28. On day 28, 8 patients had positive blood smears, on day 35, 5 patients, and on day 42, 2 patients were positive. Reinfection could not be excluded.

There was no difference in parasite clearance between the 750- and 1000-mg doses, but 750 mg of mefloquine was better tolerated than the 1000-mg dose. The incidence of vomiting and diarrhoea was not increased by the addition of primaquine.

In June 1983, the Malaria Division of the Ministry of Public Health in Thailand began a large-scale field trial of mefloquine/sulfadoxine/pyrimethamine combination for the curative treatment of falciparum infections in several “hard-core” areas of multi-drug resistance. The trial was designed as an operational research project to evaluate the tolerance and efficacy of the combination and, at the same time, to reduce the malaria case-load in the most affected parts of the country. In an attempt to reduce transmission in the study areas, operational measures have been intensified, including residual insecticide spraying, improved health education, and the involvement of the community in malaria control activities such as measures for personal protection and larval control.

The drug study is a phased trial, beginning in several sector clinics (the most peripheral units of the malaria service), located near the Kampuchean border. In the study areas, patients with \textit{P. falciparum} infections diagnosed by blood smear are treated with either a single dose of the combination (formulated in the ratio of 10 parts of mefloquine to 20 parts of sulfadoxine to 1 part of pyrimethamine) or, in the case of the control group, with quinine/tetracycline (10 mg of quinine per kg 3 times per day for 3 days) together with 7 days of tetracycline (5 mg/kg 4 times a day). In the first group, all individuals in whom \textit{P. falciparum} parasites are detected, except pregnant women and children below the age of one year, receive the combination at a dosage of mefloquine of 12.5–15 mg/kg body weight. Pregnant women and infants (there are very few patients in these categories) receive quinine alone (10 mg/kg 3 times a day) for 7 days. All patients are followed for 12 weeks to observe drug tolerance and the incidence of recrudescence or reinfection. To date, approximately 100 patients have been admitted to each group of the study. Tolerance of both regimens has been excellent. There have been two failures in the group treated with the combination, one recrudescence (or reinfection) detected on day 28, and one RII-type persistence of parasitaemia. Serum from both of these individuals showed that the drug had been absorbed normally.
When 200 patients have been admitted to the sector-based phase of the study, the zone-based comparative trial will begin. Approximately 1500 of the 50 000 patients expected to be treated with either of the two regimens will be followed up. Upon successful completion of this phase, a full-scale trial will begin in 6 zones along the Kampuchean border and in a highly resistant area of the northeast. It is expected that the combination will be used to treat an additional 60–75 000 patients, of whom 1000 will be followed for 3 months; the trial should be completed by the end of 1984.

For the cure of resistant falciparum malaria, the following dosage recommendation can be made for the combination of mefloquine/sulfadoxine/pyrimethamine (ratio 10 : 20 : 1):

- in areas where resistance to sulfadoxine/pyrimethamine is rare, two tablets of the fixed combination, each containing 250 mg of mefloquine, 500 mg of sulfadoxine, and 25 mg of pyrimethamine given as a single dose;
- in places where resistance is high, three tablets given as a single dose.

The dosage used in children for the cure of *P. falciparum* infections in the Thai field trial is presented in Table 12.

<table>
<thead>
<tr>
<th>Body weight (kg)</th>
<th>No. of tablets (single dose treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6–10</td>
<td>1</td>
</tr>
<tr>
<td>10–15</td>
<td>2</td>
</tr>
<tr>
<td>15–19</td>
<td>3</td>
</tr>
<tr>
<td>20–30</td>
<td>1½</td>
</tr>
<tr>
<td>31–45</td>
<td>2</td>
</tr>
</tbody>
</table>

4.2.4 Monitoring mefloquine trials

4.2.4.1 Methods of monitoring for adverse drug reactions. Methods of evaluating adverse drug reactions are mainly based on careful observation of the patients. Signs and symptoms caused by the drugs must be differentiated from those that are not. The disease process itself may produce symptoms, and the mere fact that the patient is being observed sometimes gives rise to symptoms. Patients receiving placebos often complain of adverse effects.
Various steps need to be taken to ensure the valid identification of drug-induced reactions. Patient randomization, double-blind studies, comparison with other treatments, careful recording of “baseline symptoms” before drug administration, basic laboratory data (haematology and serum biochemistry) obtained before drug administration, and repeated clinical and laboratory observations made after drug administration are all necessary and must be part of the protocol.

The severity of symptoms should be recorded as mild, moderate, or severe. If any specific treatment is needed to counteract adverse drug reactions, it should be recorded.

The monitoring of adverse drug reactions involves judgement on the part of the investigator regarding the causal relationship between administration of a drug and the observed adverse reactions. This relationship could be causative, probable, possible, or coincidental. (1) “Causative” is the term used when high levels of the drug can be demonstrated in blood or body fluids in association with a toxic drug reaction.

(2) The relationship is “probable” if the reaction pattern is similar to that observed with previous use of the drug, provided only one drug has been used at a time.

(3) The relationship is considered to be “possible” if the reaction pattern is similar to that seen with previous use of the drug, but if similar reactions could also be due to the disease process.

(4) If no comparisons are available from previous experience with the drug and if the association of reactions seen can neither be definitely attributed to the drug nor definitely ruled out, then the association is termed “coincidental”.

In some cases, a more definite relationship can be established, either by “re-challenging” the patient with the same drug at a later date and noting if the reaction recurs, or by “de-challenging”, i.e., by stopping the use of the drug, and noting if the reaction subsides.

4.2.4.2 Monitoring for adverse reactions to mefloquine in clinical trials.

(1) The clinical trials monitored and the methods used. Eleven clinical trials have so far been monitored; details are given in Table 13. Patients suffering from severe attacks of malaria and patients with severe accompanying diseases were excluded from the trials.
<table>
<thead>
<tr>
<th>Centre</th>
<th>Type of trial</th>
<th>No. of subjects</th>
<th>Drugs, dosage, and schedule</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belém (Pará), Brazil</td>
<td>1. Phase I</td>
<td>10</td>
<td>Mefloquine 1000 mg, single oral dose + Sulfadoxine 1500 mg + pyrimethamine 75 mg, single oral dose</td>
<td>adult male inpatients</td>
</tr>
<tr>
<td></td>
<td>double-blind, randomized,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>comparative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2. Phase II/III</td>
<td>49</td>
<td>Mefloquine 1000 mg, single oral dose + Sulfadoxine 1500 mg + pyrimethamine 75 mg, single oral dose</td>
<td>adult male inpatients</td>
</tr>
<tr>
<td></td>
<td>double-blind, randomized,</td>
<td>58</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>comparative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. Phase III</td>
<td>50</td>
<td>Mefloquine 1000 mg, single oral dose + Quinine 600 mg 3 times a day + 3 days + single dose sulfadoxine 1500 mg + pyrimethamine 75 mg on 1st day</td>
<td>adult male inpatients</td>
</tr>
<tr>
<td></td>
<td>open, randomized,</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>comparative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N'dola, Zambia</td>
<td>4. Phase I</td>
<td>5</td>
<td>Mefloquine 1000 mg, single oral dose + Chioroquine 900 mg on 1st day + 300 mg daily for next 2 days</td>
<td>adult male inpatients</td>
</tr>
<tr>
<td></td>
<td>double-blind, randomized,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>comparative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5. Phase II</td>
<td>13</td>
<td>Mefloquine 750 mg + 250 mg in one day + Chioroquine 900 mg on 1st day + 300 mg daily for next 2 days</td>
<td>adult male inpatients</td>
</tr>
<tr>
<td></td>
<td>double-blind, randomized,</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>comparative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6. Phase III</td>
<td>49</td>
<td>Mefloquine 1000 mg, single oral dose + Chioroquine 1500 mg in 3 days</td>
<td>adult male inpatients</td>
</tr>
<tr>
<td></td>
<td>double-blind, randomized,</td>
<td>49</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>comparative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study Location</td>
<td>Study Type</td>
<td>Patients</td>
<td>Treatment Details</td>
<td>Patients Description</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>----------------------------------</td>
<td>----------</td>
<td>-----------------------------------------------------------------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Bangkok, Thailand</td>
<td>Double-blind, randomized, dose-finding, comparative</td>
<td>50</td>
<td>Mefloquine 500 mg, single oral dose</td>
<td>Adult male inpatients with P. falciparum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>49</td>
<td>Mefloquine 750 mg, single oral dose</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>48</td>
<td>Mefloquine 1000 mg, single oral dose</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Open, randomized, comparative</td>
<td>36</td>
<td>Mefloquine 1000 mg, single oral dose</td>
<td>Adult male inpatients</td>
</tr>
<tr>
<td></td>
<td></td>
<td>34</td>
<td>Quinine 600 mg 3 times a day × 3 days + tetracycline 250 mg 4 times a day × 7 days</td>
<td>With P. falciparum fever</td>
</tr>
<tr>
<td></td>
<td>Open, non-comparative</td>
<td>30</td>
<td>Mefloquine 750 mg, single oral dose</td>
<td>Adult females (non-pregnant) of childbearing</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>age inpatients with P. falciparum fever</td>
</tr>
<tr>
<td></td>
<td>Open, paediatric (6-12 years old), non-comparative</td>
<td>43</td>
<td>Mefloquine 25 mg/kg body weight</td>
<td>Children inpatients with P. falciparum fever</td>
</tr>
<tr>
<td>Kuchinarai District, Thailand</td>
<td>Double-blind, randomized, comparative</td>
<td>47</td>
<td>Mefloquine 750 mg, single oral dose</td>
<td>Adult male outpatients</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>Mefloquine 750 mg + sulfadoxine 1500 mg + pyrimethamine 75 mg, single oral dose</td>
<td></td>
</tr>
</tbody>
</table>

**Total no. of patients treated with each drug or drug combination:**

1. Mefloquine: 436 adults + 43 children
2. Sulfadoxine + pyrimethamine: 68 patients
3. Chloroquine: 68 patients
4. Quinine + sulfadoxine + pyrimethamine: 50 patients
5. Quinine + tetracycline: 34 patients
6. Mefloquine + sulfadoxine + pyrimethamine: 50 patients
Malarial fever is associated with subjective symptoms such as headache, bodyache, weakness, nausea, dizziness, vomiting, abdominal pain, and sometimes mild diarrhoea, which all have to be carefully differentiated from the possible side-effects of drugs. Baseline symptoms were recorded, if possible, for 1 or 2 days before drug administration.

Laboratory tests for haematology, serum biochemistry and stool and urine analyses were also carried out prior to drug administration. The following tests were carried out in most of the patients:

(a) **Haematology:** haemoglobin, red blood cell count, haematocrit, reticulocyte count, leukocyte count and differential, platelet count, partial thromboplastin time.

(b) **Urine:** volume, frequency, pH, specific weight, albumin, glucose, urobilinogen, bilirubin, blood, sediment, pre-trial tests for 4-aminoquinolines and sulfonamides.

(c) **Stools:** blood, parasites, and eggs.

(d) **Biochemistry:** serum glucose, total bilirubin, urea, calcium, sodium, potassium, iron, magnesium, phosphate, chloride, proteins, alkaline phosphatase, aspartate aminotransferase,¹ alanine aminotransferase,² glucose-6-phosphate dehydrogenase.

Bioavailability studies of mefloquine and sulfadoxine/pyrimethamine were carried out in some patients.

The clinical and laboratory parameters of symptoms and laboratory data were recorded daily for the first 7 days after drug administration, and then once a week for the next 8 weeks. If any symptoms developed in the meantime, they were followed up daily until the patient improved. The laboratory test values were compared to those obtained in the same laboratory for the healthy target population of the particular region. If any other drug was administered during the trial, details were recorded.

At the end of the trials, the investigator filled out a form for adverse reactions indicating the symptom he considered to be drug related, its severity, and the extent to which it was considered to be related to drug administration (definite, probable, possible). If, at

¹ Previously known as glutamic oxaloacetic transaminase.
² Previously known as glutamic pyruvic transaminase.
any moment, any serious drug reaction was suspected, the central clinical monitor was informed immediately and, at the same time, blood was collected to determine the drug levels.

If the patient vomited within a few hours of drug administration, blood was collected 6 hours after this incident to determine the level of drug concentration in the blood. Loss of drug in vomited material may reduce the effective blood levels, leading to therapeutic failure.

(2) Incidence of adverse reactions to mefloquine. Compared with standard antimalaria treatment, mefloquine was well tolerated and no serious adverse reactions were observed. The pattern of adverse reactions seen in healthy volunteers was similar to that seen in patients.

The main adverse reactions reported, all of them mild to moderate in nature, were nausea, dizziness, abdominal pain, vomiting, and diarrhoea. Itching or skin rash was seen in a few cases. Sinus bradycardia, which was symptomless and needed no treatment, was observed in some patients. Neuropsychiatric symptoms were observed in 4 patients and this reaction needs further study.

The overall incidence of the various reactions was: nausea 17.7%, vomiting 13.3%, diarrhoea 14.9%, dizziness 14.7%, abdominal pain 8.3%, sinus bradycardia 8.9%, skin itching or rash 1.4%, and neuropsychiatric changes 0.9%. Most of these symptoms needed no particular treatment and were reversible.

In different countries, although the pattern of adverse reactions was similar, the actual incidence varied (see Table 14). The incidence of nausea was lowest in Zambia, while the incidence of diarrhoea was highest in Belém (Brazil). Dizziness was more common in Brazil and Zambia than in Thailand. In Thailand, inpatients in Bangkok had a very low incidence of dizziness whereas outpatients in the Kuchinarai District trial had a higher incidence of dizziness and vomiting (see Table 15). This may be attributed to the fact that the outpatients were ambulant, attending the outpatient clinic daily for follow up, whereas the inpatients remained in bed at least during the first few days after admission to hospital. Incidence of abdominal pain was higher in Belém (Brazil) and Ndola (Zambia) where concomitant multiple helminth infection was present in 90–100% of the patients.

Sinus bradycardia, which was only observed in patients in Ndola and Bangkok, usually occurred 5–7 days after mefloquine administration and reverted back to normal in 7–14 days. A number
Table 14. Adverse reactions to mefloquine in clinical trials in different areas
(1980 until April 1983)

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Belem (Brazil) (105 patients)</th>
<th>Thailand (263 patients)</th>
<th>Ndola (Zambia) (87 patients)</th>
<th>Total (436 patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Nausea</td>
<td>24</td>
<td>22.0</td>
<td>49</td>
<td>18.8</td>
</tr>
<tr>
<td>Vomiting</td>
<td>13</td>
<td>11.9</td>
<td>37</td>
<td>14.2</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>35</td>
<td>32.1</td>
<td>21</td>
<td>8.1</td>
</tr>
<tr>
<td>Dizziness</td>
<td>35</td>
<td>32.1</td>
<td>11</td>
<td>4.2</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>20</td>
<td>18.3</td>
<td>7</td>
<td>2.7</td>
</tr>
<tr>
<td>Sinus bradycardia and minor ECG changes</td>
<td>0</td>
<td>0</td>
<td>30</td>
<td>11.5</td>
</tr>
<tr>
<td>Skin itching/rash</td>
<td>3</td>
<td>2.8</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>Neuropsychiatric changes</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>1.2</td>
</tr>
<tr>
<td>Tinnitus</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Others</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Sinus bradycardia and some minor ECG changes, symptomless and reversible.
*One case of suspected myoglobinuria, probably not related to drug.

Table 15. Adverse reactions to mefloquine in clinical trials in Thailand
(1980 until April 1983)

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Bangkok (213 inpatients)</th>
<th>Kuchinarai District (47 outpatients)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Nausea</td>
<td>39</td>
<td>18.3</td>
</tr>
<tr>
<td>Vomiting</td>
<td>24</td>
<td>11.3</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>17</td>
<td>8.0</td>
</tr>
<tr>
<td>Dizziness</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Sinus bradycardia and minor ECG changes</td>
<td>30</td>
<td>14.1</td>
</tr>
<tr>
<td>Skin itching/rash</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Neuropsychiatric changes</td>
<td>3</td>
<td>1.4</td>
</tr>
<tr>
<td>Tinnitus</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Others</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Three cases of behavioural disorder.

of children under treatment showed symptomless sinus arrhythmia occurring most frequently between day 3 and day 10 (see Table 16).

Neuropsychiatric changes, characterized by behaviour disorder, paranoid ideas, and hallucinations, were seen in 3 inpatients in Bangkok and in 1 patient in Ndola. Two more patients in another
Table 16. Adverse reactions to mefloquine in clinical trials (open)
Bangkok paediatric study (1980 until April 1983)

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Incidence of reactions among 43 children aged 6–12 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Nausea</td>
<td>0</td>
</tr>
<tr>
<td>Vomiting</td>
<td>3</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>3</td>
</tr>
<tr>
<td>Dizziness</td>
<td>0</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>0</td>
</tr>
<tr>
<td>Sinus arrhythmia</td>
<td>26</td>
</tr>
<tr>
<td>Skin itching/rash</td>
<td>0</td>
</tr>
<tr>
<td>Neuropsychiatric changes</td>
<td>0</td>
</tr>
<tr>
<td>Tinnitus</td>
<td>0</td>
</tr>
<tr>
<td>Others</td>
<td>0</td>
</tr>
</tbody>
</table>

*Day of sinus arrhythmia

Number of patients | D0 | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D10 | D14 | D28 |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>9</td>
<td>12</td>
<td>17</td>
<td>20</td>
<td>23</td>
<td>19</td>
<td>18</td>
<td>16</td>
<td>13</td>
<td>10</td>
</tr>
</tbody>
</table>

A series of trials in Bangkok also exhibited similar changes; one of these patients had received a mefloquine dose of 2000 mg. All these patients recovered following symptomatic treatment and remained well. No such reactions were seen in Brazil or in outpatients in Thailand.

Mefloquine was found to compare well with the various antimalarials used in different centres regarding the pattern of sensitivity of the malaria parasites. In Ndola, mefloquine was compared with chloroquine; in Belém it was compared with sulfadoxine + pyrimethamine and the combination of quinine + sulfadoxine + pyrimethamine (see Tables 17 and 18). The incidence of abdominal pain, diarrhoea, and vomiting following mefloquine treatment was higher than that observed after sulfadoxine + pyrimethamine, whereas the incidence of dizziness was higher following sulfadoxine + pyrimethamine.

The incidence of nausea, vomiting, diarrhoea, abdominal pain, and sinus bradycardia was similar with chloroquine and mefloquine. The incidence of dizziness was increased with mefloquine. The incidence of itching or skin rash was 35.2% in patients receiving chloroquine compared with 3% in those receiving mefloquine. The combination of quinine + sulfadoxine + pyrimethamine resulted in a higher incidence of nausea (48%) and vomiting (28%) compared to that seen after mefloquine, while the incidence of dizziness was lower (20%) than after mefloquine (32.1%). No tinnitus or hearing loss was observed after mefloquine, but 34% had tinnitus and 8% had hearing difficulty following quinine.
<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Incidence of reactions</th>
<th>Overall mefloquine trials</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mefloquine (109 patients)</td>
<td>Sulfadoxine/pyrimethamine (58 patients)</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Nausea</td>
<td>24</td>
<td>22.0</td>
</tr>
<tr>
<td>Vomiting</td>
<td>13</td>
<td>11.9</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>35</td>
<td>32.1</td>
</tr>
<tr>
<td>Dizziness</td>
<td>35</td>
<td>32.1</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>20</td>
<td>18.3</td>
</tr>
<tr>
<td>Sinus bradycardia and</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>minor ECG changes</td>
<td>3</td>
<td>2.8</td>
</tr>
<tr>
<td>Skin itching/rash</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Neuropsychiatric changes</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Tinnitus</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Others</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*One case of suspected myoglobinuria, probably not drug-related.
Table 18. Adverse reactions to mefloquine in clinical trials: comparison with chloroquine Ndola, Zambia (1980 until April 1983)

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Mefloquine (67 patients)</th>
<th>Chloroquine (68 patients)</th>
<th>Overall mefloquine trials (Total: 436 patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Nausea</td>
<td>4</td>
<td>6.0</td>
<td>6</td>
</tr>
<tr>
<td>Vomiting</td>
<td>8</td>
<td>11.9</td>
<td>6</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>9</td>
<td>13.4</td>
<td>7</td>
</tr>
<tr>
<td>Dizziness</td>
<td>18</td>
<td>26.9</td>
<td>7</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>9</td>
<td>13.4</td>
<td>12</td>
</tr>
<tr>
<td>Sinus bradycardia and minor ECG changes</td>
<td>9</td>
<td>13.4</td>
<td>10</td>
</tr>
<tr>
<td>Skin itching/rash</td>
<td>2</td>
<td>3.0</td>
<td>24</td>
</tr>
<tr>
<td>Neuropsychiatric changes</td>
<td>1</td>
<td>1.5</td>
<td>1*</td>
</tr>
<tr>
<td>Tinnitus</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Others</td>
<td>1*</td>
<td>1.5</td>
<td>1*</td>
</tr>
</tbody>
</table>

*Epileptic-form convulsions.
*Three cases of behavioural disorder.
*Suspected myoglobinuria, probably not drug-related.

Mefloquine did not produce any adverse haematological or biochemical reactions in any of the patients.

On the whole, the incidence of adverse reactions after mefloquine was comparable to that seen with other standard antimalarial drugs. Most of the reactions were mild to moderate, and self-terminating.

The need for a well-planned protocol recording “baseline symptoms” before drug administration and for the careful recording, reporting, and follow-up of suspected adverse drug reactions is self-evident. Regional, racial, and genetic differences in reactions must be collected in well-planned trials in different countries, as an antimalarial drug is likely to be used widely in different parts of the world. A correct assessment of adverse reactions following the use of a new drug requires properly collected data that is evaluated with great care.

4.2.5 Deployment of mefloquine

It took a decade of intensive laboratory and clinical investigations to develop mefloquine. This new compound is highly effective in the management of multiple drug-resistant falciparum malaria. It is, in principle, also effective for the prophylaxis and treatment of infections in man due to all species of malaria parasite. However, the
risk that *P. falciparum* may develop resistance to this valuable compound is great, and every precaution must be taken to ensure that it is deployed in such a way that maximum use can be made of it, while the danger of the development of resistance is minimized.

4.2.5.1 *Hazards*. The hazards involved in the free use of mefloquine come from three sources:

1) *The patient*. The problem of individual intolerance to a recommended dose of mefloquine (as to any drug) will always be present, but the experience gained in Phase II and III clinical trials will help to identify individuals who may be particularly at risk, so that appropriate warning can be given. It is not yet possible to define the level of intolerance that may be expected, and more studies are needed on individuals with metabolic disorders, abnormal excretion, etc.

Overdosage can be a hazard when using mefloquine, since it has an unusually prolonged half-life in man. While it is not anticipated that the recommended dosage will result in accumulation, it is well known that, once any effective drug becomes freely available (on the open market, or as a drug supplied by a health service), some individuals are likely to take or administer an excessive dose believing that what one tablet cures, two will cure twice as well.

2) *The parasites*. The ability of malaria parasites in the laboratory to become resistant to mefloquine and other aminoalcohols, especially if they are already resistant to chloroquine, has already been well documented. It is not yet known, however, whether this phenomenon also applies to *P. falciparum* as well as the other malaria parasites of man. The potential for resistance seems possible, given the increasing evidence that *P. falciparum* can become resistant to quinine, which is chemically and functionally analogous to mefloquine and other new aminoalcohol antimalarials. Another open question is whether or not mefloquine resistance will prove to be of a stable nature. From experimental work in rodent malaria on compounds of this type, it seems likely that resistance is transmissible through the invertebrate vector.

3) *The drug*. The greatest problem in the use of all drugs is the gross misuse and abuse than can result from the lack of drug controls, overprescribing, use of "shotgun therapy" or, in some cases, a black market in drugs. The loss of effectiveness of one antibiotic after another is a good example of this problem. Another is the rapid appearance of resistance to antifolate antimalarials such
as sulfadoxine/pyrimethamine, which is rapidly losing its effectiveness in an increasing number of endemic areas.

4.2.5.2 Precautions. The following precautions should be taken to avoid the misuse of mefloquine, taken either alone or combined with other drugs.

(1) **Strict drug control.** One of the main precautions to be taken before releasing mefloquine should be the establishment of strict governmental regulations concerning its importation and distribution. This is a counsel of perfection, but popular demand from the public and sales pressure from suppliers should be resisted by the governments of developing countries so that effective control on the use of mefloquine can be established.

(2) **Limitation to areas where the use of mefloquine is indicated.** Measures should be taken through WHO, national governments, and manufacturers to make supplies of mefloquine available only to those countries where there is a clear indication for its use.

(3) **Time limit for the use of mefloquine.** It would be a serious mistake to depend on either mefloquine or any other antimalarial to control malaria indefinitely, in the absence of other control measures. A time limit should therefore be set for its use, particularly for community protection as described in section 3.3.

(4) **Sensitivity monitoring.** Before introducing mefloquine to any new area, all possible steps should be taken to determine the baseline sensitivity of local strains of *P. falciparum* to this drug, using the *in vitro* microtest (see section 2.4.2). After the introduction of mefloquine, monitoring should be carried out on a regular basis for as long as transmission continues, and again at any time that a change in clinical response to the drug is suspected.

(5) **Integrated control.** Mefloquine or any other antimalarial, should be used only as one of the tools in an integrated attack on malaria, either for the emergency handling of an epidemic, or as part of a larger, longer-term control programme. Reliance on antimalarials alone has always failed and has often led to the emergence of drug resistance.

4.2.5.3 **The use of mefloquine with other antimalarials.** There is strong evidence that the use of mefloquine in combination with pyrimethamine and sulfadoxine or other drugs that increase its effectiveness will also help to retard the development of resistance to these compounds, and an appropriate triple combination has
already been evaluated clinically (section 4.2.3). Indications for the use of this and other combinations are given below.

(1) To optimize the therapeutic action of mefloquine. It has been suggested that, in serious falciparum infections, it would be advisable to give one or more doses of the short-lived, but rapidly acting quinine, together with mefloquine, to avoid delay due to the time required for mefloquine to achieve a therapeutic blood level. The validity of this suggestion requires confirmation and the possibility that toxicity may be enhanced needs to be investigated.

(2) In order to interrupt transmission. A single dose of 45 mg of primaquine base (adult dose) may be given with a therapeutic dose of mefloquine in order to sterilize any existing gametocytes of P. falciparum. This may minimize the transmission of parasites in areas where vectors are present. Further research is needed on the optimum dosage and timing of primaquine administration.

(3) Combinations with mefloquine. There is accumulating experimental evidence that resistance may develop more slowly to a combination of mefloquine with an antifolate-sulfonamide combination such as sulfadoxine/pyrimethamine, than to any of the three components used alone, or to the sulfadoxine/pyrimethamine combination. This triple combination would be a far more rational application of mefloquine for malaria prophylaxis in an area of continuing transmission than the widespread use of mefloquine alone. There is an urgent need to find other compounds or drug mixtures to combine with mefloquine.

4.2.5.4 Indications for use of mefloquine and/or a triple combination of mefloquine, pyrimethamine, and sulfadoxine. Several studies on mefloquine and the triple combination are still in progress. In particular, treatment studies involving special risk groups, such as pregnant women and infants, are not yet complete. Furthermore, the use of the triple combination for malaria suppression has not yet been extensively studied. However, based on the available information detailed in sections 4.2.2 and 4.2.3, the following general recommendations can be made:

(1) Treatment. The objective of treatment should always be effective cure. Mefloquine should be used in patients infected with strains of P. falciparum likely to be resistant to chloroquine, particularly if the parasites are already resistant to sulfadoxine/pyrimethamine or similar combinations.
For residents of endemic areas it should be administered in the triple drug combination, except for patients who may be sensitized to any of the components. Until the completion of current clinical studies, mefloquine cannot be recommended for use during pregnancy or in infants. Mefloquine should not be deployed in areas where resistance to other available drugs is not a problem, unless there is a specific indication for its use in individual patients.

(2) *Suppression*. For the control of malaria, chemoprophylaxis should be replaced by the effective treatment of malaria, except under special circumstances. In such circumstances mefloquine should be used (preferably as the triple combination) only in areas where the presence of chloroquine-resistant *P. falciparum* is a problem. The use of mefloquine for suppression cannot be justified where small foci of chloroquine resistance appear to be emerging, but where adequate prophylaxis can still be obtained with alternative preparations such as sulfadoxine/pyrimethamine.

Mefloquine (preferably as the triple combination) may be needed instead of chloroquine in the following cases:

(a) To prevent or reduce morbidity and mortality in high-risk groups such as pregnant women, if investigations confirm that it is safe for them, in special groups such as labour forces, military personnel, and refugees and in non-immune immigrants, visitors, and pilgrims.

(b) To reduce the impact of malaria in communities by using it to control epidemics and to control foci of *P. falciparum* that are resistant to chloroquine and sulfadoxine pyrimethamine.

(c) In eradication programmes to eliminate residual foci, and to prevent the establishment of new foci (autochthonous or introduced).

4.3 Other new antimalarial drugs

In addition to the quinolinemethanols, several other classes of compound have been developed and tested.

4.3.1 *Phenanthrenemethanols*

The most promising phenanthrenemethanol is halofantrine (WR 171669) (Formula No. 20 in Annex 1). Like mefloquine, this drug was very active in mouse screening tests, was not phototoxic,
and was effective in single doses in *Aotus* monkeys with falciparum malaria. Early studies indicated that this compound showed cross-resistance with mefloquine, but recent studies in a rodent model and with clones of *P. falciparum* indicate that cross-resistance is not absolute.

Decreasing dose-finding studies in volunteers with induced malaria showed that failures occurred with single doses of 1000 or 1500 mg. The drug was curative in 8 out of 8 subjects at doses of 1500 mg, provided that it was given in two divided doses. However, when a 1000-mg loading dose was given, followed 6 hours later by 500 mg, 8 out of 8 subjects were also cured (25). Field studies with halofantrine began in Thailand in 1982. The treatment of naturally-acquired falciparum malaria with the dose regimen used in volunteers showed only 13 out of 20 cures; consequently the dose regimen was modified to prolong absorption by giving 500 mg at 6-hourly intervals. With this new regimen the cure rate increased to 28 out of 29 (E.F. Boudreau et al., personal communication, 1983). It would appear that a formulation problem exists which results in poor bioavailability. Further research is being carried out by the company that intends to develop and market the drug.

4.3.2 *Pyridinemethanols*

The most advanced pyridinemethanol is WR 180409 (Formula No. 21). This drug has undergone extensive pre-clinical studies and is now being tested in volunteers with induced malaria (T.M. Cosgriff et al., personal communication, 1983).

Decreasing dose-finding studies showed that a single 500-mg dose failed to cure the infection but 750 mg given in divided doses appeared to be curative: the drug is scheduled for clinical trials in naturally-acquired infections later in 1983.

4.3.3 *Qinghaosu*

Qinghaosu (artemisinine) is the active antimalarial component of the Chinese herb Qinghao (*Artemisia annua* L.). Although the herb has been used for malaria therapy in the People's Republic of China for over 1000 years, the active component of the herb was not isolated and characterized until 1972, when Chinese scientists
showed it to be a novel antimalarial compound with the structure of a sesquiterpene lactone (Formula No. 22). Subsequently, the compound and several of its derivatives have been studied by Chinese scientists for their efficacy in laboratory malaria models, and as regards their pharmacology, pharmacokinetics, and toxicology.

Qinghaosu and its derivatives represent a new chemical series of antimalarial compounds with a high level of blood schizontocidal activity against chloroquine-resistant malaria parasites in laboratory models.

Qinghaosu is only sparingly soluble in water and oils, and therefore attempts have been made to increase solubility by the synthesis of derivatives. Two of these, the methyl ether (artemether) (Formula No. 23) and the hemisuccinate derivative (artesunate) (Formula No. 24), have been selected for extensive pharmacological and toxicological examination. These substances are considerably more active and more soluble than the parent compound, artemether being lipid soluble and artesunate being water soluble.

Pharmacokinetic studies in laboratory animals have shown that qinghaosu and the two derivatives are widely distributed in the tissues and eliminated fairly rapidly. More prolonged plasma levels of qinghaosu and artemether can be obtained by intramuscular injection, and absorption of the sparingly soluble qinghaosu can be increased by the use of an oily, instead of an aqueous suspension. The plasma levels of both of these drugs fit a two-compartmental open model, whereas that of the water-soluble salt artesunate fits a one-compartmental model, showing an even shorter half-life and lower apparent volume of distribution.

Acute toxicity studies in mice have shown that qinghaosu and its two derivatives have higher LD₅₀ values (dose lethal for 50% of test subjects) and chemotherapeutic indices than chloroquine. In all animal species examined, the three drugs were well tolerated, adverse effects occurring only when very large doses were given. Subacute toxicity experiments in monkeys have indicated that the toxic effect of qinghaosu may be exerted mainly on the erythroid haemopoietic cells of the bone marrow. Liver damage was also observed at the higher doses. These toxic effects appeared to be reversible.

This group of antimalarial drugs has been used extensively in China on an investigational basis and a considerable amount of knowledge has been accumulated on the clinical efficacy of different formulations and dosage regimens.
4.3.3.1 Clinical studies on the treatment of malaria with qinghaosu and its derivatives. Clinical evaluation of qinghaosu and its derivatives has been undertaken by Chinese scientists in Yunnan Province, Henan Province, and Hainan Island from 1973 to 1980. Of the 2099 malaria cases treated with qinghaosu, 588 were falciparum malaria and 1511 vivax. Artemether has been used to treat 1088 cases, of which 829 and 259 were falciparum and vivax malaria, respectively. Artesunate (as the sodium salt) was used to treat 181 cases of falciparum malaria. Most of the cases of falciparum malaria were chloroquine-resistant at levels varying from RI to RIII.

All falciparum patients who received treatment with preparations of qinghaosu were clinically cured. However, among those treated with a tablet formulation, cure rates did not exceed 55%. Higher cure rates (between 72–90%) were observed with an oily solution or suspension, or an aqueous suspension administered intramuscularly.

Patients treated with artemether in an oily solution over a 3-day period, with total doses of 0.24–0.64 g, showed cure rates of 94%. These results suggest that 900 mg of qinghaosu in oily solution and 600 mg of artemether in oily solution, given over a 3-day period, are the most effective doses. Similar results were obtained with qinghaosu and artemether when used for the treatment of vivax malaria.

Artesunate is water soluble and can be administered intravenously. However, a total dose of 400 mg in 3 days produced a lower radical cure rate of 47.6% for falciparum malaria.

Comparative studies carried out in Yunnan Province and Hainan Island have shown that qinghaosu and artemether clear asexual parasites from the blood much more rapidly than chloroquine.

4.3.3.2 Clinical studies on the treatment of cerebral malaria with qinghaosu and its derivatives. In an endemic area of chloroquine-resistant P. falciparum malaria, 157 cases of cerebral malaria were treated with qinghaosu or its derivatives, artemether and artemesunate; 145 cases were cured and 12 died, giving an overall cure rate of 92.4%. Among the 157 cases, 106 were treated with qinghaosu, 29 with artemether, and 22 with artemesunate. Initial parasite counts ranged from 128 to $1600 \times 10^9/mm^3$, and the clinical state of cerebral malaria varied from mild to very severe; cerebral oedema was reported in 39 cases.
A suspension of qinghaosu, prepared from tablets, was given through a nasogastric tube in the earlier cases treated, but later this method was replaced by the intramuscular injection of either an oily solution or an aqueous suspension. Most patients received a total dose of 1.5 g of the oily suspension during a 2-day period or 1.2 g of the aqueous suspension over 3 days. Artemether was administered at a total dose of 640 mg over 3 days, and artesunate by slow intravenous or intramuscular injection at a total dose of 400 mg over 3 days. Cure rates of over 90% were obtained in all groups and the results indicate that the schizontocidal action of qinghaosu and its derivatives is significantly more effective and rapid than that of chloroquine or quinine.

The regimens produced few, if any, side-effects (notably less than curative regimens of chloroquine and quinine), but pharmacokinetic and toxicological data are incomplete. The most serious aspect of the toxicity of qinghaosu appears to be its reported embryotoxicity in rats and mice. Therefore for pregnant women, or women who may be pregnant, the use of qinghaosu and its derivatives should be restricted to individual cases for whom potential benefits would outweigh the risks involved. This applies particularly to artesunate, where the documented results indicate that its remarkable rapidity of action and life-saving value are superior to those of parenteral chloroquine and quinine for the treatment of cerebral malaria.

4.3.4 Triazines: WR 99210

The triazine series of compounds, including WR 99210 (Formula No. 9), are of interest since some are apparently long-acting and are effective against both chloroquine- and pyrimethamine-resistant parasites (59–62).

4.3.5 Acridines

These compounds are of interest because they have causal prophylactic as well as blood schizontocidal activity (103). One of these compounds, floxacrine (Formula No. 26), is unlikely to be developed further owing to its toxicity in laboratory animals. Work on this series of compounds continues, but so far those analogues of floxacrine that have been studied have shown similar general toxicity to that observed with the parent compound.
4.3.6 Pyronaridine

Compounds of the pyronaridine series (Formula No. 27) have been shown to be highly active blood schizontocides, effective against chloroquine-resistant strains of parasites in animal models and man. They are being developed in China, but as yet insufficient information is available on the pharmacokinetics, toxicity, and resistance patterns of these drugs. Studies have shown pyronaridine to have embryotoxic effects, but it does not appear to be mutagenic or to affect spermatogenesis (83, 84). More information should be available in the future to allow the significance of these drugs in malaria chemotherapy to be assessed. However, patterns of cross-resistance with other known antimalarials and the ease with which resistance to the pyronaridine series arises should be further studied.

4.3.7 Piperaquine

Piperaquine is a symmetrical compound in which two molecules of 7-chloro-4(1-piperazinyl)-quinidine are linked through a propyl group. It has been shown to possess blood schizontocidal activity similar to that of chloroquine. At first, little interest was shown in this compound and its analogues (12278RP, 12394RP) because field trials failed to show any advantages over chloroquine. Field studies in Hainan Island, China, showed that piperaquine had high suppressive and therapeutic activity (27), but no comparisons were made with chloroquine. Hydroxy-piperaquine (Annex 1, formula No. 28) has been synthesized in China. Levels of acute, subacute, and chronic toxicity in animals are reported to be less than those associated with chloroquine. Animal studies demonstrated that parasite clearance rates were somewhat slower than with chloroquine, but there was no marked difference in the absolute clearance time. Field studies carried out in China indicate that the drug is active against chloroquine-resistant falciparum malaria, but laboratory studies suggest that there is some degree of cross-resistance.

A further derivative, tripiperaquine, has been synthesized in China and its activity studied in mice and monkeys. In mice infected with *P. berghei* the compound was more active than chloroquine and the suppressive blood schizontocidal activity persisted for longer against both *P. berghei* and *P. cynomolgi*. However, in established infections with *P. cynomolgi* and *P. inui*, blood schizontocidal activity was incomplete.
4.3.8 Dabequine

Dabequine \((\text{benzo(g)-4-(diethylaminoethylamino)}\text{)quinoline})\) (Annex 1, No. 29) is a 4-aminquinoline derivative in which the quinoline nucleus has been expanded by the addition of a benzene ring. It was synthesized in the Soviet Union. Its activity on the blood schizonts of \(P. berghei\) is similar to that of chloroquine. However, its plasma half-life in mice is longer than chloroquine and tolerance is better. Animal studies and clinical trials indicate that there is cross-resistance with chloroquine.

4.3.9 Naphthoquinones

Research has been conducted for several years on the development of naphthoquinones as potential antimalarial drugs. These drugs may have tissue as well as blood schizontocidal activity. One of the more promising compounds is being considered for clinical trials.

4.3.10 Aminoquinolines

The 8-aminoquinoline derivative WR 238605 (Formula No. 31) is being developed by the Walter Reed Army Institute for Research and evaluated as an alternative to primaquine for the radical cure of relapsing malaria. It has been known for some time that primaquine has several drawbacks; the curative dose is very close to the toxic dose and in certain populations it causes methaemoglobinemia or haemolytic anaemia. In 1972 a number of drug classes that had been reported to have exoerythrocytic antimalarial activity were evaluated (27). These included 6-, 7-, and 8-aminoquinolines, naphthoquinones, lincomycins, and a pyrocatechol derivative. Many new drugs were synthesized and tested in a sporozoite mouse model and against \(P. cynomolgi\) in rhesus monkeys. Although many of the compounds had some degree of exoerythrocytic activity, no class was found to be better than the 8-aminoquinolines. However, using lead-directed synthesis, 8-aminoquinoline derivatives with higher activity than primaquine were discovered, and attention was concentrated on WR 238605. This drug was found to be 13 times more active than primaquine by measuring the dose required to cure \(P. cynomolgi\) infection in monkeys. The selection of this drug for development occurred only recently and no data are yet available for man.

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4.4 Additional chemotherapeutic research needed as a result of problems in malaria control

Experience gained in malaria eradication programmes has revealed certain technical and scientific difficulties whose solution will require new research.

Chemotherapy is an important element of malaria control, especially in areas where insecticide spraying is not carried out for one reason or another, and in areas where the effectiveness of the insecticide is reduced owing to the resistance of the vector or to the insufficient surface area of sprayable surface. However, technical and scientific problems have limited the efficacy of chemotherapy in malaria control. The following are considered to be priority areas of research:

(1) Resistance of *P. falciparum* to 4-aminoquinolines. This problem has caused many difficulties: not only poor individual response to the treatment, which may result in the development of severe clinical forms of the disease, but also, at the epidemiological level, where the chances of the vectors becoming infected are increased because the duration of the infection is longer.

There is a continuing need for new, inexpensive drugs (or associations of drugs) that are effective against resistant strains of *P. falciparum*, that are safe for field use, and that can be administered orally in a single dose.

(2) Mechanisms of drug action and drug resistance and the prevention of resistance. At present, multiple-drug resistance in *P. falciparum* is the major practical problem. This problem is intensified by the observed tendency of chloroquine-resistant parasites to replace sensitive ones and to spread rapidly in any geographical area in which foci become established. It is therefore important to elucidate the way in which these parasites become resistant to 4-aminoquinolines and other compounds, and to find possible means of retarding or preventing these processes. This will involve research into the mode of action of the drugs, mechanisms and genetics of resistance, ways of intervening in the development of resistance and its geographical spread, and the discovery of new drugs or drug combinations that are effective against resistant parasites. Further studies are needed in the laboratory and in the field on the dynamics and stability of resistance to various drugs.

(3) Assessment and monitoring of *P. falciparum* sensitivity to antimalarial drugs. Much progress has been made in the
standardization of techniques to determine the sensitivity of \textit{P. falciparum} to certain drugs \textit{in vitro} (section 2.4). However, there is an urgent need for the development of test kits for other antimalarial drugs.

(4) \textit{Relapses of \textit{P. vivax} infection.} The available regimens for the radical treatment of \textit{P. vivax} infections are difficult to administer in the field. The 14-day course of primaquine is practically impossible to follow in some areas, while the 5-day regimen is not so effective. Therefore, a radical curative drug (or a combination of drugs) is needed that can be given in a single dose or on a single day. This might be a new drug or a new formulation of primaquine.

(5) \textit{Treatment of severe and complicated cases of malaria.} This is a serious problem requiring expert medical care in hospital. It is compounded by the increasing incidence of drug failures with the use of quinine (either used alone or in combination with tetracycline) in South-East Asia, and by the difficulties in obtaining quinine in some other areas of the world where the parasite is still sensitive to the drug. Quinidine is often more widely available than quinine and further clinical studies are needed to assess the possible clinical advantages of quinidine in the light of its known action on the myocardium.

(6) \textit{Causal prophylaxis of malaria.} Prevention of malaria using drugs for causal prophylaxis has not been sufficiently explored. This is particularly true for multidrug-resistant falciparum malaria where the chemotherapeutic options are severely limited. The usefulness of proguanil and chlorproguanil combined with a sulfonamide or sulfone should be investigated. In addition, studies on the value of tetracycline or doxycycline in preventing malaria in the non-immune traveller should be considered.

(7) \textit{Prevention of transmission.} The action of primaquine on gametocytes is not sufficiently understood. The infectivity of gametocytes that emerge after various regimens of primaquine should be studied and the optimal dosage needed for the sterilization of gametocytes determined.

(8) \textit{Pharmacokinetics.} Further research is needed on the pharmacokinetics of antimalarial drugs—in particular, studies that relate blood levels of a drug to the \textit{in vitro} sensitivity of parasites in order to determine rational dosage regimens.

Studies are also needed on the pharmacokinetics of antimalarials in children, in the various races, and in the presence of a concomitant disease or malnutrition. Any possible interaction between
antimalarial drugs and compounds given for other medicinal purposes also needs further investigation.

(9) Effect of chemosuppression on immunity. The effects of chemoprophylaxis and chemotherapy on immunity and on the mortality and morbidity (including the incidence of cerebral malaria) of target population groups in areas with different degrees of malaria endemicity are not well known. Studies to investigate these processes are required.

4.5 Future chemotherapeutic research

Although it can be expected that mefloquine and a mefloquine combination will be introduced into operational use in the near future, the resources available to malaria chemotherapy in the future are still meagre. Most of the standard antimalarial drugs have been in use for 30 years or more and the increasing problem of drug resistance and the failure to reduce the transmission of malaria in many regions have emphasized the limitations of these drugs and made the search for new and more effective compounds imperative.

The problem of drug resistance in *P. falciparum* has dominated chemotherapeutic research for almost two decades and, although several compounds that show activity against existing strains of multiresistant falciparum malaria are being developed, others whose structure and mode of action are different from those currently used and under development are needed. The empirical approach to drug development has worked relatively well in the past, but it remains risky, cumbersome, and costly. If new drugs are to be developed in the future, some more rational form of development will be necessary. Although it is still impossible to predict which chemical structures will act reliably as antimalarials, it seems reasonable to suggest that future approaches to drug design will have to rely to a greater degree on the fundamental and specific biochemical characteristics of *Plasmodium*.

Phytochemistry may also provide leads for the development of novel compounds. Research in this field has been revived by the reports of antimalarial activity of the Chinese plant extract, qinghaosu, a compound that has a novel structure and mode of action, and is active against strains of *P. falciparum* known to be multiresistant.

It is recognized that the development and production of new drugs from such sources will take many years and therefore it is
important that research on improving the efficacy of existing compounds should continue to be carried out, particularly since the incomplete pre-clinical and clinical development of registered drugs may have resulted in their suboptimal use in the past. For example, it has been recognized in the last few years that insufficient attention has been paid to the chemotherapy of P. vivax infections for which primaquine or other equally toxic and closely related 8-aminquinolines are the only radically curative drugs available.

The optimum suppressive use of antimalarial drugs requires the maintenance of effective concentrations of the drug in the blood of the individual. The same applies to drugs which for curative purposes have to be given over a longer period of time, e.g., primaquine. Although several useful drugs are still available, they are effective only for brief periods and frequent dosing is generally required. This has led to immense problems of logistics, costs, and compliance. In addition, a fundamental limitation common to all conventional dosage forms is that the drug is released to the body tissues or fluids at rates that vary with time, i.e., the release rates are highest initially and decline thereafter. This leads to high/low blood level variation between each drug intake. Such fluctuations are particularly serious when the toxic level is close to that required for suppression. For many years it has been thought that this problem might be overcome by the development of drugs with a repository or long duration of action, but despite many years of research such formulations are not available for use in man. This approach, however, is still valid, particularly since new techniques for drug formulation are now being developed.

In addition, recent advances in cell biology, immunology, and other related areas have suggested that the toxicity problems of drugs may be overcome by the use of carrier systems that will protect the host tissues from the drug they carry (and vice versa) and at the same time “target” the compounds to where they are required or facilitate their release at their site of action.

There have been major technological advances in the last decade and more success may be achieved by the rational development of drugs in the future than has been the case in the past.

4.5.1 Chemotherapeutic approaches based on parasite biochemistry

Nearly 70 years ago Paul Ehrlich first advocated the rational development of drugs by a study of the biochemistry of parasites.
He concluded that a complete and exhaustive knowledge of all the different chemoreceptors was the *sine qua non* for success in chemotherapy. Although knowledge of the biochemistry of malaria parasites is neither complete nor exhaustive, there is suggestive evidence that unique biochemical determinants do exist, and that these might provide a basis for the successful development of chemotherapeutic agents.

Attempts have been made in the past to rationalize drug development. Folate biosynthesis and metabolism have provided a fruitful area for antimalarial drug development and generally this research has been backed by biochemical studies on the appropriate enzyme. However, other results have not been as good for a variety of reasons. One major factor has been the use of inappropriate test systems. For example, the occurrence of a ubiquinone (coenzyme Q₈) in malaria parasites that differs from that found in their mammalian hosts (coenzyme Q₁₀) was thought to be the reason for the activity of the naphthoquinones. Early assays of inhibition of respiration were made using *P. gallinaceum* but most work was carried out on beef heart mitochondria and correlation with final antimalarial activity was poor (120). Similarly, potential inhibitors of hypoxanthine-guanine phosphoribosyltransferase were tested using the enzyme from mammalian tissue culture cells, and no activity against the malaria enzyme, or the parasites, *in vivo* was observed (94, 95).

The techniques developed for the cultivation of the erythrocytic stages of *P. falciparum* and for the fractionation and isolation of parasite material have provided a better test system. Thus, unless large quantities of parasites are required, biochemical and drug inhibition studies for blood schizontocides can now be carried out on the target organism. The *in vitro* culture method has also provided a simple and cheap screening system that has led to the identification of many compounds with antimalarial activity. Unfortunately, such an *in vitro* system does not yet exist for the study of the exoerythrocytic phase of *P. falciparum*, although complete erythrocytic development of *P. berghei* has been obtained in W 138 lung cells (52), and more recently in hepatocytes (66, 96).

### 4.5.1.1 Biochemical targets

(1) *Energy metabolism.* Glucose metabolism might seem an unlikely target for malaria chemotherapy since this metabolic
pathway is similar in all living cells. However, the plasmodial enzymes involved in this pathway can be quite distinct from those of the host. Almost 20 years ago (113) it was shown that there was heterogeneity of lactate dehydrogenase in *P. lophurae* and that the active site of the parasite enzyme was different from that of the host's enzyme. This has now been observed in *P. falciparum* (125). To date, there have been no reports on the kinetic properties of any purified glycolytic enzyme, simply because it has been difficult to grow parasites in large enough quantities for conventional biochemical analyses, and for some unexplained reason the plasmodial enzymes are exceedingly labile. However, detailed characterization should provide evidence for parasite-specific receptors.

(2) Protein synthesis. It is generally agreed that there are three potential sources of amino acids for the erythrocytic stages of plasmodia: (a) carbon dioxide fixation; (b) the free amino acid pools of the plasma and red cell; and (c) red cell haemoglobin (114).

Carbon dioxide fixation can supply only a limited quantity of amino acids for parasite protein synthesis but, despite this, one of the carbon dioxide fixing enzymes, i.e., phosphoenolpyruvate carboxylase (EC 4.1.1.31), identified in *P. berghei*, has never been identified in other eukaryotes (116).

Despite the large number of studies that document the increased uptake of amino acids by malaria infected cells, there has been no detailed description of the mechanisms by which this is accomplished. If it is due to parasite-specific carriers, it may be possible to produce protein imbalance by specifically blocking such transport systems. In this context, it is interesting to note that a new permeability pathway for anion transport in human red cells infected with *P. falciparum* has been partially characterized (65).

There has been no estimate of the relative importance of free amino acids and haemoglobin as sources of amino acids for the parasite although it is presumed that the haemoglobin of the red cell is the major source. Parasite-specific proteases have been identified in various malaria parasites. If it is true that malaria parasites obtain the majority of their amino acids by proteolysis of haemoglobin, then it is possible that the parasite-specific cathepsin D and aminopeptidases that have been identified (20, 48, 115) could be specific targets for chemotherapy.

How the free amino acids derived from blood plasma or haemoglobin enter the parasite is unknown. Until now, it has not been possible to study these uptake mechanisms, mainly because the
parasite is very unstable when it is removed from the host cell. Unfortunately, a medium has not yet been developed that will maintain “free” parasites intact for long enough to carry out investigations of substrate transport.

The mechanism of protein synthesis in malaria parasites appears to be typically eukaryotic but little research has been carried out on this subject and nothing is known of any specific requirements for synthesis.

(3) Nucleic acid synthesis. Although protein synthesis may be typically eukaryotic, RNA from malaria parasites is typically protozoan having a guanine + cytosine content of 35% in contrast to that of 65% in the host (114). In addition, recent studies have now shown that the DNA of *P. falciparum*, like that of *P. berghei*, has a unique base composition of approximately 17–19% guanine + cytosine (42, 100). If the ribosomes and the DNA of the parasite are more than inert structures on which parasite molecules are assembled, then the specific base compositions of parasite RNA and DNA might provide suitable targets for new drugs.

The purines and pyrimidines necessary for the formation of parasite nucleic acids are derived by two distinct routes. Purines cannot be synthesized *de novo* but are obtained preformed, preferably as hypoxanthine, by purine salvage pathways similar to those of the host cell. There may, however, still be possibilities for identifying specific plasmodial chemoreceptors in this pathway since adenosine deaminases from the parasite and host cell appear to have structural differences based on their isoelectric points and reaction to inhibitors.

In contrast to their dependence on preformed purines, malaria parasites synthesize pyrimidines *de novo*. The enzymes required for the synthesis of thymidylate have been identified in several malaria parasites and the inhibition of one of these dihydrofolate reductases has been shown to be the basis of the action of pyrimethamine and other antifolates. There is reason to believe that dihydrofolate reductase (EC 1.5.1.3) and thymidylate synthase (EC 2.1.1.45) form a bifunctional complex in malaria parasites whereas the mammalian enzymes exist separately (36). It is not known if these differences could be exploited in the development of drugs.

All of the enzymes required for the *de novo* synthesis of deoxyuridylate (dUMP) have been identified in extracts of *P. berghei* (51). The possible existence of a complex made up of the first three enzymes of this pathway, i.e., carbamoyl-phosphate synthetase
(EC 6.3.5.5), aspartate carbamoyltransferase (EC 2.1.3.2), and dihydroyorotate (EC 3.5.2.3), suggest parasite characteristics similar to eukaryotes. However, the fourth enzyme, dihydroyorotate oxidase (EC 1.3.3.1) is of great interest. It is sensitive to cyanide, antimycin A, and menometone suggesting that, during the oxidation of dihydroyorotate, electrons are fed into a cytochrome chain at the ubiquinone level. Cytochrome oxidase, which is also inhibited by cyanide, is the only cytochrome that has been identified in plasmodia but there is no evidence for a tricarboxylic acid cycle in mammalian malarials. It could be that the main reason for having an electron transport chain in the blood stages of malaria parasites is its involvement in orotate biosynthesis. If this is the case, the microaerophilic requirements and the presence of cytochrome oxidase may be related, i.e., oxygen is involved in the synthesis of pyrimidines (47). Some of the enzymes of pyrimidine biosynthesis have been identified in *P. falciparum*, and have been shown to be different from those in the host (40, 104). The latter study (104) suggests that in contrast to the mammalian system, *P. falciparum* metabolizes orotate first to the free intermediate orotidine-5'-monophosphate and then to uridylicate monophosphate. This area of parasite metabolism is obviously promising for drug development.

4) Folate metabolism. It is well known that sulfonamides and sulfoxides interfere with the biosynthesis of dihydrofolate and that inhibitors of dihydrofolate reductase, e.g., pyrimethamine, block the formation of tetrahydrofolate. These are the only drugs for which the mechanism of antimalarial action is known. It has been generally accepted that the malaria parasite, like bacteria and in contrast to the host, uses para-aminobenzoic acid (PABA) and not folic acid for the synthesis of folates. Recently, studies with *P. falciparum in vitro* indicate that both folic acid and PABA interfere with the activity of sulfadoxine. These results suggest that another metabolic pathway may exist in the parasite by which, in the absence of plasma folates and PABA, the parasite is capable of utilizing red blood cell folate present in the form of polyglutamated 5-methyl tetrahydrofolate (R.E. Desjardins, personal communication, 1983).

The origin of the pteridine for folate biosynthesis in the malaria parasite is unknown.

5) Parasite invasion of red cells. Major advances have been made in the last few years in determining the mechanism of parasite invasion of the red cell. An understanding of merozoite invasion at the molecular level could lead to new methods of chemotherapeutic
attack. Several studies have produced evidence that strongly suggests that the red cell sialoglycoproteins, glycophorin A and B as well as the Wright antigen (Wr⁺) are the host cell receptors for merozoites (53, 86, 87, 106).

In addition, it has been shown that the interaction between erythrocytes and merozoites of P. falciparum involves highly specific, lectin-like binding of the parasite to these complementary carbohydrate determinants on the red cell surface (57, 58). Four potential receptors on the surface of merozoites with relative molecular masses of 210 000, 140 000, 70 000, and 35 000, have been identified and shown to bind to red cell sialoglycoproteins or N-acetylglucosamine. Three of them, with relative molecular masses of 140 000, 70 000, and 35 000 seem to be specifically bound by N-acetylglucosamine. It is not possible to say at this stage how many sugar-binding proteins are present on the merozoite surface, since three of the proteins appear to be related, perhaps representing a tetramer, dimer, and monomer form of the same protein. However, if any of these proteins can be shown to be unique, they are obvious targets for the design of new antimalarial compounds.

(6) Oxidant killing of malaria parasites. The involvement of nonspecific factors in the killing of P. vinckei and P. chabaudi and the possibility of these factors being macrophage products has been suggested by Clark and his coworkers for many years. These studies on the nonspecific factors involved in parasite death in rodent malaria have now produced leads that may have application in malaria chemotherapy. Recently, this group has produced new data (22, 23), supported by parallel studies in other laboratories (10, 31), implicating toxic oxygen radicals in the killing of malaria parasites. Their experiments show that both P. vinckei, in vivo, and P. falciparum, in vitro, are killed when their host red cells are exposed to generators of free oxygen radicals such as alloxan and t-butyl hydroperoxide. Although this was not accomplished without side-effects, notably haemolysis of the host cell, parasite death was not secondary to haemolysis. These results suggest that infected cells are under oxygen stress and, therefore, are more susceptible to the effects of oxygen radicals. They also suggest that compounds that generate free oxygen radicals may be shown to be suitable antimalarials. This may be of interest in connection with several, mainly plant-derived, candidate compounds that may exert oxidative activity. While this approach merits further study, caution should be exercised since it
is possible that the margin between efficacy and toxicity with such compounds may be narrow.

4.5.2 Exploitation of potential biochemical targets for drug action

The preceding section has outlined some areas of parasite biochemistry and biology that may be potential targets for chemotherapeutic action. This information, provided it is reliable and well documented, can be exploited in several ways for the development of new antimalarial drugs. While traditional approaches should continue to be used, it is possible that sufficient biochemical information on the potential target molecules of *P. falciparum* could be produced to enable the more efficient development of inhibitors or antagonists by the new methods that are becoming available. For example, computer-assisted molecular modelling can give information that is useful in the development of new drugs (45). Although these systems have not been widely used in antimalarial drug design, they have proved useful for other drugs. Computer programmes are available for generating 3-dimensional chemical structures, determining preferred conformations, comparing such structures and, where receptor models are available, fitting them to a receptor site. These capabilities enhance the chemist's ability to understand the relationship between structure and activity of current antimalarial compounds and to predict the properties of hypothetical drugs before they are synthesized.

However, basic structural information must be available before such techniques can be used. This can be obtained from physicochemical and X-ray crystallographic studies of the target molecules, but as yet these latter techniques have not been applied to biochemical targets in the malaria parasite. The absolute configuration of compounds and the position of carbon, oxygen, and hydrogen atoms can be determined with a high degree of accuracy. Although there are major difficulties with compounds with a relative molecular mass of 1000–2000, it is relatively easy to determine the structures of smaller compounds (Rmm less than 1000) or to deal with larger molecules such as proteins and nucleic acids by the use of isotope markers.

If the reaction mechanism of a target enzyme is known, then it becomes possible to develop "suicide" substrates to inactivate that enzyme selectively. Such substrates must be structural analogues of the normal substrate, but contain a latent, hidden functional group
that is unreactive until the target enzyme uncovers it, when it can then react rapidly with the active site (128). True suicide substrates irreversibly inhibit the enzyme; some compounds such as the anticancer agent methotrexate show pseudo-reversible inhibition of enzyme activity and therefore act similarly to suicide substrates.

An example of the feasibility of this approach is illustrated by the effect of α,α-difluoromethylornithine, a suicide substrate for ornithine decarboxylase (EC 4.1.1.17) which has been shown to have antitrypanosomal activity in vivo (13). As malaria parasites synthesize pyrimidines de novo, it has been argued that this biosynthetic pathway and those for folate and thymidylate synthesis, are suitable synthetic pathways for the use of suicide substrates. Preliminary results suggest that D-3-fluoroalanine and 5-ethenylorotate may act as suicide substrates for serine hydroxymethyltransferase (EC 2.1.2.1) and dihydroorotate oxidase (EC 1.3.3.1), respectively. Therefore, in this area, further detailed studies with *P. falciparum* may be profitable.

4.5.3 *Natural products*

Natural products have always been used as a source of medicinal chemicals. In antimalaria chemotherapy quinine is the best known example, but others have also been isolated and studied. Febrifugine, known to the Chinese for over a thousand years, was first isolated in the 1940s and, although many analogues were synthesized, unfortunately none could be developed. More recently, as a result of the efforts of Chinese scientists, interest is now centred on qinghaosu, the active component of the medicinal plant *Artemisia annua* L. (Qinghao). This antimalarial compound has been shown to be a sesquiterpene lactone of novel structure and mode of action (101). Molecular modifications that destroy the peroxide structure result in a loss of activity but derivatives of the carbonyl group have shown increased activity and solubility. One of these derivatives, artesunate, a water-soluble succinyl formulation, seems to be a promising drug for the treatment of cerebral and other complicated forms of falciparum malaria, including infections with multidrug-resistant parasites. Other sesquiterpene compounds, Yinghaosu A and B, have been isolated by Chinese scientists from the plant *Artabotrys uncinatus* and have also shown antimalarial activity (69, 70). These studies confirmed the importance of the peroxide bridge for activity.
The above studies have, moreover, stimulated a re-examination of other plant products. It has been known for some time that extracts from plants of several families including Amaryllidaceae, Saxifragaceae, and Simarubaceae, exhibit antimalarial activity. Unfortunately, the active principle in these plants is often present in such low concentrations that the crude extract may not always show activity in drug screens. Thus, the difficult and time-consuming task of isolating and purifying the active component must be carried out. This has been performed with certain quassinoids from the Simarubaceae. One of these, bruceantin, has been shown to have both antitumour and antiprotozoal, including antimalarial, activity and is presently undergoing clinical trials as an anticancer agent. Antimalarial activity of quassinoids has been demonstrated against chloroquine-resistant \textit{P. falciparum in vitro} (121). A number of quassinoids were tested and the antimalarial activity \textit{in vitro} appeared to parallel the \textit{in vivo} antitumour activity. In this study, it was found that the concentrations for \textit{in vitro} growth inhibition of malaria parasites were considerably lower than those required for the inhibition of tumour growth in culture; this suggested that the therapeutic index \textit{in vivo} might therefore be better for antimalarial activity than for antitumour activity. These results appear promising. Toxicity studies of these compounds indicate that their toxic effects are generally dose-related and reversible.

4.5.4 \textit{Long-acting formulations}

There are two basic approaches to the development of long-acting antimalarial drugs. One is concerned with the search for substances that after oral administration, are fixed by host tissues and are then slowly released. The second involves developing methods for prolonging the effects of known antimalarial drugs. Drugs from different classes of antimalarial compounds persist in the tissues for relatively long periods following oral administration although none has yet been demonstrated to have useful repository antimalarial activity in man. Several groups have studied the chemical modification of drugs and the development of sustained release formulations as possible mechanisms of producing long-acting medicaments.

4.5.4.1 \textit{Chemical modification}. Chemical modification of a drug to extend its release characteristics can be achieved in several ways. For example, the drug can be converted from a bioactive base or acid
into an appropriate insoluble salt and then injected in a suitable carrier. This was the basis of the pioneering studies of P.E. Thompson and his colleagues that led to the development of the pamoate salt of cycloguanil demonstrating the potential value of this type of compound. Unfortunately, these formulations had to be abandoned because of cross-resistance problems and local tissue reactions that made their application under field conditions unacceptable. However, the formulation of pamoate salts to extend the action of antimalarial drugs may still be a valid concept, particularly if they can be incorporated in carriers that are easily standardized and acceptable for use in man.

Although the development of an extended formulation of pyrimethamine alone or in combination with another drug would probably not be acceptable operationally, it has been used as a model drug in several studies to test the feasibility of the production of various extended release formulations. Pyrimethamine also forms an insoluble salt with pamoic acid (33) and this salt as well as others formed from bis-naphthoic acids protected mice from challenge with *P. berghei* for more than 8 weeks. Equally important was the absence of irritation at the site of injection in mice (32). More recently, single intramuscular injections of 50 and 200 mg/kg body weight of pyrimethamine pamoate in peanut oil/benzyl benzoate have protected rhesus monkeys for 2 and 4 months respectively against *P. cynomolgi* infections, again without any local irritation (L. Werbel, personal communication, 1981).

Formulations of pyrimethamine have also been made in pure, synthetic oils prepared from dimeric ethoxytetrahydropyran ester (Et) and its alkyl analogues. These oils are novel and "non-toxic" solvents that appear to be ideally suited to the formulation of insoluble compounds with characteristics shared by many antimalarial drugs. The advantage of their use is that they are reported to be biodegradable and can be obtained in sufficient quantity and quality whereas other oils, e.g., peanut oil used in the preparation of the pyrimethamine pamoate injection referred to above, vary from batch to batch. In addition, the Et-oils may have advantages when emulsified with aqueous solutions and may guarantee a more regular drug release. At present, comparisons between pyrimethamine base and pyrimethamine pamoate in Et-oils and in peanut oil/benzyl benzoate are being carried out in a rodent model. To date, complete protection against challenge with *P. berghei* has been obtained for up to 56 days in mice given 30%
pyrimethamine pamoate in both peanut oil and Et, and all mice
given pyrimethamine pamoate in peanut oil and 3 out of 5 mice given
the drug in Et were protected at 70 days. However, the applicability
of the "non-toxic" Et-oil formulation for potential use in man
requires further toxicological evaluation.

Whether the principle of pamoate salt formation can be extended
to include the action of candidate antimalarials to which resistance
or cross-resistance to existing antimalarials does not exist, remains
to be seen. If practical these salts would require formulation in an
acceptable carrier. The use of biodegradable synthetic oils seems to
be promising but, before their true potential can be assessed, further
research is needed into their degradation in man, their toxicity, and
the drug/carrier interactions.

Acylation of amino and hydroxy groups has also been shown to
be a way of extending the duration of action of drugs. The
assumption made is that the active species of the drug is regenerated
by in vivo deacylation and this has proved to be successful with
aspirin and N,N'-diacetylandaminodiphenylsulfone (DADDS). Such
compounds have different chemical and physical properties and have
to be considered as novel drugs. Several N,N'-acylated derivatives
of pyrimethamine have been synthesized some of which exhibited
antimalarial action (A. Brossi, personal communication, 1982).

Latentiation, a molecular modification method, is the means by
which a biologically active compound is incorporated into an
inactive carrier or "pro-drug" so that its therapeutic action develops
only after biotransformation through enzymatic or non-enzymatic
processes. This method, using polymers as the carrier, can be used
to prolong drug activity, but there is only one report of the
application of this method to antimalarial drugs.

Pro-drugs of dapsone and sulfadimethoxine have been produced
through the covalent bonding of the parent drug to starch polymeric
dialdehyde (Starch – 190) (63). The results obtained suggested that
the antimalarial activity of the compounds was increased because of
a prolonged duration of action and improved absorption of the
formulation. These results require further evaluation.

4.5.4.2 Sustained-release formulations. The duration of action of
drugs can also be extended by incorporating them into biologically
"inert" matrices thus delaying their release. Such systems known as
sustained-, extended-, or controlled-release formulations are a
relatively new development based on a concept that encompasses
many mechanisms. All systems that are currently under development have at least two features in common. All have a drug reservoir that stores the drug in a stable form and in sufficient quantity for the prescribed treatment. In this way, the drug is also protected from degradation by the host. In addition, all formulations contain some system for controlling the release of the drug so that a prescribed level is maintained throughout the operational life of the formulation. This rate controller can be based on physico-chemical principles such as controlled diffusion or erosion of solid dosage forms. The success of these types of formulation depends on the extent to which the various rate controlling mechanisms can be defined and optimized.

Two biodegradable polymer systems have been studied in an attempt to develop sustained-release formulations of antimalarial drugs. These systems involve the release of drugs incorporated in erodible and biodegradable polymers. One is based on the use of polyglycolic acid and polylactic acid polymers and the other uses a polymer based on dihydropyran and glycerol. The former polymer has been employed for almost 20 years for synthetic resorbable sutures and is now proving useful as a potential erodible matrix and membrane material for a variety of sustained-release formulations.

Injectable formulations of the quinazoline, WR 158122, in a copolymer of glycolic and lactic acids protected mice against challenge with *P. berghei* for a period of 14 weeks compared with 12 days when the drug was administered alone (134–136). In addition, it has been shown that implants of the dihydropyran/glycerol polymers containing 20% pyrimethamine and 20% sulfadoxine protected mice against *P. berghei* for 20 and 35 weeks respectively (56). It has also been found (134–136) that each drug must have a specifically developed individual polymer matrix to obtain optimum release characteristics, which in turn will be influenced by relative molecular mass and homogeneity of the matrix.

Although these preliminary results indicate that the development of biodegradable sustained-release formulations of antimalarial drugs may be feasible, the techniques needed are still in their infancy and many questions must be answered before these formulations can be confirmed to be operationally useful. The matrices themselves have to be fully characterized both chemically and physically and their production standardized using methods compatible with their use in man. Although the matrices used in the development of
antimalarial formulations are known to be biodegradable, surprisingly little is known of the various stages of degradation and the resultant end products. The effects of their long-term use in experimental animals and man have also to be determined. In addition, the question of which drug or drugs should be selected for use in such systems is still unresolved and will depend on future operational needs, the spread of multiresistant strains of falciparum malaria, and the availability of new suitable compounds. The drug(s) chosen should show high activity against current drug-resistant parasites and ideally should be rapidly excreted so that it is concentrated only in the formulation and not in the tissues. The drug formulation should also be injectable and give protection for a period of at least 3 months in the case of a blood schizontocide. Which drug is chosen will also depend on the compatibility of the drug with the matrix selected and its pharmacokinetics and metabolism in the host.

Finally, in spite of the obvious advantages of such systems, sustained-release formulations may have a potential limitation in that the sustained, low-level release of a drug might facilitate the emergence of drug-resistant strains of malaria parasites. This is not known, but the systems described above, although not necessarily offering operationally useful formulations, provide a way of verifying this hypothesis.

4.5.5 Targeting of antimalarial drugs

Side-effects and the inability to reach specific targets in the body are major obstacles to the successful use of many drugs. For malaria chemotherapy, this is particularly true of the 8-aminoquinoline, primaquine, which is the only operationally useful tissue schizontocide in current use. Experimental studies indicate that such toxic manifestations may be partially overcome by the use of carrier systems that “target” the drug to the parasite. The principle of such systems is that the drug-carrier complex should preserve its integrity, avoid association with normal cells, penetrate interposing membranes, and selectively recognize, and associate with, the target. This should then facilitate both the release of the drug from its carrier and the disposal of the carrier itself. Research, particularly in the field of cancer, has shown that macromolecular, cellular, and synthetic carrier systems can be developed (44). At present, however,
despite the large amount of data published, the significance of targeting and its practical therapeutic potential are unknown.

Two methods have so far been used in experimental chemotherapy of malaria, i.e., the use of liposomes and glycoproteins as carriers for targeting primaquine. Primaquine incorporated into multi-lamellar liposomes, consisting of phosphatidylcholine, phosphatidyserine, and cholesterol, has been shown to be about 3.5 times less toxic in mice than free primaquine, although activity was not enhanced when compared with the free drug (97, 98). In addition, it was shown that the chemotherapeutic index of primaquine was increased against sporozoite-induced infections of P. berghei when the drug was linked to the hepatotropic glycoprotein asialofetuin, using a peptide spacer of alanine-lysine-alanine-lysine (99, 124). While it is not suggested that either of these preparations would be suitable for use in man, the results show that the toxicity of an antimalarial drug can be reduced by the use of target carriers. A great deal of further work has to be done before such experimental studies can be assessed fully and translated into operational use. There may be other methods of targeting drugs. For example, serum albumin to which galactose is linked by lysine residues appears to be selectively recognized by hepatocytes both in vitro and in vivo. In addition, specific monoclonal antibodies to sporozoites, asexual blood stages, and gametes of malaria parasites have recently been developed and may be considered as possible carriers for drug targeting in the future.

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5. RECOMMENDATIONS

5.1 Drug resistance

(1) The Group, recognizing the need for information on drug response for the appropriate deployment of antimalarial drugs and appreciating the efforts of the member governments in obtaining information concerning the drug sensitivity of malaria parasites using the WHO standard test systems, RECOMMENDS:

(a) that Member States continue to participate fully in, and give support to, the WHO Global Monitoring Programme for determining *in vitro* and *in vivo* susceptibility of human malaria parasites to antimalarial drugs used in the treatment and prevention of malaria;

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(b) that local monitoring systems continue to be developed to facilitate the dissemination of information through the WHO Global Monitoring Programme.

(2) The Group, appreciating that the current WHO standard in vivo and in vitro test systems have been widely accepted globally, realizing that efforts should be made to use the data provided by the programme to simplify the test procedures and acknowledging the time it takes to introduce new test systems or radically change existing systems, RECOMMENDS:

(a) that efforts are continued to simplify and reduce the costs of the existing in vitro/in vivo test procedures to ensure the widest possible use globally;

(b) that the existing test systems be modified and expanded to permit the testing at field level of all current first-, second-, third-line, and new antimalarial drugs as they become available for widespread use;

(c) that studies be made to ascertain whether the existing, modified, or new in vivo test systems can provide information from short duration tests (ideally 7 days or less) on drug response with drugs that normally require extended follow-up to detect recrudescences.

(3) The Group, recognizing the importance of chemotherapeutic intervention measures through the health service delivery systems of the member countries, RECOMMENDS that intensive information activities should be encouraged to make health services aware of the presence and risk of drug-resistant malaria, the need for timely diagnosis and treatment, the importance of following up all treated cases, and the prompt reporting of relevant information to all the authorities.

(4) The Group, recognizing the danger of introducing drug-resistant *P. falciparum* malaria and reintroducing *P. falciparum* into receptive areas, RECOMMENDS that countries appreciate the potential risks involved and develop appropriate measures to ensure the early recognition of such an occurrence and the timely application of remedial measures.

### 5.2 Drug use

(1) The Group, recognizing that the mass administration of antimalarial drugs to large segments of a population is not a cost-effective malaria control method because it monopolizes
manpower and financial resources of the health services, that high levels of compliance in the population are difficult to sustain, and that such programmes may favour the development of resistance to essential drugs, RECOMMENDS that countries eliminate programmes of mass drug administration, chloroquinized salt, and other programmes of drug distribution that are not time limited and targeted at defined high-risk groups or the control of epidemics, and that they continue to develop a health care infrastructure for the provision of effective treatment of malaria infections.

(2) The Group, recognizing the increased danger of malaria in non-immune persons moving to malarious areas, as well as in some special groups exposed to malaria, RECOMMENDS that adequate information on the risk of acquiring malaria in different countries and on the appropriate preventive measures should be provided.

(3) The Group, recognizing the danger to the mother and fetus of acute falciparum malaria, and on the basis of existing evidence of the safety of the drug, RECOMMENDS that, for pregnant women living in areas with highly chloroquine-resistant *P. falciparum*, the use of sulfadoxine/pyrimethamine at the appropriate dose should be considered for the prophylaxis and treatment of falciparum malaria in these women if, and as soon as, restrictions are lifted by the drug regulatory authorities.

(4) The Group, recognizing the urgent need to protect mefloquine and ensure its optimal deployment, strongly RECOMMENDS:

(a) that governments should legislate for strict control of the importation, distribution, and utilization of mefloquine alone or in drug combinations;

(b) that the use of mefloquine by communities in endemic areas should be restricted to the treatment of acute malaria attacks that are likely to be due to multiple drug-resistant *P. falciparum* in specific groups;

(c) that, when available, drug combinations known to delay the development of drug resistance (as might be the case with the mefloquine/sulfadoxine/pyrimethamine combination currently under development) should be used for prophylaxis or treatment, when necessary, instead of mefloquine;

(d) that mefloquine should *not* be distributed for use as a single prophylactic drug by residents in endemic areas.

(5) The Group, considering that the decision to change a blood schizontocide in general use as a first-line drug should not be based only upon the presence of parasite resistance to that drug but also
upon the degree and frequency of resistance, its geographical
distribution, the malaria endemicity, and immune levels, that
together determine the severity of infection and response to
treatment, and taking into consideration the accessibility of a
referral system and its ability to cope with non-responding and
severe cases, RECOMMENDS that the approach to alternative
drugs be based upon the concept of making available appropriate,
effective first-, second-, and third-line antimalarial drugs at different
levels of a health infrastructure to ensure the best possible clinical
treatment for the population and at the same time preserve the
effectiveness of the available drugs for as long as possible.

(6) The Group, considering that global acceptance of primary
health care as the strategy for health development offers the best
opportunity to ensure the establishment of malaria diagnostic
capabilities at the periphery and the continued provision of
appropriate treatment of malaria cases, RECOMMENDS that in
areas of high malaria endemicity where primary health care coverage
is not yet fully developed and where the disease is a dominant health
problem, treatment posts and malaria clinics should be established
and the community mobilized for antimalaria action in such a way
as to provide a basis for the development of primary health care.

(7) The Group, recognizing that malaria services are under an
obligation to provide the best possible treatment for patients, that
resistance to widely used drugs may be exacerbated when large
numbers of a population receive insufficient treatment, and that
limited resources, particularly of drugs, must be used as efficiently
as possible, RECOMMENDS:

(a) that every attempt be made by national health services to
provide appropriate diagnostic services at the most peripheral level
possible and to treat malaria with fully effective drug doses,
following up patients for evidence of successful therapy;

(b) that referral procedures be well established in order that
patients requiring more complex or sophisticated treatment may be
appropriately cared for;

(c) that chemoprophylaxis be given to pregnant women living in
malarious areas;

(d) that infants and young children be protected by early
diagnosis and appropriate treatment through the primary health
care system.

(8) The Group, recognizing the frequent commercial and medical
misuse of antimalarial drugs and a need to ensure appropriate
treatment of clinical malaria and to prolong the effective life of drugs, RECOMMENDS that national governments develop well-defined policies regarding the importation, local production, distribution, use, pricing, and control of essential antimalarial drugs, and the recommendations for treatment in different clinical situations that are appropriate for achieving effective treatment in accordance with the advice of WHO.

5.3 Drug research

(1) The Group, recognizing the need for diagnosis as a basis of effective treatment, and realizing that the current routine diagnostic methods for malaria will remain impracticable for some time in large parts of the malarious areas of the world, RECOMMENDS that efforts be made to develop simple and rapid diagnostic tools that can be used at the peripheral level of primary health care.

(2) The Group, recognizing that experience both in the laboratory and the field has highlighted the ease with which some species of malaria parasite, notably *P. falciparum* of man and various rodent malarials, develop mechanisms to overcome the action of antimalarial drugs and that this remarkable adaptability of the parasites may soon diminish or negate the value of each new drug, RECOMMENDS that basic laboratory research into this problem should be intensified and should include the following areas:

(a) mode of action of antimalarial drugs;

(b) mechanisms of drug resistance;

(c) ways of limiting the development and spread of drug resistance;

(d) development of new drugs active against resistant parasites.

(3) The Group, recognizing the urgent need to provide drugs for the treatment of severe and complicated falciparum malaria, RECOMMENDS that further clinical studies of quinidine in comparison with quinine be carried out and that the development of qinghaosu and its derivatives, particularly artesunate, be accelerated to assess as quickly as possible the relevance of these compounds to the treatment of severe malaria.

(4) The Group, recognizing the need to characterize further the pharmacokinetics of currently available drugs and those at present under development, RECOMMENDS that studies in this field should be encouraged to:
(a) determine appropriate dosage schedules, particularly in children and in individuals suffering from cerebral and other severe forms of malaria or from complications caused by other diseases and clinical disorders prevalent in areas where malaria is endemic;

(b) investigate drug interactions that may occur with combinations of such substances in use, or under development;

(c) develop sensitive and specific chemical and/or bioassay tests to measure the level of drugs in body fluids so that the results of in vivo and in vitro tests for the sensitivity of the parasite to drugs can be correlated with the drug concentrations obtained in vivo, especially in patients who do not respond to treatment.

(5) The Group, recognizing the operational difficulties experienced with the application of currently used regimens of primaquine for the treatment of P. vivax, RECOMMENDS that studies should be carried out to develop a drug (or combination of drugs) that is fully effective and can be given as a single dose or on a single day. This might be a new drug or a new formulation of primaquine.

(6) The Group, recognizing the lack of knowledge of the value of gametocytocides and sporontocides in limiting the geographical spread of drug-resistant parasites, RECOMMENDS that field and laboratory studies should begin without delay to determine the optimum dosage and timing of administration of primaquine for its gametocytocidal action, and to assess the value of this drug in reducing transmission.

(7) The Group, recognizing the limited number of antimalarial drugs currently under development in laboratory and clinical studies, and the continuing need for additional drugs, RECOMMENDS that research be intensified to identify new drugs to be used for:

(a) the radical cure of multiple drug-resistant P. falciparum;

(b) the radical cure of P. vivax;

(c) the causal prophylaxis of all strains of these and of other species of human malaria parasites.

Support for this work should be made available from international, national, and industrial sources, with WHO continuing to offer supportive laboratory facilities through its existing International Reference Centres.

The Group also RECOMMENDS that basic laboratory research aimed at identifying rational approaches to antimalarial drug
design, and improved drug screening models (especially for new hypnozoitocides) should be intensified and that the necessary support be made available.

ACKNOWLEDGEMENTS

The Scientific Group wishes to acknowledge the special contributions made by the following WHO staff members: Dr P. Beales, Chief, Programming and Training, Malaria Action Programme; Dr E.B. Doberstyn, WHO Senior Malarialogist, The Malaria Division, Ministry of Public Health, Devavesm Palace, Bangkok, Thailand; Dr F.J. Lopez-Antuñano, Coordinator, Tropical Diseases Program, WHO Regional Office for the Americas/Pan American Health Organization, Washington, DC, USA; Dr O. Losev, Programming and Training, Malaria Action Programme, Geneva, Switzerland; Dr L. Molinaux, Epidemiological Methodology and Evaluation, Malaria Action Programme, Geneva, Switzerland; Dr F. Onori, Chief, Epidemiological Methodology and Evaluation, Malaria Action Programme, Geneva, Switzerland; Mr D. Payne, Research and Technical Intelligence, Malaria Action Programme (UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases) Geneva, Switzerland; Dr U.K. Sheth, Research and Technical Intelligence, Malaria Action Programme (Consultant to the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases), Geneva, Switzerland; Dr P.I. Trigg, Research and Technical Intelligence, Malaria Action Programme (UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases), Geneva, Switzerland.
Annex 1

STRUCTURAL FORMULAE OF ANTIMALARIAL DRUGS

4-Aminoquinolines

1. Chloroquine<sup>*</sup>
   7-chloro-4-(4'-diethyl-amino-1'-methylbutyl-amino)quinoline

2. Amodiaquine<sup>*</sup>
   7-chloro-4-(3'-diethylamino-methyl-4'-hydroxyanilino)-quinoline

8-Aminoquinolines

3. Primaquine<sup>*</sup>
   6-methoxy-8-(4'-amino-1'-methylbutylamino)quinoline

Dihydrofolate reductase inhibitors

4. Proguanil<sup>*</sup>
   N<sup>1</sup>(p-chlorophenyl)-N<sup>5</sup>-isopropylguanidine

5. Chlorproguanil<sup>*</sup>
   N<sup>1</sup>(3,4-dichlorophenyl)-N<sup>5</sup>-isopropylguanidine

6. Cycloguanil embonate<sup>*</sup>
   4,6-diamino-1-(p-chlorophenyl)-1,2-dihydro-2,2-dimethyl-s-triazine
   with 4,4'-methylene-bis(3-hydroxy-2-naphthoic acid (2:1)

<sup>*</sup> International Nonproprietary Name (INN).
(7) **Pyrimethamine**
2,4-diamino-5-\(p\)-chlorophenyl-6-ethylpyrimidine

(8) **Trimethoprim**
2,4-diamino-5-(3',4',5'-trimethoxybenzyl)pyrimidine

(9) **WR 99,210**
4,6-diamino-1-\{(3,4,6-trichlorophenoxy)propoxy\}-1,2-dihydro-2,2-dimethyl-1,3,5-triazine

**Sulfonamides**

(10) **Sulfadoxine**
\(N^{'-(5,6-dimethoxy-4-pyrimidinyl)}\)-sulfanilamide

(11) **Sulfalene**
\(N^{'-(3-methoxy-2-pyrazinyl)}\)-sulfanilamide

**Sulfones**

(12) **Dapsone**
4,4'-diaminodiphenylsulfone

**Antibiotics**

(13) **Tetracycline**
4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide

*International Nonproprietary Name (INN).*
(14) **Doxycycline**
- 4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydroxy-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide

(15) **Minocycline**
- 4,7-bis(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydroxy-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide

(16) **Clindamycin**
- methyl 7-chloro-6,7,8-trideoxy-6-trans-(1-methyl-4-propyl-2-pyrrrolidinecarboxamido)-1-thio-1-threo-o-d-galacto-octopyranoside or 7-(5S-chloro-7-desoxylincomycin)

(17) **Erythromycin**

* International Nonproprietary Name (INN).
Quinolinemethanols

(18) Quinine
6-methoxy-α-(5-vinyl-2-quinuclidinyl)-4-quinolinemethanol

(19) Meltoquine* (WR 142,490)
α-(2-piperidyl)-2,8-bis(trifluoromethyl)-4-quinolinemethanol

Phenanthrenemethanol

(20) Halofantrine*
1,3-dichloro-α-[2-(dibutylamino)-ethyl]-6-(trifluoromethyl)-9-phenanthrenemethanol

Pyridinemethanol

(21) WR 180,409
2-trifluoromethyl-4-[α-hydroxy-α-(2-piperidyl)-methyl]-6-(4-trifluoromethylphenyl)-pyridine

* International Nonproprietary Name (INN).
Sesquiterpene lactones

(22) **Qinghaosu**  
(artemisinine)

(23) **Artemether**  
(methyl ether derivative of artemisinine)

(24) **Artesunate**  
sodium salt  
(sodium succinyl salt of artemisinine)

Acridines

(25) **Mepacrine**  
2-methoxy-6-chloro-9-(4'-diethylamino-1'-methylbutylamino)-acridine

* International Nonproprietary Name (INN).
(26) **Flexacrine**  
7-chloro-3,4-dihydro-10-hydroxy-3-(α,α,α-trifluoro-p-tolyl)-1,9(9H)-acridandione

**Miscellaneous compounds**

(27) **Pyronaridine** (7351)  
2-methoxy-6-chloro-9[3,5-bis(1-pyrrolidinylmethyl)-4-hydroxy]anilino-1-aza-acridine

(28) **Hydroxypperaquine**  
1,3-bis[1-(7-chloro-4-quinyl)-4-piperazinyl]-2-hydroxypropane

(29) **Dabequine**  
benzo(g)4-(diethylaminoethylamino)quinoline

168
(30) *Menotone* (WR 49,808)
2-(8-cyclohexyloctyl)-3-hydroxy-1,4-naphthoquinone

(31) WR 238,605
2,6-dimethoxy-4-methyl-5-(3-trifluoromethyl)-phenoxy-8-(4-amino-1-methylbutyl-amino)quinoline

* International Nonproprietary Name (INN).
Annex 2

INTERNATIONAL NONPROPRIETARY NAMES
OF SYNTHETIC ANTIMALARIALS AND SOME
SYNONYMS, PROPRIETARY NAMES,
AND BASE EQUIVALENTS OF ANTIMALARIAL DRUGS

(1) Quinine
Quinimax 3394 R.P.
Quinoforme (formiate) SN 359
         WR 2,976

4-Aminoquinolines

(2) Chloroquine* (diphosphate)  Chloroquine* (sulfate)
Aralen  Resochin  Nivaquine
Avloclor  Resoquine  Nivaquine B
Bemaphate  Sanoquin
Chinamine  Tanakan
Delagil  Tresochin
Gontochin  Trochin
Imagon  3377 R.P., diphosphate
Iroquine  SN 7618
Klorokin  Win 244
Luprochinn  WR 1,544

(3) Amodiaquine* (dihydrochloride)  Amodiaquine* (base)
Cam-aqi  Fluroquine  Basoquin
Camquin  Miaquine  CAM-1201
Camoquinal  SN 10,751
Flavoquine  WR 2,977

(4) Amopyroquine* (dihydrochloride)
Propoquin  CI-356
         PAM-780
         WR 4,835

(5) Cycloquine
Ciklochin
Halochin

170
8-Aminoquinolines

(6) *Primaquine* (diphosphate)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Manufacturer Code 1</th>
<th>Manufacturer Code 2</th>
<th>Manufacturer Code 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neo-Quipenyl</td>
<td>4516 R.P., diphosphate</td>
<td>SN 13,272</td>
<td>WR 2,975</td>
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</table>

(7) *Quinocid* (dihydrochloride)

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<th>Drug</th>
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<tbody>
<tr>
<td>Chinocid</td>
<td>CN 1,115</td>
<td>Win 10,448</td>
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</table>

Dihydrofolate reductase inhibitors

(8) *Proguanil* (hydrochloride)

<table>
<thead>
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<th>Drug</th>
<th>Manufacturer Code 1</th>
<th>Manufacturer Code 2</th>
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</thead>
<tbody>
<tr>
<td>Balusil</td>
<td>Lepadina</td>
<td>M 4888</td>
</tr>
<tr>
<td>Bigumal</td>
<td>Paludrine</td>
<td>3359 R.P., hydrochloride</td>
</tr>
<tr>
<td>Chlorguanide</td>
<td>Palusil</td>
<td>SN 12,837</td>
</tr>
<tr>
<td>Cloriquane</td>
<td>Plasin</td>
<td>WR 3,091</td>
</tr>
<tr>
<td>Diguanyl</td>
<td>Proguanide</td>
<td></td>
</tr>
<tr>
<td>Drinupal</td>
<td>Tirian</td>
<td></td>
</tr>
<tr>
<td>Guanatol</td>
<td></td>
<td></td>
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</tbody>
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(9) *Chlorproguanil* (hydrochloride)

<table>
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</thead>
<tbody>
<tr>
<td>Lapudrine</td>
<td>M 5943</td>
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(10) *Cycloguanil embonate*

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<tr>
<td>Camolar</td>
<td>CI-501</td>
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<tr>
<td>Cycloguanil pamoate</td>
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</table>

(11) *Pyrimetamine* (base)

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<th>Manufacturer Code 1</th>
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</thead>
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<tr>
<td>Chloridin</td>
<td>B-W 50-63</td>
<td>D. R. 16,056</td>
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<tr>
<td>Darapram</td>
<td>NSC-3061</td>
<td>Erbaprinia</td>
</tr>
<tr>
<td>Daraprim</td>
<td>4753 R.P.</td>
<td>Malocide</td>
</tr>
<tr>
<td>Erbaprinia</td>
<td></td>
<td>WR 2,978</td>
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<tr>
<td>Tindurin</td>
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</tr>
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</table>

(12) *Trimethoprim*

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<th>Manufacturer Code 1</th>
<th>Manufacturer Code 2</th>
</tr>
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<tbody>
<tr>
<td>Syraprim</td>
<td>B-W 56-72</td>
<td>Ro 5-6846</td>
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<tr>
<td></td>
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<td>20,932 R.P.</td>
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<tr>
<td></td>
<td></td>
<td>WR 5,949</td>
</tr>
</tbody>
</table>

* International Nonproprietary Name (INN).
### Sulfones

(13) *Dapsone*  
- Avlosulfone  
- Croysulfone  
- Damitome  
- Daphone  
- Diaphenylsulfone  
- Diatox  
- Diphenason  
- Diphone  
- Disulone  
- Eporal  
- Novophone  
- Sulfadione  
- Udolac  
- DDS  
- PAM-1111  
- 1358 R  
- 2466 R.P.  
- WR 0448

(14) *Acetadapsone*  
- Camilan  
- Hansolar  
- Rodilone  
- Sulfadiamine  
- CI-556  
- DADDS  
- 1555 F  
- PAM-1165  
- SN 759

### Sulfonamides

(15) *Sulfadiazine*  
- Adiazine  
- Codiazine  
- Cremodialazine  
- Debenal  
- Diazine  
- Diazyl  
- Eskadiazine  
- Eustral  
- Keladiazine  
- Primal  
- Pyrimal  
- Sterazine  
- Sulfazine  
- 2616 R.P.  
- SN 112  
- WR 7,557

(16) *Sulfafurazole*  
- Gantrisin  
- Gantrosan  
- Neazolin  
- Sulfazin  
- Sulfoxazole  
- Soxisol  
- 6437 R.P.

(17) *Sulfadimethoxine*  
- Levisul  
- Madribon  
- Madriquid  
- Sulfadimethoxypyrmidine  
- 10,659 R.P.

* *International Nonproprietary Name (INN).*
(18) Sulfamethoxypyridazine*  
<table>
<thead>
<tr>
<th>Drug</th>
<th>Common Name</th>
<th>Code</th>
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<tr>
<td>Davosin</td>
<td>Myasul</td>
<td>CL 13,494</td>
</tr>
<tr>
<td>Deposulfal</td>
<td>Spofadiazine</td>
<td>7522 R.P.</td>
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<tr>
<td>Depovernil</td>
<td>Sulfadurazin</td>
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<tr>
<td>Kynex</td>
<td>Sulfalex</td>
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</tr>
<tr>
<td>Lederkyn</td>
<td>Sulitreine</td>
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<tr>
<td>Midicel</td>
<td>Unosulf</td>
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<tr>
<td>Midikel</td>
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(19) Sulfadoxine*  
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<tr>
<td>Fansil</td>
<td>Sulformethoxine</td>
<td>Ro 4-4393</td>
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<tr>
<td>Fansulf</td>
<td>Sulforthidemethoxine</td>
<td>13,114 R.P.</td>
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<tr>
<td>Fanzil</td>
<td>Sulforthomidine</td>
<td>WR 4,873</td>
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(20) Sulfalene*  
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<th>Code</th>
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<tr>
<td>Kelfzina</td>
<td>11,070 R.P.</td>
</tr>
<tr>
<td>Kelfzine</td>
<td>WR 4,629</td>
</tr>
<tr>
<td>Sulfamethoxypyrazine</td>
<td></td>
</tr>
<tr>
<td>Sulfametopyrazine</td>
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</tr>
</tbody>
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(21) Tetracycline*  
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<th>Drug</th>
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<th>Code</th>
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</thead>
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<tr>
<td>Achromycin</td>
<td>Polycycline</td>
<td>5598 R.P.</td>
</tr>
<tr>
<td>Agromicina</td>
<td>Puracyclina</td>
<td>WR 6,527</td>
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<tr>
<td>Ambromicina</td>
<td>Sanelomycin</td>
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</tr>
<tr>
<td>Cyclomycin</td>
<td>Tetrabon</td>
<td></td>
</tr>
<tr>
<td>Hostacyclin</td>
<td>Tetracyclin</td>
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<tr>
<td>Omegamycin</td>
<td>Tetradecin</td>
<td></td>
</tr>
<tr>
<td>Pannycin</td>
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</tr>
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</table>

(22) Doxycycline*  
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<tr>
<th>Drug</th>
<th>Common Name</th>
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<tbody>
<tr>
<td>Bassado</td>
<td>Doxityrex</td>
</tr>
<tr>
<td>Biocelina</td>
<td>Novelciclinia</td>
</tr>
<tr>
<td>Cirenyl</td>
<td>Rodomiclinia</td>
</tr>
<tr>
<td>Dosil</td>
<td>Parvidoxil</td>
</tr>
<tr>
<td>Doxacin</td>
<td>Sincromycin</td>
</tr>
<tr>
<td>Doxilina</td>
<td>Vibradina</td>
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<tr>
<td>Doxipan</td>
<td>Vibramycin</td>
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</tbody>
</table>

(23) Minocycline*  
<table>
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<tr>
<th>Drug</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minocin</td>
<td>WR 87,781</td>
</tr>
</tbody>
</table>

* International Nonproprietary Name (INN).
Base equivalents of antimalarial drugs

Chloroquine

(a) 167 mg of diphosphate corresponds to 100 mg of base.
(b) 136 mg of sulfate corresponds to 100 mg of base.

Amodiaquine

130 mg of dihydrochloride dihydrate corresponds to 100 mg of base.

Mefloquine

110 mg of hydrochloride corresponds to 100 mg of base.

Primaquine

17.6 mg of diphosphate corresponds to 10 mg of base.

* International Nonproprietary Name (INN).
Annex 3

LIST OF ESSENTIAL ANTIMALARIAL DRUGS

<table>
<thead>
<tr>
<th>Main list</th>
<th>Complementary list</th>
<th>Route of administration, dosage forms, and strengths</th>
</tr>
</thead>
<tbody>
<tr>
<td>chloroquine</td>
<td>tablet, 150 mg (as phosphate or sulfate)</td>
<td>syrup, 50 mg (as phosphate or sulfate)/5 ml</td>
</tr>
<tr>
<td>primaquine</td>
<td>tablet, 7.5 mg, 15 mg (as phosphate)</td>
<td>tablet, 300 mg (as bisulfate or sulfate)</td>
</tr>
<tr>
<td>quinine</td>
<td>injection, 300 mg (as dihydrochloride)/ml in 2-ml ampoule</td>
<td></td>
</tr>
<tr>
<td>amodiaquine</td>
<td>suspension, 150 mg (as hydrochloride)/5 ml</td>
<td></td>
</tr>
<tr>
<td>sulfadoxine + pyrimethamine</td>
<td>tablet, 500 mg + 25 mg</td>
<td></td>
</tr>
<tr>
<td>tetracycline</td>
<td>capsule or tablet, 250 mg (as hydrochloride)</td>
<td></td>
</tr>
</tbody>
</table>

*This list has been taken from the WHO Technical Report Series, No. 685, 1983 (The use of essential drugs: report of a WHO Expert Committee). It has been modified by the addition of tetracycline, which is included in the list of essential antinflective drugs.

*This is an example of a therapeutic group; various drugs could be used as alternatives.

*For use when drugs in the main list are known to be ineffective or inappropriate for a given individual.

*Tetracycline together with quinine is used for the treatment of infections with multi-resistant P. falciparum.
Annex 4

PRACTICAL ASPECTS OF THE IN VIVO TESTING FOR SENSITIVITY OF HUMAN PLASMODIUM SPP. TO ANTIMALARIALS

The principles and methodology of in vivo testing of human Plasmodium spp. to determine their sensitivity to antimalarials is well documented. This Annex is an attempt to provide a practical guide to the organization and conduct of such studies in the field.

1. Selection of a study area

The area to be studied is often preselected since evidence of incipient resistance may have been indicated by health workers who report treatment failures with drugs at normal therapeutic levels. In such a case, it is merely necessary to plan a study that will give a representative picture of the status of drug sensitivity in the area. The other possibility is to create a monitoring service to define the current status of sensitivity to particular drugs, or drug combinations, with a view to establishing baseline data. These data can then be compared with those of other areas, or followed up longitudinally to detect changes that may occur with time.

Unfortunately, it is frequently the areas where studies are most needed or desired that are the furthest from well developed laboratory facilities and, moreover, often have unstable populations such as migrants, project workers, and other unsettled people. Therefore, when selecting an area, the following questions must be considered:

(1) Is the area accessible by normal transportation methods? If not, can special arrangements be made to transport staff and material to the area and provide adequate logistic support for the duration of the study?

(2) Is the population sufficiently numerous and the level of malaria prevalence/incidence sufficiently high to provide a significant sample?

(3) Are the local administrative agencies able and willing to give advice on how the people of the area can be approached and motivated to participate in the study, and are these agencies willing to provide the necessary support to make the study a success?

(4) Are the aims of the study strictly relevant to the situation prevailing in the area, or should baseline malarialmetric or demographic surveys be carried out first to define the situation more clearly?

(5) Are there any activities currently in progress or planned (e.g., mass drug administration for the prevention or control of malaria or other diseases) that could compromise the results of the study?

(6) Is the sale or distribution of antimalarial drugs in the area sufficiently controlled, or controllable, so that a valid study can be performed?

(7) Are the dynamics of transmission of malaria in the area well understood and defined or, if not, are there facilities for the isolation of patients to eliminate the possibility of reinfection during longer-term studies (i.e., more than 7 days)?

(8) Can arrangements be made to provide adequate facilities for the various kinds of laboratory work that must be conducted on the spot or at a nearby location?

2. Selection of cases

2.1 Source of cases

Basically, there are two types of area in which in vivo studies are possible:

(1) Areas of endemic malaria where cases will be found at most times of the year but often with considerable differences in frequency between the transmission and non-transmission season(s). The parasite density of the infections will be generally low, with higher parasite densities being found predominantly in the younger age-groups.

(2) Areas of epidemic malaria where there will be considerable annual variation in prevalence and, whilst cases will occur more evenly throughout the population, many infections will be of such a high parasite density that they are life-threatening and require immediate curative treatment. The treatment needed may not match the drug regimens under study, or it may require supportive treatment not included in the protocol of the study.
Accordingly, in areas of endemic malaria the most abundant source of cases for study is usually children attending school or pre-school clinics, while in epidemic areas the source of cases is usually patients attending outpatient clinics. (Outpatient studies in areas of endemic malaria are less productive since patients frequently attend only after self-medication has failed, and many otherwise suitable cases have to be rejected on these grounds.)

2.2 Required characteristics

(1) Single species infections. Mixed infections are not normally satisfactory for testing and great care must be taken during selection so that they are eliminated from the study. The most common mixed infections are *P. falciparum*/*P. malariae* in tropical Africa and *P. falciparum*/*P. vivax* in South America and southern Asia. Other combinations, however, are by no means rare in areas where two or more species occur together.

(2) Parasite density. A minimum threshold of 1000 asexual parasites per mm$^3$ blood (the sexual forms are also studied, though separately, in *P. falciparum* infections) is required for a meaningful *in vivo* test and, theoretically, any parasite density above this is acceptable. Some patients with parasite densities higher than 10 000 asexual parasites may be excluded on ethical grounds, especially if they are non-immunes, since clinical considerations are always paramount. The advice of a physician is, therefore, essential and consequently every team should have a physician available whenever patients are being screened, monitored, and treated.

(3) Medical history. The patient must not have received any 4-aminoquinolines, quinine, or tetracycline during the previous 14 days, while drug-free intervals of 4 weeks and 6 weeks are required in the case of sulfadoxine/pyrimethamine and mefloquine, respectively.

(4) Urine test. The patient should have urine tests for 4-aminoquinolines and sulfonamides. A positive result for either or both, excludes the case.

3. Screening procedures

The method employed to screen potential candidates for the test will differ according to the situation. Some general guidelines are given below.
3.1 Schools and pre-school clinics

In endemic areas, a sufficient number of infections with high parasite densities may be obtained through the systematic blood-slide screening of large numbers of schoolchildren or children attending pre-school clinics. A preselection of children with fever (38 °C or more) will usually enhance the detection rate and reduce the workload, but this advantage is usually offset by the fact that children with fever are far more likely to have taken drugs recently. A typical yield from non-selected children in an area with a crude parasite rate of 40% in the 5–9 years age-group would be for 300 children examined:

- 115 positive for *P. falciparum*;
- 90 single infections of *P. falciparum*;
- 45 with an asexual parasite density of 1000 parasites or more per mm$^3$ blood;
- 35 with no history of treatment with antimalarials or antibiotics;
- 30 with negative urine tests for 4-aminoquinolines and sulfonamides.

3.2 Outpatient clinics

The medical workers at the clinic are requested to refer, before treatment, all patients with fever (38 °C or more) or signs and symptoms of malaria. These patients are re-questioned about recent prophylaxis and treatment and their urine is tested for 4-aminoquinolines and sulfonamides. If all these inquiries are negative, a blood slide is prepared and the normal selection procedure is followed.

3.3 Organization of screening procedures

A preparatory visit is paid to the appropriate local authorities to explain to them the plan of work, and inform them of the objectives and duration of the study. Whenever possible, letters of introduction/authority are obtained and local officials are nominated as liaison workers.

In collaboration with these officials a pre-selection of possible sources of candidate cases is reviewed, and from this a timetable is prepared indicating the hours of school or clinic attendance of the selected collection points. Based on this timetable a programme of
work is established to adjust the volume of the following activities to the team’s working capacity:

— identification of potential candidates (school rosters, clinic attendance registers, etc.);
— entry of names of potential candidates on a survey form (identification of fever cases, if this procedure is followed);
— preparation of blood film;
— staining and examination of blood film;
— recording of result;
— selection of suitable potential candidates;
— questioning about history of taking antimalarials and antibiotics;
— urine testing;
— weighing of patient (dosage is always determined by body weight);
— calculation of appropriate dosage (mg of base per kg body weight);
— preparation of another blood slide (day 0 pre-treatment slide);
— administration of test drug;
— instructions given on further treatment and follow-up.

4. Preparation of blood slides

Although opinions vary on the best format for blood slides for the study of malaria parasites, there is little doubt that for in vivo studies the presentation of both thick and thin films on the same slide is to be preferred.

4.1 Thick films

Ideally, a thick film should have about 20 leukocytes per microscope field at a total magnification of 700 ×. The shape of the film is not very important but, as will be seen from section 7.2.1 of this Annex, an oblong film approximately 1 cm long and 0.5 cm wide can be more easily examined systematically and because of this it is to be preferred. The film should not be defibrinated by stirring.

Maximum adhesion of the film to the slide is ensured by using only well cleaned slides (pre-cleaned slides can be purchased) and by carefully cleaning the patient’s finger with 70% alcohol.
4.2 Thin films

Most diagnoses can be made with a well prepared and stained thick film but in the case of doubtful species diagnosis, or mixed infections, the thin film can often be the final arbiter. It also serves as a location for numbering the slides (see section 5 of this Annex).

The thin film should be about 3 cm long. It is prepared from a small drop of blood placed in the centre of the slide and spread along the length of the slide: this leaves the remaining half of the slide for the thick film as described above.

5. Labelling of blood slides

In vivo studies produce an enormous number of blood slides and, unless a simple and clear system of labelling them is employed, chaos will ensue. There are two principal sources of blood slides in in vivo studies: (a) screening surveys; and (b) day 0 and follow-up days.

The following is an example of a basic labelling system:

(a) Screening survey

| Serial number | 22 |
| Location      | UBI |
| Date          | 22.3.1982 |

Serial number: this number is restricted to one particular person and, if this person is selected, the number is retained throughout the test. At each location the numbering will start with 1 and continue until the screening in that locality is complete. At the next locality it will start with 1 again and so on.

Location: a two or three letter code identifies the locality where the study is made. It is usually derived from the first letters of the name of the locality.

Date: the date is that on which the particular slide was made; screening may take place over several days.

(b) Day 0 (zero) and following days

| Day of study | Day 2 |
| Serial number | 22 |
| Location      | UBI |
| Date          | 24.3.1982 |

Day of study: once a case is selected for study a second slide will be taken just before treatment is given. This is the day 0 slide. Thereafter slides will be taken each day, or at predetermined
intervals, and these will be labelled according to the number of days that have elapsed since day 0. Therefore, at the end of a 7-day test, one should have the original screening slide, the day 0 slide taken just before treatment, and slides for days 1, 2, 3, 4, 5, 6, and 7.

**Serial number**: this is the original number given to the patient when screened. It never changes and will not be given to another patient in the same location.

**Location**: the same as for the screening slide.

**Date**: the date is that on which the particular slide was made; if the day 0 slide is dated 22.3.1982, then the day 7 slide would be dated 29.3.1982.

Labelling can be carried out with a diamond pencil, waterproof pen, or any other durable marker. However, experience has shown that dermographic (wax) pencils are generally unsuitable and should not be used.

In fact the simplest and most effective method is to write directly on the thin film with an ordinary lead pencil. This provides an easily read and permanent record, providing the thin film is “fixed” with methanol (laboratory grade) before staining. This method of labelling does not damage the slide and comes off when the slide is washed with detergent.

**6. Staining of blood slides**

It is almost universally accepted that Giemsa aqueous stain provides the most consistent standard of colour differentiation for the routine diagnosis of malaria. Moreover, it is stable, easily transported, and reasonably priced.

However, the best method of using the stain in the field is disputed. The general practice of increasing the concentration of the stain (10%) so that shorter staining times (10 minutes) are needed is very difficult to justify. In fact, with a reasonable degree of organization, the routine use of a 3% Giemsa stain for 30 minutes will give consistent and acceptable results in almost all foreseeable situations.

Giemsa stain does, however, require a strict pH control; the water used to dilute the stock solution should be pure (distilled or deionized) and adjusted with standard phosphate buffers to a pH of 7.1–7.3 depending on the results of trial and error staining with each particular batch of stain (these batches vary considerably and the pH
that gives the best coloration and differentiation of cytoplasm and chromatin should be chosen).

7. Examination of blood slides

For in vivo studies three types of blood-slide examination are required: (a) for screening of patients for potential inclusion in the study; (b) for verification of species and an estimation of the parasite density; (c) for definitive parasite-density counting. Each of the above requires a special technique. Before considering these techniques, it is necessary to describe the optical equipment required to carry them out.

7.1 Optical equipment

Differential diagnosis of human malaria and parasite-density counts are best achieved at a total magnification of ×600–700. This is routinely obtained by using a standard ×100 oil immersion objective and ×6–7 eyepieces. At higher magnifications, such as the commonly-used combination of ×10 eyepieces and a ×100 oil-immersion lens, definition is lost and colour aberration is usually enhanced. Moreover, the amount of transmitted light that is required for proper illumination at ×1000 magnification is double that required for ×700 and in many standard binocular microscopes this is at, or beyond, the upper limit of the illumination system.

Similarly, wide-field lenses are not essential for this work. They frequently give poorer definition and are usually only available at magnifications of ×8 and above.

A mechanical stage and an optical grid or graticule are essential for the counting of parasites. Ideally, the grid should have 25 squares although one with 100 squares is also acceptable. In the absence of a manufactured grid one can be easily made by marking out a good quality coverslip with a fine mapping pen and Indian ink or arranging a pattern with human hair glued to the edges of the coverslip. Plastic coverslips are easier to handle but are usually too opaque and may reduce definition.

Daylight filters are essential when artificial light is used.
7.2 System of examination

7.2.1 Thick films. Parasites are not evenly distributed in a blood film and the scanning process used should ensure that complete cross-sections of the film are examined. Oblong thick films (as described in section 4.1 of this Annex) simplify this procedure. The edge of the thick film is located with the ×10 objective and the ×100 oil-immersion lens moved into position. Then by judicious use of the mechanical stage, the film is scanned by what has been called “the farmer ploughing his field” technique: across the film to the opposite edge, a slight lateral move, then back across the film, a slight lateral move, and the process is repeated.

7.2.2 Thin films. In thin films the parasites tend to congregate around the “tail” of the film which, if it has been properly made, is much thinner than the rest of the film. Here a “battlement” technique is used traversing the edge of the tail in short vertical and horizontal tracks. These two methods are illustrated in Fig. 1.

Fig. 1. Scanning movements for thick and thin blood films

Thick film

Thin film

"farmer ploughing"

"battlement"

7.3 Screening of patients

The objective is to select potential candidates for testing as quickly as possible from the population under study. When schoolchildren or village populations are screened (see section 2.1 of
this Annex) this may involve the microscopical examination of blood films from 300 persons within about 2 hours. The selection procedure should, therefore, be geared to identify only those patients who have parasite densities likely to meet the threshold requirement of 1000 parasites per mm$^3$ of blood. Assuming that thick films have 20 leukocytes per microscope field and that there are approximately 8000 leukocytes per mm$^3$ of blood, then, if a patient has 1000 parasites or more per mm$^3$, one would expect to find on average 2.5 parasites or more per microscope field. For example, if the number of parasites seen in a sample of 10 fields is counted, one would expect to find at least 25 parasites. Consequently, after very little practice, it becomes easy to select out such cases very rapidly and one person can routinely screen 100 slides per hour. Of course, this only gives an approximate count and a further examination is required to establish more accurately the true parasite count and to eliminate the possibility of mixed infections. In terms of the screening process this does not require a lot of time or effort and with sufficient staff and good advance planning, the whole selection procedure can run concurrently.

7.4 Counting of parasites

As explained above, the basis for counting parasites in in vivo studies is the determination of the number of parasites per given volume of blood. Since the sample examined is dried blood, it is not possible to estimate the quantity of blood present unless a pre-measured amount has been placed in a predetermined area of the slide. Although techniques have been developed for this, a much simpler approach is to assume that certain components of the blood occur at a particular frequency and make these the standards against which parasites can be quantified.

There are two obvious possibilities:
(a) erythrocytes (RBCs), about 5 000 000 per mm$^3$; and
(b) leukocytes (WBCs), about 8000 per mm$^3$.

Since the erythrocytes are not defined in a thick film, the leukocytes are the logical choice.

A simple mathematical formula should be used to convert the counts into the number of parasites per mm$^3$ of blood. The counts can be total counts (all forms of the parasite) or counts of particular forms (asexual or sexual, trophozoites, schizonts, or gametocytes), for example:
P. falciparum asexual parasite count per mm$^3$ of blood =

\[
\frac{P. \text{falciparum} \text{ assexual parasites counted} \times \text{standard leukocyte count}}{\text{per mm}^3 \text{ of blood}} \times \frac{\text{leukocytes counted}}{1420 \text{ asexual parasites} \times 8000} = 4297 \text{ asexual parasites per mm}^3 \text{ of blood}
\]

e.g.,

\[
\frac{2644 \text{ leukocytes}}{1250 \text{ red blood cells (RBCs)} + 2000 \text{ white blood cells (WBCs)}} = 4 \text{ parasites per mm}^3 \text{ of blood}
\]

The next consideration is how many leukocytes and parasites must be counted to obtain a reliable sample.

Theoretically, if 0.25 mm$^3$ of blood is examined, it should contain 1 250 000 red blood cells (RBCs) and 2000 white blood cells (WBCs) and the threshold of parasite detection would be 4 parasites per mm$^3$ of blood. This level of detection is accurate enough for routine in vivo studies.

It is rather tedious and time consuming to count 2000 WBCs, so the normal practice is to count microscope fields, and if one assumes a mean count per microscope field of 20, then 0.25 mm$^3$ of blood is equivalent to 100 fields and this is frequently taken as the examination norm. However, if thick films are not consistently made to provide densities of 20 WBCs/field, then standardization can only be achieved by using a correcting factor such as:

\[
\frac{8000}{\text{mean number of WBCs in 10 fields}} \times \frac{\text{number of fields to be examined}}{0.25 \text{ mm}^3 \text{ of blood}} = \text{that are equivalent to 0.25 mm}^3 \text{ of blood}
\]

This lengthy examination procedure is used to determine whether a slide is positive or negative and to provide a reliable species diagnosis.

Normally a slide is not considered to be “negative” nor is the possibility of “a mixed infection” ruled out until 0.25 mm$^3$ of blood has been examined. Counting parasites is a completely separate activity and only begins when the routine examination is complete.

Parasite density is determined using different criteria. White blood cells and parasites are counted simultaneously on two hand tally counters (or a multiple counting device) and counting stops when the parasite count reaches 500 or the leukocyte count 1000, whichever figure is reached first. (Obviously, if either the parasite total (500) or the WBC total (1000) is reached before the count of a particular field is complete, counting continues until all the
leukocytes and parasites have been counted in that particular field.)
Then by means of the following formula the parasite density is calculated:

\[
\frac{\text{parasites counted} \times 8000}{\text{leukocytes counted}} = \text{parasite count per mm}^2 \text{ of blood}
\]

The use of this formula will produce counts suggesting a degree of precision beyond the actual scope of the method used. Consequently, the figures are usually rounded off to the first two digits, i.e., 16 would remain 16, but 161 would be 160, 1611 would be 1600 and so on.

8. Calculation of drug dosages and administration of medication

8.1 Drug schedules and regimes

Most antimalarials in common use are chemically stable if properly stored but, whenever possible, only drugs of known provenance should be used. Special arrangements should be made to ensure that drugs are handled with due care in the field and that extra precautions are taken when necessary. Abraded or accidentally broken tablets should not be used. If any doubt exists about the quality of the drug under test, assays should be made of a sample taken directly from drugs used in a trial and a sample should always be set aside for this. Since the active part of the drug is the base, dosages are always calculated in terms of the base of the drug and the weight (in kg) of the test subject. The weight of the base of the drug (in mg) is usually given on the container or enclosed leaflet. The smallest practicable fraction of a tablet is a quarter so the availability of tablets containing various amounts of the base is very convenient when preparing treatment regimes.

Chloroquine is available in tablets containing 150 mg, 100 mg, and 50 mg of base, and with this variation treatment regimes within the range of ± 12.5 mg are possible. However, it should be noted that the dose given is always equal to or slightly greater than the required dose, never less. For example, a patient weighing 36 kg who is to be given 10 mg of chloroquine per kg requires a total of 360 mg
of base. Thus, if only tablets containing 150 mg of base are available the best schedule is:

- 2 whole tablets = 300 mg
- and 1 half tablet = 75 mg
thus: total dose of base = 375 mg (15 mg of base excess)

If 50-mg tablets are also available
- 2 whole 150-mg tablets = 300 mg
- 1 whole 50-mg tablet = 50 mg
- \(\frac{1}{4}\) of 50-mg tablet = 12.5 mg
thus: total dose of base = 362.5 mg (only 2.5 mg of base excess).

Obviously, the smaller dosage tablets are particularly useful when children are being treated as any excess is proportionally less when there is a choice of different dose formulations. The tablets should be carefully divided with a sharp knife, or razor blade, and it is useful to do this in advance, so that care can be taken to assure that the divisions are properly made.

Defective portions should be discarded. Once an adequate supply of a reliable drug is available, it is useful to prepare a standard treatment chart whereby the appropriate dosages for all the possible body weights are pre-calculated and given in terms of tablets and mg of base of the drug. For example, starting at 10 kg body weight, the schedule for chloroquine treatment regimes of 5 mg and 10 mg would be as shown in Table 1.

<table>
<thead>
<tr>
<th>Body weight (kg)</th>
<th>5 mg of base/kg body weight</th>
<th>10 mg of base/kg body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total base required (mg)</td>
<td>Number of tablets of:</td>
</tr>
<tr>
<td></td>
<td>150 mg 100 mg 50 mg</td>
<td>150 mg 100 mg 50 mg</td>
</tr>
<tr>
<td>10</td>
<td>50 0 0 1</td>
<td>100 0 1 0</td>
</tr>
<tr>
<td>11</td>
<td>55 0 0 1½</td>
<td>110 0 1 ½</td>
</tr>
<tr>
<td>12</td>
<td>60 0 0 1½</td>
<td>120 0 1 ½</td>
</tr>
<tr>
<td>13</td>
<td>65 0 0 1½</td>
<td>130 0 1 ½</td>
</tr>
<tr>
<td>14</td>
<td>70 0 0 1½</td>
<td>140 1 0 0</td>
</tr>
<tr>
<td>15</td>
<td>75 0 0 1½</td>
<td>150 1 0 0</td>
</tr>
<tr>
<td>16</td>
<td>80 0 0 1½</td>
<td>160 1 0 ¼</td>
</tr>
<tr>
<td>17</td>
<td>85 0 0 1½</td>
<td>170 1 0 ½</td>
</tr>
<tr>
<td>18</td>
<td>90 0 1 0</td>
<td>180 1 0 ½</td>
</tr>
<tr>
<td>19</td>
<td>95 0 1 0</td>
<td>190 1 0 1</td>
</tr>
<tr>
<td>20</td>
<td>100 0 1 1</td>
<td>200 1 0 1</td>
</tr>
<tr>
<td>30</td>
<td>150 0 1 0</td>
<td>300 2 0 0</td>
</tr>
<tr>
<td>40</td>
<td>200 1 0 1</td>
<td>400 2 1 0</td>
</tr>
<tr>
<td>50</td>
<td>250 1 0 1</td>
<td>500 3 0 0</td>
</tr>
<tr>
<td>60</td>
<td>300 2 0 0</td>
<td>600 4 0 0</td>
</tr>
</tbody>
</table>

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Often, drugs are given in divided doses over 3 days. Chloroquine, for example, is never given in one individual dose greater than 10 mg of base per kg. Therefore, to provide a total dose of 25 mg/kg, 10 mg/kg is given on days 0 and 1 and then 5 mg/kg on day 2. It is dangerous to exceed the recommended dosage. Drug schedules and regimes should always be approved by a qualified medical practitioner (see section 8.2 of this Annex).

8.2 Administration of medication

Antimalarial drugs in tablet form are usually well tolerated provided they are not taken on an empty stomach. Occasionally, patients who are ill may vomit. Therefore, milk, biscuits, and other such bland foods should always be given before the administration of the drug.

Moreover, patients should be encouraged to rest under observation for at least 30 minutes after the drug has been ingested to ensure that no vomiting occurs. All drug treatments should be approved and actively monitored by a qualified medical practitioner who should provide supportive treatment if the course of the malaria infection requires further intervention.

Ethical considerations should never be compromised and the welfare of the patient must always be the primary concern.

9. Recording results

The maintenance of careful records is essential if useful results are to be obtained from in vivo studies.

Under the auspices of the Applied Field Research in Malaria component of the UNDP World Bank WHO Special Programme for Research and Training in Tropical Diseases, the Malaria Action Programme (WHO, Geneva) has developed a computer form that can be used to record systematically the results of in vivo tests of antimalarials against human Plasmodium spp. The use of this form eliminates the need to keep extensive personal records although, of

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1 Form WHO 5418 EME/MAP. A copy of this 3-page form and instructions for its completion may be obtained from: Director, Malaria Action Programme, World Health Organization, 1211 Geneva 27, Switzerland.
course, additional records can be kept if required. (It is essential that they be compiled at the time of testing.) Records of the population screening process are more difficult to keep. Appendix 1 is an example of a summary form for this purpose; it can be modified where necessary to suit local requirements. As discussed in section 3.3 of this Annex, local records, such as school and clinic attendance registers, should be used as a source of reference to ensure that names, ages, residential data, etc., are correct. This is particularly useful when dealing with young children or illiterate adults; the omission or juxtaposition of part of a name may well lead to confusion between two or more individuals. However, once patients are selected, the computer forms should always be used to ensure comparability of data.

When the studies are reasonably straightforward, these two forms provide all the information that is required for the routine follow-up of cases. If the studies become more complicated because the screenings have to be spread over several days to obtain sufficient cases for a significant sample, or if two or more drug regimes or combinations are being studied at once, it may become very difficult to keep track of what has to be done each day. In such cases, a special form should be devised; an example of a possible format for this is given in Appendix 2. Such a form will simplify the follow-up of cases and avoid possible omissions and errors.

10. Interpretation

As discussed in section 7 of this Annex, the compilation of standard computer forms will permit the detailed analysis and comparison of data on a global basis and should eventually result in the production of malaria maps showing the status of resistance to antimalarials throughout the world. However, the data produced can also be interpreted at local levels by means of simple graphs.

A single failure to respond to normal malaria therapy is not in itself significant unless this finding is supported by serum level estimations and other corroboratory evidence. What is significant is when a trend away from the normal response to treatment can be spotted in a significant sample of the population of a given area. The size of the sample required to be significant depends on the prevalence of resistance, but in general, a minimum sample would be 30 cases with complete follow-up.
10.1 Organization of data

As the material from the tests is processed, the results should be entered directly on to computer forms which also have provision for entering raw data in a way that such information can easily be extracted for either computer or “manual” processing. For instance, the daily parasite and leukocyte counts can be entered on the computer forms referred to above as raw numerical data or as the number of parasites per mm$^3$ of blood.

These results are then readily consolidated in an appropriate format (see Appendix 4) so that a longitudinal review can be prepared for each case. The individual results can then be combined to show the daily mean parasite density of all the cases under study. This mean can in turn be plotted on a graph (Appendix 3) to give a pictorial representation of the effect of treatment on the malaria infections of the cases studied.

For various reasons, these data are best produced in the form of a geometrical mean either by using Napierian Logarithms or a parasite index such as Bruce-Chwatt’s parasite density index (PDI) (see Table 2).

<table>
<thead>
<tr>
<th>Parasite count per mm$^3$ of blood</th>
<th>100+</th>
<th>101–200</th>
<th>201–400</th>
<th>401–800</th>
<th>801–1600</th>
<th>1601–3200</th>
<th>3201–6400</th>
<th>6401–12,800</th>
<th>12,801–25,600</th>
<th>25,601+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
</tr>
</tbody>
</table>

Example: Results of the counting of 10 *P. falciparum* positives (asexual forms only):

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Count/mm$^3$</th>
<th>Class</th>
<th>Serial No.</th>
<th>Count/mm$^3$</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,600</td>
<td>5</td>
<td>6</td>
<td>31,000</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>3,300</td>
<td>7</td>
<td>7</td>
<td>11,000</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>120</td>
<td>2</td>
<td>8</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>540</td>
<td>4</td>
<td>9</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>16,000</td>
<td>9</td>
<td>10</td>
<td>190</td>
<td>2</td>
</tr>
</tbody>
</table>

Therefore the frequency at which the classes occur is:

Class 1 = 2
Class 2 = 2
Class 3 = 0
Class 4 = 1
Class 5 = 1
Class 6 = 0
Class 7 = 1
Class 8 = 0
Class 9 = 1
Class 10 = 2

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To find the parasite density index from this data, the class number should be multiplied by the frequency and the results added, for example:

Class 1 × 2 = 2; Class 2 × 2 = 4; Class 3 × 0 = 0; Class 4 × 1 = 4;
Class 5 × 1 = 5; Class 6 × 0 = 0; Class 7 × 1 = 7; Class 8 × 0 = 0;
Class 9 × 1 = 9; Class 10 × 2 = 20;

or

2 + 4 + 0 + 4 + 0 + 7 + 0 + 9 + 20 = 51.

Dividing this total by the number of infections counted we get:

\[ \frac{51}{10} = 5.1 \] which is the parasite density index, i.e., PDI = 5.1

The table in Appendix 4 shows how this is done. Also, from this table the “mean parasite clearance time” can be calculated by adding up the number of days it took each infection to clear and dividing by the number of cases, i.e., for the 31 cases in Appendix 3: 1 day 2 cases; 2 days 9 cases; 3 days 15 cases; 4 days 5 cases;

mean parasite clearance time = \[ \frac{85}{31} = 2.7 \text{ days} \]

In this way, long-term trends in drug sensitivity can be compared by annual studies in the same localities.
Appendix 1

Summary form for *in vivo*/*in vitro* tests for the determination of the sensitivity of *Plasmodium* spp. to antimalarial drugs

<table>
<thead>
<tr>
<th>Identification number (Serial No.)</th>
<th>Sex</th>
<th>Age</th>
<th>Weight (kg)</th>
<th>Name</th>
<th>Microscope examination result</th>
<th>Result urine test</th>
<th>Drug(s) under test and dose in mg of base/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pos./neg. If positive species</td>
<td>Asexual parasites/mm³ of blood</td>
<td>Day 0</td>
</tr>
</tbody>
</table>

Locality: ___________________________ Date: __________________

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### Appendix 2

Example of a form to detail the status of each person in *in vivo* test by date, day of trial, and activity(ies) to be performed that day.

<table>
<thead>
<tr>
<th>Identification number</th>
<th>Drug regime</th>
<th>Name</th>
<th>Age</th>
<th>Sex</th>
<th>Weight in kg</th>
<th>Date of activity and corresponding day of trial: coded activity for each day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20/7</td>
<td>21/7</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>Fatima</td>
<td>6</td>
<td>F</td>
<td>140</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>mg/kg</td>
<td>Ali</td>
<td>6</td>
<td>F</td>
<td>140</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>C</td>
<td>Marcus</td>
<td>10</td>
<td>M</td>
<td>260</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>mg/kg</td>
<td>Aurelius</td>
<td>10</td>
<td>M</td>
<td>260</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>C</td>
<td>John</td>
<td>7</td>
<td>M</td>
<td>240</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>mg/kg</td>
<td>Williams</td>
<td>7</td>
<td>M</td>
<td>240</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>C</td>
<td>Pablo</td>
<td>10</td>
<td>M</td>
<td>270</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>mg/kg</td>
<td>Picasso</td>
<td>8</td>
<td>M</td>
<td>270</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>C</td>
<td>Mini</td>
<td>10</td>
<td>F</td>
<td>130</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>mg/kg</td>
<td>Le Rue</td>
<td>4</td>
<td>F</td>
<td>130</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>A</td>
<td>Dinh</td>
<td>25</td>
<td>M</td>
<td>160</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>mg/kg</td>
<td>Aasowe</td>
<td>25</td>
<td>M</td>
<td>160</td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>A</td>
<td>Nyanka</td>
<td>22</td>
<td>M</td>
<td>63</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>mg/kg</td>
<td>Aashika</td>
<td>22</td>
<td>M</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>52</td>
<td>A</td>
<td>Patel</td>
<td>18</td>
<td>F</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>mg/kg</td>
<td></td>
<td></td>
<td></td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

* M = Male; F = Female;  
U = Urine test as appropriate for drug under test;  
S = Blood slide, thick and thin film;  
? = Questions concerning vomiting, diarrhoea, pruritus, etc. in proceeding 24 hours;  
C = Chloroquine; A = Amodiaquine.  
*To be extended as required.
Appendix 3

Graph showing sensitivity of *P. falciparum* (asexual forms) to chloroquine in 31 malaria patients at Maun hospital, Botswana, February 1974
Appendix 4

Parasite density counts (expressed arithmetically, logarithmically, and by Bruce-Chwatt’s parasite density index) of 31 cases followed up in a chloroquine sensitivity trial of *P. falciparum* in Botswana in February 1974

<table>
<thead>
<tr>
<th>Serial No./ sex/age</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parasites</td>
<td>Log</td>
<td>PDI</td>
<td>Parasites</td>
</tr>
<tr>
<td>3 F 05</td>
<td>128 000</td>
<td>5.1072</td>
<td>8</td>
<td>152 000</td>
</tr>
<tr>
<td>11 F 07</td>
<td>15 000</td>
<td>4.1761</td>
<td>9</td>
<td>8 000</td>
</tr>
<tr>
<td>13 M 04</td>
<td>8 000</td>
<td>3.9031</td>
<td>8</td>
<td>4 000</td>
</tr>
</tbody>
</table>

<p>| | | | | | | | | | | | | | | | | | | | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>M</td>
<td>12</td>
<td>5 000</td>
<td>5.9996</td>
<td>7 200</td>
<td>3.3010</td>
<td>6</td>
<td>300</td>
<td>2.4771</td>
<td>3</td>
<td>20</td>
<td>1.3010</td>
<td>1</td>
<td>Neg.</td>
<td>Neg.</td>
<td>Neg.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>M</td>
<td>78</td>
<td>30 000</td>
<td>4.4771</td>
<td>10</td>
<td>7 000</td>
<td>3.8451</td>
<td>8</td>
<td>4 000</td>
<td>3.6021</td>
<td>7</td>
<td>10</td>
<td>1.0000</td>
<td>1</td>
<td>Neg.</td>
<td>Neg.</td>
<td>Neg.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Mean parasite density (arithmetic) | 24 800 | 11 100 | 920 |
| Mean parasite density (geometric) | 4 900 | 1 900 | 70  |
| Parasite density index | 7.0 | 5.8 | 2.6 |
| Parasite clearance time | 2.7 days | 0 0 0 0 0 0 |

The table above lists various data points related to parasite density and clearance times. Each row represents a different data point, with columns indicating the date, sex, and various measurements such as the parasite density and clearance times. The last row provides the mean values for the parasite density and clearance times.
CLINICAL TRIALS FOR NEW ANTIMALARIAL DRUGS

1. General aspects of clinical trials

Clinical pharmacology has undergone extensive development in recent years and the techniques used in clinical trials have been greatly improved. If useful and meaningful data are to be collected, trials must be planned and conducted with great care, the first consideration being the safety of the patient to whom a new compound is administered. The testing of new antimalarial drugs poses special problems; the drugs must be very safe because, if given for prophylaxis they may be used for long periods of time by people living in tropical areas where intercurrent infections and poor nutritional standards are common. No other class of medicinal compound is expected to match the criteria demanded for the ideal antimalarial drug. There is, moreover, a multiplicity of species, strains, and parasite stages, all of which must be considered when assessing the value of new antimalarial compounds.

1.1 Considerations for administering a new drug

The prerequisites for administering a new antimalarial for the first time in man have been summarized (24). Official documents including the Guidelines for evaluation of drugs for use in man (43), the Clinical guidelines edited by the United States Food and Drug Administration (16–18) as well as recommendations issued in the corresponding country on this topic, must be consulted by investigators (3). When developing new antimalariais, special consideration must be given to the following:

(1) The target populations that need such drugs live in tropical and subtropical environments, where malnutrition and associated infections are common. Certain genetic factors known to interfere with drug metabolism and/or with the disease (e.g., deficiency of glucose-6-phosphate dehydrogenase in the erythrocyte, haemoglobinopathies, rapid acetylator phenotypes) are highly prevalent in some areas.

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(2) Antimalarials are usually administered without medical supervision or may even be distributed prophylactically for a prolonged period. Antimalarials must therefore be very safe. They should also be non-mutagenic, non-carcinogenic, and of low toxicity for the fetus; pregnant women, infants and small children are the groups the most vulnerable to malaria.

1.2 Definition of phases for clinical trials

Some discrepancies exist regarding the definition of the phases of clinical trials, depending on the regulatory bodies of various countries.

In the report of a WHO Scientific Group (42) six phases were described. However, this publication described steps established according to the stage of development of the parasite as well as to the types of research performed either in hospitalized individual subjects or in the field.

The usual classification into four clinical phases is recognized by most of the national regulatory authorities. Phase zero includes all pharmacological and toxicological pre-clinical research.

(1) Phase I trials correspond to the initial administration of a new drug to man. At this stage, healthy or oligosymptomatic volunteers will be selected to determine whether the drug is well tolerated and whether a beneficial effect is to be expected from it (16). For this purpose a qualified clinical pharmacologist will study:

(a) the safety of the new compound;
(b) its bioavailability, mode of elimination, pharmacokinetics and, if possible, its metabolism (16, 17, 20);
(c) its pharmacological effect and, when appropriate, evidence of tolerance and effectiveness including early dose-ranging studies (16, 17).

For antimalarials, it is essential to perform phase I trials also in endemic areas of Africa, Latin America, and Asia in healthy or oligosymptomatic volunteers who belong to the target population, and who are potential carriers of malaria parasites.

(2) Phase II consists of controlled clinical trials designed to demonstrate effectiveness and safety. During this phase it is essential to determine the optimal dosage schedule. Depending on the drug in question properties that must be tested include its effectiveness as a causal prophylactic, its anti-relapse activity, as well as its blood
schizontocidal, gametocytocidal, and sporontocidal activity (8, 29, 35).

(3) *Phase III* includes expanded, usually comparative trials in which effectiveness and safety are confirmed in larger groups of patients. More severe cases and patients at particular risk such as those with associated diseases, associated medication, genetic anomalies and special groups (children, infants, and women of childbearing age) will be included. The main objective of this phase is to assess the safety of the drug when given in an optimal dosage to a larger group of patients, and to compare its activity with standard treatments.

(4) *Phase IV* applies to all aspects of drug investigation following the approval of a New Drug Application (6).

The most important data to be collected at this stage concern the side-effects, especially the rare adverse reactions occurring after prolonged use under special conditions or in special categories of patients.

The efficacy of the drug must be constantly reassessed because of the possible development of resistance among parasites (section 2 of the Report).

### 1.3 Choice of phase I and II trial centres in malarious countries

The trial centre should be sited close to a malarious region, preferably outside the transmission zone. This facilitates the recruitment of suitable patients and makes a prolonged follow-up possible. Care must be taken to avoid reinfection: the patients should be kept in a mosquito-proof environment especially in the evening and during the night.

The trial centre should also be associated with a hospital where there are the best possible treatment facilities in different fields, preferably including an intensive care unit. Whenever possible, specialists in internal medicine, gynaecology, surgery, paediatrics, and psychiatry should be available as consultants.

This association with a general hospital provides many advantages, especially regarding the safety of the patients. Furthermore, the recruitment of patients for therapeutic trials may be facilitated.

There should be good laboratory facilities available and also sufficient space to add complementary techniques, primarily in the fields of parasitology (e.g., *in vitro* sensitivity testing for *Plasmodium*...
falciparum, and malaria serology), haematology, blood chemistry, etc. The clinical trial centre should, inter alia, have facilities for electrocardiography and electroencephalography. Ideally, it should also have equipment to perform the chemical analyses of samples (e.g., high-performance liquid chromatography). However, such analytical work could be carried out elsewhere if good storage and transport facilities are available (large freezers for storage at $-20^\circ$C and possibly freezers for storage at $-60^\circ$C). These make the storage of blood, urine, and other samples possible and interesting parasitic strains can be cultivated at a later stage or tested in animal models.

1.4 Monitoring of clinical trials

A qualified physician (the trial monitor) with experience in the fields of clinical pharmacology and parasitology must be available to monitor the implementation of the study (see section 4.2.4 of the report).

He will determine whether a principal investigator has the necessary competence and the required facilities, including medical personnel, supporting staff, transport facilities, adequately equipped wards and laboratories, and the possibility of recruiting enough patients or volunteers for the study.

Usually, the physician will help to overcome most of the basic problems. He will establish contacts with the staff of the medical centre. There must be good collaboration with other medical departments of the hospital and the creation of an ethical committee in the institution (if such a structure does not yet exist) may be one of the goals of his first visit to a centre.

Quality control of the results produced by the laboratory is essential before the initiation of any clinical study. Particular attention must be paid to malaria parasitology and to the diagnosis of other infectious diseases. The standard of haematology must be high; blood chemistry and serology may not be essential because samples can be transported to a neighbouring centre.

For each laboratory parameter to be assessed during the trial, the normal range and mean values in the locality must be determined. Before the trial, tests will have to be performed on a large number of subjects belonging to the same ethnic group and living in the same socioeconomic and epidemiological environment as the patients expected to participate in the study.
The adaptation of a laboratory to a particular research programme may last for up to one year after the necessary instruments have been purchased. The speed of adaptation to new techniques depends on the ability and training of the laboratory technicians.

During this preparation time a trial centre will be treating malaria patients with standard medicaments; the principal investigator will try to fulfil all the requirements of the study with regard to clinical and laboratory tests and adequate follow-up of the patients should be ensured.

This will provide important basic knowledge on the course of the disease and will facilitate the determination of inclusion and exclusion criteria for the therapeutic trial.

The trial monitor must remain permanently in contact with the investigators by correspondence or telephone. He will need to visit the centre at least twice a year, when he should have access to all the patients' records and medicaments. He will also have to check the work performed in the laboratory.

The monitor will check that the patients' records have been properly kept, obtain any missing data, and have the documents dated and signed by the principal investigator.

The monitor should make sure that the principal investigator adheres to the protocol. He needs to have the authority to make minor amendments to the trial plan if there are imperative reasons for doing so. He also reports regularly on adverse reactions.

1.5 Ethical considerations

Most nations have ratified the Declaration of Helsinki of 1975, adopted by the 18th World Medical Assembly in Helsinki, Finland, in 1964, and revised by the 29th World Medical Assembly in Tokyo, Japan, in 1975.

The basic principles regarding the ethical aspects of any biomedical research involving man have been clearly stated and must be considered as an essential minimum. Some of the principles laid down in Helsinki may need to be quoted in detail in trial protocols:

"2. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol which should be transmitted to a specially appointed independent committee for consideration, comment and guidance."
“5. Every biomedical research project involving human subjects should be preceded by careful assessment of predictable risks in comparison with foreseeable benefits to the subject or to others. Concern for the interests of the subject must always prevail over the interests of science and society.”

“9. In any research on human beings, each potential subject must be adequately informed on the aims, methods, anticipated benefits and potential hazards of the study and the discomfort it may entail. He or she should be informed that he or she is at liberty to abstain from participation in the study and that he or she is free to withdraw his or her consent to participation at any time. The doctor should then obtain the subject's freely-given informed consent, preferably in writing.”

“10. When obtaining informed consent for the research project the doctor should be particularly cautious if the subject is in a dependent relationship to him or her or may consent under duress. In that case the informed consent should be obtained by a doctor who is not engaged in the investigation and who is completely independent of this official relationship.”

“12. The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present Declaration are complied with.”

In phases I and II, the informed consent of volunteers or patients must be obtained in every case. In tropical countries, this means that the information to be given to the patient must be written down or translated into a language and a form that is well understood by the patient. The above stipulations apply also to phase III trials, but since children or severely ill patients might be included in these trials, informed consent should be obtained from relatives in accordance with national legislation.

Each protocol has to be submitted to an ethical committee. The safety of the volunteers or patients is the primary consideration of the ethical committee in giving approval for a study. However, scientific aspects, such as quality of the design of the study, competence of the investigator, and the chance of acquiring useful knowledge from the study, must also be considered before approving any new project.

1.6 Trials in experimentally infected subjects

In the United States of America, especially during the Second World War and up to the end of the war in Viet Nam, and in some other countries, e.g., Australia and Brazil, healthy prison inmates or military personnel were allowed to volunteer for safety and phase II efficacy studies. Experimental infections in non-immune volunteers
or in neurosyphilitic patients have made an important contribution to our knowledge of human malaria (8).

The details for conducting clinical trials in non-immune infected volunteers are given in Annex 4 of *Chemotherapy of malaria and resistance to antimalarials* (42).

2. Phase I clinical trials

The FDA guidelines indicate that “Phase I studies should include determination of absorption, excretion and plasma clearance of the drug, and, if feasible, metabolic pathways” (16).

2.1 Objectives

The aim of early trials is twofold: the assessment of safety and a study of the pharmacokinetics of the new compound, including absorption. Studies are made of the bioavailability, distribution, and the rate and mechanism of elimination of the active compound and its main metabolites. The potential activity and toxicity of a given dose or concentration obtained in human plasma may be calculated on the basis of corresponding concentrations measured in animals in which toxicity (and efficacy) trials have been performed (19).

For antimalarials, healthy volunteers from developed countries may be involved in early trials, but more representative subjects, such as oligosymptomatic patient volunteers living in the endemic area, must be included in phase I trials (43).

2.2 Initial dosage

The choice of the initial dose will be based on toxicological findings in the most susceptible animal species. For instance, 5 mg/kg per day of mefloquine proved to be non-toxic when given for 28 days to the most susceptible species (dogs). The first dose given to a volunteer was about 1 mg/kg body weight, i.e., a single dose of 50 mg (20).

If the first dose does not provoke any relevant unwanted effect, a second higher dose, chosen arbitrarily, can be given after a “wash-out” period of 3–7 days (20). The doses may be increased according to toxicological considerations. If toxicity for a given organ has been noticed, adequate laboratory tests must be used to determine very early changes in the function of that organ.
If bioavailability is being studied, the “wash-out” period between two administrations should be equal to approximately 6 biological plasma half-lives \((t_{1/2})\) of the drug, which may be very time-consuming e.g., for mefloquine with a \(t_{1/2}\) about 20 days, the “wash out” would be 120 days.

2.3 Pharmacokinetics

The results of pharmacokinetic studies may indicate the rate of absorption, the bioavailability, the distribution in various compartments, and the type of metabolic transformation of a drug, as well as its mode and rate of elimination. To determine whether the plasma concentrations are dose dependent and whether repeated administration will modify the way the body deals with the drug, the effects of different dose levels and multiple doses have to be studied.

Penetration of the active substance into the erythrocyte may be essential for schizontocidal drugs.

The concentration of active compound required to inhibit schizogony of \(P. falciparum in vitro\) can be compared with the concentration reached in the plasma of volunteers. For most schizontocidal compounds there is a good correlation between in vitro activity and clinical activity \((42, 44)\).

2.4 Tolerance study

During phase I pharmacokinetic studies, volunteers must be kept under very close medical supervision because all pharmacological or toxicological effects must be assessed. Clinical examination must be carried out at least twice daily. This may require electrocardiography, electroencephalography, and other investigations, depending on the organs that seem most likely to be affected by the drug. Laboratory tests performed daily should include a representative series of haematological and biochemical measurements. Complementary tests may be needed if the substance is thought to be potentially toxic for a given tissue or system.

Physiological variations and a relatively broad range for normal values in any given laboratory test must be taken into account. Furthermore, external factors such as strenuous physical activity, associated drugs, alcohol consumption, and heavy smoking may affect the pharmacokinetics and the tolerance of the test medicament \((27, 28)\).
Bioavailability, pharmacokinetics, and tolerance studies of a new drug must be repeated as soon as possible in apparently healthy or oligosymptomatic subjects belonging to the target population in malarious areas. Oligosymptomatic subjects should be semi-immune, in good physical condition, and carriers of \textit{P. falciparum} or \textit{P. vivax}. Parasitological follow-up should be carried out in order to determine the dose required for radical cure. In areas with ongoing malaria transmission, the subjects must be protected from reinfection in a mosquito-proof environment.

In trials with drugs possessing potential sporontocidal activity, repeated (indirect) feeding of \textit{Anopheles} mosquitoes on the blood of treated volunteers who present a high gametocytinaemia, before and repeatedly after medication, permits assessment of this activity in the test compound. Direct feeding of mosquitoes on infected patients produces a higher percentage of infection in the vector than feeding through a membrane, but it may not be ethically acceptable.

2.5 Repeated dosage

If a single dose of the new drug proves to be safe and effective, repeated administration may be undertaken. This requires complementary subacute or chronic toxicity data in two animal species. The dosage interval for repeated administration of an antimalarial drug in a suppressive trial should correspond approximately to the biological half-life of the drug in man. Each dose administered should be about one-quarter of an effective, well-tolerated single dose.

These trials should be designed whenever possible as randomized, double-blind, comparative trials \textit{versus} a standard drug such as chloroquine. Good acceptability and tolerance are essential properties of drugs that are to be used prophylactically. These repeated dosage studies make it possible to demonstrate whether unexpectedly high accumulation, or a change in the $t_{1/2}$, occurs after repeated doses.

3. Phase II clinical trials

The aim of phase II studies is to determine the optimal dosage required to achieve a given antimalarial response. The activity of the drug may vary depending on the geographical origin of the parasite strain.
All relevant information concerning the chemistry, pharmacy, pharmacology, and toxicology of the drug together with the results of the phase I clinical trials should be summarized in the "Phase II investigational drug brochure" given to the investigator.

3.1 Trials in experimentally infected patients or volunteers

Drug trials in healthy volunteers deliberately infected with selected strains of *P. falciparum* or *P. vivax* were undertaken between the Second World War and the end of the war in Viet Nam, in the United States of America, Australia, and some other countries (1, 4, 5, 9, 39, 46). These studies provided "a steady flow of invaluable information on the course of malaria in the non-immune subjects and on the action of drugs against the various stages of the parasite. This information could not have been obtained in any other way" (41).

There are many advantages in using experimentally infected subjects for dose-finding efficacy trials:

1. Such volunteers, living far from endemic areas, are non-immune, whereas naturally infected subjects have some degree of immunity that may influence the course of infection and enhance the antiparasitic effect of the drug.
2. Reliable long-term follow-up observations can be made because there is no risk of reinfection.
3. The parasite strains used for experimental infections have a predictable pattern of response to the standard antimalarials, whereas "wild", naturally acquired strains may exhibit different resistance patterns, even when originating from the same transmission area.
4. Mixed infections, which are frequently encountered in endemic areas, are often difficult to detect and they represent a potential source of error. This difficulty does not occur with experimental infections.
5. Comparison of the protective activity of a compound against mosquito- and blood-induced infection can be used to indicate whether or not a drug has causal prophylactic activity as in the case of proguanil or its metabolite (14).
6. To test malaria vaccines, it will be necessary to study the immunological changes that occur in non-immunes. Vaccinated volunteers with a positive antibody response may have to be challenged with repeated bites from infective mosquitoes (12, 13).
3.2 Trials in naturally infected subjects

Problems arise when naturally infected subjects are selected for early therapeutic trials:

(a) Since the immune status of the patients will often be unknown, any with severe infection should be excluded in order to reduce the risks. Whenever possible, serological tests should be performed prior to therapy.

It has been claimed that non-immunes require higher doses than semi-immunes for the clinical cure of malaria. However, if the follow-up period of the semi-immune patients is long enough, the differences measured in clinical response may be less marked where radical cure is concerned. For instance, the mefloquine dose required for the radical cure of falciparum malaria in non-immune patient volunteers and in semi-immune, naturally infected subjects, is of the same order of magnitude, i.e., a single dose of 750–1250 mg of mefloquine base for adult patients.

For greater clinical safety during early phase II therapeutic trials in falciparum malaria, the new compound can be given together with a short-term course of quinine (21–23, 33).

(b) Patients in areas with ongoing malaria transmission should be kept in a mosquito-free environment. The follow-up period required for studying radical cure of P. falciparum infections depends on the kinetics of the trial drug and is usually within the range of 4–9 weeks; follow-up of radical cure of P. vivax infections is essentially longer.

(c) In vitro sensitivity tests to various antimalarials should be performed prior to therapy and especially in the case of recrudescence (36–38, 40). In cases of failure, cryopreservation and transport of the isolate to specialized laboratories for further in vitro tests or in vivo tests in monkeys should be considered.

(d) The parasitologists must be experienced; parasitaemia must be assessed quantitatively and qualitatively. If possible, two separate technicians should check the blood slides independently. Should discrepancies be found, unstained preparations collected for this purpose must be re-examined and, if necessary, sent to a reference centre. The laboratory diagnosis of malaria from thin and thick

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1 Instructions for use of the WHO test kit for the assessment of the response of Plasmodium falciparum to chloroquine (unpublished WHO document MAP/79.1.)
blood films has been well described in textbooks of parasitology and elsewhere (15, 45).

Mixed infections can cause problems. Young ring forms of *P. vivax* emerging some weeks after treatment of a *P. falciparum* infection may be described erroneously as a recrudescence of the primary infection. In naturally infected subjects from Latin America or South-East Asia, mixed infections with *P. vivax* and *P. falciparum* are common; in Africa *P. malariae* is often associated with *P. falciparum*.

When treating naturally transmitted *P. vivax* infections, *P. falciparum* may also emerge when the more susceptible *P. vivax* has been eliminated.

(e) During the assessment of the activity of a new drug against the erythrocytic forms of naturally transmitted *P. vivax* infections, an antirelapse compound such as primaquine, known to have low schizontocidal activity, must be given.

(f) The gametocytocidal and sporontocidal activity of a compound is not easy to assess. Although the usual blood schizontocidal drugs do not destroy mature gametocytes of *P. falciparum*, it is possible that they may inhibit the early stages. Sulfadoxine/pyrimethamine has no sporontocidal activity, except when the strain is fully susceptible to pyrimethamine (2).

After long-term suppressive treatment with schizontocidal drugs, the gametocytamia progressively decreases and disappears. This should always be carefully recorded.

(g) Non-compliance with therapy is a source of error in therapeutic trials. In severely ill patients, vomiting or incomplete absorption of the active substance may occur. Malabsorption may be a consequence of the malaria infection itself or of an underlying disease (25).

One way of discovering if there has been non-compliance or malabsorption is to collect a sample of plasma from each treated patient at the beginning of the β-phase of elimination of the drug. A correlation has been demonstrated between poor absorption and drug failure in patients treated with new phenanthrenemethanol derivatives (10).

3.3 Trial plans

In phase II trials, the greatest attention must be paid to the trial protocol. To avoid the risk of a potentially dangerous infection,
clinical severity of the disease is a reason for exclusion. The optimal
dosage of a new compound in phase II trials is, at first, unknown;
therefore two or more different dosage regimens are compared at
this stage.

In any plan the rationale of the study must be summarized from
the background knowledge already described in greater detail in the
"Investigational drug brochure". New publications should be quoted,
and all recent information, especially on tolerance, must be given.

The objectives of the study must be precisely stated, for example:
"The aim of this randomized, double-blind, comparative trial is to
determine the efficacy and tolerance of a single administration of
either 500 mg or 1000 mg of mefloquine base compared with those
of a standard treatment (quinine + sulfadoxine + pyrimethamine)
in symptomatic, uncomplicated, falciparum malaria in semi-immune
patients, living in an area where chloroquine-resistant falciparum
malaria is highly prevalent".

The main goals may include a comparison of:

— the rapidity of clearance of asexual parasitaemia;
— the radical cure rate (eradication of asexual forms of *P.
falciparum*) within a given follow-up period, the patients not
being exposed to reinfection;
— the speed of the clinical response in symptomatic patients, using
clinical parameters listed in the record form;
— the safety and acceptability of the drugs (clinical adverse
reactions and changes in the laboratory parameters).

Ethical considerations must be clearly stated in the protocol and
the records must note that informed consent has been obtained from
each patient.

The criteria for the selection of a patient must be adequate to
provide results that are comparable in homogenous groups:

— the age-range should be narrow, and adult male and female
patients should be grouped separately;
— the immune status should be the same in all patients
(epidemiological considerations);
— the severity of the disease should be of the same order;
— patients with severe malaria or severe underlying disease should
be excluded.

Exclusion criteria must be precise: the unacceptable age groups
or sex must be stated, as well as signs associated with severe
prognosis (e.g., temperature above 40°C, fever lasting for more than 7 days, parasitaemia exceeding 100 000 mm³, signs of meningism or encephalitis, cardiovascular involvement such as systolic blood pressure below 100 mmHg (13.3 kPa), blood dyscrasias including platelet counts below 60 000 mm³, low haemoglobin values, haemorrhagic diathesis, jaundice, severe dehydration, severe renal involvement) or any severe underlying disease or accompanying infection requiring specific treatment.

Whenever possible, the trial will be comparative, randomized, and double-blind. The principal investigator receives sealed, numbered key envelopes. He will open the key only if this is required for safety reasons. The sealed envelopes are sent back, unopened, together with the corresponding completed record forms at the end of the trial.

All adverse reactions that occur during the study must be reported on the case-record forms. The side-effects are graded on a three-point scale (mild/moderate/severe) and reported in detail, including date of onset and date of disappearance. In cases of severe adverse reactions, the monitor must be informed immediately by telephone or by cable. A special questionnaire must be filled in by the investigator with a precise description of the event (the FDA provides a suitable questionnaire). Samples of 4 ml of plasma should be taken and kept in suitable, correctly labelled containers at −20°C.

Should unexplained changes in the results of laboratory tests occur, corroborative tests must be carried out until values have returned to normal and or an adequate explanation is found.

Every effort must be made by the investigator to keep all the subjects in the study until the end of the follow-up period. All treated patients must be assessed, even if the follow-up is incomplete. The reasons for removing patients from the study must be clearly stated on the record forms. Such reasons would include treatment failure, adverse reaction, intercurrent illness, administrative reasons, etc.

It is essential that adequate case-record forms (questionnaires) be given to the investigators. These should facilitate the recording of all relevant information and findings.

The completed questionnaires should be sent in batches of ten to the monitor after having been signed by the chief investigator. A copy of the patient consent forms and the corresponding sealed key envelopes should also be attached.

These documents will be checked by the monitor and thereafter assessed, whenever possible, with the help of a computer.
4. Phase III clinical trials

Phase III clinical trials are designed to confirm that the drug is effective and safe when it is administered to all patients, including children, infants, women during pregnancy, and severely ill patients who may be semi-immunes or non-immunes, as well as patients with associated diseases requiring concomitant medication. Interactions of the new compound with other medicaments administered to the patient should be studied.

These investigations can only be initiated when the optimal dosage schedule, the finalized pharmaceutical preparations, and all statutory toxicological data are available. Complementary information on pharmacokinetics in special conditions (younger age-groups, patients with impaired renal or liver function, and malnourished subjects) must be acquired early in phase III.

Although the design of a phase III trial will not differ basically from that of phase II, it may be simplified. The follow-up period may be reduced to 28 days and the list of laboratory tests may be considerably shortened.

Control groups treated with standard medicaments should be included: the trials will usually be designed as randomized, prospective, comparative studies.

4.1 Selection of patients and risk factors

4.1.1 Infants and children. During studies in children, risks should be avoided as far as possible. The optimal existing treatment will be taken as standard for the control group (26). Infants may be included in the trials only if favourable results have already been obtained in adults and children. The FDA (18) summarizes the special problems of paediatric drug therapy and emphasizes that the investigator and the review board must ensure that any risks involved are minimal, and that the research is scientifically sound and significant. It must be stressed that children have the greatest need for better, safe, and well-accepted antimalarials as they represent the most vulnerable group with the highest morbidity and mortality. “The rights of the child to receive treatment with adequately tested drugs must not be abridged” (34).

4.1.2 Genetic blood dyscrasias. During phase III, pilot tolerance studies may be conducted among subjects with genetic anomalies of
the red blood cells (glucose-6-phosphate dehydrogenase deficiency, thalassaemia, sickle cell anaemia, and other haemoglobinopathies), if competent haematologists are available to undertake the required controls.

4.1.3 Pregnancy. The deleterious effect of malaria on the outcome of pregnancy has been well established; there is a significant decrease in resistance to malaria in pregnant women (30, 31). Unfortunately, in some areas of South-East Asia and Brazil, *P. falciparum* does not respond to either 4-aminoquinoline derivatives or antifolate-sulfonamide combinations. Even prolonged treatment with quinine may not eliminate the asexual forms and there is an increase of RII resistance to the standard treatment with quinine. It is therefore ethically acceptable, and indeed even compulsory and scientifically justified, to administer new life-saving drugs that may protect the fetus or the baby, as well as the mother, in controlled studies during any stage of pregnancy in such areas.

5. Phase IV clinical trials

Drug trials undertaken after registration of a drug, especially when it is in widespread clinical use, may be defined as phase IV trials (6). No fundamental differences exist between phase III and IV clinical studies except regarding the stage of registration of the compound.

6. Field trials in malaria

Field trials must be considered independently of the four clinical trial phases. Pilot field trials may be begun during phase II, and extended field trials usually take place during phase III or IV. Field trials are planned studies not confined to modern clinics with continuous close medical surveillance. Suppressive treatment of groups of subjects in field conditions or curative treatment in less sophisticated centres, such as malaria clinics, in conditions resembling those found in other rural hospitals provides an opportunity for careful study of a drug in a larger number of subjects.

The objectives of pilot field trials were summarized in the report of the first Technical Meeting on Chemotherapy of Malaria (47).
They are to establish optimum dosage and regimen, further evidence of side-effects, evidence of possible resistance and cross-resistance, and acceptability of the drug to the population.

Extended field trials are an attempt to observe the consequences of mass drug administration in a given population (8). Their objectives are the following:

— to confirm the antiparasitic property of the drug and to compare its activity with that of a well established standard antimalarial;
— to assess the acceptability and tolerance of the drug in a larger population group, special attention being paid to discovering possible rare or delayed adverse reactions;
— to determine whether some strains of plasmodia are resistant to the drug (in such cases in vitro sensitivity testing would be needed);
— to evaluate the effect of mass drug administration on the degree of transmission in the area. This is possible when all groups of the population (including infants and pregnant women) can be treated. This will rarely be the case before the end of phase III or the registration of the drug (in phase IV). Such extended field trials may provide basic operational data for planning large-scale chemotherapeutic measures (8).

6.1 Design and selection of participants for field trials

Fewer than 200 selected subjects participate in pilot field trials, whereas in extended field trials usually more than 1000 subjects are involved. At the end of phase III, the selection criteria for the participants become less stringent. Phase IV studies may be conducted in all types of subjects, including infants and pregnant women, unless contraindicated in any particular group. Bruce-Chwatt (7), Clyde (11), and McGregor et al. (32) were pioneers in conducting such trials.

As field trials will always be prospective, randomized, comparative trials versus a standard antimalarial, the groups to be compared need to be similar from the parasitological, epidemiological, and immunological points of view.

Bruce-Chwatt (8) insists upon the importance of having “the full understanding and cooperation of the community. The success of such a trial depends on the goodwill of the whole population and must be carried out with every consideration for local customs and mores”.

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The inclusion of non-immune subjects is essential to determine the dosage schedule to be used for travellers entering endemic areas. However, there are few papers that report such trials. Army personnel and labour groups transferred from a non-malarious into an endemic area represent relatively homogeneous groups of non-immunes that are easy to supervise.

6.2 Extended field trials during phase III

Only a limited number of parameters can be measured in field trials, the most important being the asexual parasite and gametocyte counts. For the determination of the parasite clearance time, counts should be made once or twice daily for at least 7 days. Thereafter, blood slides must be examined at weekly intervals up to the fourth week; more prolonged examinations up to the eighth week of therapy are required when long-acting drugs such as mefloquine are used in order to assess the radical cure rate in cases where there is no risk of reinfestation.

The viability of gametocytes can be checked to determine whether the drug itself, or the concomitant administration of a single dose of 30 or 50 mg of primaquine, has sporontocidal properties. Locally bred mosquito vectors fed on blood from the patients can be used for this purpose.

Simple laboratory tests may be performed if suitable techniques are available, e.g., microhaematocrit, red and white blood cell counts, and urinalysis. The size of the spleen and the body weight may also be recorded. Urine samples can be tested to check whether or not the drug has actually been taken. It is essential to know whether any adverse reaction or any intercurrent disease occurs during prolonged suppressive treatment, and medical examinations should, therefore, be made at regular intervals.

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