and taken into this study when they presented themselves at the Hospital Antenatal Clinic. Selection of patients was based on their willingness to participate in the study, to deliver their babies in hospital rather than at home and to be seen with their babies for regular assessment including blood tests for a period of up to 2 years during lactation. Intake of patients was staggered over 12 months.

Assessment of leprosy was made during pregnancy and after delivery at 6-month intervals whenever possible. This included inspection of skin lesions, clinical drawings, palpation of nerves and regional lymph nodes, slit skin smears and biopsies; full details are recorded elsewhere. When a patient was suspected of having developed dapsone-resistant leprosy, a biopsy of an active skin lesion, with a positive morphological index (MI), was taken and tested for dapsone resistance in all 11 cases by the mouse foot-pad technique.

Resistance to dapsone was defined as multiplication of M. leprae in mouse foot pads at a concentration of dapsone 0.0001% or more in the diet.
Results

Sixty-seven patients were included in the study (35 were BL, 32 LL); 3 of them were followed through 2 pregnancies. At the start of the study 6 patients were already diagnosed as dapsone resistant, and were taking clofazimine or rifampicin plus thiacetazone and dapsone. An additional 4 patients were suspected of dapsone resistance, and were receiving dapsone 100 mg daily under trial conditions with the maximum possible supervision and frequent assessments. Thus the initial prevalence of proven and suspected dapsone-resistant leprosy was 10/70, 14%; that is, much the same as the general prevalence among lepromatous patients at that time. The remaining patients were receiving dapsone 100 mg daily under routine outpatient clinic supervision.

During the course of the study an additional 24 patients showed clinical and/or bacteriological or histological deterioration despite apparently continuing to take dapsone, and were therefore considered to have prima-facie evidence of dapsone-resistant leprosy. In the majority of cases the diagnosis was clinical; the patients showed new active skin nodules, in which the BI and MI were raised. However, nearly half of the patients (10/24) gave indications of relapse on routine skin smears and/or biopsies before new skin lesions became evident, and an additional 3 cases showed definite relapse on smears/biopsies when there was only minimal clinical evidence of relapse. A striking feature of clinical relapse in these patients was the rapidity of development and increase in number of skin lesions after routine smears gave indications of relapse. The most rapid deterioration occurred toward the end of pregnancy when 7/24 women showed marked clinical deterioration during a 3-month period including part or all of the third trimestre. An additional 5 patients showed moderately rapid deterioration starting in the third trimestre and extending into the first 6 months of lactation.

It was not possible to perform mouse foot-pad tests for dapsone resistance in all cases. In some cases, because of shortage of mice, foot-pad tests were only done with DDS in low dietary concentrations. There were 4 ‘technical failures’ on account of delays in the biopsied material reaching the laboratory; in these cases repeat biopsies were not carried out as alternative dual therapy had been instituted on account of rapid deterioration during pregnancy. Four of the 6 patients, already designated as DDS resistant, had this confirmed by mouse tests prior to their present pregnancies. Results of the 7 patients tested successfully during the study are shown in Table 1. Three of these (Nos. 1, 2 and 7) were from the 4 patients suspected of being DDS resistant, but improving on dapsone 100 mg daily monotherapy under trial conditions prior to pregnancy; the remaining 4 were from the 24 patients relapsing for the first time during pregnancy. Of the patients whose detailed results are known 11/11 were resistant at 0.0001% DDS in diet, 6/8 were resistant at 0.001% DDS in diet and 6/6 were sensitive at 0.01% DDS in diet. None of the patients tested was proved dapsone sensitive.
Table 1. Results of mouse foot-pad assessment of dapsone sensitivity

<table>
<thead>
<tr>
<th>No. of Patients</th>
<th>Patient No.</th>
<th>Concentration of DDS in Mouse Diet</th>
<th>Human Equivalent Dose</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.01%</td>
<td>0.001%</td>
<td>0.0001%</td>
</tr>
<tr>
<td>Tested during study</td>
<td>7</td>
<td>1</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

ND = Not done.
R = Resistant.
+ = Growth of bacilli.
0 = No growth of bacilli.

The timing of relapse is shown in Table 2. Half the patients (14/28) relapsed clinically in the third trimester (in some cases a rising BI was detected earlier in the pregnancy) and most of the remainder within 6 months after delivery.

In addition to these patients, all 4 patients already suspected of dapsone resistance and being treated under trial conditions showed further deterioration during this study (3 in the third trimester or lactation, 1 at the end of the first trimester).

The complaint of 'rheumatism' was commonly associated with relapse, it preceded relapse in about half the patients, and was almost always a complaint at the time of relapse. ENL and neuritis preceded relapse in about half the patients (but were not uncommon in smear positive cases who did not relapse). At the time when relapse became clinically evident ENL was observed in half (14/28) of the patients. ENL was much more common than might be expected in BL patients, being recorded during pregnancy in 25% (9/36) of them: ENL was seen more frequently in BL patients who relapsed (33%: 5/15) than in BL patients who did not relapse (19%: 4/21).

Three BL patients downgraded to LL in association with relapse due to probable dapsone resistance; 2 downgraded during the third trimester of pregnancy, 1 at 6 months postpartum. The diagnosis was made on histological grounds. The 2 patients who downgraded during pregnancy at the time of clinical relapse, together with another patient (initially classified LL) who had also relapsed during the third trimester of pregnancy, all upgraded during lactation (1 to BL, 1 to BB/BL and 1 to BB/BT). The upgrading reaction was associated with new nerve enlargement or frank neuritis in all 3 cases and was confirmed by histology. The upgrading reaction was most marked at 6 months postpartum when all 3 women were still lactating and amenorrhoeic. In addition, and as reported elsewhere,4 3 patients downgraded to polar lepromatous
Table 2. Timing of clinical relapse due to probable dapsone resistance

<table>
<thead>
<tr>
<th>Classification of mother’s leprosy</th>
<th>BL</th>
<th>LL</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of pregnancies studied</td>
<td>34</td>
<td>34</td>
<td>70</td>
</tr>
<tr>
<td>No. of relapses</td>
<td>15</td>
<td>13</td>
<td>28</td>
</tr>
<tr>
<td>Timing of clinical relapse:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy trimestre – first</td>
<td>2</td>
<td>_</td>
<td>2</td>
</tr>
<tr>
<td>– second</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>– third</td>
<td>8</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>Lactation (3-month periods)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–3</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>4–6</td>
<td>1</td>
<td>_</td>
<td>2</td>
</tr>
<tr>
<td>7–9</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>10–12</td>
<td>1</td>
<td>_</td>
<td>2</td>
</tr>
<tr>
<td>13–15</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>16–18</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>19–21</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>22–24</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
</tbody>
</table>

leprosy (LL_p) showing for the first time, the histological features of LL_p during late pregnancy or the puerperium in association with relapse.

During the course of the study it became evident that there was an unexpectedly high incidence of probable dapsone-resistant leprosy associated with pregnancy. Therefore, case records of women already diagnosed as suffering from dapsone-resistant leprosy were reviewed, and the patients interviewed. An obstetrical history was obtained from 42 patients; 36 of them had had children after starting anti-leprosy treatment, of whom 31 first noticed new relapse nodules during pregnancy or soon after delivery or after a spontaneous abortion. Only 5 relapsed independently of pregnancy. The patients themselves were well aware that pregnancy had made their leprosy worse.

Discussion

Emergence of dapsone-resistant leprosy occurs more frequently when the dosage of dapsone is low or irregular; thus it will be associated with poor-patient compliance in taking dapsone regularly. Studies in Ethiopia have indicated that outpatients swallow approximately half the dapsone issued to them. This is the usual finding in such studies, though figures as high as 89% have been reported.

In the study we are reporting, less than 10% of women stated they had stopped taking dapsone for a few weeks during the first trimestre on account of emesis gravidarum. They all stated they had resumed treatment during the second trimestre. The rest of the women said they never stopped taking dapsone. Furthermore, it appeared to be generally believed that dapsone (unlike some other drugs) would not harm the foetus. The degree of patient—
doctor contact was high, and we considered it probable that the women took treatment regularly. However, from the results of the mouse foot-pad tests (Table 1) there is evidence that patients 2–7 could not have been taking dapsone regularly or fairly regularly, otherwise the lower level of dapsone-resistant mutants would have been killed. The failure of compliance demonstrated by these patients is disturbing. Nevertheless, it follows the pattern of other diseases; attempts to improve compliance by educating diabetic, hypertensive or tuberculosis patients about the importance of regular treatment have all failed.10

In Ethiopia, where dapsone resistance has become a major problem, dapsone resistance at a concentration of dapsone 0.0001% in the diet is referred to as low-grade resistance and has been shown to respond, for a period of up to 4 years, to treatment with dapsone 100 mg daily.11 But as these patients harbour a number of more highly resistant dapsone mutants12 in time resistance to higher dosage of dapsone emerges in a stepwise fashion.11, 12 This is in contrast with the single-step emergence of resistance to rifampicin.13 Recurrent pregnancies by providing periods of physiological suppression of CMI could well be a factor in contributing to the progression of dapsone resistance among women.

The suppression of CMI during pregnancy is also probably responsible for the extremely rapid deterioration observed during the third trimester of pregnancy—3 to 6 months for half of our patients compared with 12 months for clinical relapse in a male patient under closely controlled conditions.14 Downgrading and upgrading in association with relapse, occurring during pregnancy and lactation respectively is further evidence of the increased immunological instability associated with pregnancy.

The association of pregnancy and the emergence of dapsone-resistant leprosy is clear from the obstetrical histories of women already diagnosed as having developed dapsone-resistant leprosy. It is fully confirmed by this prospective study. Indeed, the difficulty is not to establish the relationship but to account for the excessively high incidence in the trial patients during the study period. Possible sources of error include:

(1) Selection of patients. Although to the best of our knowledge no special selection of patients occurred, it is possible that patients who were already feeling that all was not well regarding their leprosy opted to be in the study, thus applying some degree of self-selection.

(2) Overdiagnosis of relapse. This is not a serious possibility. The clinical and laboratory findings supported each other in most cases, as most of the patients showing (at first) only laboratory evidence of relapse, had relapsed clinically by the end of the study.

(3) Overdiagnosis of resistance. This again is unlikely. Four of the 6 patients already dapsone resistant (following relapse in a previous pregnancy) and
7 patients in the present study were tested by mouse foot pad tests and none showed dapsone-sensitive bacilli.

(4) One possibility is that in the early stages of emergence of dapsone-resistant leprosy the clinical signs are labile, and that relapse lesions might resolve between pregnancies, the condition progressing in a stepwise fashion. The relatively short period of this study prevents any definite conclusion but when last seen only 3 of the patients suspected of dapsone-resistant leprosy, but not tested in mice, were still improving on dapsone mono-therapy.

**Practical applications**

There are 3 important areas of application of these findings to the practical management of women with lepromatous leprosy.

(1) The possibility of giving supplementary chemotherapy in effective dosage during pregnancy and lactation might be considered: this would aim both to prevent the emergence of dapsone-resistant leprosy and also to lessen the risk of infecting the baby before and after delivery. Clofazimine (in the dosage of 100 mg at least 3 times a week) for 1 year starting at the beginning of the second trimestre would probably be the most suitable drug for the purpose, and would have the additional advantage of possibly reducing the amount of ENL occurring during pregnancy and lactation.

(2) There is a clear risk that pregnancy will make leprosy worse; patients frequently develop ENL and neuritis, which could be damaging even in the absence of dapsone resistance. It would be reasonable to advise women with lepromatous leprosy to limit the size of their families by whatever means are locally acceptable. However, it should be remembered that the role of exogenous oestrogens (such as are found in the contraceptive ‘pill’) in the causation of relapse is as yet unknown, and use of oral contraceptives should be carefully monitored. Personal interviews with patients who had suffered relapse or reaction in association with pregnancy not only revealed that the patients were aware of the adverse effect of pregnancy on leprosy, but in addition many patients volunteered the information that they wished for no more than 1 or 2 children at most.

(3) Should any of the children of women with dapsone-resistant leprosy develop leprosy at an early age, it is highly likely that they will have dapsone-resistant leprosy — and hence would require alternative therapy.

**Acknowledgements**

We thank Dr D S Ridley for independent histological assessment. The mouse foot-pad tests were carried out in the Armauer Hansen Research Institute.
(AHRI), Addis Ababa, Ethiopia and at the National Institute for Medical Research, Mill Hill, London. Drs R St C Barnetson, R A Marshall and D S Ridley’s comments on the script are appreciated. Above all we thank the patients and staff of the Addis Ababa Leprosy Hospital for their co-operation throughout this study. M E Duncan was supported for part of the study by a research grant from the British Leprosy Relief Association (LEPRA).

References


Leprosy and the community

COLOUR POSTERS ON LEPROSY. THE LEPROSY CONTROL PROGRAMME: SIERRA LEONE

Father Franci Fiori has very kindly drawn our attention (and sent examples) to the excellent colour posters on leprosy produced by this secretariat. These are of large size, suitable for public information, but also useful in clinics and for the teaching of para-medical staff. At least in the African context, they are perhaps the best so far produced and deserve a wide circulation. Although free of charge, it should be noted that reasonable mailing costs must be met. Apply to Father Fiori at the Leprosy Control Programme, PO Box 673, Freetown, Sierra Leone, Africa.

A ‘COMMUNICATION PROFILE’ FOR MEDICAL OFFICERS INVOLVED IN LEPROSY CONTROL. MISS TERESITA POSIS, HEALTH EDUCATION ADVISER, LEPROSY CONTROL SERVICES, DERMATOLOGY RESEARCH AND TRAINING, THE MINISTRY OF HEALTH, MANILA, THE PHILIPPINES

During a recent seminar in Cebu, the Philippines, organized by the Sasakawa Memorial Health Foundation, Teresita Posis introduced the questionnaire which appears below, with the object of obtaining systematic information from those in leprosy control on (1) their attitudes towards leprosy control and (2) their current techniques for disseminating information about leprosy to various sections of the community. It was also thought that its use would help in the identification of further needs in the field of health education in the Philippines.

It may in fact have a wider application in other countries where health education in leprosy is either non-existent or patently ineffective. We reproduce some sections in the hope that readers may try it out or adapt it — and report on their experiences to the pages of this journal.

A Study of the ‘Communication Profile’ of Medical Officers in Leprosy

I. PERSONAL BACKGROUND

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>M</th>
<th>F</th>
<th>Civil Status</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Religion</th>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Official Position</th>
</tr>
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</table>

<table>
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<tr>
<th>Length of Service in this Position</th>
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<table>
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<tr>
<th>Area of Coverage as per Agency Assignment</th>
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<table>
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<tr>
<th>Length of Appointment to the present Area Assignment</th>
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</table>

<table>
<thead>
<tr>
<th>Main/Major responsibility (Pls Check)</th>
</tr>
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</table>

Training | Research | Clinical |

<table>
<thead>
<tr>
<th>Administration</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Highest Edu. Attainment</th>
</tr>
</thead>
</table>
272  Leprosy and the community

Training/Seminar/Workshops Attended/Organized (Pls mention all those related to Leprosy/TB only)

a. As participant Date/Duration Place and Sponsoring Agency

b. As Resource Speaker/Organizer

II. ATTITUDE TOWARDS THE LEPROSY CONTROL PROGRAM

You may agree, disagree or be undecided with each of the following statements. Please put a (/) on the blank corresponding to your attitude. There is no correct or wrong answer.

STATMENTS                  AGREE   DISAGREE   UNDECIDED
1. Leprosy is one of the most serious public health problems in the country today and therefore deserves high government priority attention.
2. Integration with the basic health services is the best possible approach to the prevention and control of leprosy in the Philippines.
3. To create greater impact on the community, the total leprosy control program should be implemented by only one appropriate agency.
4. To improve casefinding and case-holding, leprosy control program should utilize all peripheral health workers of the RHU.
5. Monitoring and supervision of the leprosy control program by the national staff will help attain program objectives.

Section 3 is entitled ‘Communication Behaviour’ and deals mainly with communication media, and Section IV with ‘Communication Contacts’ – how do you obtain information about leprosy? The full document may be obtained from Teresita Posis at the above address.
Field Workers’ Forum

A PRACTICAL CLASSIFICATION OF LEPROSY FOR FIELD WORKERS

In the recent past there has been much discussion among Indian leprologists on the need to review the Indian classification of leprosy and on the form the new classification should take. There is a body of opinion in India that the Ridley–Jopling classification, though valuable to immunologists and research workers, is unsuitable for field workers; for example, Dharmendra complains that ‘all clinical forms of leprosy cannot be fitted into it’, and Desikan writes ‘it is scientifically sound, but cannot be adopted by field workers’. As regards Dharmendra’s criticism I am satisfied that all clinical forms can be fitted into it if we exclude indeterminate leprosy, a transient form which can only very rarely be proved,

Table 1. A classification of leprosy for field workers (based on the Ridley–Jopling classification)

<table>
<thead>
<tr>
<th>Observation or test</th>
<th>TT</th>
<th>BT</th>
<th>BB–BL</th>
<th>LL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of skin lesions</td>
<td>Single usually</td>
<td>Single or few</td>
<td>Several or many</td>
<td>Very many</td>
</tr>
<tr>
<td>Size of lesions</td>
<td>Variable</td>
<td>Variable</td>
<td>Variable</td>
<td>Small</td>
</tr>
<tr>
<td>Surface of lesions</td>
<td>Very dry, sometimes scaly</td>
<td>Dry</td>
<td>Shiny</td>
<td>Shiny</td>
</tr>
<tr>
<td>Hair growth in lesions</td>
<td>Absent</td>
<td>Moderately diminished</td>
<td>Slightly diminished</td>
<td>Not affected</td>
</tr>
<tr>
<td>Sensation of lesions (not face)</td>
<td>Completely lost</td>
<td>Moderate—marked loss</td>
<td>Slight—moderate loss</td>
<td>No loss</td>
</tr>
<tr>
<td>AFB in smears from lesions</td>
<td>Nil</td>
<td>Nil or scanty</td>
<td>Several—many</td>
<td>Many (plus globi)</td>
</tr>
<tr>
<td>AFB in noseblows or in nasal scrapings</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil (scanty rarely)</td>
<td>Many (plus globi)</td>
</tr>
<tr>
<td>Lepromin test</td>
<td>Strongly positive (+ + +)</td>
<td>Weakly positive (+ or + +)</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

This scheme applies whether skin lesions are flat (macules) or thickened (plaques). If you find a single macule, lighter in colour than the surrounding skin (in a dark-skinned patient), with normal sensation and no AFB, refer the patient to the Medical Officer in case the diagnosis is indeterminate leprosy. Do likewise if the patient has one or more thickened nerves but no skin lesions, in case the diagnosis is pure neural leprosy. AFB = acid-fast bacilli. TT = tuberculoid. BT = borderline-tuberculoid. BB–BL = mid-borderline and borderline-lepromatous. LL = lepromatous.

and as regards Desikan’s comment the Ridley–Jopling classification can, with one minor modification, be easily understood and used by field workers. The modification involves not giving a separate description to mid-borderline (BB) leprosy but including it with borderline-lepromatous (BL) leprosy as these 2 groups have skin lesions which are not easily differentiated clinically, acid-fast bacilli (AFB) are present in skin lesions, and both are negative on lepromin testing — see Table 1. On the vexed subject of indeterminate leprosy — a type of

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leprosy which appears prominently in Indian and South American classifications — my opinion is that it has no place in a classification for field workers (just as Dr Ridley and I excluded it when we drew up our classification). What field workers need to know about it is that it presents, usually in children, as a single hypopigmented flat lesion (macule) which may be self-healing, that they will not be able to demonstrate sensory impairment in the lesion, nor will they find AFB in it, and therefore it would be dangerous for them to make a diagnosis of leprosy; the patient should be referred to the Medical Officer. Similarly, pure leprosy can be excluded from a classification for field workers on the grounds that there are no skin lesions, and a good deal of experience is required to identify thickened nerves with certainty, but they should suspect it if the thickened nerve feels harder and stiffer than the nerve on the opposite side, or if its surface feels irregular, especially if there is associated evidence of nerve damage in the form of anaesthesia with or without muscle weakness or wasting. Such cases should be referred to the Medical Officer. However, these pure neural cases can be readily included in the Ridley–Jopling classification with the help of a lepromin test and a nerve biopsy. I have deliberately included a lepromin test in my classification for field workers, for it gives great help in classification, it is easy to carry out and interpret and increasing quantities of lepromin are likely to become available, thanks to the countless millions of leprosy bacilli supplied by infected armadillos.

References

1 Ridley DS, Jopling WH. Classification of leprosy according to immunity: a five-group system. *Int J Lepr*, 1966, 34, 255.


WH JOPLING

INFORMATION CONCERNING AUDIO-VISUAL LOAN PROGRAMME OF NATIONAL HANSEN'S DISEASE CENTER, CARVILLE, LOUISIANA

We gratefully acknowledge permission to publish the following information about audio-visual teaching and learning material from Carville, recently reviewed from the Director of Education and Training, Dr Richard O'Conor.

A primary function of the National Hansen's Disease Center, Carville, Louisiana, is to promote an increased awareness of Hansen's disease among the medical community. Accordingly, the Center has instituted an audio-visual loan programme by which instructional materials produced for in-house use may be loaned to other medical and educational institutions for teaching purposes. Materials are loaned at no cost for a 2-week period. A list of materials available for loan is attached.

Further information concerning this programme, as well as availability of other training and educational material in Hansen's disease, may be obtained by contacting:

Director of Education and Training, National Hansen's Disease Center, Carville, Louisiana 70721, (504) 641-7771 Ext. 281 (FTS) 687-0205

A. 35 mm Slide Series (Typescripts Included)

**CARE OF THE HAND IN LEPROSY**

A set of 57 slides developed by Ms Helen Ramsammy, Chief Occupational Therapist at Carville. The series focuses on the need to adapt tools, cooking utensils and other objects in
everyday use to protect persons with insensitive hands from injury. Illustrations are also provided of proper splinting techniques and other procedures used by professional healthcare specialists in leprosy.

**Clinical Aspects of Leprosy**
This set of 42 slides has been developed at Carville to visualize an introductory lecture on leprosy for medical students and other health professional personnel attending 1-day seminars. The accompanying script is lengthy and should be reviewed thoroughly by the instructor before presenting the slides.

**General Concepts in Hansen's Disease**
A set of 40 slides and typescript depicting various skin lesions and other manifestations of leprosy. Slides of the Carville facility and various research activities in leprosy are included. The slides are in no particular order and should be integrated individually into other slide presentations.

**Histopathological Aspects of Leprosy**
This series of 48 teaching slides and typescript was developed by Dr Richard Mansfield and is intended to provide an overview of histopathological concepts important in the diagnosis of leprosy.

**Ophthalmological Aspects of Leprosy**
The series of 53 slides on eye care in leprosy has been developed by Dr Margaret Brand to illustrate her lecture to resident dermatologists attending Carville 2-day seminars. It is quite specific in content and should be used only by those already quite familiar with fundamental concepts in leprosy.

**Prescription Footwear**
This set of 50 slides is designed to acquaint persons with methods involved in the fabrication of prescription footwear.

**Skin Smear Techniques**
This set of 37 slides demonstrates the procedure for obtaining skin smears, staining slides and microscopic examination of skin smears for leprosy bacilli.

**B. Instructional Television Programmes (Available in ¾" U-matic or ½" Beta formats)**


**Care of the Inensitive Hand** (22 min., colour, Oct. 1979, English and Spanish). Programme visually demonstrates recognition signs, evaluation methods and management practices utilized in care of the insensitive hand by Occupational Therapy Department.

**Diagnosis of Hansen's Disease** (16 min., colour, 1977, English and Spanish). Discusses signs, symptoms and areas of involvement. Emphasizes diagnostic techniques. Shows clinical manifestations and presents differential diagnosis aspects.

**Effects of Mechanical Stress** (46 min, colour, 1977). Discusses and illustrates 4 types of force destructive to insensitive foot. Research and thermographic studies shown. Lecture by Dr Paul W Brand, Chief, Rehabilitation Branch.

REHABILITATION IN HANSEN'S DISEASE (60 min., colour, 1976) Programme illustrates disease processes and associated aspects of physical rehabilitation. Programme taped during lecture to Tulane medical students by Paul W Brand, FRCS, Chief, Rehabilitation Branch.

PRESSURE ASSESSMENT METHODS (13 min., colour, 1977, English and Spanish). Programme demonstrates procedures to be followed in conducting: 1. The Harris Mat Test. 2. Slipper Sock Test. Tests are used for evaluation of uneven force distribution and pressure on insensitive feet.

SKIN SMEAR TECHNIQUES (20 min., colour, 1980). Programme features laboratory technique unique to Hansen's Disease, used for diagnostic confirmation, classification and treatment response. Includes patient selection, skin scraping, slide staining and interpretation, i.e. morphological and bacteriological index readings.

SO, YOU HAVE HANSEN'S DISEASE (44 min, colour, 1979, English and Spanish). Basic patient education/orientation programme for newly diagnosed HD patients at Carville. Presents medical orientation by Carville staff members and a video tour of activities and services for Carville patients.

THE EYE IN HANSEN'S DISEASE (40 min., colour, 1977). Illustrates slide presentation by Dr Margaret Brand, Chief, of the Ophthalmology Department, on common eye problems in Hansen's disease, and their management. Programme stresses preventive aspects of eye care in Hansen's disease.

THE TOTAL CONTACT CAST (17 min., colour, 1978, English). Practical demonstration of plaster-casting technique used at Carville. Contact cast enables patient with plantar ulcer to be ambulatory and promotes healing of ulcer.


VISITOR'S INTRODUCTION TO HANSEN'S DISEASE (9 min., colour, 1976, English and Spanish). A non-medical orientation to Hansen's disease for Carville visitors. Programme cites several common misconceptions about the disease and provides general descriptive information about contemporary management techniques.

C. Audiotapecs

CLINICAL ASPECTS (60 min., 1980). Lecture by Robert R Jacobson, MD, PhD, Chief, Clinical Branch.

TREATMENT OF UNCOMPPLICATED LEPROSY (60 min., 1980). Lecture by Robert R Jacobson, MD, PhD, Chief, Clinical Branch.

PREGNANCY IN LEPROSY: EFFECTS ON MOTHER AND CHILD (60 min., 1980). Lecture by Dr ME Duncan, National Institute for Medical Research, London, England.

MICROBIOLOGY OF LEPROSY (60 min., 1980). Lecture by EJ Shannon, PhD, Immunologist, Pharmacology Research Department.
SPECTRUM OF LEPROSY (60 min., 1980). Lecture by Roy E Pflatzgraff, MD, Garkida Hospitals, NE State, Nigeria.

RECENT PROGRESS IN BIOMEDICAL LEPROSY RESEARCH (60 min., 1980). Lecture by WF Kirchheimer, MD, PhD, Chief, Laboratory Research Branch.

WHO TROPICAL DISEASE RESEARCH AND TRAINING PROGRAM (60 min., 1980). Lecture by Barnett L Cline, MD, PhD, Tropical Medicine Tulane University, New Orleans, LA.

REHABILITATION CONCEPTS IN LEPROSY (60 min., 1980). Lecture by Paul W Brand, FRCS, Chief, Rehabilitation Branch.

CARE OF THE EYE (60 min., 1980). Lecture by Margaret Brand, MB, BS, Chief, Ophthalmology Department.

REACTIONS AND THEIR TREATMENT (60 min., 1980). Lecture by Robert C Hastings, MD, PhD, Chief, Pharmacology Research Department.

IMMUNOLOGICAL RESEARCH (60 min., 1980). Lecture by Robert C Hastings, MD, PhD, Chief, Pharmacology Research Department.

PROSPECTS FOR LEPROSY CONTROL IN THE NEXT DECADE (60 min., 1980). Lecture by Robert R Jacobson, MD, PhD, Chief, Clinical Branch.

DERMATOLOGICAL ASPECTS (60 min., 1980). Lecture by SL Moschella, MD, Lahey Clinic, Boston, Massachusetts.

THE INTERNATIONAL PROBLEM OF HANSEN’S DISEASE (60 min., 1980). Lecture by W Felton Ross, MB, Medical Director, American Leprosy Mission.

IMPACT OF INSENSITIVITY (60 min., 1980). Lecture by Paul W Brand, FRCS, Chief, Rehabilitation Branch.

FUNCTIONAL ANATOMY OF THE FOOT/ANKLE COMPLEX (60 min., 1980). Lecture by Thomas McPoil, Jr, RPT.

PATHOMECHANICS OF SOFT TISSUE (60 min., 1980). Lecture by Paul W Brand, FRCS, Chief, Rehabilitation Branch.

MATERIALS USED IN FOOT MANAGEMENT (60 min., 1980). Lecture by Ronald S Brocato, RPT.

BIOMECHANICAL CONSIDERATIONS OF THE FOREFOOT TO REARFOOT DURING GAIT (60 min., 1980). Lecture by William C Coleman, DPM.


SHOE MODIFICATION TECHNIQUE FOR THE HYPO AND HYPERSENSITIVE (60 min., 1980). Lecture by Freddie Childress, CPed and John O McMahan, CPed.

ROCKER BOTTOM vs ROLL-OVER TO MODIFICATION IN SHOE DESIGN (ANKLE ON THE FLOOR CONCEPT) (60 min., 1980). Lecture by Paul W Brand, FRCS, Chief, Rehabilitation Branch.
EUROPEAN LEPROSY SYMPOSIUM, 1–3 MAY 1981

Under the auspices of the Italian Leprosy Relief Organization ‘Amici di R Follereau’, and with the participation of WHO and ILEP, a Symposium on leprosy in light-skinned people was held in Santa Margherita Ligure, near Genoa, Italy, on 1–3 May 1981. The objectives of the Symposium were two-fold: first, to give up-to-date information about the most important aspects of leprosy, with emphasis on clinical presentation as seen in Europe, and secondly, to publish the proceedings together with an atlas of clinical manifestations of leprosy in patients with light skins. Five thousand copies of these proceedings are to be sent to dermatology and infectious diseases departments of hospitals in Europe and the USA.

During the first two days of the Symposium papers were given by F Cottenot (France) on symptomatology and clinical diagnosis. KF Schaller (ILEP Medical Commission) on differential diagnosis, J Terencio de las Agas (Spain) on laboratory procedures, SR Pattyn (Belgium) on culture of Mycobacteria leprae, MF Lechat (Belgium) on epidemiology, ADM Bryceson (Kenya) on immunology, RH Cormane (Netherlands) on mechanisms of nerve damage, J Convit (Venezuela) on immunotherapy and immunoprophylaxis, WH Jopling (UK) on clinical classification, J Bodingius (Netherlands) on patient compliance with treatment, and SK Noordeen (WHO, Geneva) on chemoprophylaxis. The third day was devoted to short case histories, illustrated by colour transparencies, in which 20 speakers took part; a selection of these will be reproduced in an atlas.

The organizers are to be congratulated on the success of this well attended Symposium held in one of Italy’s beauty spots, and a debt of gratitude is owed to Lepeit Pharmaceuticals Ltd for financial backing and for providing a memorable Dinner on 2 May for participants and guests.

SASAKAWA MEMORIAL HEALTH FOUNDATION, TOKYO, JAPAN

We gratefully acknowledge receipt of two important booklets from Mr Suminori Tsurusaki, the General Secretary of SMHF.

1. The Way Toward Eradication of Hansen’s Disease. This is the text of an address delivered by Professor Michel Lechat (Belgium) on the occasion of a special meeting held in Tokyo in September 1980, under the theme ‘Health and Peace’.

2. Health for All by the Year 2000. Text of an address delivered by Dr H Mahler, Director General of WHO, who was another main speaker at the above meeting. (It is of particular interest since it has a section dealing specifically with leprosy and primary health care.)
ROYAL IRISH ACADEMY: IRISH SCIENTISTS WIN THE 1980 UNESCO SCIENCE PRIZE

The UNESCO Science Prize for 1980 has been awarded jointly to a group from the Laboratories of the Medical Research Council of Ireland, Dr JG Belton, MRIA, Dr ML Conalty, MRIA, Dr JF O'Sullivan and Dr D Twomey, MRIA, for the discovery of the anti-leprosy agent clofazimine, and to Dr L Mata of Costa Rica for his work on malnutrition and infection.

The Prize, which was presented at UNESCO House in Paris at the end of May, is awarded biennially to an individual or group to acknowledge ‘an outstanding contribution, through the application of science and technology, to the development of a developing Member State or region’. It is open to all Member States of the United Nations Organization, each government being entitled to nominate one candidate. This is the first occasion an Irish nomination has been made.

In 1944 a Medical Research Council of Ireland team, headed by the late Dr Vincent C Barry, was set up to develop new antituberculosis agents and, since then, has also been engaged in the development of compounds for other bacterial diseases and for the treatment of cancer.

In the course of their investigations a series of compounds (rimino-phenazines) was discovered, many of which were found to be active against tuberculosis in experimental animals. Because it was observed that these agents concentrated within cells of a type in which leprosy bacilli were known to develop, arrangements were made for a clinical trial of the most active compound, B663 (clofazimine), in leprosy, by Drs SG Browne and LM Hogerzeil in Nigeria. This and other extensive trials established clofazimine as a first line drug in the treatment of leprosy. In this way the group’s efforts have contributed to the health of people living in all developing regions where leprosy is endemic and it is hoped it will play a major role in the eventual eradication of this disease.

Three members of the group (Dr ML Conalty, Dr JF O'Sullivan and Dr D Twomey) visited India in March/April 1980 under the auspices of the Department of Foreign Affairs, to advise on the manufacture of clofazimine by IDL Chemicals Ltd, of Hyderabad and Bangalore, who, in collaboration with the Central Drug Research Institute of the Indian Government, are to undertake the production of clofazimine for sale in India on a non-profit basis.

In addition to the foregoing, Drs O'Sullivan and Conalty, in collaboration with Dr NE Morrison of Johns Hopkins University, Baltimore, USA, are now in the fourth year of a project, funded in part by the World Health Organization, to develop new analogues of clofazimine which would be active against clofazimine-resistant strains of Mycobacterium leprae, should these emerge.

The members of the Irish research team, who were presented with the Prize at 5.00 p.m. on 25 May 1981, during the 112th session of the Executive Board of UNESCO, were nominated to UNESCO by the Irish Government in consultation with the Irish National Commission and on the advice of the Royal Irish Academy.

THE ROYAL SOCIETY FOR THE ENCOURAGEMENT OF ARTS, MANUFACTURES AND COMMERCE, LONDON, LECTURE BY DR RJW REES 'THE APPRAISAL OF MEDICAL RESEARCH IN THE TREATMENT AND CONTROL OF LEPROSY'

This lecture was delivered in London on 24 March 1981 by Dr RJW Rees, Head of the Laboratory for Leprosy and Mycobacterial Research, the National Institute for Medical Research and Chairman, Medical Advisory Board of LEpra. The meeting was chaired by the Dean of the London School of Hygiene and Tropical Medicine, Professor CE Gordon Smith and attended by a distinguished audience of medical and scientific workers, mainly from the London area.
THE INTERNATIONAL UNION AGAINST TUBERCULOSIS: XXVth WORLD CONFERENCE, BUENOS AIRES, ARGENTINE REPUBLIC, 21–24 APRIL 1982

Programme: 1982 is the centenary of the discovery of the tubercle bacillus by Robert Koch. In view of the significance of the year 1982 for tuberculosis and renewed efforts against this disease, an important session will be that in which the WHO and the IUAT will express their views on the antituberculosis campaign in the next two decades and their own part in it. Presentations will also be made on recent progress and future prospects in epidemiology, bacteriology, chemotherapy and prevention of tuberculosis.

Other sessions will combine tuberculosis and non-tuberculosis respiratory disease, with special reference to immunology and the problems of diagnosis, treatment and prevention of tuberculosis and acute respiratory disease in children.

They will also include clinical, diagnostic and therapeutic advances in tuberculosis, respiratory disease and leprosy, simplified standardized techniques and their efficacy, the delivery of services and the application of control programmes at community level, criteria for defining cases and examination norms, epidemiological procedures, data gathering and forecasting and evaluation procedures and surveillance and their significance.

All enquiries to: International Union Against Tuberculosis, 3 rue Georges Ville, 75116 Paris, France.

EDITORIAL NOTE: PLANS FOR THE JOURNAL IN 1982

It was our original intention to publish a special number or a supplement with Number 4 of this volume (1981) on the subject of 'Leprosy and Primary Health Care'. Despite a great deal of correspondence with many experts in the field of leprosy in different parts of the world, it has, however, become clear that most of those who are most likely to contribute original material will need longer for its preparation. Taken with our decision to issue a supplement on the papers given at the International Symposium on the Epidemiology of Leprosy in Oslo, Norway, September 1981, we have therefore decided to postpone further publication on the subject of leprosy in relation to primary health care until 1982. Meanwhile we would greatly appreciate original papers or correspondence on this subject.

During 1982, we plan to publish editorials from invited contributors on the subjects of rifampicin, steroids, thalidomide and teaching and training in leprosy. Here again, related material in the form of original articles or correspondence will be welcome.

LEPROSY REVIEW: CIRCULATION GOES OVER 1,000 IN 1981

We record with pleasure that the fact that the circulation of this journal is now well over 1,000, and steadily increasing. It is issued to 108 different countries with a wide distribution over the leprosy-endemic world. Thanks to the packaging and posting direct from the LEPRA office in Colchester, it is clear that delivery has greatly improved, with concomitant reduction in previous misunderstandings about subscriptions and payment.

ARMAUER HANSEN RESEARCH INSTITUTE, PO BOX 1005, ADDIS ABABA, ETHIOPIA. CONFERENCE ON IMMUNOLOGICAL ASPECTS OF LEPROSY, TUBERCULOSIS AND LEISHMANIASIS, 27–30 OCTOBER 1980, ADDIS ABABA

In drawing attention to this conference in Leprosy Review, 52 (1981) we apologise for the completely wrong list of papers, 1–10. The main subject headings were in fact as follow: Basic Immunology; Antigenic Structure of Mycobacteria; Mycobacteria and Leishmania; Clinical and Immunological Aspects; Mechanisms of Tissue Damage; Effector and Escape Mechanisms; Experimental Aspects of Leprosy, Tuberculosis and Leishmaniasis; Immunogenetics and Epidemiology; Vaccines; Present and Future. Editor
Letters to the Editor

CHROMOGENS FOR THE ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) USING HORSE-RADISH PEROXIDASE

Sir,

Horse-radish peroxidase (EC1.11.1.7) is rapidly gaining popularity as a cheap and stable enzyme for use in the enzyme-linked immunosorbent assay (ELISA). There are at least three easy and efficient ways to conjugate it onto immunoglobulin, or antigens.\(^1\)-\(^3\) However, there is still some confusion as to the best way to quantitate the enzyme.

The presence of peroxidase is revealed by the development of a coloured product, when the enzyme interacts with hydrogen peroxide (H\(_2\)O\(_2\)) and a chromogen. The chemistry involved is not fully understood and is bewilderingly complex.\(^4\) The ideal chromogen should be cheap, stable, easily used and non-carcinogenic and the change in optical density should be large in relation to the amount of enzyme present (e.g. the extinction co-efficient should be high).

The last point deserves amplification. If the optical density achieved in the presence of a certain quantity of enzyme is low, the sensitivity of the assay is obviously also low. At first sight this might seem unimportant for most purposes, because in ELISA assays it is usually possible to increase the concentration of enzyme conjugate used until readable results are obtained. However, not only does this waste reagents, but it is not even a satisfactory solution to the problem because the use of an insensitive chromogen has another more serious disadvantage. This becomes apparent when the optical density achieved is plotted against the quantity of enzyme present. With a poor chromogen this dose-response curve is relatively flat and the test therefore achieves very poor discrimination between 'positive' and 'negative' samples.\(^1\)-\(^2\), \(^5\)

The available chromogens

A number of possible chromogens have become obsolete because of carcinogenicity and insolubility (o-dianisidine), or instability of the coloured product, (o-tolidine), and need not be considered further.

Those currently in use are ortho-phenylene diamine (OPD), 2,2'-azino-di (3 ethyl) benzothiazoline-6-sulphonic acid (ABTS) and 5-amino salicylate (5-AS).

Of these three 5-AS should probably now be regarded as obsolete. When compared with OPD\(^1\)-\(^2\) or o-tolidine\(^5\) it proves so insensitive and gives such a flat dose-response curve that its use for the type of assay described by Huikeshoven and his colleagues\(^6\)-\(^7\) cannot be recommended. The ratio of positive to negative results in a number of assays can be 5 to 20 times lower with 5-AS than with more efficient chromogens\(^1\), \(^5\) and higher concentration of conjugate and longer incubation periods are needed.\(^5\) Moreover, the relative insolubility both of 5-AS and its coloured reaction product is a major problem with many batches of the compound.
There is now general agreement that the greatest sensitivity is achieved with OPD\textsuperscript{1,2} (own unpublished data). This compound is available cheaply from Sigma (Cat. No. P-3888). Optimal results are obtained when 34 mg of OPD are dissolved immediately before use, in 100 ml of citrate/phosphate buffer (0.15 M, pH 5.0) containing 30 $\mu$l of 6\% (20 vol\%$) H$_2$O$_2$. The reaction can be stopped with 12.5\% H$_2$SO$_4$ and the results are read at 492 nM. This chromogen does, however, have two minor disadvantages. First, it has been said to be mutagenic\textsuperscript{8} but the author is not aware of any reports of carcinogenicity, and there are no restrictions on its use. It is recommended by most suppliers of reagents and equipment for ELISA assays. The second disadvantage is its photosensitivity and the tendency for the colour to darken slowly even after the reaction has been stopped. This means that tests should be read immediately.\textsuperscript{2}

An alternative to OPD is ABTS (Sigma, Cat. No. A-1888). This was developed by Boehringer as a non-toxic chromogen, and no reports of toxicity were revealed by a recent computer search of the Cancerline and Toxline data bases (although there is a report\textsuperscript{8} that like OPD, ABTS is mutagenic in the fluctuation test and the Ames test, when used at high concentrations). Fifty milligrams of ABTS should be dissolved in 100 ml of citrate/phosphate buffer (0.1 M, pH 4.0) with 30 $\mu$l of 6\% H$_2$O$_2$. The reaction can be stopped with sodium fluoride (final concentration of 0.64 mg/ml). Used in this way, both the substrate and the reaction product are remarkably stable and the tests do not have to be read at once. There are two absorption peaks, one at 414 nM and one at 650 nM. The latter results in a blue colour which is particularly easy to read visually. It also allows the design of a very simple battery-powered photometer for accurate through-the-well reading of the plates,\textsuperscript{9} because monochromatic light of approximately this wavelength can be obtained with a red light-emitting diode. Most workshops should be able to construct such a device.

In spite of all these advantages, ABTS is somewhat less sensitive than OPD, which in our hands gives absorbance values two to four times higher. This difference is not enough to matter and it is vastly superior to 5-AS. Its other advantages may lead to acceptance of ABTS as the chromogen of choice for many applications.

In summary, there are now two safe, soluble, inexpensive and sensitive chromogens for use in peroxidase-based ELISA systems. These are o-phenylene diamine (OPD), and 2,2' azino-di (3 ethyl) benothiazoline-6-sulphonic acid (ABTS).

G A W ROOK

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References


BACTERIAL AND MORPHOLOGICAL INDICES IN LEPROSY

Sir,

We wish to make some comments on the letter published in Leprosy Review, 1980, 51, 361 by Schreuder and Colpa.

It appears that some time can be saved for an experienced laboratory assistant by recording his findings along the line of Ridley's SFG index (Leprosy Review, 1971, 42, 96–7) because if, for example, the proportion of solids is (at one glance) between 1 and 19%, then he does not have to decide in each doubtful case whether he sees a solid AFB or a fragmented AFB. We feel, however, that by using Ridley's SFG index a great deal of potentially useful information is lost. For instance, one would no longer know whether the proportion of solids was 2% or 20% (both would give the value 1 for Ridley's index) or whether a patient has 25% fragmented AFB and 75% granules or 75% fragmented AFB and 25% granules (both situations would give the value 2 for the SFG index), which surely is of interest.

Secondly, it appears useful to us to observe a shift to the right (i.e. from solid towards granular) in the bacterial morphology during the course of treatment, as a sign that the compliance of the patient is adequate and that there is no primary resistance. This is only possible if the relative percentages of solid, fragmented and granular AFB are recorded in detail, as has been done for the past 15 years in Malawi in a standardized way. A typical smear result of a new, well-established lepromatous patient would be recorded by us as follows: 5+, 15, 47, 38 (BI SFG%). In the case of a low BI we record the actual numbers of S, of F and of G seen, rather than their percentages.

Ridley's SFG index would only allow a relatively rough monitoring of the patient's progress, although it would probably pick up the emergence of secondary resistance as quickly as a more detailed recording of the proportion of solids, fragments and granules.

Another question we have been asking ourselves recently is whether it is more meaningful to record the average of the BI and of the proportion of S and F (and G) AFB, or the highest value, if smears are taken from several sites, which is the usual case. In relapsed patients with new lesions, the results of slit-skin smears from these new lesions are of critical importance. We should not allow the results of concurrent smears from routine sites (such as the earlobes, which might still be low in BI and might have no solid AFB yet) to depress the alarming facts into a smoothing average. We would very much welcome comments on this question.

LEPRA Control Project
P.O. Chilumba
Malawi

G BOERRIGTER
J M PONNIHAUS

LEPRA Control Project
P.O. Chilumba
Malawi
Letters to the Editor

'SGF INDEX': REPLY TO 'BACTERIAL AND MORPHOLOGICAL INDICES IN LEPROSY'

Sir,

Drs Boerrigter and Ponnighaus are correct in saying that each of the code values (2, 1, 0) for solid, fragmented and granular bacilli represents a wide range of percentages of each of those forms. Any one who needs to know the actual percentage of particular forms of bacillus is recommended to do an arithmetic count, always provided that he has the time to do so, that he has accurately defined the 3 forms and has demonstrated reproducibility of counts among observers and reproducibility of morphology in spite of vagaries of stain technique. This would be more accurate and there may be research requirements which would demand it, but it is not easy.

The rationale of the SFG index is that the latitude of any code value is limited by the other two code values. This may operate through the obvious fact that the actual percentages have to add up to 100, and if one or two go up something else must come down. And it may operate through the probability that a redistribution between, say, fragmented and granular forms will not come about without some change also in the number of solids. In the example given by your correspondents, the number of solids would be unlikely to remain at 20% or 2% while a preponderance of fragmented forms was replaced by a preponderance of granular forms. This would be the situation when the index fell from 5 to 3. Thus the SFG index is most useful for showing a shift to the right, during treatment, or the left in relapse. This is just what Drs Boerrigter and Ponnighaus say requires an actual percentage count, which makes one wonder if they have ever tried the SFG index in practice.

The index was originally proposed for routine clinical use, but during 20 years experience it has been found satisfactory by us for at least some research purposes.

As regards the last point raised in the letter, it is correct to record indices as the average of all sites, but in the special situation of a reappearance of solid forms at one site, indicative of relapse, most people would surely draw attention to the fact and record it separately.

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DS RIDLEY

TRAINING IN LEPROSY OF MEDICAL STUDENTS IN ETHIOPIA

Sir,

As long as leprosy control depends mainly upon early detection and adequate treatment of patients the battle cannot be won until a satisfactory distribution of health services and a proper training of all health personnel in the recognition and management of leprosy patients has been established. The training of such personnel is particularly important where leprosy work is rapidly becoming integrated into the general health services. In the training programmes high priority should no doubt be given to the medical students.

Most students will, after their graduation, be posted to rural hospitals and health centres. For many of them this will sooner or later imply a teaching task in the district – or regional training schools for other cadres of health personnel such as nurses, medical assistants, health assistants, rural medical aids, sanitarians, etc. The young doctor will also become responsible for the proper management of leprosy patients referred to the hospital because of complications of the disease.

It follows, therefore, that the medical schools should provide a thorough training in leprosy not only to prepare future doctors for this clinical task but also to enable them to teach various cadres of health personnel, upon whose participation the success of leprosy control so much depends.
In 1978 the Medical Faculty of Addis Ababa University took the decision to have the medical undergraduates trained in leprosy and dermatology at the All Africa Leprosy and Rehabilitation Training Centre (ALERT) for a period of 4 weeks instead of the usual 2 weeks. In addition, training in leprosy field work was included in their 6 weeks assignment to a rural health centre, so that they could study community health activities at first hand.

During the 4 weeks at ALERT the students undergo an intensive course consisting of supervised clinical practice, lectures with case demonstrations, and slide presentations.

The objectives of the course primarily aim at enabling the students to satisfactorily deal with the great majority of leprosy complications and all forms of uncomplicated leprosy. That ability is an indispensible prerequisite for their teaching leprosy to other cadres of health personnel. As regards dermatology, the emphasis is more on the principles of examination, diagnosis and treatment than on the pathology and treatment of individual skin diseases, with the exception of the most common dermatoses.

In the outpatients department the students are therefore from the start made to take histories, examine the skin and record their findings. These and their proposals as to treatment are subsequently discussed with each of them by one of the staff dermatologist-leprologists.

In classroom sessions during the first 2 weeks a great variety of leprosy patients is demonstrated to the class and extensively discussed. Much attention is given in those sessions to the early recognition and the prevention of disabilities. During the second 2 weeks the students themselves demonstrate and discuss cases to the group and members of staff. To this end the students are given inpatients to examine without access to the clinical records. During the 2 weeks in the field the students participate in the running of rural leprosy clinics and undertake school, village and contact surveys. Up to 1981, 92 students were trained according to this new programme.

Going by their scores in the final test and by their evaluation reports, it seems to produce satisfactory results. The students particularly appreciate their active participation in the running of the various clinics. The 4 weeks of intensive involvement with a good many leprosy patients not only gives them the required confidence and abilities but also liberates them from the fear of the disease with which, many admit, they entered the course. The change of attitude is a major achievement of the course and also a major prerequisite for becoming sound teachers of leprosy.

An important criticism from the students on the courses is about the large amount of time spent on leprosy as compared to dermatology. This criticism is valid. The students are very much aware of the fact that although patients with skin diseases form a great proportion of the patients attending clinics and hospitals many if not most of the medical officers and other health professionals are ill- or not at all trained in the management of those patients. They therefore see the need for a better gearing of their medical education to the actual needs of the community. It is, however, not the intention to make alterations in the ALERT courses in favour of dermatology as this would not solve their problem and only detract from their leprosy training. To meet the criticism of the students, lectures and case demonstrations on dermatology are going to be held in the University hospital for the students while they are doing their medical internships. The medical faculty will release the students for those sessions for 2 hours each week.

Ethiopia seems to be setting a commendable example which might well be followed by others.

ALERT,
PO Box 165,
Addis Ababa, Ethiopia

J A WARNDORFF
Director of Training
Abstracts

[The Abstracts which follow are reprinted from the *Tropical Diseases Bulletin* through the courtesy of the Director, the Bureau of Hygiene and Tropical Diseases, London.]

1. Microbiology

9. GOYLE S, VIRMANI V (1979) 'In vitro' studies on biopsies from leprosy cases. *Indian Journal of Medical Research* 69 (June), 919–25.

Organotypic cultures of skeletal muscle, skin and subcutaneous fat were set up from biopsies of 4 patients with leprosy, and in successful cultures there was good growth of tissue elements and macrophages. Acid-fast bacilli were observed in the culture medium in all cases and maintained without morphological change for over a year. They were present also in macrophages, spindle cells (macrophages?) and muscle cells, and in the parent mass of a subculture. There were no organisms in control cultures and no growth on Lowenstein-Jensen medium.

D S Ridley

2. Immunology, pathology


It is suggested that continuous leakage of bacilli into the circulation from a primary focus of intraneural infection may simultaneously initiate bacillary dissemination and the suppression of cell-mediated immunity. Both these features are essential for the development of lepromatous leprosy. Nerve involvement in leprosy, previously thought of as a diagnostic feature of the disease and as a complication of therapy, may represent an essential phase in the cycle of infection and reinfection by *Mycobacterium leprae*.

This closely reasoned hypothesis warrants reading in full. It deserves careful weighing against what is currently known of the loss and gain in specific cell-mediated immunity in human leprosy patients and also in experimental leprosy due to *Mycobacterium leprae* and in rat leprosy infection due to *Mycobacterium lepraemurium*.

M FR Waters

11. LAGRANGE PH (1979) Active or passive acquired resistance after *Mycobacterium lepraemurium* infection in C57BL/6 and C3H/HeN mice. *Annales d’Immunologie* 130C (4), 561–79.

C57Bl/6 mice and C3H/HeN mice, known respectively as responders and non-responders to infection with *Mycobacterium lepraemurium*, were tested for specific and non-specific resistance after primary infection.

After subcutaneous infection, C57Bl mice controlled *M. lepraemurium* multiplication during the first weeks of challenge infection. They produced non-adherent lymph-node cells which transferred (a) delayed hypersensitivity to specific antigen and transient resistance to challenge to normal recipients; (b) a more prolonged resistance to cyclophosphamide-treated normal recipients. They developed also a non-specific resistance to infection with *Listeria monocytogenes*, and a potentiated delayed hypersensitivity response to sheep red blood cells after immunization with the heterologous antigen.

C3H mice, infected subcutaneously, could not resist challenge infection. They produced non-adherent lymph node cells which transferred only delayed hypersensitivity to normal recipients and, which could not transfer any specific resistance to challenge to either normal or cyclophosphamide-treated normal recipients. They were less resistant to non-specific challenge with *L. monocytogenes* than
C57Bl mice; and infection did not potentiate delayed hypersensitivity to sheep red blood cells.

In both mouse strains, preimmunization with low doses (10^4 and less) of mycobacteria given intravenously was very inefficient and with high doses (10^6) facilitated growth of challenge mycobacteria.

The author suggests that the two mouse strains (1) differ in the type of cellular hypersensitivity they develop; (2) produce different types of T lymphocytes so that C57Bl mice can recruit and activate macrophages while C3H mice cannot.

[The role of macrophage activation in controlling infection was not investigated.]

P Preston


The footpad reaction to autoclaved whole Mycobacterium leprae-murium organisms (MLM lepromin) in high-resistance (C57BL) and low-resistance (BALB/c) mice was studied. Infected C57BL mice gave a prolonged footpad response persisting for 4 weeks after skin testing with high and low doses of lepromin. This was accompanied by mononuclear cell infiltration. Uninfected C57BL mice gave no response. The majority of infected BALB/c mice gave no increase in footpad thickness. However, a high proportion of infected and control BALB/c mice tested with the high dose showed mononuclear cell infiltration which resembled that in C57BL mice. The low dose caused little infiltration in infected or control BALB/c mice. The course of infection in the two strains was different. Dissemination of organisms from the infected footpad was minimal in C57BL mice 5 months after infection. In BALB/c mice, dissemination to the draining lymph node and to some extent to the liver had occurred by 5 months. The draining lymph node of BALB/c mice showed histological evidence of local antibody formation, which was not found in C57BL mice. On the basis of these findings, it was possible to fit murine leprosy in these two strains into a classification similar to that used for human leprosy.'


‘To study further the pathogenesis of Lucio’s phenomenon, we have made a comparative histological study of 11 patients with Lucio’s phenomenon and 12 with ENL.

‘Confirming the findings of others, Lucio’s reaction could be distinguished from ENL by epidermal necrosis and by necrotizing vasculitis manifesting necrosis in the walls of superficial vessels and severe, focal endothelial proliferation of mid-dermal vessels. Furthermore, in Lucio’s phenomenon large numbers of AFB were found in evidently normal and in swollen or proliferating endothelial cells.

We hypothesize that patients with Lucio’s phenomenon have an exceptionally deficient defence mechanism, allowing unrestricted proliferation of AFB in endothelial cells, facilitating contact between bacterial antigen and circulating antibody and leading to infarction; also, this nadir of resistance allows unimpeded dissemination of AFB, accounting for the clinical features of diffuse non-nodular leprosy. Thus, an explanation is offered for the restriction of Lucio’s phenomenon to patients with diffuse non-nodular lepromatous leprosy.’

[AFB = acid fast bacilli.]


‘In studies aimed at the development of an antileprosy vaccine for use in man, Mycobacterium leprae suspensions were pre-
pared from livers of experimentally infected armadillos. The 2 methods of purification in chief use, carried out after irradiation of the tissue with 2.5 megarads of gamma irradiation from $^{60}$Co, involved treatment with 0.1 N NaOH for 2 h at room temperature, trypsin and chymotrypsin digestion for 24 h at 37°C, and separation in a 2-phase liquid polymer (dextran: polyethylene glycol) system. All vaccines were autoclaved and injected intradermally in mice. Earlier studies have shown that heat inactivation does not interfere with the immunogenicity of $M$. leprae. Immunogenicity was measured by foot-pad enlargement (FPE) after challenge with heat-killed $M$. leprae suspensions or by protection against infectious foot-pad challenge. The results indicated that the irradiation and 2-phase separation did not decrease immunogenicity but the NaOH treatment and enzyme digestion did.\textdagger}


3. Clinical


The appearance of Volume 1 of Leprosy, edited by Dr Dharmendra, is an event in the world of medical publishing. Let it be said at the outset that this volume bears little resemblance to the slimmer works that preceded it, valuable though these were. It is an entirely new production, and the editor has called on the collaboration of 31 leprologists and social workers, most of whom have commendably high reputations beyond their native countries. In point of fact, most of the contributors are nationals from India itself or have spent a large part of their working lives in India, whereas only 5 contribute from their experience outside India.

The first volume, then, may be regarded as a worthy exposition of leprosy from a land that has the unenviable distinction of having the greatest number of leprosy sufferers. This fact will do much to account for the value of the book, and also its Indian ‘flavour’.

As might be expected, the sections on clinical manifestations, diagnosis and differential diagnosis are well done, excellently descriptive and authoritative; they present a very good review of the established disease, as seen in India. In the section (pp. 319–51) on classification, the details of historic battles fought largely around the Indian claims appear somewhat irrelevant to workers conversant with modern ideas on immunology and host-parasite relations.

The section on treatment (pp. 355–682) is reasonably up to date, but more cognizance could have been taken of recent work on the advantages of administering dapsone in high doses from the beginning of treatment, uninterrupted during episodes of acute exacerbation.

There is an abundance of clinical photographs in this volume, mostly in black and white; their definition and contrast are not always above criticism.

All in all, though, this first volume is a commendable and workmanlike production, a safe guide and a useful handbook for doctors working in leprosy. Despite its largely Indian origin and emphasis, it should appeal to a larger audience.

S G Browne


The paranasal air sinuses of 16 patients in Dichpalli, south India, with untreated lepromatous leprosy were investigated. 15 of these patients complained of nasal symptoms such as crusting and airway obstruction, but pain was not a feature. On examination, all 16 had clinical evidence of nasal involvement; nose-blow specimens collected from 14 patients were positive
for acid-fast bacilli (AFB) and all 16 showed radiological evidence of sinus involvement (details are presented separately, Barton, *Journal of Laryngology and Otology*, 1979, 93, 597). Biopsies of maxillary sinus mucous membrane were taken from 2 patients and were positive for AFB; in 1 of these biopsies there were numerous bacilli in the lamina propria, globi were plentiful, and the morphological index was 20. These microscopical findings are shown in a black-and-white photograph.

The authors give details of the surface area and the temperature of maxillary sinuses; they stress the importance of sinus involvement in lepromatous leprosy and recommend further studies.

*W H Jopling*


‘Two patients with lepromatous leprosy presenting initially because of lepromatous orchitis are reported. These cases are unusual because they were diagnosed as lepromatous orchitis at a stage when no other evidence of leprosy was present. Generalized skin lesions characteristic of lepromatous leprosy subsequently developed in one of these patients. It is suggested that lepromatous orchitis should be actively considered in the differential diagnosis of orchitis and infertility.’

These two cases from the Department of Pathology and Internal Medicine, King Faisal Specialist Hospital and Research Centre, Saudi Arabia, are mainly of interest because of the claim that lepromatous orchitis was found ‘at a stage when no other evidence of leprosy was present.’ The clinical and laboratory findings are not, however, completely reassuring on matters such as the examination of peripheral nerves and eyes, anaesthesia, and the results of multiple-site slit-skin smears. Many leprologists of experience may wonder if such data, perhaps accompanied by a skin biopsy at the outset, would have revealed more a widespread involvement of the body by a lepromatous process.

*A C McDougall*

4. Therapy


‘Seventy-one Burmese adult patients with lepromatous leprosy were treated with various regimens of rifampicin monotherapy, 450 mg daily for 60 days or 900 mg once weekly for 12 weeks or 450 mg daily for six months. Of the patients, 18 had relapsed after stopping DDS therapy, 20 were intolerant of DDS, 18 were DDS resistant and 15 had received no previous treatment.

Rifampicin produced a 75% reduction in the size of skin nodules in two thirds of the patients and a complete disappearance of nodules in the others. After one month drug treatment the MI fell to zero but the BI remained unchanged. The once weekly regimen was as effective as the daily treatment. Four patients had to be withdrawn due to ENL reactions.’


Due to lack of space Book Reviews and further Abstracts have been retained for the next issue. *Editor*
Nederlandse Stichting voor Leprabestrijding
(Netherlands Leprosy Relief Association)

have the following vacancies which urgently require filling.

**NGERIA — MEDICAL OFFICERS**
Positions are vacant in Zaria, Katsina, Garkida and some other parts in Northern Nigeria for leprosy work in hospitals, field clinics and training.

**TANZANIA — REGIONAL TB/LEPROSY CONTROL OFFICERS**
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For full job descriptions of the above posts please write to:

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Antileprosy drug with anti-inflammatory properties

effective in the prevention and treatment of lepra (ENL) reactions

indicated as a part of combined therapy for the prevention and treatment of dapsone resistance in lepromatous and borderline leprosy.

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Printed in Great Britain at the Alden Press

Oxford, London and Northampton