Drug-Resistant Malaria

The Report of a meeting held under the auspices of the World Health Organization (WHO) Regional Offices for South-East Asia and the Western Pacific, and the Scientific Working Groups on the Chemotherapy of Malaria and on Applied Field Research in Malaria of the UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases.

Kuala Lumpur, Malaysia, 10-15 August 1981

Edited by

Walter Wernsdorfer,
Chief Medical Officer,
Research and Technical Intelligence,
Malaria Action Programme, WHO

and

Secretary, Scientific Working Groups on Malaria,
Special Programme for Research and Training in Tropical Diseases

UNDP/WORLD BANK/WHO
Special Programme for Research and Training in Tropical Diseases
Geneva, 1982
Preface

The Special Programme is a goal-oriented research and training programme with two objectives:

- Research and development to obtain new and improved tools for the control of major tropical diseases, and

- Strengthening of the research capability of the tropical countries.

The research is conducted on a global basis by multidisciplinary Scientific Working Groups; the training and institution strengthening activities are limited to the tropical countries where the diseases are endemic.

The six diseases initially selected for attack are: malaria, schistosomiasis, filariasis (including onchocerciasis), trypanosomiasis, (both African sleeping sickness and the American form called Chagas' disease), leishmaniasis and leprosy. Scientific Working Groups are also active in trans-disease areas: Biomedical Sciences, Vector Control, Epidemiology, and Social and Economic Research.

Scientists interested in participating in the Special Programme are invited to write for further information.

Dr Adetokunbo O. Lucas, Director
Special Programme for Research and Training in Tropical Diseases
\( \text{c/o World Health Organization} \)
Geneva, Switzerland
Foreword

A meeting on drug-resistant malaria was held in Kuala Lumpur, Malaysia on 10-15 August 1981. It was organized jointly by the WHO Regional Offices for South-East Asia and the Western Pacific, and the Scientific Working Groups on the Chemotherapy of Malaria and Applied Field Research in Malaria, UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

The main objectives of the meeting were to review the problems of drug-resistant Plasmodium falciparum in the countries of the Western Pacific and South-East Asia Regions and to elaborate recommendations on the monitoring and containment of drug resistance and on appropriate laboratory-based and field research activities.

The meeting was opened by Y.B. Tan Sri Chong Hon Nyan, Minister of Health, Malaysia. His address was preceded by messages from the Regional Director of the WHO South-East Asia Region, Dr U. Ko Ko, and the Western Pacific Region, Dr H. Nakajima, and an address by Dr T. Lepes, Director, Malaria Action Programme, WHO, Geneva (see Chapter II).

Dr P.J. Jacob, Deputy Director, Malaria Eradication Programme, Malaysia, was elected chairman of the meeting. Professor R.E. Desjardins and Dr A.N.A. Abeyesundere held the offices of Vice-chairman and Rapporteur respectively. The proposed agenda was adopted, and the Chairmen and the Rapporteurs for the various working groups were nominated. The list of participants is given in Appendix 1 and the list of documents in Appendix 2.
Table of Contents

I. INTRODUCTION ........................................ 1
   Objectives of the Meeting. ....................... 2

II. OPENING ADDRESS AND MESSAGES ..................... 5
   Opening Address by Y.B. Sri Chong Hon Nyan
   Minister of Health, Malaysia .................... 5
   Message - Dr U. Ko Ko, Director
   Regional Office for South-East Asia, WHO ........ 8
   Message - Dr H. Nakajima, Director
   Regional Office for the Western Pacific, WHO .... 9
   Address - Dr T. Lepes, Director
   Malaria Action Programme, WHO, Geneva .......... 10

III. REVIEW OF DRUG RESISTANCE IN P. FALCIPARUM ...... 13
    South-East Asia Region ......................... 13
    Western Pacific Region ........................ 20
    The Westward Spread of Drug-Resistant P. falciparum 28
    American Region ............................... 30

IV. MONITORING OF DRUG RESPONSE OF P. FALCIPARUM ...... 33
    Test Systems, Recent Experiences ............... 33
    Recording, Processing and Analysis of Results .... 42

V. RECENT RESEARCH RESULTS RELATED TO THE CHEMOTHERAPY
    OF MALARIA ........................................ 53
    Mechanism of Drug Resistance .................... 53
## CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug Pressure and Resistance</td>
<td>54</td>
</tr>
<tr>
<td>Drug Combinations and Synergism in Relation to the Prevention of the Development of Drug Resistance</td>
<td>55</td>
</tr>
<tr>
<td>Exo-Erythrocytic Development of Malaria Parasites.</td>
<td>56</td>
</tr>
<tr>
<td>Chloroquine Retinopathy.</td>
<td>56</td>
</tr>
<tr>
<td>Clinical Pharmacological Studies</td>
<td>56</td>
</tr>
<tr>
<td>VI. CONTROL OF DRUG-RESISTANT P. FALCIPARUM</td>
<td>59</td>
</tr>
<tr>
<td>Control of Transmission and Prevalence</td>
<td>59</td>
</tr>
<tr>
<td>Drug Use Aiming at Preventing, Delaying or Reversing the Selection of Resistant P. falciparum</td>
<td>63</td>
</tr>
<tr>
<td>VII. FIELD AND LABORATORY RESEARCH FOR THE CONTROL OF RESISTANT P. FALCIPARUM</td>
<td>75</td>
</tr>
<tr>
<td>GENERAL TOPICS</td>
<td>75</td>
</tr>
<tr>
<td>SPECIFIC TOPICS</td>
<td>76</td>
</tr>
<tr>
<td>PRIORITY TOPICS</td>
<td>81</td>
</tr>
<tr>
<td>VIII. RECOMMENDATIONS</td>
<td>85</td>
</tr>
<tr>
<td>National and International Cooperation</td>
<td>86</td>
</tr>
<tr>
<td>Parasite Genetics</td>
<td>86</td>
</tr>
<tr>
<td>Evaluation and Development of Antimalarial Drugs</td>
<td>87</td>
</tr>
<tr>
<td>Training</td>
<td>88</td>
</tr>
<tr>
<td>IX SUMMARY</td>
<td>91</td>
</tr>
</tbody>
</table>

### APPENDICES

1. List of Participants, Observers and Secretariat                        | 93
2. List of Documents                                                      | 103
3. Response of P. falciparum to Chloroquine and Mefloquine                | 107
4. Sample Size in Studies on Drug Response of Plasmodium falciparum       | 111
5. Antimalarial Drugs and Drug Regimens (Falciparum Malaria)              | 113
6. Future Deployment of Mefloquine and Essential Measures for Protecting Mefloquine Against Resistance | 123
7. Outline Research Proposals                                             | 129
8. Considerations Related to the Practical Performance of the Assessment of Drug Sensitivity in Plasmodium falciparum | 135
Antimalarial drugs have always played an important role in malaria control. Quinine was the first drug to be employed in this context, followed later by the synthetic drugs such as 4-aminoquinolines, dihydrofolate reductase (DHFR) inhibitors and sulfonamides.

The World Health Organization convened several Scientific Group meetings to consider different aspects of malaria chemotherapy, the use of antimalarials, the status of research in this field and the resistance of plasmodia to antimalarial drugs. The deliberations of these groups have been published in the WHO Technical Report Series. In 1955, WHO published a monograph on the Chemotherapy of Malaria, the second edition of which has just been published (WHO Monograph Series No. 27, second edition, Geneva, World Health Organization, 1981).

In its 8th, 9th, 12th, 14th, 16th and 17th meetings, the WHO Expert Committee on Malaria referred to antimalarial drugs and stressed the importance of their use for accelerating the elimination of the parasite reservoir, for interrupting malaria transmission and for dealing effectively with residual foci and with imported cases.

The Malaria Eradication Programmes initiated in the late 1950s therefore laid emphasis on the strategic use of antimalarials, but did not usually employ them as attack measures. However, it has to be realized that in certain countries the use of drugs may be a major, if not the only method available for depopulating the malaria reservoir.
INTRODUCTION

Differences in drug response between plasmodia of the same species, but originating in various geographical areas, have long been recognized by investigators first in relation to quinine and later to other antimalarial drugs, pointing to the existence of distinct strains of plasmodia. Although this concept has been accepted for a long time, there are no adequate techniques for the biological characterization of strains.

True resistance of plasmodia to antimalarial drugs has been known since the beginning of the 20th century, but it is only recently that the problem of resistance of *Plasmodium falciparum* to antimalarials has reached a level at which hopes for the efficient control of malaria in some countries of the South-East Asia and Western Pacific Regions are fading. In the 20 years since chloroquine resistance of *P. falciparum* was first detected and confirmed, an important geographical spread of resistance has been observed in Asia and the Pacific, involving practically all malarious countries from India eastwards with the exception of Sri Lanka. In South America recent geographical spread was less marked although the problem has grown in intensity. Resistance of *P. falciparum* to chloroquine in East Africa was detected 3 years ago, but seems still to be limited both in extent and degree. The successful in *vitro* selection, under chloroquine pressure, of a resistant line of *P. falciparum* from an isolate from Senegal is indicative of the potential future problems of resistance in Africa.

With financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases an extensive cooperative programme for the assessment and monitoring of drug resistance was initiated a few years ago in both the South-East Asia and Western Pacific Regions. Considerable progress has been made with the *in vivo* and *in vitro* testing of the response of *P. falciparum* to chloroquine and some other drugs, but there is an urgent need for a precise as possible assessment of the problem of drug-resistant malaria in order to develop a realistic policy for the use of antimalarial drugs in the immediate future. At the same time, the methods of sampling, testing and evaluation deserve attention. Most of the past activities were devoted to baseline assessment; monitoring, i.e. the longitudinal qualitative and quantitative follow-up of drug sensitivity under different epidemiological conditions, has only recently commenced.

OBJECTIVES OF THE MEETING

The general objective of the meeting was to review the problems of drug-resistant *P. falciparum* in the Western Pacific and South-East Asia Regions and the research activities related to the containment of drug resistance. Based on this review the meeting was to make specific recommendations concerning the monitoring and
control of drug-resistant malaria, and appropriate research. The following specific objectives were identified.

(a) To review the countries' field research projects related to *P. falciparum*.

(b) To review the distribution in space and time of the response of *P. falciparum* to antimalarial drugs in the two regions and to take note of the situation of drug resistance in the Americas. This review includes an updating of available information, a tentative analysis and projections;

(c) To review the monitoring systems, both in terms of the capacity and the factual application of the tests, the processing and analysis of data, and sampling. Recommendations made regarding monitoring include the future of current projects and their financing;

(d) To review the current and planned methods of containing the problem of drug resistance, including the use of alternative drugs, the control of transmission, the reduction of prevalence of drug-resistant *P. falciparum*, and policy decisions about drug use, with a view to making appropriate recommendations.

(e) To identify research subjects relevant to the control of drug-resistant *P. falciparum* malaria, with an emphasis on questions amenable to field research, and to identify priorities for the implementation of appropriate projects.
II.

Opening Address and Messages

OPENING ADDRESS

Dr. T.T. Tan Sri Chong Hon Nyan,
Minister of Health, Malaysia

On behalf of the Ministry of Health and the Government of Malaysia, I welcome all the participants to this meeting on the problems of "drug-resistant malaria". We are happy that this should attract experts from both the South-East Asia and Western Pacific Regions as well as from Europe and the United States. As a responsible member of the Community of Nations, we have always enjoyed close working relations with the World Health Organization. I am happy that we are able once again to act as the host for this WHO sponsored meeting in the interests of health.

During the next few days in this country, you will have an opportunity to know about some of our antimalarial measures to control this disease here. You will be able to judge for yourselves whether these measures, some of which are being applied in other countries, can be equally effective in your own environment. We expect similarly to learn from all of you. Our fight against malaria goes back over a century and a half. The Malaysian history of malaria is coincident with the history of the opening up of this country to modernization. The first reported case of malaria was in Penang Island in 1829. It was then known as "Penang Fever" as its origins were unknown. Penang now owes its worldwide fame to its beauty and tranquility as an island holiday resort rather than to any fever of unknown origin. We have progressed since then.
It is a fact, however, that malaria was a major health obstacle to the physical and economic development of this country especially in the earlier years. As we opened up our forests for rubber estates and tin mines, so did we expose the population to the malaria disease-carrying mosquito. Our workers were defenceless against it and deaths from malaria were consistently high. We had no preventive measures and very few curative ones. Our major port is Port Keland - it was then known as Port Swettenham. Its early development was nearly abandoned because of malaria. The first permanent antimalarial measures were started in Kelang in 1901. The port at Kelang flourishes today and is no longer menaced by malaria, neither are the majority of our rubber estates and tin mines.

Malaysia is still learning how to cope with this disease out of its own past experience in preventive and curative medicine backed up by continuing research in our Institute for Medical Research. We have paid for this hard experience in terms of human wastage to this disease. We must continue to be vigilant against it although as the incidence of this disease decreases, there is danger of complacency creeping in where our attitude is concerned. Health workers and the population must be continually reminded that the danger still exists although it is controlled. The very effectiveness of the present programmes here in reducing the incidence of malaria from more than 300,000 cases a year to 9,110 reported cases in 1980, can lead to the erroneous conclusion amongst planners that we can now devote our resources to other health activities. Statistics alone do not hide the fact that malaria still exists here and in the region. It can flare up again as it has flared up elsewhere.

We share the experience of other countries in that particular problems over eradication and control measures still remain. We now face a new anxiety that you have all gathered here to tackle in greater depth - the emergence of the drug-resistant malaria parasite. We have identified this resistant strain in various parts of our country since its first detection in the State of Perlis in Northern Malaysia in 1967. Unless we can effectively counter it, the growing emergence of this strain will pose serious curative problems for us, particularly in Sabah where there is a tradition of migrant labour moving in and out. This is why we have always stressed the vital importance of bilateral health programmes between us and our immediate neighbours of Indonesia, Thailand and Singapore. We have established so-called "border health committees" with all these friendly neighbours to further develop preventive measures for the control of tropical communicable diseases. We cannot remain isolated or secure in the knowledge that our own preventive measures are effective. We must also be reasonably certain that we are not transmitting this disease to others, just as much as we would expect others to be reasonably careful that they are not transmitting the disease to us. The malaria parasite does not recognize political or geogra-
physical boundaries. With the rapid development of this whole region, and the increasing mobility of our total population, we have to widen our horizons as well.

The World Health Organization and the Ministry of Health are now conducting a joint study on this particular problem of drug-resistant malaria in Malaysia. We shall be happy to share our knowledge with others as I am certain that you would wish to share yours with us. We have a common enemy in our midst. It is in this context that I am particularly happy that the World Health Organization has proposed the establishment of a network of National Malaria Training Centres in Asia, which will receive training and research support from a Permanent Secretariat which will also give assistance on request to any of the countries concerned.

We are particularly glad to accept its further proposal that such a Permanent Secretariat should be established in Kuala Lumpur. I have already obtained the endorsement of the Malaysian Government to this proposal, together with the formal agreement to establish such a Permanent Secretariat at the Institute for Medical Research. Arrangements are being made for this agreement to be signed shortly. When this Permanent Secretariat is established here, with the benefit of both local and foreign expertise, I hope for the further strengthening of the health links that already exist in this Region. Some of the delegates here may belong to different Regions in the World Health Organization purely because of administrative divisions. This does not necessarily mean, however, that we cannot or should not have bilateral arrangements or other health groupings that can serve our common interests. We already have such a health grouping in the Association of South-East Asian Countries. The establishment of this Permanent Secretariat for Malaria for the whole of Asia gives us yet another dimension to work in.

As the Minister of Health, I have readily recommended the acceptance of these proposals to our Government as I believe that they will be of immense benefit to Asia. We regard this as also being a singular honour to Malaysia in recognition of our own efforts to control this disease, if not to eradicate it altogether. We look forward to the early establishment of this Permanent Secretariat accordingly just as we look forward to continued cooperation amongst us all to fight the mosquito and the malaria parasite.

We cannot afford the wastage of human energy to this debilitating disease, especially when it affects our rural people whose social and economic betterment is of priority concern to us all. I wish this meeting every success accordingly.
It gives me much pleasure to greet participants in this important meeting, which is being sponsored by the World Health Organization's South-East Asia and Western Pacific Regions, and by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. The welcome extended by the host Government of Malaysia to the delegates from the countries of these Regions, and to the international experts and advisers, is most gratefully acknowledged. We hope that the conclusions reached at the meeting will prove useful to Malaysia as well as to other countries where drug-resistant falciparum malaria has increasingly been a problem interfering with control and eradication of the disease.

It is well known that chloroquine-resistant falciparum malaria, which has proved to be so serious an obstacle to the use and mass distribution of this safe and reliable chemotherapeutic agent, was first identified in a border area between the two Regions more than 30 years ago. At first thought to be of relatively little operational significance, the resistant parasites have spread with astonishing rapidity, aided no doubt by the drug pressure provided by increasingly widespread and popular use of chloroquine at a time when the successes of the insecticide-based eradication campaigns were faltering. Our dependence these days on chemotherapy, in particular on distribution of presumptive treatment through peripheral health centres and community volunteers, has brought the problem to the forefront in both regions.

Identification and monitoring of the chloroquine-resistant parasite by in vivo and in vitro testing, determination of its degree of susceptibility to other drugs, and mapping its ever-widening distribution, are vital activities of the national teams that have to a considerable extent been trained and supported by the World Health Organization. Our South-East Asia Regional Advisory Committee on Medical Research has for several years endorsed the importance of, and authorized us to support to the fullest possible extent, our Regional Collaborative Studies on Plasmodium falciparum resistant to 4-aminoquinolines, and I am glad that one part of this meeting will be a review of these ongoing studies; in fact for our Region this will be the second such review. The information that will be made available will enable the national malaria programme directors, as well as the international experts gathered here, to recommend appropriate tactics, such as intensification of parasite containment measures and a flexible use of alternative drugs or combinations of drugs.
to preserve for as long as possible those few compounds that are still effective.

Greater urgency is lent to these discussions by identification in Thailand of \textit{P. falciparum} relatively resistant to the combination of pyrimethamine with a long-acting sulfonamide, our first-line backup as presumptive treatment where the 4-amino-quinolines fail. What may we use to replace these drugs? Is sufficient attention being paid to the indigenous antimalarials? For clinical curative purposes, it is true that quinine remains to a large extent effective, and quinine with tetracycline fully effective. But the prospects for presumptive treatment, let alone mass drug administration give little cause for optimism, and we are well aware that pressures are mounting to throw the new compound mefloquine into the arena. Some of your time will no doubt be devoted to consideration of how best to protect this powerful schizontocide from premature exploitation.

Since critical decisions by the concerned governments will rest on your conclusions, it is gratifying to note the breadth of expertise assembled at this meeting. The health of the inhabitants of countries where drug-resistant \textit{falciparum} malaria occurs is very much in your hands, and I wish you every success in your deliberations.

\underline{MESSAGE}

Dr H. Nakajima, Director, Regional Office for the Western Pacific, World Health Organization

Allow me, on behalf of the WHO Regional Office for the Western Pacific, to extend a warm welcome to you all and to express my appreciation to the Government of Malaysia for kindly agreeing to hold this important meeting on drug-resistant malaria in Kuala Lumpur. I am particularly grateful to the Honourable Minister of Health, Tan Sri Chong Hon Nyan, for the support extended to this meeting.

The issue addressed by this meeting is considered to be the most serious and intractable of the technical problems facing antimalaria programmes in many countries. It is not by accident therefore that the participants in this meeting include a distinguished group of scientists, directors of antimalaria programmes, and research workers from both the South-East Asia and Western Pacific Regions. It is hoped that the resulting exchange of information and experience will lead to a better understanding of the problem.
Almost two decades have elapsed since the malaria parasites were observed to be resistant to 4-aminoquinolines. Since then, Plasmodium falciparum has developed a stronger resistance to chloroquine, and its resistant strains have spread, gaining a wider geographical distribution. Chloroquine-resistant P. falciparum is now known to occur in all endemic malarious countries of the Western Pacific Region, as well as in the neighbouring region. There is thus an urgent need today to fill in the gaps in our knowledge concerning antimalarial drugs and the use of more effective drug combinations. Such knowledge would be invaluable to both clinicians and malariologists in the prevention and control of malaria.

I am persuaded that, in selecting the most effective regimen of antimalarial drugs, active cooperation is necessary among countries. P. falciparum resistant strains do not observe political boundaries. In this connexion, we anticipate the fullest cooperation with our neighbouring South-East Asian countries and will welcome any efforts to achieve closer collaboration on this pressing problem.

The ultimate objective of this meeting is to make practical recommendations with regard to the monitoring of the problem of drug-resistant Plasmodium strains, the methods of containment and relevant field research.

You have several days of hard work before you in which to review the progress made and determine the means of overcoming the difficulties involved. I hope you will find the discussions stimulating and fruitful, and I wish you every success in your deliberations.

Thank you.

ADDRESS

Dr T. Lepes, Director,
Malaria Action Programme,
World Health Organization, Geneva

On behalf of the Director-General of the World Health Organization, of Dr A.O. Lucas, Director of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical diseases and on my own behalf, I have pleasure in welcoming you to the Meeting on Drug-Resistant Malaria.

It is very gratifying to see the number of investigators participating from countries in the South-East Asia and Western Pacific Regions and to know that the cream of the scientific world
is also here and ready to continue their assistance in the field of malaria chemotherapy. This, in itself, is indicative of the importance and gravity of the problems being encountered in so many countries in this field. It is true that drug resistance is not exclusively linked to malaria and that other diseases, mainly communicable, have problems connected with resistance of the causative agents to drugs. Drug resistance is not only a problem to present-day malariologists, since it had already been observed in the past that infections, particularly those with Plasmodium falciparum, did not respond to the same drug in the same manner, in different geographical areas. Marchiafava was the first, with Bignami, to note the difference in the clinical symptoms caused by P. falciparum in East Africa and in Italy. This was confirmed by Robert Koch in the early part of this century. It was again observed by workers at the Malaria Therapy Station in Horton, UK, in 1920. More detailed studies carried out in the United States during the Second World War confirmed previous observations and also indicated that this problem could be resolved by adjusting the doses and regimens of drugs existing at that time.

Since the initiation of malaria eradication programmes in most countries affected by malaria, with the exception of Africa south of the Sahara, the use of antimalarial drugs in malaria control/eradication was advocated mainly for the depletion of the parasite reservoir. Looking back at the late 1950s and 1960s, it is difficult to escape the impression that the resistance of P. falciparum to 4-aminoquinolines detected in South-East Asia and South America was considered more as a problem of academic interest rather than as an obstacle to the eradication of the disease. However, the resurgence of the disease in the early 1970s, particularly in South-East Asia and parts of Central and South America, coupled with the ever-increasing spread of P. falciparum resistance to 4-aminoquinolinos and more recently, to a combination of sulfadoxine/pyrimethamine, has become such an important problem that many health administrators are wondering how to continue with malaria control in areas where this problem occurs.

Research on the development of antimalarial drugs has been pursued for many years and, in the last 10 to 15 years, more than 250,000 compounds have been screened and tested but only a few could be considered as suitable candidates. But, even though the future does not seem very promising in this respect, there is no place for pessimism. We must continue with our efforts and with activities such as this meeting which has been organized to review every possibility available in the utilization of the existing antimalarial drugs and their combinations.

It is a pleasure to see the enthusiasm with which so many scientists have accepted to undertake the monitoring of drug resistance. In this respect the number of susceptibility tests carried out in the last two years speaks for itself. However, the
monitoring of drug resistance should not be confined simply to testing the resistance of *P. falciparum* to antimalarials, but should include trials with different combinations of existing drugs and it is hoped that you will make many suggestions on this subject in your recommendations.

You have a difficult task ahead of you and I am sure that with the knowledge and experience accumulated by all of you, there are good hopes for finding efficient solutions to our problems.

I wish you every success in your deliberations.
III.

Review of Drug Resistance in *P. falciparum*

**SOUTH-EAST ASIA REGION**

**General**

The Regional Collaborative Studies on drug-resistant *falciparum* malaria in the WHO South-East Asia Region commenced in 1978. The first review meeting was held in Chiangmai, Thailand, 8-13 October 1979, and highlighted the achievements of the first year of operation. At this, the second review meeting, it was intended to review the progress made over the period of two years since the first review meeting, to discuss problems that had hindered the progress, and to review work plans for the years 1981-1983.

**Objectives of the South-East Asia Regional Collaborative Studies**

The objectives listed below are those that were defined at the Regional Workshop held in SEARO in August 1977 and endorsed by seven countries - Bangladesh, Burma, India, Indonesia, Nepal, Sri Lanka and Thailand - of the Region in 1978.

(a) assessment of the status of susceptibility of *P. falciparum* to 4-aminoquinolines throughout the Region, by application of the *in vivo* and/or *in vitro* techniques and establishment of a baseline for the current geographical distribution, prevalence and degree of resistance;

(b) monitoring of the spread, relative prevalence and degree of 4-aminoquinoline resistance in *P. falciparum* in the countries of
the Region with the aim of facilitating the implementation of operational counter measures;

(c) assessment, on a regional scale, of the response in vivo and in vitro of *P. falciparum* to (i) currently used antimalarial drugs other than 4-aminoquinolines, so as to determine their clinical and operational usefulness, and (ii) to candidate antimalarial compounds, in order to establish baseline levels of sensitivity of the local strains prior to introduction of the drugs for general use;

(d) provision of guidelines on the effective clinical management of malaria, with particular reference to areas where *P. falciparum* resistance to 4-aminoquinolines is present or suspected; and

(e) development of systems of operational measures aiming at the prevention of the propagation of 4-aminoquinoline-resistant *P. falciparum* in vulnerable and receptive areas, and the elimination or suppression of existing 4-aminoquinoline-resistant *P. falciparum* foci.

**Progress, Baseline Studies and Monitoring**

The studies have moved beyond the initial stage and have begun to yield data and information in accordance with the objectives. Assessment of the sensitivity levels and response of *P. falciparum* to chloroquine in vivo and in vitro has been in continual progress. Some countries have either completed or partially completed the collection of baseline data on which to plan a future monitoring programme. Others have made an attempt to reach this goal but failed to do so due to various administrative and technical constraints. Results of the sensitivity tests of *P. falciparum* to chloroquine conducted during the period 1978-1981 in the various countries of the Region are presented in Table 1.

Research capabilities have been developed in all seven countries concerned. Training at the Regional and National level has been conducted and teams trained to assess baseline levels and monitor *P. falciparum* sensitivity to chloroquine by applying the in vivo and in vitro techniques.

Field studies have been considerably expanded in most countries. In Thailand, assessment of the response of *P. falciparum* to chloroquine in vitro has been completed throughout the country. Chloroquine in vivo tests were considered ethically unacceptable when it was known that a very strong resistance to this drug was present. In Burma, assessment by applying the in vivo and in vitro techniques was completed in all but two states. Bangladesh has produced information on the response in vivo and in vitro in the
Table 1. Sensitivity Tests 1978-1981 (Chloroquine)\(^1\)

<table>
<thead>
<tr>
<th>Country</th>
<th>In vivo</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>In vitro</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>S/RI</td>
<td>RI</td>
<td>RII</td>
<td>RIII</td>
<td>Total</td>
<td>S</td>
<td>R</td>
<td>Total</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>-147</td>
<td>27</td>
<td>67</td>
<td>15</td>
<td>256</td>
<td></td>
<td>85</td>
<td>44</td>
<td>79</td>
</tr>
<tr>
<td>Burma</td>
<td>19</td>
<td>136</td>
<td>26</td>
<td>20</td>
<td>0</td>
<td>201</td>
<td>6</td>
<td>118</td>
<td>124</td>
</tr>
<tr>
<td>India</td>
<td>982</td>
<td>610</td>
<td>118</td>
<td>27</td>
<td>13</td>
<td>1750</td>
<td>83</td>
<td>63</td>
<td>146</td>
</tr>
<tr>
<td>Indonesia</td>
<td>80</td>
<td>51</td>
<td>39</td>
<td>33</td>
<td>2</td>
<td>205</td>
<td>49</td>
<td>94</td>
<td>143</td>
</tr>
<tr>
<td>Nepal</td>
<td>12</td>
<td>9</td>
<td>6</td>
<td>23</td>
<td>0</td>
<td>50</td>
<td>0</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Sri Lanka</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Thailand</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>19</td>
<td>537</td>
<td>556</td>
</tr>
<tr>
<td>Total</td>
<td>1093</td>
<td>953</td>
<td>216</td>
<td>170</td>
<td>30</td>
<td>2462</td>
<td>212</td>
<td>874</td>
<td>1086</td>
</tr>
</tbody>
</table>


In India extensive studies have been carried out and as a result some information is available on the response of *P. falciparum* to chloroquine in many of the falciparum areas of the country. This information has mostly been collected by conducting in vivo tests but wherever possible the macro in vitro test has been applied. The country has reported a further westward extension of chloroquine resistance in the States of Uttar Pradesh, Madhya Pradesh, Maharashtra, and Andhra Pradesh as well as in the Andaman-Nicobar islands.

Nepal continues to report the absence of indigenous cases resistant to chloroquine. Subjects returning from the North Eastern States of India have been shown to be carrying resistant strains. However, it is encouraging to note a considerable...
decrease in the percentage of imported falciparum malaria cases from India over the last year.

Sri Lanka experienced difficulty in conducting the sensitivity tests due to the limitations of the macro in vitro technique, the low numbers of cases available and self-medication with 4-aminoquinolines by the population. Information was finally obtained in three areas by applying the micro in vitro technique.

In Indonesia national training courses were conducted in East Kalimantan, East Java and Central Java. This training allowed for team expansion and extension of the study programme to Java, Sumatra and other islands. The studies during the period under review were carried out in East Kalimantan, West Kalimantan, Irian Jaya, South Sumatra, Riau and Central Java.

A single resistant case of P. falciparum to chloroquine has been located in Lampung, South Sumatra. A focus of chloroquine resistance at the RI-RII level has been found in the Regency of Jepara, Central Java. A more recent study has also confirmed the presence of resistant strains in the province of Timor Timur. Field studies have indicated that P. falciparum resistant to chloroquine is also prevalent on the southern coast of Irian Jaya.

In Thailand in vivo studies confirmed the increasing occurrence in many areas of P. falciparum resistant to the sulfadoxine-pyrimethamine combination. Macro in vitro studies were initiated in the country on the response of P. falciparum to mefloquine. Investigations have already been carried out in five provinces and a further five provinces will be investigated during the latter part of 1981 (for results see Table 2).

<table>
<thead>
<tr>
<th>Province</th>
<th>Total tests</th>
<th>Growth 2 n=101 (2x10^{-6} M/l) (end point)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chanthaburi</td>
<td>107</td>
<td>2</td>
</tr>
<tr>
<td>Tak</td>
<td>100</td>
<td>5</td>
</tr>
<tr>
<td>Yala</td>
<td>102</td>
<td>0</td>
</tr>
<tr>
<td>Kalasin</td>
<td>85</td>
<td>3</td>
</tr>
<tr>
<td>Petchaboon</td>
<td>92</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>486</td>
<td>10</td>
</tr>
</tbody>
</table>
Other Studies

Validation and development studies were carried out on the micro in vitro test on the response of *P. falciparum* to antimalarial drugs. Studies using this technique with chloroquine were conducted in Burma, India, Indonesia, Sri Lanka and Thailand. In Sri Lanka the technique was used to compare the response to chloroquine and amodiaquine, while in Thailand it was employed to study the response to chloroquine, quinine and mefloquine.

In Burma studies on the drug combinations sulfadoxine-pyrimethamine and sulfalene-pyrimethamine have indicated that both combinations produce similar clinical results although with a higher number of early recrudescences in the sulfalene/pyrimethamine group. A small percentage of patients included in the studies failed to respond a priori.

A scientist from Thailand was given the opportunity of visiting Sri Lanka to collect *P. falciparum* isolates for characterization studies. Using the continuous culture method the isolates have been shown to be highly sensitive to chloroquine, amodiaquine, quinine, pyrimethamine and mefloquine.

Under a research grant, a scientist at Chiangmai University, Thailand, has commenced a study on the metabolic fate of sulfadoxine and on the correlation between the host's metabolic activity and the effect of the sulfadoxine/pyrimethamine combination.

A scientist of the Department of Medical Research, Rangoon, Burma, was given the opportunity of carrying out a comparative study of the in vivo and in vitro micro tests for the response of *P. falciparum* to chloroquine.

Coordination

The Regional Office has continued to provide coordination and technical guidance and to assist in training and in the planning of studies. Exchange of information between the countries is arranged by the Regional Office through correspondence and by conducting review meetings. These review meetings are planned on a bi-annual basis.

Training

National training has been stepped up in Indonesia with the assistance of a WHO team. This has enabled the country to expand the number of monitoring teams. In India further training was postponed for various reasons, but a priority programme is being developed to introduce the micro in vitro technique. An increase of the monitoring teams from 6 to 12 has been envisaged.
Distribution of Chloroquine-Resistant
*Plasmodium falciparum* in Asia and Australasia.
Status on 31 December 1981.
Distribution of Chloroquine-Resistant Plasmodium falciparum in South America.
Status on 31 December 1981.
A regional workshop was held in Prabhudhabat, Thailand, in March 1981 with the participation of technical officers from all seven participating countries. During this course the participants were introduced to the micro in vitro technique.

At present the Region counts 25 teams for the assessment and monitoring of drug sensitivity of \textit{P. falciparum}, namely six teams each in India and Indonesia, five in Thailand, four in Bangladesh, two in Burma and one each in Nepal and Sri Lanka.

Cooperation was extended to the training of scientists from Indonesia and Sri Lanka in the techniques of continuous \textit{in vitro} cultivation of \textit{P. falciparum}. The training was conducted in Bangkok, Thailand.

Recording and Reporting

The new computer form prepared by the Malaria Action Programme, WHO, Geneva, has been introduced into the Collaborative Studies. Data and information obtained from this reporting system will be utilized in the global monitoring programme.

WESTERN PACIFIC REGION

General

In the Western Pacific Region a reliable malaria prophylactic regimen is no longer on hand and a widely effective radical treatment for a \textit{P. falciparum} infection has become nearly impossible to prescribe. Such a situation is also being found in areas where other methods of malaria control have serious operational or technical limitations. As a result, attempts to contain the present problem of drug resistance through vector control are bound to fail at least in a number of areas.

Resistance to 4-aminooquinolines has now been observed in all countries of the Region where \textit{P. falciparum} is endemic; imported resistant cases have been reported from Australia, Japan and Singapore, where malaria is no longer endemic. It appears that chloroquine resistance started in this part of the world in the early 1960s in the Kampuchea/Thai border area. It remains unclear whether resistance spread from this focus over the Indochinese peninsula or whether new foci occurred spontaneously. Biological differences of the resistant isolates identified in the area may point to the latter. Resistance in Peninsular Malaysia is thought to have resulted from direct spread from either Thailand or Viet Nam; that in Sabah from Pensinsular Malaysia or the East Kalimantan focus in Indonesia. Resistance in Sarawak is clearly imported from either Sabah or East Kalimantan; that in the Philippines is less easily explained as imported although there
are signs to suggest this origin. Resistance in Papua New Guinea is clearly caused by importation from Irian Jaya, the subsequent spread into the Solomon Islands by importation from Papua New Guinea, and the spread into Vanuatu originated obviously from either Papua New Guinea or the Solomon Islands.

It may be noted that resistance to 4-aminoquinolines occurred in several countries of the Region originally as a resistance to amodiaquine rather than to chloroquine. The reported difference in the response to these drugs appears to have, therefore, little practical significance insofar as the Western Pacific Region is concerned.

The continued massive use of 4-aminoquinolines for presumptive treatment, clinical cure or mass drug distribution has undoubtedly contributed to the rapid dissemination of resistance once a resistant strain has been introduced. While due caution regarding the projection of results in rodent malaria to human malaria is required, it appears that chloroquine resistance is of a very stable nature. Genetic studies carried out in Edinburgh and epidemiological data on *P. falciparum* seem to indicate that resistant mutants have a distinct biological advantage over sensitive parasites. The former are stable and also overgrow the latter. This seems to be borne out by the epidemiological observations in the Western Pacific Region and it would indeed be useful to carry out observations in an isolated confined area (island) with established chloroquine resistance and see whether and when the parasite would revert to a sensitive state upon the withdrawal of all 4-aminoquinolines.

There are indications from several countries in the Region that a continuous high degree of transmission has been of greater importance than drug pressure for the ultimate spread of resistance.

Other specific questions which need to be answered in relation to the present situation, arise from our lack of knowledge about the continued sensitivity of *P. falciparum* to alternative drugs including quinine and combinations such as quinine/sulfadoxine/pyrimethamine and chloroquine/sulfadoxine/pyrimethamine. At the same time the preferential susceptibility or refractoriness of vectors to chloroquine-resistant strains of *P. falciparum* deserves attention, particularly in the members of the *Anopheles punctulatus* complex, *A. sinensis* and *A. balabacensis* complex.

Failures with combinations of DHFR inhibitors and sulfonamides have been reported in small numbers from most countries in the Region. The phenomenon appears to be rather common in parts of Kampuchea.
Objectives of the Western Pacific Regional Collaborative Studies

The objectives listed below are those that were defined at the Regional Workshop held in WPRO in May/June 1978 and attended by participants from six countries of the Region: Australia, Malaysia, Papua-New Guinea, Philippines and Socialist Republic of Viet Nam.

(a) assessment of the response in vivo and in vitro of *P. falciparum* to chloroquine to (i) establish the geographical distribution in the Region of chloroquine-resistant *P. falciparum*, preparing maps to show its baseline response in countries of the Region, and (ii) monitor the spread, frequency and degree of chloroquine resistance in *P. falciparum* to assess any changes in the situation and, to this purpose, undertake a longitudinal study particularly in areas where transmission of malaria continues or is increasing and in areas where *P. falciparum* is still sensitive to chloroquine and which are adjacent to areas where chloroquine resistance is known to occur;

(b) the evaluation and promotion of vector control and of measures other than drug administration in an attempt to interrupt transmission especially in areas where drug resistance has been established and where chemoprophylaxis and treatment are only part of the answer to the resistance problem;

(c) establishment of baseline data on the response of *P. falciparum* to alternative drugs, particularly in areas where such drugs have been widely used against other diseases, by undertaking clinical studies to, for example, (i) establish the dosage of Fansidar required to produce a radical cure, (ii) make a comparison between sulfadoxine/pyrimethamine and sulfaflene/pyrimethamine, (iii) make a comparison between Fansidar and sulfamonomethoxine/pyrimethamine in controlled population groups, and (iv) test the combination of Fansidar/primaquine against Fansidar alone in mass drug administration, giving special attention to the effect of the combination in G-6-PD deficient subjects, and similarly test the dapsone/pyrimethamine combination;

(d) assessment of the sporontocidal effect of Fansidar and of other sulfonamide/pyrimethamine combinations in areas where the asexual blood forms are already resistant to these drugs;

(e) confirmation of the safety of the combined therapeutic administration of Fansidar and primaquine;

(f) determination of the minimum dosage of primaquine needed for a gametocytocidal/sporontocidal effect, i.e. a single dose of 30 mg primaquine versus 45 mg;

(g) elucidation of the effect of chloroquine pressure on the spread of chloroquine resistance;
(h) specialized investigation of the pharmacokinetics, pharmacodynamics and side effects of specific drugs with a view to optimizing drug dosage and regimen.

Progress, Baseline Studies and Monitoring

Following the workshop on drug-resistant malaria held in Manila in May 1978, Contractual Technical Services (CTS) agreements were signed between the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) and several institutions in the Region during 1979/1980 for the assessment and monitoring of drug resistance, in particular the response of *P. falciparum* to 4-aminoquinolines. The studies carried out to date are summarized below.

(a) Australia. Malaria was eradicated several years ago. Of the 628 cases of malaria reported in 1980, nearly all were imported (625, with one death) 2 were introduced and there was one relapsing introduced case. The majority of cases (529) were due to *P. vivax*; 89 were *P. falciparum* infections. Since all *P. falciparum* cases were treated with quinine/sulfadoxine/pyrimethamine, the number of chloroquine-resistant infections is unknown. The Australian Army Malaria Research Unit carried out macro in vitro tests on 7 cases from various hospitals: 2 were sensitive to chloroquine, 3 resistant and 2 tests failed.

(b) China. In 1980 some 3.3 million cases of malaria were reported, among which 12,867 were due to *P. falciparum*. All *falciparum* malaria cases came from the southern provinces of Guizhou, Hunan, Guangxi, Guangdong and Yunnan. In Guangdong Province most cases are confined to Hainan Island. Chloroquine resistance has been detected in border areas in Yunnan, the southern part of Guangxi Autonomous Region and the hill areas of Hainan Island. Of 396 cases tested in vivo between 1973 and 1980, 124 were sensitive and 272 resistant. Of 982 cases followed up for 7 days only, 258 were resistant (RII, RIII).

In vitro tests using microplates provided by WHO and plates from the Institute of Parasitic Diseases in Shanghai showed no difference in growth. Chloroquine resistance in Hainan was confirmed; resistance to pyrimethamine was also observed in this area.

Pyronaridine and Qinghaosu are used for the treatment of chloroquine resistant *P. falciparum* and complicated *falciparum* malaria. Piperaquine (13228 R.P.) is being used for chemoprophylaxis and treatment of uncomplicated cases. While piperaquine is currently in general production, pyronaridine and Qinghaosu and its derivative 804-Na are in trial production only.

(c) Lao People's Democratic Republic. Chloroquine
resistance has been known since RI type resistance was reported among expatriates working at the Nam Ngum dam in 1970. Later, in vivo studies by the antimalaria programme confirmed the existence of chloroquine-resistant strains, apparently sensitive to sulfadoxine/pyrimethamine, among a limited number of people examined in Vientiane. In a recent study undertaken in the Vientiane area all of the 12 successful in vitro tests showed chloroquine resistance, 2 at $1.5 \times 10^{-6} \text{M/l}$ and 5 each at $3.0 \times 10^{-6} \text{M/l}$ and at high concentrations. Simultaneous in vivo tests in the 10 patients who could be followed showed 4 cases of RI, 5 of RII and one of RIII resistance. Limitations in staff restricted the geographical coverage.

(d) Malaysia. Among the total of 9110 cases of malaria reported from Peninsular Malaysia in 1980, 7000 were due to *P.falciparum*. In vitro testing was recently started by the malaria eradication programme in Kelantan: one successful macro test showed sensitivity; three micro tests showed all resistant cases. In Sabah the annual number of malaria cases is estimated at around 40 000, over 75% of which are due to *P.falciparum*. Chloroquine resistance has been known since 1970 when it was demonstrated by the malaria control programme in the Keningau, Tenom and Beaufort districts by in vivo sensitivity testing. Further in vivo studies in 1973-1975 showed resistant strains in Tawau, Labuan and Papar. Since 1978, in vitro tests have shown chloroquine resistance to be widespread throughout the State. Of 135 successful macro tests, 109 showed chloroquine resistance, 14 being fully inhibited at $1.5 - 2.0 \times 10^{-6} \text{M/l}$, 24 at $2.0 - 3.0 \times 10^{-6} \text{M/l}$ and 71 at more than $3.0 \times 10^{-6} \text{M/l}$. Of 23 micro tests performed in 1981 only 2 were successful, the failure being ascribed to faulty growth medium. Drug sensitivity tests have so far been carried out in 11 of the 23 districts of Sabah. Malaria in Sarawak appears to be on the decrease. Among 765 cases reported in 1980, 235 were due to *P.falciparum*, mostly imported and introduced cases. The 1981 figure is expected to be even lower. In 1978, in limited foci, 16 cases were tested in vivo, 8 showing RI and 8 RII response to chloroquine. Five macro tests were performed in 1979: 3 of these failed and 2 showed sensitivity to chloroquine. Among 6 in vivo tests carried out in 1980, there were 4 sensitive and 2 resistant responses. One of the latter was an RI originally from local transmission, the other an RIII imported from Vietnam. Of 5 in vitro tests 2 were sensitive, 2 resistant and there was one failure. Findings in Sarawak reflect closely the situations in Sabah and parts of Kalimantan, Indonesia.

(e) Papua New Guinea. Sensitivity testing is carried out by the Malaria Control Programme and the Institute of Medical Research. The first cases of chloroquine resistance were found in 1975 in the western part of the country bordering Irian Jaya, Indonesia. By 1977 chloroquine resistance had been reported from Western Province, West Sepik and Madang. From 1978 a systematic
TABLE 3. Comparison of in vivo and in vitro Results of Chloroquine Sensitivity Testing, Papua-New Guinea

<table>
<thead>
<tr>
<th>In vitro tests (critical concentration in nanomols)</th>
<th>In vivo results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Children below 10 years of age (87)</td>
</tr>
<tr>
<td></td>
<td>S</td>
</tr>
<tr>
<td>Less than 1.0</td>
<td>28</td>
</tr>
<tr>
<td>1.0 or 1.25</td>
<td>4</td>
</tr>
<tr>
<td>1.5 and above</td>
<td>7</td>
</tr>
</tbody>
</table>

study was undertaken using both in vivo and macro in vitro methods. It showed chloroquine resistance to be geographically widespread throughout most of the country and at all clinical levels. Among 226 parallel tests carried out, 111 showed resistance in vivo and 115 a sensitive response, as against 143 cases of resistance in vitro, and 83 sensitive isolates. These results show a considerable degree of correlation between the two methods but with a number of "false" resistant cases in the in vitro tests. This finding may be at least in part explained by the high degree of immunity prevailing among the schoolchildren included in these studies, as there are indications that the discrepancy in the test results increased with age (see Table 3).

Of 32 cases tested in vitro only, 28 were found resistant; of 102 others tested in vivo only, 56 were found resistant. In 1981, 33 tests conducted concurrently in Central Province showed 28 cases resistant in vitro against 20 in vivo. Twenty others tested in vitro only, were all resistant; 8 others tested in vivo only, showed 4 sensitive and 4 resistant (RI) responses to chloroquine.

(f) Philippines. Investigations started in 1974 and were conducted by the Central Headquarters team, Manila, using comparative in vivo and in vitro tests on the same infections in preliminary investigations. Patients were residents of Manila, where malaria transmission is almost unknown. A total of 27 in vitro and in vivo tests on the same infections were carried out and an in vivo 28-day follow-up completed in the absence of
transmission. The in vivo tests revealed 13 sensitive and 14 resistant responses to chloroquine. Among the resistant infections, 13 gave an RI response with remission periods varying from Day 13 to Day 28, while one case was a clear RIII response. Among the 13 cases in which the in vivo response indicated chloroquine sensitivity, the corresponding in vitro tests showed a complete inhibition of schizont maturation at the 0.25 n-mol concentration (0.25 x 10^{-6} M/1) 0.5 n-mol (0.5 x 10^{-6} M/1) in 2 tests at 0.75 n-mols (0.75 x 10^{-6} M/1) in 4 tests, and at 1.0 n-mol (1.0 x 10^{-6} M/1) in 2 tests. In one further test schizonts were present at the 1.0 n-mol (1.0 x 10^{-6} M/1) concentration. From the above results, it was concluded that isolates which showed complete inhibition of schizont maturation up to the 1.0 n-mol (1.0 x 10^{-6} M/1) concentration were sensitive to standard dosages of chloroquine in vivo. Those with schizont maturation at or above the 1.0 n-mol (1.0 x 10^{-6} M/1) concentration were considered to be resistant. The comparative in vivo and in vitro tests showed that a close correlation exists between the two, permitting the use of the in vitro test alone for assessing the presence and extent of chloroquine resistance throughout the country.

Since 1974 a total of 1159 macro in vitro tests have been performed. Of the 742 successful tests, 393 have shown resistance, with the highest proportion of resistant cases in the southern provinces, where 70 to 80% resistance occurs in Palawan. Limited attempts made in 1981 with the micro test have remained so far unsuccessful.

(g) Singapore. Malaria has been eradicated from Singapore since 1975. In 1980 there were 195 imported and 5 introduced cases, predominantly due to P. vivax and originating in India and Indonesia. Chloroquine-resistant P. falciparum cases have been reported originating from Burma, Indonesia and Thailand (in vivo tests only). These were treated successfully with a combination of quinine, sulfadoxine and pyrimethamine.

(h) Solomon Islands. The first clinical reports suggesting chloroquine resistance dated from 1978. In 1980, with the cooperation of the Australian Army Malaria Research Unit, a special team began its investigations and obtained the following results: out of 157 in vitro tests performed 37 failed, 47 showed sensitivity and 73 resistance to chloroquine. Of 62 in vivo tests performed, 54 showed sensitivity and 8 resistance. Although one case in Malaita proved to be resistant, most of the resistance appeared to be restricted to Western Province, bordering Papua New Guinea. In 1981 a remarkable shift towards P. falciparum had occurred in the parasite formula and chloroquine resistance proved to be well established in Guadalcanal (9 resistant against 5 sensitive cases) and Malaita (33 resistant against 17 sensitive cases) besides Western Province where 90% of cases tested proved to be resistant to chloroquine.
Of 22 mefloquine in vitro tests performed in 1980, 6 failed, 13 showed full sensitivity but 3 showed schizogony at 1.5 nanomol (1.5 \( \times 10^{-6} \text{M/1} \)) concentration. (Note: This is still below the plasma levels prevailing during the first 10 days after the administration of a 1g mefloquine base dose.)

(i) Vanuatu. One in vitro macro test carried out by the Australian Army Malaria Research Unit in Australia in 1980, showed resistance at 2.0 nanomols (2.0 \( \times 10^{-6} \text{M/1} \)) concentration. The infection originated in the tiny but fairly malarious island of Aoba in the northern part of the archipelago. Studies undertaken with WHO cooperation of suspected cases in Aoba and Efate islands confirmed 2 resistant cases in vivo (RI) and 3 in vitro (schizogony at 1.5 nanomols = 1.5 \( \times 10^{-6} \text{M/1} \)). Other suspected cases have been reported from the islands of Pentecost and Santo, near Aoba, where \( P. falciparum \) is thought to have recently become predominant over \( P. vivax \).

(j) Viet Nam. Strains of \( P. falciparum \) resistant to 4-aminoquinolines were first recognized in 1963. The distribution remained very much restricted to the malarious areas of the southern provinces. The frequency of chloroquine resistance varied between 40 and 50%, and had soon reached 80 to 90% in some areas. In 1966 the first cases were noted in the northern provinces, where the occurrence of \( P. falciparum \) remained restricted to limited foci.

Prior to 1980 a locally developed in vitro test kit was used. Since 1980 in vitro tests have been performed using the WHO macro test kit. Among 288 tests performed, 179 were successful, 151 showing resistance. Of 14 in vivo and in vitro tests run in parallel, all showed resistance in vitro but 2 indicated sensitivity in vivo; 5 gave RI and 7 RII responses. A comparison between quinine and chloroquine in 53 tests showed 11 to be sensitive to chloroquine and 34 to quinine, all others being resistant at various levels.

Other Activities

The Malaria Eradication Services of the Philippines started the global production of the macro in vitro test kit in 1979. Quality control is carried out by the Communicable Disease Centers, Atlanta, USA; The WHO Western Pacific Regional Office is handling the overseas shipment of kits.

The Sabah Health Services, the University of Malaya and the Philippine Malaria Eradication Service are cooperating with WHO and TDR in the development of a micro in vitro test kit, the production of which is expected to start in 1982.
Training

Following the introduction of the in vivo and macro in vitro testing techniques at an interregional seminar conducted at the Institute for Medical Research in Kuala Lumpur in 1975, the techniques were demonstrated and discussed in detail with principal investigators at the regional workshop on drug-resistant malaria in Manila in 1978. With the cooperation of WHO country and regional staff, senior laboratory technicians of Australia, Papua New Guinea, Philippines and Sabah, Malaysia, were trained in the techniques on an ad hoc basis. A formal course was organized in 1979 for technicians from Peninsular Malaysia, Sarawak and Singapore. The Australian Army Malaria Research Unit was instrumental in the training of technicians from the Solomon Islands.

Nationally and/or internationally trained staff is currently available in Australia (5), China (exact number unknown), Lao People's Democratic Republic (7), Peninsular Malaysia (approximately 12), Sabah (7), Sarawak (3), Papua New Guinea (2), Philippines (6), Singapore (2), Solomon Islands (2) and Viet Nam (55). In some countries difficulties are experienced with the recruitment of suitable candidates for training.

The micro in vitro technique first demonstrated at the Manila workshop in 1978, was introduced in China during two courses in 1979. In 1980 WHO enabled a scientist of the Philippines to study the method in detail in Thailand. The technique will be introduced to the principal investigators and senior technicians during a regional workshop scheduled at the University of Malaya in Kuala Lumpur immediately after the conclusion of the meeting.

THE WESTWARD SPREAD OF DRUG-RESISTANT P.FALCIPARUM

In vivo Predictors

In vivo methods of testing drug resistance have rarely proved useful as an early warning system in Asia, not only because information from practitioners has mostly been erratic and unreliable, but also because 4-aminoquinolines, being a component of the eradication campaigns, were not assessed for minimal dose trophozoite clearance when they were introduced, and scientific comparisons of former and present dose effects cannot be made. However, by analogy with existing estimates of quinine sensitivity patterns in Asia, it may be assumed that strains of P.falciparum in Thailand and Indonesia were, prior to the emergence of resistance in the late 1950s, sensitive but not hypersensitive to chloroquine, whereas those in India and Sri Lanka were hypersensitive.

In areas adjacent to foci of resistance, epidemiological and
operational conditions govern further spread and must be taken into consideration in attempting to forecast such spread. Where falciparum malaria is being contained and reduced by measures directed against transmission, namely anti-vector and anti-gametocyte measures, then it may be assumed that drug-sensitive and resistant strains will be equally inhibited. In contrast, where falciparum malaria is spreading, there is every indication that chloroquine-sensitive strains are gradually being replaced by chloroquine-resistant strains. Progressive movement of resistant parasites is historically apparent in South-East Asia, as they have spread during the past 20-25 years through much of the Region from their point of emergence at the Thai-Kampuchean border.

Trends in the Spread of Resistance Westwards in Asia

It is reasonable to project this historical spread beyond 1980 along the lines of flow that may be expected on the basis of general increases in *P. falciparum* incidence in receptive areas, particularly those visited by migrants carrying resistant gametocytes. In the past two years resistant strains of *P. falciparum* have continued to replace sensitive strains in Burma, eastern Bangladesh and north-east India; foci have been identified more or less sequentially across central India (see page 14) and this progression may be expected to continue in the direction of Rajasthan and Pakistan rather than southwards where the prevalence of *P. falciparum* is slight.

Similarly, the degree of resistance has increased, with a shift occurring from RI towards RIII as resistant strains become more prevalent. It appears that the periphery of a zone of resistance is composed of RI and S grades, both of which are carried to the adjacent susceptible-grade zones where they hybridize with the indigenous S-strains and, if conditions are favourable, give rise to RI foci. Meanwhile, the central part of a zone of resistance will have lost its S-strains through hybridization or overgrowth, and continual hybridization among the concentrating R gametocytes progressively shifts the grade towards RIII. Intermediate zones between the periphery and the centre are occupied mainly by RI and some RII, with a small residue of S on the verge of extinction. Such free hybridization occurs because several strains of parasite may coexist in an individual host, all producing gametocytes picked up together by a feeding mosquito.

**In vitro Predictors**

In vitro testing affords a more comprehensive measurement of the spread and degree of resistance than in vivo testing. If on the periphery of the advancing wave of resistance there are areas where the degree of susceptibility of the parasite is decreasing, but has not yet reached RI, this should be identifiable by in vivo
or in vitro testing. The changing status of the parasite from the peripheral ("RI") to the central ("RIII") area may similarly be observed. However, in vitro methods provide better resistance predictors, since the particular studies may be set up more adequately to satisfy statistical prerequisites of sample size and randomization.

Analysis of the small number of in vitro tests that have so far been done in Asia confirms long-standing in vivo indications of the westward trend of chloroquine resistance. While at present more information is available from macro than from micro testing, the greater operational convenience of the latter promises to provide more comprehensive data. Recent macro in vitro results from individuals in Thailand, Burma, Bangladesh, India and Sri Lanka have been grouped by study locale, and weighted means calculated from the maturation percentages that occur at the discriminating dose levels of 0.25, 0.75, 1.25 and 3.0 nanomol (0.25; 0.75; 1.25; 3.0 x 10^-6 M/l). These weighed means show in a single figure the degree of parasite susceptibility in an area. Prediction of development of or increase in level of resistance may be obtained by charting the geographical flow of resistance into receptive areas, or from repetitive studies in fixed foci.

The geographical range of resistance in South-East Asia in 1978-1980, in terms of these weighted means, diminishes towards the west, from the high levels of 70 to 97 observed throughout Thailand, through the somewhat lower levels around 65 in Burma and 27 to 63 in Bangladesh and north-east India, to a borderline S/RI situation across central India where the means range from 23 in Orissa westwards to 5 in Gujarat. In Sri Lanka in 1980 the mean was 4, indicating hypersensitivity. In some areas of Thailand, tests carried out in 1977 gave weighted means of 30 to 45; by 1980 in nearby, epidemiologically similar places the weighted means had reached values of 85 to 91.

In order to indicate the direction of flow of chloroquine-resistant P. falciparum, and determine changes in the degree of resistance, it is necessary to increase geographical coverage of in vitro testing operations and design and to undertake repetitive testing in selected indicator areas. Only with such information will the rational planning and deployment of appropriate containment measures be possible.

The model propounded above depends essentially on the application of Mendel's law, given R and S. It is therefore somewhat surprising that RIII is relatively rarely seen while RI and RII responses seem to remain more prevalent even in the oldest known foci. This observation is not readily explained although some of the reasons could be differences in the biological advantages between moderately and highly resistant parasites and
the possible influence of prevailing immunity in the populations tested. In this connexion, further detailed studies are apparently required to specify the immunogenic differences, if any, between resistant and sensitive P.falciparum strains, and to investigate the biological characteristics of P.falciparum in relation to the degree of resistance.

AMERICAN REGION

General

In the American Region falciparum malaria is a major problem in 13 countries with a population of approximately 141 million, where 185,068 cases were reported in 1980. Factors like multi-resistant vectors to insecticides in Central America and serious problems related to migration, new or shifting settlements, mining, prospecting, pastoral or agricultural activities, construction of dams and hydroelectric power stations, are impeding or hindering the control of falciparum malaria.

Presumptive treatment is widely applied, using approximately 10 mg/kg chloroquine or amodiaquine base and in some countries a single dose of 0.75 mg/kg primaquine base is also added in order to curb transmission.

Radical cure of P.falciparum infections is obtained with ± 25 mg/kg chloroquine or amodiaquine three days together with 0.75 mg/kg primaquine in a single dose or divided over three days.

After the advent of chloroquine resistance a combined 1.25 mg/kg pyrimethamine plus 25 mg/kg sulfadoxine treatment has been used in South American countries since the mid-1960s when the cure rate was close to 100%. Recently, the efficacy of this combination has decreased by probably 30-50%.

Mass drug administration in large-scale projects has been difficult to evaluate. In small foci different drugs have proved to be very useful in mass drug administration.

In vivo and in vitro tests performed between 1959 and 1981 suggest that countries of North and Central America and the Caribbean have so far only chloroquine-susceptible P.falciparum, while chloroquine-resistant parasites are prevalent in South American countries.

Control of Drug-Resistant P.falciparum

Since the recognition of the problem of drug resistance, efforts have been made for the establishment of a system to assess it in the field. Up to the late 1970s this was mainly pursued
through the confirmation of field reports. However, this approach proved to be inadequate for fully assessing the distribution and the spread of drug resistance in South America. Therefore, an urgent need was felt to strengthen the regional monitoring capability. With the collaboration of TDR and the Gorgas Memorial Laboratory in Panama a plan for the study for *P. falciparum* susceptibility in the region was developed in 1977/1978 which includes the following activities:

(a) Training in susceptibility testing in vitro and in vivo. Three courses were given in 1978 in Brazil, Colombia and El Salvador and two in 1980 in Brazil and Colombia.

(b) Participation in the global programme for the monitoring of drug sensitivity in *P. falciparum*.

(c) Collaboration with national malaria and specialized institutes in the field evaluation of alternative standard antimalarial drugs and of new drugs.

(d) Establishment of a surveillance system designed to study the epidemiology of drug-resistant *P. falciparum*.

(e) Containment of drug-resistant *P. falciparum* and its spread.

This new system should be capable of monitoring drug sensitivity throughout the Americas, of containing resistant *P. falciparum* in South America, and of preventing its spread in Central America and the Caribbean. It should also be conducive to regulating the use of new drugs and those already existing.

The above-mentioned programme has completed the primary training activities and is in the process of consolidating the establishment of its national components.

The revised "Continental Plan of Action against Malaria" considers that the study of drug resistance is an integral part of the study of the epidemiology of malaria. It is therefore an important responsibility of the national antimalaria programmes.
IV.

Monitoring of Drug Response of *P. falciparum*

**TEST SYSTEMS, RECENT EXPERIENCES**

The main effort in the testing of antimalarial drugs is currently centred on the blood schizontocidal drugs, and techniques are available for assessing them by *in vivo* and *in vitro* methods.

The *in vivo* method assesses the results of a standard radical cure treatment on plasmodial infections over a period of 7 days - the WHO Standard Field Test, - or over a period of 28 days - the WHO Extended Field Test. The standard test provides an assessment of resistance at the RII and RIII levels; RI resistance can only be detected with the extended 28-day test.

The development of an *in vitro* test system permits a truly objective assessment of the sensitivity of *P. falciparum* to various blood schizontocidal drugs. The *in vitro* test systems currently available (all limited to *P. falciparum*) are: a macro culture technique in which glucose supported defribinated blood samples are incubated at approximately 38 °C over 24 hours; a micro culture technique using culture medium to support very small quantities of heparinized patient-blood at similar incubation conditions; and *in vitro* tests using material from continuous culture.

The macro *in vitro* technique is simple and relatively easy to carry out under field conditions. The simplicity and long-term dependability of the test may make its continued use desirable in hospitals, for instance, where blood collection by venipuncture is not a problem and when only a few tests are required during a year.
The micro in vitro test permits a wider application in field studies. The technology involved is definitely more demanding than that of the macro-test but the method has been successfully utilized as a field test and has proved to be reliable under extreme conditions of application. The test is more readily adapted to mass surveys than the macro-test and the testing of 20 or more patients per day is easily achieved when sufficient positive cases are available.

The comparison of the results of macro and micro tests demands that the two test systems be reduced to compatible terms: e.g. chloroquine concentration as multiples of $10^{-6}$ mol/litre blood.

Test systems using continuous in vitro culture have application in laboratories with high levels of expertise and competence in handling culture material. They are widely used in experimental and screening work with new drugs and formulations.

A 48-hour test, as it is known, has been developed on the basis of a continuous culture system but it has been adapted for use with fresh parasite material from patients. It has been successfully tried in the field and opens up distinct possibilities for testing with DHFR inhibitors (pyrimethamine, proguanil). This test system requires further validation in the field.

Measurement of *P. falciparum* Response to Chloroquine In vivo

An impressive number of in vivo tests have been performed in recent years (see pages 14 & 22).

Advantages and disadvantages of the method are well known. With a 7-day follow-up period, it is not possible to distinguish between sensitivity and RI type responses. Extension of the observation period to 28 days is generally difficult and expensive in terms of money and manpower. Moreover, in areas with ongoing malaria transmission it is difficult to distinguish between recrudescences and re-infections. There is considerable evidence from Papua New Guinea and other areas of the world that a substantial proportion of recrudescences may occur beyond Day 28, even up to Day 45, possibly related to the high degree of immunity in the persons tested. Counting these late recurrences as genuine recrudescences would reduce but not completely remove the discrepancy between the results obtained in simultaneously conducted in vivo and in vitro tests.

In vitro Test, Macro System, 4-Aminoquinolines

A considerable number of macro in vitro tests have been carried out in the South-East Asia and Western Pacific Regions
during recent years (see pages 14 & 22) especially in Papua New Guinea, the Philippines, Thailand and Viet Nam.

Problems

Prior antimalarial treatment of the patients and failure of the electrical power supply, even where portable generators were used, rank highest among the problems encountered with the method. Other difficulties pertain to the scarcity of suitable cases (trophozoites too young, parasite densities too low or too high), the need for the blood sample to be taken by venipuncture, the scarcity of qualified manpower, the cost of travel and of fuel for the generators as well as bacterial contamination and the failure of equipment other than the generators. In several instances the exact cause of test failure could not be determined. Of a total of 1588 tests carried out during 1978-1980 in various countries of the Regions from which such information is available, 540 (34%) have failed. There was no significant improvement in this rate over the years, suggesting that this failure rate is more or less inherent to the technique.

Delays in obtaining government agreement and signatures on the CTS agreements were a major obstacle to the implementation of planned activities. While availability and despatch of testing material have been quite satisfactory, long delays in clearing at air and sea ports have further hampered progress in a number of instances.

Technical Suggestions

(a) For defibrination, the McCartney bottle of 25 ml capacity is preferable to the easily breakable Erlenmeyer flasks.

(b) For the same reason sterile plastic disposable pipettes of 1 ml capacity should replace the glass pipettes.

(c) Fluctuations of electric power and portability and cost of mobile power sources proved to pose major technical difficulties. A suitable incubator or waterbath operated from a rechargeable battery would probably resolve some of these problems.

(d) Temperature fluctuations during incubation should be monitored routinely at least with a maximum/minimum thermometer. An electric clock wired into the incubator/waterbath circuit should be used to indicate the duration of power failure.

(e) The correlation between the age of the ring stages and the time required for schizont maturation in the control vials should be studied in order to provide guidelines for optimum incubation.

(f) The definition of a "successful" in vitro sensitivity
test needs to be determined. Based on the minimum number of schizonts found in the controls the WHO Regional Office for South-East Asia considers 15 schizonts per 300 white blood cells as a criterion while WHO Headquarters stipulate a minimum of 20 schizonts (related to 300 or, if required, 1000 leukocytes). At present, many workers use lower numbers particularly with lower parasite concentrations. In these cases it is always indicated to count the schizonts on the basis of 1000 white blood cells.

(g) As a rule cases are accepted for macro in vitro testing if the parasitaemia is between 400 or 100 000/µl. Both figures seem to be somewhat high, as tests with lower parasite densities are frequently successful and tests with densities at the higher range tend to fail. In spite of the relatively high failure rate of 50-100 000 parasites/µl it is suggested to maintain the critical upper level at 100 000 parasites/µl, but the lower critical level should be fixed at 200 parasites/µl. If good schizont maturation is achieved at this density, the test would still produce valid results if it were read on the basis of 1000 leukocytes.

(h) Use of a portable ice pack carrier and the storage of wrapped bottles of defibrinated blood on wet ice for at least two hours and up to 48 hours before incubation seem to enhance the chances for successful growth. This observation appears to contrast somewhat with the experience gained in short-term storage (up to two hours) of parasitized blood which is best carried out at temperatures between 25 and 35 °C. Storage on wet ice is the method of choice when samples have to be transported over great distances and/or relatively long periods.

(i) Staining with a 1% to 2% Giemsa solution at a pH of 6.8 for 30 minutes appears to yield better results than the saline Giemsa technique and the standard Giemsa staining method at pH 7.2.

(j) Information on test methods and their modification takes a long time to reach the field workers. Apart from securing rapid collection of field information and its dissemination to member countries, the WHO Regional Offices may provide relevant technical data on a regular basis through a newsletter or another appropriate medium.

In vitro Test, Micro System, 4-Aminoquinolines

Experiences in the South-East Asia Region

The micro in vitro test system is being developed through the coordinated efforts of various field workers and institutions in collaboration with the Research and Technical Intelligence Unit, Malaria Action Programme, WHO, Geneva. A South-East Asia Regional programme has been initiated to familiarize the monitoring teams with the performance of the micro test. Teams in Burma, Indonesia,
India, Sri Lanka and Thailand have already been given this introduction to the technique. The Region has also conducted a workshop on the micro in vitro technique that was attended by technical officers from all seven countries participating in the Regional Collaborative Studies.

A. Material

Sterile tissue culture plates were pre-dosed with chloroquine in Bangkok or WHO, Geneva. Well A was left untreated as the control and wells B-H dosed with 1 to 32 p.mol chloroquine, corresponding to 0.2 - 6.4 mol/l blood. Plates were stored at 4 °C until taken to the field areas.

RPMI 1640 medium was pre-weighed in lots of 260 mg and placed in sterile containers. Hepes buffer/gentamycin and sodium bicarbonate were used as sterile stock solutions. In the field, after dissolving 260 mg RPMI 1640 in 23 ml sterile double distilled water (injection ampoules), and adding 1 ml each of the Hepes gentamycin and sodium bicarbonate stock solutions, the resulting medium was passed through a sterile Millipore filter (0.2μm) into 5 ml plastic holding vials. These vials were stored at 4 °C or in a thermos flask containing wet ice until required; normally the medium was discarded if not used within 3-4 days.

B. Method

Screening for cases was conducted at the village level and in hospitals, health centres or malaria clinics. Cases were selected on the basis of parasite counts of 1000 - 60 000 per μl of blood and of negative urine tests for 4-aminquinolines and sulfonamides. From each case three thick blood films were prepared for pre-culture counting.

Using a sterile disposable tuberculin syringe, 0.9 ml of the prepared medium was transferred from the holding vial to a 2 ml sterile plastic vial, one vial for each selected patient. By finger/toe prick 100 μl of blood was drawn using a sterile heparin-coated 100 μl capillary tube and ejected into the vial containing the 0.9 ml medium. Thus 1 ml of medium-blood suspension was obtained. Defibrinated blood may be used when the micro test is conducted together with the macro in vitro test.

The vials containing the medium-blood mixture were conveyed in a slotted box to the field laboratory for processing, usually within three hours. In the laboratory, after ensuring that the blood was well suspended in the medium, 50 μl of the suspension was transferred from the vial to each well of the test line on the microtitre plate using a fixed-volume Eppendorf pipette starting with well A and descending to well H. The microtitre plate can be used for 12 tests. Whenever possible an effort was made to assess the age of the trophozoites on the pre-culture slides before
distributing the medium-blood mixture to the plate. When conducting several tests simultaneously, those showing a majority of small rings and those with a majority of large rings were placed on separate plates. This separation is most important for choosing an optimal incubation period.

Each column of the test plate representing a single test series requires a total of 400 μl medium-blood mixture, leaving a balance of 600 μl. This amount is amply sufficient for challenging the parasites against another drug, e.g. amodiaquine, quinine or mefloquine.

After setting up the micro cultures the plates were placed in a candle jar. This was incubated at 38.5 °C in a dry incubator or submerged in a waterbath. Incubation continued for 24-28 hours. Using 20 μl capillary tubes the supernatant was removed from each well and blood films prepared. After a 48-hour drying period the blood films were stained with 1.5% Giemsa, pH 7.1, for 30 minutes. Schizonts were counted against 200 asexual parasites and findings in the drug wells expressed as a percentage of control maturation.

C. Results

The studies were carried out in areas of known chloroquine resistance such as East Kalimantan, Indonesia, and Chantaburi, Thailand, and in areas where P. falciparum was considered to be sensitive such as Orissa, India; Puttalum, Sri Lanka; and Central Java, Indonesia.

Values of ED50, ED90, ED95, ED99, and ED99.9, i.e. the doses effecting a 50%, 90%, 95%, 99% and 99.9% reduction of schizont formation are given in Table 4.

From the information obtained, it may be noted that the micro in vitro tests have reconfirmed the presence of resistance to chloroquine in the province of East Kalimantan, Indonesia, and in Chantaburi, Thailand. The tests have also shown the presence of resistant strains in the areas of Orissa, India, and Central Java, Indonesia, where P. falciparum had previously been considered to be sensitive to chloroquine. The results from these two areas were correlated with and reconfirmed through in vivo and macro in vitro tests. Strains in Puttalum, Sri Lanka, were confirmed to be sensitive to chloroquine.

D. Summary

The in vitro micro technique, modified as described, can be performed by trained technicians. The technique requires only a small quantity of blood that is obtained from the conventional finger prick or toe prick. Limitations as experienced in the macro in vitro technique are greatly reduced. With the use of
Table 4. Results of Micro In Vitro Tests (Chloroquine/P. Falciparum) in Various Areas of the South-East Asia Region*

<table>
<thead>
<tr>
<th>Location</th>
<th>ED$_{50}$</th>
<th>ED$_{90}$</th>
<th>ED$_{95}$</th>
<th>ED$_{99}$</th>
<th>ED$_{99.9}$</th>
<th>In vivo response in study area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Puttalum (Sri Lanka)</td>
<td>0.3</td>
<td>0.4</td>
<td>0.46</td>
<td>0.6</td>
<td>0.8 x 10$^{-6}$ M/I</td>
<td>S</td>
</tr>
<tr>
<td>Central Java (Indonesia)</td>
<td>1.14</td>
<td>2.4</td>
<td>2.8</td>
<td>4.0</td>
<td>6.4 x 10$^{-6}$ M/I</td>
<td>RI/RII</td>
</tr>
<tr>
<td>Oriissa (India)</td>
<td>1.0</td>
<td>2.4</td>
<td>3.0</td>
<td>6.2</td>
<td>&gt; 6.4 x 10$^{-6}$ M/I</td>
<td>RI/RII</td>
</tr>
<tr>
<td>East Kalimantan (Indonesia)</td>
<td>0.6</td>
<td>3.0</td>
<td>6.0</td>
<td>6.4</td>
<td>&gt; 6.4 x 10$^{-6}$ M/I</td>
<td>RI/RII</td>
</tr>
<tr>
<td>Burma</td>
<td>2.0</td>
<td>&gt; 6.4</td>
<td>&gt; 6.4</td>
<td>&gt; 6.4</td>
<td>&gt; 6.4 x 10$^{-6}$ M/I</td>
<td>RI/RII/RIII</td>
</tr>
<tr>
<td>Chantaburi (Thailand)</td>
<td>3.4</td>
<td>&gt; 6.4</td>
<td>&gt; 6.4</td>
<td>&gt; 6.4</td>
<td>&gt; 6.4 x 10$^{-6}$ M/I</td>
<td>RI/RIII</td>
</tr>
</tbody>
</table>

* In vitro results for chloroquine are expressed in M/I blood (not suspension!).

ED = effective dose for reducing shizont formation by 50%, 90%, 95%, 99% or 99.9%.
heparin-coated capillary tubes and pre-dosed plates the technique is relatively simple. The use of dry incubators and waterbaths produces comparable results, the incubator being ideal for use at a fixed station whereas the waterbath is more suitable for tests in the field.

The waterbaths supplied under the Regional Collaborative Studies require heavy generators as a power source. Although capable of producing good results, they have in any instances proved to be quite cumbersome to transport to field stations. Experience in Indonesia has shown that the inter-island transport of the generators, by air freight, adds considerably to the cost of the studies. The problem may possibly be solved by the introduction of miniature waterbaths, operated on a small portable generator or a 12 volt car battery. These waterbaths have yielded good results in Thailand and are at present being tested in Burma and Sri Lanka.

Monitoring of the sensitivity level of strains of P.falciparum to antimalarial drugs, using the in vitro micro technique, could now be considered as an operational component for inclusion in malaria programmes. The monitoring teams could with very little extra work produce data and information not only on the response of the parasites to chloroquine but also to other drugs already available or new drugs such as amodiaquine, quinine and mefloquine.

The use of various EDs (i.e. the effective doses required to inhibit schizont maturation at given degrees), could be a lead parameter for the interpretation of results, as it permits a quantitative comparison of baseline and monitoring data, and thus an analysis of the dynamics of drug response.

Experiences in the Western Pacific Region

Erratic results obtained with the micro in vitro test in early trials appear to have been associated with factors inherent in the plate material. These problems have now been solved.

In China, Malaysia and Papua New Guinea limited series of micro in vitro tests have been carried out successfully. Where this test is carried out simultaneously with the macro in vitro test on the same individuals, results obtained with the two systems showed a very close correlation.

Recent studies in Malaysia and the Philippines were less successful when freeze-dried formulations of the growth medium were used, indicating that some components of the test kit may require further selection before a fully standardized, reliable test kit can be brought on the market. The difficulties with the growth medium have recently been resolved through the introduction of factory-made minipacks of RPMI 1940.
The superiority of the micro test over the macro test is beyond doubt. However, it requires a greater skill of the technician because of the more refined techniques involved. But this does not appear to be a serious obstacle. The micro test will permit the screening of statistically valid samples of communities. This is virtually impossible with the macro test because of frequent failures, the need for venipuncture and the large proportion of material that has to be discarded on account of unsuitable trophozoite stages.

Tests for Alternative Drugs

Precise knowledge about the response of \textit{P. falciparum} to alternative drugs and drug combinations is patchy and for several countries not available at all.

There is an urgent need for a detailed study of the response to alternative drugs and combination of drugs \textit{in vivo} and, as far as adequate techniques are available, also \textit{in vitro}. Such \textit{in vitro} techniques do not yet exist for all of the widely used alternative drugs and their development deserves a high priority. \textit{In vitro} tests for sulfonamides and pyrimethamine using the occurrence of morphologically abnormal schizonts as an end point or measuring the inhibition of schizont maturation are currently under investigation.

Reports from Thailand suggest that the alarming increase in resistance to the sulfadoxine/pyrimethamine combination is in the process of spreading through population movements from the Thai-Kampuchean border to other parts of the country in a way similar to that which appears to have been instrumental in the earlier spread of chloroquine resistance. Regimens based on a 7-day course of quinine remained fortunately highly effective in the affected areas.

The priority given to the testing for chloroquine sensitivity and the difficulties involved in the simultaneous \textit{in vitro} assessment for both chloroquine and mefloquine sensitivity in the macro test (high blood volume) explain the very limited number of tests performed so far on the response of \textit{P.falciparum} to mefloquine. Early attempts undertaken in Sabah, Malaysia failed due to inadequate reagents. Among the 22 \textit{in vitro} tests performed with mefloquine in 1980 in Solomon Islands (see page 22) there were six failures and 16 successful tests. Of the latter, 12 cases proved to be sensitive to chloroquine and mefloquine; one chloroquine resistant case showed growth at 1.0 nanomol of mefloquine/ml (1.0 x 10^{-6} M/l), one chloroquine sensitive case at 1.5 nanomol mefloquine/ml (1.5 x 10^{-5} M/l) and two cases, one chloroquine sensitive, one chloroquine resistant, at 2.0 nanomol mefloquine/ml (2.0 x 10^{-5} M/l). From these results and similar preliminary experiences in Papua New Guinea and Thailand, it is
concluded that definite criteria for the interpretation of the sensitivity tests with mefloquine still need to be determined in correlation with in vivo observations. Appropriate studies could be undertaken in the framework of the current clinical trials of mefloquine in Brazil, Thailand and Zambia.

Measurement of Plasma Levels of Antimalarial Drugs

The measurement of the plasma levels of antimalarial drugs is of considerable importance, particularly in the investigation and interpretation of apparent discrepancies between the results of in vivo and in vitro tests. Besides the degree of parasite sensitivity to the specific drug and the influence of immunological host factors, the in vivo response may depend on individual variations in the plasma levels of the drug. It is realized that appropriate studies cannot be undertaken as a routine. But their performance in groups representative of ethnic origin and age class should be envisaged with all operational blood schizontocidal drugs, and in parallel with in vitro and in vivo observations.

Unless simplified techniques for the measurement of plasma levels of antimalarial drugs become available, this application will remain restricted to a limited number of adequately equipped institutions. Some of these specialized laboratories are willing to receive and to test samples from abroad. Only a relatively limited number of such samples could be processed. They should therefore be selected carefully and originate from a well designed study.

RECORDING, PROCESSING AND ANALYSIS OF RESULTS

A revised system of global monitoring supported by electronic data processing is currently being developed.

Record Forms

A precoded form for recording the results of the in vitro tests of P. falciparum to chloroquine and/or mefloquine has been adopted and widely distributed (see Appendix 3.1).

Also, a precoded form for recording the results of in vivo tests has been developed (see Appendix 3.2). The record forms were designed for:

- use as primary records;
- immediate manual processing of the results by the individual investigator;

1 See Summary of Guidelines for the assessment of the response of Plasmodium falciparum to antimalarial drugs (Appendix 8).
- input of the results into the global monitoring system;
- retrieval and analysis by computer.

Processing and Analysis (at WHO Headquarters)

33 000 in vitro test forms have been printed; 30 150 have been distributed, and 341 completed forms have been returned to WHO Headquarters.

The number of completed forms received by mid-1981 at WHO Headquarters is quite small. The majority of the forms (177 out of 341) pertain to results obtained in 1978-1980. The proportion of reported failures is high on the average, but varies widely between countries.

Some of the forms received are incomplete, and some are incorrectly filled.

Table 5 shows the number of schizonts in the control counts of the in vitro macro test. There is wide variation and there is obviously a need for defining the minimum acceptable number of schizonts in the controls (see also page 35, paragraph f).

The analysis of the data in WHO Headquarters is being undertaken jointly by the Malaria Action Programme and the Units of Data and Text Processing and Health Statistical Methodology. The following technical points are under consideration.

- the definition of cut-off points for the classification of individual test results;
- the study of alternative methods of combination of the results from a group of tests;
- the development of alternative models of the dose-response relationship (e.g. the log-normal distribution of the effective dose) and of methods for fitting such models to the data (e.g. the method used for the response of vectors to insecticides);
- the selection of alternative tests of significance of the difference between two or more groups of tests;
- the mapping of results.

In order to function optimally, closest coordination will be required at national level in transmitting relevant information to the WHO Regional Offices and Headquarters. Whether a summary of information about the distribution and level of resistance could be supplied more frequently to workers in the field remains to be determined. The central (global) monitoring involves an inevi-
Table 5. Number of Schizonts in the Control Counts of the in vitro Macro Test

<table>
<thead>
<tr>
<th>No. of schizonts in control</th>
<th>Bangladesh</th>
<th>Burma</th>
<th>Laos</th>
<th>Sarawak</th>
<th>Solomon Islands</th>
<th>Sri Lanka</th>
<th>Thailand</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>52</td>
<td>26 b</td>
<td>22 b</td>
<td>4 b</td>
<td>6</td>
<td>38 b</td>
<td>8</td>
</tr>
<tr>
<td>1-10</td>
<td>5</td>
<td>1</td>
<td>-</td>
<td>8 c</td>
<td>19 d</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>11-20</td>
<td>6</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>21-50</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>30</td>
</tr>
<tr>
<td>51-100</td>
<td>7</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>5</td>
<td>-</td>
<td>42</td>
</tr>
<tr>
<td>101-200</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>-</td>
<td>3</td>
<td>-</td>
<td>41</td>
</tr>
<tr>
<td>201-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>94</td>
</tr>
</tbody>
</table>

Range (excl. 0) 2-180 | 9-652 | 25-517 | 1-238 | 1-122 | 4-29 | 16-517

Total control readings 81 | 47 | 32 | 20 | 35 | 40 | 177

No. of tests 79 | 24 | 16 | 10 | 18 | 20 | 89

---

a Per 300 or per 1000 leucocytes.
b "No growth" interpreted as no growth in two controls.
c Two tests with controls (4;1) and (5;2) rejected by the investigator; two tests with controls (8;8) and (9;8) accepted.
d Four tests with controls (0;7), (3;1), (1;0), and (1;2) rejected by the investigator; seven tests with controls (5;4), (7;5), (6;4), (10;9), (5;9), (3;2), and (3;2) accepted.
table time lag and cannot be expected to replace the investigators' immediate analysis of their own results.

Guidelines for Sampling

In principle, the selection of cases for study should follow the basic precepts for the selection of a statistically valid sample. However, it is appreciated that the prevalence of suitable positive cases for testing in a particular study area may make the application of this process difficult or impossible.

Population to be Investigated

The population to be investigated needs to be selected and specified in accordance with the objectives of the study. Appropriate population groups might be:

- inhabitants of a given geographical area at a given time;
- labourers and migrants in camps within a given geographical area;
- migrants, domestic and international, between specified areas (indicate also routes of migration);
- others.

Questions to be Answered by the Investigation

(a) Is confirmed resistance present or are there reports on the lack of clinical response ("resistance" being defined in terms of the test, e.g. growth of schizonts in the presence of 1.5 n-mol (= 1.5 x 10^{-6} M/l) or more of chloroquine in the macro in vitro test, and 5.7 p-mol (=1.14 x 10^{-6} M/l) in the micro in vitro test)?

(b) Does the frequency of resistance reach a certain level and distribution at which some specific decision may be called for (e.g. change to alternative drug(s) or drug combinations, introduction of intensive vector control measures, etc.)?

(c) What is the response of the parasite to the drug (in the population)?

(d) Was the response of the parasite to the drug changed significantly from that observed in earlier investigations of the same population?

Estimate of the Required Sample Size

It is appreciated that the estimation supposes that the sample
is random. The desirable sample size will vary with the answers to the above-mentioned questions.

(a) Question (a), page 45, is best approached through non-random sampling, namely through the detection and investigation of drug failures reported by clinicians. However it can also be approached through random sampling; if the time prevalence of resistance is \( p \) and one wishes to detect its presence with probability \( P \), then the required sample size would be:

\[
N = \frac{\log (1-P)}{\log (1-p)}
\]

\( N \) is easily calculated with a pocket calculator, for example: if \( p = 0.01 \), i.e. the true prevalence of resistance is 1%, and \( P = 0.95 \), i.e. a desired 95% probability of finding at least 1 resistant infection, then

\[
N = \frac{\log (1-0.95)}{\log (1-0.01)} = 298
\]

One can also calculate \( P \), given \( p \) and \( N \):

\[
P = 1 - (1-p)^N
\]

Table 6 gives values of \( P \) for \( p = 0.01, 0.02 \ldots 0.10 \) and \( N = 10, 20 \ldots 100 \).

(b) Question (b), page 45. If the "critical level" is \( p = 0.20 \) (i.e. 20%) and if the sample prevalence from a population where the prevalence is actually \( p \), should fall between \((p-d)\) and \((p+d)\) (e.g. between 0.10 and 0.30) with a probability of \( P = 0.95 \), then the desirable sample size \( N = p \frac{(1-p)}{(2 d/4)^2} \) or in the example \( N = 0.2 \times 0.8 = 64 \).

If the sample prevalence is to fall between 0.15 and 0.25, then

\[
N = \frac{0.2 \times 0.8}{(0.1/4)^4} = 256
\]

If the sample prevalence is to fall between \((p-d)\) and \((p+d)\) with a probability \( P \) of 0.99, the formula becomes \( N = p \frac{(1-p)}{(d/2.6)^2} \)

(c) Questions (c) and (d), page 45, both require a method of describing the drug response on a population basis. In the case of in vivo tests, the results form a frequency distribution into 4 classes (S to RIII). The required sample size depends on the true frequencies and on the precision required (see Table 6). Different population samples can be compared by the Chi-square test.
Table 6. Probability of Detecting the Presence of Resistance as a Function of Its True Prevalence and of the Sample Size
(all probabilities rounded to the nearest 0.01)

<table>
<thead>
<tr>
<th>Sample size (n)</th>
<th>True prevalence (p) of resistance (R) expressed as a proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>10</td>
<td>0.10</td>
</tr>
<tr>
<td>20</td>
<td>0.18</td>
</tr>
<tr>
<td>30</td>
<td>0.26</td>
</tr>
<tr>
<td>40</td>
<td>0.33</td>
</tr>
<tr>
<td>50</td>
<td>0.39</td>
</tr>
<tr>
<td>60</td>
<td>0.45</td>
</tr>
<tr>
<td>70</td>
<td>0.51</td>
</tr>
<tr>
<td>80</td>
<td>0.55</td>
</tr>
<tr>
<td>90</td>
<td>0.60</td>
</tr>
<tr>
<td>100</td>
<td>0.63</td>
</tr>
</tbody>
</table>

(Figures falling below thick line represent 95% probability of detecting resistance)

Calculated as follows: If p = true prevalence of R
Then the probability that a random case is R = 1-p
Therefore the probability that 2 random cases are both = (1-p)^2
Therefore the probability that n random cases are all = (1-p)^n
Therefore the probability that among n random cases there is at least
1R = 1-(1-p)^n

These probabilities are easily computed with a pocket calculator, as follows:
Enter 1, subtract p, take log, multiply by n, take antilog, change sign, add 1.
In the case of the in vivo tests, it is commonly assumed that the effective dose follows the log-normal distribution. This assumption underlies the computation of the population parameters (ED 50, ED 90, etc.) and of their confidence limits, as well as the statistical tests of the significance of the difference between the parameters of two groups of results. The same applies to computations of the sample sizes required to estimate the parameters with a predetermined precision, or to detect a given difference with a given confidence. It is not yet proven that the assumption of a log-normal distribution of the effective dose is generally correct, and this needs to be tested on relatively large data sets from a variety of sources. If one is not willing to assume a specific underlying distribution, results of a group of in vitro tests may also be described as a frequency distribution; the frequency distribution has two dimensions, drug concentration and parasite maturation (in percent of the control), and the total number of classes is the product of the number of concentration classes (several concentrations may be grouped) by the number of maturation classes (e.g. 0%, 1-25%, 26-50% etc.). Two groups of test results can be compared by the chi-square test.

Empirically, sample sizes of 30 for the macro test and 24 or 48 for the micro test, corresponding to the number of tests available in standard test kits, seem to provide a meaningful description of the response of a population to infections. Annex 9 describes a simple empirical exploration of the effect of sample size on the answer to question (c), page 45.

Sample selection

(a) Representative sample

A representative sample of the whole population is taken as follows:

- setting a target for the number of tests, e.g. N = 48, 24;
- estimating the size of the population to be sampled, e.g. 20 000, 50 000, 100 000.
- identifying the relevant population clusters and numbering them, e.g. 100 villages are identified on a map and numbered sequentially from 1 to 100.
- making the best guess of the prevalence of P. falciparum infections suitable for the test, e.g. 1%, 10%
- calculating the population to be screened, e.g. prevalence = 1% and N = 48, screen 48 x 100 = 4800
prevalence = 1% and N = 24, screen 24 x 100 = 2400
prevalence = 10% and N = 48, screen 48 x 10 = 480
prevalence = 10% and N = 24, screen 24 x 10 = 240
(if the number to be screened is prohibitively large, see (b) below).

- calculating the number of clusters (e.g. villages) to be screened by dividing the population by the average village size, e.g. 2400/1000 = 3 (rounded up).

- determining the sampling fraction n by dividing the total number of clusters by the number of clusters to be screened (e.g. n = 100/3 = 33). Continuing with the sample a random number is drawn between 1 and 33 (e.g. 17); this (17) is the number of the first village to be surveyed. In order to obtain the others, 3 is added to the base number as often as required, i.e. the villages to be surveyed are numbered 17, 50 and 83.

(b) **Substitutes**

It may not be possible to select a representative sample, e.g. if the number of people to be surveyed is prohibitively large or the number of falciparum infections too small; in this case substitutes for general population samples may be used, the most common of which are:

- Young age-groups (under 10 years) in the general population, i.e. within a sample selected along the lines indicated above. The obvious reason for their selection is that they are expected to have a higher prevalence of suitable parasitaemias. It would be useful to investigate, if possible from available data, whether and how the drug response varies as a function of the age of the infected person (variation in vivo would be expected, but may not be present in vitro).

- Schoolchildren, selected for the same reason and for their accessibility. This may introduce a bias, e.g. in terms of the frequency of drug failure, absence from school due to current illness, prior radical treatment or presumptive treatment (perhaps given by school authorities under voluntary collaboration). It is not easy to determine how seriously this bias may influence the results.

- Fever cases detected by survey, e.g. within villages selected above.

- Outpatients. These may also introduce a bias towards drug failure if drugs are available peripherally and the patients therefore already pre-selected.

**Documenting the samples actually collected**

Irrespective of whether the guidelines are followed or not,
the pre-coded forms provide for the recording of several data concerned with the identification of the population sampled and the method of selection. In reporting the results of studies on drug response it is always recommended to describe clearly how the sample was drawn (recruited) and to discuss possible biases.

Development of the Collaborative Programme

In South-East Asia, a plan of work was prepared following the Regional Workshop on the Collaborative Studies of Drug-Resistant Malaria in 1977, in which seven countries participated. Training was organized in 1977 in Thailand, and subsequently in Sri Lanka, Bangladesh, India and Indonesia. Regional funds were allocated to this programme as from 1978 through the mechanism of Contractual Technical Services (CTS) Agreements. The UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) supported the regional course in Thailand (19 participants), provided test kits to the collaborating institutions and gave financial support to a follow-up meeting of principal investigators in 1979.

In the American Region TDR supported studies started in 1978. The coordinating institution was located in Panama, and collaboration developed gradually between this institution and the malaria services of 18 countries in the Region. Courses were organized in Brazil, Colombia and El Salvador with a total of 43 participants.

In the Western Pacific Region, a regional plan of work was developed following the 1978 Manila Workshop, in which 9 countries or areas participated. TDR has concluded CTS agreements with individual institutions or services in these countries, and test kits, additional laboratory equipment and supplies as well as limited operating costs were provided. A sub-regional training course was organized in 1979 with 12 participants, complemented later by the organization of national training activities.

In the Eastern Mediterranean and African Regions, coordinated regional programmes do not yet exist, the initial emphasis having been placed on the training of personnel in two courses in the Sudan in 1978 and 1981 respectively (31 participants). Studies have started in three countries in Africa with which CTS agreements were concluded.

In addition to these countries and institutions, a limited number of test kits was provided to institutes in Africa, the Americas and Europe, mainly for special studies and individual clinical diagnostic procedures.

The production of macro test kits is carried out by the Malaria Eradication Service in the Philippines with the support of
TDR. At the end of 1980, the total number of test kits produced amounted to 200 "A", 400 "B" and 160 "C". The cost per kit was US$180, US$90 and US$90 respectively, and the capacity per kit is 30, 15 and 18 tests respectively.

The micro test kits are for the time being in limited production. Kit "A" containing material for 48 tests each against chloroquine and mefloquine, costs US$390 and kit "B", for 24 tests each against chloroquine and mefloquine, costs US$130 including air-freight charges.

Programme implementation was most intense in the South-East Asia and the Western Pacific Regions, followed by the American Region. Programmes still need to be planned and implemented in the African and in the Eastern Mediterranean Regions.

Coordination and evaluation meetings on regional collaborative studies on drug-resistant malaria have proved to be a major catalyst of programme planning and implementation in the South-East Asia and Western Pacific Regions. They are recommended for other Regions as well.

Training should be related to the plans for institutional manpower development and to the description of the functions and duties of the personnel. At international level two types of personnel should be trained, i.e. epidemiologists and senior laboratory technicians, and they should be able in turn to train the other members of national teams. This was the pattern followed so far in the South-East Asia and Western Pacific Regions.

Both regional voluntary funds and TDR funds are limited, and they cannot cover all the needs of the programmes for the monitoring of drug sensitivity. These activities should therefore be gradually integrated with those of the operational routine work of the national malaria services. Moreover, other field research activities need to be developed which are directed at producing methods for the containment or the elimination of drug resistant malaria.
Among the biological phenomena of the malaria parasite, resistance to antimalarial drugs is probably the one which is causing the most important interference with the control of the disease. It helps the parasite to overcome the challenge posed by antimalarial drugs. However, knowledge of the biological basis of drug resistance and of the mode of action and the pharmacokinetics of antimalarials may help to overcome the resourcefulness of the parasite and through a well-planned tactical deployment of antimalarial measures, it may be possible to limit the spread and to eliminate or at least reduce the impact of drug-resistant P.falciparum in exposed populations and antimalaria programmes.

Antimalarial drugs directed against human-pathogenic plasmodia show marked stage-specific and often also species-specific action. There are moreover important natural strain differences in the drug sensitivity of particular species and stages, without necessarily implying natural drug resistance in less susceptible strains. In the case of P.falciparum, a natural parasite population is composed of individuals showing different susceptibility to the drug, within a relatively limited range if the isolate is sensitive, within a wider range if it is resistant.

In order to be effective, a drug has to interfere with one or several essential life functions, usually metabolic functions, of the parasite. To do so, the drug has to be available at the place of action for a long enough time and in an adequate quantity/concentration to ensure damage to the parasite. Pharmacokinetic
characteristics of drugs in conjunction with tolerability are therefore playing a major role in defining the most appropriate dosages and regimens, as are metabolic processes which may produce active metabolites (e.g. in the case of proguanil) or inactive drugs, or make them liable to faster elimination.

The major groups of blood schizontocidal drugs such as 4-aminoquinolines, DHFR inhibitors and quinine type drugs have different mechanisms of action. Their efficacy depends on their ability to pass through the membrane of the infected erythrocyte. With some drugs, such as 4-aminoquinolines a significant selective uptake of the drug by the parasite occurs. Resistance to 4-aminoquinolines is marked by a reduced drug binding of the parasite, that against DHFR and dihydropteroate synthesis (DHPS) inhibitors by the production of enzymes, which are less susceptible to drug-induced inhibition.

Resistance to antimalarial drugs appears to be caused by spontaneous mutation only and genetically expressed in multiple loci. Chloroquine resistance is quite stable, apparently dominant upon gene recombination, and seems to confer a biological advantage to the parasite.

Selection of resistant parasites is effected under drug pressure. In the absence of adequate complementary defense mechanisms, this may be more evident in nonimmune individuals in contrast to semi-immune individuals.

Based on pharmacological and pharmacokinetic properties of a drug or a drug combination, on the mechanisms and genetics of drug resistance and the factors governing the selection of drug-resistant parasites, it is possible to arrive at practical guidelines for a rational use of drugs which should postpone the occurrence of resistance and maintain the operational usefulness of the medicaments. However, it is necessary to underline the important role of other operational, especially vector control, measures for the containment of drug resistance. Further research is required for the development of new generations of antimalarial drugs since the armamentarium of effective alternative medicaments has virtually been depleted in some areas of the world.

DRUG PRESSURE AND RESISTANCE

The biological advantage of resistant *P. falciparum* populations is considered to be an important factor in the selection of resistance under drug pressure, e.g. through chemoprophylaxis.

Resistance is not introduced by the drug itself but occurs naturally at a certain frequency. The selection of these resistant mutants seems to be responsible for occurrence and
spread of drug resistance. The importance of baseline differences in drug sensitivity of different P.falciparum strains in relation to the development of resistance is not known. There may be differences in the rate of spontaneous mutations of different P.falciparum strains. Thus, mass drug administration with chloroquine was practised in Central America from 1960-1967 without emergence of resistance. However, in other areas with malaria control programmes the amount of chloroquine required to clear parasitaemia has increased year by year. This applies to several countries including India.

The role of immunity in the selection is not known. Non-immunes often require higher drug doses for radical cure. The aggregation of nonimmune migrants in areas with naturally infected semi-immunes may therefore favour the expression of drug resistance. Another important factor is the presence of areas of malaria transmission which, for political reasons, are unreachable for normal control operations.

DRUG COMBINATIONS AND SYNERGISM IN RELATION TO THE PREVENTION OF THE DEVELOPMENT OF DRUG RESISTANCE

The synergistic effect of DHFR inhibitors and sulfonamides/sulfones cannot be solely explained on the basis of an inhibition at two different steps of the same linear metabolic pathway to tetrahydrofolate which should be additive only. Perhaps another interaction, related to thymidine synthesis or the formation of another metabolite may explain the well known synergistic effect of these two classes of compounds.

The use of drug combinations may be important in delaying the appearance of drug resistance. However, compounds need not be synergistic for this purpose. Conversely, when synergistic drugs are used, the emergence of resistance may only be delayed but not totally prevented. The most important application of these principles may be the use of non-synergistic compounds with mefloquine to prevent the development of resistance to this new drug, as it is of supreme importance to protect mefloquine.

Data from rodent models using P.bergheï have demonstrated that the rate of the development of resistance is slowed when sulfonamides are given with mepacrine, and that there is no such effect when chloroquine and pyrimethamine are given in combination. However, the use of chloroquine/sulfonamide combinations inhibits resistance. Perhaps the best protection against the development of resistance is the use of a chloroquine/sulfadoxine/pyrimethamine combination. It was noted that it has taken 10 years or more for resistance to pyrimethamine/sulfadoxine to emerge, although the combination may not have been used widely in some areas. This combination is synergistic despite the presence of moderate levels of pyrimethamine resistance.
EXO-ERYTHROCYTIC DEVELOPMENT OF MALARIA PARASITES

The hypnozoite stage of parasite development was recently discovered with a fluorescent antibody technique in sections of liver from rhesus monkeys infected with *P. cynomolgi*. It is a single cell liver stage which is thought to be a dormant precursor of tissue schizonts responsible for relapses in this and similar infections such as *P. vivax*. Hypnozoites have been found in retrospective examinations of liver sections from chimpanzees infected with *P. vivax*. While more information is needed, knowledge of the hypnozoite may help to understand better the phenomenon of relapses and the mode of action of antirelapse drugs. Experimental evidence with *P. simiovale*, *P. cynomolgi* and *P. vivax* suggests that the ability of a particular sporozoite to cause a primary attack, an early or late relapse may be genetically predetermined. At present, the 8-aminoquinolines (e.g. primaquine) are the only group of drugs available to prevent relapses. Primaquine probably clears hypnozoites but, as yet, dose relationships are not known. Primaquine has a sporontocidal action as well. However, it is difficult to relate the dose levels required for sporontocidal action to those needed for preventing relapses.

It is quite possible that a screening model for gametocytocidal activity (e.g. *Haemoproteus*) could also be used for finding new antirelapse drugs which are effective against hypnozoites.

CHLOROQUINE RETINOPIATHY

It is difficult to distinguish between chloroquine specific and other types of retinopathy. In susceptible individuals the deficiency of sphingomyelinase or other lysosomal enzyme systems could cause the development of this toxic manifestation in a shorter period of time. This fact could explain isolated reports of rapidly developing retinopathy. Retinopathy is known to develop rather fast in patients taking high daily doses of chloroquine, e.g. for the treatment of collagenoses. With regard to chloroquine for malaria chemoprophylaxis, there are several extended studies in large populations which were exposed to a prolonged use of this drug either in the form of tablets or as medicated salt. No retinopathy has been observed in these groups. Chloroquine retinopathy is time and dose dependent. It is generally accepted that chloroquine retinopathy very rarely occurs under routine chemoprophylaxis at total cumulative doses of less than 100 g base. In most persons, retinopathy will not develop even after an intake of more than 100 g (base).

CLINICAL PHARMACOLOGICAL STUDIES

The importance of pharmacodynamic and pharmacokinetic factors in relation to the efficacy of particular drugs and dose regimens
has been recognized. In the field of malaria, the administration of specific drugs is not yet in tune with clinical pharmacological requirements and knowledge of the latter is still scanty. Moreover, pharmacokinetics and pharmacodynamics may differ within the same patient.

Studies in Thai children with multiresistant *P. falciparum* infections and treated with quinine for 14 days at 10 mg quinine/kg body weight suggested that peak blood concentrations were reached within 2 or 3 days and that minimum inhibitory concentrations (MIC) need to be maintained for at least six days in order to produce radical cure. This may not be possible with the indicated dose level since the blood levels tend to fall when the fever disappears. Thus the levels may drop even below the MIC within one week.
VI.

Control of Drug-Resistant *P. falciparum*

**CONTROL OF TRANSMISSION AND PREVALENCE**

A review of the problems encountered by antimalaria programmes as a consequence of drug resistance of *P. falciparum* indicates that attention should be focused on the following types of areas:

- where the existence of resistant *P. falciparum* is well established and widespread;
- where limited foci of resistant *P. falciparum* are already established;
- where resistant *P. falciparum* has not yet been recognized.

Nevertheless, the management of problems related to drug resistance is considered to be an integral part of the general epidemiological study and control of malaria. It should therefore be an important responsibility of the antimalaria programme or of the general health services, wherever malaria services do not exist within which resources should be made available, to cope with this specific problem. These activities must be adequately funded and the services be given the necessary administrative flexibility to deal with the emergency.

The available information should be analysed in order to stratify the malarious areas of the country within the three categories of areas defined above.

Migratory movements should be defined in order to evaluate various degrees of vulnerability and to assess the potential of
the spread of drug resistance. As these movements cannot be controlled, the aim should be to intensify individual protective measures through health education, blood examination of suspected malaria cases and radical treatment of confirmed cases. In addition, and depending upon the epidemiological situation in the locality, appropriate protective measures should be organized for the community.

In the context of Health for All by the Year 2000, drug resistance should be considered as a problem of high priority and an emergency requiring the mobilization of resources from all possible sources, national, bilateral and international. This includes intersectorial cooperation within the country and technical cooperation between countries so as to meet operational and other requirements.

A coordination mechanism between countries and Regions should be established in order to provide:

- essential exchange of technical information;
- technical cooperation where and when needed;
- resources for emergencies.

It is well recognized that the control of drug-resistant falciparum malaria may be extremely difficult in some areas in view of constraints of a technical, social, political, behavioural and other nature; nevertheless, control measures known to be effective or partially effective should be applied whenever feasible.

Table 7 summarizes the range of actions which might be included in a programme for the control of transmission and prevalence of drug-resistant P.falciparum.

The programme provides the opportunity of enhancing the knowledge of the epidemiology and thus ultimately the control of drug-resistant malaria. Whenever possible, resource groups should be established to study critically the results obtained and to design and conduct complementary field studies as and when required.

It is recognized that one of the main obstacles to the study of drug-resistant malaria and its control is the lack of well trained and motivated personnel; the realization of this programme is dependent on an adequate training programme for all levels of the services dealing with malaria and on the strengthening of career opportunities.
DRUG USE AIMING AT PREVENTING, DELAYING OR REVERSING
THE SELECTION OF RESISTANT P. FALCIPARUM

Introduction

Among the factors to be considered before attempting to formulate policies regarding the management of drug resistance in falciparum malaria are the type of drug deployment most likely to be associated with the emergence of resistance, the currently available antimalarials, the events responsible for the development of resistance and its spread, and possible measures that can be adopted to deal with the problem. Insofar as drug resistance is due to the selection under drug pressure of potentially resistant mutant organisms, it follows that the rate of selection will be directly proportional to (a) the dose of drug used, including the total amount in a given geographical area, (b) the number of parasites exposed to the drug, (c) the frequency with which resistant mutants are present in a given parasite population, and (d) the influence, if any, of the drugs on parasite transmission through anopheline vectors.

Dose and Pattern of Drug Use

This will depend upon several factors such as:

- the drugs given for prophylaxis or treatment. In the former case a smaller quantity may be given per dose, but over a longer period of time; in the latter, a larger dose will be given on a single occasion only;

- the number of people receiving prophylaxis or treatment;

- the duration of prophylaxis.

Number of Parasites Exposed to Drugs

Since the antifols, proguanil and pyrimethamine, may affect developing pre-erythrocytic stages of P. falciparum, at least in sensitive strains, the number of parasites exposed to such compounds is comparatively small when these compounds are used for prophylaxis. However, this advantage has been widely lost since most of the P. falciparum isolates are now resistant to DHFR inhibitors. Chloroquine is active only against asexual erythrocytic stages. Thus when it is used for prophylaxis it is available to attack the relatively few intra-erythrocytic parasites originating from merozoites that first enter the circulation from the liver, as well as subsequent generations that

Antimalarial drugs and their use are reviewed in Appendix 5.
Table 7. Specific Actions for the Control of Transmission and Prevalence of Drug-Resistant *P. falciparum*

<table>
<thead>
<tr>
<th>Areas where the existence of resistant <em>P. falciparum</em> is well established and widespread</th>
<th>Areas with limited foci of resistant <em>P. falciparum</em> already established</th>
<th>Areas where resistant <em>P. falciparum</em> infections have not been recognized</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Map the distribution and intensity of <em>P. falciparum</em> resistance by in vitro/in vivo studies.</td>
<td>1. Map the distribution and intensity of <em>P. falciparum</em> resistance, and delineate the foci, by in vitro/in vivo studies.</td>
<td>1. Assess the sensitivity of local <em>P. falciparum</em> to current antimalarial drugs to establish a baseline.</td>
</tr>
<tr>
<td>2. Introduce anti-vector measures expected to be more effective. 1</td>
<td>2. Intensify appropriate anti-vector measures in the established limited foci and new foci backed by a suitable evaluation system.</td>
<td>2. Stratify these areas in relation to their vulnerability in order to establish priorities for action.</td>
</tr>
<tr>
<td>3. Introduce additional vector control measures appropriate to the bionomics of the main vector(s) and to the other possible vectors responsible for the propagation of the resistant strain(s).</td>
<td>3. Provide effective radical treatment of <em>P. falciparum</em> including an effective gametocytocide.</td>
<td>3. Establish an effective vigilance system including a warning system involving the private and public health sectors, designed to reveal the importation and establishment of drug-resistant <em>P. falciparum</em> cases. Compulsory notification of malaria treatment failures is recommended.</td>
</tr>
<tr>
<td>4. Provide effective radical treatment for <em>P. falciparum</em> including an effective gametocytocide.</td>
<td>4. In the event that manageable foci exist, and as soon as new foci emerge, efforts should be directed towards the elimination of the resistant <em>P. falciparum</em> infections.</td>
<td>4. Establish the capability to investigate reported cases of malaria treatment failures including in vitro/in vivo studies.</td>
</tr>
<tr>
<td>5. Provide active health information and education to the community to encourage early appropriate treatment and to prevent transmission by personal protection, especially of children.</td>
<td>5. Provide active health information and education to the community to encourage early appropriate treatment and to prevent transmission by personal protection, especially of children.</td>
<td>5. Provide effective and rapid radical treatment for <em>P. falciparum</em> cases, including an effective gametocytocide.</td>
</tr>
<tr>
<td>6. Introduce and enforce a more radical use of antimalarial drugs.</td>
<td>6. Introduce and enforce a more rational use of antimalarial drugs.</td>
<td>6. Where appropriate to local conditions and technically feasible and justified, establish diagnostic and treatment check posts on known migration routes from drug resistant areas.</td>
</tr>
</tbody>
</table>

1 This includes all types of measures (chemical, biological, environmental) applied either singly or in combination.
Table 7. Specific Actions for the Control of Transmission and Prevalence of Drug-Resistant *P. falciparum* (Continued)

<table>
<thead>
<tr>
<th>Areas where the existence of resistant <em>P. falciparum</em> is well established and widespread</th>
<th>Areas with limited foci of resistant <em>P. falciparum</em> already established</th>
<th>Areas where resistant <em>P. falciparum</em> infections have not been recognized</th>
</tr>
</thead>
<tbody>
<tr>
<td>7. Monitor the extent and degree of resistance by annual in vitro/in vivo studies in fixed indicator areas and on an ad hoc sampling basis.</td>
<td>7. Monitor the susceptibility of <em>P. falciparum</em> to drugs in selected indicator areas, annually, by in vitro/in vivo techniques within the already established foci and also on an ad hoc sampling basis.</td>
<td>7. Introduce and enforce a rational use of antimalarial drugs throughout the country.</td>
</tr>
<tr>
<td>8. Assess the sensitivity of local <em>P. falciparum</em> to current antimalarial drugs to establish a baseline.</td>
<td>8. Assess the sensitivity of local <em>P. falciparum</em> to current antimalarial drugs to establish a baseline.</td>
<td>8. Intensify appropriate anti-vector measures in areas where imported cases of resistant <em>P. falciparum</em> malaria have been detected and where the receptivity is sufficiently high to support local transmission.</td>
</tr>
<tr>
<td>9. Establish an information system to monitor the clinical response to alternative antimalarial drugs in use.</td>
<td>9. Establish an information system to monitor the clinical response to antimalarial drugs in use throughout the public and private medical sectors. This information can be used to determine when and where to carry out ad hoc studies.</td>
<td></td>
</tr>
</tbody>
</table>
may develop from any surviving individual asexual forms. When any of these compounds are used for the treatment of established infections (in practice this will usually only refer to chloroquine), far larger numbers of asexual parasites will be exposed to them. Ultimately the number of parasites exposed to a drug is the product of the number of people taking the drug multiplied by the mean number of parasites per individual.

**Frequency of Resultant Mutants in Parasite Populations**

Various estimates have been made of the frequency with which mutants resistant to various antimalarial drugs occur in different species of malaria parasites of birds and of rodents, but no good estimates are available for *P. falciparum*. While the frequency of mutations resulting in parasites resistant to DHFR inhibitors appears to be greater than that for chloroquine, this depends very much on the parasite species. Estimates for pyrimethamine seem to suggest that resistant mutants are present in the order of 1 in $10^9$ parasites or less in natural populations of both *P. gallinaceum* and *P. falciparum*. While no estimate is available for chloroquine, the relative frequencies would appear to be $P. berghei > P. falciparum > P. gallinaceum > P. vivax$. Moreover, there seems to be a geographical variation in the mutation rate in *P. falciparum* as regards resistance to chloroquine, the rate being apparently higher in South-East Asia and South America than in Africa. However, host as well as parasite factors must play a role in the ease of manifestation of chloroquine resistance, and this may well account for the long delay before this phenomenon has become apparent on the African continent.

**Influence of Drugs on Parasite Transmission**

While antifols have a sporontocidal action against drug-sensitive strains of *P. falciparum*, this action is lost in parallel with decreasing sensitivity of the asexual stages. Chloroquine which has neither a sporontocidal action nor any action against mature gametocytes even of drug-sensitive strains of *P. falciparum*, appears to have a paradoxical action on the transmission of chloroquine-resistant parasites, and may actually enhance their infectivity to vector anophelines. However, this may be related to the biological advantage of chloroquine-resistant *P. falciparum*. Primaquine which is, of course, not used for the prophylaxis or therapy of falciparum malaria, possesses a potent gametocytocidal action against both chloroquine or antifol-sensitive or -resistant parasites.

**Antimalarial Drugs Available and Their Roles**

Table 8 shows drugs or combinations in current use (see Appendix 5 for further details)
Table 8. Drugs or Combinations in Current Use

<table>
<thead>
<tr>
<th>Name</th>
<th>Type</th>
<th>Role</th>
<th>Problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proguanil</td>
<td>DHFR inhibitor (antifol)</td>
<td>Prophylaxis</td>
<td>Resistance common (P.f. and P.v.)</td>
</tr>
<tr>
<td>Pyrimethamine</td>
<td>DHFR inhibitor (antifol)</td>
<td>Prophylaxis</td>
<td>Resistance common (P.f. and P.v.)</td>
</tr>
<tr>
<td>Pyrimethamine/sulfadoxine</td>
<td>DHFR and DHPS inhibitors</td>
<td>Therapy of</td>
<td>Resistance appearing in parts of Brazil, Kampuchea, Thailand and elsewhere</td>
</tr>
<tr>
<td>Pyrimethamine/dapsone</td>
<td>DHFR and DHPS inhibitors</td>
<td>chloroquine-resistant P.f.</td>
<td>May cause agranulocytosis</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>4-aminoquinoline blood</td>
<td>Prophylaxis</td>
<td>Resistance widespread in P.f. except in Western Asia and large parts of Africa</td>
</tr>
<tr>
<td></td>
<td>schizontocide</td>
<td>Therapy</td>
<td></td>
</tr>
<tr>
<td>Amodiaquine</td>
<td>4-aminoquinoline blood</td>
<td>Prophylaxis</td>
<td>Resistance slightly less in P.f. as compared to chloroquine</td>
</tr>
<tr>
<td></td>
<td>schizontocide</td>
<td>Therapy</td>
<td></td>
</tr>
<tr>
<td>Quinine</td>
<td>Blood schizontocide</td>
<td>Therapy of</td>
<td>Expensive, relatively toxic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>multiple-resistant P.f.</td>
<td></td>
</tr>
<tr>
<td>Primaquine</td>
<td>8-aminoquinoline</td>
<td>Antirelapse</td>
<td>Toxic especially in G-6-PD deficient subjects when used in daily administration for antirelapse treatment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>in P.v.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gametocytocide in P.f.</td>
<td></td>
</tr>
<tr>
<td>Mefloquine</td>
<td>Quinoline-methanol blood</td>
<td>Therapy of</td>
<td>Clinical trials not yet completed (see Annex 11)</td>
</tr>
<tr>
<td></td>
<td>schizontocide</td>
<td>multiple-resistant P.f.</td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>Antibiotic blood</td>
<td>Therapy of</td>
<td>Slow action. Usually given concurrently with or subsequent to quinine administration</td>
</tr>
<tr>
<td></td>
<td>schizontocide</td>
<td>multiple-resistant P.f.</td>
<td></td>
</tr>
</tbody>
</table>

Note: P.f = Plasmodium falciparum; P.v. = Plasmodium vivax; G-6-PD= glucose-6-phosphate dehydrogenase
The list is not very impressive, especially when one views the levels of resistance that exist to proguanil, pyrimethamine and chloroquine, and the geographical spread of P.falciparum resistant to one or more of these compounds. Resistance to Fansidar is being recognized increasingly in such countries as Thailand and Brazil. The response to amodiaquine is not sufficiently superior to the response to chloroquine to make it a serious contender as a replacement for the latter. While quinine retains its place as the drug of choice for the treatment of serious falciparum malaria, especially where multiple drug resistance is present, a number of falciparum infections have recently been noted not to respond as readily to this drug as would be expected, and it seems likely that some degree of quinine resistance is beginning to emerge, at least in parts of the Indochinese peninsula.

Recommendations for the deployment of currently available drugs are summarized in Table 9.

Mefloquine is not yet available, although advanced clinical trials currently under way should permit the release of limited supplies of this new drug for therapy under controlled conditions in the near future. There is a serious danger however, that the potentially valuable clinical life of mefloquine will be fore-shortened if it is freed for unrestricted use alone, and measures are being sought to protect it by using it in combination with other drugs. These studies still require several years for their completion (see Annex 11). In the meantime the use of mefloquine alone should be rigorously restricted to indications where there is no ethically acceptable alternative.

Possible Measures to Combat Drug Resistance

Prevention or Delay of the Emergence of Resistance

(a) Limiting the use of drugs

The major obstacle to limiting the use of antimalarial drugs is their ready availability on the open market. It is probably a council of perfection to suggest that antimalarial drugs should only be imported and supplied through national health authorities. The least that should be aimed for is government control of the distribution and/or sale of antimalarials in affected countries, even if laws exist for this purpose. While it is certainly too late to do much to manage the use of existing antimalarial drugs, every effort should be made to restrict the importation and distribution of any new compound that becomes available. If this is not done, new compounds will certainly meet the same fate as the older ones, and in a short time they will be rendered more or less obsolete by the emergence of malaria parasites that are resistant to them. The current fate of
Table 9. Suggested Use of Antimalarial Drugs in Areas With or Without Drug Resistance (1)
Adult doses, to be adjusted for age, See Appendix 5 for detailed description of alternative drug regimens

<table>
<thead>
<tr>
<th>Type of treatment</th>
<th>Areas where chloroquine-resistant P.falciparum is well established (2)</th>
<th>Areas where chloroquine-resistant P.falciparum is emerging</th>
<th>Areas without chloroquine-resistant P. falciparum and not seriously threatened by its potential spread</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presumptive treatment and single dose treatment of fever cases (3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Where there is no established sulfadoxine/pyrimethamine resistance</td>
<td>Sulfadoxine 1.5 g + pyrimethamine 75 mg + primaquine 30-45 mg</td>
<td>(5)</td>
<td>(Chloroquine - 600 mg + (B) or (amodiaquine - 600 mg + (primaquine - 30-45 mg)</td>
</tr>
<tr>
<td>2. Where there is established sulfadoxine/pyrimethamine resistance</td>
<td>Mefloquine: In accordance with WHO Policy Statement</td>
<td>(Not applicable)</td>
<td>(Chloroquine - 600 mg + (B) or (amodiaquine - 600 mg + (primaquine - 30-45 mg)</td>
</tr>
<tr>
<td>Mass drug administration (3)</td>
<td>Sulfadoxine 1.5 g + pyrimethamine 75 mg + primaquine 30-45 mg</td>
<td>B or A on a focal basis</td>
<td>B or A on a focal basis</td>
</tr>
<tr>
<td>Radical cure (3)</td>
<td></td>
<td></td>
<td>(Chloroquine - 600 mg + (B) or (amodiaquine - 600 mg + (primaquine - 30-45 mg)</td>
</tr>
<tr>
<td>P. falciparum (sulfadoxine + pyrimethamine sensitive)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Uncomplicated</td>
<td>Sulfadoxine 1.5 g + pyrimethamine 75 mg + primaquine 30-45 mg</td>
<td>D or C on a focal basis</td>
<td>(Chloroquine 1.5 g base in (3 days</td>
</tr>
<tr>
<td>2. Acute complications</td>
<td>Quinine 2g/day p.o. x 3-7 days or initially 20-30 mg/kg/day i.v. as indicated + sulfadoxine 1.5 g + pyrimethamine 75 mg</td>
<td>(D (amodiaquine 1.4 g + (primaquine 30-45 mg base x 1)</td>
<td>(Quinine 2g/day p.o. x 7 days (or initially 20-30 mg/kg/day (i.v. as indicated (followed by chloroquine or (amodiaquine as above</td>
</tr>
</tbody>
</table>
Table 9. Suggested Use of Antimalarial Drugs in Areas With or Without Drug Resistance (1) (cont’d)
Adult doses, to be adjusted for age, See Appendix 5 for detailed description of alternative drug regimens

<table>
<thead>
<tr>
<th>Type of treatment</th>
<th>Areas where chloroquine-resistant <em>P.falciparum</em> is well established (2)</th>
<th>Areas where chloroquine-resistant <em>P.falciparum</em> is emerging</th>
<th>Areas without chloroquine-resistant <em>P.falciparum</em> and not seriously threatened by its potential spread</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P.falciparum</em> (sulfadoxine + pyrimethamine resistant)</td>
<td>Quinine 2 g/day p.o. x 3-7 days + tetracycline 2 g/day x 7-10 days or Mefloquine: In accordance with WHO Policy Statement</td>
<td>(Not applicable)</td>
<td>(Not applicable)</td>
</tr>
<tr>
<td>1. Uncomplicated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Acute complications</td>
<td>Quinine 2 g/day p.o. x 3-7 days or initially 20-30 mg/kg/day i.v. as indicated</td>
<td>Tetracycline 2 g/day x 7-10 days or Mefloquine: In accordance with WHO Policy Statement</td>
<td>(Not applicable)</td>
</tr>
<tr>
<td><em>P.vivax</em> and <em>P.ovale</em></td>
<td>Chloroquine 1.5 g or amodiaquine 1.4 g base for 3 days + primaquine 15-22 mg/day x 5-14 days. (In G-6-PD deficient individuals give chloroquine or amodiaquine as above + primaquine 45 mg once a week x 8 weeks.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P.malariae</em></td>
<td>Chloroquine or amodiaquine as for <em>P.vivax/P.ovale</em>.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prophylaxis for non-immunes</td>
<td>Sulfadoxine 500 mg + pyrimethamine 25 mg one weekly or Dapsone 100 mg + pyrimethamine 12.5 mg once weekly (with chloroquine 300 mg weekly as protection against species other than <em>P.falciparum</em> With personal anti-vector precautions.</td>
<td>(As in column at the left)</td>
<td>Chloroquine 300 mg weekly or Amodiaquine 400 mg weekly</td>
</tr>
</tbody>
</table>
### Table 9. Suggested Use of Antimalarial Drugs in Areas With or Without Drug Resistance (1) (cont'd)

Adult doses, to be adjusted for age. See Appendix 5 for detailed description of alternative drug regimens.

<table>
<thead>
<tr>
<th>Type of treatment</th>
<th>Areas where chloroquine-resistant <em>P. falciparum</em> is well established (2)</th>
<th>Areas where chloroquine-resistant <em>P. falciparum</em> is emerging</th>
<th>Areas without chloroquine-resistant <em>P. falciparum</em> and not seriously threatened by its potential spread</th>
</tr>
</thead>
<tbody>
<tr>
<td>(sulfadoxine + pyrimethamine resistant)</td>
<td>Sulfadoxine 500 mg + pyrimethamine 25 mg once weekly or dapsone 100 mg + pyrimethamine 12.5 mg once weekly (with chloroquine 300 mg weekly as protection against species other than <em>P. falciparum</em> where they are resistant to pyrimethamine) With personal anti-vector precautions. Consult experienced physician if fever occurs.</td>
<td>(Not applicable)</td>
<td>(Not applicable)</td>
</tr>
</tbody>
</table>

**Note**

1. Intended primarily for the South-East Asia and Western Pacific Regions and not necessarily applicable to other Regions.
2. At present it is not possible to define a generally applicable criterion for discontinuation of the use of chloroquine in clinical presumptive treatment. Research is urgently required. For the time being this decision should be made by health authorities based on the best local information.
3. For definitions, see WHO TRS No. 529 (1973).
4. In areas where treatment of clinical cases is the only measure of malaria control, it may be essential to retain chloroquine in addition.
5. If option A has to be used in areas with a significant proportion of species other than *P. falciparum*, chloroquine 600 mg should also be used.
6. Mefloquine by itself must not be used for mass drug administration.
Fansidar in Thailand is a case in point, and the history of antibiotic abuse in the field of antibacterial chemotherapy gives further support to this argument.

From the statements above (see page 63), it is evident that the greater the drug selection pressure that is exerted on a parasite population, the sooner will resistant mutants be selected. Thus it is logical to suggest that antimalarial drugs should be used as little as possible. In this connexion the following major areas of drug deployment need to be considered:

Chemosuppression and prophylaxis. Drug administration may be considered for certain groups of people exposed to high-intensity transmission. These groups include populations under high risk of severe or complicated disease, e.g. children under five and pregnant women. Other groups may also be considered eligible, for example some migratory labour forces, certain closed communities such as military camps in transmission areas, as well as nonimmune migrants, visitors and pilgrims going to endemic areas. With very minor exceptions, large-scale prophylactic use of antimalarial drugs (as the sole control measure) has never succeeded in making a lasting impact on the intensity of transmission or the level of endemicity of malaria. On the contrary such a use has inevitably been followed after a shorter or longer period of time by the emergence of parasites of one or more species that are resistant to the drugs that have been used. Prophylactic drug use should therefore be restricted.

Treatment of fever cases. Much restraint should be exercised by antimalaria services in the use of single dose treatment of fever cases and it is to be hoped that, with the further development of services in the regions, the need for such treatment will decrease. Radical treatment where drugs are only given after microscopic diagnosis, is the preferred method of drug administration.

However, in the meantime, it is recognized that single dose treatment of fever cases needs to be administered by the following malaria workers: primary health care personnel such as community health workers, malaria volunteers, active case detection teams and malaria survey teams.

Mass drug administration (MDA) for the control of epidemics in situations where vector control measures cannot be applied, and for the elimination of residual foci in control eradication programmes. For maximum benefit, mass drug administration should in principle only be carried out under the cover of parallel vector control operations. In instances in which services cannot adhere to this principle, it is wise to keep to a minimum the period during which MDA is being carried out.
Radical therapy of microscopically diagnosed cases. It is self-evident that a patient suffering from symptomatic malaria should be radically treated, be the illness of an acute nature or simply manifested by relatively minor clinical symptoms.

The question arises as to whether in holoendemic areas, semi-immune asymptomatic parasite carriers should be given treatment.

The question is particularly pertinent in areas holoendemic for malaria where a major portion of the population may be asymptomatic carriers. With the exception of those individuals who fall under the heading of high risk or special groups as referred to in the previous section, the answer, in the absence of intensive parallel control measures (e.g., effective vector control), is probably not to treat them unless they develop symptoms attributable to their parasitaemia.

(b) Use of drug combinations

There is abundant evidence that the use of appropriate combinations of drugs can at least delay the rate at which microorganisms, including malaria parasites, become resistant to them, as compared to the components when used alone. Combinations can be of several types:

- Complementary
  - acting on different stages of the parasites, e.g., chloroquine plus primaquine in the treatment of vivax malaria or in the prevention of transmission of malaria by the gametocytocidal action of primaquine.
  - acting on the same stages, e.g. sequential quinine and tetracycline in the therapy of multiple-resistant P. falciparum infection.

- Additive
  - acting on the same stages, e.g. chloroquine plus pyrimethamine used prophylactically to prevent resistance from developing in the asexual stages (apart from a complementary, sporontocidal effect of the pyrimethamine)

- Potentiating
  - sulfonamides or sulfaones with pyrimethamine. The potentiating mixture retaining its activity against all stages of the cycle which are fundamentally susceptible.
In relation to the prevention of drug resistance, the complementary use of primaquine together with blood schizontocides is probably the most valuable measure that can be taken since this compound is equally effective against the gametocytes of drug-sensitive or multiple drug-resistant P. falciparum, a single 30 mg base (adult) dose being sufficient for this purpose.

The large-scale deployment of an additive mixture of chloroquine with pyrimethamine which was selected purely empirically, with no substantiating experimental background proved, in the event, not to prevent the emergence of pyrimethamine-resistant and, in some areas, multiple drug-resistant strains of P. falciparum. There is good experimental evidence, however, to suggest that other additive drug mixtures may be effective in delaying the selection of resistant mutants.

Potentiating mixtures of DHFR inhibitors with sulfonamides or sulfones may delay significantly, but do not entirely prevent the development of parasites that are resistant even to these mixtures, and the example of Fansidar resistance in Brazil and Thailand bears this out. It should be noted that the development of a serious problem of resistance to Fansidar required at least a decade of large-scale drug use even in a situation where resistance to pyrimethamine alone was already present. However, once established, resistance to Fansidar appears to spread quite rapidly.

**Drug Monitoring**

In order to be forewarned of any possible change in the drug response pattern in a given area, to interpret its significance and to plan and implement any essential, appropriate operational answers, it is necessary to establish baseline data and an effective monitoring system. Such a system should monitor the sensitivity status related to all operational and advanced candidate blood schizontocidal drugs and be based on the use of in vivo and in vitro methods, and, if so indicated, be complemented by pharmacokinetic studies. The development of standardized methods of in vivo and in vitro tests for drugs other than 4-aminoquinolines, the provision of standardized test material, and the establishment of threshold levels of in vitro data as related to in vivo response require urgent attention.

**Management of Established Drug Resistance**

Drug-resistant micro-organisms as a general rule suffer from a number of biological disadvantages over the drug-sensitive parent stocks. In the absence of drug selection pressure there is generally a reversion towards a normal response to the drug due to the overgrowth of resistant organisms by sensitive ones. For antimalarial DHFR inhibitors there appears to be evidence that after the withdrawal of pyrimethamine from a pilot area in
Tanzania several years ago, there was within this area a gradual return to pyrimethamine sensitivity of *P. falciparum*. A more recent investigation detected pyrimethamine resistance outside the original pilot area despite the absence of pyrimethamine pressure. This however may well be due to the use of other DHFR inhibitors in the area, not normally employed for the treatment of malaria.

In contrast, chloroquine-resistant *P. falciparum* appears to have biological advantages over chloroquine-sensitive parasites and, once established, resistant parasites usually manifest a rapid geographical spread into receptive areas. Resistance is stable in the absence of drug selection pressure in experimental models and, possibly also in *P. falciparum* in nature. It may thus not be sufficient simply to withdraw chloroquine and chemically related drugs and hope that the problem will resolve itself.

Chloroquine resistance now goes hand in hand with resistance to DHFR inhibitors in most countries and, in some, even with resistance to potentiating combinations of pyrimethamine with sulfonamides. Multiple drug-resistance thus poses the most serious threat to health and life in such areas.

**Conclusions**

The above consideration of the factors related to drug resistance in *P. falciparum* suggest policies that may help to minimize the further development of resistance to existing compounds, and to safeguard any new drugs that may be introduced in the future:

(a) The rate of emergence of drug resistance is a function of the number of parasites exposed to the drug. It is therefore indicated to:

- limit the distribution of antimalarials through national health legislation
- select priority groups for prophylaxis
- consider whether semi-immune asymptomatic carriers require treatment or not.

(b) Restriction of the transmission of gametocytes of resistant *P. falciparum* by the judicious use of single-dose primaquine along with providing radical cure with blood schizontocides.

(c) Establishing an effective drug monitoring system including *in vitro* testing.

(d) Avoiding attempts to control the transmission of malaria by drug distribution alone. Deployment of drugs for
control only as part of an integrated campaign including effective vector control measures.

(e) Establishing and imposing government control on import and use of new antimalarials, e.g. mefloquine, so as to minimize their abuse.

(f) Encouraging the use of judiciously selected and tested drug combinations. This does not necessarily mean the acceptance of empirically formulated antimalarial combinations proposed by members of the pharmaceutical industry, as has happened in the past.

(g) Encouraging research on antimalarials involving national health research centres and universities, especially in collaboration with WHO and other international research organizations.
VII.

Field and Laboratory Research for the Control of Resistant *P. falciparum*

There are several areas in which field research workers can contribute to the study of drug resistance in *P. falciparum* and to the evaluation of measures aimed at its containment. These areas of study and investigation include the parasites' response to drugs by *in vivo* and *in vitro* tests, sensitivity monitoring, drug influence on parasite transmission, pharmacogenetic variations in drug metabolism, the relation of immunity and nutrition to drug response, the importance of population movement, the provision of data on drug usage and drug control, and the control of advanced clinical trials of new drugs and drug formulations.

**GENERAL TOPICS**

Problems requiring field and laboratory research could be broadly listed as follows:

**Epidemiology**

General epidemiology of drug resistance needs to be studied, including those factors of a biological, ecological, social, economic, cultural, or political nature which have a bearing on the emergence of drug-resistant malaria. This may include an analysis of geographical spread and the identification of causative factors of the current distribution of resistant *P. falciparum*.

**Control**

An evaluation of the effectiveness of vector control and the assessment of the potential of drugs in reducing the reservoir and
the transmission of resistant *P. falciparum* are important subjects. Similarly, measures aimed at the reduction of man-vector contact need to be assessed for their effectiveness and appropriate technologies that can be applied by the community need to be developed.

**Biological Factors**

Parasite strain characteristics, the infectivity of resistant parasites to vectors as compared to that of sensitive parasites and the efficiency of various vectors in transmitting resistant parasites need to be assessed.

**Assay Techniques**

The simplification of *in vivo* tests, the development of *in vitro* test kits for all clinically and operationally used drugs as well as the development of a simplified warning test for the early detection of suspected resistance are required.

**Practical Aspects of Research Implementation**

It is recognized that the research topics listed under Epidemiology and Control (see page 75) are of such complexity that single projects are not expected to yield the essential information. It would therefore be necessary to promote and assist in the design and evaluation of multiple research projects in a variety of ecological, vectorial and epidemiological situations. The resources needed to implement these projects exceed those available through TDR and should be sought from national, bilateral and international sources. WHO could assist in the identification of appropriate sources for support.

**SPECIFIC TOPICS**

Specific field research subjects which should be investigated in TDR supported projects are listed below:

**Factors Associated with the Parasite**

- baseline responses in different areas to chloroquine and other drugs
- techniques for early detection of drug resistance
- mapping of resistant strains
- monitoring of changes with time, dynamics of drug resistance
- epidemiological tracing of origins of resistant strains
- ability of local vectors to transmit sensitive as compared to resistant parasites
- parasite resistance in relation to biological characteristics.
In vivo and in vitro procedures are now available or are being developed for establishing the baseline responses of *P.falciparum* to chloroquine, amodiaquine, quinine, mefloquine, pyrimethamine and sulfadoxine. While some countries, e.g. Malaysia and Thailand, have taken steps so that these techniques are utilized to determine the response of the parasites to some of these drugs, other countries should be encouraged to institute teams capable of undertaking this work in a number of key centres.

A good procedure must be to ensure the very early detection of new foci of drug resistance.

The drug sensitivity teams should be organized in such a way that they can be deployed to monitor the responses to standard antimalarials by repeated testing in selected areas, and by an in-depth assessment of responses where resistance is suspected on clinical grounds. In this way changes in drug responses on a country and regional basis can be plotted both geographically and in relation to time.

When resistance to a particular drug is first confirmed in a new area (e.g. chloroquine resistance in East Africa), it would be invaluable to be able to determine whether the source of the resistant parasites is autochthonous or imported in order to plan appropriate containment measures. At present, no certain method exists for pinpointing the geographical origin of a parasite. However, ongoing studies on the biochemical characterization of *P.falciparum* based on isoenzyme typing may, in the near future, provide such a method. This could then be used by field teams for relevant epidemiological investigations, or field workers could collect isolates for subsequent examination in reference laboratories.

The epidemiological impact of single-dose primaquine for gametocytocidal action needs to be assessed in order to determine the desirability of deploying this drug for limiting the spread of drug-resistant *P.falciparum*.

Experiments both in laboratory models and with *P.falciparum* in Thailand have suggested that the use of chloroquine may enhance the transmission of chloroquine-resistant parasites. This requires further investigation, not only in relation to chloroquine but also to other blood schizontocides. Appropriate studies could be carried out conjointly between laboratory and field workers in endemic areas. In view of the currently increasing use of and resistance to sulfonamide/antifol combinations, it is especially important to determine whether the enhancement of gametocyte production that sometimes accompanies the administration of DHFR inhibitors, sulfonamides or sulfones results in a similar phenomenon. The remarkably rapid rate at which Fansidar resistance has recently been increasing in Thailand, for instance, suggests that such a factor may be playing a role.
Factors Associated with the Human Host

- ethnic variations in drug metabolism
- relation of immunity to drug-resistance
- importance of nutritional status
- importance of population movement, refugees, etc.

A number of workers have suggested that individuals belonging to different ethnic groups may metabolize certain antimalarials in different ways, or at different rates, e.g. coastal Nigerians who, it seems, commonly develop pruritus on use of this drug may be metabolizing it by unusual pathways. Individuals with a deficiency of G-6-PD develop acute haemolysis when given repeated doses of primaquine, while the excretion half-life of this compound may be shorter in Thais than in Caucasians. All such variations may influence the drug regimen that is optimal for the inhabitants of a given area. While the detailed study of genetic influences of drug metabolism is clearly a function of specialized laboratories, field workers can play a role in the provision of patients, specimens and clinico-epidemiological data in association with this type of investigation.

Chemotherapy helps the human organism to overcome infection, and a substantial part of killing malaria parasites and their removal from the circulation is effected by immune mechanisms of the host. The "semi-immune" host can cope with and tolerate a higher parasite load than a nonimmune individual. Thus resistant falciparum parasites may be present in a population of "semi-immunes" for some time before the introduction of nonimmunes in the form of new-born infants, or immigrants - acting as "sentinels" - exposes the presence of resistance by leading to acute infections that patently do not yield to standard antimalarial therapy. The level of individual and communal immunity can be shown by malariometric surveys that include serological as well as parasitological parameters. Surveys of this nature could be organized in parallel with surveys of drug response that utilize a suitable in vitro technique.

The transfer of resistant parasites by people moving from one locality to another is clearly of major importance in the spread of drug resistance. Consequently it is essential to determine the origin of resistant parasites when they are first detected in a new area. There may be numerous situations where it is possible for field teams to monitor the movement of resistant parasites from area to area, by checking blood films from travellers at frontier posts, refugee centres, etc., by recording the clinical and parasitological responses of such individuals following therapy, and by conducting in vitro tests on blood samples from them.

In certain situations, it may be necessary to establish a system for giving presumptive treatment to certain categories of
migrants or immigrants (see Table 9) in order to limit the spread of resistant parasites, and field studies should be established to determine the possible ways of achieving this objective.

Not only infected people but also infected anophelines may spread resistant falciparum from one area to another. Field teams should study the ways in which this may occur and methods for prevention, e.g. insistence on vector control measures on aircraft and ships, sanitation of receptive areas around possible ports of entry, etc.

Factors Related to Drug Deployment

- types and quantities of antimalarial drugs used
- total drug consumption in the country
- logistics of drug control and supply

Some of the most difficult questions to answer have proved to be related to the types of drugs used, the population groups using these drugs and the source of drugs. Similarly the quantities of drugs imported into or produced for home consumption in a country and their distribution (private enterprise and/or government), their selling prices, the existence of a black market for antimalarial drugs and the degree of real government control over drug distribution are factors which may have a major bearing on the occurrence and the spread of drug resistance.

This type of information which can be gathered by field researchers would be invaluable in trying to understand the reasons underlying the origin and spread of resistance of P.falciparum to various drugs. At present, accurate data on these points are extremely scarce.

Factors Related to the Containment of Resistance

- alternative drugs to be used
- criteria for changing and/or withdrawing drugs
- role of drugs and/or control measures, in the containment of drug-resistant malaria, e.g. in the elimination of isolated foci
- determination of the effect of withdrawing drugs on the resistance status
- assessment of the effect of the alternation of drugs.

The question of which drugs can be used as an alternative to chloroquine or Fansidar must be tested in the field. There are, unfortunately, only too few alternatives at present and reference is made to page 63 of this report.

To what degree other control measures are applicable in a given area as part of an integrated control programme, in
association with chemotherapy, can only be determined by a careful malariaiological field study of each situation following which a plan of action can be "tailor-made" to fit the epidemiological circumstances and the potential of the local health authorities.

A change of policy regarding the use of chloroquine (or sulfadoxine/pyrimethamine), particularly at a peripheral health service level, when resistance emerges, is a particularly difficult question on which no blanket recommendation can be made. Appropriate investigations should be carried out in the field to determine at what level of resistance a drug should be withdrawn and replaced by an alternative drug if, indeed, for practical reasons, it is at all possible to withdraw it. For example, chloroquine may still be essential to relieve symptoms or even be life-saving, despite a moderate, yet well established level of resistance in a particular area. It may also not be possible or desirable to replace completely chloroquine with sulfadoxine/pyrimethamine for presumptive treatment. A further problem arises in areas where a significant degree of resistance has emerged also to sulfadoxine/pyrimethamine.

Research is needed to determine the part that drugs should play in relation to other control measures, for example in the elimination of isolated foci of resistant parasites.

While experimental and epidemiological evidence suggests that chloroquine resistance is a highly stable, if not a dominant character of P. falciparum, no data exist to show whether parasites could revert to normal sensitivity if drug selection pressure were to be totally withdrawn for a period of time. Field studies to determine this could be established, e.g. in island populations.

No data are available from the field on the possible influence of the alternation of drugs on the development of drug resistance. Both retrospective and prospective studies are required on this question.

Factors Related to Clinical Pharmacology

- optimal dosage schedules of available drugs
- value of drug combination
- clinical trials of new drugs

Certain questions still remain concerning the use of existing drugs, e.g. the optimal dosage of chloroquine for presumptive treatment, dosage and timing of primaquine administration as a gametocytocide. These should be addressed in appropriate field research projects.

In the course of the development of new drug formulations, drug combinations or entirely new drugs, there comes a time when the preparation, having passed the hurdles of pre-clinical studies
and Phase 1 and 2 clinical trials, enters the ultimate phases which are (ref. TRS.529):

- Phase 3: Pilot field trials in partially immune subjects
- Phase 4: Treatment of hospital patients
- Phase 5: Extended field trials
- Phase 6: Observations on mass drug administration

While dose range-finding studies, and tolerability studies in different ethnic groups are essentially part of Phases 3 and 4, and the responsibility of specialized clinical workers, field research in drug development will be required with the release of a new preparation for Phase 5. Detailed protocols must be drawn up in close collaboration with investigators who have been responsible for the earlier phases of development, and, in the context of the present meeting, with the Secretariat of CHEMAl.

**PRIORITY TOPICS**

The following research topics were selected as being appropriate for field or laboratory based research and deserving priority consideration.

**Effect of Gametocytocidal Drugs**

The effect of single doses of gametocytocidal drugs (e.g. primaquine) on the transmission of *P. falciparum*, including drug-resistant *P. falciparum*, as a complementary measure to vector control needs to be evaluated.

The place of gametocytocidal treatment in malaria control should be assessed in various epidemiological situations. This could be done in circumscribed populations which are sufficiently stable and accessible to allow adequate evaluation.

When second-line drugs are being used (quinine or sulfadoxine/pyrimethamine or mefloquine) for the treatment of chloroquine-resistant (or sulfadoxine/pyrimethamine-resistant) malaria, it becomes necessary to investigate whether the spread of these resistant strains will be restricted by the addition of primaquine to this regimen. Furthermore, it would be indicated to study the impact of the periodic (e.g. weekly) administration of primaquine on the spread of drug-resistant strains.

**Comparative Vector Competence for Resistant and Sensitive Parasite Strains**

With regard to vector competence it has been suggested in the past that the transmission of chloroquine-resistant *P. falciparum* has been associated preferentially with one or more species of *Anopheles*. While there seems to be only questionable evidence to
support this theory, field studies should be conducted in affected areas to determine whether a preferential receptivity exists in certain anopheline species or strains favouring the transmission of drug-resistant malaria. If such is the case, the selective control of the more receptive species could be advantageous.

The approach of such research would include studies on the natural infection rates of anophelines in the field and the experimental comparative feeding of anophelines incriminated or suspected as vectors in an affected area, using known resistant and sensitive parasites (preferably from the same area). Complementary laboratory research, using anophelines colonized from various areas and drug sensitive and resistant parasite isolates cultured in vitro, can provide predictive information on the potential receptivity of vector species prior to the natural emergence of resistance.

Early Detection of Resistance

A simplified warning test for the early detection of resistance is urgently required. Studies should be made in areas adjacent to those in which drug resistance has already been detected as well as in all others in which transmission of *P. falciparum* persists in spite of vector control measures. (A summary outline proposal of such a research project is given in Appendix 7, proposal 1).

Drug Screening and Evaluation

(a) Continued screening and evaluation by the in vivo and in vitro techniques of natural and synthetic compounds for antimalaria activity is required to identify much needed new drugs.

(b) Continued evaluation of the efficacy of various combinations and dosage regimens of available antimalarial compounds needs to be carried out in different areas.

(c) The usefulness of amodiaquine in areas with low level in vivo or in vitro resistance to chloroquine, should be evaluated with regard to presumptive therapy, radical treatment and chemoprophylaxis.

(d) It should be determined whether the sulfone/pyrimethamine combination is still effective for chemoprophylaxis when there is resistance to sulfonamide plus pyrimethamine combinations. (A summary outline is given in Appendix 7, proposal 3.)

Parasite Strain Characteristics and Sensitivity

(a) Techniques for strain characterization of the malaria parasite need to be developed and reference resources established.
(b) Methods and test kits for assessing the sensitivity of malaria parasites to available antimalarials need to be further developed.

Clinical Pharmacology and Pharmacokinetics

(a) The clinical pharmacology and pharmacokinetics of antimalarial drugs such as chloroquine, amodiaquine, sulfadoxine plus pyrimethamine, quinine, and sulfalene plus pyrimethamine should be investigated particularly in indigenous semi-immune populations including children, patients with acute malaria, patients with impaired renal and/or hepatic function, malnourished persons, and persons with other concomitant infectious diseases. (A summary outline is given in Appendix 7, proposal 2.)

(b) Prospective and retrospective investigations should be undertaken in order to assess an eventual risk of sulfonamide/pyrimethamine and sulfone/pyrimethamine combinations to pregnant women (first and third trimesters) and their offspring as well as to infants under 6 weeks of age.

Drug Development

The development of more effective gametocytocidal and sporontocidal regimens is needed. In particular the dose related response of P.falciparum gametocytes to primaquine should be studied in various geographical areas so as to determine the minimum dose required for both gametocytocidal and sporontocidal effect.
VIII.

Recommendations

Problems of a technical and operational nature and more particularly the multiple resistance of *P. falciparum* infections to drugs have created an alarming situation. For instance, in some large areas of the Western Pacific and South-East Asia, *P. falciparum* malaria prevention through the use of antimalarials is no longer feasible, a problem which is likely to spread rapidly. Moreover, the implementation of reinforced attack measures for the control of malaria in general and for the prevention of the further spread of resistant *P. falciparum* malaria is beyond the reach of national resources of malaria stricken countries. Consequently, the containment and control of malaria require not only international and bilateral assistance but also coordination at country, inter-country, regional and global levels.

It is believed that the uncontrolled widespread use of antimalarial drugs, particularly 4-aminoquinolines and antifols, has led to the present serious situation of drug-resistant malaria. To deal with this situation research efforts are required in several areas. There is a lack of both basic and applied knowledge concerning the genetics of plasmodia which play an important role in the emergence, spread and control of drug-resistant malaria. There is also a lack of precise information in most countries on the distribution and intensity of drug resistance in *P. falciparum* as well as uncertainty concerning the correlation between *in vivo* and *in vitro* tests for monitoring the resistance to some drugs and a need to relate *in vitro* and *in vivo* drug response to the actual serum levels in treated subjects. Other needed investigations cover the variation in the response of strains of *P. falciparum* to drugs in different geographical areas, the increase in spread and intensity of resistance to the available antimalarials (particularly 4-aminoquinolines, pyrimethamine
and sulfonamides), and further examination of the mode of action efficacy and dosage of existing antimalarial compounds, including traditional preparations. Finally, there remains the difficult task of developing new and effective antimalarial compounds that are needed in view of the limited number of alternative drugs available.

The control of communicable diseases in general, and of malaria in particular, requires well trained and experienced national staff. Priority needs to be given to the development of national expertise and, in this connexion, joint efforts have been made by national institutions, the scientific community as a whole and the World Health Organization in promoting specialized training enabling national staff to undertake studies on the resistance of P.falciparum to antimalarials. However, the development of suitably qualified manpower requires further efforts at national and international levels.

In view of the above situation which, it is felt, requires the urgent implementation of exceptional measures to contain the actual malaria foci and prevent their further spread, thereby enabling attainment of the goal of "Health for all by the year 2000", the Meeting made the following recommendations:

NATIONAL AND INTERNATIONAL COOPERATION

(i) Countries affected by Plasmodium falciparum malaria should make every effort to strengthen and intensify all the appropriate control measures currently available in order to achieve maximum containment of this disease.

(ii) WHO should continue to cooperate with member states by stimulating and facilitating rapid exchange of information regarding drug-resistant malaria, by disseminating relevant technical documentation, and by organizing meetings at inter-country, regional and interregional levels.

(iii) Recognizing the problem of drug resistance as one of top priority, WHO should assist member states in mobilizing additional financial resources from bilateral and other international agencies.

PARASITE GENETICS

(iv) WHO should convene a meeting of experts to examine existing knowledge on the genetics of plasmodia, and determine lines of study in the laboratory and field, relevant to the matter.
DEVELOPMENT OF IN VIVO AND IN VITRO TESTS FOR MONITORING DRUG RESISTANCE

(v) In vivo tests should be broadened and adapted to include drugs other than 4-aminoquinolines, redefining, if necessary, the levels of drug response S, RI, RII and RIII.

(vi) In vitro tests for chloroquine and other drugs should be further developed and adapted for field use, and efforts should be made for their early introduction into the monitoring programme;

(vii) In certain countries studies on the correlation of the results of the in vitro microtechnique and the in vivo tests for assessing the response of P. falciparum to chloroquine, mefloquine and other antimalarials should be conducted and related to actual serum (plasma) drug levels;

(viii) In respect of mefloquine, baseline in vitro sensitivity data should be obtained and, in any country where this drug becomes operational, the response of P. falciparum should be continuously monitored in vitro and in vivo;

(ix) The monitoring of the response of P. falciparum to chloroquine and other antimalarials, in respect of the degree of drug sensitivity and the distribution of drug resistance should be continued and extended on a scientific basis.

EVALUATION AND DEVELOPMENT OF ANTIMALARIAL DRUGS

(x) Efforts should be increased to provide new compounds, including indigenous drugs and combinations of compounds, and to determine optimal drug regimens, and their efficacy should be evaluated in vivo and in vitro in areas of multiple drug resistance. Particular attention should be paid to the provision of drugs suitable for use at Primary Health Care (PHC) and community levels.

(xi) Attention should be paid to the continuing need for experimental in vivo testing systems, for example Aotus and rodent models, in addition to in vitro techniques.

(xii) Additional research should be undertaken by appropriate institutes into the mode of action of currently available and new antimalarials, as well as into mechanisms of the development of resistance to drugs by P. falciparum.

1 In view of the importance of the search for new drugs and the more effective utilization of already available and new compounds, these recommendations complete those made in Chapter VII of this report.
(xiii) Simple techniques should be developed, if possible, for the measurement of blood levels of antimalarial drugs in order that chemotherapy may be applied more rationally.

(xiv) Simple urine tests should be developed for antimalarial drugs including quinine and mefloquine.

(xv) Field trials should be undertaken to establish the optimal dosage levels of primaquine for producing a full sporontocidal effect against *P. falciparum* in different areas.

(xvi) A protocol should be designed to monitor any increase in the prevalence of blackwater fever in areas where the use of quinine is being increased.

(xvii) An appropriate study should be designed in certain island situations where chloroquine-resistant *P. falciparum* is predominant in order to determine whether or not *P. falciparum* remains resistant to chloroquine in areas where this drug has been withdrawn. A tentative design of the study has been proposed, including the size of the population to be studied and measures of intervention that are required to protect the population at risk. Details of the study design, however, would depend on specific epidemiological features of areas that may be considered for the proposed study. Considering this recommendation to be of priority, further efforts should be made for the development of a study design which would cover all scientific, epidemiological and ethical aspects of the study.

(xviii) Any new antimalarial drugs that become available in the future for the treatment of multiple-resistant falciparum malaria, e.g. mefloquine, should be used only under the auspices of the National Health Authorities, and if necessary, legislation should be provided to control the importation and distribution to the public of such compounds.

(xix) Guidelines on drug use should be provided to the medical profession and public by the National Health Authorities.

**TRAINING**

(xx) The establishment of the WHO Secretariat for the interregional training centre at Kuala Lumpur should be accelerated in order to promote among others the following training activities:

- assistance to national malaria training centres in augmenting the teaching staff and training facilities required for conducting local courses;
- organization of workshops on technical subjects of high relevance at national and/or international level, including in vitro micro-techniques, monitoring of drug-resistant *P. falciparum*, and containment of drug-resistant malaria;

- organization of workshops for the promotion/training of staff (to be) engaged in field applied research.

(xxix) The UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases should continue to support specialized training and field applied research relative to the problem of drug-resistant malaria.
IX.

Summary

The Meeting on Drug-Resistant Malaria held in Kuala Lumpur on 10-15 August 1981, has promoted the exchange of experience between programme directors and field research workers confronted with the problem of drug-resistant falciparum malaria in countries of the South-East Asia and Western Pacific Regions, and has strengthened their contact with the Scientific Working Groups on the Chemotherapy of Malaria and Applied Field Research in Malaria. This cohesion is required in order to accelerate and intensify research aimed at practical solutions to the problems of drug resistance.

Chloroquine resistance of Plasmodium falciparum involves by now all malarious countries of the Western Pacific Region, and has shown further westward and southward spread in the South-East Asia Region, besides an increase of frequency and degree of resistance in the extensive, earlier affected areas. The advent of resistance to second-line antimalarial drugs, such as long-acting sulphonamides combined with pyrimethamine in several countries of both Regions, and of South America, is a cause for special concern, especially where combined with other technical problems favouring malaria transmission, such as insecticide resistance of vectors and their evasive behaviour as well as population movement.

Besides financial, administrative and technical constraints, which limit the application and effectiveness of major malaria control efforts in many of the affected areas, the very limited range of operationally applicable antimalarial drugs poses the most serious obstacle to the elimination of foci and the reduction of the problem in its epicentres.

The meeting reviewed the monitoring of drug sensitivity, the dosages and schedules of drugs for the treatment and suppression
of *P. falciparum*, the strategies and methods for the control of drug-resistant malaria, and the policies for drug use. It established guidelines in these areas and proposed research lines for the clarification of urgent issues and the solution of pressing operational problems which are reflected in the recommendations.

The meeting also underlined the need for a speedy flow of information on all aspects of drug-resistant malaria, and for the training of personnel in basic research, applied field research and the interface of both research disciplines.

A substantial part of the meeting was devoted to the epidemiology and control of drug-resistant malaria; but an important concern was also the relevant aspects of basic research, which has its major role in the improvement of the use of existing drugs and the development of new candidate drugs, of which several have already been identified and one – mefloquine – is expected to be released for well directed operational use in the near future.
APPENDIX 1

List of Participants, Observers and Secretariat

PARTICIPANTS

South-East Asia Region

Bangladesh

Dr Mohi-Uddin-Ahamed
Parasitologist
Malaria Control Programme
Ministry of Health
(Principal Investigator)
Dacca

Dr A.I. Chowdhury
Chief Epidemiologist
Ministry of Health
Dacca

Dr S.M. Nuruzzaman
Deputy Director In-charge
Malaria and Parasitic Diseases
Control Programme
Dacca

Burma

Dr Franco Tin
Maliariologist
Malaria Control Division
Department of Health
(Principal Investigator)
Rangoon
Dr U. Nyunt Hlaing  
Deputy Director  
Malaria Control Division  
Department of Health  
Rangoon

Dr Myint Lwin  
Head, Parasitology Research Division  
Department of Medical Research  
Rangoon

India

Dr S. Pattanayak  
Director, National Malaria Eradication Programme  
Ministry of Health  
(Principal Investigator)  
New Delhi

Dr C. Chawngdinga  
Assistant Director (Malaria)  
Directorate of Health Services  
Mizoram, Aizawl

Indonesia

Dr Nyoman Kumara Rai  
Chief, Malaria Control Programme  
Directorate General P3M  
Percetakan Negara 1  
Jakarta

Dr Cyrus Simandjuntak  
Research Officer  
Division of Diseases Ecology  
Health Research Centre  
Ministry of Health  
(Principal Investigator)  
Jakarta

Dr Poerwokoesoemo Roos Arbani  
Medical Officer  
Malaria Control Programme  
Directorate General of CDC  
Percetakan Negara 1  
P.O. Box 223  
Jakarta
Nepal

Dr M.K. Banerjee
Regional Malaria Officer
Nepal Malaria Eradication
Organization
(Principal Investigator)
Baneshwar
Kathmandu

Dr M.B. Parajuli
Chief Officer
Nepal Malaria Eradication
Organization
Baneshwar
Kathmandu

Sri Lanka

Dr P.B.R. Dias
Medical Officer
Laboratories & Transport
Anti-Malaria Campaign
(Principal Investigator)
Narahenpita
Colombo-5

Dr A.N.A. Abeyesundere (Rapporteur)
Superintendent & Head
Anti-Malaria Campaign
Narahenpita
Colombo-5

Thailand

Mrs Laksami Yisunsri
Assistant Chief, Epidemiology
Section
Malaria Division
Department of Communicable
Diseases Control
Ministry of Public Health
(Principal Investigator)
Bangkok

Dr Surin Pinichpongse
Director of Malaria Division
Department of Communicable Disease
Control
Ministry of Public Health
Bangkok
Western Pacific Region

Australia

Lt. Col.(Dr) A.D. Parkinson
Research Officer
1 Army Malaria Research Unit
Milpo, Ingleburn 2174
New South Wales

China, People's Republic of

Professor Zhou Zhujie
Deputy Director
Institute of Parasitic Diseases
Chinese Academy of Medical Sciences
Beijing

Lao People's Democratic Republic

Dr Sap Detvongsà
Chief, Malaria Control Programme
Department of Hygiene and Preventive Medicine
Ministry of Health
Vientiane

Mr Phommaly Sychangthavong
Senior Laboratory Technician
Department of Hygiene and Preventive Medicine
Ministry of Health
Vientiane

Malaysia

Dr P.J. Jacob (Chairman)
Deputy Director
Malaria Eradication Programme
Ministry of Health
Young Road
Kuala Lumpur

Dr S.P. Palaniapan
Assistant Director of Medical Services (Malaria)
Kota Kinabalu, Sabah

Dr Patau Rubis
Deputy Director of Health
Medical and Health Department
Kuching, Sarawak
Papua New Guinea

Dr Isabelo S. Dulay
Specialist Medical Officer
(Epidemiologist)
Department of Health
Malaria Control Headquarters
P.O. Box 2249
Konedobu

Philippines

Dr Cesar Valera
Chief of Medical Division II
Malaria Eradication Service
Ministry of Health
San Lazaro Compound
Sta. Cruz
Manila

Singapore

Dr Ling Mun Kin
Registrar (Epidemiology)
Quarantine and Epidemiology
Department
Ministry of the Environment
Princess House
Alexandra Road
Singapore 0315

Solomon Islands

Dr Nathan Kere
Chief Medical Officer
(Communicable Diseases)
Ministry of Health and Medical Services
P.O. Box 349
Honiara

Viet Nam, Socialist Republic of

Dr Nguyen Van Kim
Chief, Laboratory of Experimental Biology
Institute of Malariology, Parasitology and Entomology
Ministry of Health
Hanoi
Viet Nam (cont'd.)

Dr Nguyen Phouc Hong
Head, Department of Planning and Techniques
Institute of Malariology, Parasitology and Entomology
Ministry of Health, Hanoi

Fieldmal/Chemal Scientific Working Groups

Professor R. E. Desjardins
Department of Clinical Investigation
The Wellcome Research Laboratories
Burroughs Wellcome Company
3031 Cornwallis Road
Research Triangle Park
North Carolina 27709
United States of America

Professor Geoffrey Jeffery
US Department of Health, Education and Welfare
Public Health Service
Centres for Disease Control
Atlanta, Georgia 30333
United States of America

Dr H. C. Spencer
Clinical Research Centre
P.O. Box 20778
Nairobi, Kenya

Professor H. J. van der Kaay
Laboratory of Parasitology
State University of Leiden
Institute of Tropical Medicine
Rapenburg 33
Leiden, The Netherlands

CONSULTANT

Professor W. Peters
Director
Department of Medical Protozoology
London School of Hygiene and Tropical Medicine
Keppel Street (Gower Street)
London WC1E 7HT, United Kingdom
TEMPORARY ADVISERS

Professor Khunying Tranakchit Harinasuta
Professor of Tropical Medicine
Department of Clinical Tropical Medicine and
Hospital for Tropical Diseases
Faculty of Tropical Medicine, Mahidol University
Bangkok 4, Thailand

Dr A.P. Ray
Chief Coordinator, WHO/SIDA - P.f. CP/ARA
National Malaria Eradication Programme
Ministry of Health
New Delhi, India

Professor V. Thomas
University of Malaya
Kuala Lumpur, Malaysia

Dr W.J.O.M. van Dijk
142 van Beverningkstraat
The Hague, The Netherlands

OBSERVERS

Dr T.A.B. Björkman, Physician
Roslagstulls Hospital
Institute of Infectious Diseases
114 89 Stockholm, Sweden

Dr Tan Chongsuphajaisiddhi
Head, Department of Tropical Pediatrics
Faculty of Tropical Medicine, Mahidol University
Bangkok, Thailand

Dr Surinder Singh Dhillon
Malaria Eradication Programme
Ipoh, Malaysia

Dr K.E. Dixon
Chief, Department of Epidemiology
USA Medical Component, Armed Forces Research
Institute of Medical Sciences
Bangkok, Thailand

Dr Gurcharan Singh Gill
Malaria Eradication Programme
Kuala Lumpur, Malaysia

Dr R.S. Kumar
Orang Asli Hospital
Gombak, Malaysia
Dr R. Mariappan  
Public Health Institute  
Kuala Lumpur, Malaysia

Dr Lee Cheow Peng  
Malaria Eradication Programme  
Kuala Lumpur, Malaysia

Professor Tin Ohn  
University Kebangsaan  
Kuala Lumpur, Malaysia

Dr Lars Börje Rombo  
Medical Officer  
Roslagstulls Sjukhus  
Box 5901  
114 89 Stockholm, Sweden

Lt.Col. Dr Cheah Phee San  
Ministry of Defence  
Kuala Lumpur, Malaysia

Dr Lloyd L. Smrkovski  
Immuno-parasitologist  
U.S. Naval Medical Research Unit No.2  
Sarmiento Building, 6782 Ayala Avenue  
Makati, Metro Manila  
Philippines

Dr K.D. Sukumaran  
Institute for Medical Research  
Kuala Lumpur  
Malaysia

Dr (Mrs) Kew Siang Tong  
General Hospital  
Kuala Lumpur  
Malaysia

SECRETARIAT

WHO Headquarters, Geneva

Dr T. Lepes  
Director  
Malaria Action Programme

Dr S. Goriup  
Secretary  
Steering Committee of the Scientific Working Group on Applied Field Research in Malaria
Dr L.E. Molinesaux  
Epidemiologist  
Malaria Action Programme

Dr J.H.A. Pull  
Chief, Epidemiological Methodology  
and Evaluation (EME)  
Malaria Action Programme

Dr W.H. Wernsdorfer (Secretary)  
Chief, Research and Technical Intelligence (RTI)  
Malaria Action Programme

WHO, American Region

Dr F.J. Lopez-Antunano  
Malaria Adviser  
PAHO/WHO  
Washington, D.C.

Dr J.A. Najera-Morrondo  
Chief, Malaria, Parasitic Diseases  
and Vector Control  
PAHO/WHO  
Washington, D.C.

WHO, South-East Asia Region

Dr David F. Clyde  
Senior Regional Malaria Adviser  
New Delhi, India

Dr H.G. Cardenas  
Senior Malariologist  
Colombo, Sri Lanka

Mr J.R. Cullen  
WHO Technical Officer (Research)  
Malaria and Vector Control Project  
THA MPD 001  
Thailand

Dr E.B. Doberstyn  
Senior Malariologist  
Bangkok, Thailand

Dr T. Matsushima  
Medical Research Officer  
Special Programme for Research and Training in Tropical Diseases  
New Delhi, India
Mr W. Rooney  
Laboratory Specialist  
Intercountry Project ICP/MPD/001  
New Delhi, India

WHO, Western Pacific Region

Dr C.T. Ch'en  
Regional Malaria Adviser  
Manila, Philippines

Dr G. Farid  
Medical Officer  
Special Programme for Research and Training in Tropical Diseases  
Manila, Philippines

Mr J. Storey  
Technical Officer (Parasitology)  
Regional Antimalaria Team  
Kuala Lumpur, Malaysia
APPENDIX 2

List of Documents

A. WORKING PAPERS

<table>
<thead>
<tr>
<th>Document Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAL/DRUG/81.WP.1 (HQ)</td>
<td>Resistance of malaria parasites to drugs in the context of the malaria action</td>
</tr>
<tr>
<td></td>
<td>programme; studies to be undertaken in this respect to better define a realistic policy for the future. Dr T. LEPES</td>
</tr>
<tr>
<td>MAL/DRUG/81/WP.la (WPRO)</td>
<td>Chloroquine resistance studies in Papua New Guinea. Dr I.S. DULAY</td>
</tr>
<tr>
<td>MAL/DRUG/81.WP.1a, (SEARO)</td>
<td>Progress and planning report Bangladesh</td>
</tr>
<tr>
<td>MAL/DRUG/81/WP.1b (SEARO)</td>
<td>Progress and planning report Burma</td>
</tr>
<tr>
<td>MAL/DRUG/81/WP.1c (SEARO)</td>
<td>Progress and planning report India</td>
</tr>
<tr>
<td>MAL/DRUG/81/WP.1d (SEARO)</td>
<td>Progress and planning report Indonesia</td>
</tr>
<tr>
<td>MAL/DRUG/81/WP.1e (SEARO)</td>
<td>Progress and planning report Nepal</td>
</tr>
<tr>
<td>MAL/DRUG/81/WP.1f (SEARO)</td>
<td>Progress and planning report Sri Lanka</td>
</tr>
</tbody>
</table>
Progress and planning report
Thailand

Review of the Regional Collaborative Studies on Drug-Resistant *Plasmodium falciparum* malaria.

Analysis of answers to questionnaire.
Dr. L. Molineaux

Predicting the westward spread of drug-resistant *falciparum* malaria in Asia.
Dr. D. F. Clyde

A synthesis of the current situation of the drug response of *P. falciparum* in WPR including tentative explanation and projection.
Dr. W. J. O. M. van Dijk

A synthesis of the current distribution of drug response of *P. falciparum* malaria in the American Region.
Dr. F. J. López-Antunano and Dr. J. A. Najera-Morrondo

The role of population migration in the spread of drug-resistant *falciparum* strains.
Dr. S. Pinichpongse and Dr. E. B. Ooberstyn

The role of population migration in the spread of drug-resistant *falciparum* strains.
Dr. Nyunt Hlaing

Chloroquine and mefloquine sensitivity testing in the field.
Dr. A. D. Parkinson et al.

Studies on drug-resistant *P. falciparum* with the *in vitro* and *in vivo* tests carried out on the same infections.
Dr. C. Valera

Technical aspects of interest noted during the studies on the response of *P. falciparum* to chloroquine in the Philippines, with the *in vitro* technique (macro test).
Dr. C. Valera

Application of the micro *in vitro* technique on the response of *P. falciparum* to antimalarial drugs in South-East Asia.
Mr. W. Rooney
Comparative study on the micro in vitro and the in vivo tests on the response of *P. falciparum* to chloroquine in Burma. Dr Myint LWIN et al.

Monitoring of drug response: tests, data processing and analysis, sampling. Dr L. MOLINEAUX and Mr D. PAYNE

Development of drug sensitivity monitoring programmes: future needs and funding. Dr S. GORIUP

Biological aspects related to drug resistant *P. falciparum* and its control. Dr W.H. WERNSDORFER

Alternative drugs to be used against *P. falciparum* malaria; practical guidelines and suggestions. Professor R.E. DESJARDINS

Alternative drugs to be used against multi-resistant *P. falciparum* malaria with special reference to the present situation in Thailand. Professor T. HARINASUTA

Alternative drugs to be used against multi-resistant *P. falciparum* malaria, with special reference to drug combination comparative trials in Burma. Dr Franco TIN

Control of transmission of drug-resistant *P. falciparum* with particular reference to the containment programme in India. Dr A.P. RAY

The effect of *P. falciparum* resistance on the application of the four tactical variants and suggestions to overcome expected implications. Dr G.A. FARID

Policies on drug use aiming at preventing, delaying or reversing the selection of resistant *P. falciparum* parasites. Professor W. PETERS

Field research on the control of transmission of drug-resistant *P. falciparum*. Professor G. JEFFERY
Suggestions for field research relating to drug-resistant \textit{P.falciparum}.

\textbf{Professor W. PETERS}

Format for outlining field research projects.

\textbf{Dr L. MOLINEAUX}

\section*{B. INFORMATION PAPERS}

\begin{itemize}
  \item Draft agenda \hspace{1cm} \textit{MAL/DRUG/81/INF.1 (HQ)}
  \item Objectives and Detailed agenda \hspace{1cm} \textit{MAL/DRUG/81/INF.2 (HQ)}
  \item List of documents \hspace{1cm} \textit{MAL/DRUG/81/INF.3 (HQ)}
\end{itemize}

Extracts from the Minutes of the joint CHEMAL/FIELDMAL Steering Committee meeting held on 4 and 5 June 1981.
### RESPONSE OF Plasmodium spp TO ANTI-MALARIAL DRUGS (IN VIVO TEST) PAGE 1

#### A COUNTRY AND PLACE OF TEST:

<table>
<thead>
<tr>
<th>Institution</th>
<th>City/Town</th>
<th>Serial No.</th>
<th>Country Code</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Province/State</th>
<th>District/Country</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### B COUNTRY AND PLACE INFECTION PROBABLY CONTRACTED

<table>
<thead>
<tr>
<th>Country</th>
<th>Province/State</th>
<th>District/Country</th>
<th>Locality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### C SPECIES OF Plasmodium UNDER TEST

- [ ] falciparum
- [ ] vivax
- [ ] malariae
- [ ] ovale

#### D DATE TEST STARTED (DAY 0)

<table>
<thead>
<tr>
<th>Drug</th>
<th>[ ] Oral</th>
<th>[ ] I.M.</th>
<th>[ ] I.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### E PATIENT

<table>
<thead>
<tr>
<th>Name</th>
<th>Sex: [ ] MALE</th>
<th>[ ] FEMALE</th>
<th>Age (in years)</th>
<th>Weight (in kilogrammes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### F DRUG IDENTIFICATION (DRUG[S] TESTED)

<table>
<thead>
<tr>
<th>DRUG 1</th>
<th>Chemical name</th>
<th>Country of manufacture</th>
<th>Manufacturer</th>
<th>Batch number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DRUG 2</th>
<th>Chemical name</th>
<th>Country of manufacture</th>
<th>Manufacturer</th>
<th>Batch number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DRUG 3</th>
<th>Chemical name</th>
<th>Country of manufacture</th>
<th>Manufacturer</th>
<th>Batch number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DRUG 4</th>
<th>Chemical name</th>
<th>Country of manufacture</th>
<th>Manufacturer</th>
<th>Batch number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### G DOSAGE (to nearest mg) AND ROUTE

<table>
<thead>
<tr>
<th>Drug</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### H REASON FOR SCREENING

- [ ] Resist. n area of origin
- [ ] Routine monitoring
- [ ] Resist. in area of origin (abroad)
- [ ] Other
- [ ] Resist. in adjacent area
- [ ] Resist. in other rel. area
- [ ] Resist. in area of origin (abroad)
- [ ] Other

#### I SAMPLE

- [ ] General pop.
- [ ] Labour force
- [ ] Inpatient
- [ ] Other

#### J HISTORY OF TREATMENT TAKEN DURING LAST 2 WEEKS

- [ ] Yes
- [ ] No

If "Yes" specify drug(s): (box 222)
RESPONSE OF *Plasmodium* spp TO ANTI-MALARIAL DRUGS (IN VIVO TEST)  PAGE 2

<table>
<thead>
<tr>
<th>COUNTRY OF TEST</th>
<th>Investigator</th>
<th>Serial No.:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(Copy from Page 1)</td>
</tr>
</tbody>
</table>

**K URINE TESTS Results of tests on DAY 0 and DAY 2**

- [ ] pos.  [ ] Doubtful  [ ] neg.  [ ] Not done
- 4-aminoquinolines (box 224-225)
- Sulphonamides (box 226-227)

**L IN VIVO TEST RESULTS**

<p>| Daily parasite counts asexual forms and gametocytes against leucocytes: parasite densities |</p>
<table>
<thead>
<tr>
<th>DAY 0</th>
<th>DAY 1</th>
<th>DAY 2</th>
<th>DAY 3</th>
<th>DAY 4</th>
<th>DAY 5</th>
<th>DAY 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASEXUAL FORMS</td>
<td>LEUCOCYTES</td>
<td>P. falciparum gametocytes</td>
<td>LEUCOCYTES</td>
<td>ASEXUAL FORMS PER mm³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asex. forms</td>
<td>Leucocytes</td>
<td>P. f. gams.</td>
<td>Leucocytes</td>
<td>Asex/mm³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>340</td>
<td>372</td>
<td>400</td>
<td>430</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>226</td>
<td>258</td>
<td>294</td>
<td>356</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>288</td>
<td>312</td>
<td>358</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>332</td>
<td>370</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>312</td>
<td>340</td>
<td>378</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>320</td>
<td>350</td>
<td>388</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330</td>
<td>360</td>
<td>390</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- If test not completed state why not
  - 1 = Non-compliance
  - 2 = Clinical condition
  - 3 = Other (state why)

**M SIDE EFFECTS OF TREATMENT OTHER THAN VOMITING?**

- [ ] Yes  [ ] No

**N WAS RE-INFECTION POSSIBLE?**

- [ ] Yes  [ ] No  [ ] ?

**Q Were the slides referred for checking?**

- [ ] Yes  [ ] No

**P Has the patient travelled and where (during the last 12 months)?**

**Q Conclusion:**

**R If in vitro test was conducted simultaneously enter serial number**
# RESPONSE OF P. FALCIPARUM TO CHLOROQUINE AND MEfloQUINE (IN VITRO-TEST)

## A COUNTRY AND PLACE OF TEST

<table>
<thead>
<tr>
<th>Institution</th>
<th>City/Town</th>
<th>Serial No.:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

| Country Code | 5         |

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Country</th>
<th>Institution:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Province/State</th>
<th>District/Country</th>
</tr>
</thead>
</table>

## B COUNTRY AND PLACE INFECTION PROBABLY CONTRACTED

<table>
<thead>
<tr>
<th>Country Code</th>
<th>Prov Code:</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Lat. in box 15</th>
<th>Long. in box 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 = N</td>
<td>1 = E</td>
</tr>
<tr>
<td>2 = S</td>
<td>2 = W</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>City/Town</th>
<th>Locality</th>
</tr>
</thead>
</table>

## C DATE AND TIME BLOOD TAKEN

<table>
<thead>
<tr>
<th>Started:</th>
<th>Terminated:</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Day</th>
<th>Month</th>
<th>Year</th>
<th>Hour</th>
<th>Min</th>
</tr>
</thead>
</table>

## D INCUBATION TIME

<table>
<thead>
<tr>
<th>Date</th>
<th>Duration (hours)</th>
</tr>
</thead>
</table>

## E PATIENT

<table>
<thead>
<tr>
<th>Sex</th>
<th>Less than 1 year</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>M/F</td>
<td>00</td>
<td>44</td>
</tr>
</tbody>
</table>

## F REASON FOR SCREENING

<table>
<thead>
<tr>
<th>Reason for screening</th>
<th>General pop.</th>
<th>Labour force</th>
<th>Inpatient</th>
<th>Migrant labour</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 = Resist. or suspected case</td>
<td>2 = Collateral case of resist. or suspect. resist. case</td>
<td>3 = Resist. in area of origin</td>
<td>4 = Resist. in area of origin, (abroad)</td>
<td>5 = Resist. in adjacent area</td>
</tr>
</tbody>
</table>

## G SAMPLE

<table>
<thead>
<tr>
<th>General pop.</th>
<th>Labour force</th>
<th>Inpatient</th>
<th>Migrant labour</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 = General pop.</td>
<td>2 = School</td>
<td>3 = Labour force</td>
<td>4 = Outpatient</td>
</tr>
</tbody>
</table>

## H DRUG TAKEN DURING LAST 2 WEEKS

<table>
<thead>
<tr>
<th>Any anti mal. drug taken</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 = Yes</td>
<td>2 = No</td>
<td>3 = ? (box 48)</td>
</tr>
</tbody>
</table>

## I PRE-CULTURE SLIDE EXAM. ASEXUAL P. FALCIPARUM

<table>
<thead>
<tr>
<th>Asexual P. Falciparum per mm³ blood:</th>
<th>No. counted:</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>small</th>
<th>medium</th>
<th>large</th>
</tr>
</thead>
</table>

## J RESULT OF MACRO-TEST

<table>
<thead>
<tr>
<th>Chloroquine n mol/well</th>
<th>Mefloquine n mol/well</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCHIZONT./300 leuc.</td>
<td>SCHIZONT./1000 leuc.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotype 1</th>
<th>Genotype 2</th>
<th>Genotype 3</th>
<th>Genotype 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>0.50</td>
<td>0.75</td>
<td>1.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mean K</th>
<th>%</th>
<th>%</th>
<th>%</th>
<th>%</th>
<th>%</th>
<th>%</th>
<th>%</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## K RESULT OF MICRO-TEST

<table>
<thead>
<tr>
<th>Chloroquine p mol/well</th>
<th>Mefloquine p mol/well</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCHIZONT./200 paras</td>
<td>SCHIZONT./200 paras</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotype 1</th>
<th>Genotype 2</th>
<th>Genotype 3</th>
<th>Genotype 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>0.50</td>
<td>0.75</td>
<td>1.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mean K</th>
<th>%</th>
<th>%</th>
<th>%</th>
<th>%</th>
<th>%</th>
<th>%</th>
<th>%</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## L Were the slides referred for checking? | yes | no |

## M Has the patient travelled and where (during the last 12 months)? |

## N Conclusion:
APPENDIX 4

Sample Size in Studies on Drug Response of *P. Falciparum*

(EXERCISE)

The first 85 results of the *in vitro* macro tests with mefloquine from Thailand give the following distribution of results in terms of the number of cases with specified levels of schizont maturation at given drug concentrations (figures in brackets are explained below):

```
n-mol/vial (= x 10^-6 M/l)  

<table>
<thead>
<tr>
<th>n-mol/vial (x 10^-6 M/l)</th>
<th>0.25</th>
<th>0.5</th>
<th>0.75</th>
<th>1.0</th>
<th>1.5</th>
<th>2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>76 -</td>
<td>24 (20)</td>
<td>3 (-)</td>
<td>1 (-)</td>
<td>- (-)</td>
<td>- (-)</td>
</tr>
<tr>
<td>0.5</td>
<td>51 - 75</td>
<td>35 (45)</td>
<td>7 (5)</td>
<td>2 (5)</td>
<td>- (-)</td>
<td>- (-)</td>
</tr>
<tr>
<td>0.75</td>
<td>26 - 50</td>
<td>17 (15)</td>
<td>25 (20)</td>
<td>7 (-)</td>
<td>2 (5)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>1.0</td>
<td>1 - 25</td>
<td>9 (5)</td>
<td>39 (60)</td>
<td>34 (40)</td>
<td>19 (25)</td>
<td>7 (10)</td>
</tr>
<tr>
<td>1.5</td>
<td>0 - (-)</td>
<td>11 (-)</td>
<td>41 (40)</td>
<td>64 (55)</td>
<td>77 (70)</td>
<td>80 (75)</td>
</tr>
<tr>
<td>2.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
```

A systematic subsample of 1/5, i.e. test results numbers 5, 10, 25... 85, or 17 tests out of 85 gave the following distribution:
The shapes of the two distributions can be compared in a simple way by multiplying each figure in the second table by 5; this yields the figures in brackets in the first table. The two distributions are remarkably similar.

<table>
<thead>
<tr>
<th></th>
<th>0.25</th>
<th>0.50</th>
<th>0.75</th>
<th>1.00</th>
<th>1.5</th>
<th>2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>76 -</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>51 - 75</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>26 - 50</td>
<td>3</td>
<td>4</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>1 - 25</td>
<td>1</td>
<td>12</td>
<td>8</td>
<td>5</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>0</td>
<td>-</td>
<td>-</td>
<td>8</td>
<td>11</td>
<td>14</td>
<td>15</td>
</tr>
</tbody>
</table>

The shapes of the two distributions can be compared in a simple way by multiplying each figure in the second table by 5; this yields the figures in brackets in the first table. The two distributions are remarkably similar.
APPENDIX 5

Antimalarial Drugs and Drug Regimens
(Falciparum Malaria)

1. MEDICAMENTS FOR THE TREATMENT OF UNCOMPPLICATED FALCIPARUM MALARIA

1.1 Chloroquine

7-chloro-4-(4'-diethylamino-1'-methylbutylamino) quinoline

Trade names:

Phosphate salts - Aralen, Avloclor, Arechin, Arquin, Baraquine, Chinophen, Clorkin, Delagil, Diroquine, Fetoquin, Kalquin, Kwinemal, Lagaquin, Malarex, Malarivon, Mexaquin, Quinalen, Quinora, Resochin, Rivoquine, Roquin, Siragan, Teraquin.

Sulfate salt - Nivaquine

Formulations available: Tablets of 150 mg and 300 mg base. Syrups of various strengths.

Regimen:

Adults$^1$ - 600 mg$^2$ (base) initially then 600 mg at 24 hours and 300 mg 48 hours later

---

$^1$ Adult is defined as a person of 60 kg body weight or over.

$^2$ In some areas, 2.4 g base over 4-5 days have been used.
Chloroquine is effective against the asexual blood stages of susceptible strains of *Plasmodium falciparum*, resulting in a radical cure of this infection. It is generally well tolerated in a therapeutic regimen, occasionally causing nausea, vomiting, dizziness, hypotension, headache, blurring of vision and pruritus. The more serious ocular damage due to drug induced retinopathy occurs only with repeated high doses for months or years. The maximum safe dose has been estimated to be a total of 100 g over 2 1/2 to 6 1/2 years. There are no reports of teratogenic effects in humans when chloroquine has been used as an antimalarial drug and it may therefore be administered to women of childbearing age and during pregnancy. Severe hypotension and cardiovascular collapse have been reported following parenteral administration of chloroquine, especially in infants and young children. The drug is metabolized by dealkylation in the liver and excreted by the kidneys with a half-life of 55 hours in healthy normal adults. Dosage reduction may be necessary in patients with severe hepatic or renal disease, though there are little data on this point and some authorities have recommended that the standard therapeutic regimen be employed in these patients.

1.2 Amodiaquine

7-chloro-4-(3'-diethylaminomethyl-4'-hydroxyanilino) quinoline

Trade names:

Camoquin, Basoquin, Flavoquine

Formulations available: Tablets of 150 mg and 200 mg (base) and a suspension of 150 mg/5 ml

Regimen:

Adults - 600 mg (base) initially then 400 mg at 24 and 48 hours

Children - 10 mg/kg initially then 5 mg/kg at 24 and 48 hours

The efficacy and side effects of amodiaquine are essentially the same as chloroquine. Recommendations and precautions may be regarded as equivalent. There are in vivo and in vitro data suggesting that amodiaquine is more active than chloroquine against strains of *P. falciparum* resistant to the latter drug, but

1 With 150 mg tablets formulation, the same regimen as with chloroquine is used.
not of sufficient magnitude to support recommendation of its use in known resistant infections, without additional research.

1.3 **Quinine**

6-methoxy-δ-(5-vinyl-2-quinuclidinyl)-4-quinolinemethanol

**Trade names:** Quinine, Coco-quinine, Quinimax

**Formulations available:** Quinine sulfate tablets of 130 mg, 200 mg, and 325 mg; suspension 110 mg/5 ml

**Regimen:**

- **Adults** - 650 mg every 8 hours for 7-14 days
- **Children** - 10 mg/kg every 8 hours for 7-14 days

Quinine is active against asexual erythrocytic stages of all forms of human malaria. Quinine is regarded by many experienced clinicians as the most rapidly active drug for the treatment of severe cases of falciparum malaria. A suppressive cure can be achieved with quinine alone but this drug is less efficient than 4-aminoquinolines for that purpose. A common syndrome of characteristic side effects known as cinchonism occurs in many patients taking quinine. Giddiness, lightheadedness, transient hearing loss, tinnitus, tremors, amaurosis and blurred vision may appear during the first 2-3 days of therapy. These do not necessitate discontinuation of treatment and subside quickly when the drug is stopped. Other less frequent but more serious side effects of quinine include urticaria, "asthma", fever, pruritus, thrombocytopenia, haemolysis, and oedema of the eyelids, mucous membranes and lungs. These may occur following a single dose and require immediate discontinuation of the drug. Cardiovascular collapse may follow rapid intravenous infusion of quinine. There is a limited risk of inducing abortion with the use of quinine in pregnancy. Pregnant women are frequently hypersusceptible to acute haemolytic anaemia or thrombocytopenia due to the drug. Less than 5% of an administered dose of quinine is excreted unchanged in the urine. Most of the drug appears in the urine as hydroxylated metabolites in patients with normal hepatic function. Hepatic dysfunction due to liver disease or sustained fever can prolong the half-life of quinine, which is normally less than two hours. Monitoring blood levels is recommended when the drug is used in patients with impaired renal or hepatic function.

---

1 Experience in some areas indicates that use of quinine beyond 7 days is of limited additional benefit.
1.4 Sulfonamide plus a dihydrofolate reductase (DHFR) inhibitor

(a) Sulfadoxine (sulformethoxine)
\[ N^{\prime}-\left(5,6 \text{ dimethoxy-4-pyrimidinyl}\right)-\text{sulfanilamide} \]

(b) Pyrimethamine
\[ 2,4\text{-diamino-5-p-chlorophenyl-6-ethylpyrimidine} \]

Trade names: Fansidar, Pyrixine

Formulations available: Tablets of 500 mg sulfadoxine + 25 mg pyrimethamine

Regimen:

- **Adults** - 3 tablets as a single dose
- **Children** - 9-14 years - 2 tablets
  - 5-8 years - 1 tablet
  - 0.5-4 years - 1/2 tablet

Sulfadoxine and pyrimethamine are active blood schizontocides with marked synergism due to their respective modes of action in the folate metabolic pathway. The use of quinine for 1-3 days in conjunction with the use of Fansidar has been advocated to accelerate the clinical response.

The effectiveness of the combination (sulfadoxine plus pyrimethamine) when administered as a single dose is attributable to the long half-lives of both components, 205 and 92 hours respectively. Side effects which occur in patients treated for acute malaria consist mainly of headache, nausea, vomiting, abdominal pain and skin reactions. Occasional haematotoxic effects such as leucopenia, granulocytopenia, thrombocytopenia, anaemia and eosinophilia may be encountered. While the safety of this combination for use in pregnancy has not been established, it has been used in a large number of cases with no apparent adverse effect on the foetus. Sulfadoxine undergoes limited hepatic metabolism consisting of acetylation and glucuronide conjugation. Both sulfadoxine and pyrimethamine are excreted by the kidneys and their half-lives may be greatly prolonged in patients with renal impairment.

Sulfonamides are theoretically capable of inducing haemolysis in individuals with a genetic deficiency of glucose-6-phosphate dehydrogenase (G-6-PD). This does not, however, appear to have been recognized as a clinical problem with sulfadoxine.
Note: A fixed combination of sulfalene 500 mg and pyrimethamine 25 mg is available under the trade name Metakelfin. Its cost, regimen, efficacy and tolerance may be similar to the combination of sulfadoxine plus pyrimethamine. It is available in tablets (500 mg + 25 mg) and suspension (200 mg + 10 mg per ml).

1.5 Tetracycline

4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide

Trade names:

HCl salts - Achromycin, Agromicina, Ambramicina, Bristacycline, Cyclapar, Cyclomycin, Hostacyclin, Omegamycin, Panmycin, Polycycline, Purocyclina, Robitet, Sanclomycin, Sumycin, Tetra-Bid, Tetrabon, Tetracap, Tetrachel, Tetracycin, Tetradecin, Tetralan, Tetram

Formulations available - Tablets or capsules of 500 mg, 250 mg, 100 mg; syrup of 125 mg/5 ml

Regimen:

Adults 1-2 g per day in 4 equal doses for 7-10 days

Children (over 8 years) - 25-50 mg/kg in 2-4 equal doses for 7-10 days

Tetracycline has been shown to have blood schizontocidal activity in man. Its activity against an established infection with P. falciparum is, however, too slow and should never be relied upon alone. For that reason, it is generally used in combination with conventional doses of quinine for at least the first three days of therapy. Because of the widespread use of tetracyclines for many various infections numerous side effects have been reported. When used in children less than 8 years old tetracyclines may cause permanent discoloration of teeth and hypoplasia of enamel. Impaired bone growth may also occur in very young children. For these reasons tetracyclines should not be used in young children or pregnant women. Gastrintestinal intolerance is not uncommon including nausea, vomiting, abdominal pain and diarrhoea. Less commonly oesophageal ulceration and pseudomembranous colitis have been reported. When used in high doses or in patients with impaired renal function, liver toxicity may occur. Outdated tetracycline should never be used because it is nephrotoxic.

Infrequent reactions include fever, urticaria, pseudotumor cerebri, headache, impaired vision, vertigo, haemolytic anaemia, leukopenia and phototoxicity.
Various tetracycline analogs have also been evaluated as antimalarial drugs, as for example doxycycline and minocycline. While just as effective and perhaps more convenient because of a longer half-life, these analogs are very much more expensive and have a similar or greater incidence of adverse effects.

2. TREATMENT OF COMPLICATED FALCIPARUM MALARIA

Definitions: Patients with life-threatening acute falciparum malaria, or patients who are otherwise unable to take antimalarial medication orally.

Injectable formulations

Quinine dihydrochloride - 540 mg (base)/2 ml

Chloroquine, hydrochloride, diphosphate or sulfate - 40 mg (base)/ml in 5ml ampoules

Sulfadoxine (500 mg) + pyrimethamine (25 mg) per 2.5 ml ampoule.

2.1 Quinine

Adults: 60 mg in isotonic solution as an intravenous infusion over a period of 2-4 hours, every 8 hours. Change to oral administration as soon as possible. Children 10-15 mg/kg.

2.2 Chloroquine

Where intravenous quinine is not available, chloroquine may be administered in a dose of 5 mg/kg in isotonic solution as an intravenous infusion over a period of 4 hours every 12-24 hours for a total of 1-5 doses. A dose by the intramuscular route is not to exceed 5 mg/kg. Parenteral chloroquine has been associated with shock and sudden death in children.

2.3 Sulfadoxine plus pyrimethamine

A single dose of 5 ml (2 ampoules) by intramuscular injection. Corticosteroids have been shown to be of no therapeutic benefit and may be deleterious to patients with cerebral malaria.

3. CHEMOPROPHYLAXIS (CHEMOSUPPRESSION) OF P. FALCIPARUM

The prophylactic administration of antimalarial drugs should start one week prior to exposure in persons who have not yet taken the selected medicament in the past. In others it should commence latest shortly before arrival in the malarious area. To be
effective, prophylaxis needs to be continued for at least 4 weeks, better still, 6 weeks after exposure. 1

3.1 Chloroquine

See section 1.1 for chemical name, trade names and formulations.

Regimen:

**Adults** 300 mg (or 5 mg/kg) weekly

**Children:**
- up to 1 year 37.5 - 50 mg (5 mg/kg) weekly
- 1-4 years 50 - 100 mg (5 mg/kg) weekly
- 5-8 years 150 - 200 mg (5 mg/kg) weekly
- 9-12 years 200 - 300 mg (5 mg/kg) weekly

A loading dose of 300 mg for adults or 5 mg/kg in the younger age groups, given on the second day of prophylaxis is recommended. It will provide effective blood concentrations earlier.

Chloroquine is highly effective as a suppressive chemoprophylactic drug for falciparum malaria and side effects are uncommon at the recommended dosage regimen. It may be used safely in children and pregnant women. While a therapeutic regimen for an acute attack may be tolerated by patients with impaired renal function, a reduced dosage regimen may have to be employed for long term prophylaxis. Retinophathy is a function of the total administered dose and may appear if a total dose in excess of 100 g is administered over 2 1/2 to 6 1/2 years.

3.2 Amodiaquine

See section 1.2 for chemical name, trade names and formulations.

Regimen:

**Adults**: 400 mg weekly

**Children**: up to 1 year 50 mg weekly
- 1-4 years 50-100 mg weekly
- 5-8 years 150-200 mg weekly
- 9-12 years 200-300 mg weekly

1 The use of proguanil or pyrimethamine alone for chemoprophylaxis is not recommended in view of the very widespread resistance to DHFR inhibitors of *P. falciparum* and *P. vivax* throughout the world.
A loading dose on the second day of prophylaxis identical to that of chloroquine is recommended (see 3.1).

The slightly greater activity of this 4-aminoquinoline against chloroquine-resistant \textit{P.falciparum} is not sufficient to recommend its use where resistance is recognized to be present. Its use and limitations as a chemoprophylactic drug are therefore the same as chloroquine.

3.3 Dapsone plus pyrimethamine

Dapsone: 4,4'-diaminodiphenylsulfone

Trade names: Maloprim

Formulations available: Tablets of dapsone 100 mg plus pyrimethamine 12.5 mg, and syrup 20 ml = 1 tablet.

Regimen:

- Adults: 1 tablet weekly
- Children:
  - 2 months - 6 months: 2.5 ml syrup weekly
  - 6 months - 5 years: 5 ml syrup weekly
  - 5 years - 10 years: 1/2 tablet or 10 ml syrup weekly.

The combination of dapsone plus pyrimethamine is an effective prophylactic drug for falciparum malaria. The half-life of dapsone is 27.5 hours and that of pyrimethamine is 95.7 hours. The combination is therefore not pharmacokinetically well balanced. Some authorities have recently advocated twice the standard dosage regimen, i.e. 2 tablets weekly or 1 tablet twice weekly. There are, however, insufficient clinical data on tolerance of this regimen to support such a recommendation at this time. Side effects at the recommended dosage are uncommon. Occasionally cyanosis attributed to methaemoglobinaemia and haemolysis in individuals with G-6-PD deficiency have been reported. Rare idiosyncratic reactions to dapsone, including agranulocytos, have occurred. Reversible bone marrow suppression and megaloblastic anaemia, especially in folate deficient individuals, may develop. While the safety of this combination for use in pregnant women has not been established, it has been used in a large number of cases with no apparent adverse effect on the foetus. Reduction in dosage may be required for patients with impaired renal and hepatic functions. Recent reports of carcinogenicity only in male rats are probably of no clinical importance.

Note: Those in charge of the treatment of leprosy patients are concerned about the potential role of malaria prophylaxis with dapsone in the production of drug resistant \textit{Mycobacterium leprae}. 

3.4 Sulfadoxine plus pyrimethamine

See 1.4 above for chemical names, trade names and formulations.

Regimen:

Adults: 1 tablet weekly
Children: 4 years 1/4 tablet weekly
5-8 years 1/4 tablet weekly
9-12 years 3/4 tablet weekly

4. PRESUMPTIVE TREATMENT OF FEVER CASES

1. Chloroquine
   Adults 600 mg (base) single dose
   Children 10 mg/kg (base) single dose

2. Amodiaquine
   Adults 600 mg (base) single dose
   Children 10 mg/kg (base) single dose

3. Sulfonamide plus a DHFR inhibitor
   Same as radical treatment.

5. GENERAL CONSIDERATIONS

5.1 Gametocytocidal use of primaquine (falciparum malaria)

Primaquine administered as a single oral dose of 30-45 mg
(0.9 mg/kg in children) to patients who are known or likely to
harbour mature gametocytes and who reside in or will return to an
area with malaria transmission, may be used as an adjunct to all
treatment modalities.

5.2 Dose calculation of antimalarial drugs

The following methods are suggested as alternative approaches
for calculating the dose of most antimalarial drugs to be adminis-
tered to children under 12 years of age, and adults under 50 kg.

5.2.1 Body surface area (m²) method:

<table>
<thead>
<tr>
<th>Age</th>
<th>Body weight (kg)</th>
<th>Surface area (m²)</th>
<th>Fraction of adult dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 months</td>
<td>4.5</td>
<td>0.26</td>
<td>1/6</td>
</tr>
<tr>
<td>4 months</td>
<td>6.5</td>
<td>0.34</td>
<td>1/5</td>
</tr>
<tr>
<td>1 year</td>
<td>10.0</td>
<td>0.42</td>
<td>1/4</td>
</tr>
<tr>
<td>3 years</td>
<td>15.0</td>
<td>0.56</td>
<td>1/3</td>
</tr>
<tr>
<td>7 years</td>
<td>23.0</td>
<td>0.85</td>
<td>1/2</td>
</tr>
<tr>
<td>12 years</td>
<td>40.0</td>
<td>1.23</td>
<td>3/4</td>
</tr>
<tr>
<td>20 + years</td>
<td>65</td>
<td>1.7</td>
<td>1</td>
</tr>
</tbody>
</table>
The drug should be administered according to the same schedule as for adults and at the fraction of the adult dose for the nearest body weight.

5.2.2 Fractional dose per kilogram method

The drug should be administered according to the same schedule as for adults and at a dose per kilogram of body weight equivalent to the adult dose divided by 50.
1. INTRODUCTION

Detailed accounts of the chemical and pharmacological properties of mefloquine were presented at a recent meeting of the Scientific Working Group on the Chemotherapy of Malaria of the UNDP/World Bank/WHO Special Programme on Research and Training in Tropical Diseases. For the purpose of this annex the following aspects of mefloquine should be borne in mind:

- action restricted to asexual blood stages (and developing gametocytes?)
- no action on mature gametocytes of Plasmodium falciparum
- no action on tissue schizonts of any species, nor on mosquito stages
- no action on secondary tissue stages of P. vivax and P. ovale (hypnozoites?)
- blood schizontocidal action present against all Plasmodium species
124 / APPENDICES

- fully active against chloroquine and multi-drug-resistant *P.falciparum*
- fully active against parasites resistant to antifols
- long half-life in man, therefore cumulative in excessive dosage

2. INDICATIONS

Mefloquine, in principle, is indicated for treatment or for prophylaxis of all species of human malaria.

2.1 Treatment

(i) The major indication for the use of mefloquine in treatment should be in patients infected with strains of *P.falciparum* resistant to chloroquine, particularly if the parasites are also resistant to Fansidar or similar combinations.

(ii) Patients infected with chloroquine-resistant *P.falciparum* who are sensitized to sulfonamides or sulfones could also be treated with mefloquine.

(iii) Mefloquine should not be deployed in areas where chloroquine resistance is not a problem, but should be reserved for the positive indications above.

2.2 Prophylaxis

Here too mefloquine should only be used in areas where chloroquine-resistant *P.falciparum* is a problem. It is not justified to deploy mefloquine for prophylaxis, for example, at the present time in East Africa, where small foci of chloroquine resistance appear to be emerging, but where prophylaxis is afforded by other alternative preparations such as Fansidar or Maloprim. (Even these should not be used for large-scale prophylaxis in such areas, since there is a danger that resistance may develop relatively quickly to them in such circumstances.) Mefloquine should be used for prophylaxis only with the precautions indicated below, even in areas of chloroquine and multiple drug resistance.

Three categories should be considered in relation to the prophylactic use of mefloquine: individuals, special groups and indigenous communities. Because of the relative ease of controlling the sale, distribution and application of drugs in the first two categories, mefloquine (again with the precautions noted below) may justifiably be released for them in the near future. It is believed that mefloquine should not be made generally available for large communities until the conditions outlined below can be fulfilled.
More specifically, mefloquine may be required:

(i) To prevent or reduce morbidity and mortality in
   - high risk groups (under-fives, pregnant women, etc.)
   - special groups (indigenous labour forces, military personnel, refugees, etc.)
   - nonimmune immigrants and visitors, pilgrims, etc.

(ii) To reduce endemity in communities
   - holding operation when eradication measures are not possible
   - control of epidemics
   - control of localized foci

(iii) In eradication programmes
   - to eliminate residual foci
   - to prevent establishment of new foci (autochthonous or introduced)

3. HAZARDS

Hazards facing the free use of mefloquine lie in three directions:

3.1 Individual people

The problem of individual intolerance to a recommended dose of mefloquine (as to any drug) will always be present, but experience gained in Phase II and III clinical trials will help to define any possible types who may be particularly at risk, so that appropriate warnings can be given. Available data seem to be insufficient to define either the level of intolerance that may be expected, or the particular groups which would be most at risk, e.g. individuals with disorders of metabolism, excretion, etc.

Overdosage is a special hazard with mefloquine, which has an unusually prolonged half-life in man. While it is not anticipated that normal dosage would lead to accumulation, it is well known that, once any effective drug becomes freely available (be it on the open market, or as a drug supplied by, e.g. a government malaria service), some individuals are likely to take or administer an excessive dose on the grounds that what one tablet cures, two will cure twice as well.

3.2 The parasites

The ability of certain species of malaria parasites to become resistant to mefloquine and other aminoalcohols, especially if
they are already resistant to chloroquine, has been well docu­mented. It is not yet known, however whether this hazard applies also to P. falciparum, nor to the other malaria parasites of man. That the potential for resistance does exist seems possible, in view of increasing evidence showing that P. falciparum can become resistant to quinine which is, chemically and functionally speaking, anal­ogous to mefloquine and the other, new, aminoalcohol antimalarials. Another open question is whether mefloquine resistance will prove to be a stable character. That it is transmissible through the invertebrate vector appears very likely from experimental work in rodent malaria on compounds of this type.

3.3 The drug

The greatest curse of all drugs, especially in developing countries, is their gross misuse and abuse due to lack of drug controls, overprescribing, the use of "shotgun therapy" or, in some cases, a black market in drugs. The loss of effectiveness of antibiotic after antibiotic is a good example of this problem. Another is the rapid appearance of resistance to antifol antibacterial drugs such as Fansidar, which is rapidly losing its effectiveness in Thailand, for example.

4. PRECAUTIONS

4.1 Strict drug control

From several of the above comments, it is obvious that one of the main precautions to be taken before releasing mefloquine should be the establishment of strict governmental regulations concerning its importation and distribution. That this is a counsel of perfection is clearly understood and it is likely that, in the face of popular demand from the public and in some cases, sales pressure from suppliers, few developing countries will succeed in establishing any meaningful control on the use of mefloquine.

4.2 Limitation to areas where mefloquine is indicated

Measures should be taken through WHO, national governments and manufacturers to limit the supply of mefloquine to those countries where there is a clear indication for its use as outlined in 2.1 and 2.2 above.

4.3 Time limit for use of mefloquine

It would be a serious mistake to depend on mefloquine any more than on any other antimalarial to control malaria indefi­nitely, in the absence of other control measures. A limit should therefore be set for its use if it is decided to use mefloquine,
particularly for the protection of indigenous communities in the indications set out above.

4.4 Sensitivity monitoring

Prior to introducing mefloquine to any new area, all possible steps should be taken to determine the baseline sensitivity of local strains of *P. falciparum* to this drug, using the Rieckmann *in vitro* microtest\(^1\) as further developed by WHO\(^2\). Repeated monitoring should be carried out on a regular basis after its introduction as long as transmission is continuing, and at any time that a change in clinical response to mefloquine is suspected.

4.5 Use drug only in integrated control

Mefloquine and, indeed, any other antimalarial should only be used as one tool in an integrated attack on malaria, be it for the emergency handling of an epidemic, or part of a larger, longer-term control programme. Reliance on antimalarials alone has always resulted in failure and has often led to the emergence of drug resistance.

5. USE OF DRUG COMBINATIONS

5.1 In therapy

Drug combinations including mefloquine should be considered for two purposes:

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

(ii) To interrupt transmission. A single dose of 45 mg primaquine base (adult dose) should be given with mefloquine used for therapy in order to sterilize any existing gametocytes, especially those of *P. falciparum*. This will minimize the transmission of parasites in areas where vectors are present, and hence also minimize the risk of the emergence of drug resistance.

---


5.2 In suppressive regimens

(i) To prevent transmission. The use of a primaquine/mefloquine combination to minimize transmission merits consideration. A single adult primaquine dose of 45 mg base with a fortnightly or monthly dose of mefloquine might be of value for this purpose.

(ii) To delay the possible emergence of resistance to mefloquine. There is accumulating experimental evidence to indicate that resistance may develop more slowly to a combination of mefloquine with an antifol-sulfonamide combination such as Fansidar, than to any of the three components used alone, or to the Fansidar combination. This triple combination would be a far more rational application of mefloquine for malaria prophylaxis in an area of continuing transmission than the wide-scale use of mefloquine alone.

(iii) Search for other mefloquine combinations. There is an urgent need to find other compounds or drug mixtures to combine with mefloquine in view of the fact that, in at least one of the most problematic areas, Thailand, a measure of resistance already exists to Fansidar.

6. SUMMARY

The major problems in the deployment of mefloquine appear to be as follows:

(i) Mefloquine is virtually the only alternative to currently used drugs.

(ii) Mefloquine belongs to the same chemical class as quinine, to which some resistance is already known.

(iii) It is going to be almost impossible to limit the use of mefloquine once it is released, since it will be in great demand once its value is recognized by the general public.

(iv) The cost of mefloquine is still high (although this may change if it becomes more widely used).
Appendix 7

Outline Research Proposals

Outline research proposal 1
Development of a simplified warning system for the early detection of Plasmodium falciparum resistance.

(1) Carry out malariometric surveys in selected villages on age-group 5-15 years (most accessible), possibly at the peak of the transmission season.

(2) Subject positives to full dose radical treatment with chloroquine plus single dose of primaquine.

(3) Select several minimal schedules for blood slide examination between days 3 and 7.

(4) In case of effective parasite clearance, re-visit the village within four weeks and enquire about possible recrudescences, and ascertain situation parasitologically.

(5) In case of confirmed resistance or doubt of the effect of treatment, undertake in vitro testing.

In areas already affected by resistance, make further attempts for closer cooperation with medical professionals in general and health services, to obtain early warning of possible resistance to alternative drugs.
Outline research proposal 2
Clinical pharmacological and pharmacokinetic investigations of 4-aminoquinolines in target populations

1. Objectives

The work on the response to chloroquine has so far mainly concentrated on the parasite in the host (in vivo test) or in vitro without giving sufficient consideration to the impact on the control of infection of different concentrations of the drug used. A possible increase of the amount of drug or a change of the time period of administration might convert clinical or parasitological response from R to S.

2. Study design

2.1 Intervention

Oral administration of chloroquine in standard dosage (i.e. 10 mg base/kg body weight single dose or 25 mg/kg body weight in a 3-day course).

2.2 Target population and area

The target population consists of indigenous inhabitants living in endemic areas, where the malaria parasites are sensitive to 4-aminoquinolines and drug pressure is low.

2.3 Patient selection

Some 40-50 febrile adults (over 18 years old) coming to the hospital or malaria service for treatment, are examined for malaria. If asexual parasites are present and there is no evidence of recent antimalarial medication and no serious diseases other than malaria, the patients are asked to participate in the study which will be explained to them in order to obtain informed consent.

2.4 Organization

The study should be conducted in collaboration between the Malaria Service and the health authorities. Advantage should be taken of any previous investigations of a similar kind.

2.5 Investigation

The study requires the analysis of chloroquine and concomitant assessment of certain biochemical parameters at various time
Chloroquine analysis follows the spectrofluorometric method of McChesney et al. The millilitres of venous blood should be withdrawn before treatment and at 3, 6, 12, 24, 48, 96, 168, 336 and 772 hours after the drug is taken (first dose in the case of radical treatment). Blood should be centrifugated and serum stored in deep freeze (-20°C) until analysis. If the blood samples are to be transported, this should be done on dry ice.

Thick blood films from finger prick should be examined daily during the whole period of observation, i.e. four weeks. Patients should be questioned for side-effects.

3. Analysis

Chloroquine concentrations should be presented in a graphic form and related to the clearance of asexual parasitaemia and other symptoms of patients. The results should also be related to the minimum concentration of the drug believed to be therapeutically effective in the area under study and to any side-effects recorded. The latter should be compared with side effects obtained in similar investigations in other areas.

4. Personnel, equipment and budget

The studies require the services of an ad hoc special team headed by a physician, assisted by a well qualified laboratory technician. A list of equipment and supplies required is available from the laboratories which are practising the McChesney method of chloroquine analysis in various parts of the world. In addition, ordinary laboratory supplies and materials are required in accordance with the appropriate study protocol. The estimated cost is approximately US$ 20 000.

Outline research proposal 3
Determination of effectiveness of the sulfone/pyrimethamine combination for chemoprophylaxis in areas with resistance to the sulfonamide/pyrimethamine combination.

1. Objectives

Short-term

To determine effectiveness of sulfone/pyrimethamine combinations for chemoprophylaxis in areas with known *Plasmodium falciparum* resistance to sulfonamide/pyrimethamine combinations.

---

Long-term

To prevent the spread of multiple drug-resistant P.falciparum isolates to other areas and to protect immigrants and other persons entering areas with multiple drug-resistant P.falciparum from acquiring malaria infections.

2. Study design

2.1 Intervention

To provide chemoprophylaxis with either sulfone/pyrimethamine or sulfonamide/pyrimethamine to matched groups entering areas with multiple drug-resistant P.falciparum and to compare the results in both groups.

2.2 Target population and area

The target population consists of male adults entering an area with current transmission of multi-resistant P.falciparum, e.g. Thai districts along the Kampuchean border. The area needs to be selected on the basis of existing data and the continued validity of the latter, and any modification of the sensitivity status of P.falciparum needs to be monitored throughout the study.

2.3 Selection of participants

The selection of participants for the study should be based on the ease of follow-up, e.g. soldiers, border police, camp personnel, etc. Attribution to one of the two study groups will be randomized, but both groups will be equal in size and matched for age and area (exposure). Participants in the study will be fully briefed on the purpose and the conduct of the investigations in order to obtain their informed consent.

2.4 Trial groups and medication

The size of the trial groups will depend on any observed difference between both groups. The study will therefore be conducted open-ended, i.e. with the continuous inclusion of new participants until such a time that a statistically different result has been obtained. If such a result is not obtained in the course of 2 years and a sizeable sample has been studied (e.g. 2 x 500 participants with a 10+% breakthrough rate in the sulfadoxine/pyrimethamine group), the study will be terminated and judged non-conclusive.

Participants in Group A will receive 500 mg sulfadoxine and 25 mg pyrimethamine (combined tablet) once weekly, those in Group
B will receive 100 mg dapsone and 12.5 mg pyrimethamine (combined tablet) once weekly. Drug administration will be effected in the presence of the investigator or his deputy.

2.5 Safeguards

The study population will be followed up intensively and in the case of a breakthrough of infection, appropriate radical treatment will be given without delay.

2.6 Evaluation

2.6.1 Blood films (thick films) are taken prior to enlistment in the study and once monthly during the study from all participants. This will also help the detection of symptomatic cases. Results will be duly recorded. Positives will be radically treated and discharged from the study.

2.6.2 Urine samples will be collected once monthly for a urine test for sulfonamide and sulfones and for the measurement of drugs and their metabolites.

2.6.3 Apart from the monthly routine examination, blood slides are taken and immediately examined whenever malaria symptoms appear. If positive, a blood sample is collected for in vitro sensitivity test and drug assay and a urine sample for drug measurement. The patient will receive radical treatment without delay and be discharged from the study.

3. Analysis

The rate of P. falciparum infections in both groups is the basis of analysis which follows accepted statistical practice. In addition, the incidence of P. vivax will be compared in both groups.

4. Personnel, equipment and budget

Running cost and involvement of personnel will depend on the area and the population groups selected and the required duration of the study. Cost of equipment will depend on the institution/services conducting the investigation and its already available facilities.
Considerations Related to the Practical Performance of the Assessment of Drug Sensitivity in *P. falciparum*

Guidelines for the sampling and selection of cases for the assessment of drug response in *Plasmodium falciparum* have been developed in order to assist investigators in their studies and to ensure conformity with accepted statistical methods (see page 45). This annex has been prepared with a view to amplifying some of the contents of Section on Guidelines for Sampling, page 45, and to provide guidance for approaches in circumstances in which classical statistical sampling procedures are not easily applicable. This refers equally to the detection of foci of drug resistance and to the quantitative measurement of sensitivity levels as well as the longitudinal observation (monitoring) of drug sensitivity.

1. **Sample size**

Sample size will be related to the actual availability of *falciparum* infections. In areas with a high prevalence it will be possible to apply classical statistical methods of sampling and stratification. This may not be possible in areas where both prevalence and incidence of *P. falciparum* infections are low (either naturally or as a result of intervention). In these circumstances it will be necessary to resort to "opportunistic" sampling, aiming at collecting as much information as possible, when and wherever it is available.
2. **Selection of area**

Monitoring of drug sensitivity is essentially a country-wide activity. Within the areas selected for study a stratification may be applied according to the following:

- Epidemiological strata
- Administrative divisions
- Geographical and ecological strata
- Domestic and international borders
  - Migration areas
    - Domestic
    - Seasonal
    - Pilgrim
    - International
  - Political
  - Labour
  - Illegal
- Construction and projects
  - Roads
  - Hydro-electric works
  - Canals and irrigation systems
  - Agricultural development
  - Others

3. **Case selection**

Baseline data are a *sine qua non* for a meaningful, longitudinal (sequential) comparison which is the essence of monitoring. The baseline needs to be established in a way that is representative of the area as a whole and of its various strata (see 2 above). In areas with a high prevalence and incidence this will be possible by following the usual sampling procedures for obtaining representative samples; in areas with low prevalence/incidence of *P. falciparum* baseline assessment needs to be exhaustive, i.e. covering the maximum available number of cases.

The subsequent analysis of results should aim at clarifying the origin of infection and at defining the sensitivity status on a geographical basis. In this connexion, the following classification may be useful:

**Locally contracted**
- Within the village
- Within the study area
- Within the province/zone/state
- Within the country

**Imported**
- Across international border
- Across international border adjacent to the study area,
  - short range movements
- Arising from neighbouring countries, long range movements
- Arising from distant countries
4. Screening points

The screening points are selected according to epidemiological considerations and the objectives of the study and may include the following:

- Villages
- Schools
- Malaria clinics
- Health centres
- Hospitals
- Others
- Disciplinary camps/centres
- Private practitioners
- Border check-posts
- Sea and airports
- Labour aggregations
- Army, police

5. Screening approaches

Screening to locate and select suitable cases to be included in the study would essentially consist of:

- Mass blood surveys
- Mass fever case surveys
- Out/in patients (passive case detection)
- Active case detection

as appropriate under the specific circumstances.

6. Test methods

The testing methods will greatly depend on practicability of the various techniques available, but it is to be noted that in vitro tests are required for the monitoring of sensitivity levels especially in areas where there is still a satisfactory in vivo drug response.

(a) **in vivo**
   - 7-day test
   - 28-day test

(b) **in vitro**
   - Macro test
   - Micro test

The above-mentioned tests have been described in WHO Technical Report Series 529 (1973) an document WHO/MAP/80.2.

It is realized that certain operational, technical and administrative constraints may render the large-scale performance of these tests, especially of in vivo tests, quite difficult. In these circumstances a simplified procedure for preliminary orientation would be required especially for areas adjacent to those with resistant P.falciparum, other areas which are exposed to the occurrence of drug resistance and areas where the prevalence/ incidence of P.falciparum remains high in spite of intensive malaria control measures. Such a preliminary orientation could be carried out as follows:
(a) Performance of malariometric surveys in selected villages on age group 5-15 years (most accessible), possibly at the peak of the transmission season.

(b) Full dose clinical (radical) treatment of positives \textit{(P.falciparum only)} with cloroquine plus single dose of primaquine.

(c) Examination of blood slides on days 0 and 7, recording of results (grouping S, RI/RII, RIII).

(d) If parasite clearance on day 7 is observed in all cases, re-visit of the village in 4 weeks' time and enquiry about possible recrudescences and examination of blood slides.

(e) In case of confirmed resistance or doubtful results follow-up by \textit{in vitro} testing.

In addition to the above-mentioned orientation procedures it will be important, in areas which are not yet affected by drug resistance, to establish close cooperation with the health professions - government services and private - in order to obtain an early warning of possible resistance.