

Update Le point

Articles in the *Update* series give a concise, authoritative, and up-to-date survey of the present position in the selected fields, and, over a period of years, will cover many different aspects of the biomedical sciences and public health. Most of the articles will be written, by invitation, by acknowledged experts on the subject.

Les articles de la rubrique *Le point* fournissent un bilan concis et fiable de la situation actuelle dans le domaine considéré. Des experts couvriront ainsi successivement de nombreux aspects des sciences biomédicales et de la santé publique. La plupart de ces articles auront donc été rédigés sur demande par les spécialistes les plus autorisés.

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Drug-resistant malaria—occurrence, control, and surveillance*

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Chloroquine-resistant strains of Plasmodium falciparum were first reported in the early 1960s, and are now found in many areas of Asia and South America. Prevalence and degree of resistance are increasing in all affected areas. This represents a serious setback to antimalaria programmes, since alternative drugs are considerably more expensive and often more cumbersome to use. A concerted effort therefore has to be made to arrest the spread of resistant strains by developing standardized national policies on drug use.

This report considers the probable genetics and epidemiology of drug resistance and highlights the problems involved in its control. It also describes the WHO global programme which has recently been established to monitor the prevalence and degree of drug resistance and to develop effective operational countermeasures.

Resistance of human plasmodia to modern synthetic antimalarials was observed soon after these drugs had become widely used. Although pyrimethamine and proguanil resistance was first reported in the early 1930s, this did not present a serious challenge to antimalaria programmes while malaria parasites continued to be highly susceptible to 4-aminoquinolines, especially to chloroquine and amodiaquine, which could be used for both malaria treatment and suppression.

Resistance of *Plasmodium falciparum* to 4-aminoquinolines was reported from Asia and South America in the early 1960s, but the extensive and regular use of effective residual insecticides kept chloroquine-resistant *P. falciparum* to a few small and rather isolated foci. However, over the last 10 years, a drastic reduction in the use of residual insecticides because of financial and technical difficulties has led to the spread of malaria. Thus, chloroquine-resistant strains of *P. falciparum* are now found in many areas where the prevalence of the disease had been greatly reduced and where chemotherapeutic measures had consequently become the main and often the only measures of malaria control.

Autochthonous infections with chloroquine-resistant *P. falciparum* have since been reported from 21 American and Asian countries. There are also strong indications that

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chloroquine resistance has established a bridgehead on the east coast of Africa. In some countries, resistance has become so widespread and intense that existing drug regimens have had to be changed and chloroquine has been replaced by far more expensive alternative drugs. These developments, and the slow progress in the development of new antimalarials, emphasize the importance of the rational use of existing drugs and the deployment of efficient measures against drug-resistant malaria.

BIOLOGY AND GENETICS OF DRUG RESISTANCE

Antimalarial drugs interfere with important physiological functions of the parasites. Their action may be confined to particular stages of the parasite life-cycle if stage-specific metabolic systems are inhibited or interrupted, or may extend over several developmental phases if common systems are affected.

Chloroquine and mepacrine apparently block acid proteases and peptidases in the phagosomes of intraerythrocytic parasites. This disruption of the digestive process leads to amino acid deprivation. Pigment clumping is an outward sign of the disturbed metabolism. *P. falciparum* has two binding sites for chloroquine, one of which is identical with the quinine receptor. Chloroquine is selectively taken up by the chloroquine-susceptible parasite and the concentration of the drug found in infected erythrocytes is more than 100 times higher than in non-infected erythrocytes.

Quinine, like chloroquine, is believed to intercalate with deoxyribonucleic acid (DNA), but it probably binds to more than one site.

Pyrimethamine and proguanil inhibit dihydrofolate reductase (DHFR) and thus the biosynthesis of nucleotides. There are marked quantitative differences in the binding to parasite and host DHFR.

Sulfonamides and sulfones compete with 4-aminobenzoic acid (PABA) and inhibit dihydropteroate synthetase and thus the biosynthesis of nucleotides. They show a potentiating effect with pyrimethamine, acting in principle on the same pathway.

Primaquine disrupts the structure and function of the parasite mitochondria.

Baseline susceptibility data for commercially available antimalarial drugs do not exist as suitable assay methods were not available at the time of their introduction. Nevertheless, circumstantial evidence from *in vitro* tests suggests that strains of *P. falciparum* from various parts of the world, although primarily susceptible to chloroquine, exhibit, *a priori*, different sensitivities. Thus, *P. falciparum* in the Sobat valley of Ethiopia and in central Sudan appears to be significantly less susceptible to chloroquine than the Uganda I strain, although *in vivo* tests confirmed clinical and parasitological efficacy corresponding to an S response.^a

There are no indications yet of chloroquine resistance in *P. vivax*, *P. malariae*, or *P. ovale*. However, in the absence of *in vitro* assay techniques for these species it is difficult to assess quantitative differences in the response of isolates from different geographical regions.

While there is no evidence of primary resistance of human pathogenic plasmodia to DHFR inhibitors, it is evident that *P. vivax* responds very little or not at all to dihydropteroate synthetase inhibitors.

Primary differences in the tissue schizontocidal action of primaquine are known to exist in *P. vivax*. Higher primaquine doses are required for the radical treatment of infections with the Chesson strains found in Papua New Guinea than for treatment of infections with *P. vivax* strains from other parts of the world.

^a Details of the spectrum of response of malaria to drugs are given in WHO Technical Report Series, No. 529, 1973 (*Chemotherapy of malaria and resistance to antimalarials*: report of a WHO Scientific Group), p. 30.

Chloroquine-resistant malaria parasites show a defect of chloroquine binding. Consequently their chloroquine uptake is markedly reduced in comparison with susceptible parasites. All quinine-resistant *P. falciparum* isolates are also chloroquine resistant, and vice versa, thereby confirming the existence of common binding sites.

Resistance to DHFR inhibitors is associated with the production of new DHFR isoenzymes with a considerably reduced drug binding capacity. The reduction of binding may be drug-specific, so that resistance to pyrimethamine is not necessarily accompanied by resistance to other DHFR inhibitors, e.g., proguanil or trimethoprim.

In *P. falciparum* infections, the potentiating action of PABA competitors on DHFR inhibitors often yields full clinical and parasitological effects even when pyrimethamine resistance is present.

The clinical and parasitological response of malaria infections to blood schizontocidal drugs is, in practice, often influenced by the immune status of the patient, especially in the case of RI resistance. Parasite isolates, whether susceptible or resistant, usually show a wide range of responses within a given population. In non-immune subjects, a resistant response is to be expected even in the presence of a relatively small absolute number of resistant parasites; in contrast, semi-immune subjects may still show full clinical and parasitological response in the presence of a small proportion of resistant parasites.

Investigations of the genetics of drug resistance in cloned populations of rodent malaria parasites suggest that resistance to both DHFR inhibitors and 4-aminoquinolines is the result of the spontaneous mutation of nuclear genes. Subsequent drug pressure results in the selection of the mutants. In resistance to DHFR inhibitors, multiple loci seem to be involved, some of which may produce mutants that are simultaneously resistant to DHFR inhibitors and dihydropteroate synthetase inhibitors. Genetically, resistance to chloroquine is apparently independent of resistance to DHFR inhibitors, and both are highly stable in the absence of drug pressure. In mixed populations of chloroquine-resistant and chloroquine-sensitive rodent plasmodia the former show a distinct biological advantage, even in the absence of drug pressure; such advantage is not seen in the case of DHFR inhibitors.

Recombination of genes determining drug resistance frequently occurs in mosquitos when female gametes are fertilized, but it has not been observed at any other stage of the plasmodian life cycle.

DISTRIBUTION AND SPREAD OF DRUG RESISTANCE

Quinine-resistant strains of *P. falciparum* were first encountered at the beginning of this century, in Brazil. Sporadic cases of quinine resistance, although rarely substantiated, continued to be reported from various countries until widespread use of this drug was superseded by modern synthetic antimalarials. While mepacrine was in use, resistance of *P. falciparum* to this drug was reported only once, in Papua New Guinea.

Resistance of *P. falciparum* to DHFR inhibitors usually appears shortly after starting their use in mass drug distribution schemes, once considerable drug pressure has built up. Parasites resistant to one type of DHFR inhibitor are often resistant to others. It was demonstrated in East Africa that resistant strains were spreading rapidly in areas adjacent to the operational zones, and gradually mixing with the sensitive strains. Today, foci of falciparum malaria resistant to DHFR inhibitors are scattered in most areas where the disease is endemic. Their distribution is patchy and their exact location is often unknown, since systematic monitoring was not attempted at the time when 4-aminoquinolines were the drugs of choice and their efficacy was not threatened by the occurrence of resistance. So far, DHFR inhibitors are the only drugs to which resistance has been reported in human plasmodia other than *P. falciparum*.

Resistance of *P. falciparum* to chloroquine was first reported from Colombia and Thailand in the early 1960s, and has since spread to other areas of South America and Asia. In Asia, chloroquine resistance has recently been reported from Papua New Guinea, from the state of Orissa in India, from Solomon Islands, and from Hainan Island off the southern coast of China; it probably occurs also in other parts of southern China. In South America, recent reports substantiated the presence of chloroquine-resistant *P. falciparum* in Ecuador, French Guiana, and Suriname. Although earlier reports of chloroquine-resistant falciparum infections in Africa have invariably proved to be unsubstantiated, recent reports from Kenya and the United Republic of Tanzania have caused great concern and require urgent investigation.

The chronological sequence of occurrence and the distribution of chloroquine-resistant falciparum infections are shown in Fig. 1. The relative prevalence of chloroquine-resistant infections and the degree of resistance are still on the increase in all affected areas. This process is more pronounced in countries of South-East Asia than in South America where the level of resistance is usually lower. With the exception of a few areas in Brazil and Venezuela, the proportion of resistant cases is still moderate in South America, and RI responses constitute the majority of all resistant cases. However, in the Lao People's Democratic Republic, Thailand, and Viet Nam, RII and RIII responses are more frequent and the majority of falciparum cases are resistant to 4-aminoquinolines.

Various theories have been advanced to explain the appearance and the dynamics of the spread of chloroquine resistance in these two areas of the world. Reviewing the situation in South-East Asia, Verdrager et al.^b concluded that there was a direct relationship between the occurrence of chloroquine-resistant falciparum malaria and the presence of *Anopheles balabacensis*. However, Tigertt and Clyde^c saw the major reason for this occurrence in the genetic predisposition of the local strains of malarial parasites, which had initially shown a low response to quinine. These authors postulated that the development of resistance to chloroquine had by now occurred in all areas where *P. falciparum* was genetically capable of producing resistant strains and that any future spread of resistance would probably be due to importation. Studies carried out on local vectors in South-East Asia suggested that they were able to transmit chloroquine-resistant strains more readily than sensitive strains. This ability was apparently enhanced after the patients in the area had been treated with chloroquine.

The other factors that may have facilitated the appearance and spread of chloroquine-resistant strains in these areas are:

(a) the reduction in the use of residual insecticides, because of technical and operational problems;

(b) the relatively high potential for the spread of falciparum malaria.

EPIDEMIOLOGICAL CONSIDERATIONS

The development of drug resistance in areas with previously susceptible parasites has so far always been associated with the use of the particular medicaments. Four main factors seem to be involved:

- the degree of drug pressure
- the degree of host/parasite contact
- the duration of drug pressure
- the type of drug used.

^b VERDRAGER, J. ET AL. Chloroquine-resistant falciparum malaria in East Kalimantan Indonesia. *Journal of tropical medicine and hygiene*, **79**: 58-66 (1976).

^c TIGERTT, W. D. & CLYDE, D. F. Drug resistance in human malaria. *Antibiotics and chemotherapy*, **20**: 246-272 (1976).

Drug pressure is highest in areas where antimalarials are routinely used for suppressive purposes, on the basis of full coverage of all age-groups. If such drug pressure is maintained over a sufficiently long period, it may lead to the selection of resistant parasites, especially if measures are not taken to protect against the development of drug resistance, for example, in the case of prophylaxis with pyrimethamine alone. Resistance is apt to appear and spread faster in human populations with naturally low or artificially reduced immunity, especially in areas where little or no vector control is applied.

Exclusively therapeutic use of 4-aminoquinolines for the purposes of radical cure seems to exert little selection pressure. Wherever chloroquine resistance has developed, it has been in areas where the drug was massively employed for suppressive purposes, often in a haphazard way. Nevertheless, a considerable number of successful malaria eradication programmes have used chloroquine uniquely and systematically for presumptive and radical treatment without any indication of drug resistance.

Laboratory experience with rodent plasmodia has shown that the development of specific drug resistance can be induced against practically all antimalarials. This process is relatively rapid with DHFR inhibitors but takes longer with chloroquine, quinine, and mefloquine. *In vitro* observations with *P. falciparum* in continuous culture confirm these findings, which are also borne out by the results of field studies on the occurrence of pyrimethamine and chloroquine resistance.

The distribution of chloroquine-resistant *P. falciparum* in South-East Asian and Western Pacific regions largely coincides with that of *A. balabacensis balabacensis*. While a specific, advantageous biological adaptation of the chloroquine-resistant variant to this vector species cannot be ruled out, it is more plausible to explain this feature in terms of the vector's exophilic behaviour. Most of the areas concerned were originally meso- or hypodemic for malaria. The attack measures in areas with endophilic vectors consisted mainly of intradomiliary spraying with residual insecticides, while in areas with exophilic vectors, increasing use was made of suppression with drugs. The same applied to moving populations and groups with temporary dwellings. These initial attack measures were successful in curbing transmission and thus in reducing the malaria reservoir to a low level, except in forested hill tracts where *A. b. balabacensis* was one of the main vectors. Immunity to malaria in the population dropped in consequence, and this, combined with the high drug pressure and lack of effective vector control promoted the occurrence and spread of drug resistance.

The apparent arrest of the westward spread of chloroquine-resistant *P. falciparum* in Bangladesh and north-eastern India seemed to be circumstantial evidence of its specific association with *A. b. balabacensis*, until a new focus of resistance was detected in the state of Orissa in 1979, and in other areas outside the distribution of this vector species. Until further investigations have been made on the susceptibility of *P. falciparum* in West Bengal it is difficult to decide whether the occurrence of resistance in Orissa was the result of contiguous spread or "leapfrogging". However, observations in various countries of eastern Asia indicate that the transmission of chloroquine-resistant *P. falciparum* in those areas is by no means confined to *A. b. balabacensis*; other vector species are also involved wherever they are not adequately controlled.

Most of the vectors in the South American areas with chloroquine-resistant *P. falciparum* are endophilic and could be controlled by intradomiliary spraying with suitable insecticides, except in a rather limited area with exophilic *A. nuneztovari*. However, suppressive drug administration has usually been the only form of malaria control in areas where residual spraying could not be applied for financial, logistic, or security reasons or where major population movements offered little prospect of success for this type of measure. In these areas, chloroquine-resistant *P. falciparum* occurred in the presence of several vector species, without signs of a specific adaptation of the resistant variant to a particular vector.

The sporadic occurrence of resistant (RI) *P. falciparum* infections in non-immune subjects who have visited Kenya or the United Republic of Tanzania cannot yet be ascribed to the selection of resistant strains from autochthonous parasite populations, although several local factors favour such a selection, e.g., high drug pressure, moderate prevalence of malaria in comparison with other areas of tropical Africa, and absence of systematic vector control. However, East Africa is exposed to the importation of malaria cases from South-East Asia and it is conceivable that foci of chloroquine-resistant *P. falciparum* may have originated from imported cases, especially in the presence of the above-mentioned conditioning factors.

Isolated findings of clinical (*in vivo*) resistance to 4-aminoquinolines need to be thoroughly investigated, as deviations from the standard schedule of radical treatment or, occasionally, individual differences in absorbing, metabolizing, and excreting drugs may be responsible for an inadequate response. In such cases the drug sensitivity of *P. falciparum* should be determined *in vitro* whenever possible.

CONTROL OF DRUG RESISTANCE

The consequences of chloroquine resistance affect the treatment and control of malaria and also have repercussions on research into the chemotherapy of the disease.

The occurrence of chloroquine-resistant falciparum malaria, whether imported or indigenous, requires the urgent attention of the health authorities, since inadequate treatment of these infections may result in death. Therefore, several operational measures have to be undertaken, and important decisions will have to be made.

The first group of measures involves:

- (i) informing all health institutions about the dangers posed by chloroquine-resistant malaria to the patient and the community;
- (ii) providing alternative antimalarial drugs to all institutions treating malaria cases;
- (iii) elaborating simple and explicit instructions for the administration of these drugs.

The notification of resistant cases to the public health authorities is necessary and a system for the investigation of alleged chloroquine resistance should be developed.

The occurrence of chloroquine resistance is a serious setback to antimalaria programmes, since it precludes the use of a cheap and previously effective drug. None of the currently available alternatives, used alone or in combination, has the efficacy of chloroquine on strains of normal sensitivity. Alternative drugs are considerably more expensive than chloroquine, and some are also more cumbersome to use. There are also indications that resistance may develop relatively rapidly to the most acceptable alternative drugs—a combination of sulfonamides and pyrimethamine.

Instructions must be given concerning the principles of drug use in antimalaria programmes in the event of the spread of drug resistance:

(a) Chloroquine or other 4-aminoquinolines, if they continue to be employed by the programme for presumptive, mass, or radical treatment, should not be used alone, but in combination with gametocytocidal drugs.

(b) Alternative drugs, i.e., those not belonging to the 4-aminoquinolines, should not be employed for mass prophylaxis. This is particularly important in areas where the presence of chloroquine-resistant *P. falciparum* has already been confirmed, as it is essential to preserve the drug's efficacy in the treatment of malaria cases.

Important decisions then have to be made concerning the development of national policies on the rational utilization of antimalarial drugs, in particular prevention of their

uncontrolled use, selection of appropriate drugs and suitable drug dosages and schedules, standardization of indications for treatment, and the establishment of reliable means of drug supply and distribution.

Finally, an effective strategy must be decided upon for arresting the spread of chloroquine-resistant malaria, and for selecting appropriate countermeasures. The type and the complexity of the countermeasures will obviously depend on many factors, such as the extent and degree of resistance, the prevailing epidemiological conditions, the response of malaria to other attack measures, and the capability of the programme and the health services to find and to apply flexible and diverse alternative methods. The following principles should be considered in the planning and implementation of operational countermeasures:

(a) In countries or areas subject to the continuous importation of chloroquine-resistant malaria cases, antimalaria activities should be directed towards their early detection. They should also aim to prevent the establishment of foci of chloroquine-resistant disease wherever conditions for malaria transmission exist. The countermeasures should therefore include intensive case-detection activities at points of entry into the country and other strategic points; radical treatment of all imported malaria cases with suitable drugs in combination with gametocytocidal drugs; intensification of vigilance in countries where malaria has been eradicated; and the establishment of surveillance operations in areas of high receptivity and vulnerability to malaria. In areas where these measures are impracticable on account of a high malaria prevalence or incidence in the population, effective antivevector measures, epidemiological investigation of all alleged autochthonous cases of chloroquine-resistant malaria, and prompt administration of focal measures should be implemented.

(b) In countries or areas where the presence of chloroquine-resistant foci is confirmed, the antimalaria activities should aim at their elimination. After the size of the foci and the extent of the problem (frequency and degree of resistance, pattern of response to other drugs, conditions that favour occurrence of drug resistance) have been assessed, intensive vector control measures, whether directed against the larval forms or adults or both, should be applied. These should aim to reduce malaria transmission, and consequently the reservoir of infection, to the lowest possible level, and at least to a level low enough for surveillance to become a meaningful and effective task. Mass drug administration, using a combination of alternative and gametocytocidal drugs, may be used for the speedy elimination of small foci that are easily amenable to control. Whenever technical or operational reasons do not permit the elimination of chloroquine-resistant foci, antimalaria measures should be instituted in order to prevent the spread of chloroquine resistance to other areas. The practical application of a rational policy on the use of antimalarial drugs, and the intensification of malaria control measures, both call for increased participation by the community and by individuals. Communal and individual cooperation in leaving insecticide residues undisturbed, in avoiding creating mosquito breeding places and in reducing existing ones, in the daily use of knock-down insecticide sprays, in mosquito-proofing of sleeping quarters, and in the use of bed-nets could be of considerable value in determining the success of the general measures. It should, however, be remembered that indiscriminate use of drugs for prophylaxis may render them useless and lead to disaster for the community in the absence of a third group of easily applicable medicaments.

(c) In countries or areas where chloroquine-resistant foci of *falciparum* malaria are widespread, the antimalaria programme should aim at minimizing the effects. This requires the development of a national policy on the use of antimalarial drugs, the supply of alternative drugs for treatment, even in the most remote areas, and the intensification of antivevectorial measures. In view of the threat of chloroquine-resistant *P. falciparum*, the affected countries should strive for malaria eradication rather than control, a goal which most of them are capable of achieving.

The occurrence and spread of resistance of *P. falciparum* to 4-aminoquinolines, the limited number of alternative drugs, their limitations and high cost, all underline the need for research in the field of malaria chemotherapy. The development of operationally useful alternative medicaments is a priority within the programme of the Scientific Working Group on the Chemotherapy of Malaria of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

MONITORING OF DRUG SENSITIVITY

Methods

Sensitivity to blood schizontocidal drugs can be assessed by *in vivo* and *in vitro* methods. These have practical limitations, but the evaluation of sensitivity is feasible for the main drugs used by applying one or both techniques.

In the *in vivo* methods a standard radical cure treatment is given and the response of the plasmodial infection is assessed over a period of seven days when using the WHO Standard Field Test, or preferably over 28 days with the WHO Extended Field Test.^d

The interpretation of results is summarized in Fig. 2. The Standard Field Test permits a quick observation of the occurrence of RII and RIII responses, but the Extended Field Test is required to differentiate between S and RI responses. The results of the *in vivo* tests may be influenced by the immune status of the patients and this should be taken into consideration when conducting this type of test in areas with high malaria endemicity.

In vitro tests permit, in principle, an objective measurement of the sensitivity of *P. falciparum* to all directly-acting blood schizontocidal drugs. There are three major *in vitro* test systems: the macrotechnique and the microtechnique in short-term culture, based on the methodologies of Rieckmann et al.,^{e,f} and an *in vitro* test using material grown in continuous culture.

The macrotechnique is suitable for use with 4-aminoquinolines and mefloquine and is carried out with blood obtained through venepuncture. After defibrination, aliquots of 1 ml of blood are added to vials containing glucose alone (controls) or glucose and different concentrations of the drug. The vials are incubated for 24–28 hours at 38.5 °C and thick films are made from the contents of each vial. After staining with saline Giemsa, schizont counts are made on all slides and growth inhibition in the drug vials is calculated relative to the control readings.^g Full inhibition of schizont maturation at a concentration of 1 nmol of chloroquine per ml (1×10^6 mol/litre) indicates chloroquine sensitivity; schizont growth at 1.5 nmol of chloroquine per ml indicates resistance. The *in vitro* test results do not permit definite conclusions regarding the degree of resistance, but schizont maturation at 2.5 nmol chloroquine per ml (2.5×10^6 mol/litre) is usually a sign of RII or RIII resistance.

The microtechnique employs the same growth medium as the continuous culture and can be used for sensitivity tests with 4-aminoquinolines, mefloquine, and quinine. The test is performed on 8 cm × 12 cm flat-bottom, plastic tissue-culture plates containing samples of different drug concentrations and controls. Blood is taken by means of a heparinized or EDTA-treated capillary and suspended in reconstituted growth medium. Aliquots of 50 µl of the suspension are added to the test wells; the plate is placed in a candle jar^h and after

^d WHO Technical Report Series, No 529, 1973 (*Chemotherapy of malaria and resistance to antimalarials: report of a WHO Scientific Group*).

^e RIECKMANN, K. H. ET AL. Effects of chloroquine, quinine and cycloguanil upon the maturation of asexual erythrocytic forms of two strains of *Plasmodium falciparum* *in vitro*. *American journal of tropical medicine and hygiene*, 17: 661-671 (1968).

^f RIECKMANN, K. H. ET AL. Drug sensitivity of *Plasmodium falciparum*. An *in vitro* microtechnique. *Lancet*, 1: 22-23 (1978).

^g For full technical details of the macrottest, see WHO document WHO/MAP/79.1, 1979 (Instructions for use of the WHO test kits for assessment of the response of *Plasmodium falciparum* to chloroquine).

^h TRAGER, W. & JENSEN, J. B. Cultivation of malaria parasites. *Nature (London)*, 273: 621-622 (1978).

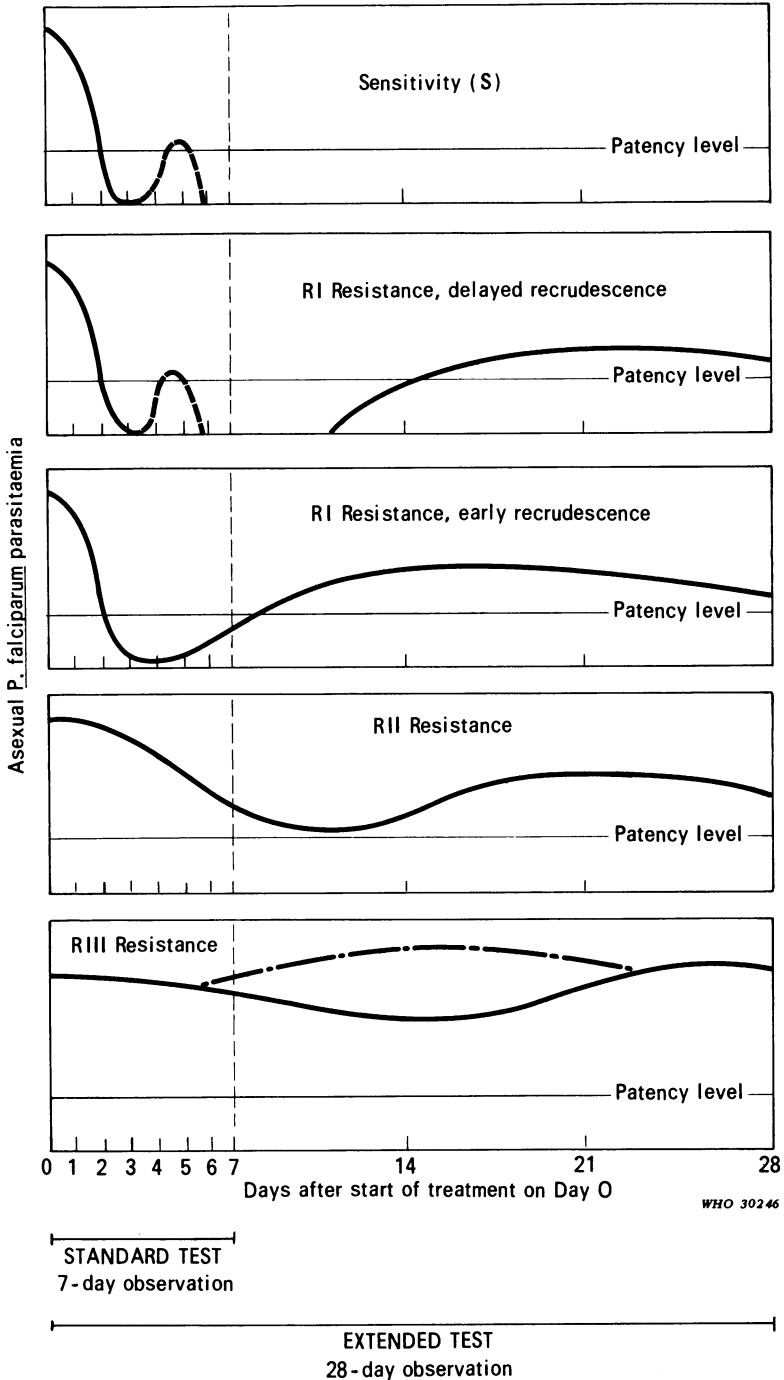


Fig. 2. Response to field test for sensitivity of falciparum malaria to chloroquine. Drug is administered during first 3 days. In sensitive and RI resistant strains, parasitaemia may reappear by day 5. There may be an increase in parasitaemia in RIII resistance. Note that it is not possible to distinguish between S and RI in the standard (7-day) test (from WHO Technical Report Series, No 529, 1973).

lighting the candle and closing the jar, the jar is placed in an incubator. Alternatively a sealed candle water bath can be used. After incubation at 37.5 °C for 24–26 hours the plates are taken out and the supernatant removed from the individual wells. Thick films are prepared from the erythrocyte sediment, stained and read for schizont maturation, the findings in the drug wells being expressed on the basis of the controls. The interpretation of the results is similar to that in the macrotechnique, the results being expressed in pmol of drug per μl of blood.

A comparison of the macrotechnique and the microtechnique (Fig. 3) shows nearly identical base values and regression coefficients. It can therefore be expected that the microtechnique will eventually replace the macrotechnique since it has certain advantages, such as the need for a smaller volume of blood, which can be obtained from a fingerprick instead of venepuncture, and a generally higher success rate. Moreover, the application of the technique does not depend on the availability of particular growth stages of trophozoites, nor is it limited by a particular parasite density.

The microtechnique is currently undergoing extensive evaluation and this should yield essential information on the shelf-life of the materials used in the test, interplate variability, drug dose/parasite density correlation, and other important factors. Based on this experience it is hoped to standardize test material and to produce test kits for general use.

An *in vitro* technique using long-term culture has been described by Richards & Maples.⁷ With this technique total growth inhibition is used as a measure of drug effect—as opposed to the inhibition of schizont maturation in the short-term tests—and it is possible to test DHFR inhibitors. As parasites produced by continuous culture are used, it is restricted to research laboratories. The results obtained with this system need to be interpreted with some caution since adaptation to culture could selectively eliminate parts of the original parasite population.

None of the *in vitro* systems is as yet adapted to testing with drugs which act through metabolites.

Global programme of monitoring

As drug resistance of malaria parasites is a problem of current or potential concern to all malarious countries and to those exposed to the importation of malaria cases, WHO has developed a global monitoring programme. Planning and execution are coordinated at regional level, usually through the regional advisory Committees on Medical Research and the WHO regional offices. The programme was first implemented in 1977 in the South-East Asia Region. It is now also well advanced in the Region of the Americas and the Western

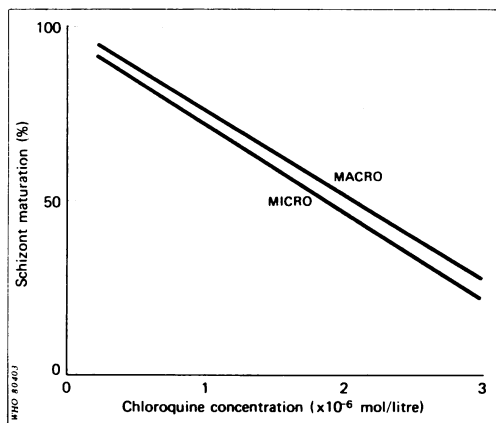


Fig. 3. Chloroquine-induced inhibition of schizont maturation in chloroquine-resistant *P. falciparum*: comparison of the macrotest and the microtest in eight isolates from Prabudhabat, Thailand. Linear regressions.

ⁱ LÓPEZ ANTUÑANO, F. J. & WERNSDORFER, W. H. *In vitro* response of chloroquine-resistant *Plasmodium falciparum* to mefloquine. *Bulletin of the World Health Organization*, 57: 663-665 (1979).

^j RICHARDS, W. H. G. & MAPLES, B. K. The effect of chloroquine and pyrimethamine on parasite growth and viability. *Annals of tropical medicine and parasitology*, 73: 99-108 (1979).

Pacific Region, and is in its early stages in the African and Eastern Mediterranean Regions. Research activities are carried out by the malaria services and collaborating institutes in the countries concerned. Global coordination is effected through the Scientific Working Groups on Applied Field Research and on Chemotherapy of Malaria and their secretariats, as the programme has components of both field and basic research.

The results obtained are collated and evaluated at country, regional, and global levels. Data on baseline assessment and monitoring of parasite sensitivity are published once a year in the *Weekly epidemiological record*. Other results are circulated in the form of WHO documents or published in WHO serial publications.

The global programme has the following objectives in relation to falciparum malaria:

- Assessment of the current geographical distribution, prevalence, and degree of resistance to 4-aminoquinolines.
- Monitoring of sensitivity levels and the spread, relative prevalence, and degree of resistance to 4-aminoquinolines, with the aim of facilitating the implementation of operational countermeasures.
- Assessment of sensitivity to currently used drugs other than 4-aminoquinolines and to candidate antimalarial compounds in order to determine their clinical and operational usefulness and baseline data.
- Methodological research on the determination of drug sensitivity.
- Studies on the epidemiology of drug-resistant malaria.
- Elaboration of guidelines on the clinical management of malaria resistant to 4-aminoquinolines.
- Development of operational measures for limiting the spread and eliminating foci of drug-resistant malaria.

While all of these objectives apply to the global programme, individual countries have selected specific sets of objectives and a schedule of implementation which are relevant to the local epidemiological situation.

In view of the importance of *P. vivax* in many parts of the world, studies related to vivax malaria are being conducted in some countries. However, large-scale testing of drug susceptibility is envisaged only at a later stage of the programme, after improvement of methods, and after major progress has been made in the priority area of drug-resistant falciparum malaria.
