



7294

UNDP/WORLD BANK/WHO SPECIAL PROGRAMME FOR
 RESEARCH AND TRAINING IN TROPICAL DISEASES
 Geneva, 3-5 June 1985

over
 malaria - the r
 Antimalarials -
 Plasmodium - f. eff



SCIENTIFIC WORKING GROUP ON THE CHEMOTHERAPY OF MALARIA

THIRD PROGRAMME REVIEW

CONTENTS

	<u>Page</u>
1. INTRODUCTION	2
2. ADVANCES IN THE PAST DECADE	4
2.1 Experimental Parasitology	4
2.1.1 Parasite structure and organelle function.	4
2.1.2 Biochemistry	5
2.1.2.1 Glycolysis	5
2.1.2.2 Pentose phosphate pathway	5
2.1.2.3 Oxygen utilization and electron transport	5
2.1.2.4 Lipids	5
2.1.2.5 Haemoglobin utilization and pigment formation	6
2.1.2.6 DNA synthesis	6
2.1.2.7 Purines and pyrimidines	6
2.1.2.8 Histidine-rich protein	7
2.1.3 Modes of drug action and mechanisms of resistance	7
2.1.3.1 Antifolates	7
2.1.3.2 8-Aminoquinolines and naphthoquinones	8
2.1.3.3 4-Aminoquinolines and related blood schizontocides	8
2.1.3.4 Antibiotics	10
2.1.3.5 Artemisinin (qinghaosu)	10
2.1.4 <u>In vitro</u> tests for drug activity against malaria parasites	11
2.1.4.1 Asexual erythrocytic parasites	11
2.1.4.2 Exoerythrocytic parasites	11
2.1.4.3 Gametocytes	11
2.1.5 Parasite genetics	12
2.1.6 DNA probes: Potential applications to malaria diagnosis	12

This report contains the collective views of an international group of experts convened by the UNDP/WORLD BANK/WHO SPECIAL PROGRAMME FOR RESEARCH AND TRAINING IN TROPICAL DISEASES (TDR). It does not necessarily reflect the views of TDR/WHO. In the interests of rapid communication it has been submitted to only minimal editorial revision. Moreover, any geographical designations used in the report do not imply the expression of any opinion whatsoever on the part of TDR or WHO concerning the legal status of any country, territory, city or area or of its authorities concerning the delimitation of its frontiers or boundaries.

Ce rapport exprime les vues collectives d'un groupe international d'experts réuni par le PROGRAMME SPECIAL PNUD/BANQUE MONDIALE/OMS DE RECHERCHE ET DE FORMATION CONCERNANT LES MALADIES TROPICALES (TDR). Il ne représente pas nécessairement les vues du TDR/OMS et, en vue d'une diffusion accélérée, il n'a pas été l'objet d'une mise en forme particulièrement soignée. En outre, les noms géographiques utilisés dans le présent rapport n'impliquent, de la part du TDR ou de l'OMS, aucune prise de position quant au statut juridique de tel ou tel pays, territoire, ville ou zone, ou de ses autorités, ni quant au tracé de ses frontières.

2.2	Drug Development	13
2.2.1	Clinical research centres	14
2.2.2	Clinical pharmacology	14
2.2.3	New formulations	14
2.2.4	Mefloquine and its combinations	15
2.2.5	Qinghaosu	15
2.2.6	Other new blood schizontocides	16
2.2.7	Primaquine	16
3.	RECOMMENDATIONS FOR FUTURE CHEMAL-SUPPORTED STUDIES	17
3.1	Parasite Biology in the Rational Development of New Drugs	17
3.2	In Vitro Systems	17
3.3	Genetics	18
3.4	Modes of Drug Action and Mechanisms of Resistance	18
3.5	Drug Development	18
3.6	Clinical Research Centres	19
3.7	Drugs: Candidates for Immediate Development	20
4.	REFERENCES	20
5.	PARTICIPANTS	21

1. INTRODUCTION

The Scientific Working Group (SWG) on the Chemotherapy of Malaria (CHEMAL) of the UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases (TDR) met in Geneva, Switzerland, on 3-5 June 1985 to review the CHEMAL programme and make recommendations on future directions for research.

CHEMAL's research programme was established in 1975. The following brief review of the topics of its SWG meetings reflects some of the major research directions since 1978, when the second SWG reviewed the objectives and progress of the CHEMAL programme, identified problems in its implementation and determined its future direction (1). In October 1980, the third SWG focused on tissue schizontocides (2). An SWG Section Meeting in May 1981 reported on the development of mefloquine (3) and the fourth SWG in October 1981 considered the development of qinghaosu and its derivatives as anti-malarial drugs (4). The proceedings of an SWG meeting on the modern design of antimalarial drugs, held in Bethesda, MD, USA, in June 1982, were published in 1983 (5). A second publication comprising papers on the pharmacokinetics, metabolism, toxicity and activity of primaquine, presented at an SWG meeting held in Geneva in February 1984, is in press (6).

Noteworthy advances made during the past decade were highlighted in TDR's Seventh Programme Report (7) and included the following:

- Mefloquine, the first new antimalarial to be clinically tested in thirty years, has been registered in Switzerland for the prophylaxis and treatment of malaria in adult males and nonpregnant females and in children over two years of age. The triple drug combination comprising mefloquine, sulfadoxine and pyrimethamine has been registered in Switzerland and Thailand. Restricted use of the combination in adults and in children over two years of age began in Thailand in late 1984.
- Kits for the in vitro testing of Plasmodium falciparum for susceptibility to sulfadoxine and pyrimethamine are being standardized in a

multicentre study in Switzerland, Thailand and the United States and field-tested in several malaria-endemic countries. Kits for testing susceptibility to chloroquine, amodiaquine, mefloquine and quinine have been widely used for at least five years.

- One of the most noteworthy accomplishments of the CHEMAL programme has been the extensive collaboration established between WHO, government institutions, pharmaceutical companies and other laboratories. Particular note is made of the role of the Walter Reed Army Institute of Research (WRAIR), Washington, DC, USA, and Hoffmann-La Roche and Company, Basel, Switzerland, in the development of mefloquine.
- Negotiations are under way with five pharmaceutical companies on the clinical development of new blood schizontocidal compounds belonging to a variety of chemical classes. Several simple trioxane-ring compounds have also been shown to possess blood schizontocidal activity and several new 8-aminoquinolines show tissue schizontocidal activity. Primaquine itself has been the subject of intense research, essentially on pharmacokinetics, metabolism and mode of action, and significant findings are emerging.
- Exoerythrocytic stages of *P. vivax* have been successfully grown in culture, thus paving the way to the development of *in vitro* screens for tissue schizontocidal activity of primaquine, its metabolites and other 8-aminoquinolines.

The promising development of DNA probes for malaria diagnosis must be added to these advances. When made sufficiently selective and sensitive for use in field surveys, these probes are likely to transform the technology used to detect the presence of malaria parasites in humans and mosquitos, making it possible for a single technician to process more than a thousand prepared specimens a day.

Modern laboratory techniques have led to significant progress in understanding the basic biology and metabolism of malaria parasites and in discovering leads for designing new antimalarial drugs. In the coming years the main source of new and effective drugs is likely to be the rational selection of chemotherapeutic compounds.

The present meeting reviewed the objectives of the CHEMAL programme in the light of the above advances and the current malaria epidemiological situation, which has deteriorated in many parts of the world since the last programme review was held in July 1978. The effectiveness of available therapeutic agents has diminished with the increased intensity and extent of drug-resistant falciparum malaria. Chloroquine-resistant *P. falciparum*, previously absent from the African continent, was reported in East Africa in 1978 and has since rapidly spread through Africa south of the Sahara; the distribution of chloroquine-resistant *P. falciparum* in India has also been extended throughout the subcontinent. The need for effective antimalarials remains the same as that stated by the SWG in 1978: to relieve suffering and save lives; to prevent malaria infections; to produce a radical cure of established infections.

CHEMAL's priorities, however, have changed with the altering demands of the malaria situation (see Box 1 for important future research directions). There is an urgent need for safe, well-tolerated drugs which would ideally be active against all species of malaria parasites and especially against multi-resistant ones. Such compounds must possess one or more of the following types of activity: blood schizontocidal; tissue schizontocidal; hypnozoitocidal; sporozoitocidal; gametocytocidal/sporontocidal. In addition, they should be inexpensive, stable for long periods under suboptimal storage conditions and, where possible and appropriate, long-acting.

Box 1. Important areas recommended for future studies

- Compounds already known to inhibit parasite enzymes should be developed as antimalarials and new biochemical targets identified.
- In vitro tests for drug action on all stages of cloned malaria parasites should be refined and standardized to improve precision and accuracy.
- Modern biochemical and genetic techniques should be further developed to explore mechanisms of drug resistance.
- Specific, sensitive DNA probes, especially those that do not involve the use of radioisotopes, should be developed, particularly for use in epidemiological surveys.
- Clinical research centres already established for the assessment and safety of mefloquine and its combinations should be maintained and strengthened.
- Collaboration with governments, academia and industry should be maintained and intensified in order to bring promising new antimalarial compounds to clinical trial as soon as possible.

2. ADVANCES IN THE PAST DECADE

2.1 Experimental Parasitology

2.1.1 Parasite structure and organelle function

Electron microscope studies on the morphological effects of drugs on malaria parasites have yielded information on drug sites and modes of action. Such studies have been mainly carried out on erythrocytic stages of the parasite.

After chloroquine administration, morphological changes are first detected in the food vacuoles, which become larger. The particles of malarial pigment (haemozoin) within them become smaller and more amorphous. Electron microscope autoradiography has demonstrated that the food vacuoles are the primary site of chloroquine concentration within the parasite.

Mefloquine has a similar effect in impairing the action of the food vacuoles. Since it binds to haemoglobin and to the erythrocyte membrane, the food vacuoles, which contain haemoglobin, may be the primary site of mefloquine concentration within the parasite.

Observations, using light microscopy, of parasites after exposure to pyrimethamine reveal that the drug's effect on the erythrocytic stages occurs at the schizont stage of development. When observed through the electron microscope, many more nuclei show bundles of spindle fibres than those in normal controls and, because of interference with nuclear division, a large nucleus is often seen in an intermediate stage between the newly forming merozoite and the developing schizont. Pyrimethamine arrests or slows down the nuclear division of Plasmodium spp., apparently interfering at metaphase or anaphase.

After the administration of qinghaosu, mitochondrial swelling occurs in the erythrocytic stages of P. inui, but not in host tissue cells. The changes

are first observed two hours after exposure to qinghaosu; the degree of mitochondrial swelling increases in relation to exposure time and drug concentration, reaching a maximum swelling at eight hours. Qinghaosu also causes changes in the parasite ribosomes and membranes of the erythrocytic stages of P. berghei.

Electron microscopy of the exoerythrocytic stages of P. fallax exposed to primaquine also shows swollen mitochondria within 24 to 48 hours of exposure; the degree of swelling depends on exposure time and drug concentration. Electron microscope autoradiography using tritiated primaquine indicates that this compound and/or its metabolites are concentrated in parasite mitochondria.

2.1.2 Biochemistry

2.1.2.1 Glycolysis

P. falciparum is a micro-aerophilic homolactate fermenter that completely metabolizes glucose to lactate. All of the known glycolytic enzymes have been identified in extracts of erythrocytic stages of P. falciparum but, except for D-lactate dehydrogenase (LDH) (EC 1.1.1.28),* no plasmodia-specific enzyme has yet been sufficiently characterized to suggest that it could be exploited as a chemotherapeutic target.

2.1.2.2 Pentose phosphate pathway

Considerable controversy has arisen concerning the pentose phosphate pathway: the second enzyme, 6-phosphogluconate dehydrogenase (PGD) (EC 1.1.1.44), has routinely been identified in plasmodial extracts but evidence for the first enzyme, glucose-6-phosphate dehydrogenase (G6PD) (EC 1.1.1.49), has rarely been found. This may be explained by the recent observation that when parasites are adapted to red cells low in G6PD, a net synthesis of G6PD by P. falciparum occurs; however, when grown in red cells with normal amounts of G6PD, the parasite-specific enzyme is not produced.

2.1.2.3 Oxygen utilization and electron transport

In 1979 it was suggested that the oxygen utilized by Plasmodium spp. may be coupled to pyrimidine biosynthesis. Recent findings support this: the dihydroorotate dehydrogenase (EC 1.3.99.11) from P. yoelii is highly sensitive to 2-(4-t-butylcyclohexyl)-3-hydroxy-1,4-naphthoquinone (BW58C) with a median effective dose (ED₅₀) of 3×10^{-9} M; and BW58C inhibits pyrimidine synthesis of P. falciparum in vitro with an ED₅₀ of 0.5×10^{-9} M, due to the direct inhibition of dihydroorotate dehydrogenase.

The mitochondrial swelling produced by naphthoquinones and primaquine may indicate that the drugs affect parasite oxygen utilization and electron transport.

2.1.2.4 Lipids

Since plasmodia are incapable of de novo synthesis of cholesterol and fatty acids, the parasites are entirely dependent on the host for the supply of these materials. Plasma fatty acids and lysophospholipids both serve as fatty-acid precursors for phospholipid synthesis in P. knowlesi-infected cells. Phosphatidylethanolamine (PE) is formed by decarboxylation of phosphatidyl-

* Source used throughout this report: Enzyme Nomenclature (Recommendations (1984) of the Nomenclature Committee of the International Union of Biochemistry). Orlando-London: Academic Press, 1984.

serine (PS), and phosphatidylcholine (PC) is derived from the methylation of PE. High levels of activity of PS decarboxylase (EC 4.1.1.65) and PE methyltransferase (EC 2.1.1.17) have been found in red cells infected with *P. knowlesi* and *P. falciparum*; these enzymes represent the first examples of specific plasmodial membrane markers that might be targets for chemotherapeutic attack.

2.1.2.5 Haemoglobin utilization and pigment formation

As the malaria parasite grows within the red cell, there is an increase in malarial pigment (haemozoin) and a decrease in intraerythrocytic haemoglobin. It is presumed that haemoglobin digestion takes place within the food vacuoles through the concerted action of a series of proteolytic enzymes. A cathepsin D-like endopeptidase inhibited by 10 μ M pepstatin and 100 mM chloroquine has been identified in *P. yoelii* and *P. falciparum*. Cathepsin D (EC 3.4.23.5) has not yet been localized within the parasite; however, it is likely to be active within the food vacuole. Thiol aminopeptidase activity identified in *P. falciparum* is inhibited noncompetitively by chloroquine, mefloquine and mepacrine. According to a recent ultrastructural study, aminopeptidase and endoarylamidase seem to be processed on the cytoplasmic ribosomes and appear later in the pinocytotic vesicles; both mefloquine and chloroquine are concentrated in and produce enlargement of the food vacuoles, suggesting that they impair haemoglobin digestion.

Using a non-invasive technique (Mössbauer spectroscopy), the haemozoin of rat erythrocytes infected with *P. berghei* was shown to contain trivalent and high-spin iron. The iron component consisted of 10 Å diameter micro-aggregates which were further aggregated to form the 2000-3000 Å crystals of haemozoin. Since the Mössbauer spectrum revealed pigment iron in both chloroquine-sensitive and -resistant isolates (in spite of the fact that haemozoin was visually obvious in only the sensitive strain), it was suggested that both isolates degraded haemoglobin but that the macroscopic aggregation properties of the two isolates were dissimilar. Thus, it was concluded that chloroquine resistance could not be attributed to a lack of haemoglobin digestion. Two questions remain highly controversial and require further study: Is malarial pigment itself (or some constituent such as ferriprotoporphyrin) involved in the biochemical mechanism of chloroquine resistance in malaria parasites? Can observations from studies on chloroquine resistance in the rodent model be applied, by extrapolation, to the human model in which pigment formation appears to be unaffected?

2.1.2.6 DNA synthesis

During the asexual cycle, DNA synthesis begins in the trophozoites and continues until schizogony is complete. Pyrimethamine slows down or arrests nuclear division, especially metaphase.

2.1.2.7 Purines and pyrimidines

A functional de novo purine biosynthetic pathway has not been demonstrated for malaria parasites; consequently, it is assumed that they require an exogenous supply of preformed purines. The preferred purine for both *P. lophurae* and *P. falciparum* appears to be hypoxanthine, which is derived intraerythrocytically through the following pathway: adenosine triphosphate \rightarrow adenosine diphosphate \rightarrow adenosine monophosphate \rightarrow inosine monophosphate \rightarrow inosine \rightarrow hypoxanthine. *P. falciparum* isolated from human erythrocytes has been found to possess the following purine-salvage-pathway enzymes: adenosine deaminase (ADA) (EC 3.5.4.4), purine nucleoside phosphorylase (PNP) (EC 2.4.2.1), hypoxanthine phosphoribosyltransferase (HPRT) (EC 2.4.2.8) and adenosine kinase (EC 2.7.1.20). The adenosine deaminase from *P. lophurae* and *P. falciparum* is unique in its lack of sensitivity to the inhibitor erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA).

Purified HPRT and PNP from P. lophurae and P. falciparum differ distinctly from those of the red cell in several kinetic parameters, e.g. the parasite HPRT is competitively inhibited by formycin B. Inosine monophosphate (IMP) dehydrogenase (EC 1.1.1.205) and adenylosuccinate synthase (EC 6.3.4.4) activity has also been identified in P. lophurae. Bredinin, an inhibitor of IMP dehydrogenase, arrests P. falciparum growth in vitro.

Thymidylate synthase (EC 2.1.1.45) exists in Plasmodium as a bifunctional protein in combination with dihydrofolate reductase (EC 1.5.1.3), with a relative molecular mass (Mr) of 125 000. The synthase has a subunit relative molecular mass of 65 000. The precise functional significance of a bifunctional protein in malaria parasites is not yet understood.

2.1.2.8 Histidine-rich protein

One of the best-characterized proteins from a malaria parasite is the histidine-rich protein (HRP) isolated from membrane-bound cytoplasmic granules of P. lophurae. HRP has been successfully translated in a cell-free system and the translation product synthesized as a large unglycosylated precursor of Mr 63 000. In the presence of dog pancreas microsomal membranes, a larger glycosylated protein was synthesized and subsequently processed, after transport from the rough endoplasmic reticulum to the granule, to a protein of Mr 58 000.

The nucleotide sequence for the HRP gene of P. lophurae has been determined; the gene is encoded in two exons separating the signal-peptide encoding sequence from the prosequence. The signal sequence is present in multiple copies. HRP genes have also been identified in P. falciparum. 2- and 4-fluorohistidines have been synthesized and shown to have antimalarial activity. However, these compounds are likely to be systemically toxic to the host, as are the α -fluoromethyl and difluoromethyl analogues. The precise function of HRP is at present unknown.

Ultrastructural studies suggest that the apical organelles of the merozoite (rhoptries and micronemes) are involved in the invasion of the erythrocyte. In P. yoelii, the rhoptry protein has a Mr of 235 000 and in P. falciparum, of 140 000. The presence of protein antigens within the rhoptries was recently demonstrated using monoclonal antibodies against a protein of Mr 235 000.

2.1.3 Modes of drug action and mechanisms of resistance

2.1.3.1 Antifolates

The antifolates include dihydrofolate reductase inhibitors, such as pyrimethamine and proguanil, and dihydropteroate synthase inhibitors, such as sulfonamides and sulfones. These compounds have an antimalarial action on all multiplying stages of the parasite and are normally used operationally in combination with each other as blood schizontocides.

Studies on the development of pyrimethamine resistance in P. chabaudi clones and on the analysis of P. falciparum clones from the field suggest that the development of resistance could arise from a mutation which results in the production of a dihydrofolate reductase enzyme with reduced affinity for the drug and its natural substrate, dihydrofolate. However, a comparison of two P. falciparum isolates from different geographical areas showed that the dihydrofolate reductase enzyme of the resistant P. falciparum isolate showed no qualitative differences from the enzyme of the sensitive isolate (although it was produced in 30- to 80-fold greater amounts). This could indicate that gene amplification is a mode of development of resistance in malaria. Gene amplification has been shown to occur in methotrexate resistance in mammalian cells grown in vitro and in Leishmania spp.

Sulfonamide-resistant parasite lines are often crossresistant to dihydrofolate reductase inhibitors but the reverse is not true; isolates resistant to dihydrofolate reductase inhibitors may be hypersensitive to sulfonamides and sulfones. This and the obligatory localization of sulfonamide-resistant lines of *P. berghei* in immature mouse erythrocytes indicate that resistant parasites may take advantage of the availability of reduced folate intermediates in the red cell. It has recently been suggested that *P. falciparum* can utilize monoglutamated folic acid in culture medium, which would explain the antagonism shown *in vitro* by folate against sulfadoxine. It would be of interest to know whether the polyglutamated tetrahydrofolate derivatives that constitute the major component of the erythrocytic folate pool can be utilized by sensitive or resistant parasites.

2.1.3.2 8-Aminoquinolines and naphthoquinones

These compounds have activity against primary tissue stages, hypnozoites, gametocytes and asexual blood stages. Resistance to 8-aminoquinolines is not a serious operational problem, although South-East Asian and Pacific strains of *P. vivax* may require increased dosage regimens for radical cure.

The biochemical basis of the mode of action of primaquine (an 8-aminoquinoline derivative) is unknown. Nor is it known whether its therapeutic and toxic effects are due to the parent drug and/or to the metabolites. However, significant advances have been made in the identification of primaquine metabolites, one or more of which may be responsible for the antimalarial or toxic effects. The carboxylic acid metabolite of primaquine, 8-(3-carboxy-1-methylpropylamino)-6-methoxyquinoline, has been shown to be a major metabolite in blood and urine. 8-Amino-6-methoxyquinoline and the 5-hydroxylated and 6-demethylated metabolites of primaquine, predicted from earlier studies on primaquine, have also been identified. Antimalarial naphthoquinones that have similar morphological effects on the parasite mitochondrion may provide a lead to the mode of action of primaquine metabolites. The naphthoquinones (e.g. the newly developed, highly active BW58C) probably interfere with electron transport in the parasite mitochondrion and inhibit ubiquinone-linked dihydroorotate dehydrogenase, which is vital for pyrimidine synthesis. The strong redox potential of hydroxylated primaquine metabolites may also increase the oxidation stresses in infected liver cells or erythrocytes and in the parasite.

The chemotherapeutic distinction between drugs with anti-hypnozoitocidal and anti-tissue schizontocidal activity should be elucidated.

2.1.3.3 4-Aminoquinolines and related blood schizontocides

The selective toxicity of certain blood schizontocides is based on their concentrative uptake by infected erythrocytes. It has been shown that drug-resistant isolates of malaria parasites take up chloroquine and closely related drugs less efficiently than drug-sensitive isolates, in which uptake is glucose-dependent. Chloroquine uptake is saturable, with a diffusion constant (K_d) varying from 10 to 100 nmol/L. Chloroquine has a rapid effect on the parasite's digestive vesicles, resulting in their fusion and formation of autophagic vacuoles, observed as pigment clumping. Malarial pigment does not contain any appreciable amount of the drug. Mefloquine, as with other arylamino alcohols, causes enlargement of the food vacuoles but no pigment clumping takes place. Chloroquine-induced clumping is competitively inhibited by antimalarial arylamino alcohols.

Lysosomotropic activity. The 4-aminoquinoline blood schizontocides, being weak bases, are able to gain or lose protons from their amino groups depending on the pH of the environment. Each molecule may thus be positively charged (protonated) or neutral (uncharged base). Such agents would be expected to permeate biomembranes in the uncharged state but not when

charged. Consequently, one would expect them to be localized in acidic (proton-rich) membrane-bound vacuoles such as lysosomes. Quinine and chloroquine localize in lysosomes of mammalian cells in culture, and several studies indicate that intralysosomal digestion is inhibited in such cells. Concentrations of chloroquine in excess of 10 $\mu\text{mol/L}$ of medium are needed in mammalian cell cultures for such effects to appear, yet malaria parasites are affected at 1/1000 of this concentration. There is some evidence that the intralysosomal localization of blood schizontocides such as chloroquine is related to their antimalarial action, but in general the structural specificity of such antimalarials is not explained by this otherwise attractive hypothesis.

Membrane association. Chloroquine at low concentrations is known to stabilize erythrocyte biomembranes. Cations such as calcium are inhibited from binding to or permeating artificial membranes containing antimalarials such as chloroquine, quinine, quinidine or mepacrine. The presence of a protonated nitrogen atom is necessary for competition with cations; the potency of drugs in such tests is related to their lipophilic character. These and other observations suggest that chloroquine and related drugs may have specific reactions with parasite membranes. Mefloquine associates strongly with membrane phospholipids, which may be an important feature of its activity on mefloquine-resistant isolates. However, chloroquine and the related blood schizontocides have little, if any, effect on the malaria parasite when it is not digesting haemoglobin.

Interactions with ferriprotoporphyrin IX. Intraerythrocytic asexual parasites digest up to 75% of host cell haemoglobin. Digestion is carried out in a low pH medium by endo- and exopeptidases found within the food vacuoles. In mammalian cells that digest haemoglobin, the toxic residue, haem (ferroprotoporphyrin IX), is detoxified by haem oxygenase (EC 1.14.99.3), producing the bile pigment biliverdin and releasing iron for use in further haemoglobin synthesis. Since malaria parasites do not possess haem oxygenase, they apparently have a different method of detoxifying the haem residues. It has been suggested that haem is sequestered as an inert complex, the malarial pigment haemozoin, by a protein or proteins synthesized by the parasite for this purpose, i.e. the haemin-binding protein or haembinder.

Quinine and chloroquine bind with high affinity to both ferriprotoporphyrin IX and its dimeric form and there is some evidence that interactions between these antimalarials and the porphyrin moiety are involved in the toxicity of blood schizontocides to the intraerythrocytic parasite. It has been suggested that the formation of complexes with chloroquine may prevent ferriprotoporphyrin IX from being detoxified since it cannot then bind to the haemin-binding protein. The iron-porphyrin/chloroquine complex is known to be as damaging to membranes as iron-porphyrin alone. The similarity between the K_d of chloroquine absorbed by malaria-infected erythrocytes and that of chloroquine bound to the ferriprotoporphyrin dimer has been used to support the suggestion that this is a factor in the drug's mode of action. Antimalarials of the arylamino alcohol type, including quinine, quinidine, cinchonine, cinchonidine, mefloquine and the phenanthrene methanols, form coordination complexes with ferriprotoporphyrin IX, whereas the inactive epimer of quinine, 9-epiquinine, does not.

The mechanism of chloroquine resistance, however, is difficult to explain on the basis of the above ferriprotoporphyrin hypothesis. It is now known that digestion of haemoglobin is carried out by chloroquine-resistant *P. berghei* and *P. falciparum*; therefore, ferriprotoporphyrin IX should be available to form complexes with drugs. It is possible that intracellular proteins capable of sequestering the iron-porphyrin may be present in larger quantity in resistant parasites, otherwise the process of sequestration into haemozoin would probably be accelerated. Another possibility is that alterations in membrane permeability might explain resistance to blood

schizontocides, irrespective of their modes of action. Mechanisms of resistance are not necessarily linked to mechanisms of activity, but may relate as much to changes in membrane lipid composition or in carrier proteins as to other factors.

2.1.3.4 Antibiotics

It was discovered many years ago that chlortetracycline was active against avian malaria. Other antibiotics of the tetracycline family, most recently minocycline and doxycycline, have been shown to have some activity against developing liver stages and marked activity against blood stages of the parasite. In order to prevent recrudescence of antifolate- and quinine-resistant P. falciparum malaria, since the effects of treatment take 48 hours or so to appear, the antibiotics are generally given following a course of quinine to reduce parasitaemia.

Lincomycin has weak antimalarial activity but chlorinated derivatives, such as clindamycin, are much more active and have been used in conjunction with quinine. Clindamycin, however, is associated with a high risk of inducing pseudo-membranous colitis. Erythromycin, which has weak anti-malarial activity, shows potentiative synergism with chloroquine against chloroquine-resistant isolates of rodent malaria in vivo and P. falciparum in vitro, but in clinical trials against chloroquine-resistant P. falciparum in Thailand, the effectiveness of combinations of chloroquine with erythromycin or tetracycline was not confirmed.

Antibiotics of the above types are inhibitors of ribosomal protein synthesis in the prokaryote. It seems likely, therefore, that their anti-malarial action takes place within the mitochondrion, which presumably has a similar type of ribosome. This would account for their slow antimalarial action, since mitochondrial replication may be restricted to a limited part of the cell cycle.

2.1.3.5 Artemisinin (qinghaosu)

This sesquiterpene lactone was isolated in China from the plant Artemisia annua, which has been used there as antipyretic for over 1000 years. Artemisinin has been shown to be active against chloroquine-resistant P. falciparum in humans, although some crossresistance with chloroquine has been observed with P. berghei in rodents. Antimalarial activity depends on the presence of an endoperoxide bridge in one of the compound's ring systems, which was thought initially to indicate that the mode of action, apparently unique, was related only to the peroxide group. However, more recent evidence suggests that full activity depends on involvement of the whole molecule.

The first biochemical effect observed is the cessation of protein synthesis, which occurs within 30 to 60 minutes of drug exposure. Alterations in the ribosomes, and particularly in membranes such as the rough and smooth endoplasmic reticulum, the limiting membrane and the mitochondrion, have been observed in P. berghei soon after drug administration. Autoradiography has demonstrated that ³H-dihydroartemisinin is localized in P. falciparum membranes and it is possible that most other observed effects are secondary to changes in membrane permeability. Erythrocytes infected with P. falciparum concentrate labelled dihydroartemisinin from culture medium with a K_d of 10.5 nmol/L.

In vitro studies with P. falciparum and in vivo studies with rodent malaria have demonstrated that artemisinin and mefloquine form a potentiating combination but that artemisinin antagonizes the effect of antifolates. Qinghaosu also forms synergistic combinations with halofantrine.

Resistance to artemisinin has been induced in P. yoelii in mice. The artemisinin-resistant line shows some crossresistance to quinine and mefloquine, but remains sensitive to the hemisuccinyl derivative of artemisinin, artesunate. The mode of action and crossresistance pattern of qinghaosu and its derivatives require further study.

2.1.4 In vitro tests for drug activity against malaria parasites

2.1.4.1 Asexual erythrocytic parasites

Since its introduction in 1976, the technology for the continuous cultivation of P. falciparum in vitro has opened the way for major contributions to experimental chemotherapy, drug development and epidemiology. The most commonly applied techniques used in these studies are the microtest for testing drug susceptibility in the field, which uses morphological criteria as a measure of parasite growth, and an automated method using ³H-hypoxanthine uptake as a measure of parasite growth, which is mainly used in the laboratory for drug screening.

Most laboratory studies require the cloning of parasites using techniques of limiting dilution or, preferably, micromanipulation. A modified RPMI 1640 medium containing 0.5 µg/L p-aminobenzoic acid and 10 µg/L folic acid (LPLF), approximating the concentration of these nutrients in human serum, has recently been used successfully for assessing the activity of antifolate drugs.

Susceptibility testing of in vitro P. falciparum to chloroquine, amodiaquine, quinine and mefloquine is now performed routinely under field conditions. With the recent availability of LPLF culture medium, it is now possible to assess the susceptibility of parasites to pyrimethamine and sulfadoxine combinations.

Parasite cultivation for periods exceeding 30-32 hours requires supplementation of the medium with human serum. Rabbit or goat sera have been substituted under certain circumstances. Non-serum substitutes such as adenosine, C-18 unsaturated fatty acids and fatty-acid-free bovine serum albumin may prove useful in the future.

2.1.4.2 Exoerythrocytic parasites

Important technical advances have been made over the last five years in cultivating the exoerythrocytic stages of mammalian plasmodia. The complete exoerythrocytic cycle of P. berghei has now been established in W138 lung cells, in a continuous transformed hepatoma cell line and in primary hepatocyte cultures. Similar development of P. yoelii has been obtained in primary hepatocyte cultures of Thamnomys gazellae and of adult rats. P. vinckei and P. chabaudi have also been cultivated in hepatocytes.

These studies finally led to the successful cultivation of exoerythrocytic stages of P. vivax in a hepatoma cell line and in primary human hepatocytes and of P. falciparum in human hepatocytes. Since it appears that hypnozoites may be formed in P. vivax cultures, it should be confirmed whether the system is applicable for testing drugs for hypnozoitocidal activity. Preliminary studies on the mode of action of primaquine and its metabolites and analogues and of pyrimethamine have been carried out using the P. berghei model. These cultivation methods need to be standardized for routine drug screening.

2.1.4.3 Gametocytes

Appropriate in vitro cultivation methods have led to the isolation of P. falciparum clones that regularly produce gametocytes. These methods and

clones are being used to compare the activities of antimalarial compounds on the numbers of gametocytes produced, their fine structure and their infectivity to mosquitos. The test will be useful for assaying the gametocytocidal and sporontocidal activity of 8-aminoquinoline compounds and their metabolites. These methods need to be standardized and their precision and accuracy improved.

2.1.5 Parasite genetics

Studies on parasite genetics may be essential for understanding the spread of drug resistance in the field. Appropriate genetic markers are required for these studies. Asexual erythrocytic forms of P. falciparum, maintained in vitro, have been characterized by the following:

- enzymes [such as glucose 6-phosphate isomerase (GPI) (EC 5.3.1.9), adenosine deaminase (ADA) (EC 3.5.4.4), D-lactate dehydrogenase (LDH) (EC 1.1.1.28), phosphogluconate dehydrogenase (PGD) (EC 1.1.1.44), peptidase E (PEP) and glutamate dehydrogenase (GDH) (EC 1.4.1.4)], using acetate and starch gel electrophoresis;
- antigens (S-antigens, schizont surface antigens and merozoite antigens), using monoclonal antibodies and immunofluorescence microscopy;
- proteins, using two-dimensional polyacrylamide gel electrophoresis (PAGE);
- drug-susceptibility response markers for chloroquine, mefloquine, amodiaquine, quinine and pyrimethamine, using in vitro drug assays.

Studies of these markers in parasite clones have been conducted to test their stability over long periods of cultivation. Testing for the stability of drug susceptibility response markers depends on an accurate assay method and work is in progress towards standardization of such tests, using methods such as semi-automated microdilution techniques. Markers have been used successfully to identify genetic crosses with rodent malaria models and will soon be used with P. falciparum clones that produce gametocytes. The stability of biological characteristics, such as gametocyte formation in clones, requires further study.

In vivo studies with rodent malaria parasites and with clones of isolated P. falciparum in vitro indicate a classical Mendelian basis for the development of drug resistance. However, recent observations indicate that the development of resistance to some drugs may be slow, relatively unstable and stepwise. Such resistance reverts to sensitivity on removal of drug pressure but rapidly returns on reapplication. It is tempting, therefore, to speculate that gene amplification may play a part in the development of resistance. Further studies on the molecular biological basis of drug resistance are needed.

2.1.6 DNA probes: Potential applications to malaria diagnosis

Malaria diagnosis is currently performed by microscopical examination of a stained blood film. The proposed use of DNA probes as an alternative to blood film examination offers several advantages, although there are some technical difficulties to overcome. The major advantage is that DNA probes can be used for the simultaneous screening of multiple samples, which would be especially useful in large surveys of malarious populations requiring the screening of thousands of samples. The sensitivity and specificity of DNA probes are already approaching those obtained by microscopical analysis of blood film.

DNA probes with potential for diagnosing P. falciparum malaria have been developed in several laboratories. Cloned DNA fragments have been isolated that are repeated many times in the genome and contain a short sequence which repeats several times within the cloned fragment. It is claimed that a detection level of 0.001% parasitaemia in a 50 μ L sample of blood can be achieved with these probes. The probes do not crossreact with P. vivax or with human DNA.

The major remaining hurdle is that radioactive-labelled DNA must be used, but isotopes with a short half-life are unsuitable for use in a clinical or field setting. There is, therefore, an urgent need for non-radioactive methods of labelling DNA probes. Such methods are being developed at a rapid rate in other fields and will be tested in clinical settings in the near future. The most widely used method is the biotin-labelling of DNA by nick translation. Other methods may also be applicable, such as the use of fluorescent labels, magnetic beads and antibodies directed at specific modifications of DNA (e.g. the glucosylation site of T4 DNA).

Practical field testing of DNA probes specific to P. falciparum is now needed. Several important considerations must enter into the design of such a test. For example, do the DNA probes recognize every strain of P. falciparum or are they limited to the strain from which they are derived? This question is especially relevant since the currently available probes are derived from highly repeated DNA sequences which may undergo relatively rapid evolutionary changes, similar to the changes that occur in the kinetoplast DNA minicircle of Leishmania.

A second consideration involves the sensitivity of the test. In laboratory tests, currently available probes can detect a 0.001% parasitaemia in a 50 μ L sample of infected blood. It may be possible to increase the sensitivity of the assay by using a combination of several different probes within a single hybridization.

Using the standard diagnostic technique of stained thick film examination, a good microscopist can detect a parasitaemia of 0.0002% in a 2 μ L blood sample, but this takes skill and time; a sensitive probe technique enabling a technician to process 1000 samples a day would be of great value in malaria surveys.

DNA probes have other potential applications, such as the detection of infected mosquitos. This application should be explored and compared with other available methods. The question of the stability of both the probe and the sample must be addressed in any field test. In general, the stability of DNA is one of the major advantages of this technology over immunological methods, where protein stability may be a problem. The overall practicability of the method must be assessed and compared with other tests under controlled conditions.

2.2 Drug Development

In 1978 the CHEMAL SWG recognized four specific problems of drug development:

- lack of available clinical research centres in endemic areas;
- difficulty finding suitable human subjects for clinical testing of new drugs and associated ethical considerations;
- shortage of clinical pharmacologists, particularly in endemic countries;

- the need to develop new formulations of antimalarials, including those appropriate for sustained-release preparations.

The first three of these difficulties have been largely overcome.

2.2.1 Clinical research centres

A notable achievement of the CHEMAL programme has been the establishment of well staffed and equipped clinical research centres in Brazil, Thailand and Zambia. These centres have performed 26 high-quality Phase I, II and III clinical trials in adults and children during the past three years. They have also provided the basis for extensive field trials of mefloquine alone and in combination with other antimalarials. Other clinical studies have been performed with chloroquine, sulfadoxine/pyrimethamine, sulphadoxine/pyrimethamine/quinine and tetracycline/quinine combinations. In vitro drug sensitivity testing has been carried out in these centres in conjunction with in vivo studies.

Extensive pharmacological studies have also been conducted by these centres and by several other collaborating laboratories not directly supported by the CHEMAL programme. For example, Phase I, II and III studies on mefloquine and its combinations have been performed outside TDR at centres in Burma, Gabon, India, Malaysia, Nigeria and the Philippines and in other centres in Brazil and Thailand.

The successful performance of clinical trials in all centres, using standardized protocols, a closely monitored and coordinated reporting system and computerized data-processing facilities, have been invaluable in drug efficacy and safety assessment and have provided objective means for determining geographical differences in the manifestations of adverse reactions to antimalarial drugs. The clinical trial monitor, who coordinates all the trials, has also played a major role in the successful and expeditious clinical evaluation of mefloquine and its combinations. These studies also produced the documentation necessary for the registration of these formulations.

The clinical research centres and other collaborating laboratories have effectively overcome the difficulty of finding healthy volunteers and patients for clinical studies. Under the most stringent of ethical conditions, mefloquine has been tested in more than 2000 individuals and the mefloquine combination with sulfadoxine/pyrimethamine in more than 3000 patients.

2.2.2 Clinical pharmacology

The need for clinical pharmacologists has been partially satisfied by TDR through collaboration with its Research Strengthening Group. These training efforts should be further intensified to increase the availability of appropriately qualified and experienced personnel needed to carry out the required clinical pharmacological investigations on antimalarial drugs.

Since 1978 the programme has also supported and encouraged the application of modern techniques to pharmacokinetic studies on antimalarials in animals and humans. Highly sensitive, specific and reproducible methods have been developed for and applied to kinetic and metabolic studies of chloroquine, amodiaquine, primaquine, mefloquine, quinine, quinidine, qinghaosu, sulfadoxine, pyrimethamine and several novel candidate antimalarials in centres in industrialized countries and endemic areas.

2.2.3 New formulations

Pyrimethamine has been used as a model drug to evaluate sustained-release preparations based on biodegradable polymers and simple oils.

Polymeric preparations of lactic acid/glycolic acid (9:1) and a series of matrices prepared from dihydropyran and triols, diols and simple alcohols have been evaluated in animal studies. Antimalarial protection of three month's duration was achieved in animals, but no system tested was appropriate for further development. Pyrimethamine is not an ideal drug for operational use in such formulations and questions have arisen with regard to the toxicity of the carriers. The ideal blood schizontocidal drug for inclusion in such formulations should have high antimalarial activity, be effective against drug-resistant strains present in the field and be rapidly excreted, thus being concentrated in the formulation and not in the tissues. The blood schizontocidal formulation should be protective for a period of at least three months. If a potent compound with an appropriate mode of action should be discovered, efforts should be made to produce long-lasting formulations, using materials and technology of high scientific quality.

The development of a sustained-release formulation for the radical treatment of vivax infection would also be appropriate if a suitable 8-aminoquinoline or similar compound were developed for use in humans.

2.2.4 Mefloquine and its combinations

Extensive Phase I and II clinical pharmacological studies and Phase III clinical trials with mefloquine alone and in combination with sulfadoxine/pyrimethamine have been conducted. Mefloquine has been tested for efficacy and safety, alone and in combination with sulfadoxine/pyrimethamine, and compared with several other antimalarial drugs. These studies have demonstrated that mefloquine alone, given in a single oral dose, is highly effective against chloroquine-resistant P. falciparum. Single-dose kinetic studies of mefloquine in humans have shown that the drug has a long half-life ranging from 6 to 33 days.

Formulation of the mefloquine combination was undertaken to minimize the risk of P. falciparum developing resistance to this valuable compound. In 1974 the monosubstance was registered in Switzerland and the combination in Switzerland and Thailand; registration is also foreseen in other countries where its operational use is justified. Guidelines for the control and use of mefloquine and its combinations were developed by a WHO Scientific Group on the Chemotherapy of Malaria (8) and were considered by the Executive Board of the World Health Assembly in January 1985. The guidelines were subsequently sent to all WHO Regional Offices for distribution to Member States. [See (7) for a more detailed account of the development of mefloquine.]

2.2.5 Qinghaosu

The limited availability of the novel antimalarial agent qinghaosu (artemisinin), discovered by scientists in China, has restricted the preclinical and clinical investigation of this compound. Qinghaosu has been shown to be a very rapidly acting blood schizontocide in multidrug-resistant P. falciparum infections. Sufficient Artemisia annua L. is currently under cultivation outside China to produce an adequate amount of purified compound for preclinical pharmacological and toxicological studies and to provide material for the synthesis of analogues in 1986. Studies are also under way to improve the yield of the active principle from the plants.

Since qinghaosu is only sparingly soluble in water and oils, attempts have been made to produce more soluble derivatives. The water-soluble hemisuccinate derivative, artesunate, was considered a leading candidate for development for the treatment of cerebral malaria; however, it has recently been shown to be unstable under certain conditions and other derivatives are therefore being considered for clinical development. The current leading candidate is the ethyl ether derivative (artether), a lipid-soluble compound.

Sensitive and specific high-pressure liquid chromatography (HPLC) and gas chromatography/mass spectrometry techniques have been developed for the quantitative determination of qinghaosu and dihydroqinghaosu blood levels. Total synthesis programmes have been initiated to facilitate the preparation of radioactive-labelled compounds.

2.2.6 Other new blood schizontocides

Halofantrine

This phenanthrene methanol is highly effective against chloroquine-resistant P. falciparum in humans and is unlikely to possess clinically significant crossresistance with mefloquine. Clinical studies carried out in Thailand by WRAIR have indicated that the current formulation's bioavailability in humans is poor. Further development of this compound is being carried out by WRAIR in collaboration with a pharmaceutical company.

Quinidine

Quinidine is being evaluated in clinical trials conducted in Thailand outside the CHEMAL programme. Animal and in vitro studies conducted by CHEMAL have shown that quinidine is a more effective antimalarial, but a more cardioactive drug, than quinine.

4-Aminoquinolines

The effectiveness of amodiaquine against chloroquine-resistant P. falciparum in vitro, in monkeys and in humans has stimulated, primarily through WRAIR support, the synthesis of more than 200 analogues. One of these compounds, WR 228,258, is more than 10 times as active as amodiaquine and has a very long half-life. The compound is under consideration for clinical study.

Hydroxynaphthoquinones

These antimalarial compounds were derived from rational drug development programmes carried out by the pharmaceutical industry. Further studies on such compounds are in progress outside the CHEMAL programme.

A new, rapidly acting, effective and safe drug is needed that can be given intravenously or intramuscularly as an alternative to quinine for treating severe malaria.

2.2.7 Primaquine

Primaquine is at present the only clinically acceptable, antirelapse (hypnozoitocidal) drug available for radical cure of P. vivax infections. The compound is also gametocytocidal and sporontocidal against P. falciparum. It is among the oldest, but least understood, of all antimalarials. Since 1978 CHEMAL and WRAIR have supported a major research effort on primaquine and on the search for alternative tissue schizontocides. The development of HPLC and other sensitive analytical techniques and their application to detailed pharmacokinetic investigations in humans and animals have provided a greater understanding of the kinetics of primaquine and identified the major metabolite in blood and urine as 8-(3-carboxy-1-methylpropylamino)-6-methoxyquinoline. This derivative, plus an extensive series of putative metabolites of primaquine and several primaquine analogues (initially identified by studies on the metabolism of primaquine by microorganisms), have now been synthesized within the CHEMAL programme. Subsequent testing of the carboxylic acid metabolite in vivo and in vitro against asexual and sexual stages of P. falciparum, and against the exoerythrocytic stages of P. cynomolgi in rhesus monkeys and of rodent malarials in mice and in vitro, has demonstrated that it is inactive. Testing of the series of putative metabolites has so far failed to identify a

highly active derivative of the drug, but new derivatives with promising antimalarial activity have emerged in the course of this work and a clearer insight into the activity of primaquine on all stages of malaria parasites has been obtained.

The synthesis of radioactive-labelled primaquine has been achieved in two centres and the ring-labelled compound is being used in pharmacological and other studies.

3. RECOMMENDATIONS FOR FUTURE CHEMAL-SUPPORTED STUDIES

3.1 Parasite Biology in the Rational Development of New Drugs

The major advances made during the past decade in understanding malaria parasite biochemistry indicate that rational drug development based on an exhaustive knowledge of potential chemotherapeutic target sites in Plasmodium will play an increasingly important role in new drug development. The Group made three major recommendations:

3.1.1 Drugs that are known to inhibit already identified parasite-specific enzymes should be developed, particularly: enzymes of the purine-salvage pathway (hypoxanthine phosphoribosyltransferase and purine-nucleoside phosphorylase); those involved in de novo synthesis of pyrimidines (thymidylate synthase/dihydrofolate reductase and dihydroorotate dehydrogenase); lipid biosynthetic enzymes (phosphatidylserine decarboxylase and phosphatidylethanolamine methyltransferase); those involved in haemoglobin breakdown (cathepsin D and aminopeptidase).

3.1.2 Special emphasis should be given to the identification and characterization of novel parasite targets for which chemotherapeutic agents might be developed. This should include but not be limited to: proteins involved in invasion; parasite mitochondrial proteins; parasite and host cell membrane-transport proteins; factors involved in parasite sequestration; repeating epitopes of proteins from sporozoites and asexual stages; molecules involved in release of the parasite from the host cell; pigment-associated proteins; parasite-associated microtubules. Attention should be paid not only to asexual erythrocytic stages but also to sporozoites, exoerythrocytic stages and gametocytes.

3.1.3 Adequate amounts of purified plasmodial enzymes should be provided so that structural and functional studies can be carried out. They should be produced by recombinant DNA technology or purified directly from isolated parasites. It is important for the future rational development of drugs to characterize and crystallize parasite-specific enzymes, so that computer graphics studies on quantum structure/activity relationships can be carried out to determine optimal drug-ligand binding characteristics.

3.2 In Vitro Systems

The successful cultivation of malaria parasites in vitro has made it possible to measure in the field the susceptibility of P. falciparum to antimalarial drugs, to screen new antimalarial compounds and to undertake studies of the parasites' basic biology and biochemistry. The SWG made the following recommendations:

3.2.1 In vitro tests for screening antimalarial drug effects against asexual erythrocytic and exoerythrocytic stages and against gametocytes of P. falciparum should be further refined and standardized to improve their precision and accuracy. The tests would then be applicable to studies of structure/activity relationships and clone characterization.

3.2.2 Potential antimalarial compounds should be screened in vivo and in vitro for activity against all appropriate parasite stages.

3.2.3 Methods for continuous in vitro cultivation of erythrocytic stages of P. vivax should be developed.

3.2.4 The fine structure of hypnozoites should be studied in both in vitro and in vivo systems.

3.2.5 Factors governing sporozoite invasion of cultured cells should be identified.

3.2.6 The basic biology and metabolism of exoerythrocytic parasites should be investigated.

3.2.7 Clones should be used whenever possible for drug assays and for other appropriate experimental work.

3.3 Genetics

The development of parasite cloning techniques and the application of DNA technology have opened up an exciting new field in malaria research. The SWG recommended that:

3.3.1 Molecular and genetic methods should be applied to investigations of the mechanisms of drug resistance in malaria parasites.

3.3.2 Genetic drug-resistance studies should be carried out not only with P. falciparum but also with rodent models, especially P. vinckei and P. chabaudi.

3.3.3 Specific DNA probes for diagnosing all human forms of malaria should be developed, particularly for use in epidemiological studies. Special attention should be given to the development of tests using DNA probes that do not involve the use of radioisotopes.

3.4 Modes of Drug Action and Mechanisms of Resistance

Understanding the ways in which compounds with antimalarial activity exert their effects and how resistance to them develops can assist in designing new drugs and in indicating how best to deploy them so that they are not rendered useless by the rapid development of resistance. The Group made the following recommendations:

3.4.1 The modes of action of and mechanisms of resistance to antimalarial compounds should be studied, the sesquiterpene lactone qinghaosu and its derivatives being of special importance.

3.4.2 In vivo and in vitro studies should be carried out to identify potentiating drug combinations and to assess the development of resistance to these combinations. The possibility that such combinations might prove toxic to the host should be borne in mind and drug interactions, including those with antimalarials currently used in ad hoc and new combinations, should be studied.

3.4.3 Radioactive-labelled drugs should be synthesized for pharmacokinetic studies in animals.

3.5 Drug Development

Collaborative links with government research laboratories, the pharmaceutical industry and academic institutions have resulted in several

promising new antimalarial compounds in various stages of development. This collaboration will be maintained and new links actively sought. Novel, chemically defined compounds, both synthetic and natural, will be solicited for submission to CHEMAL for screening. Active collaboration with any interested party developing new antimalarial drugs or formulations will be encouraged, and the resources of the clinical research centres will be made available for the fullest evaluation of suitable products. In addition, priority will be given to the following:

3.5.1 Mefloquine alone and in combination with sulfadoxine/pyrimethamine. Both drugs are at an advanced stage of clinical assessment and have been registered in some countries. Future clinical investigations of mefloquine and its combination with sulfadoxine/pyrimethamine need to be carried out in high-risk groups in whom drug tolerance, bioavailability, metabolism and elimination may be affected (e.g. persons with impaired liver and kidney function, nutritionally deficient patients and those receiving medication for other conditions). Phase III studies need to be extended to infants and pregnant women suffering from multidrug-resistant *P. falciparum* infections. Further studies should also be made of combinations of mefloquine with drugs other than sulfadoxine/pyrimethamine.

3.5.2 Sesquiterpene lactones. CHEMAL will continue investigating the toxicology, formulation, development, clinical pharmacology and efficacy of qinghaosu and its derivatives, particularly the ethyl ether of dihydroqinghaosu (artether). This project will be developed with maximum coordination with governmental agencies and other institutions. Programmes for the preparation of other qinghaosu derivatives and the synthesis of simple analogues of qinghaosu, along with studies to optimize the yield of the parent compound from plant material, have been initiated and will continue.

3.5.3 Primaquine. Research will continue on the pharmacodynamics and mode of action of primaquine and its metabolites. To permit the fullest evaluation of the properties of the optical isomers of primaquine and to provide comparison with the racemate, CHEMAL should procure 100 g quantities of both (+) and (-) primaquine from laboratories producing fine chemicals.

3.5.4 Selected 8-aminoquinoline derivatives. CHEMAL will continue investigating the toxicology, formulation development, clinical pharmacology and antimalarial efficacy of selected 8-aminoquinoline compounds. The project will be pursued with maximum coordination with WRAIR.

3.5.5 Proguanil and chlorproguanil. The clinical effectiveness of these drugs for treating multidrug-resistant malaria should be investigated and *in vitro* tests for parasite sensitivity developed, using the parasite's cyclic metabolic products.

3.6 Clinical Research Centres

The highest priority will be given to the maintenance and, where necessary, further strengthening of CHEMAL clinical research centres in Africa, Asia and South America. These centres are of the greatest importance to the development of new antimalarial drugs and may play a future role in the evaluation of vaccines. They should be encouraged to undertake further relevant projects on the pharmacology of antimalarials and should not be restricted to routine clinical trials. The centres should evolve to fully self-sufficient units capable of undertaking complete clinical pharmacological evaluation of antimalarial drugs. Links and collaborative programmes between the centres and clinical pharmacology laboratories in endemic and nonendemic areas should be further strengthened.

3.7 Drugs: Candidates for Immediate Development

The following drugs have been identified as candidates for immediate development under the CHEMAL programme:

- artether, in a suitable vehicle for intramuscular administration;
- a new, stable, water-soluble qinghaosu derivative;
- halofantrine;
- an appropriate naphthoquinone derivative;
- an 8-aminoquinoline derivative to be selected from the WRAIR programme;
- M & B 35769;
- proguanil and chlorproguanil.

Work being conducted outside TDR may lead to the discovery of other promising compounds. The above list may therefore be modified to include alternative or additional promising compounds offered by other research groups or laboratories.

4. REFERENCES

1. * TDR. Report of the second Scientific Working Group on the Chemotherapy of Malaria, Geneva, Switzerland, 3-6 July 1978. Document TDR/CHEMAL-SWG(2)/78.3.
2. * TDR. Report of the third meeting of the Scientific Working Group on the Chemotherapy of Malaria: The development of tissue schizontocides, Geneva, Switzerland, 6-8 October 1980. Document TDR/CHEMAL-SWG(3)/80.3.
3. * TDR. Report of a section meeting of the Scientific Working Group on the Chemotherapy of Malaria: Development of mefloquine, Geneva, Switzerland, 28-30 May 1981. Document TDR/CHEMAL-SWG(MEFLO)/81.3.
4. * TDR. Fourth meeting of the Scientific Working Group on the Chemotherapy of Malaria: The development of quinhaosu and its derivatives as antimalarial drugs, Beijing, China, 6-10 October 1981. Document TDR/CHEMAL-SWG(4)/81.3.
5. * WERNSDORFER, W.H. & TRIGG, P.I. (eds.) Modern Design of Antimalarial Drugs. Proceedings of a Meeting of the Scientific Working Group on the Chemotherapy of Malaria held in Bethesda, Maryland, USA, 31 May - 2 June 1982. Geneva: UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases, 1983, 183 pp.

* TDR technical documents (marked with a single asterisk in the above list) are available upon request from the Office of the Director of the Special Programme, HQ/TDR, World Health Organization, 1211 Geneva 27, Switzerland

6. * WERNSDORFER, W.H. & TRIGG, P.I. (eds.) Primaquine: Pharmacokinetics, Metabolism, Toxicity and Activity. Proceedings of a Meeting of the Scientific Working Group on the Chemotherapy of Malaria held in Geneva, Switzerland, 27-28 February 1984. New York - Chichester: John Wiley & Sons, 1986 (in press).
7. ** TDR. Tropical Disease Research - TDR. Seventh Programme Report, 1 January 1983 - 31 December 1984. Geneva: World Health Organization, 1985, 278 pp.
8. ** WHO. Advances in malaria chemotherapy. Report of a WHO Scientific Group. Technical Report Series, 711: 218 pp. (1984).

5. PARTICIPANTS

AIKAWA, Prof M., Institute of Pathology, Case Western Reserve University,
2085 Adelbert Road, Cleveland, OH 44106, USA

ANAND, Prof N., Central Drug Research Institute, Chattar Manzil Palace,
P.O. Box 173, Lucknow, Uttar Pradesh 226001, India

BROSSI, Dr A., Chief, Medicinal Chemistry, Department of Health and Human
Services, Public Health Service, Bethesda, MA 20205, USA

CANFIELD, Dr C.J., 3508 Old Largo Road, Upper Marlboro, MA 20772, USA

DESJARDINS, Dr R., Executive Director, Domestic Clinical Research, American
Cyanamid Co., Lederle Laboratories, Pearl River, NY 10965, USA

FERNEX, Prof M., Department of Parasitology, F. Hoffmann-La Roche & Co.,
Grenzacherstrasse 124, 4002 Basel, Switzerland

GOODWIN, Dr L.G., Shepperlands Farm, Finchampstead, Wokingham, Berkshire,
RG11 4QF, UK

HEIFFER, Dr M.H., Division of Experimental Therapeutics, Walter Reed Army
Institute of Research, Walter Reed Army Medical Center, Washington,
DC 20307, USA

HOWELLS, Dr R.E., Department of Parasitology, Liverpool School of Tropical
Medicine, Pembroke Place, Liverpool, LS 5QA, UK

LANDAU, Dr I., Laboratoire de Zoologie (Vers), Muséum National d'Histoire
Naturelle, 61 rue de Buffon, 75231 Paris Cédex 05, France

PINICHPONGSE, Dr S., Director, Malaria Division, Ministry of Public Health,
Devavesm Palace, Bangkok, Thailand

RIECKMANN, Dr K., 7 Crown St., Henley, NSW 2111, Australia

ROSARIO, Dr V.E. do, Department of Parasitology and Laboratory Practice,
University of North Carolina, School of Public Health, Chapel Hill, NC
27514, USA

** WHO publications (marked with a double asterisk in the above list) are available through the WHO network of designated sales agents (listed in all WHO publications) or may be obtained directly from the World Health Organization, Distribution and Sales Service, 1211 Geneva 27, Switzerland.

SALAKO, Prof L.A., Department of Pharmacology and Therapeutics, University of Ibadan, Ibadan, Nigeria

SHERMAN, Prof I.W., Dean, College of Natural and Agricultural Sciences, University of California, Riverside, CA 92521, USA

TAUIL, Dr P.L., Assessoria Parlamentar, Senado Federal, PCA Dos 3 Poderes, Brasilia DF, Brazil

WARHURST, Dr D.C., Department of Medical Protozoology, London School of Hygiene and Tropical Medicine, Keppel St., London, WC1E 7HT, UK

WIRTH, Dr D.F., Department of Tropical Public Health, Harvard Medical School, 665 Huntington Avenue, Boston, MA 02115, USA

WHO Secretariat

DOBERSTYN, Dr E.B., Secretary, CHEMAL Steering Committee, Special Programme for Research and Training in Tropical Diseases

LUCAS, Dr A.O., Director, Special Programme for Research and Training in Tropical Diseases

SHETH, Dr U.K., Consultant, Special Programme for Research and Training in Tropical Diseases

TRIGG, Dr P.I., Malaria Action Programme and Co-Secretary, CHEMAL Scientific Working Group

WERNSDORFER, Dr W.H., Malaria Action Programme and Co-Secretary, CHEMAL Scientific Working Group

.....