RESISTANCE
OF MALARIA PARASITES
to drugs

Report of a WHO Scientific Group

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WORLD HEALTH ORGANIZATION

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WHO SCIENTIFIC GROUP ON
RESISTANCE OF MALARIA PARASITES TO DRUGS

Geneva, 13-20 October 1964

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RESISTANCE
OF MALARIA PARASITES TO DRUGS

Report of a WHO Scientific Group

INTRODUCTION

The meeting of the WHO Scientific Group on Resistance of Malaria Parasites to Drugs was held in Geneva during the period 13-20 October 1964. It was opened by Dr P. Dorolle, Deputy Director General, who welcomed the participants and stated that this meeting seemed to be particularly appropriate at a time when the Organization is paying increased attention to those problem areas that are impeding somewhat the remarkable progress of eradication of malaria.

In such an endeavor, aiming at eliminating malaria from the world, some obstacles are not only to be expected but should be considered as normal, and they provide a challenge for the greater organizational, financial, operational and scientific effort needed to reach the goal of malaria eradication.

From the dawn of chemotherapy, the progress of the treatment of bacterial and protozoan infections has been marked by the appearance of drug resistance due to the modified response of the parasite to the chemical compound.

The concept of malaria eradication, which has been developing since the early 1950’s when the residual insecticides were introduced in many fields of public health, was accepted by the World Health Organization and embodied in an integrated plan nearly ten years ago. At that time, the reports on resistance of human plasmodia to proguanil and pyrimethamine created some disappointment, but the importance of this phenomenon from the point of view of malaria eradication was small because these drugs, particularly valuable for prevention of infection, are rather imperfect therapeutic agents.

Since 1961, the need for appraisal of observations indicating the possibility of resistance of P. falciparum to chloroquine — the most widely used drug in chemotherapy of malaria and a powerful weapon in the service of malaria eradication — has been recognized by the World Health Organization and a close watch on the situation has been maintained. It has also been recognized that knowledge of the true distribution of resistance, characteristics of drug-fast parasites, and interpretation of
the biochemical or genetic mechanisms involved is insufficient. There is need for more research in these fields and especially for new antimalarial compounds, suitable for alternative use should resistance to drugs present an obstacle to malaria eradication.

The discussions of the meeting were guided by two priorities: (1) careful and sober assessment of the present situation with regard to drug resistance in malaria parasites, (2) agreement on realistic future action.

The Group elected unanimously Dr Leon Schmidt as Chairman, Dr S. P. Ramakrishnan as Vice-Chairman, and Dr L. G. Goodwin as Rapporteur.

1. DIFFERENCES IN THE RESPONSE OF SPECIES AND STRAINS OF MALARIA PARASITES TO DRUGS

Since the assessment of the present situation with regard to the reported cases of resistance of malaria parasites to drugs is possible only when the available data on the differences in the response of various species and strains of malaria parasites are fully known, a brief survey of this subject might be of value.

1.1 Observations made before 1939

Prior to any experimental work on human malaria some malariologists had realized, on purely clinical grounds, that malaria infections in different parts of the world varied in severity and sometimes also in their response to quinine. As early as 1894 Marchiafava and Bignami had observed that falciparum malaria in the Roman Campagna was a much more severe disease than in Northern Italy. Robert Koch, after having studied falciparum infections in Italy and in East Africa, reported that although the two parasites causing the disease could not be distinguished morphologically and belonged to the same species the infections caused by them differed markedly in their clinical effects. Subsequently Koch concluded that falciparum malaria of East Africa was not as dangerous a disease as was commonly accepted, whereas infections caused by the same species of plasmodium encountered in certain parts of Italy, e.g., Grosseto, nearly always presented a severe clinical picture. Moreover, while in African falciparum malaria 1 g of quinine was generally sufficient to bring about disappearance of parasites from the peripheral blood, in infections found at Grosseto much higher doses of this drug were needed to obtain the same effect.

Since then many observations have been reported indicating that falciparum infections in one part of the world are more serious and require more quinine for effective treatment than infections with the same species elsewhere. As a possible explanation of such discordant results obtained
in different areas with the same treatment, it has often been suggested that different strains of the same parasite species might not respond in the same way to a given treatment.

More definite evidence of differences in strains of parasites of the same species has been obtained since 1920 from the practice of malaria therapy which, for the first time, made it possible to study parasite strains of very diverse geographical origin under controlled and reasonably comparable conditions in the human host.

At the Horton Malaria Therapy Centre, established in the United Kingdom in 1925, it was first demonstrated under controlled conditions that infections with different strains of *Plasmodium falciparum* differ in their clinical severity, tendency to relapse, susceptibility to quinine, and other properties. Infections produced by the Italian and Romanian strains of *P. falciparum* were much more severe than infections due to Indian and West African strains: the fever periods lasted twice as long and recrudescences occurred over a much longer period (21 weeks) than in infections with the Indian strains (maximum 11 weeks). Furthermore, the amount of quinine necessary to control a primary attack caused by falciparum strains from the Roman Campagna and Sardinia was, on the average, eight times as great as that required for infections with Indian and other strains.

Moreover, patients who had become immune to an Indian strain of *P. falciparum*, as the result of repeated infections, were yet highly susceptible to infections with a Roman strain. Still another difference between Indian and Italian strains was their infectivity to mosquitoes: the *Anopheles atroparvus* in the United Kingdom readily became infected with the Italian and Romanian strains of *P. falciparum*, but all attempts to infect the same vector with the Indian and African strains regularly failed.

Similar observations have been reported by other workers. In Italy, a study of two morphologically indistinguishable strains of *P. falciparum*, one from Ethiopia and another from the Roman Campagna, showed that they differed considerably in their pathogenicity, in the amount of quinine required to control clinical attacks, and in the number of recrudescences following treatment. In infections with the Ethiopian strain, fever attacks were short, symptoms mild, and two doses of 1 g of quinine were sufficient to bring about temporary disappearance of fever and also, in most cases, of parasitaemia. In infections with the Roman strain, paroxysms and symptoms were much more severe and quinine treatment had to be started much earlier. Fever did not disappear until 4 to 6 doses of 1 g of quinine had been given, and asexual parasites persisted in all cases. Recrudescences were likewise more numerous in infections with the Roman strain and the total amount of quinine needed for adequate treatment of this infection was about four times as great as that required for infections with the Ethiopian strain. From the clinical point of view, the latter
strain is similar to the tropical strains of Africa and India described by Koch and James.

Analogous observations have been made with different strains of *P. falciparum* at the Socola Malaria Therapy Centre in Romania.

In Italy, three strains of *P. vivax* originating from northern, central and southern Italy were compared and it was found that the southern strain had a much less pronounced susceptibility to quinine. In patients inoculated with *P. vivax* from northern or central Italy, a single dose of 0.3 g of quinine, given during the primary attack, generally resulted in a temporary disappearance of fever and parasitaemia; to obtain the same result in patients infected with the strain from southern Italy, two to six doses of 0.3 g of quinine had to be given.

Comparison of the Madagascar strain of *P. vivax* with the *P. vivax* strain encountered in Holland showed that the virulent Madagascar strain was highly susceptible to neosarsphenamine, while the mild Dutch strain proved relatively resistant to this drug. Immunity acquired against the Dutch strain did not protect against attacks with the Madagascar strain.

In the USA, similar studies were carried out with American strains of *P. vivax* and *P. falciparum* and it was found that strains belonging to the same species differed in their clinical activity, antigenic properties, and also in their ability to infect certain anopheline vectors.

The discovery that within each parasite species there are multiple strains which differ in pathogenicity and in susceptibility to drugs has had an important bearing on certain therapeutic concepts. The Third General Report of the Malaria Commission of the League of Nations, based mainly on this new knowledge acquired in the practice of malaria therapy, pointed out the need for determining locally and individually the optimum dosage of drug to be used. Any scheme of treatment should take into consideration:

(a) the species of parasite;
(b) the virulence of the particular strain;
(c) the size of the infective inoculum;
(d) the degree of immunity that the patient may have acquired by reason of former attacks;
(e) the stage at which the infection is treated.

1.2 Studies carried out in the USA

The extensive malaria research programmes initiated during the Second World War have provided many further examples of the varying response to drugs of different species of malaria parasites as well as of different strains within the same species. In the USA, in view of the

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need for improved regimens for malaria suppression and treatment of acute attacks, an important part of the programme was devoted to the quantitative clinical testing of already existing antimalarials, such as quinine, mepracrine, various 4-aminoquinolines and proguanil. For these studies, selected strains of *P. vivax* and *P. falciparum* and highly standardized testing procedures were used.

Many of the dosage regimens subsequently recommended for malaria suppression and treatment have been chosen on the basis of results obtained in these investigations. Since some of the results of these studies are frequently quoted as representing the “normal” values applicable to fully susceptible parasite strains, the relevant experimental conditions should be outlined briefly.

The objective of the studies was to evaluate the antimalarial activity of the various drugs, measured by their ability to terminate acute attacks of blood-induced *P. vivax* and *P. falciparum* infections. The infections were established in neurosyphilitic patients by the intravenous injection of 500,000 parasites. For the studies on *P. vivax* malaria there was systematic exclusion of all individuals who might have had natural or acquired immunity.

Four parasite strains were employed: two strains of *P. vivax*, the McCoy strain, isolated in 1931 from an indigenous infection in Florida, USA, and the Chesson strain, isolated in 1944 from a soldier who had probably acquired the infection in New Guinea. The two strains of *P. falciparum* used were both of local origin: the McLendon strain, obtained in 1940 from a Negro patient in South Carolina, USA, and the Costa strain, isolated about 1932 from an indigenous infection in Florida, USA.

In *P. vivax* infections, therapy was started on the 4th and 5th day after the onset of fever; in *P. falciparum* infections on the first or second day of fever, or when parasitaemia reached 50,000 per mm³ in the absence of fever.

The drugs to be tested were administered by dosage schedules designed to achieve and to maintain fairly stable plasma drug concentrations of the desired levels during a four-, six- or eight-day therapeutic period. Each course of treatment was initiated by a loading dose and continued with smaller maintenance doses at four- to six-hour intervals. Blood samples for the estimation of the plasma drug concentrations were obtained at sufficiently short intervals (several times daily) to permit an appraisal of the individual mean concentrations each day.

The results of treatment were classified in three classes on the basis of the course of parasitaemia and fever following therapy:

Class I: No effect — drug administration had no detectable effect on either the parasite count or the course of the fever paroxysms.
Class II: Partial or temporary effect — a temporary suppression, either partial or complete, of parasitaemia and/or fever.

Class III: Permanent effect — a complete interruption of the asexual cycle, i.e., an absence of parasitaemia for 14 days (P. vivax) or 21 days (P. falciparum) after the last effective plasma drug level, followed by a positive reinoculation with 1 million parasites to demonstrate continuous host susceptibility to the infection.

Early in the studies, investigation of the relationship between oral dosage, plasma drug concentration and antimalarial effect had shown that the correlation between oral dosage and therapeutic result was not close, since in different individuals receiving the same oral doses a wide range of plasma drug levels was obtained. On the other hand, the relationship between mean plasma drug concentrations of quinine maintained for four or six days and the therapeutic effect achieved was found to be sufficiently consistent to permit the definition of a "critical" plasma drug concentration above which permanent interruption of the erythrocytic cycle of a given parasite strain may be expected.

Using the procedures outlined above, the suppressive antimalarial effect of quinine, mepacrine, proguanil and various 4-aminoquinolines was determined in terms of oral dosage and minimal plasma drug concentrations required for a given therapeutic effect.

Over-all results showed that the response of the erythrocytic parasites to treatment was generally (but not always) correlated with the mean plasma drug concentration and with the period during which an effective concentration was maintained. In agreement with the observations reported previously from the United Kingdom, Italy and other countries, the results of workers in the USA showed that the plasma drug concentrations and the duration of therapy necessary to achieve a Class III effect vary from species to species and from strain to strain within a given species; consequently, data obtained with a single strain of plasmodium are not necessarily applicable to treatment of malaria due to other strains.

1.2.1 Susceptibility to quinine

The results confirmed previous observations that P. falciparum infections are, as a rule, less amenable to quinine than P. vivax infections, and that within the P. falciparum species the susceptibility to quinine may differ considerably in different strains of the parasites. It was found that the Costa strain of P. falciparum, although more resistant to quinine and to other drugs than the McLendon strain, is generally much less severe in its clinical course; thus a higher resistance to the action of drugs is not necessarily linked with a higher virulence.

The results obtained with quinine against the two P. vivax and two P. falciparum strains are summarized in Table 1.
### TABLE I. RELATIONSHIP OF THERAPEUTIC EFFECT TO QUININE DOSAGE, MEAN PLASMA QUININE LEVELS AND DURATION OF THERAPY IN BLOOD-INDUCED P. VIVAX AND P. FALCIPARUM INFECTIONS*  

<table>
<thead>
<tr>
<th>Species and strains</th>
<th>Number of subjects</th>
<th>Days of treatment</th>
<th>Total dose (g of base)</th>
<th>Mean plasma levels (mg/litre) maintained during days of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Highest</td>
<td>Range of Class II</td>
</tr>
<tr>
<td>P. vivax</td>
<td></td>
<td></td>
<td>Class I</td>
<td>Class III</td>
</tr>
<tr>
<td>McCoy</td>
<td>54</td>
<td>4</td>
<td>1.2</td>
<td>0.5-2.5</td>
</tr>
<tr>
<td>Chesson</td>
<td>7</td>
<td>4</td>
<td>—</td>
<td>1.4-6.5</td>
</tr>
<tr>
<td>P. falciparum</td>
<td></td>
<td></td>
<td>Class I</td>
<td>Class III</td>
</tr>
<tr>
<td>McLendon</td>
<td>15</td>
<td>4</td>
<td>0.8</td>
<td>0.8-8.0</td>
</tr>
<tr>
<td>13</td>
<td>6</td>
<td>—</td>
<td>2.7-4.7</td>
<td>3.6</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>6</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Costa</td>
<td>16</td>
<td>6</td>
<td>3.1-18.0</td>
<td>8.4</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>8.1</td>
<td>7.8-14.3</td>
<td>7.8</td>
</tr>
</tbody>
</table>

* One subject.

Note: Under "Total dose," are given: (a) the highest dose that failed to produce a detectable effect on parasitaemia and/or fever (Highest Class I); (b) the lowest and highest doses that produced only partial or temporary suppression of parasitaemia and/or fever (Range of Class II); (c) the lowest dose that resulted in disappearance of parasites for 14 days (P. vivax infections) or 21 days (P. falciparum infections) (Lowest Class III).

Under "Mean plasma levels," the same effects are recorded in relation to the mean plasma quinine concentrations maintained during the period of therapy.

* Data compiled from:

### 1.2.2 Susceptibility to mepracine

Susceptibility of *P. vivax* and *P. falciparum* to mepracine was studied on the same two strains of each species and the results obtained were in keeping with those seen in the quinine series; plasma mepracine concentrations and duration of therapy necessary to achieve Class III effects in blood-induced *P. vivax* and *P. falciparum* infections varied according to species and to strains.

In infections with the McCoy strain of *P. vivax*, a mean plasma mepracine concentration of 31 μg/litre or higher, maintained for not less than four days, resulted in a Class III effect in all five subjects. The Chesson strain of *P. vivax* seemed to be more resistant to mepracine; in order to obtain a Class III effect a plasma concentration of about 30 μg/litre had to be maintained for at least six days (3 subjects). A Class II effect was obtained in two other subjects in whom a similar plasma concentration of the drug was maintained for four days.

The two *P. falciparum* strains tested — McLendon and Costa — appeared to be about twice as resistant to mepracine as the McCoy
Strain of *P. vivax*, as judged by the plasma mepacrine concentrations required to produce consistently a Class III effect.

The division between Class II and Class III effects in relation to plasma drug concentration was not very well defined; particularly in *P. falciparum* infections there was a considerable overlap between plasma levels associated with Class II or Class III effects.

The relationship between total doses and therapeutic results obtained was even less consistent. In one subject, a Class III effect was achieved with a total dose as low as 590 mg, whereas in four other subjects having received each a total dose of 930 mg, Class II effects were obtained. Similar inconsistencies could be observed in the results obtained in infections due to the Costa strain of *P. falciparum* (Table 2).

### 1.2.3 Susceptibility to proguanil

Comparative data on the suppressive activity of proguanil were obtained for the two *P. vivax* strains and for the Costa strain of *P. falciparum*.

*P. vivax* infections due to the McCoy strain were found to be very susceptible to proguanil: total doses of 50 mg, or mean plasma levels of at least 10 µg/litre maintained for four days regularly produced Class III effects. The Chesson strain of *P. vivax* appeared to be slightly less susceptible to proguanil. In contrast, the Costa strain of *P. falciparum* proved to be much more resistant. Nine patients received total doses of proguanil ranging from 338 to 750 mg, and in only two of them was a Class III effect obtained, although plasma levels ranging from 55 to 106 µg/litre were maintained for six days.

### 1.2.4 Susceptibility to 4-aminoquinolines

Using the same standard procedures, various 4-aminoquinolines were tested for their ability to terminate acute attacks of blood-induced *P. vivax* (McCoy) and *P. falciparum* (McLendon) infections. However, comparable data for other strains of these species are not available.

In vivax malaria due to the McCoy strain of the parasite, a total dose of 300 mg of chloroquine base and plasma levels ranging from 14 to 42 µg/litre maintained for four days produced a Class III effect in all six patients so treated.

Again, *P. falciparum* was found to be less responsive than *P. vivax*, as shown by the longer duration of therapy (six days instead of four) and by the higher plasma drug levels needed to achieve a Class III effect.

In *P. falciparum* infections with the McLendon strain the lowest total dose of chloroquine which produced a Class III effect in all patients was 650 mg of base and mean plasma levels maintained for six days ranged

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1 Doses of 4-aminoquinolines and 8-aminoquinolines given in this and succeeding sections are in terms of base.
<table>
<thead>
<tr>
<th>Drug</th>
<th>Species and strains</th>
<th>Number of subjects</th>
<th>Days of therapy</th>
<th>Total dose (mg of base)</th>
<th>Mean plasma levels (µg/ml) sustained during days of therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Highest Class I</td>
<td>Highest Class II</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Range of Class II</td>
<td>Range of Class III</td>
</tr>
<tr>
<td>Mepacrine</td>
<td><strong>P. vivax</strong></td>
<td>32</td>
<td>4</td>
<td>380</td>
<td>300 - 630</td>
</tr>
<tr>
<td></td>
<td><strong>McCoY</strong></td>
<td>2</td>
<td>4</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td><strong>Chosson</strong></td>
<td>3</td>
<td>4</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td><strong>Falciparum</strong></td>
<td>24</td>
<td>6</td>
<td>—</td>
<td>590 - 930</td>
</tr>
<tr>
<td></td>
<td><strong>McLendon</strong></td>
<td>22</td>
<td>6</td>
<td>—</td>
<td>450 - 1100</td>
</tr>
<tr>
<td>Primaquine</td>
<td><strong>P. vivax</strong></td>
<td>28</td>
<td>4</td>
<td>—</td>
<td>12.5 - 25</td>
</tr>
<tr>
<td></td>
<td><strong>McCoy</strong></td>
<td>16</td>
<td>4</td>
<td>—</td>
<td>25 - 100</td>
</tr>
<tr>
<td></td>
<td><strong>Chosson</strong></td>
<td>28</td>
<td>4</td>
<td>—</td>
<td>340 - 750</td>
</tr>
<tr>
<td></td>
<td><strong>Falciparum</strong></td>
<td>22</td>
<td>6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td><strong>McLendon</strong></td>
<td>22</td>
<td>6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Chloroquine</td>
<td><strong>P. vivax</strong></td>
<td>25</td>
<td>4</td>
<td>225</td>
<td>130 - 225</td>
</tr>
<tr>
<td></td>
<td><strong>McCoy</strong></td>
<td>25</td>
<td>4</td>
<td>—</td>
<td>225 - 350</td>
</tr>
<tr>
<td></td>
<td><strong>Falciparum</strong></td>
<td>25</td>
<td>6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td><strong>McLendon</strong></td>
<td>25</td>
<td>6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Artemisinine</td>
<td><strong>P. vivax</strong></td>
<td>7</td>
<td>4</td>
<td>300</td>
<td>225 - 300</td>
</tr>
<tr>
<td></td>
<td><strong>McCoy</strong></td>
<td>7</td>
<td>4</td>
<td>—</td>
<td>650 - 750</td>
</tr>
<tr>
<td></td>
<td><strong>Falciparum</strong></td>
<td>7</td>
<td>6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td><strong>McLendon</strong></td>
<td>9</td>
<td>6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Sulfadoxine</td>
<td><strong>P. vivax</strong></td>
<td>19</td>
<td>4</td>
<td>—</td>
<td>350 - 600</td>
</tr>
<tr>
<td></td>
<td><strong>McCoy</strong></td>
<td>19</td>
<td>4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td><strong>Falciparum</strong></td>
<td>25</td>
<td>6</td>
<td>1400</td>
<td>700 - 1700</td>
</tr>
</tbody>
</table>

a One case.
N.D. = No data available. See also note to Table 1.

* Data compiled from:
1. Taggart, J. Y. et al. (1948) J. clin. Invest., 27, 93.
from 20 to 54 μg/litre. A mean plasma level of 18 μg/litre maintained for six days produced only a temporary (Class II) effect in three out of four patients.

**TABLE 3. APPROXIMATE TOTAL DOSES, MEAN PLASMA DRUG LEVELS AND DURATION OF THERAPY AND OF EFFECTIVE PLASMA LEVELS REQUIRED TO ACHIEVE CONSISTENTLY CLASS III EFFECTS IN BLOOD-INOCULATED INFECTIONS DUE TO DIFFERENT STRAINS OF P. VIVAX AND P. FALCIPARUM**

<table>
<thead>
<tr>
<th>Drug</th>
<th>P. vivax strains</th>
<th>P. falciparum strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>McCay</td>
<td>Cheeson</td>
</tr>
<tr>
<td>Quinine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total dose (g base)</td>
<td>2.5 g</td>
<td>9.0 g</td>
</tr>
<tr>
<td>Mean plasma level</td>
<td>5.2 mg/l</td>
<td>8.0 mg/l</td>
</tr>
<tr>
<td>Duration of therapy and of effective plasma levels</td>
<td>4 days</td>
<td>6 days</td>
</tr>
<tr>
<td>Mepronil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total dose (mg base)</td>
<td>720 mg</td>
<td>1100 mg</td>
</tr>
<tr>
<td>Mean plasma level</td>
<td>30 μg/l</td>
<td>50 μg/l</td>
</tr>
<tr>
<td>Duration of therapy and of effective plasma levels</td>
<td>4 days</td>
<td>6 days</td>
</tr>
<tr>
<td>Pregnanil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total dose (mg base)</td>
<td>50 mg</td>
<td>150 mg</td>
</tr>
<tr>
<td>Mean plasma level</td>
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<td>20 μg/l</td>
</tr>
<tr>
<td>Duration of therapy and of effective plasma levels</td>
<td>4 days</td>
<td>4 days</td>
</tr>
<tr>
<td>Chloroquine</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>N.D.</td>
</tr>
<tr>
<td>Mean plasma level</td>
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<td>N.D.</td>
</tr>
<tr>
<td>Duration of therapy and of effective plasma levels</td>
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<td>N.D.</td>
</tr>
<tr>
<td>Antimalumin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total dose (mg base)</td>
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</tr>
<tr>
<td>Mean plasma level</td>
<td>11 μg/l</td>
<td>N.D.</td>
</tr>
<tr>
<td>Duration of therapy and of effective plasma levels</td>
<td>4 days</td>
<td>N.D.</td>
</tr>
<tr>
<td>Sontafine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total dose (mg base)</td>
<td>1100 mg</td>
<td>N.D.</td>
</tr>
<tr>
<td>Mean plasma level</td>
<td>90 μg/l</td>
<td>N.D.</td>
</tr>
<tr>
<td>Duration of therapy and of effective plasma levels</td>
<td>4 days</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

> indicates that this dose or plasma level did not always produce a Class III effect, but no higher doses were tested, or no higher plasma levels were obtained.

N.D. — No data available.

* Data compiled from:
Amodiaquine and sontoquine appeared to be less effective than chloroquine in terms of total dosages and plasma levels required to obtain similar effects. No *P. falciparum* strains other than the McLeod strain have been tested against 4-aminoquinolines under the same conditions.

Tables 2 and 3 give comparative data regarding the suppressive activity of various antimalarial drugs against different parasite species and strains. (The figures reported in the three tables represent minimum rather than average requirements and apply to the four strains employed in these studies but not necessarily to other strains.)

1.3 Studies carried out in Australia

Further experimental evidence of strain differences as regards severity of infection and response to drugs has come from the studies carried out during and after the Second World War at Cairns, Australia. In the course of these investigations, several parasite strains were discovered which differed significantly from strains previously described: New Guinea strains of *P. vivax* and New Guinea strains of *P. falciparum*, including the Aitaipe-Wewak strain.

New Guinea strains of *P. vivax* were found to differ in two respects from the *P. vivax* strains encountered in the Eastern Mediterranean area and from those that had previously been used in experimental work in the United Kingdom and USA: (a) relapses appeared with great regularity within four to eight weeks after the primary attack or after withdrawal of mepacrine, and (b) though acute attacks were readily controlled by drugs, the subsequent relapse rate was very high. (These strains may be identical with the Chesson strain of *P. vivax* used in experimental work in the USA, which is thought to have originated in New Guinea.)

The suppressive activity of quinine, various sulfonamides, mepacrine, proguanil and two 4-aminoquinolines (sontoquine and chloroquine) was tested in volunteers experimentally infected with sporozoites of New Guinea strains of *P. vivax* and *P. falciparum*.

*Quinine sulfate*, at the recommended dose of 10 grains daily (540 mg of quinine base), was shown to be completely ineffective as a suppressant of New Guinea strains of *P. falciparum* and very often failed to suppress *P. vivax*, overt attacks occurring during the period of daily drug administration. With a daily dose of 20 grains (1.1 g of quinine base), suppression of *P. vivax* was complete, but 20 to 30 grains daily (1.1-1.6 g of quinine base) were required for complete suppression of *P. falciparum*. As these doses cannot be tolerated for any prolonged period, quinine proved to be of little value as a suppressant in New Guinea.

*Sulfonamides* differ from other antimalariais in that they are more effective in *P. falciparum* than in *P. vivax* malaria. Sulfadiazone, sulfamerazine and sulfamethazine in a dosage of 1 g daily suppressed sporo-
zoite-induced \textit{P. falciparum} malaria in 20 out of 21 volunteers. When the
daily dose was continued for three to four weeks after the last exposure,
radical cure of \textit{P. falciparum} malaria was obtained in 17 out of 21 volun-
teers. But the same group of sulfonamides given at the same dosage
failed to suppress \textit{P. vivax} and overt attacks occurred in 21 out of 24 volun-
teers who were taking a daily dose of 1 g.

Mepacrine at the originally recommended total weekly dose of 400 mg
(200 mg twice weekly) was found effective in suppressing New Guinea
strains of \textit{P. vivax}, but not those of \textit{P. falciparum}. In volunteers infected
with both \textit{P. falciparum} and \textit{P. vivax} and taking only 400 mg of mepacrine
weekly, \textit{P. vivax} infections remained suppressed whereas \textit{P. falciparum}
malaria broke through with great regularity.

Extensive investigations demonstrated that mepacrine in a dosage of
100 mg daily completely suppressed sporozoite-induced \textit{P. vivax} and \textit{P. fal-
ciparum} malaria in volunteers repeatedly infected with the two species,
provided that the administration of the daily dose was started at least four
weeks prior to the first infective bite and was continued regularly during
the whole period of exposure. \textit{P. falciparum} infections were radically
cured if the daily dose was continued for three to four weeks after the last
mosquito bite. (It is of interest to note that in two volunteers infected
with \textit{P. falciparum}, 300 or 400 mg of mepacrine weekly, though continued
for 28 days after the last infective bite, failed to cure the infection.)

In most subjects, a daily dose of 100 mg of mepacrine was found to
maintain a mean plasma concentration of 20 to 30 \(\mu\)g/litre, considered to
be necessary for the suppression of either vivax or falciparum malaria in
New Guinea. Great variations were found to occur in the mean plasma
levels of individuals receiving the same regimen of mepacrine.

Studies of the "build-up", equilibrium level, and "die-away" con-
centrations of mepacrine observed with prolonged administration of
certain dosage regimens in a group of individuals showed that an equili-
brium level of about 20-30 \(\mu\)g/litre is not reached for some six weeks after
beginning the treatment with a constant dosage of 100 mg of mepacrine daily.

Subsequent field experience in New Guinea and other malarious areas
in the South West Pacific confirmed on a vast scale the effectiveness of
mepacrine prophylaxis in the dosage of 100 mg daily. Apparent failures
of mepacrine suppression were almost invariably shown to be failures to
take the drug regularly. There was, however, one exception: in the
Aitape-Wewak area on the northern coast of New Guinea, it was found
that in addition to the prevalent mepacrine-sensitive strain of \textit{P. falci-
parum}, a relatively insensitive strain was present in a certain number of
patients. In volunteers infected with the Aitape-Wewak strain of \textit{P. fal-
ciparum} and taking 100 mg of mepacrine daily, parasitaemia was not sup-
pressed and break-throughs occurred when the plasma mepacrine level
was well above 30 \(\mu\)g/litre.
Overt attacks due to this strain were also more resistant to therapy; larger doses of mepacrine than the usual 2.8 g were required to produce radical and sometimes even clinical cure. Following the standard course of treatment, recrudescences occurred while a maintenance dose of 100 mg of mepacrine daily was being taken.

To suppress the Aitaipe-Wewak strain of *P. falciparum*, a daily dose of at least 200 mg of mepacrine was required. Even with this dose overt attacks were not always prevented, and radical cure was not necessarily achieved when the 200 mg regimen was continued for 28 days after the last infective bite.

*Proguanil* was found to be very effective against the New Guinea strains of *P. falciparum* and *P. vivax*. No parasites could ever be found in the blood of volunteers repeatedly infected with sporozoites of *P. falciparum* and *P. vivax* while taking a daily dose of 100 mg of proguanil. A course of proguanil consisting of 100 mg thrice daily for ten days (total dose = 3.0 g) produced radical cure in 99% of overt falciparum infections.

4-aminoquinolines: The suppressive effects of chloroquine and sontoquine have been investigated on a small scale at Cairns. In volunteers heavily infected with sporozoites of *P. falciparum* (Aitaipe-Wewak and other New Guinea strains), parasitaemia remained completely suppressed while a daily dose of 100 mg of chloroquine or sontoquine was being taken. If the daily dose was continued for 28 days after the last exposure, radical cure of falciparum malaria resulted. During the four days preceding the first infective bite, a “build-up” was given consisting of a daily dose of 400 mg of chloroquine or sontoquine.

1.4 Relevance of studies to treatment of malaria

It appears from this brief survey that different parasite species and different strains of the same species undoubtedly exhibit differences in virulence and in their response to drugs which have an important bearing on the results of treatment.

It has been shown that *P. falciparum* requires higher doses of drugs and higher plasma levels for effective suppression than *P. vivax*, and that treatment of acute attacks of *P. falciparum* requires not only higher doses and higher plasma levels but also a longer duration of therapy than is needed for acute attacks of vivax malaria.

Furthermore, it is now well established that in addition to interspecific differences in susceptibility to drugs, there are also intraspecific differences, since each plasmodium species consists of numerous strains which may show wide variations in their response to the same drug and dosage.

Differences in susceptibility to drugs of various parasite strains greatly increase the difficulties of assessing the true value of antimalarial com-
pounds, since doses that prove effective in one area may be insufficient in another, where the prevailing parasite strain is inherently less sensitive to the drug. It follows that to prevent misleading conclusions being reached, strains of different geographical origin should be used in evaluating antimalarial drugs.

Despite their obvious importance, species and strain differences are not the only factors that influence the results of treatment. It has been recognized that individuals may differ greatly in their reactions to malaria infection and to a given course of treatment. Even under highly standardized experimental conditions, when similar subjects, inoculated on the same occasion with the same dose of parasites of the same strain, are treated at the same stage of their infections with the same drug and dosage, the results obtained are never the same in all subjects; identical drug regimens may produce different plasma levels in different individuals and this may be associated with different therapeutic results. Even greater individual variations may therefore be expected when natural infections are treated in the hospital or under field conditions.

The response of any subject to treatment depends not only on the species of the parasite and its strain but on the interaction of a great number of factors, most of which are imperfectly known, so that their relative importance cannot be adequately assessed. If follows that the routine application of a rigid, standard dose or standard treatment cannot be expected to be effective in all instances and the recommended therapeutic regimen should be considered as a guide, to be modified within a reasonable range in relation to the circumstances.

2. THE PRESENT SITUATION IN REGARD TO THE REPORTED CASES OF RESISTANCE OF MALARIA PARASITES

2.1 Proguanil and related compounds

Reduced susceptibility of human plasmodia to proguanil (and more recently to chlorproguanil) has been observed since 1948 in Assam, Cambodia, Ghana, Indochina, Indonesia, Kenya, Malaya, New Guinea, Tanganyika and Thailand.

It had previously been found that various strains reacted somewhat differently to the same dosage schedules. Thus, the McCoy strain of *P. vivax* (of American origin) was susceptible in non-immune hosts to 200 mg given over four days and the Chesson strain of this species (from New Guinea) was only slightly less susceptible; the latter strain was subsequently made resistant to a daily dose of 1.6 g of proguanil and was then found to multiply at the same rate as the original strain and to be
equally infective to the vector. Other New Guinea strains were unfail-
ingly suppressed by a daily dose of 100 mg. Infections with the Costa
strain of *P. falciparum* (of American origin) were not, however, always
radically cured in non-immune hosts by total dosage schedules ranging
from 340-750 mg of proguanil. New Guinea strains were suppressed by
a daily dose of 100 mg and, in all but one instance, the infection was cured
by 300 mg taken daily for ten days. The recrudescences required a fur-
ther course of no less than 1.0 g daily for 14 days to achieve a radical
cure. In 1948 and 1949 it was reported that infections with some Indian
and Nigerian strains of *P. falciparum* were not cured by courses of 300 mg
of proguanil given daily for 10 and 14 days respectively.

Despite these findings of variable response of malaria parasites to
proguanil, it has recently come to be accepted that infections, particularly
those due to *P. falciparum*, which could not be radically cured by a course
of treatment consisting of 300 mg of proguanil daily for five days should
be considered relatively resistant. At the end of 1948, one year after
proguanil had been introduced into Malaya, infections with *P. falciparum*
in the Tampin area failed to respond to single doses of 100-300 mg and,
in 1949, they could not be cured by a course of treatment consisting of
300 mg of proguanil daily for 7-10 days. It also appeared that some
*P. vivax* infections were not responding to proguanil. In 1961-62, in
northern Perlis (Malaya), 10% of adult non-immunes reputedly receiving
200 mg daily developed falciparum malaria.

In some instances, primary resistance has developed to pyrimethamine
in the course of community-wide administration of that drug, cross-resis-
tance to proguanil being noted only incidentally (Cambodia, Ghana, Tan-
ganyika, West Irian); in Tanganyika, such cross-resistance to proguanil
and chlorproguanil did not become apparent until 1959, when pyrimetha-
mine resistance had been widespread for three years. This apparent
lack of cross-resistance occurs also in Malaya, where it is believed that
some strains of parasite are resistant to both drugs whereas others are
resistant to only one. Experimental work with non-human plasmodia
has shown that the phenomenon is very variable: some proguanil-resistant
strains are resistant to pyrimethamine whereas others are not, but all
pyrimethamine-resistant strains have been resistant to proguanil. This
is probably because pyrimethamine is a much more potent antimalarial
than proguanil; a strain of parasite completely resistant to proguanil
may still be sensitive to tolerated doses of pyrimethamine.

Resistance to the dihydrotriazine metabolite of proguanil, adminis-
tered as an injection of cycloguanil pamoate (CI 501), a long-acting reposi-
tory drug, has appeared in *P. falciparum*. In the course of a field trial
in West Pakistan, the drug was given to 40 subjects with patent parasita-
emia. In all of them parasite clearance was obtained; however, three
children had a recrudescence. Parasitized blood from one of these chil-
dren was later inoculated into non-immune subjects and was found to be resistant to proguanil and to pyrimethamine.

In the USA, a Southern Rhodesian strain of *P. falciparum* was found on subinoculation to be resistant to both cycloguanil and proguanil. In studies among volunteers, cycloguanil failed to exert a long-term protective effect against the patenty of mosquito-induced infections with Thailand (JHK) and South Viet Nam (Sn) strains, which had been shown to be resistant to proguanil and chloroquine.

A strain of *P. gallinaceum* made highly resistant to cycloguanil was found to be resistant to proguanil but sensitive to pyrimethamine, and a strain of *P. berghei* rapidly made resistant to more than 800 times the normally effective dose of cycloguanil, showed some degree of cross-resistance to pyrimethamine and primaquine, while showing hypersensitivity to sulphadiazine and diaphenyl-sulfone (Dapsone) and a normal response to chloroquine and mepacrine.

The responses of some chloroquine-resistant strains of *P. falciparum* to various dosage schedules of proguanil that have failed to cure chloroquine-resistant *P. falciparum* after subinoculation into non-immune volunteers are shown in Tables 4 and 5.

2.2 Pyrimethamine

2.2.1 Distribution of known foci of resistance

Resistance of human plasmodia (mainly *P. falciparum*) to pyrimethamine has been observed since 1953 in Kenya, Cameroun, Ghana, northern and south-western Nigeria, Senegal, southern Sudan and Upper Volta. It has also occurred in Venezuela, Cambodia and West Irian and, recently, as part of multi-drug resistance, in the Rupununi District of British Guiana, Cambodia, Malaya and Thailand. Preliminary observations in many of these places had indicated that the parasites were initially highly sensitive to the drug, a single dose of 25 mg given once a week being adequate.

In Kenya pyrimethamine was given in 1952 in three mass treatments at intervals of six months to the inhabitants of a hyperendemic area of Makuori in Machakos district. Each treatment consisted of a single dose of 100 mg of the drug or the equivalent for children. All schoolchildren were given an additional dose at monthly intervals over a period of eight months. From the sixth month onwards an increasing number of infections with *P. falciparum* and some with *P. malariae* were detected. The infections that were not susceptible to pyrimethamine were also resistant to proguanil but, since this drug had been used in the area before the pyrimethamine trial began, there is no proof that cross-resistance between these compounds had occurred.
In Tanganyika in 1953, in a holoendemic area where *P. falciparum* predominated, single doses of 100 mg of pyrimethamine or the equivalent dose for children were given monthly to inhabitants of the village Mgeza near Muhaza. Although an initial treatment at this dosage had apparently been successful, within three months many *P. falciparum* infections were found among the treated population, and after six months the incidence of this parasite was almost as high as before treatment. In a locality 12 miles away, pyrimethamine in the same quantity was administered to several thousand inhabitants of the Mkuzi area once a week for 20 months, initially with success but ultimately, after five months had elapsed, with the reappearance of *P. falciparum* in people treated regularly. This strain did not respond to treatment with the maximum permissible dosage of 150 mg of pyrimethamine given to semi-immune children aged ten; however, in a few cases thus treated it appeared that a much smaller dose of 25 mg given two weeks later suddenly resulted in parasite clearance. This was attributed to ill-defined factors of high host immunity. In contrast to experimental studies on *P. gallinaceum* the Tanganyika strain of pyrimethamine-resistant *P. falciparum* continued to respond in the normal manner to proguanil and not for a further three years was resistance to proguanil and chlorproguanil apparent. *P. malariae, P. vivax* and *P. ovale* were cleared with the first treatments and did not recur until drug administration ceased. It is important to note that in the two above-mentioned trials in Kenya and Tanganyika the standard of drug administration and investigation was good. Confirmatory chemical tests of the absorption of pyrimethamine were made on blood samples obtained 3 hours after the ingestion of a single dose of 150 mg by semi-immune children about ten years of age, who were carriers of the resistant strain of the parasite.

In Katsina Emirate, Northern Nigeria, where *P. falciparum* was the commonest malaria parasite, a marked reduction in the parasite rate was observed at first when pyrimethamine was distributed in a dosage of 25 mg at monthly intervals. However, after three months had elapsed the incidence of *P. falciparum* again increased and this species regained its former position eight months after the commencement of treatment. In Senegal, where pyrimethamine had been used since 1958, there was a sharp rise in *P. falciparum* infections among children receiving the drug every second week in the equivalent of an adult dose of 50 mg. In a rural community in Ghana given pyrimethamine once a week at a dosage of 12.5 mg for infants and 25 mg for older people, the marked initial fall in parasitaemia proved to be transitory; *P. falciparum* reappeared by the 37th week of treatment and was refractory to twice the original dosage.

In a holoendemic area of the Northern Camerouns, combined treatment with 75 mg of chloroquine and 6.25 mg of pyrimethamine weekly was instituted for children aged less than nine years. After three months
### TABLE 4. RESULTS OF STUDIES ON STRAINS OF P. FALCIPARUM

<table>
<thead>
<tr>
<th>Strain</th>
<th>Chloroquine (1.5 g base over 3 days)</th>
<th>Amodiaquine (1.4 g base over 3 days)</th>
<th>Mepacrine (2.5 g salt over 7 days)</th>
<th>Proguanil (1.5 g salt over 5 days)</th>
<th>Pyrimethamine (50 mg base)</th>
<th>Quinine sulphate (9.7 g over 5 days)</th>
<th>Primaquine (30 mg base daily for 7 days)</th>
<th>Other Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colombia</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S?</td>
<td>S?</td>
<td>R to hydroxychloroquine, 1.2 g base</td>
</tr>
<tr>
<td>Thailand (JHK)</td>
<td>R</td>
<td>R (600 mg)</td>
<td>R</td>
<td>R</td>
<td>R (100 mg)</td>
<td>S</td>
<td>S?</td>
<td>R to amopyrinquin, 300 mg i.m. over 3 days; R to 377 C 54, f 1.2 g</td>
</tr>
<tr>
<td>Cambodia (I)</td>
<td>S (but R to 600 mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cambodia (II)</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malaysia (I)</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td></td>
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</tr>
<tr>
<td>Malaysia (II)</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
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</tr>
<tr>
<td>Malaysia (III)</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td></td>
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</tr>
</tbody>
</table>

* Studies conducted by the US National Institutes of Health, Washington, USA.
R = asexual erythrocytic forms resistant to drug.
S = asexual erythrocytic forms sensitive to drug (and radical cure affected).
R = Prevented sporozoite infections when combined with chloroquine.
\(^a\) Appeared normally sporontocid.
\(^b\) 377 C 54 is a code name for 1,6-dihydroxy-2,5-bis (cyclohexylaminomethyl)naphthalene.
<table>
<thead>
<tr>
<th>Strain</th>
<th>Chloroquine (1.5 g base over 3 days)</th>
<th>Chloroquine (1.0 g base over 3 days)</th>
<th>Hydroxychloroquine (1.5 g base over 3 days)</th>
<th>Amodiaquine (14.4 g base over 3 days)</th>
<th>Pyrimethamine (150 mg base over 3 days)</th>
<th>Primaquine (200 mg base over 10 days)</th>
<th>377 C-54 (1.5 g base over 3 days)</th>
<th>Quinine sulfate (13.6 g over 7 days)</th>
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<tbody>
<tr>
<td>Colombia</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
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<td>R</td>
<td>R</td>
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<tr>
<td>Thailand (And.)</td>
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<td></td>
<td>R</td>
<td>R</td>
<td>R</td>
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<td>R</td>
<td>R</td>
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<tr>
<td>Thailand (Wa.)</td>
<td>R</td>
<td></td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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</tr>
<tr>
<td>Vietnam (Sao)</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
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</tr>
<tr>
<td>Vietnam (CV)</td>
<td>R</td>
<td></td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Malay (Comp.)</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

* Studies conducted by the Army Medical Research Unit, Department of Medicine, University of Chicago, USA.
R = asexual erythrocytic forms resistant to drug.
S = asexual erythrocytic forms sensitive to drug (and radical cure affected).
^ Only limited, preliminary studies conducted with these strains.
^ Two of six non-immune volunteers treated with 10 grains (647 mg) of quinine sulfate every 8 hours for 7 days had recrudescence (total dose = 13.6 g of quinine sulfate).
of treatment a marked fall in malarialometric rates was observed, but this was not maintained. Subsequent observations suggested the presence of *P. falciparum* resistant to pyrimethamine.

Resistance to pyrimethamine has been observed in West Irian (New Guinea) where cooking salt containing the drug was distributed in a holoendemic malarious area. Resistance appeared in *P. falciparum* within six months from commencement of the medicated salt distribution, but *P. malariae* and *P. vivax* were eliminated. This pyrimethamine-resistant strain was also insusceptible to proguanil in a dosage of 600 mg daily.

Evidence concerning pyrimethamine resistance of south-east Asian and south American strains of *P. falciparum* (showing a refractory response to chloroquine) is summarized in Tables 4 and 5. In the course of these investigations resistance of *P. vivax* to pyrimethamine has been observed.

It should be noted, however, that in some areas pyrimethamine has been used for long periods among non-immunes and semi-immunes alike without resistance becoming apparent. For example, in Liberia 25 mg of the drug were distributed monthly for more than a year; in a holoendemic area of Nigeria 50 mg were given monthly for 5 years to pregnant women and a smaller dose to children (febrile cases being treated with chloroquine); near Lagos 25 mg were given monthly to village inhabitants for two years; and in Tanganyika the equivalent of the adult dose of 100 mg successfully suppressed all species of malaria parasites among schoolchildren living some 400 miles west of the focus of pyrimethamine resistance near Mkuzi. No resistance to pyrimethamine has been reported from the USSR, where the drug was given prophylactically.¹

The rapid development of pyrimethamine resistance upon exposure to a high dose has been observed in non-immune subjects infected with *P. falciparum*, *P. malariae* and *P. vivax* and treated with two 100-mg doses of pyrimethamine, and in the field this has commonly occurred with *P. falciparum* and at least once with *P. malariae*. In experimental work, it has been repeatedly observed that selection of resistant strains is more rapid when a large parasite population is exposed to large drug doses with frequent sub-passages. Resistance in *P. gallinaceum* developed at least as rapidly in strains treated with large doses (0.05 mg/20 g) as in those treated with one-fiftieth the amount. Laboratory strains resistant to pyrimethamine have always proved to be cross-resistant to proguanil, and this has recently been confirmed with *P. cynomolgi bastianellii*. It is significant that procedures that succeeded in selecting strains resistant to pyrimethamine and to diaphenylsulfone separately failed to produce resistance to either drug when the two were used in combination.

2.2.2 Geographical spread of resistance

All the factors that affect the geographical spread of drug-sensitive parasites (gametocyte densities in the host, vector capacity for transmission, response on the part of the semi-immune recipient, etc.) are also operative in respect of the resistant parasite. In addition, special situations arise when the drug pressure ceases: first, resistance may be an unstable characteristic of the strain and soon decrease; second, the strains then have to compete with a resurgent wave of drug-sensitive parasites which dilutes the resistant strain and reduces its chances of propagation. With regard to the first point, laboratory studies on non-human species of plasmodia indicate that resistance to pyrimethamine, once manifested, remains stable in contrast to that of resistance to chloroquine. Knowledge concerning dominance of genes transmitting drug resistance might be helpful in understanding the spread of drug-resistant parasites in nature.

The spread of pyrimethamine-resistant *P. falciparum* from a single focus has been followed over a ten-year period in a holoendemic area of Tanganyika. Despite the difficulties and imperfections of an investigation of this size under constant variations of natural conditions, certain attributes of the Tanganyika strain or strains are now apparent.

Parasite densities of the resistant strain, even when existing alone in the semi-immune host under the pressure of continuous pyrimethamine treatment, remain so low that many infections are missed in single-film surveys. Serial films as a part of a longitudinal survey are accordingly particularly desirable when dealing with resistant parasitaemia, but such a procedure may not be practicable on a large scale. Moreover, clinical attacks of malaria appear to be less frequent in the investigated population. When drug pressure is removed and drug-sensitive strains reappear at the higher density, usual for the particular degree of immunity, resistant parasites become even scarcer and the proportionate reduction in the number of gametocytes suggests that transmission must suffer competitively. Multiple strains differing one from another in degree of resistance (as revealed by provocative drug testing) appear to co-exist with susceptible strains in the same host. Laboratory studies of this aspect are somewhat contradictory: the growth rate of a pyrimethamine-resistant strain of *P. berghei* is frequently less than that of a wild susceptible strain, with the result that the resistant strain is soon swamped. This has not proved true in respect of simian malaria, where the multiplication rates of resistant strains and their sensitive counterparts have been identical. However, sulfadiaizine-resistant strains of *P. cynomolgi bastianelli* and *P. berghei*, when mixed with equal numbers of the sensitive parent strains and inoculated into new hosts, have lost their resistant characters within a few weeks. Such exact studies are not entirely applicable to the situation
in the field because in the holoendemic area of human malaria described here there is continuous transmission of resistant and susceptible strains of *P. falciparum*, the inoculation rate being about one infective bite every night for each person.

These observations suggest that pyrimethamine-resistant *P. falciparum* might have difficulty in becoming established in areas already densely populated by the normal strains of the four species of human malaria parasite. In Tanganyika the spread of the resistant strain from its original focus has taken place through holoendemic malarious country, the human inhabitants of which are infected with pyrimethamine-susceptible *P. falciparum*. The presence of the resistant strain is ascertained by administration, under careful supervision, of a single dose of 25 mg of pyrimethamine to semi-immune carriers of *P. falciparum*, 6-12 years of age. Seven days after drug administration, their blood is examined for persistent trophozoites; repeated testing has shown that they are consistently cleared of drug-sensitive trophozoites when given this quantity of pyrimethamine.

Study of the spread of resistance which has occurred in Tanganyika in the absence of treatment with pyrimethamine has indicated that the resistant strain that emerged in 1953 in the Mnageza-Mkuzi area was present five years later in localities 20 miles away. In 1964 the pyrimethamine-resistant strain constitutes approximately two-fifths of the entire *P. falciparum* population, and it is slowly spreading to places more than a hundred miles away.

### 2.3 4-aminoquinolines

Decreased susceptibility on the part of the malaria parasite to the 4-aminoquinolines, with particular reference to chloroquine, has been reported during the past few years from Brazil, British Guiana, Cambodia, Colombia, Malaya, Thailand and Viet Nam.

**Colombia**

In 1959 and 1960, two infections of *P. falciparum* were acquired in the Magdalena valley in Colombia. One was repeatedly treated with chloroquine, from July to November 1959, but the plasmodia were not transmitted to volunteers for further study. The other was encountered a year later. This second patient had received a first course of chloroquine (dosage unknown) in Colombia in May 1960. About 6 weeks later, on his return to the USA, he had a relapse and was treated with a five-day course of 2.1 g of chloroquine base; three weeks later, when ring forms were found again, the strain was passed into neurosyphilitic patients. In six of these patients the parasites were not cleared by a single dose of 600 mg and a course of 1.5 g of chloroquine, or they disappeared slowly
only to reappear within ten to fourteen days. This tardy or incomplete response was observed after mosquito passage, and the administration of 300-400 mg of chloroquine once a week failed to suppress parasitaemia. The strain responded favourably to proguanil and pyrimethamine but not to amodiaquine, and although mepraquine removed patent parasitaemia temporarily, recrudescence occurred in four out of six patients. This strain of *P. falciparum* appeared insensitive also to standard doses of hydroxychloroquine and amodiaquine, but radical cure was obtained with quinine. The actual drug regimens used in the investigations carried out on human volunteers are given in Tables 4 and 5.

Although a brief survey carried out in the Magdalena Valley failed to reveal the presence of infections insensitive to chloroquine, there have been recent reports of relatively resistant *P. falciparum* infections in the departments of Córdoba, Magdalena and Norte de Santander.

**Brazil**

It has been reported recently from localities in the following states and territories in Brazil that some infections of *P. falciparum* malaria do not appear to be yielding to courses of chloroquine considered by the field investigators normally fully effective: Amapá, Amazonas, Goiás, Mato Grosso, Para, Rio Branco, and Rondônia.

Several patients with *P. falciparum* infections apparently acquired in the Amazon area were treated in medical centres in Brazil and showed unusual responses to 4-aminoquinoline and other antimalarial drugs. Some of these strains were subinoculated into neurosyphilitic patients and it is reported that infections in two subjects given 3.0 g chloroquine orally over three days and in one given 2.54 g parenterally over 11 days did not recrudesce; in one patient given the 3.0-g oral course, the infection recrudesced in 19 days.

It has also been reported that subinoculation of Brazilian strains of *P. falciparum* considered resistant to chloroquine has been carried out in neurosyphilitic patients at the Ribeirão Prêto Screening Centre: at least two strains of falciparum malaria (Rondônia and Boa Vista) recrudesced within 11 to 33 days after treatment with exceptionally high doses of chloroquine (2.4-4.95 g in two to three days).

**British Guiana**

It has been reported from British Guiana that *P. falciparum* infections with decreased susceptibility to chloroquine have appeared since 1962 in the Lethem area of the Rupununi district near the Brazilian border, where a medicated salt project has been in operation since 1961. Higher incidence of clinically mild malaria was observed in this area during 1963 and 1964 and it was tentatively suggested that the strain of *P. falciparum* with an abnormal response to chloroquine was brought to this area from
the Roraima Federal Territory where the operational difficulties of medicated salt distribution were particularly great. Further investigation is in progress.

Thailand

*P. falciparum* from an infection contracted in Thailand in 1961 has been studied intensively. The subject, an American serviceman, was treated with chloroquine at irregular intervals for three months. He received a total of 2.1 g of chloroquine and 200 mg of primaquine in November 1961 and when parasitaemia reappeared a month later he was given an additional 4.2 g of chloroquine over 21 days. One month later, after he had been transferred to a malaria-free country, parasitaemia reappeared and he received a course of 1.5 g of chloroquine. Parasites again appeared in the blood and the strain (Thailand (JHK)) was studied in human volunteers. The results summarized in Tables 4 and 5 indicate poor response to chloroquine, amodiaquine, mepacrine, pyrimethamine and proguanil, but quinine proved effective.

Since this discovery, recrudescences of *P. falciparum* infections among Thai people treated with chloroquine have been observed at the Bangkok hospital. Following treatment with a course of 1.5 g, nine of 48 *P. falciparum* infections relapsed in two to three weeks. When the course was repeated, one patient relapsed once more and another twice. These patients came from various parts of Thailand, but some lived near the Malaysian or Cambodian border. A brief field survey carried out in a village where one of the hospital patients might have been infected, indicated the possibility of the presence in two children of *P. falciparum* relatively resistant to chloroquine.

It has been reported recently that several other Americans in Thailand acquired *P. falciparum* infections which did not respond fully to chloroquine. Parasites from two of these individuals have been transferred to non-immune volunteers in a non-malarious area, and preliminary studies indicate that they are resistant to 1.5 g of chloroquine given over three days (Table 5).

Cambodia

In October 1962, three research workers were infected with *P. falciparum* during a brief visit to the Pailin area of Cambodia. Although they were supposed to have been taking chloroquine as a suppressive whilst in Cambodia, this was not continued when they returned to a non-malarious area. One of these patients was given 1.8 g of chloroquine over four days, but parasites reappeared 15 days later. He was then given a course of 1.5 g; recrudescence occurred 17 days later, when a further course of 1.5 g was given. Parasites reappeared 20 days after this last course, at a time when the plasma chloroquine was reported as
being 80 µg/litre. The second patient showed a recrudescence after two courses of combined chloroquine and pyrimethamine; the third case occurred in a subject who had taken 900 mg of chloroquine over eight days as a suppressive; 900 mg given in one day eliminated the infection.

*P. falciparum* from two of these patients were inoculated into volunteers, with the results shown in Table 4. It was found that the Cambodia (I) strain was susceptible to the 1.5 g course but not to 600 mg, while Cambodia (II) was resistant to chloroquine, mepacrine, proguanil and pyrimethamine in the doses shown, but susceptible to quinine.

A brief field survey carried out among the semi-immune inhabitants of the village in the Pailin area, where these infections occurred, gave reason to suspect that some *P. falciparum* and *P. vivax* infections did not fully respond to a single adult dose of 600 mg of chloroquine.

**Viet Nam**

In 1962 an American serviceman, apparently taking 300 mg of chloroquine weekly as a suppressive whilst at Nha Trang in south Viet Nam, developed a clinical attack of falciparum malaria. He received chloroquine treatment on three occasions at intervals of 2-3 weeks because of recrudescences. Studies on this parasite strain (Viet Nam (Sn)) inoculated into volunteers gave the results shown in Table 5.

It has been reported recently that several other Americans in South Viet Nam acquired *P. falciparum* infections which did not respond fully to chloroquine. Parasites from one of these subjects were transferred to non-immune volunteers and studies on this strain (Viet Nam (CV)) are in progress (Table 5).

**Malaya**

A series of reports on high incidence of malaria in 1962 in non-immune troops operating in northern Malaya, together with observations at the military base hospital situated in a transmission-free area, indicated that treatment with 2.4 g of chloroquine in 5 days was proving inadequate in some patients. Response to a number of other synthetic antimalarials was also inadequate, although 20 g of quinine given over ten days resulted in a cure.

Inoculation of four Malayan strains into human volunteers has been carried out in the USA. The resistance spectrum for each of the four strains (Malaya I, Malaya II, Malaya III and Malaya (Camp)) is shown in Tables 4 and 5. With regard to the Malaya (Camp) strain, it is noted that 10 grains (647 mg) of quinine sulfate given every eight hours for seven days was successful in six out of seven non-immune volunteers, the seventh having a recrudescence. Pyrimethamine did not effect radical cure in any of the four volunteers treated. In contrast, the administration of
pyrimethamine (25 mg every 12 hours for three days — total amount 150 mg) concurrently with sulfadiazine (500 mg every six hours for five days — total amount 10.0 g) effected radical cure in five out of six non-immune volunteers treated during their first acute attacks, although this combination acted slowly.

A field study was carried out in north Perlis in 1963 among the indigenous semi-immune population, using a single adult dose of 300-600 mg of chloroquine. Neither dose effected radical cure in a proportion of subjects. Those not cleared were then given 1.2 g over three days (adult dose) and 12 of 50 showed parasites within four weeks, two at the same time as the chloroquine content of their plasma was 18 µg/litre and 23 µg/litre, respectively. However, a similar field test carried out in 1964 suggested that *P. falciparum* in the coastal plains of southern Perlis and northern Kedah, and in Pulau Aur responds to single doses of 600 mg of chloroquine.

*Other observations*

Although decreased susceptibility to chloroquine on the part of *P. falciparum* has been reported from Upper Volta and Liberia, confirmation has not been obtained and investigations continue. Careful studies have disproved a number of alleged findings of chloroquine resistance in Guinea, south-eastern Nigeria and Tanganyika.

It is notable that the prolonged use of chloroquine on a large scale in Ghana, Madagascar and Tanganyika by direct administration of the drug and as chloroquinized salt has not resulted in the appearance of resistance. This is particularly relevant in Madagascar, where once-weekly chloroquine has been distributed for 12 years as an effective suppressant to 1 ½ million children.

Points of considerable importance in the epidemiology of chloroquine-resistant malaria concern the speed of development and the rate of loss of resistance. It is apparent from experimental work that although chloroquine resistance has been obtained by several workers in *P. berghei* it is much more difficult to produce than resistance to proguanil or pyrimethamine. Repeated attempts under a variety of conditions have been unsuccessful with *P. gallinaceum*; nor has resistance been produced in *P. cynomolgi*. One of the strains of *P. berghei* has been found to show marked cross-resistance to amodiaquine and mepracrine, some cross-resistance to quinine, and slight cross-resistance to primaquine, but it is sensitive to proguanil and pyrimethamine and hypersensitive to sulfadiazine and diaphenylsulfone. When drug pressure is removed, chloroquine resistance in *P. berghei* is rapidly lost.
3. PROCEDURE FOR ASSESSING THE RESPONSE OF MALARIA PARASITES TO TREATMENT

3.1 Definition of drug resistance

The reference to malaria parasites resistant to drugs has been considered by the Group principally with regard to clinical and public health problems that it may create. The seemingly simple expression "drug-resistant malaria" conceals a complicated biological phenomenon due to the flexible response of living organisms to various adverse conditions. The Group discussed the problem of resistance of human malaria parasites in relation to drugs "which, administered to the vertebrate host in adequate and safe doses, normally destroy or contribute to the destruction of malaria parasites at some stage or other in their life cycle".1

Drug resistance has been defined as the "ability of a parasite strain to multiply or to survive in the presence of concentrations of a drug that normally destroy parasites of the same species or prevent their multiplication. Such resistance may be relative (yielding to increased doses of the drug tolerated by the host) or complete (withstanding maximum doses tolerated by the host)".2

Some geographical strains of malaria parasites may be inherently resistant to drugs; other strains may show a changed response to the drug or to its metabolite only after a significant contact with it, due to the treatment of the human hosts.

A distinction between relative and absolute degrees of resistance may be difficult, if not impossible, to evaluate in cases of human malaria. *P. falciparum* infections, for example, are often of a severity that precludes any quantitative assessment of the level of their drug sensitivity.

Taking into account the fact that strains of malaria parasites may differ greatly in their response to drugs, the definition of relative resistance might read: "Ability of a parasite strain to survive and/or to multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within the limits of tolerance of the subject".

3.2 Assessment of the response of malaria parasites to treatment

The Group assessed the evidence available concerning the resistance of human malaria parasites to drugs and considered the relevant published data as well as the information contained in unpublished reports.

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The serious potential implication of any report on higher tolerance or resistance of *P. falciparum* to chloroquine were emphasised. In this respect the Group fully endorsed the statement made previously by the WHO Technical Meeting on Chemotherapy of Malaria.\(^1\)

"It is evident that [such] . . . records demand the fullest and strictest investigation, before reports on them are circulated or published. The relevant investigation should cover all the circumstances under which the alleged resistance appeared, its degree, geographical distribution and transmissibility through the vector. Evidence of resistance of malaria parasites to any drug can only be obtained if the response of the relevant strain to the specific drug is assessed under rigorous conditions excluding reinfection and assuring that the drug has been ingested and reached adequate blood level. Final proof of resistance requires the transfer of such a strain of malaria parasite to an uninfected, non-immune host."

Despite this warning, given three years ago, knowledge concerning resistance of plasmodia to antimalarials continues to be bedevilled by a number of reports, usually of one or two cases, in which resistance has been claimed merely on the grounds that the patient was reputedly taking the antimalarial in prophylactic doses and had developed fever diagnosed as malaria. The Group therefore stressed that in assessment of the response of malaria parasites to treatment the strictest possible criteria should be applied.

Numerous variables and potential sources of error must be taken into account in determining the response of malaria parasites to drugs. This discussion concerns chiefly evaluation of the response of asexual erythrocytic forms of *P. falciparum* to 4-aminoquinolines. Many conditions necessary for adequate evaluation apply, however, regardless of whether blood schizontocidal, tissue schizontocidal, gametocytocidal, or sporontocidal properties of various drugs to any species of human plasmodia are under consideration. Although certain of these conditions may be more easily satisfied in a clinical research centre, they are no less essential in the field if satisfactory data are to be obtained. Factors that merit consideration include those pertaining to (a) the drug, (b) administration of the drug, (c) assessment of absorption of drugs given orally, and (d) the response of the parasite in the individual receiving the drug.

(a) *Factors pertaining to the drug.* Administration of the drug must be justified and the particular drug employed must be appropriate for the situation at hand. Many important precautions must be taken to be reasonably certain that causes of drug failure other than the response of the parasite are excluded. The drug must be of satisfactory quality and the formulated product must be stable, corresponding in chemical

formula and in dose to the information given on the label. The dose
should be expressed in terms of the base and the particular salt employed
should be noted. Solubility may be important when various salts of the
same drug are available; markedly different blood levels and therapeutic
responses may be obtained, depending upon whether salts of high or low
absorbability are administered. Solubility and particle size of the drug,
and the nature of the vehicle may be important, particularly when drugs
are administered parenterally. Preparations of drugs administered orally
must not be such that tablets fail to disintegrate, or disintegrate too low
in the bowel for adequate absorption. Attention should be given to the
quality of drug tablets; some may have been stored for considerable periods
under tropical conditions and chemical or physical changes may have
taken place as a result of humidity and heat and the growth of moulds.

(b) Administration of the drug. The dose must be appropriate to
the weight of the recipient. There must be close and direct supervi-
sion of administration, so that both ingestion and retention of orally
administered compounds are assured. It is important to recognize that
many people are prejudiced against acceptance of drugs and that even
when given tablets to swallow they may hide them under the tongue or
elsewhere and later spit them out. In many areas, it is unrealistic to
expect people to take tablets regularly, or indeed at all, without direct
supervision. Such tablets are frequently thrown away, or find their way
to other persons or may be sold. Immediate or delayed vomiting may
occur after the tablets have been swallowed; their absorption may also
be limited by abnormal conditions affecting the bowel. Children who
swallow drugs on an empty stomach frequently vomit; this can be preven-
ted by giving some water, sweets or food at the same time as the drug.

(c) Assessment of absorption. Tests made on urine and on plasma
are useful methods of checking that a drug has been taken and some of
it absorbed. Appropriate precautions are necessary to ensure that no
other drugs are administered that may interfere with the results of the
plasma or urine examinations, because other drugs may give positive
Haskins tests.1 It is possible that renal disease may affect somewhat
the excretion of antimalarials. In this connexion the work on the associa-
tion between malarial infection and nephrosis is noteworthy. Urinary
excretion of 4-aminoquinolines varies and may be affected by fluctuations
of urinary pH.

(d) Assessment of the response of the parasite. Accuracy of parasit-
ological observations is of critical importance. Facilities and personnel
should be such that there is no doubt of the validity of observations con-
cerning the stage or species of malaria parasite detected in blood films.

1 Details of methods for assessment of urinary excretion of 4-aminoquinolines
are given in Annex 2.
Artefacts are especially liable to occur in the tropics and may pose a problem in the examination of thick and thin blood films, particularly the former. Faulty techniques of staining and improper storage of stains are generally responsible for such errors. It should be remembered that artefacts and platelets, mistaken for parasites, have been in the past major causes of misconceptions regarding resistance. Care is also required not to mistake early gametocytes for schizonts.

The frequency and extent of observations that may be appropriate for the investigation of suspected drug resistance depend on the situation. Key questions concern evaluation of the effects of 4-aminoquinoline drugs administered to patients with clinical attacks of falciparum malaria. If possible, such patients should be admitted to hospital, observed closely and kept under conditions that preclude reinfection. The welfare of the patient with malaria is of paramount importance and assessment of resistance must be subservient to the medical needs at all times. Any possible emergency measures or alternative therapy should be anticipated.

Experience of the effect of 4-aminoquinoline compounds in the treatment of *P. falciparum* infections of normal sensitivity has shown that after administration of the appropriate dose (or doses) fever, if present, usually abates within two to three days and patent parasitaemia should subside within three days. More rapid abatement of symptoms and disappearance of parasitaemia often occur in individuals treated during mild clinical attacks and in partially-immune patients. However, complete subsidence of fever and of patent parasitaemia may at times require 4-6 days, especially in severe infections attended by high levels of parasitaemia in non-immune persons.

Clinical observations are of considerable value in assessing response to treatment, but clinical findings may at times be misleading when there is dissociation between severity of symptoms and levels of parasitaemia. Non-immune individuals may experience a severe clinical attack of malaria although the density of *P. falciparum* parasites detectable in blood smears is very low. Wide and rapid fluctuations in levels of parasitaemia in patients with falciparum malaria, particularly in non-immune individuals, often make it necessary to take blood films for examination several times a day if a relatively accurate assessment of the course of parasitaemia is to be obtained. Persistence of pyrexia or other symptoms may well result from coincidental disease unrelated to malaria. It is noteworthy in this connexion that multiple causes of fever in tropical work are the rule rather than the exception. It is therefore suggested that, whenever possible, parasite counts should be done after drug administration in order to provide more precise data.

It should be recognized that despite the absence of patent parasitaemia, asexual erythrocytic forms may at times persist in the blood at very low (subpatent) levels and give rise to a subsequent recrudescence. Follow-
up observations are necessary after administration of "standard" doses of 4-aminoquinolines and during "follow-up treatment" (weekly administration of a 4-aminoquinoline drug for one month after the treatment of the acute attack). It may be impossible to distinguish between a recrudescence and a reinfection unless the conditions under which the observations are made preclude reinfection.

If a legitimate suspicion arises of drug-resistance of a malaria parasite it is imperative that detailed, carefully-recorded observations be made while the patient is in hospital. All blood films (which should be taken in duplicate), laboratory data, and other pertinent documents should be retained so that they are available for subsequent scrutiny.

Any investigation of the response of malaria parasites to drugs generally and to 4-aminoquinolines in particular must be based on a standardized procedure described in section 4 of this report.

4. CRITERIA OF RESISTANCE OF MALARIA PARASITES TO 4-AMINOQUINOLINES

4.1 Range of response to drugs of naturally occurring strains

It has long been known that naturally occurring strains of human malaria parasite species vary in their response to antimalarial drugs. If a dosage of a particular antimalarial, standardized in relation to body weight, frequency and duration of administration, were given to persons with infections of the same species from various parts of the world it is probable that, using standard criteria of response to the drug and in the absence of complicating factors such as immune response, abnormal haemoglobin, G6PD abnormality, and the possibility of reinfection, a continuous range of "sensitivity" would be demonstrated.

The result of such a hypothetical study is illustrated in the figure on page 34.

The term "drug resistance" is somewhat loosely applied to any observed gradual or sudden decrease of sensitivity to a drug and is usually suspected in the field when parasites are found that tolerate a "standard drug dosage" previously regarded as sufficient to eliminate the infection.

One of the most important problems facing malariologists today is the recognition of true resistance in the field on the basis of the results of treatment. It is obvious that more attention should be given to some agreement on arbitrary criteria of resistance to chloroquine and other 4-aminoquinolines in the same way as criteria for insecticide resistance have been adopted by the entomologist.

In practice a field worker may be alerted by changes in the expected effect of the drug, shown either in failure to prevent malaria infection or
in disappointing effects of the drug on symptoms of the disease, parasitaemia and the achievement of radical cure.

The use of radical cure as a criterion of the sensitivity of malaria parasites is very difficult to assess. When a malaria infection has been apparently cured, a recurrence of symptoms means that the treatment was inadequate to eliminate all the asexual forms or that it failed to destroy persisting tissue forms. Relapses in *P. vivax* and *P. malariae* infections are known to originate from secondary tissue forms which resist the schizonticidal drugs and require the use of 8-aminoquinolines.

It is doubtful if persisting tissue stages of *P. falciparum* exist and recrudescences are therefore attributed to the persistence of a few remaining asexual parasites which survive the treatment. The pattern and duration of recrudescences of *P. falciparum* infections after treatment are far from being fully understood, especially as they appear to vary in relation to the strain of the parasite.

To infer that drug resistance is present because an apparently regular administration of a suppressive drug fails to prevent parasitaemia is often unsound, because supervision of the taking of the drug is possible only under exceptional conditions.
However, the persistence of parasitaemia, accompanied or not by symptoms, is certainly one of the best indications that resistant strains may be present, provided there is the certainty that the relevant drug was taken and absorbed. In every case of a disappointing response to treatment, it is prudent to suspect drug failure and possible causes of failure should be investigated.

The notification from the field of suspected drug resistance should immediately lead to the initiation of studies to detect other cases in the area.

The final conclusion can only be arrived at by transference of the parasite to a new and susceptible individual, either by inoculation of the blood or by the bite of a mosquito infected from the original subject, followed by demonstration of the persistence of parasitaemia despite normally adequate treatment or after administering higher than normal dosage of the drug. Such an investigation is not practicable in the field but can be carried out in a research centre where the allegedly resistant parasites can be studied with precision.

4.2 Standard procedure for determining the response of malaria parasites to chloroquine

In view of the serious implications of the development and spread of chloroquine-resistant strains, it is important that a uniform standard should be applied to all areas in which such strains are suspected to exist. It is also important to survey all areas in which malaria occurs, in order to provide basic information on the sensitivity of local strains of parasites to this drug.

At present several different procedures are being used for this purpose and adequate interpretation of the results on a comparative basis is impossible.

The standard procedure proposed below is carried out under field conditions. It is important that the method should be simple and interfere as little as possible with the normal lives of the people. Its proper application may indicate the presence of suspected resistance to an amount of chloroquine that has been selected for the purpose of the test. It will exclude a number of causes of drug failure that might otherwise give the impression that a resistant strain is present in the area.

Further investigations will be necessary to confirm or refute the presence of resistant strains; these can only be carried out in centres with the necessary specialized facilities.

The proposed test is applicable to trophozoite carriers found among the rural or urban populations, or the subjects may be found in dispensaries or hospitals. It may be carried out:

(a) during surveys for the investigation of local parasite strains; or
in areas where suspicion has been aroused that the doses of chloroquine usually administered for suppression or treatment are not producing the usual response.

In the test described chloroquine is given orally. In spite of some possibility of vomiting after the first dose of chloroquine by mouth, this route has been chosen for the standard test procedure in preference to injection of the drug because oral administration is easier to carry out under field conditions.

Injection of chloroquine has the advantage that vomiting is avoided and the dose is known to be retained. The results of testing the sensitivity of parasites to chloroquine given by injection may not be directly comparable with the standardized test procedure for oral administration. It is therefore important that the two methods should be compared side by side under both field and laboratory conditions.

The results of the proposed test are assessed by examination of thick blood films. It is considered that pyrexia is not a reliable criterion of the drug activity. In the field, transmission cannot be excluded and recrudescence cannot be differentiated from reinfection. For this reason parasite observations are confined to a few days immediately following drug administration.

All stages of the test must be supervised by a responsible medical officer; the technical staff must be experienced and reliable.

4.3 Field test for strain sensitivity to a standard regimen of chloroquine

4.3.1 Object of the test

The object of this test is to determine the response of a strain of malaria parasites to a standard test dose of chloroquine. The test may be performed on all persons irrespective of age, degree of immunity, parasite count and previous suppressive therapy. However, it is not applicable to a person who is seriously ill.

4.3.2 Procedure

The test is performed in one or two stages: the first requires the presence or the daily reporting of the subject for three to five days; if the second stage is necessary the subject will have to be observed for a further six days (total 11 days).

First stage. The standard test dose of 10 mg of chloroquine (base) per kg body weight will be given by mouth. Body weight will be measured to the nearest 3.25 kg (equivalent to one quarter of a 150-mg tablet); thus an adult of 60 kg receives 600 mg of chloroquine. In areas where 100-mg chloroquine tablets are used the weight of the subject can be measured to the nearest 2.5 kg (equivalent to one quarter of a 100-mg tablet).
The chloroquine tablets must not be coated and must comply with the standards laid down by the International Pharmacopoeia or the national pharmacopoeia of the country.

The subject must be kept under observation to ensure that the proper dose of the drug is ingested and retained. To avoid nausea or vomiting, the drug should not be given on an empty stomach. If the subject vomits he should not be used for the test. Duplicate thick blood films will be made immediately before the test dose is given and daily throughout the first stage of the test. One of each set of duplicate films must be kept for reference. Parasite counts will be made and the species of parasite identified. The film will not be considered negative unless 400 satisfactorily stained fields have been examined. Urine will be collected on the day prior to the first drug administration (day —1) and at least on day 1 and day 3 and tested for the presence of chloroquine by a suitable method. It must be noted that the clinical condition of the patient will at all times take precedence over the conduct of the test.

Alternative parenteral test. Workers who use this procedure give an intramuscular dose of 5 mg of chloroquine per kg body weight in sterile aqueous solution. This is followed by a second intramuscular dose of 5 mg/kg six hours later. This procedure replaces the first test dose of 10 mg/kg given on day 0 in the oral test described in the preceding paragraphs. In all other respects the test is carried out by the same methods and for the same purposes as the oral test.

Assessment of first stage. After the administration of chloroquine, the trophozoite count may diminish, remain the same, or increase.

(a) If the trophozoite count falls to zero within 72 hours of the ingestion of the drug, as is likely in the majority of subjects, the parasite strain may be considered to be sensitive to the test dose of chloroquine.

(b) If trophozoites are still present 72 hours after drug ingestion, the subject will be observed for a further two days.

(c) If at the end of day 5 trophozoites are absent from the blood films, the parasite may be regarded as sensitive to the test dose of chloroquine. If trophozoites are still present in the blood films, the second stage of the test will be carried out as follows:

Second stage. A course of four doses of chloroquine (a total of 1.5 g of base for a 60-kg adult) will be given over three days, according to the following scheme:

Day 6: 1st dose — 10 mg/kg (600 mg for a 60-kg adult)
2nd dose — 5 mg/kg (300 mg for a 60-kg adult) 6 hours after 1st dose
Day 7: 3rd dose — 5 mg/kg (300 mg for a 60-kg adult) 18 hours after 2nd dose
Day 8: 4th dose — 5 mg/kg (300 mg for a 60-kg adult) 24 hours after 3rd dose

1 See Annex 2.
At the time of each drug administration precautions will be taken to ensure that the drug is swallowed and retained. Blood films will be made on day 11, three days after the last dose of the course.

Assessment of second stage. If trophozoites are found in the blood film taken on day 11, the parasites may be suspected to be resistant to the standard course of treatment. A suggested outline for recording the results of the test is shown below.

**SUGGESTED OUTLINE FOR RECORDING THE RESULTS OF THE FIELD TEST FOR STRAIN SENSITIVITY TO A STANDARD DOSE OF CHLOROQUINE**

Name of subject: ..........................................................
Age: ............ Sex: ................................ Weight (kg): ............
Locality: .......................................................... Date of day 0: ............

Particulars of chloroquine tablets:
Brand and origin: ..........................................................
Dose of base per tablet: ..........................................................
Salt: ..........................................................

<table>
<thead>
<tr>
<th>Day</th>
<th>Parasites</th>
<th>Drug dose (mg base)</th>
<th>Urine test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Species</td>
<td>Trophozoite count/mm²</td>
<td></td>
</tr>
<tr>
<td>-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>8</td>
<td></td>
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<td>9</td>
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<td></td>
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<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Result: ..........................................................................

Action: .........................................................................

*a Two doses administered on day 6.
4.3.3 Application of the test

The numbers of trophozoite carriers to whom the test is to be applied will depend upon the circumstances. It is, of course, applicable on an individual basis, but if information on the base-line sensitivity of the local parasites is being sought adequate numbers (10-20) should be studied. If a detailed search is being made for the presence or absence of resistant strains much larger numbers should be submitted to the test.

On the basis of the results obtained resistance can only be suspected, as this field test may not entirely eliminate a number of possible forms of drug failure. To corroborate the evidence of drug resistance the strain must be further examined in a specialized reference centre.

4.4 Reference centre test for drug resistance

Laboratories with experience in the study of induced malaria have advantages over field studies in that expert staff and the necessary facilities and equipment are available. Infections can be studied in an isolated group of non-immunes in the absence of natural transmission. Facilities are available for mosquito transmission of selected strains of parasites, which can be stored frozen in liquid nitrogen for future reference, and there is more control and supervision of subjects. The response of the strain to the drug can be examined in a number of test subjects.

The strain of parasite suspected to be resistant to chloroquine is transported from the field to the laboratory in a human subject or a blood sample preserved in crushed ice or in infected mosquitoes. It may then be transferred to non-immune subjects by blood inoculation or by mosquito transmission and tested for response to chloroquine.

The clinical condition of the patient will at all times take precedence over the conduct of the test.

The following course of chloroquine is administered by mouth:

1st dose : 10 mg/kg
2nd " : 5 mg/kg 6 hours after 1st dose
3rd " : 5 mg/kg 18 hours after 2nd dose
4th " : 5 mg/kg 24 hours after 3rd dose.

Either of the following results indicates resistance of the parasites to the drug:

(1) Failure to reduce the parasite density, determined at least every six hours, within 48 hours of the first dose of drug, or an over-all increase in parasite density within that time.

1 The technique for the collection and transport of blood is described in Annex 1.
(2) Persistence at patent levels or the reappearance of asexual parasites with or without clinical symptoms within 2 months of the first dose of the drug. The presence of asexual parasites should be determined in at least two films, taken at different times. At least 0.1 mm$^3$ of blood should be examined.

It is recommended that studies be conducted with at least five individuals infected with each strain. The consistency or inconsistency of findings should be taken into account in interpretation of the data before reaching a conclusion as to drug resistance of the parasite.

When appropriate, tests may then be made on the effect of longer courses or of higher doses of chloroquine to assess the degree of resistance of the strain. Unduly large doses, especially if repeated at short intervals, should not be given without consideration of the possible toxic effects of the drug. Plasma concentrations of drug may be determined to obtain additional information. Studies of the response to other drugs and of cross-resistance patterns may be carried out.

In view of the present uncertainties regarding the relationship between therapeutic response and plasma concentrations of chloroquine (as determined by the methods at present available), the estimation of drug in the plasma can be regarded as qualitative evidence of absorption but is not a reliable criterion of drug resistance.

Advice on a suitably prolonged chloroquine regimen for suppression and treatment, which could be recommended for use in the area of origin of the strain, and on suitable alternative schizonticides should be made available to the field workers concerned at an early date.

5. EVALUATION OF THE PRESENT AND FUTURE IMPACT OF Drug RESISTANCE IN MALARIA ERADICATION

The potential importance of the impact on malaria eradication of any spread of resistance of human plasmodia to drugs might be assessed fully by taking into account the state of the global programme at the end of 1963. It appears that in countries from which reports are available, malaria eradication has already been achieved over areas inhabited by 343 million people; 762 million people live in areas where eradication programmes are still in progress; 275 million where pre-eradication programmes are being carried out and 122 million people live in areas where no plans for malaria eradication have yet materialized.¹

It is well known that the importance of chemotherapy in malaria eradication has increased considerably during the past few years. Drugs

are now used in all phases of malaria eradication programmes. Chemotherapy may not be essential during the first year of the attack phase but the supplementary use of drugs may accelerate the interruption of transmission. During the second or third year of the attack phase, as soon as case detection is in operation, the use of drugs becomes increasingly important. Their role at that stage is to provide immediate relief of clinical symptoms of malaria, and to make the patient non-infective to mosquitoes until the result of the blood examination becomes available. Radical treatment of relapsing infections has generally already come into operation during the last one or two years of the attack phase, in order to eliminate the largest possible number of infections before the cessation of spraying.

During the consolidation phase, the use of antimalarial drugs is of primary importance. Presumptive (single dose) treatment of all persons suspected of malaria and subsequent radical treatment of all confirmed cases are the principal measures for the elimination of all remaining infections and for prevention of the establishment of new foci of transmission.

Finally, during the maintenance phase antimalarial drugs are essential for the rapid radical cure of every imported case of malaria in order to prevent any new spread of the disease.1

The importance of chemotherapy is equally great if not greater in "problem areas" which, according to a recent definition of the WHO Expert Committee on Malaria,2 comprise "a defined geographical area within which an adequate epidemiological evaluation shows that the transmission of malaria persists despite total, complete, regular and sufficient coverage with residual insecticide, and where careful studies have revealed that administrative or operational factors are not responsible for the persistence of transmission and where additional measures are required in order to prevent the occurrence of new cases."

Although up to now the extent of recognized problem areas is relatively small, involving only about 16 million persons and representing only a minor part of the territory under the eradication programmes, these areas are having an untoward effect out of all proportion to their size. Generally, any special measures so far undertaken to stop transmission in these problem areas have been on a small scale and the results have not been rapid or conclusive. This delay in quickly eliminating known problem areas prevents the completion of the programmes now in operation and requires large additional expenditure.

Among the remedial measures, such as the use of new insecticides and larvicides, the increased scope for chemotherapy has been repeatedly stressed and it is obvious that the appearance of strains of malaria para-

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sites resistant to drugs would seriously impair the efficacy of chemotherapy.

Moreover, any unconfirmed reports on "drug resistance" are prejudicial, even if they cannot be substantiated in the course of later and more thorough investigation, as they undermine the reliance on an otherwise excellent drug and may be used as an easy excuse for administrative or operational deficiencies of some malaria eradication operations.

Significant problems of drug resistance arose in practice 15 years ago with the large-scale use of proguanil. A few years later the same problem appeared with regard to pyrimethamine. Disappointing though they were, these findings never had any important effect on the progress of malaria eradication. Proguanil, on account of its short-lived action, is not recommended in eradication programmes and pyrimethamine is used only for its sporontocidal activity and on a relatively modest scale.

Since 1961, the problem of drug resistance in malaria eradication has attracted renewed attention following reports of the occurrence of chloroquine-resistant *P. falciparum* in various parts of the world. The occurrence of such resistance may be of relatively little importance if the area is very limited or if transmission can be interrupted by the use of residual insecticides. On the other hand, any spread of this resistance may have most serious consequences for malaria eradication, the more so since cross-resistance to other types of antimalarials has been demonstrated.

There is usually considerable delay before the appearance of drug resistance is detected after it has first occurred and as a result of this there is a serious possibility of the spread of the resistant strain by the *Anopheles* vector or through movements of infected populations to new areas. Although with discontinuation of the drug the resistance may perhaps disappear as a result of dilution or biological disadvantages of the drug-fast strain of the parasite in competition with sensitive strains, very little evidence on this point is available.

Admittedly, knowledge of the mechanism of drug resistance in malaria parasites is inadequate, to say the least, but nevertheless some ways of preventing the appearance and spread of this phenomenon in eradication projects can be indicated.

Observations from the field show that resistance develops when the selection of drugs used for a particular chemotherapeutic purpose is not properly considered. The use at any dosage of essentially prophylactic or sporontocidal drugs for treatment of acute cases of malaria or for suppression of high parasitaemia should be especially avoided. It has been noted that the administration of unduly low or widely spaced doses of antimalarials increases the probability of drug resistance but in some instances experience has shown that administration of high doses of antimalarials is not a guarantee that drug resistance will not occur. With
drugs having a relatively long duration of action it is important that regularity and adequacy of dosage be maintained. Generally speaking, it seems that the combined use of drugs with different types of action and at adequate dosage may prevent the development of resistance.

Practical advice with regard to some currently used drugs is as follows. Pyrimethamine, biguanides or 8-aminoquinolines given for the purpose of gametocytocidal, sporontocidal or anti-relapse action should always be used together with a schizontocidal drug (4-aminoquinolines). Pyrimethamine alone should not be used for mass drug administration and especially not in medicated salt. For treatment of established infections 4-aminoquinolines are undoubtedly the best drugs (followed by 8-aminoquinolines when a radical cure of relapsing malaria is aimed at). The new long-acting injectable cycloguanil pamoate has as an active principle a dihydrotriazine derivative (a metabolite of proguanil) and is now being investigated in at least eight field trials in various parts of the world. The future value of this drug cannot be forecast in highly endemic areas where resistance to proguanil and pyrimethamine is known to appear relatively easily. The recent reports from small-scale field trials of this drug in New Guinea and West Pakistan suggest the presence of resistance in these areas, but full accounts of these trials are not yet available.

The most serious setback would be the widespread development of resistance to chloroquine and other 4-aminoquinolines; these drugs are today the main chemotherapeutic weapons used for presumptive treatment of suspected cases and (with the addition of 8-aminoquinolines) for radical treatment of confirmed malaria.

Every malaria eradication project should be viewed as a separate problem, with due regard to the species of parasite and vector, intensity of transmission, selection of proper drug or drugs, practicability of regular, frequent dosage, and the extent and nature of previous drug administration. Steps should be taken to ensure the early discovery of resistance; any possible needs for adequate quantities of a different drug should be anticipated and suitable action must be planned in advance.

6. PROPOSALS FOR COUNTERMEASURES
WHEN DRUG RESISTANCE IS CONFIRMED

When quinine was the only drug available for the suppression and treatment of malaria, deaths from *Plasmodium falciparum* infections were frequent. The daily dose of quinine necessary to avoid malaria fever in many parts of the tropics was high and well within the range that caused toxic side-effects; blackwater fever was an important cause of ill health and death. The treatment of many *P. falciparum* infections needed doses of quinine
that exceeded the limit tolerated by the host and such strains (assessed according to the previous definition) could be justifiably classified as relatively resistant to quinine.

With the advent of synthetic antimalarials, blackwater fever disappeared, and for the past 25 years, non-immunes have been able to live in highly endemic areas with little thought of the danger of malaria. It is likely that in some limited areas strains of malaria parasites less susceptible to these drugs are now liable to gain the ascendancy.

With the intelligent use of the drugs at present available, this can be avoided. But drugs cannot be used intelligently in the absence of a continuous supply of information on the developing pattern of degrees of sensitivity of malaria parasites to chemotherapy. Such information is essential if decisive action is to be taken to deal with strains that may be refractory to treatment or may constitute an obstacle to the control and eradication of malaria.

A warning note has recently been sounded and parasites are appearing that survive after drug regimens which a few years ago were generally used with success. The group considered the measures that should be taken to prevent the spread of resistant infections and to eliminate them from the area where their presence has been confirmed.

The Group agreed that, as in all public health activities, before effective steps can be taken, an estimate must be obtained of the urgency and extent of the problem. A survey must be carried out to determine whether the drug resistance is limited to a single focus or is more widely distributed; this can be done by means of the field test for strain sensitivity described in section 4.

Should the problem appear to be local, facilities available to the national health service might be sufficient for this survey, but any evidence of involvement of a large area would make it desirable for assistance to be obtained from WHO. A special team experienced in applying the field test for strain sensitivity would prove of value. This team could undertake an extended survey and should be able to give guidance on the dosage and nature of the drugs likely to be required in the areas concerned. The advisory activities of the team should be planned with the following objectives in mind:

(a) Spread of the resistant parasite must be limited as quickly and as effectively as possible.

(b) The resistant parasite must be eliminated in the host and in the vector so that the focus does not remain as a threat to malaria eradication.

(c) Future management of the area must be planned in such a way that effective schizontocides are available for the treatment of acute infections.
6.1 Measures applicable to foci of drug resistance

These objectives are not easy to attain and the measures that can be taken will depend upon the epidemiological, climatic, geographical, social and economic conditions of the area. Every focus of drug resistance must be treated as a separate problem and the past history of local malaria control measures must be taken into account. The following measures must be applied, with due consideration of the phase reached by any malaria eradication programme in progress in those areas where the resistant strain has been demonstrated:

(a) **Treatment of confirmed cases.** An effective drug must be used for treatment. This may mean the use of a combination of drugs (including quinine), but an increase in the dosage of chloroquine may cautiously be made unless it is apparent that resistance to this drug persists into higher dosages. It must be realized, however, that the limits of tolerance to chloroquine are not well known. Patients must not be discharged from this treatment until every effort has been made to ensure that they no longer constitute a source from which the infection can be transmitted by mosquitoes. For this purpose, a sporontocidal drug will be required; the choice of this drug will be guided by the epidemiological considerations and the information provided by the reference centre.

(b) **Presumptive treatment.** In areas where there is a high prevalence of resistant strains and transmission cannot be reduced by insecticide applications, the use of presumptive treatment with the drug to which the parasites are resistant will be ineffective; indeed, it may provoke increased resistance. In such circumstances any other drug and dose used for presumptive treatment must be able to cure the patient and render him non-infectious to mosquitoes. In some areas or foci of resistance and where administrative facilities are present, consideration should be given to a change from presumptive to radical treatment based on rapid diagnosis of malaria.

(c) **Mass drug distribution.** If the drug to which the parasites have been shown to be resistant is being used for mass distribution (for example in salt, or in regular dosage by other means), consideration must be given to its discontinuation and another drug may have to be substituted. This is not difficult in the case of proguanil and pyrimethamine which can readily be replaced by 4-aminooquinolines and primaquine. But the replacement of 4-aminooquinolines by other drugs is not easy, since according to available observations none of the synthetic antimalarials, including mepacrine, show striking curative effects when used against *P. falciparum* strains that are chloroquine resistant. The use of quinine combined with 8-aminooquinolines in such cases is feasible in small foci, but should be
given only under strictly supervised conditions. Other drug combinations may also prove useful.

The resistance may eventually diminish or disappear and a return can subsequently be made to the original drug. However, in some conditions steps may have to be taken with regard to the administration of drugs available in the area: thus there may be need to prevent the unrestricted public sale of malaria prophylactics through government control of import licences and by legislation. Existing supplies of drugs should be examined with regard to their authenticity and quality.

(d) Other measures. In order to prevent the spread of the focus various intensified insecticidal measures will be necessary to interrupt transmission and to destroy mosquitoes infected with the resistant strain. Residual spraying and space spraying must be extended to adjacent areas in an attempt to provide a barrier to the spread of resistant parasites. All these operations should be adapted to the phase which the given malaria eradication programme has reached.

Consideration should be given to the effects of population movement across the insecticide barrier in both directions. If possible, similar measures should be adopted to those that are applicable to the management of population movement with respect to reimportation of malaria in areas from which malaria has been eradicated.1

(e) Difficult areas. A special difficulty is presented by those areas in which communications are poor, and the terrain and habits of the vector make the economic application of insecticides by present techniques expensive and incomplete. The plan of operations for areas of this type should be reviewed and improved as much as possible. Health education of the public is of primary importance, and information on the risk of using inadequate regimens of drugs for individual prophylaxis should be given together with advice that people developing symptoms of acute infection should obtain medical care as soon as possible.

Special treatment centres may have to be organized in selected places within the area where radical cure and treatment of severe infections can be carried out. These centres should be provided with the necessary facilities for the management of serious P. falciparum infections.

In circumstances in which residual insecticides fail to give adequate control of the vector, the introduction of larvicidal and other operations must be considered.

7. RESEARCH ON DRUG RESISTANCE

As in the case of other chemotherapeutic agents, progress of research on the problem of resistance to antimalarials has been held back by lack of knowledge of the biochemical mechanisms by which these drugs act. The study of the mechanisms of drug resistance at the biophysical or biochemical level, which is proving so promising in bacteria, is difficult to apply to malaria parasites because of their obligatory relationship to red blood cells and the lack of a method for continuous cultivation of plasmodia in vitro. In fact, although the definition of drug resistance stresses the loss of response to the effect of the active compound, the degree of this response is difficult to assess in malaria as it can only be ascertained by tests in the infected host.

The effectiveness of the drug on the parasite depends on its rate of multiplication, the degree of the parasitaemia and the status of immunity of the host. Moreover, it is conceivable that some nutritional and other factors in the host play an important part in the response of the parasite to the drug or its metabolite and consequently influence the development of specific resistance.

Compounds such as proguanil and pyrimethamine, whose mode of action appears to be directed against some stage of folic acid metabolism of the parasite and which affect the division of the parasite nucleus, favour the appearance of resistant strains relatively rapidly under experimental conditions, whereas compounds such as mepacrine, chloroquine, pamaquine and quinine either do not produce resistance or do so only after prolonged contact with the strain.

The basic principle of specificity of drug action stresses the relationship between chemical structure and biological activity, and according to this principle parasites might be expected to show cross-resistance to chemically related compounds. However, this is not always the case and the occurrence of cross-resistance among compounds not related chemically underscores the lack of full knowledge of the biochemical action of specific drugs.

It is likely that the development of drug resistance in plasmodia, as in other micro-organisms, depends on a number of different mechanisms by which the parasite may escape the inhibitory effect of the drug. These include:

(a) A drug-sensitive metabolic path essential for growth in susceptible strains may be either absent or supplemented by one that performs the same function and is unaffected by the drug.

(b) The properties of the surface membrane of the cell or of intracellular organelae may be such that the drug cannot penetrate.
(c) The protozoan cell may possess a means of destroying the drug before it exerts its effect.

(d) The protozoan cell may produce a greater amount of the normal metabolite which effectively competes with the drug.

These four mechanisms are not mutually exclusive and the differences between sensitive and resistant organisms are not absolute; it is usually a question of degree.

The concept of drug resistance in micro-organisms is often applied to a strain, meaning by this a population not necessarily derived from a single organism. Speculation on the methods by which resistance develops involves two different but not necessarily mutually exclusive concepts. One concept postulates gene mutation in the sensitive organism which occurs sporadically and may involve changes in the metabolism of the cell, for instance in the type or amount of a particular enzyme produced or in the permeability of the cell membrane to the drug. There is evidence from work on bacteria that such mutations may occur irrespective of the presence of the drug, which acts only as a selective agent by killing the drug-sensitive organisms and allowing the growth and multiplication of the insensitive organisms. The other concept postulates an adaptive response of the organism to the drug and the resulting formation of new or increased amounts of enzymes, so that ultimately growth is able to take place in the presence of concentrations of drug which formerly were inhibitory. Usually, gene mutations occur rarely (1 in a million to 1 in 10,000 million cells) and independently of any known factor in the environment, but in some organisms the mutation rate can be increased by radiation, certain chemical compounds, or other mutagenic agents. Recombination of genetic material may occur as a result of a sexual union between cells and the genetic composition of cells may be changed by transfer of DNA from cells bearing some different character. Although gene mutations normally affect only a small proportion of a population, small differences between the survival rate of mutant and non-mutant organisms in the presence of the relevant drug may rapidly change the character of the population. Gene mutations have great stability although the probability of reverse mutation cannot be excluded.

The general consensus is that variations in the sensitivity of microorganisms to drugs are controlled by genes. However, the possibility that resistance may arise by an adaptive process cannot be dismissed; since drug resistance may be transmitted through many generations, the adaptive method must imply the replication of the resistant character.

To what extent these mechanisms of drug resistance in bacteria are applicable to protozoa generally and to malaria parasites in particular cannot be stated with any certainty, but the evidence suggests that resistance arises by mutation.
The two proposed mechanisms of the resistance phenomenon have the following characteristics:

<table>
<thead>
<tr>
<th></th>
<th>Genetic theory</th>
<th>Adaptive theory</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Origin</strong></td>
<td>Spontaneous</td>
<td>Induced by the action of the drug</td>
</tr>
<tr>
<td><strong>Frequency</strong></td>
<td>Rare with gradual increase of frequency</td>
<td>Frequent in the majority of cells</td>
</tr>
<tr>
<td></td>
<td>through selection. Does not occur in</td>
<td>Might arise in conditions that do not</td>
</tr>
<tr>
<td></td>
<td>conditions preventing growth</td>
<td>allow growth</td>
</tr>
<tr>
<td><strong>Stability</strong></td>
<td>Stable unless reverse mutation occurs</td>
<td>Less stable and related to the period of</td>
</tr>
<tr>
<td></td>
<td></td>
<td>contact with the drug</td>
</tr>
</tbody>
</table>

A rational approach to the practical problems of preventing drug resistance or combating it when it appears may include the following:

(a) Selection pressure in favour of drug-resistant mutants may be reduced by the avoidance of low doses of (infrequently administered) drugs, especially those that have shown great capacity for favouring the emergence of drug-resistant strains. Since the development of resistance may be related to the size of the population of organisms exposed to the action of the drug, a reduction in the number of organisms exposed to drug action may reduce the rate of the development of resistance.

(b) The frequency of emergence of drug-resistant mutants may be greatly decreased by the simultaneous use of two unrelated drugs, since any gene mutations occur independently of each other and the chances of mutation towards resistance against two drugs presumably having different loci of action will be remote.

(c) Drug resistance may be prevented by substances that inhibit specifically the formation or the action of enzymes responsible for destroying the drug or for providing the cell with an alternative drug-resistant metabolic pathway. However, substances having such “enzymic repression” activity are known at present only in a very limited field of antibiotics.

The amount of research work on the mechanism and genetics of drug resistance in malaria parasites of mammals generally and of primates in particular is strikingly small considering the importance of the problem, and greater activity in this field should be stimulated.

7.1 New antimalarial drugs

The advice “have a stock of other equivalent drugs for use when resistance occurs” seems to be a counsel of perfection as far as chloroquine is concerned, because no drug fully equivalent to chloroquine or other 4-aminoquinolines exists at the present time. This shows how narrow is the margin of security in the chemotherapy of malaria and how great is the need for new and better drugs.
The delay in the introduction of new antimalarial drugs has been due in part to the absence of an urgent need for additional therapeutic agents and in part to the absence of facilities for screening and for the pharmacological and toxicological evaluation of new compounds. Independent investigators who may be able to prepare a number of synthetic compounds of potential value are often unable to assess their antimalarial action on avian or rodent plasmodia, because well-established routine screening laboratories are relatively scarce and are normally available only to the research departments of pharmaceutical industry. The Group discussed the possibilities of extending the facilities for experimental chemotherapy now available and felt that an increased participation of research institutes and university laboratories in this field would be most beneficial and deserves much encouragement and assistance.

However, investigations of the biochemistry or genetics of drug resistance or search for new antimalarial compounds based on avian or rodent malaria must be interpreted with caution; there is need for a method of reproducing and treating the infection, in all its stages, in a convenient experimental animal more closely related to man, and recent developments in the field of simian malaria have stressed the importance of this approach. For this reason, research on simian malaria should be encouraged and actively supported by WHO and by other agencies.

The Group welcomed the recent creation of centres for research on primates and stressed their importance for solution of those problems of chemotherapy of malaria that require study of the response of higher animals susceptible to plasmodial infection.

The difficulty in subjecting any new antimalarial drugs first to a clinical trial and then to a field trial has been mentioned previously by the Technical Meeting on Chemotherapy of Malaria \(^1\) and the Group felt that much research now carried out on the experimental chemotherapy of malaria cannot be fully utilized and rapidly developed without better facilities for the trial in man of promising and relatively non-toxic candidate antimalarial compounds.

The possibility of testing new drugs by treating induced malaria in neurosyphilitic patients has now practically disappeared. On the other hand, early field trials of promising drugs in tropical countries are complicated by the factors of immunity, multiplicity of strains, possibility of reinfection etc., and the interpretation of the results of such trials is often difficult.

At the present time, human malaria research centres employing non-immune volunteers exist only in the USA. The amount and quality of scientific data obtained in these centres on the characteristics of drug-resistant strains of malaria parasites and on their response to drugs is

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invaluable, and the Group felt that medical science owes an immense debt of gratitude to the administrators of these institutions, to the research workers concerned, and above all to the courage and devotion of the volunteers.

The Group stressed that in view of the present urgent requirements of the chemotherapy of malaria, more of such human malaria research centres are needed in other parts of the world and considered that the setting-up of such centres should be stimulated and assisted by WHO.

7.2 Suggested research projects

In considering various aspects of the problems created by the resistance of malaria parasites to drugs, the Group felt that the following research projects would be of particular interest and practical importance in relation to the prevention and treatment of plasmodial infections of man:

1. The amount of field research on drug resistance should be increased. Studies on the epidemiology of transmission due to drug-fast strains are of importance and may be assessed by mathematical analysis.

2. There is an urgent need for the application to chloroquine of the standard procedure for determining the response of malaria parasites in order to provide base-line data in countries where resistance has not appeared and to delimit the affected areas where it has.

3. The response of resistant strains to increased dosage of drugs and means of rendering the subjects infected with these strains non-infectious to mosquitoes should be carefully studied.

4. Precise investigation of drug-resistant strains of malaria parasites whose presence in the field has been confirmed should be carried out in reference laboratories or human malaria research centres.

5. Strains resistant to one drug may yield to various combinations of drugs (particularly to pyrimethamine with a long-acting sulphonamide or sulfone) and intensive studies should be carried out on the response of various strains to mixtures of drugs available at the present time. There is a need for preliminary investigation of cross-resistance patterns in human malaria, in particular the resistance patterns produced by associations of two or more drugs.

6. Investigations are needed on the value of pyrimethamine and 8-aminoquinoline compounds as sporontocides in chloroquine-resistant P. falciparum infections.

7. Research on the improvement of drugs available at present and on the development of new drugs should be greatly increased with a view to discovering schizontocides and sporontocides with a rapid action, simi-
lar drugs with a prolonged action, and an anti-relapse drug free from side-effects and radically curative at a single dose or, at most, after a three-day treatment.

(8) There is need for studying a number of active drugs previously rejected as antimalarials on the grounds that they are uneconomic to manufacture or that they are too toxic to laboratory animals for consideration as long-term prophylactics in man. A drug with a narrower range of safety might be acceptable for the treatment of acute malaria which does not respond to any of the standard drugs. Moreover, much progress may be achieved through a search for drugs — not necessarily themselves antimalarials — which (a) reinforce the action of antimalarials, or (b) reverse the process that causes parasites to become resistant through enzymic modification or changes in membrane permeability.

(9) Research upon the physiology and biochemistry of malaria parasites is greatly needed in order to make possible an understanding of the mode of action of antimalarial drugs and the changes that the parasites undergo when they become resistant to drugs. One of the difficulties in undertaking such work is the inability to obtain adequate culture of the parasite, and investigations on the in vitro cultivation of mammalian plasmodia should be stimulated and assisted. Such a culture technique could be used both for a study of the parasite’s metabolism and for direct assessment of the activity of drugs and their metabolites. These studies should be carried out with plasmodia both of laboratory animals and of man.

(10) Studies of the mechanism of development of drug resistance by malaria parasites should continue on avian malaria, on rodent malaria, and particularly on simian malaria. The method of study should be based on the follow-up of the effect of the drug on the clones of the parasites maintained by serial inoculations. The trend and degree of the induced resistance to the drug (or drugs) should be studied, as also the stability of the resistance after passage through a mosquito vector and the presence of cross-resistance to related compounds.

(11) Much new and valuable knowledge can be expected from studies based on the transmission to other primates of plasmodia that infect man and on the investigation of the therapeutic action of promising compounds on these infections.

(12) It is suggested that in some circumstances sensitive P. falciparum schizonts may escape effective plasma concentrations of drug. Further studies on drug concentrations in plasma, on the confirmed presence of parasites (e.g., in P. coatneyi) in deep capillaries, and on the availability of drug fixed in the lungs, liver and other tissues may help in the understanding of relapses after chloroquine treatment. Radioisotope-labelling techniques may be of value for the elucidation of this problem.
(13) Injectable cycloguanil has shown promise in preliminary trials as a repository preparation. However, for the large scale use of this compound investigations are needed on the advisability of previous effective schizonticidal therapy and on the response to this drug in an area where proguanil and/or pyrimethamine were previously widely used.

(14) A simplified form of the present quantitative test for chloroquine in the plasma is greatly needed. The availability of such a test would allow the study of concentration curves of the drug in relation to therapeutic effects.

Further studies of the physiological disposition of chloroquine and other antimalarial drugs (including quinine) are required using modern analytical methods. Preliminary investigations should include studies on the rate and degree of absorption from the gastrointestinal tract after the administration of various doses up to those maximally tolerated; the rate of renal excretion should be measured concurrently. The metabolism of the drug should also be studied in various ethnic groups. A new method for the determination of small quantities of chloroquine in plasma and urine should be developed with a view to the eventual development of a simple, sensitive and accurate test to be used in the field.

(15) Parenteral administration of chloroquine ensures that the patient receives the dose and that it is not lost by vomiting. The use of this route as a standard procedure for the assessment of the response of parasites to treatment should be studied in comparison with the field test for strain sensitivity in which the drug is given orally.

(16) There is a great need to increase the speed and reliability of detection of malaria parasites in the blood. Three methods are of potential promise: (a) development of an electronic scanning apparatus which would select only positive (or probably positive) slides for further checking; (b) development of a new, simple and selective staining technique (such as fluorescent stain); (c) development of "enrichment methods."

(17) There is need for further development and application of the fluorescent antibody method and other immunological techniques for identification of various drug-resistant and drug-sensitive strains of human malaria parasites.

(18) Rodent plasmodia in which chloroquine resistance has been induced experimentally lose this characteristic rapidly after a few passages when the drug pressure is removed. The study of the rapidity of disappearance of chloroquine resistance in P. falciparum of man when the drug administration ceases may yield valuable data.

(19) The relationship between pigment production and the resistance to chloroquine of various mammalian plasmodia should be investigated to assess the recently postulated antagonistic action of ferrihemic acid.
(20) There is need for a new and sensitive method of measuring the activity of antimalarial drugs; the "respiratory test" used for immunological studies of trypanosomes may perhaps be adapted for this purpose.

(21) Comprehensive study of the stability of tablets containing antimalarial drugs and stored under tropical conditions is necessary. Such a study should also comprise preparations containing two or more antimalarials.

(22) Adjustment of the effective dosage of antimalarial drugs to the size of the patient requires investigation. It is possible that the adequate dose of antimalarial compounds in children should be calculated in terms of relative surface area and not by body weight or age group.\(^1\)

8. QUALITY CONTROL OF ANTIMALARIAL DRUGS

In discussing the problem of unconfirmed and at times unconfirmable reports on resistance of malaria infections to various drugs, the Group stressed repeatedly the need for taking into account the possibility of drug failure. It is necessary to discriminate between such causes of drug failure as deliberate or accidental omission of drug administration, vomiting, deficient absorption, etc., and the attention of the Group was drawn to two important problems related to the quality of pharmaceutical preparations generally and antimalarial compounds in particular.

It is now obvious that with the great increase in the number of manufacturers of pharmaceutical preparations it has become difficult to ensure that drugs comply with the specifications of the International Pharmacopoeia or national pharmacopoeias. Some countries import in bulk the active compound that complies with these requirements and use it to compound a pharmaceutical preparation that may not undergo the same thorough quality control.

Moreover an increased consumption of antimalarials in tropical areas has created the relatively new problem of deterioration of drugs during storage in inadequate conditions.

Experience in the pharmaceutical industry has shown that the instability of some drugs is of importance, as there can be considerable delay between the manufacture of a medicated product and its use by the population. Although antimalarial compounds are relatively stable, they can nevertheless deteriorate unless proper conditions of storage are provided in tropical countries.

\(^1\) See Annex 3.
The Group understood that WHO had already devoted some attention to these problems\textsuperscript{1} and that the present situation is as follows:

(1) Arrangements for an adequate quality control of drugs which exist in exporting countries may at present not be available in some of the importing countries. Attempts are now made to ensure that the pharmaceutical preparations exported from any country should comply with the same drug requirements as apply to drugs for domestic use.\textsuperscript{2} However, there may be no effective quality control for these preparations in their country of origin.

(2) An effective and practical way of controlling the quality of imported drugs is by checking representative samples in an official control laboratory of the importing country. Moreover, bilateral arrangements between the exporting and importing country can assist in solving some of the difficulties.

(3) WHO provides assistance for the establishment of national laboratories for pharmaceutical quality control and prepares specifications for the quality control of a large number of pharmaceutical preparations. While the Organization does not undertake the control of the quality of pharmaceutical preparations, it can supply names of national laboratories that could carry out such control. The Organization may assist with the quality control of drugs used in WHO projects but for other projects limits itself to giving names of laboratories that could be asked to carry out the analysis of the relevant drug.

The Group believes that in order to provide antimalarial drugs of satisfactory quality, some comprehensive pharmaceutical quality control must be exercised not only by the country exporting the drug but also by the one importing it. There is also need for some quality control at a more peripheral level when antimalarials can be obtained by unrestricted sale to the public. Such control would be helpful in eliminating preparations that have deteriorated through bad storage. Information on storage and package requirements of antimalarial drugs should be provided; this subject may be given increased attention by WHO and the information made available to the health authorities of tropical countries.


9. RECOMMENDATIONS

(1) The Group noted the available information concerning the varying response to treatment by antimalarials of different parasite species and of several strains of the same species. The Group stressed that the strain differences in susceptibility to drugs have a bearing on the results of application of standard treatment and recommended that more information should be sought on the dosages of drugs required for effective treatment of infections with diverse strains of malaria parasites in various parts of the world.

(2) Having reviewed the present situation with regard to the reported emergence of resistance to drugs in malaria parasites, the Group agreed that proguanil and pyrimethamine are the agents most frequently implicated.

Evidence for the local occurrence of resistance of *P. falciparum* to chloroquine has been obtained in some parts of the world.

(3) The Group noted that in some instances information concerning the apparent failure of treatment of malaria infections by 4-aminoquinolines is difficult to interpret. The Group stressed that certain observations in the field seem to be based on insufficient evidence and require careful checking.

The Group recommended that investigations be carried out in the field with the assistance of WHO to secure fuller information on the response of different species and strains to 4-aminoquinolines in various parts of the world.

(4) The Group having agreed that a precise definition of drug resistance applicable to all cases is most difficult, approved the following definition of relative resistance to drugs as adequate for practical purposes:

"Ability of a parasite strain to survive and/or to multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within the limits of tolerance of the subject."

(5) The Group agreed on the general importance of establishing a suitable method for assessment of the response of malaria parasites to treatment, approved the details of such a procedure in the field,\(^1\) and stressed the need for strict adherence to its recommendations.

(6) Having considered that there is need for establishing some definite criteria for recognition in the field of possible resistance to chloro-

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\(^1\) Sections 4.2 and 4.3 of the present report give the details of the standard procedure.
quine and other 4-aminodiquinolines the Group proposed a two-step field test for the determination of the response of malaria infection to a standard dose of chloroquine.

The Group agreed that the failure of the asexual parasitaemia to respond to the administration of the test dose of chloroquine should be regarded as the criterion of suspected drug resistance. The Group indicated that the field test will be of value only if uniformly applied with strict attention to details.

The Group stressed that confirmation of drug resistance of a parasite strain and the study of all its characteristics can be done only through transfer of the strain to a special reference centre having the facilities for inoculation of the relevant strain to non-immunes not exposed to reinfection.

(7) Having reviewed the available information on resistance observed in some species and strains of malaria parasites to certain drugs, the Group considered that resistance to proguanil or pyrimethamine is of relatively small importance for malaria eradication programmes. On the other hand, any confirmed drug resistance to chloroquine and other 4-aminodiquinolines may create a potentially serious problem for some malaria eradication programmes.

The Group stressed that at the present time the areas where chloroquine resistance has been reported and confirmed are few and that some observations still await confirmation.

Chloroquine and other 4-aminodiquinolines are the most valuable drugs used in all phases of malaria eradication and there is no evidence that the over-all situation with regard to the importance of these drugs has shown any significant change.

(8) The Group recognized the need for special measures in areas where resistance to antimalarial drugs has been confirmed and proposed that the main lines of action be directed toward eliminating the focus of the resistant strain or preventing its spread and at future management of the area taking into account its epidemiological conditions.

The Group emphasized that in some conditions the proposed action should be considered as an emergency measure which may require rapid and full technical assistance from WHO. Such an urgent action might be of particular importance in areas where operational difficulties exist in addition to technical problems encountered during the execution of a malaria eradication project.

(9) The Group reviewed the available information on the progress of research on antimalarial drugs and on the mechanism and prevention of drug resistance.

The Group drew attention to the slow progress of research on new antimalarial compounds and urged that research on the improvement of
existing drugs and on the development of new drugs be vastly increased, with the encouragement and assistance of WHO and of national governments.

The Group wished to express its full agreement with the relevant recommendations of the Technical Meeting on Chemotherapy of Malaria, convened by the World Health Organization in 1960.1

(10) The Group noted that present facilities for experimental chemotherapy of malaria are manifestly insufficient, especially with regard to the screening of potentially valuable antimalarials on plasmodia of birds, rodents and primates. The Group suggested that WHO should facilitate, co-ordinate and assist the development of better facilities for the screening of antimalarial compounds.

The Group stressed the importance of simian malaria for research on new drugs and for the study of many aspects of drug resistance, and it welcomed the possibilities now available in this field through the creation of primate research centres.

The Group expressed the conviction that any substantial future progress in the chemotherapy of human malaria will depend on the knowledge gained in human malaria research centres through the investigations carried out in the course of treatment of neurosyphilitic patients or human volunteers. The Group wished to pay tribute to the work carried out in the existing research centres and to emphasize the urgent need for the creation of other human malaria research centres of equal value.

(11) The Group agreed that there are large gaps in our knowledge of the biochemical action of antimalarial drugs and of the mechanism of drug resistance and that both fundamental and applied research in this field should be greatly expanded; this expansion should be encouraged, co-ordinated and assisted by WHO.

The Group considered the needs of basic and applied research in the field of chemotherapy of malaria and drug resistance and submitted for the attention of the Organization and national research institutes a list of projects that deserve technical and financial assistance.

The Group stressed the exceptionally good possibilities available to the Organization for field research on the recognition of suspected drug resistance and on its geographical distribution and spread.

(12) The Group considered the possible relationship between drug failure and the quality of drugs for prevention or treatment of malaria and concluded that the present arrangements for quality control of antimalarials freely available to populations living in malarious areas should be improved.

The Group agreed that quality control schemes should be extended to the countries receiving the preparation containing the active compound

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and that deterioration due to improper storage of these drugs must be avoided.

The Group recognized that WHO is already giving attention to the general problem of quality control of drugs and stressed the need for giving high priority to the specific problem of antimalarials in order to provide the necessary guidance to the national governments concerned.

(13) The Group recognized with appreciation the present scope of scientific information on chemotherapy of malaria and on the problem of resistance of malaria parasites to drugs gathered, assessed, consolidated and distributed by WHO.

The Group recommended that this valuable activity of the Organization be maintained and, if necessary, expanded in order to co-ordinate the world-wide effort devoted to chemotherapy of malaria.
Annex 1

DISPATCH OF PARASITIZED BLOOD
TO SPECIAL MALARIA REFERENCE CENTRES

A. Materials

1. Containers and anticoagulant

Venules or "Vacutainers" of 5 or 10 ml capacity which already contain the anticoagulant are preferable for drawing the blood and for dispatch of the sample. They are provided with an appropriate sterile needle for venepuncture and the vacuum speeds up the flow of blood.

As an alternative, a 10-ml sterilized, dry syringe with an appropriate sterile, dry needle may be used for drawing blood.

As anticoagulant heparin (2 mg to 1 ml of blood) or 2% sodium citrate in 0.85% sodium chloride solution should be added in the proportion of 1 part of citrate solution to 4 parts of blood.

For dispatch of the blood sample, 5-10-ml rubber-capped sterile serum (or vaccine) vials can be used when the blood is drawn with a syringe.

2. Protective wrapping

The venules or vials should be wrapped in about 50 cm of surgical gauze, secured with a length of thin but strong string.

3. Refrigerant

For the field, a portable ice-box filled with chipped ice is needed. If blood is collected in a hospital, the normal refrigerator can be used for storage of the sample before dispatch.

4. Shipping container

For long-distance dispatch, a vacuum flask (thermos flask) is needed. It must be large enough to contain the samples together with at least 20 times their bulk of chipped ice. The vacuum container should be inspected before use for the good fit of the stopper and firm support of the inner flask. Good adhesive tape is need to secure the cap.

5. Packing material

The vacuum flask should be packed in a small wooden crate or lined wicker basket. As an alternative, corrugated cardboard and strong string may be used. Gummed labels are also required.
B. Procedure

1. A blood film must be examined immediately before the collection of the blood sample to confirm that the donor has an adequate degree of parasitaemia. (Success in the transfer of infection by this method is much more certain when the parasite count is about 5000 per mm$^3$.)

2. Blood is taken from a vein under aseptic conditions and 5 to 10 ml of blood are drawn into the venule (“Vacutainer”). If a syringe is used, the blood can be mixed with the anticoagulant in the syringe before it is gently transferred into the vial. Utmost care must be taken to avoid contamination at this stage.

3. The vial is labelled with the identity of the donor, date, and place of origin. The label and ink must be waterproof.

4. The sample is put immediately into the ice-box for transport from the field and then in the refrigerator (not in the freezing compartment) to await dispatch.

5. Before dispatch, the vial is wrapped in several layers of gauze and securely tied. A length of thin string is attached to it for easy removal from the vacuum flask.

6. The vacuum flask is half filled with crushed ice (not with solid carbon dioxide, also known as “dry ice”) and the sample is placed on top of the ice; the flask is then filled to the top with more ice. It is then corked so that a length of the string attached to the vial will protrude and permit its easy withdrawal. The cap is screwed on tightly and secured with adhesive tape.

7. The vacuum flask should be packed in a light wooden crate, a lined basket, or a cardboard parcel to protect it against damage during transport. The whole parcel must be adequately labelled and should be sent by the fastest route (usually by air) to reach the laboratory in not more than 5 days from the time the specimen was taken.

8. A cable should be despatched to the laboratory indicating the flight number and estimated time of arrival of the plane.

9. It is advisable to make preliminary arrangements with the receiving laboratory before specimens are sent, and to confirm these arrangements by cable if necessary.
Annex 2

ASSESSMENT OF URINARY EXCRETION
OF SOME ANTIMALARIAL DRUGS

Methods concerned with the assessment of urinary excretion of proguanil and pyrimethamine are today of limited interest. A description of the estimation of proguanil in urine under field conditions in Assam by the method of Gage & Rose (1946) has been given by Gilroy (1952). Pyrimethamine can be assayed in biological fluids by one of the methods introduced by Brodie & Udenfried (1945) and Brodie et al. (1947), their modification by Schmidt et al. (1953), or the method described more recently by Clyde & Shute (1956). For the determination of pyrimethamine in urine, especially in the field, these methods are not suitable, although Smith & Ihrig (1957) obtained reasonably good results. Generally speaking none of these methods is adequate for quantitative or even qualitative assessment of pyrimethamine given in the usual doses.

On the other hand, the qualitative and, in some instances, quantitative estimation of chloroquine in urine are of far greater practical importance. It should be remembered that the excretion of antimalarials depends not only on their actual concentration in body fluids but also on the acid-base balance of the individual. The urinary elimination of chloroquine is higher when the urine is acid and lower when the urine becomes alkaline; these changes may be due to the type of food consumed and also to other physiological or pathological factors. For rapid recognition of chloroquine in samples of urine under field conditions, qualitative tests are adequate inasmuch as they simply indicate whether a subject (or a group of people) is ingesting the administered drug. References to quantitative methods have been given by Bruce-Chwatt (1959), Fuhrman & Koénig (1955), Pereira & Paulini (1956), and in Guide-Lines for the Use of Medicated Salt (Pinotti’s Method) in Malaria Eradication Programmes.¹

The following qualitative tests for detection of the presence of chloroquine in urine can be easily applied in the field:

1. Wilson & Edeson’s test (1954)

   Reagent:
   
   Mercuric chloride (HgCl₂) .... 6.8 g
   Potassium iodide (KI) .......... 24.9 g
   Distilled water ................ 500 ml

   Mayer-Tanret’s reagent

Method: A few drops of Mayer-Tanret's reagent are added to a few millilitres of fresh clear urine. A white turbidity appears which disappears on heating and reappears as the urine cools down. If albumin is present the turbidity increases on heating. The test can be carried out, however, if the albumin is removed by boiling and subsequent filtration. The test is more sensitive if the urine is cold (half an hour in the refrigerator) (Pille & Lambourg, 1958). The sensitivity of the test is about 0.4-1.0 mg per 100 ml. This test becomes positive within 12 hours after the administration of a single dose of 600 mg of chloroquine base and remains positive in most subjects for 5-6 days.

2. Haskins' test (1958)

Reagents:

Sodium hydroxide solution, 10%

Chloroform or ethylene dichloride, purified

Methyl orange solution (prepared by dissolving 0.1 g of methyl orange, indicator grade, in 100 ml of 5% boric acid solution). Shake the mixture for a few minutes. Allow the solution to stand for a few hours or overnight and filter it. The clear filtrate is used as a reagent. If a precipitate forms again it may be removed by filtration without loss of efficiency. This reagent is stable for some time.

Method: To a test-tube containing 5 ml of urine, 1 ml of sodium hydroxide solution and 5 ml of chloroform are added. The tube is corked and shaken for 1 minute and the layers are then allowed to separate (centrifugation may be necessary). The supernatant layer is pipetted off and the chloroform is carefully transferred to a clean tube; 0.5 ml of the methyl orange solution is then added to the chloroform, the tube is corked and shaken for 20 seconds, and the layers are allowed to separate. Urine containing 0.2 mg of chloroquine per 100 ml gives a perceptible reaction. 0.5 mg per 100 ml gives a definite yellow colour and 1.0 mg per 100 ml gives an intense yellow colour. This test becomes positive within 4-5 hours after the administration of a single dose of 300 mg of chloroquine base; it remains positive for 4-5 days and slowly declines in intensity until it becomes indistinguishable from the blank on the tenth day.

In carrying out the tests for detection of chloroquine in urine it is advisable to compare the urine sample to be examined with a control urine sample from a person who has not been taking the drug. There are other basic drugs that may give false positive tests for chloroquine, such as quinine, pamaquine, primaquine, codeine, amphetamine, ephedrine and pethidine.

It should be stressed that neither of these two tests is reliable for the detection of amodiaquine excretion in urine, since the amodiaquine-
methyl-orange complex is nearly colourless and cannot be distinguished from the blank.

References

Pille, G. & Lambourg, J. (1958) Med. trop., 18, 131

Annex 3

ADJUSTMENT OF THE DOSAGE OF ANTIMALARIALS

It has been suggested that in adjusting the dose of a drug to the size of a patient when testing strain sensitivity, it is preferable to modify the dose in accordance with surface area rather than with body weight. It is claimed that for many drugs the validity of adjusting the doses in accordance with relative surface areas is widely confirmed by clinical experience, and that if the adult dose of a drug is adjusted for a child according to body weight the child will be underdosed.1

This could clearly have an important effect when chloroquine is given to children in the standard test for strain sensitivity in malaria. If the children are “underdosed” on the body-weight basis, there is a possibility that a strain of parasite fully sensitive to standard dosage in the adult might persist in the child and be recorded as relatively resistant.

The surface areas of animals are approximately proportional to their respective weights raised to the power 2/3. In order to assess the effect that such an adjustment would make on the dosage of chloroquine to

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children, the relative figures for the standard single dose of 600 mg of chloroquine base for a 60-kg adult have been calculated and are shown in Table 1. The dose of 600 mg is the maximal single dose (10 mg/kg) recommended for adults and children by the International Pharmacopoeia.

**TABLE 1. DOSAGE OF CHLOROQUINE BASE CALCULATED IN RELATION TO THE WEIGHT AND TO THE SURFACE AREA OF THE SUBJECT**

<table>
<thead>
<tr>
<th>Body weight (kg)</th>
<th>Dose (mg) adjusted to weight (10 mg/kg)</th>
<th>Dose (mg) adjusted to relative surface area</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>800</td>
<td>730</td>
</tr>
<tr>
<td>70</td>
<td>700</td>
<td>660</td>
</tr>
<tr>
<td>60</td>
<td>600</td>
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<td>380</td>
</tr>
<tr>
<td>20</td>
<td>200</td>
<td>292</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td>180</td>
</tr>
</tbody>
</table>

The adjustment in terms of the number of chloroquine tablets (containing either 100 mg or 150 mg of base), given to the nearest quarter-tablet, is shown in Table 2.

**TABLE 2. DOSAGE OF CHLOROQUINE TABLETS IN RELATION TO THE WEIGHT AND SURFACE AREA OF THE SUBJECT**

<table>
<thead>
<tr>
<th>Number of 100-mg tablets</th>
<th>Number of 150-mg tablets</th>
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</thead>
<tbody>
<tr>
<td>Dose adjusted to body weight</td>
<td>Dose adjusted to surface area</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>5 1/2</td>
</tr>
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<td>3 1/2</td>
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<tr>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>1 1/2</td>
</tr>
</tbody>
</table>

The desirability of ensuring that subjects of all age-groups receive pharmacologically equivalent doses of the drug is recognized. However, no tolerance data are available to support the absolute increase in the dosage of chloroquine that would result in small children from adopting the adjustment in relation to surface area. A general recommendation for such an increase of dosage could not be justified. Nevertheless, it would be highly desirable to pursue as a research project a study of the therapeutic advantages weighed against possible side effects of dosages of chloroquine related to surface-area measurements.
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