Advances in malaria chemotherapy

Report of a
WHO Scientific Group

World Health Organization
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WHO SCIENTIFIC GROUP ON THE CHEMOTHERAPY OF MALARIA

Geneva, 12-16 September 1983

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ADVANCES IN MALARIA CHEMOTHERAPY

Report of a WHO Scientific Group

A WHO Scientific Group on the Chemotherapy of Malaria met in Geneva from 12 to 16 September 1983. The meeting was opened on behalf of the Director-General by Dr S.K. Litvinov, Assistant Director-General.

INTRODUCTION

In recent years, the emphasis of the worldwide struggle against malaria has changed from an attempt to eradicate the infection to an attempt to control the disease.

The use of chemotherapy is very important in alleviating the suffering and in reducing the mortality caused by malaria in tropical countries. A few doses of an effective antimalarial drug can have a dramatic effect on the condition of the patient. Unfortunately, malaria is again on the increase in many countries mainly owing to the natural ability of malaria parasites and their insect vectors to acquire resistance to the chemicals used against them, but also as a result of other factors, including the unwise use of such chemicals, the movements of populations, and political instability.

In the report of a WHO Scientific Group that met in 1972 (1) and in earlier reports, concern was expressed regarding the development of resistance by Plasmodium falciparum to the 4-aminoquinoline compounds. During the past ten years this resistance has increased and spread and, in addition, resistance has developed in some areas to the hitherto effective alternative treatment involving the combination of pyrimethamine with long-acting sulfonamide derivatives.

The problems of malaria chemotherapy have previously been discussed at a Technical Meeting on Chemotherapy of Malaria in 1960 (2), the meeting of the WHO Scientific Group on Chemotherapy of Malaria in 1967 (3), and the above-mentioned WHO Scientific Group on Chemotherapy of Malaria and Resistance to Antimalarials in 1972 (1).
The problem of drug resistance also received special attention in the report of the WHO Scientific Group on Resistance of Malaria Parasites to Drugs (4) and in the more recent reports of the WHO Expert Committee on Malaria (5–9). A special meeting on clinical management of acute malaria was held in New Delhi, India, in 1978 (10), and others on drug-resistant malaria were held in Kuala Lumpur, Malaysia, in 1981 (11), in Albuquerque, USA, in 1982, and in Jakarta, Indonesia, in 1983. In view of the urgent need for new and effective compounds, a meeting on the modern design of antimalarial drugs took place in Bethesda, Maryland, USA, in 1982 (12).

The present report provides advice on the use of drugs for the suppression and treatment of malaria taking into account the presence of drug-resistant parasites and on the best ways in which existing and new antimalarials may be used to counter the further development and spread of such resistance. The development, clinical assessment, and future deployment of the new drug, mefloquine, have received special attention. Emphasis is placed on the need for standardized techniques for testing parasite sensitivity by in vitro and in vivo methods, and on the efficient conduct and monitoring of clinical trials.

REFERENCES

10. The clinical management of acute malaria. New Delhi, WHO Regional Office for South-East Asia, 1980 (WHO Regional Publications, South-East Asia Series, No. 9).
1. PROBLEMS FACING MALARIA CONTROL

With the recognition that the worldwide eradication of malaria is not an attainable goal in the foreseeable future given existing resources, many countries have tried to develop realistic programmes for antimalaria action adapted to their local conditions and available resources. The technical requirements, the considerable increase in the cost of supplies and labour, and the economic crisis of the last few years have meant that it has been necessary to make important readjustments to the control operations of antimalaria programmes.

Resurgences of malaria have occurred in many parts of the world. Most, including the massive resurgence of the disease in the Indian subcontinent in the mid-1970s, were controlled by the mobilization of extraordinary resources and the redeployment of traditional control methods. The numbers of malaria cases reported from 1974 to 1981 are given in Table 1. Although in some instances these figures (in thousands) do not cover the total population at risk, they clearly indicate the impact of the resurgences as well as the situation of relative global stagnation since 1979 with progress in some areas being counterbalanced by deterioration in others.

Table 1. Numbers (in thousands) of malaria cases reported

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Africa*</td>
<td>5.120</td>
<td>4.209</td>
<td>5.390</td>
<td>4.477</td>
<td>6.682</td>
<td>5.647</td>
<td>1.119</td>
<td>2.039</td>
</tr>
<tr>
<td>Americas</td>
<td>269</td>
<td>357</td>
<td>379</td>
<td>399</td>
<td>469</td>
<td>515</td>
<td>603</td>
<td>638</td>
</tr>
<tr>
<td>Europe</td>
<td>7</td>
<td>13</td>
<td>41</td>
<td>41</td>
<td>119</td>
<td>93</td>
<td>34</td>
<td>38</td>
</tr>
<tr>
<td>Eastern Mediterranean</td>
<td>480</td>
<td>429</td>
<td>348</td>
<td>227</td>
<td>162</td>
<td>125</td>
<td>137</td>
<td>144</td>
</tr>
<tr>
<td>Western Pacific</td>
<td>173</td>
<td>188</td>
<td>211*</td>
<td>4.457</td>
<td>3.422</td>
<td>2.706</td>
<td>3.654</td>
<td>3.450</td>
</tr>
<tr>
<td>Total (excl. Africa)</td>
<td>5.091</td>
<td>7.092</td>
<td>8.283</td>
<td>10.742</td>
<td>8.936</td>
<td>7.038</td>
<td>7.993</td>
<td>7.643</td>
</tr>
</tbody>
</table>

*Mainly clinically diagnosed cases; does not include the majority of chronic infections.
*Excluding China.
Case-reporting is known to vary considerably among countries and areas. Based on country evaluations of data reliability and, in some instances, on survey data, the number of malaria cases in the world in 1981 is estimated to have been of the order of 90 million.

Specific technical problems, such as vector resistance to insecticides and particularly parasite resistance to drugs, have increased in extent and intensity, considerably diminishing the effectiveness of vector control and chemotherapy as antimalaria measures.

The development of primary health care is seen as the only means of achieving health promotion, and consequently the eventual control of endemic diseases. Malaria control as a component of primary health care must first aim to prevent the mortality and reduce the human suffering associated with the disease in all areas where malaria is a serious health problem, through the provision of appropriate facilities for diagnosis and treatment.

The impact of the problem of drug resistance on the capacity to provide adequate treatment is becoming more marked as more countries try to provide treatment facilities for their entire population.

The need for research to discover new drugs with novel mechanisms of action and to find ways of preventing or delaying the development of drug resistance has become increasingly urgent.

2. DRUG RESISTANCE IN HUMAN PATHOGENIC PLASMODIA

2.1 Mechanisms of drug resistance in plasmodia

In analysing the mechanisms of drug resistance in malaria parasites four factors must be considered:

(a) How do the drugs exert their action on the target organisms?
(b) How do some of these organisms survive this action while others succumb?
(c) How is this ability to survive transmitted to the progeny in successive generations?
(d) What are the population dynamics of the sensitive and resistant organisms in a mixed population containing both?
Most of the rather limited knowledge concerning these factors in
the genus *Plasmodium* has been obtained from studies on species
infecting birds, or mammals other than man. In recent years it has
also become possible to study the malaria parasites of man, either
by infecting susceptible species of monkeys (e.g., *Aotus trivirgatus*),
or by using *Plasmodium falciparum* in continuous culture in vitro.
However, some answers to point (d) have been derived from studies
on the various species of *Plasmodium* in naturally infected human
populations in the field or in experimentally infected volunteers.

The study of malaria chemotherapy is inevitably and inextricably
linked with that of the biochemistry, physiology, molecular biology,
and genetics of the parasites and their intimate host–parasite
relationships. With few exceptions these studies have been limited
to the intraerythrocytic stages. With the recent description of
techniques to culture pre-erythrocytic schizonts of rodent malaria
parasites (23, 31) it will now be possible to extend these
investigations to the tissue stages. The biochemistry and
chemotherapy of malaria have been extensively reviewed in recent
years (6, 24, 50, 51, 53, 55, 77, 78, 81). These topics and the
pharmacology of antimalarials (including mechanisms of drug
action and resistance) are discussed in a recent monograph (56).

2.1.1 Modes of drug action

2.1.1.1 Blood schizontocides. The compounds that exert their
action primarily on the asexual intraerythrocytic stages of human
plasmodia may be classified as shown in Table 2.

The inclusion of these compounds in a particular group is based
in some cases on incomplete knowledge of their mode of action. For
example, haematin (ferriprotoporphyrin IX), a transient breakdown
product of haemoglobin within the parasite, readily disrupts
plasmodial and host membranes. It has been suggested that the
parasites synthesize a “segregating protein” which aggregates with
haematin to form the innocuous malaria pigment, haemozoin. On
the other hand, it is claimed that chloroquine readily binds to
haematin to form an even more haemolytic complex that disrupts
the parasite–host membranes, thus killing the plasmodia (20). While
it is clear that chloroquine does influence haemoglobin digestion by
the malaria parasite, the molecular mechanisms involved are still
controversial. The first change observed in parasites exposed to
chloroquine is a rapid aggregation of all the existing haemozoin
Table 2. Classification of blood schizontocides

<table>
<thead>
<tr>
<th>Main site of action</th>
<th>Compound</th>
<th>Chemical class</th>
</tr>
</thead>
<tbody>
<tr>
<td>para-aminobenzoic acid</td>
<td>dapsone</td>
<td>sulfone</td>
</tr>
<tr>
<td>incorporation* (PABA blockers)</td>
<td>sulfadoxine</td>
<td>sulfonamide</td>
</tr>
<tr>
<td>folate metabolism</td>
<td>sulfatone</td>
<td>sulfonamide</td>
</tr>
<tr>
<td>(dihydrofolate reductase</td>
<td>proguanil*</td>
<td>biguanide</td>
</tr>
<tr>
<td>inhibitors)</td>
<td>pyrimethamine</td>
<td>pyrimidine</td>
</tr>
<tr>
<td>haemoglobin digestion products</td>
<td>chloroquine</td>
<td>4-aminoquinoline</td>
</tr>
<tr>
<td></td>
<td>amodiaquine</td>
<td>4-aminoquinoline (and Mannich base)</td>
</tr>
<tr>
<td></td>
<td>quinine</td>
<td>aminoaicohol</td>
</tr>
<tr>
<td></td>
<td>mefloquine</td>
<td>aminoaicohol</td>
</tr>
<tr>
<td></td>
<td>halofantrine</td>
<td>aminoaicohol</td>
</tr>
<tr>
<td>? protein metabolism</td>
<td>artemisinine (qinghaosu)</td>
<td>sesquiterpene lactone</td>
</tr>
<tr>
<td></td>
<td>artesunate (hemisuccinyl derivative of qinghaosu)</td>
<td>sesquiterpene ester</td>
</tr>
<tr>
<td>? protein synthesis</td>
<td>tetracyclines</td>
<td>naphthacene derivatives</td>
</tr>
</tbody>
</table>

*Little if any action on P. vivax.
*Through its active metabolite, cycloguanil.

granules into a large clump. The drug may then complex with the haematin but this has not yet been shown. Similarly it is not yet certain to which sites compounds such as mefloquine and artemisinine (qinghaosu, Annex 1, No. 22) bind to exert their effects on the target organism. The affinity of mefloquine for haematin is 1000 times less than that of chloroquine.

The sites where the drugs act in the parasites are known mainly for the compounds that block the incorporation of para-aminobenzoic acid (PABA) or the synthesis of folate. Moreover, it has long been recognized that the simultaneous use of 2 drugs, one of each type, results in a potentiated effect: not only are the required doses of each compound reduced, but the speed at which the parasites succumb is increased. In addition, these drug combinations effectively kill parasites that are already moderately resistant to one or other component.

Compounds such as the 4-aminoquinolines that interact with haemoglobin digestion products are rapidly acting blood schizontocides, but several of those drugs that interrupt protein synthesis (probably as their primary mode of action) such as the artemisinine series (2I) act even more rapidly. Mefloquine has a slow onset of action which is probably related to its pharmacokinetics.
Artemisinin and artesunate, like chloroquine, are concentrated in parasitized red blood cells, but the binding sites responsible for their concentration differ. While chloroquine has been shown to intercalate between the dimers of haematin (ferriprotoporphyrin IX, a degradation product of haemoglobin within the parasite food vacuoles) (41), the other compounds appear to be bound at different sites within the parasite. Quinine and mefloquine have been shown to modify the ultrastructure of the malaria pigment (53), but the molecular basis for this effect is still uncertain. Unlike all the other compounds, the sesquiterpenes rapidly produce structural changes in the parasite nuclei, but again the exact molecular mechanism involved is unknown.

2.1.1.2 Tissue schizontocides. These are of two types, primary tissue schizontocides (causal prophylactic drugs) that affect the pre-erythrocytic (PE) schizonts, and hypnozoitocides1 that affect the latent forms, i.e., hypnozoites, now believed to be responsible for the production of true relapses in *P. vivax* infection (and *P. cynomolgi*) (30) and also possibly in *P. ovale* infection. The available drugs are listed in Table 3.

<table>
<thead>
<tr>
<th>Stage affected</th>
<th>Site of action</th>
<th>Compound</th>
<th>Chemical class</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE schizont</td>
<td>mitochondria</td>
<td>primaquine*</td>
<td>8-aminoquinoline</td>
</tr>
<tr>
<td>PE schizont</td>
<td>folate metabolism</td>
<td>proguanil</td>
<td>biguanide</td>
</tr>
<tr>
<td>hypnozoite</td>
<td>PABA incorporation*</td>
<td>pyrimethamine</td>
<td>pyrimidine</td>
</tr>
<tr>
<td>hypnozoite</td>
<td>unknown</td>
<td>sulfadoxine</td>
<td>sulfonamide</td>
</tr>
<tr>
<td>hypnozoite</td>
<td>unknown</td>
<td>primaquine*</td>
<td>8-aminoquinoline</td>
</tr>
</tbody>
</table>

*Possibly through an active metabolite.
*Not confirmed in any human species of *Plasmodium*.

While experimental compounds from numerous chemical classes have been shown, particularly in rodent malaria and *P. cynomolgi*, to exert a causal prophylactic effect (12), relatively few of them have been critically examined in the only valid model for *P. vivax*, namely *P. cynomolgi* in the rhesus monkey. The only clinically useful drugs with definite hypnozoitocidal activity to have emerged from this work are the 8-aminoquinolines, of which only primaquine is currently employed. It is postulated, but not yet proven, that it is a

1 This term is proposed to denote activity against the dormant liver forms, hypnozoites, that are responsible for relapses, in contrast to agents that act against tissue schizonts developing immediately from sporozoites.
metabolite of primaquine and not the parent compound that is active against PE schizonts and hypnozoites. The absence to date of a laboratory model more convenient than *P. cynomolgi* to test for hypnozoitocidal action makes the possible resolution of this question very difficult at present.

Evidence for the blocking action of primaquine and other related 8-aminoquinolines on the mitochondria of PE schizonts has only been obtained so far in rodent malaria (4, 61). A similar action on the mitochondria of intraerythrocytic stages has also been noted in these parasites when exposed to primaquine and to mefloquine, a naphthoquinone (25).

2.1.2 *Mechanisms by which parasites survive drug action*

Almost all the investigations carried out on this subject have been made in non-human plasmodia with the exception of a few studies on *P. falciparum in vitro.*

2.1.2.1 *Blood schizontocides.*

(1) *PABA blockers and antifols.* The resistance of microorganisms to these drugs may result from different mechanisms. These may include one or more of the following:

— modifications of drug transport mechanisms;
— gene amplification leading to an increase in the synthesis of blocked enzymes;
— production of mutant enzymes with reduced drug affinity;
— increase in drug-inactivating enzymes;
— use of alternative pathways.

So far the only mechanism to have been clearly identified in *Plasmodium* is the production of a mutant dihydrofolate reductase in pyrimethamine-resistant *P. berghei.* While several reports have indicated that sulfonamide-resistant rodent parasites can thrive in the absence of PABA (indeed, sulfonamide resistance has been produced simply by passaging parasites in PABA-deficient animals), it is not yet known how the parasites compensate for the lack of this essential metabolite (19). It was suggested that avian erythrocytes containing *P. lophurae* synthesize more folate than unparasitized red

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1 Antifols are drugs that selectively inhibit dihydrofolate reductase (EC 1.5.1.3) and/or dihydropterotate synthase (EC 2.5.1.15).
cells (83), but the evidence is not clear. It has not yet been shown whether mammalian or avian host red cells containing pyrimethamine-resistant or sulfonamide-resistant plasmodia also synthesize or transport abnormally large quantities of folate or PABA to compensate for any deficit in the "guest" parasites' own supplies of these substances—a deficit brought about by the presence of antimetabolic drugs. When considering this question it has to be remembered that resistance to an antifol (e.g., pyrimethamine), once established in the asexual intraerythrocytic stage, seems to continue through all the other stages of the life cycle.

(2) Compounds affecting haemoglobin digestion products. The classical example of this group of compounds is chloroquine. While the mode of action of chloroquine is still disputed, several aspects are certain. First, the drug is concentrated to a high level in parasitized erythrocytes from very low external chloroquine concentrations. Another point of significance is the recently reported increase in surface area of the resistant parasites, probably permitting more efficient pinocytosis to take place across the membrane separating the parasite from the host in the red cell (C. Slomianny et al., personal communication, 1983). Associated with this membrane change, there appears to be an increased production of the proteolytic enzymes required to metabolize haemoglobin (35), and this may compensate for any disturbance of amino-acid production from haemoglobin that might result from the hypothetical interaction of chloroquine with haematin.

It has been suggested, but not yet proven, that the malaria parasite synthesizes a protein that binds with haematin and segregates it in the form of the malaria pigment, haemozoin (93). Parasites in immature red cells produce less haemozoin and digest less haemoglobin compared to those in mature erythrocytes. Highly chloroquine-resistant *P. berghei* appear to produce no haemozoin, although they do live in reticulocytes. However, the presence of parasites in reticulocytes does not automatically result in the plasmodia becoming chloroquine-resistant.

Chloroquine is unusual in that it appears to be beneficial to the survival of chloroquine-resistant parasites. Moreover, there is both experimental evidence in *P. chabaudi* and circumstantial evidence in *P. falciparum* to suggest that the resistant organisms are biologically "tougher" than their drug-sensitive parents. In both *P. berghei* (65) and *P. falciparum* (92), chloroquine appears to enhance the infectivity of resistant parasites to vector anophelines. In the latter
case, this may facilitate the spread of the resistant organisms in nature to areas where chloroquine (or compounds with a similar action) is still in use.

When *P. berghei* is exposed to quinine or mefloquine the haemozoin appears to be collected into small aggregates in membrane-lined vesicles; these aggregates are quite different from the large clumps of pigment produced by 4-aminoquinolines. Moreover each granule of haemozoin seems to be relatively translucent compared with normal haemozoin. One possible explanation investigated was that haemozoin actually dissolved in quinine or mefloquine, but this could not be demonstrated experimentally *in vitro* (D.C. Warhurst, personal communication, 1983).

Whatever the means by which parasites resist chloroquine, this resistance is maintained through the whole life-cycle and is transferred to the progeny. Cross-resistance can be demonstrated when such parasites are exposed to other 4-aminoquinolines or to mepacrine, but not when exposed to quinine, mefloquine, PABA blockers, or antifols. Moreover, it has been found that the level of cross-resistance may be less against amodiaquine. This compound, in addition to being a 4-aminoquinoline, may be considered to have the structure of a Mannich base. It is interesting to note that other Mannich bases, e.g., pyronaridine (see Annex 1, No. 27) and the experimental drug WR 228, 258 (62) also show good activity against chloroquine-resistant *P. berghei* and *P. falciparum*

The disturbing feature of mefloquine resistance is that it develops more readily in parasites that are already resistant to chloroquine. This has been shown clearly in several lines of *P. berghei* (37, 57). The resistance to both compounds is retained through cyclical transmission without drug selection pressure. Reports of mefloquine resistance in *P. falciparum* are already appearing in areas where this parasite is showing resistance to chloroquine (3). The most disturbing report is that of an infection which, when treated with mefloquine, relapsed several times, showing a decreased response to mefloquine on each occasion (7). Chloroquine resistance is increasing very rapidly in the area of Africa where this patient lived (54).

(3) **Drugs possibly acting by inhibition of protein synthesis.** Resistance to these compounds could arise in a variety of ways. These may include:

— changes in drug transport mechanisms into the parasites;

16
— increase in drug-inactivating enzymes;  
— increase in production of "segregating protein", thus reducing free haematin that would otherwise disrupt parasite and host membranes.

Unlike mefloquine, artemisinine is concentrated in parasitized red cells in a somewhat random fashion and this compound does not produce the same ultrastructural changes in haemoglobin that are seen with mefloquine. Artemisinine resistance has readily been produced in P. berghei by the use of the "relapse technique" (D. James, unpublished observations, 1983), but no studies have been carried out to date on the way in which this resistance is produced.

2.1.2.2 Tissue schizontocides. It has already been indicated that resistance to proguanil and pyrimethamine is carried through all stages of the life cycle, although there is some suggestion that the causal prophylactic action of proguanil may be retained in spite of a mild degree of resistance of the blood stages. So far there is only limited resistance to primaquine, but the relative toxicity of primaquine nevertheless makes even this degree of resistance a serious matter. The mechanism of this resistance is unknown. Studies on the blood schizontocidal action of primaquine have shown that the initial disruptive effect of the compound on parasite mitochondria appears to be rapidly compensated for by an increase in the synthesis of these organelles (25). No studies have yet been made on the ultrastructure of the drug-resistant blood stages, nor of the PE schizonts from such a strain.

2.1.3 The genetics of drug resistance

Investigation of this topic was pioneered by Bishop in Cambridge and Beale and his colleagues in Edinburgh, who made extensive use of various avian and rodent malaria models (for reviews see 1, 85). So far all investigations have shown that resistance to sulfonamides, pyrimethamine, and chloroquine is transferred by classical Mendelian inheritance during sexual reproduction of the parasite in the anopheline vector. Multiple mutations at one or more gene loci seem to be involved. At present, there is no evidence for either mutational effects of any clinically used antimalarial drugs on the plasmodia, or confirmed evidence of any extranuclear transmission of resistance factors. While pyrimethamine-resistant clones appear to have a selective disadvantage compared with
sensitive *P. chabaudi*, the converse is true with chloroquine-resistant clones of this parasite. The rapid geographical spread of chloroquine-resistant *P. falciparum* in some areas suggests that the same may be true of this parasite.

Experimentally, it is easy to hybridize clones carrying resistance to one drug with clones of the same *Plasmodium* species carrying resistance to another compound. While this may account for the common occurrence in nature of *P. falciparum* that is highly resistant to both chloroquine and antifols, it is also possible that, once able to survive the presence of each compound, the parasites compensate for metabolic deficiencies created by the drugs by stimulating their host cells to increase their production or transport of the missing metabolites. Thus the continuing growth of the parasites under multiple drug pressure may be dependent upon a common factor in the new host–parasite relationship (59). This hypothetical stimulation of the host cells may facilitate the development by the parasites of resistance to other compounds. This could explain the observation that in areas of highly chloroquine-resistant *P. falciparum*, such as the Thai-Kampuchean border area, resistance to the combination of pyrimethamine and sulfadoxine is widespread and resistance to quinine increasingly common (54), while isolated cases of resistance to mefloquine have already been reported from such areas. The outlook, in short, is bleak unless other compounds with entirely new modes of action can be developed and deployed. The development of a chloroquine-resistant line of *P. falciparum in vitro* (43), another resistant to mefloquine (5), and reports of the spontaneous selection of other resistant lines in continuous *in vitro* culture (32) suggest that, at least in some areas, resistant mutants may be present in nature at a relatively high frequency.

2.1.4 The population dynamics of drug-sensitive and drug-resistant parasites

Few studies have been made on the population dynamics of mixed infections of drug-sensitive and drug-resistant malaria parasites either in animals or man, or on the influence of drug mixtures on the process of acquiring resistance. Earlier studies have been reviewed by Peters (30). Using a simple rodent model in which the rate of acquisition of drug resistance by *P. berghei* and other rodent plasmodia can be monitored in successive blood passages
(49), several authors have reported that pre-existing resistance to chloroquine facilitates the acquisition of resistance to other, unrelated compounds such as mefloquine (38, 57). In several cases pre-existing pyrimethamine resistance has facilitated the production of chloroquine resistance [e.g., P. vinckei, (64)]. By infecting mice with a known mixture of cloned chloroquine-sensitive and chloroquine-resistant lines of P. chabaudi and then cloning and identifying the surviving parasites, it has been shown that the resistant organisms later predominated (75).

Several studies have been made in avian and rodent parasites on the influence of drug mixtures on the rate of acquisition of resistance to the individual components of the mixture. The first of these studies involved various mixtures of pyrimethamine with sulfonamides or sulfones (50). In rodent models, it has been shown that while a combination of chloroquine and pyrimethamine did not delay the acquisition of resistance, the substitution of a sulfonamide for chloroquine significantly prolonged the time required for resistance to develop (59). A combination of the three compounds chloroquine, pyrimethamine, and sulfadoxine was even more effective (52). Subsequently, it was shown that either pyrimethamine or sulfadoxine were effective in combination with mefloquine (60) and that the triple combination of mefloquine, pyrimethamine, and sulfadoxine was even more effective in “protecting” all of the three components (38, 57).

2.1.5 Field observations

In the “classical” field observations, longitudinal studies were made on the geographical spread and retreat of pyrimethamine resistance in naturally acquired P. falciparum infections in Tanganyika (now the United Republic of Tanzania) (9, 10). It was found that in the absence of drug selection pressure, the resistant parasites appeared with time to be outnumbered by sensitive parasites. However, further surveys in the same area 20 years later showed the presence of pyrimethamine-resistant parasites. Until recent years little work has been carried out in the field to monitor the rates of change in the proportions of sensitive and resistant

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infections, but the development of *in vitro* drug sensitivity tests for *P. falciparum* has allowed a more detailed study to be made of clinically responsive, as well as unresponsive infections. In the United Republic of Tanzania, a large shift was recorded in the *in vivo* responsiveness of *P. falciparum* in semi-immune indigenes to chloroquine after a period of about 10 years (47). This phenomenon has been paralleled by a rapid increase in the number of reports of clinical chloroquine resistance from this and neighbouring countries, first of all in non-immune visitors and expatriate residents and, more recently, in indigenes. In addition, the level of resistance has recently been increasing. Also, there has been a parallel increase in reports in this region of resistance not only to antifols used alone for prophylaxis, but also to antifol/sulfone or sulfonamide combinations used either for prophylaxis or therapy.

In the South-East Asia Region the rapidly deteriorating situation regarding the resistance of *P. falciparum* to chloroquine, and antifol/sulfonamide combinations has been well documented, but little information is available on the quantitative changes occurring in terms of time, or on the rate of geographical spread. Moreover, no observations appear to have been made that might correlate changes in drug sensitivity to the utilization of these or related drugs (e.g., the combination of trimethoprim and sulfamethoxazole for bacterial infections) in the respective areas. Only in Thailand has an attempt been made to record the rate of change of drug response, the drug involved being quinine which is gradually beginning to lose its effectiveness in certain localities (8). Resistance to pyrimethamine appeared in the field within a year of it first being used. Significant resistance to the potentiating pyrimethamine/sulfadoxine combination only became apparent after it had been widely used for well over a decade, at least in South-East Asia.

With respect to the use of drug combinations, it seems that the association of pyrimethamine with chloroquine (both used mainly in low doses) has not delayed the appearance of pyrimethamine and chloroquine resistance; on the other hand, current observations suggest that the association of primaquine with chloroquine may have prevented or delayed the selection of resistance to chloroquine.

No field studies have been made (partly for ethical, but also for logistic reasons) to record the changes that would occur in parasite drug response if one or the other compound were to be deliberately withdrawn from an area in which resistance to it had already become established. There are verbal reports that in certain geographically
isolated areas—e.g., Zanzibar Island, some of the islands of the Solomons group, and Vanuatu—chloroquine resistance, once present, spreads extremely rapidly; it would seem that such areas might be suitable for field studies aimed at elucidating the dynamics of this phenomenon if no alternative methods are available to reduce or eliminate malaria transmission there.

As stressed at a recent WHO meeting (86), it must be remembered that the rate of selection of resistance is directly proportional to drug pressure, numbers of parasites exposed, mutation rate of the parasites, and concurrent measures (if any) taken to limit parasite transmission.

2.2 Known geographical distribution, frequency, and intensity of drug-resistant malaria

Drug resistance has been defined as the “ability of a parasite strain to multiply or to survive in the presence of concentrations of a drug that normally destroy parasites of the same species or prevent their multiplication. Such resistance may be relative (yielding to increased doses of the drug tolerated by the host) or complete (withstanding maximum doses tolerated by the host)” (88).

The resistance of *P. falciparum* to quinine was reported from Brazil as early as 1910 (42, 46), long before the advent of synthetic antimalarials.

During the 1930s mepacrine started to replace quinine as a blood schizontocidal drug, especially in the suppression (prophylaxis), but also in the treatment of acute malaria attacks. Until mepacrine was replaced by new and easier-to-use blood schizontocidal compounds in the late 1940s and early 1950s, there was no indication that resistance had developed to any significant extent or intensity in any part of the world.

Drug research programmes conducted during and after the Second World War produced highly active antimalarial compounds such as the 4-aminoquinolines (chloroquine and amodiaquine) and the dihydrofolate reductase inhibitors (proguanil and pyrimethamine) which were all introduced in the late 1940s or early 1950s.

Resistance to proguanil and pyrimethamine first occurred in the early 1950s, shortly after their wider use, especially for suppression. At the time this resistance was not considered to be of major operational importance since the 4-aminoquinolines retained their
efficacy both for curative and suppressive use. However, the almost simultaneous occurrence of chloroquine resistance in southern Asia and in South America at the end of the 1950s was considered to be serious from the beginning since it threatened to invalidate the operationally most useful drugs. This resistance stimulated drug research, and the development of the sulfadoxine/pyrimethamine combination provided the first alternative drug: it is still widely used, although resistance to it is increasing quite rapidly in various parts of the world. The occurrence of multiresistant *P. falciparum* has refocused attention on quinine which is now being used, usually together with tetracycline, as a third-line antimalarial. This is an additional function since quinine is still used as an emergency drug for the management of severe and complicated cases of falciparum malaria.

New antimalarial drugs should soon become available. Mefloquine, the most advanced, is expected to become available late in 1984, both on its own and in combination with sulfadoxine and pyrimethamine. It is in the interest of those requiring or administering antimalaria treatment to help maintain the usefulness of mefloquine and other new drugs by the judicious use of these medicaments.

2.2.1 *Drug resistance in P. falciparum*

The introduction of *in vitro* techniques for the assessment of drug sensitivity of *P. falciparum* has facilitated testing in individuals and communities; the correlation between *in vivo* and *in vitro* findings is very close in individuals and communities with moderate, little, or no immunity. However, this may not be the case in persons with longstanding, intensive contact with malaria, i.e., in semi-immunes from areas that are hyper- or holoendemic for malaria. In these persons the *in vivo* test may give a sensitive response in the presence of drug concentrations that are not curative in non-immunes, as shown by the numerous examples of sentinel cases originating from East Africa.

The *in vitro* test provides a means for the quantitative assessment of drug response related to individual (and grouped) parasite isolates. The results are therefore independent of the influence of immunity which largely determines the *in vivo* response and makes the interpretation of *in vivo* test results difficult.
Although the signs suggesting the presence of drug resistance have so far come mostly from in vivo observations, i.e., drug failures, in vitro tests are a useful additional method for monitoring the quantitative drug response. The in vitro system has the particular advantage of allowing the rapid testing of a large number of cases and the simultaneous testing of a parasite population with several drugs.

2.2.1.1 Resistance to 4-aminquinolines. Chloroquine resistance of P. falciparum was first suspected in South America in the late 1950s (50) and confirmed in 1959 in Thailand (22). Almost simultaneously the resistance occurred in a totally independent focus in Colombia. In the following years more countries in eastern Asia and South America became affected; the marked centrifugal tendency observed in eastern Asia (with the Indochina Peninsula as the centre) was less evident in South America. In 1978 East Africa also became affected, beginning in Kenya and the United Republic of Tanzania, and now parasite resistance to chloroquine is also expanding in this part of the world. The chronological sequence of detection of chloroquine resistance is shown in Fig. 1.

(1) Present distribution of chloroquine-resistant P. falciparum. The known distribution of chloroquine-resistant P. falciparum by 30 April 1983 is shown in Fig. 2. The distribution in Asia and Oceania forms a solid band extending from Vanuatu (formerly New Hebrides) to central and southern India. In South America all malarious countries are affected with the exception of Argentina, Paraguay, and Peru, which have practically no falciparum malaria. The northern limit of the distribution of chloroquine-resistant falciparum malaria is Panama, east of the Canal. This limit has been maintained since 1969. Chloroquine-resistant P. falciparum has also been found in Africa.

A list of countries where chloroquine-resistant P. falciparum is at present being transmitted is given in Table 4.

Nepal is not included in Table 4 since there is as yet no evidence that chloroquine-resistant P. falciparum is being transmitted in this country although the local drug-sensitive strain of P. falciparum is transmitted and a substantial number of resistant cases of infection are imported from India. An incompatibility between the local anopheline vectors and chloroquine-resistant P. falciparum has been proposed to explain this phenomenon.
Fig. 1. Chronological sequence of the reporting of chloroquine-resistant P. falciparum in the world.
Fig. 2. Distribution of chloroquine-resistant *P. falciparum* in the world (status as of April 1983)
Table 4. Countries and areas with chloroquine-resistant *P. falciparum* (1983)

<table>
<thead>
<tr>
<th>America</th>
<th>Asia &amp; Oceania</th>
<th>Africa</th>
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<tr>
<td>Bolivia</td>
<td>Bangladesh</td>
<td>Comoro Islands</td>
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<td>Brazil</td>
<td>Burma</td>
<td>Gabon</td>
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<tr>
<td>Colombia</td>
<td>China</td>
<td>Kenya</td>
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<td>Ecuador</td>
<td>Democratic Kampuchea</td>
<td>Madagascar</td>
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<td>French Guiana</td>
<td>East Timor</td>
<td>Malawi</td>
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<td>Guyana</td>
<td>India</td>
<td>Sudan</td>
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<td>Panama</td>
<td>Indonesia</td>
<td>Uganda</td>
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<tr>
<td>Suriname</td>
<td>Lao People's Democratic Republic</td>
<td>United Republic of Tanzania</td>
</tr>
<tr>
<td>Venezuela</td>
<td>Malaysia</td>
<td>Zambia</td>
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<td></td>
<td>Papua New Guinea</td>
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<td>Philippines</td>
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<td>Vanuatu</td>
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<td>Viet Nam</td>
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</table>

(2) **Degree and frequency of chloroquine resistance.** The frequency and degree of chloroquine-resistant cases tend to increase with the length of time drug resistance has been present in the area concerned and are also related to the intensity of drug pressure and malaria transmission after the initial selection/introduction of resistant parasites. Thus a high frequency and high degree of parasite resistance are observed in Democratic Kampuchea, Thailand, and Viet Nam, areas in which chloroquine resistance was first noted in the 1960s.

In areas where chloroquine resistance has occurred only recently, both the frequency and degree of resistance tend to be low or moderate. An example is Zambia, where the *in vivo* and *in vitro* response to chloroquine among more than 100 patients treated during clinical trials under environmentally controlled conditions was normal (S) in all cases. The two cases of confirmed chloroquine resistance were treatment failures observed outside the clinical trials.

Between the two above extremes there are all degrees of transition. It must be remembered that this grading reflects mainly the *in vivo* responses which—for areas with a high endemicity of malaria—may not provide a precise picture of the drug sensitivity of the parasite.

It is advisable, therefore, to characterize drug sensitivity using the data derived from schizont maturation or growth inhibition tests *in vitro*. The regressions obtained through probit analysis of log dose/
Fig. 3. Chloroquine sensitivity of *P. falciparum* in vitro (WHO standard test)

response tests provide a graphic representation;\(^1\) the "effective concentrations", especially the EC\(_{50}\), EC\(_{90}\), EC\(_{95}\), and EC\(_{99}\) (i.e., the drug concentrations producing 50%, 90%, 95%, or 99% inhibition of schizont maturation, respectively) are useful parameters. Sensitive isolates normally produce steep regressions with an EC\(_{50}\) well below 0.5 \(\times 10^{-6}\) mol/litre chloroquine, and an EC\(_{99}\) below 1.0 \(\times 10^{-6}\) mol/litre chloroquine. The flatter the regression line and the higher the EC\(_{99}\) (greater than 1.0 \(\times 10^{-6}\) mol/litre chloroquine) the more resistant is the isolate.

A number of representative regression lines are given in Fig. 3 to illustrate this point. The data from Malindi, Kenya (1981/82), indicate sensitivity to chloroquine. The regression from Mto Wa Bu, United Republic of Tanzania (1981), is relatively steep, but the EC\(_{99}\)

exceeds $1.0 \times 10^{-6}$ mol/litre chloroquine. The regression line for *P. falciparum* from Lambarene, Gabon (1982/83), is relatively flat and even the EC$_{95}$ exceeds $1.0 \times 10^{-6}$ mol/litre chloroquine. Although not yet expressed in gross *in vivo* drug failures in the local population (only RII and RIII responses can be identified in local circumstances), there is a high probability that sentinel cases will also show resistance *in vivo*. The regressions from Burma and Zanzibar are quite flat, indicating substantial resistance. Here the EC$_{50}$ values are near to or higher than $1.0 \times 10^{-6}$ mol/litre chloroquine, and the EC$_{95}$ values are beyond the $1.0 \times 10^{-5}$ mol/litre chloroquine level.

The *in vitro* tests also permit longitudinal comparison. The example in Fig. 4 shows the regression lines of chloroquine sensitivity of *P. falciparum* from Shirati, United Republic of Tanzania, in 1979/80 and in 1982. In this area since 1979, there has been intensive suppressive administration of chloroquine to children up to the age of 12 years. The graph shows clearly a shift to the right from drug sensitivity in 1979/80 to resistance in 1982. Moreover, the
comparison shows that significant changes in chloroquine sensitivity may occur rapidly under drug pressure in areas with intensive, unabated malaria transmission. It should also be borne in mind that the 0–5 years age-group constitutes the major gametocyte reservoir in the hyper- and holoendemic zones of tropical Africa.

(3) Resistance to amodiaquine. Despite the evidence that amodiaquine is more effective than chloroquine, this drug has not been used to the same extent. It would be logical to exploit this therapeutic advantage at least in the areas affected by low-grade chloroquine resistance, but this has not been done so far.

Recent studies in Thailand (63), where more than 85% R responses were recorded for chloroquine, indicate that radical cure was obtained with amodiaquine (total adult dose 1600 mg, over 3 days) in 43% of the cases, with regional variations between 13% (Sadao, Thai/Kampuche inference) and 76% (Petchabun, northern Thailand). RI responses accounted for 46%. The performance of amodiaquine was therefore significantly better than that of chloroquine, although the therapeutic advantage is no longer useful in practice in this area of very high chloroquine resistance.

A comparative in vivo study in Colombia in 1982 showed an absence of RIII responses under amodiaquine. RII responses were dramatically reduced compared with chloroquine, and the proportion of infections sensitive to amodiaquine was much higher.

2.2.1.2 Resistance to sulfadoxine/pyrimethamine. The combination of sulfadoxine and pyrimethamine is being extensively used as an alternative drug for the treatment of chloroquine-resistant falciparum malaria. It is also widely used for suppression and this may have accelerated the occurrence of resistance, especially in the Thai/Kampuche inference border area where its use became operationally significant in the early 1980s (26, 63). The sulfadoxine/pyrimethamine combination does not, however, provide a 100% cure rate even in persons infected with fully sensitive parasites, either as a result of drug loss (vomiting or diarrhoea) or because of individual abnormalities in sulfonamide metabolism, disposition, and elimination. The percentage of these "natural" non-responders has been given as 10–20% (63). In studies carried out in various regions of Thailand in 1980–81, the cure rates obtained varied between 32% (Thai-Kampuche inference border) and 90% (northern Thailand). On average there were 31% RI, 5% RII, and 9% RIII responses.
Resistance to sulfadoxine/pyrimethamine was most pronounced on the eastern (Kampuchean and Lao) and the western (Bur- mese) borders. Drug response to the sulfadoxine/pyrimeth- amine combination is most impaired in children (Tan Chongsuphajaisiddhi, personal communication, 1982). Although observations in Viet Nam indicated a satisfactory response to the combination with 90% S and 10% RI responses in 1981/82 (Vu Thi Phan, personal communication, 1982), there are reports of increasing failure rates from that country and from Burma and Sabah (Malaysia).

In South America, sulfadoxine/pyrimethamine appears to have maintained its efficacy better than in the “hard core” areas of eastern Asia. In clinical trials conducted with patients from Paragominas, Brazil (1980/81), some 25% R responses were recorded, most of them at the RI level. Increasing failure rates are now being reported from the Amazon basin and Colombia.

Failures of prophylaxis and treatment with sulfadoxine/ pyrimethamine have also occurred in persons who had contracted *P. falciparum* infections in East Africa, especially in Kenya and the United Republic of Tanzania (17, 29, 36, 79, 82). In view of the usual proportion of “natural” non-responders to sulfonamides (up to 20% of the population) and the large number of persons visiting East Africa who have been successfully protected by taking sulfadoxine/pyrimethamine, these reports do not necessarily reflect an increasing resistance to sulfadoxine/pyrimethamine in East Africa. However, a medium level of pyrimethamine resistance does in fact exist in this area.

Precise information on the status of *P. falciparum* sensitivity to sulfadoxine/pyrimethamine will only be available after a suitable *in vitro* test for field-collected specimens has been developed. Work in this direction is in an advanced state and a test system may soon be widely available.

2.2.1.3 Resistance to quinine. Although resistance of *P. falciparum* to quinine was reliably reported at the beginning of this century from Brazil (42, 46), the phenomenon showed virtually no signs of aggravation in spite of the widespread and intensive use of this drug before the era of synthetic antimalarials, which started in earnest only in the late 1930s. Despite wide regional variation, a dosage of 30 mg/kg body weight/day for 7–10 days produced a radical cure of most infections.
Reports of quinine resistance are still quite rare, and the alleged quinine failures are mostly the result of too short a course of treatment and/or inadequate doses. However, studies in eastern Thailand have shown true quinine resistance in this area (66). In five different areas of Thailand, an overall cure rate (S) of 90% was obtained with quinine given at an adult dose of 3 × 650 mg per day for 7 days (63). Cure rates varied between 84% at Mae Sot on the Burmese border and 96% in Kuchinarai in north-eastern Thailand. Among the ten drug failures, there were nine RI responses and one RII. When quinine was used at the same dose together with a standard dose of sulfadoxine pyrimethamine (1500 mg sulfadoxine + 75 mg pyrimethamine) or with a 7-day course of tetracycline (4 × 250 mg per day), the cure rates were 96% and 95%, respectively, with no RII or RIII responses.

In a limited study of quinine use in children with falciparum malaria in Viet Nam, administration of a daily total dose of 25 mg of quinine per kg body weight for 5 days resulted in a cure rate of only 15%, with 40% RI, 21% RII, and 23% RIII responses (Vu Thi Phan, personal communication, 1982). This poor result may not reflect true resistance since the regimen was relatively short, the dosage probably insufficient, and no loading dose was used. It is known from pharmacokinetic studies that minimum inhibitory concentrations (MIC) have to be maintained for at least 4, probably even 6 days in order to produce a radical cure. Quinine (3 × 500 mg daily for 10 days) plus the standard dose of sulfadoxine/ pyrimethamine in adults yielded 92% radical cure and 8% RI type responses, i.e., a result not much different from that of the Thai studies, especially if one considers the smaller daily dose of quinine.

Apart from South-East Asia there are only a few sporadic reports of quinine failures; however, because of progressing multiresistance in *P. falciparum*, it is important to conduct baseline assessment and monitoring of quinine sensitivity *in vitro*. An appropriate *in vitro* micro-test has been developed, validated, and recently introduced into routine use (see section 2.4.2).

2.2.1.4 Resistance to mefloquine. Clinical trials have not yet shown any evidence of a major problem of primary mefloquine resistance in *P. falciparum*. Cure rates in the dose range of 12.5–20 mg of base/kg body weight (single dose) were consistently higher than 95%; the trials have included to date more than 2000 patients in Asia, Africa, and South America. Most of the failures
(only RI type responses up to now) were associated with drug loss (early vomiting) with resulting drug concentrations below the MIC. However, two cases of primary drug resistance have been observed, one in a patient in Thailand (3) and the second in a patient who contracted the infection in East Africa (7). It has been assumed that there may be cross-resistance between quinine and mefloquine, but this must be substantiated. Similarly, the baseline *in vitro* response of *P. falciparum* should be assessed before the drug is more widely used, and monitoring should be continued.

*In vitro* macro-test and micro-test systems developed for the quantitative assessment of drug response to mefloquine have been introduced into routine use with an increasing emphasis being placed on the latter test system for standardization purposes. Some examples of regression lines obtained with grouped data from these tests in Gabon, Kenya, Thailand, and the United Republic of Tanzania are given in Fig. 5; all show sensitivity to mefloquine.

![Fig. 5. Mefloquine sensitivity of various *P. falciparum* isolates](image-url)
2.2.2 Drug resistance in other human pathogenic plasmodia

Apart from *P. falciparum*, the only other species for which some drug sensitivity data are available is *P. vivax*. In many parts of the world, *P. vivax* is known to have become resistant to dihydrofolate reductase inhibitors such as proguanil and pyrimethamine. Therefore, these drugs are of little value for the suppression of *P. vivax*, which, however, is still fully sensitive to the 4-aminoquinolines as blood schizontocides. Since sulfadoxine is a poor blood schizontocidal drug for *P. vivax*, there are many drug failures with the sulfadoxine/pyrimethamine combination in areas where *P. vivax* is pyrimethamine-resistant.

In the absence of *in vitro* test systems it is not yet possible to assess the quantitative response of *P. vivax* to antimalarial drugs.

2.3 The assessment of drug sensitivity *in vivo*

The *in vivo* techniques used at present for determining the drug susceptibility of malaria infections mostly involve the response of *P. falciparum* to chloroquine and were formulated by WHO Scientific Groups in 1965 and 1967 (89, 90). Further modifications in the interpretation of these tests were proposed by another WHO Scientific Group in 1973 (91).

During the meeting on drug-resistant malaria in the South-East Asia and Western Pacific Regions held at Kuala Lumpur in August 1981 (86) and a workshop on the epidemiology and control of *P. falciparum* malaria in the American Region held at the University of New Mexico, Albuquerque, in October 1982, a detailed review was made of the standard and alternative treatments (95). Recommendations were made on the monitoring and containment of drug resistance and on appropriate research activities.

The practical aspects of carrying out the *in vivo* test are given in Annex 4.

The performance and evaluation of *in vivo* tests for the assessment of the sensitivity of plasmodia to blood schizontocides is limited by many factors.

The lack of simple methods for the estimation, under field conditions, of the concentrations of antimalarial drugs in biological

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Footnote: 1 Annex 4 is a shortened version of an unpublished document WHO/MAL 82.988 (Payne, D. *Practical aspects of the in vivo testing for sensitivity of human Plasmodium spp. to antimalarials*).
fluids prevents a fuller understanding of the results of \textit{in vivo} tests, and their proper correlation with those of \textit{in vitro} tests. The application of a recently developed, and very efficient, \textit{in vitro} test is accumulating interesting data, but correlative information is needed on the condition of the patients and their response to the infection and treatment.

Self-medication to mitigate or prevent symptoms attributable to malaria, represents a serious constraint to performing \textit{in vivo} testing in certain areas.

Even though the antimalarial drugs available are few in number, there is little scientific information to support sound policies and strategies for the rational use of drugs, and the methodology to extend diagnosis, treatment, and information as part of primary health care strategy is often lacking. In endemic areas great difficulties exist in ensuring completion of treatment schedules and follow-up because of logistic, cultural, economic, migratory, or other obstacles. In addition, there is sometimes a lack of awareness among the population as to the threat posed by malaria and the importance of its prevention, timely diagnosis, and effective treatment.

Improved methods of obtaining information on mortality, case fatality rates, prevalence, and incidence of malaria are needed to assess the impact of intervention with antimalarial drugs.

It is important to stimulate interest among biomedical and social scientists, health service workers, and the public in the development of monitoring and surveillance systems, and in the creation of a suitable climate for effective public health action with the necessary efficacy, efficiency, and equity at a local level.

National educational and research institutions should develop an interest in field research into the chemotherapy and epidemiology of malaria.

2.4 \textit{In vitro} methods for assessing the drug sensitivity of \textit{P. falciparum}

In the late 1960s, a simple \textit{in vitro} test was developed that could assess the susceptibility of \textit{P. falciparum} to chloroquine and other antimalarial drugs by using a 10–12 ml specimen of venous blood (71).

This test measures the extent to which the maturation of ring forms to normal schizonts is inhibited after the incubation of parasitized blood at various drug concentrations for a period of
24-30 hours. In this short-term culture system, a marked difference in the maturation of sensitive and resistant parasites is observed in the presence of drug plasma levels comparable to those observed after administration of the drug in vivo. Early studies showed that this technique was a quick and reliable method for estimating the presence, prevalence, or degree of chloroquine resistance under field conditions (11, 16, 48, 58, 70, 80, 84).\textsuperscript{1}

After considerable work, a standard test kit and procedure were developed for chloroquine and mefloquine.\textsuperscript{2} The "macro-test" became the first test to be used for the routine assessment of the drug susceptibility of \textit{P. falciparum} and was incorporated into the WHO global monitoring programme (87).

Under field conditions, the main problems in carrying out this test have been the scarcity of suitable candidates for testing because they have had too many, too few, or too young parasites, and the need for venepuncture to collect specimens.

In 1978, another \textit{in vitro} field test was introduced (74). With this test, parasite susceptibility to a number of different drugs can be determined from a single specimen of capillary blood. The "micro-test" has been evaluated quite extensively under different conditions by WHO and other investigators and it will probably eventually replace the macro-test except in individual studies.

Other \textit{in vitro} tests that have been developed are (a) the "48-hour test" (44), used mainly for determining the susceptibility of \textit{P. falciparum} to pyrimethamine and (b) a semi-automated test (13) that measures the effect of a drug by the inhibition of incorporation of radioactive hypoxanthine into the parasite. This sensitive method has been widely used experimentally to study the effects of antimalarial drugs and drug interactions. However, it can only be used in well-equipped laboratories and does not have direct field applications.

The principal problems encountered with the \textit{in vitro} field test systems have been self medication, lack or failure of electricity supplies, and bacterial contamination.


\textsuperscript{2} \textit{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).
2.4.1 The macro-test

2.4.1.1 Procedure. The test procedure involves the following steps:

(a) Thick and thin blood films are collected from a patient suspected of having falciparum malaria and are examined for the presence of asexual forms of *P. falciparum*.

(b) Patients excluded from the test are those who have taken antimalarial drugs recently, are severely ill or vomiting, have parasite counts lower than 1000/mm$^3$ or higher than 80,000/mm$^3$, have predominantly young ring forms, or have only gametocytes of *P. falciparum*.

(c) Urine specimens, collected from suitable patients who are willing to participate in the test, are examined for the presence of chloroquine and, if possible, other antimalarials. Patients whose urine contains drugs are excluded from the test.

(d) About 10–12 ml of blood are collected from the patient, transferred immediately to a sterile flask containing glass beads, and defibrinated by rotation of the flask for 5 minutes.

(e) 1-ml aliquots of blood are placed in sterile, screw-capped, flat-bottomed glass vials (1.5-cm internal diameter) containing glucose (5 mg) and either no drug (control) or increasing quantities of drug.

(f) The mixture in the vials is swirled gently to mix the contents well, the caps are unscrewed about half a turn, and the vials are placed in an incubator or water bath at 38–40°C for about 24 hours.

(g) After incubation, the vials are shaken to resuspend the erythrocytes in plasma, thick films are prepared and stained for 20 minutes with 5% Giemsa stain (pH 7.0).

(h) Drug-induced inhibition of the maturation of ring forms to normal schizonts is determined by comparing the degree of maturation in control samples with that in samples containing various concentrations of the drug. The percentage of ring forms that mature to schizonts of normal appearance containing more than two nuclei is determined by microscopic examination and provides a useful end-point for the quantitative assessment of maturation. Usually, the number of schizonts per 300 or 1000 leukocytes is counted in each sample and the results are expressed by dividing the number of schizonts in a drug sample by the corresponding value observed in the control samples. For example, if the drug sample contained 12 schizonts per 300 leukocytes and the
mean of the two control samples was 60 schizonts per 300 leukocytes, then 20% of parasites were able to mature to schizonts in the presence of the drug.

2.4.1.2 Effects of chloroquine. The in vitro susceptibility of different strains of *P. falciparum* to chloroquine has been studied more thoroughly and extensively than for other antimalarial drugs. The results of some of these investigations are shown in Fig. 6. As comparative in vitro—in vivo testing was carried out in a few studies, it is now possible to relate the parasite sensitivity in vitro to the response of non-immune individuals treated with chloroquine. Complete inhibition of schizont formation at a chloroquine concentration of 1.0 or 1.1 μmol/litre of blood indicates an infection with chloroquine-sensitive parasites, whereas chloroquine-resistant parasites mature to schizonts at these drug concentrations. Maturation to schizonts is often observed at concentrations up to 1.5 or 2.0 μmol of chloroquine per litre of blood with an RI level of resistance, at chloroquine concentrations up to 2.4 or 3.0 μmol/litre with an RII level of resistance, and above 4.0 μmol of chloroquine per litre with an RIII level of resistance in non-immune individuals.

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Fig. 6. Chloroquine sensitivity of *P. falciparum* in vitro and in vivo*

2.4.1.3 Effects of other antimalarial drugs.

(1) Amodiaquine. The effects of amodiaquine upon the morphological appearance of parasites of *P. falciparum* is identical to that observed with chloroquine. The most striking effect of both of these 4-aminoquinolines is an inhibition of maturation at successively earlier stages of development as ring forms are exposed to increasing concentrations of the drugs. Mole for mole, the activity of amodiaquine *in vitro* is greater than that of chloroquine against a strain of *P. falciparum* with a high level of resistance to chloroquine. Subsequently, this was also shown to be the case *in vivo* (67).

(2) Quinine. The effects of quinine upon the morphological appearance of parasites are similar to those observed with the 4-aminoquinolines. In chloroquine-sensitive strains, the quinine concentration required to inhibit the maturation of schizonts is about 10 times greater than the chloroquine concentration needed to produce a similar effect in parasites that are fully sensitive to both drugs (71). After administration of curative doses, the quinine levels in plasma are ten times higher than those observed under chloroquine.

(3) Mefloquine. The new antimalarial drug, mefloquine (WR 142490), exerts an effect upon the morphology of the parasites *in vitro* similar to that of chloroquine. However, it is considerably more effective than chloroquine, both *in vitro* and *in vivo*, against strains of *P. falciparum* with a high level of resistance to chloroquine (73).

(4) Dihydrofolate reductase (DHFR) inhibitors. The effects of DHFR inhibitors, such as cycloguanil and pyrimethamine, upon the maturation of parasites differ from those observed with the above drugs (71); even high concentrations of the DHFR inhibitors do not prevent the maturation of ring forms to trophozoites. The most conspicuous effect of DHFR inhibitors is the formation of parasites with an abnormal appearance (71). This had been previously observed in patients treated with proguanil, presumably because the parasites were exposed to the dihydrotriazine (cycloguanil) metabolite of the drug (2, 34). After exposure to DHFR inhibitors, the chromatin of the parasites, does not divide normally to form schizonts, but is split into indistinct fragments of varying size and shape. The difference in sensitivity to DHFR inhibitors between drug-sensitive and drug-resistant strains is usually much greater than that observed with other antimalarial drugs, e.g., 4-aminoquinolines.
(5) Drugs that show no activity in vitro. Maturation of parasites is not affected by the addition of drugs that exert their antimalarial activity only after metabolic transformation by the host. Thus, proguanil shows no activity in vitro, but its dihydrotriazine metabolite, cycloguanil, shows a marked DHFR inhibitor effect upon maturing parasites (71). This characteristic could be used to compare the relative antimalarial activity of a parent compound and its metabolites in the in vitro test.

In addition, the determination of parasite susceptibility to some slow-acting drugs, such as the sulfonamides, sulfones, and tetracyclines, is not possible in a short-term in vitro system that cannot support parasite maturation for longer than 30 hours. If a slow-acting drug does not exert its activity through its metabolite(s), it should be possible to determine the antimalarial activity of the drug by the in vitro micro-test (see section 2.4.2.2). As parasite growth can be supported through one or more complete life cycles with this procedure, the effects of a slow-acting drug can be studied over a longer period of time.

2.4.2 The micro-test

The introduction of a micro-test for assessing the drug susceptibility of *P. falciparum* (74) has many advantages; among other things it has made it easier to carry out sensitivity testing in young children. The decreased volume of blood required for this technique means that specimens can be collected by finger-prick rather than by venepuncture. In contrast to the macro-test, parasites are able to mature to schizonts when counts exceed 80,000/μl and, as parasites do not degenerate when incubated for longer than 30 hours, the period of incubation can be extended to enable young rings to develop into schizonts (68). The period of incubation can be extended to 48 hours without a medium change, so that the effects of a drug on the parasite reinvasion of erythrocytes can be studied (94).

2.4.2.1 Procedure. The test procedure involves the following steps:

(a) Preliminary examination of patients suspected of having *falciparum* malaria should follow the macro-test procedure (see section 2.4.1.1).
(b) 100-μl sample of blood is collected from the tip of a finger and transferred immediately into a presterilized plastic vial containing 0.9 ml of culture medium. The medium consists of powdered RPMI 1640 (10.4 mg/ml), sodium bicarbonate (2 mg/ml), HEPES buffer (6 mg/ml), and gentamycin sulfate (4 μg/ml). This quantity of blood is sufficient to determine the susceptibility of parasites to at least two drugs.

(c) The blood–medium mixture (1:9; for 48-hour cultures, 1:19) is then transferred, in 50-μl quantities, to flat-bottomed wells (diameter, 6.5 mm) or a plastic microculture plate containing a range of concentrations of the selected drugs.

(d) If necessary, the blood–medium mixture can be transported to the laboratory in the vials, keeping the vials fairly close to body temperature (e.g., in a shirt pocket) during transportation, and transferring the blood–medium mixture to the culture plates within 6 hours of collecting the blood specimens.

(e) The plate, covered with a lid, is then agitated gently and placed in a jar containing a pure paraffin candle and a damp sponge. After lighting the candle, the air-tight lid of the jar is replaced and the culture plate incubated at 37–38 °C for 24–48 hours. The duration of incubation will vary depending on the maturity of rings at the start of culture and on the stage of parasite growth desired at the end of culture.

(f) After incubation, about 30–40 μl of supernatant culture medium are removed from each well and thick films are prepared from the sediment.

(g) Giemsa-stained thick films are examined for parasite maturation. As a higher proportion of ring forms mature to schizonts than is observed in the macro-test, it is usually sufficient to count only 100 or 200 asexual parasites to obtain an accurate estimate of the extent of maturation. Results may be expressed as described previously for the macro-test.

2.4.2.2 Advantages and applications of the micro-test.

(1) Advantages. The main advantages of the micro-test are that parasitized specimens of blood can be obtained by finger-prick and they can be used irrespective of the stage of development or density of the parasites.

Following the initial laboratory studies with two strains of P. falciparum (74), a number of studies have shown the value of this technique in assessing the sensitivity of P. falciparum to chloroquine
and other drugs under both field and laboratory conditions (28, 33, 68). The micro-test is largely replacing the macro-test as the standard procedure for assessing the presence, prevalence, and degree of drug resistance in various parts of the world. The development of inexpensive field incubators, operated by rechargeable batteries, makes it more feasible to apply the test in remote areas (14).

The drug sensitivity of parasites in patients with acute falciparum malaria can be determined more readily by the micro-test because young ring forms, the stage that predominates during acute febrile episodes, are able to mature to schizonts during 30–42 hours of incubation. As the results of the sensitivity test are available within 2 days of the start of treatment, the initial chemotherapeutic regimen can be modified if the in vitro results show that alternative drugs might be more effective in achieving a complete cure.

(2) Determination of baseline sensitivity to chloroquine and other drugs. In areas where falciparum infections are still sensitive to chloroquine treatment, this test should be valuable in obtaining baseline values against which subsequent changes in chloroquine sensitivity can be measured. In addition, the sensitivity of local strains of *P. falciparum* to new drugs, e.g., mefloquine, can be determined in areas where these drugs might be used in the future. The minute quantities of drug required for the micro-test are particularly advantageous when only a limited quantity of a new drug is available for experimental studies and also when values of parasite sensitivity in natural isolates are required.

(3) Determination of sensitivity to sulfonamide/pyrimethamine combinations. Since a single dose of sulfadoxine and pyrimethamine is becoming less effective as an alternative antimalarial medication in many chloroquine-resistant areas, it is important to be able to determine parasite sensitivity to the sulfonamides and pyrimethamine. Although sulfonamides alone have no effect on parasites cultured in ordinary RPMI 1640 medium, the antimalarial activity of pyrimethamine in the micro-test is increased by the presence of sulfonamides (15, 94).

Recent studies have shown that sulfonamides alone can exert a marked antimalarial activity in vitro when used with culture media containing little or no folate (R. E. Desjardins and R. Reber-Liske, personal communications, 1983). Because of these findings, it was suggested at a recent WHO meeting in Bangkok (April 1983) that the use of a culture medium containing approximately the concentrations of PABA and folate found in the blood might
provide the most accurate way of determining the sensitivity of parasites to sulfadoxine/pyrimethamine in the micro-test. As relatively inexperienced microscopists may have difficulty in differentiating between “normal” and “abnormal” schizonts (see section 2.4.1.3, DHFR inhibitors), an easier end-point for determining drug action may be to use the extent to which parasite reinvasion of erythrocytes is inhibited during 48 hours of incubation.

(4) Determination of sensitivity to slow-acting antimalarial drugs.

The activity of slow-acting antimalarials, e.g., tetracyclines, can be determined using this in vitro test because the parasites can be exposed to the drug for more than one developmental life cycle. The only disadvantages are that it is necessary to replace the medium and drugs on one or more occasions and to continue the observations for a longer period.

2.4.3 The 48-hour test

This test is very similar to the micro-test (44, 45); however, it uses about 4 times as much blood and 10 times as much culture medium per well (diameter, 16 mm). The concentration of blood in the medium is lower than that for the micro-test, i.e., 4% as opposed to 10%. In addition, the medium is supplemented by 10% group AB serum. The end-point for assessing drug activity is parasite reinvasion of erythrocytes as determined by examination of thin films prepared after an incubation period of 48 hours. Initially, the medium was changed after the first 24 hours of incubation, but this is no longer considered necessary (P. Nguyen-Dinh, personal communication, 1982). Although the test has been used to evaluate parasite sensitivity to chloroquine, it is felt that its main value may be for determining parasite sensitivity to DHFR inhibitors such as pyrimethamine.

2.4.4 Visual micro-test

Using parasites obtained from continuous culture, a technique was developed recently that can detect drug activity without the use of a microscope (69). This test is based on the principle that malaria pigment, produced during the maturation of rings to schizonts, forms a precipitate after the addition of an alkali. The precipitate formed in the microculture is visible to the naked eye. In the presence of effective drug concentrations precipitates are not observed,
presumably because the rings are unable to mature to pigment-producing schizonts.

A field study was carried out under the auspices of WHO in Thailand during May 1983 to determine whether this visual test could be used to assess the drug sensitivity of parasites from malaria patients. Unfortunately, normal and malaria-infected blood produced precipitates in wells containing no drug as well as in those containing effective drug levels. The removal of leukocytes by cellulose column filtration removed the precipitate from normal blood but not entirely from malaria-infected blood. (Leukocytes are usually present at low levels in stored blood used for the continuous culture of parasites.) Further studies are needed to determine whether modification of the technique might yield a visual test that can be used for parasites obtained directly from malaria-infected individuals.

2.4.5 Pitfalls

2.4.5.1 Mixed P. falciparum-P. malariae infections. In areas where both P. falciparum and P. malariae are prevalent results obtained with the in vitro test must be interpreted with caution.1 “Unidentified” young ring forms of P. malariae (easily confused with more mature P. falciparum rings) may mature to schizonts at chloroquine concentrations that invariably prevent schizogony of chloroquine-sensitive parasites of P. falciparum. In mixed infections, with only a few P. malariae parasites, the mistaken diagnosis of chloroquine resistance can only be avoided by a careful search for typical P. malariae parasites in pre- and post-culture thick and thin blood films.

2.4.5.2 Comparison of results obtained by macro-test and micro-test. At equivalent blood concentrations of chloroquine, inhibition of growth in the micro-test is similar to that observed in the macro-test (74, 94). Blood specimens containing more than 80,000 or 100,000 parasites per μl of blood require higher

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concentrations of chloroquine to achieve a similar inhibition of growth (68, 74, 96). Since growth at such high parasite densities is not possible in the macro-test, this difference in drug effect need only be considered in relation to the micro-test.

Although comparable results for chloroquine are obtained with the macro- and micro-test per unit of blood or erythrocytes, this is not necessarily the case with other drugs. For example, the activity of pyrimethamine is related to the concentration of the drug per unit volume of fluid (i.e., medium, serum).

2.4.5.3 Comparison of effective in vitro concentrations of different drugs and their application in vivo. Recently, attempts have been made to predict the in vivo response to drugs, on the basis of in vitro findings. It is likely that the in vivo response to a specific drug can be predicted with a certain amount of confidence on the basis of extensive comparative in vitro—in vivo studies carried out in non-immune and immune patients. However, such predictions should not be made without these essential comparative studies.

Consideration of the drug amodiaquine illustrates this point. As is well known, most of the isolates of *P. falciparum* so far examined are more sensitive to amodiaquine than to equivalent doses of chloroquine in vivo. However, the degree of this greater sensitivity to amodiaquine in vivo cannot be predicted on the basis of in vitro findings alone. Comparative in vitro—in vivo studies are needed to clarify the situation. For example, the Viet Nam (Marks) strain has an RIII level of resistance to chloroquine (25 mg/kg body weight) and an RI level of resistance to amodiaquine (25 mg/kg body weight) in non-immunes. In vitro studies showed that 4.0 or 5.0 μmol of chloroquine and about 0.3 μmol of amodiaquine per litre of blood were needed to inhibit schizont formation completely.¹ The Uganda I strain, which is sensitive in vivo to 10 mg/kg, required 0.5 μmol of chloroquine and 0.1 or 0.15 μmol of amodiaquine per litre of blood for complete inhibition of schizont formation.¹ Mole for mole, amodiaquine is obviously more active in vitro than chloroquine in an isolate with comparable in vivo susceptibility.

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).
2.5 Monitoring the response to antimalarial drugs

In principle, monitoring of the drug response in malaria by in vivo and in vitro tests should consist of the systematic and standardized measurement and recording of the response of malaria parasites to drugs, the geographical variation of the response, and its evolution with time. This monitoring process is aimed at improving the control of malaria by delineating the appearance and spread of drug resistance.

The need for some form of monitoring to improve malaria control is generally recognized, but there are several unresolved problems regarding what is technically desirable and what is operationally feasible.

2.5.1 Objectives of monitoring drug sensitivity

The final objective of monitoring is to improve malaria control and this can be achieved via various intermediate objectives. Fig. 7 shows a tentative schematic representation of the links between monitoring of drug sensitivity and malaria control.

The aim of descriptive epidemiology is to describe the distribution of drug resistance, while the aim of analytic epidemiology, mainly on the basis of associated variables, is to explain the distribution of drug resistance in an attempt to identify possible ways of modifying it. As indicated in Fig. 7, the explanation of the distribution of drug resistance includes, in addition to the findings of analytic epidemiology, those of basic research on the mechanisms, genetics, and selection of drug resistance. Experimental epidemiology evaluates the effects on drug resistance of interventions in a controlled (experimental) setting. It is the object of short-term drug policies to adapt recommended therapeutic and prophylactic drug regimens to the existing requirements, with the aim of preventing morbidity and mortality that might result from the use of a drug regimen to which the parasite is insensitive; this also applies to the advice given to travellers. As shown in Fig. 7, the formulation of short-term drug policy uses, in addition to the findings of descriptive epidemiology, the results of drug trials. Long-term drug policies are the measures used to delay the selection of drug resistance.

Specific questions that might be resolved by monitoring are:
— is resistance present in an area or a population?
— has resistance, in an area or a population, reached a critical level requiring some specific action?
— how does the parasite population, in an area or a population, respond to a given drug?
— is there a difference in drug response between parasite populations in different places or at different times?
— what are the factors and their variations that may be associated with a variation in drug response?

So far, monitoring has been used mainly to guide palliative measures, and at present, it is almost impossible to apply preventive measures. Existing knowledge of the epidemiology of drug resistance is not yet adequate to define a rational policy and, even if a completely rational policy could be defined and agreed upon, implementation and compliance would be limited by the attitude of the general public, the behaviour of the drug suppliers (public and private), and by commercial interests.

2.5.2 Current monitoring activities

The assessment of the sensitivity of malaria parasites to drugs has been carried out for a long time and is currently performed in numerous places by many investigators and supported by several sponsors. All of these activities are relevant to the development of a true monitoring system. Several institutions, mainly WHO sponsored, attempt to gather together the results of as many of these activities as possible to include them in an up-to-date description of the situation. Within WHO, the recently developed computerized global monitoring project tries to build upon and coordinate these activities.

The technical objectives of monitoring are to standardize the recording and analysis of test results, to provide a comprehensive and accessible data bank, and to produce periodic reviews of the situation. Governments, public health authorities, and individual investigators are provided with pre-coded forms and are invited to send their test results to WHO Headquarters in Geneva, where processing and analysis are carried out at present. It is hoped that in the future facilities will be developed at WHO Regional Office and country levels, while still maintaining standardization and a global data bank. Two standardized forms have been developed for the recording of results, one for in vitro testing of the sensitivity of *P. falciparum* to chloroquine and mefloquine (macro-, and/or micro-test) and one for in vivo testing of the sensitivity of any species of malaria parasite to most drug regimens, including combinations, together with instructions for their use. The practical aspects of sensitivity testing are dealt with in more detail in Annex 4.
Computer programmes are available for checking, editing, and analysing both in vitro and in vivo results.¹

Regions and countries are regularly consulted and some problems have been identified. Participation has not, as yet, reached the required level and there is a problem in obtaining information from all existing sources. Methods of sampling, the provision of staff, and running costs for the field activities and for the computer analysis of data are all difficulties that will need to be resolved.

2.6 Criteria for the assessment of drug resistance

The criteria used up until now to classify the in vivo drug response of *P. falciparum* to 4-aminoquinolines are still valid (6). However, the definitions are sometimes not well understood and have occasionally resulted in misinterpretations. A review of the criteria is urgently needed to identify and eliminate any existing ambiguities.

The criteria used at present for the assessment of drug response to 4-aminoquinolines have been widely adopted for other drugs. However, before these criteria are accepted and standardized, more in-depth observations are urgently required, especially regarding the route of drug administration, the regimen employed, the physical condition of the patient, and the duration of post-treatment observation.

2.7 Measures to prevent or restrict the occurrence and spread of drug resistance

2.7.1 Measures to be taken

The prevention of the further spread of drug-resistant *P. falciparum* requires that the boundaries of areas already affected be known and that the eventual diffusion and severity of the phenomenon be monitored. For this purpose, the World Health Organization has organized the global monitoring programme (87) whose main objectives have been reviewed in section 2.5 above.

¹ Two reports have been prepared: Molineaux, L. Global monitoring of susceptibility of malaria parasites to drugs. Preliminary report, June 1982 (unpublished WHO document MAP/SCGM/INF/83.1); and Molineaux, L. Global monitoring of susceptibility of malaria parasites to drugs. Second report, October 1983 (unpublished WHO document MAP/83.3). These reports are available on request from the Malaria Action Programme, World Health Organization, Geneva, Switzerland.
In selecting the specific measures necessary to prevent the introduction or development of drug-resistant *P. falciparum* parasites in an area, a distinction can be made between regions where malaria has been eradicated or has never existed but where the potential for transmission is still high, and regions where the disease is endemic.

2.7.1.1 *Malaria-free but highly receptive and vulnerable areas.* Preventive action should include the three elements that are involved in malaria transmission, namely the vector, the parasite, and the human host. However, if the measures are to be effective, there are certain prerequisites that must be observed.

(a) The vector. In areas with a previous history of malaria, information on the distribution, the density, and the bionomics of the vector population should be brought up to date (assessment of receptivity).

The susceptibility of vectors to the most commonly used insecticides should be monitored at regular intervals.

The international sanitary regulations relating to the disinsection of aircraft transporting passengers and goods should be rigorously applied.

(b) The parasite. Centres for a prompt and precise microscopic diagnosis should be available.

*In vivo* and *in vitro* studies should be carried out to investigate reported cases of failure of malaria treatment and to follow the treatment of cases imported from areas with drug-resistant *P. falciparum*.

Adequate knowledge and competence for the provision of effective and rapid radical treatment of *P. falciparum* infections must be immediately available.

Immediate notification of *P. falciparum* resistant cases to national authorities and to WHO should be encouraged.

Microscopic examinations and treatment should be performed on particular groups (refugees, students, migrant labourers, etc.) at their port of entry or in reception camps, especially if they arrive from countries affected by drug resistance.

(c) The human host. Training courses for academic and technical personnel, specialized in malaria and malaria control should be promoted.

A specialized group of technical personnel, together with the necessary specific infrastructure should be available in the national
health system to permit rapid and efficient intervention should local foci of transmission occur.

Strict collaboration should be established between the medical services and the immigration authorities in order to deal promptly and efficiently with parasite carriers upon their arrival.

2.7.1.2 Areas where malaria is endemic. Most of the measures that have been recommended above, will also be applicable to areas where malaria is endemic. In these areas, however, other measures should be introduced in an attempt to prevent the introduction, development, and spread of isolates of drug-resistant P. falciparum. These measures are:

— rational use of antimalarial drugs;
— provision of an effective radical treatment of P. falciparum;
— establishment of “monitoring/sentinel” posts in representative geographical areas for obtaining baseline information on P. falciparum sensitivity to antimalarials and for conducting more detailed in vivo and in vitro studies whenever there are signs of changes in sensitivity;
— assessment of sensitivity of local P. falciparum to antimalarial drugs currently in use and monitoring of the situation in subsequent years;
— gathering of knowledge on migratory movements of the population and establishment of diagnostic and treatment facilities on known migratory routes from areas where there is drug resistance.

In areas where resistant P. falciparum is well established and widespread, intervention should be aimed at controlling transmission and reducing the prevalence of the parasite.

Specific action should include:

(a) mapping the distribution and intensity of P. falciparum resistance by in vivo and in vitro studies;
(b) introduction of effective antivector measures, such as house-spraying with residual insecticides and the use of other chemical or biological agents, or environmental manipulations or modifications, applied either singly or in combination;
(c) monitoring the extent and degree of resistance by periodic in vivo and in vitro studies in indicator areas and on an ad hoc sampling basis;

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(d) assessment of the sensitivity of local *P. falciparum* to current antimalarial drugs in representative geographical areas;
(e) establishment of an information system to monitor the clinical response to alternative antimalarial drugs;
(f) promotion of health education in order to create and eventually modify population attitudes and beliefs to give a better community awareness of malaria, its prevention, and control, and to obtain community acceptance of medication, attendance at treatment posts, and use of self-protective measures;
(g) establishment of diagnostic/treatment centres in strategic positions for a prompt diagnosis and early effective treatment of *P. falciparum* infections, including an effective gametocytocide;
(h) introduction and enforcement of a rational use of antimalarial drugs.

2.7.1.3 Combinations of drugs. Combinations of drugs for antimalarial chemotherapy and prophylaxis have been used for many years. Some of these have been developed rationally, such as the potentiating combination of dihydrofolate reductase inhibitors with sulfa drugs, while others (e.g., chloroquine with pyrimethamine) lacked a sound scientific basis.

Recent experimental studies in rodent malaria suggest that combinations of certain antimalarial compounds may retard the development of resistance to the individual compounds (38, 57, 59, 60). This has been the rationale behind the development of the combination mefloquine pyrimethamine sulfadoxine. This combination is considered to be the best available at present but others may be developed in the future. Not all drug combinations will be beneficial and the following principles should be considered when developing combinations of antimalarial drugs:

--- antimalarial activity may be: potentiating
additive
antagonistic;

--- development of resistance to the compounds may be: delayed
unchanged
enhanced;

--- toxicity may be: reduced
additive
potentiating.
Pharmacokinetic considerations must also be taken into account. The individual drug components should be matched correctly so that active concentrations of all the components are maintained during the dosage interval. The interaction of the compounds regarding bioavailability, distribution, metabolism, and elimination must also be considered.

Thus, an ideal combination is one that is potentiating and well matched, has reduced toxicity, and delays the emergence of resistance to the individual components. In this way it may be possible to produce formulations that are more acceptable, have fewer side-effects, and result in improved logistics and patient compliance.

2.7.2 Difficulties in implementing the recommended measures

From the list of measures suggested it can be concluded that several possibilities exist to deal with and counteract the phenomenon of drug resistance. It must be realized, however, that while it is relatively easy to draw up a list of possible interventions, serious difficulties may sometimes be encountered in their implementation. The type and adequacy of the remedial measures applied depend quite often on the health facilities and the financial and human resources available in the country concerned. Moreover, some of the measures that have been repeatedly recommended over the years, sound more like dogmatic statements than realistic and implementable remedial actions. Attention is drawn to the following points:

(a) The rational use of antimalarial drugs. In endemic areas where *P. falciparum* is still sensitive to 4-aminoquinolines, these drugs can and must continue to be used provided, however, that they are given at doses high enough to obtain a radical cure. In many areas of tropical Africa, complete elimination of the erythrocytic parasite forms is no longer possible with a single-dose treatment (10 mg of base/kg, adult dosage) as was the case a few years ago (47), and the full 3-day treatment (25 mg/kg) should be given, although this may create difficulties with regard to patient compliance.

(b) Impossibility of controlling the distribution and utilization of drugs. In many areas where *P. falciparum* drug resistance is well established, antimalarials of all types and in different combinations are readily available in local markets, where they can be purchased easily; but they are usually taken at sub-curative doses.

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Warnings have been repeatedly issued to prevent the use of antimalarials for mass drug administration or presumptive treatment since, in the absence of well-conducted vector-control operations, they are unlikely to reduce the prevalence of the disease.

The second-line antimalarial, sulfadoxine/pyrimethamine, is quite often prescribed for the protection of non-immune individuals visiting areas where *P. falciparum* chloroquine resistance has not yet been reported, or only a few cases have been found at the RI resistance level. There is also a growing and dangerous tendency to replace the 4-aminoquinolines with sulfadoxine/pyrimethamine for the treatment of *P. falciparum* infections in areas where the parasites are still sensitive to the 4-aminoquinolines.

(c) *Difficulty in providing effective treatment for chloroquine-resistant* *P. falciparum*. The provision of an adequate and effective radical treatment of *P. falciparum* infections in large areas of the Mekong river basin has become a major problem owing to the presence of resistance to 4-aminoquinolines, sulfadoxine/pyrimethamine, and quinine. When mefloquine becomes available, the situation may improve, provided, however, that the new drug is properly used (see section 4.2.5).

(d) *Difficulty in implementing effective vector-control operations*. In large areas of South-East Asia, where transmission is maintained by *Anopheles dirus* (formerly *A. balabacensis balabacensis*) and in vast areas of South America where *A. nuneztovari*-transmitted malaria prevails, it is extremely difficult to conduct effective vector-control operations because of the exophagic and exophilic tendencies of the vectors. This problem is often complicated by the presence of political turmoil that results in security problems and uncontrollable movements of the population.

The examples referred to above are far from exhaustive, but explain, at least in part, the continuing increase in drug resistance and the limited opportunities available to control its spread.

### 2.7.3 Gaps in our knowledge

Although the problem of *P. falciparum* drug resistance has been known to exist for more than two decades, a great deal remains to be learned about the epidemiology of this phenomenon. Consequently, the present policies for controlling the selection and development of drug-resistant parasites and preventing their spread can only be improved if certain gaps in our knowledge are filled or clarified.
Experience accumulated over the years seems to indicate that an important correlation exists between drug pressure and the selection of resistant parasites. The appearance of drug resistance following the implementation of medicated salt schemes (18, 39, 40) and the recent discovery of chloroquine resistance among semi-immunes in the United Republic of Tanzania (27, 47), Zanzibar (76), and Madagascar (V. Pansini, personal communication, 1983) where intensive chloroquine pressure has existed in past and recent years, seem to be more than a coincidence.

What remains to be ascertained is the mechanism by which selection occurs. In this connection, improved knowledge of the genetics and molecular basis of drug resistance is required as well as more information on the presence of different parasite populations (highly resistant, resistant, sensitive, highly sensitive) and their proportions in the same isolate. This involves the cloning of P. falciparum from the field, both from areas where drug resistance has not yet been established, and from areas affected by different degrees of resistance.

More information is needed on whether and how chemotherapy and chemoprophylaxis influence the immune response of the patient, and on their role in selecting drug-resistant parasites. Similarly, it should be assessed whether and to what degree the immune response modifies the result of chemotherapy and chemoprophylaxis.

The susceptibility of vectors to infection with drug-resistant or drug-susceptible P. falciparum isolates also needs further investigation.

It is not yet known whether there is any relationship between the resistance of parasites to DHFR inhibitors, especially pyrimethamine, and their resistance to chloroquine. It is also not known whether there is any potential value in adding a potent gametocytocidal and sporontocidal (primaquine) (72) to an effective blood schizontocidal to prevent the transmission of drug-resistant parasites in the absence of other measures to control malaria transmission.

Laboratory and field studies are still required to establish the optimal dosage and regimen of primaquine needed to produce a full sporontocidal and gametocytocidal effect, to investigate parasitologically and entomologically its sporontocidal and gametocytocidal effect on populations from areas of different endemicity, and to confirm epidemiologically the effect of primaquine on malaria transmission in an endemic area.
REFERENCES


71. RIECKMANN, K. H., ET AL. Effects of chloroquine, quinine, and cycloguanil upon the maturation of asexual erythrocytic forms of two strains of Plasmodium
3. USE OF ANTIMALARIAL DRUGS

3.1 Clinical treatment of falciparum malaria

3.1.1 Treatment of acute falciparum malaria

The clinical features of acute falciparum malaria are highly variable and depend upon the level of immunity of the host, the duration and hence the level of parasitaemia, and the occurrence of complications (cerebral malaria, algid malaria, hyperpyrexia, etc.). Treatment will depend upon these factors as well as the sensitivity of the particular parasite population to antimalarial drugs. Special care is needed when treating acute falciparum malaria in pregnant women; the incidence of severe forms of infection and complications is significantly increased during pregnancy (see section 3.1.3).

Acute falciparum malaria is a potentially lethal disease, and complicated cases should be considered medical emergencies. Whenever possible, a parasite count should be made to assist treatment and prognosis, and serial counts should be made to assess the effect of treatment on the parasitaemia. The choice of the antimalarial drug used for treatment should be guided by the geographical origin of the infection.

3.1.1.1 Infections sensitive to the 4-aminoquinolines. Adults should receive a course of treatment totalling 25 mg/kg body weight of chloroquine or amodiaquine base in several doses over a period of 3 days. Variations of this regimen are used in different countries,
but most are based on the administration of 900 mg (in divided doses) on the first day followed by smaller doses on subsequent days (see Table 5).

In areas where the parasites are sensitive, treatment with a single dose of 600 mg of chloroquine or amodiaquine base has been in many cases enough to terminate a mild attack of uncomplicated falciparum malaria in partially immune adults. However, the use of this low dose may preferentially select less sensitive parasites; the objective of treatment should be to bring about a complete cure.

If the patient is vomiting or has severe diarrhoea, or if he is comatose, or has a high parasite count (>100,000/mm³), therapy must be started intravenously. Quinine dihydrochloride is the drug of choice and may be administered to either adults or children in a slow intravenous drip (over 2–4 hours, repeated if necessary after 6–8 hours). Oral administration should be started as soon as possible. Following successful drug treatment, clinical improvements will be observed within 24–48 hours. The blood films will show a concomitant fall in the parasite count and after about 4 days they will become negative for trophozoites. (The continued presence of gametocytes does not indicate drug failure.)

3.1.1.2 Infections resistant to the 4-aminoquinolines. If the parasites do not respond to chloroquine, the condition of the patient may deteriorate rapidly if alternative treatment is delayed. Therefore, treatment with blood schizontocides of more certain efficacy than the 4-aminoquinolines must be started immediately. At present, a limited and imperfect range of antimalarials is available, and the choice made will depend upon the length of time the patient is available for treatment and his condition when chemotherapy begins.

Whichever course of treatment is selected it is important that intravenous quinine be used at the beginning when treating severe and complicated cases (as described above).

(1) Quinine. Patients are treated under supervision with prolonged courses of quinine, either alone or in association with other antimalarial drugs. Quinine sulfate, or another salt, is given daily at a dose of 2 g for 7–14 days. In areas where the infection is known to recrudesce after this regimen, or in those very rare cases where asexual parasitaemia persists, the drugs listed below may be used in conjunction with a second course of quinine or even used with the initial course.
(2) Antifolate-sulfonamide combinations. Pyrimethamine, 75 mg (or 1.5 mg/kg of body weight), may be given together with 1500 mg of sulfadoxine (25 mg/kg) or 1500 mg of sulfalene (25 mg/kg).

The combination of trimethoprim–sulfamethoxazole (cotrimoxazole) is not recommended for the treatment of malaria.

For infections resistant to both 4-aminoquinolines and antifolate–sulfonamide combinations, quinine in combination with antibiotics may be used.

(3) Antibiotics. Tetracyclines usually cure falciparum infections if given for seven or more consecutive days. Tetracycline hydrochloride (1 or 2 g daily in 4 equal doses), doxycycline (0.2 g daily), and minocycline (0.1–0.4 g daily) are equally effective, but, because the infection does not usually respond for 3 or 4 days, it is essential to administer a rapidly acting blood schizontocide, such as quinine or amodiaquine, at the beginning of the course of treatment (see also section 3.3).

Other antibiotics, notably clindamycin and erythromycin, have been used for the treatment of malaria. Clindamycin alone acts slowly and when it is used together with quinine (for rapid reduction of parasitaemia), the incidence and intensity of gastrointestinal side-effects are exacerbated. For this reason, clindamycin is not recommended. The evaluation of the efficacy of erythromycin is not yet complete.

(4) Mefloquine. (See section 4.2.)

(5) Complementary medication. The prevention of further transmission of the infection from patients suffering from falciparum malaria can be ensured only by complementary blood schizontocidal treatment with primaquine, a single gametocytocidal adult dose of 30–45 mg is suitable for this purpose.

---

Table 5. Drugs used in the therapy of malaria

| A. Treatment of *P. falciparum* infections of mild to moderate severity |
|-------------------|----------------|----------------|
| (1) *In areas of 4-aminoquinoline sensitivity* |
|                  | Day 1            | Day 2            | Day 3            |
| chloroquine      | 900 mg in 2–3 doses | 300 mg           | 300 mg           |
| (base)           |                  |                  |                  |
| OR               | 600–800 mg in 2–3 doses | 400 mg           | 400 mg           |
| amodiaquine      |                  |                  |                  |
| (base)           |                  |                  |                  |

The total dose of 1.5 g is equivalent to 25 mg/kg. This regimen is also fully effective for infections caused by *P. malariae*. 

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(2) In areas of sensitivity to antifolate–sulfonamide combinations but where 4-aminouquinolines are considered ineffective

<table>
<thead>
<tr>
<th>Drug</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Days 4-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>sulfadoxine</td>
<td>1.5 g</td>
<td>1.5 g</td>
<td>1.5 g</td>
<td></td>
</tr>
<tr>
<td>pyrimethamine</td>
<td>75 mg</td>
<td>75 mg</td>
<td>75 mg</td>
<td>mg (single dose)</td>
</tr>
<tr>
<td>OR</td>
<td>1.5 g</td>
<td>1.5 g</td>
<td>1.5 g</td>
<td>1.5 g daily</td>
</tr>
<tr>
<td>sulfonamide</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pyrimethamine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75 mg (single dose)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Whenever possible in cases of greater severity, it is highly advisable to administer a short oral course of quinine (3-9 doses at 8-hour intervals) in association with these combinations for more rapid reduction of parasitaemia and relief of symptoms. Sulfonamides should not be prescribed to persons known to be sensitive to these drugs.

(3) In areas where both 4-aminouquinolines and antifolate–sulfonamide combinations are considered ineffective

<table>
<thead>
<tr>
<th>Drug</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Days 4-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>quinine</td>
<td>1800 mg</td>
<td>1800 mg</td>
<td>1800 mg</td>
<td>1800 mg daily</td>
</tr>
<tr>
<td>OR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tetracycline</td>
<td>1-2 g</td>
<td>1-2 g</td>
<td>1-2 g</td>
<td>1-2 g daily</td>
</tr>
<tr>
<td>PLUS</td>
<td>1800 mg</td>
<td>1800 mg</td>
<td>1800 mg</td>
<td>1800 mg daily</td>
</tr>
</tbody>
</table>

Quinine is usually given divided into 3 doses at 8-hour intervals for 3 days but may be continued for 7 days in areas of South-East Asia where diminished sensitivity to quinine has been noted. Other tetracyclines (e.g., doxycycline or minocycline) may be acceptable alternatives.

(4) Treatment of severe P. falciparum infections when parenteral drug administration is required

Quinine dihydrochloride, 10 mg/kg body weight in physiological saline solution (10 ml/kg body weight), administered by intravenous infusion over 2-4 hours, given every 6-12 hours until the patient is able to take oral medication.

(a) If it is known with certainty that the patient has not taken prior medication, a loading dose of quinine may be given, i.e., 20 mg/kg, as above, as the initial dose.

(b) Parenteral chloroquine is not recommended because of its potential toxicity, and parenteral formulations of the tetracyclines and antifolate–sulfonamide combinations have no role in the therapy of malaria.

(c) Blood glucose monitoring is desirable during the course of drug administration in the event of intravenous quinine administration and/or high parasitaemia since in both cases there may be associated hypoglycaemia.

(d) Corticosteroid therapy is contraindicated in the treatment of cerebral malaria; anti-coagulant therapy is also contraindicated in the absence of proof of disseminated intravascular coagulation.

B. Treatment of P. vivax and P. ovale infections

Chloroquine or amodiaquine is given as described for the therapy of sensitive infections of P. falciparum on days 1, 2, and 3 followed by 15 mg of primaquine base daily on days 4-17 (if radical cure is desirable, e.g., in malaria-free areas and those with a low endemicity).

(a) Primaquine should not be used according to this schedule when glucose-6-phosphate dehydrogenase deficiency is known to be present or suspected. An alternative regimen (45 mg of primaquine base weekly for 8 weeks) may be considered in such cases.

(b) Routine administration of primaquine may not be indicated in malaria control programmes or in the early stages of malaria eradication programmes.

(c) Antifolate–sulfonamide combinations should not be used for the therapy of P. vivax infections because of widespread resistance to antifolates and the inadequate efficacy of sulfonamides against this parasite.

C. Therapy of suspected cases of malaria where parasitological diagnosis is not possible

(a) In areas of 4-aminouquinoline sensitivity, the therapy is the same as for P. falciparum (above).

(b) In areas with 4-aminouquinoline resistant P. falciparum, but where P. vivax also occurs, a single dose of one of the antifolate–sulfonamide combinations (as indicated above) may be combined with a single 600-mg dose of chloroquine (for the vivax infection).
3.1.2 Paediatric therapy schedules

The therapy of *P. falciparum* infections in children is similar to the treatment for adults, with the proviso that children with severe infections may be more sensitive to parenteral medication. Intravenous and intramuscular injections of chloroquine are particularly hazardous and should not be used. For treatment schedules see Table 6.

3.1.3 *Falciparum* malaria in pregnancy

In endemic areas, clinical episodes of malaria are more frequent and more severe during pregnancy. Consequently, antimalarial drugs for chemoprophylaxis should be given during pregnancy.

Asymptomatic *P. falciparum* infections during pregnancy have been associated with severe placental infection, infarcts, and inadequate fetal nutrition (7). The resulting low birth weight predisposes to increased infant mortality.

In areas of chloroquine sensitivity, 4-aminoquinolines may be used, at the dosages recommended for suppression (section 3.3.2.1) or treatment (section 3.1.1.1).

In many areas of chloroquine resistance, the sulfadoxine/pyrimethamine combination is used for malaria prophylaxis, but there has been some confusion as to whether or not this combination of drugs should be recommended for this purpose in pregnant women. When the product was first introduced in 1970, the manufacturers indicated in the accompanying literature that it should not be used during pregnancy because of the known teratogenic effect of pyrimethamine in rats (preventable by a folate supplement) and the fear that the sulfadoxine component might cause kernicterus in the neonate if given in late pregnancy. There appears to be no real foundation for either of these fears (1).

Pyrimethamine, alone or together with a sulfonamide or sulfone has, during the past 30 years, been taken by millions of women during pregnancy for malaria prophylaxis without any report of teratogenicity. This course of treatment has saved many babies and their mothers from the effects of malaria attacks. Pyrimethamine, given with sulfonamides throughout pregnancy has also been used very successfully to prevent abortion in women with toxoplasmosis (2, 6). A recent analysis that assesses the human and animal data on the safety of sulfadoxine/pyrimethamine in pregnancy concludes
Table 6. Dosage of antimalarial drugs for oral treatment of moderately severe malaria in non-immune children according to age*

<table>
<thead>
<tr>
<th>Drug</th>
<th>Up to 1 year (3/4 of adult dose)</th>
<th>1–3 years (1/2 of adult dose)</th>
<th>4–6 years (1/3 of adult dose)</th>
<th>7–11 years (1/4 of adult dose)</th>
<th>12–15 years (1/5 of adult dose)</th>
<th>Regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinine</td>
<td>100–200 mg</td>
<td>200–300 mg</td>
<td>300–500 mg</td>
<td>500–1000 mg</td>
<td>1000–2000 mg</td>
<td>Daily dose to be divided into 2–3 parts continued for 7–10 days (i) Initial dose</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>(i) 75 mg (1 tablet)</td>
<td>(i) 150 mg (2 tablets)</td>
<td>(i) 300 mg (2 tablets)</td>
<td>(i) 300 mg (2 tablets)</td>
<td>(i) 450–600 mg (3–4 tablets)</td>
<td>(i) Initial dose</td>
</tr>
<tr>
<td></td>
<td>(ii) 75 mg (1 tablet)</td>
<td>(ii) 115 mg (2 tablets)</td>
<td>(ii) 150 mg (2 tablets)</td>
<td>(ii) 150 mg (2 tablets)</td>
<td>(ii) 225–300 mg (1–2 tablets)</td>
<td>(ii) Second dose, following initial dose after 6–24 hours</td>
</tr>
<tr>
<td></td>
<td>(iii) 37 mg (1 tablet)</td>
<td>(iii) 75 mg (1 tablet)</td>
<td>(iii) 150 mg (1 tablet)</td>
<td>(iii) 150 mg (1 tablet)</td>
<td>(iii) 150–300 mg (1–2 tablets)</td>
<td>(iii) Daily dose for the next 2 days</td>
</tr>
<tr>
<td>Amodiaquine</td>
<td>(i) 50 mg (1 tablet)</td>
<td>(i) 100 mg (2 tablets)</td>
<td>(i) 150 mg (2 tablets)</td>
<td>(i) 200–300 mg (2 tablets)</td>
<td>(i) 400–600 mg (2 tablets)</td>
<td>(ii) Dose for the first day</td>
</tr>
<tr>
<td></td>
<td>(ii) 50 mg (1 tablet)</td>
<td>(ii) 100 mg (2 tablets)</td>
<td>(ii) 150 mg (2 tablets)</td>
<td>(ii) 250–400 mg (2 tablets)</td>
<td>(ii) 250–400 mg (2 tablets)</td>
<td>(ii) Dose for the next 2 days</td>
</tr>
<tr>
<td>Sulfadoxine</td>
<td>250 mg</td>
<td>500 mg</td>
<td>1000 mg</td>
<td>Single dose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pyrimethamine</td>
<td>12.5 mg (1 tablet)</td>
<td>25 mg</td>
<td>50 mg</td>
<td>(ii)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfalene</td>
<td>Same as for sulfadoxine + pyrimethamine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: The dosage of chloroquine and amodiaquine is expressed in terms of base. The upper limit of the adolescent dose constitutes the generally adopted adult dose. Dosages of chloroquine are adjusted for fractional use of the common formulation of the drug containing 150 mg of base per tablet. In some countries chloroquine is formulated in tablets of 100 mg of base. Mepacrine, now considered an obsolete drug, has not been included in the table.

*Based on Bruce-Chwatt, L.J., ed. (3).
that this drug combination has no teratogenic effect on the human fetus and is unlikely to cause kernicterus at the doses used for malaria treatment and prophylaxis.\textsuperscript{1} The manufacturers are now seeking permission from regulatory authorities to remove the warnings from their literature. If this application is successful, there would seem to be no logical objection to a recommendation that sulfadoxine/pyrimethamine should be given as a prophylactic, or for the treatment of malaria, at appropriate dosage levels at any stage of pregnancy in the areas where it is needed. In such cases the importance of not exceeding recommended prophylactic doses should be emphasized. It should not be used in circumstances where drug compliance is questionable. In addition, it would be a sensible precaution to give a folinic acid supplement during the first trimester, especially during the treatment of toxoplasmosis which requires high dosage levels and prolonged administration.

Tetracycline and primaquine are not recommended for use in pregnancy. \textit{P. vivax} infection should be treated with an effective dose of a 4-aminoquinoline. Weekly suppression with a 4-aminoquinoline should then be given until delivery when primaquine can be administered. Mefloquine has not yet been assessed for use in pregnancy or in the treatment of infants.

Severe anaemia commonly occurs in pregnant women suffering from malaria and should be effectively treated.

3.1.4 \textit{Severe and complicated malaria}

3.1.4.1 \textit{Anaemia.} Severe progressive anaemia in falciparum malaria, causing reduced blood viscosity and oxygen carriage, with increased cardiac output, may pose a major clinical problem. Haemolysis is related both to malaria and, separately, to oxidant antimalarial drugs. Deficiency in glucose-6-phosphate dehydrogenase (G-6-PD) and other red cell enzymes together with possibly abnormal haemoglobins may increase susceptibility to oxidant-induced haemolysis. Blackwater fever (defined as severe malaria infection in the absence of G-6-PD deficiency, with intravascular haemolysis, haemoglobinuria, and renal failure) is probably due to an auto-immune process triggered by quinine and is seldom observed nowadays. Massive haemoglobinuria usually

\textsuperscript{1} Scholer, H.J. \textit{Assessment of the safety of Fansidar to pregnancy, Animal and human data}, 1983 (unpublished WHO document MAP/SGCM/INF/83.6).
results from G-6-PD deficiency. It is essential to maintain the
haematocrit level above 20% by the transfusion of fresh blood
(collected within 24 hours) in which both clotting factors and
platelets are present. In a few life-threatening cases of falciparum
malaria, removal of parasitized blood and its replacement with a
mixture of non-infected erythrocytes in the patient’s plasma has been
effective. Insufficient data are available to justify a general
recommendation for the use of this technique.

3.1.4.2 Cerebral malaria. This is the most important form of severe
falciparum malaria and intravenous quinine should be given without
delay (see section 3.1.1.1). Parasitized red cells may be impeded in
their passage through the cerebral (and other) vascular beds because
of their decreased deformability (8, 9). In addition, blood flow may
be sluggish owing to the adherence of parasite-containing red cells
to the capillary endothelium. Both phenomena are probably
responsible for stagnant cerebral hypoxia.

The commonest neurological signs are unconsciousness, convul-
sions, and upper motor neurone and brain stem abnormalities.
Retinal haemorrhages and exudates were observed in 10% of cases
in Chantaburi, Thailand.

Although cerebral oedema has been described frequently at
autopsy, the clinical evidence for its occurrence during life is not
convincing; most fatal cases pass through a terminal phase of
cerebral anoxia, which could explain the autopsy finding. Because
cerebral oedema was thought to be an important feature of cerebral
malaria, corticosteroid treatment has been widely used since the
mid-1960s. A double blind, placebo controlled trial of 100 cases of
strictly-defined cerebral malaria was recently carried out in
Chantaburi, Thailand (15). Mortality was the same in both
treatment groups, but patients treated with dexamethasone suffered
significantly prolonged unconsciousness and increased incidence of
complications. Dexamethasone is therefore contraindicated for the
treatment of cerebral malaria. Other methods for reducing cerebral
oedema seem unlikely to be helpful and since the blood viscosity is
often already reduced by anaemia, the additional effects of dextrans
would not be helpful. The use of heparin treatment was supported
by a single study from Indonesia (10, 11), but most studies published
in the last 5 years have suggested that disseminated intravascular
coagulation (DIC) is relatively uncommon in severe malaria and that
heparin is either unhelpful or positively dangerous (13, 14).
The unconscious patient needs special nursing care. Rehydration of the patient should be carried out with caution particularly in the first 24 hours. Fluid intake and output should be accurately recorded because over-hydration may precipitate pulmonary oedema. In general, 2-3 litres of fluid are required on the first day. Anticonvulsants and antimicrobials (for infections of the urinary and respiratory tracts) are often required. Corticosteroids are harmful (15). Although coma and parasitaemia are pathognomonic of cerebral malaria, care should be taken to exclude any other possible cause of unconsciousness. Examination of cerebrospinal fluid is mandatory in patients suspected of having cerebral malaria.

3.1.4.3 Management of severe and complicated malaria.

(1) Renal failure. Although more than 50% of patients with severe malaria have raised blood urea and serum creatinine levels on admission to hospital, the cause is usually pre-renal. In the 5-10% with true renal failure the cause is almost always acute tubular necrosis, presumably resulting from impaired renal perfusion. This may be due either to hypovolaemia or reduction in blood flow through the renal microcirculation with adequate central blood volume. The distinction between pre-renal and established renal failure is very important for correct clinical management. Measurement of urine output, specific gravity, and sodium or urea concentration is most helpful, together with clinical evidence of dehydration, including clinical assessment of the jugular venous pressure, postural effects on blood pressure, tissue turgor, and challenge with fluid or a diuretic. Central venous pressure monitoring is recommended to control fluid replacement in all patients with severe malaria.

A history of oliguria (300 ml of urine or less during the previous 24 hours) with vomiting suggests renal failure. Initially urine examination, obtained if necessary by urethral catheterization, is important. A specific gravity of 1.010 or less, suggests acute tubular necrosis. A concentrated urine with normal microscopic appearance suggests dehydration. Thus, in assessing oliguria, 1000 ml of isotonic (0.9%) saline are infused with the first dose of quinine (10 or 20 mg/kg) over 4 hours. In most cases, central venous pressure will not rise above 10 cm and urine will start to flow. If the amount of urine passed in 8 hours is less than 200 ml, another 1000 ml of saline (with the second dose of quinine 10 mg/kg) and a diuretic such as
furosemide (80 mg) or bumetanide (2 mg) is given by intravenous injection.

Patients who fail to produce more than 200 ml of urine in 16 hours should be placed on a strict fluid balance and the amount of fluid given should be regulated to the central venous pressure. Dialysis (peritoneal or renal) is essential. If the side-effects of quinine are pronounced, the amount of quinine given can be reduced to 5 mg/kg every 8 hours. In patients who revert to the diuretic phase the fluid balance must be adjusted accordingly.

(2) Hypoglycaemia. Hypoglycaemia has been observed quite frequently in patients with moderate or severe falciparum malaria. It may contribute to, or cause the neurological disturbances attributed to cerebral malaria. Hypoglycaemia appears to be most common in pregnant women. It may present during early convalescence, and can occur even while the patient is receiving 5% dextrose by intravenous infusion; it is not due to starvation and liver glycogen depletion. Possible explanations of this condition include glucose consumption by the malaria parasites and stimulation of insulin secretion by some antimalarial drugs (19).

The possibility that hypoglycaemia is present should always be considered for any patient with the appropriate symptoms (anxiety, breathlessness, sweating, convulsions, impaired consciousness, or severe neurological signs).

Prompt intravenous glucose is essential and life-saving in these cases. A second attack of hypoglycaemia may occur.

(3) Pulmonary oedema. The clinical features contributing to the development of pulmonary oedema are over-hydration, pregnancy, cerebral malaria, high levels of parasitaemia, hypotension, acidosis, and uraemia. Patients with pulmonary oedema should be nursed propped up, given oxygen to breathe, and their fluid balance should be regulated by central venous pressure monitoring. Pulmonary oedema is often confused with bronchitis and bronchopneumonia; chest X-ray is helpful for diagnosis. In some patients with a mild presentation, the response to diuretics is dramatic; however, most of the more severely affected patients die within a few hours. The use of heparin should be discouraged.

(4) Hyperpyrexia. Hyperpyrexia is controlled with high doses of antipyretics, ice-cold sponging, and fanning, or a cooling blanket if available.

(5) Jaundice. Severe jaundice is not uncommon in patients with falciparum malaria but hepatic failure has not been observed. In
cases not otherwise affected, symptomatic treatment is all that is required; uneventful recovery takes from 1 to 3 weeks. However, the condition is often associated with acute tubular necrosis; in this situation, the prompt management of renal failure is necessary.

3.1.5 Recent developments in the clinical treatment of malaria

In addition to the development of new antimalarial agents, older drugs have been the subject of recent basic clinical investigation. Results are available on the use of quinidine, and on techniques for optimizing quinine and chloroquine administration.

Several studies have emphasized the importance of monitoring the blood levels of antimalarials when measuring their efficacy and in diagnosing resistance. In Sweden, recrudescence of falciparum malaria in one patient was ascribed to low serum concentrations of chloroquine (4).

A method has been developed to determine chloroquine levels in the blood utilizing 0.1-ml samples taken by fingerprick and preserved on filter paper (12).

In Thailand (17), the acute pharmacokinetics of intravenously infused quinine was evaluated in 25 patients with cerebral malaria and in 13 with uncomplicated falciparum malaria. The results obtained emphasize that the benefits of high plasma concentrations (15 or 20 mg/litre) in the acute phase of treatment of cerebral malaria outweigh the risks of toxicity and it may be beneficial to give an initial loading dose of 20 mg/kg in patients known to be previously untreated (18).

In another recent study in Thailand (16), 14 men with uncomplicated falciparum malaria were treated with quinidine. Six had recrudesced after previous treatment, including two who had received quinine. All 14 infections were cured with quinidine. The minimal inhibitory concentration (MIC) in vitro of 16 isolates of P. falciparum was lower for quinidine than quinine in every isolate. This work suggests that where quinine is not available, quinidine may be an acceptable alternative; it is a drug commonly found in the cardiology departments of hospitals. However, particular care should be taken to monitor any cardiotoxic effects of quinidine.

3.2 Treatment of vivax, ovale, and quartan malaria

Resistance to 4-aminoquinolines has not been reported in P. vivax, P. malariae, or P. ovale infections. 900 mg of chloroquine
(base) is given in 2 or 3 divided doses on the first day and 300 mg as a single dose on each of the 2 following days. Latent exoerythrocytic forms of *P. vivax* and *P. ovale* in the liver which are unaffected by 4-aminoquinolines. In order to prevent relapses, 15 mg (base) of primaquine should be given daily for 14 days (see Table 5).

### 3.3 Drugs for suppression (prophylaxis)

The range of drugs available for suppression is quite limited. It includes the 4-aminoquinolines (chloroquine and amodiaquine) and combinations of dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS) (EC 2.5.1.15) inhibitors such as pyrimethamine with sulfadoxine or dapsone. The status of proguanil is uncertain, but prophylaxis using pyrimethamine alone is no longer considered to be adequate, since resistance of both *P. falciparum* and *P. vivax* to it is widespread throughout the tropics and subtropics. However, even in the presence of pyrimethamine resistance, sulfadoxine may potentiate the effect of pyrimethamine to such an extent that a full suppressive effect may be obtained. *P. vivax* is inherently less sensitive than *P. falciparum* to sulfadoxine, and this may explain the disappointing results obtained with the sulfadoxine/pyrimethamine combination against pyrimethamine-resistant *P. vivax*.

If a drug is to be an effective suppressant, it must reach, and be maintained at, a suitable concentration in the blood, preferably in the form of a steady state, and this level must be attained by the time the parasites enter the blood, i.e., 6–10 days after the start of exposure to infection. With chloroquine this is usually achieved with a weekly regimen of 5 mg of base per kg body weight (300 mg for adults of normal weight and in proportion to body weight for children) if the first dose is repeated on the second day of prophylaxis. This drug intake provides a blood concentration that is generally adequate to sustain parasite suppression. A weekly (adult) dose of one tablet containing 500 mg of sulfadoxine and 25 mg of pyrimethamine is enough to maintain suppressive blood levels against pyrimethamine-sensitive *P. falciparum*. With this medicament the steady state is usually reached by beginning prophylaxis 1–2 weeks before exposure to infection. This procedure also identifies those individuals who cannot tolerate sulfonamides.
Side-effects associated with chloroquine and amodiaquine administration in prophylactic doses are rare and are usually restricted to mild gastric discomfort. Individuals who experience pruritus associated with chloroquine prophylaxis frequently tolerate amodiaquine. Long-term administration of chloroquine is associated with a risk of retinopathy once the total cumulative dose exceeds 100 g of base.

Retinopathy is irreversible, in contrast to crystalline deposits in the cornea and lens which disappear after the withdrawal of chloroquine. Chloroquine-induced retinopathy has occurred frequently in patients who received high doses of chloroquine for the treatment of collagen diseases, but a small number of cases of retinopathy have also occurred among those who have taken chloroquine for malaria prophylaxis in weekly doses of 600 mg, or daily doses of 100 mg for prolonged periods. All age groups can be given 4-aminoquinolines; amodiaquine is more acceptable to infants than chloroquine because it has a less bitter taste. Pregnancy is not a contraindication to prophylaxis with chloroquine or amodiaquine.

For the use of sulfadoxine/pyrimethamine in pregnancy, see section 3.1.3.

3.3.1 Potential of prophylaxis

There is at present no drug that guarantees malaria suppression. Anybody seeking advice on drug prophylaxis should be made aware of this fact and of the need for the immediate use of diagnostic and therapeutic facilities if and when fever occurs while under, or after having terminated malaria prophylaxis. Similarly, physicians must remember that malaria may occur in all persons exposed to infection regardless of the type and regularity of drug prophylaxis.

The risk of exposure to infection may vary, ranging from sporadic inoculation to several potentially infectious bites every night. Complementary preventive measures are always useful and sometimes necessary in order to reduce the risk of infection. Such complementary measures consist of the screening of premises, the use of bed-nets and repellents, staying indoors from dusk to dawn, spraying the room with a "knock-down" insecticide, and wearing clothing that limits body access to mosquitoes.

The choice of prophylactic drug will depend on the parasite species involved and their response to drugs in the area of exposure. Wherever *P. vivax*, *P. ovale*, or *P. malariae* are transmitted to a
significant extent, 4-aminoquinolines are the medicaments of choice for prophylaxis, unless there is a valid contraindication such as intolerance or risk of retinopathy.

Similarly, the 4-aminoquinolines are suitable drugs for prophylaxis wherever \textit{P. falciparum} is fully sensitive to chloroquine. This also applies to areas with low-grade and/or low frequency of chloroquine-resistant \textit{P. falciparum}, but as a precaution, one or more treatment doses of sulfadoxine/pyrimethamine should always be carried. This can be used if fever occurs and diagnostic/therapeutic facilities are not within easy reach.

In areas with a high frequency of highly chloroquine-resistant \textit{P. falciparum}, sulfadoxine/pyrimethamine should be used as the suppressive drug. Since practically all these areas are also affected by \textit{P. vivax}, it will be necessary to use chloroquine simultaneously. This may have some additional benefit in suppressing \textit{P. falciparum}, but breakthrough of infection may occur in all areas with multiresistance, necessitating treatment with the third-line drugs, quinine and tetracycline, or when available, mefloquine or the combination of mefloquine, sulfadoxine, and pyrimethamine.

3.3.2 \textit{Recommendations for prophylaxis}

Three major groups of persons need to be considered in connection with malaria drug prophylaxis: non-immune travellers visiting malarious areas for relatively short periods, non-immune residents in malarious areas, and semi-immunes residing in malarious areas.

3.3.2.1 \textit{Non-immune visitors to malarious areas} (staying up to one year). These are persons visiting malarious areas for relatively short periods, and include international travellers, businessmen, and tourists, and also persons normally residing in non-malarious areas of the same country (e.g., persons from non-malarious cities), or members of special groups (e.g., army, police) assigned on temporary duty to malarious areas. The recommendations for drug prophylaxis in non-immune visitors are summarized in Table 7, according to the drug sensitivity status of the areas to be visited. (Fig. 1 and 2 show the malarious areas of the world and the areas in which chloroquine-resistant \textit{P. falciparum} occurs.) Drug prophylaxis in this group is not considered to have a significant epidemiological impact regarding the selection of drug resistance in
Table 7. Chemotherapeutic doses for malaria suppression (1983)

<table>
<thead>
<tr>
<th>(1) Chloroquine-sensitive <em>P. falciparum</em> and/or other species (Americas north of the Panama Canal, Argentina, Paraguay; north, west, south, and central Africa; Asia west of central India)</th>
</tr>
</thead>
<tbody>
<tr>
<td>chloroquine OR amodiaquine</td>
</tr>
<tr>
<td>Adults</td>
</tr>
<tr>
<td>9–12 years</td>
</tr>
<tr>
<td>200–300 mg of base weekly</td>
</tr>
<tr>
<td>5–6 years</td>
</tr>
<tr>
<td>150–200 mg of base weekly</td>
</tr>
<tr>
<td>1–4 years</td>
</tr>
<tr>
<td>50–100 mg of base weekly</td>
</tr>
<tr>
<td>1 year</td>
</tr>
<tr>
<td>37.5–50 mg of base weekly</td>
</tr>
</tbody>
</table>

The first dose should be repeated on the second day of prophylaxis, which should be continued weekly for 4–6 weeks after leaving the endemic area.

<table>
<thead>
<tr>
<th>(2) Incipient and low-grade chloroquine-resistant <em>P. falciparum</em> (Burundi, Comoros, Kenya, Madagascar, Somalia, Sudan, Uganda, United Republic of Tanzania, Zambia)</th>
</tr>
</thead>
<tbody>
<tr>
<td>chloroquine OR amodiaquine</td>
</tr>
<tr>
<td>Dosage as for chloroquine-sensitive <em>P. falciparum</em> malaria.</td>
</tr>
<tr>
<td>A dose of sulfadoxine/pyrimethamine should be kept in readiness for the treatment of an attack and taken on leaving the endemic area.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(3) Highly chloroquine-resistant, sulfadoxine/pyrimethamine-resistant <em>P. falciparum</em> with <em>P. vivax</em> also present (Asia east of central India; Australia; Oceania; America south of the Panama Canal, except Argentina and Paraguay)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sulfadoxine (500 mg)/pyrimethamine (25 mg) PLUS chloroquine OR mefloquine</td>
</tr>
<tr>
<td>Adults</td>
</tr>
<tr>
<td>1 tablet</td>
</tr>
<tr>
<td>plus 300 mg of base or 250 mg of base</td>
</tr>
<tr>
<td>9–12 years</td>
</tr>
<tr>
<td>1 tablet</td>
</tr>
<tr>
<td>plus 200–300 mg of base or 125 mg of base</td>
</tr>
<tr>
<td>5–6 years</td>
</tr>
<tr>
<td>1 tablet</td>
</tr>
<tr>
<td>plus 150–200 mg of base or 125 mg of base</td>
</tr>
<tr>
<td>1–4 years</td>
</tr>
<tr>
<td>1 tablet</td>
</tr>
<tr>
<td>plus 50–100 mg of base or 63 mg of base</td>
</tr>
<tr>
<td>1 year</td>
</tr>
<tr>
<td>–</td>
</tr>
<tr>
<td>plus 37.5–50 mg of base or 30 mg of base</td>
</tr>
</tbody>
</table>

The above doses should be administered at weekly intervals. Mefloquine, if available, should only be used for non-immune, short-term travellers to malarious areas. Young children should be given supplementary calcium folinate/cyanocobalamin when sulfadoxine/pyrimethamine is administered.

Breakthrough of multiresistant *P. falciparum* may occur, even under combined prophylaxis, necessitating treatment with quinine plus tetracycline or another third-line antimalarial drug.

the target area since the suppressive regimens are usually followed quite strictly and there is little chance of short-time visitors developing gametocytopenia under regular prophylaxis.

3.3.2.2 Non-immune residents of malarious areas. The recommendations for prophylaxis in non-immune residents depend on the length of their planned stay in the malarious area. If the total stay is less than 6 years with continuous prophylaxis, or correspondingly longer if only seasonal malaria prophylaxis is needed, then chloroquine may be taken as recommended in Table 7.
This will be adequate in areas with chloroquine-sensitive *P. falciparum* and with *P. vivax, P. malariae,* and *P. ovale*; in areas with *P. falciparum* with low-grade resistance to chloroquine, the combination of sulfadoxine and pyrimethamine should be available for emergency treatment.

In areas with a high frequency of highly chloroquine-resistant malaria, sulfadoxine/pyrimethamine can be taken. Up to 2 years of continuous prophylaxis with sulfadoxine/pyrimethamine has been reported to be well tolerated, but leukocyte differential counts should be controlled at 6-monthly intervals.

If the stay in the malarious area is long, the continued use of chloroquine and the long-term administration of sulfadoxine/pyrimethamine may become more detrimental to health than an early diagnosed and immediately treated attack of malaria. In these cases, it will be preferable to rely exclusively on methods for avoiding infection, which have now become the mainstay of prevention, and on reliable standby drugs that can be taken if an infection occurs.

For non-immune individuals submitted to long-term or intense exposure to a relapsing species of malaria (*P. vivax, P. ovale*), radical curative therapy with 14 days of primaquine should be undertaken during the last 2 weeks of the suppressive regimen. Examination for glucose-6-phosphate dehydrogenase deficiency or close clinical observation for evidence of haemolysis is mandatory during primaquine therapy.

3.3.2.3 *Semi-immune residents of malarious areas.* The only semi-immunes in malarious areas for whom systematic malaria suppression is indicated are pregnant women. Recommendations for the management of suppression and treatment of malaria in pregnancy are given in section 3.1.3.

Mass prophylaxis in children under 5 years old is not recommended because:

— it is impossible to achieve continuous suppression in a significant proportion of the population;
— it may interfere with the development of protective immunity;
— it may accelerate the development of drug resistance;
— it utilizes scarce resources that may better be used for treatment.

In these circumstances, it is preferable to provide curative treatment whenever and wherever it is required, ideally through a primary health care system that possesses the facilities for
microscopic diagnosis. In the absence of the latter, fever should be considered as the criterion for antimalarial treatment.

3.3.2.4 Special risk groups. Aggregations of labour, police forces, army units, refugees in camps, and other compact groups are particularly prone to malaria infection if they are composed of non-immunes and deployed in endemic areas. Wherever possible their protection should not be based exclusively on drug prophylaxis, but also on systematic local vector control covering an adequate perimeter around the camp. The appropriate siting of camps should also be given due consideration.

Continuous drug prophylaxis in large groups staying in areas of intensive malaria transmission leads to the risk of setting up a substantial selection pressure for resistance. All infections occurring in such groups should therefore receive appropriate blood schizontocidal and gametocytocidal treatment, and be followed up after treatment so that any recrudescence can be detected and treated.

If groups carrying malaria infections come to work or stay in a receptive area (e.g., labour forces or refugees), it is necessary to screen them for cases of malaria and if necessary, to administer radical and gametocytocidal treatment. Vector-control measures within a safe perimeter may be necessary for the protection of the surrounding area.

3.4 Operational use of antimalarial drugs

Antimalarial drugs are used in the fight against malaria with two main objectives: to treat individual malaria patients and to reduce the collective parasite reservoir. The treatment of patients in an operational malaria service necessarily differs from that provided in a hospital because immediate medical supervision is usually lacking and nurses are not available. Treatment becomes the responsibility of personnel with limited education and training, and therefore the drug regimens used must be easily comprehensible to staff and patients alike. Gametocytocides should also be given in order to prevent the transmission of the parasite. The reduction of parasite reservoirs achieved by the mass distribution of drugs is very difficult to maintain in the absence of an effective reduction in the transmission potential of the disease. Nevertheless, the short-term rewards of mass administration of blood schizontocides are often
tempting to managers eager to improve their statistics. Depending on the degree of organization (and the budget) of the service, drug practices vary greatly in the quality and the nature of the treatment given.

Antimalarial drugs are often freely available on the open market without adequate guidance for their use. Commercial distribution is not necessarily bad, but it is imperative that a way is found to provide the community with advice on the appropriate use of antimalarial drugs.

3.4.1 Development of national policy for the use of antimalarial drugs

A clearly defined national policy on the use of antimalarial drugs is essential in order to:

— ensure the successful implementation of malaria control;
— prevent or delay the selection of drug resistance;
— ensure the availability of appropriate antimalarial drugs at all levels of the health care system;
— minimize toxicity of antimalarials by preventing inappropriate use;
— avoid the use of obsolete antimalarials and/or the premature and uncontrolled use of second- and third-line drugs and new compounds;
— adopt chemotherapeutic measures suitable for the epidemiological situation and consistent with the social and economic conditions;
— place in perspective the requirements of the health service for antimalarial and other drugs;
— place in perspective the role of chemotherapy in the malaria control strategy and other health activities implemented through the health care structure.

At present, in many countries no national policy for the use of antimalarial drugs exists. The planning and establishment of such a policy depends upon:

— the current situation regarding the drugs available, regimens in use, quantity of drug required, sources of drug, availability and cost of drugs at each step in the drug delivery system;
— the national resources available;
— the efficacy of drug dosages and regimens;
— the capacity of the government to exert control over the importation, distribution, use, and cost of antimalarials;
— the stage of development of the health care infrastructure and its ability to support drug distribution;
— the capability of monitoring changes in the situation and the ability to modify the operation accordingly.

3.4.2 Clarification of the terminology and concepts of malaria control

The application of terminology developed for time-limited malaria eradication to malaria control is now causing considerable confusion. In the operational use of antimalarial drugs for eradication, the predominant concept was one of malaria as an infection implying elimination of malaria parasites from the community. This concept has less relevance to malaria control, which is concerned with malaria as a disease necessitating treatment with drugs on clinical grounds.

There are several terms used in malaria eradication that are now being misused and require review in the light of present knowledge and practice.

(1) Presumptive treatment. In malaria eradication terminology, this meant treatment given to a presumptive malaria case (generally a fever case) at the time when a blood sample was taken for examination. It usually consisted of a single dose of a schizontocide, often with a sporontocide, and its aim was to relieve symptoms possibly due to malaria and to prevent the patient from being a source of mosquito infection until a fully curative treatment could be given if the malaria infection was confirmed.

Presumptive treatment was thus a precautionary measure, particularly indicated during the consolidation phase of eradication when malaria prevalence had been sufficiently reduced. Its use was limited to selected individuals in whom malaria infection was suspected.

The term “presumptive treatment” should be used only in that context. A clear distinction should be made between this term and the current practice in some areas of giving patients suspected of having malaria a single dose treatment without diagnosis and without follow-up.

(2) Active and passive case detection. These terms as understood and used in malaria eradication have less relevance to the operational use of antimalarial drugs in malaria control, where a major emphasis is placed on diagnosing the disease and treating the patient.
(3) *Clinical treatment.* In the context of malaria control, clinical treatment of malaria must be effective, whether it is provided at health centres immediately after microscopic diagnosis, or by health service workers in peripheral areas where microscopy is impossible.

*Effective clinical treatment* implies the administration of schizontocidal drugs at a dosage sufficient to produce clinical cure. R1 resistance does not necessarily preclude the continued use of a given regimen. Lower doses should not be given even if it is anticipated that additional treatment will follow a delayed diagnosis.

(4) *Radical treatment.* This is treatment of confirmed malaria cases so that complete elimination of parasites is achieved, thus preventing a relapse or recrudescence and hence ensuring that the patient is non-infective to mosquitoes. Its use is indicated in non-malarious areas to protect the individual from relapse and in receptive non-malarious areas to protect the community. Radical treatment may also be used in areas under extensive control where malaria transmission has been greatly reduced. Mass radical treatment of a population is only indicated in areas where malaria has been previously eradicated, for the elimination of limited foci (thus preventing epidemics and the re-establishment of malaria).

(5) *Antirelapse treatment.* This is given in areas where an advanced stage has been reached in the control of *P. vivax*. A tissue schizontocide is used to treat either the whole community, or that part of it known to have been infected during the previous transmission season.

(6) *Mass drug administration.* In the context of malaria eradication, mass drug administration is aimed at the elimination of foci of infection by the distribution of a drug to every member of a population, infected or not. This approach led to the design of policies that have little application in the context of the current strategy of malaria control. Mass drug administration to specified populations is still useful in the control of epidemic malaria and is used in conjunction with effective antivectoral measures. There is no indication for the use of medicated salt.

3.4.3 *Use of antimalarial drugs in malaria control*

Policies for the utilization of antimalarial drugs will depend on the degree of development of and the future plans for primary health care in a given area. The quality and training of health personnel at the peripheral level, the efficiency of their relationship with the
community, the adequacy and accessibility of a functioning referral system, and the political commitment to primary health care will all influence the use of antimalarial drugs. The availability of facilities for microscopic diagnosis will determine which particular antimalarial treatment can be introduced.

3.4.3.1 Essential drugs. From the list of essential drugs recommended in this report for antimalarial treatment, each country should select those that are appropriate for the prevailing conditions. Countries should, as far as possible, control the importation and distribution of second- and third-line drugs. National policy should provide guidelines regarding where, and at which level in the referral system these alternative drugs should be available.

3.4.3.2 The approach to malaria therapy in endemic areas. Wherever possible, patients should be identified and the diagnosis of malaria confirmed by blood examination. The administration of blood schizontocidal and gametocytocidal (or sporontocidal) drugs should be supervised and the patients followed up to ensure that they have been successfully treated.

However, the realities of the operational use of antimalarial drugs will often mean that effective treatment will have to be given on the basis of clinical signs and symptoms only, in the absence of confirmed parasitological diagnosis. Attempts must be made to educate the community and to ensure that all patients receive effective treatment.

The facilities and strategies for diagnosis and treatment in a malaria control (not eradication) programme may include the following:

— malaria clinics, located in highly malarious areas, either within the most peripheral health unit, or as a satellite;
— mobile malaria clinics, that bring a team, including a microscopist, to areas of case concentration;
— health care facilities such as midwifery centres, health centres, and district hospitals;
— house visiting;
— community health workers;
— treatment facilities outside the health services.

Irrespective of how many of the above systems for diagnosis and treatment may be present, their activities should be coordinated so
that the best use is made of the resources available. An information system is essential for providing feedback from the periphery to the centre on drug utilization and requirements, as well as changes in the epidemiological situation which may demand prompt action.

Within the framework of the developing health care infrastructure there are certain key points where antimalarial treatment could contribute significantly to the control of malaria as a disease.

1. *Malaria clinics.* A malaria clinic is a specialized peripheral unit of the health service designed to provide on-the-spot parasitological diagnosis and effective clinical treatment. It should be established at a strategic, accessible point, in or near an area of high prevalence of life-threatening *P. falciparum* malaria and where no other appropriate services are available. It may well provide a point of entry for the further development of the health care infrastructure.

Important elements in the successful operation of a malaria clinic include:

— positive rapport between the clinic and the population served. The workers must be trusted by the patients, and the service provided must be effective, resulting in a good reputation and word-of-mouth advertising;
— regular operation during hours convenient to the population served—during market hours, and rest days, when patients and their relatives are able to attend;
— convenient location near a transmission area and served by public transport if possible;
— service by well-trained and well-supervised microscopists to ensure accurate diagnosis;
— provision of a reliable supply of effective drugs, preferably distributed without cost to the patient.

2. *Mobile clinics.* The most appropriate use of a mobile clinic is in providing diagnosis during an unexpected outbreak. The concept of providing periodical visits by a mobile malaria clinic on a regular basis (which often means a single individual) is both too inefficient and too expensive to be recommended.

3. *Health care facilities.* In support of the general health services and with the prospect of providing malaria treatment through primary health care facilities in areas of high endemicity, the training of staff to prepare and read malaria smears, and to administer effective treatment, must be considered a priority. These technicians represent the first level of referral for the community health worker.
and they must be prepared to deal with patients who have not responded to treatment. Therefore, they must be able to provide differential diagnosis and there should be a system to measure drug resistance. Facilities for the management of severe malaria and for the referral of patients for further treatment must be available.

(4) **House visiting.** Home visiting by health service workers has a very limited role in malaria control. Special situations such as focal epidemics may require extraordinary efforts for the provision of treatment and education through home visiting, but the aim should be to provide education and treatment on a permanent basis through interaction with the community and the community health worker.

(5) **Community health workers.** Community health workers are usually the principal agents for providing antimalarial treatment at the periphery. As such they are an indispensable part of malaria control through primary health care. They must be adequately trained in the management of fever and the use of the drug regimens supplied, and they must be supported by a good referral system. Their main functions in the context of malaria control are effective clinical treatment, provision of basic health education, and reporting abnormal situations to the health services. The use of these workers for the mass collection of blood slides should be discouraged.

3.4.3.3 **Drugs distributed outside the health services.** Family self-diagnosis and medication frequently occurs and often results in inappropriate and inadequate treatment. The tendency for self-medication needs to be controlled as far as possible by ensuring that the population receive adequate advice and education. In some areas, the only community source of antimalarial drugs is from the open market. The health service should make efforts to control this type of distribution so that good quality, effective drugs, appropriate for the treatment of malaria in that particular area, are available at a reasonable cost and are accompanied by the correct information. In addition, the health service should try to ensure that diagnostic facilities and guidance in the use of antimalarial drugs are available to the community. The distribution of injectable chloroquine outside the health service should be prevented.

3.4.3.4 **Drugs to be used for malaria control.** The choice of drugs for malaria control is influenced by the epidemiology of malaria in the area, the availability of diagnostic facilities, the effectiveness of antimalarial drugs in that particular area, the cost, and the level of the primary health care system at which the drug is to be used.
(1) *4-aminoquinolines.* Chloroquine and amodiaquine are valuable drugs for the control of malaria in areas where they are effective because they are well-tolerated, safe, inexpensive, rapidly acting, and equally effective against all species of the parasite. Even though they have to be administered over a period of 3 days, the patient usually continues to have symptoms for this time and therefore remembers to take the drug as prescribed.

(2) *Antifolate-sulfonamide combinations.* The fixed combination of sulfadoxine/pyrimethamine has the important advantage that it may be given in a single dose. The drug is safe, generally well tolerated (although it should not be given to persons with a history of hypersensitivity to sulfonamides) and can be administered at the periphery. Uncontrolled commercial distribution of sulfadoxine/pyrimethamine and other similar combinations and their use for malaria suppression, as well as in the treatment of other diseases, may have been important in the development of the parasite resistance that has limited their value in parts of South-East Asia and South America. Antifolate/sulfonamide combinations are less effective for vivax than for falciparum infections. The combination should never be used as the first-line drug for malaria control in areas where the parasites are sensitive to 4-aminoquinolines. It should be available and reserved as a second-line drug.

(3) *Quinine.* Quinine should be reserved for the initial treatment of severe cases of malaria; the injectable form will usually be required (see section 3.1). Quinine should also be available as a second- or third-line drug in areas where chloroquine- and pyrimethamine/sulfadoxine-resistant *P. falciparum* exists. When treatment with pyrimethamine sulfadoxine is to be given, quinine may be administered for 2 or 3 days to clear parasitaemia and clinical symptoms more rapidly; this use of quinine is not required in the majority of cases and it should not be employed at the periphery. The use of quinine at the periphery is limited by its side-effects and because multiple daily doses for 3–10 days are required. Although it may be thought desirable to use quinine with tetracycline at the periphery in areas with a high frequency of RIII multiple drug resistance, recent studies have shown that outpatients almost never complete a course of medication lasting more than 3 days and this is particularly the case when quinine is used. The inevitable side-effects become more distressing than the symptoms of malaria after the first few days of treatment; patients know that tinnitus, visual disturbances, and nausea are related to taking the
drug and therefore stop taking it. The doses not taken may be saved for the next attack, used for prophylaxis, given to others, sold, or simply discarded. Even when simple visual diagrammatic instructions are distributed to patients indicating how the drugs are to be taken, they are usually ignored. When, because of expense or resistance, quinine cannot be used alone, the problem is compounded. When a 7-day course of tetracycline is accompanied by a short course of quinine, patients find the instructions even more complicated and the likelihood that the regimen will be completed diminishes.

(3) Mefloquine. The use and deployment of mefloquine are described in section 4.2.

(4) Primaquine. The use of primaquine as a gametocytocide for falciparum infections is important in areas where the reservoir of the parasite has been considerably reduced but receptivity remains. A small, safe, single dose is highly effective in killing the fully or almost mature gametocytes. In some areas, it is given as a daily dose for up to 5 days, thus increasing the probability that it will eliminate all gametocytes. The standard 14-day course of primaquine employed for the radical cure of vivax infections should not be used as a gametocytocidal regimen for falciparum malaria; the risk of providing patients with a packet of potentially toxic pills that may be accessible to small children must be weighed against its theoretical benefit. Unsupervised patients seldom complete a full course of treatment.

(5) Tetracycline. Tetracycline has well-recognized antimalarial activity and is readily available at all levels of the health care system as an essential drug. It should be used only in combination with quinine as a second- or third-line treatment for malaria.

3.4.3.5 Approach to alternative drugs. In areas where resistance to the 4-aminoquinolines is not too great, these compounds should be used as first-line drugs; second- and third-line drugs need to be available only at the first referral level.

In the presence of significant resistance to the first-line drug, a second-line compound should be provided to the periphery, with clear instructions on its use.

In areas where RII and RIII resistance are present, severe cases of falciparum malaria should a priori be treated with alternative (second-, or if applicable, third-line) drugs.
A decision to change the first-line drug should be based not only upon the presence of a high local frequency of RIII resistance, but also upon the capability of the health service to cope with the disease problem. The threat to the population should be considered when deciding whether or not second- and third-line drugs should be made available at the periphery, or at the first referral level.

Unless the health care system can cope adequately with a large number of referred patients and prevent mortality, it may be better to use second- and third-line drugs at the periphery.

3.5 Global requirements of antimalarial drugs

While determining the role of chemotherapy and chemoprophylaxis in malaria control, it is helpful to review periodically the use and requirements of antimalarial drugs throughout the world, since fluctuations and trends in usage reflect current national situations and the strategies being applied. This review can provide insights into the potential drug pressure on the parasite population, purchasing practices of governments, the cost involved in drug procurement, and future production requirements.

Data on the use of antimalarial drugs in the six WHO Regions from 1978 to 1979 and on the anticipated demands for 1980–1984 have been obtained from 56 countries and supplemented with estimates for the African Region and for part of the European Region. The data provide a reasonably good indication of the trend in drug requirements for national malaria control/eradication programmes until 1984. Subsequently, this list was augmented to 72 countries and information was obtained on the drugs used up to 1982 in most of these countries; the anticipated requirements were calculated for 1985. No analysis has been made of the reliability of the data received, except in some obvious cases of conflicting reports. In some instances, the data given represent demand and not consumption (see Table 8).

The quantities of antimalarial drugs, both those consumed prior to 1982 and the anticipated demands rose steadily from 1978 to 1985. The figures for the drugs used in 1982 appear to be higher than those anticipated for the future. An irregular increase occurred in each group of drugs. The 4-aminoquinoline group accounts for the greatest proportion of all the antimalarial drugs used in 1978–79 (94% in 1978) and it is expected that this trend will be maintained in the coming years, with a slight decrease every year (82% in 1985).
Table 8. Antimalarial drugs for national malaria control/eradication programmes within each of the six WHO regions: estimated consumption and requirements, 1978–85 (expressed in kg of the base)

<table>
<thead>
<tr>
<th>WHO Region*</th>
<th>Drug</th>
<th>Quantity used</th>
<th>Estimated quantity required</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFR</td>
<td>4-amino-quinolines</td>
<td>131 800</td>
<td>131 800</td>
</tr>
<tr>
<td>AMR</td>
<td></td>
<td>5 271</td>
<td>5 956</td>
</tr>
<tr>
<td>EMR</td>
<td></td>
<td>7 806</td>
<td>14 825</td>
</tr>
<tr>
<td>EUR</td>
<td></td>
<td>4 343</td>
<td>3 615</td>
</tr>
<tr>
<td>SEAR</td>
<td></td>
<td>102 600</td>
<td>72 613</td>
</tr>
<tr>
<td>WPR</td>
<td></td>
<td>13 685</td>
<td>25 638</td>
</tr>
<tr>
<td>Total</td>
<td>265 052</td>
<td>254 447</td>
<td>264 975</td>
</tr>
<tr>
<td>AFR</td>
<td>8-amino-quinolines</td>
<td>201</td>
<td>215</td>
</tr>
<tr>
<td>AMR</td>
<td></td>
<td>73</td>
<td>88</td>
</tr>
<tr>
<td>EMR</td>
<td></td>
<td>175</td>
<td>265</td>
</tr>
<tr>
<td>EUR</td>
<td></td>
<td>450</td>
<td>2 631</td>
</tr>
<tr>
<td>SEAR</td>
<td></td>
<td>169</td>
<td>170</td>
</tr>
<tr>
<td>Total</td>
<td>1 061</td>
<td>3 369</td>
<td>1 218</td>
</tr>
<tr>
<td>AMR</td>
<td>DHFR-inhibitors</td>
<td>27</td>
<td>36</td>
</tr>
<tr>
<td>EMR</td>
<td></td>
<td>326</td>
<td>445</td>
</tr>
<tr>
<td>EUR</td>
<td></td>
<td>543</td>
<td>489</td>
</tr>
<tr>
<td>SEAR</td>
<td></td>
<td>740</td>
<td>730</td>
</tr>
<tr>
<td>WPR</td>
<td></td>
<td>179</td>
<td>920</td>
</tr>
<tr>
<td>Total</td>
<td>1 815</td>
<td>2 601</td>
<td>3 452</td>
</tr>
<tr>
<td>AMR</td>
<td>Sulfonamides</td>
<td>75</td>
<td>99</td>
</tr>
<tr>
<td>EMR</td>
<td>and sulphones</td>
<td>25</td>
<td>17</td>
</tr>
<tr>
<td>SEAR</td>
<td></td>
<td>310</td>
<td>781</td>
</tr>
<tr>
<td>WPR</td>
<td></td>
<td>848</td>
<td>1 664</td>
</tr>
<tr>
<td>Region</td>
<td>Quinine</td>
<td>20000</td>
<td>20000</td>
</tr>
<tr>
<td>----------</td>
<td>---------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>AFR</td>
<td></td>
<td>2000</td>
<td>2000</td>
</tr>
<tr>
<td>AMR</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>EMR</td>
<td>610</td>
<td>610</td>
<td>610</td>
</tr>
<tr>
<td>SEAR</td>
<td>8262</td>
<td>10524</td>
<td>10786</td>
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<tr>
<td>WPR</td>
<td>4224</td>
<td>9945</td>
<td>2653</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>12506</strong></td>
<td><strong>14469</strong></td>
<td><strong>34249</strong></td>
</tr>
</tbody>
</table>

*AFR = African Region; AMR = Region of the Americas; EMR = Eastern Mediterranean Region; EUR = European Region; SEAR = South-East Asia Region; and WPR = Western Pacific Region.*
The average annual consumption for 1978 and 1979 was about 260 metric tons (tonnes) of base. After an expected increase in use in 1984 (353 tonnes), the annual forecast of demand is expected to diminish slightly to 351 tonnes in 1985.

The available data do not always specify the name of the 4-aminooquinoline compound but the bulk of the foreseeable demand is for chloroquine (about 90%), followed by amodiaquine (9%–11%).

The consumption of primaquine in the world (except for China) has increased from 1061 kg of base in 1978 to around 1200 kg in 1980. An increase observed in 1979 was due to the high consumption of primaquine in India. The figures in Table 8 include data from China since 1981.

The increase in the use of pyrimethamine was marked for the period 1978–79. Requirements continued to rise until 1981 and then diminished slightly. All Regions remained in the same relative position regarding the amounts used throughout the period 1978–85. The inclusion of data from China since 1981 indicates a change in quantity but not in the general trend.

Growing interest in the use of pyrimethamine, normally in combination with sulfonamides or sulfoones, probably reflects its increased use against P. falciparum resistant to 4-aminoquinolines.

The consumption of sulfonamides and sulfoones in recent years has shown a corresponding sharp increase. About 1300 kg of these drugs were used in 1978 throughout the world in antimalaria programmes; this quantity doubled in 1979 and doubled again in 1980 to reach 6 tonnes. The maximum demand had been estimated to be about 16 tonnes in 1982 and 1983, but further estimations indicated a reduction in the figure to 7–8 tonnes. The available data do not always specify the name of the drug, but sulfadoxine constitutes the bulk of the requirements. Among other drugs, sulfalene and dapsone are included in this group.

Sulfonamides and sulfoones alone are not sufficiently effective for the treatment of malaria, and as antimalarias they are therefore usually marketed in combination with pyrimethamine or another DHFR inhibitor. A reasonable ratio of sulfadoxine/pyrimethamine is 1:20 and annual figures for requirements of pyrimethamine and sulfa drugs are very close to this ratio.

The quantity of quinine used for antimalaria purposes accounted for 12.5 and 20.5 tonnes in 1978 and 1979, respectively. Estimates were provided only by the South-East Asia and Western Pacific
Regions, the main users of quinine; the total quantity for all the Regions would not be expected to exceed twice that amount.

No major increase in demand for quinine was anticipated in 1980–84; however, it should be pointed out that quinine is becoming increasingly important as an antimalarial.

An analysis of the updated figures of the global drug consumption revealed no significant changes in the trends already observed.

Despite a slight increase in the annual amount of 4-aminoquinolines used, the dominant role of this group of drugs would appear to be decreasing. The global picture, however, can be easily altered by significant changes in the drug practices of a single major consumer. For instance, the inclusion of data from China led to an increase in the role of 4-aminoquinolines and DHFR inhibitors. Similarly, a decrease in sulfones and sulfonamides was brought about by a change in utilization in Viet Nam.

It is regrettable that the data are incomplete but attempts to document the trends will continue and it is hoped that in time the quality and quantity of data will improve so that they will provide a more realistic picture.

The results of this survey demonstrate the variety in antimalarial drug usage and requirements throughout the world. Although no major overall changes in antimalarial drug usage are anticipated for national malaria control eradication programmes during the period 1980–84, these forecasts may underestimate the effects of a further expansion of resistance to antimalarial drugs. Moreover, although the capabilities of existing drugs do not yet seem to have been exhausted, a need for more effective and less expensive new drugs has become an important requirement for the global antimalaria campaign.

REFERENCES


4. RECENT PROGRESS IN CHEMOTHERAPEUTIC RESEARCH

Major advances made in many areas of biomedical research have greatly improved our knowledge of the chemotherapy of malaria. One such area is the pharmacokinetics of drugs. It is now widely recognized that the safe and effective use of drugs requires an understanding of their absorption, distribution, metabolism, and excretion as well as their possible interactions with other drugs. All these aspects are covered in the now rapidly advancing field of pharmacokinetics. The application of this discipline to the study of antimalarial drugs used at present is resulting in a more rational approach to their use, especially regarding the frequency of dose, the route of administration, and the problems of special groups such as children and persons with coexisting malnutrition, liver disease, or renal insufficiency.

The limitations in efficacy and safety of the currently available drugs and the increasing spread of resistance to some of them have encouraged further research on the development of new drugs. Some of these are of novel chemical structure while others are active substances isolated from traditional herbal remedies; these new compounds have reached various stages of pre-clinical and clinical evaluation. Pre-eminent among these new drugs is mefloquine which is now at an advanced stage of development.

Finally, it is recognized that a number of gaps still exist as regards malaria in current chemotherapeutic research and new approaches in basic and applied research are needed to fill them.

4.1 Pharmacokinetics of antimalarial drugs

4.1.1 Chloroquine

The basic pharmacokinetics of chloroquine were described in the 1940s (12, 16, 81). Further pharmacokinetic studies on chloroquine using various oral doses of the drug were reported in the 1960s (74–79). During the last 5 years the pharmacokinetics of chloroquine have been reinvestigated in a number of laboratories throughout the world, using more sensitive and more specific techniques for the
determination of the drug and its metabolites in body fluids and tissues (1, 11).

4.1.1.1 Absorption. Recent studies in healthy volunteers and children with malaria have shown that chloroquine is rapidly and almost completely absorbed from the intestinal tract. In one study (46), 300 mg of chloroquine base were given orally in the form of tablets or solution to healthy male adults weighing between 65 and 91 kg. Peak plasma concentrations of 56–102 μg/litre (mean 75 μg/litre) were reached in 1–6 hours (mean 3 hours), with a bioavailability of 75%. In another study (5), 10 mg of chloroquine per kg body weight was administered in the form of tablets to children with malaria. The peak plasma concentration (about 250 μg/litre) was reached in 2 hours with an absorption half-life of 0.56 hour. The concentration reached in the plasma within the first 30 min after administration of an oral dose of 10 mg/kg is usually substantially higher than the therapeutic level for sensitive P. falciparum (i.e., 30 μg/litre) (5, 127). In the study by Gustafsson et al. (46) 300 mg of chloroquine was given by slow intravenous infusion over a period of about 25 min. The peak plasma concentration was reached 5–15 min (mean 12 min) after starting the infusion and was 10 times the peak plasma concentration attained after an oral dose. Therefore, there is a very real danger of toxicity if chloroquine is given intravenously. In developing countries, where chloroquine is readily available without prescription and is often taken as the first-line home remedy in the presumptive treatment of fevers, toxic quantities of the drug may be taken. It was found that approximately 50% of children diagnosed as having malaria in a teaching hospital in Nigeria had high plasma chloroquine levels before they were given the drug, suggesting that it had already been taken as self-medication at home (127). If chloroquine is given intravenously to such patients, the plasma level attained may be high enough to produce severe or even fatal cardiotoxicity. Although there is no recent data on the rate of absorption of chloroquine after intramuscular administration in man, it has been shown (108) that the peak plasma concentration after intramuscular administration of 10 mg/kg in the rabbit was reached in 30 min, and was approximately twice the peak plasma concentration attained after the same dose was administered orally. Since chloroquine is rapidly and almost completely absorbed after oral administration, even in the presence of pyrexia, there is no need to administer the drug parenterally. A patient with a severe infection, unable to take
chloroquine by mouth should be given quinine intravenously as described in section 3.1.

4.1.1.2 Distribution. After absorption, chloroquine is distributed throughout the body. Early studies showed that the drug accumulated in the tissues: the concentrations found in the spleen, kidney, lungs, heart, and liver were 300–500 times higher than those found in the plasma (16, 74, 77). The drug has a particularly high affinity for the melanin-containing tissues of the skin and eye. These findings have been confirmed by more recent studies (2, 3). Because of the extensive tissue distribution and binding, chloroquine has an exceptionally large apparent volume of distribution—in one study a mean value of 204 litres/kg was found after intravenous dosing (46). Since the malaria parasite is intraerythrocytic during blood schizogony, i.e., during the phase of its life cycle that is responsible for the clinical manifestations of malaria, the concentration of chloroquine in red blood cells has always been of interest to investigators. Early studies (72, 129), showed that the erythrocyte/plasma chloroquine concentration ratio was approximately 100 in mice infected with P. berghei and only 10 in non-infected mice. The ratio was also less in mice infected with chloroquine-resistant parasites than in those infected with sensitive strains. The ratio of the chloroquine concentrations in erythrocytes and plasma has been investigated in children with acute malaria (5). The erythrocyte concentration was found to be higher than the plasma concentration during the sampling period of 7 days. At the height of the parasitaemia the ratio was 21 and this fell to a steady value of about 5.3 after 4 days when parasitaemia had completely disappeared. This study confirms that in man, there is preferential concentration of chloroquine in red blood cells and that this concentration is further increased by the presence of malaria parasites in the red cells. The value of 5.3 for the unparasitized erythrocyte plasma chloroquine concentration ratio obtained in African children (5) was similar to the value of 4.8 obtained in normal Swedish volunteers (46). The other cellular elements of blood—leukocytes and thrombocytes—have also been shown to concentrate the drug, even more so than erythrocytes (15) and, because of the elution of chloroquine into the serum during clotting, serum concentrations are usually higher than those found in the plasma.

In all studies on the kinetics of the distribution of chloroquine in different tissues, peak concentrations have been found to occur in erythrocytes and plasma at the same time (5, 46). In contrast, peak
concentrations were reached in different organs (e.g., liver, kidney, lungs, heart) at different times, which also differed from the peak concentration times in the plasma and red blood cells (2, 3).

The binding of chloroquine to plasma proteins is much less than would be expected from its extensive tissue binding, being only a little over 50% (4). The percentage binding to human serum albumin is less than the percentage binding to plasma, suggesting that chloroquine is bound to plasma proteins other than albumin (4). The non-albumin proteins to which chloroquine is bound have been shown to include α₁-acid glycoprotein (126). The percentage of chloroquine binding to protein is not affected by aspirin which is often given at the same time as chloroquine during the treatment of acute malaria.

4.1.1.3 Metabolism. Chloroquine is 7-chloro-4(4'-diethylamino-1'-methylbutylamino) quinoline (Annex 1, formula 1). It is metabolized by side-chain de-ethylation leading successively to first desethyl- and then bisdesethylchloroquine, which is a primary amine. This compound can be deaminated to form an alcohol (the 4'-hydroxy compound) which is oxidized to the 4'-carboxylic acid derivative. Successive dealkylation of the side-chain ultimately produces the compound 7-chloro-4-aminoquinoline (64, 77). The quinoline nucleus is resistant to degradation. Metabolism of chloroquine occurs slowly and the main metabolite varies in different species. In man it is desethylchloroquine (37, 46, 79).

The peak plasma concentration of desethylchloroquine was found to be 2–5 μg/litre after intravenous administration of 300 mg of chloroquine, this peak concentration being reached in about 6 hours (46). The peak plasma concentration of the metabolite after oral administration of the same dose was 10–20 μg/litre and the time to reach the peak concentration was approximately the same as for the parent compound. Walker et al. (127) also monitored the plasma concentrations of chloroquine and desethylchloroquine in children after a dose of 10 mg of chloroquine per kg. Desethylchloroquine was detected in the plasma after 30 minutes and reached a peak level within 2–12 hours—the same as for the unchanged drug. In both these studies the concentration of the metabolite remained at a value of 25–40% of that of chloroquine after the peak levels had been reached. The desethyl metabolite of chloroquine has the same profile of distribution and tissue binding as the parent drug (37). Individual variations in the pattern of distribution of chloroquine and its
desethyl metabolite may have an important bearing on the pattern of adverse reactions to the drug. The concentrations of chloroquine and desethylchloroquine in the plasma and skin have been studied in two groups of African subjects—those who itched after chloroquine intake and those who did not (85). Plasma concentrations were found to be similar in the two groups. However, the skin from subjects prone to chloroquine-induced pruritus contained higher concentrations of chloroquine and lower concentrations of the metabolite than skin from other subjects.

Recent studies have shown that desethylchloroquine has a similar activity against chloroquine-sensitive plasmodia as the parent drug (6).

4.1.1.4 Elimination. Chloroquine is eliminated from the body so slowly that, after a single dose of 300 mg, the drug and its metabolites can be detected in the plasma for up to 56 days, depending on the sensitivity of the assay method used. The decline in the concentration of chloroquine in the plasma with time after a single dose is polyexponential, suggesting a multi-compartmental distribution. The decline of the erythrocyte concentration after the peak level has been reached parallels that of the plasma concentration, showing that equilibrium is reached quickly and is subsequently steadily maintained between these two media which can therefore be regarded as behaving pharmacokinetically as a single compartment with regard to chloroquine (5, 46). In contrast, the decline phase of the log concentration–time curves for the various tissues diverges from that of the plasma curve with time. The tissues therefore behave as separate compartments with slower elimination rate constants than plasma (2, 3). The determination of the real half-life of a drug depends on the identification of the true terminal log linear elimination phase. With chloroquine this is virtually impossible to achieve in view of its continuous redistribution from the large tissue stores to the plasma over weeks or even months. Estimates of the plasma half-life of chloroquine would thus vary, depending on the duration of sampling. The longer the sampling period, the greater the number of exponential functions of the log concentration–time curve revealed, the flatter the “terminal slope” and the longer the half-life. This probably explains the wide variations in the values for the plasma half-life obtained by different authors over the years. These values have ranged between 2½ days on sampling for 7 days (12), and about 10 days on sampling
for up to 56 days (46). It has been suggested (38) that the kinetics of chloroquine are dose-dependent on the basis of a study in which 3 different doses (250, 500, and 1000 mg) of the drug were given and 3 significantly different half-lives (3.1, 42.9, and 312 hours, respectively) were obtained. However, examination of this report reveals that sampling lasted for only 50 hours after the 500 mg dose and for 1176 hours after the 1000 mg dose. It is therefore obvious that different exponential functions of the concentration–time curves were being analysed, a procedure that was bound to produce different half-lives. Indeed, recent studies have failed to confirm that chloroquine kinetics are dose-dependent (7, 46).

The total plasma clearance of chloroquine varies between 750 and 1050 ml/min and the renal clearance is between 400 and 450 ml/min (46). Since the value for renal clearance is more than the glomerular filtration rate, the drug is probably excreted by both glomerular filtration and tubular secretion. The kidney is the main route of elimination of chloroquine from the body and the drug is detectable in the urine for 120 days after a dose of 300 mg. Maximum excretion was found to occur in the first 24 hours, about 10% of the administered dose being excreted in that interval (46). Subsequently, urinary excretion decreased exponentially, with a computed total quinoline urinary recovery of between 50 and 60% of the administered dose. Chloroquine is predominantly excreted unchanged, the desethylchloroquine metabolite accounting for only about 25% of the excreted drug.

4.1.1.5 Effect of race. A comparison of the pharmacokinetics of chloroquine in whites and blacks found them to be basically the same (78). The pharmacokinetic data obtained in the recent studies of Salako and his colleagues on Africans (5, 126) do not differ substantially from the values given in the literature for whites. In one study (34), the rate of excretion of chloroquine in the first 7 hours after administration of the drug was compared in 4 racial groups—British, Gambians, Sudanese, and Thais. The values obtained for the British, Gambians, and Sudanese were similar, but the Thais excreted significantly more drug than the other 3 racial groups. The proportion of excreted desethylchloroquine to chloroquine was, however, similar in all 4 groups.

4.1.1.6 Effect of disease. Wharton & McChesney (130) studied the effect of malnutrition on the metabolism of chloroquine. The subjects studied were African children suffering from kwashiorr
a condition characterized by a fatty liver. The malnourished children excreted less desethylchloroquine (as a percentage of excreted drug) in the urine before treatment of their kwashiorkor condition than after, suggesting that less chloroquine was being metabolized because of the presence of kwashiorkor. There is as yet no definitive study to compare chloroquine kinetics in normal subjects and in those with hepatic insufficiency.

The kinetics of elimination of chloroquine in patients with renal insufficiency after a single oral dose (600 mg) of the drug have been investigated (109). It was found that the rate of decline of the plasma concentration with time in patients with renal failure was less than in normal subjects. It was estimated that the elimination half-life of chloroquine would be longer in patients with renal insufficiency than in patients with normal renal function. Other studies have not shown malaria infection to have any effect on the pharmacokinetics of chloroquine except for the increased concentration of the drug in parasitized red blood cells (5, 126).

4.1.2 Quinine

Quinine is rapidly and almost completely absorbed from the intestine. Peak plasma concentrations are reached 1–3 hours after a single oral dose. After absorption, quinine is distributed throughout most of the body fluid. The concentration in red blood cells is approximately one-fifth of that in plasma and, in cerebral malaria, the concentration in the cerebrospinal fluid is approximately 7% of that in plasma. The apparent volume of distribution is about 2.5 litres/kg. Quinine is metabolized in the liver and is excreted partly unchanged but mainly as the hydroxylated metabolite. Elimination from the body is rapid. The drug and its metabolite appear in the urine within 1 hour of its administration and little remains in the body after 48 hours (49, 131). The decline of plasma quinine concentration with time after a single dose of the drug is monoexponential, with an elimination half-life of 10–12 hours in normal individuals. The main method of quinine elimination is via hepatic metabolism, renal clearance of unchanged quinine accounts for only about 20% of the total clearance. There is some evidence that renal excretion of quinine is by both glomerular filtration and tubular secretion (131).

A recent study shows that the pharmacokinetics of quinine are altered significantly by malaria infection (131). Clearance and the
apparent volume of distribution are lower in patients with falciparum malaria, the reduction being greater as the severity of the infection increases. Renal clearance is decreased with malaria infection, but the reduction does not appear to be related to its severity. Renal insufficiency does not alter the disposition of quinine significantly although it is usual to recommend a reduction in the dose for a patient with impaired renal function. Hepatic metabolism of quinine is reduced when there is hepatic insufficiency (123). Since there is reduced hepatic blood flow as well as histopathological evidence of liver damage in experimental malaria (9, 117), the reduction in the total clearance of quinine and the prolongation of its half-life are probably related to impaired liver function in the acute phase of the infection.

4.1.3 Primaquine

Primaquine is rapidly absorbed from the intestine. After a single oral dose of 45 mg of primaquine base, a peak plasma level of up to 250 μg/litre is reached within 1–3 hours. The drug is distributed throughout the body fluid with an apparent volume of distribution of about 3.5 litres/kg. Only a very small amount is fixed in the tissues. Primaquine is eliminated rapidly from the body. The elimination phase of the plasma concentration–time curve is monoexponential with a half-life of about 6 hours, and the drug is almost completely eliminated from the body within 24 hours of administration. The plasma clearance is probably due predominantly to metabolic degradation since only 1% of an administered dose is recovered in the urine as unchanged primaquine in 24 hours (43). 6-Methoxy-8-aminoquinoline was identified as one of the metabolites of primaquine (14), but the major one found in the plasma of rodents and man has been identified as a carboxylic acid metabolite of primaquine in studies conducted at the University of Mississippi and in Chicago. The studies of Greaves et al. (43) did not find any significant differences between primaquine kinetics in British and Thai subjects, neither were the kinetics altered in subjects deficient in the enzyme glucose-6-phosphate dehydrogenase.

4.1.4 Pyrimethamine

Pyrimethamine is absorbed relatively rapidly from the intestine so that after an oral dose, the peak plasma level is reached between
2–6 hours. The absorption is almost complete. The drug is distributed throughout the body fluid and is moderately bound to tissues with an apparent volume of distribution of about 3 litres/kg. It is about 80% bound to plasma protein. Salivary concentration is about 20% of that of plasma and reflects the unbound drug concentration in the plasma (8). The drug persists in the plasma and continues to be excreted in the urine for as long as 2 weeks after the administration of a single oral dose of 25 mg. The elimination half-life in a number of studies has been of the order of 80–100 hours (8, 55).

4.1.5 Proguanil

Proguanil is rapidly absorbed from the intestine, the peak concentration after a single oral dose is reached in about 4 hours. It is concentrated in the red blood cells where the concentration is 4–8 times greater than that of plasma. It is converted in the body to a triazine metabolite, cycloguanil, which is thought to be the active compound. Excretion is slow and takes place mostly via the kidney. About 40% of a given dose can be recovered from the urine and faeces in the unchanged form, the rest as the metabolite. The plasma elimination half-life is about 24 hours.

4.1.6 Sulfadoxine and sulfalone

These two compounds are long-acting sulfonamides. They are rapidly and well absorbed but slowly excreted. The elimination half-life of sulfadoxine is estimated to be between 100 and 200 hours while that of sulfalone is about 65 hours. They are highly bound to plasma protein. Only a small proportion is metabolized, about 5% to the acetyl derivative and 2–3% to the glucuronide. Acetylation of sulfonamides depends on the type of sulfonamide and is also known to be genetically determined, individuals being either slow or rapid acetylators. However, because such a very small percentage of sulfadoxine and sulfalone is subject to acetylation, differences in acetylator phenotypes should not affect the activity of these drugs in man.

4.1.7 Dapsone

Dapsone is well absorbed from the intestine, the peak plasma concentration is reached 3–6 hours after oral intake. It is distributed
throughout all the tissues. It is about 75% bound to plasma protein. Salivary concentration, which reflects the free plasma dapsone concentration, is about 25% of the total plasma concentration. The concentration in red blood cells is practically the same as that in plasma.

Dapsone is polymorphically acetylated in man, initially to monoacetyldapsone (39), but the pharmacokinetics of dapsone are similar in both fast and slow acetylators. The half-life for the elimination of dapsone from the plasma is about 26 hours (8, 39, 41). Dapsone and its metabolites are excreted into the bile and reabsorbed from the intestine. Urinary excretion ultimately eliminates about 90% of an administered dose of dapsone, mainly in the form of metabolites, the remainder being excreted in the faeces.

4.1.8 Fixed-dosage combinations

Some of the antimalarials most widely used at present are fixed-dosage combinations of two different antimalarial drugs. The advantages of such combinations are:

(a) the two drugs potentiate the actions of each other. The potentiation may be such that the combination is effective against parasites resistant to either individual component and active against stages of the parasite’s life cycle not normally sensitive to either component;

(b) there is the possibility of administering reduced doses of each component, with a consequent reduction in dose-related adverse reactions;

(c) the likelihood of resistance developing to the component drugs is reduced;

(d) combinations may also provide simultaneous activity against different stages of the parasite’s life cycle, e.g., against asexual blood forms and gametocytes.

It is important that certain pharmacokinetic parameters, particularly absorption rate and elimination half-lives, of the individual drugs in a combination should be as close as possible. This is not always the case. Thus, although the elimination half-lives of pyrimethamine and sulfadoxine (the components of Fansidar) are similar (about 100 hours), those of dapsone (25 hours) and pyrimethamine (the components of Maloprim) are dissimilar.

100
There are very few studies on the effects of one member of a combination on the pharmacokinetics of the other. One such study (8) investigated the interaction between dapsone and pyrimethamine. They found that the pharmacokinetics of pyrimethamine were not affected by dapsone. On the other hand, the peak concentration of dapsone was decreased and its apparent volume of distribution increased by pyrimethamine. Pyrimethamine also displaces dapsone from plasma protein binding sites, thus increasing the free plasma dapsone concentration, but has no effect on its elimination half-life. It is therefore obvious that studies on the pharmacokinetic interaction between the individual drugs in a combined preparation should prove helpful in evaluating their optimal proportion in the combination.

4.2 Mefloquine

4.2.1 Origin and preliminary studies

During the Second World War there was an active programme within the United States of America to discover and test new antimalarial drugs. Some 16 000 chemicals were tested for antimalarial activity during this time. One chemical class of interest was the quinolinemethanols and the drug with the most activity in this class was SN 10275. This drug was developed and tested in 5 volunteers with induced vivax malaria and all 5 were clinically cured.

Despite this encouraging evidence of activity, the development of SN 10275 was not pursued because of phototoxicity. One month after a number of volunteers had taken the drug to assess its tolerance, they participated in a baseball game and developed an erythematous reaction to the sun. This reaction persisted for several months because of the long half-life of the drug.

When the United States Army began working on new drugs in the 1960s, this old lead was re-examined and it was decided to attempt to synthesize, on a selective basis, new compounds that might retain the antimalarial activity of SN 10275, but which would not have phototoxic characteristics.

A series of new quinolinemethanols was synthesized and tested. Initially, ring modifications of the basic structure of SN 10275 in the
2, 6, and 8 positions showed that antimalarial activity or efficacy was directly related to phototoxicity—the more active, the more phototoxic. With continued synthesis, activity and phototoxicity began to separate. One of the more active compounds to be produced during these studies was WR 30090. This was a very active drug with minimal phototoxicity in the mouse test system.

WR 30090 had advanced to the stage of clinical trials in the late 1960s and was used to treat patients whose malaria infections recurred repeatedly despite treatment with all available drugs in a variety of combinations. In these cases the drug was uniformly successful and a limited field trial in Viet-Nam showed that it was about 90% successful in treating primary infections. This cure rate was equivalent to the standard treatment which, at the time, consisted of a combination of quinine, pyrimethamine, and a sulfonamide or sulfone. The major disadvantage of WR 30090 was its poor bioavailability and resulting erratic absorption, and the need for at least 6 days of drug administration.

Meanwhile, other quinolinemethanols had been synthesized and tested and some of them had even greater antimalarial activity than WR 30090. The next drug selected from this class for advanced testing was WR 142,490 (racemic erythro-α-(2-piperidyl)-2,8-bis(trifluoromethyl)-4-quinolinemethanol which later was named mefloquine (Annex 1, No. 19). The initial development and testing of mefloquine was performed by the United States Army; since 1976 it has been developed by WHO, in collaboration with the Walter Reed Army Institute of Research and Hoffmann-La Roche.

In the mouse screening tests, mefloquine was about as active as WR 30090 but was not at all phototoxic. Further tests in Aotus monkeys were even more encouraging and showed that the drug could be curative when given in single large doses.

Extensive pre-clinical toxicological studies demonstrated sufficient safety for continued development. Using ¹⁴C-labelled mefloquine, the absorption, the distribution in different organs, and the elimination of the labelled material were determined in mice, rats, and Aotus monkeys (71, 82, 107, 118). The structure of several metabolites in rats was elucidated (34) and new methods of assessing the concentration of the parent (active) substance and of its main metabolite in various body fluids were developed (110).

Following a single-dose kinetic study of mefloquine in man (28), the fate of unchanged mefloquine and of its main metabolites in laboratory animals and volunteers (Africans and Europeans) has
been described (110, 111). The absorption was rapid, the apparent half-life of absorption being 0.36–2.0 hours. The plasma concentration of mefloquine was about 1.0 mg/litre 2–12 hours after administration (Fig. 8). The decline of the plasma concentration was extremely gradual, the calculated terminal half-life of elimination ($t_{1/2}$) ranging from 15 to 33 days (mean 21.4).

In the plasma more than 99% of mefloquine is bound to protein; however, the concentration in or on erythrocytes is nearly 170% of that in the plasma. About half of the mefloquine retained by the red blood cells appears to be bound to the cell membrane (82).

Fig. 8. Plasma levels of unchanged mefloquine and of its main metabolite following oral administration of 1000 mg of mefloquine base*

*Reproduced from Schwartz et al. (111) by permission of the publisher.

Assuming that the dose administered was totally absorbed, it has been estimated (111) that the volume of distribution of mefloquine ranges between 27–50 litres/kg, indicating that the drug is widely distributed in tissue compartments.
The elimination of unchanged mefloquine in the urine is very slow, being about 5% of the dose within the first 4 weeks. The drug is mainly eliminated by biotransformation and only 4 hours after oral intake measurable amounts of the main metabolite (2,8-trifluoromethyl-quinoline-4-carboxylic acid) can be detected in the plasma. After a few days, the concentration of this metabolite exceeds that of mefloquine and the area under the plasma concentration–time curve is about 3.4 times greater than that of the active unchanged drug. However, the volume of distribution of the metabolite is small (in the dog about 30 times smaller than that of mefloquine); it does not diffuse into tissue compartments or into the erythrocytes. Toxicological trials performed in animals with the main metabolite indicate that this substance does not markedly affect the toxicity of mefloquine.

Plasma levels gradually increase when repeated weekly doses of mefloquine are administered, reaching the steady state level after only a few months. In a tolerance trial in which volunteers received 250 mg of mefloquine base at weekly intervals, mean mefloquine steady-state plasma level minima of 0.56–1.25 mg/litre were found. Mean plasma levels of the metabolite, measured at the same time, were 3.5–8.6 times greater than those of mefloquine. The half-life of elimination of the metabolite from the plasma was about equal to or slightly shorter than that of mefloquine. After 6 months of weekly administration the elimination half-life of mefloquine from plasma was assessed (multiple-dose kinetics) and was found to range from 17 to 35 days (mean 22.5), i.e., the same as after the administration of a single dose (110, 111). This indicates that no enzyme induction or inhibition is to be expected after prolonged therapy.

4.2.2 Clinical trials of mefloquine

Clinical trials of mefloquine started in 1972 within the research programme of the Walter Reed Army Institute of Research. In addition to rising-dose tolerance studies, special studies were undertaken because of early complaints of dizziness, and toxicological observations that showed histological changes in the epididymis of a single animal species after very high doses and prolonged administration. These special studies included neurological examinations, cold pressure tests, caloric stimulation and orthokinetic nystagmus testing, audiograms, and electro-encephalograms. In addition, repeated monthly spermatograms
were performed during a long-term study in healthy volunteers. The results of these tests remained within the normal range both in the mefloquine-treated group and in subjects receiving placebo at weekly intervals for 22 weeks.

Many study reports on mefloquine have been published (24, 26, 29, 30, 50, 122). The tolerance and pharmacokinetics were assessed in healthy volunteers and the efficacy against various malaria parasites studied in more than 1000 patients. Performance of these clinical trials has largely followed the procedures outlined in Annex 5.

The curative activity of mefloquine in 854 patients with falciparum malaria is shown in Table 9. The main side-effects observed were dizziness, nausea, vomiting, diarrhoea, and headache (see section 4.2.4).

<table>
<thead>
<tr>
<th>Mefloquine dose</th>
<th>No. of patients</th>
<th>Type of response</th>
<th>Cure rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S</td>
<td>RI</td>
</tr>
<tr>
<td>Adults</td>
<td>500 mg</td>
<td>63</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>750 mg</td>
<td>215</td>
<td>206</td>
</tr>
<tr>
<td></td>
<td>1000 mg</td>
<td>235</td>
<td>226</td>
</tr>
<tr>
<td></td>
<td>1250-1500 mg</td>
<td>181 (NI)</td>
<td>180</td>
</tr>
<tr>
<td>Children</td>
<td>20 mg/kg</td>
<td>65</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>25 mg/kg</td>
<td>37</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>30 mg/kg</td>
<td>58</td>
<td>53</td>
</tr>
<tr>
<td>Total</td>
<td>854</td>
<td>826</td>
<td>24</td>
</tr>
</tbody>
</table>

*Pooled results from 17 clinical research projects.
*One patient was a case of reinfection and two patients suffered from vomiting.
*One patient suffered from vomiting.
NI: Non-immune subjects.

A suppressive prophylactic field trial carried out by the Walter Reed Army Institute of Research in Thailand was performed at the end of the 1970s, before the spread of strains of \emph{P. falciparum} resistant to the sulfadoxine/pyrimethamine combination in this area (90). The study population comprised the semi-immune inhabitants of five hamlets in the Bhu Phram Valley, Prachinburi Province,
150 km northeast of Bangkok. All eligible and consenting villagers
over 10 years of age were included in the study group of 990 subjects.
Assignment to one of the treatment groups was made on a stratified
random number basis.

The suppressive treatment lasted for 26 weeks, each subject
being visited weekly at his home on a pre-arranged day. The drug
was always taken under the supervision of a technician. Besides
weekly parasitological tests, haematological and immunological
parameters were checked to assess the safety of the drug. All
participants were re-examined 1, 3, and 15 weeks after the end of the
suppressive treatment. The blood slides were examined
independently by three technicians. When discrepancy between the
findings occurred, the slides were sent to and examined by the
principal investigator.

During the follow-up period, all subjects who had experienced
falciparum parasitaemias were given a therapeutic dose of
sulfadoxine (1500 mg)/pyrimethamine (75 mg), and those with vivax
or malariae parasitaemias were treated with the standard regimen of
chloroquine (1500 mg given over a 3-day period), followed by
primaquine, 15 mg daily for 14 days, for those study subjects known
to be normal for the enzyme glucose-6-phosphate dehydrogenase.
The results of 6 months of chemosuppression were analysed
separately for *P. falciparum* and *P. vivax*. Good protection was
achieved by the test drug: there was practically no failure in the
subjects treated with 180 mg or 360 mg of mefloquine hydrochloride
once a week or once every two weeks, respectively.

Sulfadoxine (500 mg) plus pyrimethamine (25 mg) taken once a
week was the most effective commercially available drug in
preventing falciparum malaria in this area where chloroquine-
resistant malaria was already highly prevalent. Mefloquine was more
active than the standard drugs used in the 1980s for preventing
falciparum and vivax parasitaemia. Tolerance and acceptance were
very satisfactory when the drug was given for suppressive treatment
for up to 26 weeks.

Tolerance trials with 500 mg of mefloquine weekly for 1 year
showed that the drug was well tolerated by 20 volunteers, except for
a slight loss of hair in two subjects. The laboratory findings in this
double-blind study were identical in the placebo and in the
mefloquine group. An additional double-blind suppressive study in
53 non-immune Europeans travelling to Africa showed that 250 mg
of mefloquine weekly was as well tolerated as 300 mg of chloroquine.
Based on the results of the clinical trials, the following dose recommendations can be made for the effective cure of *P. falciparum* infections:

*(a)* adults: 18–20 mg/kg body weight (750–1250 mg) as a single dose unless the dose is higher than 750 mg, when 250 mg or 500 mg should be given 8 hours after the first dose of 750 mg;

*(b)* children: 25 mg/kg body weight as a single dose.

Half these dose levels is sufficient to eliminate *P. vivax* from the blood; however, hypnozoites will not be eliminated unless primaquine is given. If in acute falciparum malaria parenteral therapy is required, quinine infusion should be administered initially and followed by mefloquine 8–12 hours after the last infusion.

For suppressive treatment, 250 mg of mefloquine base is the standard dose for adults, given at weekly intervals. Half of this dose might be sufficient in the steady state (i.e., after about 2 months). In children, suppressive treatment will require 4–6 mg of mefloquine base per kg body weight weekly (Table 10).

<table>
<thead>
<tr>
<th>Adults and children above 45 kg body weight</th>
<th>1 tablet weekly</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children*: 15–19 kg</td>
<td>¼ tablet weekly</td>
</tr>
<tr>
<td>20–30 kg</td>
<td>¼ tablet weekly</td>
</tr>
<tr>
<td>31–45 kg</td>
<td>⅐ tablet weekly</td>
</tr>
</tbody>
</table>

*No clinical experience is available with mefloquine in infants.

4.2.3 *Clinical trials of the combination of mefloquine with sulfadoxine pyrimethamine*

One of the major concerns when introducing a new drug for malaria treatment is the emergence and rapid spread of strains of *P. falciparum* resistant to the new drug. Type II mefloquine resistance has been observed in Thailand (17). In this area, mefloquine was available only for very limited clinical trial use, but it is possible that resistance to quinine already existed. The discovery of mefloquine resistance in a Tanzanian isolate of *P. falciparum* (19) was more surprising. The systematic *in vitro* testing of *P. falciparum* strains in Gabon (18) indicated that there was, in some instances, reduced sensitivity to mefloquine in strains highly resistant to
chloroquine. Reduced sensitivity was also found among *P. falciparum* isolates tested in the Philippines (119).

These observations have convinced many malariologists that it would be unwise to make widespread use of mefloquine alone and that a rational combination should be developed that will delay the emergence of mefloquine resistance. It has been shown that specific combinations of antimalarials delay the emergence of resistance in experimental models (91) (see section 2.1). The pharmacokinetics of the different components have to be well matched, so that the 2 or 3 active compounds remain together and in contact with the parasites in the erythrocytes for a sufficient length of time. In suppressive treatment, all of the drugs administered should remain in the blood at effective levels during the dosage interval (35). These requirements are fulfilled by a combination of mefloquine with sulfadoxine/pyrimethamine.

The development of resistance to mefloquine is easy to induce experimentally, but strains of *P. berghei* resistant to mefloquine appear to be more susceptible than expected to the combination of mefloquine with sulfadoxine/pyrimethamine. This indicates potentiation (see Table 11) rather than the additive effect observed with strains of *P. berghei* that are normally sensitive to mefloquine.

Table 11. Blood schizontocidal activity of the combination (1:3) of mefloquine and sulfadoxine/pyrimethamine compared with the activity of these two components given separately on a strain of *P. berghei* resistant to mefloquine *

<table>
<thead>
<tr>
<th></th>
<th>Mefloquine</th>
<th>Sulfadoxine/ pyrimethamine</th>
<th>Combination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mefloquine resistant</td>
<td>100</td>
<td>11</td>
<td>6.4</td>
</tr>
</tbody>
</table>

*A* After Leiner & Weidemann (68).

The risk of selecting primary resistant strains of *P. falciparum* exists when mefloquine is used alone because such strains are known to have been present in some areas even before the drug was deployed (17–19, 119).

When using the *P. berghei* model for inducing resistance to mefloquine, it has been shown that if the sulfadoxine/pyrimethamine combination is given together with mefloquine, there is a considerable reduction in the level of resistance induced to the drugs (and the
rate of induction) as compared with the rapid development of the high degree of resistance induced by mefloquine alone (80, 92, 93).

These findings were convincing enough for studies to be made of the activity, toxicology, and pharmacokinetics of such a triple combination in several animal models. The additive antimalarial activity of these three compounds, in the absence of an increase in toxicity, allowed clinical trials to be initiated using tablets containing 250 mg of mefloquine, 500 mg of sulfadoxine, and 25 mg of pyrimethamine.

The animal toxicology study was the most intensive ever undertaken for an antimalarial drug. The pharmacokinetics of all the components were studied in the animal species used for toxicology, including pregnant animals for reproductive studies. The results of some of these studies in animals have been published (80, 105, 132).

Tolerance studies in healthy volunteers using doses of 500 mg of mefloquine, 1000 mg of sulfadoxine, and 50 mg of pyrimethamine produced mild symptoms in 4 out of 8 subjects (loose stools, flatulence, tinnitus) lasting for not more than one day. One subject had abdominal cramps on the 28th day after the dose.

In a double-blind study on patients suffering from symptomatic falciparum malaria in Thailand, the triple combination was compared with mefloquine (68). One hundred male patients aged 15–51 years were included in this study. Efficacy was assessed in 47 patients who received 750 mg of mefloquine alone and in 49 who received the triple combination (mefloquine 750 mg + sulfadoxine 1500 mg + pyrimethamine 75 mg). The follow-up lasted for 42 days. Parasitaemia was cleared in all patients, but in 3 of them P. falciparum reappeared.

In the group treated with mefloquine alone, recrudescence with low parasitaemia occurred in one patient on day 14. In a second patient parasites reappeared on day 42, which could well have been a reinfection.

In the group treated with the triple combination, there was a single recrudescence; this patient vomited shortly after treatment and the amount of drug absorbed might have been insufficient.

A similar study was performed in Brazil with groups of 25 patients who received mefloquine, sulfadoxine/pyrimethamine, or the combination of these two drugs. Sulfadoxine/pyrimethamine treatment was less effective than the others; there were two RII and three RI-type failures. In both groups treated with mefloquine, alone
or in combination, asexual parasitaemia was cleared within 3.3 or 3.0 days, respectively, without recrudescence.

In double-blind, randomized, comparative, dose-finding studies, three dose levels of the combination were compared in groups of 50 patients with *P. falciparum* infections: doses were one tablet containing 250 mg of mefloquine + 500 mg of sulfadoxine + 25 mg of pyrimethamine, two tablets, or three tablets. The studies were performed in Ndola (Zambia) and in Belém (Brazil). The results of the African study showed that tolerance was satisfactory and there were no recrudescences in any group after a follow-up period of 63 days. This very high radical cure rate for *P. falciparum* with a single tablet may be due to the fact that there is no resistance to sulfadoxine/pyrimethamine in this area. In Brazil where resistance to sulfadoxine/pyrimethamine is common, recrudescence occurred in 6 out of 35 patients treated with 1 tablet only, whereas there was complete cure in the groups treated with 2 or 3 tablets (35 patients each).

An outpatient study was conducted in the Kuchinarai District in Thailand, on adult male patients suffering from *P. falciparum* malaria, in which mefloquine alone was compared with the combination of mefloquine and sulfadoxine/pyrimethamine. A double-blind, randomized study was performed: 47 patients received mefloquine (750 mg) alone, and 49 patients received mefloquine (750 mg) + sulfadoxine (1500 mg) + pyrimethamine (75 mg). In the mefloquine treated group one patient showed a low level of parasitaemia on day 14 that cleared without any further treatment. One patient was found to be positive on day 42; in this case reinfection might have occurred. No recrudescence occurred in any other patient during a follow-up period of 42 days. In the mefloquine + sulfadoxine/pyrimethamine treated group, recrudescence occurred on day 21 in one patient who had vomited 70 minutes after receiving the drug. No recrudescence occurred in any other patient. In both groups tolerance was good and there was no significant difference in parasite clearance and clinical response.

In another study in the same area, mefloquine was given at two dose levels, 1000 mg to one group of 150 patients and 750 mg to another group. Both groups also received a single dose of primaquine, 45 mg of base, half of the patients in each group on day 0 and half on day 3. Only 3 patients failed to complete at least 28 days follow-up. All of the patients responded clinically and no recrudescence of parasitaemia occurred in any of them before day