Studies on the Diagnosis and Treatment of Human Filariasis in Rhodesia

J. M. GOLDSMID, SUE ROGERS

SUMMARY

Experiences in Rhodesia with various recovery techniques available for the laboratory diagnosis of infections with Dipetalonema perstans and Wuchereria bancrofti are discussed. A diagnostic laboratory regimen for routine filarial investigations is suggested. Included are preliminary observations on the use of mebendazole (Vermox) for the treatment of D. perstans infections.


In Rhodesia, only two endemic species of filarial nematodes have been recorded in man — Dipetalonema perstans, which is found in Lupani and in the Zambesi and Burma valleys, and Wuchereria bancrofti, which is limited to the Zambesi Valley. As a result of the paper by Dukes et al., Orihel postulated that Meningonema peruzzi, as described by Orihel and Esslinger, occurs here. However, to date no definite cases have been found in Rhodesia in either the vervet monkey (Cercopithecus aethiops) or in man. Occasional imported cases of Onchocerca volvulus are recorded from Malawi, and recently 2 imported cases of Loa loa from Nigeria were reported by Sparrow and Goldsmid.

Of the two species of filarial worm recorded as being endemic in Rhodesia, D. perstans is generally considered to be a commensal, although a number of authors ascribe to this species allergic reactions which may be severe, especially in Whites. W. bancrofti causes lymphangitis, and, in the late chronic stages where repeated long-term infection has occurred, elephantiasis of the penis, scrotum, legs, arms and breasts.

This article is a compilation of our work in Rhodesia on the problem of the diagnosis of human filarial infections with D. perstans and W. bancrofti, together with preliminary observations on the treatment of D. perstans infections with mebendazole (Vermox).

DIAGNOSIS

An accurate clinical diagnosis of filariasis is not possible, for even elephantiasis may be due to many causes apart from Bancroftian filariasis. Diagnosis of filariasis therefore depends upon the demonstration of microfilariae in the blood.

Department of Medical Microbiology, University of Rhodesia, Salisbury, Rhodesia
J. M. GOLDSMID, B.SC. HONS, M.SC., PH.D., M.I. BIOL., M.R.C. PATH., Reader in Medical Parasitology
SUE ROGERS, B.N.C., DIP. MED. TECH.

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The laboratory diagnosis of human filariasis involves the use of staining techniques, concentration techniques and sometimes indirect methods of diagnosis such as serology or the filarial skin test.

METHODS

Methods used to diagnose human filarial infections in Rhodesia have included wet drop preparations, thick and thin blood films stained with Romanowsky stains, haematoxylin or acridine orange O utilising ultraviolet microscopy.

Concentration techniques which have been used are the Knott concentration, the buffy coat, the microhaematocrit, the counting chamber, the swinney filter, the cytocentrifuge and the saponin method.

Indirect diagnostic methods have involved the use of the skin test with Dirofilaria immitis antigen.

Blood for investigation of D. perstans was usually collected during the day, but blood for W. bancrofti investigations was collected at about midnight, the Rhodesian strain being nocturnal.

RESULTS AND DISCUSSION

When the microfilaraemia is high, thick, or even thin, blood films are quite satisfactory when stained with such conventional stains as Leishman's, Giemsa, Wright's or May-Grünewald-Giemsa. Field's stain, however, was found to give poor results for microfilariae, even when modified. Slightly better results were obtained with acridine orange O stain and ultraviolet microscopy. This method gave results which were slightly superior to Giemsa-stained smears and made screening less tiring (Table I). Species identification with this technique was as easy as with the Romanowsky-stained smears, since the same morphological features were utilised. In our experience, a thick smear obtained directly from the patient's finger shows less film peeling and minimal loss of parasites.

The use of the wet drop or laking procedure has been found useful for rapid scanning when parasitaemia is

<table>
<thead>
<tr>
<th>Method</th>
<th>Number of patients examined</th>
<th>Acridine</th>
<th>Giemsa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>134</td>
<td>53</td>
<td>46</td>
</tr>
<tr>
<td>Negative</td>
<td>187</td>
<td>141</td>
<td>187</td>
</tr>
</tbody>
</table>

TABLE I. NUMBER OF CASES OF DIPETALONEMA PERSTANS INFECTATION DIAGNOSED BY MEANS OF BLOOD SMEARS STAINED WITH GIEMSA AND WITH ACRIDINE ORANGE
high (Table II) and this has been improved upon by the counting chamber method of Denham et al. — a technique which we have also found satisfactory.

**TABLE II. NUMBER OF POSITIVE FINDINGS ON BLOOD SPECIMENS CONTAINING D. PERSTANS AND W. BANCROFTI EXAMINED BY 4 RECOVERY TECHNIQUES**

<table>
<thead>
<tr>
<th>Technique</th>
<th>Micro-</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>haemocrit</td>
<td>findings</td>
</tr>
<tr>
<td></td>
<td>drop</td>
<td>Wet</td>
</tr>
<tr>
<td></td>
<td>film</td>
<td>Thick</td>
</tr>
<tr>
<td></td>
<td>film</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Micro-</th>
<th>Saponin</th>
<th>Microhaematocrit</th>
<th>Cytocentrifuge</th>
<th>Wet drop</th>
<th>Thick film</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. perstans</td>
<td>105</td>
<td>77</td>
<td>53</td>
<td>41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W. bancrofti</td>
<td>47</td>
<td>31</td>
<td>25</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total specimens</strong></td>
<td><strong>152</strong></td>
<td><strong>108</strong></td>
<td><strong>78</strong></td>
<td><strong>54</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Where the microfilaraemia is low, concentration methods may have to be used. The Knott concentration technique has always proved very satisfactory in this respect, as has the saponin technique.

The use of the full buffy coat technique is another good method for recovery of microfilariae and the modification of Clarke makes the whole process much easier. This method involves the use of disposable 5-ml syringes, into which the blood and anticoagulant are drawn. The base of the barrel with the lug and plunger in position is then cut off, allowing the whole syringe to be centrifuged until the buffy coat has separated. The syringe is then placed over a peg and the buffy coat is placed on a slide for examination after expulsion of the plasma layer.

A very sensitive microtechnique which is accurate and time-saving is the microhaematocrit centrifuge technique. This method is easy, quick and accurate (Table II), although, since it is a micromethod and therefore uses less blood than the full buffy coat procedure, it is perhaps not as sensitive as the latter method. However, an advantage of the microhaematocrit technique is that it can, by the use of heparinised tubes, be utilised on a finger-prick sample.

It is possible to make permanent smears for staining and confirmation of species identification from the buffy coat and microhaematocrit technique, but it is not satisfactory, and thus the finding that the cytocentrifuge can concentrate the buffy coat onto a slide for staining and examination was a distinct advance. The sensitivity of this method has not yet been fully evaluated, but the preliminary results are given in Table III.

In cases where the microfilaraemia is very low or in which microfilarial counts are to be used, the membrane filter technique is very useful but, in our opinion, rather tedious. We have tried it, however, and the results of the investigation are given in Table IV. This involved the filtering of blood haemolysed with Teepol in the collecting syringe through a swinney filter and staining the filter paper with Giemsa stain as for a routine thick film.

Indirect methods of diagnosis may have to be used where the microfilaraemia is very low, or where the infection is still prepatent or if the worms have died. This often happens in W. bancrofti infection, where symptoms such as elephantiasis can develop after the death of the adult worms and microfilariae. These indirect methods for filariasis can include provocation tests such as the Mazotti test (mostly used for onchocerciasis), serological tests such as the indirect fluorescent antibody test, or the filaria skin test. However, the serological tests have not proved practical, since commercial antigen has not been available and the skin test, which depends on a cross-reaction with Dirofilaria immitis antigen has not proved very successful for D. perstans, either in our experience or in that of other investigators in Rhodesia.

It is worth noting that calcified adult W. bancrofti are sometimes reported as having been visualised on X-ray.

**TABLE III. RESULTS AFTER THE USE OF VARIOUS RECOVERY TECHNIQUES IN 7 PATIENTS WITH D. PERSTANS INFECTION AND 1 WITH W. BANCROFTI INFECTION**

<table>
<thead>
<tr>
<th>Technique</th>
<th>Knott concentration</th>
<th>Saponin technique</th>
<th>Microhaematocrit technique</th>
<th>Cytocentrifuge technique</th>
<th>Wet drop</th>
<th>Thick film</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>D. perstans</td>
<td>7</td>
<td>6</td>
<td>7</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>W. bancrofti</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>8</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>

**TABLE IV. POSITIVE CASES OF D. PERSTANS AND MEAN NUMBER OF MICROFILARIAE RECOVERED BY VARIOUS TECHNIQUES**

<table>
<thead>
<tr>
<th>Technique</th>
<th>Membrane filter</th>
<th>Microhaematocrit technique</th>
<th>Wet drop</th>
<th>Thick film</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number positive</td>
<td>10</td>
<td>10</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Mean number microfilariae recovered</td>
<td>400</td>
<td>6</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>
films. The diagnosis of filariasis in humans in Rhodesia is sometimes made in rather unusual circumstances — thus microfilariae of *D. perstans* have been found in bone marrow smears, ascitic fluid, liver aspirates and even in the warm stools of patients with haemorrhoids suspected to be amoebic dysentery. It is not unusual to find various species of microfilariae in urine. Thus, in East Central Africa, urine samples often contain microfilariae of *Onchocerca volvulus* and in Rhodesia we have found both *D. perstans* and *W. bancrofti* in the urine of patients with bilharzial haematuria. The latter species can sometimes be recovered from chylous urine samples.

### TREATMENT

While the treatment of *W. bancrofti* infections with diethylcarbamazine is well established, studies in Rhodesia have indicated that this drug is virtually useless for the treatment of infections with *D. perstans*.24

In recent years, a number of new anthelmintics which have been reported to have some filaricidal activity have appeared. Zaman and Fung and Zaman and Lal obtained promising results with levamisole. However, this drug was unavailable to us and we decided to make preliminary studies with a new broad-spectrum anthelmintic, mebendazole (Vermox), which is extremely safe and free from side-effects, and which has been shown to have a wide range of efficacy against intestinal nematodes,cestodes and acanthocephala.25

On the recommendation of the manufacturers, 2 White men, aged 28 and 72 years respectively, were treated with mebendazole tablets (400 mg twice a day) for 14 days. Despite the very high dosage, neither subject complained of any side-effects and the initial results showed a marked reduction in the number of microfilariae in both. The one patient who was exhibiting symptoms (blackouts, eye troubles, etc.) which were believed to be caused by the *D. perstans* said that he felt much better, and his physician reported an improvement in his general condition.

Long-term follow-up failed to reveal a disappearance of microfilariae and, in one case at least, the count mounted again after 5 months and 12 months, and was again associated with deterioration in the condition of the patient. (It is worth noting that he reported the deterioration before we had shown a rise in the microfilarial count, thus suggesting that his improvement was not psychological.)

However, in both these patients, there has been a definite reduction in microfilariae counts.

These preliminary trials would thus suggest that, while it has some antifilarial activity, mebendazole is not effective in achieving a cure for *D. perstans* infections at the dose regimen tried. The recent report of the effectiveness of trichlorophone against *D. perstans* in Mexico is of great interest and should be followed up in Africa.29 The fact that mebendazole caused only a reduction in filarial count and not a total cure in our trials might be owing to the fact that only sublethal concentrations in the blood were achieved because of a low intestinal absorption of the drug, despite the high dosage regimen used.

In a recent paper, Maertens and Wery claim to have achieved success in treating *D. perstans* infections in Zaire with a combination of mebendazole and levamisole. They found, however, that mebendazole alone was less successful, but that in one patient so treated microfilariae disappeared after 7 weeks of treatment at a dose of 200 mg twice daily.

### CONCLUSIONS

As a result of our studies we conclude that the following investigations for the detection of filariae are to be recommended at present:

(a) thick blood films and venous blood in citrate collected at any time for *D. perstans* and at about midnight for *W. bancrofti*;

(b) thick blood film stained with acridine orange and examined on an ultraviolet microscope;

(c) a wet drop preparation examined under a coverslip by light microscopy;

(d) the microhaematocrit concentration technique using 3 - 6 microhaematocrit tubes of blood.

We find that these investigations are adequate for a routine laboratory investigation. Where more detailed investigations are needed, especially if microfilarial counts are needed (e.g. in anthelmintic trials), then we have further incorporated either the Knott concentration technique, the membrane filter technique or the counting chamber technique.

As regards treatment, the treatment available for infections with *Dipetalonema perstans* is still unsatisfactory and it remains a matter of urgency to confirm reports from Mexico of the effectiveness of trichlorophene and the report from Zaire of the effectiveness of mebendazole and levamisole in combination.

We should like to thank Dr David Clain for his help and comments regarding the preliminary trials with mebendazole, and also Ethnor Laboratories for supplying the mebendazole used in the study.

### REFERENCES

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Isoniazid Acetylator Status of Black South African Tuberculosis Patients

P. H. BACH, SUSAN B. HIGGINS-OPITZ, BARBARA BIMA, W. P. LEARY

SUMMARY

Black male tuberculosis patients (106) were phenotyped into 59% fast and 41% slow acetylators with isoniazid (INH) plasma half-lives. The antimode time dividing the two acetylator groups was longer than that previously reported for Whites. The therapeutic significance of INH acetylator phenotyping per se is questioned since it provides neither a measurement of the amount of INH absorption nor of its distribution within the body.


Tuberculosis remains common among the inhabitants of Southern Africa despite the considerable attention that has been paid to its prevention and treatment. Notwithstanding the availability of several alternatives, therapy relies heavily upon the use of isoniazid (INH). This potent tuberculous drug is rapidly absorbed after oral administration, and undergoes hepatic metabolism to its bacteriologically inactive N-acetyl derivative, which is then excreted via the kidney. The activity of N-acetyltransferase, the inactivating enzyme, is controlled by simple Mendelian inheritance as a result of which patients show a polymorphism and can be classified as fast or slow acetylators of the drug. The proportion of fast and slow phenotypes varies in different ethnic groups: whereas 70-80% of Semitic populations are slow acetylators, only 5-10% of Eskimos, Japanese and Chinese are of this phenotype. Other ethnic groups vary between these extremes.

The clinical significance of the acetylator status of patients is controversial. Fast acetylators respond less favourably to a high dose of INH administered once a week than do patients who acetylate the drug slowly, but this difference is no longer statistically apparent if INH is administered more than twice weekly. Intermittent therapy is often unsuccessful in those ethnic groups with a low proportion of slow acetylators. The accumulation of INH in slow inactivators may exacerbate adverse reactions that arise from pyridoxine deficiency in malnourished patients, but these reactions are easily controlled by the co-administration of the vitamin.

To date, no detailed study of INH pharmacokinetics among Black tuberculosis patients has been reported in Southern Africa; this article reports the first of a series of such detailed studies carried out at the University of Natal.

PATIENTS AND METHODS

One hundred and six Black male tuberculosis patients aged 13-70 years (mean 36) were studied. Patients had been hospitalised for between 6 and 349 days (mean 87) and all were ambulant at the time of study, except 2 patients who were confined to wheelchairs. None had clinical evidence of other serious disease.

Daily ward routine and drug therapy regimens were continued for the duration of the study, except in the case of patients who were taking pyrazinamide (PZA). In such cases PZA was not given on the day of investigation until after the last blood sample had been drawn for analysis, since this preparation interferes with the spectrophotometric determination of plasma INH levels.

The prescribed morning dose of INH was given under...