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WHO EXPERT COMMITTEE ON ONCHOCERCIASIS

Geneva, 21–29 April 1986

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WHO EXPERT COMMITTEE ON ONCHOCERCIASIS

Third Report

1. INTRODUCTION

A WHO Expert Committee on Onchocerciasis met in Geneva from 21 to 29 April 1986.

Opening the meeting on behalf of the Director-General, Dr S.K. Litvinov, Assistant Director-General, stated that onchocerciasis remains a public health and socioeconomic problem of considerable magnitude in many tropical countries, particularly in Africa, and that it appears to be spreading in Latin America. Onchocerciasis affects people in rural areas, particularly in remote places. It causes severe itching, disfiguring skin lesions, and a variety of eye lesions, the sequelae of which produce the feared “river blindness”.

Hitherto, few satisfactory control methods have been available against this disease either by way of the primary health care system or by means of community involvement. The outstanding success in onchocerciasis control over the last 10 years has been the Onchocerciasis Control Programme (OCP) in West Africa. This vertically structured, costly, single-disease control programme, with the World Health Organization as its executing agency, has successfully used long-term aerial larviciding against Simulium to interrupt the transmission of onchocerciasis over an area of 764 000 km² in one of the worst afflicted savanna zones of West Africa.

Some 16.5 million people in seven West African countries are no longer at risk of infection with Onchocerca volvulus. It is planned that the Programme will soon extend its coverage to the west and south to include another 8 million people in 4 additional countries, thus bringing the total area under control to 1 235 000 km². Because of this Programme, onchocerciasis has been, or shortly will be, controlled over about one-quarter of the total global endemic area.

Much progress has been made in the areas of diagnosis, pathogenesis, treatment, and epidemiology of onchocerciasis as a result of research sponsored during recent years by the UNDP/World Bank/WHO Special Programme for Research and Training
in Tropical Diseases and by the Onchocerciasis Control Programme, including the Onchocerciasis Chemotherapy Project. In addition, recent clinical trials of ivermectin show promise of improved chemotherapy in the future.

Against this background, this report will reassess the global public health and socioeconomic importance of onchocerciasis, and review the advances, in all aspects of the disease, that have taken place during the past 10 years, relating them to feasible and practical improvements in treatment and control and to future research needs. Suggestions for further research are made at the end of each section. Special attention is paid to methods and strategies for the control of onchocerciasis that can be applied through the primary health care system with the aid of health education and community involvement. The recommendations are designed to help the national administrations of those Member States that are afflicted by this disease, as well as international health organizations, to formulate health policies and establish standards that will enable them to cope with the problem and thus contribute, in this respect, to the goal of health for all by the year 2000.

2. DISTRIBUTION, PREVALENCE, AND SOCIOECONOMIC EFFECTS

The prevalence and severity of onchocerciasis as well as the magnitude of the associated social and economic effects vary widely in different geographical areas where the disease occurs. Some of these variations as well as the distribution and prevalence of the disease are discussed in this section, while the socioeconomic consequences of the disease are considered further in section 14. However, the Committee wishes to stress its view that onchocerciasis can be a devastating disease, producing intolerable misery as a result of its effects on the eyes and skin, and that it can lead to the disintegration of social structure and the abandonment of homes and land. In severely endemic zones, onchocerciasis is, without question, the main public health problem facing affected communities.

2.1 Reliability of data

This section is subdivided by WHO region and by country. In Tables 1–3 estimates of total population, number of persons at risk of onchocerciasis, number infected, and number blind as a result of
the disease are presented by country and by region, and the global totals are given. The approximate overall distributions of the endemic areas in the African and Eastern Mediterranean Regions and in the Region of the Americas are shown in Fig. 1 and 2. A more detailed map showing the area covered by the Onchocerciasis Control Programme is shown in Fig. 12 (section 15).

In many countries the total figures for the prevalence of infection and blindness have been estimated by extrapolation from relatively small sample surveys and without an accurate knowledge of the local distribution of endemic foci. Total figures derived in this way often underestimate the true level of prevalence. Furthermore, on the small-scale maps that have to be used in this report, it is not possible to show the detailed focal distribution that exists within the broad, shaded endemic areas.

The Committee has drawn on a variety of sources in compiling this section, together with the tables and maps, including formally published literature (embracing OCP documents) and information supplied by locally experienced individuals. In some areas, there may be an apparent discrepancy between the number of people infected and the number of blind. This can be due to many factors and may represent either an underestimation of the number of infected or an overestimation of the number of blind. Furthermore, several definitions of blindness may have been used for these assessments, and they may not conform to the internationally accepted definition of blindness as vision of less than 3/60 corresponding to the inability to count fingers at a distance of 3 m (1). When available, summarized information is included on the socioeconomic effects of onchocerciasis in each country. The Committee has designated an area as endemic for onchocerciasis only if there is evidence or good reason to believe that transmission continues or would restart if vector control were not maintained. This latter proviso applies at present (1986) particularly to the well-controlled parts of the Onchocerciasis Control Programme area, that are included in the cross-hatched area in Fig. 1, and it will continue to apply in this area until such time as the human reservoir of infection has died out.

2.2 The African Region

No cases of onchocerciasis have been reported during the last few years from The Gambia, and infection has not been recorded in Rwanda (see Table 1 and Fig. 1). A single, apparently
autochthonous, case of onchocerciasis has been recorded recently in a child from southern Zambia (if confirmed, this would represent the most southerly known focus of the disease).

2.2.1 Distribution by country and the socioeconomic consequences

For the seven countries in the original area covered by the Onchocerciasis Control Programme (Benin, Burkina Faso, Côte d'Ivoire, Ghana, Mali, Niger, and Togo) the socioeconomic consequences before and after control are discussed in sections 14 and 15. Persons living in the previously endemic areas of these countries, where transmission is currently (1986) well controlled by the Programme, have not been included in Table 1 as being “at risk

<table>
<thead>
<tr>
<th>Country</th>
<th>Total population (UN Year Book 1983)</th>
<th>Number at risk in onchocerciasis endemic areas</th>
<th>Number infected with O. volvulus</th>
<th>Number blind as result of onchocerciasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angola</td>
<td>8,349,000</td>
<td>2,000,000</td>
<td>100,000</td>
<td>2,000</td>
</tr>
<tr>
<td>Benin (1985)</td>
<td>3,720,000</td>
<td>600,000</td>
<td>300,000</td>
<td>7,800</td>
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<td>Burkina Faso (1985)</td>
<td>6,810,000</td>
<td>0</td>
<td>160,000</td>
<td>9,000</td>
</tr>
<tr>
<td>Burundi (1985)</td>
<td>4,420,000</td>
<td>60,000</td>
<td>12,000</td>
<td>400</td>
</tr>
<tr>
<td>Cameroon</td>
<td>9,150,000</td>
<td>4,636,000</td>
<td>1,200,000</td>
<td>20,000</td>
</tr>
<tr>
<td>Central African Republic</td>
<td>2,450,000</td>
<td>1,630,000</td>
<td>390,000</td>
<td>19,000</td>
</tr>
<tr>
<td>Chad (1985)</td>
<td>4,790,000</td>
<td>592,000</td>
<td>128,000</td>
<td>11,000</td>
</tr>
<tr>
<td>Congo</td>
<td>1,650,000</td>
<td>100,000</td>
<td>20,000</td>
<td>500</td>
</tr>
<tr>
<td>Côte d'Ivoire (1985)</td>
<td>9,160,000</td>
<td>100,000</td>
<td>200,000</td>
<td>10,400</td>
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<tr>
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<td>380,000</td>
<td>30,000</td>
<td>4,000</td>
<td>100</td>
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<tr>
<td>Ethiopia (1984)</td>
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<td>7,300,000</td>
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<td>Gabon (1985)</td>
<td>1,150,000</td>
<td>100,000</td>
<td>60,000</td>
<td>3,000</td>
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<tr>
<td>Ghana (1984)</td>
<td>12,700,000</td>
<td>1,200,000</td>
<td>278,000</td>
<td>10,500</td>
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<tr>
<td>Guinea (1985)</td>
<td>5,180,000</td>
<td>2,100,000</td>
<td>560,000</td>
<td>20,000</td>
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<tr>
<td>Guinea Bissau (1985)</td>
<td>860,000</td>
<td>132,000</td>
<td>30,000</td>
<td>1,400</td>
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<tr>
<td>Liberia (1995)</td>
<td>2,060,000</td>
<td>1,300,000</td>
<td>600,000</td>
<td>2,900</td>
</tr>
<tr>
<td>Malawi (1985)</td>
<td>6,430,000</td>
<td>456,000</td>
<td>120,000</td>
<td>1,000</td>
</tr>
<tr>
<td>Mali (1985)</td>
<td>7,530,000</td>
<td>860,000</td>
<td>360,000</td>
<td>15,000</td>
</tr>
<tr>
<td>Niger (1985)</td>
<td>5,770,000</td>
<td>0</td>
<td>5,000</td>
<td>2,400</td>
</tr>
<tr>
<td>Nigeria (1986)</td>
<td>89,020,000</td>
<td>38,919,500</td>
<td>6,962,000</td>
<td>113,600</td>
</tr>
<tr>
<td>Senegal</td>
<td>8,328,000</td>
<td>186,000</td>
<td>44,000</td>
<td>1,500</td>
</tr>
<tr>
<td>Sierra Leone</td>
<td>3,470,000</td>
<td>1,030,000</td>
<td>300,000</td>
<td>10,000</td>
</tr>
<tr>
<td>Togo</td>
<td>2,760,000</td>
<td>1,000,000</td>
<td>160,000</td>
<td>8,500</td>
</tr>
<tr>
<td>Uganda</td>
<td>14,630,000</td>
<td>200,000</td>
<td>30,000</td>
<td>900</td>
</tr>
<tr>
<td>United Republic of Tanzania (1985)</td>
<td>20,380,000</td>
<td>1,300,000</td>
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<td>Zaire (1985)</td>
<td>31,150,000</td>
<td>12,000,000</td>
<td>3,384,000</td>
<td>27,000</td>
</tr>
</tbody>
</table>

Regional subtotal          | 293,750,000                          | 78,272,500                                    | 17,120,500                     | 326,600                                |
in onchocerciasis endemic areas", although the risk would be renewed if *Simulium* control were to break down. In these same areas the number of persons infected with *O. volvulus* has been calculated in Table 1 as 25% of those infected at the beginning of the Control Programme.

*Angola.* The disease occurs in the north of the country in the provinces of Cuanza Norte, Lunda, Malanje, Uige, Zaire, in the Bie plateau area, and in the Cabinda enclave. No recent information is available on its socioeconomic consequences.

*Benin.* The northern part of the country is included in the area covered by the Control Programme, although there is still some transmission on the borders of the Programme area. There is widespread infection in the rest of the country except in the coastal area.

All the infected areas will be included in the southern extension of the Onchocerciasis Control Programme beginning in 1987. In some of the southern areas, the socioeconomic effects are similar to those previously encountered in the north.

*Burkina Faso.* The entire country is within the Control Programme area and there is now virtually no transmission, and although many thousands of individuals remain infected, their microfilarial levels are falling steadily.

*Burundi.* The disease is confined to the southern, less-populated parts of the country in the Ruzizi valley and along the shores of Lake Tanganyika. New foci have recently been discovered in Bururi and Bubanza provinces. No information is available on the socioeconomic consequences.

*Cameroon.* There is a hypoendemic focus in the north (Maroua), a hyperendemic region in the centre (especially round Poli, Tcholliré, and Touboro), and the disease is widespread in numerous foci in the south and south-west of the country. The hyperendemic region of Poli has recently received a large number of refugees from Chad. In the region of Touboro, an agricultural development project is threatened because of emigration from the area.

*Central African Republic.* There is a wide distribution of the disease in all areas of the country except possibly for the area around Bangui; there is particularly high endemicity along the Oubangui river and tributaries, and the Ouham and Bamingui rivers. No detailed information is available, but infection appears to be widespread and severe, and the effects seem to be similar to those observed in the Programme area before control.
**Chad.** There are 3 major endemic zones in the country all containing hyperendemic and mesoendemic villages of blinding savanna onchocerciasis—the Fianga-Léré zone on the Mayo Kebbi; the Baidokoum zone on the Logone basin, which is contiguous with the endemic areas in Cameroon and the Central African Republic; and the Mossafoyo-Marou zone in the Chari river basin. The endemic areas are sparsely populated despite having good soils and being rich in game and fish.

**Congo.** Endemic zones are found in the subprefectures of Minoulé, Boko, Mayombé, and Mouyondzi as well as along the Congo and Djoué rivers. No recent information is available on the socioeconomic consequences of the disease.

**Côte d’Ivoire.** Most of the country is included in the area covered by the Control Programme. In the forest foci, both inside and outside the Control Programme area, the disease does not appear to have serious socioeconomic effects.

**Equatorial Guinea.** There is a hyperendemic focus on Malabo; in Rio Muni infection occurs along the Campo, Benito, and Congo rivers near the coast, as well as in the north and east of Bioko. No recent information is available.

**Ethiopia.** Infection is widely distributed in the southwestern and northwestern parts of the country in the regions of Keffa, Wallega, Illubabor, Gamo Gofer, Gondar, and probably in parts of five other regions. Infection is particularly associated with coffee growing in the south-west. The large-scale resettlement of drought refugees in endemic areas may increase future problems.

**Gabon.** Seven separate foci are known, Lastourville, Ndjobé and Boné, Fougamou Sindara, Makokou, Nyanges, Lebamba, and Minongo, but nodules may be found in individuals from any part of the country. There are no known socioeconomic consequences, but the constant attention of the medical services is necessary in endemic areas, particularly where foci traverse the transgabonais highway construction.

**Ghana.** The northern regions of the country are in the area covered by the Control Programme, but uncontrolled transmission occurs in Brong-Ahafo, Ashanti, Volta, and a small part of the eastern region. Infection still occurs along the Pra river in the central region, probably also along the Ochi river, and along the Tano and Ankobra rivers in the western region. The Brong-Ahafo and Volta regions will be included in the southern extension of the Control Programme in 1987.
Guinea. Infection is widely distributed in Upper and Middle Guinea with many hyperendemic foci (e.g., Kankan, Farañah, and Dinguiyaye regions) and also occurs in parts of Forest Guinea (Yomou, N'Zérékoré, and Lola), and Lower Guinea (Fria and Forécaria). Almost all savanna foci will be included in the western extension of the Onchocerciasis Control Programme in 1987. In Upper Guinea several fertile valleys of the Niger basin have been abandoned in strips 15–20 km wide and there is a high prevalence of blindness. An inverse relationship has been established between the average population of a village and the level of endemicity. The disease does not appear to be so important in forest areas.

Guinea Bissau. Two principal endemic zones in the basin of the Corubal and Geba rivers in the east will be included in the western extension of the Control Programme in 1988. Both zones contain mesoendemic foci.

Kenya. The last remaining focus of the disease was on the slopes of Mount Elgon, but no cases were found during a small survey carried out in 1975. The possibility of recrudescence of transmission still exists.

Liberia. Infection occurs in some parts of all nine counties with important hyperendemic areas along the St Paul and Lofa rivers. Prevalence appears to be increasing. No deserted villages have been found. However, the limited number of blind have a reduced life expectancy and approximately 0.5–1.0% of the inhabitants of hyperendemic areas have severe chronic onchoderatitis and have become social outcasts.

Malawi. The principal disease focus is in the Thyolo district in the south; satellite foci probably also occur. The infection causes a significant reduction in productivity in the tea industry. Skin lesions account for many hospital and dispensary visits and sufferers believe the disease reduces their ability to work in their maize gardens.

Mali. The east of the country is within the Control Programme area although there is still transmission on the western borders of the area. In the west of the country, to be included in the western extension of the Control Programme in 1987/88, foci are found in all the valleys of the Niger, Baoulé, Bakoyé, and Bafing-Senegal rivers, as well as the Falémé and its tributaries. Blindness rates are similar in the eastern and western areas of the country.

Niger. The formerly endemic area is entirely covered by the Control Programme and microfilarial levels in previously infected individuals are falling markedly.
Nigeria. Infection is widespread in parts of all states except Lagos and Rivers states, and is found in both savanna and forest zones; infection is found in almost all parts of Anambra, Kaduna, Kwara, Ondo, and Oyo states. No new information is available on the socioeconomic consequences of the disease, but prevalence and blindness rates in many savanna foci are high. Nigeria is the country with the largest number of infected persons in the whole of Africa.

Senegal. Infection is found in the southeast in the department of Kédougou and parts of Tambacounda and Bakel departments. The entire endemic zone will be included in the western extension of the Control Programme in 1988. There are high rates of blindness in the hyperendemic zone in the east of the country and there are many depopulated strips along the fertile river valleys.

Sierra Leone. Onchocerciasis occurs in the northern province, which will be included in the western extension of the Control Programme in 1988, and infection is found over most of the rest of the country except the coastal plain. No information is available on socioeconomic consequences of the disease, but the savanna form of the disease with high blindness rates, probably exists in the north.

Togo. The north of the country is in the Control Programme area, but some transmission is still taking place near the southern limits of the Programme coverage. Infection is found over almost all the rest of the country; this area will be included in the southern extension of the Control Programme in 1987. In some places in the south the disease appears to be as severe as it was in the north before control.

Uganda. There is still widespread infection at the Mount Elgon focus. Other probable foci of infection include Budongo, Bugoma, West Nile, Ruwenzori, Kigezi, and Kabarega National Park. It is not known whether infection has returned to the area around the Nile below the dam where transmission was eliminated in 1973. No information is available on the socioeconomic consequences of the disease.

United Republic of Tanzania. There are numerous foci of infection at altitudes between 500 and 1500 m along a line from the Usambara mountains in the northeast to Lake Nyasa in the south. No socioeconomic consequences are known. Blindness prevalence rates range from 0.7% to 1.0% in villages with medium and low endemicity; these rates do not differ from those observed in villages with no onchocerciasis. Hyperendemic foci probably do not exist in Tanzania, and there is no migration away from infected villages. It
is therefore doubtful that the 7500 cases of blindness recorded for Tanzania in Table 1 were caused by onchocerciasis.

Zaire. Infection is widespread over most of the country except for the Shaba region in the south and the north of the Bandundu region. Particularly high prevalence rates are found in east and west Kasai, central and southern Equateur, Bas-Zaïre, and along the rivers of Haut-Zaïre. A high prevalence of blindness (6.5–8%) is found in some hyperendemic areas such as the diamond mining region of Sankuru. The success of new agricultural development in Haute Inzia (Bandundu) is threatened by an increasing prevalence of blindness.

2.3 The Eastern Mediterranean Region (Table 2)

A few possibly autochthonous cases of infection have been reported from the Tuban district of Lahej Governorate and the Lodar district of Abyan Governorate in the Democratic Republic of Yemen. Transmission may be taking place near wadis in the Asir region in southwest Saudi Arabia; more than a dozen cases have been reported recently in that country.

<table>
<thead>
<tr>
<th>Country (Assessment date)</th>
<th>Total population (UN Year Book 1983)</th>
<th>Number at risk in onchocerciasis endemic areas</th>
<th>Number infected with O. volvulus</th>
<th>Number blind as result of onchocerciasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sudan (1983)</td>
<td>20,350,000</td>
<td>2,000,000</td>
<td>520,000</td>
<td>8,400</td>
</tr>
<tr>
<td>Yemen (1985)</td>
<td>5,230,000</td>
<td>60,000</td>
<td>20,000</td>
<td>None reported</td>
</tr>
<tr>
<td>Regional subtotal</td>
<td>25,590,000</td>
<td>2,060,000</td>
<td>540,000</td>
<td>8,400</td>
</tr>
</tbody>
</table>

2.3.1 Distribution by country

Sudan. Known endemic zones extend over the whole of Bahr el Ghazal, the western part of Equatoria, the Radom area of southern Darfur, southern and eastern parts of Upper Nile, the Khor Yabus area of Blue Nile, parts of the Atbara and Setit rivers in Karsala, and the Abu Hamad focus in Nile Province. In northern Darfur infected refugees from Chad are settling in large numbers and there is a danger that the southern Darfur focus will expand further. However, the real extent of onchocerciasis infection may be greater than this.
In the southwestern foci, there has been large-scale desertion of fertile river valleys equivalent to that seen in the area covered by the Onchocerciasis Control Programme before the initiation of vector control. In the Raga area, where people have been settled close to rivers, up to 25% of the adult population is blind.

Yemen. Infection appears to be limited to a few permanent, major wadis at altitudes of 300–1200 m: Wadi Ghayl, Rasyan, Zabid, Rima, and Surdud, and possibly Siham and Harad. Severe onchoceratitis occurs in 0.5–2.0% of infected persons and this reduces working capacity as well as being physically and socially disabling.

2.4 The Region of the Americas

In the last 10 years new foci have been discovered in the Americas (see Table 3 and Fig. 2) in the province of Esmeraldas in the north of Ecuador where the prevalence of infection appears to be increasing. A focus among Amerindians has been confirmed on the north-west border of Brazil (Amazonas and Roraima territories) and has been shown to extend to the neighbouring area of Venezuela.

2.4.1 Distribution by country

Brazil. Onchocerciasis is found in Yanomami and Makiritari Indians living near the Auaris, Parima, and Toototobi rivers adjoining Venezuela. There may be a new focus in Goiás State where a single autochthonous case has recently been identified at Minazu. Infection is at present confined to isolated groups of Amerindians. However, the area contains gold and mineral ores, including cassiterite, and the population is, therefore, likely to increase in the future.

Colombia. Infection was confined to the Micay river area in the municipality of Lopez, department of Caucá, but transmission has now ceased and only a few people remain infected. A few cases of the disease may also occur in other areas of southern Colombia adjoining the focus in Ecuador. The disease has no socioeconomic consequences. The number of persons at risk may be smaller than indicated in Table 3.

Ecuador. Infection occurs in Esmeraldas province and there is a major focus on the Santiago river basin with satellite areas on the
Table 3. The Region of the Americas: estimated number of persons at risk, infected, and blind

<table>
<thead>
<tr>
<th>Country (Assessment date)</th>
<th>Total population (UN Year Book 1983)</th>
<th>Number at risk in onchocerciasis endemic areas</th>
<th>Number infected with <em>O. volvulus</em></th>
<th>Number blind as result of onchocerciasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brazil (1985)</td>
<td>129,660,000</td>
<td>8,000</td>
<td>5,500</td>
<td>100</td>
</tr>
<tr>
<td>Colombia (1985)</td>
<td>27,520,000</td>
<td>732,300</td>
<td>Not known</td>
<td>0</td>
</tr>
<tr>
<td>Ecuador (1984)</td>
<td>9,250,000</td>
<td>11,180</td>
<td>7,000</td>
<td>500</td>
</tr>
<tr>
<td>Guatemala (1985)</td>
<td>7,930,000</td>
<td>441,100</td>
<td>49,000</td>
<td>500</td>
</tr>
<tr>
<td>Mexico (1980)</td>
<td>75,100,000</td>
<td>190,500</td>
<td>24,700</td>
<td>100</td>
</tr>
<tr>
<td>Venezuela (1965)</td>
<td>16,390,000</td>
<td>3,486,200</td>
<td>20,000</td>
<td>100</td>
</tr>
<tr>
<td><strong>Regional subtotal</strong></td>
<td>265,650,000</td>
<td>5,251,280</td>
<td>97,200</td>
<td>1,400</td>
</tr>
<tr>
<td><strong>Global total</strong></td>
<td>586,190,000</td>
<td>85,583,780</td>
<td>17,757,700</td>
<td>336,400</td>
</tr>
</tbody>
</table>

*Addition of the Regional subtotals from Tables 1, 2, and 3.

Verde, Canande, Viche, Sucio, Cojimies, and Vilsa rivers. So far no socioeconomic consequences are recorded but prevalence and, presumably, severity are increasing and blindness may become a problem. Levels of infection seem to be higher among Chachi Amerindians than among Negroes living in the same villages.

**Guatemala.** Endemic foci are recognized in the northwest bordering Mexico (Huehuetenango department), and in the south and east, affecting parts of six departments (Suchitepéquez, Solola, Chimaltenango, Escuintla, Guatemala, and Santa Rosa). The disease is associated principally with coffee, sugar, and cardamom plantations which often employ a number of migrant labourers. Blindness and severe ocular lesions affect agricultural productivity and make individuals a burden to their families. In addition, the threat of eye lesions makes it necessary to maintain a costly nodulectomy service.

**Mexico.** There are two areas of infection in southeastern Mexico, one in the State of Chiapas and one in Oaxaca. The Soconusco focus is continuous with the endemic area in western Guatemala and is the largest in terms of both area and number of people at risk. There are also many infected individuals in northern Chiapas around San Cristóbal de las Casas who have become infected while harvesting coffee in the Soconusco region. The disease is particularly associated with the labour force working on coffee fincas. Regular nodulectomy and diethylcarbamazine treatment are carried out by specialized teams.
Venezuela. The two northern foci have been known for many years; one is towards the east in the States of Sucre, Anzoategui, and Monagas; the other is more central involving areas of the States of Aragua, Carabobo, Miranda, and Guarico, and small parts of Cojedes, Falcon, and Yaracuy. In addition, there is the recently discovered focus in the State of Bolivar and the Federal Territory of Amazonas among Amerindians of the Yanomami, Makiritari, and Piaroa tribes. Infection is mild in the coastal area where there is a high population density and the prevalence is falling due to chemotherapy programmes. There seem to be few or no socioeconomic consequences of the disease. The disease is more serious in the southern focus amongst Amerindians and is important at the local level. At present, this area is sparsely populated but is scheduled for future economic development.

2.5 Conclusions

Based on the data available, the present Expert Committee has estimated that the total number of people exposed to the risk of onchocerciasis is 85.5 million and that the total number infected is about 17.5 million, the great majority of them living in Africa. However, the disease appears to be spreading in Latin America. This estimate of persons infected is lower than those made in 1947, 1966, and 1976 when the estimate was 20 million.

In the last decade, tremendous advances have been made against onchocerciasis in the Onchocerciasis Control Programme area in West Africa where an estimated 1.25 million people have lost their infection and some 3 million children born in the Programme area since the onset of control have so far lived free from the risk of infection. On the other hand, in several major endemic zones with high blindness rates, such as Nigeria, Cameroon/Chad/Central African Republic, Sudan, and Zaire, little progress has been made and little information is available about the true extent and severity of onchocerciasis. Recent surveys in a number of countries have revealed a higher number of infected persons than was previously realized. It is therefore reasonable to postulate that the figure of 17.5 million for the total number of people infected with *O. volvulus* throughout the world is an underestimate. From the available data, there are approximately 340 000 people who are blind as a result of onchocerciasis. On the basis of this figure, it can be estimated that there are probably one million people in the world who have suffered
a significant visual loss (either reduced visual acuity and/or constricted visual fields) leading to, at least, partial disability as a result of onchocerciasis. Just as the total number of people infected may be an underestimate, this estimated number of people with blindness or visual disability is also probably too low.

2.6 Suggestions for further study

Efforts should be made to obtain more data on the prevalence and intensity of infection in all countries where onchocerciasis is endemic, and a watch should be kept for the development of new foci of infection.

3. CLINICAL MANIFESTATIONS

There is a wide spectrum of clinical manifestations associated with onchocerciasis and there are marked geographical variations; signs and symptoms may be characterized as dermal, lymphatic, systemic, and ocular.

3.1 The prepatent and incubation periods

The prepatent period, i.e., the period between infection with infective larvae and the production of detectable microfilariae by fertilized adult female worms, varies from 7 months to more than 2 years; most commonly it is 12–15 months. It is not known whether growing worms cause clinical symptoms during this period, but this possibility cannot be excluded. The immature worms certainly stimulate immunological responses, and pruritis has been reported in the prepatent period. Even without fertilization the female worms may induce the formation of small nodules and, provided there are no other productive macrofilariae present, patients in whom this occurs will not have microfilariae. The clinical incubation period, i.e., the time from invasion by infective larvae to the development of clinical symptoms or signs, is generally longer and more variable than the prepatent period. It may last for many years and is normally longer for ocular than for dermal disease.
3.2 Dermal onchocerciasis

The skin manifestations are common and may be of major concern to the patient. The type and severity of the skin lesions produced vary between different geographical areas. However, it is difficult to describe clear patterns of skin disease that are typical of each bioclimatic zone, such as African savanna or rain-forest, because of the wide range of skin changes observed among individuals living in different areas of these zones and even within the same community.

3.2.1 Early skin lesions

The skin lesions associated with onchocerciasis result from inflammatory reactions around damaged or disintegrating microfilariae. Antigens released by microfilariae probably damage vessels and connective tissue in the skin and, therefore, the clinical manifestations vary according to the microfilarial density in the skin, the immune responsiveness of the human host, and the duration of infection. Most microfilariae are found in the upper dermis, but they can be found at all skin levels, and also in the subcutis.

Most infected persons complain of pruritus that is intermittent at the beginning, but later becomes severe and permanent, sometimes so severe that it causes insomnia and reduced working capacity. Pruritus is not necessarily related to microfilarial density and some people do not complain of any itching despite the fact that they have a significant microfilarial load in their skin.

Visible skin changes include an alteration in the pigmentation with areas of hyper- and hypopigmentation. Hypopigmented macules can, at times, be confused with the early signs of leprosy. Other patients may develop subacute relapsing or chronic inflammatory skin disease. In the beginning, this is usually localized to one part or anatomical quarter of the body. Later, it may spread to other parts or, rarely, to the entire body. Inflammatory manifestations include urticaria, macules, papules, and, less frequently, pustules, scaling, oedema, lichenification, and pachydermia. Severe itching and scratching may lead to superficial excoriations and crusts. Secondary bacterial infection may develop, sometimes leading to ulceration and, rarely, sepsis.

Recurrent dermatitis, usually of the face and upper trunk, is sometimes found in young people in Mexico and Guatemala and this condition is known as erisipela de la costa. It presents with fever,
painful deep-red swelling of the skin, oedema, papules, and inflammation of the eyes. In Africa, an acute urticarial reaction may occur, with headache, general malaise, fever, and musculo-skeletal pains, that clinically resembles the Mazzotti reaction.

3.2.2 Late skin disease

After years of chronic infection, with or without visible inflammatory lesions, atrophy of the skin develops. In severe cases the thin epidermis has a shiny fragile appearance that has been described as parchment-like (lizard skin). The appendages of the skin are reduced and the skin is dry. The normal dermal structure is replaced by scar tissue, and the alteration of collagenous and elastic fibres leads to loss of elasticity. The skin may be wrinkled and hang in loose folds in elderly people. Such folds occur on the face (leonine facies), in the axilla, above and below the inguinal folds (hanging groin), and on both sides of the knees. In some foci, dermal atrophy may be seen, even in young people. In onchodermatitis, periods of quiescence and exacerbation may alternate, and different forms of inflammatory and atrophic skin disease may be combined with varying severity.

A spotty depigmentation (leopard skin) is a striking feature on the shins. Rapid assessment of its prevalence in a village population may permit a preliminary estimation of the prevalence of onchocerciasis. Much less frequently, leopard skin is observed at other sites of the body, such as over the trochanters, iliac crests, inguinal folds, Scarpa’s triangles, scrotum or elsewhere.

A less common purplish discolouration of the skin (mal morado) occurring in older people with high microfilarial loads is found in Mexico and Guatemala.

Chronic hyperreactive, localized onchodermatitis, often called sowda, was first described in the Yemen, but it also occurs with low prevalence in Africa and America. Yemenis with typical sowda present with an intensely itching, usually asymmetrical, well-circumscribed onchodermatitis with papules, and often with pustules and crusts, oedema, pachydermia, darkening of the skin, and considerable enlargement of the local lymph nodes. Microfilariae are usually restricted to the area of the skin lesions and their density is always very low. These patients have high levels both of blood eosinophils and antifilarial antibodies. In most cases, administration of a microfilaricidal drug causes a distinct papular
Mazzotti reaction in patients with sowda. In Africa, the area of skin disease in people with sowda is often more extensive than in Yemen. Sowda usually occurs in children and young persons, for whom it represents not only a severe physical disease, but also causes psychic and social problems that require therapy.

Dermal onchocerciasis presents a spectrum of manifestations from low to high reactivity against microfilariae. One pole is represented by a chronic hyperreactive onchodermatitis, an ability to kill microfilariae, and an active immune response to parasite antigens that is associated with severe dermal pathology. The other pole is more common and is represented by the generalized form of onchocerciasis, usually associated with high microfilarial loads with relatively little skin disease, especially during the earlier years, and a reduced immunological response. Intermediate types occur frequently and one type may change into the other. Patients with untreated intermediate-type dermal onchocerciasis may suffer from transient inflammatory attacks of comparatively mild dermatitis with intermittent pruritus, oedema, papular rashes, and changes of pigmentation. The symptoms are usually milder than those of sowda, and are associated with moderate to high microfilarial densities. Later, these cases may progress to a state of nonreactivity to their invading parasites as the level of infection increases and the generalized form of onchocerciasis develops.

3.2.3 Skin manifestations in expatriates

In expatriates and other people who enter endemic areas and first become infected in adult life or after early childhood, acute pruritic skin rashes may occur. These may be red and papular, and are often confined to one anatomical quarter of the body. The rash can be associated with mild local lymphadenopathy and with an intermittent dull aching pain in the muscles of the affected part.

Because of the length of the prepatent period of onchocerciasis these signs and symptoms may not start until some 1–3 years after the patient has left the endemic area, particularly if the stay there was short. This long time-lag may cause diagnostic problems when the patient presents to a physician outside the endemic area.
3.3 Nodules (onchocercomata)

Fibrous nodules form around the female macrofilariae. The nodules usually contain 1–10 adults worms, but in some cases, up to 60 have been observed. The ratio of male to female worms present in the nodules is usually between 1:1 and 1:2. Palpable nodules are found on various sites of the body either lying subcutaneously or occasionally attached to the skin. Many others are deeper and impalpable lying near to joint capsules (especially the hip), bones, fasciae, and rarely in or between muscles. Apart from a single reported case in the aorta, adult *Onchocerca* have not been found in body cavities or inner organs.

The distribution of palpable nodules on the body varies in different geographical regions. In most endemic areas in Africa, the majority of nodules are found on the pelvic girdle, especially near to the iliac crests, the greater trochanters, the sacrococcygeal region, and the ischial tuberosities. A few are also found on the head, over the ribs, scapulae and vertebrae, on both sides of the knees, and rarely elsewhere. In young children in Africa, relatively more nodules are found over the upper part of the body than elsewhere, especially on the head, and some are seen over the abdominal wall. In Guatemala and Mexico, nodules are more frequent on the head in all age groups. In South America, nodules are generally less common and are usually found over the lower part of the body. Typically nodules measure 0.5–2 cm in diameter; a few are smaller and some are larger with diameters exceeding 6 cm. They are firm, well-defined masses, round or elongated, often lobulated, and they are not tender or painful; a few are hard because they contain only calcified worms, and at times they may inconvenience the patient. Occasionally after chemotherapy, nodules become painful.

Often, several nodules are closely attached to one another and in these cases it is difficult to count them accurately. To assess the intensity of infection it is preferable to count the number of palpable nodules and also to record their distribution on the body. In order to locate as many nodules as possible by palpation, the patient should always be questioned, since he will often be able to point out his own nodules, particularly small ones that would otherwise be difficult to detect, e.g., under the hair of the head or in the muscles of the thighs and calves. During surgery, additional nodules are often found lying beneath those that were palpable from the surface. An unknown, and probably substantial percentage of the total
nodule load lies in the deeper tissues and cannot be detected clinically by inspection and palpation. The average number of palpable nodules on adults living in hyperendemic areas in Africa is 5–10, and an additional 3–4 nodules may be found deeper in the body during nodulectomy; individual nodule loads may be much higher, and cases of individuals with more than 100 nodules have been reported. There are many people with microfilariae, especially children, in whom no nodules can be detected; conversely there are a small number of individuals with nodules but no detectable microfilariae.

Onchocercal nodules may be confused with lymph nodes around the ear, in the suboccipital region, near to the axilla, between the ribs, or in the inguinal region. Nodules must also be distinguished from lipomata, foreign body granulomas, sebaceous and dermoid cysts, ganglia and, in some regions, from histoplasmosis nodules or cysticercosis. The diagnosis can be confirmed by removal and examination of the nodule or by needle aspiration and demonstration of filarial material.

3.4 Lymphatic onchocerciasis

In endemic areas, those infected with *O. volvulus* often present with enlarged, firm, discrete superficial lymph nodes that are not painful or tender. In onchocerciasis patients the lymph nodes draining areas of filarial dermatitis may be affected, the inguinal and femoral nodes being those most commonly involved. Most of these lymph nodes contain small numbers of microfilariae and in long-standing infections severe fibrosis develops. Clinically they differ little from the lymph nodes of uninfected persons, since slight inguino-crural adenopathy is common in endemic areas and has several causes including infections of the legs and feet. After treatment with a microfilaricidal drug the regional lymph nodes usually become larger, soft, tender, often painful, and contain increased numbers of disintegrating microfilariae.

Patients with severe, chronic hyperreactive dermatitis or sowda show clusters of strikingly enlarged, soft, and homogeneous lymph nodes draining the affected skin; it is mostly the femoral lymph nodes that are involved.

Sowda patients often have considerably swollen, oedematous legs for several years. This swelling may resemble elephantiasis but it is mostly reversible and in only a relatively few cases does a mild
residual swelling of the legs persist in later years when the dermatitis has improved. An association between elephantiasis of the genitalia and onchocerciasis has been described in some endemic areas (Chad, Sudan, Zaire), but not in others. However, it has never really been shown to what extent onchocerciasis is the cause of elephantiasis.

3.5 Systemic manifestations of onchocerciasis

Small numbers of microfilariae can be found not only in skin, eyes, and lymph nodes, but also in other organs. In hyperendemic villages microfilariae may be detected in the peripheral blood of up to one-third of the infected population and a similar number of infected persons may excrete microfilariae in their urine. Microfilaraemia and microfilaruria increase after the administration of filaricidal drugs; microfilariae have also been observed in sputum, tears, synovial fluid, hydrocoele fluid, vaginal smears, and, most importantly, in the cerebrospinal fluid after treatment. In one study, 3% of the newborn babies of mothers heavily infected with *O. volvulus* were found to have microfilariae in the skin, in umbilical cord blood, or in the tissues of the cord. The significance of the presence of microfilariae in the inner organs, for the well-being of the patient, is not known. However, the clearance of microfilariae in the liver that is observed after treatment with diethylcarbamazine, may cause the significant increases of serum aspartate aminotransferase (EC 2.6.1.1), serum alanine aminotransferase (EC 2.6.1.2), and lactate dehydrogenase in the blood.

Blood eosinophilia may be found in patients with onchocerciasis and very high numbers of eosinophils may be seen in those with hyperreactive disease.

In hyperendemic areas, patients with severe onchocerciasis may have lower body weights than uninfected persons or those with mild infections; the significance of this relationship is unknown. Epilepsy and dwarfism with delayed sexual development have been associated anecdotally with onchocerciasis in patients from hyperendemic areas. In particular, the association of dwarfism and onchocerciasis has been suggested following observations made on patients in Uganda.

The significance of the systemic manifestations of *O. volvulus* infection is not clear nor is it possible to assess their public health importance at this time.
3.6 Ocular onchocerciasis

The main pathological changes that occur as a result of onchocerciasis appear to be directly or indirectly related to the local death of microfilariae; blinding lesions result, at least in part, from the death of microfilariae in the eye. There are several routes by which microfilariae can enter the eye. They may enter the cornea from the skin by way of the conjunctiva; other routes of entry include the bloodstream and direct invasion of the posterior part of the eye particularly along the sheaths of the ciliary vessels and nerves.

In the eye, as elsewhere in the body, live microfilariae generally stimulate very little inflammatory response and appear to pass undetected by the host’s immune system. Live microfilariae may be found in the conjunctiva, cornea, sclera, anterior and posterior chambers, vitreous, the whole of the uveal tract, retina, and the optic nerve and its sheath. In the untreated disease, a relatively small number of microfilariae are dying at any given time and each initiates only a limited inflammatory response. With time, many microfilariae die, and cumulatively this produces considerable local inflammation and scarring. Because the eye depends on optical clarity for its normal function, even minor scarring can cause significant visual impairment.

3.6.1 Geographical variations in ocular onchocerciasis

Blindness as a result of onchocerciasis can be caused by a variety of lesions that affect different parts of the eye. Regional geographical differences occur in the clinical picture of ocular onchocerciasis. In general, in the savanna areas of West Africa, blindness is common (2–15% of the population in hyperendemic areas) and is mainly attributable to sclerosing keratitis, whereas, in the rain-forest areas of West Africa, blindness is less common (less than 2%) and is often due to posterior-segment lesions. At present, in Guatemala and Mexico, blindness is relatively rare and usually results from anterior uveitis. Other endemic areas, both in Africa and South America, may exhibit other patterns. The most salient feature, however, is not the regional differences, but the striking similarities that exist in the ocular changes found in different areas.
3.6.2 Early ocular lesions

In onchocerciasis infection, the earliest sign of ocular involvement is the invasion of the eye by microfilariae. Microfilariae in the anterior chamber can be seen easily with a slit lamp, especially if, before examination, the patient sits with his head between his knees for at least 2 minutes to allow any microfilariae in the anterior chamber to sink towards the centre of the cornea. The microfilariae are seen as small, thin, wriggling, white worms approximately 0.3 mm long, glistening in the light beam. Often they can be seen to circulate in convection currents in the aqueous environment.

 Conjunctival reactions with hyperaemia, chemosis, and epiphora are often present in severely infected patients and may be particularly severe during the early stages of treatment with some microfilaricides or suramin sodium.

 In the cornea, dead microfilariae are relatively easy to see because they lie straightened out and are opaque. Live microfilariae can be more difficult to find because they are almost transparent and are often coiled. Dead microfilariae can be seen with direct illumination and often form the nucleus of a punctate opacity. Live microfilariae are best seen with high magnification (25x) using retroillumination produced by an oblique beam reflected from the iris. Whether they are alive or dead, microfilariae are most common in the peripheral cornea, especially temporally and nasally, but they may be found anywhere in the eye.

 In the cornea, dead microfilariae are usually surrounded by an inflammatory infiltrate that appears as an ill-defined punctate, "fluffy" or "snowflake" opacity, about 0.5 mm in diameter. Although punctate keratitis occurs in people who have not received treatment, it is more commonly observed in people treated with a microfilaricidal drug, such as diethylcarbamazine, when many microfilariae are killed simultaneously. Initially, a dead microfilaria can be recognized in the centre of a punctate opacity but, over a period of several weeks, the remains of the microfilaria disappear and then over several months the opacity clears without visible sequelae. Because the opacities form around dead microfilariae, their distribution is similar to that of the microfilariae themselves.

 Recently, intraretinal microfilariae have been recognized clinically. They are best seen with slit-lamp contact lens examination. Occasionally they can be seen lying under the internal limiting membrane of the retina, but more commonly they appear as small, pale
metallic green, slender, intraretinal opacities near retinal blood vessels; they can be seen to move when observed over a period of several minutes (2). No retinal inflammation is visible around them. Microfilariae may be found in the anterior vitreous and, at times, they may be attached to the lens capsule or to Descemet’s membrane.

3.6.3 *Late ocular lesions*

(a) **Cornea.** An increased limbal haze seen through the slit-lamp is the earliest sign of sclerosing keratitis. A fibrovascular pannus and inflammatory infiltrate, composed mainly of lymphocytes and eosinophils, develops at the level of Bowman’s membrane. Over a period of years this opacification advances in an arc being most marked at each side and below where it becomes vascularized and partially pigmented. Usually the remaining areas of clear cornea show heavy infiltration of microfilariae. The opacity progresses to cover the central cornea, and eventually the whole cornea may become opaque so that the presence of sclerosing keratitis can be recognized even from a distance.

(b) **Anterior uveal tract.** The presence and severity of anterior uveitis (iritis and iridocyclitis) in onchocerciasis is extremely variable. A mild non-granulomatous uveitis, with a minimum flare and only an occasional cell, commonly occurs when there are microfilariae in the anterior chamber or the cornea. At times, the eye may be heavily infiltrated by microfilariae and yet there may be no detectable anterior chamber reaction. However, severe anterior uveitis, that may develop in some patients even in the presence of relatively few microfilariae, often leads to the formation of inferior, anterior, and posterior synechiae and then to the development of a characteristic pear-shaped deformity of the pupil, retropupillary fibrosis, and pseudohypopyon. Chronic granulomatous anterior uveitis also causes a loss of the iris pigment frill and a pumice stone appearance of the iris develops. Free granules of pigment are commonly seen in the aqueous, especially after a patient has been positioned head down. Mild anterior uveitis has been related to microfilarial invasion of the iris, and more severe granulomatous anterior uveitis to invasion of the ciliary body. Extensive synechiae may cause *seclusio* and *occlusio pupillae*, secondary cataracts, and secondary glaucoma.
The association between onchocerciasis and glaucoma has been controversial. Although the two conditions may coexist, onchocerciasis usually causes a marked lowering of intraocular pressure. However, intraocular pressure may be acutely elevated during onchocerciasis-induced anterior uveitis, and severe secondary glaucoma can occur owing to the extensive formation of synechia. There does not seem to be a direct relationship between onchocerciasis and chronic open-angle glaucoma.

(c) Choroid and retina. The wide spectrum of fundal changes observed appears to result from a few basic pathological processes. Numerous descriptive names and eponyms have been given to the different clinical pictures they present. However, it is simpler to consider the chorioretinal changes in terms of the basic pathological processes involved, affecting the inner retina, the outer retina and retinal pigment epithelium, and the choroid.

In the earlier stages of retinal involvement, the abnormalities seen are limited to the inner retina. Active retinitis appears in focal areas as an inner retinal whitening, often with small haemorrhages. Fluorescein angiography reveals leakage of retinal capillaries and veins in these areas. Transient small intraretinal haemorrhages independent of retinitis have also been observed during the early stages of the disease. Cotton wool spots (indicative of local ischaemia or infarction of the nerve fibre layer) also occur in onchocerciasis. These findings are usually subtle, transient, and isolated; their precise significance requires further study. Rarely, severe retinal vasculitis may occur.

Several different types of white retinal dot occur early in the evolution of onchocercal chorioretinitis. Some of these are intraretinal deposits in the mid- and outer retina and occur in the macula region as well as at the periphery. Small, local areas of retinal pigment epithelial atrophy are also common and may be widespread throughout the retina. The significance of these findings in the natural history of the disease and their relevance to chemotherapy, are not clear.

Another common early chorioretinal change is a granular atrophy or mottling of the retinal pigment epithelium. Although areas of severe atrophy are easy to see, milder forms may only be revealed by fluorescein angiography. Intraretinal pigment migration also occurs frequently, and small clumps of brownish pigment can be seen in the retina, anterior to the retinal pigment epithelium. In more severe forms of the disease, large distinct areas of chorioretinal
atrophy are common in the posterior pole, particularly temporal to
the macula and nasal to the optic nerve; they are usually associated
with retinal pigment clumping. Well-defined, white, plaque-like
areas of subretinal fibrosis, with fibrovascular scarring, may occur in
these regions. In the advanced stages of the disease, there can be
extensive chorioretinal atrophy and scarring involving the entire
posterior pole. In these cases, there is often optic atrophy and visual
acuity is extremely poor.

Each of the three changes just described—retinal pigment
epithelial atrophy, chorioretinal atrophy, and subretinal fibrosis—is
believed to represent an end stage of, or the structural change caused
by, previous active inflammation induced either by the local death of
microfilariae or, possibly, by circulating antiretinal antibodies or
immune complex deposition. There does not seem to be a clear
 correlation between the presence of intraretinal microfilariae and
these features of intraretinal inflammation.

Pale, ill-defined areas of swelling in the choroid may also occur;
they show leakage on fluorescein angiography and are thought to
be choroidal granulomas. Transitory retinal pigment epithelial
abnormalities have also been seen following diethylcarbamazine
therapy. In addition to the abnormalities directly attributable to
onchodercal chorioretinopathy, other vitreo-retinal abnormalities
are common in patients with onchocerciasis. It seems likely that
many of these changes may be secondary to ocular inflammation
with premature vitreous degeneration.

Optic nerve. Optic neuritis and optic atrophy are often seen in
patients with onchocerciasis. Microfilariae have been found within
the optic nerve and its sheaths, and it is reasonable to assume
that the occasional death of these microfilariae initiates an
inflammatory response and subsequent neuronal atrophy. Optic
atrophy would also be expected to occur due to the loss of ganglion
cells in areas of retinal atrophy and from secondary glaucoma.
However, optic neuritis and optic atrophy have been found to occur
more commonly in persons who have received treatment, either
with diethylcarbamazine or suramin sodium, than in those who
have not been treated with these drugs. It is unclear, therefore, how
much of the optic atrophy seen in patients with onchocerciasis is
caused by the disease itself and how much results from the
inflammation caused by treatment. Optic atrophy is usually
associated with peripheral visual field loss and may lead to tightly
constricted visual fields.
3.7 Suggestions for further study

(a) Identify the factors that determine the host response and the subsequent clinical manifestations of hyperreactive disease and hyporeactive, generalized disease.

(b) Study the interrelationship and biological balance between the host and the adult worms established in the nodule, paying attention to the stimulus for nodule formation, the nutrition of the parasite, and drug uptake.

(c) Establish the relative importance of *O. volvulus* compared with other filarial parasites as a cause of lymphoedema.

(d) Carry out detailed autopsies on heavily infected onchocerciasis patients to establish the distribution of deep nodules, the occurrence of free-living worms, the involvement of deeper organs, and the evolution of ocular changes.

(e) Study the distribution of deep nodules using modern non-invasive techniques, such as ultrasonography, computer-assisted tomography, and magnetic resonance imaging.

(f) Ascertain the public health impact of the systemic manifestations of onchocerciasis, including the general debilitation caused and immuno-unresponsiveness.

(g) Elucidate the association, if any, between *O. volvulus* infection and epilepsy and hypossexual dwarfism.

(h) Define the pathogenesis of ocular lesions, especially chorioretinitis and optic neuritis, including investigations on individual and geographical risk factors and variations in disease manifestations.

(i) Make detailed natural history studies on the evolution of ocular lesions in areas with and without ongoing transmission.

(j) Produce an up-to-date publication with clear illustrations describing the clinical and pathological manifestations of onchocerciasis, particularly the dermal and ocular changes, for general distribution to physicians and workers in the field.

4. GEOGRAPHICAL VARIATIONS IN ONCHOCERCIASIS

Although the clinical manifestations of onchocerciasis are generally similar throughout its range, there are characteristic and readily appreciable differences in the pattern of disease that are encountered in different parts of the world. These differences apply mainly to the clinical picture of the fully developed disease.
4.1 Clinical patterns in different parts of the world

4.1.1 West Africa

In West Africa the most striking difference in the clinical picture occurs between that seen in the savanna and that seen in the rain-forest belt. In the former, the blindness rates in heavily-infected villages can reach 15% and can cause severe socioeconomic consequences. In the forest areas, the disease can also be of considerable public health importance, but the blindness rates rarely exceed 2% and do not lead to migration from the endemic zones.

In the forest zone of Liberia where *Simulium yahense* and the *S. sanctipauli* subcomplex are the vectors, there is little blindness due to onchocerciasis, despite annual transmission potentials (ATPs) in excess of 5000.1 Similarly, in Côte d'Ivoire, there is almost no blindness in the forest, although transmission by *S. yahense* is intense. The onchocerciasis in the savanna of this country, transmitted by *S. damnosum* s.s. and *S. sirbanum*, results in a higher prevalence of severe ocular lesions in both the anterior and posterior segments of the eye, with more ocular microfilariae, greater number of nodules, and higher microfilarial concentrations, as well as more dermatitis, vitiligo, lymphadenopathy, and hanging groin.

In Cameroon, onchocerciasis in the savanna is characterized by higher skin concentrations of microfilariae than in the rain-forest and by more skin atrophy; in the eye there is intense microfilarial invasion of the cornea leading to a much higher prevalence of sclerosing keratitis, a somewhat higher prevalence of iritis, and 2.5 times more blindness. By contrast, in the most heavily-infected forest populations, more nodules are seen than in the savanna, as well as more skin depigmentation, groin lymphadenopathy, and hanging groin. The number of microfilariae in the anterior chamber of the eye and the prevalence of optic atrophy and chorioretinitis are similar in the two zones. It is very noticeable that the high rate of blindness seen in the savanna, where transmission is by *S. damnosum* s.s. and *S. sirbanum*, has been associated with annual transmission potentials of only 1000–3000, whereas in the forest, with *S. squamosum* as the main vector, figures of up to about 90,000 have been recorded, but the severity of the ocular onchocerciasis observed has not approached that seen in the savanna.

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1 See section 13.2.4 for explanation.
4.1.2 East and Central Africa and Yemen

Over most of East and Central Africa the clinical picture of onchocerciasis is not particularly severe. Local variations that have been reported include the high prevalence of severe eye lesions, particularly in the posterior segment, in the southern Sudan, where the vectors are known to be *S. damnosum* s.s. and *S. sirbanum*; the severe skin and lymphatic lesions seen along the Uele River on the border between Zaire and the Central African Republic, where gross elephantiasis of the scrotum and/or legs is often present; and the large number of head nodules and high prevalence of blindness seen in the Sankuru focus in the Zaire forests. In Kenya, before eradication occurred, severe blinding onchocerciasis was transmitted by *S. neavei* s.s. Finally, in the Yemen a very severe form of sowda occurs.

4.1.3 The Americas

Compared with that in Africa, the clinical picture in Guatemala and Mexico is characterized by a higher proportion of head nodules and much milder skin and lymph-node lesions. Before the nodullectomy campaign started there was a high prevalence of severe ocular onchocerciasis (see section 7).

In South America (Brazil, Colombia, Ecuador, Venezuela) relatively few serious eye lesions occur and there is little skin or lymph-node pathology.

4.2 Possible explanations for geographical variations

4.2.1 Parasite variants

There is ample evidence to show that there are genetically distinct populations of *O. volvulus*. The evidence for the existence of "savanna" and "forest" forms of *O. volvulus* in West Africa, each associated with different vectors of the *S. damnosum* complex, is based mainly on the results of crossed transmission experiments using human volunteers and experimental chimpanzees, and on the differing pathogenicity of the microfilariae of the two forms when inoculated into the eyes of rabbits. It appears that these entities differ in their "virulence" or in the extent to which their microfilariae produce pathological lesions in different organs or tissues (pathogenicity).
Over the last 10 years no conclusive evidence has been produced to refute the hypothesis that different forms of *O. volvulus* exist. However, there has been suggestive, although far from conclusive, evidence from acid phosphatase staining studies on microfilariae that there are significant differences in this variable between forest and savanna parasites in West Africa. Although individual microfilariae cannot yet be allotted to one or other form of *O. volvulus*, the frequency distributions of the five different microfilarial acid phosphatase staining patterns vary significantly between the savanna and forest populations (3). Using biochemical and immunological methods it has been shown that there are clear differences between the antigen preparations from adult *O. volvulus* from savanna (Mali) and forest (Cameroon) (4). Likewise, electrophoretic characterization of isoenzymes in adult worms has revealed differences in allele frequencies found at certain loci that may be great enough to permit the biochemical identification of *O. volvulus* populations from different habitats. Studies are now in progress to determine whether differences can be detected in the DNA of the different forms, and whether the forms can be distinguished using monoclonal antibodies.

The assumption that "savanna" and "forest" forms of *O. volvulus* exist in West Africa has practical implications for control programmes in the area, particularly the Onchocerciasis Control Programme (see section 15). An important priority is to develop a reliable means of distinguishing between these two forms of *O. volvulus*, both in the individual human host and in the fly at the infective larval stage.

While it is clear that transmission by *S. damnosum* s.s. and *S. sirbanum* is associated with savanna onchocerciasis in West Africa and across to the Sudan, this is not necessarily a causal relationship. It may be that both the clinical picture of savanna onchocerciasis and successful breeding by *S. damnosum* s.s. and *S. sirbanum* depend on the same ecological factors. It must be considered that the present geographical limits of the two forms of the disease may not be stable. The limits may change if the recent decline in annual rainfall persists and deforestation continues.
4.2.2 Vector biting habits

It is difficult to assess to what extent the differences in clinical patterns may reflect the biting habits of the vector Simulium species. For example, there may be a marked seasonal pattern to the level of transmission, even in the West African forest belt, whereas in the savanna zone, there is no transmission in many severely afflicted communities for several months each year. In Guatemala and Mexico, S. ochraceum bites mainly on the head and upper parts of the body and this may account for the relatively high incidence of head nodules. Most of the other South American vector species tend to bite low on the body, but it is interesting to note that in the Brazil–Venezuela lowland focus the recently studied vector, S. oyapockense also shows a similar tendency to bite high on the body.

4.2.3 Host factors

In some areas, it may be that characteristics of the human host, particularly those affecting immunological responses, could account for the observed variations in the clinical picture. This may be the explanation for the relatively high prevalence of sowda lesions in the Yemeni foci. Other human factors (ecological, nutritional, etc.) may also influence the clinical pattern in different areas, as may multiple infections with other parasites.

4.3 Suggestions for further study

(a) Identify and classify the putative forms of O. volvulus and determine their relative pathogenicity.

(b) Describe in detail the geographically distinct clinical patterns of onchocerciasis and investigate the role of parasite, host, and vector in determining these patterns.

(c) Monitor the changes in the geographical distribution of clinical patterns and vector species following ecological changes.
5. PATHOLOGY OF ONCHOCERCIASIS

5.1 Dermal onchocerciasis

5.1.1 Untreated persons

(a) Early skin lesions. At the microscopic level, the essential feature of this condition is the presence of microfilariae in the dermis. Prior to treatment, microfilariae occur in all layers of the skin, but are most numerous in the upper dermis. Live microfilariae appear to cause no observable reaction, but skin biopsies from patients with many microfilariae may contain a few dead as well as live parasites. Such microfilariae usually have eosinophils or eosinophilic material attached to the cuticle. The morphology of some parasites is well preserved while others begin to disintegrate and only the remains of the cuticle and pyknotic nuclei are found. These disintegrating parasites may also be surrounded by neutrophils and macrophages.

(b) Late skin lesions. The chronic or late changes of onchocercal dermatitis probably result mainly from microfilarial death. Initially, inflammatory cells (mainly lymphocytes, histiocytes, plasma cells, mast cells, and a variable number of eosinophils) are found in the perivascular spaces of the upper dermis. Later, hyperkeratosis, focal parakeratosis, and acanthosis are seen as well as more advanced changes in the dermis including pigmented incontinence, dilation of lymphatics, tortuous dermal vessels, and an increase in mucosaccharides between the collagen fibres. Dermal fibroblasts increase in number and this increase leads to fibrosis and the gradual replacement of the normal dermis by hyalinized fibrous tissue which has a striking pattern of concentric perivascular fibrosis. “Fibrinoid degeneration” of dermal collagen, characterized by the attachment of amorphous eosinophilic material to some collagen fibres, may also be seen when tissue sections are processed with Russell-Movat stain. There is a loss of elastic fibres and a decrease in the number of skin appendages.

(c) Severe onchocercal dermatitis

(i) Sowda. The most striking histopathological feature of sowda is the presence of an extensive inflammatory cell infiltrate of the upper dermis that is composed of many plasma cells, a variable number of eosinophils, and a few lymphocytes and histiocytes. The
infiltrate forms a broad cuff around dermal vessels and appendages, greatly thickens the dermis, and extends into the subcutaneous adipose tissue. Sclerosis and oedema of the dermis may be prominent features. There is discontinuity of melanin, and Russell-Movat staining reveals reduced elastica in the upper dermis. Changes in the epidermis include hyperkeratosis, parakeratosis, follicular plugging, acanthosis with elongation of rete ridges, and scattered foci of intraepidermal oedema. Microfilariae are extremely difficult to find in tissue sections.

(ii) Acute inflammatory reactions. Skin biopsies taken from individuals (usually expatriate visitors to areas where onchocerciasis is endemic) with the hyperresponsive type of clinical presentation (section 3.2.3) generally show more acute inflammatory skin changes than those from other types of onchocerciasis. These include prominent intraepidermal and dermal oedema, dilated lymphatics surrounded by eosinophils, increased number of dermal fibroblasts, and perivascular inflammatory cell infiltrates consisting predominantly of eosinophils with a few lymphocytes and plasma cells. The natural evolution of these acute inflammatory skin changes (in the absence of treatment) has not been described.

5.1.2 Treated patients

A few minutes after the administration of diethylcarbamazine, eosinophils collect around the dermal microfilariae and then degranulate; the "fibrinoid" material that surrounds the microfilariae has been shown by immunofluorescence staining to include the eosinophil granule product—major basic protein (MBP). This major basic protein, and perhaps other toxic proteins from the eosinophil may play an important role in killing the microfilariae. Nuclei of the degenerating microfilariae become hyperchromatic and fragmented and finally disappear; disintegration of the microfilariae follows. These degenerating fragments of microfilariae are generally found in the upper dermis and they may be taken up by macrophages and small multinucleate cells.

Less commonly, some microfilariae are seen to migrate into the epidermis where they provoke the formation of eosinophil abscesses that mature or progress to become parakeratotic nests that can persist for up to 7 days after treatment with diethylcarbamazine. Clinically, this microfilarial death causes papules, or oedema and
urticaria, depending on the number present, the suddenness of the microfilarial killing, and the host's reaction.

Similar histological changes are seen after treatment with other microfilaricidal drugs. After metrifonate, the killed microfilariae are surrounded by eosinophils and later ensheathed by eosinophilic material. Fragments of disintegrating microfilariae are surrounded by macrophages and multinucleated cells, and some of the microfilariae are found in epidermal microabscesses. During suramin therapy, microfilariae in the dermis exhibit similar features of degeneration and disintegration. Information on the skin changes that occur following ivermectin treatment is not yet available.

Ultrastructural studies of onchocercal dermatitis before and during treatment with diethylcarbamazine have been carried out and changes in the cuticular layers of the microfilariae observed.

5.2 Onchocercal nodules

5.2.1 Untreated persons

Adult worms live either singly or, more often, as coiled entangled masses in the deep fascia and subcutaneous tissue. They become encapsulated by fibrous tissue to form discrete onchocercal nodules (onchocercomata) in the deep dermis and subcutaneous tissue.

The nodule has three essential components: an outer capsule of fibrous tissue; an inflammatory cell exudate; and the enclosed adult worm(s). The cut surfaces of nodules show a variety of appearances, reflecting different stages of host reaction and worm degeneration. Some nodules have a solid matrix of granulation tissue surrounding the coiled worms. Others have a soft central fibrin lake that surrounds portions of worm. Older nodules may contain calcified worm fragments.

Microscopically, a variety of inflammatory components (granulomatous, suppurative, and chronic) may all be present. The central cavity is rich in fibrin and contains portions of adult worms. A thick layer of fibrinoid material surrounds the cuticle of the female worm, and sometimes there is mucosaccharide in the matrix. Clusters of neutrophils attack and remove this fibrinoid material from the worm's cuticle, while histiocytes and giant cells phagocytose the cuticle and other portions of the dead adult worms. Even in mineralized nodules, fragments of cuticle can usually be identified. Nodules also contain microfilariae outside the adult
worm; some degenerate with degranulation of eosinophils as in the
dermis, but others are phagocytosed by small multinucleate cells.

There appears to be an intimate relationship between the cuticle
of the adult worms and the capillaries of the host. Capillaries
proliferate in the granulation tissue around the worms, with vessels
from the outer capsule arborizing into capillary beds that form a
"jacket" or "sleeve" around the adult worms. There is also a space
around the worm that is continuous with blood vessels and the
central fibrin lake of the nodule. Immunoperoxidase staining for
Factor VIII antigen (von Willebrand's factor) reveals proliferation
of the endothelium of host vessels around the worms, and histo-
chemical staining indicates iron deposits along the cells lining the
worm's intestine.

In sowda, untreated patients usually show few or no palpable
nodules and the nodules present contain very few adult worms.
Nodules that do contain microfilariae-producing female worms
show an extensive host reaction with eosinophils, and the nodule is
rather large in relation to the few adult worms it contains.
Microfilariae at all stages of degeneration and disintegration are
found, either embedded in eosinophilic material and surrounded by
macrophages or in neutrophil-containing microabscesses. The
tissues around the nodules may show considerable infiltration by
eosinophils and, if muscles are involved, degenerating muscle fibres.

5.2.2 Treated patients

Diethylcarbamazine treatment may cause microfilariae to be
discharged from the uterus of the female worm; and in a young
nodule, removed during therapy with this drug, a violent reaction,
not to the worm, but to the many microfilariae discharged from the
worm, has been described.

A few weeks after the initiation of suramin therapy, some nodules
become enlarged supplicative cysts accompanied by inflammatory
reactions. Rarely, this leads to perforation of the skin and the
formation of a fistula, through which pus and worms may exude.
During the first months following treatment, purulent cysts
sometimes form around dying worms. The number of microfilariae
outside the adult females decreases and after about 3 months this
number approaches zero; 4-8 months after treatment many nodules
become fibrotic, containing fragments of calcified worms and the
remains of other adult worms surrounded by giant cells. Concentric
rings of fibrosis indicate sites where the adult worms have been absorbed.

5.3 Lymphatic onchocerciasis

5.3.1 Untreated persons

The histological basis for the concept of onchocercal lymphadenitis is two-fold: (a) the presence of onchocercal microfilariae in the nodes; (b) a progression of characteristic histopathological features leading to complete lymph-node fibrosis. These changes correlate with special clinical manifestations of severe onchocerciasis, including hanging groin, cutaneous oedema, elephantiasis, and severe Mazzotti reactions.

At first the lymph nodes are enlarged, but eventually they become sclerotic. These changes may be followed by lymphatic obstruction and the development of overlying hanging groin when femoral or inguinal nodes are involved, or rarely hanging armpit when axillary nodes are involved. The nodes show varying degrees of fibrosis of the capsule, pericapsular tissues, and interior of the node, with up to 80% fibrous replacement of the cross-sectional area. Nodes in the “early stage” have follicular hyperplasia, sinus histiocytosis, and varying numbers of plasma cells, eosinophils, and neutrophils. Nodes in the “intermediate stage” show moderate fibrous replacement, but have less pronounced sinus histiocytosis. Nodes in the “late stage” show atrophy with replacement of almost all the lymphoid cells by fibrous tissue. The pattern of fibrosis is perivascular. The Russell-Movat connective tissue stain reveals “fibrinoid material” that by immunofluorescence staining has been shown to include eosinophil granule major basic protein; the same protein has also been found in specimens of skin.

Microfilariae can often be identified in lymph nodes, most frequently in the fibrous capsule and interstitial connective tissue, but sometimes in the medullary and cortical regions.

In contrast to the atrophic and fibrotic nodes from Africans with longstanding generalized onchocerciasis, Yemenis with sowda of the legs have greatly enlarged femoral lymph nodes. Indeed, clusters of 3–6 lymph nodes may present an accumulation of 50–80 g of highly active lymphatic tissue. Histologically, these nodes exhibit a typical strong humoral response, i.e., follicular hyperplasia with activation of germinal centres; increased number of plasma cells (staining for
IgG and IgE) in the medullary cords; many eosinophils, mast cells, and immunoblasts within the sinuses; and often a vasculitis with increased number of eosinophils and mast cells. No microfilariae are seen in these hyperreactive lymph nodes.

5.3.2 Treated patients

Nodes removed from patients with generalized onchocerciasis during treatment with diethylcarbamazine have shown an early (first 3 days), massive infiltration with large numbers of microfilariae and eosinophils. These cells adhere to the microfilariae, and their degranulation products are prominent in the local inflammatory reactions. Plasma cells, again staining for IgE and IgG immunoglobulins, and many mast cells also infiltrate these nodes following the administration of diethylcarbamazine. Changes in the lymph nodes of patients with sowda-type onchocerciasis after diethylcarbamazine treatment have not yet been described.

Histological changes in the lymph nodes have not yet been studied after treatment with other drugs.

5.4 Systemic onchocerciasis

5.4.1 Untreated persons

There are very few data available on the involvement of internal organs because few autopsies have been performed on patients with onchocerciasis. In those that have been carried out, onchocercal nodules have not been found in the lungs, intestine, liver or other visceral organs but in one case a coiled, non-gravid adult female, believed to be *O. volvulus*, was found in the wall of the thoracic aorta. Microfilariae, on the other hand, have been found in many internal organs in autopsies of untreated patients; sometimes they are seen within the blood vessels, but at other times they lie in the parenchyma away from the vascular channels.

5.4.2 Treated patients

Following treatment with diethylcarbamazine, microfilariae are mobilized and using histological methods they can be seen at autopsy in the vessels as well as in the parenchyma of internal organs including the liver, kidney, lungs, and pancreas. In these organs, they
have been found degenerating in microabscesses containing lymphocytes, plasma cells, and polymorphonuclear leukocytes. Since such autopsy tissue has only rarely been obtained, it is unclear if these findings are typical of most patients treated with diethylcarbamazine.

5.5 Ocular onchocerciasis

5.5.1 Untreated persons

The scarcity of human eyes for pathological examination has severely limited the number of studies carried out on the histopathology of ocular onchocerciasis. Most of the human case material available represents advanced stages of the disease of many years’ duration, and conclusions drawn from these studies about the early stages of the disease process are unreliable. Animal models have been used to determine the types of early reaction that may take place, but the conclusions that can be drawn from these studies are limited by the lack of comparable investigations of eye pathology in definitive hosts of *Onchocerca*.

Pathology in the conjunctiva has been studied in human onchocerciasis by means of biopsies. Inflammatory cells may be present in small numbers but they do not appear to interact directly with living microfilariae. However, dead microfilariae are usually surrounded by eosinophils and lymphocytes. Punctate keratitis is a transient change seen in the early stages of ocular involvement, and collections of eosinophils and chronic inflammatory cells surround the dead microfilariae in the corneal stroma. These reactions usually resolve without residue once the microfilarial debris has been removed.

Sclerosing keratitis is a permanent opacification that develops progressively. It consists of chronic inflammatory cells, with occasional eosinophils and neutrophils, in a fibrovascular pannus of the superficial stroma near the level of Bowman’s membrane. Redistribution of pigment may occur, and subepithelial bullae can develop overlying the chronic inflammatory infiltrate. Vascularization, fibroblast proliferation, and chronic inflammatory infiltrates may occur in the deep stroma as well as superficially; limbus with perivascular plasma cell cuffs is usually present. A florid interstitial keratitis has been described in human corneas heavily infected with microfilariae, and this may be an early stage of
sclerosing keratitis. The shift from acute inflammation to chronic inflammation and pannus may result from the summation of many individual acute reactions to microfilariae in the cornea; autoimmune mechanisms also may play a role, since microfilarial antigens may be retained within the cornea.

Nongranulomatous anterior uveitis occurs, with plasma cells and eosinophils infiltrating the iris and ciliary body; these cells may also be present in the anterior chamber and iris. In long-standing infections fibrosis, necrosis, hyalinization and pigmentary changes of the iris, and anterior synechiae, may occur in addition to the chronic inflammatory cell infiltrate. However, in cases of severe uveitis, microfilariae may not be demonstrable in the anterior uvea.

Posterior segment lesions are of particular importance in the development of blindness due to *O. volvulus* infection. Retinal changes may include microcystic degeneration of photoreceptor and ganglion cell layers, atrophy of nerve fibre and ganglion cell layers, and obliteration of the choriocapillaris and neuroretina, leaving chorioretinal scars. Pigmentary alterations may also occur, including pigment epithelial hyperplasia, heaping-up of pigment granules in the pigment epithelium (drusen), and migration of pigment-containing macrophages into the photoreceptor layer. The optic disc may be severely cupped secondary to glaucoma, and the optic nerve head may be atrophic or infiltrated with chronic inflammatory cells. Plasma cell perivascular cuffs may occur around the ciliary arteries. Choroidal pathology may include round cell and eosinophil infiltration, obliteration of the choriocapillaris, and hyalinization of the choroidal stroma.

5.5.2 Treated patients

The only ocular tissues specifically studied histopathologically after treatment have been the globular infiltrates that develop at the conjunctival limbus during the first hours following the administration of diethylcarbamazine. These globules consist of amorphous eosinophilic material surrounded by inflammatory cells comprising eosinophils, mast cells, and basophils showing appreciable degranulation, together with perivascular collections of these same cells and plasma cells. Sometimes microfilariae can be identified in the centre of these lesions.
5.6 Suggestions for further study

(a) Study, at autopsy, the pathology of systemic onchocerciasis involving internal organs.

(b) Conduct further studies of the histopathology of ocular onchocerciasis including immunohistology and electron microscopy.

(c) Use immunohistology and electron microscopy to study further skin pathology in onchocerciasis.

(d) Study the histopathological changes of the skin of heavily infected patients after ivermectin treatment.

(e) Investigate the pathological effects of multiple filarial infections, especially the effects on the lymphatic system.

6. IMMUNOLOGICAL ASPECTS OF ONCHOCERCIASIS

Onchocerciasis is generally viewed as an immune-mediated disease, in which the host response to the parasite, particularly microfilariae in the skin and eye, leads to tissue damage. However, the present understanding of the immune response to *O. volvulus* infection in man and of the immunopathology of the disease is quite limited.

6.1 Antigens

There is little published information available about fractionated extracts or purified antigens of *O. volvulus*. Much serological work and antisera production has been carried out with crude extracts, sections or homogenates of *O. volvulus* or, more commonly, with antigenic preparations of heterologous filarial parasites. Newer approaches are being used, however, to define and purify individual antigens relevant to immunodiagnosis, immunopathology, and protective immunity. These include analysis of excretory–secretory (E–S) antigens and parasite surface molecules as well as the use of various electrophoretic techniques, affinity and other types of chromatography, immunoblotting, development of monoclonal antibodies, and molecular biology techniques.

Such studies have recently defined surface antigens of microfilariae and third-stage larvae that are important in the antibody-mediated cellular adherence of inflammatory cells to parasites; antigens that are apparently distinctive for *O. volvulus* worms originating from the West African savanna or forest areas;
antigens with potential diagnostic significance; and antigens found circulating in the blood of infected patients. However, none of these antigens has yet been put to practical use.

6.2 The humoral immune response

Infection in man leads to the production of antibodies against many parasite antigens. Because the time of onset of the infection is usually unknown and there are few studies in children, data are not available to define the evolution of the antibody response. However, it is clear that with chronic infection, antibodies develop against a multiplicity of parasite antigens. In addition, immunoglobulin levels of various isotypes (especially IgG, IgM, and IgE), are elevated nonspecifically, and it is likely that *O. volvulus* infection leads to polyclonal B-cell activation.

The IgE response in onchocerciasis deserves special mention. In many people with onchocerciasis, IgE levels are raised to extremely high levels (probably higher than in any other helminth infection), and a greater proportion of IgE antibody (compared to IgG antibody) is directed against parasite antigens. The significance of the high IgE levels is unclear, but IgE antibody is almost certainly important in the acute inflammatory complications of *O. volvulus* infection.

The diagnostic utility of the antibody responses in onchocerciasis has been limited, partly due to cross reactions with antigens of other parasites, especially filariae, and partly because antibody analysis is unable to distinguish between past and present infection.

There is no evidence for a protective role of antibodies in the human immune response to *O. volvulus* infection. However, *in vitro* experiments show that sera from chronically infected persons promote both granulocyte adherence to microfilariae and infective larvae, and the destruction of microfilariae. The presence of degranulating eosinophils around dying microfilariae suggests that eosinophils may be a major effector cell in antibody-dependent killing of microfilariae, and also raises the possibility that local and perhaps even distant tissue damage may result from repeated, massive eosinophil degranulation. It is possible that similar processes take place in the eye.

Circulating antigen–antibody complexes are found in the sera of a high percentage of persons infected with *O. volvulus*, but the
significance of this observation is unclear. It is possible that immune-complex deposition leads to acute inflammation and tissue damage, or that the complexes contribute to a down-regulation of the host immune response.

6.3 Cell-mediated immune responses

Persons infected with *O. volvulus* are known to have an impaired cell-mediated immune response as assessed both by skin testing and *in vitro* lymphocyte blastogenic response to *O. volvulus* antigens. This phenomenon appears to be more marked in persons with little cutaneous reaction (and microfilariae distributed throughout the skin) than in those with highly reactive skin disease (and few demonstrable microfilariae). The precise mechanisms by which dampening of the cell-mediated immune response occurs are as yet unknown.

Because cellular immune responses exert regulatory effects on the function of diverse cell types (e.g., granulocytes, fibroblasts), variations in cellular responsiveness may be of foremost importance in determining variations in the tissue response to infection both between individuals and in a single individual over time. The existence of a direct relationship between lymphocyte response to *O. volvulus* antigen and skin disease (see above) would be consistent with this hypothesis.

6.4 Variations and modulation of the immune response

It is thought that *O. volvulus* infection in man leads to a metastable immunological equilibrium in which the humoral and cell-mediated immune responses directed against the parasite are balanced by blocking antibody, suppression of lymphocyte reactivity, and probably other phenomena. Differences in, and modulation of, the host response appear to contribute to the observed wide variation in disease manifestations. This delicate balance can be upset by therapy directed against the parasite and probably by other more subtle factors, with the result that there is an enhanced or reduced expression of the disease. Efforts towards improving chemotherapy and developing a vaccine must take these considerations into account in order to minimize the possible adverse consequences of any intervention.
6.5 Immunopathogenesis and immunopathology

Antigen-specific suppression of the immune response to microfilariae and, conversely, disease exacerbation with the onset of immunorecognition of the parasite are likely to be important in the immunopathogenesis of onchocerciasis. A relationship between active severe dermatitis (sowda) and cell-mediated immune responsiveness was first noticed because of delayed positive results of the hypersensitivity skin test in patients with sowda lesions but not in patients with generalized disease. Unreactive individuals may be comparable to the parasitaemic patients with *Brugia* or *Wuchereria* infections, and the reactive ones to those with one of the amicrofilaraemic syndromes. The response to microfilariae in the skin and the eye ranges from nil to severe; this observation is consistent with the notion that host response patterns are important determinants of disease outcome. In some patients IgG antibodies may be involved in the destruction of microfilariae by sensitizing the parasites for eosinophil adherence and killing. It is these patients that are most likely to be in a "reactive state", destroying microfilariae *in vivo* and suffering intense, pruritic skin disease. It has been speculated that the relative anergy to microfilariae seen in other patients may reflect an immune tolerance induced by exposure *in utero* to microfilariae or microfilarial antigens.

The killing of microfilariae probably leads almost unnoticed to damage of the host tissues. After the administration of diethylcarbamazine, deposits of eosinophil granule proteins can be demonstrated and these proteins are known to damage tissues such as dermal collagen. This potential role of eosinophils in inducing tissue damage can be overlooked if one evaluates only the accumulation of cells, and not their toxic products. Self-inflicted trauma from scratching may lead to secondary infections.

Such observations point to differences in immunoregulatory pathways in individuals that result in qualitatively and quantitatively distinct responses, manifested in turn as variations within a spectrum of clinical disease. Unresponsiveness to microfilarial antigens has been demonstrated both in onchocerciasis and in other filariases in man, and circulating suppressive factors from some patients with onchocerciasis can inhibit the *in vitro* responses of normal lymphocytes. Similarly, excretory–secretory products from adult *O. volvulus* seem to be potently suppressive. It is not known how these factors contribute to the nonspecific immune suppression
seen in some patients or how exposure to antigens or suppressive factors in utero influences the pattern of subsequent reactivity of individuals in endemic areas.

6.6 Protective immunity

In certain experimental filarial infections some degree of protective immunity has been achieved using irradiation-attenuated infective larvae. There are no similar data from experimental or natural studies with O. volvulus, but the epidemiological pattern of infection can be interpreted as showing some measure of host-protective immune response. Infections in hyperendemic areas are acquired early in life, but the density of microfilariae in the skin does not rise indefinitely. Often, it reaches a plateau during the second or third decade, and some balance seems to be reached even during apparently high levels of continuing exposure. Much less common are the communities in which peak prevalence is reached by 16–20 years but microfilarial density continues to rise with increasing age. These patterns may be determined physiologically or behaviourally, and newly acquired parasites may also influence established parasites, but it is also possible that a protective immune response is stimulated against juvenile forms. It should be noted, however, that persons who have been cured of O. volvulus infection by chemotherapy but who continue to live in an area of ongoing infection readily become reinfected.

In the absence of good laboratory models, sequential immunological studies of chimpanzees exposed and challenged in various ways may be the most direct available experimental approach to examine protective immunity in onchocerciasis. It is possible, however, that a breakthrough in research on host-protective immune mechanisms in other filarial infections could guide work on O. volvulus more quickly towards potential types of effective immunogens.

6.7 Immunodiagnosis

Many test procedures using homologous and heterologous antigen preparations have demonstrated host antibody in onchocerciasis. However, a major limitation in their diagnostic usefulness has been the poor specificity of these assays, largely because extensive antigenic homologies exist between O. volvulus
and other filarial and helminth parasites. Furthermore, with few exceptions, serological or skin-test detection of antibody does not correlate well with either the presence or severity of *O. volvulus* infection. The exceptions are primarily those where seroconversion of previously unexposed individuals (e.g., young children in "vector-controlled" areas or visitors from nonendemic regions) accompanies a compatible clinical presentation.

Of greater potential value in accurately assessing infection serologically is the direct detection of parasite antigen in body fluids. Both the currently available polyclonal and monoclonal antibody-based techniques for antigen detection suffer from interference by host antibody, and often have poor sensitivity and specificity. However, with further development, antigen detection may be able to provide more reliable indications of the presence of infection (from prepatent to chronic stage) as well as the intensity of infection (perhaps by quantifying metabolic products of adult worms or microfilariae).

### 6.8 Effects of onchocerciasis on immune responses to other infections and antigens

In onchocerciasis the immune response to certain immunogens and other infections has been reported to be depressed. Both the cell-mediated and humoral response to heterologous (viral, bacterial, and parasitic) and homologous (onchocercal) antigens appear to be affected.

#### 6.8.1 Mycobacterial antigens and infections

In a number of areas of West Africa, a higher proportion of non-reactors to intradermal injection of a mycobacterial (tuberculin) purified protein derivative has been found in hyperendemic communities than in mesoendemic, hypoendemic or nonendemic communities. Furthermore, in one study, pulmonary tuberculosis has been reported to be more common among persons with onchocerciasis, and low reaction rates have also been observed under similar conditions in populations immunized with BCG. However, persons living in hyperendemic villages in the forest zone, where ocular lesions are appreciably less common than in the savanna zone, do not exhibit such abnormally low reactivity to the purified protein derivative and other mycobacterial antigens.
An association between lepromatous leprosy and generalized onchocerciasis has been reported from several different regions in Africa.

6.8.2 Tetanus toxoid

In Burkina Faso a trial has been carried out in which the effectiveness of antitetanus immunization using a single dose of concentrated adsorbed toxoid was compared in individuals hyperinfected with *O. volvulus* and in controls. Two months later, 44.5% of the non-onchocercal patients had antitoxin antibody levels indicating that immunization had been effective; only 7.1% of those with onchocerciasis had similar antibody levels.

6.8.3 Other parasitic diseases

Only malaria and schistosomiasis have been jointly investigated in individuals concurrently infected with *O. volvulus*. Abnormal persistence of malarial parasitaemia has been observed in adults infected with *O. volvulus* and the antischistosome complement-fixing antibody is absent in individuals concurrently infected with schistosomiasis and onchocerciasis.

6.9 Suggestions for further study

(a) Develop immunodiagnostic tests capable of detecting early (especially prepatent) infection.

(b) Define more fully the decreased immune responsiveness that results from onchocercal infection, with especial emphasis on its effect on disease susceptibility and the patient's competence to react appropriately to vaccines of public health importance.

(c) Establish longitudinal studies of affected populations to define the clinical progression of disease in relation to changing immunological patterns for individual patients. The effects of treatment or of interruption of re-exposure could be studied in the areas to be included in the western extension of the Onchocerciasis Control Programme.

(d) Define the immunological mechanisms involved in the pathogenesis of onchocercal eye disease.

(e) Define the determinants of hyporeactive and hyperreactive states in patients with onchocerciasis as well as the role that exposure
to onchocercal antigen in utero might have in altering the clinical and immunological responses to infection.

(f) Investigate the role of eosinophils both as effector cells in parasite killing and as the source of potential tissue-destroying inflammatory mediators.

(g) Investigate the mechanisms responsible for the natural development of protective immunity in man (if such occurs), as well as the mechanisms underlying the protective immune responses in experimentally immunized animal models of onchocercal and other filarial infections.

7. NODULECTOMY IN ONCHOERCIASIS

The removal and examination of nodules has played an essential part in elucidating the actions of new drugs (see sections 8.6 and 8.7), in understanding cyclical reproduction in *O. volvulus* (see section 10.1.1), in descriptive studies of the pathology of the nodule (see section 5.2), and in monitoring the long-term decline in parasite populations after interruption of transmission by vector control (see section 12.1).

7.1 Nodulectomy in the Americas

In Guatemala a systematic nodulectomy campaign to combat onchocerciasis was initiated in 1935 and has continued up to the present time. Between 1935 and 1979, 685 localities in 7 Departments were repeatedly visited by the campaign teams. In a total of 11,729 visits, 1,578,904 persons were examined. During the campaign, as many as 257,883 nodules were excised. The mean number of nodules excised per person per visit was 1.4.

The nodule carrier rate was found to decrease with time. The overall rate was 24.0% for 1935–39, 13.1% for 1940–49, 13.1% for 1950–59, 10.5% for 1960–69, and 8.7% for 1970–79. This decrease in nodule carrier rate is related to the frequency of visits by the teams but, even in places where the least number of visits occurred, the overall rate also decreased gradually. This suggests that the observed decrease in nodules is not only due to the nodulectomy campaign, but also to other factors such as the decrease in vector density or man–vector contact, which in turn are probably due to changes in socioeconomic conditions. The extent of the decrease was not the
same in each of the separate endemic areas. The number of hyperendemic areas has also decreased, particularly around Santa Rosa. At present there are only a few foci of high onchocercal endemicity remaining, and each of them is surrounded by a zone of low endemicity.

The overall impact of the nodulectomy campaign in Guatemala on ocular disease and blindness is hard to assess. However, in San Vicente Pacaya, an area of relatively high endemicity, the blindness rate is now less than 0.5%, compared with reported rates of around 7% in 1934.

A similar nodulectomy campaign has been conducted in Mexico and this has been combined recently with diethylcarbamazine treatment, but little published information is available.

7.2 Nodulectomy in Africa

A study on the effects of nodulectomy has been carried out in Liberia and Burkina Faso. It showed that 2 years after the removal of all detectable nodules from persons in areas of ongoing transmission, one-third of the people remained free of nodules and had slightly reduced microfilarial skin-snip counts. The other two-thirds showed an average of one nodule per person and slightly raised microfilarial counts at the end of the 2-year period. Persons living in the same villages who did not volunteer for operation had significantly higher nodule and microfilarial counts after 2 years. Up to the present time, no systematic nodulectomy campaigns have been initiated in Africa.

7.3 Suggestions for further study

(a) Establish the cost-effectiveness of carrying out large-scale nodulectomy campaigns.
(b) Identify the specific risks associated with the presence of head nodules and the possible advantages of removing them.

8. CHEMOTHERAPY

Despite the fact that much research has been carried out, there are still only a limited number of drugs available for the treatment of onchocerciasis. Over the last decade much has been learnt about the
adverse effects frequently associated with diethylcarbamazine (DEC) treatment; at times, these side-effects can be severe. However, diethylcarbamazine is still widely used. Suramin remains the only macrofilaricidal drug available for clinical use. Several new potential filaricidal compounds have been tested in man over the last 20 years but, with the exception of ivermectin, none has proved to be both safe and effective.

In clinical trials, there seem to be some advantages in using ivermectin instead of diethylcarbamazine and, if it is registered for clinical use in 1987 as planned, its use may well produce a significant advance in the treatment of onchocerciasis.

8.1 Diethylcarbamazine and combination therapy

8.1.1 Limitations of diethylcarbamazine

Recent investigations have shown that the use of diethylcarbamazine in the treatment of onchocerciasis has important limitations, and these can be summarized as follows:

(a) Treatment is only suppressive: only the microfilariae are killed, whereas adult worms remain essentially unaffected, and inevitably repopulation of the skin and eye occurs. Repeated administration of the drug at intervals is necessary for as long as the adult female worms are fertile.

(b) Severe and sometimes dangerous systemic reactions (Mazzotti reactions) occur, especially in heavily infected patients; they can also be seen in some lightly infected patients.

(c) Aggravation of existing ocular lesions or precipitation of new lesions may occur, especially in individuals who, because of threatening eye problems, are in great need of treatment.

8.1.2 Recommended dose schedule for diethylcarbamazine

Despite extensive studies, it has not proved possible to develop entirely safe dosage schedules for diethylcarbamazine. For an adult with a body weight of 50–60 kg the following recommended dose regimen is both effective and economical, and has been extensively used.

A low dose of diethylcarbamazine should be given for the first 1 or 2 days. This starting dose could be 25 mg (0.5 mg/kg) or 50 mg (1.0 mg/kg). The maintenance dose should be 100 mg (2.0 mg/kg)
given twice a day for 5–7 days so that the total drug dose given is approximately 1.3 g (30 mg/kg) for an adult; longer treatment is unnecessary and not recommended.

Frequently, concomitant corticosteroid treatment is indicated to reduce the severity of the Mazzotti reaction. Typically, betamethasone or dexamethasone 4 mg (80 μg/kg) for an adult per day is started 1 or 2 days before the first dose of diethylcarbamazine is given, and is continued or tapered off on an individual basis after 3 days of treatment with diethylcarbamazine. Aspirin can be used to reduce the symptoms of fever and musculoskeletal pain during the Mazzotti reaction and antihistamines may decrease pruritus and help with insomnia.

Because of the risks associated with diethylcarbamazine treatment, its indiscriminate use should be strictly avoided. It should only be given under medical supervision and systemic corticosteroids should only be used for hospitalized patients.

A major drawback of therapy with diethylcarbamazine is that sight-threatening changes can occur following treatment. A recent study involving the administration of 1.3 g of diethylcarbamazine over 8 days, with an initial dose of 50 mg, resulted in functional ocular deficit in 2 out of 20 treated patients. In heavily infected patients the incidence of severe ocular damage with similar treatment may be even higher. Despite corticosteroid coverage, severe ocular lesions can still occur following diethylcarbamazine treatment.

8.1.3 Topical application of diethylcarbamazine

The safety and efficacy of applying diethylcarbamazine lotion to the skin has not been confirmed by detailed controlled studies. Eyedrops containing the drug have not been found useful for the treatment of ocular onchocerciasis.

It has been suggested that high doses of diethylcarbamazine (30 mg/kg body weight per day for 7 days) may have a macrofilaricidal effect although conflicting evidence exists. This issue is currently being investigated in a controlled clinical trial.
8.1.4 Suppression of side-effects

The agents that have been administered concomitantly with diethylcarbamazine in an attempt to prevent or reduce the severity of the Mazzotti reaction include dexamethasone, betamethasone, triamcinolone, prednisone, methysergide, cyproheptadine, promethazine, aspirin, indometacin, and diazepam. Of these, only the corticosteroids have been shown to be beneficial; but even they only partially suppress the itching or ocular reactions. Levamisole has also been assessed in combination with diethylcarbamazine but it did not offer any significant advantage.

8.1.5 Low-dose, spaced therapy

Several studies have assessed the long-term, intermittent, suppressive use of diethylcarbamazine. These studies show that it is poorly accepted on account of the repeated Mazzotti reactions that lead to progressively decreasing compliance. Suppressive treatment does not prevent, and may even provoke, posterior segment and other ocular lesions; neither does it abolish the Mazzotti reaction nor clear all microfilariae from the skin. Severe complications, including proteinuria, were observed in one study.

8.2 The Mazzotti reaction

Minutes to hours after the administration of diethylcarbamazine to most patients with onchocerciasis, a series of events occurs that includes dermal, ocular, and systemic components known collectively as the Mazzotti reaction. Clinical manifestations are the most prominent features of the reaction and they can be severe, dangerous, and debilitating. These side-effects limit the widespread use of the drug for onchocerciasis treatment and great efforts have been made to study and control pharmacologically the underlying mechanisms involved. None of the reactions is a manifestation of direct drug toxicity since they only occur when microfilariae are killed. The same reaction may occur to varying degrees with other drugs that kill microfilariae.

8.2.1 Systemic reactions

Systemic reactions include increased itching, rash (papules, oedema, urticaria), headache, aching muscles, joint pain, pain, swelling, and tenderness in lymph nodes; fever, tachycardia and
hypotension, and vertigo. Most of these reactions begin within the first 24 hours after treatment, but increased itching may commence within minutes. It has been possible to quantify the severity of Mazzotti reactions by giving weighted scores to the commonly occurring features of the systemic manifestations (5). Use of this scoring system has shown that the level of pruritus, fever, adenopathy, and hypotension are correlated with the intensity of infection. No significant association has been demonstrated between intensity of infection and either tachycardia or joint reactions.

The severity of the reaction also depends on the dose of diethylcarbamazine employed and hence to the extent and rapidity of microfilarial killing.

In some patients, a severe acute febrile polyarthritis occurs several days after initiating therapy. Effusions, in which microfilariae may be present, may develop in large joints such as the knee.

8.2.2 Treatment of severe Mazzotti reactions

Intravenous hydrocortisone hemisuccinate (100–200 mg, 4–6 hourly) and intravenous fluids are needed to control severe reactions. Paracetamol (acetaminophen) or aspirin are needed to control fever and musculoskeletal pain. Acute febrile polyarthritis usually responds readily to paracetamol or aspirin and corticosteroids are rarely needed. The local use of corticosteroid eye drops and mydriatics are indicated for acute uveitis.

8.2.3 Ocular reactions

In the first few hours after diethylcarbamazine treatment, most patients develop transient conjunctival hyperaemia and limbitis, often with excessive watering and photophobia. Small transient globular infiltrates develop at the limbus of the conjunctiva, their number generally corresponding to the number of microfilariae found in the cornea; they resolve within a few days.

At the same time, microfilariae migrate from the periocular tissues into the cornea and die during the next 2–4 days, together with those already present before treatment. An inflammatory infiltrate forms around the dead microfilariae and produces the lesions of punctate keratitis that remain for some weeks before resolving completely.
The number of microfilariae in the anterior chamber may increase transiently but later decreases.

In the posterior segment, transient retinal pigment epithelial defects have been detected using fluorescein angiography in an appreciable number of patients during the first days of diethylcarbamazine treatment. Optic neuritis also occurs and leads to visual field loss (6).

8.2.4 Microfilarial migration

Mobilization of microfilariae occurs and they are found in increased numbers in blood, urine, and lymph nodes as well as in the cornea. They also appear in the epidermis, synovial fluid, cerebrospinal fluid, hydrocoele fluid, tears, and in the sputum.

8.2.5 Immunological changes

The most consistent and dramatic changes to occur involve the eosinophils. Early during therapy there is an eosinopenia due to the migration of these cells out of the blood and their degranulation in the tissues around the trapped microfilariae. This condition is characterized by an elevation of eosinophil-granule proteins in the serum. Later, there is an eosinophilia that persists for several weeks. This “post treatment eosinophilia”, when observed in conjunction with a reduction in the skin snip counts, provides conclusive evidence of the microfilaricidal effect of any drug regimen. Neutrophilia also occurs early and persists for a few days; a modest lymphocytopenia may occur during the first 3–4 days.

Elevations also occur in the level of IgG antifilarial antibodies and circulating immune complexes during the second week of treatment. No similar rise in specific serum IgE occurs.

8.2.6 Other laboratory changes

Elevations in serum aspartate aminotransferase (EC 2.6.1.1), serum alanine aminotransferase (EC 2.6.1.2), and lactate dehydrogenase occur, possibly as a result of the intrahepatic sequestration and destruction of microfilariae; proteinuria may also appear.
8.2.7 Current concepts of mechanisms underlying the Mazzotti reaction

The fact that the incidence and intensity of the Mazzotti reaction is usually a function of the severity of infection suggests that it is in some way related to microfilarial killing, but it is likely that many inflammatory pathways are activated. The mechanisms responsible for this killing of microfilariae are not clearly defined, but there is evidence that eosinophils are involved. It has been speculated that the Mazzotti reaction may be triggered by the activation of complement (as in the Jarisch-Herxheimer reaction), by the initiation of IgE-mediated immediate hypersensitivity responses (as in anaphylactic reactions), or by the degranulation of eosinophils during antibody-dependent, cell-mediated killing, with resultant inflammatory activity. However, careful analysis of the reactants in all 3 of these inflammatory pathways has provided little evidence that either complement or immediate hypersensitivity responses is the principal pathway involved. Eosinophil degranulation, on the other hand, with the release of inflammatory mediators from these cells into the tissues and peripheral blood, is extremely marked in all patients undergoing the Mazzotti reaction and develops with a time course generally consistent with that required of an "initiator" of the reaction.

Numerous other inflammatory mediators and pathways may be activated, especially since the reaction itself has multiple components and can continue to evolve over many days. Initial evaluation of some potential immunoreactants (e.g., kinins, prostaglandins, immune complexes) has so far failed to yield specific clues, but these mediators are often short-lived and notoriously difficult to measure. In addition, many inflammatory mediators or reactants (e.g., leukotrienes, platelets, and parasite-derived inflammatory molecules) deserve further investigation.

8.2.8 The Mazzotti test

A single small dose of diethylcarbamazine (usually 50 mg for an adult) has been used as a provocative test to diagnose onchocerciasis: this is the so-called Mazzotti test. However, because of the dangers of inducing the Mazzotti reaction even in lightly infected patients, this test should be used only in individuals in whom a diagnosis of onchocerciasis is being considered but in whom parasites cannot be detected in skin, eyes or blood. In such
individuals the Mazzotti test can be extremely helpful by inducing local pruritus and rash, clinically indicative of the parasite's presence.

8.3 Suramin therapy

Suramin is the only macrofilaricide available for use against *O. volvulus*. Although knowledge of its complications, especially those associated with the eye, has advanced during the last decade, little is known about the mode of action of this drug. Suramin inhibits a number of enzymes that play a part in the metabolism of the filariae and also of the host. One of its properties, namely that of combining with plasma and tissue proteins, especially in the kidney, explains to some extent its toxicity as well as its slow and progressive parasiticidal activity.

8.3.1 Dosage and complications

To kill all adult worms, a total of at least 6 g of suramin must be given over 6 weeks. However, such a dosage can produce significant and dangerous side-effects in 10–30% of patients and cannot be recommended for general use.

Studies have been carried out to investigate the safety and efficacy of different dosage schedules of suramin and the following regimen is now generally recommended. The total dose should be 4 g for an adult weighing 60 kg or more. This is the lower limit needed to achieve a high degree of therapeutic activity combined with acceptable safety, and is given over 6 weeks. The drug should be given as follows:

<table>
<thead>
<tr>
<th>Week</th>
<th>Dose (g)</th>
<th>Dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>0.2</td>
<td>3.3</td>
</tr>
<tr>
<td>2nd</td>
<td>0.4</td>
<td>6.7</td>
</tr>
<tr>
<td>3rd</td>
<td>0.6</td>
<td>10.0</td>
</tr>
<tr>
<td>4th</td>
<td>0.8</td>
<td>13.3</td>
</tr>
<tr>
<td>5th</td>
<td>1.0</td>
<td>16.7</td>
</tr>
<tr>
<td>6th</td>
<td>1.0</td>
<td>16.7</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>4.0</strong></td>
<td><strong>66.7</strong></td>
</tr>
</tbody>
</table>

In patients who do not show any signs of toxicity, the macrofilaricidal action can be improved by a final injection of 1 g, raising the total dose to 5 g. This dose schedule has not resulted in any serious general complications in trials in hyperendemic areas of
Africa. Anterior uveitis may develop, but can usually be controlled by topical corticosteroid therapy.

Suramin treatment has long-term effects on ocular onchocerciasis. The ocular microfilarial load (anterior chamber and cornea), which may increase slightly due to the mobilizing effect of suramin during the first month, is greatly decreased after 3 months, becomes low within a year and absent during the second year. Lesions of the anterior segment usually improve or stabilize: only occasionally are they worsened. However, advanced sclerosing keratitis does not respond to suramin, and new lesions may also appear.

Only rarely do conditions affecting the posterior segment and optic nerve improve during the course of treatment. Optic neuritis and its sequel, optic atrophy, have been observed during and after treatment with a regimen of 6 g of suramin. It is as yet unclear to what extent these complications will occur with the recommended reduced dose.

A Mazzotti skin reaction may appear during the first few weeks of suramin treatment. The reaction and the ocular changes are presently attributed to the microfilaricidal effect of the drug.

The adverse reactions specifically due to suramin treatment include anaphylactic shock following the first injection, nephropathy, exfoliation of the skin and mucous membranes, jaundice, chronic diarrhoea, and asthenia; occasionally, the treatment may result in death. In general, however, if treatment is discontinued at the first appearance of any adverse signs, they will usually disappear. They can also be alleviated by the systemic administration of corticosteroids.

Some albuminuria, desquamation of the skin, as well as pain and tenderness in the palms of the hands and soles of the feet, commonly occur during treatment; the presence of these side-effects alone does not necessitate the withdrawal of treatment.

8.3.2 Contraindications to the use of suramin

Restrictions are placed on the use of suramin because of the frequency of associated complications and because the drug is intrinsically toxic and relatively difficult to handle. Suramin should not be given to the very old, the very young, or during pregnancy; nor should it be given in the presence of other diseases, especially kidney and liver abnormalities.
8.3.3 Constraints on treatment

The special problems of suramin treatment on a community scale require strict medical surveillance so that general, renal, and ocular complications are identified during the course of treatment. The intravenous route of administration and the length of the treatment schedule mean that staff and equipment have to be available over long periods. These constraints also explain the considerable absenteeism that occurs during the course of treatment. Although little is known about the dangers of repeating suramin treatment in an area where transmission continues, a minimum period of 2–3 years between treatment courses seems desirable.

8.4 Present indications for treatment with diethylcarbamazine and suramin

8.4.1 Indications

As the sight-threatening side-effects of chemotherapy have become better known, the recommended indications for the treatment of onchocerciasis have become more stringent. The major indication for treatment with currently available drugs is the presence of onchocerciasis that is severe enough to threaten sight (Table 4). Advanced chorioretinal changes and optic atrophy are not

<table>
<thead>
<tr>
<th>Major indication: Sight-threatening disease, as indicated by:</th>
</tr>
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<tbody>
<tr>
<td>—20 or more microfilariae in either anterior chamber</td>
</tr>
<tr>
<td>—50 or more microfilariae in either cornea</td>
</tr>
<tr>
<td>—severe uveitis in patients with onchocerciasis</td>
</tr>
<tr>
<td>—early sclerosing keratitis</td>
</tr>
<tr>
<td>—visual field constriction in patients with active disease</td>
</tr>
</tbody>
</table>

**Other indications:**

—severe onchodermatitis
—high microfilariae counts
—100 microfilariae or more per mg of skin
—15 microfilariae or more per mg of outer canthus skin snip

improved by drug treatment, and their presence alone is not an indication for therapy. Severe onchodermatitis may also justify treatment.

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8.4.2 Recommended treatment

Patients being treated for onchocerciasis should be hospitalized or treated under close medical supervision.

Treatment should begin with diethylcarbamazine given under corticosteroid coverage as previously described in section 8.1.2. This should be followed by a course of suramin following the schedule given in section 8.3.1.

Patients should be monitored carefully for side-effects and the treatment modified or interrupted if major adverse reactions occur. It should be possible to assess the efficacy of treatment about 6 months after it is completed and subsequently, the patient should be followed at suitable intervals to detect reinfection.

Treatment should not be given indiscriminately or without due consideration for the patient’s present and future status and the likelihood of reinfection.

8.4.3 Interaction with other parasitic diseases

Care must be taken to evaluate individual patients who come from onchocerciasis endemic areas and who are being considered for treatment of other diseases such as loiasis or lymphatic filariasis (if treated with diethylcarbamazine), schistosomiasis (if treated with metrifonate), and trypanosomiasis (if treated with suramin). Since each of these drugs has an effect on *O. volvulus*, adverse reactions may occur during the treatment of the primary condition as a result of the killing of the *Onchocerca* parasite. For such cases, modifications may be necessary in the treatment scheme, closer medical supervision may be needed, and the use of corticosteroids considered.

8.5 The current status and potential of ivermectin

Ivermectin is a macrocyclic lactone, a synthetic derivative of avermectin B₁, which is a fermentation product of *Streptomyces avermitilis*. It has been used extensively against animal parasites, and is effective against arthropods, but in man its use has been limited initially to investigational administration for the treatment of *O. volvulus* infection (7, 8). It is anticipated that ivermectin will be available for clinical use by the end of 1987.
8.5.1 Actions of ivermectin

The predominant effect of ivermectin in *O. volvulus* infection is the rapid elimination of microfilariae from the skin following a single oral dose of the drug. This is followed by a gradual disappearance of microfilariae from the cornea and anterior chamber of the eye.

The mechanism of action of ivermectin against *O. volvulus* has not yet been clearly established. The microfilaricidal effect is thought to result from ivermectin’s activity in enhancing both the presynaptic release of gamma-aminobutyric acid (GABA), and the post-synaptic enhancement of GABA-binding to a chloride channel-linked receptor. Maintenance of the chloride channel in an open state leads to muscular paralysis, and the subsequent death of microfilariae by immune or other mechanisms.

Although ivermectin has been shown to have macrofilaricidal activity in some animal filarial species, there is no evidence to date suggesting that such an effect occurs against *O. volvulus*. However, adult female worms isolated from ivermectin treated subjects do show evidence of a suppressive effect on the release of microfilariae. This effect appears to result from the trapping of microfilariae within the female uterus, with their subsequent degeneration *in utero*. There is no evidence of any effect of the drug on the production of embryos. The duration of the effect on the release of microfilariae is probably several months.

8.5.2 Clinical effects of ivermectin

The principal effect of the drug that has been documented in trials to date is a reduction in the number of microfilariae in the skin and in the eye. Comparative studies reported so far have shown less severe and less frequent systemic reactions with ivermectin than with diethylcarbamazine. Qualitatively, the mild reaction seen in some ivermectin recipients is similar to that seen with diethylcarbamazine treatment; fever, rash, and lymph-node pain or swelling being the principal reactions.

The ocular reactions seen following ivermectin therapy have been minimal. The number of punctate opacities seen in ivermectin recipients following therapy do not increase significantly. In addition, mobilization of microfilariae into the cornea and anterior chamber of the eye was not seen in the patients given ivermectin as it was in those who received diethylcarbamazine. Following ivermectin administration, no significant changes in the posterior
segment have been detected so far by clinical examination or by fluorescein angiography; but before it can be concluded that the drug is not toxic for the posterior segment of the eye, it is essential that it be tested in subjects at high risk of developing the ocular changes associated with therapy.

To date more than 1200 people have received ivermectin, although the limited time of follow-up precludes definition of possible long-term beneficial effects of therapy. However, evidence from the ongoing Phase III trials indicates that elimination of microfilariae from the skin by ivermectin treatment is associated with a significant improvement in clinical status.

8.5.3 Ivermectin and transmission

The potential effect of ivermectin on transmission has been tested in experiments in which blackflies were fed on subjects who had received ivermectin, diethylcarbamazine, or placebo either 3 or 6 months previously. Flies that were fed on ivermectin-treated subjects showed a significantly reduced intake of microfilariae at 3 and 6 months when compared to those fed on both diethylcarbamazine and placebo treated subjects, as well as a reduced number of infective larvae at 6 months. Similar results have been obtained with *S. sirbanum* in Mali one year after ivermectin therapy. This suggests that a single dose of ivermectin given annually may significantly reduce transmission.

8.5.4 Ivermectin and prophylaxis

There are as yet no specific data available on the effect of ivermectin on third and fourth larval stages of *O. volvulus*. However, ivermectin has been shown to be highly effective as a prophylactic agent against *Dirofilaria immitis* in dogs and *O. gibsoni* in cattle. It’s possible chemoprophylactic action against *O. volvulus* is currently being assessed in chimpanzees.

8.5.5 Potential toxicity of ivermectin

Concern over the toxicity of ivermectin has centred on a potentially fatal neurotoxicity observed in certain breeds of dogs, specifically collies and collie-crosses, that appeared to be related to the concentration of ivermectin within the central nervous system. It
is thus conceivable that conditions that alter the normal blood–brain barrier in man could result in the occurrence of potentially fatal neurotoxicity. In this regard, it is important to note that no significant neurotoxicity has been observed in cattle or horses, despite the worldwide administration of several hundred million doses of the drug. Moreover toxicity has not been seen in non-collie dogs with induced bacterial meningitis.

In the more than 1200 persons with *O. volvulus* infection who have been treated with ivermectin, no major toxicity has been observed to date. However, in a small minority of subjects in two studies, minor ST–T wave changes were observed in electrocardiograms obtained on day 3 after therapy. This change may reflect an effect of the drug on repolarization, or, less likely, myocardial ischaemia possibly related to a mild Mazzotti reaction. No clinical correlates of these electrocardiographic findings have yet been observed.

Regarding the use of ivermectin in pregnancy, animal studies have not revealed any teratogenicity except at doses that are toxic to the mother. Because of possible secretion of the drug into breast milk, it is currently recommended that ivermectin should not be given to lactating women, but this subject is being investigated further. Animal studies have shown no effect of ivermectin on male or female reproductive function or fertility. There is no information on interactions between ivermectin and other drugs widely used in man.

8.5.6 Conclusions

Ivermectin shows great promise as a single-dose oral agent that is effective and well-tolerated in the treatment of onchocerciasis in man. It acts as a microfilaricide, but it also has a temporary suppressive effect on the release of microfilariae. Retreatment at 6-to 18-monthly intervals will probably be the ideal schedule in endemic areas with substantial ongoing transmission. No major toxicity has been observed in man to date, but the safety of ivermectin therapy in persons with advanced ocular disease has not yet been tested. The drug also shows promise as a means of controlling disease and transmission in populations living in endemic areas.
8.6 The current status of benzimidazoles, metrifonate, and arsenicals

8.6.1 The benzimidazoles

Most benzimidazoles have been developed as potential intestinal anthelmintics for veterinary use; therefore, by design, most are poorly absorbed when given orally. The primary effect of benzimidazoles on filarial worms is on the formation of microtubules, and this action blocks embryogenesis at the early morula stage. This effect temporarily sterilizes the adult females and allows a gradual attrition of the number of microfilariae to take place; existing microfilariae die and are not replaced. Because of their poor oral absorption, huge doses of these drugs must be given to provide adequate systemic levels.

1. Mebendazole. Mebendazole is widely used against a number of human intestinal parasites. The normal total oral dose of mebendazole for an adult is 100–600 mg. Large doses of mebendazole (30–50 mg/kg/day for 3 weeks) have had variable effects on microfilarial skin snip counts and embryogenesis. Because of its inconsistent effects on *O. volvulus*, its toxicity, and potential teratogenicity, mebendazole is unsuitable for prolonged use at these high doses for the treatment of onchocerciasis.

2. Flubendazole. Flubendazole resembles mebendazole with the addition of a single fluorine atom. It is a very expensive drug and, although effective orally against a range of intestinal parasites, it is even less well absorbed than mebendazole. Flubendazole is inactive against *O. volvulus* when given orally.

Although an intramuscular formulation of flubendazole effectively blocked embryogenesis in one trial, it also produced a marked local reaction at the injection site. Despite intensive efforts, it has not been possible to prepare a satisfactory reformulation for flubendazole.

3. Other benzimidazoles. The two other benzimidazoles that have been tested against human onchocerciasis, tiabendazole and albendazole, were both ineffective.

8.6.2 Metrifonate

Metrifonate is an organophosphorus compound with anticholinesterase activity. It is used as an insecticide and is also active against *Schistosoma haematobium*. Metrifonate is primarily a micro-
filaricidal drug although it is less effective and less consistent than diethylcarbamazine. It also precipitates a Mazzotti reaction.

8.6.3 Arsenicals

Melarsonyl potassium, mentioned as a promising new drug in the second report of the Expert Committee (9), was withdrawn from use in the treatment of onchocerciasis shortly afterwards because of the danger of associated fatal arsenical encephalopathy. Although arsenicals are effective macrofilaricides, the risk of encephalopathy cannot be assessed during preclinical toxicological testing in animals. Therefore, it is not ethically acceptable to carry out clinical trials with such compounds for use against a non-fatal disease such as onchocerciasis.

8.7 Potential new macrofilaricide undergoing clinical trials

CGP 6140, a piperazine derivative of amoscanate, is an effective macrofilaricide in all rodent filarial screens and also against O. gibsoni in cattle.

The first clinical studies of CGP 6140 at subtherapeutic levels showed no adverse clinical effects. A pharmacokinetic study has been completed in man and multicentre Phase II trials of the drug in O. volvulus-infected patients are planned for 1986.

8.8 Suggestions for further study

(a) Clarify by further study the following points regarding the use of ivermectin:

— the mode of action of ivermectin on O. volvulus;
— the possible occurrence of rare toxic manifestations;
— the possibility of adverse effects on the eye, especially the posterior segment, in cases of onchocerciasis with severe ocular involvement.
— the effects of the drug in reducing transmission when applied on a community basis;
— the possible effect of the drug as a causal or clinical chemoprophylactic for O. volvulus infection;
— the combined use of ivermectin and suramin to achieve a definitive cure for onchocerciasis; this possibility must first be studied experimentally in animals to be sure that suramin
does not dangerously lower the blood–brain barrier for ivermectin;
— the best dosage schedule to prevent the further development of ocular and dermal lesions in infected persons;
— the interaction of ivermectin with other drugs likely to be used in endemic areas.

(b) Investigate further the beneficial and possible adverse effects of corticosteroids administered in conjunction with diethylcarbamazine and/or suramin for the treatment of onchocerciasis, particularly in relation to the possible prevention or stabilization of ocular lesions.

9. EXPERIMENTAL CHEMOTHERAPY

The problems involved in experimental antifilarial chemotherapy have recently been reviewed at length (10); the present discussion concentrates on drug research targeted at *O. volvulus*.

9.1 Experimental models for primary and secondary screening

For the discovery of new leads and the early development of anti-*Onchocerca* agents, cost-effective and chemotherapeutically relevant *in vitro* and *in vivo* screens are required. Ideally, these screens should use the target species (*O. volvulus*), have low compound requirements, and rapidly generate drug activity data. In this way structure–activity relationships can be established to rationalize further chemical syntheses as well as the selection of candidate compounds for advanced lead development. However these “ideals” are not readily attained and, at present, no screening models using *Onchocerca* spp. can be maintained in the laboratory; as a result the search for new drugs for the radical cure of onchocerciasis is impeded.

9.1.1 *In vivo* models in small animals

1. *Transfer of parasites to surrogate hosts*. Attempts to establish *Onchocerca* infections in rodent hosts by injecting infective larvae or by implanting whole nodules or adult worms, have been unsuccessful. The introduction of microfilariae of *Onchocerca* spp. into rodent hosts has been achieved, however, and is the model used
to demonstrate the efficacy of ivermectin in vivo. The accuracy of such models in predicting the antifilarial activity of test compounds remains to be proven. Until alternative in vivo models have been developed, reliance must still be placed on model infections involving other filarial species in rodents.

2. Dipetalonema and Brugia in rodents. A recent review of the drug responses of Brugia spp., Dipetalonema viteae, and Onchocerca spp. showed O. gibsoni and O. gutturosa to respond in the most similar way to O. volvulus to chemotherapeutic agents, but it was not possible to determine which of D. viteae or Brugia pahangi was the best choice. Data on drug activity against these latter laboratory parasites have been assembled from screens involving a variety of hosts and infection techniques, and the pharmacokinetic relevance of these models to nodule-forming O. volvulus is unknown.

Because of the difficulty of selecting either B. pahangi or D. viteae as a screening organism, a compromise assay using dual infections of D. viteae and B. pahangi in gerbils has been developed. In this assay adult D. viteae and B. pahangi are implanted subcutaneously and intraperitoneally respectively. Drugs can then be tested against the two filariae in the same host with resultant economies in drug, animals, and labour. However, because the parasites are in different locations, no absolute comparison of drug susceptibility can be made. Another drawback of present lead-finding and development activities using these in vivo assays is that 2–3 months may be required to generate the first results because antifilarials can be slow acting.

9.1.2 In vitro assays

There are two major approaches to the interpretation of in vitro drug activity: (a) evaluation of whole organism parameters that can indicate different general modes of action (e.g., motility, moulting, mortality); (b) utilization of biochemical or morphological measurements tailored to detect activity in a particular target area (e.g., oxygen uptake for effects on electron transport or glucose utilization).

Motility may be an especially good measurement for identifying effects on energy generation and/or nervous control. A micromotility meter is now available for quantitative and automated

\footnote{Currently, there is enough published evidence to suggest that the correct name of this species is Acanthocheilonema viteae.}
assessment of worm motility and can be used not only on whole parasites but also on worm fragments. This technique may therefore be used in primary in vitro assays to evaluate drug effects against Onchocerca spp. including O. volvulus.

Recently it has been shown that the in vitro culture of O. lienalis microfilariae can be used to demonstrate 100% susceptibility of the parasite to low doses of ivermectin. This model may, therefore, have potential for assaying microfilaricides. Attempts have also been made to assess the suitability of O. lienalis third-stage larvae for screening, as they also are extremely sensitive to ivermectin in vitro.

Another new in vitro assay employs dividing cells isolated from the germinative zone of the gonducts of D. immitis or B. pahangi to detect anti-mitotic activity of test compounds. Drug effects on mitosis are ascertained using cytological methods, and the potential exists to utilize this assay in the evaluation of inhibitors of both tubulin polymerization and protein, DNA or RNA synthesis. Such methods should also be applicable to Onchocerca spp.

9.2 Models for tertiary drug screening

9.2.1 The bovine Onchocerca drug screen

Cattle naturally infected with O. gibsoni and O. gutturosa are common in the northern states of Australia, and these have been used to evaluate drugs that have shown promising antifilarial activity in laboratory screening models.

1. Description of infections. Adult O. gibsoni worms occur in fibrous nodules, similar to those of O. volvulus in man, and these are located subcutaneously or in deeper connective tissue between the pectoral muscles. Each nodule of O. gibsoni usually contains one female, and often one male worm. Nodules begin to form around immature females, probably at the time of the final moult, and continue to increase in size (up to 5 cm in diameter) as the female grows. They progressively decrease in size after the death of the worms. Because one can limit examination to nodules weighing over 2 g and guarantee that more than 80% of nodules will contain viable, fertile worms, any degenerative changes induced by drugs can be assessed with confidence and accurately. Adult O. gutturosa worms occur within connective tissue at many sites, but they are most easily collected from the surface of the nuchal ligaments. In contrast to the nodules of O. gibsoni, O. gutturosa adults occur in an
extended form with immature, mature, and degenerating worms lying together; this makes assessment of drug-induced effects more difficult than with *O. gibsoni*.

2. Methods of drug screening. Naturally infected cattle are selected on the basis of high microfilarial skin counts for *O. gibsoni* (in midline thoracic skin) and *O. gutturosa* (in midline cervical skin). Drugs are administered intravenously, orally, subcutaneously, intramuscularly or intra-abominally; for each compound formulation, the dose, route, and frequency of administration are determined by its known or anticipated pharmacokinetic properties. In general, drugs are screened at their maximum tolerated doses and are subsequently titrated if their activity warrants it. Skin snips from thoracic and cervical areas are taken to assess microfilarial numbers before, and periodically after, treatment for 10 weeks. Animals are then killed and macrofilaricidal and embryostatic effects determined by histological examination of formalin-fixed nodules. The major disadvantage of the bovine *Onchocerca* screen is the requirement for large amounts of drug to dose animals weighing approximately 350 kg.

Because of the small number of drugs with proven activity against *O. volvulus* in man, it is difficult to comment on the predictability of the bovine *Onchocerca* model for the final selection of potential drugs for clinical trials in man. However, all drugs that have shown activity in man were also effective in the cattle screen. Suramin had equal macrofilaricidal action against *O. gibsoni* and *O. gutturosa*, while the compound CGP 6140 was more effective against *O. gibsoni* than *O. gutturosa*. Ivermectin and diethylcarbamazine were highly effective microfilaricides, and ivermectin was also active as a prophylactic agent in preventing natural infection of calves with either *O. gibsoni* or *O. gutturosa*. Benzimidazoles, such as mebendazole, gave transient embryostasis, particularly in *O. gibsoni* females, and treatments at intervals of 3 months almost completely suppressed microfilariae in the skin.

Compounds such as furapyrimidone1 and organic arsenicals showed good activity as macrofilaricides against *O. gibsoni* and *O. gutturosa* respectively, but they are too toxic to be used for the treatment of onchocerciasis in man.

Direct injection of test substances into individual nodules of *O. gibsoni* has been used to test compounds that are only available

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1. N-(5-nitro-2-furfurylidene)aminotetrahydro-2(1H)pyrimidinone.
in small (mg) quantities. While showing some initial promise, problems of mechanical damage to the parasite make this assay difficult to standardize.

9.2.2 The chimpanzee

Since chimpanzees can be experimentally infected with *O. volvulus*, they have been used in a very small number of drug efficacy studies. The use of this protected animal in further chemotherapy studies should be limited to exceptional circumstances.

9.3 The absorption of nutrients and drugs by *Onchocerca*

Tissue-dwelling filarial nematodes exhibit important physiological differences from gastrointestinal nematodes in the way in which they take up nutrients. Published data on the uptake of nutrients and drugs by *Onchocerca* is limited to a single preliminary demonstration that the absorption of glycine by adult female *O. gutturosa* occurs by a transepithelial route, but this observation is consistent with similar information derived from studies of *Brugia pahangi* and *Dirofilaria immitis* and emphasizes the importance of the cuticle as an absorptive surface for the uptake of amino acids, monosaccharides, some nucleic acid precursors, and drugs. The intestine of *O. volvulus* is reduced to a microscopic, thread-like structure, possibly reflecting a reduced role of this organ in nutrient and drug uptake.

*In vitro* studies have shown that the drugs diethylcarbamazine, mebendazole, flubendazole, ivermectin, levamisole, and several arsenicals are absorbed, but not actively transported, across the cuticle and body wall of *B. pahangi* adult worms. *In vivo* studies indicate that these compounds may also be ingested orally by the worms, but the rate of drug accumulation by this route of uptake is slow. Such findings contrast with those made with suramin, the uptake of which occurs exclusively by the oral route.

9.4 Suggestions for further study

(a) Attempt to develop *in vivo* and *in vitro* systems using *Onchocerca* spp. parasites that will reflect the therapeutic effects of drugs against *O. volvulus* in man.

(b) Study the mechanisms responsible for nutrient and drug uptake by *O. volvulus* worms.
(c) Undertake studies to define the relationship between the pharmacokinetic compartmentalization of anti-filarial drugs in the \textit{in vivo} screening models and in the nodule-encapsulated \textit{O. volvulus} worms in man.

10. EXPERIMENTAL ONCHOERCIASIS

Several major areas of research on the parasite and in experimental onchocerciasis have yielded valuable and important results during the past decade. These include:

\begin{itemize}
  \item (a) description of the periodic reproductive activity of female \textit{O. volvulus} worms;
  \item (b) production in the laboratory of L\textsubscript{2} and L\textsubscript{3} larval stages of 3 \textit{Onchocerca} spp.;
  \item (c) partial success with \textit{in vitro} maintenance and culture of various stages of \textit{Onchocerca} spp.;
  \item (d) development of animal models for studies of \textit{Onchocerca} eye disease.
\end{itemize}

In addition, a potentially important line of research in the epidemiological study of onchocerciasis is the application of DNA probes to identify \textit{Onchocerca} parasites. Although in its early stages, this work must be mentioned in this report because of its potential significance.

10.1 Advances in knowledge of the parasite

10.1.1 Reproductive cycles of \textit{Onchocerca volvulus}

Young, old, and calcified adult worms may all be found in the same nodule; immature stages, and perhaps even third- and fourth-stage larvae, appear to be attracted to the nodules. In 80\% of nodules, 2–3 female and 1–2 male worms can be found, and exceptionally, accumulations of 50 or more worms may occur. The relatively immobile female worms are present in greater numbers in the nodules, possibly because the male worms frequently migrate in and out of the nodules. The mechanisms and signals by which developing worms locate nodules and those by which adult worms locate each other are still unknown. The nearly balanced sex ratio observed in nodules excised from patients in areas where
transmission has been interrupted for a long time suggests that the wandering of male worms outside the nodule decreases with advancing age.

Unlike many other filarial species, *O. volvulus* does not release microfilariae continuously, but does so in regular cycles. This conclusion is supported by the fact that in large samples of isolated worms only two-thirds of the females contain embryonic stages of microfilariae; the uteri of the other worms contain oocytes exclusively or a few microfilarial remnants, or are empty; this was found to be the same for females of all ages.

The frequency and duration of these cycles have been determined using worms isolated from patients treated with a variety of anti-onchocercal drugs that have different effects on the development of the intrauterine stages. Mebendazole, within a few days of treatment, affects all embryonic stages surrounded by an eggshell; only the already stretched-out microfilariae are unaffected and these are released within a few weeks of treatment. The subsequent reproductive cycle can develop normally 2–3 months later. After treatment with low doses of suramin, female worms are still able to complete their current reproductive cycle within 2–4 months; but a subsequent cycle does not follow until one year later, probably because of the long half-life of the drug in patients. Ivermectin does not impair embryogenesis; however, the mature microfilariae, instead of being released, accumulate in the uteri of hyper gravid worms, where they degenerate and are resorbed 3 months after treatment. This effect of sequestration of microfilariae persists in the subsequent cycles, but gradually ceases after 9 months. From such drug-related observations it has been inferred that the development of an oocyte to the mature microfilaria takes 3–4 weeks and that intrauterine microfilariae become vacuolated and degenerate when they cannot or do not leave the female worm within a few weeks.

These observations imply that each female worm undergoes 3–4 reproductive cycles of 2–4 months each year. A theoretical scheme for the reproduction of female *O. volvulus* suggests that the productive activities of the worms are correlated with the age and composition of the worm population. During one cycle a female produces 200 000–400 000 microfilariae, i.e., theoretically more than $1 \times 10^7$ progeny during her life. A daily release of 1000–3000 microfilariae per female worm would be in accordance with observations made *in vitro* and with *in vivo* estimations that total microfilarial loads of $1–2 \times 10^8$ can exist in heavily infected
patients. However, factors regulating the production and release of microfilariae have to be considered in such calculations and several host factors must also be taken into account. In a person with a fully developed microfilaridermia, a dynamic equilibrium seems to exist between the micro- and macrofilarial load.

There is some evidence that the frequency of the reproductive cycle slows as the worms age so that production and output are considerably reduced as the intervals between the cycles are gradually prolonged. Fertile females with empty uteri predominate among the aging worm populations found in the area under vector control in West Africa, and females harbouring numerous remnant or degenerating microfilariae are frequently present. It appears that many mature microfilariae are not released from such worms and that they degenerate, as has been observed after treatment with ivermectin.

Reinsemination of the female seems to be necessary for the initiation of each reproductive cycle. The number of intrauterine sperm is dramatically reduced at the end of a reproductive cycle, and they are often absent. 50% of the male worms contain young spermatids only, presumably because they have recently delivered their mature sperm. In old worm populations the proportion of such “active” male worms is reduced to 30%, a finding that is consistent with diminishing sexual activity.

10.1.2 *Microfilaria bolivarensis*

Based on measurements and certain morphological characteristics of microfilariae found in the peripheral blood of Amerindians living on the upper Caura River in Bolivar State, Venezuela, a new species, *Microfilaria bolivarensis*, has been described (11). The adults are unknown but morphological comparisons with microfilariae of *O. volvulus* failed to reveal any clear-cut taxonomic differences (12). The status of this form needs to be further clarified.

10.2 Laboratory methods for transmission of *Onchocerca* spp.

The development of effective systems for the laboratory transmission of *Onchocerca* spp. has expanded greatly during the past decade. Previously, the almost total absence of laboratory-derived vectors and adequate sources of microfilariae virtually
precluded experimentation with infective-stage larvae (L₃). Recently, however, cattle *Onchocerca* spp. as well as *O. volvulus* have been successfully manipulated using colonized flies so that the vector stages (L₁–L₃) can be obtained.

10.2.1 Laboratory rearing of vectors

Specialized approaches to laboratory rearing and colonization are needed for the Simulidae. Self-perpetuating colonies of subarctic species of both *Culicoides* and *Simulium* have been established by employing rearing techniques that emphasize proper larval nutrition, thereby ensuring synchronous adult emergence, the availability of robust female flies suitable for filarial infection, and insemination rates adequate for colony maintenance. Rearings of *Culicoides* (Ceratopogonidae) have been established for the experimental transmission of *O. gibsoni*.

10.2.2 Selection of microfilariae for experimental studies

The selection of microfilariae for study has often been dictated primarily by the local availability of *Onchocerca* spp. that parasitize livestock; i.e., *O. cervicalis* in horses and *O. lienalis*, *O. gutturosa*, and *O. gibsoni* in cattle. Large numbers of these microfilariae can, however, now be obtained from infected animals at slaughter and cryopreserved in liquid nitrogen. Similarly, *O. volvulus* microfilariae either within or free from skin biopsies, can be successfully frozen, transported to laboratories distant from foci of human onchocerciasis, and stored in liquid nitrogen until needed for subsequent laboratory experimentation. In endemic areas where onchocercomata are removed surgically, both uterine and nodular microfilariae can also be collected in large numbers. However, such microfilariae are unable to develop in the intermediate host without first being "triggered" to undergo a necessary maturation step, e.g., by migration through the skin of a murine proxy host.

10.2.3 Infection and maintenance of vectors

Infection of vectors may be *per os* by feeding directly on an infected host or through *in vitro* feeding devices using natural or artificial membranes. Intrathoracic inoculation is a better method for quantitative studies on the infection of vectors.
Regardless of the means of infection, the critical determinants of the successful production of *Onchocerca* spp. infective-stage larvae (L₃) relate to the manner in which the flies are maintained during the extrinsic incubation period. Definition of their local environmental needs (e.g., temperature, humidity) and the nutritional requirements necessary to satisfy their high metabolic demands are prerequisites for developing the appropriate rearing techniques.

10.2.4 *Harvest and storage of infective larvae*

Following larval development to the third stage, harvest of the parasite is most easily accomplished by dissecting the vector on glass slides. Here again technical details (e.g., the selection, pH, and osmolality of the dissecting medium, and the time of exposure of L₃ stage larvae to the ambient environment) are important to assure success in harvesting viable parasites, and these variables differ from those used commonly with the mosquito vectors of other filarial infections. Mass dissection, as has been employed for studies on *Wuchereria bancrofti* cannot be used for *O. volvulus* because of the sluggish movements of the infective-stage larvae.

The accumulation and storage of *Onchocerca* spp. infective-stage larvae in a viable condition is possible using cryopreservation techniques. For example, *in vitro* moulting of *O. volvulus* L₃ stage larvae to the L₄ stage can be achieved regularly at acceptable levels (70% or greater) after controlled rate freezing in liquid nitrogen.

10.3 *In vitro cultivation of Onchocerca*

The development of *in vitro* techniques for the cultivation of larval *Onchocerca* spp. would contribute in several ways to an overall strategy for the control of onchocerciasis in man:

(a) a system that promoted significant *in vitro* development could provide the parasite material necessary for biochemical and pharmacological studies directed towards the development of new chemotherapeutic agents;

(b) despite some inherent shortcomings, these methods could also provide a rapid and inexpensive method for the preliminary screening of new filaricides;
(c) such techniques would allow the collection of antigen or nucleic acid material that is free from complicating host factors for use in immunological or diagnostic assays;

(d) in vitro techniques could enable developmental biologists to investigate obscure or cryptic phases of the filarial life cycle in order to understand better the regulation of key morphogenetic events such as molting or gametogenesis.

10.3.1 Cultivation of larval stages normally found in the invertebrate host

The basic approach to this problem is illustrated by a recent study in which microfilariae of *O. lienalis* from bovine skin were inoculated directly into several commercially available tissue culture media supplemented with fetal bovine serum and, in some instances, a feeder layer of invertebrate cells. Cultures were maintained at temperatures that would be found in the arthropod vector (i.e., 26–27°C). As in all previous studies, even the best of the *in vitro* conditions tested promoted differentiation of microfilariae only to the late L₄ stage, but no further. Supplementing the culture media with reducing agents and with insect cells increased the incidence of this microfilarial differentiation and prolonged the survival of cultured larvae.

10.3.2 Cultivation of larval stages normally found in the vertebrate host

In contrast to the vector-associated stages, significant progress has been made towards the cultivation of vertebrate-stage filarial larvae. Techniques promoting *in vitro* development from stage L₃ to L₄ have been described for several species including *Dirofilaria immitis*, *Litomosoides carinii*, *Dipetalonema viteae*¹, *Brugia pahangi*, and *B. malayi*. With *Dipetalonema viteae*¹, composition of both the gas phase (relatively low partial pressure of oxygen) and the medium (defined tissue culture medium supplemented with fetal bovine serum) proved to be critical factors for successful cultivation (13, 14).

¹ See footnote on page 71.
Recently, both *O. volvulus* and *O. lienalis* have been cultured from stage L₃ to L₄ in vitro with 30%–50% of larval *O. lienalis* and a smaller percentage of larval *O. volvulus* completing the third moult. Survival of the L₄s was enhanced by cultivation in the presence of vertebrate cells and in a reduced oxygen atmosphere.

10.3.3 Cultivation of adults

Dissection of nodules usually yields a small number of intact, living adult male worms, but female *O. volvulus* can rarely be extracted intact. A larger number of intact, living adult worms can be recovered from nodules by collagenase digestion of the host tissue. This technique was developed primarily for the study of the worm population, for which it has proved very useful (see sections 7.1 and 12.1.3). Worms isolated alive in this way exhibit no recognizable morphological damage even at the ultrastructural level, and they have been used for various purposes including *in vitro* cultivation. However, only about 20% of female worms obtained in this way can be successfully maintained *in vitro* for periods of even a few days. Those isolated after only 6–24 hours of collagenase incubation have been most suitable for this purpose, but the mean survival period has been only 14.5 days (maximum 42 days) for female worms and 11 days (maximum 26 days) for males. Release of microfilariae by female *O. volvulus* may continue *in vitro* for as long as 25 days, but embryogenesis has not been demonstrated.

With the availability of good transport facilities, nodules or isolated worms removed in endemic areas can be shipped to laboratories in other countries. The transport of deep-frozen nodules is also possible, but the worms recovered are always dead, and application of the collagenase technique to such nodules may result in altered antigenic or biochemical parasite material. Since it has recently been shown that cryopreservation in liquid nitrogen with the recovery of living adult male *O. gutturosa* is possible, improvement of cryopreservation procedures and their application to adult *O. volvulus* may offer possibilities for the cultivation of adult worms independent of the time and place of their isolation.

10.4 DNA probes and the identification of *Onchocerca*

DNA probes have great potential for the identification of filarial parasites in both man and insect vectors; however, this is a new and
emerging area of research, and the future impact of such methodology on the epidemiology of onchocerciasis through the use of improved diagnostic techniques is still uncertain.

10.4.1 Current status of DNA probes for filarial infections

Two general approaches have been used to develop DNA probes for various infectious agents: (1) identification of DNA molecules unique to the organism, such as the kinetoplast DNA of *Leishmania* spp., and (2) identification of regions of DNA within the genome that are both repeated and specific for the organism in question. With filarial worms, the second approach has been used effectively.

Recently, repeated DNA nucleotide sequences specific for *Brugia malayi* have been successfully cloned. The basic method used was to isolate total DNA from *B. malayi* and then to clone DNA fragments cut with restriction endonucleases using recombinant DNA techniques. The resultant genomic library was then screened for the presence of repeated genes using total nick-translated *B. malayi* DNA. One clone reacted only with *B. malayi* or *B. timori*, and not with any other filarial species tested (*B. pahangi*, *Wuchereria bancrofti*, *O. volvulus*, *Dirofilaria immitis*, and *B. patei*), or with either human or mosquito DNA. In laboratory experiments, this DNA probe has also been found to be very sensitive, detecting a single L3-stage larva or as few as 5 microfilariae. Preliminary field testing of the probe showed it to be very useful in detecting L3 stages in mosquitoes. Other clones, similarly derived, are proving useful in the areas of phylogeny and systematics of filarial parasites.

10.4.2 Application of DNA probes to onchocerciasis

The initial experiments with *B. malayi* clearly indicate that the approach used is a reasonable one for the identification and isolation of specific DNA probes. A similar approach should be feasible for the development of probes specific for *O. volvulus* that should be useful for detecting infected insect vectors, differentiating larval *O. volvulus* from other parasite species, and for quantifying larval burdens in the vectors. An important question is whether such DNA probes can be used to differentiate between different geographical isolates or forms of the parasite, or whether a confusingly large array of differences will be detected.

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10.5 Animal models for ocular onchocerciasis

Two types of animal model have been used to help elucidate the mechanisms involved in the pathophysiology and immunology of onchocercal eye disease: those employing “normal” definitive hosts of *Onchocerca* spp. (*O. cervicalis* in horse, *O. lienalis* or *O. gibsoni* in cattle, *O. volvulus* in nonhuman primates); and those using experimentally introduced microfilariae of *Onchocerca* spp. in laboratory animals such as guinea-pigs and rabbits.

Important early animal studies were carried out in rabbits, but the usefulness of this model is limited. Recent studies have utilized a guinea-pig/*O. lienalis* model which is especially useful for the study of acute inflammatory changes.

A cynomolgus (*Macaca fascicularis*) monkey/*O. lienalis* model has also been used to study the development of posterior segment eye disease. *O. volvulus* infection will develop in chimpanzees after the inoculation of L₃ larvae, but because of restrictions on experimentation with these protected animals little invasive experimental intervention is possible.

10.6 Suggestions for further study

(a) Study further spermiogenesis and reproductive behaviour in male worms, including the mechanisms involved in locating female worms.

(b) Undertake studies of the developing larval stages of the parasite in man, including behavioural aspects.

(c) Try to define the conditions that will support *in vitro* cultivation of all stages of *Onchocerca* spp. parasites.

(d) Improve methods for isolating adult worms from nodules and cryopreserving them so that they are still viable when recovered.

(e) Develop new biotechnology techniques using recombinant DNA and monoclonal antibodies and use them as well as classical techniques to detect and characterize *O. volvulus* in the vector and in man.

(f) Develop new *Onchocerca* spp. animal experimental models that are suitable for laboratory work.

(g) Because of the difficulties in obtaining parasite material from different geographical regions, coordinate the provision and sharing of parasite material for biochemical, antigenic, and DNA analyses and the development of nomenclature arising from such studies.